Equilibration of D-Glucaric Acid in Aqueous Solution

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Abstract

The equilibrium of aqueous D-glucaric acid was investigated *via* Nuclear Magnetic Resonance (NMR) spectroscopy. The NMR spectra of all four species (D-glucaric acid, D-glucaro-1,4-lactone, D-glucaro-6,3- lactone and D-glucaro-1,4;6,3-lactone) were assigned.

A ^{1}H NMR spectroscopy method was developed to investigate the kinetics of equilibration of the starting species (D-glucaro-1,4-lactone and D-glucaro-1,4;6,3-dilactone). The equilibration was investigated under neutral conditions as well as conditions with increasing acidity.

Each experiment set contained 50-100 ^{1}HNMR spectroscopy experiments that were run on the same sample using a program that built in delays. Dimethyl sulfoxide was used as an internal standard, and its signal size was used as a scale to report the changes in relative concentration of the four species throughout the experiment sets.

Under neutral conditions D-glucaro-1,4-lactone is relatively stable against equilibration, while D-glucaro-1,4;6,3-dilactone is not. Under acidic conditions both compounds equilibrate within approximately 30,000 seconds. After equilibration under acidic conditions D-glucaric acid is the dominant species, while the relative concentration of D-glucaro-1,4-lactone is slightly higher than that of D-glucaro-6,3-lactone. The relative equilibrium concentration of D-glucaro-1,4;6,3-dilactone is low.

A mechanism for the equilibration of aqueous D-glucaric acid was proposed and equilibrium constants and estimates of rate constants were derived from the experimental data. These rate constants were used in MATLAB simulations that were compared to the experimental data. MATLAB simulations were used to alter the rate constants to improve the fits between experimental data and simulated data.

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List of Abbreviations

BTX	Benzene, Toluene and Xylene
COSY	COrrelation SpectroscopY
D_2O	Deuterium Oxide
DEPT	Distortionless Enhancement of Polarisation Transfer
DMSO	Dimethyl sulfoxide
FID	Free Induction Decay
GC/MS	Gas Chromatography/ Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Coherence
HSQC	Heteronuclear Single Quantum Correlation
Hz	Hertz
MHz	Megahertz
mL	Millilitres
MSc	Master of Science degree
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
PHPAs	Polyhydroxypolyamides
ppm	Parts per million
SELNOESY	SELective excitation Nuclear Overhauser Effect SpectroscopY
SELTOCSY	SELective excitation TOtal Correlated SpectroscopY

Chapter One - Introduction

1.1 D-Glucaric Acid

Aldaric acids are aldoses that have had both terminal groups oxidised to form a polyhydroxy dicarboxylic acid. D-glucaric acid [(2*R*,3*S*,4*S*,5*S*)-tetrahydroxyhexanedioic acid] (previously called saccharic acid) is formed *via* the oxidation of the common aldose sugar D-glucose. D-Glucose is the most abundant sugar found in nature, plays a central role in biochemistry and is a building unit of starch, cellulose, sucrose and lactose[1,2]. The main source of D-glucose on a commercial scale is the enzymatic hydrolysis of starch[3].

1.1.2 History and Production

D-Glucaric acid was first isolated as its acid potassium salt by Sohst and Tollens in 1888, *via* the nitric acid oxidation of D-glucose[4]. The acid potassium salt can be readily converted to a calcium salt, which upon treatment yields (in solution) D-glucaric acid[5]. Crystallisation of this solution gave what Sohst and Tollens termed "saccharolactone"[4]. Rehorst and Scholz reported that this "saccharolactone" was D-glucaro-6,3-monolactone[6]. However work by Smith *et al*. elucidated this "saccharolactone" to be a mixture of D-glucaro-6,3-lactone and D-glucaro-1,4-lactone[5, 7-9]. Smith also reported the existence of two D-glucaro-dilactones that reduced Fehling's solution[7]. It is now known that these "two" dilactones are actually the same compound – D-glucaro-1,4;6,3-dilactone, and can be formed from either monolactone[10, 11]. Aqueous D-glucaric acid exists in an equilibrium of the acyclic compound, the two monolactones (D-glucaro-1,4- and D-glucaro-6,3-lactone) and the dilactone (D-glucaro-1,4;6,3-dilactone)[12].

Today D-Glucaric acid is usually made by the oxidation of D-glucose, molasses, or starch. However starch, particularly corn starch, has the combination of favourable chemical structure, availability and low cost to make it an auspicious feedstock for conversion, and it is the most important raw material for the

production of D-glucaric acid[3, 13,14]. The common oxidation process utilises nitric acid as the oxidant[15]. This is the same oxidant used by Sohst and Tollens, and is also the method that Emil Fischer used in the late 1800s when aldaric acids were used to help confirm the relative configurations of the naturally occurring D-aldoses[16]. Nitric acid appears to be one of the few oxidants that is able to oxidise both termini of aldoses, yet leave the secondary hydroxyl groups unchanged. This method is also very selective, and regularly yields the analogous aldaric acid as the main product[1, 13,14,17]. Other methods of producing D-glucaric acid include the use of precious metal (Pt, Rh, Ru)[3, 18], hypohalites and 4-acetylamino-2,2,6,6-tetramethyl-1-piperidinyloxy(4-AcNH-TEMPO)[19], chlorine, bromine[2], and hydrogen peroxide in the presence of iron salts[20] catalysts. Production of D-glucaric acid via the electrocatalytic oxidation of D-gluconic acid has been described [21]. An *Aspergillus niger* strain has the ability to convert D-glucose to D-glucaric acid[22]. However, the nitric acid method represents the most economically favourable procedure due to the low cost of the oxidant.

Commercially acyclic D-glucaric acid is only available as one of its salts. These include monopotassium[23], dipotassium[24] and calcium D-glucarate. Crystalline D-glucaro-1,4-lactone, D-glucaro-6,3-lactone (not in New Zealand) and D-glucaro-1,4;6,3-dilactone are commercially available.

D-glucaric acid is naturally found in many fruits and vegetables especially oranges, apples, grapefruit and cruciferous vegetables[13].

1.1.3 Uses of D-Glucaric Acid

Given its straightforward manufacture and the low cost of D-glucose as a direct precursor, the full potential of D-glucaric acid has not yet been exploited. Most of the applications of D-glucaric acid involve consumption of this compound on relatively small scale, and therefore do not make use of the prospective economy of scale associated with its precursor D-glucose[14]. However the range of uses of D-glucaric acid is extremely diverse. All forms of D-glucaric acid are normal human metabolites involved in the metabolism of D-glucuronic acid[12, 14]. This has led to their use in medical and cosmetic preparations, with particular focus on

natural cancer treatments and preventatives [25-29], but also in a wide variety of formulations to treat and prevent conditions ranging from hair loss to heart disease[30-45]. D-Glucaric acid is also used in industrial processes [46-51], especially as an additive or builder[52-62]. The range of miscellaneous uses of D-glucaric acid and its derivatives reported in literature is virtually endless. From an energy-containing treatment for plants exposed to pesticides[63], to a low-sugar and low-flour base for food products[64] and a composition for cleaning egg shells[65], the use of D-glucaric acid is extremely widespread through many areas.

1.1.4 Physical Properties

The crystal structure of monopotassium D-glucarate was determined by X-ray analysis[66]. The crystals were monoclinic space group $P2_1$, $Z = 2$, $D_x = 1.807$ g/cm3, with cell dimensions $a = 8.55$, $b = 10.9$, $c = 4.85$ Å and $\beta = 90.0°$. All final structure parameters were reported (**Table 1.1**).

Kiely *et. al.* reported the crystal structures of *N,N'*-dimethyl-D-glucaramide, dipotassium D-glucarate and sodium potassium D-glucarate as part of work analysing the conformations of D-glucaric acid derivatives. It was found that the crystal structures of these molecules as well that of the monopotassium salt reported above, corresponded within one kcal/mol of the global minimums derived *via* MM3(96) conformational analysis[24].

The conformations of D-glucaric acid and its lactones in solution were investigated by NMR spectroscopy[12]. D-glucaro-1,4-lactone was found to exist in a conformational equilibrium between ^3E(D) and E_3(D) with the OH-5 group tending to occupy the position over the lactone ring in the favoured E_3(D),*gg* conformation. An explanation of the symbolism is contained in **appendix one**. Data for D-glucaro-6,3-lactone indicated that E_4(D)*gt* conformation was favoured with virtually none of the ^{4}E(D) conformer present (**Table 1.2**). D-glucaro-1,4;6,3-dilactone was found to favour the dienvelope conformation of ^{3}E:E_4(D), which retains the E_4(D) conformation of the 6,3-lactone ring but adopts the ^3E(D) conformation for the 1,4-lactone ring.

Atom	*x*	*y*	*z*	β11	β22	β33	β12	β13	B23
K	2155(6)	0	3430(14)	67	32	577	25	30	95
C(1)	1316(21)	7284(17)	7661(47)	27	15	296	6	-41	20
C(2)	2051(20)	6342(15)	9653(42)	26	4	206	4	-39	4
C(3)	3008(21)	5435(16)	7964(43)	22	11	197	11	-2	3
C(4)	3826(20)	4451(16)	9672(44)	17	11	211	13	-11	-3
C(5)	2719(22)	3549(17)	11050(43)	47	20	126	-1	-14	-1
C(6)	1890(21)	2705(17)	9018(47)	23	17	284	-5	-34	-23
O(1)	1790(17)	8394(12)	7658(36)	76	13	380	-17	-17	17
O(1')	217(15)	6840(12)	6103(32)	45	12	291	1	-37	25
O(2)	3002(16)	6893(13)	11744(31)	65	27	211	-7	-35	11
O(3)	4266(15)	6088(12)	6584(31)	29	21	273	-15	-6	2
O(4)	4935(15)	3735(13)	8002(32)	34	25	252	16	-19	11
O(5)	3549(16)	2863(12)	13147(31)	66	24	156	10	-28	15
O(6)	2295(17)	1559(12)	8826(34)	88	10	324	2	-19	0
O(6')	804(16)	3216(12)	7491(32)	40	21	256	-4	-46	5

Table 1.1 *Atomic Parameters and their deviations in potassium D-glucarate*[66]

The coupling constants of D-glucaric acid suggested conformational mixing involving the ${}_3G^+$ and ${}_2G^-$ sickle forms, together with the planar (*P*), zigzag conformation[12]. The suggested conformations for D-glucaric acid are similar to those reported for D-gluconic acid[12].

Crystalline D-glucaro-1,4-lactone monohydrate has the E_3 lactone-ring confirmation, with a small distortion of the ring to ${}_3T^2$. Calculations of non-bonding repulsion energy show that this conformation corresponds to an energy minimum, although comparable minima can also be obtained with the ring in the alternative ${}^3E$ conformation[67].

Solvent	**Lactone-ring Conformation (Contributions in Percent)**	
D-Glucaro-1,4-lactone	**^{3}E**	**E_3**
Deuterium Oxide	15	85
Methanol-*d*4	15	85
D-Glucaro-6,3-lactone	**E_4**	**^{4}E**
Deuterium Oxide	~100	~0
Dimethyl Sulfoxide-*d*6	~100	~0

Table 1.2 *Conformer populations for D-glucaro-1,4-lactone and D-glucaro-6,3-lactone in solution* [12]

1.2 Equilibria in Aldaric Acids

Aqueous aldaric acids can form monolactones or dilactones[1, 17]. In aqueous solution D-glucaric acid exists in an equilibrium of the acyclic acid, D-glucaro-1,4-lactone, D-glucaro-6,3-lactone and D-glucaro-1,4;6,3-dilactone. While crystalline meso-galactaric (mucic) acid is the commercially available form of this aldaric acid, D-mannaric acid is sold as its crystalline 1,4;6,3-dilactone which is relatively stable in aqueous solution[10, 14, 68]. Crystalline D-glucaric acid has never been isolated, instead D-glucaric acid is readily isolated as one of its monolactones (1,4- or 6,3-) or as its 1,4;6,3-diactone (**Figure 1.1**)[1, 12-14, 17].

1.2.1 Equilibrium in D-Glucaric Acid

In aqueous solution D-glucaric acid exists primarily in the acyclic form and its two monolactones[10-12]. The rapid mutarotation of D-glucaro-1,4;6,3-dilactone compared to its D-mannaro- counterpart is due to the instability of the 1,4-lactone ring (δ-lactone ring) in aqueous solution, compared with the two γ-lactone rings in D-mannaro-1,4;6,3-dilactone[10].

Paper chromatography was used to investigate the equilibrium of D-glucaric acid under different conditions including increased temperatures and in the presence of cation ion exchange resin[11]. Results indicated that D-glucaro-1,4;6,3-dilactone

was only detectable at elevated temperatures (70°C) and that the resin accelerated the formation of the equilibrium. Experiments starting with each of the monolactones separately and tracking the disappearance of the starting monolactone and the formation of the other monolactone, found that the amount of D-glucaro-6,3-lactone was higher (by 5-10%) at equilibrium than the corresponding 1,4-lactone. It was concluded that reciprocal transformation between monolactones was *via* the acyclic D-glucaric acid, not the dilactone, as the acid was detected whereas the dilactone was not[11].

Although Horton *et. al.*'s work focussed more on the confirmations of the species present in the equilibrium of D-glucaric acid, it was noted that D-glucaric acid lactonises spontaneously to give D-glucaro-6,3-lactone as the first product while D-glucaro-1,4-lactone forms more slowly in neutral conditions[12].

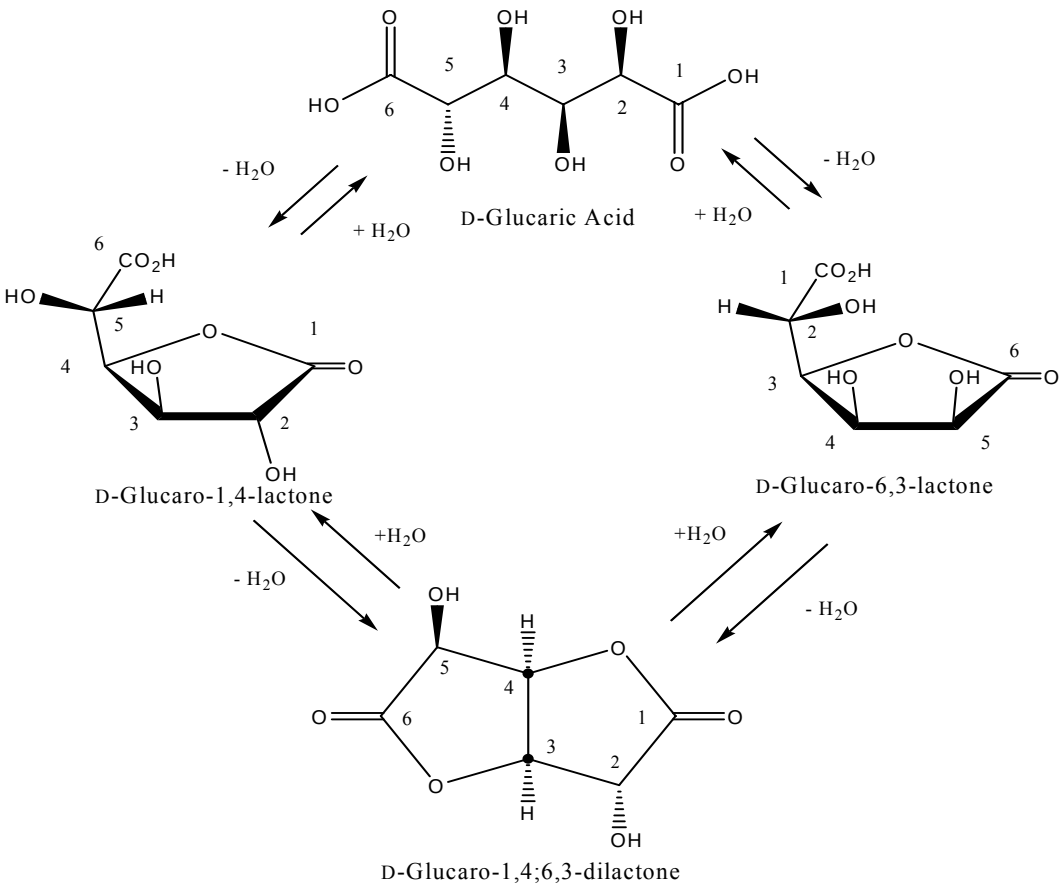


Figure 1.1 *Equilibrium of aqueous D-glucaric acid*

1.3 Use of NMR Spectroscopy for Kinetics of Carbohydrates

NMR spectroscopy is a rather under utilised technique for the study of the kinetics of carbohydrates with only four reported examples in the literature that explicitly use NMR spectroscopy to gain rate constants, and all of these using ^{13}C NMR spectroscopy.

The oxygen-18 isotope effect in ^{13}C NMR spectroscopy has been used to investigate the kinetics of oxygen exchange at the anomeric carbon of D-glucose and D-erythrose. The results produced a similar rate constant for the pseudo first order exchange of both the α and the β anomers of D-glucose as a previous chemical conversion-mass spectrometry experiment, and also provided new corresponding rate constants for D-erythose[69].

The kinetics of the jack bean alpha-mannosidase digestion of various alpha-D-mannopyranosyl linkages in hen ovalbumin glycopeptides was followed *via* ^{13}C NMR spectroscopy. It was found that ^{13}C NMR spectroscopy was a practical method for both following the kinetics of this enzymatic digestion, and determining the structures of the products of partial digestions[70].

Incorporation of a ^{13}C label ($^{13}CH_3O$) into the carbohydrate ligand methyl 3-*O*-(3,6-dideoxy-α-D-xylohexopyranosyl)-2-*O*-methyl-α-D-mannopyranoside allowed ^{13}C NMR spectroscopy to be used to investigate the kinetics of its binding to the monoclonal antibody 50 K Dalton Fab[71].

^{13}C CP-MAS spectrometry (both solid state and solution) was used to investigate the cross-polarisation kinetics and mobility of polysaccharides within primary cell walls from *Citrus* (orange) mesocarp. Using ^{13}C CP-MAS spectrometry it was possible to gain a considerable amount of motional information even though *Citrus* (orange) mesocarp represents complex and chemically diverse materials[72].

Although actual rate constants were not elucidated, the use of ^{1}H NMR spectroscopy to investigate the mechanism of the formation of polyamides by

aminolysis of D-glucaric acid esters allowed the comparison of the rates of two different reaction pathways[73].

1.4 Polymers

It is difficult to imagine life without polymers. These materials encompass human existence in the 21st century. From throwaway packaging to artificial hearts, polymers have become essential to life as we know it. If metals and inorganic compounds are disregarded, then practically everything else in the world is polymeric[74]. However with fossil fuel stocks diminishing, and global warming and pollution dominant global issues, traditional polymers can no longer be viewed as viable commodities for the long-term future.

1.5 Nylon

Nylon is the generic name for the linear saturated polyamides that feature prominently in the world polymer market. In fact during the 1990s Nylon 6,6 alone made up 4% of total polymer production in the United States of America[75]. Nylons are the product of the condensation polymerisation of difunctional acids with difunctional amines. Each type of nylon is characterised via a numbering system that describes the number of carbon atoms in the monomeric dicarboxylic acid, and the number of carbon atoms in the monomeric diamine[76]. For example, nylon 6,6 is formed from the monomers hexamethylene diamine and adipic acid (**Figure 1.2**). However, nylons based on ω-amino acids only contain one numeral in their name. The six carbon monomer of nylon 6 is the cyclic amide of ω-aminohexanoic acid (aminocaproic acid), caprolactam (6-hexanolactam)[75, 77]. Nylons, such as nylon 6, which are based on amino acids are usually synthesised via a ring-opening polymerisation rather than a condensation reaction[76, 78].

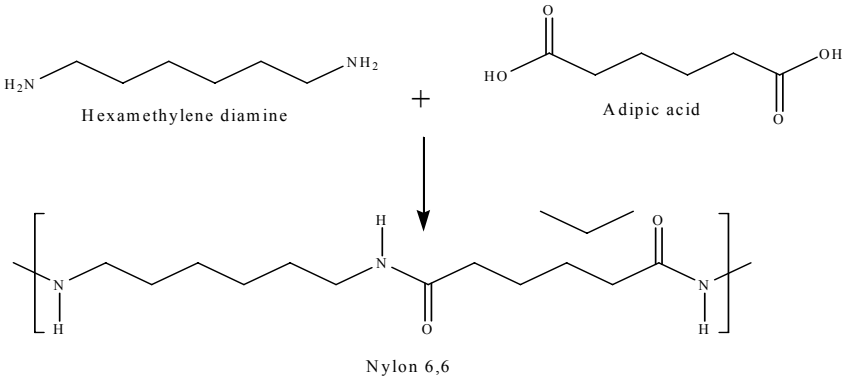


Figure1.2 *Production of nylon 6,6 from hexamethylene diamine and adipic acid*[79]

1.5.1 Development of Nylons

Development of nylons began in 1928 when Wallace Hume Carothers was employed by Du Pont de Nemours in America to initiate a program of fundamental scientific research[76]. Carothers' employment came soon after Staudinger[80] proposed his theory that the materials that are today referred to as polymers, were actually macromolecules. Carothers set about trying to prove the existence of such macromolecules by initially studying the condensation of alcohols and acids, such as ethanol and acetic acid, to give esters. He then, took the step that seems simple in hindsight, but at that time was very intuitive, and used dicarboxylic acids and dialcohols (glycols) to produce polyesters[76]. Optimisation of Carothers' method allowed "superpolyesters" (polyesters with molecular weights above 10,000) to be produced. These aliphatic polyesters were tough, opaque solids that melted at moderate temperatures to form clear, viscous liquids. Although filaments could be pulled from the liquid and these filaments drawn into strong fibres, the low melting points (below 100°C), and the fact that they were sensitive to water and soluble in dry cleaning solvent rendered them inappropriate for textile fibres[76-78]. Carothers and his group turned their attention to the chemically analogous polyamides, synthesised from diacids and diamines[76, 78]. Carothers examined a wide range of amino acids, diamines and dicarboxylic acids and found that, due to the substantial hydrogen bonding *via* the N-H groups, it was possible to produce polymers with higher melting points than their polyester counterparts[77, 78]. The biggest breakthrough in Carothers' work came in 1935 when nylon 6,6 was created. Although Carothers believed

that nylon 5,10 was the most suitable nylon for forming fibres, the monomers used in nylon 6,6 (hexamethylene diamine and adipic acid) meant that nylon 6,6 proved to be a more convenient and economic choice, and this was the nylon chosen by Du Pont for production[76].

Nylon 6,6 has excellent properties for use as a textile fibre. It has a high melting point (around 265°C) and is insoluble in most solvents. It can also be drawn into fibres that have tenacity and elasticity[78]. In 1939 nylon 6,6 stockings were first sold to the Du Pont employees in Wilmington only, until enough of this new polymer could be made. May 15 1940 marked the release of nylon stockings to the rest of America. Demand was so immense that in New York alone, four million pairs were sold in the first few hours[76]. The commercial success of nylon sparked a search for analogues that were not covered by Du Pont patents. The result was the development of the isomeric nylon 6 by I. G. Farben Industrie[77].

1.5.2 Production of Nylons

As stated above both nylon 6,6 and nylon require 6-carbon monomers. The cheapest 6-carbon feedstock is benzene from the BTX fraction of oil. This is catalytically hydrogenated to cyclohexane. For nylon 6,6 the cyclohexane is oxidised to a mixture of cyclohexanol and cyclohexanone, which are then both oxidised to adipic acid. Hexamethylene diamine is formed from adipic acid *via* the dinitrile[77, 78]. Sebacic acid (for nylon 6,10) is produced from either castor oil or electrochemically from adipic acid. Although nylon 6 is formed from caprolactam, which is the cyclic amide of ω-aminohexanoic acid, the lactam does not need to be prepared from the amino acid[77]. In fact the usual commercial process sees cyclohexane inexpensively converted to cyclohexanone oxime (*via* cyclohexanol and cyclohexanone). The caprolactam is produced from the oxime *via* the acid-catalysed Beckmann rearrangement[77, 81].

A 1:1 ratio of monomers is essential for producing nylon with a high relative mass. In the case of nylon 6,6 the achievement of this stoichiometric balance is simplified by the predisposition of hexamethylene diamine and adipic acid to

form a 1:1 salt, which can be easily isolated due to its low solubility in methanol. This salt is filtered from the boiling methanolic solution and dissolved in water. The solution is heated to 270-280°C at 250 psi for three to four hours. Acetic acid (0.5-1 mol%) is added as a cheap monofunctional end-capper to prevent excessive viscosity. Acetic acid is a monofunctional acid, therefore when it reacts with an amino group the chain is terminated. Nitrogen is used to extrude the nylon into ribbons as the presence of oxygen causes yellow discolouration. This ribbon is subsequently cut into cubes for plastic grade materials or melt-spun for fibre use[77, 78].

1.6 Hydroxylated Nylons

Much of everyday life depends on nylon and other synthetic polymers. However traditional polymers have two major drawbacks – their production relies heavily on petroleum resources and they are largely non-biodegradable. Rising fuel prices due to diminishing oil stocks will ultimately see petroleum become a non-viable feedstock before supplies run out completely. Therefore petroleum-based polymers can no longer be viewed as feasible products for the future. An alternative has to be found and significant research has gone into producing polymers that are biodegradable and that are based on monomers that are from renewable sources.

Carbohydrates are ubiquitous in life. They are found throughout the animal kingdoms and represent 75% of the dry weight of the plant world[74]. This makes them ideal candidates for a renewable chemical feedstock. As carbohydrates themselves are biodegradable, it would be sensible to hypothesize that any products encompassing carbohydrates may also exhibit biodegradable properties. Another favourable characteristic of carbohydrates is the great stereochemical diversity that even just the monosaccharides provide. This means that there are a large number of different properties available[74]. A notable amount of research in utilising carbohydrates has gone into the development and optimisation of polyhydroxypolyamides (PHPAs) (“hydroxylated nylons”). These are synthetic polyamides that are produced from aldaric acids and primary diamines[73]. The

structure of PHPAs is very similar to that of nylon (**Figure 1.3**). If the dibasic acid of nylon is replaced with aldaric acid, and this is polycondensed with a diamine, a PHPA results.

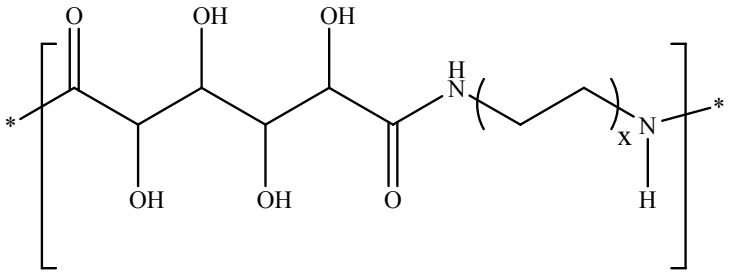


Figure 1.3 *Representative polyhydroxypolyamide structure based on a hexaric acid*

1.6.1 Development of Polyhydroxypolyamides

Incorporating carbohydrates into potentially commercially successful polyamides requires development of a method that overcomes the numerous protection/deprotection steps often associated with carbohydrate chemistry. The first carbohydrate-based polyamides produced could not be viewed as commercially viable products due to the large number of these steps required. Haworth *et. al.* reported the condensation of 1,6-diamino-1,6-dideoxy-di-*O*-methylene-D-mannitol with di-*O*-methylene-D-glucaric acid[82], while Wiggins replaced the acid with 1,2,5,6-di-anhydro-3,4-*O*-isopropylidene-D-mannitol and produced a polymer that exhibited fibre-forming characteristics[83]. Polymers, which were produced by Wolfrom *et. al.* by reacting the *O*-protected (peracetylated) aldaroyl chloride, 2,3,4,5-tetra-*O*-acetyl-galactaroyl dichloride with the diamines ethylediamine or piperazine, also used protection/deprotection steps[84].

In more recent times the synthesis of polyesters containing anhydro- and dianhydroalditols prepared by interfacial polycondensation with various aliphatic and aromatic acids has been reported. This work was then extended to the synthesis of hydrophilic polyamides via the condensation of 2,3,4,5-tetra-*O*-acetyl-galactaroyl dichloride with various heterocyclic carbohydrate-derived diamino compunds and hexamethylene diamine. These polyamides had their acetyl protecting groups removed *via* saturation with aqueous NH_3 solution[85].

Thiem and Bachmann continued this work to produce polyamides that were formed by reacting various differently modified methyl and benzyl glycosides of 2,6-diaminosaccharides with a selection of different aliphatic and aromatic carboxylic diacid dichlorides[86, 87]. García-Martín and Mancera *et. al*. used the polycondensation of *O*-methyl protected sugars to create polyamides. Pentachlorophenyl esters of 2,3,4-tri-*O*-methyl-L-arabinaric acid, 2,3,4-tri-*O*-methylxylaric acid, 2,3,4,5-tetra-*O*-methyl-D-mannarate and 2,3,4,5-tetra-*O*-methylgalactarate were reacted with aliphatic or carbohydrate based diamines[88-90].

The first polyamides based on non-protected carbohydrates appeared in the 1970s from Ogata *et. al*.[91-96]. Work in this area commenced with research into the typical conditions under which polycondensation reactions were carried out. It was noted that polycondensation reactions, including amidation reactions, consisted of an addition-elimination reaction between the carbonyl group of a carboxylic acid and the nucleophilic reagent such as the amine. The whole reaction is essentially an equilibrium[97]. Polyamide synthesis is usually accomplished by melt polycondensation of dicarboxylic acids or diesters with diamines at elevated temperatures, above the melting points of the resulting polymers, so that the polycondensation equilibrium is shifted toward the polymer formation side by driving leaving groups such as water, out of the system (**Figure 1.4**)[91].

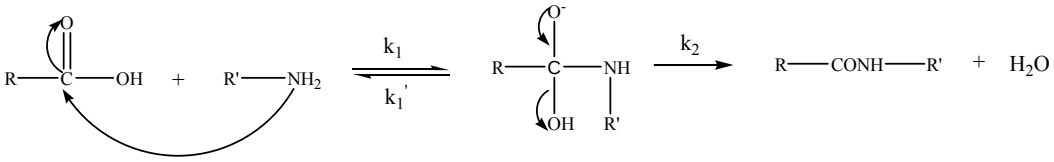


Figure 1.4 *The addition/elimination reaction of amidation*[98]

These conditions are too harsh for many monomers, carbohydrates included, to escape decomposition during the polymerisation process. Preliminary work in polycondensation[98-100] established that the reaction was strongly controlled by the surrounding media. This was emphasised by the fact that solvents or

chelating agents could influence the reaction to the extent that the equilibrium was apparently shifted. As the rate-determining step of most polycondensation reactions is the formation of the intermediate given by the rate constant k_1, it was hypothesised that the foregoing effect on the equilibrium was due to the formation of an intermediate product between the monomer and solvent. The formation of such an intermediate would accelerate the polycondensation reaction[98].

Further work into factors affecting polycondensation reactions revealed the structure of the monomers was also influential, and that it was possible to increase the reactivity of monomers by the introduction of active substituents, to such an extent that polycondensation would occur at mild temperatures regardless of the equilibrium. Esters having a thioether group β to a carbonyl group reacted with an amine at room temperature[97, 100]. It was also shown that *N*-hydroxyethyl-substituted amines could react with esters below room temperature which implied that polyamides with pendant *N*-hydroxyethyl groups could be readily prepared[99, 100]. It was presumed (through evidence provided by reactions of model compounds) that the polycondensation reaction could take place at milder than usual temperatures due to an exchange reaction between the ester and amide groups, proceeding through an oxazolidine intermediate in which the hydroxyethyl group participates (**Figure 1.5**)[99].

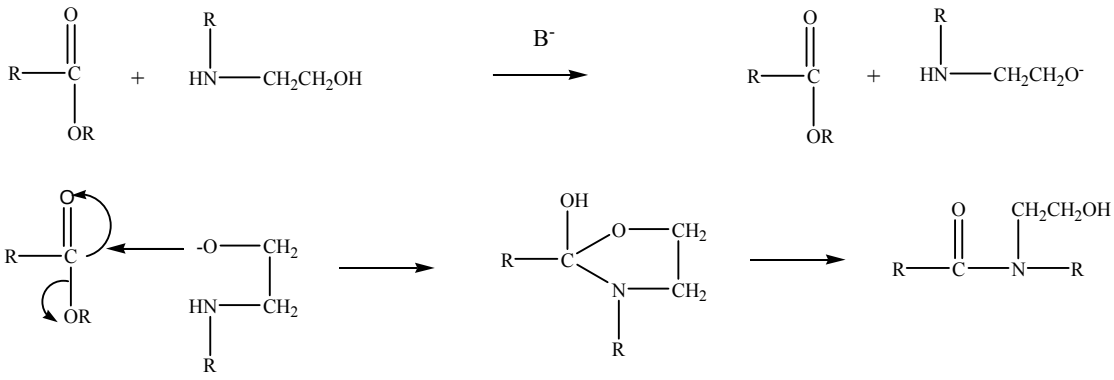


Figure 1.5 *Room temperature condensation mechanism with an oxazolidine intermediate* [99]

Ogata *et. al*. made a systematic study of the polycondensation reactions of acid derivatives having various heteroatoms at the position β to the carbonyl group.

They reported that reactivity augmentation of diesters or diacids due to the β-heteroatom could be arranged in such a way that it mimicked the order of electronegativities of the heteroatoms. The effect of the heteroatom on the reactivity was thought to be an induction effect of the heteroatom on the carbonyl group on the acid or ester. This interaction enhances the reactivity of functional groups to such an extent that the equilibrium was shifted towards the formation of polymers [95, 101-103].

The extent of this shift is apparent by the fact that active polycondensation progressed even in methanolic solution, provided that methyl esters were used as monomers[104].

Further work concluded that the reactivity enhancement of diesters by α-heteroatoms was superior to that of their β-counterparts. The cause of the superior enhancement was attributed to two possible explanations. The first being the fact that the carbonyl group becomes more electrophilic (and hence more susceptible to nucleophilic attack) when the heteroatom is in the α position, and the second was the possibility of a proximity effect of the heteroatom in the reaction intermediate (a hydrogen bond between the heteroatom and the approaching amine may form, hence forming an anchor and aiding the polycondensation reaction). In contrast, introduction of similar heteroatoms into the reacting amine hindered the polycondensation process. The most plausible cause of this is the reduction in basicity of the amine caused by the heteroatom which is turn reduces the reactivity of the amine[104].

From these studies Ogata *et. al.*[91] concluded that heterogroups such as ether or hydroxyl groups would have an enhancement effect on the reactivity of both esters and amines in polycondensation reactions. Studies on the polycondensation of carboxylic acids having pendant hydroxyl groups focussed on two main methods each producing polymers that no longer had the pendant hydroxy groups[105, 106]. Melt polycondensation at high temperatures caused cross linking due to the degradation of the monomers, while interfacial polycondensation utilising acid chlorides also caused cross linking due to the participation of the hydroxyl groups[91]. Ogata *et. al.* were able to produce

carbohydrate-based polyamides that retained the pendant hydroxyl groups by reacting α,α'-dihydroxyl diesters, particularly L-dimethyl tartarate esters[91-93, 107] and diethyl D-galactarate (mucate) esters[94, 96], with diamines in solution. Further work noted that reactivity enhancement of the diester could also be achieved by introducing a good leaving group such as *p*-nitrophenol or thiophenol[108].

Hashimoto's group produced PHPAs that were based on the dilactones of D-glucose, and D-mannose, D-glucaro- and D-mannaro-1,4:6,3-dilactones, that also featured pendant hydroxyl groups[109-111].

Hoagland's work elucidated the mechanism of the polymerisation of diethyl galactarate with ethylenediamine[112]. The pendant hydroxyl groups facilitate a fast five-membered lactonisation step before the slower aminolysis of the resulting lactone. Similar results were found during the aminolysis of diethyl xylarate[113].

In more recent times the challenge to produce commercially viable PHPAs has been taken up by Kiely *et. al*. Work from this laboratory has greatly extended both the knowledge of PHPAs, and the variety of PHPAs prepared. PHPAs have been prepared that are based on D-glucaric acid[24, 114-117], D-mannaric acid[114], galactaric acid[114, 116] and *meso*-xylaric acid[114]. A general preparation scheme for the preparation of carbohydrate-based copolymers that imitates that used for strictly petroleum-based monomers was proposed (**Figure 1.6**) [118]. A lot of Kiely *et. al*.'s work is aimed at procuding PHPAs that are commercially viable and in keeping with this end goal a list of critical characteristics for the industrial production of such polymers was formulated (**Figure 1.7**)[118].

Synthetic Copolymers Derived from Carbohydrates

-A-B-A-B-A-B-A-B-A-B-A-B-A-B- ***i.e.*.. –[A-B]$_n$–**

Polymerisation Reaction – Condensation or Step-Growth Polymerisation

$$nA + nB \longrightarrow -[A\text{-}B]_n-$$

Monomer A – derived from X-(CHOH)$_y$-X, an acyclic unprotected carbohydrate activated at both ends.

Monomer B – derived from Z-Z, a diterminally activated second monomer.

Figure 1.6 *Proposed approach for the general preparation of synthetic carbohydrate based copolymers that parallels that used for strictly petroleum-based monomers* [118]

Kiely's work has extended into developing methods of producing stereoregular head-tail polyamides[119], as well as using computer aided structural molecular modelling[120] and conformational analysis via MM3(96) software[24] to better understand the observed variances in the physical properties of PHPAs derived from D-glucaric acid[118]. Potential applications of PHPAs such as nitrogen fertilisers[121] and biodegradable adhesives[122] have been investigated, and patents concerning the manufacture of PHPAs have been created[123, 124].

Thiem *et. al*. recently published a description of polydimethylsiloxane polyamides. These polyamides were based on the hydroxyl-protected peracetylated acid chlorides of glucaric and galactaric acid[125].

Some Critical Characteristics of Idealised Carbohydrate-Based Polymerisations for Industrial Production.

1. Activated carbohydrate monomers **A** are available from any simple aldose, e.g. D-glucose, D-galactose, D-xylose, D-mannose etc.
2. Monomers **B** are relatively inexpensive and readily available.
3. Syntheses of activated carbohydrate monomers **A** and the polymers **–[A-B]$_n$–** do not require protection-deprotection steps.
4. Polymer isolation and purification utilities established industrial technology.
5. Polymers with reasonably predictable but variable properties can be synthesised by taking into account:
    a. The structure of the carbohydrate monomer **A** (i.e. the stereochemistry and number of carbons in the carbohydrate;
    b. The chain length, degree of branching, and fuctionalisation of monomer **B**.

Figure 1.7 *List of some critical characteristics of idealised carbohydrate-based polymerisations for industrial production* [118]

1.7 D-Glucaric Acid Based Polyhydroxypolyamides

Although the first unprotected polyhydroxypolyamides based on carbohydrates (L-tartrate[92, 103]and diethyl mucate[94]) appeared during the mid 1970s, it was not until the 1990s that polyhydroxypolyamides based on D-glucaric acid were produced[110, 111, 115].

Hashimoto reacted D-glucaro-1,4;6,3-dilactone (as well as its D-mannaro-counterpart) with *p*-xylylenediamine to produce poly(*p*-xylylene-D-glucaramide). The results from the model reaction between the dilactone and benzylamine in tetrahydrofuran (production of *N,N'*-dibenzyl-D-glucaramide), suggested that the reaction proceeded through the nucleophilic addition of the amino group in benzylamine to the lactone carbonyl groups accompanied with the opening of the lactone rings (**Figure 1.8**)[109].

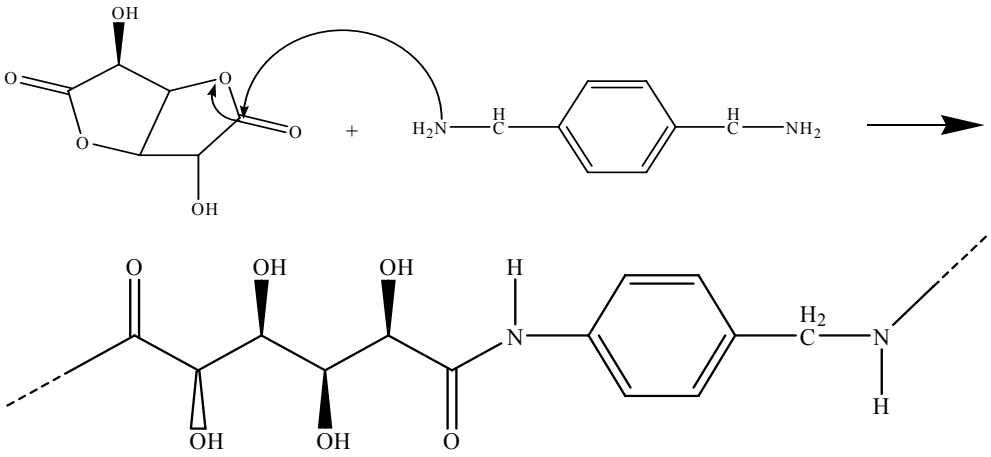


Figure 1.8 *Reaction of D-glucaro-1,4;6,3-dilactone with p-xylylenediamine to yield poly(p-xylylene-D-glucaramide)* [109]

Hashimoto extended work with D-glucaro-1,4;6,3-dilactone-based polyhydroxypolyamides by reacting the dilactone with hexamethylene diisocyanate to give a polyurethane with a dilactone skeleton in the main chain (**Figure 1.9**)[111].

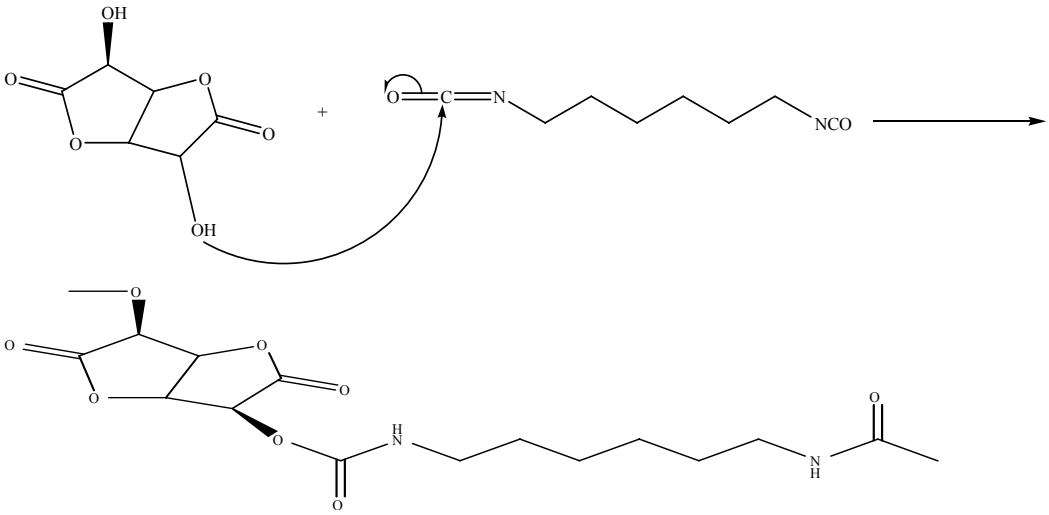


Figure 1.9 *Reaction of D-glucaro-1,4;6,3-dilactone with hexamethylene diisocynate to give a polyurethane*[110]

A significant difference between the other carbohydrate-based polyhydroxypolyamides[92, 93, 103] and their D-glucaric acid counterparts is the absence of an analogous D-glucaric acyclic diester[115]. Kiely *et. al.*[112, 113, 115] used a D-glucaric acid methanol esterification, in methanol solution containing

triethylamine, to produce poly(alkylene D-glucaramide). The triethylamine served to ensure that the base-induced lactonisation step identified by Hoagland occurred at a reasonable rate even near the end of the reaction when the concentration of the diamine was low.

A considerable driving factor in this work was to develop a process that would ultimately be commercially viable. Such a method would avoid the use of water and hence the consequent requirement of removal. This was achieved by the direct acidification/esterification of monopotassium D-glucarate in alcohol solution (**Figure 1.10**)[115].

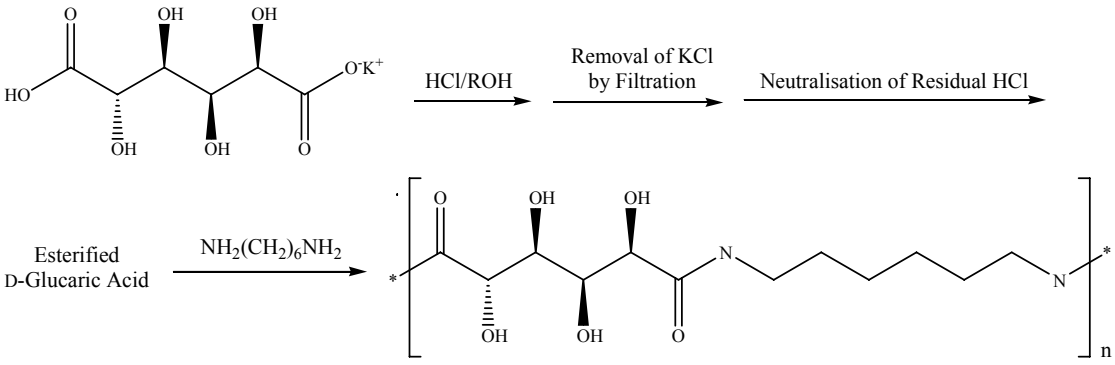


Figure 1.10 *Direct method for preparation of poly(hexamethylene D-glucaramide) from monopotassium D-glucarate. Neutralisation of residual HCl can be via bases including triethylamine, sodium carbonate, sodium methoxide or via hydroxide form anion-exchange resin*[115]

A further improvement made was to produce specific crystalline and weighable D-glucaric esters/lactones from which polyhydroxypolyamides could be produced. This allowed good stoichiometric control of the aldaric acid monomer and hence enhanced the feasibility of achieving a 1:1 monomer ratio[115]. Crystalline methyl D-glucarate 1,4-lactone, ethyl D-glucarate 6,3-lactone and D-glucaro-1,4;6,3-dilactone were all used to produce a variety of D-glucaramides from different diamines. Structures of the esterification products were determined using ^{1}H NNMR spectroscopy, ^{13}C NMR spectroscopy and GC/MS techniques. Isolation of the resulting polymers was *via* simple filtration. Differences in the physical properties of the polymers were caused by the use of

different diamines [115, 126]. Crystalline methyl D-glucarate 1,4-lactone and ethyl D-glucarate 6,3-lactone proved to be particularly useful as they have a good shelf life and are not readily hydrolysed on standing like D-glucaro-1,4;6,3-dilactone[127].

Additional work[73] investigated if the amide forming mechanism that Hoagland reported for esterified *meso*-galactaric[112] and *meso*-xylaric[113] acids applied to esterified D-glucaric acid. Methyl D-glucarate 1,4-lactone and ethyl D-glucarate 6,3-lactone respectively were reacted with *n*-propylamide to form *N,N'*-dipropyl-D-glucaramide. As well as employing ^{13}C NMR spectroscopy to track the reaction as Hoagland did, ^{1}H NMR spectroscopy was also used. This method was found to provide more detailed information about the rapid ring opening of the five-membered lactone ring due to its relatively quick FID accumulation time. As well as elucidating the mechanism of the aminolysis of both methyl D-glucarate 1,4-lactone and ethyl D-glucarate 6,3-lactone, this research investigated the contribution that direct aminolysis of the ester carbonyl makes in the aminolysis of esterified D-glucaric acid. Dimethyl L-tartrate was used as a model of an α,ά-dihyroxy esterified aldaric acid that cannot form a five-membered lactone and subsequently cannot experience the aminolysis rate enhancement that occurs because of the formation of such a lactone[73]. This was one of the compounds originally used by Ogata *et. al*. when they concluded that aliphatic esters with an alpha hydroxy group undergo aminolysis at enhanced rates compared to aliphatic esters without such a group, because of the extra polarisation of the ester carbonyl group by the hydroxy group[103]. However because of Hoagland's results[112, 113], it was speculated that dimethyl L-tartrate would undergo aminolysis at a much slower rate than methyl D-glucarate 1,4 lactone. This was what was found when both compounds were reacted with *n*-propylamine (**Figure 1.11**)[73].

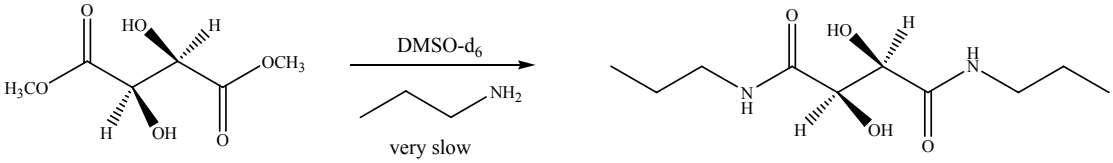


Figure 1.11 *Direct, very slow aminolysis of dimethyl L-tartrate with n-propylamine to N,N'-dipropyl-L-tartramide* [73]

Thiem and Bachmann used diamino compounds derived from D-glucosamine and D-glucose to produce polyamides[86].

1.7.1 Aminolysis of Methyl D-Glucarate 1,4-Lactone

The aminolysis of methyl D-glucarate 1,4-lactone (**Figure 1.12**) and ethyl D-glucarate 6,3-lactone was reported by Kiely *et. al.*\[73].

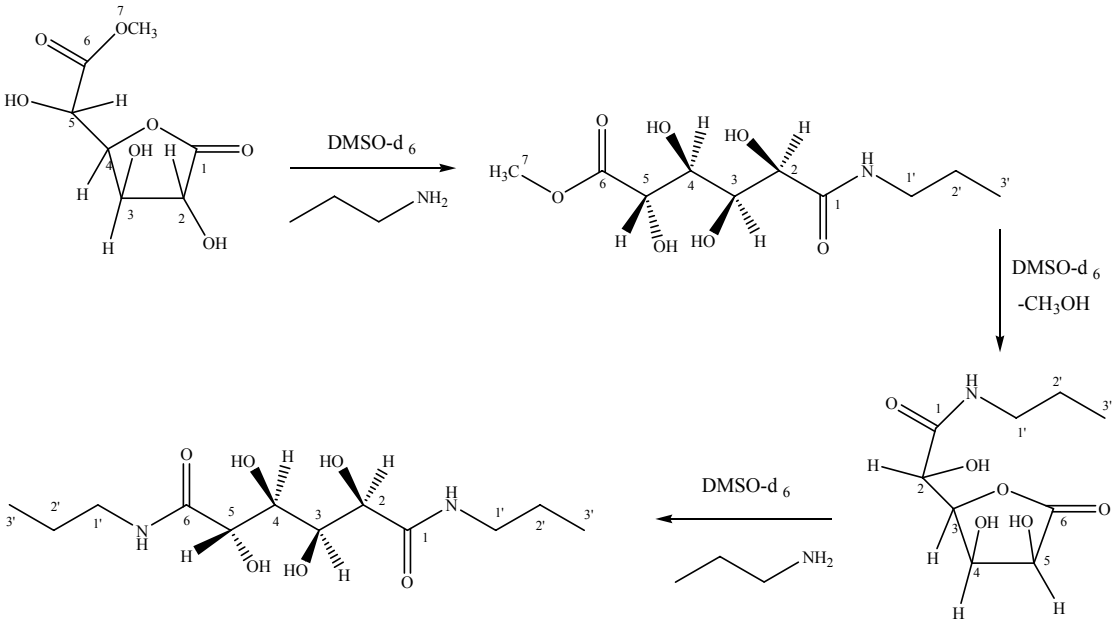


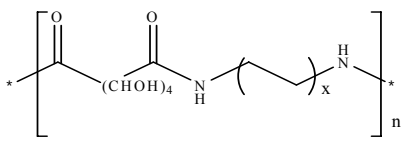
Figure 1.12 *Aminolysis of methyl D-glucarate1,4-lactone by n-propylamine to give N,N'-dipropyl-D-glucaramide* [73]

The aminolysis of ethyl D-glucarate 6,3-lactone proceeds in an analogous manner[73].

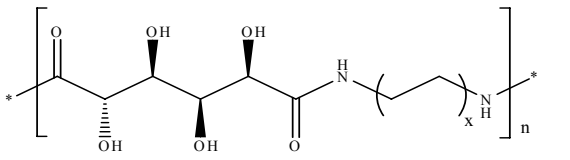
1.7.3 Comparison of the Properties of Polyhydroxypolyamides Based on D-Glucaric Acid

When the properties of polyhydroxypolyamides that are based on D-glucaric acid were compared to those based on other aldaric acids (galactaric, xylaric and D-mannaric)[114] it was found that differences in the polymer melting points and water solubilities were largely due to the conformational differences of the monomer aldaryl units. For example the extended zigzag conformation of the galactaric acid units allowed for strong intermolecular hydrogen bonding whereas the bent conformation of the D-glucaric units prevents this interaction, hence these polyamides have lower melting points and higher solubilities than their galactaric acid counterparts. Conformational analysis of D-glucaramides via MM3(96) software and x-ray crystal structures of various D-glucaric acid derivatives backed these findings by indicating that the glucaric acid portion of poly(alkylene D-glucaramides) in solution can take on a variety of conformations, mostly those with sickle arrangements which give rise to a bend in the glucaric acid monomer unit[24].

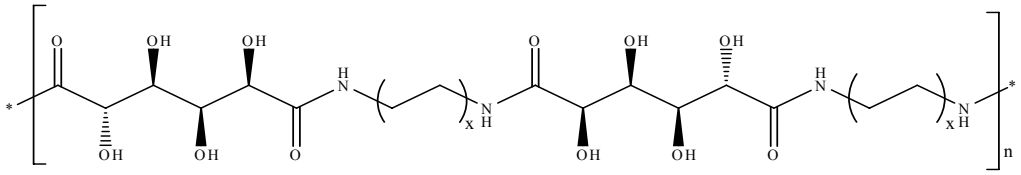
The asymmetry of D-glucaric acid allows for three classes of poly(alkylene D-glucaramides) to be assigned[24], randomly aligned polyamides[114, 115] stereoregular *Head, tail*- polyamides[119] and stereoregular alternating *Head, tail – tail, head*-polyamides (**Figure 1.13**)[24].



"Random" poly(alkylene-D-glucaramides)



Stereoregular repeating *Head, tail* - poly(alkylene-D-glucaramides)



Stereo alternating *Head, tail - tail, head* - poly(alkylene-D-glucaramides)

x = 1, 2, 3, 4, 5 or 6

Figure 1.13 *Three classes of poly(alkylene D-glucaramides)*[24]

1.8 Advantages of using D-Glucaric Acid for Polyhydroxypolyamide Production

In order for polyhydroxypolyamides to be a commercial success and compete with petroleum-derived polymers, it is crucial that starting materials are inexpensive and readily available. When all the possible carbohydrate monomers that are commercially available are considered, D-glucaric acid has the most potential in fulfilling this need. Millions of tonnes of D-glucose are produced worldwide each year from the hydrolysis of cornstarch meaning that it is the cheapest monosaccharide available (**Table 1.3**). The relatively inexpensive process of oxidising D-glucose to D-glucaric acid with nitric acid as the oxidant provides a method of producing a commercially viable source of D-glucaric acid for use in polyhydroxypolyamide production. Polyhydroxypolyamides based on D-glucaric acid also have the benefits of good biodegradability properties[14, 127].

	D-isomer Price ($USg^{-1})	L-isomer Price ($USg^{-1})
Allose	200	900
Altrose	700	N/A
Glucose	0.02	50
Mannose	0.45	80
Gulose	1300	1460
Idose	1000	N/A
Galactose	0.12	500
Talose	340	1500

Table 1.3 *Prices and commercial availability of the aldohexoses* [13]

1.9 Importance of the Equilibrium of D-Glucaric Acid in the Formation of Polyhydroxypolyamides

As discussed above, the use D-glucaric acid for the production of polyhydroxypolyamides has significant advantages. However, unlike other aldaric acids such as galactaric and xylaric, aqueous D-glucaric acid exists in an equilibrium of four species and the common esterified forms of D-glucaric acid

have ester/lactone structures[126]. It is probable that these four different forms react at different rates when used to form poly(D-glucaramides).

Investigation into the conversion of the diastereoisomeric monolactones (D-glucaro-1,4- and D-glucaro-6,3-lactones) and *n*-propylamine into poly(D-glucaramides)[73], found the process to contain three distinct and consecutive steps, two which were affected by the stereochemistry of the lactone. The steps were the aminolysis of the lactone ester to the corresponding acyclic *N*-propyl-D-glucaramide monoester, followed by lactonisation to a five-membered lactone amide (this lactone amide has the reciprocals stereochemistry compared to the starting lactone ester), followed by aminolysis of this lactone amide to *N,N'*-dipropyl-D-glucaramide. It was found that the ring opening of the 1,4-lactone ester and 1,4-lactone amide is faster than the ring opening of the corresponding 6,3-lactone ester and 6,3-lactone amide.

A convenient method of producing poly(alkylene D-glucaramides) from D-glucose involves the direct use of the mixture of activated forms of aqueous D-glucaric acid (**Figure 1.14**). This method avoids the use of water and therefore subsequent removal. It also avoids the need to separate the activated forms of D-glucaric acid[115, 118]. Factors such as these are essential if production of poly(alkylene D-glucaramides) is going to be commercially viable.

Although qualitative work into the equilibrium products of esterified D-glucaric acid has been reported[126], the equilibrium of aqueous D-glucaric acid has not been investigated (**Figure 1.15**). As this equilibrium occurs before the equilibrium of esterified products, knowledge of the aqueous equilibrium may allow it to be controlled in such a way one has control of relative amounts of the species present in the esterified equilibrium.

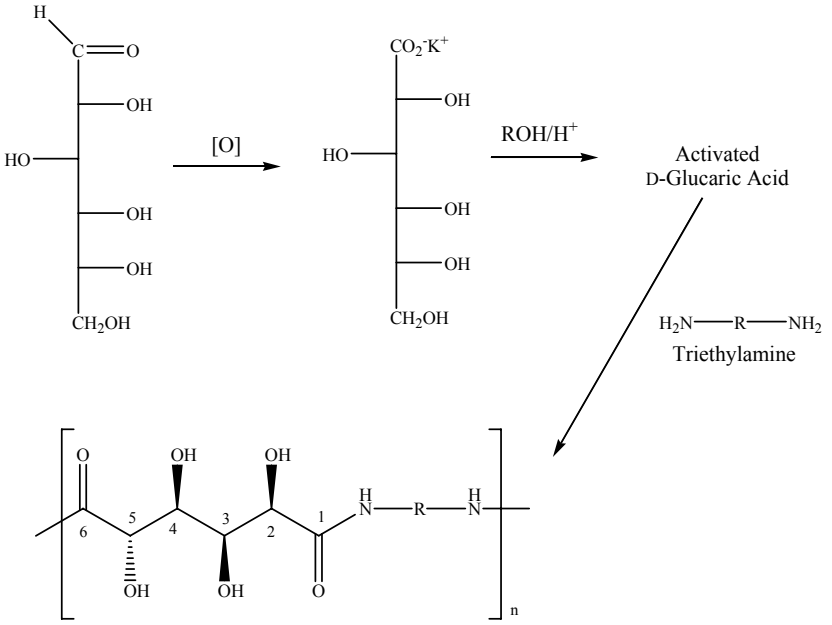


Figure 1.14 *General scheme for preparing poly(alkylene D-glucaramides)*[118]

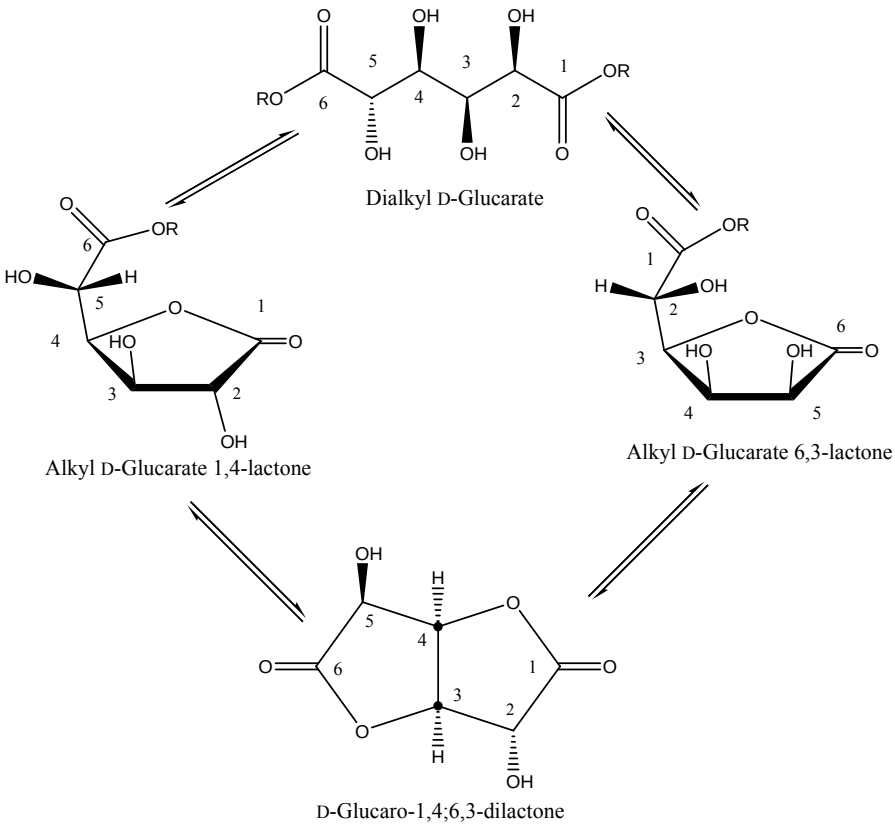


Figure 1.15 *Equilibrium of esterified aqueous D-glucaric acid*[118]

1.10 Aims of the Current Work

The purpose of this study was to investigate the equilibrium of aqueous D-glucaric acid. This began with a full NMR spectroscopy assignment of the four species involved.

A method of investigating the kinetics of equilibration *via* NMR spectroscopy was first developed, then used to investigate the equilibration of two starting species (D-glucaro-1,4-lactone and D-glucaro-1,4;6,3-dilactone) under neutral and acidic conditions.

A mechanism for the equilibration of aqueous D-glucaric acid was proposed. The experimental data was used to produce equilibrium constants, as well as estimates of rate constants involved in the mechanism proposed. The simulation software MATLAB was used to simulate the reactions involved in the mechanism. Simulations were used to adjust the estimates of rate constants and improve the fits between simulated and experiment data.

Chapter Two – Experimental

2.1 Materials

D-Glucaro-1,4-monolactone dihydrate was purchased from Sigma-Aldrich. D-glucaro-1,4;6,3-dilactone was produced at The University of Montana by Dr. Merilyn Manley-Harris. Monopotassium glucarate was prepared by a standard method[23] and supplied by The University of Montana. The purity of these chemicals was checked *via* NMR spectroscopy.

Dowex® 50WX8 Hydrogen Form resin (particle size 50-100 mesh) was purchased from Sigma-Aldrich. This was thoroughly washed with distilled water before use.

Solvents deuterium oxide (99.9 atom % deuterium, glass distilled) and dimethyl sulfoxide (99.9% purity, spectrophotometric grade) were purchased from Sigma-Aldrich

A 35% weight/weight solution of deuterium chloride (99% atom deuterium) in deuterium oxide was purchased from Sigma-Aldrich.

2.2 Sample Preparation

Starting material (either D-glucaro-1,4-lactone or D-glucaro-1,4;6,3-dilactone) (50 mg) was added to deuterium oxide (D_2O) (0.6 mL) in a vial. In the samples that had deuterium chloride (DCl) added this was added before a small drop of dimethyl sulfoxide (DMSO) was added, and the resulting solution briefly shaken to dissolve the starting material. The solution was transferred to a 5mm NMR tube.

2.3 Nuclear Magnetic Resonance Spectroscopy (NMR)

2.3.1 Spectrometer Details

NMR spectra were recorded using two spectrometers. A Bruker Avance AC-300 Fourier Transform NMR spectrometer with a 5mm inverse probe (300.1 3MHz for ^{1}H and 74.48MHz for ^{13}C) operating at 300K (27°C) was used for the ^{1}H NMR spectroscopy kinetic experiments.

A Bruker DRX400 400MHz spectrometer (^{1}H, ^{13}C, DEPT, DQFCOSY, COSY, HSQC, HMBC, SELTOCSY SELNOESY experiments) with a 5mm inverse probe (400.13MHz for ^{1}H and 100.62MHz ^{13}C) operating at 303K (30°C) was used for assigning spectra. See **appendix two** for NMR spectroscopy parameters (the parameters used for D-glucaro-1,4-lactone are given as a representative example).

2.3.2 Use of ^{1}H NMR Spectroscopy for Kinetic Information

Each sample was used in a set of ^{1}H NMR spectroscopy experiments. To produce each set of experiments, standard ^{1}H NMR spectroscopy parameters with 128 scans were used to create the first experiment in each set.

The spectrometer was shimmed and tuned on a 5mm NMR tube containing D_2O (0.6 mL), before this tube was exchanged for the tube containing the sample.

A Bruker Multi_zgvd program was used to create an experiment set containing 50-100 (depending on time available) experiments that were identical to the first one. The Multi_zgvd program was also used to build a fixed delay of five minutes between each of the experiments.

Each experiment was subsequently manually processed and integrated in the same way, with the same absolute intensity relative to the DMSO signal in the first experiment in each experiment set. Each spectrum was calibrated by means of setting the DMSO signal (singlet) to 2.71ppm[128].

Each set of experiments was performed in duplicate.

2. 4 Preparation of D-Glucaric Acid Lactone Mix

In an attempt to produce D-glucaro-6,3-monolactone, a lactone mix was made. Monopotassium D-glucarate (20 g, 0.086 mol) was added to 250 mL of distilled water in a 2.0L conical flask. Dowex 50WX8-100 ion exchange resin (70 mL, H^+), which had been pre-washed with distilled water until the wash water was colourless, was added to the flask. The mixture was shaken on a radial shaker (4 h) during which time the monopotassium glucarate gradually dissolved. Vacuum filtration was used to remove the resin and the filtrate was evaporated via vacuum filtration until an amber syrup was reached. This method is similar to that used by Bose *et. al.*[129], and it has been shown that this lactone mix contains a mixture of D-glucaric acid, D-glucaro-1,4-lactone, D-glucaro-6,3-lactone and D-glucaro-1,4;6,3-dilactone[130]. To prepare D-glucaro-6,3-lactone this lactone mix is usually seeded with a pure sample of D-glucaro-6,3-lactone [23, 129] or left to allow the D-glucaro-1,4-lactone to crystallise out before recrystallising the supernatant to form D-glucaro-6,3-lactone[7]. As there was no pure sample to seed the lactone mix with conventional crystallisation techniques were used in an attempt to produce crystals from the syrup. This included standing flask of syrup in a beaker of dicholoromethane.

Chapter Three – Assignment of Spectra

Before NMR spectroscopy could be used to investigate the equilibria of aqueous D-glucaric acid, it was essential that the spectra of the species were fully assigned and compared to literature results. This would allow confident signal selection for integration, and would give a better understanding of the species involved in the equilibrium.

3.1 Previous NMR Spectroscopy Results

The conformations of the D-glucarolactones and D-glucaric acid in solution were investigated with an aim of explaining the behaviour of these compounds in biological systems and extending the knowledge of the conformations of sugar lactones[12, 131]. Infrared and ultraviolet spectroscopy and optical rotary dispersion have been used in an attempt to assign the conformations of D-glucaric acid and its lactones. 60MHz ^{1}HNMR spectroscopy was also used[12, 132], however only D-glucaro-1,4;6,3-dilactone gave a first order spectrum. The use of a lanthanide shift reagent (praseodymium chloride) gave first order ^{1}H NMR spectra of all species at 100MHz. These conformations were confirmed with ^{13}C NMR spectroscopy results[12].

Assignments of spectra were made according to general rules that have been applied with 1,4-lactones together with others developed for furanoid sugars. As 2D experiments were not used to confirm these assignments, it is acknowledged that some of the assignments may be interchanged. The assignments made for the D-glucaric acid species were in general agreement with those made for the aldono-1,4-lactones[12, 131].

These assignments were used to assign the spectrum of D-glucaro-1,4;6,3-dilactone when it was used as an activated form of D-glucaric acid for PHPA production[115].

3.2 Current NMR Spectroscopy Results

2D correlation spectroscopy, as well as the usual 1D experiments, was used to assign the spectra of the four species of aqueous D-glucaric acid.

COSY (COrrelation SpectroscopY) experiments produce off-diagonal or cross peaks for all protons that have significant *J-J* coupling[133]. These cross peaks can then be used to determine which protons are coupled to each other. HSQC (Heteronuclear Single Quantum Correlation) (also called HMQC – Heteronuclear Multiple Quantum Coherence) is a proton-detected ^{1}H-^{13}C correlation experiment in which only directly attached proton-carbon coupling is observed[133]. The HMBC (Heteronuclear Multiple Bond Coherence) is another proton detected experiment, but instead of direct proton-carbon coupling, this experiment that capitalises on two and three bond proton-carbon couplings[133]. By utilising the above correlation experiments as well as the 1D ^{1}H and ^{13}C NMR spectroscopy experiments, it is possible to fully assign spectra.

Past literature results have assigned spectra by relying on consideration of the shielding effects felt by each proton or carbon as well as taking into account the rules developed for carbohydrate δ-lactones[12, 126]. These results and considerations make good starting points when trying to assign 2D spectra. The 2D spectra can also be used to prove or disprove these considerations.

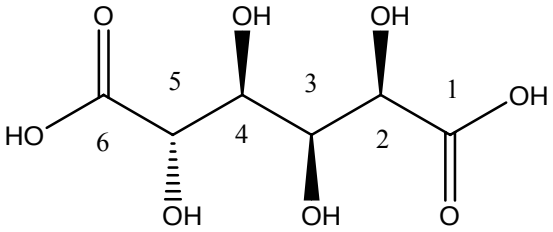
DEPT experiments were run, but because the species only had tertiary and quaternary carbons, the information provided by these spectra was not beneficial to the assignment process.

Note that in some cases the full spectrum has not been shown as because of equilibration, signals relating to the other species have started to appear. To minimise this appearance fresh samples of the species were used to run most of the NMR spectroscopy experiments instead of using the same sample for the suite of experiments.

3.2.1 D-Glucaric Acid

^{1}H NMR Spectroscopy:

It has been reported that H-2 is the most deshielded non-hydroxylic proton of D-glucaric acid and therefore resonates at the lowest field in ^{1}H NMR spectroscopy[12]. This was used to assign the doublet at 4.50 ppm as H-2 (**Figure 3.1**). As D-glucaric acid is an open chain, further NMR spectroscopy experiments such as the SELNOESY experiment that relies on interactions of the atoms through space could not be used to confirm this assignment.



D-Glucaric Acid

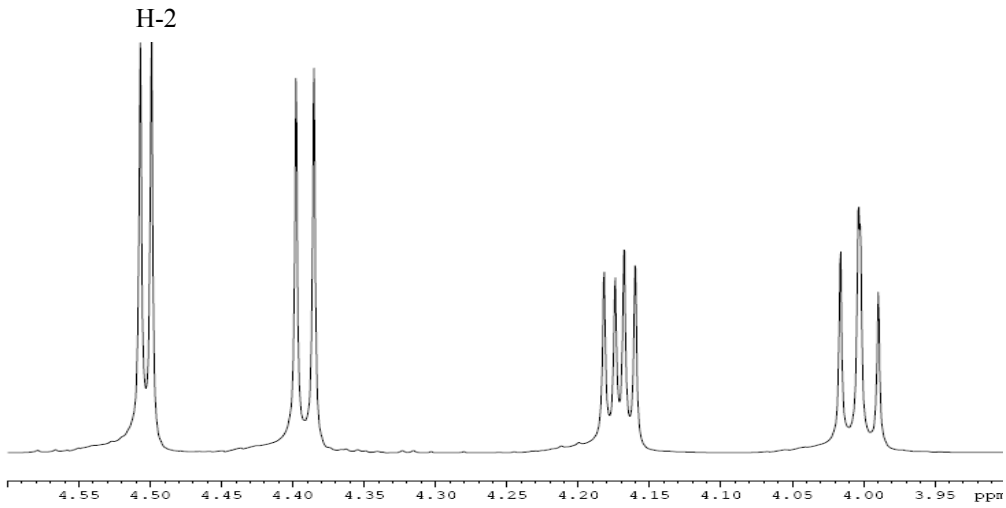


Figure 3.1 *^{1}HMR spectrum of D-glucaric acid with H-2 assigned and the structure of D-glucaric acid superimposed*

COSY:

The previous assignment of H-2 was used as a starting point. If the signal at 4.5 ppm was H-2 then this signal should correlate to only one other signal (H-3). This was the case, so the signal at 4.17 ppm was assigned H-3. If these assignments were correct, H-3 should correlate to another signal (H-4), which in

turn should correlate to another signal (H-5). This proved to be true and the signal at 4.00 ppm was assigned to be H-4 and the signal at 4.39 ppm H-5.

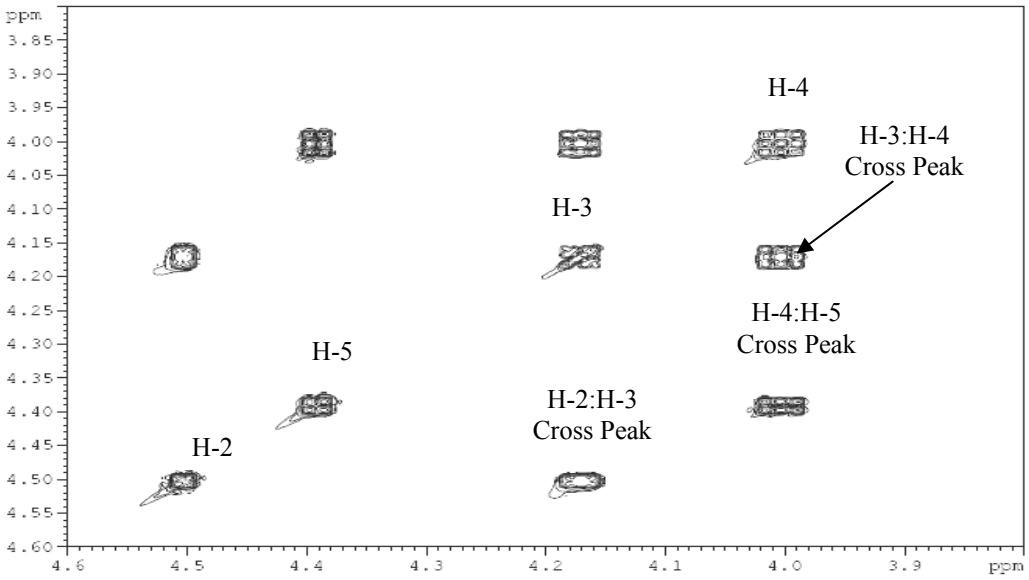


Figure 3.2 *Fully assigned COSY spectrum of D-glucaric acid*

HSQC:

The results from the COSY were used to assign the HSQC spectrum. As several of the carbon signals were very close to one another this required zooming in on the required section of the spectrum. This allowed the following assignments: 71.92ppm (C-2), 71.71 ppm (C-3), 73.43 ppm (C-4) and 71.75 ppm (C-5).

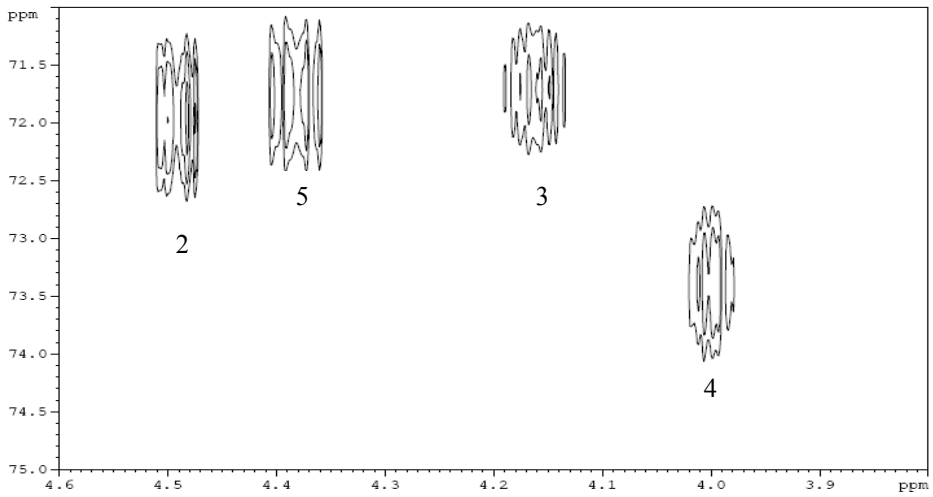


Figure 3.3 *Fully assigned HSQC spectrum of D-glucaric acid*

HMBC:

After the HSQC results were taken into account, only two signals will remain in the carbon spectrum, those resulting from C-1 and C-6. As quaternary carbons do not show in HSQC spectra, a HMBC experiment was run to discriminate between the two carbon signals at 176.13 ppm and 175.83 ppm. As the carbon resonances are very close, the required section of the HMBC was enlarged to allow accurate assignments. The possible correlations that may be seen in the HMBC spectrum of D-glucaric acid are shown (**Figure 3.4**). Careful inspection of the HMBC spectrum allowed the signals at 176.13 ppm (C-1) and 175.83 (C-6) to be assigned (**Figures 3.5 and 3.6**).

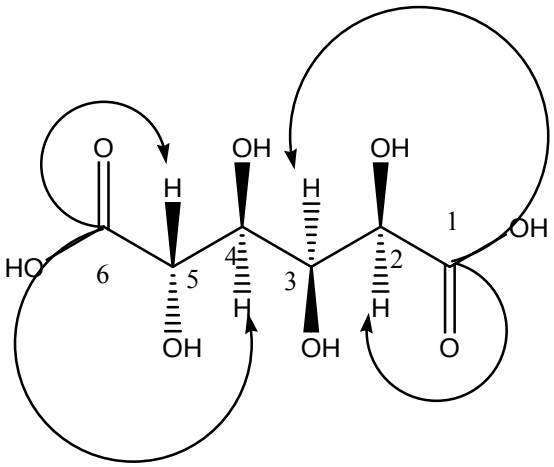


Figure 3.4 *Possible HMBC correlations of D-glucaric acid*

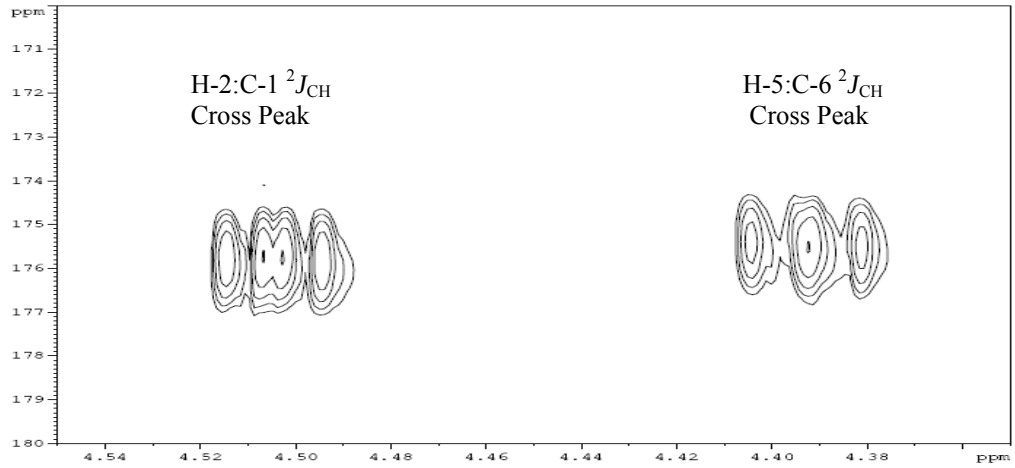


Figure 3.5 *Part of the fully assigned HMBC spectrum of D-glucaric acid*

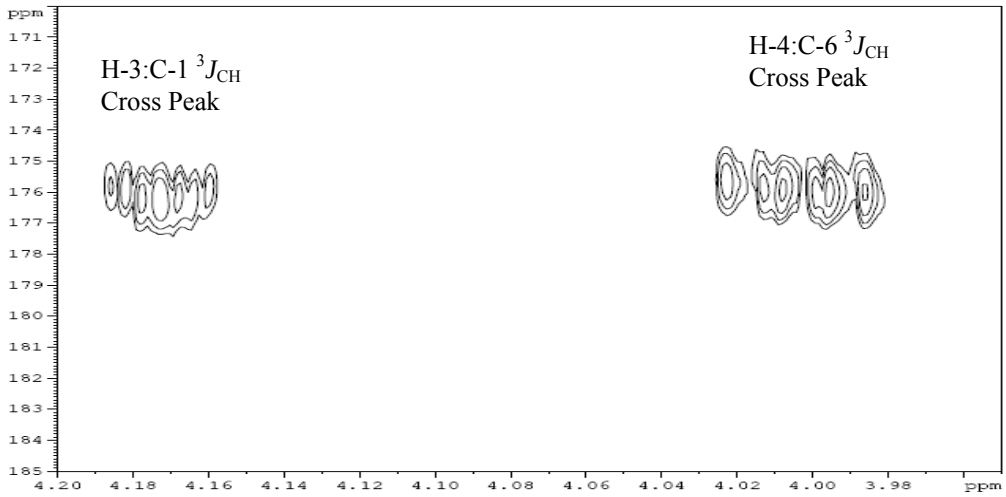


Figure 3.6 *Part of the fully assigned HMBC spectrum of D-glucaric acid*

^{13}C NMR Spectroscopy:

The results from the HSQC spectrum can be used to assign C-2, C-3, C-4 and C-5 signals on the ^{13}C spectrum, while the results from the HMBC spectrum can be used to assign C-1 and C-6. As the 13C spectrum has a large spectral window, it has been presented in two sections (**Figures 3.7 and 3.8**).

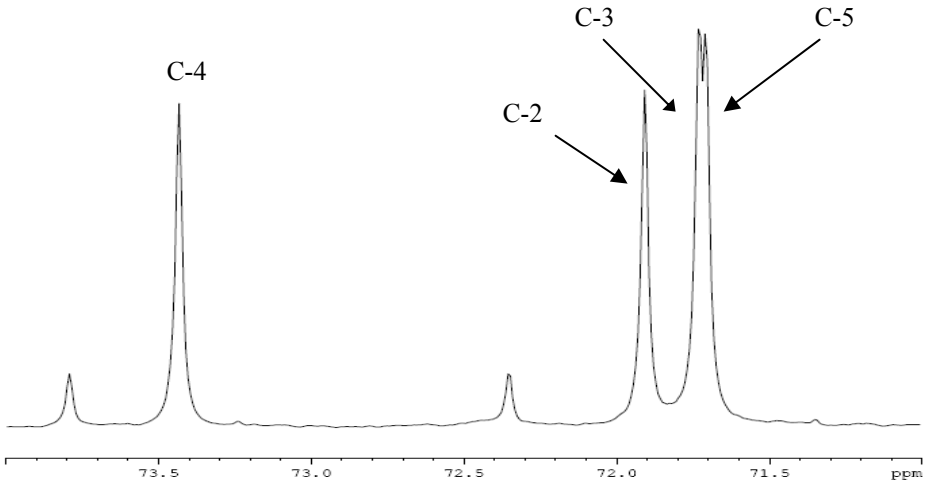


Figure 3.7 *Upfield section of the ^{13}C NMR spectrum of D-glucaric acid*

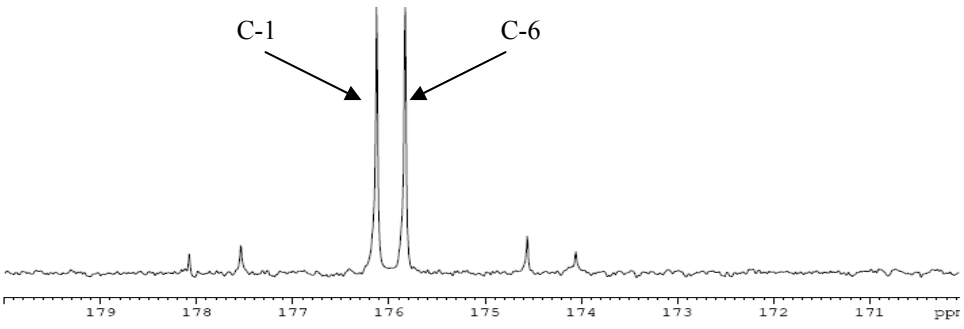
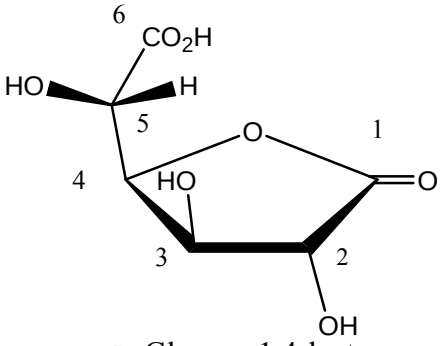


Figure 3.8 *Downfield section of the ^{13}C NMR spectrum of D-glucaric acid*

3.2.2 D-Glucaro-1,4-lactone

^{1}H NMR Spectroscopy:

The doublet of doublets at δ = 5.15 ppm was assigned to H-4 as per the rules developed for carbohydrate δ-lactones (**Figure 3.9**)[12, 126]. This proton is strongly deshielded as it is attached to the carbon adjacent to the partially positive oxygen atom of the lactone ring [12].



D-Glucaro-1,4-lactone

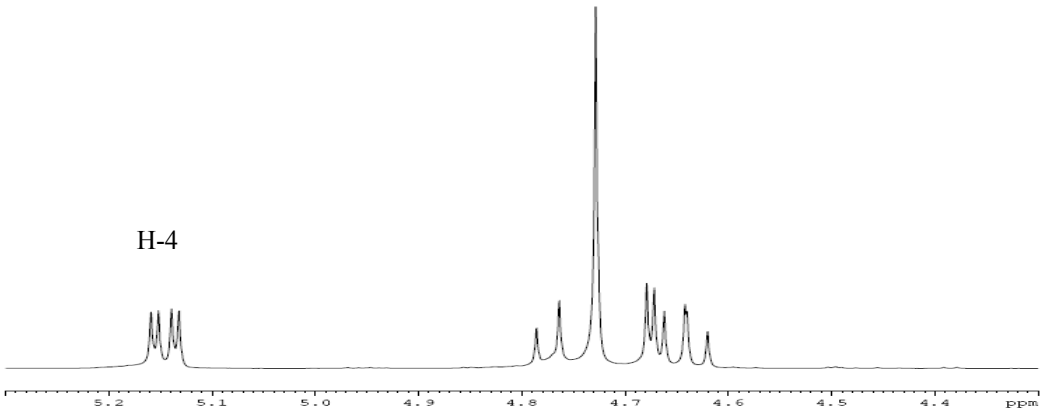


Figure 3.9 *^{1}HMR spectrum of D-glucaro-1,4-lacotne with H-4 assigned and the structure of D-glucaro-1,4-lactone superimposed*

COSY:

Using the previous assignment of H-4 as a starting point, ^{1}H:^{1}H correlations were used to assign the other three protons. H-4 correlated to two other protons. One of these had no further correlations and hence had to be H-5 (4.67 ppm) while the other proton was assigned to be H-3 (4.63 ppm). This proton (H-3) correlated to the remaining proton, which was assigned H-2 (4.77 ppm) (**Figure 3.10**).

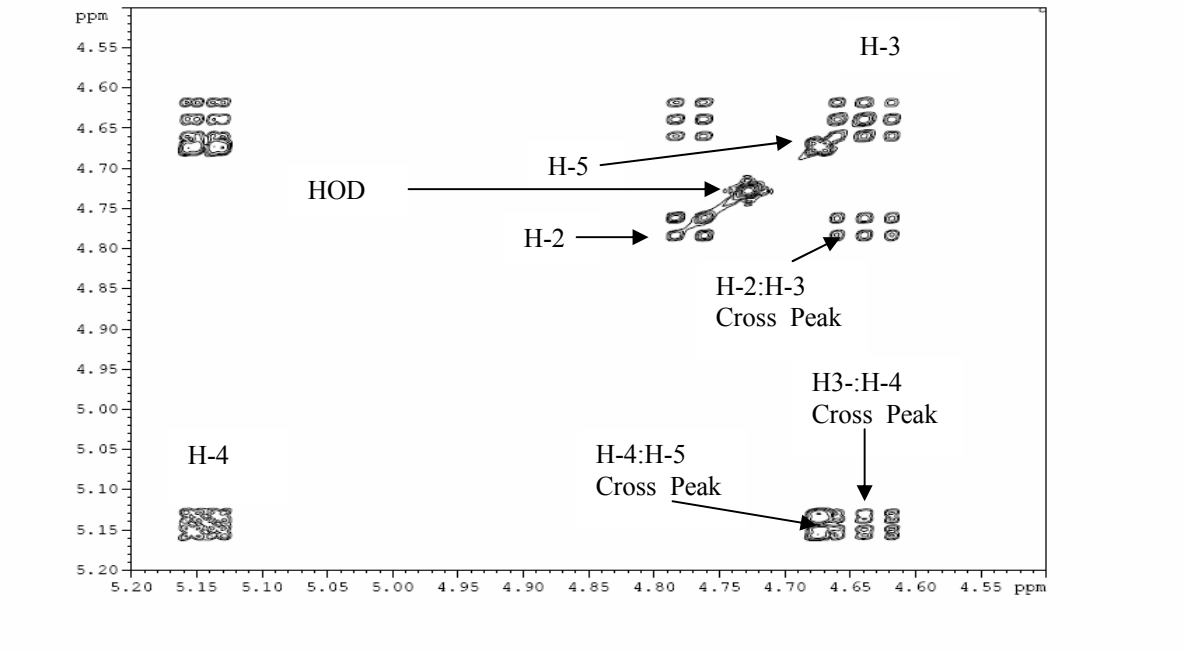


Figure 3.10 *Fully assigned COSY spectrum of D-glucaro-1,4-lactone*

HSQC:

The results from the COSY spectrum were used to assign the HSQC spectrum. This allowed the following assignments: 72.33 ppm (C-2), 73.80 ppm (C-3), 80.17 (C-4) and 69.42 ppm (C-5) (**Figure 3.11**).

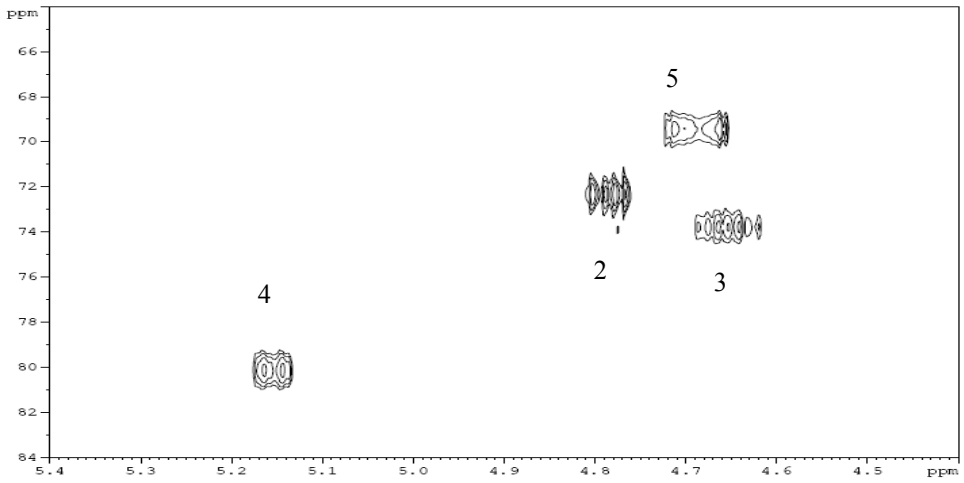


Figure 3.11 *Fully assigned HSQC spectrum of D-glucaro-1,4-lactone*

HMBC:

A HMBC experiment was run to determine which quaternary signal was C-1 and which was C-6. the possible correlations that may be seen in the HMBC spectrum of D-glucaro-1,4-lactone are shown (**Figure 3.12**). Again an enlarged spectrum was used to assign the signals: 177.52 ppm (C-1) and 174.48 ppm (C-6) (**Figure 1.13**).

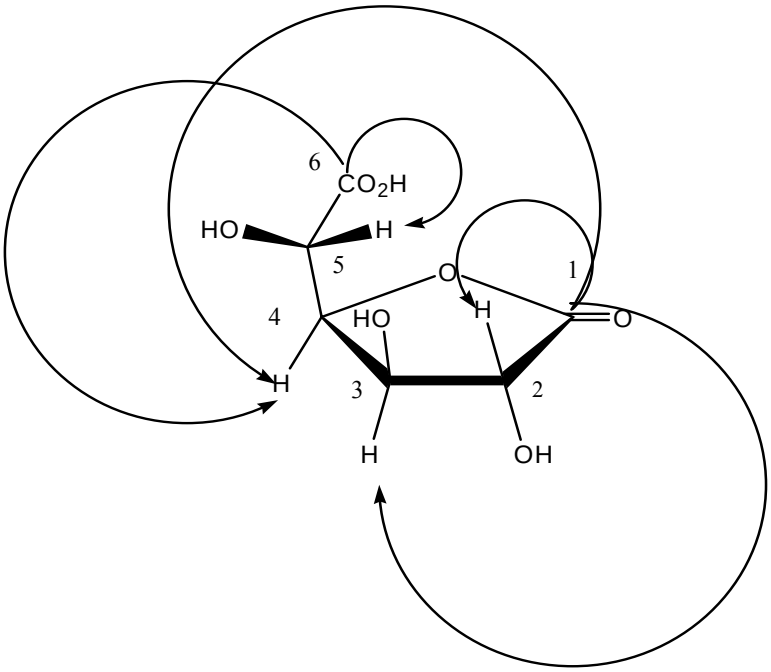


Figure 3.12 *Possible HMBC correlations of D-glucaro-1,4-lactone*

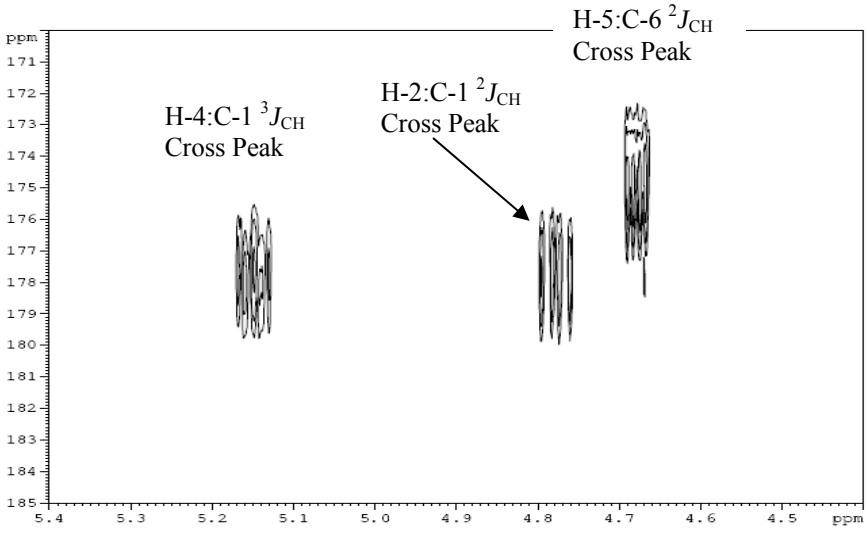


Figure 3.13 Fully assigned HMBC spectrum of D-glucaro-1,4-lactone

^{13}C NMR Spectroscopy:

The results from the HSQC spectrum can be used to assign C-2, C-3, C-4 and C-5 signals on the ^{13}C NMR spectrum, while the results from the HMBC spectrum can be used to assign C-1 and C-6. As the 13C spectrum has a large spectral window, it has been presented in two sections (**Figures 3.14 and 3.15**).

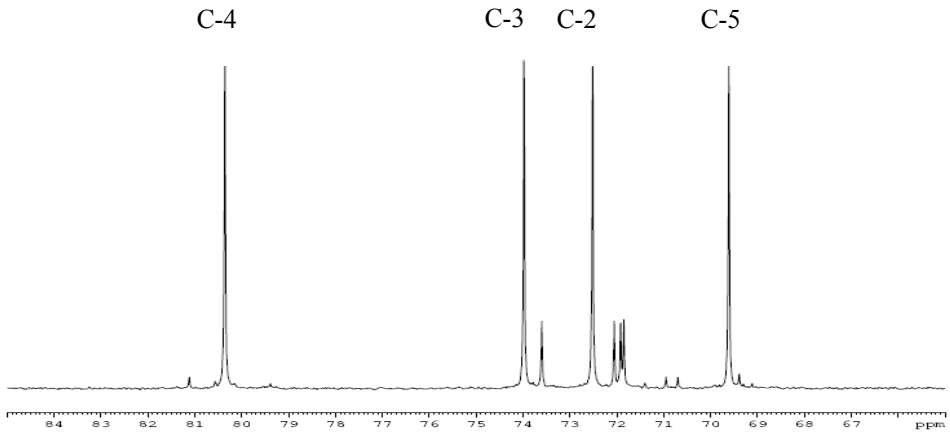


Figure 3.14 *Upfield section of the ^{13}C NMR spectrum of D-glucaro-1,4-lactone*

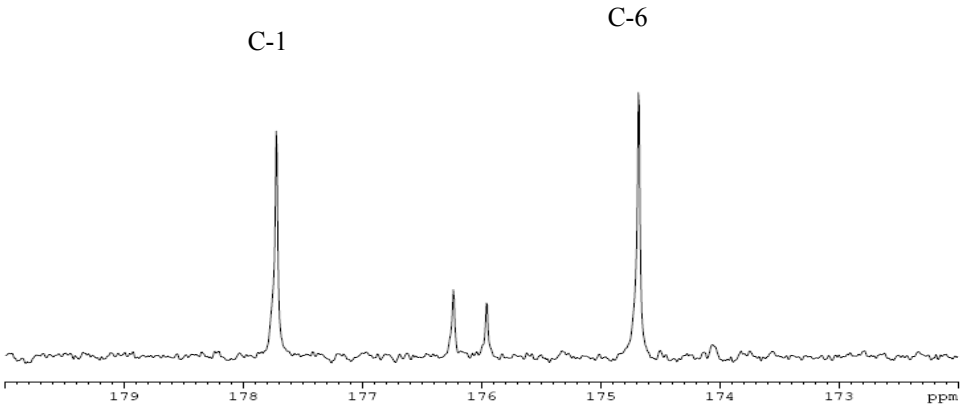


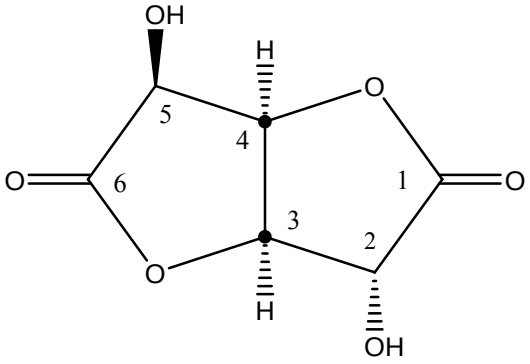
Figure 3.15 *Downfield section of the ^{13}C NMR spectrum of D-glucaro-1,4-lactone*

3.2.3 D-Glucaro-1,4;6,3-dilactone

As D-glucaro-1,4;6,3-dilactone equilbrates rapidly, in some cases the signals that relate to this species have been highlighted on the scanned spectra.

^{1}H NMR:

According to literature the quasiequatorial H-2 is the most shielded proton in D-glucaro-1,4;6,3-dilactone because of its orientation between O-1 and O-3[12], therefore the signal at 4.59 ppm was assigned to be H-2 (**Figure 3.16**).



D-Glucaro-1,4;6,3-dilactone

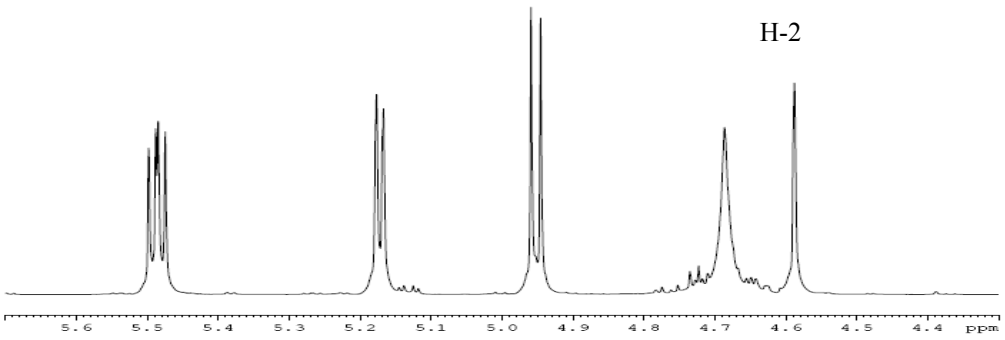


Figure 3.16 *^{1}HMR spectrum of D-glucaro-1,4;6,3-dilacotne with H-2 assigned and the structure of D-glucaro-1,4;6,3-dilactone superimposed*

COSY:

The assignment of H-2 (4.59 ppm) was used as a starting point. This signal correlated to one other signal that was assigned as H-3 (5.18 ppm). H-3 correlated to one other signal at 5.49 ppm (H-4), which in turn correlated to one other signal at 4.96 ppm (H-5) (**Figure 3.17**).

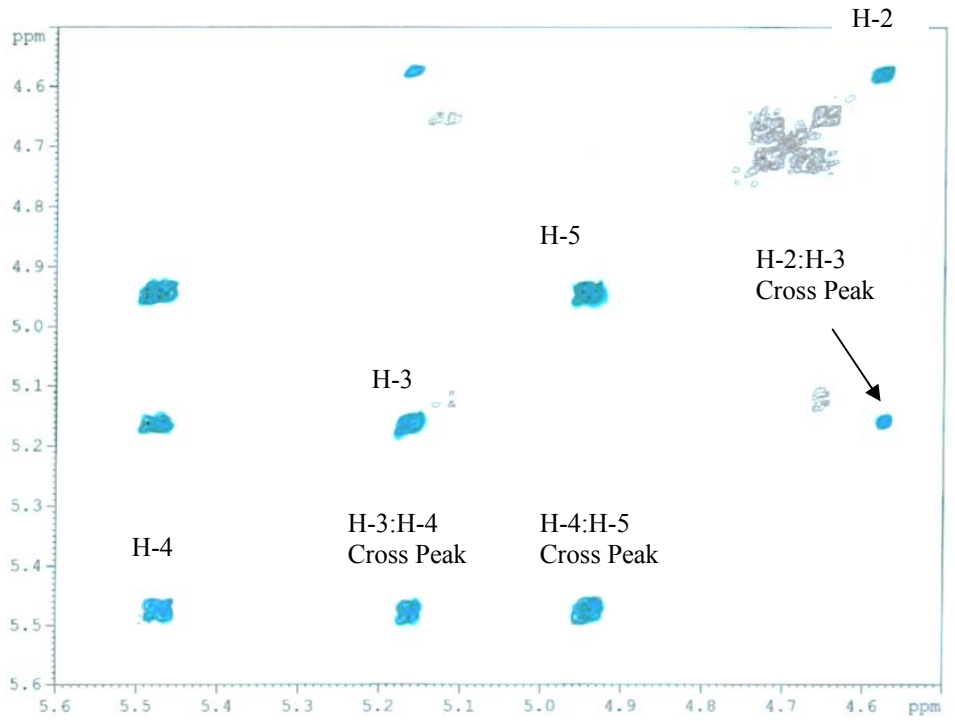


Figure 3.17 *Fully assigned COSY spectrum of* D*-glucaro-1,4;6,3-dilactone*

SELNOESY:

As D-glucaro-1,4;6,3-dilactone contains two fused rings, the interactions of the protons through space could be investigated to confirm the starting assignment of H-2. As this signal was a doublet (although it appears as a singlet) then inspection of D-glucaro-1,4;6,3-dilactone indicates that this signal could also be H-5 as this proton also only has one vicinal proton. Investigation of a model of D-glucaro-1,4;63-dilactone revealed that protons H-5, H-4 and H-3 were together on one side of the molecular place whereas H-2 was on the other side. Therefore H-5, H-4 and H-3 would have through space interactions with each other while there would be no such interactions between H-2 and the other

protons. A SELNOESY experiment irradiates a selected signal and uses the appearance of other signals to indicate through-space interactions. If the signal assigned to H-5 (4.96 ppm) is irradiated the signals relating to H-4 (5.49 ppm) and H-3 (5.18 ppm) should appear. The signal relating to H-4 should appear before the signal relating to H-3 as H-4 is closer to H-5 than H-3 is. A SELNOESY experiment was run with the signal at 4.96 ppm (H-5) irradiated. The signal at 5.49 ppm (H-4) appeared first, followed by the signal at 5.18 ppm (H-3). No other signals appeared. This result proved that the previous assignments were correct. In a second SELNOESY experiment the signal at 4.59 ppm (H-2) was irradiated. In this experiment no other signals appeared, confirming that H-2 was in a different plane with respect to the other protons. The p12 and sp2 parameters control the irradiation in a SELNOESY experiment. Both SELNOESY experiments were run using the settings for a normal multiplet (p12 = 160,000 μsec; sp2 = 74db).

HSQC:

The results from the COSY experiment were used to assign the HSQC spectrum. This allowed the following carbon signals to be assigned: 70.51 ppm (C-2), 80.88 ppm (C-3), 70.76 ppm (C-4) and 68.92 ppm (C-5).

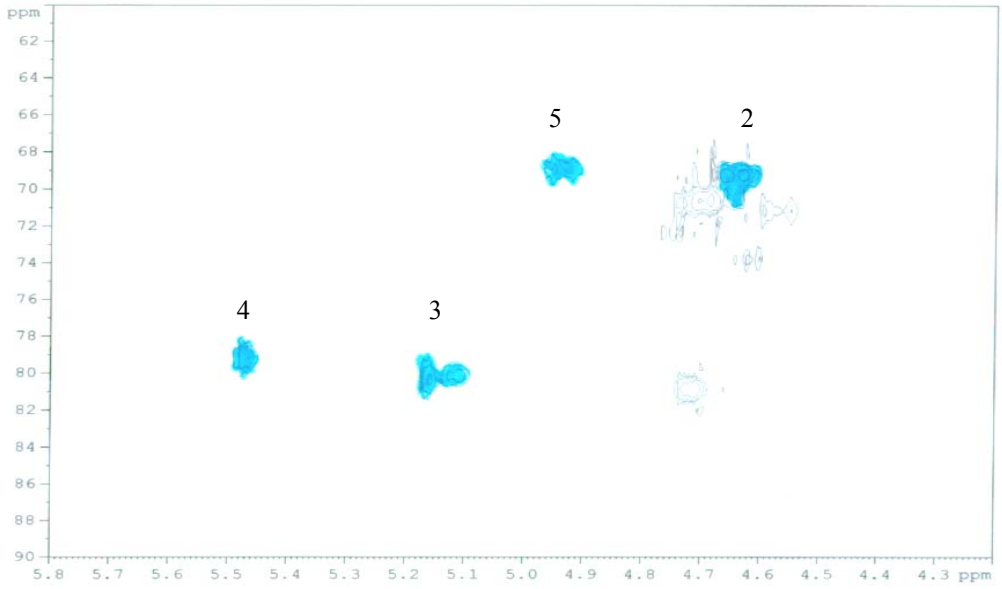


Figure 3.18 *Fully assigned HSQC spectrum of* D*-glucaro-1,4;6,3-dilactone*

HMBC:

The possible HMBC correlations for D-glucaro-1,4;6,3-dilactone are shown (Figure 3.19). However inspection of the ^{13}C NMR spectrum indicates that C-1 and C-6 resonate at the same value. Resolution enhancement (on the ^{13}C spectrum) was used to distinguish between the two signals. This was achieved by setting the line broadening to -2.00Hz and the Gaussian max position to 0.33. However these signals are indistinguishable on the HMBC spectrum.

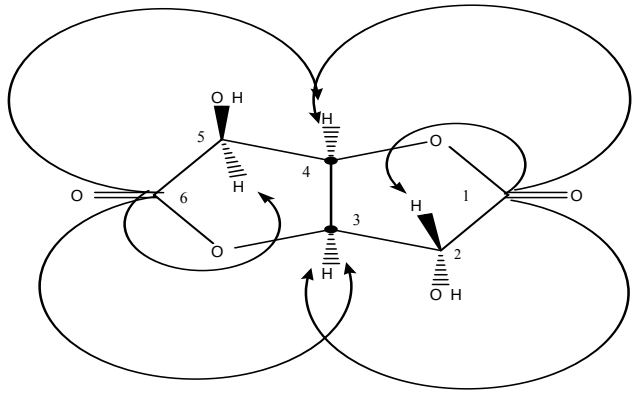


Figure 3.19 *Possible HMBC correlations of D-glucaro-1,4;6,3-dilactone*

^{13}C NMR Spectroscopy:

The results from the HSQC spectrum can be used to assign C-2, C-3, C-4 and C-5 signals on the ^{13}C NMR spectrum. As stated before C-1 and C-6 resonate at the same value (175.81 ppm), however as discussed above resolution enhancement split the signal into two (175.83 ppm and 175.81 ppm). As the ^{13}C spectrum has a large spectral window, it has been presented in three sections (**Figures 3.20 - 3.22**).

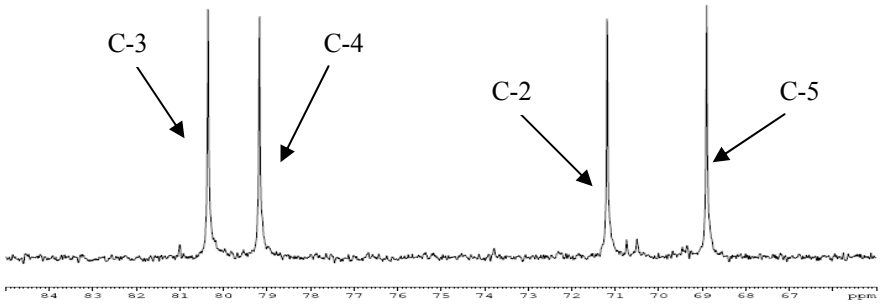


Figure 3.20 *Upfield section of the ^{13}C NMR spectrum of D-glucaro-1,4;6,3-dilactone*

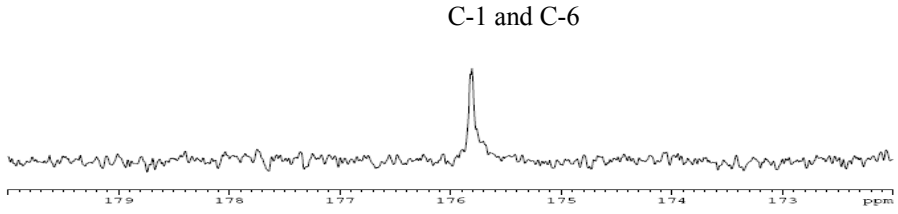


Figure 3.21 *Downfield section of the ^{13}C NMR spectrum of D-glucaro-1,4;6,3-dilactone*

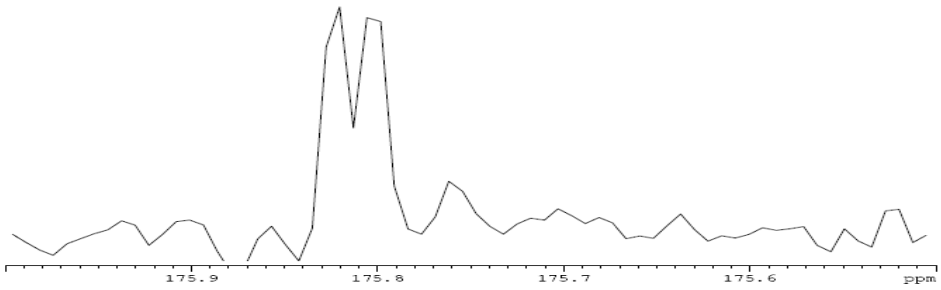


Figure 3.22 *Downfield section of the ^{13}C NMR spectrum of D-glucaro-1,4;6,3-dilactone after the described resolution enhancement*

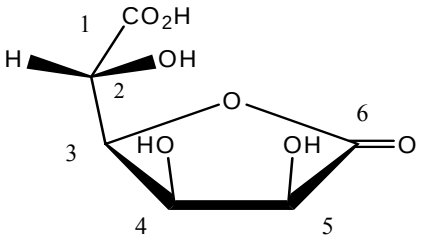
3.2.4 D-Glucaro-6,3-lactone

As no pure sample of D-glucaro-6,3-lactone was available, its NMR assignment is based on a sample of D-glucaro-1,4;6,3-dilactone plus equivalent DCl that has had time to equilibrate. Again some of the spectra have been scanned and highlighted for clarity.

^{1}HNMR Spectroscopy:

The region where the signals from D-glucaro-6,3-lactone appear on the ^{1}H NMR spectrum of the equilibrated sample can be assigned by assigning all of the

proton signals from the other three species and identifying any signals that are not assigned (see section 4.6.2).



D-Glucaro-6,3-lactone

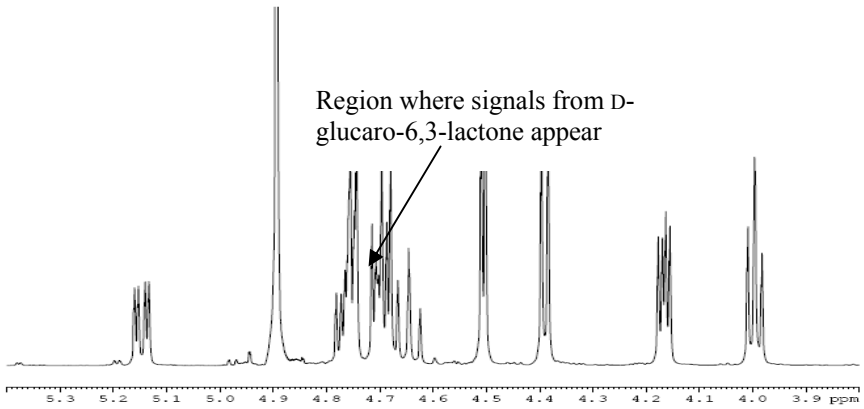


Figure 3.23 *[1]HMR spectrum of equilibrated sample with the region where the signals from D-glucaro-6,3-lactone appear labelled and the structure of D-glucaro-6,3-lactone superimposed.*

[13]C NMR Spectroscopy:

The [13]C signals of D-glucaro-6,3-lactone can be identified by assigning all of the other carbon signals in the [13]C NMR spectrum of the equilibrated mixture. Those signals remaining are those of D-glucaro-6,3-lactone. As the 13C spectrum has a large spectral window, it has been presented in two sections (**Figures 3.23 and 3.24**). As this experiment was run at the start of the equilibration process, the signals corresponding to D-glucaro-6,3-lactone are actually the largest signals in the upfield section.

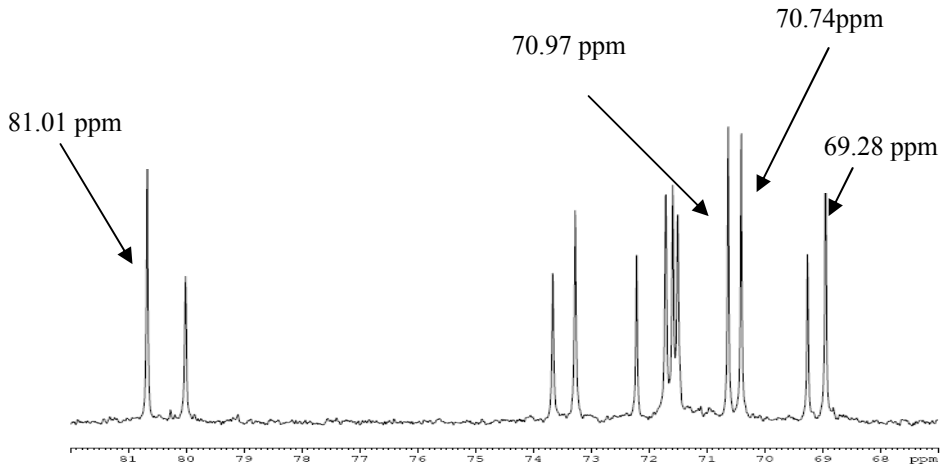


Figure 3.24 *Upfield section of the ^{13}C NMR spectrum of the equilibrated sample with the signals from D-glucaro-6,3-lactone labelled*

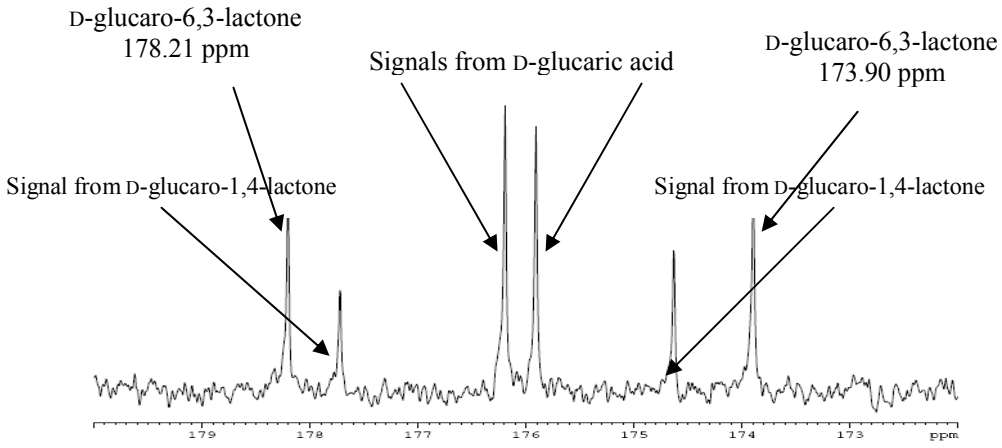


Figure 3.25 *Downfield section of the ^{13}C NMR spectrum equilibrated sample with the signals from D-glucaro-6,3-lactone labelled*

HSQC:

Assignments from the ^{13}C NMR spectrum were used in conjunction with the HSQC experiment (**Figure 3.25**) to identify the chemical shifts of the ^{1}H NMR signals of D-glucaro-6,3-lactone. The identified the following proton signals: 4.69 ppm , 4.71 ppm, 4.74 ppm and 4.76 ppm.

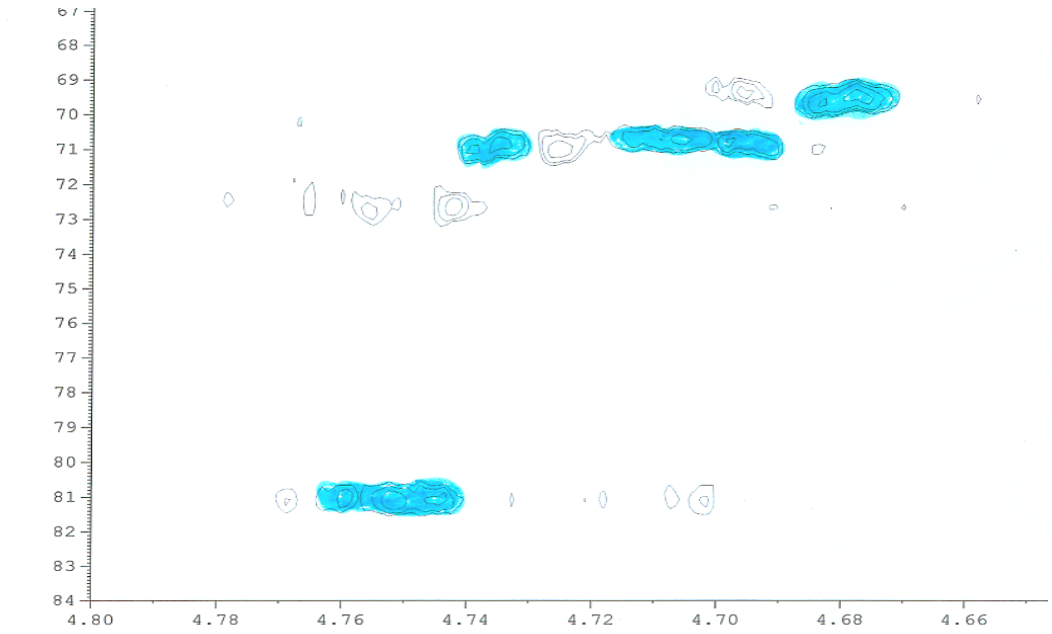


Figure 3.26 *HSQC spectrum of the equilibrated sample with the signals resulting from* D*-glucaro-6,3-lactone highlighted*

COSY:

The proton signals identified on the HSQC spectrum were highlighted on an enlarged section of the COSY of the equilibrated sample (**Figure 3.26**). It was noted that two of these signals correlated to just one other signal (therefore could be H-2 or H-5) while the other two signals correlated to two other signals (therefore could be H3 or H-4). Therefore H-2 and H-5 were either 4.69 ppm or 4.76 pp, while H-3 and H-4 were either 4.71 ppm or 4.74 ppm. The COSY spectrum was assigned after the HMBC results were interpreted.

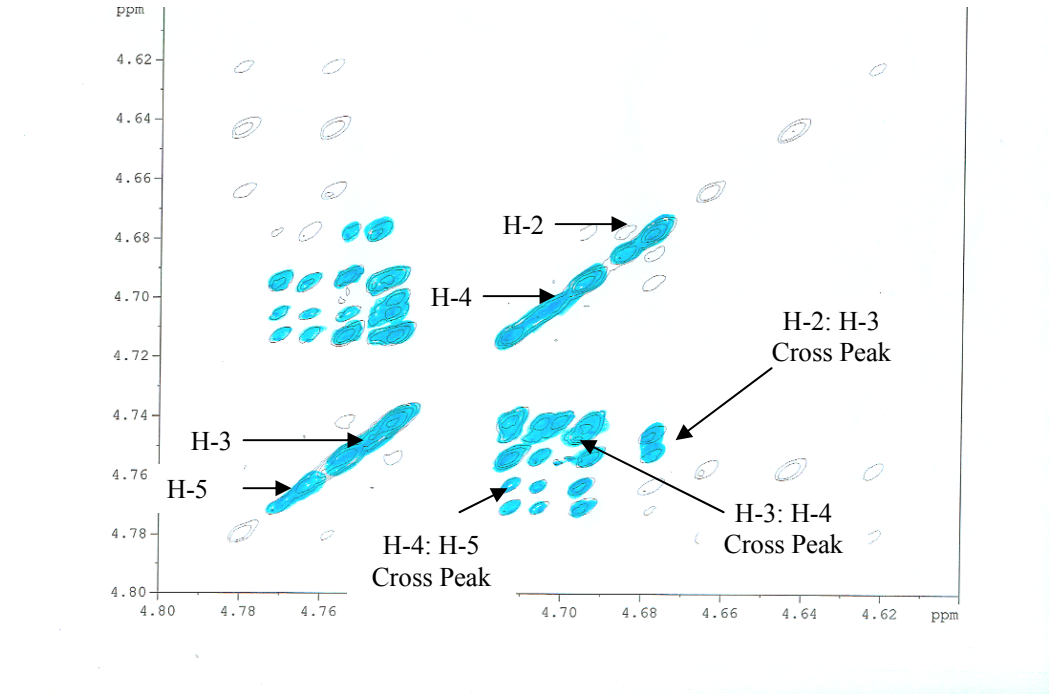


Figure 3.27 *COSY spectrum of the equilibrated sample with the signals resulting from* D*-glucaro-6,3-lactone highlighted. Note: this spectrum was assigned after the HMBC results were taken into account, however this assignment is shown here for clarity.*

HMBC:

The possible HMBC correlations for D-glucaro-6,3-lactone are shown (**Figure 3.27**). The HMBC spectrum showed the following correlations: 4.76 ppm (H) and 178.21 ppm (C); 4.74 ppm (H) and 178.21 ppm (C); 4.69 ppm (H) and 173.90 ppm (C).

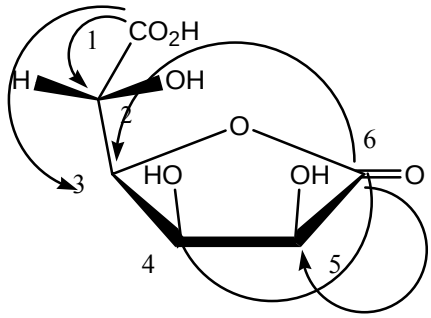


Figure 3.28 *Possible HMBC correlations of* D*-glucaro-6,3-lactone*

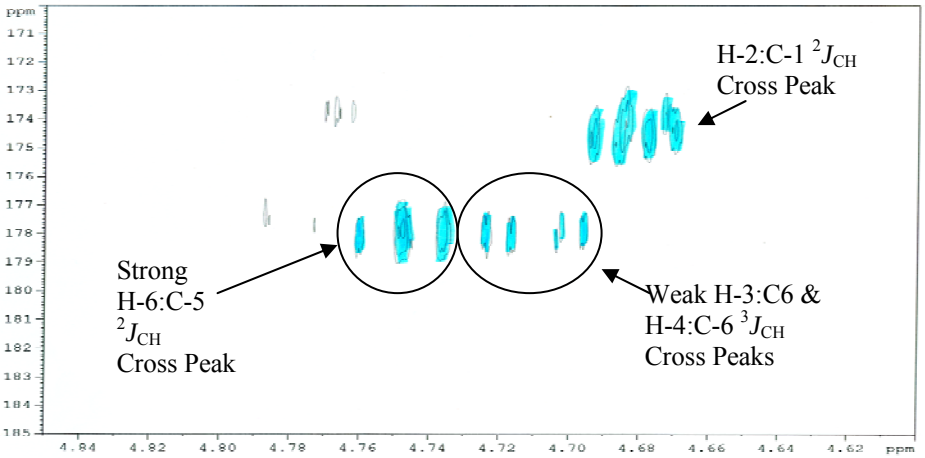


Figure 3.29 *HMBC spectrum of equilibrated sample with those signals resulting from D-glucaro-6,3-lactone highlighted. Note: the assignment of these signals was finalised after results from the HSQC and COSY spectra were considered. The assignments are included here for clarity.*

Assignment of D-glucaro-6,3-lactone spectrum:

The HMBC results indicate that the proton signal at 4.76 ppm must be H-5. If this signal was H-2, the signal at 4.74 ppm would be H-4 (from COSY). It is not possible (see **Figure 3.28**) for C-1 to be correlated to H-4 (from HMBC). Therefore the signal at 4.76 ppm must be H-5, which makes the signal at 4.74ppm H-3 (from COSY). C-6 can be correlated to H-3 and H-4 (^3J) and H-5 (^2J), therefore the signal at 178.21 ppm is C-6. The 4.69 ppm (H) and 173.90 ppm (C) correlation in the HMBC is the correlation between C-1 and H-2 (^2J).

Combining the results from the COSY, HSQC and HMBC spectra allowed the following assignments:

^{1}H Assignments:	**^{13}C Assignments:**
H-2: 4.69 ppm	C-1: 173.90 ppm
H-3: 4.74 ppm	C-2: 69.28 ppm
H-4: 4.71 ppm	C-3: 70.97 ppm
H-5: 4.76 ppm	C-4: 70.74 ppm
	C-5: 81.01 ppm
	C-6: 178.21 ppm

3.3 Comparison of Experimental Data with Literature

The experimental NMR spectroscopy assignment were compared to those reported[12]. As the reported results relied on shielding/deshielding considerations and the use of a lanthanide shift reagent to gain first order ^{1}H NMR spectra, there were a few differences between the reported assignments and the experimental results. Also the reported assignments were produced using a 100 MHz spectrometer while the experimental results were produced using a 400 MHz spectrometer, therefore this will change the absolute chemical shifts of the signals.

3.3.1. D-Glucaric Acid

The ^{1}H NMR signals from D-glucaric acid were assigned (**Table 3.1**) in the same order (i.e. same relative position) as those that were reported (**Table 3.2**), and the multiplicity of the signals matched. The absolute chemical shifts were different but this was expected, as discussed above. The ^{13}C NMR signals were assigned (**Table 3.3**). The reported results (**Table 3.4**) for the ^{13}C NMR assignments include many assignments that can be interchanged; therefore it is not worthwhile comparing these with the experimental results.

3.3.2 D-Glucaro-1,4-lactone

The relative positions of the ^{1}H assignments of D-glucaro-1,4-lactone are different between reported and experimental. H-4 is the furthest downfield signal in both sets of results but this is the only similarity. The multiplicity of the signals matched. Again the reported ^{13}C NMR assignments include many assignments that can be interchanged.

3.3.3 D-Glucaro-6,3-lactone

The relative positions for the signals of D-glucaro-6,3-lactone do not match between experimental and reported results. No multiplicities were recorded for the experimental results of D-glucaro-6,3-lactone due to the considerable overlap seen. On investigation of the structure of D-glucaro-6,3-lactone it is clear that H-

2 and H-5 should each be a doublet (they both have one vicinal proton) while H-3 and H-4 should each be a doublet of doublets(as they both have two non-equivalent vicinal protons). This is what is seen in the reported results. Again the reported ^{13}C NMR assignments include many assignments that can be interchanged.

3.3.4 D-Glucaro-1,4;6,3-dilactone

The ^{1}H signals from D-glucaro-1,4;6,3-dilactone were assigned in the same relative order as those reported. The reported results saw a octet signal for H-4 of D-glucaro-1,4;6,3-dilactone and did not include a multiplicity for the corresponding H-2 signal. The experimental results saw H-4 as a doublet of doublets, while H-2 was a singlet (probably a very fine doublet). The appearance of this singlet was also reported by Kiely *et. al*[115]. The relative position of the ^{13}C NMR signals is the same between reported results and experimental results. Resolution enhancement allowed the observation of a separate signal for C-1 and C-6 for D-glucaro-1,4;6,3-dilactone in the experimental results however lack of resolution between these signals in the HMBC spectrum prevented them being assigned to separate carbons.

Compound	H-2	H-3	H-4	H-5
D-Glucaric Acid	4.50d	4.17dd	4.00dd	4.39d
D-Glucaro-1,4-lactone	4.77d	4.63dd	5.15dd	4.67d
D-Glucaro-6,3-lactone	4.69	4.74	4.71	4.76
D-Glucaro-1,4;6,3-dilactone	4.59s	5.18dd	5.49dd	4.96d

Table 3.1 *400MHz experimental ^{1}H NMR spectroscopy data. Signal multiplicities: d = doublet; dd = doublet of doublets; s = singlet.*

Compound	H-2	H-3	H-4	H-5
D-Glucaric Acid	4.82d	4.49dd	4.32dd	4.71d
D-Glucaro-1,4-lactone	4.48d	4.87dd	5.63dd	5.42d
D-Glucaro-6,3-lactone	5.52d	5.32dd	5.17dd	5.10d
D-Glucaro-1,4;6,3-dilactone	4.98	5.57dd	5.90o	5.35d

Table 3.2 *100MHz reported ^{1}H NMR spectroscopy data[12]. Signal multiplicities: d = doublet; dd= doublet of doublets; o =octet.*

Compound	C-1	C-2	C-3	C-4	C-5	C-6
D-Glucaric Acid	176.13	71.92	71.75	73.43	71.72	175.83
D-Glucaro-1,4-lactone	177.52	72.33	73.80	80.17	69.42	174.48
D-Glucaro-6,3-lactone	173.90	69.28	70.97	70.74	81.01	178.21
D-Glucaro-1,4;6,3-dilactone	175.81[a]	71.19	80.36	79.18	68.90	175.83[a]

Table 3.3 *400MHz experimental ^{13}C NMR spectroscopy data. a = these assignments may be interchanged.*

Compound	C-1	C-2	C-3	C-4	C-5	C-6
D-Glucaric Acid	176.5	74.0[a]	72.2[a]	72.2	72.4[a]	176.25[b]
D-Glucaro-1,4-lactone	178.05	74.4[a]	72.9[a]	80.7	70.0[a]	178.6
D-Glucaro-6,3-lactone	174.3	69.7[a]	81.4	71.1[a]	71.3[a]	178.6
D-Glucaro-1,4;6,3-dilactone	176.45	71.8	80.95	79.8	69.9	176.45

Table 3.4 *100MHz reported ^{13}C NMR spectroscopy data [12]. a = these assignments may have to be reversed; b = may be interchanged with the C-1 resonance*

Chapter Four – Method Development

4.1 Introduction

For NMR spectroscopy data to be utilised in accurate kinetic analysis there are several conditions that have to be optimised. A considerable amount of time went into planning and developing a method that would not only be accurate but would make efficient use of the NMR spectroscopy resources available, keeping in mind that other users needed access to these facilities as well. When developing the method for analysing the equilibrium of aqueous D-glucaric acid both ^{1}H and ^{13}C spectroscopy were investigated, and their respective benefits evaluated.

A lot of the terminology used in this section relates to the Bruker suite of NMR spectrometers, however when such a term appears it will be described fully.

4.2 Overlap of Signals

Accurate integration is essential for kinetic data to be meaningful. If two signals overlap each other then neither can be integrated accurately. The ^{1}H NMR spectrum (**Figure 4.1**) of aqueous D-glucaric acid involves significant overlapping especially in the region between 4 ppm and 5 ppm where no individual signals can be resolved. The corresponding ^{13}C NMR spectrum involves less overlapping (**Figure 4.2**).

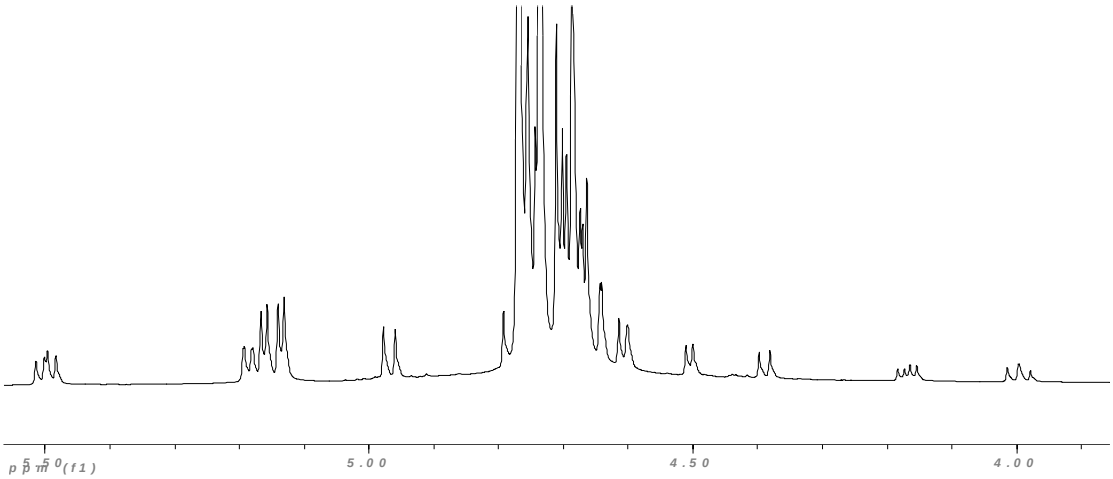


Figure 4.1 *Section of the ^{1}H NMR spectrum of equilibrated aqueous D-glucaric acid*

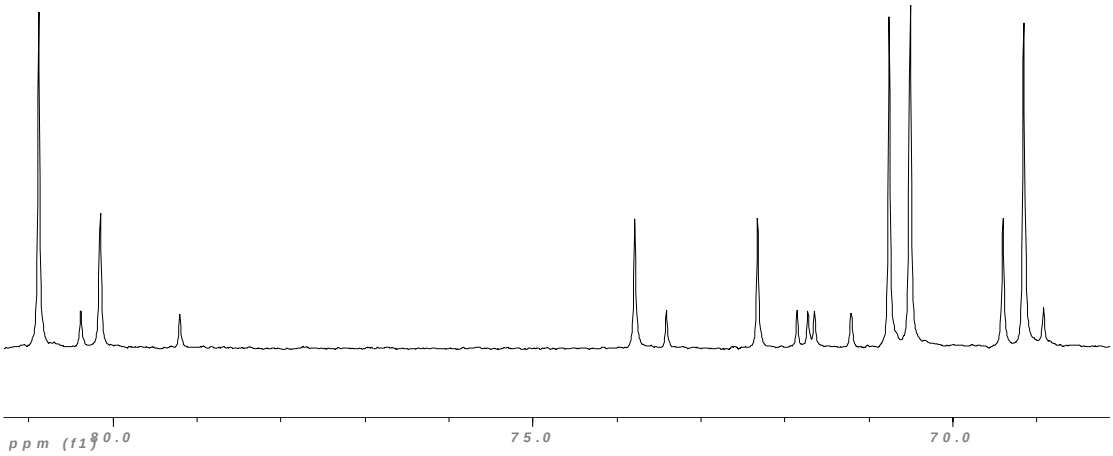


Figure 4.2 *Section of the ^{13}C NMR spectrum of equilibrated aqueous D-glucaric acid*

4.3 FID Accumulation Time

A major difference between ^{13}C and ^{1}H spectroscopy is the FID accumulation time. ^{1}H FID accumulation time is relatively quick and respectable signal to noise can be obtained in a matter of minutes; ^{13}C FID collection is much slower and experiments can take in the range of hours, even with strong samples. It was important that an inverse-gated parameter set was used for the ^{13}C experiments. This eliminates NOE variations between carbon signals and therefore allows for accurate integration. However loss of the NOE means that signal intensity decreases and more scans may be needed[133]. If FID collection takes for example one hour, then the resulting spectrum is an average of that hour. Therefore any changes in the species present are averaged for that hour. In contrast, the much

shorter FID accumulation of the ^{1}H experiment allows an experiment to be run at discrete times throughout that hour, thereby producing an accurate depiction of the changes in the species present. In preliminary ^{13}C experiments it was obvious that the sample began to equilibrate before sufficient scans had been run to gain satisfactory signal to noise as signals corresponding to the other species began to appear in the spectrum.

4.3.1 Optimisation of Delay Time

In an attempt to reduce the number of scans needed to gain good signal to noise and hopefully decrease the time needed for FID accumulation in the ^{13}C experiment, the parameters used in the ^{13}C experiment were optimised. An important parameter in ^{13}C NMR spectroscopy is the delay between acquisitions (d1). If d1 is too short then not all of the carbons will be equally relaxed. Therefore some signals will be weak and more scans would be needed. By optimising d1 all carbon atoms would be fully relaxed between scans and consequently the resulting signals would be more intense.

The results from the optimisation demonstrated that the optimum d1 for the ^{13}C inverse-gated NMR experiments on D-glucaric acid and its lactones was approximately sixty seconds. Clearly a d1 of sixty seconds is not practical due to the large amount of time needed per scan.

4.3.2 A Dynamic (Rapid) Method for ^{13}C NMR Spectroscopy

As part of a Doctoral thesis on Urea-Formaldehyde a dynamic or rapid method was developed to study the "real time" polymerisation of these resins via ^{13}C NMR spectroscopy[134]. This method involved performing NMR spectroscopy experiments using the dynamic method (^{13}C scan repetition time of 1.4 seconds) and using mathematics to calculate the expected signal integrals if a standard ^{13}C experiment (^{13}C scan repetition time of 4.9 seconds) had been performed. Although this procedure had potential, it was obvious that it had taken considerable time to optimise it for the reactions being studied. Therefore it was

decided that it would not be practical in the timeframe of a MSc to develop a corresponding procedure for the aqueous D-glucaric acid species.

4.4 HOD Signal Interference

Carbohydrate ^{1}H NMR spectroscopy routinely uses deuterium oxide as a solvent. This practise greatly simplifies what would otherwise be very complex spectra. The hydroxyl hydrogens are rapidly exchanged with deuterium so that they become "invisible" in ^{1}H NMR spectroscopy experiments. This not only reduces the number of signals that are seen, but it also greatly reduces the amount of overlap between signals that would have had very similar chemical shifts. However one of the consequences of using this solvent is the appearance of the HOD signal at 4-5ppm. This is often a broad signal and can be troublesome if sample signals occur in this region.

The ^{1}HNMR spectra of D-glucaric acid and its lactones include many signals in the region between 4-5ppm (**Figure 4.1**). Therefore the occurrence of the HOD signal affects the ability to interpret, and accurately integrate the resulting spectra.

There are means to either reduce the size of the HOD signal or to eliminate it completely. The presaturation experiment is a variation of the ^{1}H NMR spectroscopy experiment that sees the "unwanted" signal irradiated so that it collapses and becomes smaller, or disappears from the spectrum. However, when one signal is irradiated, signals close to it are also affected. This means that it would no longer be appropriate to integrate the surrounding signals as it would be impossible to tell if they were affected by the irradiation, or to what extent that they were affected. A double quantum filtered COSY experiment is a 2D experiment that removes the HOD signal. However, 2D experiments cannot be used for accurate quantitative work.

Another possible way of decreasing the effect of the HOD signal is to increase the temperature of the experiments. As the temperature increases the HOD signal moves downfield, reducing interference with the sample signals. A series of ^{1}H

NMR spectroscopy experiments were run at increased temperatures to determine the affects on the HOD signal. Each increase in temperature shifted the HOD signal slightly more downfield. However even in the experiment at 70°C the HOD signal was still not "clear" from the sample signals. Increased temperature also, of course, affects kinetics and equilibria. It was especially probable that increased temperature would cause the relative amount of D-glucaro-1,4;6,3-dilactone present to increase[11].

4.5 Choice of ^{1}HNMR Spectroscopy

When investigating the kinetics of a reaction it is vital that the data obtained provides an accurate description of the experimental situation. If the situation is changing at a considerable speed it is important that the method used to investigate is able to keep up with these changes. Although ^{1}H NMR spectroscopy is not without its problems, as discussed above, its speed is such a major advantage over ^{13}C NMR spectroscopy that it was chosen as the preferred method for this work. A similar decision was made in an investigation of the formation of D-glucaric based polyhydroxypolyamides[73].

4.6 Experimental Factors

After the decision to use ^{1}H NMR spectroscopy rather than ^{13}C had been made there were several experimental factors to be investigated and optimised to ensure the best possible results from the experiments.

4.6.1 Choice of Signals to Integrate

It was essential that appropriate signals were chosen for integration. A signal was needed for each species that did not overlap with any other species' signal or the HOD signal. Careful investigation of the spectra allowed the following signals (**Figure 4.3**) to be selected to represent each species: 4.00 ppm (H-4 D-glucaric acid), 5.15 ppm (H-4 D-glucaro-1,4-lactone) and 5.49 ppm (H-4 D-glucaro-1,4;6,3-dilactone). These signals were well resolved from other species' signals as well as the HOD signal. The signal selected for D-glucaro-1,4-lactone is

relatively close to the H-3 signal of D-glucaro-1,4;6,3-dilactone. However if this area is enlarged accurate integration is possible and there are no other signals for D-glucaro-1,4-lactone that are suitable.

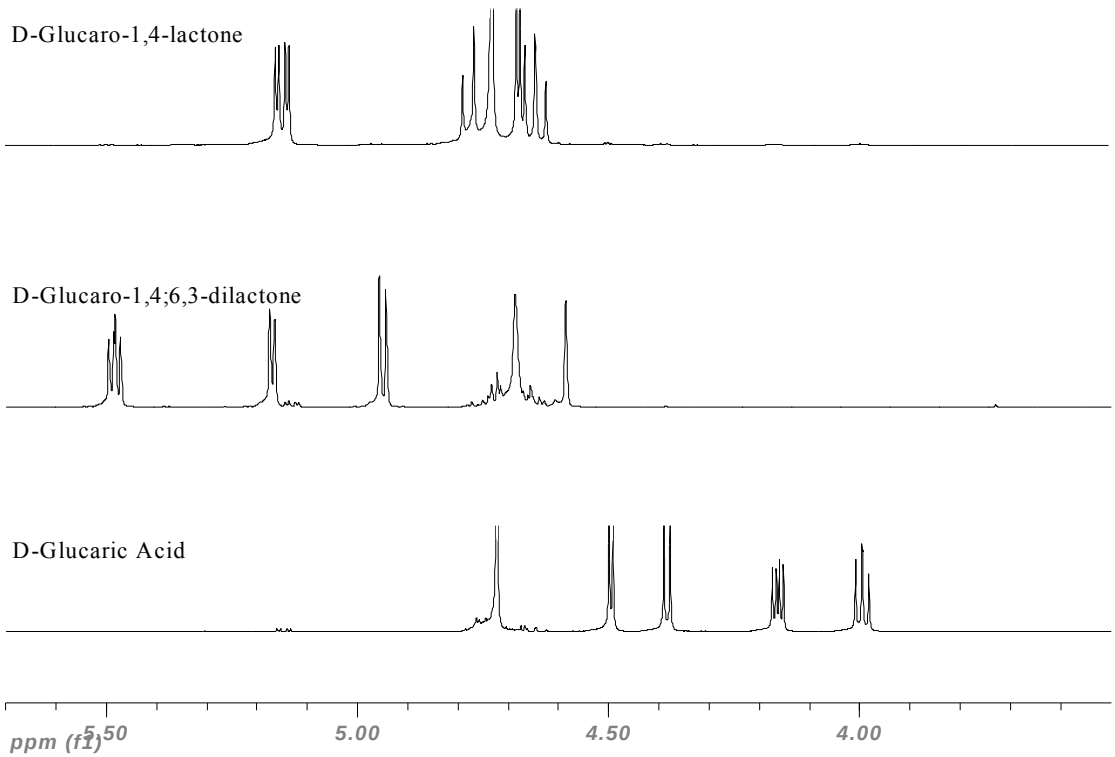


Figure 4.3 *Overlay of ^{1}HNMR spectra for selection of signals to integrate*

4.6.2 Determining the Relative Concentration of D-Glucaro-6,3-lactone

As no resolved signal could be identified for the D-glucaro-6,3-monolactone an alternative method of obtaining data for this compound had to be developed. A SELTOCSY experiment was used in an attempt to find signals that resulted from the D-glucaro-6,3-monolactone. A SELTOCSY experiment irradiates a selected signal. The appearance of other signals is used to indicate vicinal protons. However no resolved signals could be identified for D-glucaro-6,3-lactone, therefore no signal could be selected for irradiation.

After assigning the spectra of D-glucaric acid, D-glucaro-1,4-monolactone and D-glucaro-1,4;6,3-dialactone, the spectra of samples that had equilibrated was investigated and the known signals labelled. This allowed identification of the

region where the signals resulting from the D-glucaro-6,3-monolactone occurred (**Figure 4.4**).

These signals appeared between 4.6 ppm and 4.8 ppm and overlapped signals from D-glucaro-1,4-monolactone (three signals) and D-glucaro-1,4;6,3-dialactone (one signal). In experiments where one or more equivalents of DCl were added the HOD signal was shifted slightly downfield so that it was clear from this region. In experiments with less than one molar equivalent of DCl added the HOD signal occurred in this overlapping region. Therefore it was decided that in experiments where the HOD signal did not overlap it would be accurate to integrate the entire region of overlapping signals and subtract the theoretical integral value from D-glucaro-1,4-monolactone (integral for three protons) and D-glucaro-1,4;6,3-dialactone (one integral). This method proved to be reasonably precise as the graphs produced between duplicates were similar.

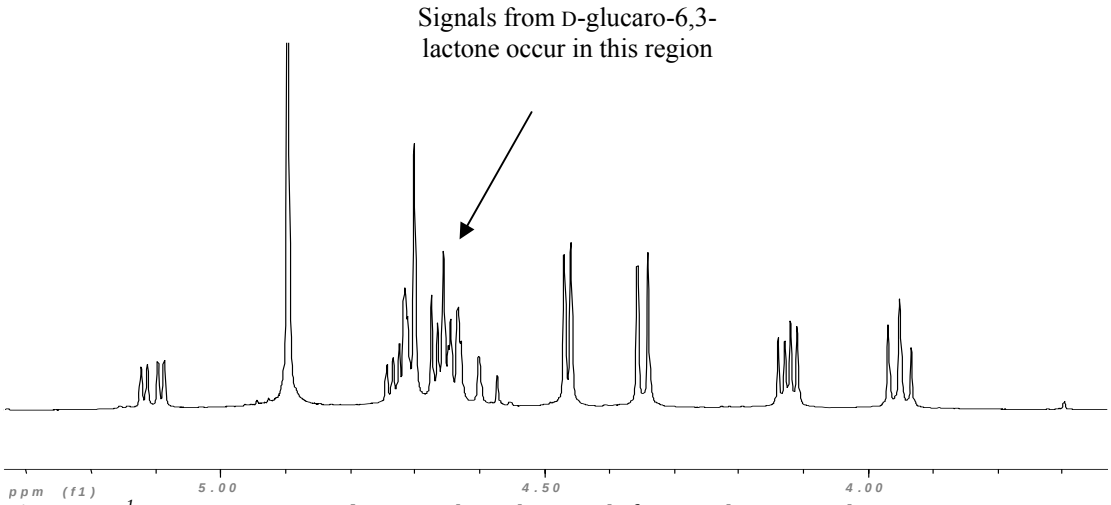


Figure 4.4 *^{1}HNMR spectrum indicating where the signals from D-glucaro-6,3-lactone occur*

When starting with D-glucaro-1,4-monolactone, D-glucaro-6,3-monolactone forms rather slowly (this is expected as the two monolactones can not directly interchange). In the early stages of these experiment sets the concentration of the D-glucaro-6,3-monolactone is so minimal that it was actually less than the errors involved with its calculation. As an area of the spectrum was integrated and then the theoretical integrals of four other signals subtracted (one from D-glucaro-1,4;6,3-dialactone and three from D-glucaro-1,4-monolactone), the integral errors

associated with five signals affect the value for the relative integral size of D-glucaro-6,3-monolactone. This is why at the start of the D-glucaro-1,4-monolactone experiment sets the integral size of the D-glucaro-6,3-monolactone appears as a negative value. However once the relative concentration of D-glucaro-6,3-monolactone increased the relative integral size became larger than the errors involved and subsequently was positive.

4.6.3 Determination of a Scale

For the investigation of all the species present at equilibrium, it was important that a suitable method was developed to allow the relative intensities to be conveyed concisely. It was more significant to be able to relate the relative concentrations of the species to each other, rather than knowing their absolute values. An appropriate way of achieving this is to "spike" each sample with a substance that will produce a signal that occurs in a region of the spectrum where no sample signals appear. A suitable additive for the current samples was dimethyl sulfoxide (DMSO). Dimethyl sulfoxide in deuterium oxide produces a singlet in ^{1}H NMR spectra at 2.71ppm[128], which is well clear from the signals of aqueous D-glucaric acid and its lactones. A small drop of DMSO was therefore added to each sample as an internal standard.

After some examination it was decided that it would be most accurate to integrate the first spectrum in each set and set the DMSO signal intensity to 1.0000. From here each subsequent spectrum in the experiment set was integrated to the scale of the first experiment. This meant that every signal was integrated to the same relative scale.

Each experiment was carried out in duplicate. As the data from each experiment was relative rather than absolute, direct comparisons between integral sizes were not possible. However comparing the graphs produced between duplicates gave an indication of how similar the duplicated results were. If the line on the graph for a particular species had a similar shape (similar slope, timescale etc) between the original experiment set and the duplicate it was concluded that the results for that species were consistent.

4.6.4 Length of Each Experiment

To ensure sufficient signal to noise it was decided that each experiment would have 128 scans. This number produced spectra that had signals that were very well resolved to the baseline and hence were able to be integrated accurately. The experimental time of approximately eight minutes (seven minutes, fifty seven seconds) was still short enough to optimise the time benefits achieved *via* ^{1}H NMR spectroscopy.

4.6.5 Multi_zgvd Development

To investigate the change in the species relative concentrations with time it was necessary to develop a system where it was possible to build in a delay between experiments. The Bruker Multi_zgvd program allowed this. This program allows a set of NMR spectroscopy experiments to be created with a delay between each subsequent experiment. Although the 'vd' in the name stands for variable delay, the user can select either variable delays (which are created *via* a desired list of delays) or a fixed delay (which is entered when the Multi_zgvd program is started).

It was decided that a fixed delay of five minutes would produce an experiment set that contained ^{1}H NMR spectroscopy experiments that would provide accurate kinetic information. As five minutes is a relatively short amount of time, the experiments in the set would be close enough together in time so that no kinetic changes were missed.

By creating the first experiment in the set and then choosing a fixed delay of five minutes and the appropriate number of experiments for that set, the Multi_zgvd program creates the required number of subsequent experiments so that they are identical to the first. Starting the Multi_zgvd program starts the first experiment running. Once this is finished the Multi_zgvd program waits the fixed delay of five minutes before starting the next experiment and subsequent experiments. This allows discrete spectra to run over a period of time, providing valuable kinetic information.

4.6.6 Length of Each Set of Experiments

The length of each set of experiments had to be decided after considering how long it would take each sample to reach equilibrium as well as taking into account how much time each set of experiments could practically take considering that other users needed to have access to the spectrometer. During prime time hours the maximum amount of time for use of the spectrometer is four hours to ensure that all users have fair access. Preliminary work showed that four hours was insufficient for the samples to reach any sort of equilibrium, therefore at least an overnight run was needed. Depending on the spectrometer time available each set of experiments contained 50-100 experiments.

4.6.7 Quick Experimental Set Up

In order for any kinetic information gained from NMR spectroscopy to be accurate with regards to the timescale it is essential that the time between sample preparation and the start of the NMR spectroscopy experiment be minimised. To help ensure this the spectrometer was shimmed and tuned on a NMR tube containing D_2O before the sample tube was inserted.

4.6.8 Errors Involved

The main source of error involved with the experiments was the accuracy with which one could integrate the signals involved.

NMR spectroscopy integration is moderately subjective which implies that errors could arise because of the way in which signals are integrated. For example the changes in the integral size of a signal between spectra could be a result of a difference in the size of area used to produce the integral, rather than a direct result of a change in the species' relative concentration. To overcome this problem careful integration is needed to ensure that the same area for each signal is integrated from spectrum to spectrum. Defining the area to be integrated *via* a dialog box was initially considered to be possibly the most accurate way in which to integrate as one could simply type in the two chemical shifts that defined the area that was to be integrated. However this method of integration

can be affected by small shifts of the spectrum. Although each spectrum was calibrated via the DMSO signal at 2.71 ppm, tiny shifts in the entire spectrum were sometimes observed between spectra. This would have affected the accuracy of the dialog method.

Instead the method of manually defining the area for integration via the mouse cursor was deemed to be more accurate than using the dialog tool. Although manual definition is a time-consuming technique, as long as each signal was suitably enlarged very accurate integration is possible. However factors such as computer screen resolution and human error impose errors on this method. Therefore a method to estimate the errors involved was required. Although the sample signals had the ability of changing throughout the set of spectra as their relative concentrations changed, the DMSO signal size remains identical as the concentration of DMSO remains unchanged between spectra in the same set of experiments. This characteristic can be exploited in a method to determine the accuracy of integration. All spectra in a set are integrated on a scale that is relative to the size of the DMSO signal in the first spectrum therefore it is not necessary to integrate the DMSO signal in consequent spectra. However if the DMSO signal in each experiment is integrated its size can be compared to the DSMO integral in the first experiment of 1.0000. Any deviations from 1.0000 can be designated as errors. Although this method of assuming errors involves a lot of extra integration it gives a reliable description of how much error is involved with manual integration. After all of the DMSO integrals were collected from each set of experiments the lowest DMSO integral was 0.97 and the highest was 1.03. Therefore it was estimated that the error in each integral was plus and minus 0.03

Chapter Five – Results and Discussion

To investigate the equilibrium of aqueous D-glucaric acid equilibration, reactions under different conditions were followed via ^{1}H NMR spectroscopy. The equilibration of both starting species (D-glucaro-1,4-lactone and D-glucaro-1,4;6,3-dilactone) in neutral aqueous solution was investigated, before the equilibration under acid conditions was considered. Investigating the affects of acidic conditions on the equilibration was deemed significant as a solution of the acid catalysed methanol esterification products of D-glucaric acid has been identified as a suitably "activated" form of D-glucaric acid for PHPA production[115, 126]. Altering the amount of molar equivalents of DCl added to the samples was used as a convenient method of creating differing acidic environments.

The signal selected for species (see section 4.6.1) in each experiment was integrated with respect to the size of the DMSO signal of the first experiment in each set. The integration vale for each signal was recorded with the time (in seconds) that represented halfway through the NMR spectroscopy experiment from which the results were obtained. The results were plotted to produce graphs. Each set of experiments was carried out in duplicate therefore producing two graphs for each experiment set.

The experiment sets with one or more molar equivalents of DCl added contain data on all four species present in aqueous D-glucaric acid (as the addition of this much acid moves the HOD signal away from the region that the signals from D-glucaro-6,3-lactone appear in so that approximated values for the 6,3-lactone can be obtained). The experiment sets with less than one molar equivalent of DCl added only have data for three of the species (the HOD overlaps the region where the signals from D-glucaro-6,3-lactone appear).

The error in the integrals size of each data point is plus and minus 0.3. Error bars have not been plotted on the graphs however, because their relatively small size causes the data points to become distorted.

Raw data is in **appendix three**.

5.1 D-Glucaro-1,4-lactone as the Starting Species

5.1.1 D-Glucaro-1,4-lactone in Deuterium Oxide

D-glucaro-1,4-lactone appeared to be relatively stable with respect to equilibration under neutral conditions (**Figures 5.1 and 5.2**). Its relative concentration dropped slightly within the first 20,000 seconds but remained relatively stable for the remainder of the time. The relative concentration of D-glucaric acid slowly rose, while the relative concentration of D-glucaro-1,4-6,3-dilactone remained stable at a relatively low value.

5.1.2 D-Glucaro-1,4-lactone Plus Quarter Equivalent DCl in Deuterium Oxide

The relative concentration of D-glucaro-1,4-lactone fell steadily within the first 20,000 seconds as the relative concentration of D-glucaric acid rose at approximately the same rate (**Figures 5.3 and 5.4**). By the end of the experiment set the relative concentrations of these two species was approximately the same. The relative concentration of D-glucaro-1,4-6,3-dilactone once again remained stable at a relatively low value.

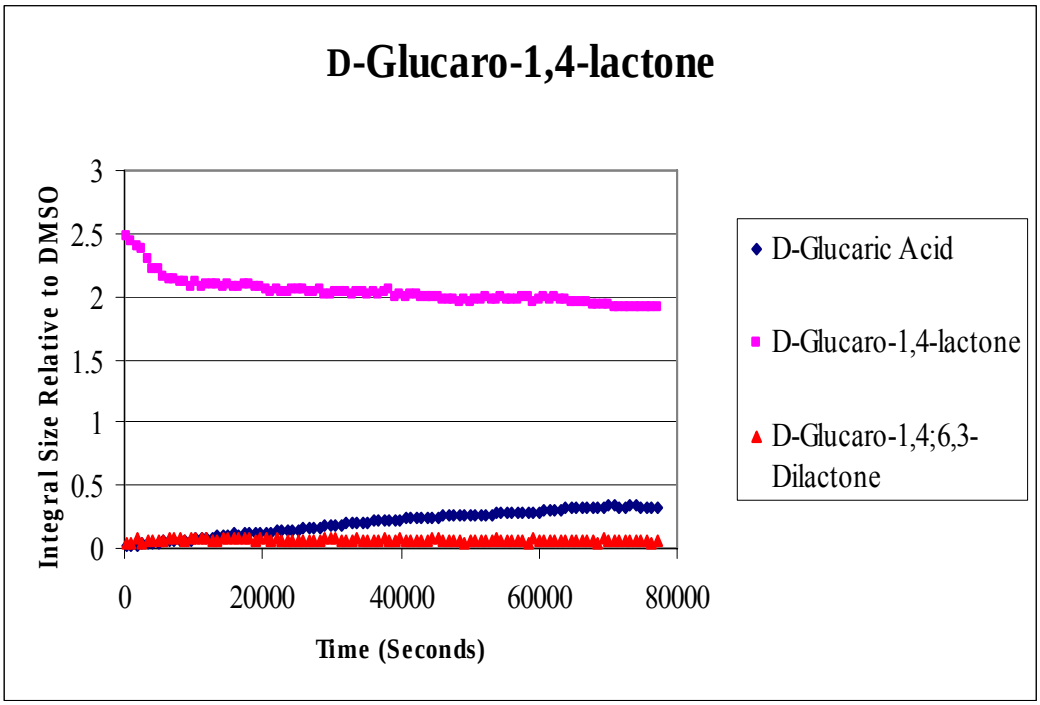


Figure 5.1 *Graph of the results from the D-glucaro-1,4-lactone experiment set*

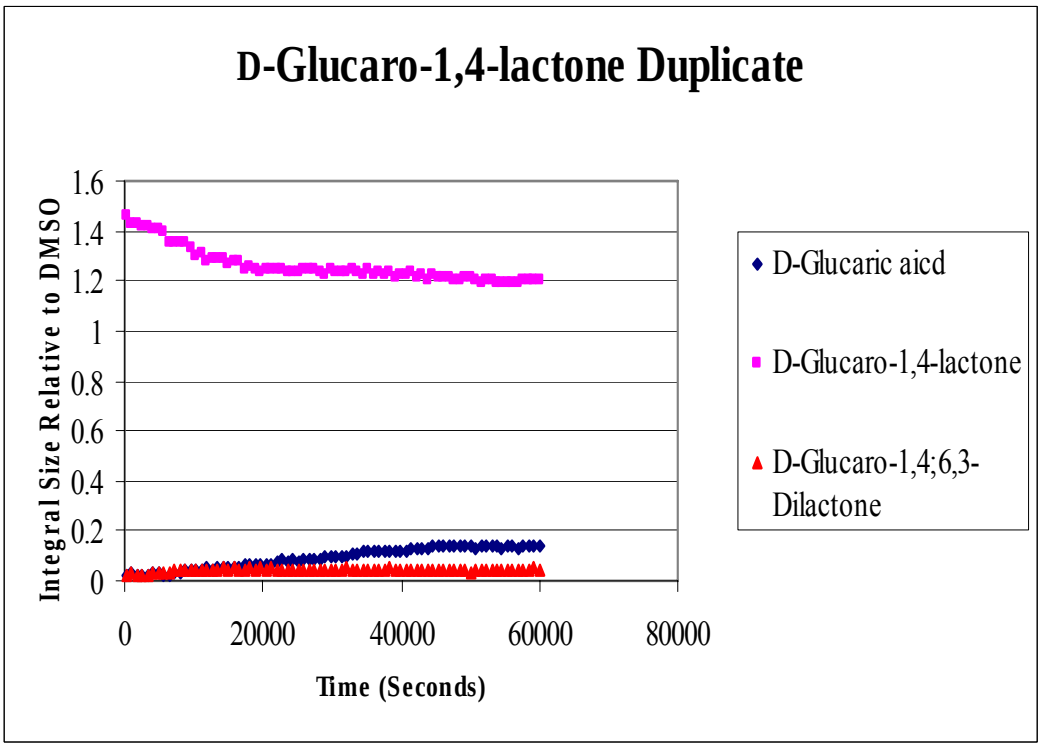


Figure 5.2 *Graph of the results from the D-glucaro-1,4-lactone Duplicate experiment set*

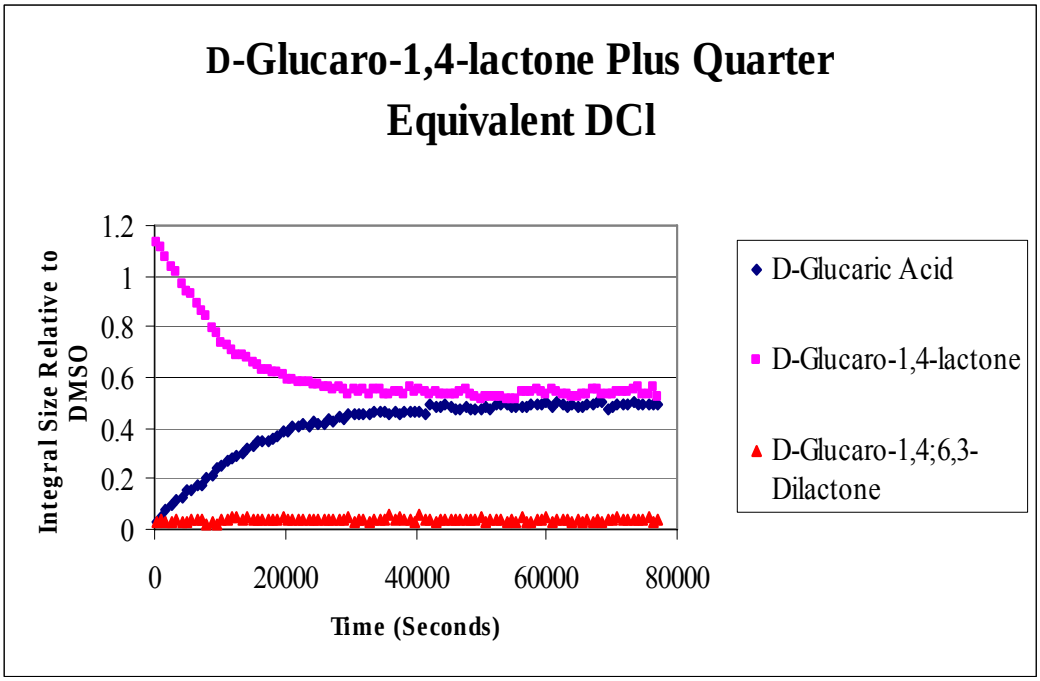


Figure 5.3 *Graph of the results from the D-glucaro-1,4-lactone Plus Quarter Equivalent DCl experiment set*

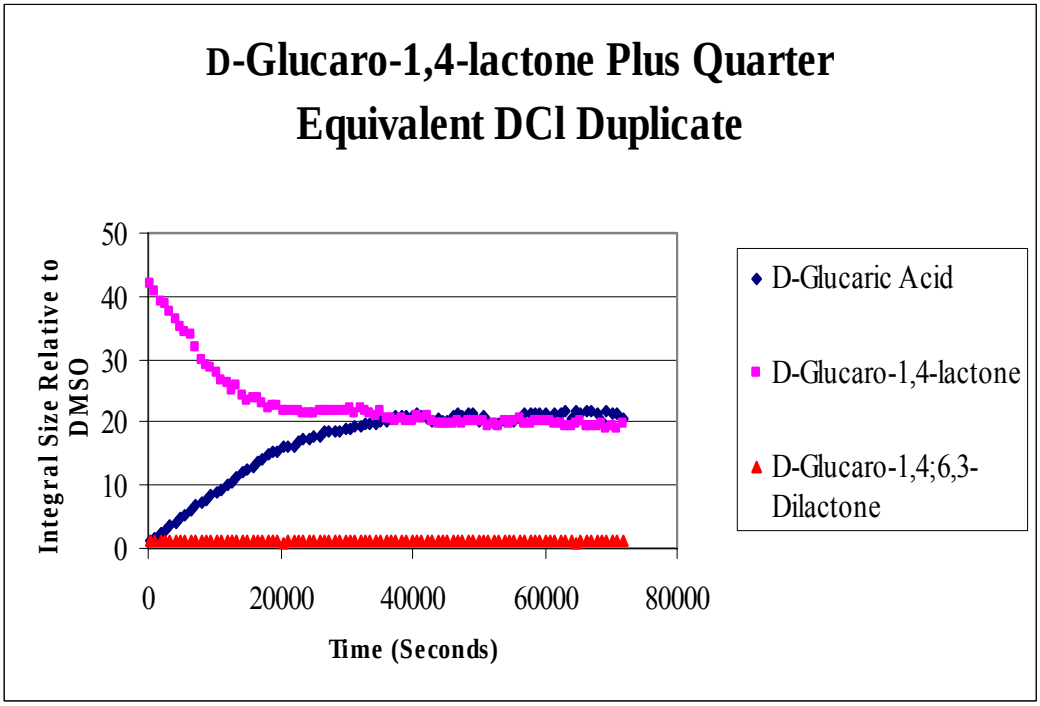


Figure 5.4 *Graph of the results from the D-glucaro-1,4-lactone Plus Quarter Equivalent DCl Duplicate experiment set*

5.1.3 D-Glucaro-1,4-lactone Plus Half Equivalent DCl in Deuterium Oxide

The results from these experiment sets (**Figures 5.5 and 5.6**) were very similar to those from the sets with a quarter equivalent of DCl. The only difference was that the relative concentration of D-glucaric acid appeared to equilibrate at a slightly higher value than that of D-glucaro-1,4-lactone.

5.1.4 D-Glucaro-1,4-lactone Plus Three Quarter Equivalent DCl in Deuterium Oxide

Again these experiment sets (**Figures 5.7 and 5.8**) produced similar results those experiment sets containing quarter and half equivalents of DCl. The difference between the relative equilibrium concentrations of D-glucaric acid and D-glucaro-1,4-lactone was slightly larger than that of the previous experiment sets. The relative concentration of D-glucaro-1,4;6,3-dilactone was once again relatively low and stable.

5.1.5 D-Glucaro-1,4-lactone Plus Equivalent DCl in Deuterium Oxide

These experiment sets (**Figures 5.9 and 5.10**) included data for the D-glucaro-6,3-lactone. This species' relative concentration slowly increased for the first 40,000 seconds before it reached its equilibration concentration. The relative concentration of D-glucaro-1,4-lactone behaved in a similar way to previous sets. The relative concentration of D-glucaric acid rose to reach an equilibrium concentration slightly higher than that of D-glucaro-1,4-lactone. The relative concentration of D-glucaro-1,4;6,3-dilactone remained similar to previous experiment sets.

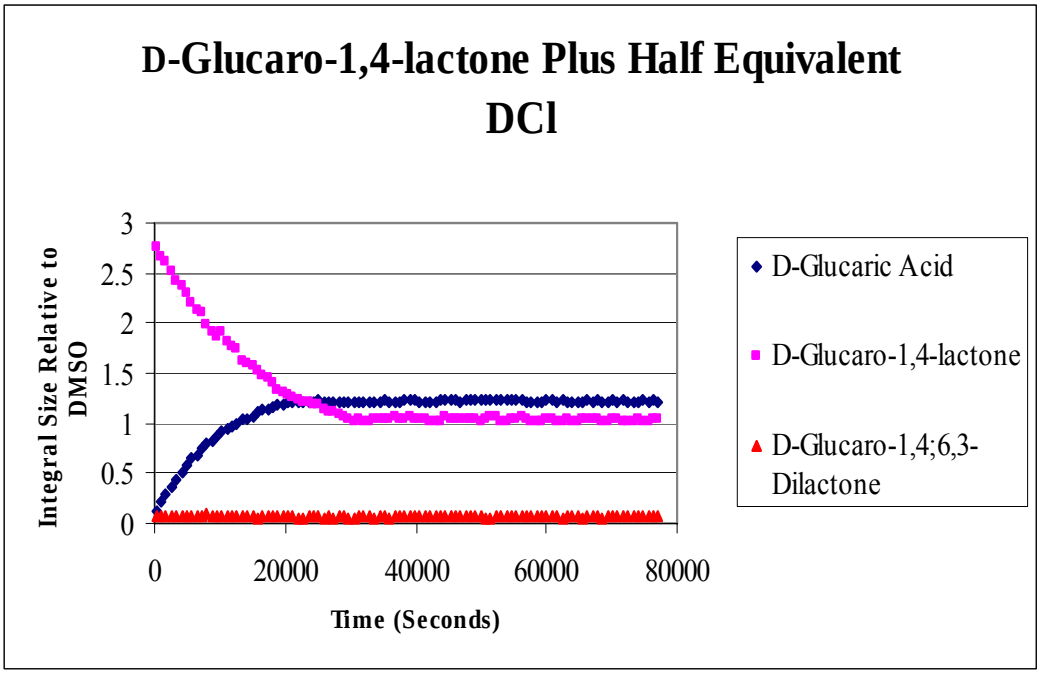


Figure 5.5 *Graph of the results from the D-glucaro-1,4-lactone Plus Half Equivalent DCl experiment set*

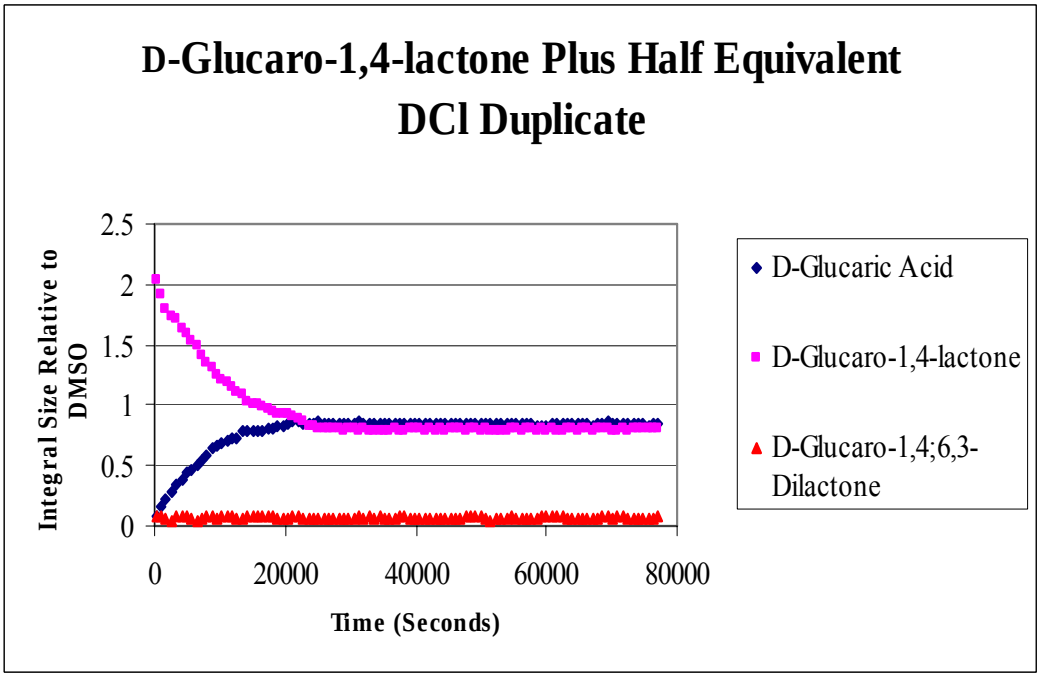


Figure 5.6 *Graph of the results from the D-glucaro-1,4-lactone Plus Half Equivalent DCl Duplicate experiment set*

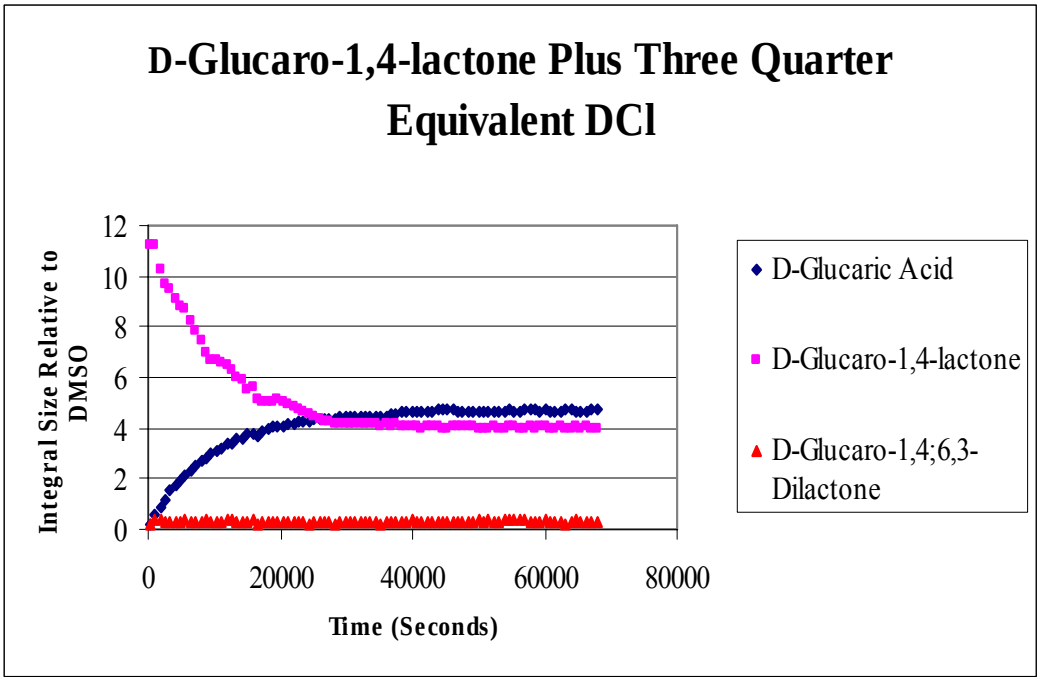


Figure 5.7 *Graph of the results from the D-glucaro-1,4-lactone Plus Three Quarter Equivalent DCl experiment set*

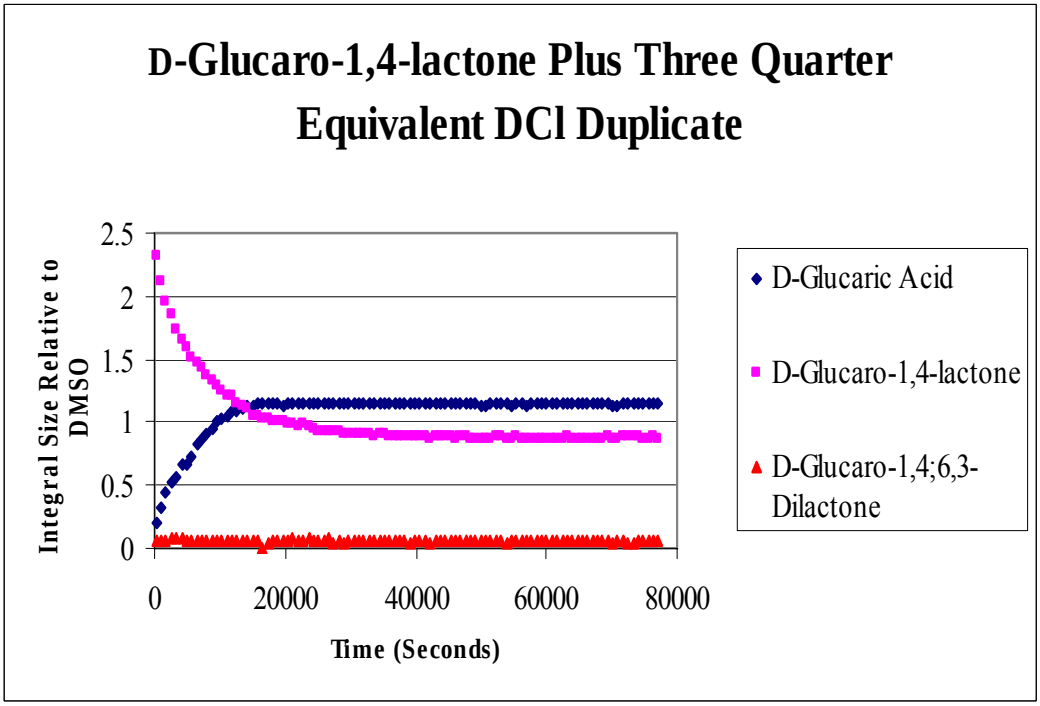


Figure 5.8 *Graph of the results from the D-glucaro-1,4-lactone Plus Three Quarter Equivalent DCl Duplicate experiment set*

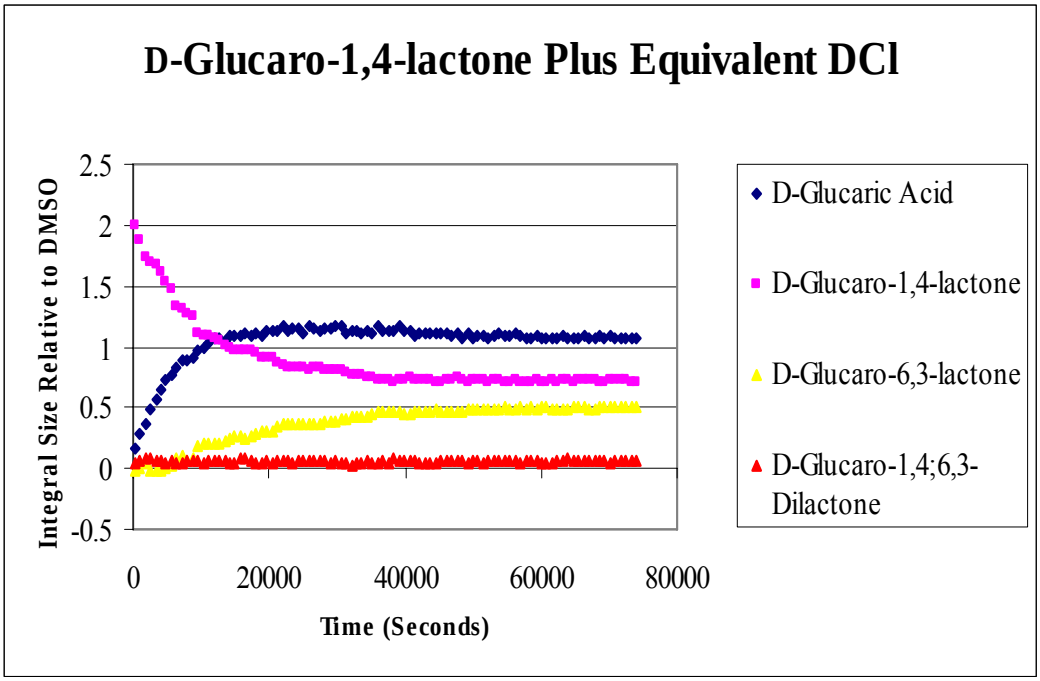


Figure 5.9 *Graph of the results from the D-glucaro-1,4-lactone Plus Equivalent DCl experiment set*

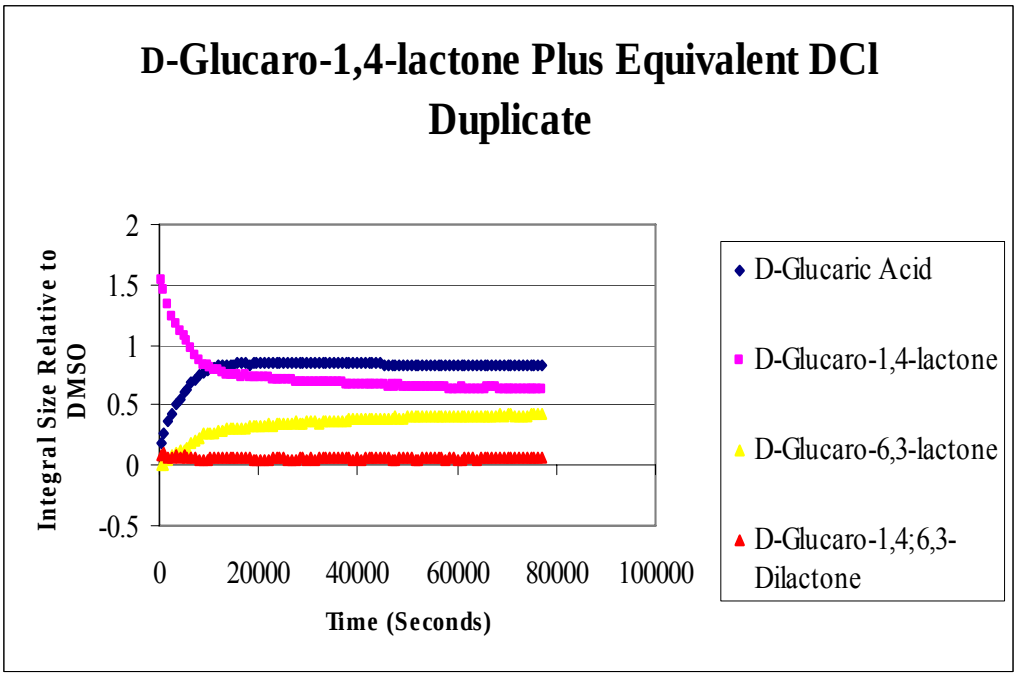


Figure 5.10 *Graph of the results from the D-glucaro-1,4-lactone Plus Equivalent DCl Duplicate experiment set*

5.1.6 D-Glucaro-1,4-lactone Plus One and a Half Equivalent DCl in Deuterium Oxide

The results from these experiments sets (**Figures 5.11 and 5.12**) were very similar to those obtained from the sets with one equivalent DCl. The only difference being that the relative concentration of D-glucaro-6,3-lactone remains at a lower level in the duplicate set than that of the same species in the original experiment set.

5.1.7 Overall Discussion for D-Glucaro-1,4-lactone as the Starting Species

The results obtained from the experiment sets with D-glucaro-1,4-lactone as the stating species were very reproducible when the duplicate sets were compared to the original sets. When the mass balance of all of the experiment sets that included all four species was investigated, it was found that this was essentially consistent throughout the experiment sets.

As a group the results obtained from each of the experiment sets were in fact very similar, especially those with added DCl. In neutral solution D-glucaro-1,4-lactone appeared to be relatively stable against equilibration. When DCl was added (even when only a quarter molar equivalent was added) the relative concentration steadily decreased to an equilibration concentration that was considerably lower than that of its starting concentration. The relative concentration of D-glucaric acid increased through the duration of all experiment sets, especially those with added DCl. In the experiment sets that had data for the relative concentration of D-glucaro-6,3-lactone, its starting relative concentration was low. This is because D-glucaro-6,3-lactone can not directly form D-glucaro-1,4-lactone, therefore it can not form until some of the D-glucaro-1,4-lactone has converted to D-glucaric acid (or D-glucaro-1,4;6,3-dilactone – it is unlikely however that much D-glucaro-6,3-lactone forms from this path as the concentration of the dilactone is relatively low throughout the experiment set).

In those experiment sets with a half equivalent or more of DCl added the relative equilibration concentration of D-glucaric acid was higher than that of D-glucaro-1,4-lactone. The relative concentration of D-glucaro-6,3-lactone (in those sets

where it was measured) was slightly lower than that of D-glucaro-1,4-lactone. The concentration of D-glucaro-1,4-;6,3-dilactone was relatively low and stable throughout all of the experiment sets.

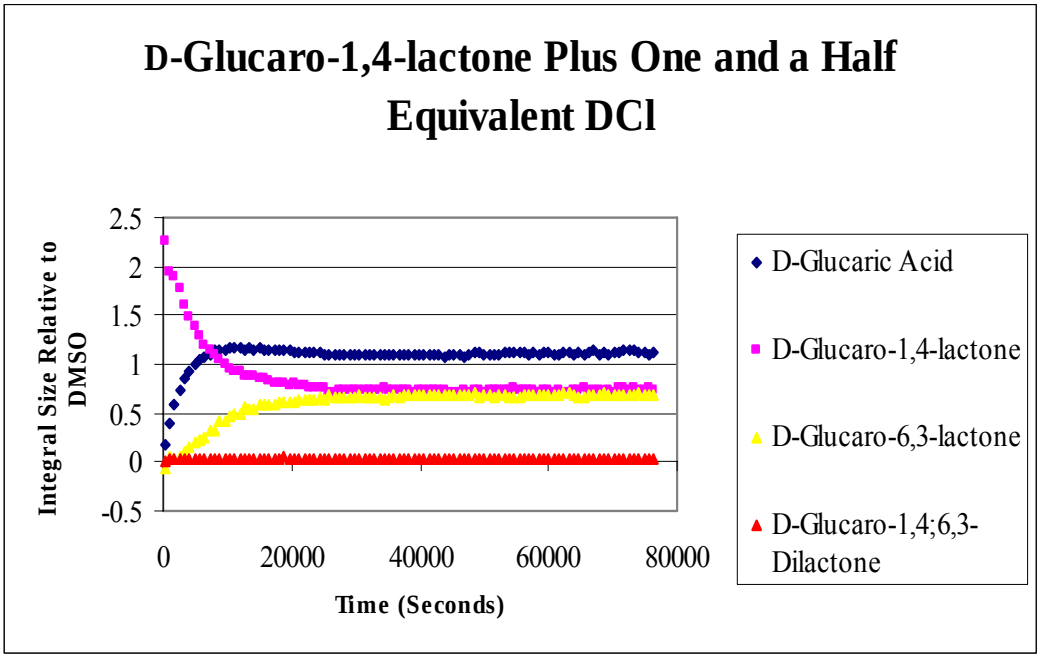


Figure 5.11 *Graph of the results from the D-glucaro-1,4-lactone Plus One and a Half Equivalent DCl experiment set*

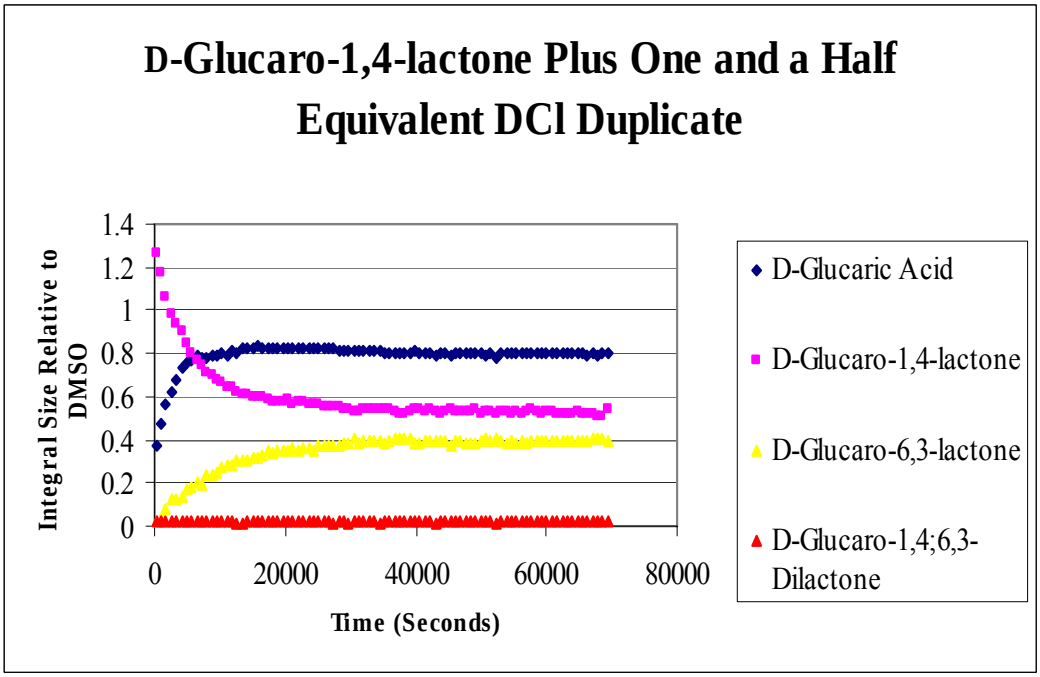


Figure 5.12 *Graph of the results from the D-glucaro-1,4-lactone Plus One and a Half Equivalent DCl Duplicate experiment set*

5.2 D-Glucaro-1,4;6,3-dilactone as the Starting Species

5.2.1 D-Glucaro-1,4;6,3-dilactone in Deuterium Oxide

The results from these experiment sets (**Figures 5.13 and 5.14**) showed that D-glucaro-1,4;6,3-dilactone is not stable against equilibration in neutral solution. The relative concentration of this species steadily decreased throughout the experiments set. The relative concentration of D-glucaro-1,4-lactone steadily increased, although in the duplicate experiment set it appears to reach its equilibration concentration within the timeframe of the experiment set. The relative concentration of D-glucaric acid started to increase once the relative concentration of D-glucaro-1,4-lactone had increased. This is because D-glucaric acid cannot form directly from D-glucaro-1,4;6,3-dilactone but can form from D-glucaro-1,4-lactone (and also from D-glucaro-6,3-lactone).

5.2.2 D-Glucaro-1,4;6,3-dilactone Plus Half Equivalent DCl in Deuterium Oxide

The relative concentration of D-glucaro-1,4;6,3-dilactone rapidly dropped (**Figures 5.15 and 5.16**) to approximately the same level that it is throughout the experiment sets with D-glucaro-1,4-lactone as the starting species. This drop occurred in around ten experiments. The relative concentration of D-glucaric acid steadily rose for the first 30,000-40,000 seconds before reaching its equilibrium concentration. The relative concentration of D-glucaro-1,4-lactone started at a higher level than the D-glucaric acid then increased for the first few experiments before remaining at a constant relative concentration for the remainder of the experiment set. The relative equilibrium concentration of D-glucaric acid is higher than that of D-glucaro-1,4-lactone.

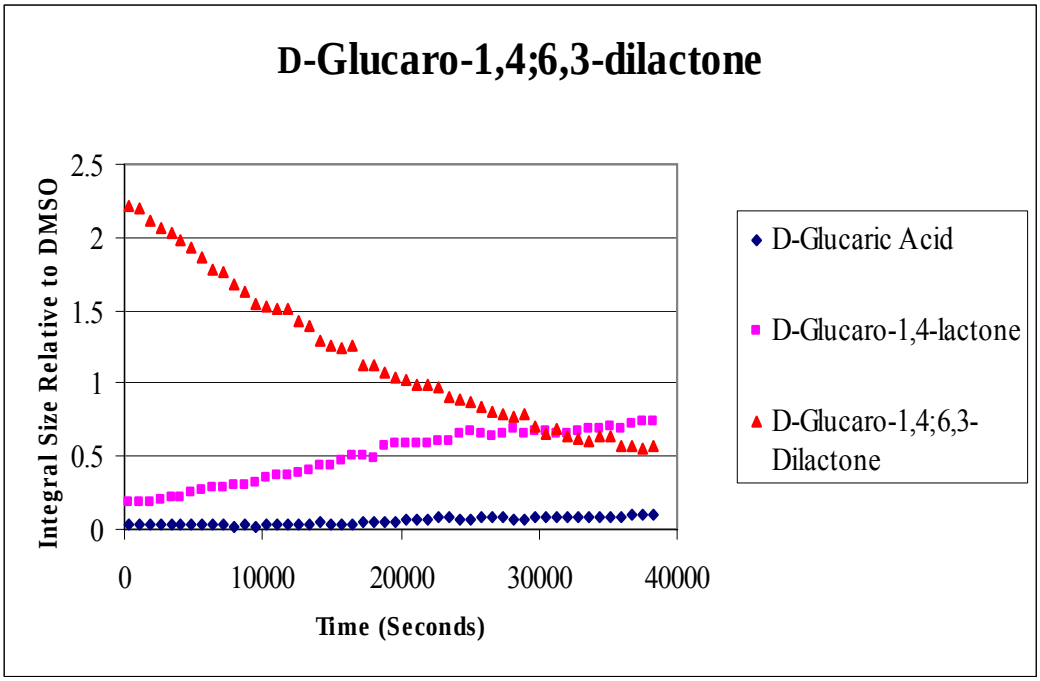


Figure 5.13 *Graph of the results from the D-glucaro-1,4;6,3-dilactone experiment set*

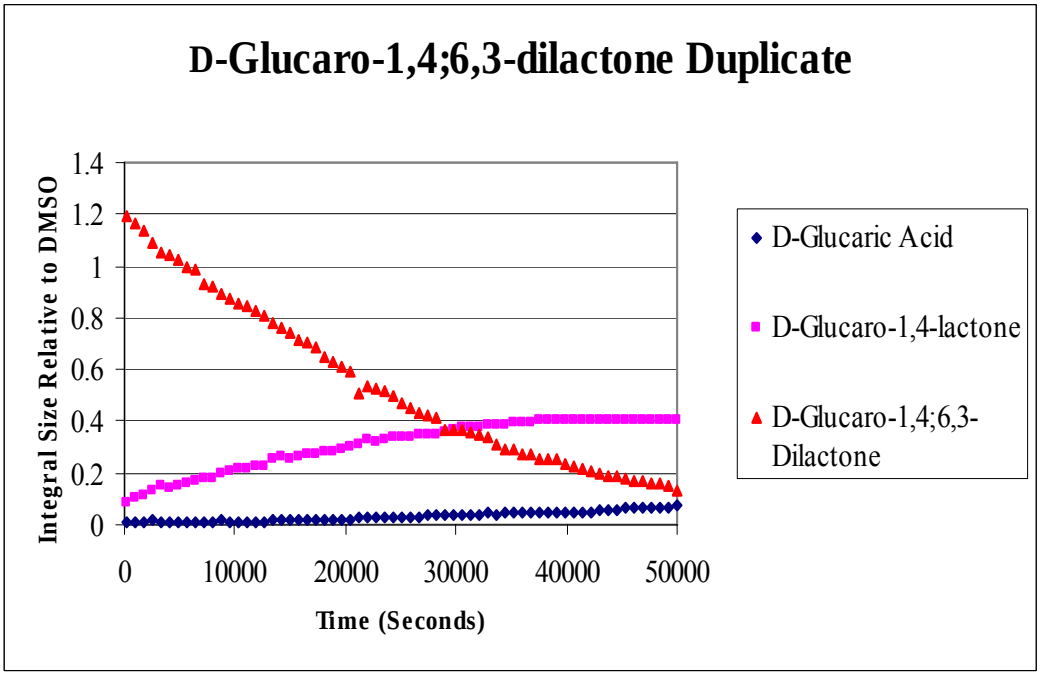


Figure 5.14 *Graph of the results from the D-glucaro-1,4;6,3-dilactone Duplicate experiment set*

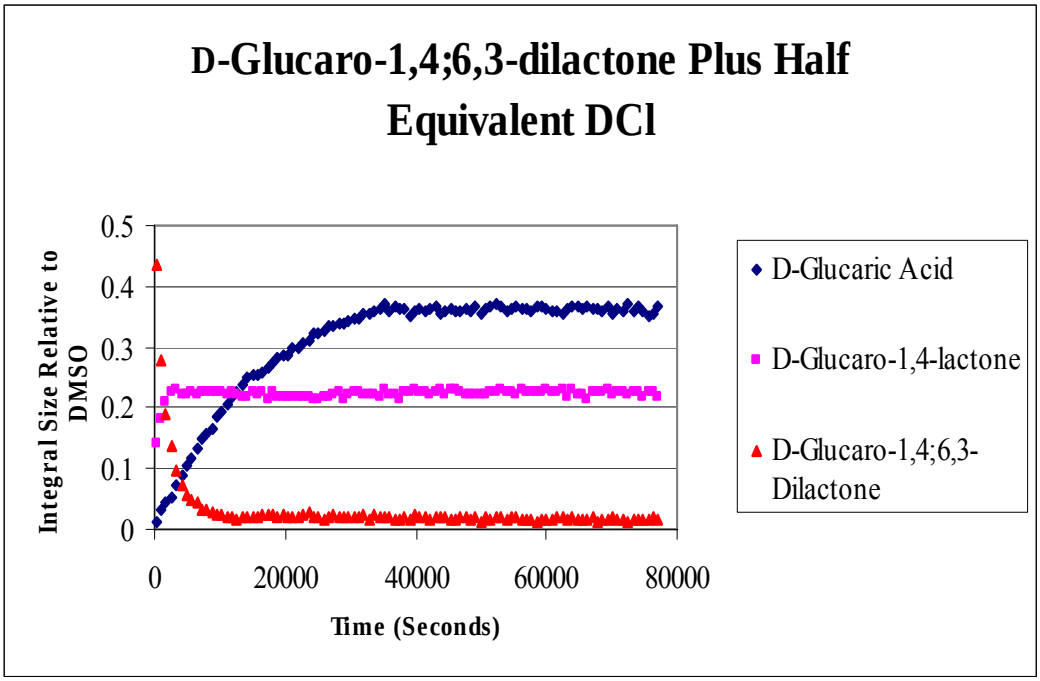


Figure 5.15 *Graph of the results from the D-glucaro-1,4;6,3-dilactone Plus Half Equivalent DCl experiment set*

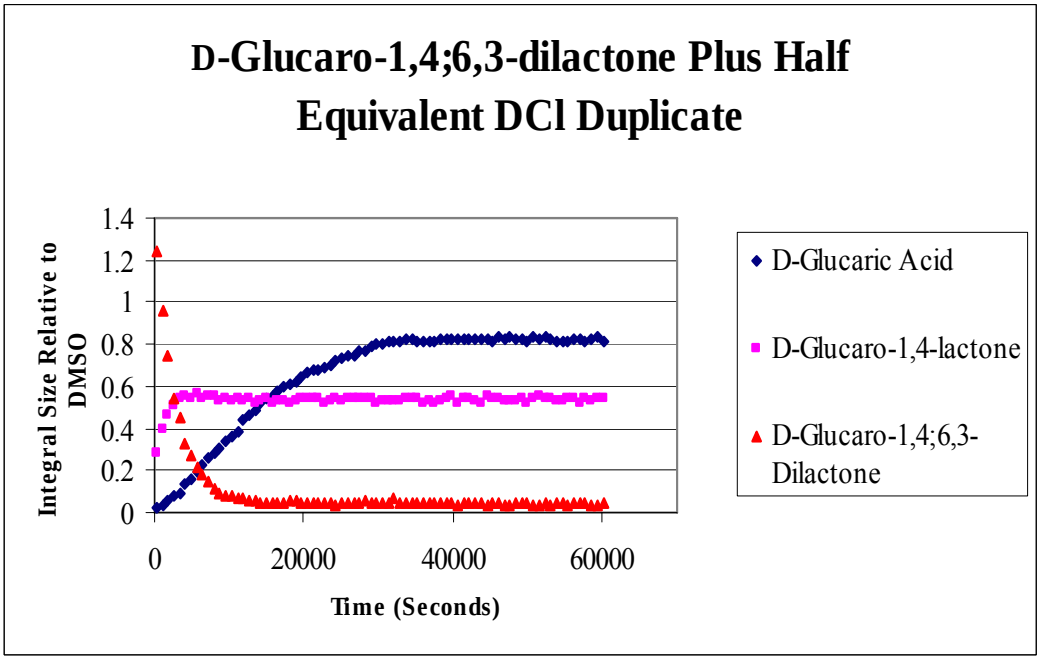


Figure 5.16 *Graph of the results from the D-glucaro-1,4;6,3-dilactone Plus Half Equivalent Duplicate DCl experiment set*

5.2.3 D-Glucaro-1,4;6,3-dilactone Plus Equivalent DCl in Deuterium Oxide

These experiments included data for the D-glucaro-6,3-lactone (**Figures 5.17 and 5.18).** Again the relative concentration of D-glucaro-1,4;6,3-dilactone rapidly dropped to approximately the same level that it is throughout the experiment sets with D-glucaro-1,4-lactone as the starting species. This drop occurred in around ten experiments. The relative concentration of D-glucaric acid steadily rose for the first 30,000-40,000 seconds before reaching its equilibrium concentration. The relative concentration of D-glucaro-1,4-lactone started at a higher level than the D- glucaric acid, then increased for the first few experiments before it remained at a constant relative concentration for the remainder of the experiment set. Again the relative concentration of D-glucaric acid at equilibrium is higher than that of D-glucaor-1,4-lactone. The relative concentration of D-glucaro-6,3-lactone increased rapidly for the first five or six experiments before steadily decreasing until approximately 20,000 seconds and remaining steady for the rest of the experiment set. In the duplicate experiment set the relative starting concentration of D-glucaro-6,3-lactone is actually higher than that of D-glucaro-1,4;6,3-dilactone.

5.2.4 D-Glucaro-1,4;6,3-dilactone Plus One and a Half Equivalent DCl in Deuterium Oxide

The results from these experiment sets (**Figures 5.19 and 5.20**) were very similar to those from the sets with one molar equivalent of DCl. The relative concentration of D-glucaro-1,4;6,3-dilactone rapidly dropped to its equilibrium level. The relative starting concentration of D-glucaro-1,4-lactone was quite high and increased for the first few experiments and then remained steady for the rest of the experiment set. The relative concentration of D-glucaric acid was low at the start but it steadily rose for the first 20,000 seconds to reach an equilibrium concentration that was higher than that of D-glucaro-1,4-lactone. Again the relative concentration of D-glucaro-6,3-lactone started at a high level before rapidly increasing for the first few experiments then steadily decreasing until it reached its equilibrium concentration. In the duplicate experiment set the relative

starting concentration of D-glucaro-6,3-lactone is actually higher than that of D-glucaro-1,4;6,3-dilactone.

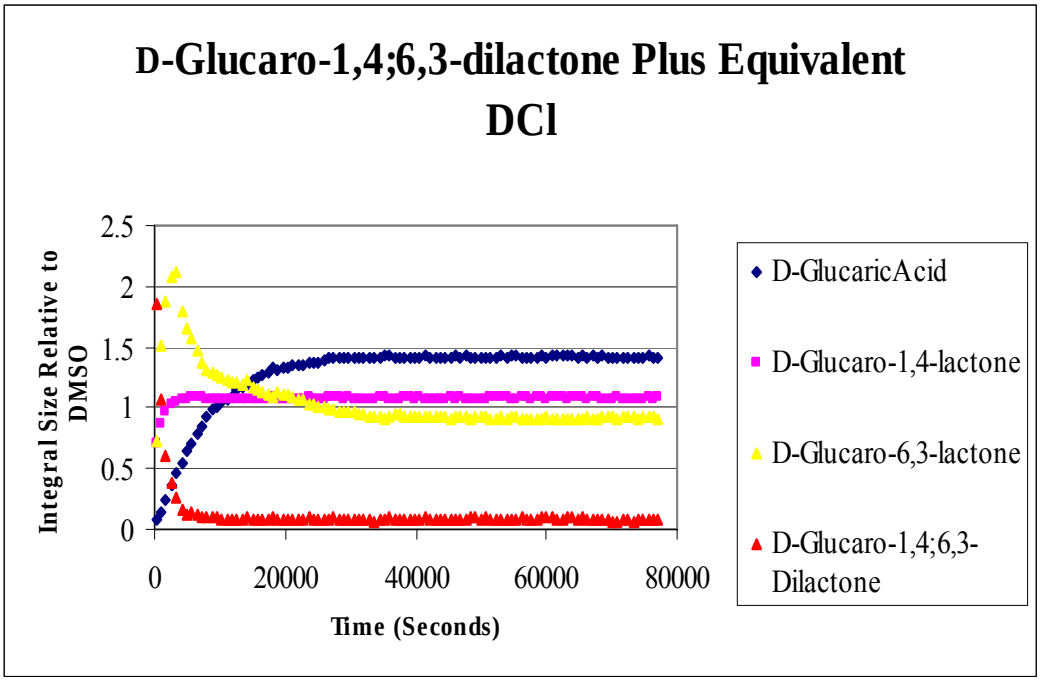


Figure 5.17 *Graph of the results from the D-glucaro-1,4;6,3-dilactone Plus Equivalent DCl experiment set*

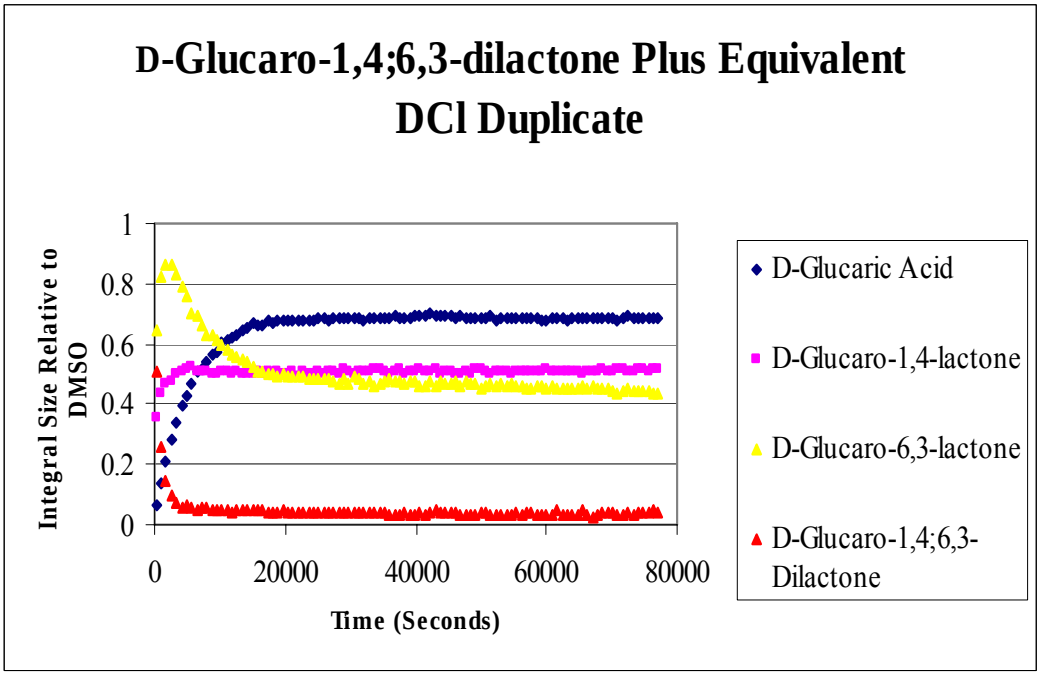


Figure 5.18 *Graph of the results from the D-glucaro-1,4;6,3-dilactone Plus Equivalent DCl Duplicate experiment set*

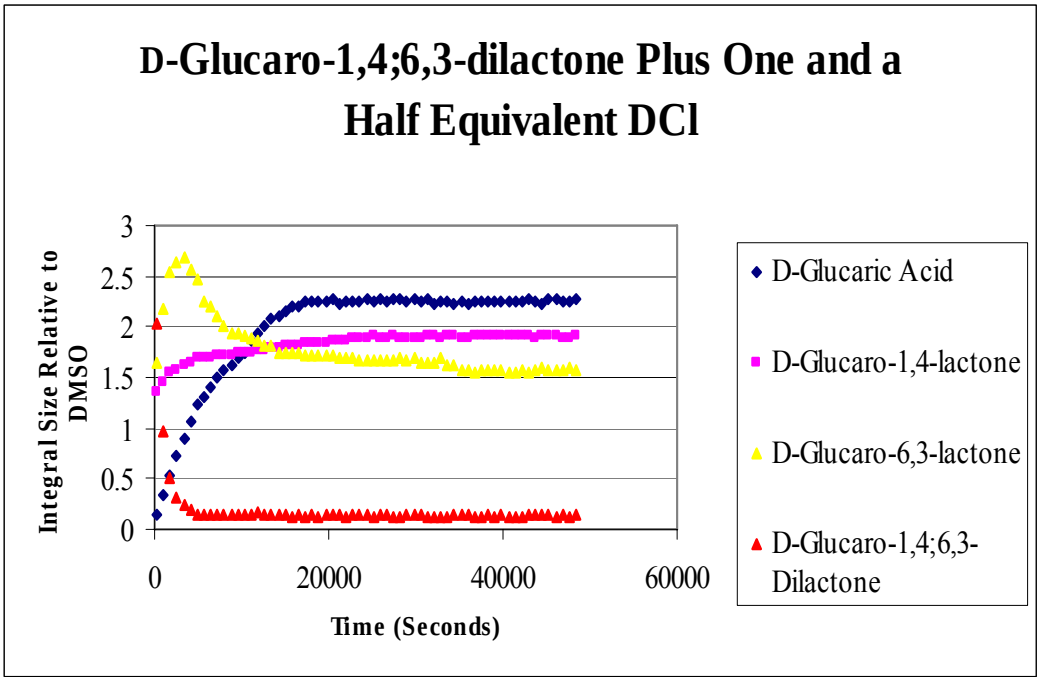


Figure 5.19 *Graph of the results from the D-glucaro-1,4;6,3-dilactone Plus One and a Half Equivalent DCl experiment set*

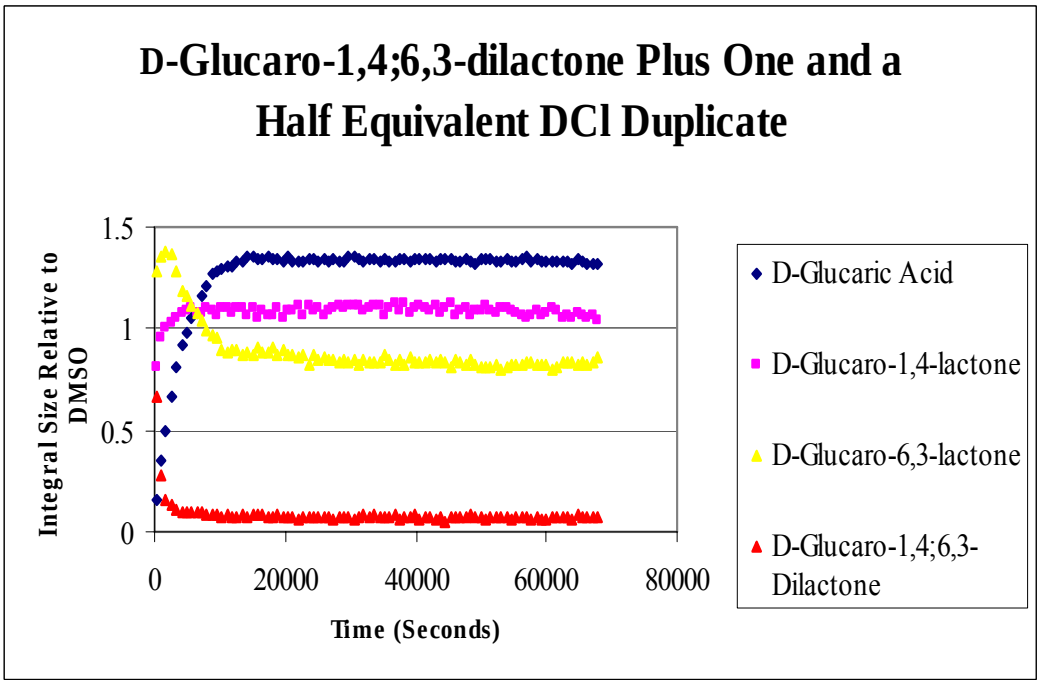


Figure 5.20 *Graph of the results from the D-glucaro-1,4;6,3-dilactone Plus One and a Half Equivalent DCl Duplicate experiment set*

5.2.5 Overall Discussion for D-Glucaro-1,4;6,3-dilactone as the Starting Species

Like the experiment sets with D-glucaro-1,4-lactone as the starting species, the results obtained from the experiment sets with D-glucaro-1,4;6,3-dilactone as the stating species were very reproducible when the duplicate sets were compared to the original sets. Again the mass balance of all of the experiment sets, that included all four species, was found to be consistent.

D-Glucaro-1,4;6,3-dilactone was not stable in neutral solution with its relative concentration dropping steadily throughout the experiment sets. The results from all of the experiment sets with added DCl were very similar. The addition of DCl caused the relative concentration of D-glucaro-1,4;6,3-dilactone to rapidly decrease and this species reached its relatively low equilibrium concentration within the first few experiments of each set. The starting relative concentration of D-glucaro-1,4-lactone was quite high in all of the experiment sets with added DCl. This relatively high starting concentration was also seen for D-glucaro-6,3-lactone, in the sets that had data for this species. The starting concentration of D-glucaric acid however was relatively low. The probable cause of this is that D-glucaro-1,4;6,3-dilactone can not directly convert to the acyclic acid and instead converts to one of the monolactones, each which can convert to the acid. At the beginning of each experiment set the relative concentration of D-glucaro-6,3-lactone rises rapidly before decreasing steadily to an equilibration concentration that is less than that of D-glucaric acid and slightly less than that of D-glucaro-1,4-lactone. The relative concentration of D-glucaro-1,4-lactone rose in the first few experiments before reaching its equilibration concentration, whereas the relative concentration of D-glucaric acid rises steadily for the first 20,000 seconds.

Chapter Six – Kinetic Analysis

6.1 The MATLAB Program

MATLAB (MATrix LABoratory) is a software package for numerical computation and visualisation[135]. The built in functions provide many useful tools for kinetic simulations of chemical data. Script files can be written to solve ordinary differential equations (as well as other mathematical equations) that would be near impossible to solve manually. A set of differential equations with estimates of the rate constants involved can be used to simulate chemical reactions.

Although the kinetic modelling program Gepasi was investigated, MATLAB was deemed to be more user-friendly than Gepasi.

6.2 Defining the Current Situation

Before any script file is produced it is vital to define the situation that one is describing. This includes labelling each reaction that is involved and applying a rate constant label to each. Also each species is assigned a number. From this a set of differential equations can be created to describe the situation.

6.2.1 Proposal of a Mechanism

Before differential equations and hence rate constants can be defined, it is essential to propose a mechanism for the reaction. Based on what is known about the equilibrium of aqueous D-glucaric acid, a list of equations describing the possible transformations that happen during the equilibration process was formed:

D-Glucaric Acid ⇌ D-Glucaro-1,4-lactone

D-Glucaric Acid ⇌ D-Glucaro-6,3-lactone

D-Glucaro-6,3-lactone ⇌ D-Glucaro-1,4;6,3-dilactone

D-Glucaro-1,4;6,3-dilactone ⇌ D-Glucaro-1,4-lactone

However when working in acidic solution it is likely that the starting species undergoes a pre-equilibrium step in which it becomes protonated:

$$StartingSpecies + H^+ \overset{K}{\rightleftharpoons} StartingSpeciesH^+ \xrightarrow{k_x} Product$$

Therefore the rate of change of the product is given by:

$$\frac{d[Product]}{dt} = k_x[StartingSpeciesH^+]$$

$$= k_x K[StartingSpecies][H^+]$$

The equilibrium constant K is given by:

$$K = \frac{[StartingSpeciesH^+]}{[StartingSpecies][H^+]}$$

Therefore:

$$[StartingSpeciesH^+] = K[StartingSpecies][H^+]$$

In the experiment sets that were used for kinetic analysis at least one molar equivalent of DCl was added. This means that $[StartingSpeciesH^+] >>> [StartingSpecies]$. By keeping the temperature constant (the NMR spectrometer has a thermostat) K is kept constant. As the concentration of DCl in each experiment set it is kept constant it is reasonable to

assume that *[StartingSpeciesH$^+$]* is directly proportional to *[StartingSpecies]*. This assumption allows the following to be correct:

$$\frac{d[Product]}{dt} = k_x[StartingSpecies]$$

This allows the reactions of the equilibration of aqueous D-glucaric acid to interpreted as pseudo-first order. This simplifies the analysis and prevents the need to determine absolute concentrations of the species.

6.2.2 Assignment of Rate Constants

Each step in the mechanism is assigned a rate constant. The present equilibrium of aqueous D-glucaric acid is a rather complicated situation as there are eight equations involved, and these reactions are strongly related to one another. Each reaction has a rate constant that has to be evaluated from experimental data before being refined via MATLAB simulation (**Figure 6.1**).

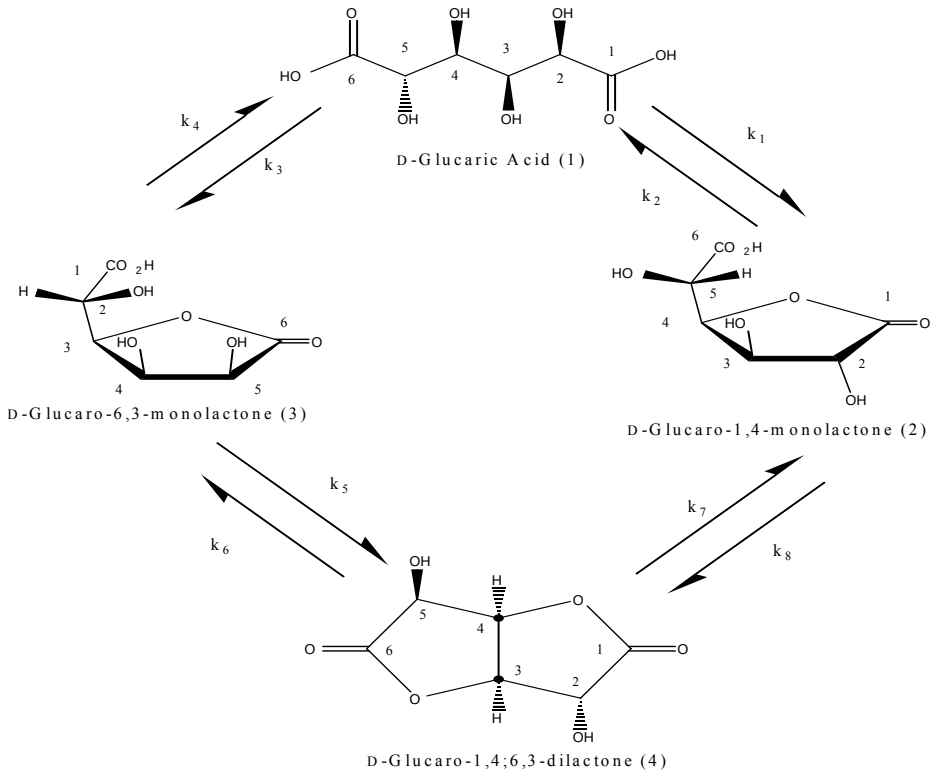


Figure 6.1 *Assignment of rate constants and species labels*

6.3 Writing the Differential Equations

The rate constants must be used to create the differential equations that one ultimately wishes to use MATLAB to solve. These equations must first be derived from the mechanism before being converted to a format that MATLAB can use.

6.3.1 Creating the Differential Equations from the Assignment of Rate Constants

The following are the differential equations, based on the above assignment of rate constants, before they were converted to the correct form for MATLAB):

d[D-glucaric Acid]/dt = k_1[D-glucaro-1,4-lactone] - k_2[D-glucaric Acid] - k_3[D-glucaric Acid] + k_4[D-glucaro-6,3-lactone]

d[D-glucaro-1,4-lactone]/dt = -k_1[D-glucaro-1,4-lactone] + k_2[D-glucaric Acid] + k_7[D-glucaro-1;46,3-dilactone]- k_8[D-glucaro-1,4-lactone]

d[D-glucaro-6,3-lactone]/dt= k_3 [D-glucaric Acid] - k_4[D-glucaro-6,3-lactone] - k_5 [D-glucaro-6,3-lactone] + k_6[D-glucaro-1,4;6,3-dilactone];

d[D-glucaro-1;4;6,3-dilactone]/dt = k_5[D-glucaro-6,3-lactone]- k_6[D-glucaro-1;46,3-dilactone]- k_7[D-glucaro-1;46,3-dilactone]+ k_8[D-glucaro-1,4-lactone]

6.3.2 Converting the Differential Equations to a Suitable Form for MATLAB

The differential equations that one wishes MATLAB to solve must be written in the correct form for MATLAB to recognise. This includes using numbers in brackets for species labels and asterisks for multiply signs. For the purposes of this script the following labels were used:

(1) = D-glucaric Acid
(2) = D-glucaro-1,4-lactone

(3) = D-glucaro-6,3-lactone

(4) = D-glucaro-1,4;6,3-dilactone

A semi colon is used at the end of each line that you do not want MATLAB to calculate separately and a % is used to designate text one wants MATLAB to ignore i.e. text labels.

The following are the differential equations in the correct form for the aqueous D-glucaric acid equilibrium:

```
dy(1) =  k1*y(2) - k2*y(1) - k3*y(1) + k4*y(3);
dy(2) =  -k1*y(2) + k2*y(1) + k7*y(4) - k8*y(2);
dy(3) =  k3*y(1) - k4*y(3) - k5*y(3) + k6*y(4);
dy(4) =  k5*y(3) - k6*y(4) - k7*y(4) + k8*y(2)
```

6.4 Writing the Script File

After the differential equations have been written, the next step is writing a MATLAB script file (M-file). A script file contains a set of valid MATLAB commands that can be executed simply by typing the name of the file into the MATLAB command line. It is the equivalent of typing all the commands the script file contains one by one[135].

Writing such a file could be near impossible if one is not intimately aware of the workings of MATLAB. It is extremely fortunate therefore, that with the current situation, the enthusiastic and very patient assistance of Professor Richard J. Field from the University of Montana was available. Professor Field has had extensive experience with chemical kinetics and modelling, and was jointly responsible for the development of the Oregonator model of the Belousov-Zhabotinskii reaction[136]. With Professor Field's assistance and the Oregonator model as a guide, it was possible after trial and error, to write the following script files. The arrows and brackets represent comments that do not actually occur in the actual script files but have been added to help explain how each script file is created.

6.4.1 Script File for Equilibrium Differential Equations:

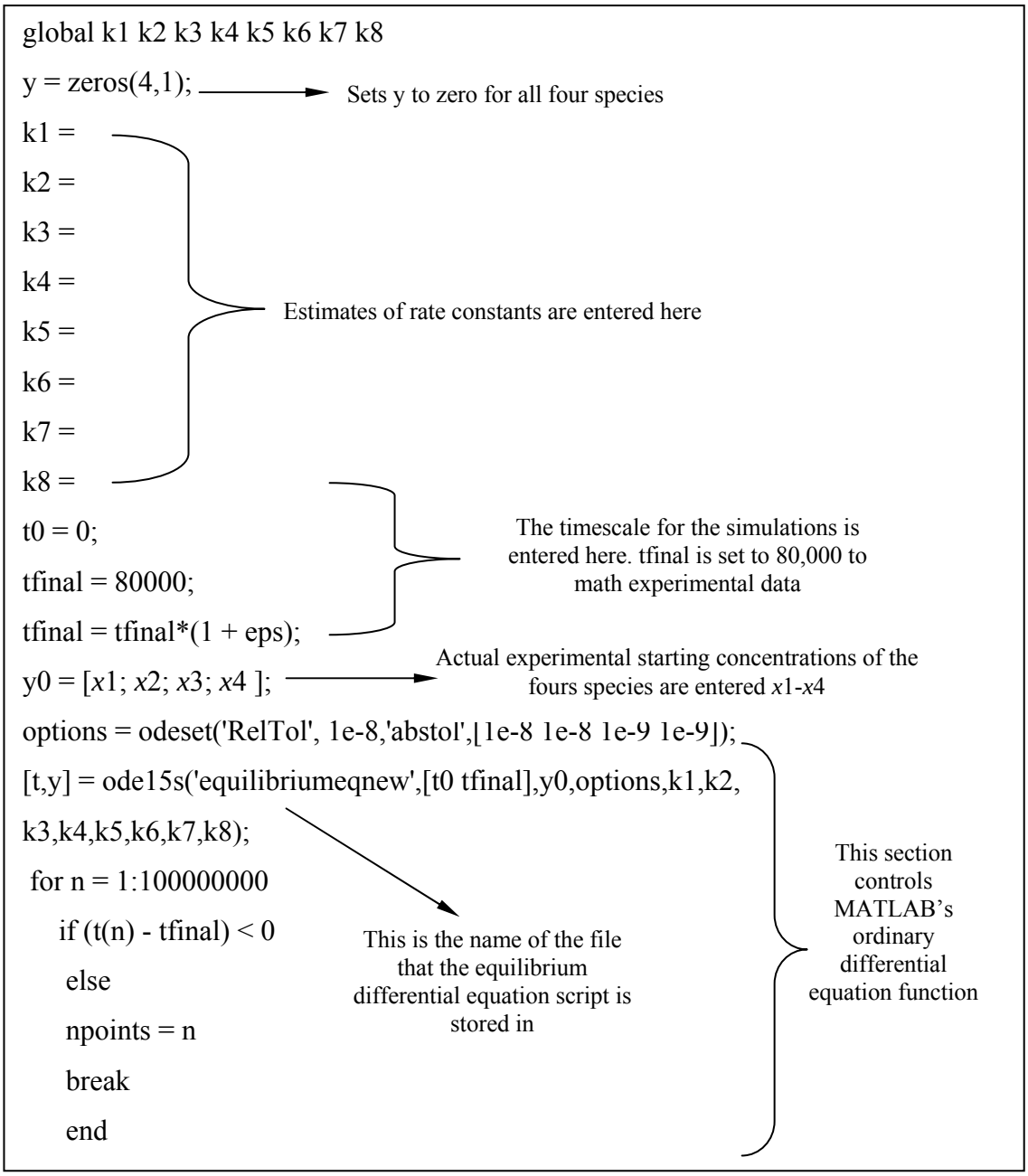
```
function dy = equilibrium(t,y)
global k1 k2 k3 k4 k5 k6 k7 k8
dy = zeros(4,1);
dy(1) =  k1*y(2) - k2*y(1) - k3*y(1) + k4*y(3);
dy(2) =  -k1*y(2) + k2*y(1) + k7*y(4) - k8*y(2);
dy(3) =  k3*y(1) - k4*y(3) - k5*y(3) + k6*y(4);
dy(4) =  k5*y(3) - k6*y(4) - k7*y(4) + k8*y(2)
```

Designates these variables to be accessible to all functions

Sets dy to zero for all 4 species

The differential equations in the correct form

6.4.2 Script File for Kinetics (Solving Equilibrium Differential Equations):



6.4.2 Plotting Commands

```
subplot(4,1,1);plot(t,y(1:npoints,1),'k')
subplot(4,1,2);plot(t,y(1:npoints,2),'k')
subplot(4,1,3);plot(t,y(1:npoints,3),'k')
subplot(4,1,4);plot(t,y(1:npoints,4),'k')
```

This creates four subplots with the simulation for one of the species on each subplot

```
subplot(4,1,4)
xlabel('Time')
subplot(4,1,1
ylabel('1,4L')
subplot(4,1,3))
ylabel('GA')
subplot(4,1,2)
ylabel('1,4L')
subplot(4,1,3)
ylabel('6,3L')
subplot(4,1,4)
ylabel('Di')
```

This labels the x-axis on each subplot with 'Time" and labels each y-axis with the correct species' name

6.5 Customising the Script File for Each Reaction

Once the script file has been created and tested to ensure that it produces simulations that have approximately correct time scales (by including estimates of starting concentrations and rate constants), the next step is to customise it to fit the reactions that one wishes to model. In the above script file, *x*1-*x*4 represent the starting concentrations of D-glucaric acid (*x*1), D-glucaro-1,4-lactone(*x*2), D-glucaro-6,3-lactone(*x*3) and D-glucaro-1,4;6,3-dilactone(*x*4). The rate constant values (k1-k8) have been left empty in the above script file. Estimates of these rate constants (from experimental data) are added and these can be altered to improve the fit between simulated data and actual experimental data.

By using the "xlsread" command in MATLAB it is possible to import experimental data from an Excel spreadsheet. This can then be plotted in MATLAB. The above plotting commands can be altered so that simulated data and the actual data can be plotted on the same graph.

6.6 Estimates of Rate Constants

The main aim of the simulation was to obtain good estimates of the eight rate constants involved in the equilibration of aqueous D-glucaric acid. To achieve this is was necessary to begin simulations with reasonable estimates of the rate constants for each reaction. Although literature was consulted[137-140] in attempt to find rate constants that may relate to the current situation, it was decided that it would be more accurate to determine estimates of rate constants directly from the experimental data. As the reactions are pseudo first order, the resulting rate constants will have s^{-1} as units.

6.6.1 Determination of Equilibrium Constants

Determining the equilibrium constant for each set of reactions makes estimating the rate constants easier, as well as providing some useful stand-alone information. If the experimental data is consistent, the equilibrium constants obtained from the data from each set of experiments should be similar after experimental error is taken into account. The following shows the relationships that make determining equilibrium constants beneficial for making estimations of rate constants of the equilibration of D-glucaric acid:

$$K_1 = \frac{[1]_{eq}}{[2]_{eq}} = \frac{k_1}{k_2}$$

$$K_2 = \frac{[3]_{eq}}{[1]_{eq}} = \frac{k_3}{k_4}$$

$$K_3 = \frac{[4]_{eq}}{[3]_{eq}} = \frac{k_5}{k_6}$$

$$K_4 = \frac{[2]_{eq}}{[4]_{eq}} = \frac{k_7}{k_8}$$

$$K_1 K_2 K_3 K_4 = \frac{[1]_{eq}[3]_{eq}[4]_{eq}[2]_{eq}}{[2]_{eq}[1]_{eq[}3]_{eq}[4]_{eq}} = \frac{k_1 k_3 k_5 k_7}{k_2 k_4 k_6 k_8} = 1$$

After visual inspection of the Excel graphs plotted from the experimental data is was decided that equilibrium was reached at 80,000 seconds. Therefore the concentration of each species at the end of each set of experiments was used in the calculation of the equilibrium constants.

Experiment Set	K_1	K_2	K_3	K_4
D-Glucaro-1,4- lactone plus Equivalent DCl	1.497	0.4765	0.1272	11.0233
D-Glucaro-1,4- lactone plus Equivalent DCl Duplicate	1.3103	0.5049	0.1560	9.6922
D-Glucaro-1,4- lactone plus One and a Half Equivalent DCl	1.537	0.6161	0.0447	23.6355
D-Glucaro-1,4- lactone plus One and a Half Equivalent DCl Duplicate	1.4804	0.4897	0.05496	25.0977
D-Glucaro-1,4;6,3- dilactone plus Equivalent DCl	1.3089	0.6375	0.0997	12.0233
D-Glucaro-1,4;6,3- dilactone plus Equivalent DCl Duplicate	1.3378	0.6321	0.0842	14.0521
D-Glucaro-1,4;6,3- dilactone plus One and a Half Equivalent DCl	1.1858	0.6956	0.0860	14.0928
D-Glucaro-1,4;6,3- dilactone plus One and a Half Equivalent DCl Duplicate	1.2611	0.6501	0.9109	13.3902

Table 6.1 *Equilibrium constants from experimental data*

Overall the equilibrium constants were very similar between experiment sets. This indicates that the data is accurate. The only equilibrium constants that deviated from what was expected was those (K_3 and K_4) involving the concentration of the D-glucaro-1,4;6,3-dilactone in the experiment sets with D-glucaro-1,4-lactone and one and a half molar equivalents of DCl. This is possibly due to the fact that 80,000 seconds is not long enough for the dilactone species to reach its equilibrium concentration in these experiment sets.

6.6.2 Use of Initial Growth and Decay Rates to Estimate Rate Constants

Estimations of rate constants can be made by calculating the initial growth and decay rates of a species. For a decaying species it is possible to get an estimate of

the rate constant for the reaction causing its decay by dividing the initial rate of its decay by the average absolute concentration of the decaying species during the time the initial rate is determined. For a growing species, the rate constant for the growth reaction is obtained by dividing the initial rate of growth of the growing species by the absolute concentration of the species it is growing from. These assumptions are based on the fact that reverse reactions are unimportant in the initial stages of a reaction.

Using data from the experiment sets that have D-glucaro-1,4;6,3-dilactone as the starting species, the initial growth rate of D-glucaro-6,3-lactone divided by the average concentration of the D-glucaro-1,4;6,3-dilactone (as the 6,3-lactone is growing from the dilactone initially) over this growth period produces an approximation of k_6. Similar calculations can be made using the data for D-glucaro-1,4-lactone to yield an approximation of k_7. The initial growth rate of D-glucaric acid can be used to estimate k_4 when it is divided the average concentration of D-glucaro-6,3-lactone over this growth period. By examining the graphs of experimental data it is sensible to conclude that D-glucaric acid is initially growing from D-glucaro-6,3-lactone. This is why the concentration of D-glucaro-6,3-lactone falls away after a period of rapid rising.

The data from the experiment sets that have D-glucaro-1,4-lactone as the starting species can be used in a similar way to produce an estimate of k_1 (from both the initial growth rate of D-glucaric acid divided by the average concentration of D-glucaro-1,4-lactone and from the initial decay rate of D-glucaro-1,4-lactone divided by its average concentration over this period). An estimate of k_3 can be obtained by dividing the initial growth rate of D-glucaro-6,3-lactone by the average concentration of D-glucaric acid over this period, because of reasons similar to those discussed above.

As the equilibrium constant of a reaction represents the ratio of the rate constants for the forward and reverse reaction, it is possible to derive estimates of rate constants for those reactions that initial growth and decay calculations cannot produce. For the current situation it is possible to gain estimations of k_1, k_3, k_4, k_6

and k_7 from the experimental data. By using the calculated equilibrium constants above it was possible to calculate estimates of k_2, k_3, k_4, k_5 and k_8.

The estimates were done per experiment set, using the equilibrium constants for that set. The results for the experiments that had D-glucaro-1,4-lactone as the starting species are shown (**Table 6.2**), as are the results for the experiments starting with D-glucaro-1,4;6,3-dilactone as the starting species (**Table 6.3**).

Experiment Set	k_1	k_2	k_3	k_4
D-Glucaro-1,4-lactone plus Equivalent DCl	$5.243e^{-5}$	$3.503e^{-5}$	$1.799e^{-5}$	$3.775e^{-5}$
D-Glucaro-1,4-lactone plus Equivalent DCl Duplicate	$6.460e^{-5}$	$4.930e^{-5}$	$1.059e^{-4}$	$2.097e^{-4}$
D-Glucaro-1,4-lactone plus One and a Half Equivalent DCl	$8.579e^{-5}$	$5.582e^{-5}$	$1.195e^{-4}$	$1.939e^{-4}$
D-Glucaro-1,4-lactone plus One and a Half Equivalent DCl	$7.891e^{-5}$	$5.330e^{-5}$	$2{,}460e^{-5}$	$5.023e^{-5}$

Table 6.2 *Estimates of rate constants from experiment sets with D-glucaro-1,4-lactone as the starting species at 27°C (units are s^{-1})*

Experiment Set	k_3	k_4	k_5	k_6	k_7	k_8
D-Glucaro-1,4;6,3-dilactone plus Equivalent DCl	$4.111e^{-5}$	$6.450e^{-5}$	$5.350e^{-5}$	$5.362e^{-4}$	$1.303e^{-4}$	$1.084e^{-5}$
D-Glucaro-1,4;6,3-dilactone plus Equivalent DCl Duplicate	$6.129e^{-5}$	$9.697e^{-5}$	$3.930e^{-5}$	$4.671e^{-5}$	$9.520e-^{5}$	$6.780e^{-6}$
D-Glucaro-1,4;6,3-dilactone plus One and a Half Equivalent DCl	$4.413e^{-4}$	$6.344e^{-4}$	$3.506e^{-5}$	$4.076e^{-5}$	$9.896e^{-5}$	$7.022e^{-6}$
D-Glucaro-1,4;6,3-dilactone plus One and a Half Equivalent DCl Duplicate	$7.107e^{-5}$	$1.090e^{-4}$	$1.508e^{-4}$	$1.656e^{-4}$	$2.065e^{-4}$	$1.542e^{-5}$

Table 6.3 *Estimates of rate constants from experiment sets with D-glucaro-1,4;6,3-dilactone as the starting species at 27°C (units are s^{-1})*

When the estimations of rate constants were compared there were no general differences between those estimated from experiment sets with one molar equivalent of DCl and those experiment sets with one and a half molar equivalents of DCl. Although acidity often affects rate constants, when one looks at the mechanism proposed above it is apparent that one molar equivalent of DCl would convert all of the starting species into its protonated form. Therefore one and a half molar equivalents of DCl represents a small excess and may not systematically affect the rate constants. Therefore values from all experiment sets were used to produce average estimations of the eight rate constants (**Table 6.4**).

Rate Constant	Averaged Estimates (s^{-1})
k_1	$7.043e^{-5}$
k_2	$4.836e^{-5}$
k_3	$1.103e^{-4}$
k_4	$1.746e^{-4}$
k_5	$6.967e^{-5}$
k_6	$1.973e^{-4}$
k_7	$1.327e^{-4}$
k_8	$1.0014e^{-5}$

Table 6.4 Average *estimates of rate constants from all experiment sets at 27°C*

6.6.3 Starting Set of Rate Constants for Simulations

When the set of estimated rate constants obtained from the initial growth and decay rates was examined it was found that the product of the equilibrium constants ($K_1K_2K_3K_4$) when these estimated rate constants were used to produce the equilibrium constants was no longer equal to one. This is due to the fact that the calculated equilibrium constants for each experiment set were used to calculate those rate constants that could not be calculated from experimental data for that set. This method produces a more accurate estimation of a particular rate constant for a particular experiment set. However because average equilibrium constants were not used, the small differences between the calculated equilibrium constants (due to experimental error) for each experiment set produce the discrepancy seen in the product of the equilibrium constants produced with the averaged rate constants. It is very fundamental that the sum of the equilibrium constants equals one; therefore it is necessary to alter one of the rate constants to ensure this. Experimental noise is more significant in smaller equilibrium constants therefore inspection of the calculated equilibrium constants suggested that the rate constants that produce K_3 should be altered so that the product of the equilibrium constants was indeed one. K_3 represents the ratio of k_5 and k_6 and its calculated value is considerably lower than the other calculated equilibrium constants. By changing k_5 from 6.967e-5 to 1.742e-5, the product of the

equilibrium constants formed by using the estimations of the rate constants is 1.07. This set of rate constants could now be used for simulations (**Table 6.5**).

Rate Constant	Rate Constants for First Simulation (s^{-1})
k_1	$7.043e^{-5}$
k_2	$4.836e^{-5}$
k_3	$1.103e^{-4}$
k_4	$1.746e^{-4}$
k_5	$1.742e^{-5}$
k_6	$1.973e^{-4}$
k_7	$1.327e^{-4}$
k_8	$1.0014e^{-5}$

Table 6.5 *Starting set of rate constants at 27°C for simulations*

6.7 MATLAB Simulations

6.7.1 Evaluation of Initial Set of Rate Constants

The above set of rate constants was entered in the appropriate place in the MATLAB script file. Separate script files were created for each experiment set. The actual starting concentrations of the species in each experiment set were entered into the correct script file. Simulations were run and the results were first plotted using the plotting commands detailed above so that each species graph was represented on a subplot. This allowed the simulation of each species to be evaluated individually. The plotting commands were then altered so that actual data and simulated data were plotted on the same graph. This allowed comparison between the simulation and actual experimental data, and the closeness of fit between the two could be evaluated. A key is provided (**Figure 6.2**) Although this set of rate constants produced relatively good fits for the experiment sets that had D-glucaro-1,4-lactone as the starting species (**Figure 6.3**), the fits produced for the experiment sets that had D-glucaro-1,4;6,3-dilactone as the starting species (**Figure 6.4**) were not as good. In these experiment sets, the simulations failed to produce that rapid rise then decay of D-

glucaro-6,3-lactone. However as a stating point gained purely from experimental data the fits between actual and simulated data were promising.

Key:
(1) = D-Glucaric Acid
(2) = D-Glucaro-1.4-lactone
(3) = D-Glucaro-6,3-lactone
(4) = D-Glucaro-1,4;6,3-dilactone

Figure 6.2 *Key to the species labels in MATLAB simulations*

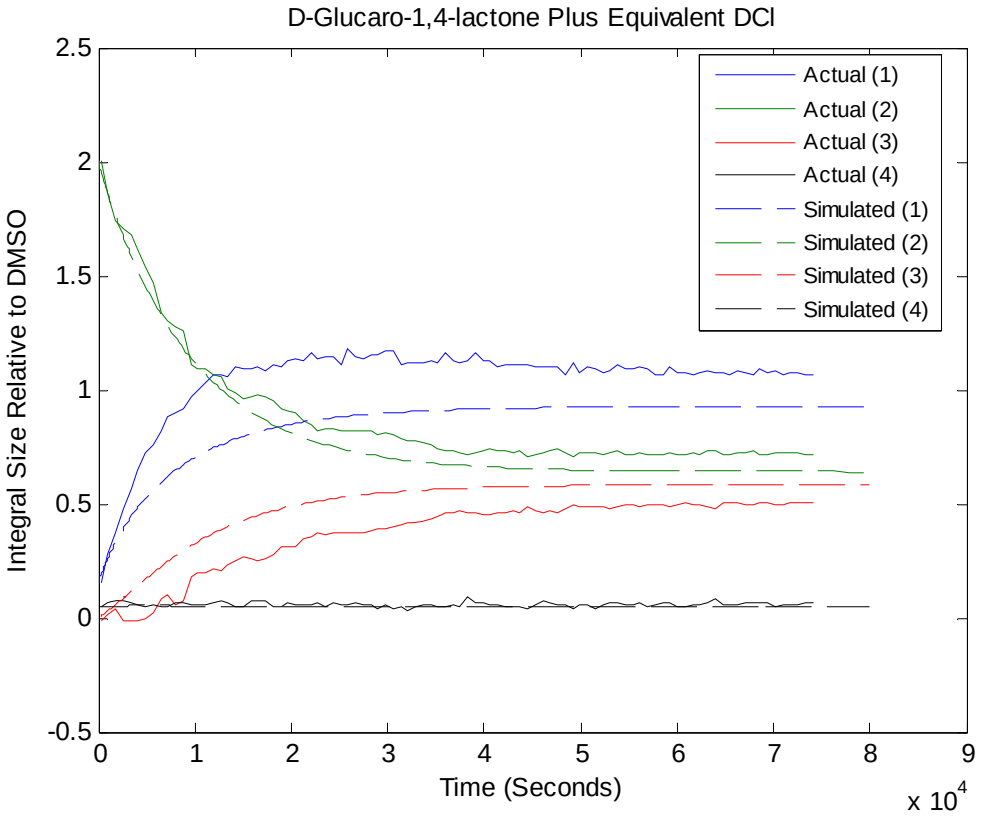


Figure 6.3 *MATLAB graph of actual data and simulated data using the first set of rate constants and experimental data from the D-glucaro-1,4-lactone Plus Equivalent DCl experiment set*

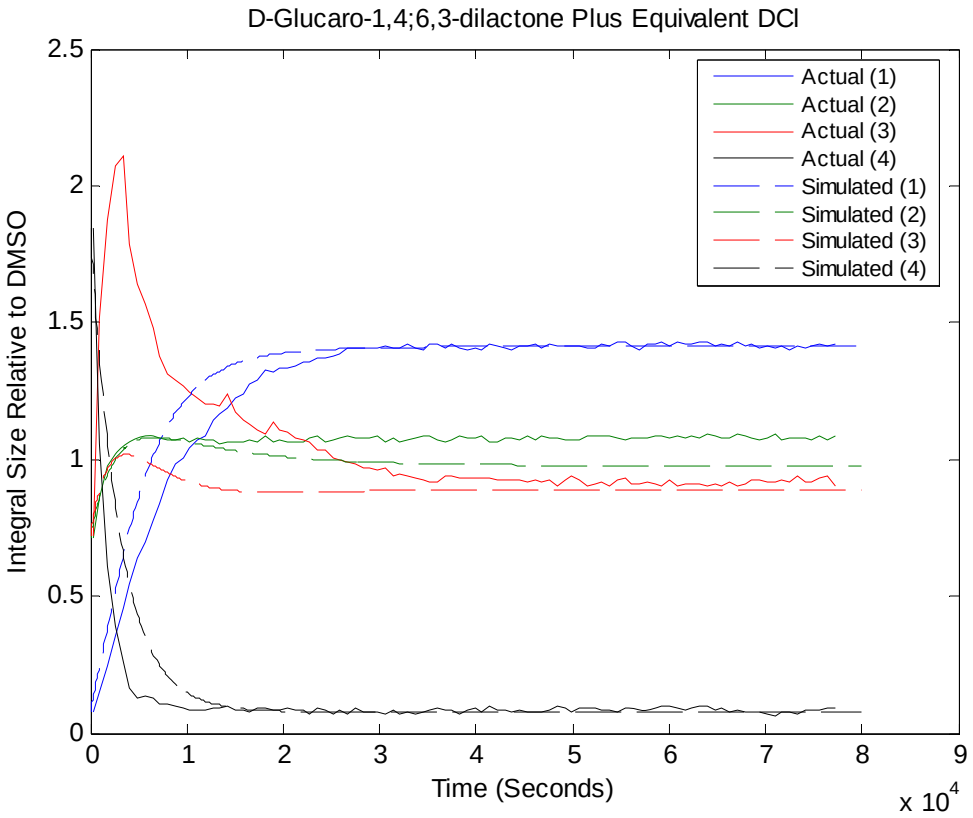


Figure 6.4 *MATLAB graph of actual data and simulated data using the first set of rate constants and experimental data from the D-glucaro-1,4;6,3-dilactone Plus Equivalent DCl experiment set*

6.7.2 Systematic Alteration of Rate Constants

To obtain an insight into how the rate constants affected the simulation, each pair of rate constants was systematically increased by 25% and decreased by 25%, and the results were plotted. By altering each pair of rate constants by the same amount the ratio (that was obtained from experimental data) was not changed and neither was the product of the resulting equilibrium constants. This process was extremely time consuming, and efficient records were needed to ensure that one did not become lost and end up making changes that altered the ratios of the pairs of rate constants. It was essential also that the first set of rate constants was kept as a reference point to go back to if the fits became worse than the original starting point

6.7.3 Trial and Error

The systematic series of increasing and decreasing the rate constants provided a base for trial and error changes. By combining these results with intuition, trial and error changes were made in attempt to improve the fits. Fitting a four-parameter system is a challenging proposition, especially when things are as strongly coupled as in this system. Therefore a lot of patience and perseverance was needed. Simulations are very sensitive to the equilibrium and rate constant values chosen, therefore minor changes can sometimes change the shape of the simulated graph completely.

6.8 Final Rate Constants and Simulations

After a considerable amount of work a final set of rate constants was arrived at. This set maintained the previous ratios, produced equilibrium constants that had a product of 1.08 and produced simulations that had good fits (**Table 6.6**).

Rate Constant	Final Rate Constants
k_1	7.043e-5
k_2	4.836e-5
k_3	5.515e-5
k_4	8.700e-5
k_5	1.742e-4
k_6	1.973e-3
k_7	6.635-4
k_8	5.007-5

Table 6.6 *Final set of rate constants at 27°C*

This set of rate constants captured the sharp peak of the rapid rise then decay of the D-glucaro-6,3-lactone, as well as producing good fits for the other species. Although it is relatively easy to fit a set of rate constants to an individual experiment, the challenge comes when that same set of rate constants used to simulate data that has come from quite different experiments. This is the case in the current situation as the different starting species produce very different graphs. This set of rate constants interprets well four sets of data from quite

different experiments as well as some of their duplicates. A key is given (**Figure 6.5**) and the results of the simulations (**Figures 6.6 – 6.11**) are shown.

Key:
(1) = D-Glucaric Acid
(2) = D-Glucaro-1.4-lactone
(3) = D-Glucaro-6,3-lactone
(4) = D-Glucaro-1,4;6,3-dilactone

Figure 6.5 *Key to the species labels in MATLAB simulations*

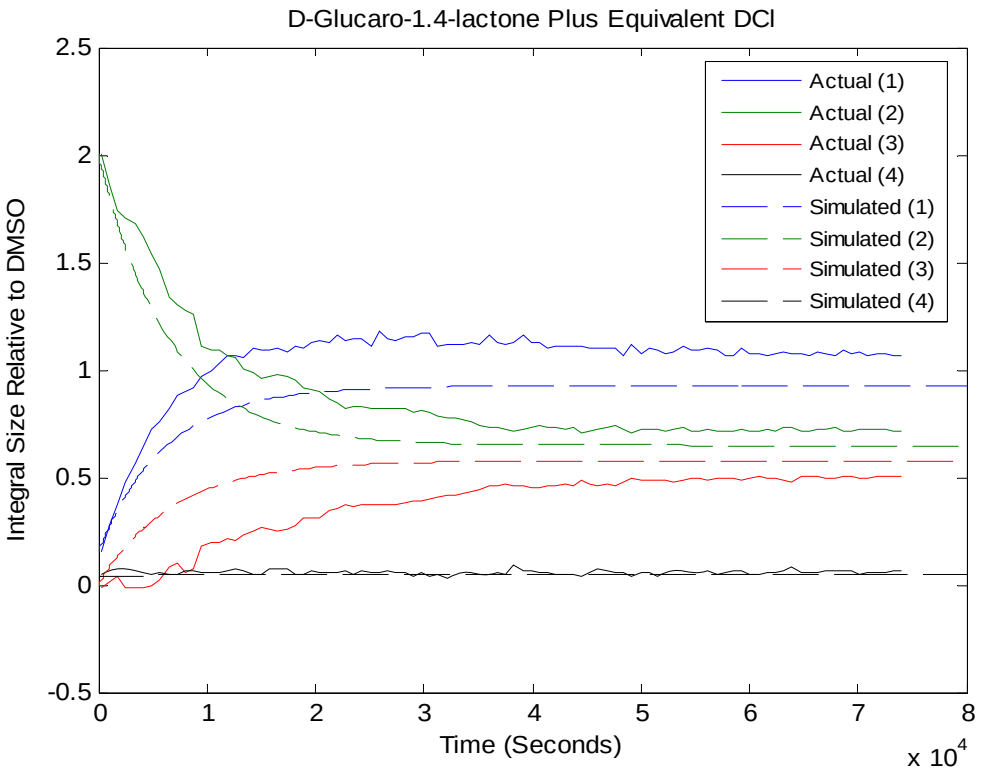


Figure 6.6 *MATLAB graph of actual data and simulated data using the final set of rate constants and experimental data from the D-glucaro-1,4-lactone Plus Equivalent DCl experiment set*

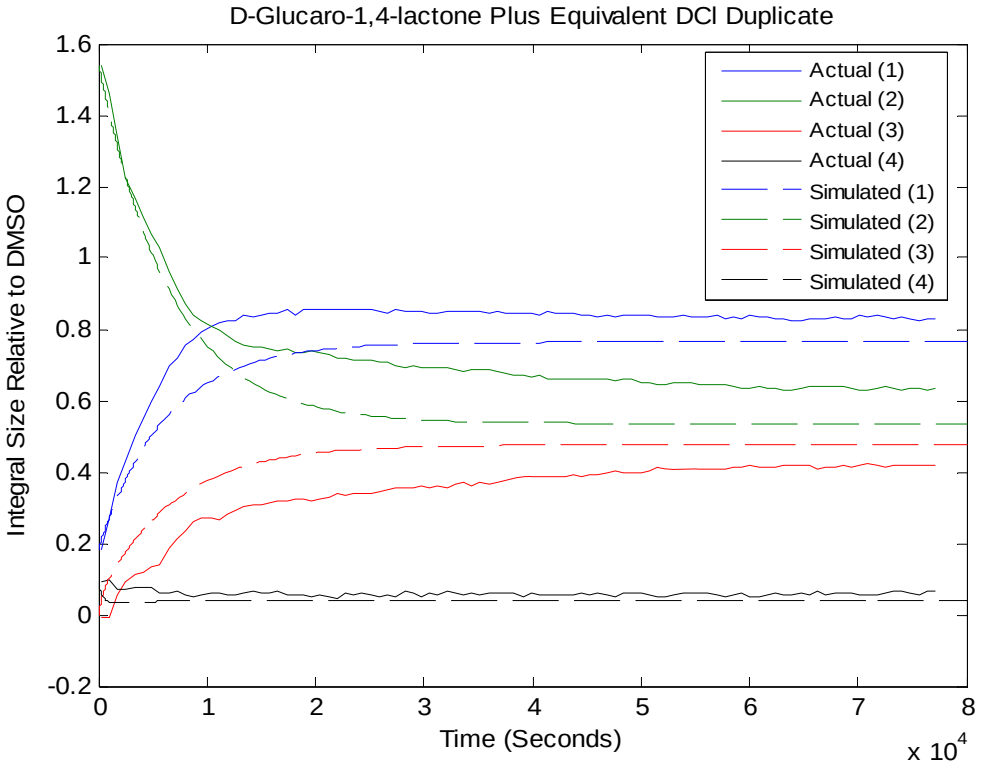


Figure 6.7 *MATLAB graph of actual data and simulated data using the final set of rate constants and experimental data from the D-glucaro-1,4-lactone Plus Equivalent DCl Duplicate experiment set*

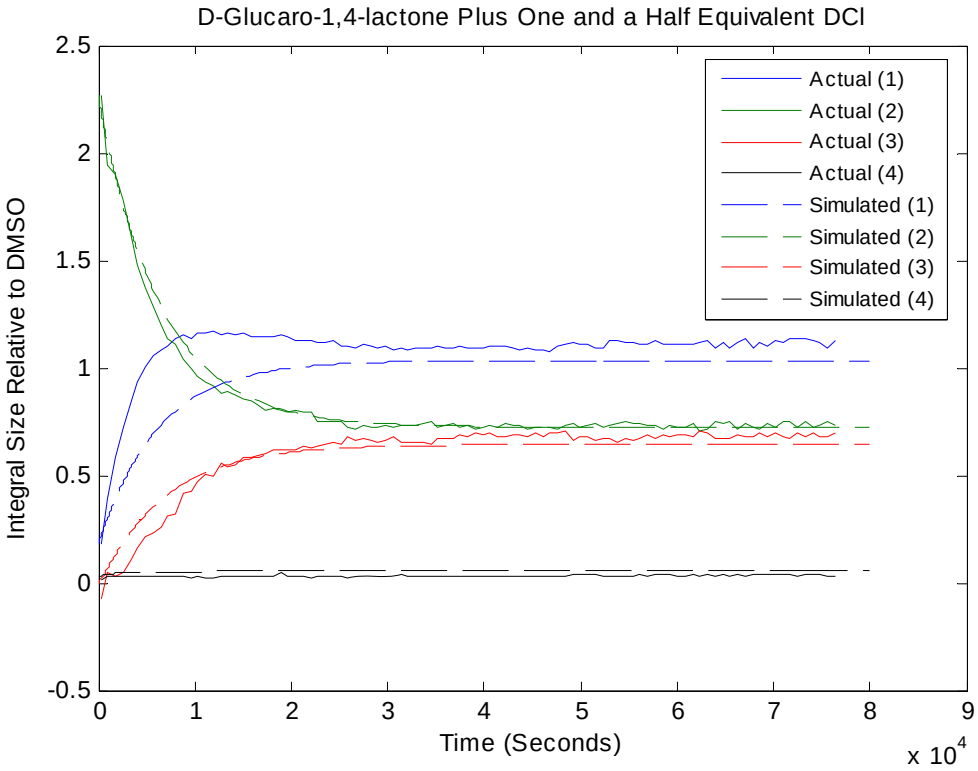


Figure 6.8 *MATLAB graph of actual data and simulated data using the final set of rate constants and experimental data from the D-glucaro-1,4-lactone Plus One and a Half Equivalent DCl experiment set*

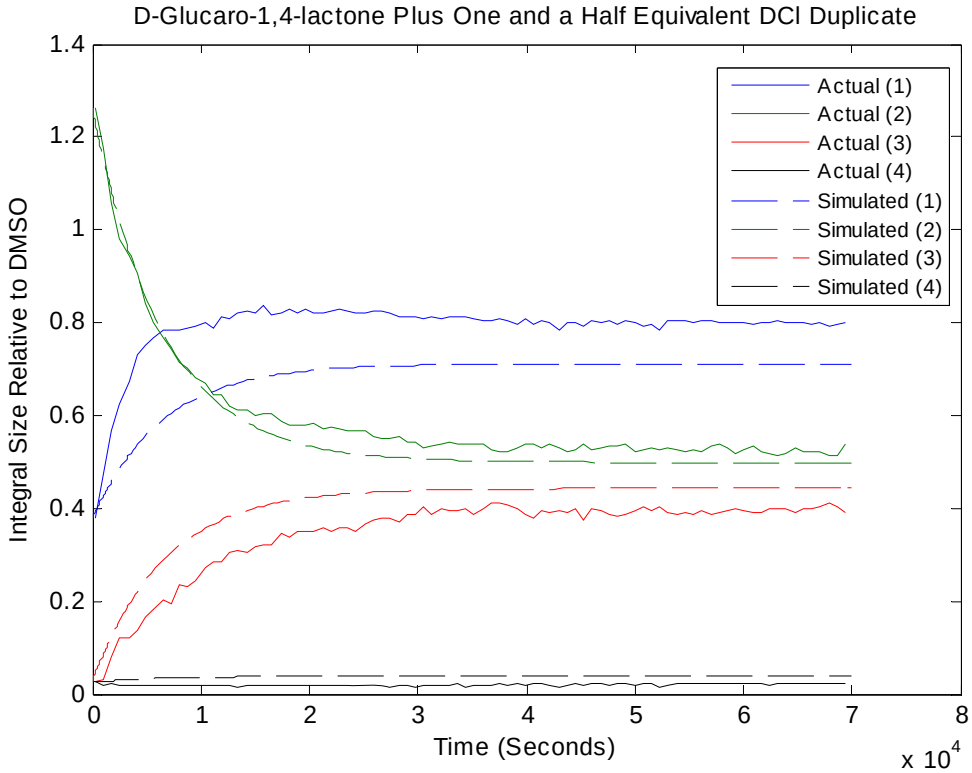


Figure 6.9 *MATLAB graph of actual data and simulated data using the final set of rate constants and experimental data from the D-glucaro-1,4-lactone Plus One and a Half Equivalent DCl Duplicate experiment set*

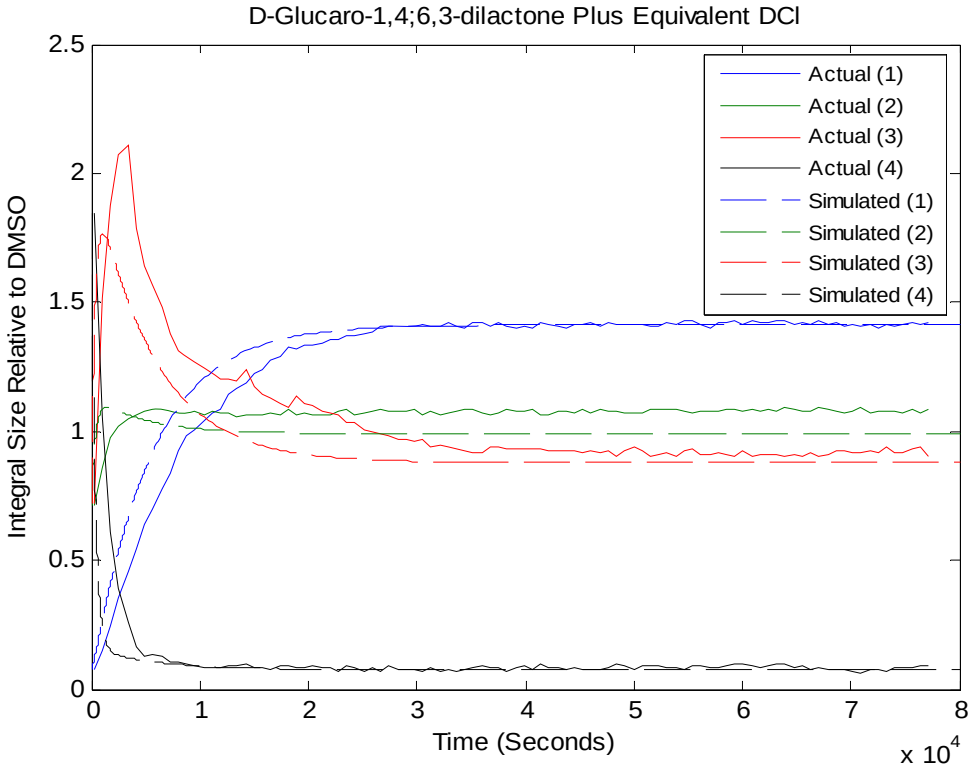


Figure 6.10 *MATLAB graph of actual data and simulated data using the final set of rate constants and experimental data from the D-glucaro-1,4;6,3-dilactone Plus Equivalent DCl experiment set*

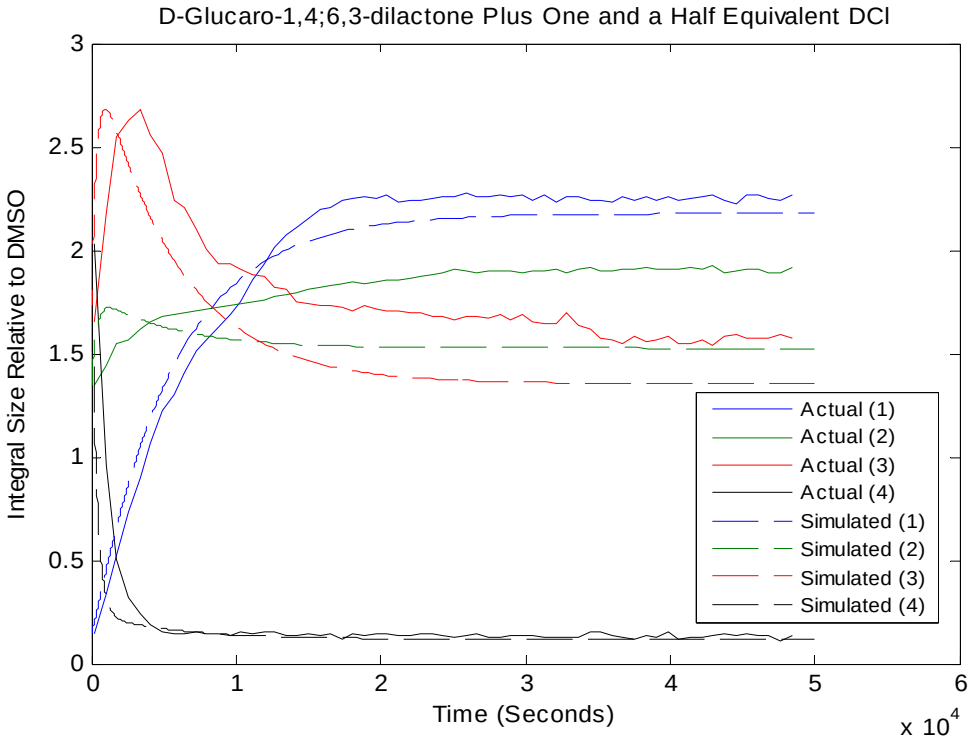


Figure 6.11 *MATLAB graph of actual data and simulated data using the final set of rate constants and experimental data from the D-Glucaro-1,4;6,3-dilactone Plus One and a Half Equivalent DCl experiment set*

6.9 Discussion and Sources of Errors

Although the final set of rate constants produced satisfactory fits for most of the data, the simulations of two experiment sets did not closely fit the experimental data for D-glucaro-6,3-lactone.

When the D-Glucaro-1,4;6,3-dilactone plus DCl duplicate and the D-Glucaro-1,4;6,3-dilactone plus one and a half Equivalent DCl duplicate experiment sets were simulated, the shape of the simulated line for D-glucaro-6,3-lactone did not match the line for the actual experimental data. As stated section 5.3 it was found that the concentration of the D-glucaro-6,3-lactone was actually higher than the concentration of the D-Glucaro-1,4;6,3-dilactone at the start of these experiment sets. Clearly this should not be the case as when one starts out with D-Glucaro-1,4;6,3-dilactone it is expected that this species will have the highest relative concentration at the start of the experiment set. This is an indication of how fast the D-Glucaro-1,4;6,3-dilactone decomposes in aqueous acidic solution.

The results from these two experiment sets suggest that there has been a slightly longer delay between sample preparation and the middle of the first experiment in these experiment sets compared to all of the other sets. This means that the experimental starting concentrations for these experiment sets are actually the concentrations after more equilibration has occurred than in the other sets. It was decided that, because of this error, these two experiment sets would not be included in the kinetic analysis. Trying to fit the rate constants to these sets may cause the set of rate constants to not produce good fits for the rest of the data.

Overall the fits between the simulated and actual data of the remaining experiment sets are very close. It would not be possible to match the simulated data exactly with the experimental data due to the errors involved with this type of calculation. There is experimental error involved in the calculation of the equilibrium constants that the rate constants are constrained by. There is also error in the actual experimental data. Ignoring the differences in acidity may also introduce error.

Although it is possible to use the final set of rate constants to "back-calculate" experimental equilibrium constants, given that the equilibrium constants from each experimental set were used to produce the ratios used to constrain the rate constants and the rate constants were averaged, back-calculation will only produce an estimate of the experimental equilibrium constant. As the value of k_5 was altered to ensure that the product of the equilibrium constants produced from the rate constants equalled one, K_3 can not be calculated *via* back-calculation.

As the simulated date and the actual experimental data fit it is likely that the proposed mechanism and the final set of rate constants are feasible. However as with any simulations, the discovery of a set of rate constants that simulates the proposed mechanism correctly may be due to chance rather than the mechanism and rate constants being accurate.

Chapter Seven – Conclusions and Further Work

7.1 General Conclusions

The development of the ^{1}H NMR spectroscopy method allowed the kinetics of the equilibration of aqueous D-glucaric acid to be investigated. The Bruker Multi_zgvd program allowed delays to occur between ^{1}H NMR experiments, which allowed 100 NMR experiments to be run on the same sample without having to manually set up each experiment.

The use of DMSO as an internal standard provided a convenient method of describing the relative concentrations of the four species. Manual processing and integration, although time consuming, provided precise data that contributed to the reproducibility between original and duplicate sets.

7.2 Summary of Results

Under neutral conditions D-glucaro-1,4-lactone was relatively stable against equilibration. This agreed with previous work[11]. In acidic conditions D-glucaro-1,4-lactone decomposed to its relative equilibrium concentration while the relative concentrations of D-glucaro-6,3-lactone (in those experiment sets with this data) and D-glucaric acid increased to their equilibrium concentrations. When D-glucaro-1,4-lactone was the starting species, the relative concentration of D-glucaro-1,4;6,3-dilactone remained low. The rate of ring-closing of the 1,4-lactone must be comparative to its ring opening as D-glucaro-1,4-lactone formed and had a relative equilibrium concentration that was slightly higher than that of D-glucaro-6,3-lactone(under acidic conditions). When k_1 and k_2 were compared it was found that they are of similar size.

D-Glucaro-1,4;6,3-dilactone was unstable in neutral conditions and rapidly equilibrated. Under acidic conditions this equilibration was even more rapid and

its relative concentration dropped to its low equilibrium concentration within the first few experiments. The relative concentration of D-glucaro-6,3-lactone increased rapidly then steadily decreased to its equilibrium concentration when D-glucaro-1,4;6,3-dilactone was the starting species. The relative concentration of D-glucaro-1,4-lactone steadily increased until it reached its equilibrium concentration.

7.3 Comparison of Results with Reported Work

As a final set of rate constants were produced, previous qualitative work can be compared to the quantitative data produced. The equilibrium (**Figure 7.1**) and set of final set of rate constants (**Table 7.1**) are included for clarity.

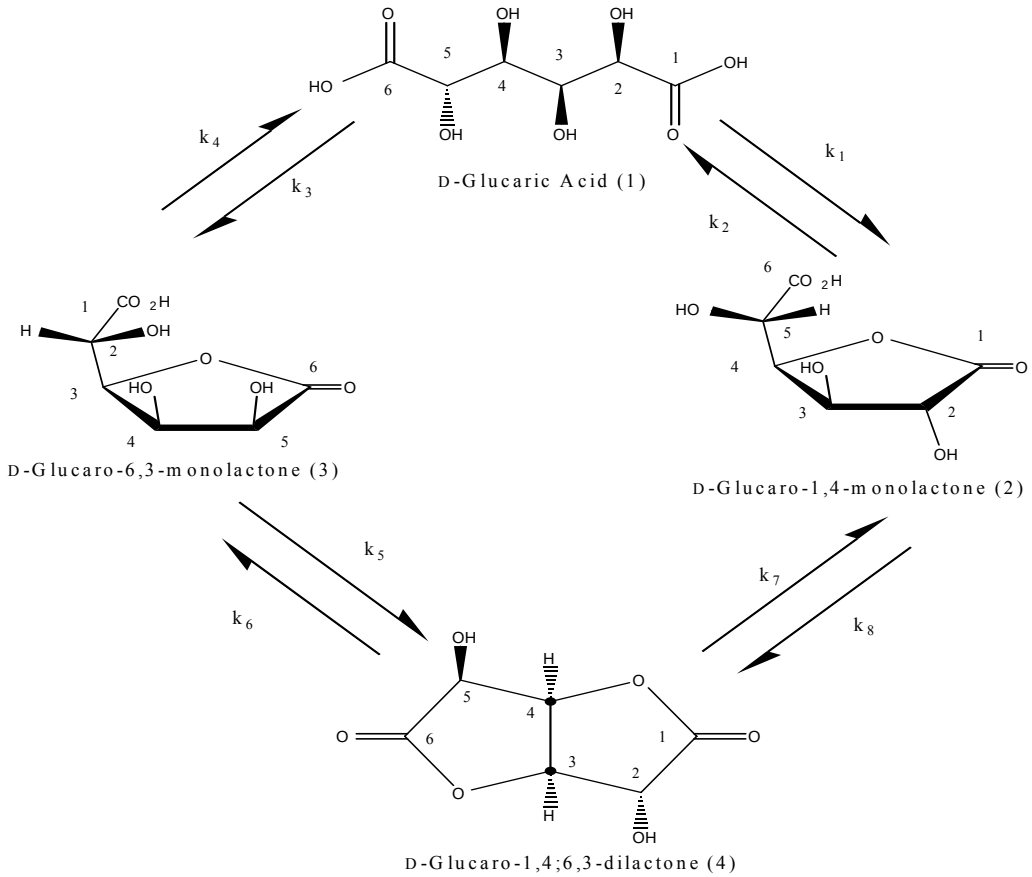


Figure 7.1 *Equilibrium of aqueous D-glucaric acid with rate constants labelled*

Rate Constant	Final Rate Constants
k_1	7.043e-5
k_2	4.836e-5
k_3	5.515e-5
k_4	8.700e-5
k_5	1.742e-4
k_6	1.973e-3
k_7	6.635-4
k_8	5.007-5

Table 7.1 *Final set of rate constants at 27°C*

It was reported that the reciprocal transformation between the monolactones was caused *via* the free dicarboxylic acid pathway, and not the dilactone[11]. The results from the experiment sets with D-glucaro-1,4-lactone confirm this. The relative concentration of D-glucaric acid rose to its equilibrium concentration but the relative concentration of D-glucaro-1,4;6,3-dilactone remained at a low level. Therefore it is possible for D-glucaro-6,3-lactone to form from the relatively high concentration of D-glucaric acid present while it is unlikely to form from the relatively low amount of D-glucaro-1,4;6,3-dilactone.

The fast mutarotation of D-glucaro-1,4;6,3-dilactone was reported to be due to the instability of the 1,4-lactone ring in aqueous solution[10]. Opening of the 1,4-lactone ring would rapidly produce the 6,3-lactone. This is what is seen in the experiment sets with D-glucaro-1,4;6,3-dilactone as the starting material. The relative starting concentration of D-glucaro-6,3-lactone is high (in some sets higher than that of D-glucaro-1,4;6,3-dilactone) and this rapidly increased over the first few experiments. This result indicates that k_6 is large. In fact k_6 is by far the largest rate constant and is approximately10-100 times larger than the other rate constants.

Previous work under neutral conditions, found that D-glucaric acid lactonised to give the 6,3-lactone spontaneously as the first product but 1,4 formed more slowly[12]. This would suggest that k_3 is larger than k_1. However when k_1 and

k_3.are compared it is found that they are similar sizes. This may be because that k_1 and k3 relate to acidic conditions, and under neutral conditions it is highly likely that the values for these rate constants would be different.

Kiely *et. al*. reported that the ring opening of the 1,4-lactone ester and 1,4-lactone amide was faster than the ring opening of the corresponding 6,3-lactone ester and 6,3-lactone amide, when the monolactones were reacted with *n*-propylamine (i.e. under alkaline conditions)[73]. A similar result was reported when the transesterification (at 90°C and under anhydrous conditions) of D-glucaro-1,4;6,3-dilactone produced 50% more of the product that required ring opening of the 1,4-lactone than the product that required the opening of the 6,3-lactone[130]. These results imply that k_6 is larger than k_7. This is what is found with k_6 being approximately 10 times larger than k_7.

Previous work investigating the equilibration of D-glucaro-1,4-lactone and D-glucaro-6,3-lactone reported that under acidic conditions (*2N* sulphuric acid or excess cation exchange resin) both monolactones had similar equilibrium concentrations[11]. This is different to the experimental results which found the relative equilibrium concentration of D-glucaro-1,4-lactone to be slightly higher than its D-glucaro-6,3- counterpart. However this work also investigated the relative equilibrium conditions of the monolactones under neutral conditions and found that the relative concentration of D-glucaro-6,3-lactone was higher than that of D-glucaro-1,4-lactone. This result indicates how the relative equilibrium concentrations of the species can change with different conditions.

Although it is useful to compare the current experimental results with results from similar investigations, it is important to note that no reports from the literature have the same conditions as the current experimental results. As kinetic results are very dependant on conditions such as temperature and pH, it is impossible to correlate results from experiments that do not have identical conditions.

7.4 Evaluation of Mechanism

The proposed mechanism and final set of rate constants provided good fits between actual experimental data and the simulated data, which suggested that these were correct. The equilibrium constants calculated from the experimental data provided useful information for the simulations as well as providing applicable information on the equilibration of aqueous D-glucaric acid.

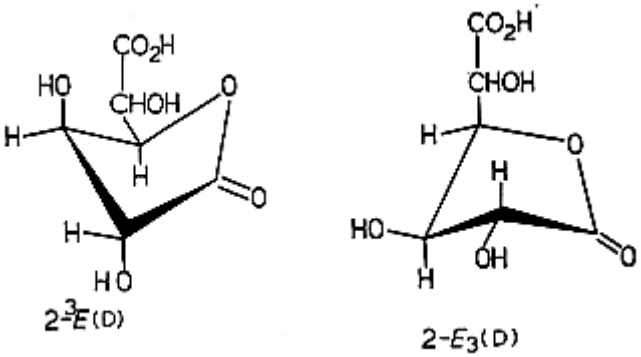
7.5 Further Work

Further work that is possible includes investigation of the equilibration under alkaline conditions and when the other two species (D-glucaric acid and D-glucaro-6,3-lactone)are used as starting species. The equilibration under alkaline conditions would be particularly useful as after the aqueous D-glucaric acid is esterified with an acid catalyst it is reacted with an amine; hence it is subjected to alkaline conditions.

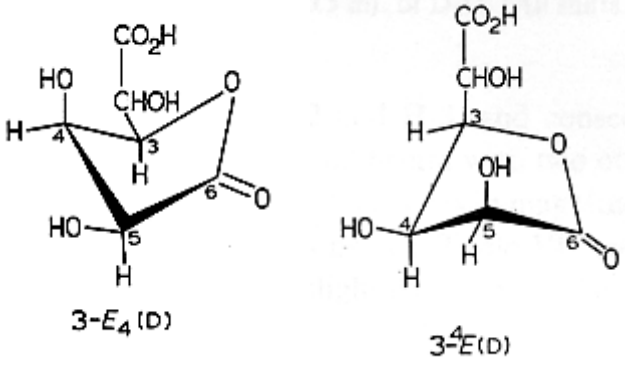
Overall this work represents a thorough investigation into some of the aspects of the equilibration of D-glucaric acid in aqueous solution. The results will be applicable to the production of poly(D-glucaramides) both as background information on the equilibrium of aqueous D-glucaric acid and more directly when activated D-glucaric acid is used to produce PHPAs.

Appendix One – Conformations

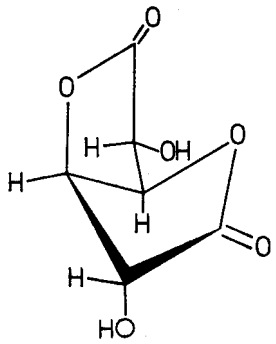
D-Glucaro-1,4-lactone[12]:



D-Glucaro-6,3-lactone[12]:

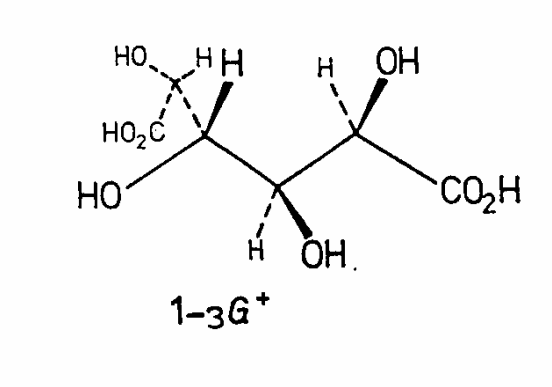


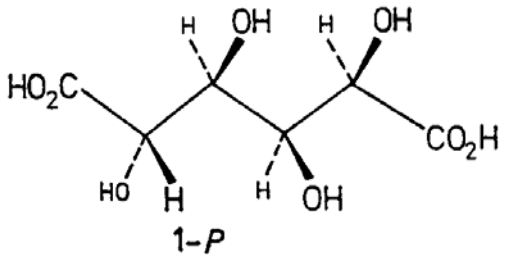
D-Glucaro-1,4;6,3-dilactone[12]:

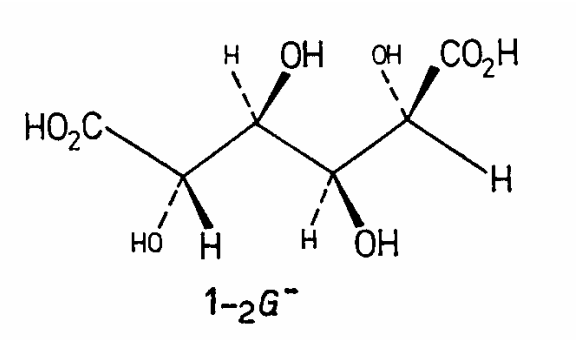


4-^{3}E:E_4(D)

D-Glucaric Acid[12]:







Appendix Two – NMR Spectroscopy Parameters

^{1}H NMR Spectroscopy:

Bruker Notation	Description	Value
TD	Size of FID	32768
NS	Number of Scans	128
DS	Number of Dummy Scans	0
SW(PPM)	Spectral Width (ppm)	20.5671
SW(Hz)	Spectral Width (Hz)	6172.84
AQ	Acquisition Time (seconds)	2.6542580
P1(μs)	F1channel – 90 Degree High Power Pulse	10.5
PL1(dB)	F1channel-Power Level for Pulse	0
FIDRES(Hz)	FID Resolution	0.188380
FW(Hz)	Filterwidth	90000
O1(Hz)	Transmitter Frequency Offset (Hz)	300.1318534
O1(PPM)	Transmitter Frequency Offset (ppm)	1853.43

^{13}C NMR Spectroscopy:

Bruker Notation	**Description**	**Value**
TD	Size of FID	32768
NS	Number of Scans	5120
DS	Number of Dummy Scans	8
SW(PPM)	Spectral Width (ppm)	238.3239
SW(Hz)	Spectral Width (Hz)	23980.814
AQ	Acquisition Time (seconds)	0.6832628
P1(μs)	F1channel – 90 Degree High Power Pulse	10
PL1(dB)	F1channel-Power Level for Pulse	-5.00
FIDRES(Hz)	FID Resolution	0.731836
FW(Hz)	Filterwidth	90000
O1(Hz)	Transmitter Frequency Offset (Hz)	10060.79
O1(PPM)	Transmitter Frequency Offset (ppm)	99.995

COSY Spectroscopy:

Bruker Notation	**Description**	**F2 Channel (^{1}H) Value**	**F1 Channel (^{1}H) Value**
TD	Size of FID	1024	256
SW(PPM)	Spectral Width (ppm)	2.0025	2.0025
SW(Hz)	Spectral Width (Hz)	801.282	801.281
AQ	Acquisition Time (seconds)	0.6390260	0.1597440
FIDRES(Hz)	FID Resolution	0.782502	3.13008
FW(Hz)	Filterwidth	90000	
NS	Number of Scans	40	
DS	Number of Dummy Scans	8	
P1(μs)	F1channel – 90 Degree High Power Pulse	10.50	
PL1(dB)	F1channel-Power Level for Pulse	3.00	

HSQC Spectroscopy:

Bruker Notation	**Description**	**F2 Channel (^{1}H) Value**	**F1 Channel (^{13}C) Value**
TD	Size of FID	2048	256
SW(PPM)	Spectral Width (ppm)	2.0025	140.006
SW(Hz)	Spectral Width (Hz)	801.282	14086.833
AQ	Acquisition Time (seconds)	1.2780020	0.0090865
FIDRES(Hz)	FID Resolution	0.391251	55.5026691
FW(Hz)	Filterwidth	90000	
NS	Number of Scans	32	
DS	Number of Dummy Scans	8	
P1(μs)/P2(μs)	F1/F2channel – 90 Degree High Power Pulse	10.50	10.50
PL1(dB)/PL2(dB)	F1/F2channel- Power Level for Pulse	3.00	-5.00

HMBC Spectroscopy:

Bruker Notation	**Description**	**F2 Channel (^{1}H) Value**	**F1 Channel (^{13}C) Value**
TD	Size of FID	2048	128
SW(PPM)	Spectral Width (ppm)	2.0025	220.0000
SW(Hz)	Spectral Width (Hz)	801.282	22137.234
AQ	Acquisition Time (seconds)	1.2780020	0.0028911
FIDRES(Hz)	FID Resolution	0.391251	172.947144
FW(Hz)	Filterwidth	90000	
NS	Number of Scans	40	
DS	Number of Dummy Scans	16	
P1(μs)/P2(μs)	F1/F2channel – 90 Degree High Power Pulse	10.50	10.50
PL1(dB)/PL2(dB)	F1/F2channel- Power Level for Pulse	3.00	-5.00

Appendix Three – Raw Data

D-Glucaro-1,4-lactone			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0244	2.4715	0.0459
1015.5	0.027	2.4355	0.0496
1792.5	0.0243	2.3976	0.08
2569.5	0.0368	2.3681	0.0503
3346.5	0.0335	2.2925	0.062
4123.5	0.044	2.2118	0.0587
4900.5	0.0447	2.2079	0.0528
5677.5	0.0513	2.1502	0.0547
6454.5	0.0546	2.1321	0.0727
7231.5	0.055	2.1282	0.0774
8008.5	0.0605	2.1196	0.0876
8785.5	0.0663	2.1217	0.0704
9562.5	0.0702	2.0811	0.0832
10339.5	0.0758	2.1184	0.0815
11116.5	0.0787	2.0771	0.0834
11893.5	0.0859	2.0858	0.0733
12670.5	0.0849	2.098	0.0623
13447.5	0.0917	2.0863	0.0665
14224.5	0.0931	2.0718	0.0807
15001.5	0.0992	2.0888	0.073
15778.5	0.1117	2.0746	0.0713
16555.5	0.1098	2.0645	0.0877
17332.5	0.1142	2.0888	0.0883
18109.5	0.1183	2.0851	0.0711
18886.5	0.121	2.0768	0.0614
19663.5	0.1213	2.0784	0.0752
20440.5	0.1241	2.0463	0.0761
21217.5	0.1302	2.0419	0.0692
21994.5	0.1353	2.0487	0.0722
22771.5	0.1391	2.0405	0.0683
23548.5	0.1426	2.0417	0.0575
24325.5	0.1457	2.0445	0.0684

25102.5	0.1483	2.0552	0.0694
25879.5	0.1534	2.0543	0.0565
26656.5	0.1585	2.0358	0.0692
27433.5	0.1638	2.0377	0.0595
28210.5	0.1642	2.0504	0.0637
28987.5	0.1733	2.0125	0.0744
29764.5	0.1769	2.0144	0.076
30541.5	0.1852	2.0337	0.076
31318.5	0.1905	2.0356	0.0596
32095.5	0.1964	2.028	0.0654
32872.5	0.2047	2.0162	0.0671
33649.5	0.2048	2.0247	0.075
34426.5	0.2105	2.0317	0.0659
35203.5	0.2101	2.0203	0.067
35980.5	0.2147	2.0293	0.0657
36757.5	0.2154	2.0104	0.0698
37534.5	0.2207	2.0274	0.074
38311.5	0.2231	2.0437	0.0636
39088.5	0.2272	2	0.0634
39865.5	0.2283	2.0121	0.079
40642.5	0.2322	1.9978	0.0621
41419.5	0.236	2.0108	0.0699
42196.5	0.2366	2.0087	0.0576
42973.5	0.2378	1.9881	0.0675
43750.5	0.2406	1.9901	0.0675
44527.5	0.2418	1.9907	0.0727
45304.5	0.246	1.9872	0.0743
46081.5	0.2576	1.9818	0.0683
46858.5	0.2577	1.9767	0.0693
47635.5	0.2545	1.9757	0.068
48412.5	0.2525	1.9586	0.0601
49189.5	0.2542	1.9727	0.0452
49966.5	0.2582	1.9592	0.0646
50743.5	0.2615	1.9727	0.0684
51520.5	0.2646	1.9737	0.0521
52297.5	0.2712	1.9954	0.0684
53074.5	0.2718	1.9767	0.0568
53851.5	0.2786	1.978	0.0717
54628.5	0.2772	1.9957	0.0579

55405.5	0.2789	1.9742	0.0647
56182.5	0.2826	1.9786	0.0531
56959.5	0.2827	1.9713	0.0584
57736.5	0.2862	1.9919	0.0601
58513.5	0.2862	1.9875	0.0424
59290.5	0.2912	1.957	0.075
60067.5	0.291	1.9769	0.056
60844.5	0.2938	1.9879	0.0572
61621.5	0.2997	1.9822	0.0672
62398.5	0.298	1.9934	0.0628
63175.5	0.3097	1.9673	0.0571
63952.5	0.3125	1.9784	0.051
64729.5	0.3161	1.9582	0.0608
65506.5	0.3165	1.9472	0.0509
66283.5	0.3154	1.9584	0.0644
67060.5	0.3141	1.9517	0.0699
67837.5	0.3172	1.9423	0.0704
68614.5	0.3206	1.9423	0.0461
69391.5	0.3186	1.9323	0.0729
70168.5	0.3366	1.9343	0.0677
70945.5	0.335	1.9161	0.0575
71722.5	0.3295	1.9203	0.0675
72499.5	0.3296	1.9127	0.0516
73276.5	0.337	1.9099	0.0697
74053.5	0.3332	1.9169	0.0591
74830.5	0.33	1.9101	0.0598
75607.5	0.3313	1.9036	0.0611
76384.5	0.3273	1.9083	0.0482
77161.5	0.3288	1.9056	0.064
D-Glucaro-1,4-lactone Duplicate			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0165	1.4571	0.0266
1015.5	0.0277	1.4308	0.0296
1792.5	0.0218	1.424	0.0256
2569.5	0.0267	1.4201	0.01894
3346.5	0.0247	1.4155	0.0249
4123.5	0.027	1.4094	0.0353

4900.5	0.0293	1.4024	0.0323
5677.5	0.0206	1.3979	0.0321
6454.5	0.024	1.3573	0.0357
7231.5	0.0309	1.3574	0.048
8008.5	0.0359	1.3489	0.0413
8785.5	0.039	1.351	0.0413
9562.5	0.0377	1.328	0.0423
10339.5	0.0386	1.298	0.0453
11116.5	0.0423	1.3048	0.0435
11893.5	0.0495	1.283	0.0384
12670.5	0.0475	1.2937	0.0404
13447.5	0.0533	1.2933	0.0407
14224.5	0.0589	1.2854	0.0491
15001.5	0.0571	1.2682	0.0472
15778.5	0.0544	1.2728	0.0492
16555.5	0.0506	1.2767	0.0452
17332.5	0.0606	1.25	0.0455
18109.5	0.0648	1.2543	0.0527
18886.5	0.0673	1.2469	0.0461
19663.5	0.0632	1.2402	0.0497
20440.5	0.0689	1.2441	0.0443
21217.5	0.0683	1.243	0.0541
21994.5	0.0738	1.2471	0.0458
22771.5	0.0809	1.246	0.0423
23548.5	0.0781	1.2345	0.042
24325.5	0.081	1.2389	0.0428
25102.5	0.0794	1.2392	0.0382
25879.5	0.0817	1.2429	0.03921
26656.5	0.0825	1.2494	0.0422
27433.5	0.084	1.2485	0.0416
28210.5	0.0848	1.2349	0.0408
28987.5	0.0921	1.2273	0.04
29764.5	0.0955	1.242	0.0459
30541.5	0.099	1.2401	0.044
31318.5	0.0995	1.2323	0.0458
32095.5	0.1001	1.2319	0.0497
32872.5	0.1059	1.2404	0.045
33649.5	0.1106	1.2329	0.0481
34426.5	0.1128	1.2239	0.0426

35203.5	0.1151	1.2465	0.0414
35980.5	0.1191	1.2231	0.0385
36757.5	0.1158	1.2398	0.0453
37534.5	0.1169	1.2249	0.0435
38311.5	0.1177	1.235	0.0518
39088.5	0.117	1.2157	0.0398
39865.5	0.1195	1.2233	0.0418
40642.5	0.1193	1.2259	0.048
41419.5	0.1269	1.2386	0.046
42196.5	0.1282	1.2138	0.047
42973.5	0.1297	1.222	0.042
43750.5	0.1329	1.2056	0.0468
44527.5	0.1346	1.2196	0.0387
45304.5	0.1352	1.2171	0.0441
46081.5	0.1388	1.2186	0.0387
46858.5	0.1383	1.2081	0.0439
47635.5	0.1344	1.2051	0.0414
48412.5	0.1362	1.2046	0.0396
49189.5	0.1343	1.2126	0.0422
49966.5	0.136	1.2112	0.0318
50743.5	0.132	1.1998	0.0404
51520.5	0.1366	1.1933	0.0407
52297.5	0.1367	1.1982	0.0411
53074.5	0.1367	1.2073	0.0436
53851.5	0.1389	1.1917	0.0424
54628.5	0.1322	1.1972	0.0453
55405.5	0.1353	1.1907	0.0458
56182.5	0.1382	1.1924	0.043
56959.5	0.133	1.1948	0.0472
57736.5	0.137	1.1986	0.0401
58513.5	0.1377	1.2059	0.0403
59290.5	0.1369	1.2038	0.0497
60067.5	0.1359	1.2055	0.0422
D-Glucaro-1,4-lactone Plus Quarter Equivalent DCl			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0271	1.1306	0.0326
1015.5	0.0474	1.1136	0.0379

1792.5	0.0765	1.075	0.0301
2569.5	0.096	1.0385	0.031
3346.5	0.113	1.0195	0.0376
4123.5	0.1289	0.966	0.025
4900.5	0.1556	0.9431	0.0304
5677.5	0.1546	0.9263	0.0372
6454.5	0.1787	0.8946	0.0342
7231.5	0.1767	0.8659	0.037
8008.5	0.2067	0.844	0.024
8785.5	0.2151	0.7932	0.0272
9562.5	0.2454	0.7783	0.0207
10339.5	0.2524	0.738	0.0371
11116.5	0.2688	0.7246	0.0372
11893.5	0.2773	0.7026	0.0448
12670.5	0.2914	0.6911	0.0461
13447.5	0.304	0.6874	0.0408
14224.5	0.3186	0.6729	0.0446
15001.5	0.3308	0.6614	0.0416
15778.5	0.3473	0.6452	0.0347
16555.5	0.3479	0.6274	0.0424
17332.5	0.3523	0.6334	0.0352
18109.5	0.3598	0.6185	0.0359
18886.5	0.3699	0.618	0.0355
19663.5	0.383	0.6091	0.0442
20440.5	0.389	0.5929	0.0409
21217.5	0.4082	0.5934	0.0412
21994.5	0.4076	0.582	0.0369
22771.5	0.4134	0.5806	0.0388
23548.5	0.4096	0.5798	0.0419
24325.5	0.4214	0.5718	0.0372
25102.5	0.4135	0.5712	0.0433
25879.5	0.4116	0.557	0.0366
26656.5	0.4382	0.5636	0.0423
27433.5	0.4243	0.5551	0.0432
28210.5	0.4415	0.5574	0.0367
28987.5	0.4359	0.547	0.0353
29764.5	0.4526	0.5344	0.0482
30541.5	0.4539	0.5501	0.0298
31318.5	0.4544	0.5449	0.037

32095.5	0.4524	0.552	0.0354
32872.5	0.4574	0.5287	0.0319
33649.5	0.4668	0.54789	0.034
34426.5	0.4691	0.548	0.039
35203.5	0.4602	0.5357	0.0372
35980.5	0.4567	0.5313	0.0578
36757.5	0.4687	0.5394	0.037
37534.5	0.4517	0.541	0.0444
38311.5	0.4655	0.5309	0.0403
39088.5	0.4604	0.5596	0.0382
39865.5	0.4651	0.5408	0.033
40642.5	0.4607	0.556	0.0536
41419.5	0.4574	0.5396	0.0378
42196.5	0.4892	0.5333	0.04
42973.5	0.4881	0.5446	0.0312
43750.5	0.4887	0.5323	0.0396
44527.5	0.489	0.5316	0.0407
45304.5	0.4807	0.5359	0.0353
46081.5	0.4782	0.532	0.0387
46858.5	0.4733	0.5462	0.0366
47635.5	0.4802	0.547	0.0413
48412.5	0.4703	0.533	0.0433
49189.5	0.4724	0.5181	0.0339
49966.5	0.4747	0.5121	0.0487
50743.5	0.4825	0.5188	0.0322
51520.5	0.4788	0.5261	0.0415
52297.5	0.4901	0.5234	0.0361
53074.5	0.4948	0.5264	0.034
53851.5	0.4978	0.5113	0.029
54628.5	0.4861	0.5171	0.0369
55405.5	0.4836	0.5104	0.0245
56182.5	0.4865	0.5379	0.0439
56959.5	0.4871	0.5382	0.0309
57736.5	0.4899	0.5447	0.0327
58513.5	0.4929	0.548	0.0373
59290.5	0.4951	0.541	0.0428
60067.5	0.4985	0.5311	0.0491
60844.5	0.4838	0.548	0.0306
61621.5	0.4998	0.5371	0.0412

62398.5	0.4925	0.5292	0.0401
63175.5	0.4859	0.5343	0.0348
63952.5	0.4921	0.5198	0.033
64729.5	0.4869	0.5218	0.0396
65506.5	0.4858	0.5286	0.0303
66283.5	0.4949	0.5329	0.0385
67060.5	0.4964	0.5491	0.0313
67837.5	0.4996	0.5484	0.0349
68614.5	0.4992	0.532	0.0336
69391.5	0.4767	0.5309	0.038
70168.5	0.4822	0.5326	0.0435
70945.5	0.4963	0.5386	0.0445
71722.5	0.4938	0.5443	0.0355
72499.5	0.4964	0.5415	0.0377
73276.5	0.4987	0.5512	0.0393
74053.5	0.4914	0.5649	0.0435
74830.5	0.4919	0.5313	0.0399
75607.5	0.4964	0.5353	0.0436
76384.5	0.4915	0.5576	0.0332
77161.5	0.4954	0.5268	0.0353
D-Glucaro-1,4-lactone Plus Quarter Equivalent DCl Duplicate			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	1.1199	42.0605	1.1238
1015.5	1.6765	40.839	1.1255
1792.5	2.3837	39.0921	1.1216
2569.5	2.9857	38.6596	1.1043
3346.5	3.6074	37.3039	1.0934
4123.5	4.2266	36.246	1.081
4900.5	4.6708	35.1891	1.0795
5677.5	5.2173	34.2368	1.1045
6454.5	6.1673	33.8397	1.0885
7231.5	6.6783	31.7239	1.1236
8008.5	7.3388	30.0337	1.1288
8785.5	7.7252	29.0665	1.1272
9562.5	8.3365	28.6366	1.0999
10339.5	8.7718	27.9224	1.1192
11116.5	9.4048	26.7063	1.1103

11893.5	9.9292	26.1993	1.1029
12670.5	10.4908	25.015	1.0752
13447.5	11.2024	25.6286	1.0611
14224.5	12.0712	24.121	1.1257
15001.5	12.5088	23.415	1.1033
15778.5	13.0594	23.9228	1.073
16555.5	13.5568	23.6204	1.1209
17332.5	14.2235	22.8284	1.0799
18109.5	14.8049	22.1363	1.1182
18886.5	15.4613	22.7105	1.0868
19663.5	15.4898	22.4792	1.0917
20440.5	15.9602	21.8263	1.0049
21217.5	16.1471	21.5782	1.0775
21994.5	16.1459	21.956	1.1113
22771.5	16.807	21.6833	1.1359
23548.5	17.1561	21.3527	1.067
24325.5	17.5126	21.4664	1.0691
25102.5	17.7657	21.1823	1.1039
25879.5	17.8505	21.8733	1.1065
26656.5	18.4217	21.704	1.137
27433.5	18.5256	21.8012	1.1147
28210.5	18.5639	21.6	1.0805
28987.5	18.6738	21.6528	1.1345
29764.5	19.039	21.7971	1.092
30541.5	19.1516	22.0224	1.098
31318.5	19.2292	21.2322	1.067
32095.5	19.2428	21.9937	1.1195
32872.5	19.7555	21.9178	1.1055
33649.5	19.5923	21.4969	1.111
34426.5	19.8538	21.0216	1.1136
35203.5	20.0553	21.9053	1.1243
35980.5	20.2089	20.7284	1.1105
36757.5	20.4029	20.4547	1.1143
37534.5	20.8041	20.2745	1.0734
38311.5	20.9071	20.6664	1.0947
39088.5	21.0679	20.1785	1.1311
39865.5	21.0026	20.2444	1.063
40642.5	21.2691	21.0514	1.1282
41419.5	20.8991	20.6846	1.1258

42196.5	20.4424	20.9979	1.1286
42973.5	20.3518	20.0137	1.0713
43750.5	20.5749	19.7289	1.1383
44527.5	20.2424	19.808	1.118
45304.5	20.0245	19.7856	1.0804
46081.5	21.0656	19.6284	1.0631
46858.5	21.4745	20.2496	1.1118
47635.5	21.0216	19.8279	1.0778
48412.5	21.1786	19.9818	1.099
49189.5	21.31	20.3312	1.0946
49966.5	20.202	20.3078	1.1185
50743.5	20.7768	20.0933	1.0712
51520.5	20.2035	19.1801	1.0687
52297.5	19.8695	19.7377	1.0816
53074.5	19.8725	19.2056	1.0669
53851.5	20.1831	20.305	1.0865
54628.5	20.0808	19.785	1.0806
55405.5	20.1178	20.0756	1.1087
56182.5	20.499	20.6759	1.0921
56959.5	21.4123	19.7587	1.0909
57736.5	21.5718	19.8459	1.0703
58513.5	21.3015	20.0285	1.0672
59290.5	21.301	19.977	1.0919
60067.5	21.4829	20.3251	1.1044
60844.5	20.4382	20.1983	1.0584
61621.5	21.4058	19.5803	1.1199
62398.5	21.5117	19.685	1.0821
63175.5	21.5912	19.4358	1.1304
63952.5	20.6268	19.435	1.1003
64729.5	21.7571	19.9079	1.004
65506.5	21.4672	20.2447	1.1252
66283.5	21.7016	19.2167	1.0633
67060.5	21.6018	19.2412	1.052
67837.5	21.1717	19.1946	1.1137
68614.5	20.2622	19.6706	1.1103
69391.5	21.6065	19.149	1.1271
70168.5	21.5263	19.4411	1.124
70945.5	21.4909	19.0361	1.0867
71722.5	20.5866	19.8612	1.1188

D-Glucaro-1,4-lactone Plus Half Equivalent DCl			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.1297	2.7546	0.0796
1015.5	0.2122	2.6706	0.0748
1792.5	0.2888	2.6147	0.0759
2569.5	0.3516	2.5167	0.0796
3346.5	0.4424	2.4107	0.0792
4123.5	0.5064	2.3775	0.0731
4900.5	0.5918	2.2984	0.0741
5677.5	0.6437	2.1968	0.0707
6454.5	0.6872	2.1404	0.0706
7231.5	0.7472	2.1001	0.0687
8008.5	0.7893	1.9769	0.0863
8785.5	0.8303	1.9198	0.077
9562.5	0.8675	1.8638	0.0676
10339.5	0.9124	1.9032	0.0826
11116.5	0.9451	1.8241	0.0751
11893.5	0.9756	1.7695	0.0616
12670.5	0.9923	1.737	0.0647
13447.5	1.0394	1.6317	0.0695
14224.5	1.0455	1.5909	0.0691
15001.5	1.0687	1.561	0.0707
15778.5	1.1225	1.5306	0.0537
16555.5	1.1362	1.4877	0.0841
17332.5	1.1454	1.4568	0.0794
18109.5	1.1703	1.4095	0.0699
18886.5	1.1748	1.3341	0.0626
19663.5	1.1889	1.3106	0.0676
20440.5	1.2017	1.2851	0.0722
21217.5	1.2152	1.2494	0.0789
21994.5	1.2152	1.2373	0.0592
22771.5	1.2102	1.22	0.0479
23548.5	1.2129	1.2012	0.0772
24325.5	1.2157	1.186	0.0767
25102.5	1.2236	1.1788	0.0617
25879.5	1.211	1.1398	0.055
26656.5	1.2188	1.1058	0.0653

27433.5	1.2073	1.1174	0.0577
28210.5	1.2183	1.0806	0.0701
28987.5	1.2217	1.0685	0.0772
29764.5	1.2147	1.0373	0.0544
30541.5	1.2155	1.025	0.0574
31318.5	1.2194	1.0432	0.0688
32095.5	1.2074	1.0253	0.075
32872.5	1.2144	1.0214	0.0626
33649.5	1.2115	1.039	0.0576
34426.5	1.2161	1.048	0.0799
35203.5	1.2253	1.0347	0.071
35980.5	1.2103	1.0472	0.0555
36757.5	1.2184	1.0559	0.0631
37534.5	1.2209	1.0284	0.0631
38311.5	1.2272	1.0454	0.0693
39088.5	1.2383	1.0577	0.0687
39865.5	1.2262	1.0365	0.0739
40642.5	1.2144	1.0287	0.0745
41419.5	1.2205	1.05	0.0605
42196.5	1.2093	1.0185	0.0615
42973.5	1.2207	1.024	0.0718
43750.5	1.2396	1.023	0.0618
44527.5	1.2259	1.0589	0.0613
45304.5	1.2328	1.0414	0.0647
46081.5	1.2292	1.0439	0.075
46858.5	1.2217	1.0373	0.071
47635.5	1.2226	1.0332	0.0743
48412.5	1.2374	1.037	0.0706
49189.5	1.2283	1.0493	0.0738
49966.5	1.2413	1.0255	0.0705
50743.5	1.2226	1.0346	0.0548
51520.5	1.2219	1.0579	0.0579
52297.5	1.2333	1.0592	0.0641
53074.5	1.2261	1.0242	0.0806
53851.5	1.2245	1.0249	0.0749
54628.5	1.2301	1.0488	0.0824
55405.5	1.2323	1.0436	0.0689
56182.5	1.2258	1.0558	0.0661
56959.5	1.2116	1.0354	0.0658

57736.5	1.2145	1.0209	0.0784
58513.5	1.2187	1.0275	0.0709
59290.5	1.2198	1.0232	0.0677
60067.5	1.2352	1.0463	0.0761
60844.5	1.2323	1.0342	0.0756
61621.5	1.2187	1.026	0.0709
62398.5	1.2224	1.0213	0.0591
63175.5	1.2172	1.0415	0.0761
63952.5	1.2108	1.0183	0.0693
64729.5	1.2097	1.0146	0.0704
65506.5	1.2091	1.0363	0.0526
66283.5	1.2247	1.0295	0.0759
67060.5	1.2163	1.0414	0.0678
67837.5	1.2287	1.052	0.0612
68614.5	1.2173	1.028	0.0601
69391.5	1.2065	1.0168	0.0737
70168.5	1.2235	1.0362	0.0653
70945.5	1.2196	1.0322	0.0733
71722.5	1.2254	1.0236	0.0711
72499.5	1.2171	1.0178	0.0733
73276.5	1.2202	1.0231	0.0605
74053.5	1.2117	1.0308	0.0742
74830.5	1.2251	1.0183	0.0715
75607.5	1.2102	1.0245	0.0694
76384.5	1.2266	1.0325	0.0819
77161.5	1.2115	1.0345	0.0706
D-Glucaro-1,4-lactone Plus Half Equivalent DCl Duplicate			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0821	2.0264	0.076
1015.5	0.1528	1.9078	0.0716
1792.5	0.2229	1.7953	0.0582
2569.5	0.2768	1.7306	0.037
3346.5	0.3425	1.7056	0.0765
4123.5	0.3815	1.632	0.0826
4900.5	0.4342	1.5871	0.0749
5677.5	0.4641	1.5342	0.0558
6454.5	0.4964	1.4916	0.0479

7231.5	0.5345	1.4135	0.0652
8008.5	0.5807	1.3512	0.074
8785.5	0.6439	1.3033	0.0748
9562.5	0.6747	1.2571	0.0612
10339.5	0.6833	1.2051	0.0757
11116.5	0.7101	1.186	0.0868
11893.5	0.7201	1.1558	0.0798
12670.5	0.7352	1.1102	0.0514
13447.5	0.7788	1.0893	0.0605
14224.5	0.7798	1.0186	0.0709
15001.5	0.7794	1.0158	0.0713
15778.5	0.7881	1.0087	0.0763
16555.5	0.7955	0.9928	0.0816
17332.5	0.811	0.9689	0.0736
18109.5	0.8124	0.9414	0.0755
18886.5	0.8184	0.9362	0.0656
19663.5	0.8304	0.9264	0.0638
20440.5	0.8502	0.9186	0.0665
21217.5	0.8596	0.9077	0.071
21994.5	0.8587	0.8825	0.0727
22771.5	0.8489	0.8613	0.0667
23548.5	0.8437	0.8313	0.0619
24325.5	0.8465	0.823	0.0692
25102.5	0.8594	0.8131	0.0573
25879.5	0.8517	0.8055	0.0679
26656.5	0.8564	0.808	0.054
27433.5	0.8512	0.8049	0.0598
28210.5	0.8536	0.8054	0.0676
28987.5	0.8553	0.7901	0.0695
29764.5	0.8501	0.8109	0.0619
30541.5	0.8512	0.8058	0.0624
31318.5	0.8571	0.7916	0.0749
32095.5	0.8516	0.8031	0.0643
32872.5	0.8465	0.7956	0.075
33649.5	0.8436	0.7914	0.0636
34426.5	0.8532	0.7895	0.0751
35203.5	0.8444	0.7893	0.0531
35980.5	0.8501	0.7955	0.0651
36757.5	0.842	0.8096	0.0655

37534.5	0.8473	0.7859	0.0733
38311.5	0.8532	0.7921	0.071
39088.5	0.8427	0.8003	0.0658
39865.5	0.8382	0.8078	0.0688
40642.5	0.8452	0.8021	0.0585
41419.5	0.8454	0.7949	0.0665
42196.5	0.8409	0.8078	0.0592
42973.5	0.8408	0.7938	0.0619
43750.5	0.8431	0.8028	0.0619
44527.5	0.8516	0.7912	0.0692
45304.5	0.8435	0.799	0.0668
46081.5	0.8561	0.8062	0.0637
46858.5	0.8425	0.8039	0.0511
47635.5	0.8406	0.795	0.0773
48412.5	0.8494	0.8055	0.0746
49189.5	0.8467	0.7986	0.0776
49966.5	0.8395	0.7942	0.0735
50743.5	0.8536	0.8007	0.0688
51520.5	0.839	0.7881	0.0417
52297.5	0.8468	0.7917	0.0687
53074.5	0.8472	0.7896	0.06712
53851.5	0.85457	0.7915	0.0644
54628.5	0.8426	0.8014	0.0716
55405.5	0.8406	0.8072	0.0582
56182.5	0.8405	0.795	0.0566
56959.5	0.8424	0.8077	0.0615
57736.5	0.8477	0.8039	0.0522
58513.5	0.8366	0.792	0.0687
59290.5	0.8352	0.8079	0.0708
60067.5	0.8341	0.7927	0.0738
60844.5	0.8556	0.8026	0.0749
61621.5	0.8406	0.7915	0.0717
62398.5	0.8471	0.7894	0.0711
63175.5	0.8503	0.7982	0.0667
63952.5	0.8511	0.8084	0.0586
64729.5	0.845	0.7915	0.0663
65506.5	0.8435	0.7989	0.0703
66283.5	0.8475	0.8021	0.0668
67060.5	0.8333	0.7924	0.07

67837.5	0.8409	0.8039	0.0675
68614.5	0.8412	0.803	0.0733
69391.5	0.8574	0.8013	0.0709
70168.5	0.8409	0.7952	0.0705
70945.5	0.8507	0.7859	0.0736
71722.5	0.849	0.8069	0.0765
72499.5	0.8468	0.7937	0.0705
73276.5	0.8368	0.799	0.0646
74053.5	0.8385	0.8054	0.0529
74830.5	0.8409	0.7998	0.0652
75607.5	0.8335	0.8061	0.0536
76384.5	0.8452	0.8012	0.0684
77161.5	0.8467	0.8029	0.0745
D-Glucaro-1,4-lactone Plus Three Quarter Equivalent DCl			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.2403	11.2584	0.2344
1015.5	0.6002	11.1955	0.3481
1792.5	0.9105	10.2367	0.343
2569.5	1.1539	9.7224	0.3364
3346.5	1.5442	9.4366	0.3118
4123.5	1.7122	9.0824	0.3147
4900.5	1.9496	8.8341	0.3221
5677.5	2.1381	8.6954	0.3494
6454.5	2.3451	8.2709	0.3062
7231.5	2.5289	7.8622	0.3109
8008.5	2.668	7.4322	0.323
8785.5	2.8434	6.9523	0.3662
9562.5	3.0387	6.6722	0.2765
10339.5	3.1427	6.67145	0.2835
11116.5	3.2085	6.58	0.2812
11893.5	3.3814	6.4448	0.4081
12670.5	3.3961	6.3095	0.3443
13447.5	3.6023	5.9856	0.3086
14224.5	3.6116	5.873	0.3345
15001.5	3.7595	5.5617	0.3022
15778.5	3.734	5.5716	0.4035
16555.5	3.7052	5.0946	0.2282

17332.5	3.8534	5.0298	0.248
18109.5	3.979	5.0215	0.3053
18886.5	4.0267	5.0643	0.258
19663.5	4.0188	5.0992	0.3035
20440.5	4.0753	5.0176	0.2753
21217.5	4.1728	4.978	0.3073
21994.5	4.2083	4.818	0.3272
22771.5	4.2211	4.7262	0.252
23548.5	4.2656	4.67	0.3275
24325.5	4.2851	4.5846	0.2292
25102.5	4.3108	4.4844	0.2851
25879.5	4.3247	4.3567	0.2748
26656.5	4.3397	4.2834	0.3164
27433.5	4.3502	4.2361	0.3366
28210.5	4.3649	4.1624	0.23
28987.5	4.4079	4.1531	0.2913
29764.5	4.4139	4.1358	0.2945
30541.5	4.4233	4.1424	0.2647
31318.5	4.4248	4.1348	0.316
32095.5	4.4411	4.127	0.2722
32872.5	4.4575	4.1332	0.3143
33649.5	4.4691	4.1196	0.2571
34426.5	4.4782	4.1328	0.3003
35203.5	4.4825	4.1057	0.2194
35980.5	4.4959	4.1215	0.3059
36757.5	4.5073	4.1095	0.2478
37534.5	4.5168	4.13	0.2811
38311.5	4.6563	4.0956	0.2714
39088.5	4.6722	4.0879	0.323
39865.5	4.6787	4.0486	0.3402
40642.5	4.6776	4.0335	0.3069
41419.5	4.6868	4.0143	0.268
42196.5	4.6913	4.0305	0.3148
42973.5	4.6921	4.0479	0.2829
43750.5	4.694	4.0252	0.2574
44527.5	4.7	4.0044	0.3074
45304.5	4.696	4.0056	0.3072
46081.5	4.6969	4.0651	0.3212
46858.5	4.6903	4.0372	0.2504

47635.5	4.6847	4.0537	0.3161
48412.5	4.6832	4.0454	0.3352
49189.5	4.6788	4.0255	0.2739
49966.5	4.6702	3.9855	0.3573
50743.5	4.6892	3.981	0.2688
51520.5	4.6863	3.9751	0.4136
52297.5	4.6827	4.0208	0.2823
53074.5	4.6818	4.0109	0.2463
53851.5	4.6802	3.9992	0.3615
54628.5	4.6954	4.0267	0.3458
55405.5	4.6916	4.0269	0.3844
56182.5	4.6854	3.9871	0.3922
56959.5	4.6939	4.0107	0.3815
57736.5	4.695	4.0266	0.318
58513.5	4.7097	4.0009	0.298
59290.5	4.6913	4.025	0.3089
60067.5	4.7047	4.0603	0.3447
60844.5	4.6887	3.9969	0.3344
61621.5	4.6916	3.9928	0.3221
62398.5	4.6902	4.0204	0.3081
63175.5	4.6988	3.9945	0.2327
63952.5	4.7055	4.0054	0.3355
64729.5	4.6895	4.0332	0.3657
65506.5	4.6933	4.0161	0.3068
66283.5	4.6799	4.0167	0.3026
67060.5	4.7097	4.0044	0.3244
67837.5	4.7002	4.0104	0.2811
D-Glucaro-1,4-lactone Plus Three Quarter Equivalent DCl Duplicate			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.2108	2.3089	0.06776
1015.5	0.3319	2.1189	0.0673
1792.5	0.4501	1.9497	0.0657
2569.5	0.5147	1.8523	0.0795
3346.5	0.5727	1.7422	0.0727
4123.5	0.6599	1.6552	0.0727
4900.5	0.6646	1.6015	0.0661
5677.5	0.7335	1.5086	0.0631

6454.5	0.8297	1.478	0.0679
7231.5	0.8634	1.4407	0.0642
8008.5	0.9009	1.3724	0.0615
8785.5	0.9548	1.3228	0.0614
9562.5	1.0033	1.2814	0.06
10339.5	1.0306	1.2471	0.0668
11116.5	1.0511	1.2135	0.0673
11893.5	1.0853	1.2022	0.0636
12670.5	1.0932	1.1517	0.0535
13447.5	1.115	1.135	0.0528
14224.5	1.1266	1.1085	0.0522
15001.5	1.1389	1.0545	0.066
15778.5	1.1405	1.0463	0.0637
16555.5	1.1499	1.0321	0.00661
17332.5	1.1438	1.0185	0.0492
18109.5	1.1499	1.0161	0.0615
18886.5	1.1399	1.0111	0.0593
19663.5	1.1344	1.0068	0.0581
20440.5	1.1446	0.9941	0.0658
21217.5	1.1451	0.9878	0.0809
21994.5	1.1402	0.9713	0.0673
22771.5	1.1455	0.9785	0.057
23548.5	1.144	0.9612	0.0743
24325.5	1.1464	0.9405	0.0616
25102.5	1.1487	0.9239	0.0552
25879.5	1.1395	0.9181	0.0559
26656.5	1.1449	0.9232	0.076
27433.5	1.1452	0.9283	0.048
28210.5	1.1453	0.9198	0.0622
28987.5	1.1422	0.9076	0.0504
29764.5	1.1414	0.9105	0.0573
30541.5	1.1476	0.9017	0.0662
31318.5	1.1475	0.9095	0.0631
32095.5	1.1422	0.9061	0.0612
32872.5	1.1431	0.9021	0.0685
33649.5	1.1484	0.8886	0.0532
34426.5	1.1481	0.8979	0.0612
35203.5	1.141	0.8975	0.0632
35980.5	1.1415	0.894	0.0649

36757.5	1.1447	0.8912	0.052
37534.5	1.1453	0.8966	0.0514
38311.5	1.1414	0.8917	0.0558
39088.5	1.1447	0.8945	0.0504
39865.5	1.1415	0.8859	0.0522
40642.5	1.1475	0.882	0.0653
41419.5	1.1416	0.8914	0.0621
42196.5	1.1433	0.8754	0.05
42973.5	1.146	0.8942	0.0516
43750.5	1.1413	0.8926	0.0641
44527.5	1.1456	0.879	0.0592
45304.5	1.1472	0.8782	0.0658
46081.5	1.1406	0.8741	0.0608
46858.5	1.1398	0.8783	0.056
47635.5	1.1446	0.8785	0.0628
48412.5	1.1413	0.8735	0.058
49189.5	1.1417	0.8756	0.0554
49966.5	1.1384	0.8752	0.0669
50743.5	1.139	0.8741	0.0612
51520.5	1.1401	0.8734	0.0536
52297.5	1.1397	0.8784	0.0595
53074.5	1.1403	0.8789	0.0612
53851.5	1.1394	0.874	0.0456
54628.5	1.1375	0.8758	0.0527
55405.5	1.1398	0.8787	0.0556
56182.5	1.1427	0.8768	0.0595
56959.5	1.1378	0.8719	0.0587
57736.5	1.1428	0.8748	0.0642
58513.5	1.1448	0.8762	0.0673
59290.5	1.1466	0.8759	0.0572
60067.5	1.1444	0.8721	0.0629
60844.5	1.145	0.8694	0.06
61621.5	1.143	0.8726	0.0516
62398.5	1.1444	0.8762	0.0655
63175.5	1.1409	0.8787	0.0595
63952.5	1.1425	0.874	0.0583
64729.5	1.1486	0.877	0.0572
65506.5	1.1432	0.8742	0.0548
66283.5	1.1419	0.8699	0.061

67060.5	1.14	0.8719	0.0666	
67837.5	1.14	0.8744	0.0588	
68614.5	1.144	0.8762	0.058	
69391.5	1.1461	0.8773	0.0553	
70168.5	1.1384	0.87	0.0494	
70945.5	1.138	0.8711	0.0556	
71722.5	1.142	0.8787	0.0545	
72499.5	1.1425	0.8783	0.0497	
73276.5	1.1419	0.8776	0.0494	
74053.5	1.1473	0.8787	0.0584	
74830.5	1.1484	0.8715	0.0517	
75607.5	1.1427	0.8744	0.055	
76384.5	1.1421	0.8771	0.0622	
77161.5	1.1481	0.8717	0.0654	

D-Glucaro-1,4-lactone Plus Equivalent DCl				
Midpoint Time	D-Glucaric aid	D-Glucaro-1,4-lactone	D-Glucaro-6,3-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.1547	2.0005	-0.0103	0.0453
1015.5	0.2755	1.8675	0.0096	0.0642
1792.5	0.3717	1.7394	0.0433	0.074
2569.5	0.4797	1.7043	-0.01037	0.0749
3346.5	0.5615	1.6789	-0.01275	0.0668
4123.5	0.6444	1.6207	-0.01075	0.0596
4900.5	0.722	1.541	-0.00575	0.0501
5677.5	0.76	1.4688	0.0249	0.056
6454.5	0.8219	1.3381	0.084725	0.0444
7231.5	0.8817	1.3052	0.101425	0.0493
8008.5	0.8986	1.2748	0.06105	0.0656
8785.5	0.919	1.2553	0.074125	0.0617
9562.5	0.9664	1.1123	0.180575	0.0577
10339.5	0.9996	1.0952	0.195	0.0534
11116.5	1.0298	1.0893	0.195175	0.058
11893.5	1.062	1.0627	0.2109	0.0657
12670.5	1.0663	1.0548	0.2049	0.0711
13447.5	1.0597	1.0073	0.2333	0.0658
14224.5	1.0992	0.9897	0.249875	0.0504
15001.5	1.0918	0.9624	0.27135	0.0517
15778.5	1.0944	0.9688	0.25795	0.0751

16555.5	1.1049	0.9761	0.253425	0.0748
17332.5	1.0853	0.9651	0.25895	0.073
18109.5	1.1089	0.9476	0.280175	0.0497
18886.5	1.0986	0.9175	0.3101	0.0475
19663.5	1.1299	0.9048	0.313	0.0651
20440.5	1.1359	0.9001	0.31305	0.0532
21217.5	1.1257	0.8603	0.347325	0.0554
21994.5	1.1628	0.8432	0.356825	0.0606
22771.5	1.139	0.8227	0.370725	0.0641
23548.5	1.1422	0.8297	0.3666	0.0521
24325.5	1.1451	0.8262	0.369475	0.0639
25102.5	1.1061	0.8234	0.37235	0.0546
25879.5	1.18	0.8181	0.374525	0.0614
26656.5	1.1449	0.8203	0.374175	0.0629
27433.5	1.1321	0.8244	0.371225	0.0569
28210.5	1.1509	0.8171	0.38055	0.0541
28987.5	1.1567	0.806	0.394	0.0382
29764.5	1.1707	0.8091	0.386825	0.0577
30541.5	1.17	0.8018	0.398025	0.0419
31318.5	1.1086	0.7884	0.409075	0.0439
32095.5	1.1222	0.7754	0.419275	0.033
32872.5	1.1224	0.7769	0.420275	0.0456
33649.5	1.1155	0.7638	0.426225	0.0532
34426.5	1.1246	0.7547	0.433325	0.0585
35203.5	1.1176	0.7399	0.443825	0.0497
35980.5	1.1611	0.7337	0.457925	0.0496
36757.5	1.1259	0.7287	0.456525	0.0557
37534.5	1.1213	0.725	0.46525	0.0508
38311.5	1.1304	0.7167	0.462425	0.088
39088.5	1.1645	0.7231	0.462825	0.0665
39865.5	1.1311	0.7325	0.455275	0.0676
40642.5	1.1239	0.7395	0.45455	0.0562
41419.5	1.0988	0.7324	0.456475	0.0602
42196.5	1.106	0.734	0.460925	0.0451
42973.5	1.1094	0.724	0.467475	0.0449
43750.5	1.1107	0.7318	0.46255	0.0501
44527.5	1.1113	0.7021	0.49075	0.0381
45304.5	1.1044	0.7155	0.473025	0.0599
46081.5	1.1015	0.7267	0.464625	0.0722

46858.5	1.1006	0.7286	0.469025	0.0651
47635.5	1.1046	0.7407	0.46405	0.0567
48412.5	1.0651	0.7269	0.4739	0.0607
49189.5	1.1186	0.7084	0.495625	0.0435
49966.5	1.0718	0.7198	0.48705	0.0602
50743.5	1.0988	0.7212	0.4846	0.0606
51520.5	1.0956	0.7256	0.485675	0.0416
52297.5	1.0776	0.7161	0.4906	0.0551
53074.5	1.0854	0.7258	0.47905	0.0642
53851.5	1.1115	0.7287	0.482625	0.0632
54628.5	1.0897	0.7107	0.498375	0.0579
55405.5	1.0911	0.7146	0.49575	0.0606
56182.5	1.1041	0.7247	0.48595	0.0669
56959.5	1.0937	0.7132	0.499475	0.0513
57736.5	1.065	0.714	0.4949	0.0599
58513.5	1.0681	0.7109	0.498025	0.0644
59290.5	1.0993	0.7203	0.4894	0.0619
60067.5	1.0715	0.7144	0.498125	0.0507
60844.5	1.0738	0.7128	0.50125	0.0482
61621.5	1.0664	0.7196	0.49625	0.0534
62398.5	1.0726	0.7176	0.496275	0.0543
63175.5	1.0844	0.7282	0.48965	0.0638
63952.5	1.0768	0.7337	0.4807	0.0794
64729.5	1.0725	0.7161	0.50065	0.0592
65506.5	1.0669	0.7192	0.5003	0.0581
66283.5	1.0808	0.7213	0.5005	0.059
67060.5	1.0779	0.7259	0.495275	0.0662
67837.5	1.0647	0.7293	0.49375	0.0634
68614.5	1.0912	0.7168	0.500375	0.0663
69391.5	1.0728	0.7122	0.507125	0.0633
70168.5	1.0822	0.7209	0.499175	0.0514
70945.5	1.0688	0.7207	0.498225	0.0551
71722.5	1.0751	0.7215	0.502125	0.0583
72499.5	1.0739	0.7229	0.50145	0.0595
73276.5	1.0701	0.718	0.50275	0.0665
74053.5	1.0641	0.711	0.507075	0.0645
D-glucaro-1,4-lactone Plus Equivalent DCl Duplicate				
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-	D-Glucaro-6,3-	D-Glucaro-1,4;6,3-

		lactone	lactone	Dilactone
238.5	0.1836	1.5405	-0.00568	0.0925
1015.5	0.2687	1.459	-0.0059	0.0973
1792.5	0.3728	1.3411	0.05365	0.0725
2569.5	0.4273	1.2263	0.093775	0.0723
3346.5	0.5001	1.1678	0.113025	0.0761
4123.5	0.5485	1.1151	0.116525	0.0744
4900.5	0.5997	1.064	0.1345	0.0749
5677.5	0.6377	1.0284	0.139525	0.0628
6454.5	0.6956	0.9624	0.18745	0.0619
7231.5	0.7181	0.914	0.211125	0.0658
8008.5	0.757	0.8703	0.232	0.0541
8785.5	0.7727	0.84	0.258	0.0518
9562.5	0.7902	0.8246	0.26975	0.053
10339.5	0.8101	0.8086	0.2709	0.0613
11116.5	0.8179	0.7996	0.266925	0.0602
11893.5	0.8216	0.7824	0.28085	0.0551
12670.5	0.8225	0.7655	0.29425	0.0562
13447.5	0.8383	0.7537	0.302625	0.0596
14224.5	0.8317	0.7481	0.307875	0.0633
15001.5	0.8397	0.7475	0.3075	0.0623
15778.5	0.8426	0.7425	0.313725	0.0581
16555.5	0.8424	0.738	0.316375	0.0638
17332.5	0.8559	0.743	0.316525	0.0547
18109.5	0.84	0.7331	0.3214	0.0568
18886.5	0.8532	0.7332	0.32375	0.0533
19663.5	0.855	0.7381	0.319675	0.0502
20440.5	0.8542	0.7343	0.322225	0.0543
21217.5	0.8555	0.7291	0.327825	0.048
21994.5	0.8573	0.7208	0.33705	0.0473
22771.5	0.857	0.7193	0.33555	0.058
23548.5	0.8551	0.7152	0.338925	0.0567
24325.5	0.8571	0.7138	0.3378	0.0593
25102.5	0.854	0.7123	0.34065	0.0559
25879.5	0.8523	0.7082	0.346075	0.0511
26656.5	0.8462	0.707	0.347425	0.0528
27433.5	0.8529	0.6987	0.357225	0.0488
28210.5	0.8477	0.6946	0.354075	0.0653
28987.5	0.8503	0.6973	0.3531	0.0592

29764.5	0.8474	0.6914	0.360375	0.0514
30541.5	0.8424	0.6906	0.3576	0.0614
31318.5	0.8446	0.6917	0.362025	0.0542
32095.5	0.8507	0.694	0.3533	0.0648
32872.5	0.85	0.6874	0.36355	0.0597
33649.5	0.8514	0.6828	0.3691	0.0562
34426.5	0.8501	0.6868	0.3629	0.0599
35203.5	0.8511	0.6888	0.371075	0.0565
35980.5	0.8465	0.6877	0.364875	0.0609
36757.5	0.8521	0.6823	0.37055	0.061
37534.5	0.8469	0.6782	0.37645	0.0599
38311.5	0.8447	0.6717	0.38065	0.0604
39088.5	0.8452	0.6709	0.38545	0.0505
39865.5	0.8455	0.6682	0.384375	0.0598
40642.5	0.8417	0.6693	0.3862	0.0532
41419.5	0.8491	0.662	0.387325	0.0572
42196.5	0.8434	0.6608	0.389275	0.0556
42973.5	0.8465	0.6629	0.3891	0.0576
43750.5	0.8461	0.6607	0.390025	0.0566
44527.5	0.841	0.6613	0.386225	0.0598
45304.5	0.8392	0.6618	0.392125	0.0558
46081.5	0.8341	0.6581	0.393975	0.0579
46858.5	0.8396	0.6608	0.3958	0.0543
47635.5	0.8348	0.6548	0.402625	0.0477
48412.5	0.8402	0.6593	0.395	0.0594
49189.5	0.8405	0.6587	0.3959	0.0591
49966.5	0.8377	0.6512	0.398475	0.0585
50743.5	0.8393	0.648	0.40285	0.0549
51520.5	0.8333	0.6434	0.410675	0.0499
52297.5	0.8367	0.6449	0.408125	0.0526
53074.5	0.8322	0.6479	0.4066	0.0572
53851.5	0.8373	0.6492	0.405925	0.0579
54628.5	0.8343	0.6476	0.406175	0.0607
55405.5	0.8324	0.6451	0.40715	0.0606
56182.5	0.8347	0.6437	0.406375	0.0623
56959.5	0.8387	0.6459	0.406175	0.0567
57736.5	0.8294	0.6429	0.40865	0.0516
58513.5	0.8325	0.637	0.4148	0.0561
59290.5	0.8305	0.6348	0.41425	0.0587

60067.5	0.8371	0.6323	0.417075	0.0516
60844.5	0.8356	0.6393	0.41395	0.0522
61621.5	0.8364	0.6349	0.415175	0.0558
62398.5	0.8294	0.6342	0.414375	0.061
63175.5	0.8291	0.6313	0.41605	0.0567
63952.5	0.8213	0.6314	0.416875	0.0527
64729.5	0.8259	0.6357	0.415925	0.0599
65506.5	0.8289	0.6385	0.416375	0.0567
66283.5	0.83	0.6416	0.4103	0.0635
67060.5	0.8296	0.6403	0.414375	0.0571
67837.5	0.8337	0.6404	0.4109	0.058
68614.5	0.8304	0.6389	0.420375	0.0583
69391.5	0.8312	0.6354	0.415075	0.0595
70168.5	0.8373	0.6315	0.419025	0.0565
70945.5	0.8323	0.6299	0.4237	0.0549
71722.5	0.838	0.6349	0.4172	0.057
72499.5	0.8338	0.6391	0.4154	0.0616
73276.5	0.8288	0.6331	0.4171	0.0651
74053.5	0.8333	0.6313	0.4168	0.0653
74830.5	0.8285	0.6331	0.420075	0.06
75607.5	0.8257	0.6358	0.417375	0.0565
76384.5	0.8291	0.6307	0.420275	0.0633
77161.5	0.8293	0.6329	0.4187	0.0653
D-Glucaro-1,4-lactone Plus One and a Half Equivalent DCl				
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-6,3-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.1761	2.2632	-0.07198	0.0174
1015.5	0.3941	1.9455	0.0525	0.0325
1792.5	0.5857	1.9021	0.027525	0.0267
2569.5	0.7273	1.7725	0.044825	0.0325
3346.5	0.8468	1.6086	0.105675	0.028
4123.5	0.9357	1.4804	0.1604	0.0291
4900.5	1.0056	1.3767	0.21301	0.02866
5677.5	1.0569	1.282	0.2293	0.029
6454.5	1.0831	1.2001	0.258175	0.0296
7231.5	1.1021	1.1379	0.313625	0.0267
8008.5	1.135	1.1053	0.31745	0.0279
8785.5	1.1514	1.0428	0.41335	0.0285

9562.5	1.1374	0.9981	0.4293	0.0206
10339.5	1.163	0.9607	0.46505	0.0288
11116.5	1.1641	0.9352	0.503775	0.0251
11893.5	1.1724	0.9209	0.49965	0.023
12670.5	1.1538	0.8825	0.5612	0.0267
13447.5	1.1597	0.8877	0.53875	0.0289
14224.5	1.153	0.8709	0.550975	0.0275
15001.5	1.1605	0.857	0.580875	0.0266
15778.5	1.1491	0.8472	0.58475	0.0292
16555.5	1.1468	0.8259	0.5961	0.0281
17332.5	1.1451	0.8066	0.598725	0.0304
18109.5	1.1442	0.8093	0.619275	0.0301
18886.5	1.1542	0.8013	0.620475	0.046
19663.5	1.1459	0.7919	0.6145	0.0279
20440.5	1.1314	0.8032	0.622175	0.0277
21217.5	1.1252	0.7901	0.63355	0.0311
21994.5	1.1231	0.79	0.626575	0.0253
22771.5	1.1228	0.7488	0.6362	0.0325
23548.5	1.1202	0.753	0.648675	0.0292
24325.5	1.1234	0.7464	0.654825	0.0319
25102.5	1.0976	0.7521	0.64485	0.0282
25879.5	1.1039	0.7206	0.675825	0.0225
26656.5	1.0917	0.7179	0.663575	0.0298
27433.5	1.1063	0.7284	0.668025	0.0293
28210.5	1.1001	0.7235	0.65935	0.0294
28987.5	1.095	0.7247	0.654525	0.0316
29764.5	1.0967	0.7357	0.6661	0.0298
30541.5	1.0867	0.7238	0.681725	0.0297
31318.5	1.0907	0.7337	0.65305	0.0361
32095.5	1.0874	0.7285	0.654225	0.032
32872.5	1.0945	0.7358	0.651625	0.0315
33649.5	1.091	0.7435	0.6562	0.0307
34426.5	1.0878	0.7534	0.6442	0.0339
35203.5	1.0998	0.7256	0.667675	0.0333
35980.5	1.0883	0.7352	0.674925	0.0324
36757.5	1.0952	0.7381	0.667625	0.0335
37534.5	1.0982	0.7248	0.6906	0.0335
38311.5	1.0909	0.7358	0.68035	0.0324
39088.5	1.0906	0.7176	0.6942	0.0342

39865.5	1.0911	0.7234	0.69005	0.0286
40642.5	1.097	0.7291	0.693625	0.0276
41419.5	1.101	0.7219	0.6814	0.0329
42196.5	1.0972	0.7376	0.681425	0.0305
42973.5	1.0901	0.7288	0.6902	0.0307
43750.5	1.0802	0.7329	0.6872	0.031
44527.5	1.0939	0.7184	0.6809	0.0324
45304.5	1.0866	0.7194	0.69535	0.0318
46081.5	1.0861	0.7198	0.68205	0.0312
46858.5	1.0789	0.7245	0.693275	0.0329
47635.5	1.1018	0.7181	0.695125	0.0307
48412.5	1.1131	0.7326	0.709575	0.0308
49189.5	1.1157	0.7379	0.662725	0.0353
49966.5	1.1058	0.72	0.682725	0.0363
50743.5	1.1054	0.7302	0.677875	0.0355
51520.5	1.0935	0.7393	0.66325	0.037
52297.5	1.088	0.7222	0.67355	0.0297
53074.5	1.1229	0.7287	0.67355	0.0313
53851.5	1.1283	0.7411	0.655575	0.0332
54628.5	1.1187	0.7469	0.6679	0.0337
55405.5	1.1202	0.7453	0.666375	0.0329
56182.5	1.1117	0.7391	0.675375	0.0371
56959.5	1.1086	0.7285	0.685375	0.0325
57736.5	1.1252	0.7367	0.6749	0.0388
58513.5	1.1086	0.7187	0.68815	0.0409
59290.5	1.1106	0.7328	0.6804	0.0384
60067.5	1.1106	0.7246	0.687625	0.0337
60844.5	1.1085	0.7201	0.692625	0.0327
61621.5	1.1088	0.737	0.67745	0.036
62398.5	1.1211	0.7054	0.704025	0.0336
63175.5	1.1281	0.712	0.698175	0.0395
63952.5	1.0949	0.7459	0.6735	0.0338
64729.5	1.122	0.7455	0.672375	0.0358
65506.5	1.0936	0.7483	0.67205	0.0318
66283.5	1.1151	0.7154	0.6922	0.0372
67060.5	1.1341	0.7449	0.677425	0.0317
67837.5	1.0962	0.7256	0.680775	0.0368
68614.5	1.1224	0.7119	0.699775	0.0356
69391.5	1.0972	0.7397	0.675975	0.0359

70168.5	1.1236	0.7193	0.695675	0.0374
70945.5	1.1171	0.7481	0.676	0.0373
71722.5	1.1392	0.7485	0.673975	0.0365
72499.5	1.1367	0.7271	0.693125	0.033
73276.5	1.14	0.7488	0.675625	0.0404
74053.5	1.1277	0.7236	0.69875	0.0377
74830.5	1.1214	0.7324	0.682925	0.0385
75607.5	1.0945	0.7491	0.67895	0.0326
76384.5	1.1261	0.7327	0.693825	0.031
D-Glucaro-1,4-lactone Plus One and a Half Equivalent DCl Duplicate				
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-6,3-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.3779	1.2617	0.027075	0.0249
1015.5	0.4714	1.1783	0.029075	0.0186
1792.5	0.5677	1.0566	0.0801	0.0223
2569.5	0.6254	0.98	0.118875	0.0201
3346.5	0.6741	0.9422	0.119975	0.0194
4123.5	0.729	0.906	0.1391	0.0197
4900.5	0.751	0.8412	0.164575	0.0192
5677.5	0.7682	0.8015	0.182225	0.0192
6454.5	0.7849	0.7688	0.203025	0.0188
7231.5	0.7841	0.7421	0.19285	0.0185
8008.5	0.7821	0.7158	0.235125	0.0179
8785.5	0.7878	0.7013	0.231975	0.0187
9562.5	0.7928	0.6805	0.24345	0.0183
10339.5	0.8013	0.67	0.270825	0.0171
11116.5	0.7875	0.6434	0.285425	0.0179
11893.5	0.8146	0.6434	0.28645	0.0182
12670.5	0.8071	0.6183	0.3051	0.0165
13447.5	0.8212	0.61	0.3072	0.0155
14224.5	0.8251	0.6101	0.304025	0.0183
15001.5	0.8209	0.6015	0.31605	0.0186
15778.5	0.8365	0.6032	0.3196	0.0186
16555.5	0.8186	0.6032	0.323175	0.0182
17332.5	0.8224	0.5874	0.3458	0.0179
18109.5	0.8288	0.5798	0.336475	0.0191
18886.5	0.8219	0.5804	0.3516	0.0182
19663.5	0.8283	0.5804	0.34965	0.0183

20440.5	0.8217	0.5838	0.3499	0.0192
21217.5	0.8214	0.5698	0.35965	0.0198
21994.5	0.8259	0.5753	0.3515	0.0197
22771.5	0.8278	0.5717	0.3586	0.0191
23548.5	0.8252	0.5665	0.356525	0.02
24325.5	0.8206	0.5669	0.352025	0.0197
25102.5	0.8191	0.5664	0.367725	0.0196
25879.5	0.8244	0.5563	0.374375	0.0203
26656.5	0.8238	0.5516	0.378175	0.019
27433.5	0.8188	0.5525	0.37725	0.0159
28210.5	0.8112	0.5538	0.371175	0.0191
28987.5	0.8133	0.5412	0.3862	0.0199
29764.5	0.811	0.5419	0.3853	0.0148
30541.5	0.8102	0.5319	0.404	0.0201
31318.5	0.8123	0.5327	0.388025	0.0183
32095.5	0.8102	0.5366	0.397575	0.0184
32872.5	0.8115	0.5411	0.396675	0.0173
33649.5	0.8126	0.5369	0.39695	0.0205
34426.5	0.8119	0.5392	0.400675	0.0163
35203.5	0.8068	0.5373	0.38775	0.0199
35980.5	0.806	0.54	0.39935	0.0198
36757.5	0.804	0.5261	0.40985	0.0195
37534.5	0.8071	0.5209	0.4105	0.0205
38311.5	0.8026	0.5225	0.40545	0.0199
39088.5	0.7971	0.5305	0.400875	0.0211
39865.5	0.8078	0.5385	0.3862	0.0203
40642.5	0.7977	0.5398	0.378675	0.0209
41419.5	0.8044	0.5292	0.39475	0.0213
42196.5	0.8002	0.5394	0.389625	0.0202
42973.5	0.7859	0.5283	0.396325	0.0145
43750.5	0.7998	0.5237	0.39095	0.0204
44527.5	0.7997	0.5262	0.400275	0.0206
45304.5	0.7915	0.5393	0.375325	0.0203
46081.5	0.8033	0.5263	0.399825	0.0207
46858.5	0.7987	0.5312	0.3945	0.0206
47635.5	0.8038	0.5341	0.386375	0.0204
48412.5	0.7964	0.5336	0.38185	0.0216
49189.5	0.8045	0.5367	0.388725	0.0209
49966.5	0.8013	0.5221	0.395725	0.0199

50743.5	0.7927	0.5253	0.4015	0.0211
51520.5	0.7973	0.5298	0.397	0.0212
52297.5	0.7819	0.5244	0.40245	0.0147
53074.5	0.805	0.5314	0.3897	0.0194
53851.5	0.8047	0.5253	0.3888	0.0207
54628.5	0.8041	0.5209	0.390525	0.0209
55405.5	0.801	0.5262	0.3852	0.0212
56182.5	0.8042	0.5238	0.3964	0.0213
56959.5	0.8027	0.535	0.3853	0.0217
57736.5	0.8015	0.5379	0.390125	0.021
58513.5	0.7998	0.5318	0.394375	0.0212
59290.5	0.8002	0.5173	0.398875	0.0217
60067.5	0.7995	0.5262	0.395175	0.0205
60844.5	0.7971	0.5325	0.39245	0.0219
61621.5	0.8016	0.521	0.39215	0.0208
62398.5	0.8019	0.5162	0.3989	0.0204
63175.5	0.8056	0.515	0.3992	0.0213
63952.5	0.8021	0.5165	0.399	0.0206
64729.5	0.8009	0.5319	0.391575	0.0211
65506.5	0.8016	0.52	0.39905	0.0212
66283.5	0.7943	0.5225	0.4003	0.0205
67060.5	0.8001	0.52	0.4015	0.0218
67837.5	0.793	0.5118	0.410925	0.021
68614.5	0.7977	0.5131	0.404875	0.0228
69391.5	0.7988	0.5396	0.3912	0.0215

D-Glucaro-1,4;6,3-dilactone

Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0258	0.1774	2.2224
1015.5	0.0291	0.185	2.1935
1792.5	0.0301	0.1863	2.1066
2569.5	0.0305	0.2063	2.0598
3346.5	0.0324	0.2265	2.0244
4123.5	0.0331	0.2214	1.9723
4900.5	0.0302	0.2449	1.9259
5677.5	0.0288	0.2696	1.856
6454.5	0.0298	0.2774	1.7759
7231.5	0.0284	0.2916	1.7604

8008.5	0.0249	0.3016	1.6731
8785.5	0.0284	0.3057	1.6354
9562.5	0.0225	0.3236	1.5493
10339.5	0.0348	0.3549	1.5186
11116.5	0.033	0.3614	1.5116
11893.5	0.0363	0.371	1.5028
12670.5	0.0397	0.3782	1.4266
13447.5	0.0332	0.406	1.3855
14224.5	0.0427	0.4303	1.299
15001.5	0.0419	0.4408	1.2596
15778.5	0.0402	0.4663	1.2455
16555.5	0.0408	0.4972	1.2585
17332.5	0.048	0.4982	1.1318
18109.5	0.0481	0.4921	1.1278
18886.5	0.0535	0.5629	1.0766
19663.5	0.0566	0.5825	1.0408
20440.5	0.064	0.5852	1.0315
21217.5	0.0754	0.5853	0.9961
21994.5	0.0754	0.5911	0.9912
22771.5	0.0773	0.606	0.9684
23548.5	0.0763	0.6019	0.9116
24325.5	0.0716	0.6523	0.8923
25102.5	0.0705	0.668	0.8777
25879.5	0.0792	0.6461	0.8402
26656.5	0.0835	0.6407	0.8108
27433.5	0.08	0.6546	0.7878
28210.5	0.0722	0.6851	0.7733
28987.5	0.0663	0.6618	0.7953
29764.5	0.0794	0.6707	0.7092
30541.5	0.0857	0.6651	0.6566
31318.5	0.0868	0.6618	0.6867
32095.5	0.0764	0.6592	0.6336
32872.5	0.081	0.679	0.6143
33649.5	0.085	0.682	0.6037
34426.5	0.0839	0.6957	0.6341
35203.5	0.0917	0.7053	0.6304
35980.5	0.0856	0.6937	0.5627
36757.5	0.1077	0.721	0.5724
37534.5	0.1006	0.7359	0.5535

38311.5	0.1025	0.7369	0.5677
D-Glucaro-1,4;6,3-dilactone Duplicate			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0104	0.081	1.1891
1015.5	0.0119	0.1069	1.1643
1792.5	0.0137	0.1124	1.1372
2569.5	0.0159	0.1342	1.0937
3346.5	0.0112	0.1459	1.0562
4123.5	0.0138	0.1453	1.0476
4900.5	0.0093	0.1477	1.0231
5677.5	0.0129	0.1635	0.9996
6454.5	0.0108	0.1691	0.9871
7231.5	0.0121	0.1791	0.9336
8008.5	0.0123	0.1822	0.9178
8785.5	0.0144	0.1961	0.8966
9562.5	0.014	0.2034	0.8776
10339.5	0.0137	0.218	0.8521
11116.5	0.0132	0.2137	0.846
11893.5	0.0125	0.224	0.8228
12670.5	0.0127	0.2257	0.8104
13447.5	0.0141	0.2517	0.781
14224.5	0.0154	0.2611	0.7637
15001.5	0.0154	0.2557	0.7437
15778.5	0.0166	0.2607	0.7101
16555.5	0.0182	0.2694	0.7046
17332.5	0.0176	0.2751	0.6871
18109.5	0.0194	0.2798	0.649
18886.5	0.0201	0.2834	0.6262
19663.5	0.0228	0.2905	0.6084
20440.5	0.0203	0.3036	0.5886
21217.5	0.0277	0.3104	0.5053
21994.5	0.0285	0.3256	0.5373
22771.5	0.0272	0.3221	0.5296
23548.5	0.0248	0.3324	0.5138
24325.5	0.0262	0.3341	0.5
25102.5	0.0289	0.3389	0.4699
25879.5	0.0284	0.3345	0.451

26656.5	0.0313	0.3447	0.4312
27433.5	0.0331	0.3474	0.4212
28210.5	0.0348	0.3486	0.417
28987.5	0.038	0.3592	0.3659
29764.5	0.0361	0.3667	0.3637
30541.5	0.0379	0.3719	0.3697
31318.5	0.0391	0.3731	0.3558
32095.5	0.0398	0.378	0.3447
32872.5	0.0423	0.3812	0.3336
33649.5	0.0421	0.3891	0.3142
34426.5	0.0439	0.3876	0.2951
35203.5	0.0433	0.3911	0.2869
35980.5	0.044	0.3923	0.2735
36757.5	0.0453	0.3945	0.2695
37534.5	0.0441	0.4053	0.2552
38311.5	0.0475	0.4074	0.2528
39088.5	0.0487	0.407	0.249
39865.5	0.0468	0.4053	0.2363
40642.5	0.0486	0.4047	0.2278
41419.5	0.0501	0.4054	0.213
42196.5	0.0516	0.4037	0.2068
42973.5	0.0557	0.403	0.1978
43750.5	0.0597	0.4008	0.1904
44527.5	0.0592	0.4022	0.1845
45304.5	0.0628	0.404	0.1775
46081.5	0.0638	0.4084	0.1703
46858.5	0.0633	0.4044	0.1694
47635.5	0.0642	0.4037	0.1607
48412.5	0.067	0.4014	0.1564
49189.5	0.0687	0.405	0.1491
49966.5	0.0715	0.401	0.1335
D-Glucaro-1,4;6,3-dilactone Plus Half Equivalent DCl			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0138	0.1394	0.4355
1015.5	0.0303	0.1832	0.2779
1792.5	0.043	0.2108	0.191
2569.5	0.0536	0.2264	0.139

3346.5	0.0714	0.2284	0.0965
4123.5	0.0894	0.2216	0.0738
4900.5	0.1057	0.2224	0.0584
5677.5	0.1166	0.2267	0.0481
6454.5	0.1337	0.2223	0.0429
7231.5	0.1507	0.2253	0.0328
8008.5	0.1577	0.2241	0.0314
8785.5	0.166	0.2272	0.0283
9562.5	0.1864	0.2261	0.0248
10339.5	0.1945	0.2266	0.0226
11116.5	0.2042	0.2202	0.0204
11893.5	0.2191	0.2252	0.0201
12670.5	0.2239	0.2222	0.0165
13447.5	0.2364	0.2194	0.0183
14224.5	0.2485	0.2182	0.0213
15001.5	0.2537	0.2252	0.0211
15778.5	0.2525	0.2202	0.0213
16555.5	0.2598	0.2245	0.0237
17332.5	0.2644	0.2134	0.0223
18109.5	0.275	0.2244	0.0222
18886.5	0.2805	0.2167	0.0214
19663.5	0.287	0.2179	0.0228
20440.5	0.288	0.2176	0.0209
21217.5	0.2998	0.2171	0.0193
21994.5	0.3003	0.2181	0.0215
22771.5	0.305	0.2172	0.0236
23548.5	0.3093	0.216	0.0274
24325.5	0.3212	0.2135	0.0202
25102.5	0.324	0.2154	0.0185
25879.5	0.3286	0.218	0.0176
26656.5	0.3339	0.217	0.0202
27433.5	0.336	0.2204	0.0225
28210.5	0.3395	0.2261	0.0208
28987.5	0.3401	0.2134	0.0204
29764.5	0.3439	0.2216	0.0199
30541.5	0.3479	0.2239	0.022
31318.5	0.3487	0.2252	0.0188
32095.5	0.3535	0.2235	0.0222
32872.5	0.3565	0.2224	0.0171

33649.5	0.3574	0.22	0.0228
34426.5	0.3614	0.2186	0.0201
35203.5	0.3705	0.2288	0.0186
35980.5	0.3606	0.2211	0.0197
36757.5	0.3662	0.2203	0.017
37534.5	0.3639	0.2142	0.0165
38311.5	0.362	0.2257	0.0193
39088.5	0.3517	0.2265	0.0157
39865.5	0.3594	0.2287	0.0228
40642.5	0.3625	0.2239	0.0198
41419.5	0.3601	0.2272	0.0214
42196.5	0.3644	0.2222	0.016
42973.5	0.3668	0.2245	0.0182
43750.5	0.3567	0.2289	0.0214
44527.5	0.358	0.2218	0.0206
45304.5	0.3613	0.2294	0.0153
46081.5	0.3574	0.2296	0.0175
46858.5	0.3575	0.2263	0.0207
47635.5	0.363	0.2208	0.0187
48412.5	0.3584	0.2218	0.0159
49189.5	0.3673	0.2219	0.0185
49966.5	0.3568	0.22	0.0136
50743.5	0.3614	0.2208	0.0213
51520.5	0.3686	0.2277	0.0181
52297.5	0.3693	0.2276	0.022
53074.5	0.3672	0.2287	0.0186
53851.5	0.3571	0.2262	0.0163
54628.5	0.362	0.227	0.0216
55405.5	0.3652	0.2229	0.0197
56182.5	0.3632	0.2284	0.0175
56959.5	0.362	0.2241	0.017
57736.5	0.3595	0.2244	0.0181
58513.5	0.3657	0.224	0.0141
59290.5	0.3664	0.2248	0.017
60067.5	0.364	0.2313	0.0144
60844.5	0.3592	0.2268	0.0172
61621.5	0.3601	0.2273	0.0186
62398.5	0.3548	0.2282	0.019
63175.5	0.3638	0.2186	0.0167

63952.5	0.3686	0.228	0.0203
64729.5	0.3685	0.2236	0.0162
65506.5	0.3609	0.2217	0.0171
66283.5	0.3674	0.2123	0.0169
67060.5	0.3613	0.2245	0.0189
67837.5	0.3633	0.2267	0.0126
68614.5	0.36	0.2252	0.0146
69391.5	0.367	0.2307	0.0162
70168.5	0.3548	0.2224	0.0182
70945.5	0.3612	0.2275	0.0171
71722.5	0.3596	0.2238	0.0147
72499.5	0.3698	0.2212	0.0138
73276.5	0.3606	0.2247	0.0172
74053.5	0.365	0.2261	0.018
74830.5	0.3589	0.2177	0.0158
75607.5	0.3521	0.2248	0.0179
76384.5	0.3564	0.2263	0.0191
77161.5	0.367	0.217	0.0177
D-Glucaro-1,4;6,3-dilactone Plus Half Equivalent DCl Duplicate			
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0235	0.2854	1.2375
1015.5	0.0355	0.3928	0.9624
1792.5	0.0553	0.466	0.7412
2569.5	0.0804	0.5062	0.5465
3346.5	0.0924	0.5431	0.4496
4123.5	0.1323	0.5476	0.331
4900.5	0.154	0.5434	0.27
5677.5	0.1882	0.5668	0.22
6454.5	0.2247	0.5446	0.177
7231.5	0.2594	0.5494	0.1453
8008.5	0.2832	0.5497	0.1091
8785.5	0.3096	0.5346	0.0852
9562.5	0.3405	0.5386	0.0755
10339.5	0.3636	0.5343	0.0748
11116.5	0.383	0.5426	0.0697
11893.5	0.4355	0.5343	0.0632
12670.5	0.4606	0.5392	0.0596

13447.5	0.4886	0.5248	0.0523
14224.5	0.5201	0.5254	0.0481
15001.5	0.5422	0.5404	0.044
15778.5	0.5504	0.5236	0.0473
16555.5	0.5753	0.5355	0.0408
17332.5	0.5955	0.5336	0.0481
18109.5	0.6126	0.5233	0.0525
18886.5	0.6221	0.5254	0.0535
19663.5	0.6429	0.5377	0.0472
20440.5	0.6659	0.5433	0.0462
21217.5	0.6718	0.5404	0.0466
21994.5	0.6761	0.54	0.0477
22771.5	0.6928	0.5241	0.0448
23548.5	0.7053	0.5293	0.0476
24325.5	0.7217	0.5437	0.0394
25102.5	0.7298	0.5254	0.0499
25879.5	0.7433	0.5384	0.0445
26656.5	0.7483	0.5386	0.0439
27433.5	0.7697	0.5466	0.0402
28210.5	0.7704	0.5465	0.0525
28987.5	0.7918	0.545	0.0452
29764.5	0.8013	0.5222	0.0482
30541.5	0.8044	0.5315	0.0458
31318.5	0.8084	0.5312	0.0488
32095.5	0.8126	0.5293	0.0696
32872.5	0.817	0.5313	0.0486
33649.5	0.8226	0.5417	0.0478
34426.5	0.8209	0.5404	0.0444
35203.5	0.8182	0.5368	0.0477
35980.5	0.818	0.5203	0.0426
36757.5	0.8132	0.5309	0.0419
37534.5	0.8173	0.5221	0.0467
38311.5	0.8223	0.5273	0.0449
39088.5	0.8227	0.5418	0.0444
39865.5	0.8296	0.5491	0.0441
40642.5	0.8198	0.5233	0.037
41419.5	0.8228	0.5453	0.0456
42196.5	0.8213	0.5392	0.0503
42973.5	0.8219	0.5285	0.0401

43750.5	0.8285	0.5239	0.0418
44527.5	0.8291	0.5479	0.0388
45304.5	0.8145	0.5415	0.047
46081.5	0.8312	0.5461	0.0421
46858.5	0.8232	0.5328	0.0389
47635.5	0.8321	0.5316	0.0346
48412.5	0.8261	0.5353	0.0441
49189.5	0.8247	0.5382	0.0437
49966.5	0.8164	0.5241	0.0409
50743.5	0.8338	0.5397	0.0391
51520.5	0.821	0.548	0.038
52297.5	0.8363	0.5402	0.0402
53074.5	0.8285	0.5381	0.0328
53851.5	0.8162	0.5362	0.0487
54628.5	0.8167	0.5325	0.0495
55405.5	0.8106	0.5384	0.0365
56182.5	0.8221	0.5413	0.0451
56959.5	0.8288	0.525	0.0406
57736.5	0.8097	0.54	0.0451
58513.5	0.8277	0.5298	0.0393
59290.5	0.8312	0.5473	0.0386
60067.5	0.8127	0.544	0.0401

D-Glucaro-1,4;6,3-dilactone Plus Equivalent DCl				
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-6,3-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0778	0.7122	0.720525	1.8485
1015.5	0.1464	0.8604	1.519075	1.0589
1792.5	0.2428	0.9742	1.877925	0.6105
2569.5	0.3529	1.0213	2.069075	0.3913
3346.5	0.4543	1.0499	2.110625	0.2609
4123.5	0.5452	1.0631	1.785525	0.164
4900.5	0.6414	1.0771	1.644475	0.1243
5677.5	0.7014	1.0854	1.57005	0.1348
6454.5	0.779	1.083	1.47715	0.1268
7231.5	0.8382	1.0812	1.3754	0.1092
8008.5	0.926	1.0698	1.31125	0.1058
8785.5	0.9808	1.0696	1.2888	0.0956
9562.5	1.0037	1.0772	1.26525	0.0945

10339.5	1.0389	1.0642	1.245475	0.0805
11116.5	1.0695	1.0748	1.226875	0.0836
11893.5	1.0861	1.071	1.202725	0.0853
12670.5	1.141	1.0675	1.2027	0.0898
13447.5	1.1691	1.0594	1.19572	0.0882
14224.5	1.1859	1.0621	1.23745	0.0978
15001.5	1.2279	1.0609	1.174825	0.0848
15778.5	1.2423	1.0629	1.14675	0.0827
16555.5	1.2764	1.0675	1.12645	0.0781
17332.5	1.292	1.065	1.1065	0.0893
18109.5	1.3294	1.0819	1.08965	0.0911
18886.5	1.3168	1.064	1.1387	0.0868
19663.5	1.3309	1.0735	1.105425	0.0812
20440.5	1.335	1.0671	1.100725	0.088
21217.5	1.3409	1.0647	1.08115	0.0837
21994.5	1.3558	1.0662	1.0717	0.0862
22771.5	1.3528	1.0778	1.062	0.0717
23548.5	1.3734	1.0833	1.0373	0.0927
24325.5	1.3721	1.0654	1.032925	0.0808
25102.5	1.3794	1.0729	1.005925	0.0709
25879.5	1.3843	1.0758	0.998125	0.0816
26656.5	1.4107	1.0881	0.989875	0.0725
27433.5	1.4059	1.0808	0.98325	0.0922
28210.5	1.4089	1.0802	0.971475	0.0787
28987.5	1.409	1.0783	0.969	0.0801
29764.5	1.4106	1.0877	0.959	0.0803
30541.5	1.4123	1.0636	0.969075	0.0724
31318.5	1.4068	1.078	0.9379	0.0856
32095.5	1.4069	1.0672	0.947075	0.0715
32872.5	1.4203	1.0745	0.936525	0.0767
33649.5	1.4103	1.0779	0.9338	0.0681
34426.5	1.4032	1.086	0.921625	0.0777
35203.5	1.4245	1.0881	0.91695	0.0865
35980.5	1.4225	1.0733	0.920675	0.0922
36757.5	1.4061	1.0615	0.939925	0.0849
37534.5	1.4202	1.0736	0.940875	0.0808
38311.5	1.4091	1.0843	0.935625	0.0724
39088.5	1.402	1.0809	0.929925	0.0853
39865.5	1.4107	1.0747	0.9321	0.0884

40642.5	1.4017	1.0886	0.9349	0.0758
41419.5	1.4217	1.0672	0.9302	0.0972
42196.5	1.4181	1.067	0.924475	0.0875
42973.5	1.4075	1.0782	0.922675	0.0828
43750.5	1.4024	1.0771	0.924375	0.0775
44527.5	1.4153	1.0718	0.92695	0.0828
45304.5	1.4115	1.0879	0.91385	0.0763
46081.5	1.4226	1.0712	0.925975	0.0742
46858.5	1.4074	1.0853	0.91865	0.0745
47635.5	1.4247	1.0759	0.921975	0.0815
48412.5	1.4144	1.0758	0.905825	0.0971
49189.5	1.4117	1.0698	0.9261	0.0923
49966.5	1.4121	1.0682	0.936125	0.0759
50743.5	1.4117	1.0719	0.921575	0.0943
51520.5	1.407	1.0836	0.903625	0.0904
52297.5	1.4098	1.0875	0.90785	0.0901
53074.5	1.4214	1.0886	0.917775	0.0846
53851.5	1.4176	1.088	0.905975	0.0817
54628.5	1.4274	1.077	0.925875	0.0773
55405.5	1.4269	1.0699	0.929475	0.0888
56182.5	1.4124	1.0804	0.912475	0.0946
56959.5	1.4032	1.0794	0.91065	0.0871
57736.5	1.4201	1.0766	0.916275	0.0822
58513.5	1.4202	1.089	0.908175	0.0895
59290.5	1.4284	1.0879	0.90145	0.0978
60067.5	1.4156	1.0763	0.922575	0.1018
60844.5	1.4289	1.0853	0.9033	0.0941
61621.5	1.4246	1.08	0.913725	0.085
62398.5	1.425	1.0819	0.9089	0.0891
63175.5	1.4278	1.0773	0.9095	0.0959
63952.5	1.4267	1.091	0.902625	0.0965
64729.5	1.4145	1.0833	0.907625	0.0843
65506.5	1.4239	1.0799	0.904725	0.0937
66283.5	1.4139	1.0769	0.920625	0.0765
67060.5	1.4295	1.0923	0.90305	0.0851
67837.5	1.4162	1.0843	0.913225	0.0802
68614.5	1.4232	1.0747	0.92675	0.0736
69391.5	1.4099	1.0724	0.927075	0.0749
70168.5	1.4174	1.0768	0.9196	0.0694

70945.5	1.4066	1.0907	0.916075	0.0627
71722.5	1.4154	1.0732	0.92555	0.0756
72499.5	1.4024	1.0762	0.92095	0.0789
73276.5	1.4149	1.077	0.9369	0.0703
74053.5	1.4072	1.0781	0.9171	0.0857
74830.5	1.4159	1.0707	0.919575	0.0837
75607.5	1.4215	1.0836	0.93145	0.0841
76384.5	1.4129	1.0723	0.93585	0.0878
77161.5	1.4211	1.0857	0.9059	0.0903
D-Glucaro-1,4;6,3-dilactone Plus Equivalent DCl Duplicate				
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-6,3-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.0672	0.3571	0.642775	0.5064
1015.5	0.1374	0.4378	0.8201	0.2596
1792.5	0.211	0.4695	0.863775	0.1474
2569.5	0.2785	0.4791	0.8639	0.0957
3346.5	0.3398	0.5001	0.8274	0.0739
4123.5	0.3938	0.51	0.7879	0.0574
4900.5	0.4288	0.5147	0.757125	0.062
5677.5	0.4682	0.5211	0.702675	0.0579
6454.5	0.5066	0.5085	0.691925	0.0516
7231.5	0.5173	0.5044	0.66465	0.0545
8008.5	0.544	0.5095	0.630325	0.0569
8785.5	0.5665	0.4984	0.627675	0.0468
9562.5	0.5703	0.5034	0.61555	0.0465
10339.5	0.6018	0.5057	0.596625	0.0503
11116.5	0.6155	0.5079	0.57955	0.046
11893.5	0.6245	0.5026	0.568	0.0437
12670.5	0.6307	0.5094	0.552675	0.0465
13447.5	0.6466	0.4994	0.5474	0.0466
14224.5	0.6516	0.5013	0.539825	0.0465
15001.5	0.6655	0.5019	0.523275	0.0505
15778.5	0.6637	0.5095	0.5085	0.051
16555.5	0.6586	0.4998	0.508525	0.0487
17332.5	0.6754	0.5067	0.496975	0.0437
18109.5	0.6733	0.4991	0.502525	0.0429
18886.5	0.6795	0.508	0.48925	0.0427
19663.5	0.6774	0.4993	0.497925	0.0457

20440.5	0.674	0.5021	0.495075	0.0416
21217.5	0.6763	0.5081	0.491	0.0417
21994.5	0.6749	0.4921	0.494175	0.042
22771.5	0.6763	0.5077	0.493175	0.038
23548.5	0.6771	0.5007	0.4871	0.0437
24325.5	0.6808	0.5031	0.48125	0.0414
25102.5	0.6847	0.5068	0.482125	0.041
25879.5	0.6836	0.5023	0.48395	0.0389
26656.5	0.6798	0.5109	0.4824	0.0376
27433.5	0.687	0.5048	0.4756	0.0403
28210.5	0.6819	0.5017	0.4708	0.0382
28987.5	0.6862	0.5145	0.484575	0.0405
29764.5	0.6847	0.512	0.46885	0.0367
30541.5	0.6891	0.5013	0.4927	0.0431
31318.5	0.686	0.5055	0.4828	0.0418
32095.5	0.6801	0.5055	0.4712	0.0433
32872.5	0.6895	0.5081	0.47195	0.0404
33649.5	0.6862	0.5174	0.462175	0.0386
34426.5	0.6845	0.5139	0.465975	0.0411
35203.5	0.6827	0.5064	0.4749	0.0393
35980.5	0.6863	0.5021	0.48124	0.0327
36757.5	0.6921	0.5041	0.47805	0.0343
37534.5	0.6853	0.5136	0.47485	0.0325
38311.5	0.6826	0.5038	0.465925	0.0408
39088.5	0.6858	0.512	0.4768	0.0349
39865.5	0.6959	0.5088	0.472275	0.0336
40642.5	0.6947	0.5171	0.4578	0.0371
41419.5	0.6942	0.5103	0.463	0.0313
42196.5	0.6985	0.5086	0.47335	0.0375
42973.5	0.6969	0.5177	0.46265	0.0487
43750.5	0.6936	0.5033	0.475325	0.0436
44527.5	0.6913	0.5068	0.469375	0.0385
45304.5	0.6904	0.5061	0.467325	0.0381
46081.5	0.689	0.5011	0.473375	0.0429
46858.5	0.6912	0.5025	0.462025	0.0346
47635.5	0.6871	0.5047	0.472475	0.0333
48412.5	0.6833	0.5036	0.4647	0.0316
49189.5	0.683	0.5125	0.464525	0.0351
49966.5	0.6877	0.5177	0.4544	0.0371

50743.5	0.6862	0.5054	0.46355	0.0366
51520.5	0.6912	0.5017	0.469425	0.0323
52297.5	0.6813	0.5092	0.460625	0.0307
53074.5	0.6859	0.5047	0.471	0.0303
53851.5	0.687	0.5042	0.4592	0.0351
54628.5	0.6835	0.5006	0.468025	0.0336
55405.5	0.6882	0.5073	0.457625	0.0437
56182.5	0.686	0.5086	0.460325	0.0349
56959.5	0.6841	0.5071	0.454525	0.0403
57736.5	0.6861	0.5101	0.455075	0.0387
58513.5	0.6826	0.5056	0.457	0.0358
59290.5	0.6782	0.5103	0.458775	0.0319
60067.5	0.6804	0.5137	0.45195	0.0321
60844.5	0.6837	0.5068	0.46245	0.0342
61621.5	0.6859	0.5043	0.45515	0.045
62398.5	0.6831	0.5081	0.4545	0.0312
63175.5	0.6812	0.5086	0.454	0.0318
63952.5	0.6888	0.5089	0.44775	0.0361
64729.5	0.6849	0.5072	0.451025	0.0362
65506.5	0.6832	0.5028	0.459475	0.0457
66283.5	0.6829	0.5072	0.4509	0.033
67060.5	0.6888	0.5117	0.46015	0.0278
67837.5	0.6836	0.5104	0.45025	0.0337
68614.5	0.6882	0.5162	0.451275	0.0374
69391.5	0.686	0.511	0.448425	0.0416
70168.5	0.686	0.5116	0.44705	0.0372
70945.5	0.6807	0.5133	0.43815	0.0332
71722.5	0.6873	0.5183	0.441075	0.0357
72499.5	0.6898	0.5114	0.455075	0.0365
73276.5	0.6882	0.5098	0.445675	0.0347
74053.5	0.6854	0.5128	0.44145	0.0388
74830.5	0.6862	0.5135	0.440825	0.0387
75607.5	0.6856	0.5073	0.441875	0.0365
76384.5	0.6844	0.5125	0.43565	0.0458
77161.5	0.6862	0.5129	0.43375	0.0365
D-Glucaro-1,4;6,3-dilactone Plus One and a Half Equivalent DCl				
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-6,3-lactone	D-Glucaro-1,4;6,3-Dilactone

238.5	0.1468	1.3445	1.65615	2.0289
1015.5	0.3414	1.444	2.183925	0.9626
1792.5	0.5242	1.5475	2.54715	0.5016
2569.5	0.7363	1.5644	2.6258	0.3177
3346.5	0.9005	1.6161	2.68215	0.2375
4123.5	1.0618	1.6559	2.558575	0.1892
4900.5	1.2263	1.6821	2.466025	0.1521
5677.5	1.3021	1.6894	2.241975	0.1491
6454.5	1.4093	1.7012	2.207925	0.1448
7231.5	1.5107	1.7069	2.101225	0.1496
8008.5	1.5698	1.7164	2.00505	0.1472
8785.5	1.6238	1.7273	1.935075	0.1445
9562.5	1.6908	1.7362	1.9336	0.1377
10339.5	1.7482	1.7372	1.908525	0.1541
11116.5	1.852	1.7508	1.88125	0.1426
11893.5	1.932	1.7592	1.870125	0.1576
12670.5	2.0159	1.7764	1.819725	0.1507
13447.5	2.0724	1.7822	1.80845	0.1349
14224.5	2.1101	1.7959	1.7467	0.1378
15001.5	2.1541	1.8089	1.74015	0.1446
15778.5	2.1962	1.823	1.73555	0.1322
16555.5	2.2044	1.8259	1.730275	0.1484
17332.5	2.2435	1.8341	1.7216	0.1185
18109.5	2.2523	1.8429	1.70905	0.1448
18886.5	2.2614	1.8414	1.729025	0.1317
19663.5	2.2539	1.8443	1.7117	0.1476
20440.5	2.2635	1.8546	1.708575	0.148
21217.5	2.2289	1.8568	1.7031	0.1338
21994.5	2.2389	1.8646	1.694025	0.1325
22771.5	2.2383	1.8765	1.696475	0.1467
23548.5	2.2512	1.8821	1.678825	0.1429
24325.5	2.2631	1.8944	1.6782	0.1367
25102.5	2.2613	1.909	1.664	0.1251
25879.5	2.2728	1.8988	1.678725	0.1348
26656.5	2.2598	1.8939	1.676975	0.1489
27433.5	2.2623	1.8993	1.67095	0.1272
28210.5	2.2681	1.8951	1.690875	0.1293
28987.5	2.256	1.8975	1.666375	0.1346
29764.5	2.2636	1.8871	1.687875	0.137

30541.5	2.2382	1.8988	1.65295	0.1342
31318.5	2.2679	1.9022	1.645925	0.1253
32095.5	2.2329	1.9099	1.648525	0.129
32872.5	2.2615	1.8904	1.693925	0.1304
33649.5	2.2599	1.9036	1.632775	0.1309
34426.5	2.2371	1.9133	1.61785	0.1498
35203.5	2.2413	1.8988	1.5783	0.1517
35980.5	2.2348	1.8985	1.563125	0.1362
36757.5	2.2553	1.9111	1.551	0.125
37534.5	2.2434	1.9077	1.579675	0.1216
38311.5	2.2617	1.9127	1.5611	0.1379
39088.5	2.2384	1.9048	1.5667	0.126
39865.5	2.2617	1.9091	1.5819	0.1493
40642.5	2.2441	1.9087	1.552425	0.1212
41419.5	2.2509	1.9174	1.54595	0.128
42196.5	2.2566	1.9115	1.562225	0.1302
42973.5	2.2646	1.9225	1.539175	0.1357
43750.5	2.2406	1.8921	1.58375	0.1483
44527.5	2.2264	1.8993	1.5884	0.1367
45304.5	2.2637	1.9038	1.578775	0.1449
46081.5	2.2634	1.9082	1.577125	0.1318
46858.5	2.2541	1.8925	1.57355	0.1346
47635.5	2.2438	1.8898	1.58965	0.1117
48412.5	2.2694	1.9138	1.5785	0.1358
D-Glucaro-1,4;6,3-dilactone Plus One and a Half Equivalent DCl Duplicate				
Midpoint Time	D-Glucaric acid	D-Glucaro-1,4-lactone	D-Glucaro-6,3-lactone	D-Glucaro-1,4;6,3-Dilactone
238.5	0.1623	0.8161	1.280425	0.6596
1015.5	0.3568	0.9547	1.3576	0.2728
1792.5	0.4957	1.0004	1.373975	0.1575
2569.5	0.6665	1.0332	1.361225	0.1328
3346.5	0.813	1.0493	1.2803	0.1127
4123.5	0.9157	1.0721	1.18255	0.1026
4900.5	0.9812	1.0833	1.159225	0.0925
5677.5	1.0509	1.1015	1.108075	0.1028
6454.5	1.0731	1.0796	1.080475	0.101
7231.5	1.1616	1.0706	1.037825	0.0978
8008.5	1.2081	1.1021	0.99515	0.0801

8785.5	1.2664	1.0888	0.964425	0.0903
9562.5	1.2806	1.0676	0.949675	0.0884
10339.5	1.2921	1.1028	0.891525	0.0749
11116.5	1.3046	1.0978	0.8792	0.0823
11893.5	1.3114	1.0762	0.893	0.0707
12670.5	1.3288	1.0954	0.89075	0.0782
13447.5	1.3303	1.0955	0.867425	0.081
14224.5	1.3514	1.0673	0.888425	0.0701
15001.5	1.3577	1.0955	0.8668	0.0888
15778.5	1.3385	1.056	0.9068	0.0796
16555.5	1.3445	1.0877	0.879725	0.0828
17332.5	1.3526	1.0696	0.88515	0.076
18109.5	1.3475	1.0602	0.9034	0.0725
18886.5	1.3396	1.0967	0.871975	0.0837
19663.5	1.3323	1.0532	0.89135	0.0754
20440.5	1.3498	1.0914	0.867925	0.0698
21217.5	1.3329	1.0866	0.8732	0.0742
21994.5	1.3287	1.1133	0.85875	0.0665
22771.5	1.3294	1.0647	0.86845	0.0783
23548.5	1.3391	1.1139	0.819775	0.0756
24325.5	1.3446	1.0924	0.873	0.0684
25102.5	1.3257	1.1031	0.8428	0.0714
25879.5	1.3394	1.0661	0.864275	0.074
26656.5	1.3365	1.0892	0.84785	0.0739
27433.5	1.3483	1.1066	0.8461	0.0662
28210.5	1.3322	1.1071	0.83825	0.0733
28987.5	1.3357	1.1	0.85135	0.0722
29764.5	1.3494	1.1137	0.83445	0.0739
30541.5	1.3497	1.1112	0.841575	0.0642
31318.5	1.3431	1.1147	0.82455	0.0785
32095.5	1.3324	1.0837	0.84785	0.0824
32872.5	1.3396	1.0996	0.84015	0.0711
33649.5	1.3435	1.11	0.83975	0.083
34426.5	1.3334	1.1144	0.83345	0.0712
35203.5	1.3439	1.0697	0.8707	0.0714
35980.5	1.3357	1.1002	0.842125	0.0723
36757.5	1.3341	1.1203	0.8233	0.0792
37534.5	1.3454	1.0898	0.8473	0.066
38311.5	1.3381	1.1256	0.826525	0.0751

39088.5	1.3301	1.0821	0.859975	0.0745
39865.5	1.3481	1.1065	0.830225	0.0814
40642.5	1.345	1.1109	0.832575	0.0642
41419.5	1.3424	1.0956	0.839525	0.0781
42196.5	1.3411	1.0878	0.8456	0.0718
42973.5	1.334	1.1148	0.82925	0.0661
43750.5	1.3423	1.0745	0.84795	0.0714
44527.5	1.3376	1.1	0.833725	0.0525
45304.5	1.3437	1.1279	0.812275	0.0736
46081.5	1.3365	1.0684	0.849625	0.0739
46858.5	1.327	1.0892	0.833225	0.0719
47635.5	1.3451	1.1063	0.8223	0.0758
48412.5	1.3357	1.0683	0.8472	0.0787
49189.5	1.3225	1.0879	0.8257	0.0669
49966.5	1.337	1.0878	0.8127	0.0712
50743.5	1.3483	1.1072	0.804575	0.0593
51520.5	1.3373	1.0934	0.814375	0.0716
52297.5	1.3309	1.0723	0.825475	0.0709
53074.5	1.3361	1.0982	0.804225	0.0746
53851.5	1.3467	1.0954	0.81055	0.0697
54628.5	1.332	1.0902	0.8253	0.0643
55405.5	1.3358	1.0757	0.819625	0.074
56182.5	1.3432	1.0667	0.82585	0.0774
56959.5	1.3562	1.0556	0.83935	0.0683
57736.5	1.3283	1.0697	0.830925	0.0631
58513.5	1.3441	1.0869	0.8166	0.0756
59290.5	1.3366	1.0699	0.8252	0.0632
60067.5	1.3304	1.0756	0.827	0.0648
60844.5	1.3321	1.1019	0.798825	0.0754
61621.5	1.3247	1.0898	0.8054	0.0693
62398.5	1.3254	1.0552	0.8307	0.0747
63175.5	1.3363	1.0604	0.83095	0.0746
63952.5	1.3227	1.0563	0.83785	0.0653
64729.5	1.3388	1.0763	0.823275	0.0808
65506.5	1.3345	1.0678	0.82895	0.0686
66283.5	1.3237	1.0571	0.828625	0.0777
67060.5	1.3234	1.0647	0.834575	0.0704
67837.5	1.3155	1.0431	0.8552	0.0779

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