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Production of aliphatic carboxylic acids during the alkali catalysed decomposition of cellulose

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Abstract

The degradation of cellulosic materials under alkaline condition (sodium hydroxide) using High performance ion exclusion chromatography (HPIEC) Dionex ICS-3000 to analyse the samples at different temperatures (at room, at 50 °C, at 90 °C), under atmosphere of N_2 during 188 hours, by using acrylic acid as internal standard, resulted in complex mixture of compounds, including isosaccharinic acid.

The retention time of aliphatic organic acids measured under the conditions outlined in experiment 1.was pyruvic (6.70),gluconic (7.90), glyceric (9.10),lactic (9.70), formic (10.50), glycolic (11.20), acetic (13.80), propionic (14.30), and acrylic acid (16.20).

As the pK_a of the aliphatic organic acid increases the retention times increases and it can also be seen in table 4.1 that as the number of carbon numbers in the compounds increase the retention time increases.

The identification of aliphatic acids formed during the alkali catalysed decomposition of cellulose in the experiments following at least six different acids are being produced in significant amounts and these are saccharinic acids, glycolic acid, lactic acid, formic acid and the final peak is that of the internal standard acrylic acid.

It is very clear that at room temperature the lactic acid is the most abundant decomposition product followed by glycolic acid with formic acid being present in much smaller amount. As the temperature is raised to be 50 ^oC, the amount of formic acid generated is significantly higher: Lactic acid glycolic acid formic acid. On a weight by weight base there is approximately the same amount of lactic acid and formic acid produced after 100 hours. In comparison, at 90 ^oC formic acid is now by far the most abundant of the small aliphatic acids, the amount of lactic acid has also increased significantly whilst there is a more modest rise in the amount of glycolic acid that is generated: Lactic acid, glycolic acid, formic acid. From these result sit is clear that the different fragmentation reactions, leading to the partitioning of the intermediate to the different small acids have different activation energies.

Chapter one

Background

1. Carbohydrates

Carbohydrates are the most abundant macromolecules in the world. There is a wide spectrum of different carbohydrates, the simplest members of the family of compounds have the empirical formula $C_n(H_2O)_m$, and contain an oxygen atom attached to each carbon atom. The most basic structural units in the carbohydrate family are monosaccharides and these include glucose, an aldose, and fructose, a ketose. The next higher analogues are disaccharides such as sucrose. Disaccharides can be hydrolysed using acid catalysts or enzymes to generate monosaccharides e.g. sucrose is hydrolysed by sucrase to give glucose and fructose. An aldose has an aldehyde CHO group at C-1, the simplest example of an aldose is glyceraldehyde, and ketoses have a keto-carbonyl and a hydroxymethylene (CH_2OH) group at C-1 dihydroxyketones (fig 1).

The common monosaccharides have an unbranched carbon chain, the majority are water soluble and are difficult to crystallise. The vast majority of carbohydrates exist as *oligosaccharides* and *polysaccharides* and these are formed by coupling *monosaccharide* units together by acetal or ketal linkages.[1]

1.1. **Monosaccharides : Aldoses and Ketoses**

In living systems, monosaccharides are used for generating energy i.e. they are an important fuel substaining biological processes through the generation of ATP, they are also important structural units e.g. as building blocks for nucleic acids. As stated above, monosaccharides are classified by their type of carbonyl group, aldose or ketose, to which is added a prefix to denote their number of carbon atoms. The suffix *-ose* is used in naming carbohydrates, although there are a number of exceptions.

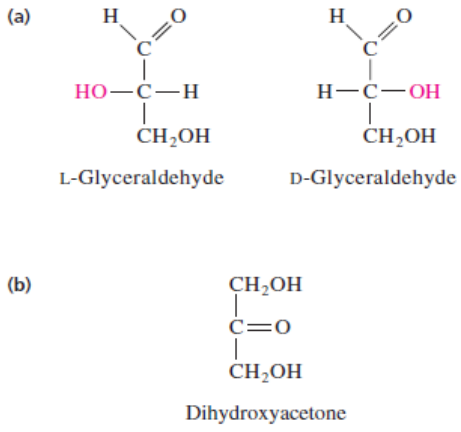


*Figure1.1: Fischer projections of **(a)** glyceraldehyde and **(b)** dihydroxyacetone.The designations L (for left) and D (for right) for glyceraldehyde refer to the configuration of the hydroxyl group of the chiral carbon (C-2). Dihydroxyacetone is a chiral* [2]

The simplest monosaccharide contains three carbon atoms (see figure 1). One of these is the carbonyl carbon, and normally each of the remaining carbon atoms bears a hydroxyl group. In aldoses, the most oxidized carbon atom is C-1. While, the most oxidised carbon of a ketose atom is usually C-2. [2,26]

Higher monosaccharides, with four, five, six, and seven carbon atoms are called tetroses, pentoses, hexoses, and heptoses. Apart from the carbonyl carbon, the majority of carbons contain four different substituents and are therefore sterogenic centres and most monosaccharides can exist as a range of stereoisomers. Because of the multiple asymmetric carbons, they exist as *diastereoisomers i.e.,* isomers that are not mirror images of each other.[15]

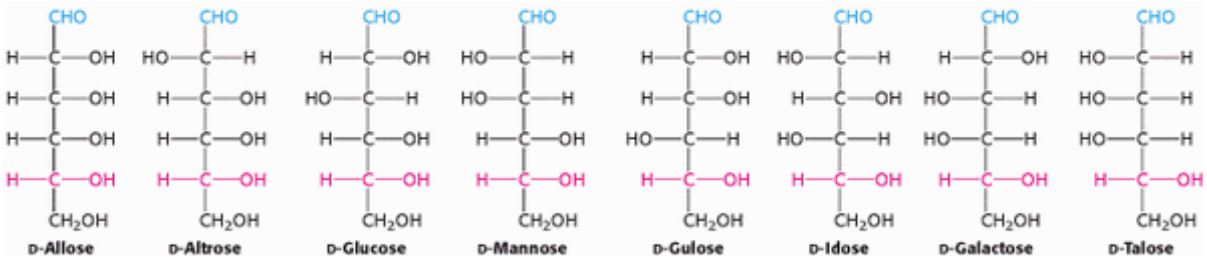


Fig 1.2 : Monosaccharides (aldoses) with six carbon atoms[2]

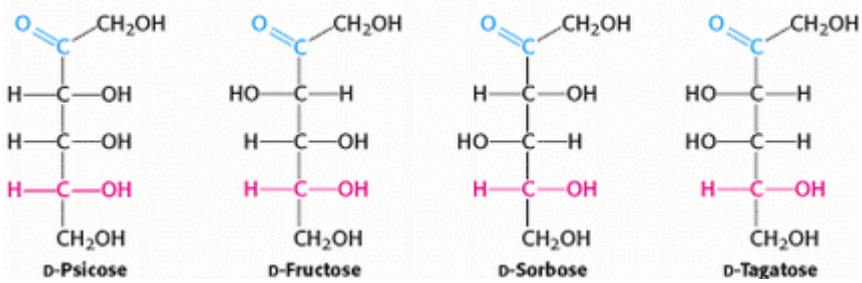
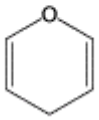


Fig 1.3 : Monosaccharides (ketoses) with six carbon atoms [2]

1.1.1. Ring conformations-Pyranose and Furanose

As monosaccharides contain both carbonyl and hydroxy functional groups they can react to form a hemiacetal or hemiketals. In aldoses such as glucose the C-1 aldehyde in the open-chain form(fig 1.4) reacts with the C-5 hydroxyl group to form a *hemiacetal.* The resulting cyclic hemiacetal, is called *pyranose* and contains a six-membered ring because of its similarity to *pyran* (fig 1.4). In ketoses, the C-2 keto group in the open-chain form such as fructose can form an *intramolecular hemiketal* by reacting with either the C-6 hydroxyl group to form a six-membered cyclic hemiketal or the C-5 hydroxyl group to form a five-membered cyclic hemiketal. The five-membered ring is called a *furanose* because of its similarity to *furan.*[2]

Furan



Pyran

Fig 1.4: Furan (five membered cycle) and Pyran (six membered cycl) rings [2]

1.1.2. Anomeric configurations of α, β Pyranose

The cyclisation of monosaccharides to form rings generates a new stereogenic centre that is frequently referred to as the anomeric centre and two different anomeric configurations are possible: alpha (α) and beta (β). When monosaccharides are drawn in the D-furanose or the D-pyranose form, the anomeric carbon atom is designation as α when the hydroxyl group (on the opposite side of the ring from the group at C-5) attached to C-1 is below the plane of the ring; β means that it is above the plane of the ring. The C-1 carbon atom is called the anomeric carbon atom (fig 1.5), and the α and β forms are called anomers.[2]

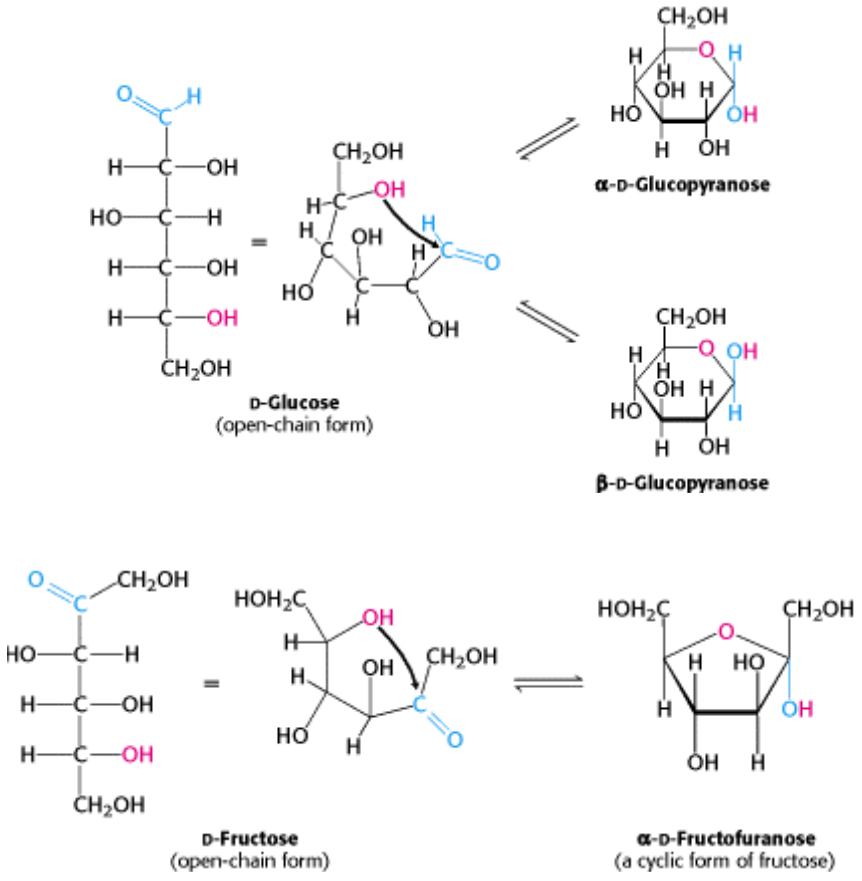


Fig 1.5: α, β (Pyranose and Furanose)[2]

1.1.3 **D and L sugars**

As stated above, the majority of sugars containing more than one stereogenic centre (chiral centre) and stereoisomers exist that are not mirror images ie. Those are diasteromers. The normal rules of stereochemistry apply and for a sugar with (***n)*** stereogenic centres (CHOH groups) there are 2^n stereoisomers. For higher sugars than glyceraldehyde, the absolute configuration of the sugar is denoted by the prefix D-*enantiomer* or L- *enantiomer*. The notation describes the absolute configuration of the secondary alcohol at the highest numbers stereogenic centre; this is R- for D-sugars and S- for L-sugars (fig 1.6).

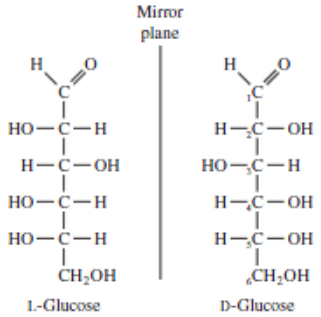


Fig1.6: L,D-Glucose [26]

In classical nomenclature,[3] sugars with the hydroxyl groups on the same side are referred to as erythrose whilst those on opposite sides are termed threose (fig 1.7)

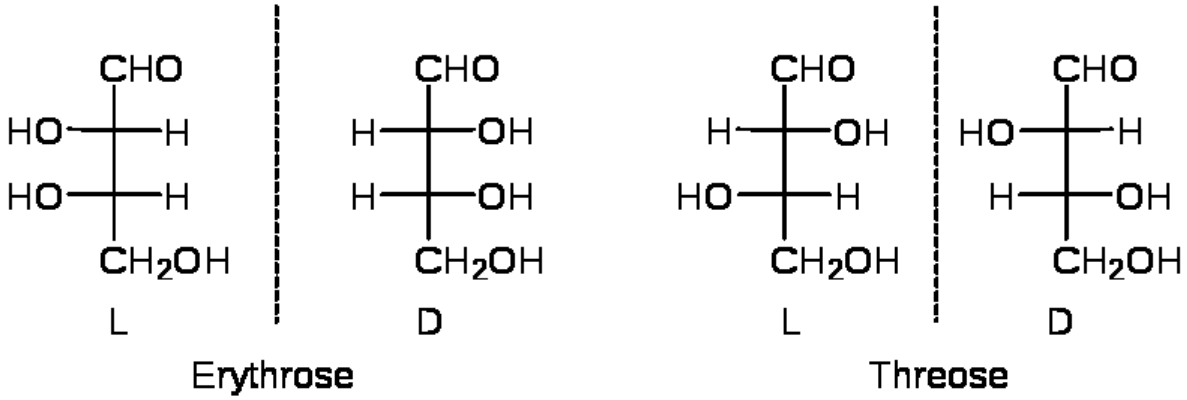


Figure 1.7: Erythtose and Threose enantiomers [3]

1.2. Disaccharides

As stated above, disaccharides consist of two sugars joined together by an O-glycosidic bond. Three of the most commonly occuring disaccharides are sucrose, lactose, and maltose (fig 1.8). Sucrose is table sugar and is extracted from cane or sugar beet. In sucrose the glycosidic bond is formed between the anomeric carbon atoms of a glucose unit and a fructose

unit and the configuration of this glycosidic linkage is α for glucose and β for fructose.

Lactose, is the major carbohydrate component of milk and consists of galactose joined to glucose by a glycosidic linkage; the four position of glucose is joined to the anomeric carbon of galactose to form a 1,4-glycosidic link. When lactose is hydrolyzed by lactase in human beings and by β-galactosidase in bacteria the two monosaccharides are released for use in the production of energy. Finally, in maltose, the anomeric carbon of one glucose is joined to the four position of a second glucose molecule in a two glucose units are α-1,4-glycosidic linkage. In humans and animals we hydrolyse starch to generate maltose which is then slowly hydrolysed by the maltase enzyme to generate glucose for use in energy production. [2]

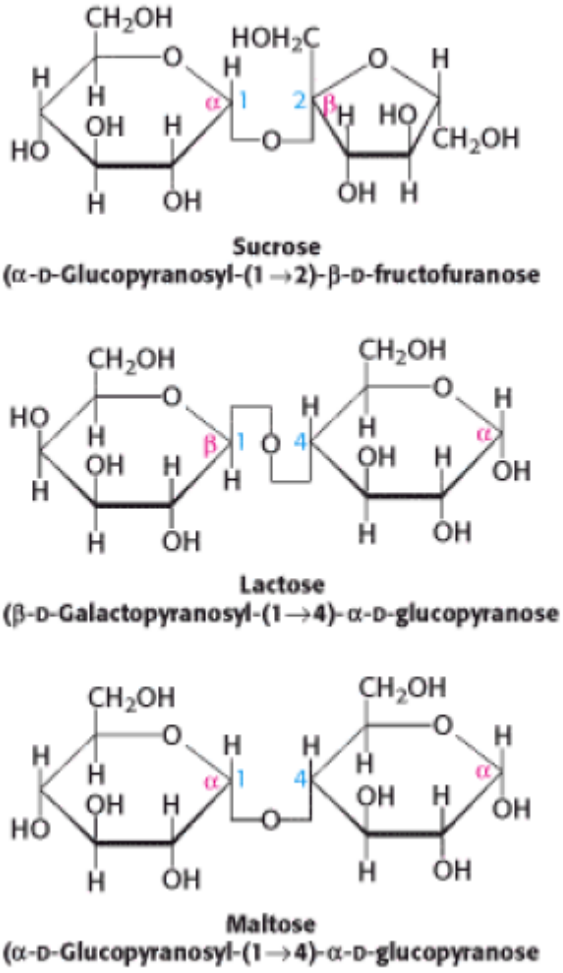


Figure 1.8: ***Common Disaccharides*** [2]

1.3. Polysaccharides

Oligosaccharides contain between three and ten monosaccharides, carbohydrates with more than ten monosaccharides joined together are termed polysaccharides. Polysaccharides are vitally important for life on earth they play major roles in energy storage, in cellular signalling processes and in maintaining the structural integrity of an organism. Polysaccharides are classified based on the types of monosaccharides they contain: if they contain only one type of monosaccharide these polymers are called homopolymers. *Glycogen* is the most common homopolymer in animal cells is *(fig 1.9)*, the storage form of glucose. glycogen is a very large, branched polymer of glucose residues. Most of the glucose units in glycogen are linked by α-1,4-glycosidic bonds. The branches are formed by α-1,6-glycosidic bonds, present about once in 10 units (this should be compared with starch-see next paragraph).[2]

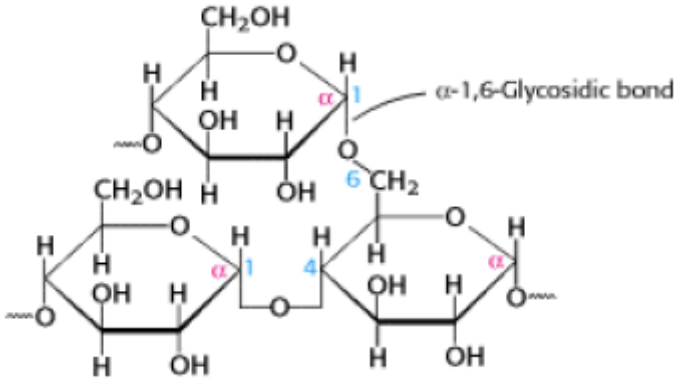


Figure 1.9: Branch Point in Glycogen [2]

In the plant kingdom the main storage polysaccharide for use as an energy source is starch, starch is actually a mixture of two homopolysaccharides: amylose and amylopectin. Amylose is simply a linear chain of α-1,4-glycopyranose units and amylopectin is the same but importantly has a significant number of α-1,6- branches. Amylopectin is crystalline, whereas amylose is generally considered to be amorphous.[1]

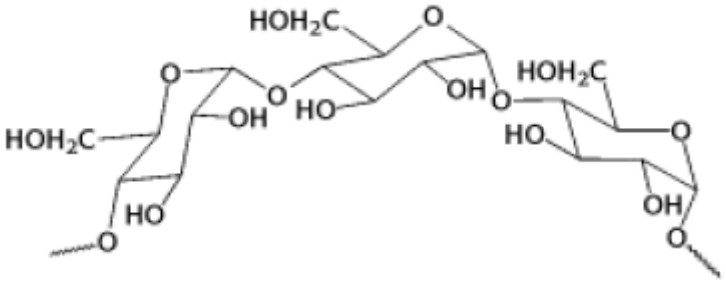


Figure 1.10: Starch structure [2]

1.4. Structural Polysaccharides-Cellulose structure

In the plant kingdom a polysaccharide is used as a major structural component of cell walls and this is cellulose and as a consequence cellulose is the most abundant organic material in the biosphere.[4] Commercially cellulose is a very important material. Cellulose derived from wood pulp is used for the manufacture of paper; cellulose derived from cotton is also used in the manufacture of cotton. In both these commercial uses of cellulose the raw material, wood pulp and cotton, is heated with alkali solution in order to transform the cellulosic material into a commercial product. Cellulose has unique properties and its abundance and the fact that is a renewable source means that it has great potential for further uses- e.g. in energy production. Given the fact that cellulose is so commercially important it is surprising that the scientific community still does not have a complete understanding of its chemical and physical properties. This is partially due to the fact that cellulose is difficult to work with, it has microfibre like structure and as a consequence it is difficult to dissolve in most common solvents**.** The large extent of hydrogen bonding between layers of fibres prevents the penetration of solvents and the enthalpy and entropy changes associated with dissolution are unfavourable. Problems associated with the dissolution of cellulose restrict its application. Development of better cellulose dissolving systems is of special importance in cellulose industry and other fields and much work including the application of ionic liquied technology has been used in an attempt to form solutions with substantial concentrations of dissolved cellulose.

The basic structure of Cellulose has glucose joined by *β*-glycosidic links i.e. it has abasic molecular unit of $C_6H_{10}O_5$, which is also called anhydroglucose unit (AGU). And is linked in the form of *β*-1,4-glucan *(fig1.11)* with adjacent glucose units rotated by 180^0 to each other. Each of the anhydroglucose monomers contains three hydroxyl groups which make cellulose a hydrophilic material. However, as was stated above, it is sparingly soluble in aqueous solutions due to the presence of large quantities of inter- and intra-molecular hydrogen bonds and

considerable van der Waals forces between the non-polar groups, the top and bottom faces of the ring are hydrophobic in nature.[5]

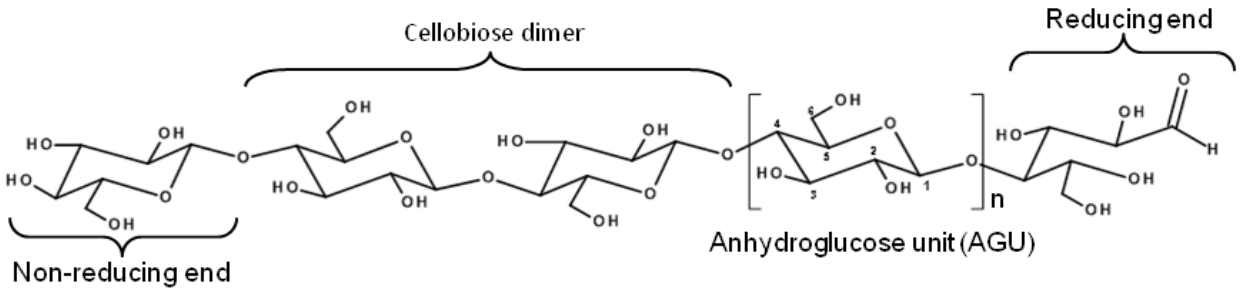


Figure 1.11:Molecular structure of cellulose, adapted from the work of Klemm et al. [9]

1.4.1. Crystalline polymorphs of cellulose

A lot of work has been devoted to determining the structure of cellulose. This area has proved problematic with a number of the early work in this area providing contradictory results and this is because cellulose can exist in a number of different structural forms i.e. different crystalline phases –polymorphs- as well as being able to adopt amorphous or non-crystalline forms. Pure cellulose exists in several crystalline polymorphs with different packing arrangements. The polymorphs are best described by consideration of their basic structural unit cells as determined by X-ray crystallography and neutron diffraction studies.[6] The unit cells are listed in Table 1, and figure 1.12

Table 1.1 Unit cells for polymorphs of cellulose

Type	Number of chains	Unit cell					
		a (Å)	b(Å)	c(Å)	α(°)	β(°)	γ(°)
Cellulose I α	1	6.717	5.962	10.40	118.08	114.80	80.37
Cellulose I β	2	7.784	8.201	10.38	90	90	96.55
Cellulose II (mercerized from flax)	2	8.01	9.04	10.36	90	90	117.1
Cellulose II (mercerized from ramie)	2	8.10	9.03	10.31	90	90	117.1
Cellulose II (mercerized from fortisan)	2	8.03	9.04	10.35	90	90	117.1
Cellulose III_1	1	4.450	7.850	10.31	90	90	105.1
Cellulose IV_1	2	8.03	8.13	10.34	90	90	90
Cellulose IV_2	2	7.99	8.10	10.34	90	90	90

To date, six different crystalline polymorphs of cellulose have been reported and these are: cellulose I (α and β), II, II_I,III_{II}, IV_I, and IV_{II}. Only cellulose I and II are found in nature and the others are derived from these either by chemical or heat treatments.[7]

1.4.1.1 Cellulose I

In the plants and microorgansims, native cellulose is a mixture of varying proportions of Iα and Iβ crystalline forms. The cellulose chain conformation is similar in Iα and Iβ, but the principal difference between them is the Iα has triclinic crystallographic symmetry while the Iβ has monoclinic symmetry-see figure below.[7] In cellulose I there are important intermolecular hydrogen bonds linking neighbouring chains along the α-axis –the hydrogen bond links O-6 of one sugar to···H-O(3) of the next glucose, so that the chains form hydrogen bonded sheets of chains parallel to the α-axis.[9] The two forms Iα and Iβ can be inter-converted by heating with the thermodynamically more stable Iβ form being preferred at a high temperature.[10]

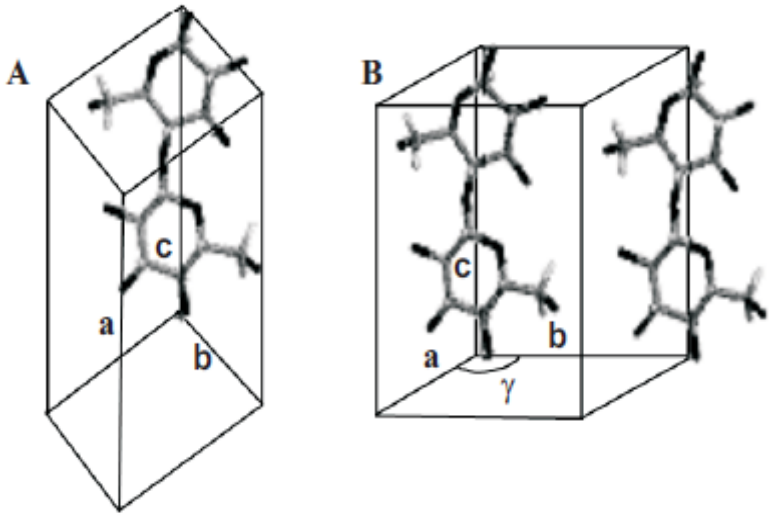


*Figure 1.12: Triclinic (A), Monoclinic (B) crystallographic symmetry of cellulose **Iα** and **Iβ** [7]*

1.4.1.2 Cellulose II

In a number of commercial processes cellulose I is converted to cellulose II by heating, Cellulose II is the most thermodynamic stable crystalline form and is generated during the manufacture of textiles in processes known as regeneration and mercerization.

As stated earlier, it is also possible to obtain cellulose in an amorphous form and this is usually associated with cellulose I. Cellulose I and II are often present together in nature. And the main difference between the two is the relative arrangement of chains and hydrogen bonding. The

inter-chain hydrogen bonding pattern differs for the two forms: as stated above, O6H—O3 inter-chain hydrogen bonding is dominant in cellulose I, in contrast the main inter-chain hydrogen bonding in cellulose II is between O6H—O2 is. The O3H –O5 intra-chain hydrogen bonding which exists in both polymorphs (I and II) is responsible for the rigid and linear shape of each cellulose chain.[7]

1.4.1.3 Parallel and Antiparallel of cellulose I and II

The most striking difference between the two natural cellulose forms is the relative arrangement of chains which are known as parallel or antiparallel arrangements. The development of the understanding comes from knowledge that Cellulose I can be converted by treatment in the solid state, so called mercerization to cellulose II. It was concluded that cellulose I and cellulose II should possess the same parallel or antiparallel packing arrangement of chains in the microfibils. However neutron diffraction studies have identified that this is not the case: it is now known that microfibrils of native cellulose I pack in a parallel chain arrangement and those of cellulose II in an antiparallel chain arrangement. On first inspection this is difficult to understand especially given that cellulose I can be converted to cellulose II without changing the appearance of the morphology or the orientation distribution of the fibers. Current theories suggest that two close cellulose I crystals with chains orientated in opposite direction undergo interdiffusion model during the mercerization to generate cellulose II. Starting with parallel packed cellulose I chains an antiparallel array of nearby mircofibils in a fibre (Fig. 1.13, left), uptake of sodium hydroxide beginning at the noncrystalline surface of the microfibrils, interdiffusion of chains, then washing and drying results in antiparallel cellulose II microfibrils (Fig. 1.13 right).[6]

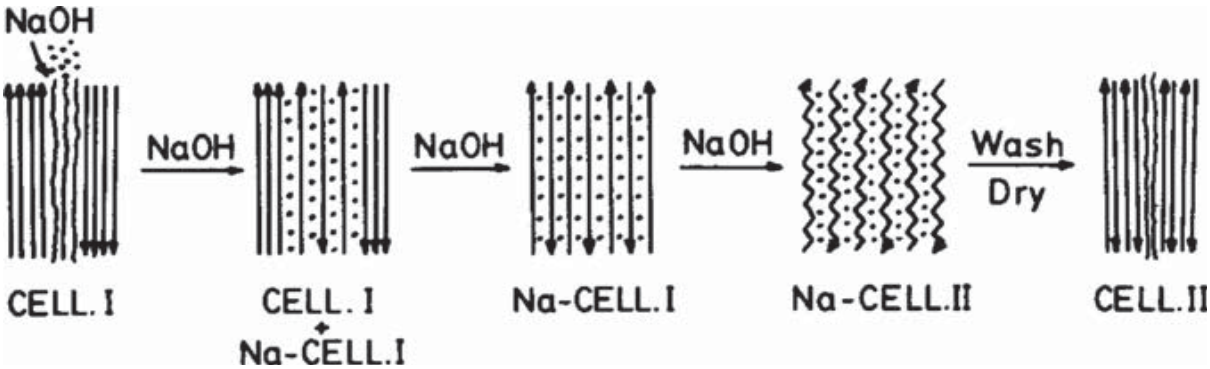


Fig 1.13: Parallel-packed arrays of microfibrils of up and down chains of cellulose I to antiparallel-packed fibrils of cellulose II.[12]

1.4.1.4 Cellulose III

The treatment of native or regenerated (mercerized) cellulose with dry liquid ammonia generates the two new crystalline cellulose structures III_1 and III_2. Simple evaporation of ammonia results in the isolation of a new polymorph called cellulose III_1.

The two forms III structures pack in quite differently with their being a parallel arrangement of chains in cellulose III_1 (fig 1.14) and antiparallel ones in cellulose III_2 (fig 1.15). Again the two can easily be converted by mild heat treatment or through a solvent complex to the parallel-packed cellulose I and that celluloseIII_2 can be converted by the same mild heat treatment to antiparallel-packed cellulose II.[6]

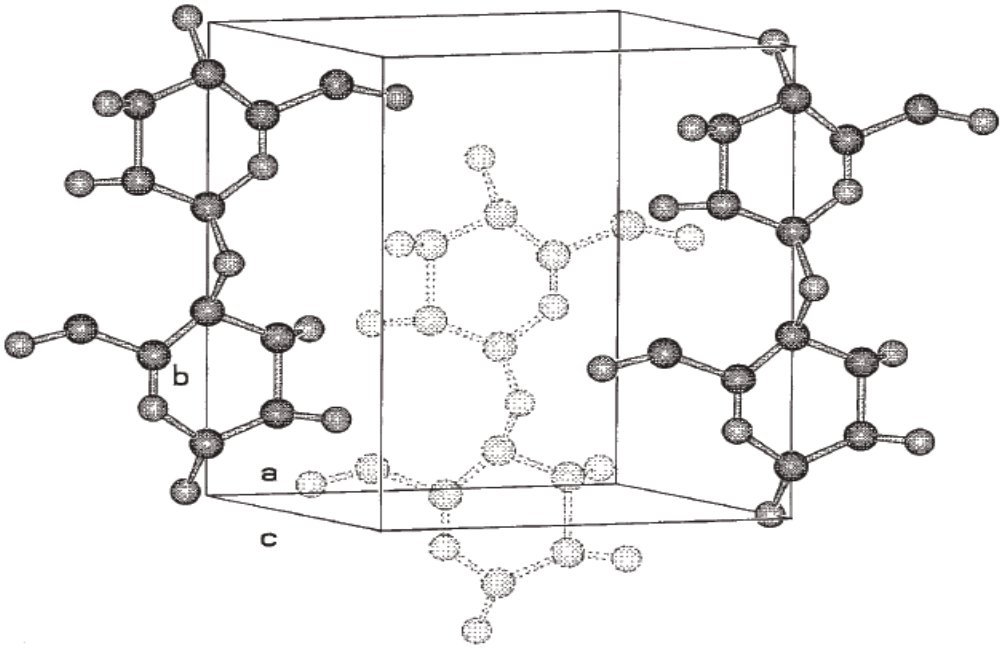


Figure 1.14: Parallel-packed arrays of microfibrils of up and down chains of cellulose I to antiparallel-packed fibrils of cellulose II. *Adapted from the work of Peter* Zugenmaier [6]

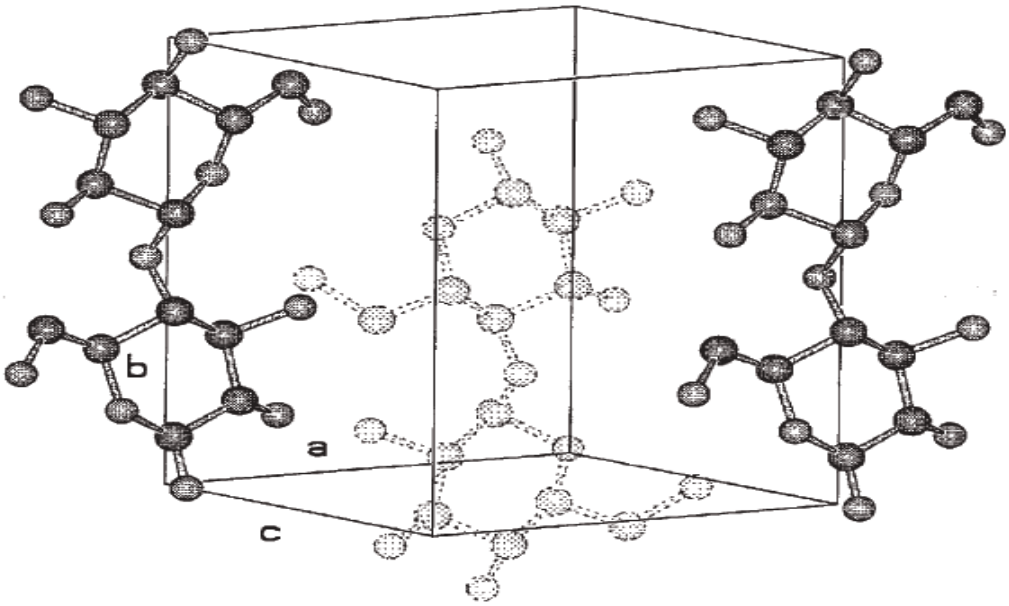


*Figure 1.15: antiparallel structure of cellulose **III** .adapted from the work of Peter Zugenmaier* [6]

1.4.1.5 Cellulose IV

The production of cellulose IV is achieved by heating cellulose III in glycerol for 20 min at 260°C and cellulose IV_1, IV_2, these two subgroups of cellulose IV (fig 1.16). Cellulose IV_1 reversibly transforms to parallel-packed cellulose I and cellulose IV_2 to antiparallel-packed cellulose II.

In summary, the different crystalline polymorphs can be divided into two families: the parallel-chain family (cellulose Iα, Iβ, III_1 and IV_1) and the antiparallel-chain family (cellulose II and IV_2) the probable antiparallel structure of cellulose III_2 has not been determined.[6]

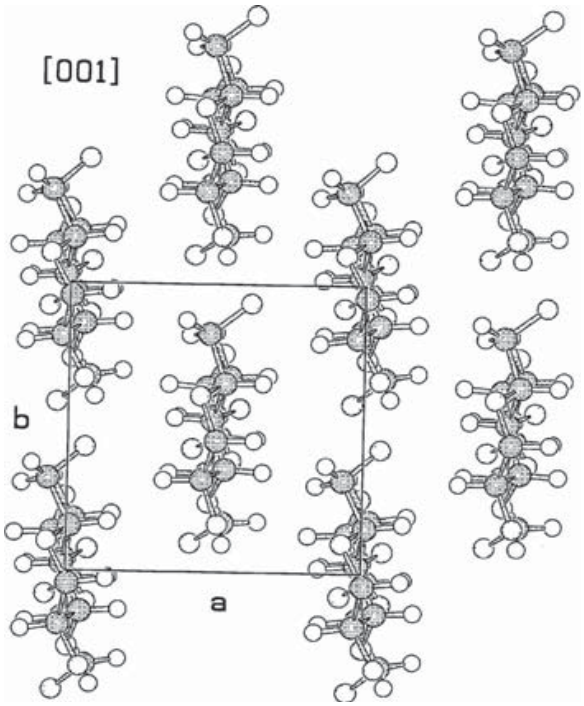
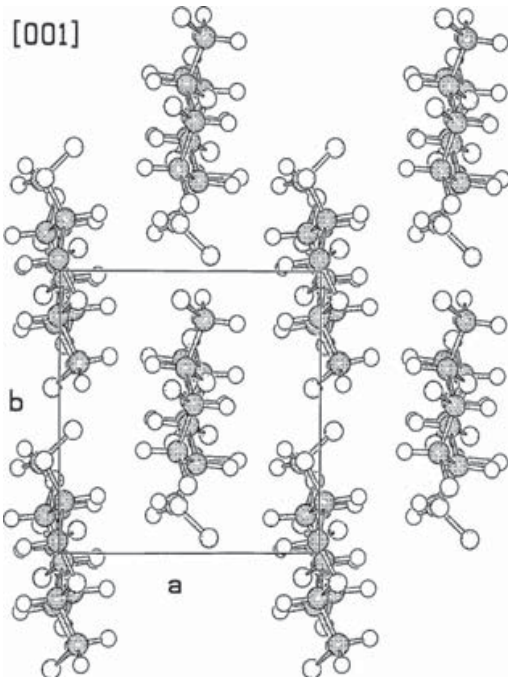


Fig 1.16:Parallel chain of cellulose IV_1



Antiparallel chain of celluloseIV_2 [6]

1.4.2. Microfibrillic structure of cellulose and its influence on chain degradation.

All cellulose chains have two ends: the one with an original C4-OH group is called the non-reducing end and the other with an original C1-OH is called the reducing end. The stability of cellulose chains is frequently determined by the number of reducing chain ends as the reducing end participates in reactions with nucleophiles such as hydroxide in the alkaline degradation of cellulose.. The cellulose molecular chains are ordered into stands as cellulose micro-fibrils through inter and intra molecular hydrogen bonding and as stated earlier these micro-fibrils often have both crystalline and amorphous regions *(fig1.17*) with a single chain passing through more than one crystalline region. It is known that the amorphous regions are available to reagents that can diffuse into the microfibril and react with the chain ends that are present; workers have shown that the alkaline degradation takes place in the amorphous region of cellulose.[4]

A lot of reactions can only take place in the amorphous regions and heating does not always improve accessibility of the cellulose chains; on heating cellulose degrades before it melts and the strong intra- and inter-molecular hydrogen bonds in cellulose prevent its molecules from dissolution in most common solvents.[5]

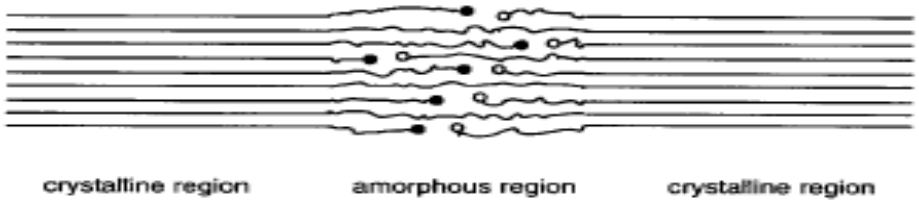


Figure 1.17.Microfibrillic structure of cellulose. • Reducing end group ° Nonreducing end group [4]

As a consequence, cellulose is a water-insoluble polymer with a rigid linear structure. The biosynthesis of cellulose generates an arrangement of extensive linear chains aligned side-by side, creating fibres of great mechanical strength.

1.4.3 Effect of Crystalline Structure on Cellulose Properties

It has been known for some time that the treatment of cellulose with sodium hydroxide at elevated temperatures during the mercerisation process changes the physical properties of the cellulose fibres. Mercerization is accompanied by swelling of the cellulose fibres and this swelling promotes absorption of moisture and of dyes. These changes in properties may reflect changes in crystallinity, Emil et al[11] have demonstrated that cellulose II crystallite can absorb water whereas cellulose I cannot.

1.4.4 Nature of amorphous regions

There is debate over the exact nature of the amorphous material in cellulose. Whereas natural rubber is completely amorphous and its characteristics are similar to those of a liquid phase, in cellulose the case as there are large variations in the amorphous regions from fibre to fibre. Occur in cellulose than are usual for rubberlike materials.

Amorphous cellulose prepared by evaporation of ammonia from ammonia cellulose.

There is no evidence that this liquid type structure is carried over into the polycrystalline cellulose fibber, and there appears to be no connection between this type of structure and the structure of amorphous regions in the untreated fibre. A completely amorphous, must be considered to contain all possible intermediate degrees of packing between the liquid state and crystalline state.[11]

1.4.5 Intra- and inter-molecular hydrogen bonds in cellulose

Cellulose possesses both, intermolecular and intramolecular hydrogen bonding (fig 1.18). As stated earlier in native crystalline cellulose *intramolecular* hydrogen bonding occurs between (O-3-H) and (O-5) which typically has a bond length of 2.707Å, and between (O-2-H) and (O-6) with bond length of 2.802 Å, This is accompanied by *intermolecular* hydrogen bonding in cellulose between (O-6-H) and (O-3) of another chain with bond length of 2.874 Å .[12]

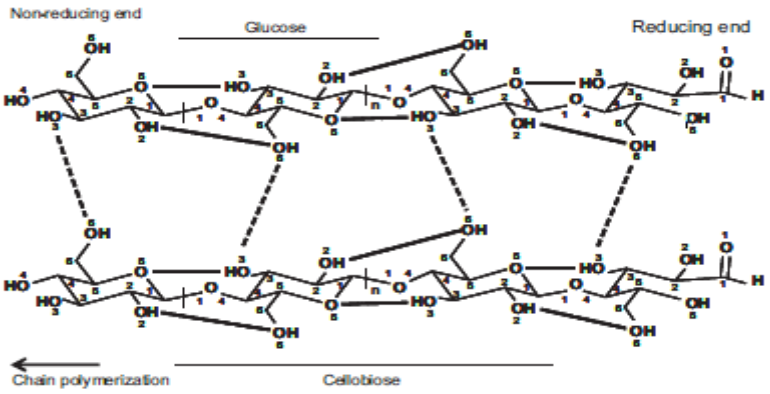


Figure1.18. Intermolecular and intramolecular hydrogen bonding in cellulose [7]

The hydrogen bonding patterns of the individual hydroxyl groups influences their reactivity, both O2 and O6 atoms show multiple possibilities of hydrogen bonding and as a consequence are the most reactive of the hydroxyl groups on the crystalline surface of cellulose. Conversely, the strength of the intra-chain O3-H-O5 bonding. Reduces the reactivity of O3

Beside hydrogen bonding, van der Waals force is also important for cellulose network forming, especially between the non-polar sheets.[12]

1.4.6 The degree of polymerization (DP)

One measure by which celluloses are classified is known as the degree of polymerisation which is a measure of the average number of anhydroglucose units per chain.[8]

The degree of polymerisation will influence the concentration of chain ends and as such is a particularly important characteristic in the analysis of materials that to be used in degradation studies; different cellulosic materials have different degrees of polymerisation.

Table (1.2) shows the degree of polymerization and the number of reducing end groups for the four cellulosic materials

Cellulose	DP	$^{*}(Gr)_{\circ}$	$(Gr)_{\circ}$
Cellulose (Aldrich)	117±3	$8.6 \cdot 10^{-3}$	$(7.8 \pm 0.8) \cdot 10^{-3}$
Tissues (Tela)	1110± 22	$9.0 \cdot 10^{-4}$	$(2.4 \pm 1.1) \cdot 10^{-3}$
Cotton (Migros)	1800±36	$5.6 \cdot 10^{-4}$	$(3.2 \pm 3.0) \cdot 10^{-4}$
Paper	290±6	$3.5 \cdot 10^{-3}$	$(1.2 \pm 0.4) \cdot 10^{-2}$

$^{*}(Gr)_{\circ} = 1/DP$, DP: Degree of polymerisation
$(Gr)_{\circ}$ see equation (1)

This is particularly true in alkali catalysed degradation studies where the amount of cellulose degraded by the peeling off reaction (see discussion below) depends strongly on the degree of polymerisation of cellulose.*(fig 1.19)* [13]

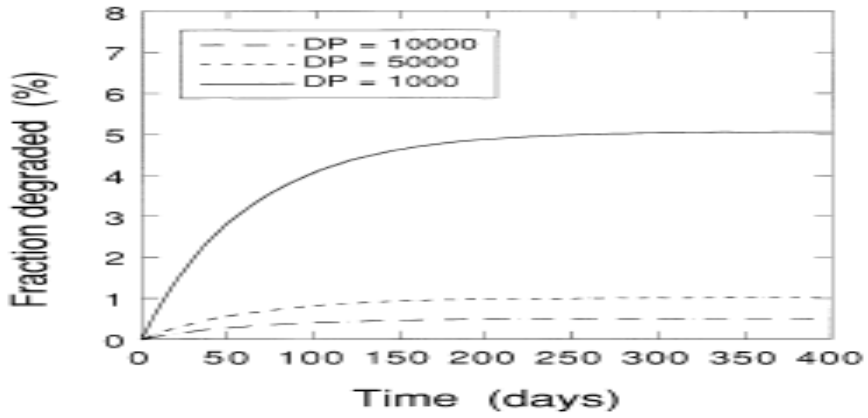


Figure 1.19: Degradation of cellulose for different degrees of polymerisation (DP)[4]

1.4.7 Number of reducing end groups in cellulose

When undergoing degradation reactions the rate of reaction will be greatly influenced by the number of reducing end groups dividing the amount of material present by the degree of polymerisation should provide a measure of the mole fraction of end groups. The larger the cellulose molecule, the lower the mole fraction of reducing end groups as can be seen from equation (1) [13]

$$(G_n)_0 = \frac{\text{moles of reducing end groups}}{\text{moles of glucose units in the chain}} \qquad (1)$$

1.4.8 Types of degradation

Cellulose can be degraded by chemical and physical processes and the degree of degradation can be measured from the change in degree of polymerisation which can be correlated to the mechanical properties of the cellulose fibre. The degradation will influence the chemical properties of the bulk material especially its reducing power which is associated with the number of chain ends. Degradation is often accompanied by the variation in the functionality present in the chain e.g. through oxidation which generates carbonyl groups mid-chain for example, exposure of cellulosic materials to the elements will result in oxidation. Degradation frequently accompanies the exposure of cellulose to variations in pH with both acids and bases degrading cellulose by hydrolytic processes. Other important degradation processes include mechanical depolymerisation; when excess physical forces applied bonds fracture via free radical processes; microbial degradation is also an important with many fungi and bacteria are able to metabolise cellulosic material. A number of workers have studied various aspects of cellulose degradation.

As there are a number of industrial processes where cellulose is contacted with hydroxide at elevated temperatures there is a significant amount of work been directed at determining the degradation that occurs at basic pHs. A substantial amount of previous work in this area has focused on the fate of cellulosic waste material that is generated in nuclear power facilities. A range of disposal facilities are currently under consideration for the storage of low level and intermediate level nuclear waste and this material contains a very substantial amount of cellulosic material. The current preferred storage method is to encase the cellulose with cement and then to store the material in a deep underground repository. A few years after closure of the repository the cement will become saturated with water and the cellulose will be subjected to cement pore water which will have a basic pH and which is expected to catalyse the degradation of the cellulosic material.

1.4.9 Cement pore water:

The composition of the pore water in the nuclear storage facilities will vary depending on the composition of the cement and the exact mineral content of the water flowing through the repository. Previous studies have demonstrated that there will be an evolution of the pore water chemistry which can be divided into three stages:
At the start, the dissolution of NaOH and KOH present in cement as "impurities" causes a (Na,K)OH cement pore water saturated with respect to $Ca(OH)_2$.

The pH of this initial pore water will be close to 13.4 (concentration of OH^- is 0.3 M) and the Ca concentration is about 2 mM.

In the next stage, after all NaOH and KOH has been leached out, the composition of the pore water will be controlled by the dissolution of $Ca(OH)_2$ .The pH of the pore water will fall to 12.5 whilst the Ca concentration increases up to 20 mM.

In the final stage when all the $Ca(OH)_2$ has dissolved the pH will falls further and hydrated Ca-Si phases (CSH-gels) start dissolving.[13]

1.5. Chemical Degradation of Cellulose

Cellulose fibres that have been treated with alkali have a reduced degree of polymerisation and there is a loss of mass in the formation of soluble small organic acids, this is a phenomenon that is well known from pulping and textile industries. As stated earlier, alkaline degradation of cellulose is a process of potential importance in the design of long-term disposal facilities for radioactive waste. The highly alkaline cement pore water in such an environment is expected to lead to either a complete or a partial degradation of the cellulose present. It is known that the degradation process generates a number of small molecules with metal complexing properties which will potentially increase the solubility and enhance the mobility of radio nuclides in the repository.[14]

Previous studies [4,13,15,21,34,35,36] have shown that the main degradation products of pure cellulose are the diastereomeric α-isosaccharinic acid (α-ISA) and *β*-isosaccharinic acid (β-ISA) *(fig 1.22),* which account for up to 80% of the total dissolved organic carbon. The remaining material consists of a very large number of short chain aliphatic acids such as formic acid, acetic acid, lactic acid and threonic acid. The degradation of the other cellulosic materials, including hemicelluloses, results in lower amounts of α-ISA and *β*-ISA and in higher yields of small organic acids (acetic acid) and of unidentified compounds.

1.5.1 Mechanism of the alkaline degradation reaction

The degradation of cellulose under alkaline conditions occurs via two main processes both of which occur simultaneously: a *peeling-off* reaction *(fig1.20)* and fragmentation. The peeling reaction generates isosaccharinic acids and the fragmentation reaction generates a series of small hydroxy-aliphatic acids.[4]

1.5.1.1 Peeling-off reaction

The mechanism of the peeling reaction is now well understood: the peeling-off reaction *(fig 1.20)* is an endwise degradation process by which a reducing end group is split-off from the cellulose chain. In alkaline media, the reducing glucose end group of a cellulose molecule is subject to a number of isomerisations that result in migration of the carbonyl group along the carbon chain from C1 to C2 generating an acidic proton at C3 which will ionize under basic conditions. The end unit is liberated from the chain by an elimination of the rest of the cellulose chain as a glycoxy anion and generates a substituted 2,3-diketohexulose as a key intermediate. [13] The vicinal dicarbonyl intermediate can then undergo a benzilic acid type rearrangement to generate isosaccharinic acids.

1.5.1.2 Peeling-off mechanism

The β-elimination occurs at the C-4 carbon atom, one hexose monomer unit is split off from the cellulose molecule, and the next glucose end group can take part in the reactions. In this way the glucose units can be released one by one from the cellulose molecule *(fig 1.20).*The process repeats along the chain in the amorphous region of cellulose.[14]

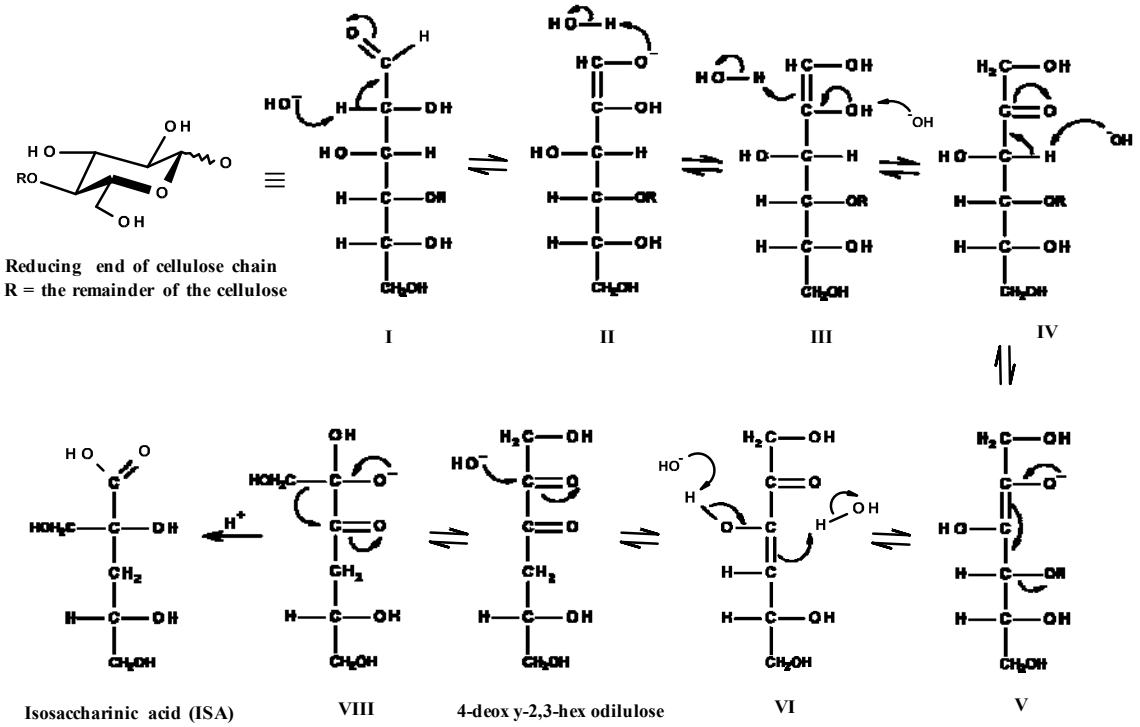


Figure 1.20: The peeling reaction[29]

1.5.1.3 Stopping reaction

The process of peeling slow and eventually stops; 'stopping' is a consequence of two separate processes i.e. it *(fig 1.21)* can be subdivided into a chemical and physical stopping reaction. The former is the transformation of a reducing end group into a stable *metasaccharinic acid* end group. The latter implies that a reducing end group reaches the crystalline region of the cellulose and is no longer accessible to alkali.

The physical stopping reaction is not an abrupt process since there is a gradual transition from the amorphous to the crystalline region rather than a distinct interface.[4]

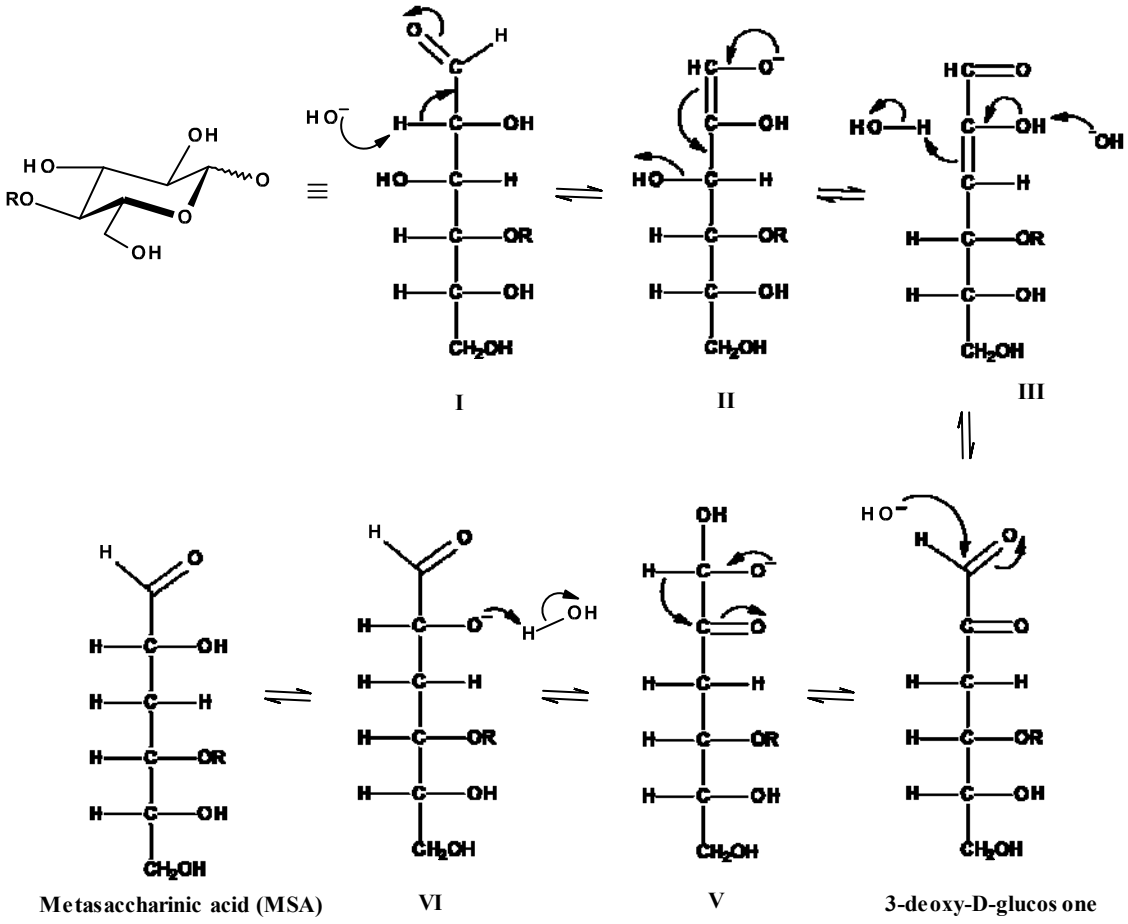


Figure 1.21. Stopping reaction[29]

1.5.1.4 Stopping reaction mechanism

The *β*-elimination can also occur at positions other than C-4 e.g. at C3; in that case the hexose unit remains attached to the cellulose molecule, which terminates the depolymerisation *(fig 1.21)*. This is called the *chemical stopping reaction*. After either type of elimination reaction, a diketo intermediate is formed that can undergo a benzilic acid rearrangement, which generates the final degradation products. Elimination at C4-gives isosaccharinic acid whereas elimination at C3 gives, after the benzilic acid rearrangement, meta-saccharinic acid covalently attached to the chain end via C4.[14]

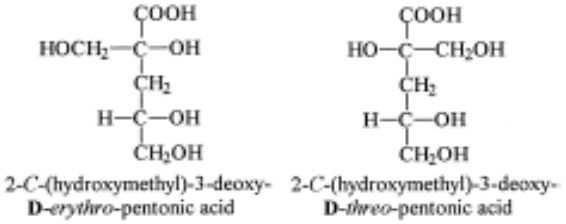
1.5.1.5 Fragmentation reactions

The vicinal dicarbonyl intermediate can also react further and authors have suggested that as the hydroxide ion and temperature increase the intermediate increasingly reacts via a fragmentation reactions that are retro-aldol reactions that generate small hydoxyl-aliphatic acids.[27]

1.5.2 Isosacharinic acid (ISA)

Two diastereomers, *α*- and *β*-D-isosaccharinic acid (*2-C-(hydroxymethyl)-3-deoxy-D-erythro-pentonic acid*) and (*2-C-(hydroxymethyl)-3-deoxy-D-threo-pentonic acid*) *(fig 1.22)* are produced in approximately equal amounts by the alkaline degradation of cellulose. Each diastereomer in turn has two corresponding enantiomeric lactone conformations.

The conformations are pH-dependent with the isosaccharinate form dominating at higher pH, and the lactone form at lower pH.[16]



(α-ISA) *(β-ISA)*

Figure.1.22. Chemical structure of (α,β- isosccharinic acid) [36]

1.5.2.1 Isosacharinic acid-Lactone

With two chiral C-atoms, four stereoisomeric configurations are possible for isosaccharino-1,4-lactone, which are shown in Fig.1.23(a–d). They can be divided into two pairs of enantiomers (RR/SS and RS/SR).

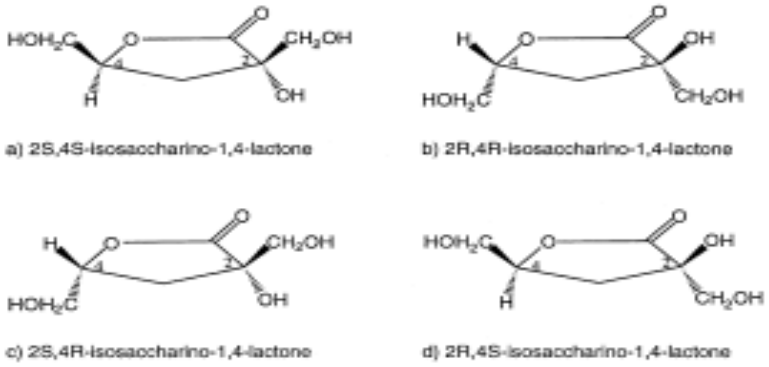


Figure 1.23: Stereoisomers of α-ISA-lactone (structures a and b) and β-ISA-lactone (Structures c and d).[15]

The NMRs of alpha and beta-ISA are not identical therefore the material cannot be enantiomers and they therefore must have a diasterotopic relationship, recent X-ray analysis of their tribenzoate esters are in agreement with the (2R,4S) configuration of β-ISA and the (2S,4S) configuration of α-ISA. Previous workers have shown that both α and β-ISAs are able to form complexes with actinides including plutonium. The presence of ISA in cellulosic waste is therefore a potential route by which radionuclides can be leached from underground repositories.[15]

1.5.2.2 Concentration of (ISA)

The rate at which metals are leached from repositories will depend on the concentration of (ISA) in the pore water. The concentration of ISA will depend on a number of factors including the cellulose loading in the waste, how much degradation of cellulose occurs, the nature of the cement, if ISA can be absorbed onto the cement, the chemical and microbial stability of ISA under the existing alkaline repository conditions and the water flow through the repository.[13] Stable complexes with tri- and tetravalent radionuclides such as Am^{3+} and Pu^{4+} will lower their sorption on the cement phase. This would lead to an enhanced release of such radionuclides to the geo and biosphere.

1.5.3 Amount of cellulosic waste

It has been estimated that about 50% of the organic waste planned to be emplaced in the repository for low and intermediate level radioactive waste (L/ILW- repository) is cellulosic.

1.5.4 Sorption Phenomena

The nature of the cellulosic material will also be influenced by the environment in which it placed; cellulose absorbs water paper and swells. This sorption process is reversible and is a generalchemical process that needs to be considering carefully when considering the safety of waste storage facilities. The sorption of radionuclides is also important and this will occur with the cement of the containers and the extent to which it occur will be determined by the presence of the complexing agents present (ISA) and the pH of the aqueous environment the pore water.[11]

1.6. Analysis of cellulose -Chromatography:

1.6.1. Theory of chromatography

The power of chromatography comes from its ability to separate a mixture of compounds.[39] Separation of two sample components in chromatography is based on their different distribution between two phases. First one, s stationary phase, a liquid or solid. The second is mobile phase[17,38].The mobile phase is a gas in gas chromatography or liquid in liquid Chromatography.[17] The stationary phase loosely interacts with each analyte based on its chemical structure, resulting in the separation of each analyte as a function of time spent in the separation column.

1.6.2. Types of chromatography

Chromatography can be divided into three basic types that include gas, liquid, and supercritical fluid (gel) chromatography (fig 1.24).
Liquid chromatography can further be divided into ion exchange, separations based on size, and even extended to gel-based electrophoretic techniques.
However, each type of chromatography is comprised of two distinct steps: *chromatography* (or separation of individual compounds in distinct elution bands) and *identification* (detection of each elution band).[39]

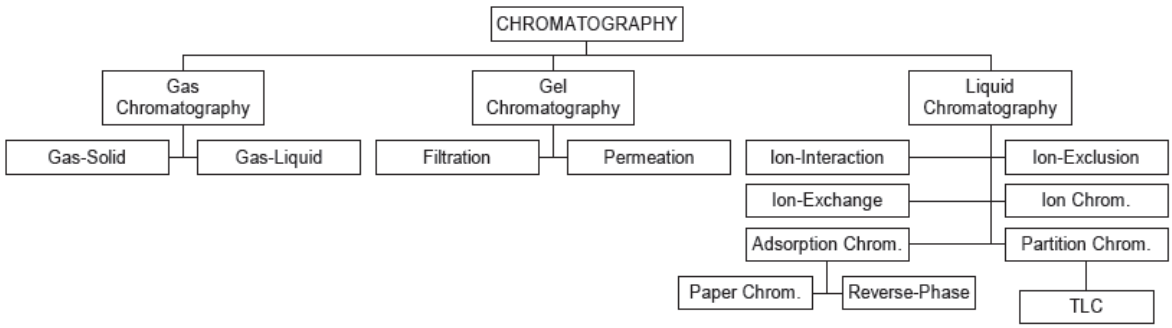


Fig:1.24 Types of chromatography [39]

1.6.3. Adsorption Chromatography

According to A.Weston, P.R.Brown, (1997)[18], the principle of adsorption chromatography is concerned mainly with classical column and thin-layer chromatography. In absorption chromatography, a relatively polar material with a high specific surface area is used as the stationary phase, normally silica or alumina. In contrast, the mobile phase is relatively non-polar (alkane or ether). The different extents to which the various types of molecules in the

mixture are adsorbed on the stationary phase provide the separation effect. A non-polar solvent such as hexane will elute an analyte more slowly than a moderately -polar solvent such as ether.[18]

1.6.4. Ion-exclusion chromatography

The alkaline catalysed hydrolysis of cellulose generates a series of hydroxylated aliphatic acids and these can be separated using either anion exchange chromatography or by ion exclusion chromatography. Previous workers in this area have used anion exchange chromatography to analyse saccharinic acids and ion exclusion chromatography to study small aliphatic acids with four carbons and less.

In ion exclusion chromatography the undissociated form of the analyte is absorbed onto a stationary phase; in anion exclusion the stationary phase chosen will be negatively charged.[19] Depending on the mobile phase pH, a weak acid will exist in a combination of its protonated and anionic form- the anionic form is excluded from the column whereas the free acid can be absorbed. The extent to which the acid is absorbed will depend on hydrophobic interactions – thus whilst small acids are not retained e.g. acetic acid and water may interact with the stationary phase to a small extent, retention time increase with increasing length of alkyl chain of the acid.[20] The separation principle in HPIEC is the Donnan exclusion of anions owing to the presence of micropores with a high degree of fixed negative charges. This causes the anions to be eluted in the void volume, whereas uncharged molecules may distribute in the micropores leading to a retarded elution separation is effected by the varying extent of different types of interaction with the stationary phase:

1- Hydrophobic interactions: This leads to a strong retention of typically fatty acids.

2-Polar interactions mainly taking place by hydrogen bonding. This leads to a differentiation between various types of hydroxycarboxylic acids, which is of high importance in the present context.

3- π-π electron interactions, being of importance in the case of aromatic carboxylic acids.[21]

1.6.4.1. Main principle in the ion exclusion mechanism:

As stated above, the ion exclusion mechanism relies on principles of retention mechanisms like Donnan exclusion (fig 1.25), steric exclusion and adsorption partition. The mobile phase composition mainly depends on analyte characteristics and extent of adsorption of the analyte to stationary phase substrate.[20]

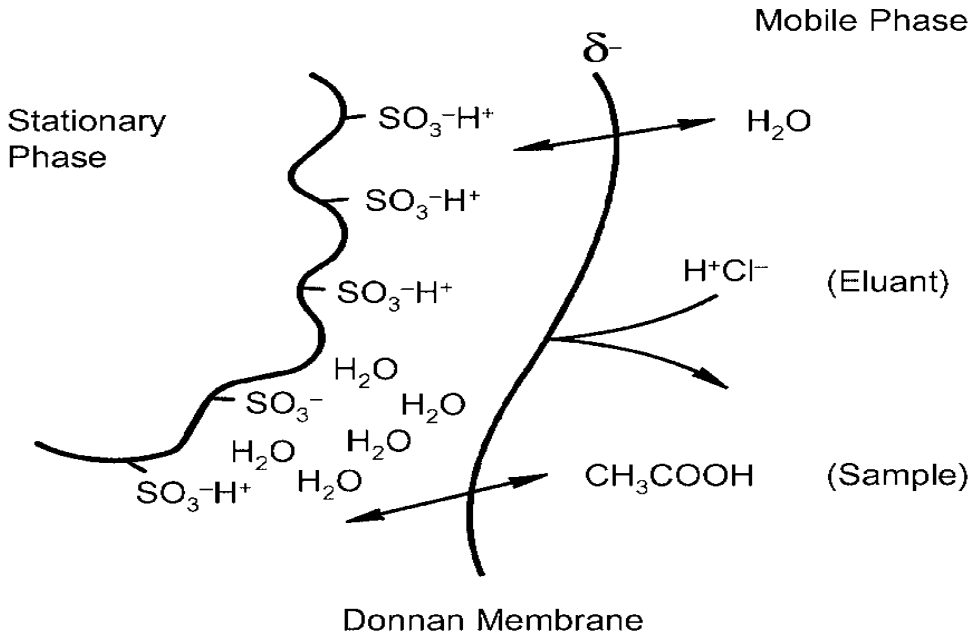


Fig 1.25 Schematic representation of the separation process on a HPICE column.[20]

1.6.5- Separation of Organic Acids

According to James **S.** Fritz, Douglas T. Gjerde,[19] in ion-exclusion chromatography (IEC), stronger acids (lower pKa,) are eluted more rapidly than weaker acids, presumably because the stronger acids are converted to the dissociated form and can interact with the negatively charged column. If this were the case, coexistence of the ionic and molecular forms might produce broader peaks; however, these compounds produce very sharp peaks. A better explanation might be that stronger acids are more polar and therefore interact less strongly with the resin matrix.

1.7. Aim of thesis

The aims of the present work are:

1) To familiarise ourselves with chromatography techniques for the analysis of decomposition of products of cellulose
2) To generate decomposition products and to identify the main C2, C3, C4, C5 and C6 hydroxyaliphatic acids and to quantify their production as a function of the reaction time.
3) To study the effect of temperature on production of cellulose degradation products.
4) To identify unknown acids.

A number of authors have identified various hydroxyaliphatic acids that are present at the end of reactions when cellulose is treated with alkali these include-see Kennedy Paper [25]. These acids are all generated by reaction of vicinal dicarbonyl intermediates (1,2 and 2,3) which can either undergo benzilic acid rearrangements to generate mainly saccharinic acids (C6) or by fragmentation which initially will generate small chain (C3-C1) hydroxyaliphatic acids. Other products are generated by decomposition and condensation of the initial hydrolysis products. The main aim of the current work was to determine how the concentrations of the different acid vary as a function of the reaction time.

There is also evidence that the flux of material through the different pathways can be influenced by the reaction conditions with high sodium hydroxide concentrations and temperatures reported to favour fragmentation. In contrast, lower temperatures and the use of calcium hydroxide as base is expected to favour the benzilic acid rearrangements.

Chapter two

Experimental

2. Materials:

Aliphatic carboxylic acids were purchased from Sigma-Aldrich Gllngha,U.K.) and were used as supplied.

Cellulose was purchased from (Sigma-Aldrich), Lot No,MKBB 4236,cat:31,069-7.

Acids and bases were general laboratory grade reagents. The purity of a number of reagents was examined using NMR spectroscopy with NMRs being recorded on a Bruker Avance 400Mhz spectrometer operating with Broker pulse programmes.

2.1 Chromatography:

The degradation reactions were analysed using ion exclusion chromatography.[22]

2.1.1. Instrumentation:

The equipment used for the analysis included:

1- an ICS-3000 Detector/Chromatography Module (DC)

The ICS3000 Chromatography Module employing both a conductivity detector and an electrochemical detector, a guard and separator columns (Column: IonPac ICE-As1 9x250 mm-7.5 μm particle size) and a suppressor (see details below)

2-an ICS-3000 Eluent Generator (EG)-Used with ISA analysis

The EG generates high purity acid (H^+) or base (HO^-) eluents online from deionized water using electrochemical oxidation.

3- an ICS-3000 Dual Pump (DP)

The DP contains two gradient pumps; those two pumps are able to operate as an isocratic isocratic pump and one gradient pump. The isocratic pump delivers one eluent, while the gradient pump can deliver a mixture of up to four eluent components. For the analysis of hydroxyaliphatic acids the system was run in isocratic mode.

4- Autosampler (AS)

The autosampler that was used to inject samplesor 100µl to 1000 µl (in 1 µl increments) via an injection valve.

Fig 2.1. Dionex ICS 3000 adapted from dionex-france[22]

Column: Ion Pac Ice-As1 9x250 mm

2.1.2. Preparation of the Mobile Phase:

a-For the acids analysis used

1-Heptafluorobutyric acid (HFBA),4mM was used as the mobile phase eluent and was prepared by dissolving 65 µl (0.4 mmol), of the acid in 1L deionised water. In the suppressor 2-tetrabutylammonium hydroxide solution (TBAOH) (5 mM) was used as a regenerant.

Both the eluent and regenerant were filtered by vacuum filtration through a nylon 0.45µm filter before use. The column temperature was maintained at 30 $^{\circ}$C and with an eluent flow rate of 0.7 ml·min^{-1} the pressure varied between (790-840 psi).

b-For cellulose analysis the following mobile phase and eluents were used

The same conditions as above were used to monitor the products generated during the decomposition of cellulose.

2.1.3. Operation of Chromatography:

The eluent (8-50 mM NaOH) was delivered at a flow rate of 0.7 $ml \cdot min^{-1}$, and the autosampler delivered an injector volume of 1ml (10µl stock sample and 990 µl of water).

2.1.3.1. Choice of Internal Standard

As samples were to be collected over extended period of times it was necessary to include a reference internal standard in the analysis. A suitable internal standard would be one whose structure was similar to the analytes under study but which is not itself generated in the reaction of interest. Ideally, the retention characteristics of the internal standard should be similar to those of the analyte i.e. have a similar retention time and response factor. A number of internal standards were tested for their suitability including benzoic acid, ribonic acid and acrylic acid.

2.2. Experiments

2.2.1 Determination of the retention time and response factors for a range of standard acids.

Stock solution of each acid (~ 10µl of liquids) and (10 mg of solids) were prepared and dissolved in ultra pure water (10 mL) at approximately 1000ppm and serial dilution was used to prepare samples for generation of linear calibration graphs.

Acids used included: acrylic acid (internal standard), formic acid, lactic acid, acetic acid, propionic acid, glyceric acid, gluconic acid, glycolic acid and pyruvic acid.

Other acids were either already available in the laboratory e.g. α-isosaccharinic acid, β-isosacharinic acid and 3,4-dehydroxybutanoic acid or were prepared using standard literature methods.

2.2.2. Preparation of standard aliphatic acids

To prepare stock solutions of acrylic acid a fixed volume (9.50 µl) of acid was taken and diluted in water (UPW 10 ml) to provide a working standard of known concentration (1000 ppm), this was then diluted (50,....,10 ppm) to provide a linear range of concentrations for use in determining the detectors response to this acid.

An example of the dilutions used: (1ml) of the stock solution (1000 ppm) was added to water (9 ml), to give a solution of 100ppm.The solutions were then injected onto the HPAEC column and the peak area ($\mu c/s^{-1}$) and the retention time of the acid were noted:

2.2.3. Attempted synthesis of C4/C5 hydroxyaliphatic acids

A number of known cellulose degradation products are not commercially available and an attempt was made to prepare a number of these following the procedures reported by Yang and Montgomery.[33]

Anhydrous glucose (180g, 1 mol) calcium hydroxide (18.5 g 0.33 mol) and magnesium oxide (26.4 g, 0.66 MOL) were added to a round bottom flask (1 L) and the resulting dry mixture was heated at 100 ^{o}C for 1h and then at 125 ^{o}C for a further 2 h. After 2 h the solid product was ground to a powder ready for purification. Before attempting to isolate the reaction products a small sample (10 mg) was dissolved in ultrapure water (5 ml) the solution was acidified by the addition of dilute sulfuric acid (5 mL, 1M) and the solution was analysed using the HPAEC system using the conditions described for the cellulose degradation reactions.

2.2.4. Extraction to purify C4/C5 acids.

The powder (20 g) recovered from experiment was carefully added to an ice cold solution of dilute sulfuric acid (100 mL) and the solution was extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were dried over sodium sulphate, filtered and the solvent removed under vacuum to give a brown tar. A small aliquot of the reaction mixture was submitted for NMR analysis.

2.2.5 Attempted synthesis of a-D-Glucosaccharino-1,4-lactone (2-C-methyl-D-ribo-pentono-1,4-lactone).

To prepare 2-C-methyl-D-ribo-pentono-1,4-lactone, a modified version of the synthetic method reported by Whistler and BeMiller was adopted. A solution of β-D-fructose (100g) in water (1L) was heated to 100 ^{o}C and calcium hydroxide (10g) was added. The mixture was cooled to room temperature flushed with nitrogen and stirred for 14 days after which time a further portion of calcium hydroxide (40 g) was added and the mixture was heated to 100 ^{o}C and stirred, under an atmosphere of nitrogen, for a further five days. The sample was cooled to room temperature and then filtered under vacuum. The solution was acidified and calcium precipitated by the addition of oxalic acid (38g) and the sample was warmed on water bath and filtered hot. The remaining calcium ions were moved by cation exchange chromatography, the solution was passed through an amberlite column (IR-120-=50 cm x 5 cm) which was conditioned when necessary by passing a dilute hydrochloric acid solution through. The final solution was placed in a fridge for crystallization. Unfortunately, after one week there was no evidence for any crystalline products. No further attempts were made to isolate the 1,4-lactone.

2.2.6 Cellulose degradation experiments

Cellulose powder (20 μm average particle cross section) (50 g) Sodium hydroxide (10 g) and water (UPW, 500 ml) were added to a three neck round bottom flask (1L) fitted with a condenser and a magnetic stirrer. The reaction was carried out under an atmosphere of N_2 atmosphere) (fig 2.2) at the room temperature, 50˚C and 90˚C respectively, and samples were removed at fixed time intervals as described below.

The samples were removed for analysis using (High performance ion exclusion chromatography) (HPIEC) Dionex ICS-3000 to analysis the samples.

1- NaOH catalysed hydrolysis of cellulose at room temperature

The samples were taken 3 times a day every day for rest of the experiment.

2- NaOH catalysed hydrolysis of cellulose at 50°C

The procedure above was repeated but the reaction was heated at 50°C, and samples

were taken every 3 hours for 5 days and then 3 times a day for the rest of the experiment

3- NaOH catalysed hydrolysis of cellulose at 90ºC

The procedure above was repeated but the reaction was heated at 90°C and samples were taken every hour for the first eight hours, every 3 hours on the second day and then 3 times a day until the end of the reaction. The samples taken for analysis (5ml) were placed into plastic centrifuge tubs (15ml) and then stored in a freezer until they were analysed.
At the end of the reaction, the suspension was then centrifuged (37.000 rpm) for 30 mins, after that which time they were filtered using a syringe- filter unit (0.22 µm).

The samples were diluted 100 fold (10 µl sample with 990 µl of a solution containing acrylic acid as an internal standard) and then analysed using the apparatus Dionex ICS-3000**.**

Fig 2.2 Cellulose degradation experiment

Chapter three

Results

3. Results

Determination of the reaction times and response factors for standard acids

High Performance Ion Exchange Chromatography (HPIEC, Dionex ICS-1500) was used to monitor the production of aliphatic acids generated during the degradation of cellulose. In order to calibrate the system, a series of solutions of standard acids of known concentration were prepared and their retention time determined and calibration graphs were constructed in order to determine response factors for each of the analytes.

3.1- Organic acids:

Details of the short chain aliphatic carboxylic acids organic acids analysed are provided in (Table 3.1) which includes their pK_a and the retention times on the HPIEC system.

Table3.1. Short chain aliphatic acids analysed by HPIEC

Organic acids	**pKa**	**Vol (1000ppm)**	**Flow rate** ml/min	**Pump**	**Concentration (ppm)**	t_{Ri} **(min)**
Acrylic acid (C3)	4.26	9.50µl	0.80	832 psi	10-50 ppm	16.20
Lactic acid (C3)	3.86	8.26µl	0.80	792 psi	50-80 ppm	9.70
Acetic acid (C2)	4.56	9.50 µl	0.80	830 psi	20-100 ppm	13.80
Formic acid (C1)	3.77	8.20 µl	0.80	835 psi	20-100 ppm	10.50
Gluconic acid (C6)	3.7	10 mg	0.80	832 psi	20-80 ppm	7.90
Glycolic acid (C2)	3.82	10 mg	0.80	832 psi	20-80 ppm	11.20
Glyceric acid (C3)	3.52	10 mg	0.80	832 psi	20-100 ppm	9.10
Pyruvic acid (C3)	2.50	7.89µl	0.80	830 psi	20-80 ppm	6.70
Propionic acid (C3)	4.67	10.07µl	0.80	832 psi	20-100 ppm	14.30

*Retention time t_{Ri} : The retention time is the time in which halve of the quantity of a solute, *i*, is eluted from the chromatographic system.[38]
*pKa : R. Williams, *pKa data compiled*, [37]

3.2. Preparation of standard aliphatic acids containing the internal standard

3.2.1 Liquid acids

A-Acrylic acid.[prop-2-enoic acid **](Internal standard)**

To prepare a stock solutions of acrylic acid a fixed volume (9.50 µl) of acid was taken and diluted in water (UPW 10 ml) to provide a working standard of known concentration (approximately 1000 ppm) this was then diluted (50,....,10 ppm) to provide a linear range of concentrations for use in determining the detector response to this acid.

An example of the dilutions used: (1ml) of the stock solution (1000 ppm) was add to water (9 ml), to give a solution of 100ppm.The solutions were then injected onto the HPAEC column and the peak area ($\mu c/s^{-1}$) and the retention time of the acid was noted.

The results of acrylic acid are presented in table 3.2. The standard calibration was constructed in (fig 3.1).

Table 3.2 Peak area of acrylic acid concentration

ppm	0	10	20	30	40	50
average	0	0.0165	0.032	0.067	0.087	0.1395
µs*min 1	0	0.020	0.030	0.054	0.084	0.146
µs*min 2	0	0.013	0.034	0.079	0.089	0.133

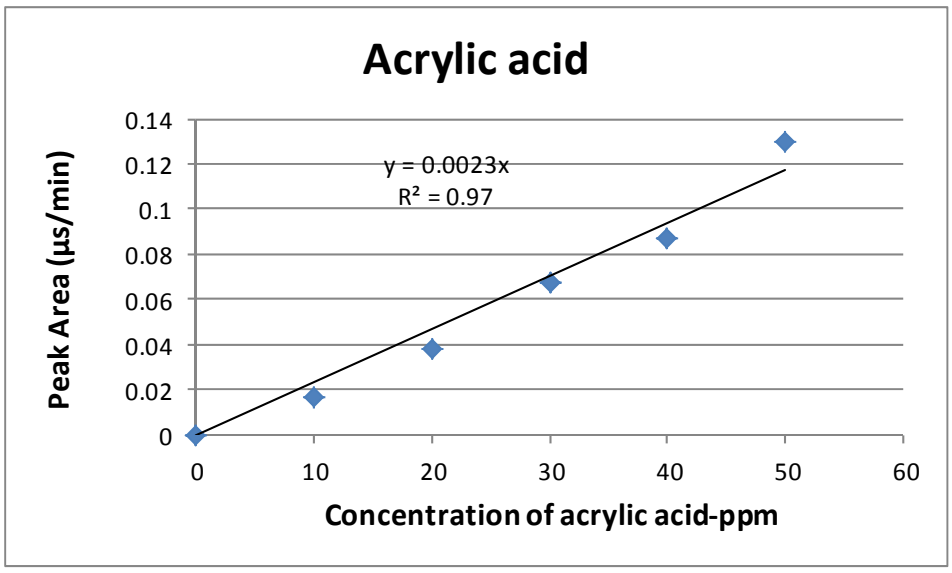


Fig 3.1- Concentration of Acrylic acid

B **-Lactic acid.** 2-Hydroxypropanoic acid

A stock solutions of lactic acid was prepared as follows: a fixed volume (9.50 µl) of acid was taken and diluted in water (UPW 10 ml) to provide a working standard of known concentration (1000 ppm), this was then diluted (80-50 ppm) to provide a linear range of concentrations for use in determining the detector response to this acid.

The results of lactic acid are presented in table 3.3. The standard calibration was constructed in (fig 3.2, fig 3.3)

Table 3.3 Peak area of lactic acid concentration

ppm	50	60	70	80
average	1.759	2.142	2.61	3.42
µs*min 1	1.767	2.065	2.832	3.347
µs*min 2	1.767	2.143	2.254	3.426
µs*min 3	1.743	2.217	2.71	3.487

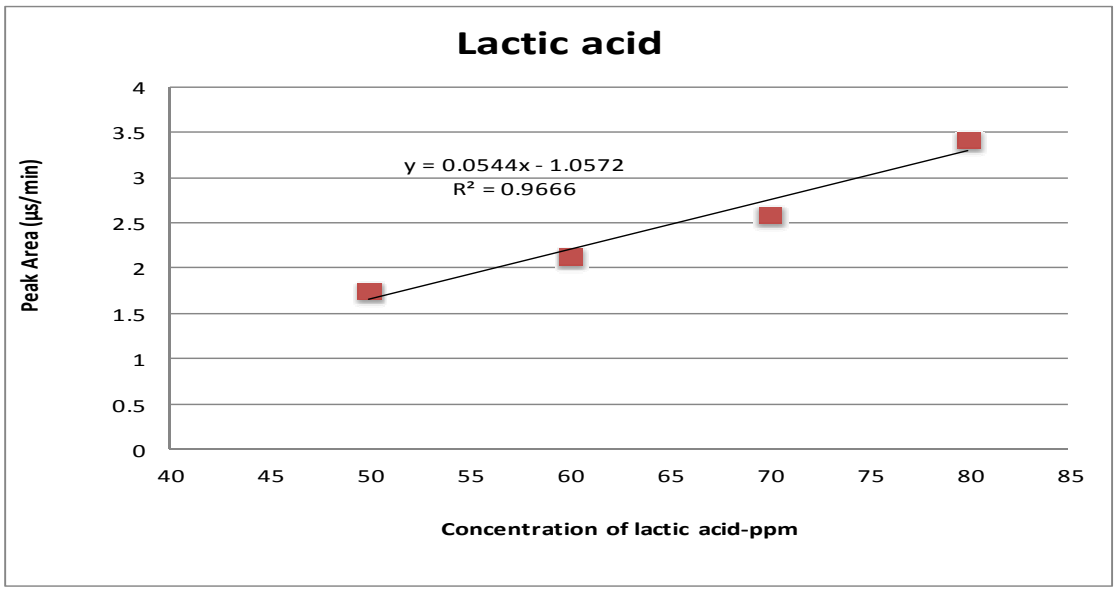


Fig 3.2- Concentration of Lactic acid

A small impurity peak was observed in the chromatograms for lactic acid and therefore it was decided to run a ^{1}H NMR of the sample in an attempt to establish the identity of the impurity:

A number of small signals were observed at 4.8, 4.4 and 1.3 ppm and the low field signals (4.8 and 4.4) are consistent with the presence of additional compounds being present containing methyne protons adjacent to methyl groups $CH_3CHX(COR)$, the high chemical shift of 4.8 ppm may indicate that there is a small amount ester (R =lactate bonded through the hydroxyl group) or anhydride (R= lactate bonded through the acid).

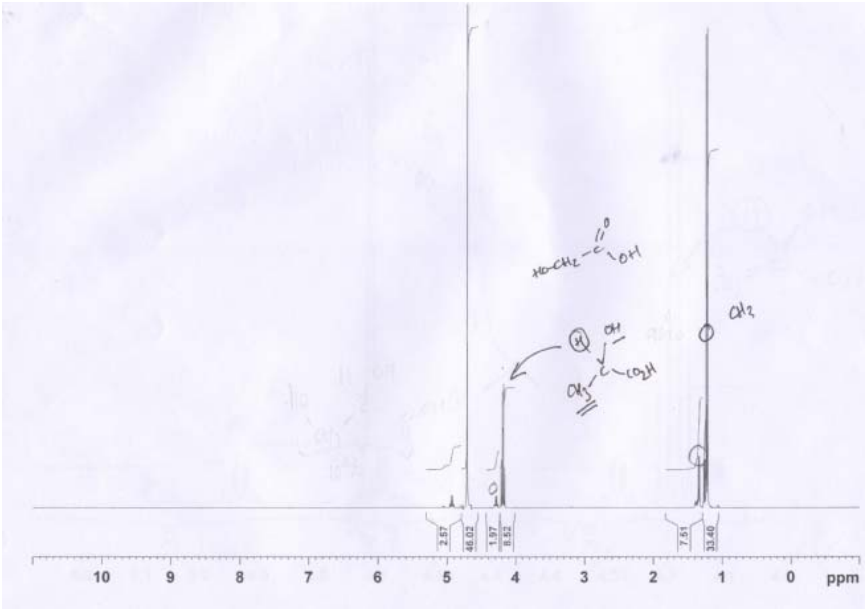


Fig 3.3-NMR result of Lactic acid

C- Acetic acid. Ethanoic acid

A stock solution of acetic acid was prepared as follows: a fixed volume (9.50 µl) of acid was taken and diluted in water (UPW 10 ml) to provide a working standard of known concentration (1000 ppm),this was then diluted (100,....,20 ppm) to provide a linear range of concentrations for use in determining the detector response to this acid.

The results of acetic acid are presented in table 3.4. The standard calibration was constructed in (fig 3.4).

Table 3.4 Peak area of acetic acid concentration

Ppm	0	20	40	60	80	100
average	0	1.39	2.65	3.15	3.85	4.95
µs*min 1	0	1.47	2.57	2.76	3.7	4.22
µs*min 2	0	1.4	2.71	3.21	4.06	5.4
µs*min 3	0	1.3	2.68	3.47	3.8	5.23

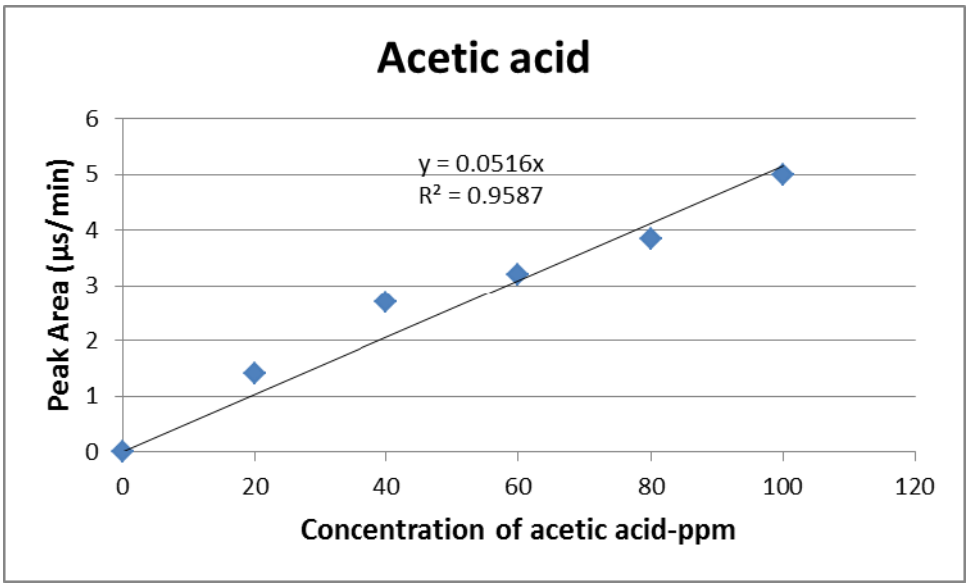


Fig 3.4- Concentration of Acetic acid

D- Formic acid. Methanoic acid

A stock solution of formic acid was prepared as follows: a fixed volume (8.20 µl) of acid was taken and diluted in water (UPW 10 ml) to provide a working standard of known concentration (1000 ppm),this was then diluted (100,....,20 ppm) to provide a linear range of concentrations for use in determining the detector response to this acid.

The results of formic acid are presented in table 3.5. The standard calibration was constructed in (fig 3.5).

Table 3.5 Peak area of formic acid concentration

ppm	0	20	40	60	80	100
average	0	1.29	2.31	3.45	4.48	5.42
µs*min 1	0	1.36	2.85	3.17	4.41	5.15
µs*min 2	0	1.42	2.06	3.72	4.38	6.35
µs*min 3	0	1.09	2.03	3.45	4.66	4.75

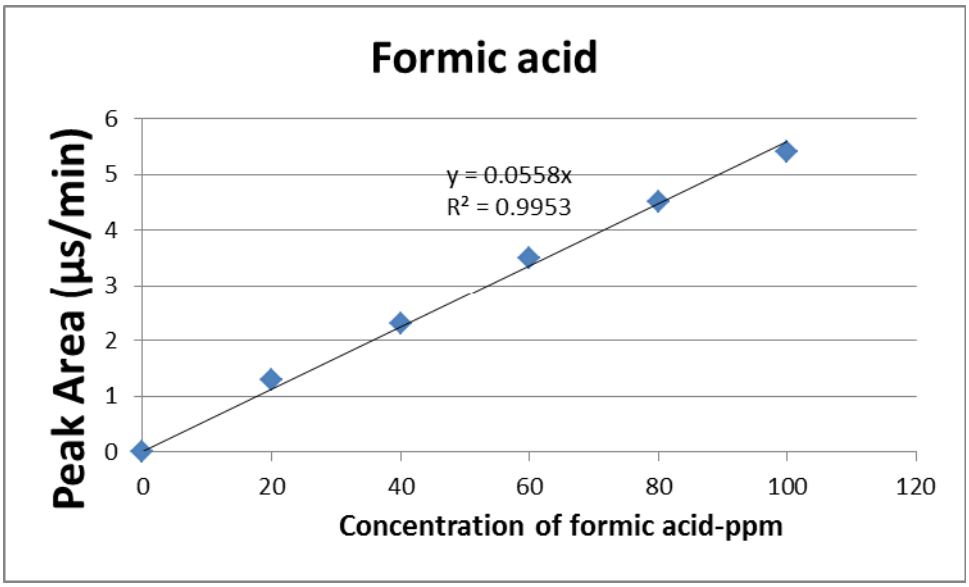


Fig 3.5- Concentration of Formic acid

E- **Propionic acid.** Propanoic acid

A standard solution of propionic acid was prepared as follows: acid a fixed volume (10.07 µl) of acid was taken and diluted in water (UPW 10 ml) to provide a working standard of known concentration (1000 ppm), this was then diluted (100,....,20 ppm) to provide a linear range of concentrations for use in determining the detector response to this acid.

The results of propionic acid are presented in table 3.6. The standard calibration was constructed in (fig 3.6).

Table 3.6 Peak area of propionic acid concentration

ppm	0	20	40	60	80
average	0	0.324	0.718	0.849	1.29
µs*min 1	0	0.309	0.722	0.745	1.201
µs*min 2	0	0.338	0.714	0.953	1.384

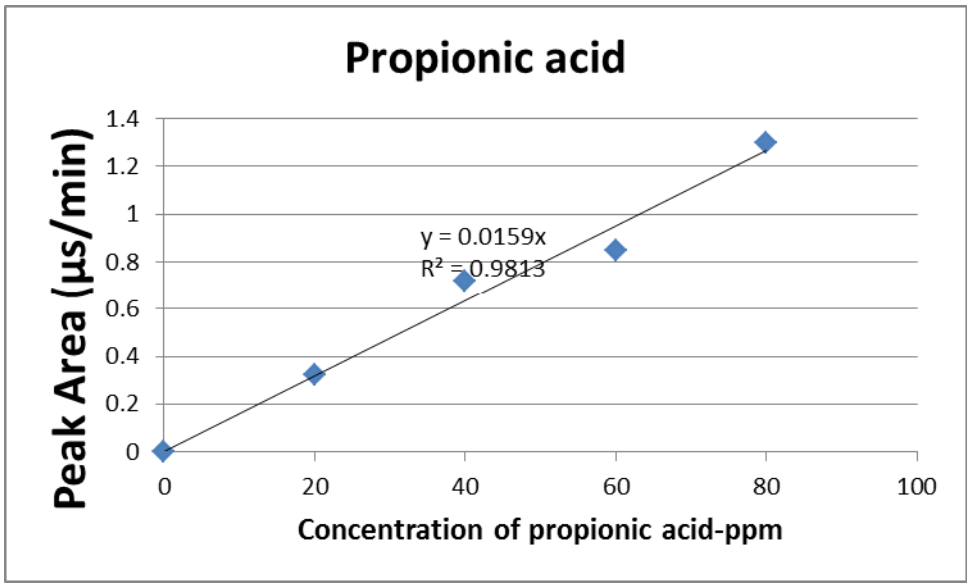


Fig 3.6- Concentration of Propionic acid

F- **Pyruvic acid** 2-oxopropanoic acid

Standard solutions of pyruvic acid were prepared as follows: a fixed volume (7.89 µl) of acid was taken and diluted in water (UPW 10 ml) to provide a working standard of known concentration (1000 ppm),this was then diluted (80,....,20 ppm) to provide a linear range of concentrations for use in determining the detector response to this acid.

The results of pyruvic acid are presented in table 3.7. The standard calibration was constructed in (fig 3.7).

Table 3.7 Peak area of pyruvic acid concentration

ppm	0	20	40	60	80
average	0	0.0.78	1.40	2.05	2.4
µs*min 1	0	0.81	1.41	2.11	2.42
µs*min 2	0	0.75	1.38	1.98	2.39

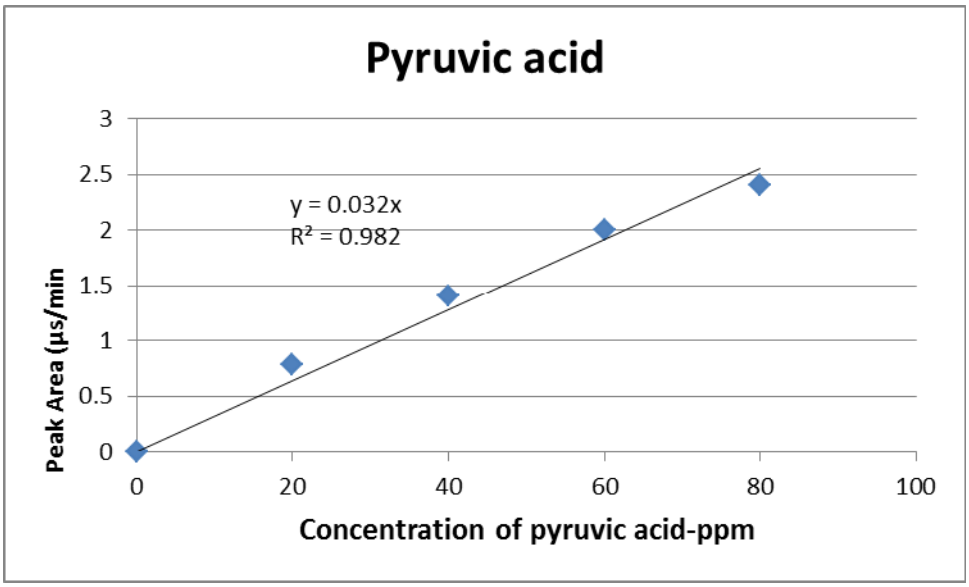


Fig 3.7- Concentration of Pyruvic acid

G- 3,4-dihydroxybutanoic acid

The original HPIEC traces observed for an aqueous stock solution of 3,4-dihydroxybutanoic acid indicated the presence of multiple peaks; as this sample had been prepared by (Mr Paul Shaw), an NMR was run to confirm if the compound had decomposed since its initial preparation.

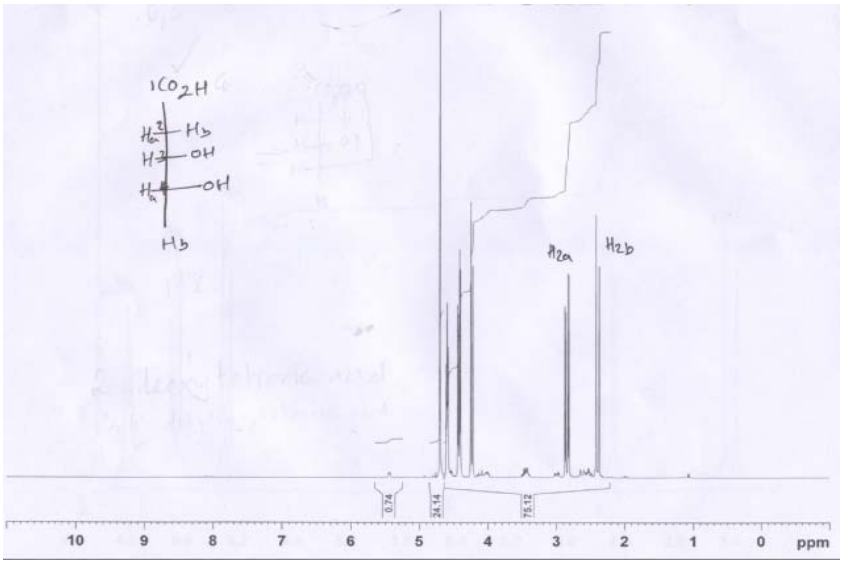


Fig 3.8- NMR of 3,4-dihydroxybutanoic acid

The ^{1}H NMR confirmed that the correct compound had been prepared and that it was still intact and of a reasonable purity**.** In aqueous solution, 3,4-dihydroxybutanoic acid can undergo spontaneous lactonisation to form a five-member ring lactone (fig 3.9)

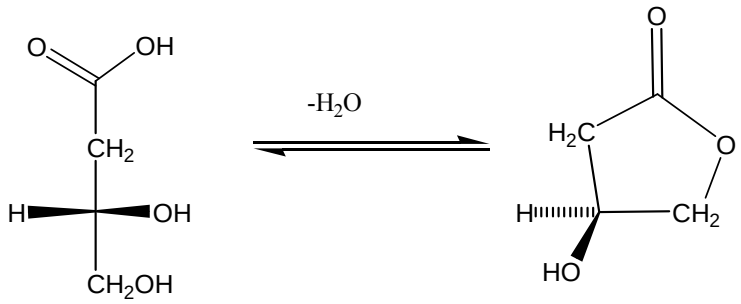


Fig 3.9 member ring lactone

When fresh solutions of 3,4-dihydroxybutanoic acid were prepared and injected directly into the HPIEC a large peak accounting for over 70% of the total peak area was observed at 10.1 mins (Fig 3.10, left hand-side) and this is thought to arise from the ring opened 3,4-dihydroxybutanoic acid; at the same time a second peak was observed at 7.3 mins. As the samples aged, a complex chromatogram was obtained but the peak at 7.3 mins increased and this was attributed to the lactone form of 3,4-dihydroxybutanoic acid (Fig 3.10, right hand-side).

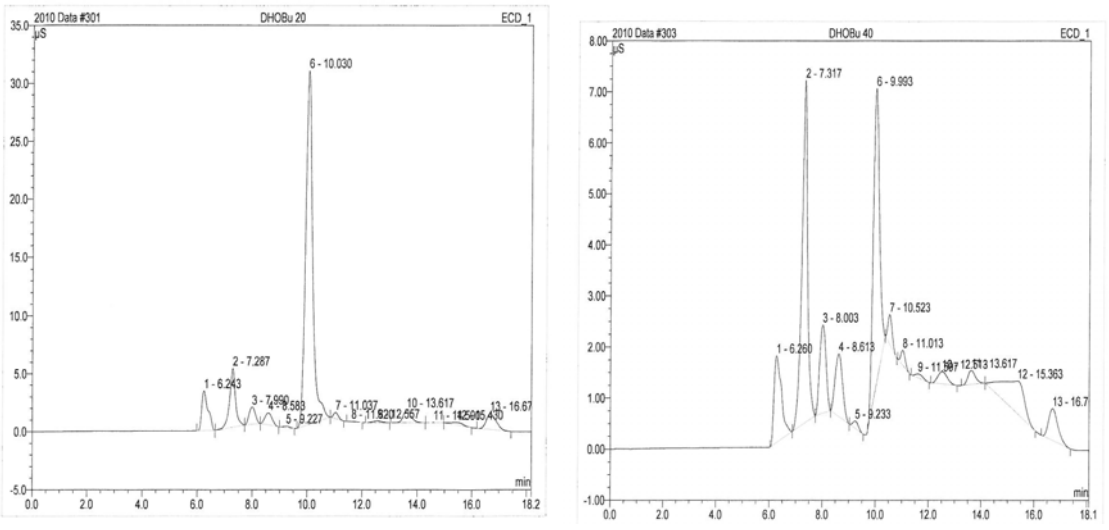


Fig 3.10: Left-Fresh Solution of 3,4-dihyroxybutanoic acid, Right-repeat injection of 3,4-dihyroxybutanoic acid after 40 mins at room temp.

3.2.2 Prepartion of standard solutions of solid acids:

A- Gluconic acid.

To prepare standards for gluconic acid a small portion of the solid (10.0 mg) was taken and the solid was dissolved in water (10 ml) to give a standard solution of concentration of 1000 ppm, and this was diluted as described for the liquid sample to give calibration standards (80,....,20 ppm)

The results of gluconic acid are presented in table 3.8. The standard calibration was constructed in (fig 3.11).

Table 3.8 Peak area of gluconic acid concentration:

ppm	0	20	40	60	80
average	0	0.056	0.112	0.236	0.323
µs*min 1	0	0.052	0.073	0.23	0.308
µs*min 2	0	0.061	0.128	0.232	0.332
µs*min 3	0	0.055	0.136	0.246	0.328

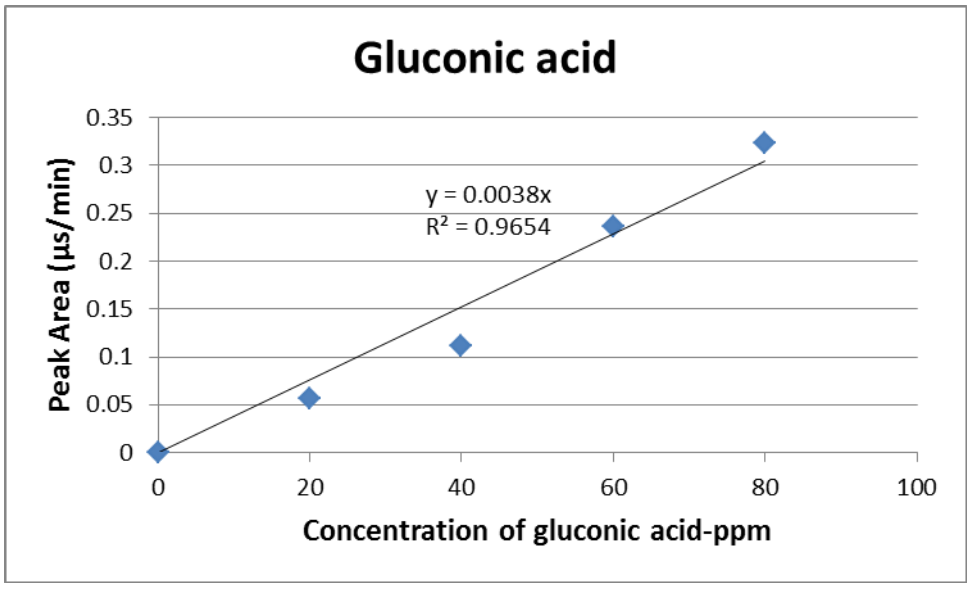


Fig 3.11- Concentration of Gluconic acid

B- **Glycolic acid**. 2-Hydroxyethanoic acid

To prepare standards for glycolic acid a small portion of the solid (10.0 mg) was taken and the solid was dissolved in water (10 ml) to give a standard solution of concentration of 1000 ppm, and this was diluted as described for the liquid sample to give calibration standards (80,....,20 ppm)

The results of glycolic acid are presented in table 3.9. The standard calibration was constructed in (fig 3.12).

Table 3.9 Peak area of glycolic acid concentration

ppm	0	20	40	60	80
average	0	0.498	1.842	2.288	3.464
µs*min 1	0	0.556	2.044	2.407	3.084
µs*min 2	0	0.489	1.678	2.308	3.659
µs*min 3	0	0.449	1.804	2.15	3.649

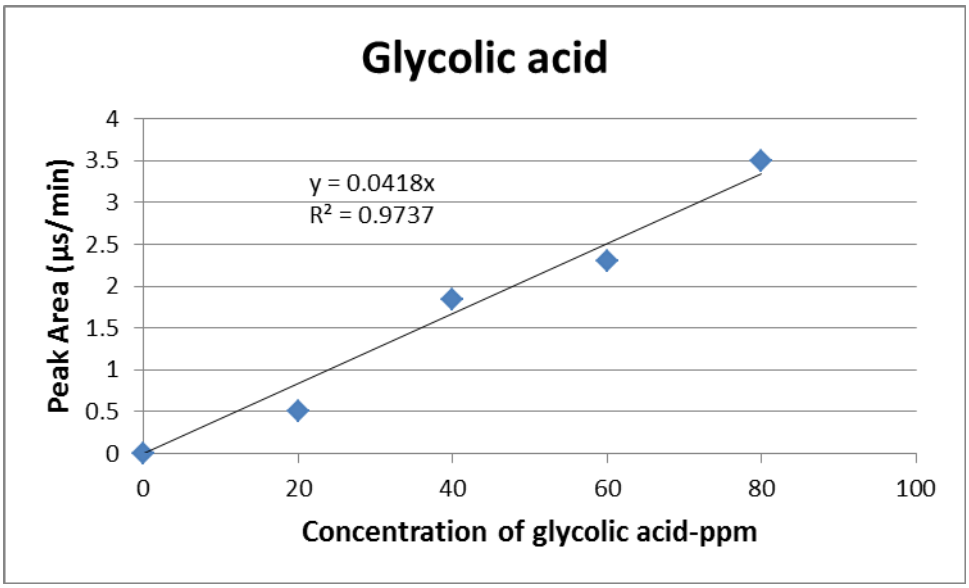


Fig 3.12- Concentration of Glycolic acid

NMR analysis of the glycolic acid indicated that the sample was pure and a single methylene CH_2 was observed at 3.9 δ.

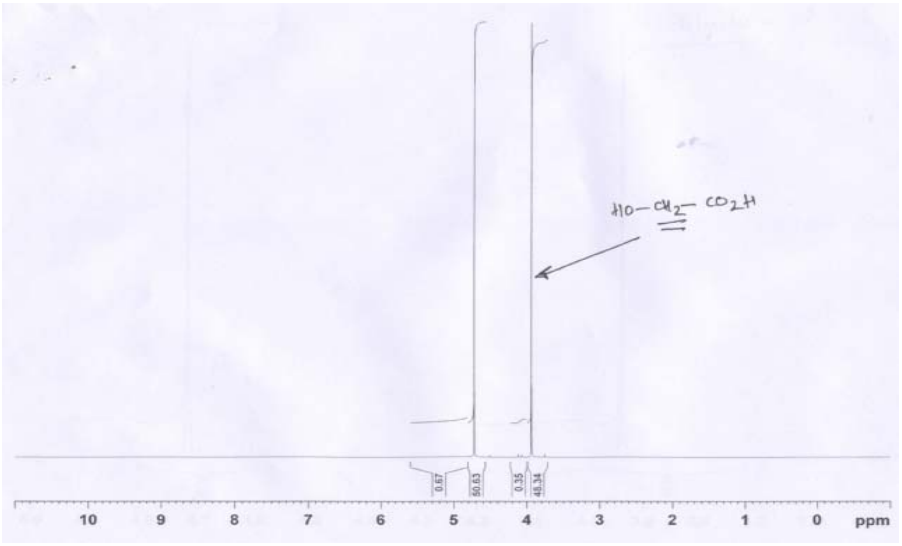


Fig 3.13 NMR analysis of Glycolic acid

C- **Glyceric acid** 2,3-Dihydroxypropanoic acid

To prepare standards for glyceric acid a small portion of the solid (10.0 mg) was taken and the solid was dissolved in water (10 ml) to give a standard solution of concentration of 1000 ppm, and this was diluted as described for the liquid sample to give calibration standards (100,....,20 ppm)

The results of glyceric acid are presented in table 3.10. The standard calibration graph was constructed (fig 3.14).

Table 3.10 Peak area of glyceric acid concentration:

ppm	0	20	40	60	80	100
average	0	0.331	0.541	0.89	1.25	1.46
µs*min 1	0	0.313	0.578	0.874	1.226	1.485
µs*min 2	0	0.349	0.503	0.897	1.274	1.439

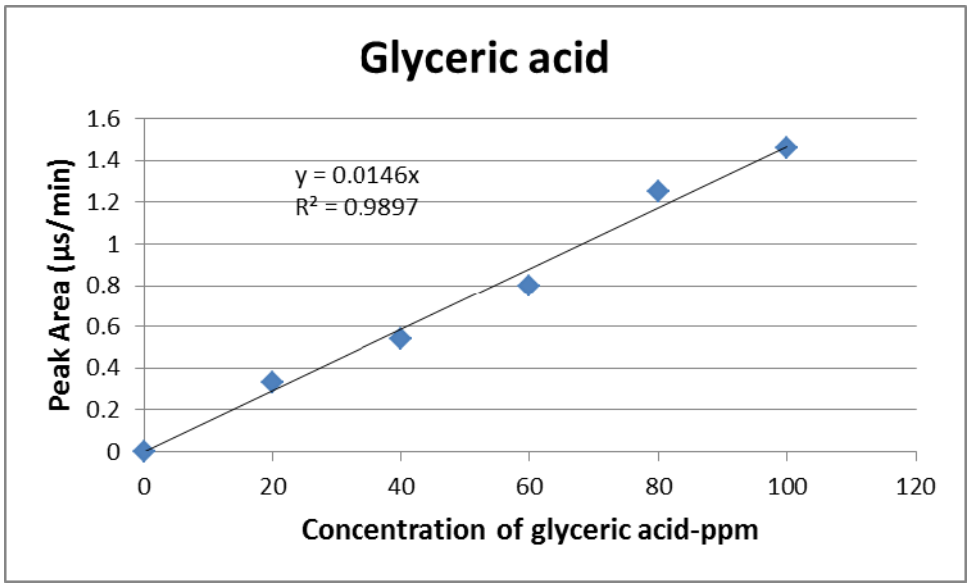


Fig 3.14- Concentration of Glyceric acid

3.2.3 Analysis of alpha and beta-isosaccharinic acid by HPIEC.

Pure samples of alpha and beta isosaccharinic acid were prepared and analysed using HPAEC and HPIEC by Mr Paul Shaw. At the pH of the mobile phase, the two acids undergo a rapid spontaneous lactonisation and only the lactone form of the two acids is visible by HPIEC. The two diasteromeric lactones coeluted and the retention times determined for the lactone forms of two acids under the conditions employed in this study was 8.1 mints see peak 2 in figure 3.15.

3.3 -Monitoring the hydrolysis of cellulose

The cellulose hydrolysis reactions were performed at three different temperatures as described in the experimental section. As the reactions were expected to proceed at different rates (very slow at room temperature, slow at 50 ^{o}C and at a moderate rate at 90 ^{o}C) different sampling intervals were used to follow each of the reactions.

At room temperature; the samples were taken 3 times a day every day, after the samples were filtered and diluted they were then analysed by HPAEC-PAD and the peak areas for each of the analytes were compared with those for the calibration standards. A representative chromatogram from the analysis of samples taken close to the start, in the middle and towards the end of the reaction that was performed at room temperature is presented in below (Fig 3.15, 3.16, 3.17).

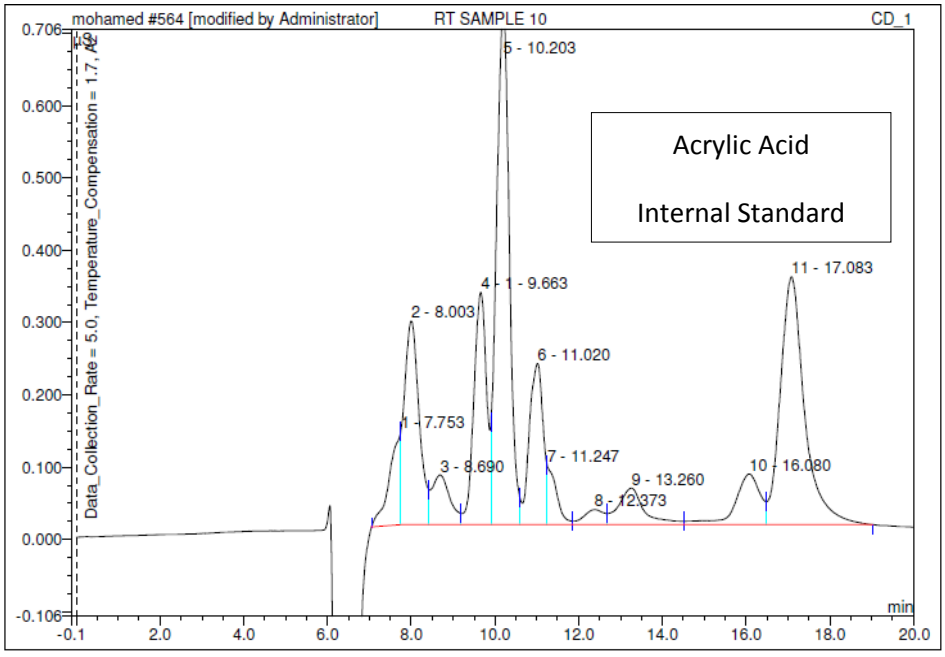


Fig 3.15-Chromatogram of cellulose degradation at room temperature

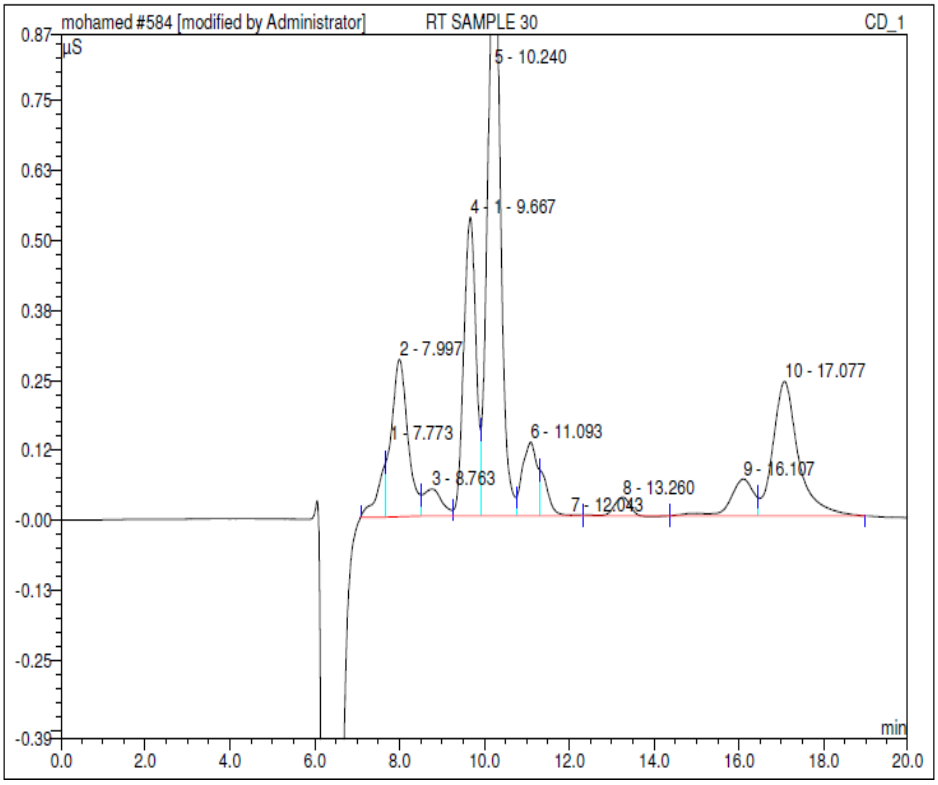


Fig 3.16- Chromatogram of cellulose degradation at room temperature

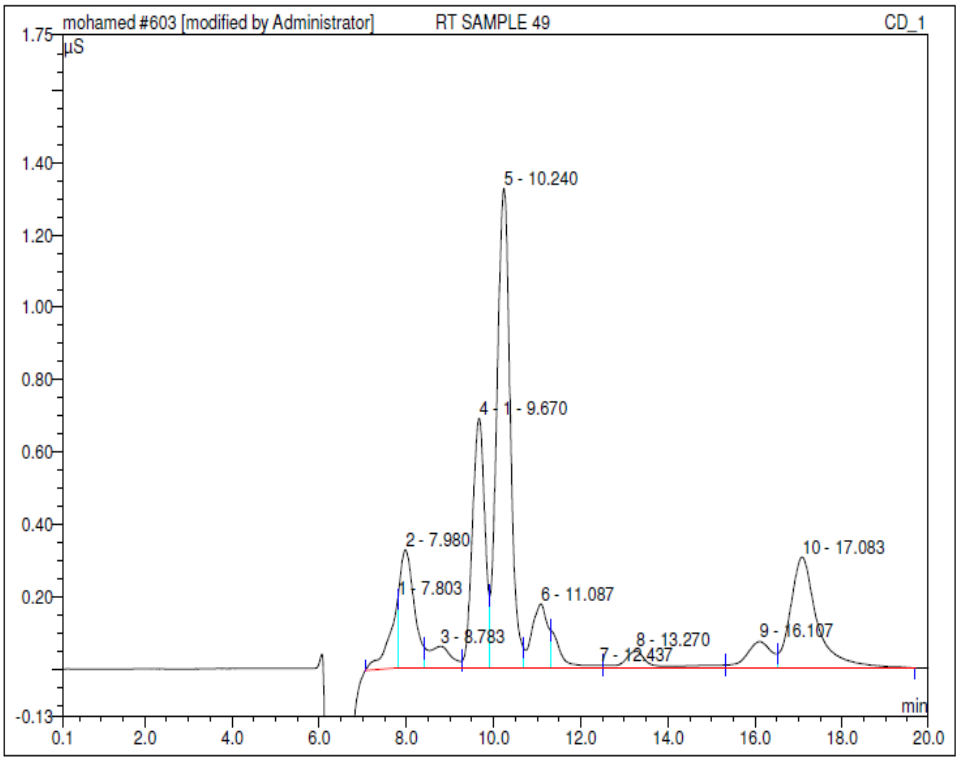


Fig 3.17- Chromatogram of cellulose degradation at room temperature

At 50˚C- samples were taken every 3 hours (5 days) and 3 times a day thereafter, after the samples were filtered and diluted they were analysed by HPAEC-PAD and the peak areas for each of the analytes were compared with those for the calibration standards. A representative chromatogram for samples taken close to the start, in the middle and towards the end of the reaction that was performed at 50ºC are presented in (Fig 3.18,3.19,3.20)

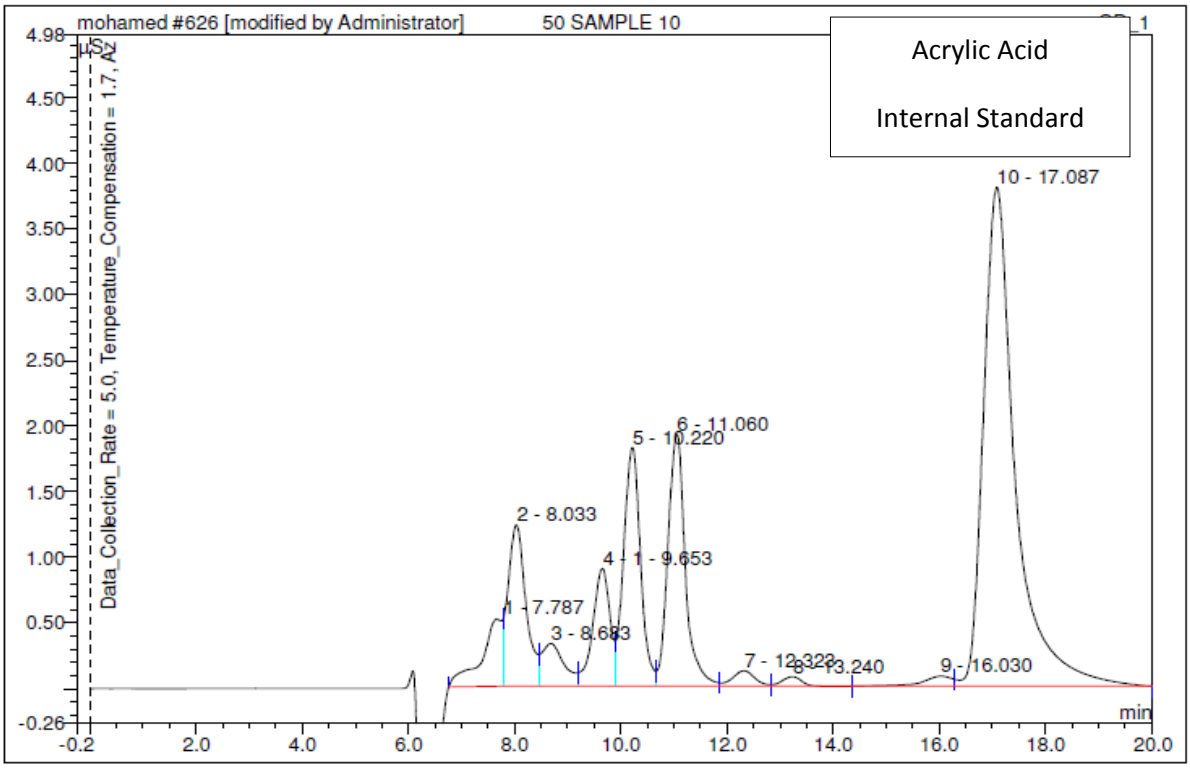


Fig 3.18- Cromatogram of cellulose degradation at 50ºC

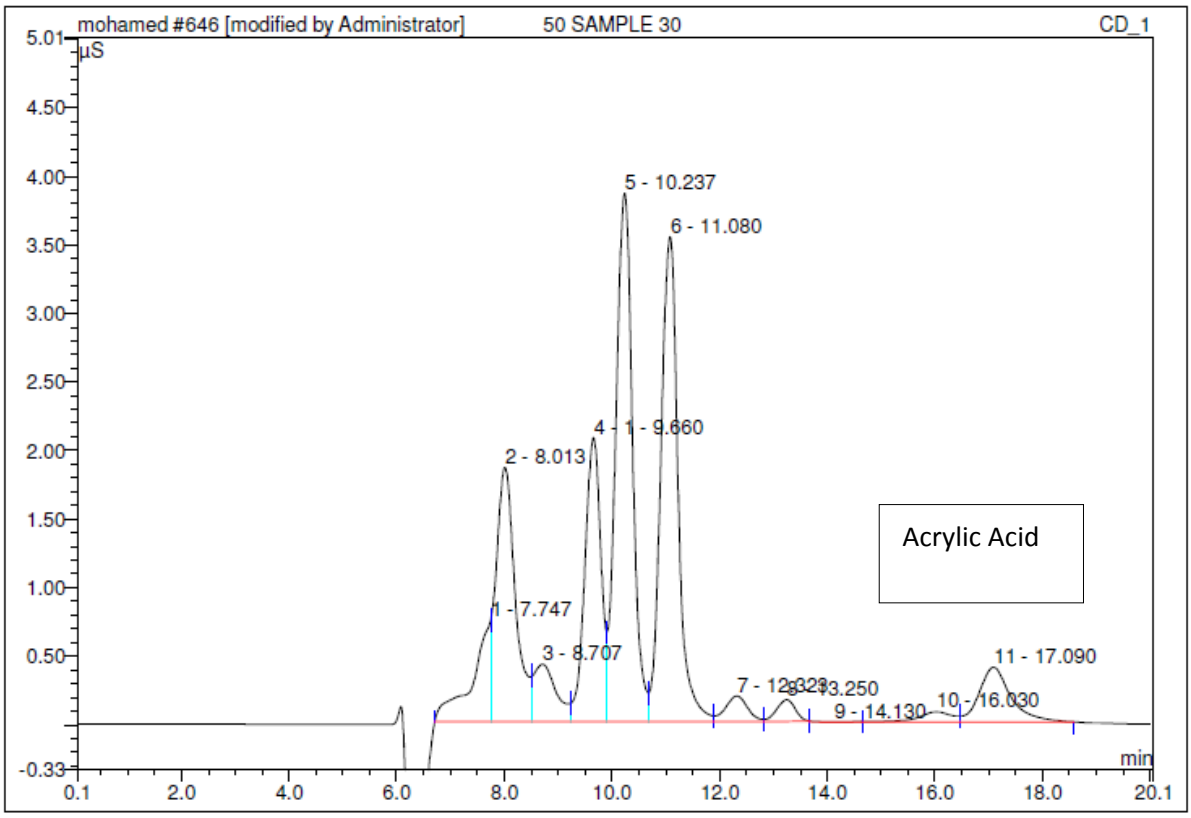


Fig 3.19- Chromatogram of cellulose degradation at 50ºC

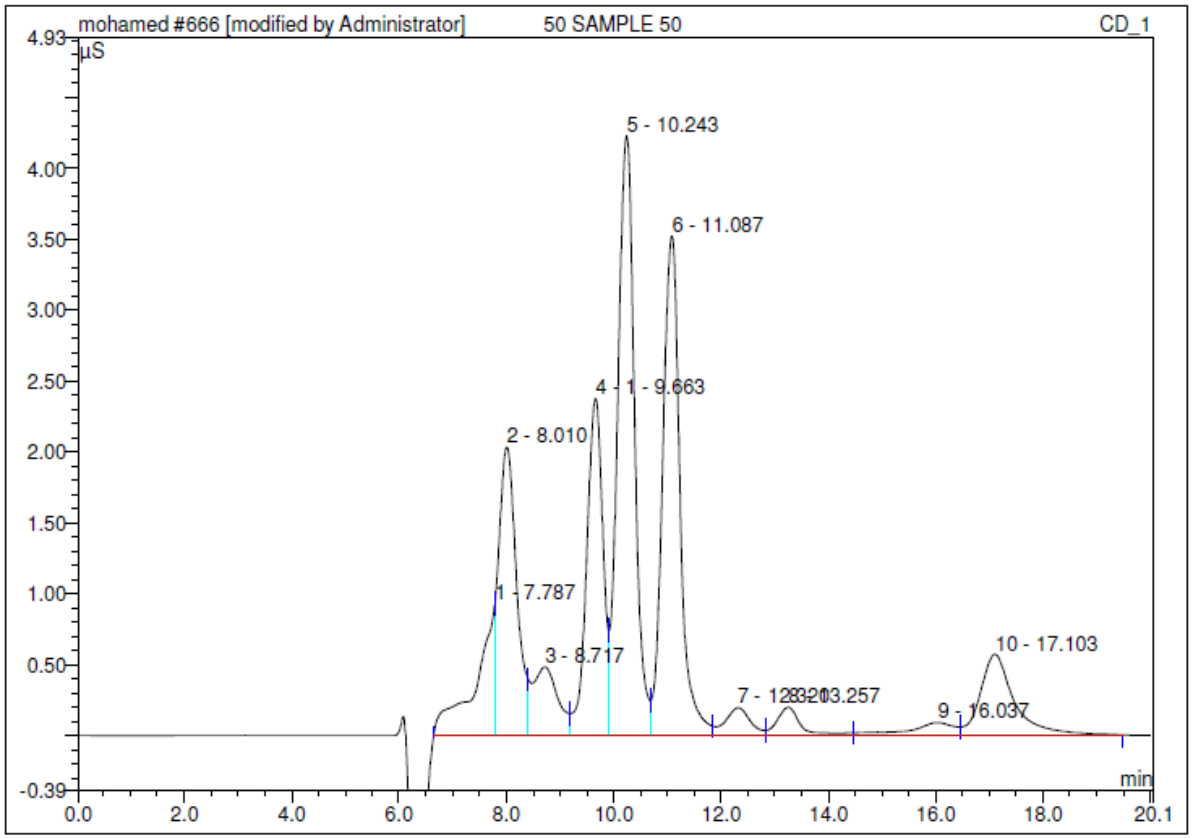


Fig 3.20- Chromatogram of cellulose degradation at 50ºC

At 90˚C -samples were taken every hour for the first day, every 3 hours for the second day, and then 3 times a day thereafter, after the samples were filtered and diluted they were then analysed by HPAEC-PAD and the peak areas for each of the analytes were compared with those for the calibration standards. A representative chromatogram described the analysis of sample at 90ºC are presented in Fig 3.21, 3.22, 3.23

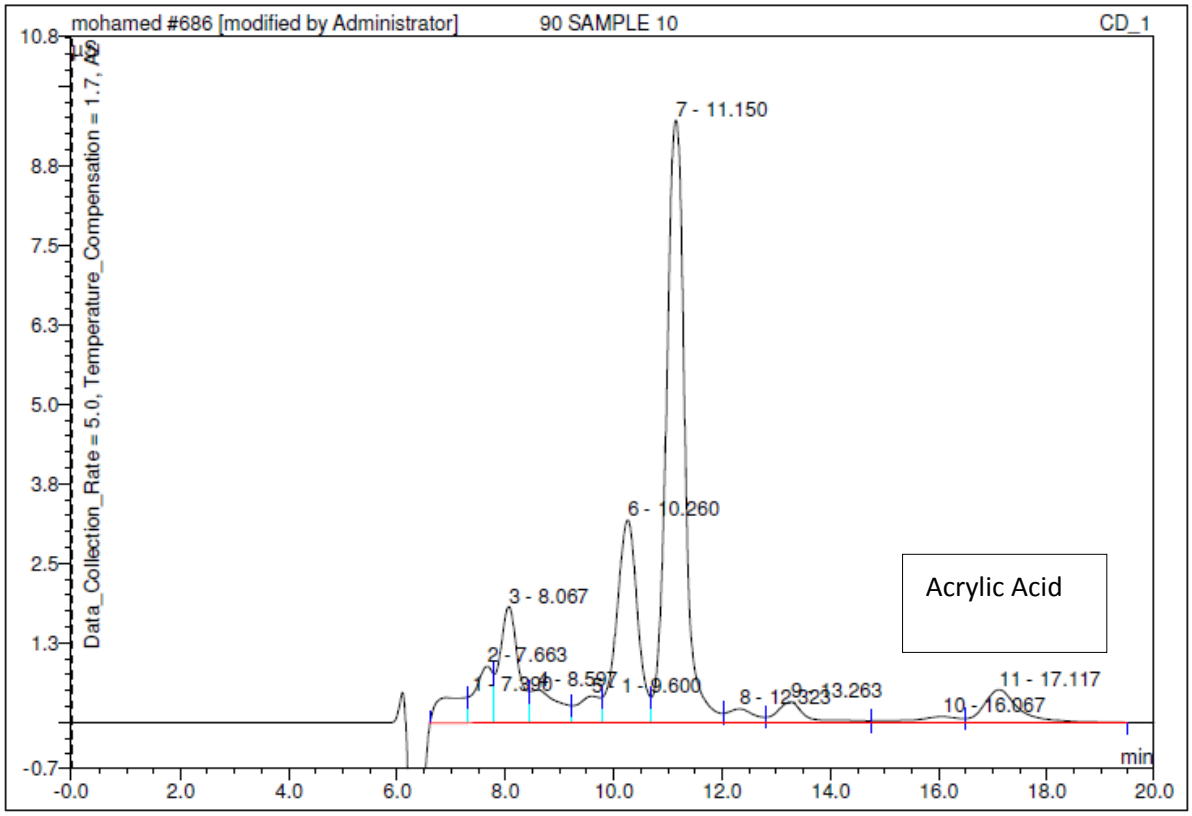


Fig 3.21- Chromatogram of cellulose degradation at 90ºC

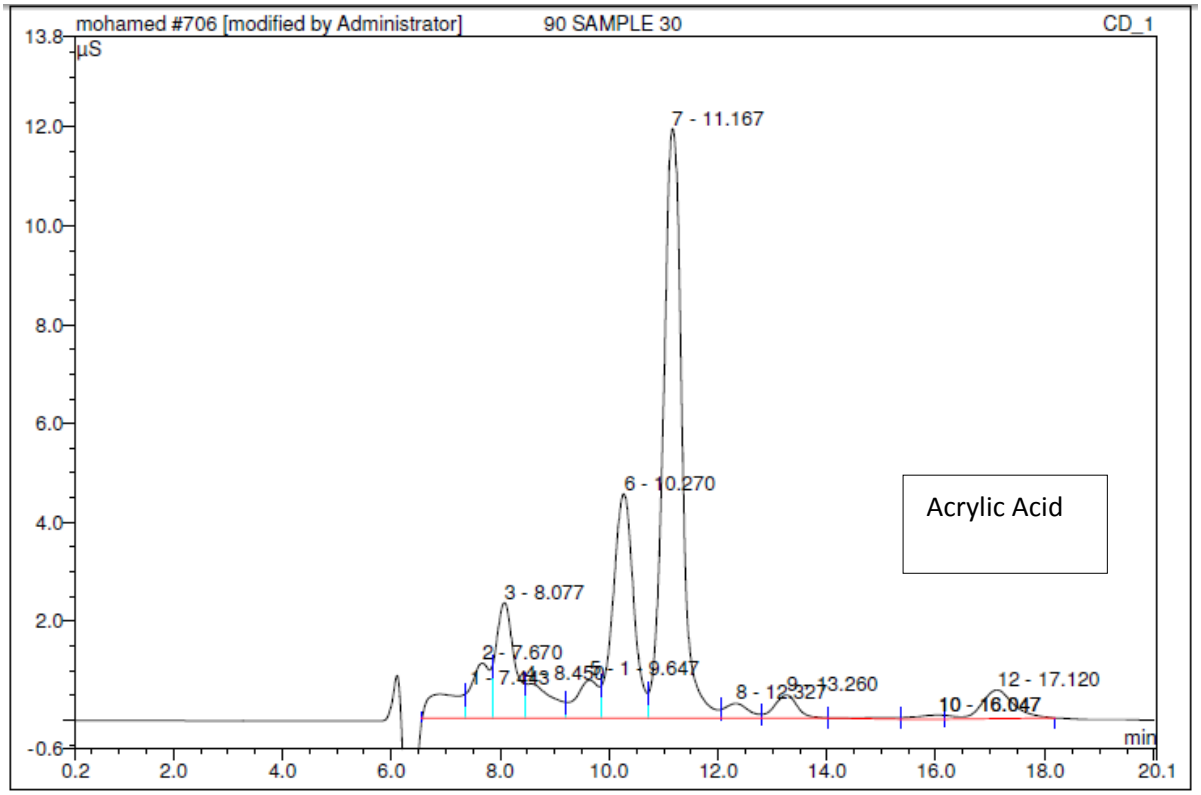


Fig 3.22- Chromatogram of cellulose degradation at 90ºC

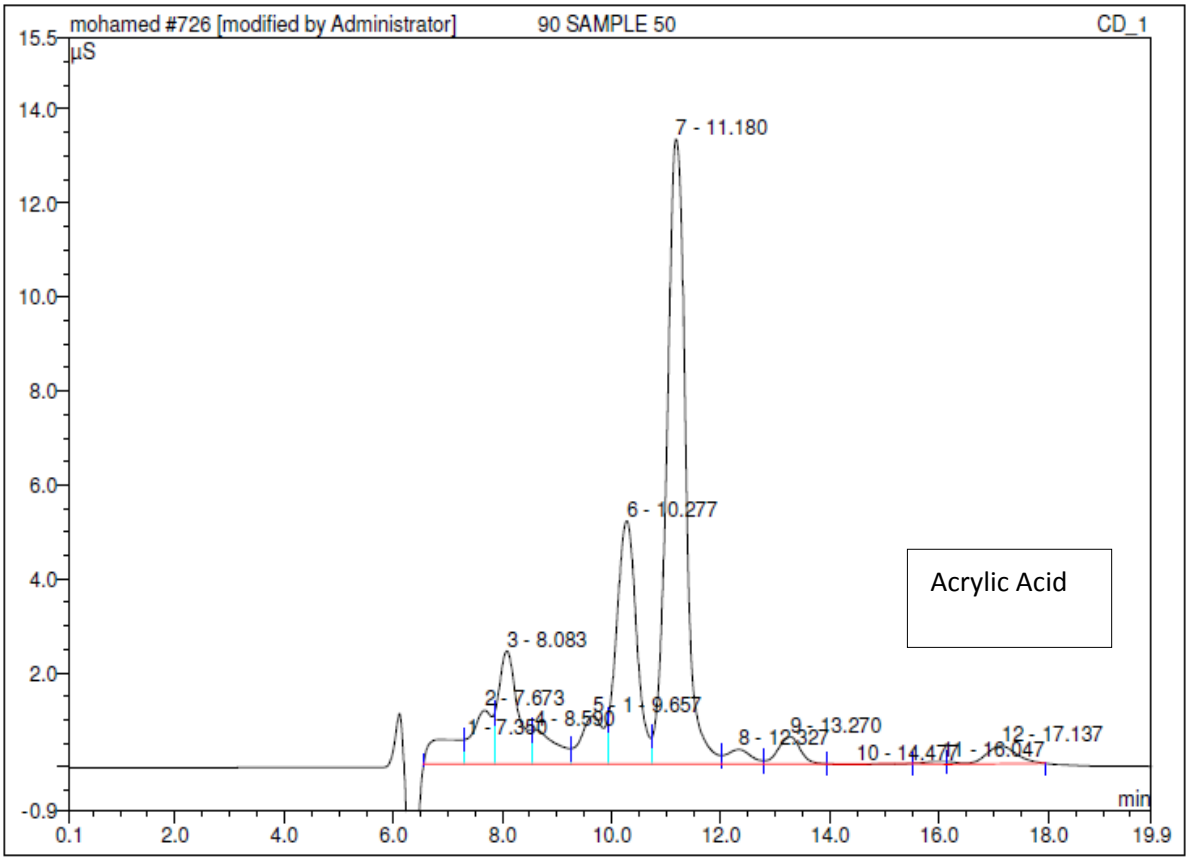


Fig 3.23- Chromatogram of cellulose degradation at 90ºC

The concentrations of the individual acids were then determined from the area underneath the individual peaks and these are presented in the following tables and the results are plotted in the corresponding graphs.

3.4. Identification of acids through the use of spiking experiments.

Solutions of the expected decomposition products (glyceric, glycolic, formic and lactic acids) were prepared at 5000 ppm and were mixed with an equal volume of the samples recovered from the decomposition of cellulose at room temperature (RT Samples 21-25) where RT is the room temperature. The ratio of the additions (v/v) was chosen such that the original acylic acid peak was visible and the peak from the spiked acid was also visible the chromatographs are shown in figures 3.24 a-e.

Figure 3.24 .1-Sample spiked with glycolic acid:

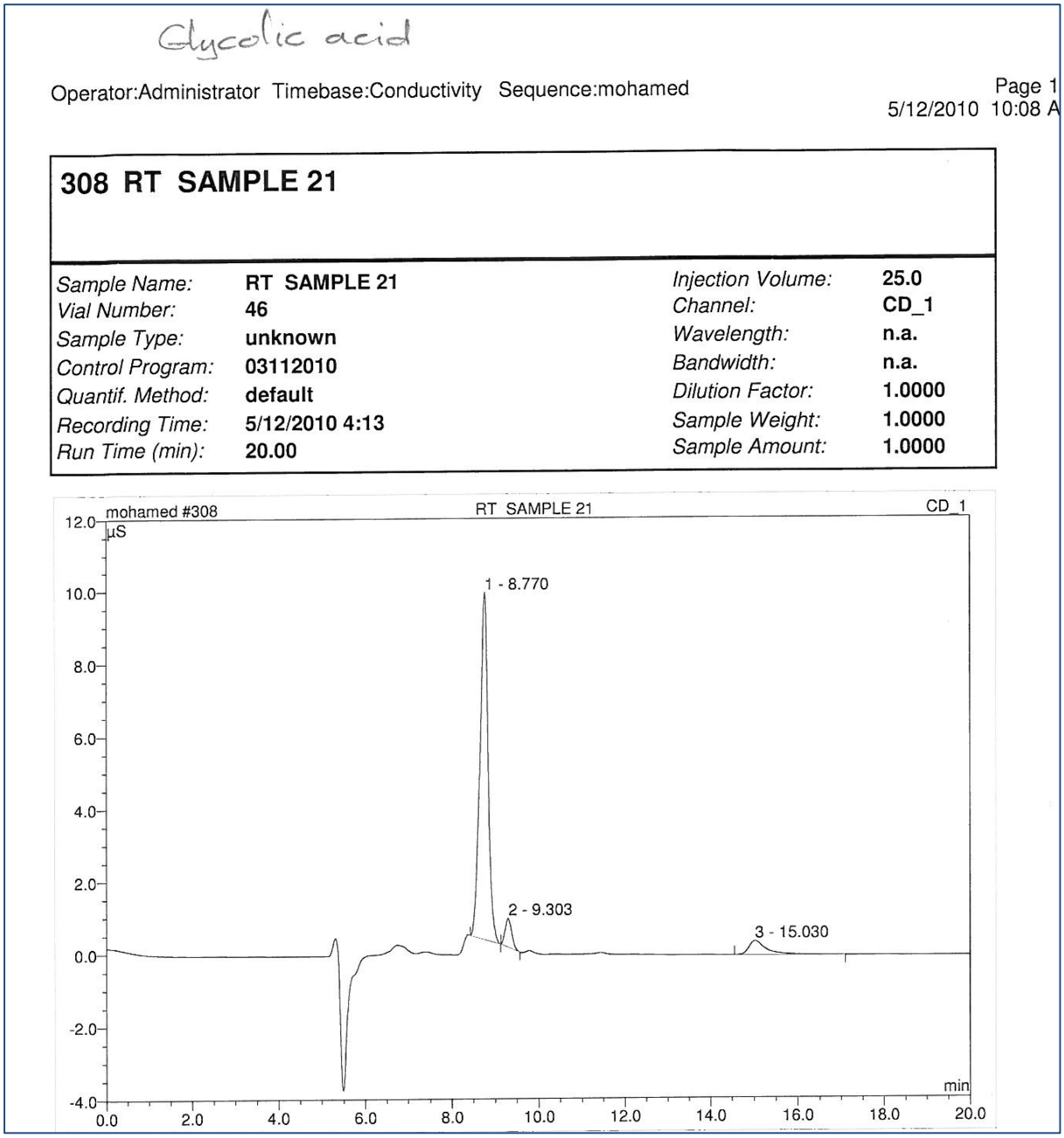


Figure 3.24 .2-Sample spiked with glyceric acid:

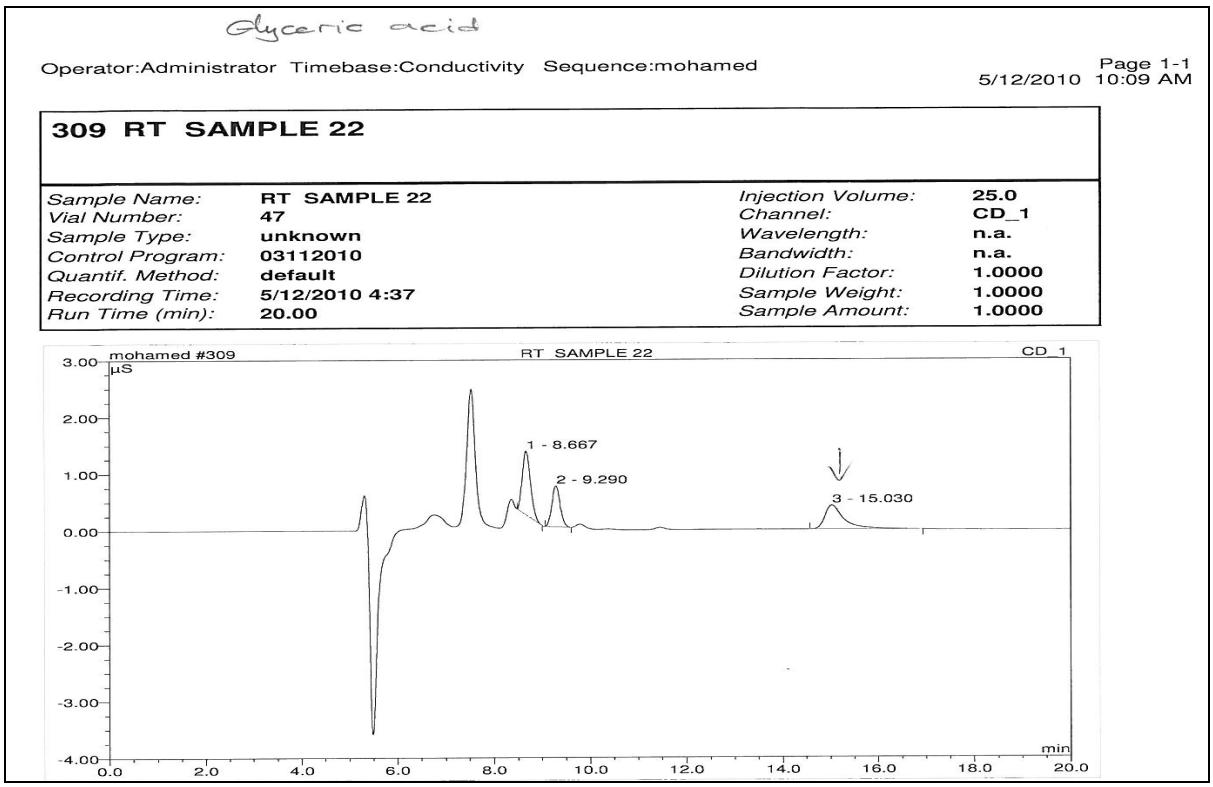


Figure 3.24.3-Sample spiked with acrylic acid:

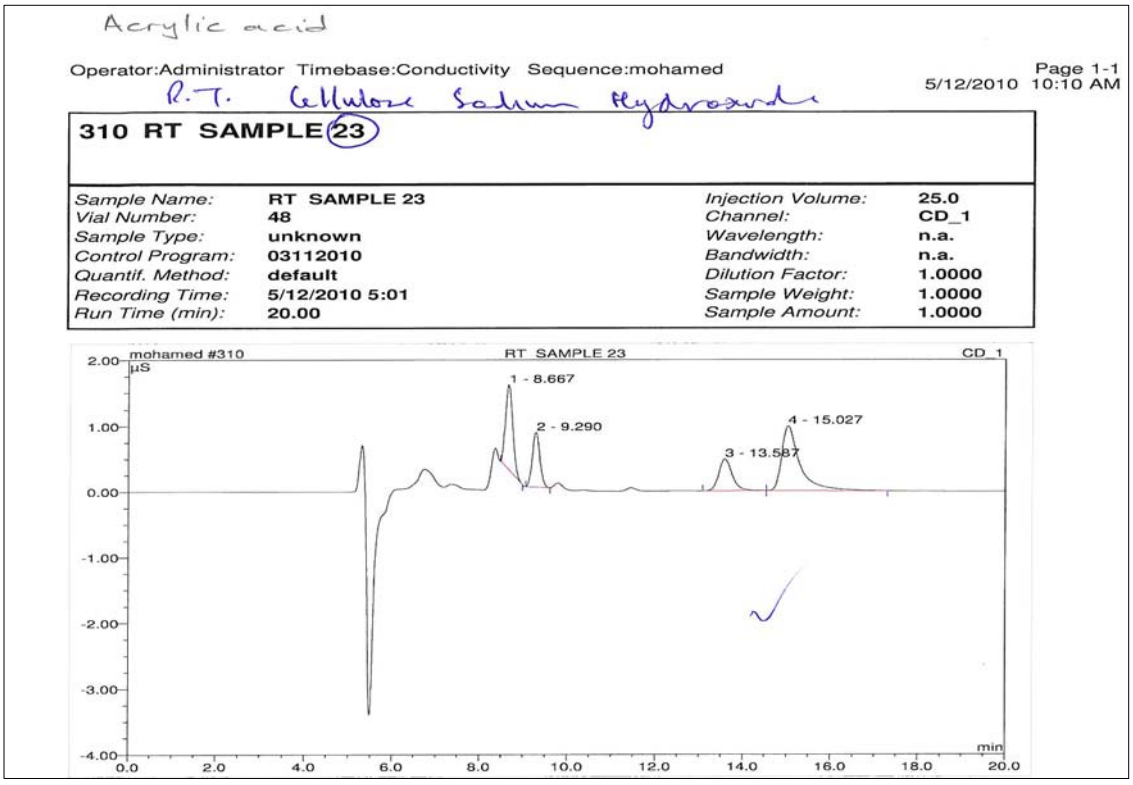


Figure 3.24.4-Sample spiked with formic acid:

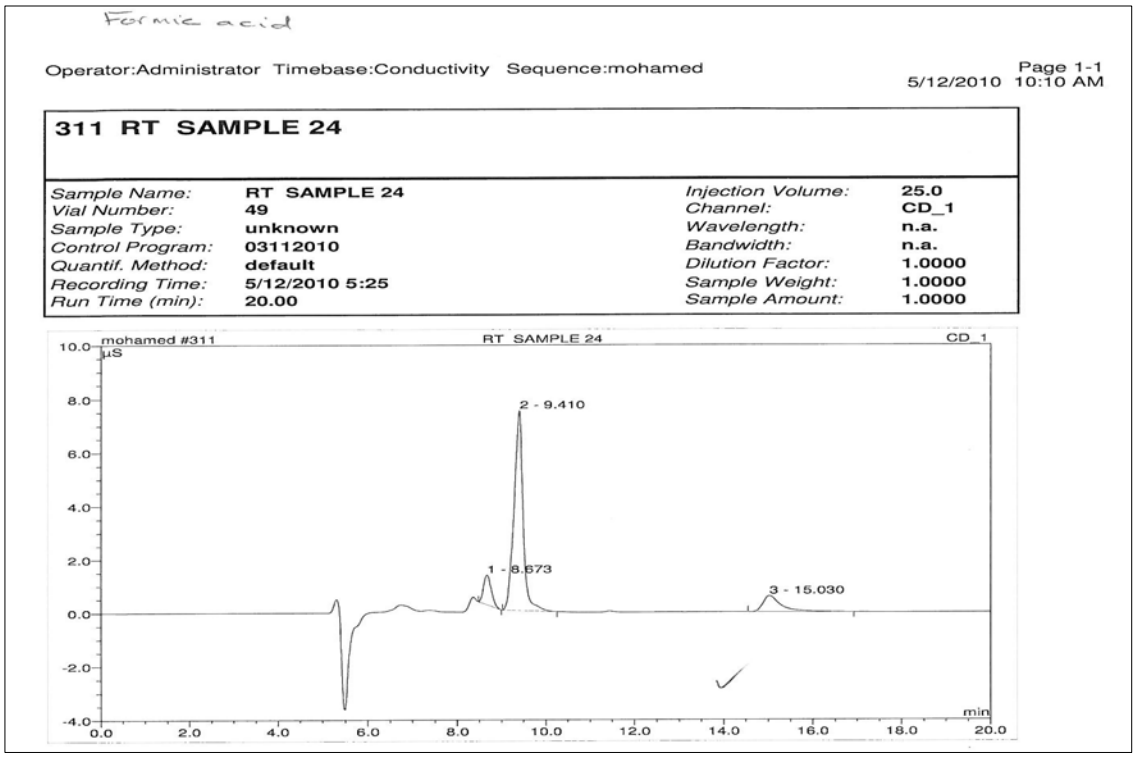
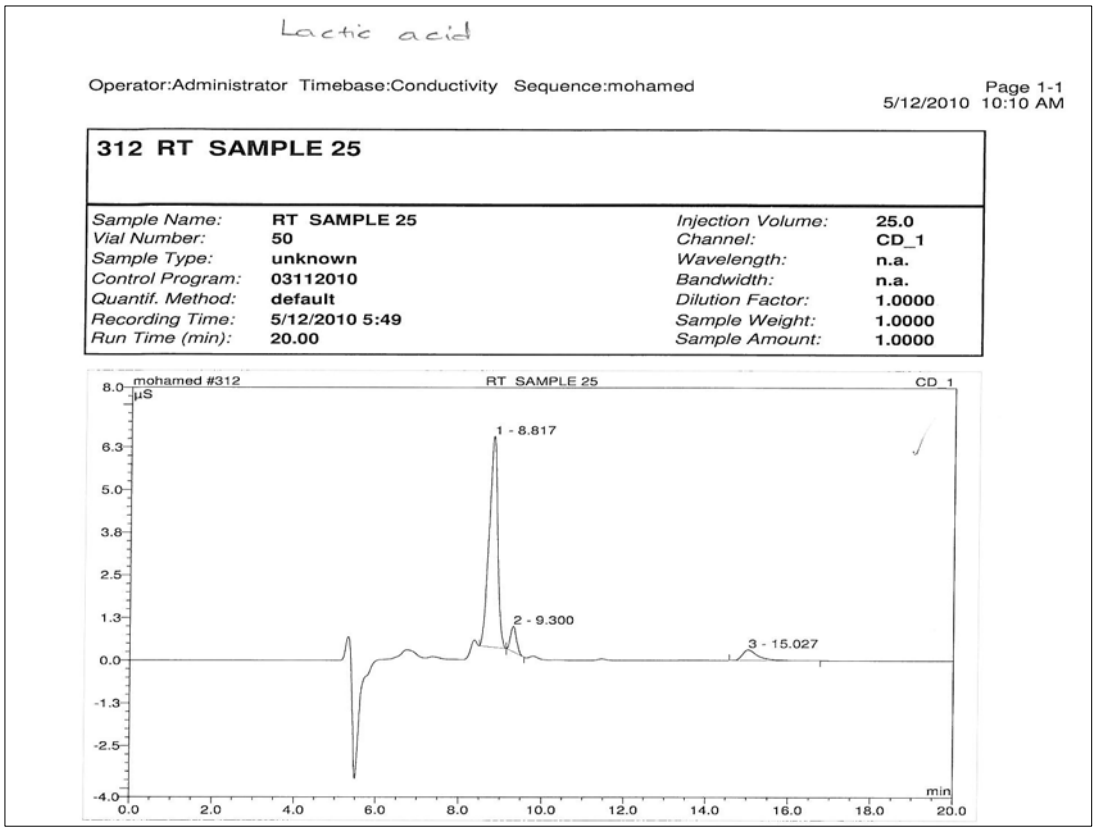


Figure 3.24.5 -Sample spiked with lactic acid:



3.5- Results-Variation of the area of peaks versus time.

Peaks 1&2 (dihydroxybutanoic acids and saccharinic acids)

Table 3.11 The results for *dihydroxybutanoic acids and α,β ISA* during deferent temperatures.

Hours	0	7	21	24	27	30	55	73	97	122	172	182	188
RT	0	2.11	2.52	2.46	2.72	2.81	3.21	3.39	3.56	3.86	4.03	4.14	4.36
50	0	3.95	4.28	5.11	4.93	6.12	6.20	7.13	6.75	7.91	8.98	9.899	10.33
90	0	11.53	11.74	12.82	14.27	16.05	23.86	31.13	34.14	38.11	41.59	45.75	47.61

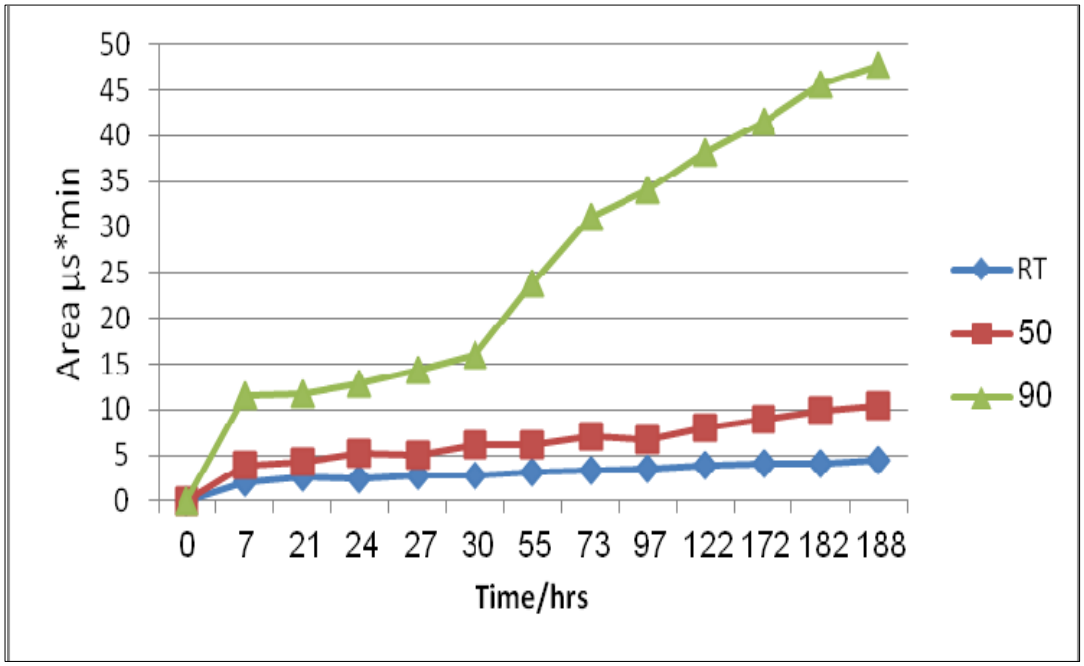


Fig 3.25- Variation of peaks 1 and 2 during decomposition at different temperatures

Peak 3 (glycolic acid)

The retention time of glycolic acid was at 9.67

Table 3.12 The results for *glycolic acid* during deferent temperatures.

Hours	0	7	21	24	27	30	55	73	97	122	172	182	188h
RT	0	1.63	1.94	2.09	2.73	3.38	3.88	3.95	4.92	4.64	4.56	4.85	5.20
50	0	2.77	3.09	2.56	3.35	4.07	4.22	4.81	4.87	5.45	7.89	8.67	9.53
90	0	3.70	4.00	4.19	4.96	5.57	8.59	10.57	11.86	14.85	17.37	22.65	23.26

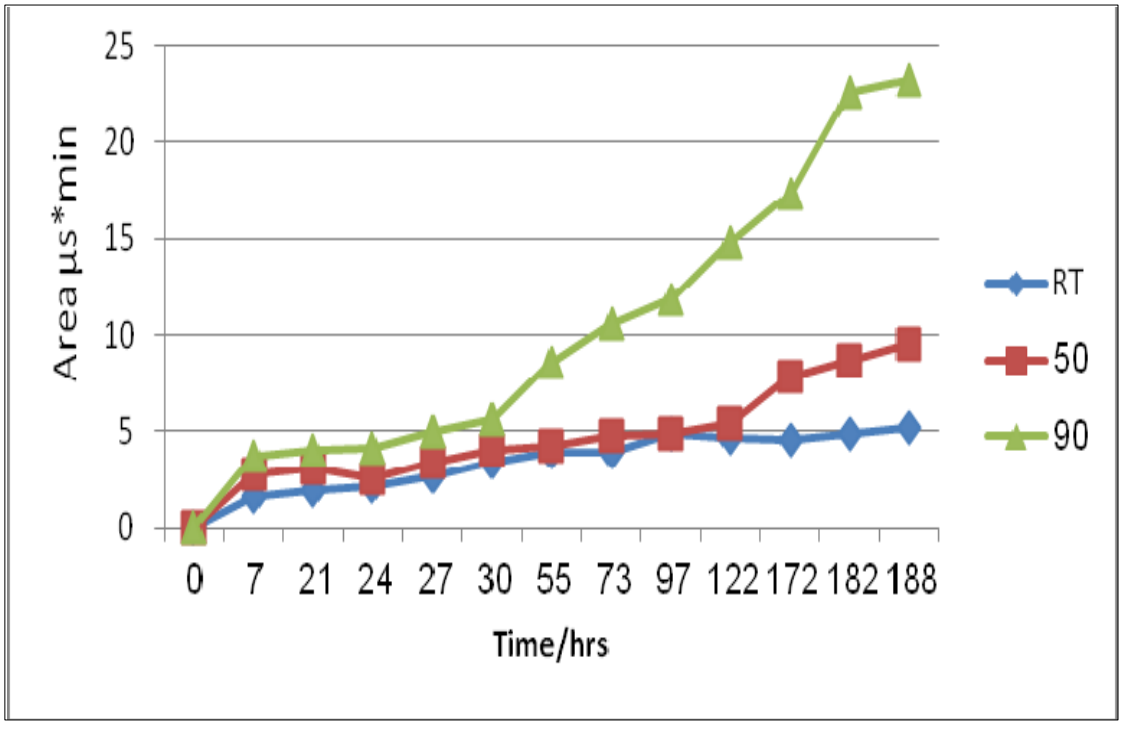


Fig 3.26- Glycolic acid during deferent temperatures

Peak 4 (lactic acid)

Table 3.13 The results for *Lactic acid* during different temperatures.

Hours	0	7	21	24	27	30	55	73	97	122	172	182	188h
RT	0	0.94	2.21	1.94	1.83	1.64	1.82	1.18	1.62	1.55	1.40	1.58	2.26
50	0	3.01	3.82	4.06	3.99	5.18	7.92	12.29	12.24	13.09	15.75	14.94	17.40
90	0	40.12	47.30	48.96	57.25	70.25	96.39	132.7	137.3	173.9	199.6	242.13	271.03

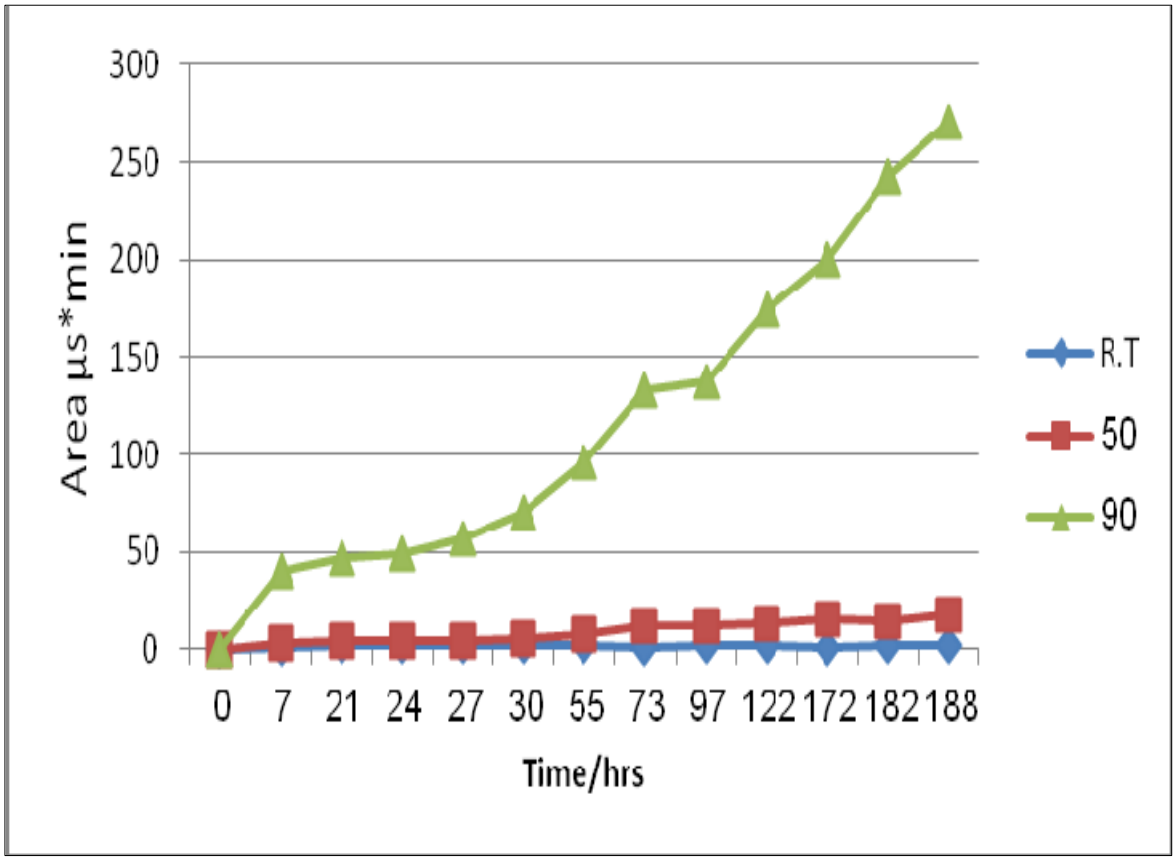


Fig 3.27- Lactic acid during different temperatures

Peak 5 (formic acid)

Table 3.14 The results for *peak 6* during different temperatures.

Hours	0	7	21	24	27	30	55	73	97	122	172	182	188h
RT	0	3.83	4.58	4.72	6.00	7.17	8.72	8.43	10.58	9.91	9.72	10.05	10.08
50	0	5.45	6.22	6.73	6.44	7.64	8.32	10.06	10.26	11.08	15.47	15.76	17.99
90	0	17.71	19.80	21.29	25.00	31.74	45.04	60.49	64.27	75.70	88.87	101.3	110.10

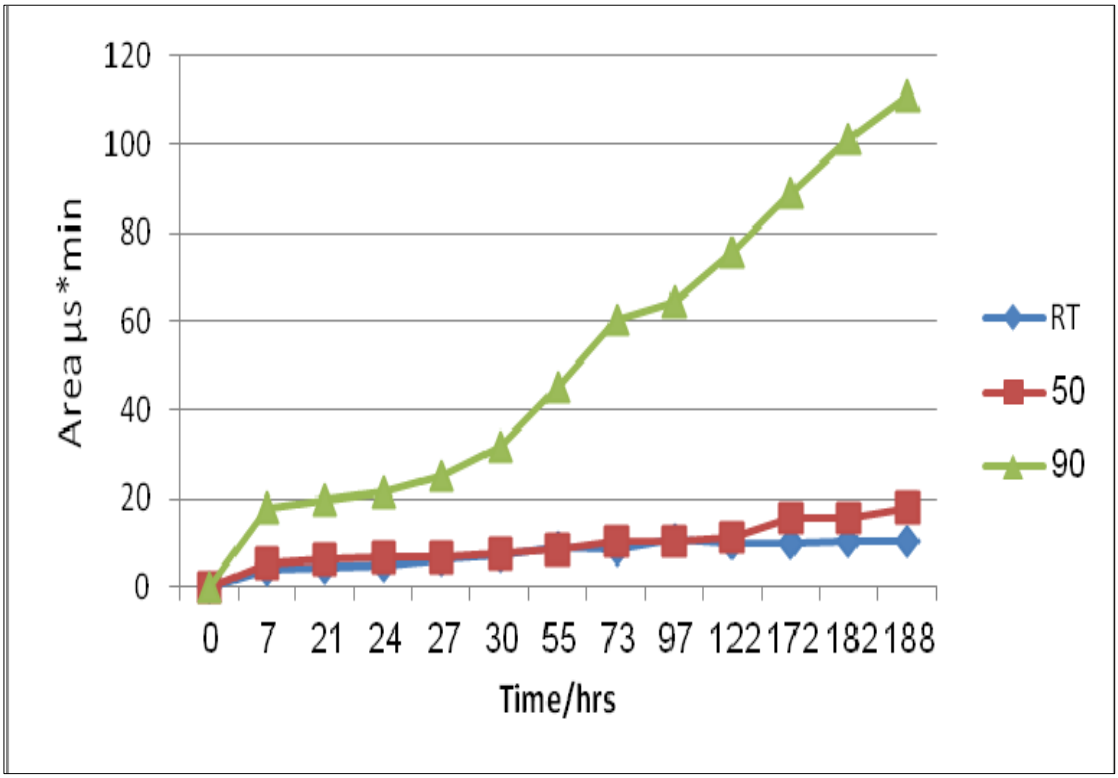


Fig 3.28- Formic acid during deferent temperatures

Peak 6 (glyceric acid)

Table 3.15 The results for *glyceric acid* during different temperatures.

Hours	0	7	21	24	27	30	55	73	97	122	172	182	188h
RT	0	0.57	0.81	0.78	0.85	0.70	0.90	0.62	0.82	0.76	0.63	0.77	0.74
50	0	1.56	1.62	1.65	1.69	1.85	1.76	1.79	2.07	2.50	2.57	2.91	2.85
90	0	5.38	5.57	5.94	7.56	8.39	9.08	11.58	12.63	12.64	14.43	16.44	22.36

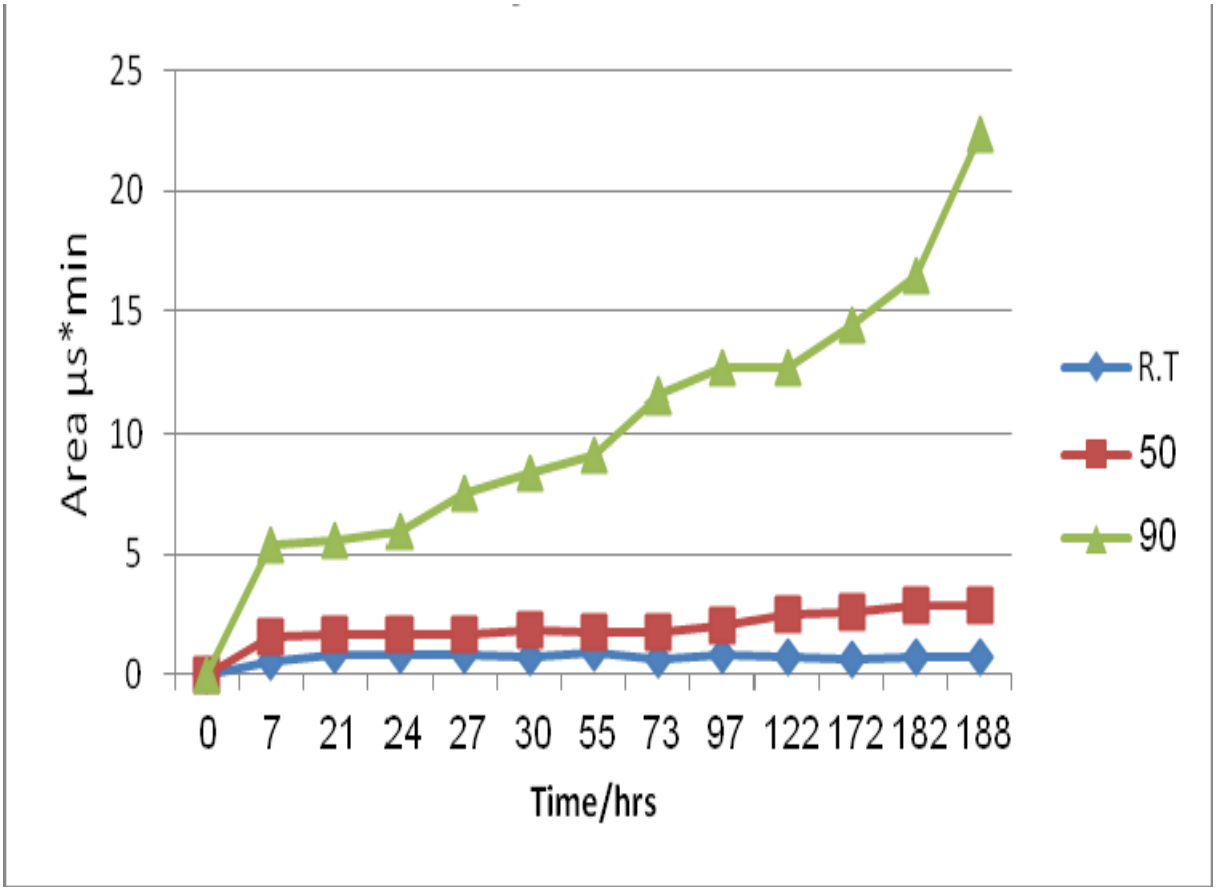


Fig 3.29- Glyceric acid during deferent temperatures

Chapter four

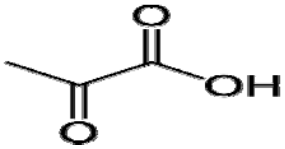
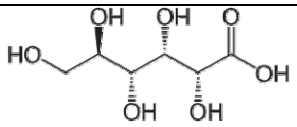
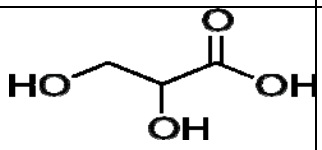
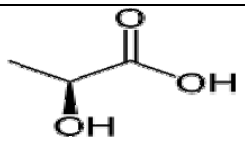
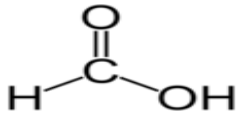
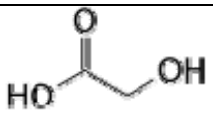
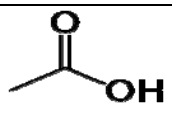
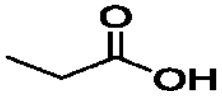
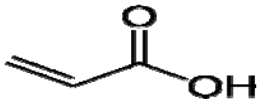
Discussion

Discussion .4

4.1_Understanding the relative Retention Times (t_{Ri}) of the different aliphatic hydroxy acids

In ion exclusion chromatography there are a number of factors which influence the retention time of carboxylic acids- most notable being the extent of ionisation of the acid in the mobile phase eluent; this can be related to the aqueous pK_a of the acid.

Table 4.1 Retention time of aliphatic organic acids measured under the conditions outlined in experiment No,1

Acids name	IUPAC naming	pKa	Acids structures	Retention time t_{Ri}	Capacity Factor	
Pyruvic	2-oxopropanoic	2.26		6.70	0.8	
Gluconic	Gluconic	3.70		7.90	2.0	
Glyceric	2,3-Dihydroxypropanoic	3.52		9.10	3.2	
Lactic	2-Hydroxypropanoic	3.66		9.70	3.8	t_{Ri} increasing
Formic	methanoic	3.75		10.5	4.6	
Glycolic	2-Hydroxyethanoic	3.83		11.2	5.3	
Acetic	Ethanoic	4.56		13.8	7.9	
Propionic	propanoic acid	4.67		14.3	8.4	
Acrylic	prop-2-enoic	4.26		16.2	10.3	

The mobile phase used in the current experiments contains heptaflurobutyric acid (HFBA) which has an aqueous pK_a of O.4-0.2 depending on the reference source used the presence of HFBA (4 mM) in the mobile phase will result in the mobile phase having a pH of 2.4. At this pH the extent of ionisation of the different analyte anions will vary enormously: acids with pK_a's between 1.4 and 2.4 acids will be between 90% and 50% ionized; in contrast, acids with pK_a's between 2.4 and 3.4 will be ionised between 50% and 10% and finally, acids with pK_a's between 3.4 and 4.4 will be ionised to a lesser extent i.e. between 10% and 1%. As can be seen from the data presented in Table 4.1, as the pK_a of the acid increases the retention times increases (see table 4.1).

It can also be seen that as the number of carbon numbers in the compounds increase the retention time increases. A more accurate measure of the extent to which an analyte is retained in liquid chromatography is the capacity factor of the analyte. The capacity factor is the observed retention time minus the time taken for an unretained analyte to pass through the column. Analysis of the chromatograms for the cellulose decomposition reactions shows that, under the present conditions, the unretained analytes elute after 5.9 mins. And this allows the capacity factors shown in table 4.1 to be calculated. A quick inspection of the results indicates that pyruvic acid is retained very weekly whilst acrylic acid is retained strongly.

Other distinguishing features of the results are:

An increase of the capacity factors of the monocarboxylic acids with increasing carbon number

An increase in capacity factor with an increase of pK_a values

A large capacity factor is observed for acrylic acid

Replacement of a hydrogen with a hydroxyl group results in a decrease in the capacity factor.

4.2 Principles of ion-exclusion chromatography

The characteristic feature of ion-exclusion chromatography is that the sign of the charge of the dissociated functional groups on the stationary phase (an ion-exchange resin) is the same as that of the potential ionic compounds to be separated. In regard to the observed retention times, by far the biggest factor which will influence this is the extent of the established dissociation equilibrium between the neutral, undissociated form and the corresponding

anionic form of the acidic solute. This equilibrium depends on the acidity (activity of the analyte): the more the analytes are dissociated the more they are excluded from the stationary phase by electrostatic repulsion (Donnan exclusion). The undissociated analyte species can penetrate through the Donnan membrane and interact with the stationary phase, causing retardation related to the flow of eluent molecules. [23]

4.3 Theory of ion exclusion chromatography

$$RCOOH + H_2O \rightleftharpoons RCOO^- + H_2O^+$$

Penetrates pores of anionic column Excluded from anionic column

Anions are excluded by electrostatic repulsion,.[24] The more of the dissociated form present i.e. the lower the pK^a of the acid, the less retained the analyte and this explains why there is a direct correlation between the pK_a and the capacity factor. However, this is not the only important mechanism of retention of analytes, another important retention mechanism involves hydrophobic interaction between the hydrophobic polymeric core of the stationary phase and the analyte: as the size of the aliphatic group increases stronger hydrophobic interactions with the column are expected and this increases the retention time: for example, the retention time of formic acid (HCOOH) is 10.45 mins and the retention time of acetic acid (CH_3COOH) is 13.76 while the retention time of propionic acid (CH_3CH_2COOH) is 14.27(Table 4.1) .Van-der-Waals forces between the solute and the polymeric resin material as well as the decrease in solubility of the solutes in the eluent also influence the distribution of the solutes between the stationary and the mobile phases. The latter point explains why the addition of hydroxyl groups which can hydrogen bond to the mobile phase constituents reduces the retention of analytes: Glyceric acid has 2-OH groups (Capacity Factor k'=3.2) elutes before lactic acid which has 1-OH (k'=3.8) which elutes before propionic acid which has 0-OH (k'= 8.4).

It was also noted that a double-bond in the carbon skeleton leads to a significantly higher retention and this is believed to be due to π-π interactions with the aromatic rings of the polymer: acrylic acid (k'=8.4) is eluted after propionic acid (k'=10.3) (Table 4.1) [20]

It should be noted at this point that during the course of the series of experiments reported in this thesis the retention times of the analytes was found to vary considerably from day to day and it is not clear why this should be the case. Possible explanations include variations in the mobile phase composition, variations in the column temperature and fluctuations in the mobile phase flow rates. A number of experiments were undertaken to try to generate more reproducible retention times; these include placing the column in an oven and adjusting the concentration of the mobile phase additive (HFBA). However, both sets of experiments did not lead to a significant improvement in the reproducibility of retention times and this may well result from the fact that the instrumentation was being used routinely for both HPAEC

and HPIEC and there was a constant exchange between the two types of chromatographies. In order to improve the robustness of the experiments reported here, an internal standard was added when trying to determine the identity of analytes (see later discussion).

4.4. Detection methods

There is no one highly sensitive, universal detector system used for HPLC. The system used is thus based on requirements which need to be met such as detection limits, expense etc. a summary of detection methods, which are used with HPLC separation, can be seen in figure 4.1. The most frequently used detector in HPLC is the UV/Vis detector.

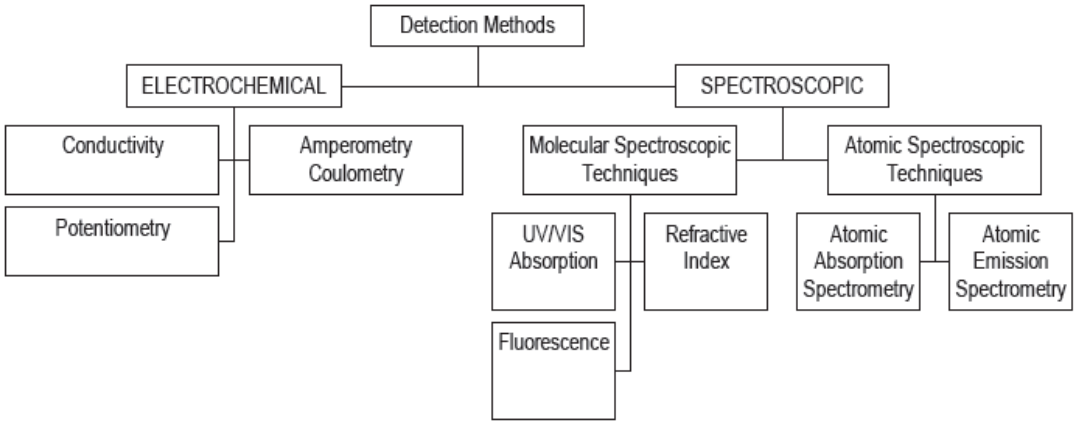


Fig 4.1 Detection methods for HPLC

4.4.1. Conductivity Detection

The polyhydroxylated aliphatic acids which are of generated in the decomposition of cellulosic materials are normally separated using ion chromatography. In Ion Chromatography detection frequently relies on measurement of the conductance of the mobile phase, conductivity is used as ions are electrically conducting (Fig 4.1), thus conductivity detection should be universal in response. Conductivity detectors are also relatively simple to use and are widely applicable. Conductivity detection is based on detection of changes in the conductance of an eluent prior to and during elution of an analyte.

The detector response equation for an anion-exchange system is:

$$\Delta G = \frac{(\lambda_s - \lambda_\varepsilon) C_s}{10^{-2} K} \qquad (2)$$

ΔG: Conductance signal

λ_s and λ_ε : Limiting equivalent ionic conductances of the analyte and eluent anions respectively; C_s: Concentration of the analyte anion; K: Cell constant

The equation (2) shows that when conductivity detection is used to monitor the effluent from an anion-exchange column the observed signal for an eluted analyte is proportional to the analyte concentration.[18] The calibration graphs generated in the work reported here were, to a first approximation are linear confirming that the conductance is proportional to the amount of analyte present.

In ion exclusion chromatography the eluent exiting the chromatography column contains both the analyte and the mobile phase additive hexafluorobutyric acid. The eluent is then passed through a suppresor membrane which exchanges tetrabutylammonium cation for the hydronium ion and which will convert undissociated acids into the tetrabutylammonium salts. The low conductance of the eluent salt and the high conductance of the analyte salt allow the analyte to be detected.

4.5 Selection of Acrylic acid- Internal standard:

One of the problems with following reactions that take place over several days and months, whilst trying to quantify the amount of each reagent present, is associated with ensuring that the detector response does not vary over the analysis time. In the present work, samples generated in a single experiment were analysed at the same time. However, this was not always possible. One way to overcome the problem of variable detector responses is to add a fixed amount of an internal standard to samples which helps eliminate random errors from the analysis. In this method a standard substance of known concentration is added to the unknown sample. The sample composition is determined by comparing the peak areas of standard and sample component. This method does not require all signals to be eluted and detected. Therefore, the method of internal standard is also suited for samples in which not all of the components must be determined.[20] In the current system a number of different internal standards were tried. Ideally, an internal standard should have a similar structure to that of the

analytes of interest, it should elute close to the analyte to make analysis times quick but it should not interfere with any of the analyte peaks. Acrylic acid was chosen as an internal standard as there are no reports that it is produced during the decomposition of cellulosic material and, being a C3 carboxylic acid, its structure is similar to that of the analytes. It was found that acrylic acid eluted immediately after the analyte peaks of interest and was ideally suited for use in the quantitative studies as a plot of concentration against detector response was linear (Fig. 3.1).

4.6 Monitoring Cellulose hydrolysis:

The intermediate product, 4-deoxy-2-oxo-D-glycero-2-hexulose (Fig 1.20), in the peeling reaction reacts by two mechanistic pathways to give the ultimate degradation products:

1- Isosaccharinic acid is formed via an internal rearrangement of the intermediate product known as a benzilic acid type of rearrangement:
2- In a fragmentation reaction the intermediate molecule, is split, via retro-Aldol reactions, into smaller organic acids such as lactic acid and formic acid.

Depending on the ionic composition of the reaction solution, temperature, atmosphere and reaction time, a variety of fragmentation products, such as C1-, C2- or C3 carboxylic acids may be formed from the intermediate product (4-deoxy-2,3-hexodiulose). Further alternative pathways starting from the intermediate product may lead to the formation of anhydroisosaccharinic acid or C4-, C5- and C6- mono- and dicarboxylic acids. Chemical stopping may also lead to the formation of other end-groups such as metasaccharinic acid (MSA).

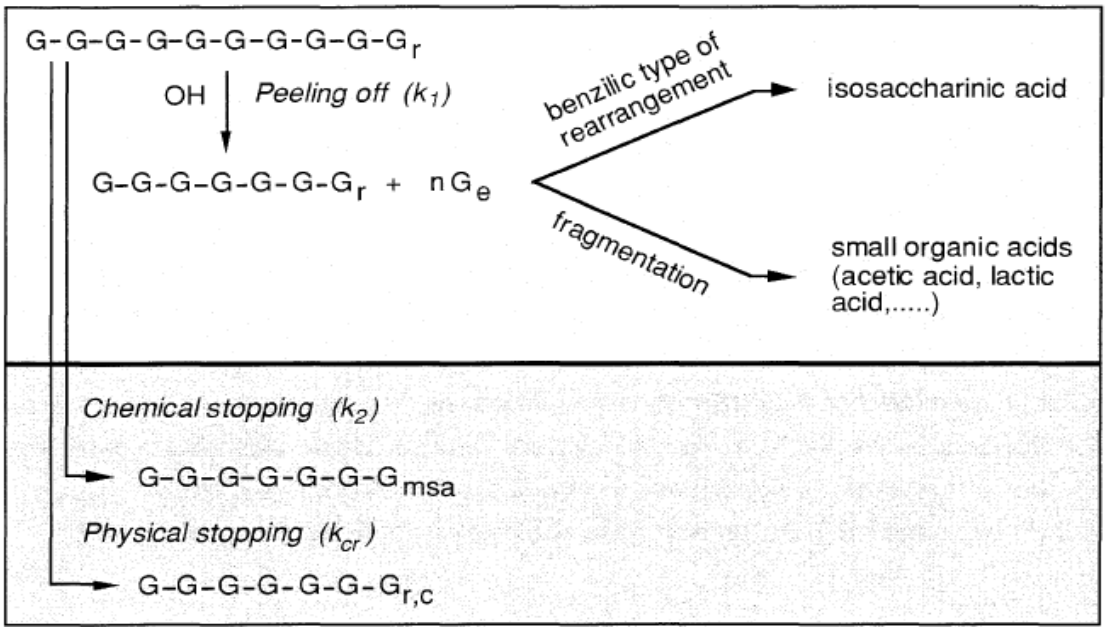


Fig 4.2: Schematic presentation of the peeling off reaction. G is a glucose monomeric unit, G_{msa} is a stable metasaccharinic acid end group, G_e is a glucose unit eliminated and n are the number of glucose units eliminated. G_r is a reducing end group in the amorphous region of the cellulose and $G_{r,c}$ is a reducing end group in the crystalline region of cellulose.[13]

Alkaline hydrolysis leads to the reformation of a reducing end group and the peeling-off process can be started again. For this reason both the peeling-of process and the alkaline hydrolysis lead to the formation of ISA, which is actually a mixture of α-ISA and β-ISA.

4.7 Complete Reaction mechanism for the formation of Saccharinic Acids:

According to the Nef-Isbell mechanism[25] which was originally proposed to explain the decomposition of monosaccharides (Fig. 4.3). The first step in the sequence involves the production of an enediol [2], via keto–enol tautomerism (i). This is followed by the production of an enediol anion [3] via deprotonation by hydroxide ions (ii). Anion isomerisation (iii) then takes place resulting in a mixture of equilibrium intermediate anions (3–5). An elimination reaction then takes place when the chain is in the position β to a carbonyl group. An important consequence of anion formation is the destruction of chirality at the C-2 position, i.e. reprotonation of anion [3] followed by conversion to the aldose form (via keto–enol tautomerism) results in the generation of D-glucose-form and its C-2 epimer (D-mannose). Similarly, protonation of anion [4] results in the production of the D-fructose-form. It is the anions [3–5] that can undergo β-hydroxycarbonyl elimination (iv), i.e. elimination of the group in the β-position to the negatively charged (carbonyl) oxygen. This results in the formation of a diketodeoxyglycitol, i.e. introducing a-carbonyl functionality relative to the enol arising as a result of the β-elimination [6–8].

The corresponding vicinal dicarbonyl compounds [9–11] are then produced via keto–enol tautomerism (v), and in the final stage of the Nef–Isbell mechanism they [9–11] then undergo a benzilic acid rearrangement (vi) to produce the corresponding deoxyaldonic (saccharinic) acids [12–14].

As stated earlier, the liberated end unit forms a 2,3-diketone intermediate then undergoes either a benzilic acid type rearrangement to give isosaccharinic acid or undergoes fragmentation by a reverse-aldol condensation to give smaller molecules. A detailed reaction scheme is given in Figure 4.2.[13]

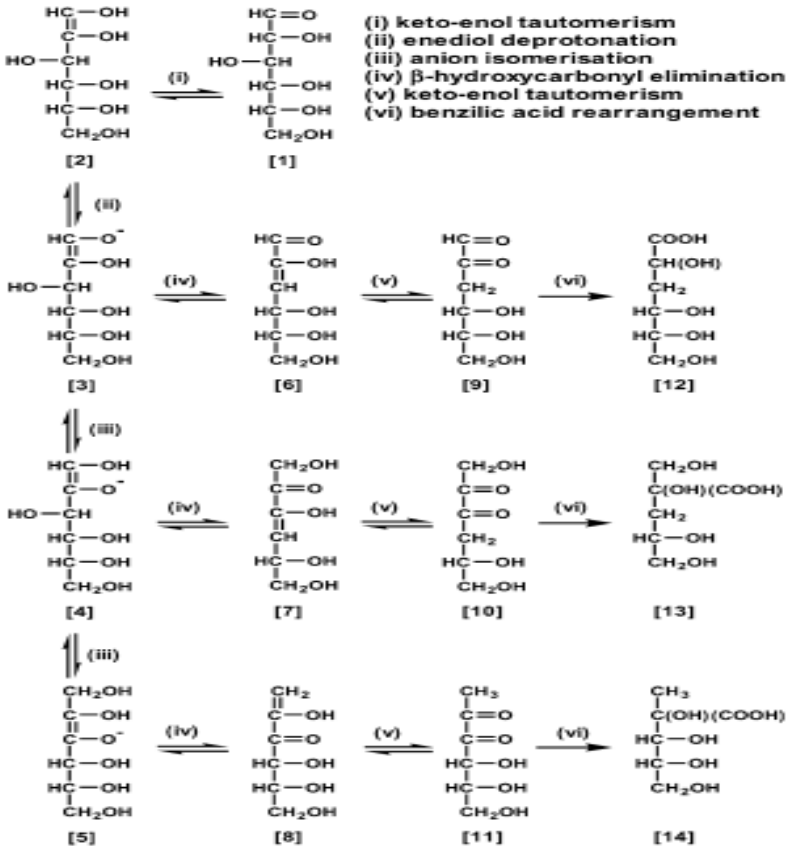


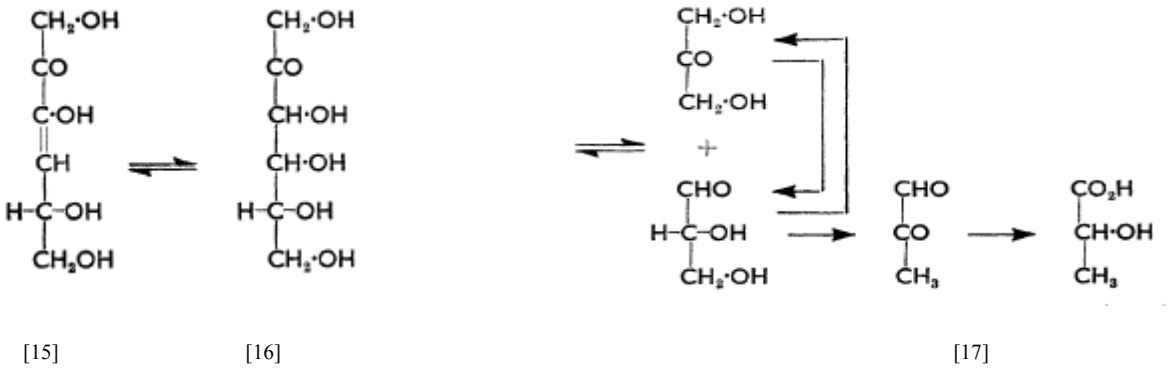
Fig 4.3 Naf-Isbell mechanism for the alkaline degradation[25]

Different products are generated from the different elimination reactions: anion [3] produces a mixture of 3-deoxy-D-ribohexonic and 3-deoxy-D-arabino-hexonic acids [12], formerly i.e. D-glucometasaccharinic acid anion: [4] produces a mixture of 3-deoxy-2-C-(hydroxymethyl)-D-erythro-pentonic and 3-deoxy-2-C-(hydroxymethyl)-D-threo-pentonic acids [13] i.e. D-glucoisosaccharinic acid; Anion [5] produces a mixture of 2-C-methyl-Derythro- pentonic and 2-C-methyl-D-threo-pentonic acids [14], known as D-glucosaccharinic acid.

Aldol and reverse aldol condensations are significant side-reactions in such alkaline reaction mixtures because of the pronounced catalytic effect of hydroxide ions with respect to such reactions.

There are additional sources of most of the other products, the most likely being degradation and rearrangement of the diketone [10], fragmentation of the reducing end-group [1], or an alternative degradation of the substituted glucosyl anion[3].

Formic acid is evidently a particularly important product and its yield both in relation to those of total acids and of acetic is quite different from that obtained similar alkaline degradation of glucose.[25]



[15] [16] [17]

Fig 4.4 fragmentation of the reducing end-group and formation of lactic acid [28]

In the case of the ketoenol [15], however, it is reasonable to suppose that a slow, competing addition of hydroxyl anion might occur, leading to formation of a ketohexose mixture [16], alkaline degradation of which would yield a mixture containing mostly lactic acid [17]. A possible route to the formation of formic acid can be envisaged in which the intermediate is initially transformed into the corresponding 1,3-dicarbonyl compound [18] via series of enolization and carbonyl migration steps, formic acid could then be generated by a retro-Claisen reaction.

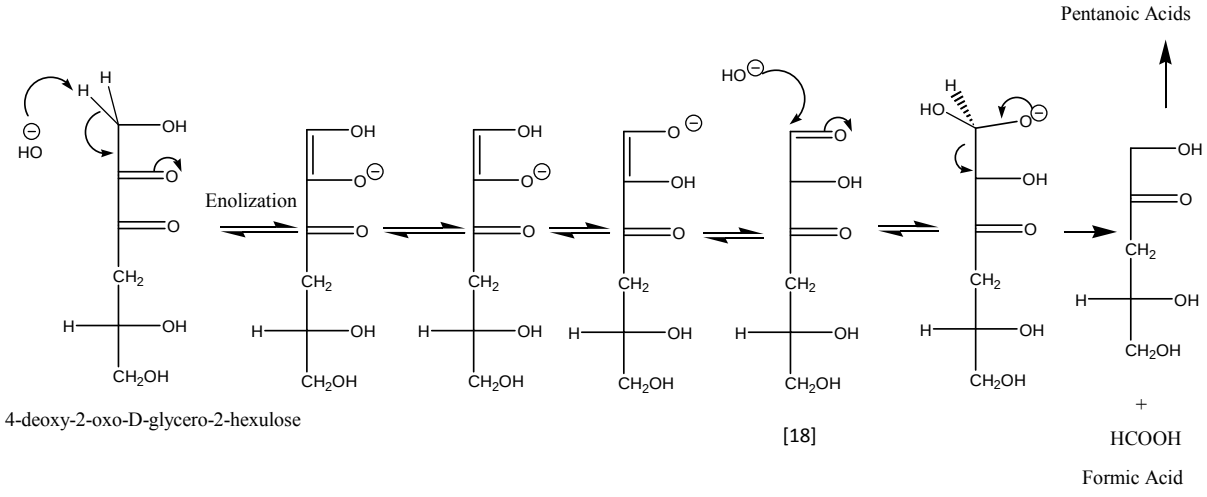


Fig 4.5 fragmentation of the intermediate and formation of lactic acid

4.8 Identification of Acids.

In the experiments following the alkali catalysed decomposition of cellulose at least six different acids are being produced in significant amounts and these are labelled A-E on the following chromatogram:

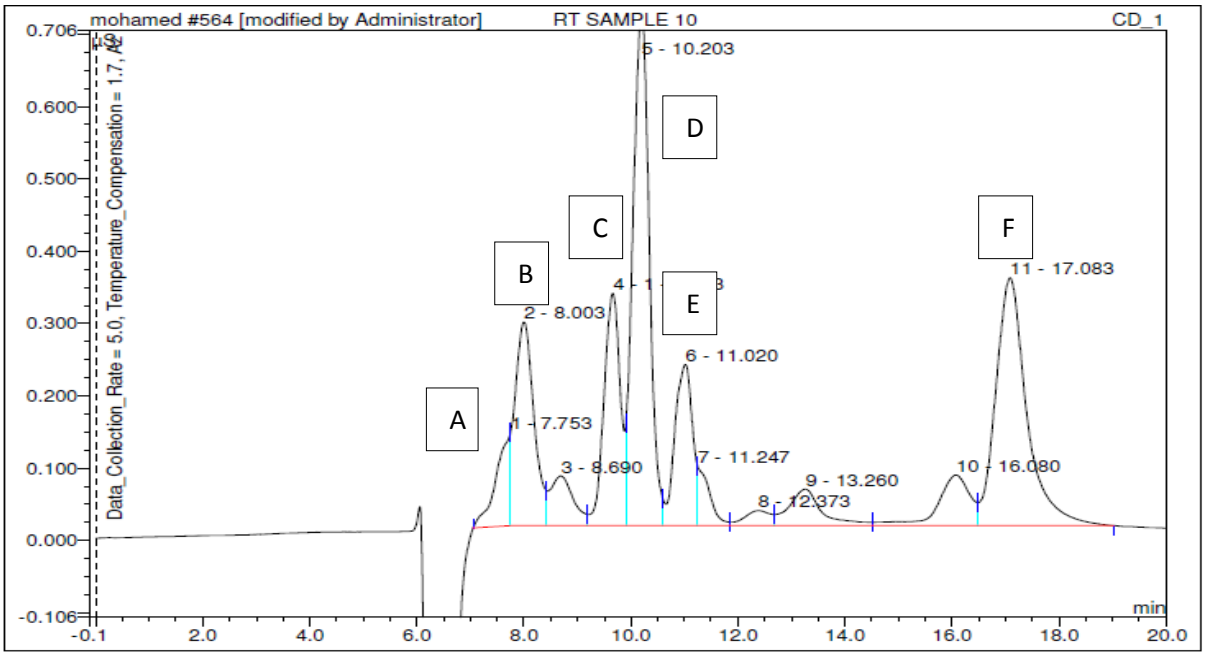
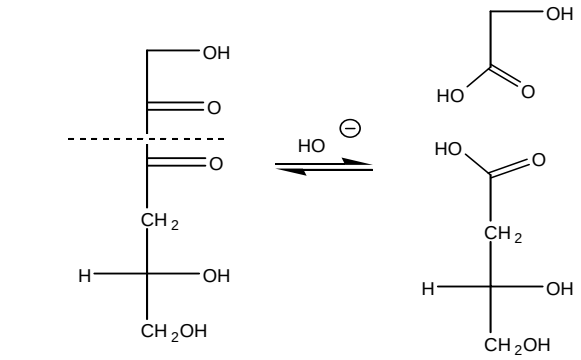


Fig 4.6. Identification of acids

The spiking experiments suggest that peaks **A** and **B** are saccharinic acids, peak **C** is glycolic acid, peak **D** is lactic acid and peak **E** is formic acid and the final peak **F** is that of the internal standard acrylic acid. It is very clear that at room temperature that lactic acid is the most abundant decomposition product (concentration at 100 h =250 ppm 2.8 mM) followed by glycolic acid (concentration at 100 h =115 ppm, 1.5 mM) with formic acid being present in much smaller amount at (concentration at 100 h =33 ppm, 0.72mM). As the temperature is raised to be 50 ^{o}C, the amount of formic acid generated is significantly higher: Lactic acid (concentration at 100 h =260 ppm 2.9 mM): glycolic acid (concentration at 100 h =135 ppm 1.8 mM): formic acid (concentration at 100 h =240 ppm 5.2 mM). On a weight by weight base there is approximately the same amount of lactic acid and formic acid produced after 100 hours. In comparison, at 90 ^{o}C formic acid is now by far the most abundant of the small aliphatic acids, the amount of lactic acid has also increased significantly whilst there is a more modest rise in the amount of glycolic acid that is generated: Lactic acid (concentration at 100 h = 1600 ppm 18 mM): glycolic acid (concentration at 100 h =275 ppm 3.6 mM): formic acid (concentration at 100 h = 3020 ppm 66 mM). From these result it is clear that the different fragmentation reactions, leading to the partitioning of the intermediate to the different small acids have different activation energies (see discussion below).

In the scientific literature[27,28] there are a number of reports describing products generating during the alkaline decomposition of cellulose and 4-substituted-hexose derivatives. The first attempt to determine the nature of the products produced from the treatment of cellulose with a boiling solution of sodium hydroxide (0.5N) was by Richards and Sephton[28] who established that the main products were saccharinic acids; lactic acid and formic acid were also determined to be present but in substantially smaller amounts and this is what we observed in the current experiments when cellulose was treated with sodium hydroxide solution at 100 ^{o}C. In a following paper,[27] reported that at lower temperatures glycolic acid was the main fragmentation product and that this changed to formic and lactic acids as the temperature was raised to 100 ^{o}C. Glycolic acid is expected to be produced along with 3,4-dihydroxybutanoic acid via the fragmentation of the 4-deoxy-D-*glycero*-3-oxo-2-hexulose:



4-deoxy-3-oxo-D-*glycero*-2-hexulose

Fig 4.7 fragmentation of the intermediate and formation of glycolic acid and 3,4-dihydroxybutanoic acid.

Glycolic acid decomposes at temperatures above 100 ^{o}C and it is more likely that the reduced percentage of the acid, isolated at higher temperatures, may result from both the loss of glycolic acid via thermal decomposition and as a consequence of the higher activation parameters for lactic acid and formic acid production.

4.9 Kinetic profiles for acid production

The degradation leading to isosaccharinic acid starts with the fast peeling-off reaction this slows and then eventually stops when all reducing end groups have been transformed to stable end groups. The further production of ISA is extremely slow and reliant on cleavage of glycosidic bonds followed by a fast peeling off reaction starting at the newly created reducing end groups. The same would also be expected for the fragmentation reactions leading to small hydroxy aliphatic carboxylic acids: the retro-Aldol reactions and retro-Claisen reactions are likely to only take place once the vicinal dicarbonyl compound has been generated.

It is impossible to know, without undertaking labelling experiments, the pathway by which individual acids are generated; it is likely that some acids are generated by more than one degradation pathway. Once acids are formed it is likely that they can undergo subsequent fragmentations and recombinations and, if this is the case, then they will only be transiently present in the reaction system. Intermediate acids will only be visible if the rate of their production exceeds the rate of their removal at some point within the degradation process.

Looking at the time profiles generated for reaction performed at 90 ^{o}C, figures 3.22 to 3.26, it can be clearly seen that over the time course in which the acid production was monitored, the production of each acid does not follow a simple kinetic profile and this is to be expected for what is a complex system.

Part of the difficulty with fitting rate data to the time profiles arises from the fact that the reaction systems are heterogeneous. As the reaction proceeds soluble cellulose will react with base to generate both fragmentation products and isosaccharinic acids. Changes in the morphology of the parent fibre will provide more accessible substrate and lead to the production of more products. What is surprising is that at 90 ^{o}C the peeling reaction slow dramatically after approximately 16 h [29] in contrast the rate of production of fragmentation products appears to remain constant for the first 188 h.

A crude measure of the rates of production of each of the acids can be obtained by treating the reactions as pseudo-zero order and ignoring the first data points as these will include product generated from the hydrolysis of any hemicelluose that is present as impurities in the cellulose. For glycolic acid, the concentration versus time plots can fit to a zero order rate equation and the slopes correspond to the rate constant at the assigned temperature (see Fig 4.8).

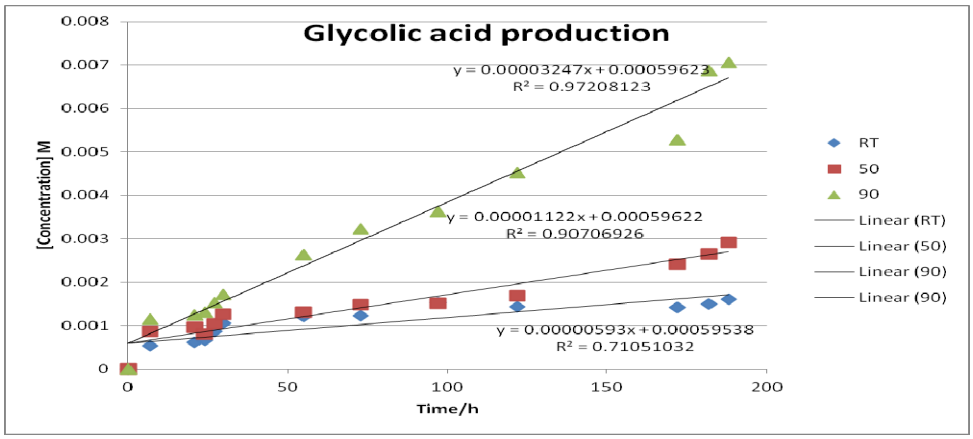


Fig 4.8 Plot of the concentration of glycolic acid measured during the decomposition of cellulose at room temperature, 50 degrees Celsius and 90 degrees Celsius as a function of time.

Table 4.2 The rate constants of glycolic acid measured during the room temperature and 50, 90 ^{o}C

Temperature ^{O}C	**1/T (K^{-1}/E-3**	**k/($molL^{-1}h^{-1}$)**
25	**3.36**	**5.93E-6**
50	**3.10**	**1.12E-5**
90	**2.75**	**3.25E-5**

The measure rate constants are shown in the table and are plotted in the Arrhenius plot in Fig 4.9

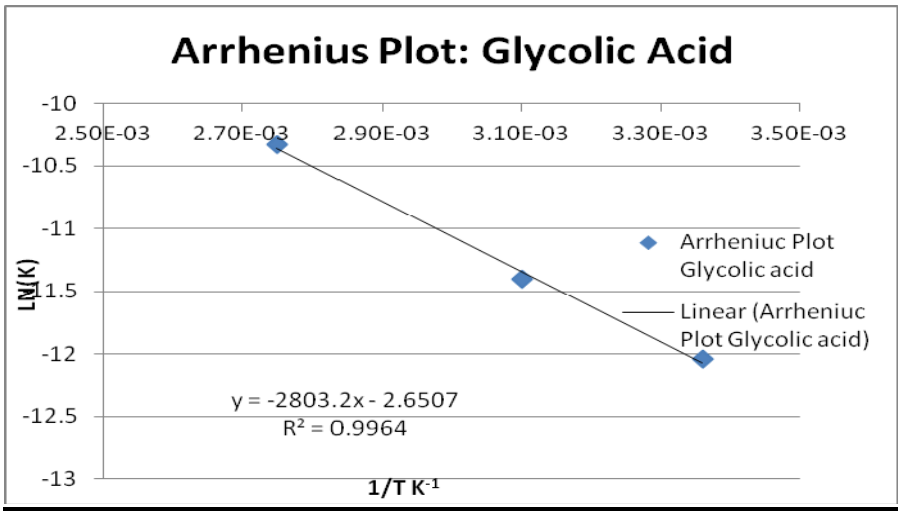


Fig 4.9 Arrhenius Plot of glicolic acid

For formic acid, the concentration versus time plots can fit to a zero order rate equation and the slopes correspond to the rate constant (see Fig 4.10).

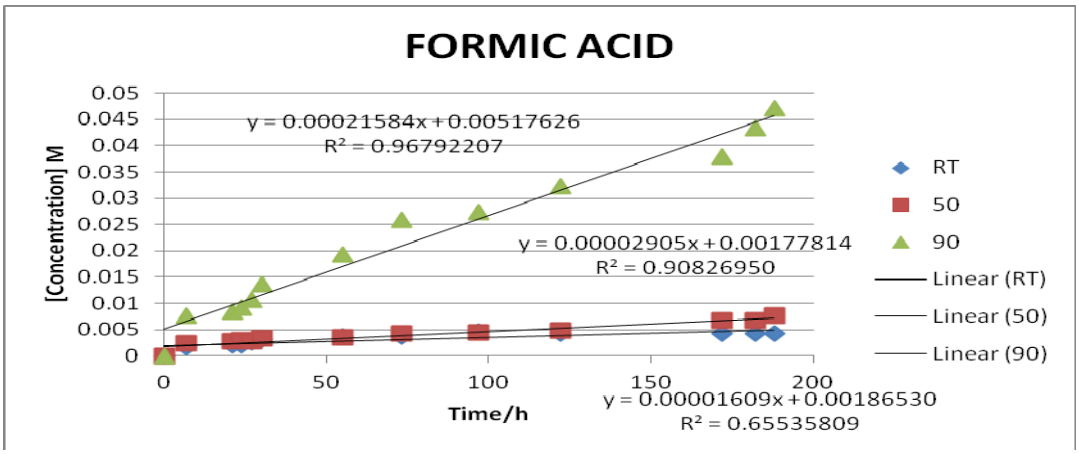


Fig 4.10 Plot of the concentration of formic acid measured during the decomposition of cellulose at room temperature, 50 degrees Celsius and 90 degrees Celsius as a function of time.

Table 4.3 The rate constants of formic acid measured during the room temperature and 50, 90 ^{o}C

Temperature ^{O}C	**1/T (K^{-1}/E-3**	**k/($molL^{-1}h^{-1}$)**
25	**3.36**	**1.61E-5**
50	**3.10**	**2.9E-5**
90	**2.75**	**2.1E-4**

The measure rate constants are shown in the table and are plotted in the Arrhenius plot in Fig 4.11

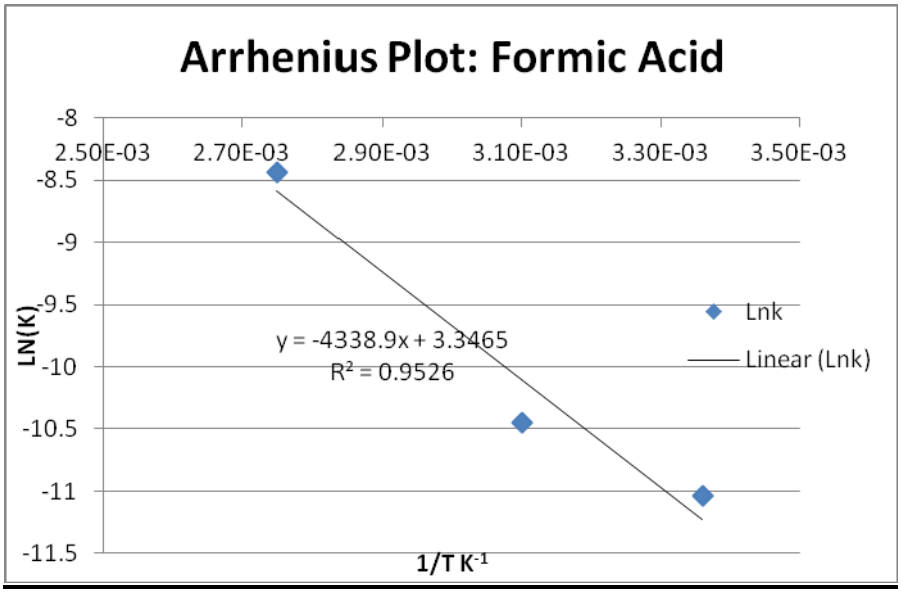


Fig 4.11 Arrhenius Plot of formic acid

For Lactic acid, the concentration versus time plots can fit to a zero order rate equation and the slopes correspond to the rate constant (see Fig 4.12).

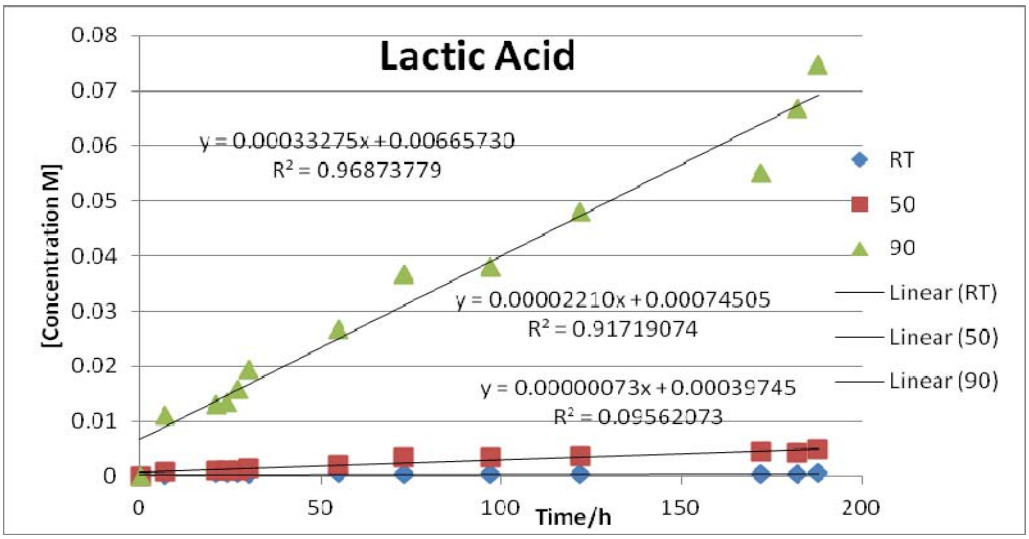


Fig 4.12 Plot of the concentration of formic acid measured during the decomposition of cellulose at room temperature, 50 degrees Celsius and 90 degrees Celsius as a function of time.

Table 4.4 The rate constants of lactic acid measured during the room temperature and 50, 90 ^{o}C

Temperature ^{O}C	**1/T (K^{-1}/E-3**	**k/($molL^{-1}h^{-1}$)**
25	**3.36**	**7.3E-7**
50	**3.10**	**2.22E-5**
90	**2.75**	**3.3E-4**

The measure rate constants are shown in the table and are plotted in the Arrhenius plot in Fig 4.13

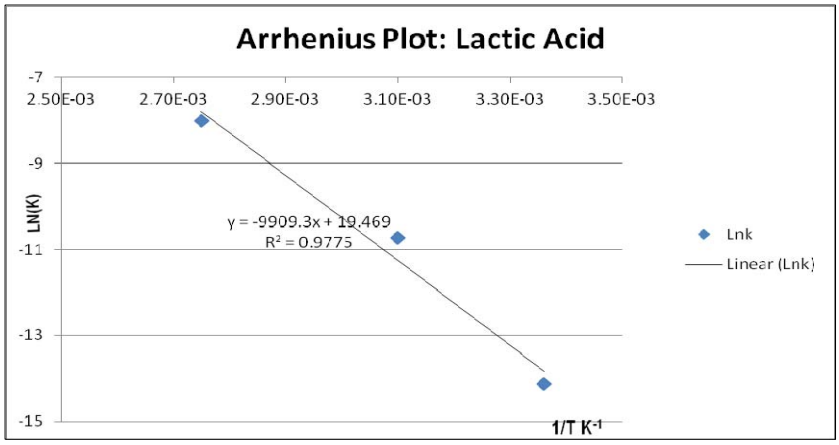


Fig 4.13 Arrhenius Plot of lactic acid

From the slopes of the Arrhenius-plots the activation energies for the production of the three acids can be determined and these are in table 4.5

Table 4.5 The activation energies for the production of the three acids

Acid	Ea/ kJmol^{-1}
Glycolic	*23.3*
Formic	36.1
Lactic	82.4

From the data presented above it can be seen that formation of glycolic acid and formic acid are favoured and have a low activation barrier whilst formation of lactic acid is more different having a considerably higher activation barrier. So the reaction producing lactic acid will only dominate at high temperatures.

Chapter five
Conclusion

Conclusion and Future Work

Whilst ion exclusion chromatography has been used by a number of authors to monitor production of small polyhydroxylated aliphatic acids during the decomposition of cellulose [15] in this study it was found that the capacity factors for a number of the acids are very similar and it was not possible to gain base-line resolution for a number of peaks.

A number of workers, mainly those studying the alkaline pulping of paper, have quantified the same analytes using GC-MS and the author [31,32] would suggest that if this work was to be repeated it may be appropriate to use a combination of the two analytical methods.

In the long-term studies of the degradation of cellulose it was noticeable that there was a measurable variation in the response factors for the detector for the different analytes and this required the use of an internal standard.

In the analysis undertaken here, acrylic acid proved to be an appropriate standard.

In future work, if a combination of analytical methods were to be used then it would be necessary to determine if the acrylic acid interfered with the quantitation of the analytes in the GC-MS system.

In the degradation studies, it is clear that glycolic acid, lactic acid and formic acid are the most abundant of the small acids that are produced and this is in agreement with the established literature. It is clear that as the temperature rises that the amount of glycolic acid observed is reduced. This may be a consequence of thermal decomposition [25].

Also, there are multiple potential reaction pathways to each of these acids (see discussion for the main routes) and as a consequence, it is difficult to reach any conclusions with regard to the significance of the measured activation parameters.

In order to determine mechanistic details it would be necessary to explore the decomposition of both model compounds i.e. the decomposition of the proposed intermediates and ^{13}C-labelled starting materials and intermediates, under the same reaction conditions.

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