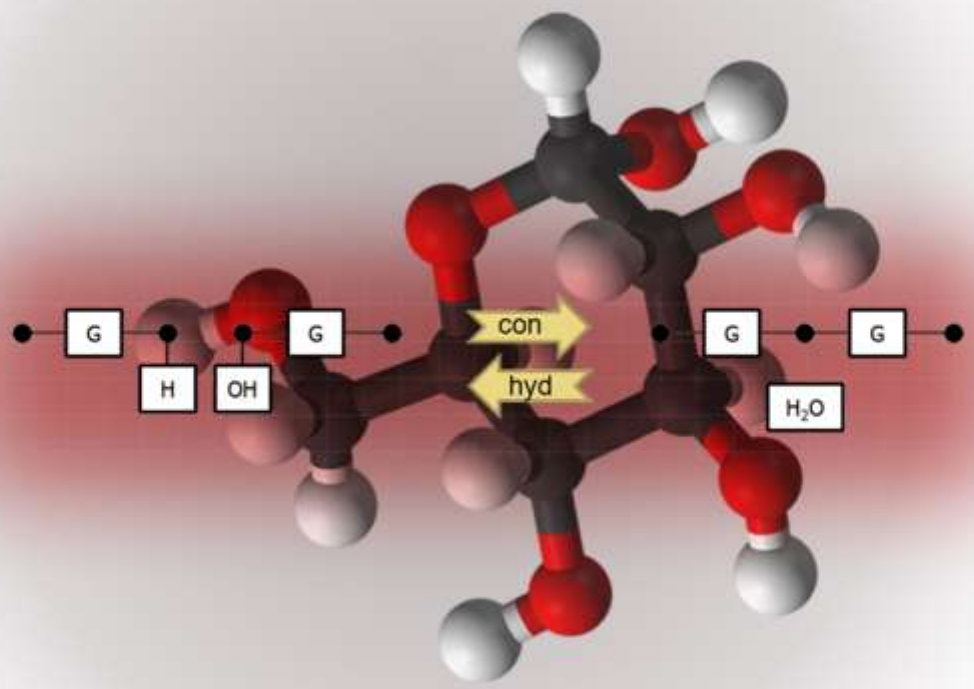
University of Leicester

Department of Computer Science

Chemical Reaction Rate Analysis Using Graph Transformations



CO3120 Computer Science Project

Final Report

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Contents

Tables and Figures

Declaration

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Abstract

The following report documents the outcome of a yearlong project aimed at the derivation of ordinary differential equations for a chemical reaction, using graph transformation techniques. While some articles have described the application of graph transformation techniques to biochemical reactions, and the use of stochastic systems to predict the kinetic profiles of reactions, very few have tried to derive these linear differential equations.

The report first describes the original motivation, aims and objectives for the project. Then, a background in chemistry is included, which describes the manual derivation of ordinary differential equations for a reaction. A summary of relevant graph transformation theory used in the project is also presented. The report briefly describes here some of the research done last term into stochastic graph transformation systems, which provide a foundation to the method eventually developed.

The project core follows, which describes in detail, our developed methodology used to generate the ordinary differential equations for a specific finite reaction network, the adaptation of existing tools that facilitates the application of this methodology, and two case studies to demonstrate this application.

In the final section of the report, there is a discussion of how functional and useful the original project plan was, finding many difficulties in the precise projection of timescales for an open-ended problem such as this. Finally, a critical appraisal focuses on the limitations of the project, such as the limited applicability of the developed methodology, problems with the software used and many suggestions for areas of further work. The project has successful results for one simple reaction (unimolecular nucleophilic substitution, SN1) with very small starting molecules, but limitations in 3rd party analysis tools rendered it useless for testing larger reaction networks and larger molecules. Further work is necessary to investigate how to make analysis faster and more efficient if it is to be of any widespread use for chemists. Nevertheless, the successful case study demonstrates the soundness of the methodology in principle.

1. Introduction

Motivation

The kinetics of any chemical reaction is important for many areas of research, whether it is deducing the reactivity of certain reagents in the lab, or for planning large scale industrial chemical synthesis. The speed at which reactions occur is vital knowledge for any such undertaking. Traditionally, chemists are able to conduct experiments in the lab that ascertain this information. The findings of such experiments relate to the observer important facets of the underlying reaction, such as the stabilities of any chemical species involved or the steps that might have occurred from the starting molecule to the product molecule. This process can however be reversed – by proposing a reaction mechanism and comparing it to experimental data, chemists can gauge the accuracy of their proposals. This offers a way of gaining a deeper understanding of the elemental chemistry behind any complex reaction.

This project aims to derive the ordinary differential equations that describe the kinetics of any reaction using graph transformation techniques. While these equations can be derived by hand, it becomes extremely difficult for unbounded and complex reaction networks. Automation of this process would make it more widely applicable and therefore more useful. Related work in this field has mainly focused on stochastic graph transformation simulations to produce quantitative data rather than the derivation of these linear algebraic equations. The results of such simulations are affected by hardware capabilities (specifically in the number of starting molecules allowed in the system). Ordinary differential equations however, are an alternative level of abstraction with a reproducible result. This result should be utilisable under different conditions to predict the progress of a reaction, whereas the simulation results are specific to the conditions and probabilistic circumstances under which it was run. The two approaches should be used together however to determine their congruence and therefore the accuracy of the proposed reaction mechanism.

While others, such as Cardelli [2], have derived these ordinary differential equations using alternative methods ([2] uses process algebra), these are not intuitive for chemists to use due to their technical content. Graph transformations remain relatively unexplored in this area, despite their visual attractiveness and simplicity. Chemists already used to using graphs (i.e. structural formula) to represent molecules would be more comfortable with an approach that bears some resemblance to this application domain. If a computer science method of deriving ordinary differential equations were to be widely applicable, graph transformation systems seem to be the most promising. Furthermore, Cardelli's process calculus approach requires the establishment of reaction rules that describe involved reactants in their entirety. Graph transformations allow the specification of rules based on functional groups i.e. only the atoms and bonds directly affected by a reaction. This more general specification makes reaction rules reusable in many molecules based on local context.

This task is not trivial, however. The first obstacle is a representation of molecules and reactions in graphs that retains as much of the real chemistry as possible while not complicating the computational analysis. The eventual balance requires an understanding of both the underlying chemistry and the computer science theory. Secondly, the derivation of ordinary differential equations by hand is a complex procedure that could require a number of sequential or repetitive steps. Such a procedure may not only be unappealing to computer scientists to replicate, but also difficult to do so in an uncomplicated way. This project aims to take the first steps towards such a methodology. This methodology once evolved and

improved upon further will aid the automated derivation of ordinary differential equations for simple finite systems, and more importantly, for open infinite reaction mechanisms too, such as polymerisation.

Aims

The project aims to develop a methodology to analyse the quantitative dynamics of chemical reactions, namely in determining the ordinary differential equations (ODE's) which define the rate of reaction. This rate is usually determined as the rate of change of concentration of one of the chemical species (which can be reactants or products) with respect to time. These differential equations will be extracted from a specification of reaction rules as local structural transformations in molecules represented as graphs. The methodology will be verified against actual case studies using appropriate tools.

Objectives

1. *To develop a methodology to model chemical reaction networks and derive ordinary differential equations for these reactions using graph transformation theory*

   This will be the major challenge of the project as the application of graph transformation theory in finding ordinary differential equations for reactions is not well documented.

2. *Case Study 1 – application of methodology derived in 1 to a simple reaction such as esterification*

   This will be an essential step in verifying the model derived in 1 and the subsequent ordinary differential equations against established empirical data. Esterification (the reaction of a carboxylic acid and an alcohol to form an ester) is a fairly simple reversible reaction which can be modelled quite easily. The network is also finite, meaning there are few intermediate steps between reactants and products. Alternate reaction pathways (causing a deviation from expected products) are limited. This, combined with a wealth of existing experimental data, will provide enough information to check our model.

3. *Case Study 2 – application of methodology derived in 1 to a complex reaction such as condensation and hydration of glucose*

   The glucose molecule can be seen as a monomer unit in this reaction, which can combine with other glucose molecules (or existing chains) to form larger and larger polymers. As the ways in which these monomers and polymers can combine are numerous, the reaction network can be immensely large. Adapting our methodology to such large and complicated networks will make this part of the project especially challenging.

4. *Implementation in AGG and other tools*

The graph transformation rules and an initial graph representing the reactant molecules can be implemented in AGG [19]. AGG is a programming tool commonly used by the graph transformation community as it has a natural, user-friendly interface. AGG can apply constructed rules to input graphs in order to test whether they produce the proper results, which is a fundamental step towards deriving ODE's. Some additional work using the AGG API will be necessary to adapt the software to our needs, and to automate certain steps in our analysis.

There is also a secondary personal learning objective outlined below:

5. *To gain a thorough understanding of graph transformation theory*

   As well as revising the background knowledge from the fields of Chemistry and Physics needed for the project, research into graph transformation theory will be necessary. This will include basic theory, stochastic theory, and also ways in which graph transformation theory can be applied to chemical reaction networks.

The aims and objectives of the project have changed somewhat since the initial inception phase (see original project description form) as understanding of the problem has become clearer through extensive reading and consultation with supervisors. In particular, the following modifications have been made:

- Universal rules governing the general reactivity of functional groups as influenced by intramolecular factors and the availability of other reacting species will not be implemented. This in itself is a large task that bears little relation to the more specialized main aim of defining reaction kinetics. Furthermore, such reaction predicting systems have already been successfully implemented by others. Therefore, only the rules directly affecting the case studies will be investigated and implemented.

- The development of an interface specifically designed for chemists constructing graph transformation systems has been abandoned as this has also been accomplished by others. In addition, development of such a system would be independent of the other major objectives of the project. In order to plan a more coherent and self-contained project, and to leave time for other more important parts of the project, existing tools (such as AGG) will be used without adapting them to a chemistry-related paradigm.

Outcomes

The project produced a methodology to derive ordinary differential equations for multistep reaction mechanisms. An implementation of the methodology was also developed to test its practicality. This was verified against a simple case study of the SN1 reaction. The methodology has some deficiencies and limits to its universal applicability as discussed in the critical appraisal. Specifically, it currently focuses on reaction networks with a finite number of rules. Due to scalability limitations in the 3rd party tools used, the implementation only works for reaction mechanisms involving molecules of bounded size and with reaction rules where local context in the left hand side of the rule is limited to a few atoms and bonds. Nevertheless the project is a useful and successful first step towards achieving our aims.

2. Background – Chemistry

This chapter outlines first the literature survey conducted as part of the "Project Plan" and provides an overview of the main ideas from Chemistry pertaining to the project. A more detailed treatment then follows explaining methodically how ordinary differential equations for a reaction can be extracted from its mechanism.

Literature survey

Before the analysis of kinetics can take place for chemical reactions, an understanding of how molecules react must be gained. Any basic course or text book in Organic Chemistry is useful here but [13] provides a thorough university-level description of reaction kinetics. In particular, it is useful because it derives formulas commonly associated with kinetics from first principles. An appreciation of this may prove fundamental when developing our model of reaction networks and especially when deriving differential equations.

In the simplest terms, chemical reactions occur when the bonds in molecules break and/or form. Some reactions are fairly simple and involve only one or very few steps. The rate laws for these reactions can be predicted fairly easily. Others however involve numerous steps, each step known as an *elementary step* in the overall *reaction mechanism*. Predicting, deriving or interpreting experimental data for such reactions can be complicated and may involve approximations and assumptions which reduce the validity of results. The mechanism is also not entirely deterministic. At any step, there may be several choices for subsequent steps. These steps may not be equally likely but probable nonetheless and could lead to by-products.

Reaction between two molecules can be prompted by collision with each other where the collision is energetic enough to cause the breaking of a bond and the formation of another bond. The minimum energy needed for reaction (provided by the collision) is denoted by the Activation Energy (this is an important concept as this energy is directly related to the rates of elementary reactions). The activation energy is greater than or equal to the difference in the stable energies of the reactants and products. In reactions where the reactants are particularly stable compared to the products, collision between the reactant molecules is often the determining factor of whether a reaction can take place or not. The collision step is known as the *rate determining step* in this case. Reactions do not always occur through collision however. If a molecule has an extremely reactive leaving group (a group in a molecule which can accept electrons from a carbon and break away) it may leave before collision. The resulting positively charged carbocation is extremely reactive and will usually react straight away. The rate determining step in this case is the leaving of the leaving group.

[13] also describes how reactions take place over potential energy minima, taking the lowest energy pathway from reactant to products. There is a potential energy maximum along these reaction coordinates, which corresponds to the activation energy. When reactant molecules overcome this maximum, they become products. However, the reverse is also true. Products can become reactants if they have enough energy to overcome the maximum from the opposite direction. As this energy is higher (remembering that products have a lower energy than reactants overall) fewer molecules have enough energy to overcome this barrier. The distribution of molecular energies within a species is given by the Boltzmann distribution, which takes the very approximate shape of a natural distribution. In a sense, all reactions are

therefore reversible, but some are not viable as the stabilization of products over reactants is so great that the reverse reaction is extremely unlikely. However, in many cases, we need to consider the reverse of elementary steps to create a more accurate model. [13] also describes the form of the rate law and its dependence on rate constants (which can be calculated using the *Arrhenius equation*) and concentrations of reactants and intermediate products. The rate law is synonymous with the ordinary differential equations we wish to derive. Its exact form depends on the details of reaction. It will be this project's objective to abstract these details to form a general graph transformation model. [13] is comprehensive in the information needed to do this.

As well as the difficulty and setbacks in analysing experimental data for more complex reactions, there are several other reasons why we may wish to model reactions rather than conduct them in a lab. A deeper understanding of exact mechanisms can be gained by simulating reactions. For example, in [5] the citric acid cycle is modelled and analysed using graph transformations (with tool support from AGG). As individual nodes are typed and can have attributes such as an ID, the movement of nodes can be traced. This led to a more in-depth knowledge of exactly where certain CH_2COO^- groups are consumed in the cycle.

Reactants may be extremely hazardous (such as radioactive material) and reactions explosive or otherwise dangerous. Modelling therefore provides a much safer alternative. Reactants or the equipment necessary for reaction may be costly, so once a model is developed, it can be very cost efficient to run. Some reactions may be too fast ([6] explains methods used in their implementation of a simulation tool that change the algorithm used in the simulation so that it is most suitable for the speed of reaction) or too slow to conduct in the lab. [13] describes some experimental methods for conducting fast reactions in the lab and still obtain meaningful results but many of these techniques are complex, require specialized equipment and can be wasteful of reactants.

What follows is a more detailed consideration of some of the more relevant theory pertaining to the project. A general example of how reaction rate laws are derived for reaction mechanisms consisting of a number of steps is given here (essentially a summary of the information from [13]).

The rate constant, k

As noted in the literature survey a reaction involving two molecules usually occurs on their collision. For a simple one step reaction involving two molecules the rate of reaction depends on two factors – the concentration of the reacting molecules and the inherent reactivity of the two reactants.

As the concentration of a species increases, the no. of molecules of that species within a particular volume increases. Intuitively (i.e. without an explicit treatment of the formal *collision theory*), this increases the probability of a collision between this molecule and the other, and therefore the rate of reaction increases. The inherent reactivity of a reaction depends on the rate constant, k, for a given reaction. As discussed in the literature survey, this depends on the activation energy of the reaction between the two reactants. The rate constant can be expressed in the following way:

$$k(T) = A\exp\left(\frac{-E_a}{RT}\right) \qquad \textit{equation 1}$$

where k is the rate constant at temperature T, A is the pre-exponential factor (unique to each reaction), E_a is the activation energy of the reaction, R is the gas constant, and T is the temperature at which the reaction is being considered.

Consider the following generalized example of a bimolecular reaction:

$$A + B \rightarrow C + D \qquad \textit{equation 2}$$

A and B collide and react to form C and D. The energy profile for these reactants is given in figure 1. Even though the energy of the products is lower than the reactants, there is still an energy expenditure barrier to overcome before the reaction can proceed. This usually corresponds to the overcoming of mutual electronic repulsion as negatively charged orbitals of electrons approach each other in order to reconfigure and become the highly unstable transition state. The higher the activation energy the more difficult it is to reach this short-lived transition state. As we can see from equation 1, the higher the activation energy the lower k becomes and therefore the lower the rate of reaction.

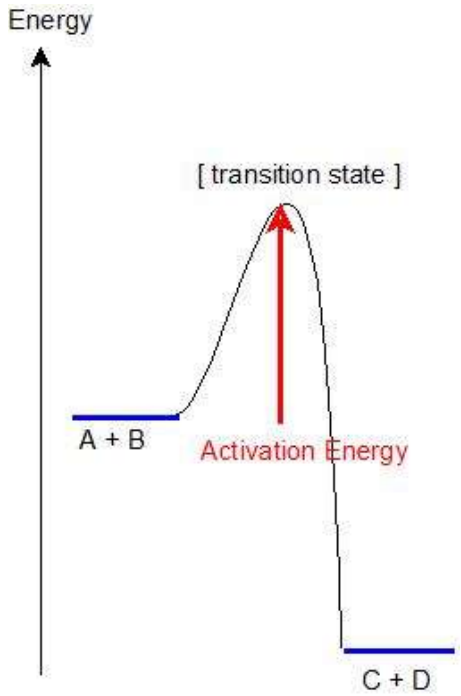


Figure 1 – energy profile depiction of activation energy

The exponential factor in equation 1 describes the Boltzmann distribution which states that the proportion of molecules with energy E_a in a mixture is proportional to:

$$\exp\left(\frac{-E_a}{RT}\right)$$

If the temperature of the system is increased, this entire exponential factor becomes larger. As the temperature is increased energy is introduced to the system. Therefore, the proportion of

molecules now possessing the energy E_a is increased. In determining the value of k, this means that more molecules have sufficient energy to overcome the minimum energy barrier; k increases, and as a result so does the rate of reaction. The pre-exponential factor A also has some minor temperature dependence, but this is often ignored due to the swamping effect of the temperature dependence in the exponential part.

The value of A can be derived purely through the application of *collision theory* (as outlined in [13]) but this ignores steric factors (such as the size and orientation of molecules) which reduce the proportion of collisions that lead to reaction and therefore leads to an overestimation of the proportion of collisions leading to reaction. Often the value of A derived from collision theory is substituted for a value of A extracted through experiment.

A simple one-step reaction

We will consider the following SN2 reaction (nucleophilic substitution involving 2 molecules) as an example:

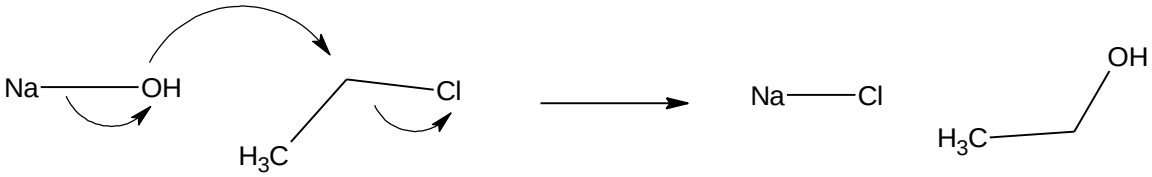


Figure 2 - simple SN2 reaction (hydrolysis of ethyl chloride to ethanol)

In this example, the negatively charged electrons on the OH group from NaOH attack the carbon atom connected to the chlorine atom in our target molecule. The carbon is slightly positively charged because the chlorine has a higher *electronegativity* and draws electrons in the mutual bond towards itself. The attack leads to a high energy transition state in which both OH and Cl are partially connected to the carbon atom in question. This is however incredibly short-lived and cannot be isolated as an intermediate in the reaction. Therefore, the reaction immediately proceeds to the products where the C-Cl partial bond is broken and the C-OH bond is completely formed. Combining the concentration dependence and k factor dependence, the differential rate of reaction can be expressed as:

$$\frac{d[CH_3CH_2Cl]}{dt} = -k[CH_3CH_2Cl][NaOH] \qquad \textit{equation 3}$$

Here the rate of reaction is written as the rate of change of the concentration of one of the starting reactants. The negative sign indicates that the reaction speeds up if there is more of the starting material but this leads to a quicker depletion of CH_3CH_2Cl. We include the concentration of both CH_3CH_2Cl and NaOH because there must be a collision between these for the reaction to proceed. The rate of change of concentration could also be written in terms of the other chemical species as follows:

$$\frac{d[NaOH]}{dt} = -k[CH_3CH_2Cl][NaOH] \qquad \textit{equation 4}$$

$$\frac{d[CH_3CH_2OH]}{dt} = k[CH_3CH_2Cl][NaOH] \qquad \textit{equation 5}$$

$$\frac{d[NaCl]}{dt} = k[CH_3CH_2Cl][NaOH] \qquad \textit{equation 6}$$

Equation 4 has the same form as that of equation 3 as both are starting reactants. Equations 5 and 6 are also the same but this time we are considering the rate of change of concentration of *products* with time. As the concentrations of starting materials increase (and hence the likelihood of a collision leading to a possible reaction) the reaction speeds up resulting in a greater rate of production of products, hence the sign of k is now positive. No matter how the rate is expressed the form of the rate law is the same and is a product of the rate constant, k, and the concentrations of the molecules involved in the reaction. If the reaction involves 2 of the *same* molecule, it appears twice in the rate law, leading to a square of the concentration of that species. The rate law therefore informs us about the mechanism behind an elementary reaction. Conversely, the mechanism immediately gives us the rate law.

More complex reaction mechanisms

The above treatment is for a simple one-step mechanism. For a more complex reaction network involving several steps from reactants to products, a differential equation for the reaction rate can still be derived in this way, but the overall reaction needs to be broken down into a series of elementary steps, bearing in mind the reversibility of any steps. Each elementary reaction will have its own energy profile and hence its own activation energy. Each reaction therefore has a unique value of k. Even for the same energy profile, a reverse reaction going from products to reactants must overcome a different energy barrier (see figure 1). Consider the following example reaction mechanism where A and B are molecules which can react to form C and D, D can then react with B to form G, or E to form F. The double headed arrows indicate reversible reactions:

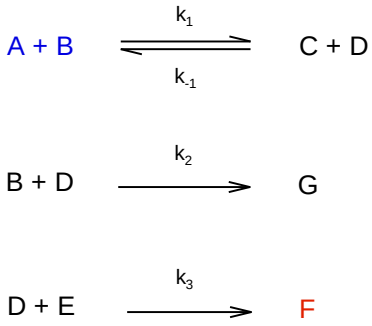


Figure 3 - example of complex reaction mechanism

Reaction 1 is reversible, reaction 2 forms a bi-product G which removes D from the system and reaction 3 forms our desired product F. Ideally, the overall rate of reaction should be expressed in the concentrations of known species which can be measured, such as the reactant molecules, or any terminal molecules such as G or F. In order to do this, we need to first formulate the rate of reaction with respect to each species in the system. The first step is deciding which elementary reactions produce or consume the species in question. For example, B is consumed in both reaction with rate constant k_1 and k_2, but is produced by the reverse reaction with rate constant k_{-1}. We would formulate the differential equation as follows:

$$\frac{d[B]}{dt} = -k_1[A][B] + k_{-1}[C][D] - k_2[B][D] \qquad \textit{equation 7}$$

Similarly, the rate can be formulated for the other species as follows:

$$\frac{d[A]}{dt} = -k_1[A][B] + k_{-1}[C][D] \qquad \textit{equation 8}$$

$$\frac{d[C]}{dt} = k_1[A][B] - k_{-1}[C][D] \qquad \textit{equation 9}$$

$$\frac{d[D]}{dt} = k_1[A][B] - k_{-1}[C][D] - k_2[B][D] - k_3[D][E] \qquad \textit{equation 10}$$

$$\frac{d[E]}{dt} = -k_3[D][E] \qquad \textit{equation 11}$$

$$\frac{d[F]}{dt} = k_3[D][E] \qquad \textit{equation 12}$$

$$\frac{d[G]}{dt} = k_2[B][D] \qquad \textit{equation 13}$$

The combination of all of the above differential reactions can then give a picture of how the rate of reaction looks with respect to a specific species (e.g. a particular reactant or product). Passing all of these individual equations to a computer math solver could then yield a single differential equation in terms of one or a few species only, perhaps those of interest or those that can be easily measured. This differential equation could also be presented graphically, showing a prediction of the kinetic profile for the reaction, or could be compared against empirical results to validate or invalidate our proposed reaction mechanism. Slight variations in the match between the two sets of results could expose overlooked details in our proposed elementary reactions or perhaps even an entire step. Our methodology will aim to produce these individual differential equations for a particular reaction.

Use of a stoichiometric matrix

Cardelli [2] proposes a method of deriving these individual ordinary reactions without explicit knowledge of the elementary reaction mechanisms, via a stoichiometric matrix. If it is known for each reaction, how many molecules of each chemical species is created or destroyed, we can build up a matrix as follows:

	A	**B**	**C**	**D**	**E**	**F**	**G**
k_1	-1	-1	1	1	0	0	0
k_{-1}	1	1	-1	-1	0	0	0
k_2	0	-1	0	-1	0	0	1
k_3	0	0	0	-1	-1	1	0

Figure 4 – stoichiometric matrix for example reaction

Each entry in the matrix corresponds to the aggregate number of molecules produced or consumed in a reaction, negative for consumption and positive for production. For example, if reaction with rate constant k_5 consumed 3 molecules of X but produced 2, the entry for k_5 and X in the matrix would be -1.

From this matrix, we can build the rate laws for the elementary reaction steps if we assume that all reactions are initiated either by collision and subsequent reaction of molecules which lead to their destruction, or by disintegration of one molecule into several. In both cases, the reaction occurs through the destruction/alteration of a molecule. If a molecule is involved in the initiation of an elementary step but is not consumed this method does not work. However, this is very rare, and even for catalysts (which are never used up by a complex reaction when considering the overall outcome) there is usually a change in form at the level of elementary reaction. Each row of the matrix informs us of the molecules involved for a particular reaction. If we multiply the rate coefficient for each row by the concentration of those species which are destroyed in the reaction we can define rate laws for our example reaction as:

$k_1[A][B]$ for the first reaction

$k_{-1}[C][D]$ for the reverse of the first reaction

$k_2[B][D]$ for the second reaction

$k_3[D][E]$ for the third reaction

If one molecule of a species is involved as reagents to an elementary reaction multiple times, it would be treated as another species and that species would be multiplied that many times e.g. if k_5 had a coefficient of -2 for X, it would appear as $k_5[X][X]$ or $k_5[X]^2$. The derivation of the rate law matrix from the stoichiometric one is not in Cardelli's methodology and he finds the rate laws in a different way, which no doubt avoids the problem specified above in assuming elementary reactions occur via the alteration of molecules. Appreciation of this method would be invaluable for further work and an enhancement of the current methodology, which has limited universal applicability.

Now we have a rate law matrix and a stoichiometric matrix, the rate law matrix taking the following form with only one row:

$\mathbf{k_1}$	$\mathbf{k_{-1}}$	$\mathbf{k_2}$	$\mathbf{k_3}$
$k_1[A][B]$	$k_{-1}[C][D]$	$k_2[B][D]$	$k_3[D][E]$

Figure 5 - rate law matrix for example reaction

A simple matrix multiplication of Rate Law Matrix by Stoichiometric Matrix then gives us a 1x7 matrix with the elementary ODE's (equations 7 to 13).

3. Background – Graph Transformations

This section provides parts of the literature survey conducted for the "Project Plan" as a summary of existing related work in the use of graph transformations to model chemical reactions.

Literature survey

[8] gives an excellent overview of basic graph transformation theory. It consists of an easy-to-follow description of graph transformation systems including the representation of systems as graphs (type and instance graphs), rules and transformations, constraints and application conditions. To explain its most basic functionality, a rule first looks for a pattern match for the left side of the rule in the input graph. Then, edges and nodes that are not in the right hand side of the rule are deleted, while edges and nodes that are newly created in the right hand side are placed into the graph. This paper will be invaluable as a reference to basic concepts throughout the project.

Modelling molecules and reaction networks using graphs is described as a very natural application by much of the literature [1,13]. [1] and [17] discuss how a form of graphs is already a fundamental part of organic chemistry, when depicting molecules by their structural formula. Atoms can naturally be seen as nodes in a graph, with the bonds between them represented as bi-directional edges. Double bonds and triple bonds could be modelled as two edges and three edges respectively (as represented in [1]) but this would only be useful if a reaction involved the breaking of one of the double bonds in one or more of its elementary steps (this may be useful for the esterification case study).

The problem with this basic approach, however, is a loss of information about spatial configurations i.e. cis and trans isomers and chirality. Furthermore, the valencies of individual atoms (the no. of other atoms it can bond to) are not automatically conserved. [5] offers an alternative approach that should be much more intuitive for chemists. By modelling the atoms as hyperedges and the bonds between them as nodes, valency and chirality can both be incorporated into the model. Each hyperedge is typed by atom, allowing for different numbers of joined nodes, therefore the concept of valency is preserved. The outgoing 'bond' nodes from a hyperedge are labelled in order to show the three-dimensional ordering (related to D-glyceraldehyde), and preserving chirality. This is particular important for reactions where one enantiomer is more reactive in an elementary step, or where only one enantiomer binds with a particular substrate. This is more prominent in biochemical systems. The example of Citrate binding with Aconitase is given in [5]. In our two case studies chirality does not need to be considered as it has no bearing on the outcome of reaction. Therefore the simpler model may prove sufficient.

From the representation of molecules as graphs, envisioning reactions between molecules as graph transformations is not a big step. Both [17] and [18] describe how reactions involve the breaking and creation of bonds in a molecule to transform it into a different molecule. In the graph, this would mean an elementary reaction step would involve the deletion of edges (bonds) and nodes not appearing in the right hand side of the rule, and the creation of edges and nodes that appear on the right hand side of the rule. As nodes and edges can have attributes, attributes may also be updated during the transformation. Such attributes could indicate energies of bonds, valencies or other useful information. Another reason why graph

transformation representations are so natural is their "inherent concurrency" [5]. This is the ability for these systems to allow simultaneous reactions of different reactants, which is obviously what happens in real chemistry. Causal dependencies can be monitored along with conflicts, using critical pair analysis. [17] attributes the strength of graph transformations in this field to their "pattern handling power". In any chemical reaction network, involving an arbitrarily large number of molecules, a multitude of simultaneous reactions are possible. However, graph transformation rules define the standard reactivity of certain functional groups. Pattern matching provides a powerful search method for where these rules can be applied within the network. This will be explored in both our case studies and a more thorough explanation is giving in later sections of this report.

[5] explains how graph transformations work in detail, by summarising the so-called double-pushout approach. The left side and right side of the *rule span* show how the molecule will change, whereas the gluing graph between the left and right hand side simply indicates which elements are involved ("read") in the rule, but not consumed. In the "toy" model of artificial chemistries given in [1] the essential idea is the same, but the gluing graph is labelled the context. The visualisation is also much more attuned to the structural formula representation used by chemists – atomic/group nodes are just labelled by element symbols and the edges replaced by standard bond representations (with double and triple bonds preserved). Although this may be more intuitive for chemists, it does not allow us to easily show attributes of nodes or edges, and also may seem alien to the way the graphs will look in their eventual implementation using tools such as AGG. Using standard graph representations should be easy enough to understand so the further simplifications in the toy model are not necessary.

[5] suggests 3 types of rules in any graph transformation system. Symmetry rules are useful for the hyperedge model. Although chiral molecules cannot rearrange their spatial configuration with respect to the other groups around the chiral carbon centre, the bond connected to this carbon can itself rotate, meaning the groups can change position. So that this is not considered a separate molecule, equivalence rules can be set up. Expansion rules allow us to expand atoms grouped together in one node/hyperedge (usually for simpler representation) into their full expanded graph. This is necessary if at some point in the graph the details of the group become important e.g. one of the atoms is involved in a reaction. Again, this contracted representation is familiar for chemists, who often contract large groups in structural formula when their exact spatial details are not contextually important. Reaction rules define the change of groups. Our model should definitely incorporate the second and third types of rules, and consider the symmetry rules if the chirality preserving hyperedge model is used. [5] provides some other invaluable information about how graph transformation theory can be applied to reactions, and how atoms can be traced throughout the reaction and will serve as good reference material throughout the project.

Stochastic graph transformation systems and rates of reaction

[10] and [11] are excellent references explaining the extension of a graph transformation system to a stochastic graph transformation system. Given a start graph and a graph transformation system, a labelled transition system can be deduced which shows all the possible states (graphs) possible. Applied to a reaction network, each state would represent all the species (reactants, products, by-products and intermediates) that would result from applying the graph transformation rules. Labels from state to state can be assigned attributes such as a probability of reaction. This is analogous to the rate of the transformation from one step to the next, hence essential to our derivation of the overall rate of reaction. [10] provides all the necessary definitions and procedures needed to convert a stochastic graph

transformation system to a Continuous Time Markov Chain, which then allows the application of stochastic temporal logic to deduce long-term non-functional stochastic properties of the system. Equivalence rules can be set up which check for certain properties of the system at any point during the progress of the simulation, thereby allowing us to monitor the presence of molecules for example. Querying the system at regular intervals can then allow us to effectively measure properties proportional to the concentration of chemical species. Paper [10], demonstrates the application of stochastic graph transformations to P2P networks. Although the model is simpler (in that the rates are more easily assigned) the example will be invaluable in understanding how stochastic systems are applied. As the theory behind stochastic graph transformations (Continuous Time Markov Chains, Q-incidence matrices, Stochastic Temporal Logic) can be quite difficult to grasp, both of these papers will be useful to return to.

In deciding the rates of elementary steps, paper [1] provides a very good starting point. As discussed earlier, the Arrhenius equation relates the change in energy between reactants and products with a rate of reaction. [1] discusses a refinement where instead of entire molecules, just the energy of hybridised orbitals involved in the reaction are considered. For complex reactions such as case study 2 we can follow the methodology described in [1] and automate the procedure of assigning energies by looking at recurring sections of molecules (characterised by different hybridised orbitals) rather than entire molecules. This generalisation step would avoid having to manually determine and assign energies for the high number of possible species involved in the reaction (i.e. chains of many different lengths). It can incorporate more complicated electronic distributions accurately into the model, such as π-stabilisation where adjacent π-bonding orbitals can overlap in a molecule, lowering their overall energy and making them more resistant to breaking and therefore reaction. [1] also describes how this method accounts for regioselectivity within a molecule i.e. if there are two places where a rule can be applied, calculating energies would force the rate of reaction at one site to be realistically higher than the other. [1] actually goes on to suggest that the calculation of energies could be used for stochastic simulations of reaction networks using the Gillespie algorithm. The background theory and ideas presented in this paper could be utilised in the project, possibly for future iterations.

There are other methods in the literature that are used to model stochastic systems. [2] and [14] both use process languages to specify the reaction rules and to derive useful properties. Cardelli's paper [2] is particularly useful as it sets out to achieve what this project does using stochastic process algebra, namely CCS and CGF. While this approach is probably more intuitive and precise for computer scientists, it loses its appeal for chemists due to its technical complexity. The visual graph representation is much more useful in this respect because the components are easily recognisable to chemists. The content of the paper is quite technical and without a background in logic and automata course requires substantial background reading to understand fully. The paper is therefore currently of limited use. Nevertheless, it would be highly advisable to understand Cardelli's approach, in order to note his assumptions and the way in which he assigns rates to elementary reaction steps. For this reason, a quick overview of π-calculus (provided by [15]) and subsequent research into CCS still needs to be undertaken.

Implementation tools

AGG [19] is widely used by the graph transformation academic community. A brief description of its utilisation to a chemical reaction setting is given in [5]. AGG does have several limitations for our purposes though. Firstly, it cannot be used for stochastic

simulations. To obtain stochastic data, a combination of PRISM and GROOVE (as described in [10] and [11]) could be used. [6] also provides a very thorough presentation of FERN, a Java framework that can be used for stochastic simulation. The API seems fairly straightforward. It does not however provide its own visualisation module. AGG also has its own Java API and a combination of the two tools could be coordinated to fit the project's needs. Further investigation of [6] and [19] will be necessary to achieve this. An appealing feature of [6] is that it provides implementations of several stochastic simulation algorithms, namely the Gillespie algorithm, extended Gillespie algorithm and a tau-leaping algorithm. The framework applies the most appropriate algorithm depending on the speed of the reaction, and can even change dynamically during runtime.

Other limitations of the software should also be considered before using AGG such as minimum requirements, known bugs and elements of graph transformation theory that are not implemented (such as the ability to input hyperedges). The user manuals, bug reports and examples which can be found at [19] should be reviewed before/while using the tool.

Consideration for more complete modelling

Despite being an overview paper that has little relevant technical content, [18] does illuminate some interesting points to consider if we are to progress to a more complete model of real chemistries. In particular, "Global Context Sensitivity" is discussed. This states that physical properties play an important role in chemical reactions, such as temperature, solvent, viscosity, catalysts and radiation to name just a few. A graph transformation model may be limited in its ability to incorporate such factors. This should be taken into account in the final stages of the project. "Local Context Sensitivity" considers for example the "three dimensional conformation of reactive groups" of a molecule and how this affects reactivity. Large groups for example may block collision with incoming molecules, thereby hindering reaction rates.

While a complete and accurate model is impossible for the scope of this project, it would be interesting to consider some of these factors, in particular temperature as the Arrhenius Equation is temperature dependent. The quality of results from the model compared to empirical results may prompt further investigation into these factors towards the end of the project, time permitting. We will return to this paper for background knowledge at this stage.

Graphs and type graphs

The graph transformation theory covered in this section provides a non-technical overview of the theory necessary to understand our methodology. For a richer, more mathematical discussion of the theory presented here, with formal definitions, please refer to [4], [5] and [8], of which the following is a summary.

Graphs are composed of a set of nodes and a set of directed edges. Each edge has a source and target node. Formally, a graph can be represented as:

$$G = (V, E, s, t) \qquad \textit{equation 14}$$

…where G is a graph, V is the set of vertices (or nodes), E is the set of edges, and s and t are the source and target functions respectively ($s,t : E \rightarrow V$).

A typed graph is much like a UML class diagram, in that it specifies the allowed types (analogous to classes) of nodes and edges, and also the allowed relations between different nodes and edges. A type graph can also be formulated in the same way as equation 14:

$$TG = (V_{TG}, E_{TG}, s_{TG}, t_{TG}) \qquad \textit{equation 15}$$

A graph is typed if it conforms to the specification laid out in the type graph, and is represented formally as:

$$G^T = (G, type) \qquad \textit{equation 16}$$

… which specifies that a typed graph consists of a graph and a graph morphism, which is essentially a function, which maps the graph to the type graph, type : G → TG. A graph morphism is a combination of two functions; one that maps all vertices from one graph to another, and one that maps the edges in the same way. All nodes and edges must be instances of the node types and edge types specified in the type graph. The graph as a whole may be considered an *instance* of a type graph, just as in UML an object is an instance of a class. As an example, consider the following simple type graph:

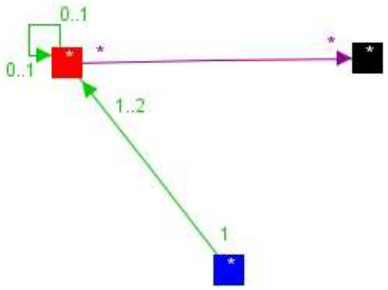


Figure 6 - example type graph

This specifies that the red node can only connect to a black node via a purple edge, and the blue node can only connect to a red node via a green edge (note the direction of the edges). Just like UML class diagrams, cardinalities can be specified on relations between nodes through their edges. Every blue node must have 1 or 2 red nodes connected to it. Every red node must have 1 blue node connected to it. The relation between the black and red node is unconstrained, i.e. there is a zero to many cardinality. As there is no edge between the blue and black node, the blue node must never be connected to the black one. A red node can be connected to no more than one other red node (including itself). An example of a valid typed graph over this type graph is given in figure 7:

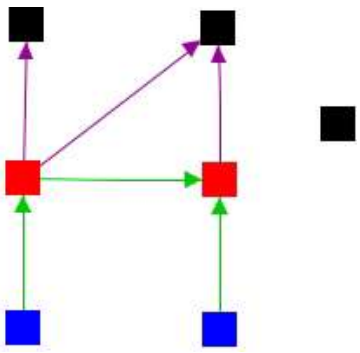


Figure 7 - example typed graph

Graph transformations

A graph transformation (also known as a rule or production) is a graph morphism that is used to specify how a graph can change. It is usually specified in the following way:

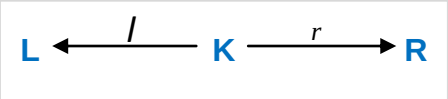


Figure 8 - definition of a graph transformation (DPO)

This is known as the double pushout approach (DPO) construction. L is the left-hand side of the rule and specifies the preconditions for the application of a rule. It is itself a graph which contains nodes and edges. K is the gluing (or sometimes called the context) graph. This specifies which nodes and edges in L are unchanged by the rule (i.e. "read" only). R is the right-hand side of the rule and specifies the postconditions for the application of the rule. Nodes and edges in L have an identity and these are mapped to nodes and edges in K and R, unless of course R introduces a new node or edge, in which case it will not have an identity. In order to perform a transformation on a given graph, there must be a *match* for the nodes and edges in L within the graph i.e. there must be an injective morphism (every member in L must be mapped to a unique member in the graph). There can be more than one such match, in which case one is non-deterministically chosen for the transformation (AGG allows interactive matching which allows you to select which match to perform the transformation with). Once a match is found, i.e. a copy of L is found in the graph, the nodes and edges not in K are deleted, and the new nodes and edges in R are copied into the graph. This can be represented pictorially as:

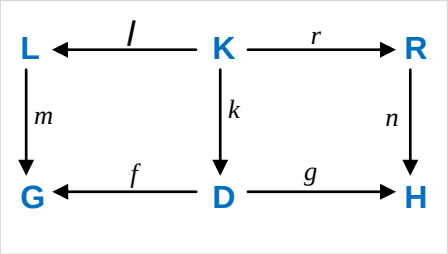


Figure 9 - definition of graph transformation with match

m, *k* and *n* all depict the embedding (injective morphism) of L, K and R respectively into G, D and H, the instance graphs. G is the graph before the transformation, D is the graph after the non-preserved nodes have been deleted, and H is the graph after the new elements in R have been copied over.

The subgraphs that constitute the left and right hand sides of a rule must also conform to type graph specifications. An example rule for the type graph given in figure 6 is:

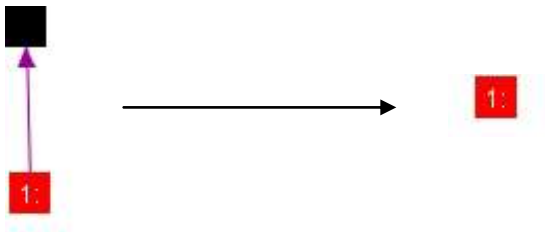


Figure 10 - example rule 1

Figure 10 shows only the left and right hand side of the rule. These diagrams were produced using AGG (a graph transformation tool), which does not require the input of a gluing graph, as this is inferred from the left and right hand sides. The gluing graph here would be the red node (1), which is the only node preserved by the rule. This rule essentially just deletes a black node, and its corresponding edge, connected to a red node. If this rule is applied to the graph in figure 7, we would get the following:

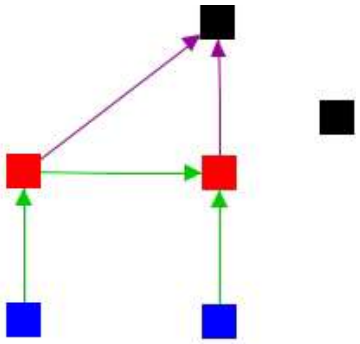


Figure 11 - rule 1 applied to example graph in figure 7

While there are three matches for the left hand side of the rule, there is only one valid match. Deletion of the second black node, connected to two red nodes, would lead to a violation of the *dangling condition*. The two remaining matches select either one of the red nodes with the black node. For either of these, if the black node and one edge are deleted, another edge is left without a target node, hence a dangling edge. There are two ways to overcome this, either to delete the dangling edge, or disallow transformations that lead to a dangling edge (AGG allows the setting of this as an option). In our case, we do not delete dangling edges, hence there is only one valid match.

Critical pairs

The theoretical background presented here is a summary of the chapter on critical pairs in [4].

A critical pair refers to a pair of rules where one rule could potentially affect the application of another. There are two main types of critical pairs: conflicts and causal dependencies.

Conflicts refer to parallel dependent pairs of rules, where the application of one rule could disable the application of another. Consider a graph, G, with the possible application of rule p_1 or rule p_2:

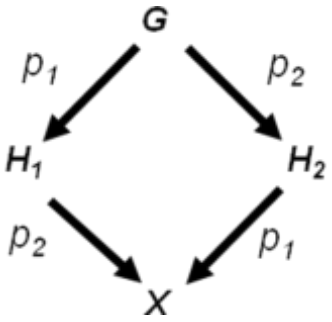


Figure 12 - parallel independence of rules [4]

If the above confluence is possible, rules p_1 and p_2 are parallel independent. However, if the transformation p_1 on graph G creates a graph H_1 such that p_2 can no longer be applied, or p_2 on graph G creates a graph H_2 such that p_1 can no longer be applied, the two rules are parallel dependent and in conflict. We can check for such a conflict by depicting the double pushout construction for each rule.

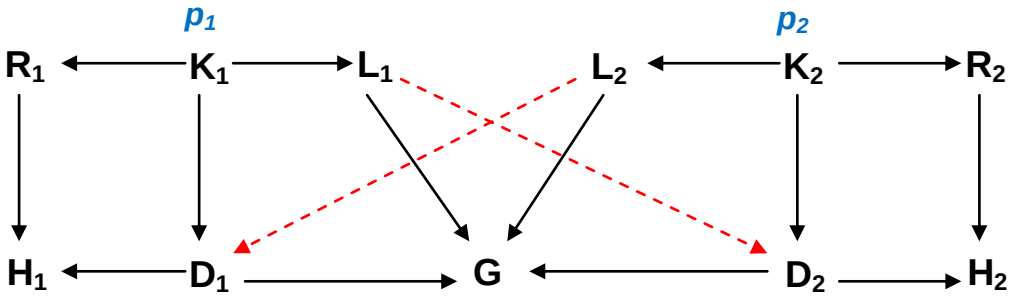


Figure 13 - double pushout depiction of parallel independence [9]

For parallel independence, each rule must not delete nodes and edges needed in the left hand side of the other rule. In other words, D_2 (the gluing graph which specifies which nodes and edges are not deleted by rule p_2) must contain L_1, and D_1 must contain L_2. Another way of considering this is to look at the union of the left hand side of both rules. There will be a number of graphs satisfying this union, each graph depending on exactly how the union takes place. If in any one of these unions, the intersection of the two left hand sides is not in the gluing graph of both rules (K_1 and K_2) the rules are in conflict.

For sequential dependence, we consider the following construction:

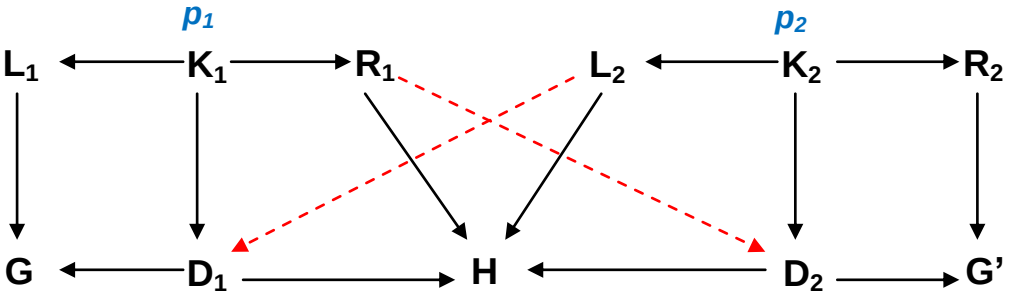


Figure 14 - double pushout depiction of sequential independence [9]

Rule p_1 has been reversed. If the order in which p_1 and p_2 are applied affects the overall outcome of the application of both rules, there is sequential dependence. Here we consider if D_2 contains R_1 instead of L_1. If we have a reaction rule p_1 such that it creates the graph in the LHS of p_2, L_2 will be in R_1 but will not be in D_1. Since p_1 creates L_2, L_2 should not exist in this gluing graph (i.e. before p_1 is applied). This is identical to saying that for some intersection of R_1 and L_2, K_1 will not contain this intersection as some nodes and elements in L_2 will not have been produced yet. The intersection of R_1 and L_2 should be in both K_1 and K_2 for sequential independence. Therefore, in this case, we have a critical pair denoting sequential dependence.

Stochastic graph transformations & reaction networks

Although stochastic techniques were not utilised in this project, considerable time was spent studying and understanding their application to chemical kinetics in the first term. As such, a very brief summary of their use is given here, gathered from [10] and [11]. As mentioned in the introduction, stochastic simulation is an alternative and better studied approach to producing reaction kinetic data. There are a number of differences between this approach and ours. While ours aims to produce equations, simulations produce quantitative data. This quantitative data depends on the circumstances under which the simulation was run, and also chance. Simulations consider every possible change from one molecule to another by a reaction in an atomic way, considering each one a unique transformation. Differential equations, however, essentially average all of these unique transformations into one overall rate law for that reaction. The stochastic simulation technique described below requires the input of a reaction *mixture* (many of each molecule) as the first state of a labelled transition system. There are scalability issues here in the number of instances of each molecule possible before the simulation can no longer run efficiently. Differential equations overcome this as generally only one instance of each molecule involved in each elementary reaction is necessary. Both of these approaches should be considered as complementary and further work would look at the connection between them. Therefore, an understanding of the theory behind stochastic graph transformation systems is very useful for the project.

While a graph transformation system describes the functional, behavioural aspects of a system, adaption of this to a stochastic graph transformation system can inform us of non-functional properties of the same system. The speed at which reactions occur is one such non-functional property. To derive kinetic information about a particular complex reaction, we would first need the rules that make up the elementary reactions for the system, and a type graph to which all rules and graphs must conform to. We can derive a labelled transition system from these rules if a start graph is used to apply these rules to. The start graph describes the initial state of the system. In our case the start graph would be the starting material molecules for the reaction.

We can describe the resulting labelled transition system as a labelled transition graph, where each graph is reachable from the start graph through the consecutive application of rules, starting at the start graph. Each transition is labelled with the rule name. This system now describes the entire reaction network, with intermediates as graphs in the labelled graph system and rules as the transitions between these graphs.

To progress from this transition system to a stochastic graph transformation system we need the notion of a Q-matrix and Continuous Time Markov Chains (CTMC's). A Q-matrix (without formal mathematical notation, which can be found in [10]), is a transition rate matrix which describes essentially the probability that a particular transition will occur in the labelled transition system, transforming one graph to another. In chemistry, this is analogous to the rate constant, k, for an elementary reaction, which transforms one set of molecules to another. Just as the Q-matrix value describes some inherent probability of transition from one graph to another, k is an inherent probability of reaction for a particular elementary reaction. The usual concentration dependence that the speed of reaction has is incorporated into the transition system model elsewhere; the more molecules there are of a particular type required in a reaction, the more likely a transition of that type will fire in any given interval if we look at the system as a whole.

Each entry in the Q-matrix describes the transition rate from one state to another. The rows and columns are both elements from the set of possible states for the system to be in. Each row must sum to 0 (a necessary normalisation prerequisite for Q-matrices). If a transition from one state to another is impossible, the Q-matrix entry where they intersect is 0. The rate from one state to the same state is usually where a negative value is inserted to normalise the matrix. For example, if A, B and C are intermediate states in a reaction, the Q-matrix might look as follows:

	A	**B**	**C**
A	-5	5	0
B	3	-11	8
C	1	0	-1

Figure 15 - example Q-matrix

Here we see that A can go to B but cannot go to C. B can go to A, but is more likely to go to C. C can go to A, although it is not very likely, but not B.

From the labelled graph system and the Q-matrix, we can define a Continuous Time Markov Chain (CTMC). The CTMC is a random process, which describes the state of a system indexed by some t. In our case, t is time, since we are considering the progress of a reaction with time. If t is from a continuous set (as it is with time), we are describing a continuous time process as opposed to a discrete time process. The "continuous time" in CTMC refers to this. The CTMC is discrete-state since the intermediate graphs are the result of concrete, finite rule applications. The current state in the CTMC only depends on the previous state.

The Q-matrix, along with the finite states provided by the labelled graph system, defines a CTMC in the following way. If the Q-matrix entry is greater than zero for any 2 sets of states, s and s' (provided $s \neq s'$), then a transition from s to s' occurs. So in our example Q-matrix above, if we start with A, A can progress to B. If there is more than one possible transition, however, a "race" between the possible transitions occurs, with the probability of any transition winning and therefore firing being the value of that entry in the Q-matrix divided by the total non-negative values in that particular row of the matrix (also given by the negative of the value for the s,s entry in the matrix). For example, once at B, we can return to A (with a value of 3) or progress to C (more likely, with a value of 8). The probability that B $\rightarrow$ A occurs is 3/11, and the probability that B $\rightarrow$ C occurs is 8/11.

Another important note about the CTMC is that the transition delay is exponentially distributed with rate –Q(s,s) i.e. the probability that a state, s, will change within time t is the same at any time and depends on the sum of the entries for any row (i.e. state) of the Q-matrix. The memoryless property of exponential distributions is particularly suited for chemical reactions, as it indicates that the proportion of molecules to undergo a particular elementary reaction in time interval, t, is constant. Again, this indicates an inherent reactivity of the molecules involved in an elementary reaction. As the sum of the rate constants for all outgoing reactions determines how fast a particular intermediate molecule will react and be used up, this is intuitively correct. These same rate constants determine the values in the Q-matrix. The changing concentration of species needed for a reaction to occur will have an

effect on the overall speed of reaction, but the rate constant and inherent reactivity of the molecules needed for a reaction should never change (assuming a constant temperature).

The stochastic graph transformation system then, attributes to each rule (i.e. transition in the labelled graph system) "an exponentially distributed delay of its application" [10], which is derived from the Q-matrix (details of how to do this are given in [11]). In chemical reactions, this is proportional to the value of the rate constant for a particular reaction. Such a system can be implemented using a tool chain such as AGG for rule specification, and GROOVE and PRISM for specifying the Q-matrix and running the stochastic simulation. Continuous Stochastic Logic (CSL) can then be used to query the stochastic system. This involves setting up atomic propositions which question the probability of certain events occurring (e.g. is the probability that there are 1000 molecules of intermediate molecule C present after 20 time units 0.1?) either throughout the time of the simulation or at long-term steady state (i.e. the end of the reaction). Using appropriately designed queries, a value (actually a probability) proportional to the number of molecules of each species can be ascertained throughout the simulated reaction. If we assume a constant volume, this is proportional to the concentration of each species. A graph of concentration against time for each species can then be plotted and checked against empirical lab results.

This was a very brief and non-mathematical overview of stochastic graph transformation systems. More details can be found in [10] and [11].

As stochastic simulations give us numerical, quantitative data that can be directly compared to existing experimental data, it may be reasonable to ask why ODE's are necessary at all. The ODE's give us a direct insight into the overall reaction mechanism for a complex reaction. A plot of the combined ODE's compared to experimental data may reveal drastic differences, which may be due to an inadequately proposed mechanism (i.e. omissions or inclusion of reactions that never occur), or highlight interesting physical properties of a reaction e.g. temperature dependence or dependence on molecular size or spatial configuration. While the methodology developed in this project does not come anywhere close to incorporating such physical influences on a reaction, the knowledge of their presence alone is invaluable.

4. Molecular Representation Using Graphs

So far this report has focused on background and related work done in this field. The following is an account of how this was used in this project. First we focus on the use of graphs to represent molecules and their reactions, and then describe the step by step derivation of ordinary differential equations for a reaction, using the concept of critical pairs. This is followed by a description of the implementation of this methodology using 3rd party and newly developed tools. Our two case studies test the methodology and its implementation for a finite reaction mechanism.

A type graph is extremely important when representing molecules using graph transformations. A type graph captures the necessary rules to restrict the bonding of atoms, their valencies (the total number of bonds a particular atom is allowed to have) and any other idiosyncrasies of molecular chemistry. During the initial stages of the project, several type graphs were experimented with. This section gives an account of the evolution of these approaches through three main stages.

1st attempt

Because molecules consist of atoms and bonds that connect two atoms, a first intuitive representation of molecules might consist of atoms as nodes and bonds as edges that directly connect them. A first approach followed this intuition and produced the following type graph (for all atoms in the esterification example):

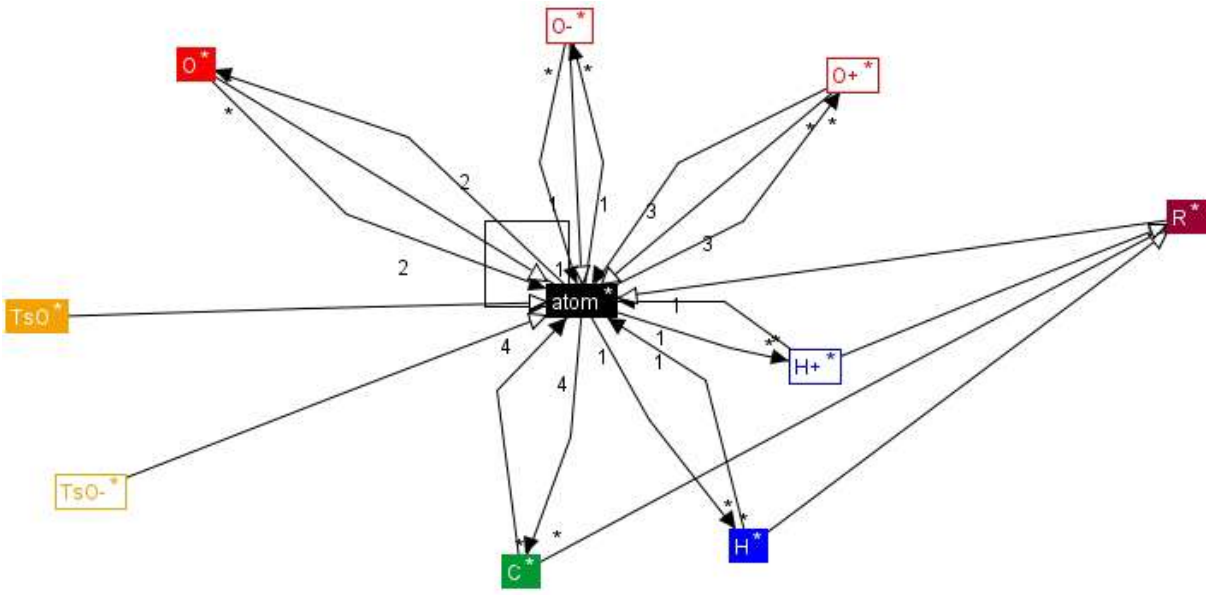


Figure 16 - esterification type graph, 1st attempt

The structure of the type graph is quite complex. Just as with UML class diagrams, we can see the use of inheritance in this type graph. All of the atoms are subtypes of the "atom" supertype. C, H and H^+ are all subtypes of "R" (used in organic chemistry to designate an arbitrary hydrocarbon chain), which in turn is a subtype of "atom". A bond between atoms must consist of a pair of edges, one in each direction. As edges are directed, making each bond a pair of oppositely directed edges avoids the added complication of specifying a direction between each pair of atoms, and allows the use of a supertype, generic atom to

specify all allowed connections. This is why all bond edges are directed to the central "atom" supertype. The cardinalities of the bond edges specify the valencies for each atom. For instance, the O type must have 2 outgoing edges to other atoms, and 2 incoming edges, therefore specifying that each O atom must have 2 atoms connected to it at all times. The "atom" supertype has a connection to itself. This is to allow connections between atoms. While the edges from the subtype atoms to the supertype "atom" should already assume this possibility, AGG did not allow it unless the "atom" to "atom" edge was added. Charged atoms were included separately as they have different valencies to their non-charged counterparts. O^+ can have 3 connections for example, whereas neutral O can only have 2.

In producing a graph over the type graph above, in AGG the type graph must be enabled. There are two options for enabling the type graph – with both minimum and maximum cardinalities enabled, or just the maximum. With the minimum enabled, this minimum is in effect throughout the lifetime of the graph, even when rules are being applied, and as most rules involve deletion of an edge before re-adding another, this minimum may be violated making the rule inapplicable. Therefore, for all type graphs, we only enable the maximum cardinalities of the type graph. Figure 17 shows an example of a graph that conforms to the type graph of figure 16. These are the starting materials in the esterification reaction studied in this project:

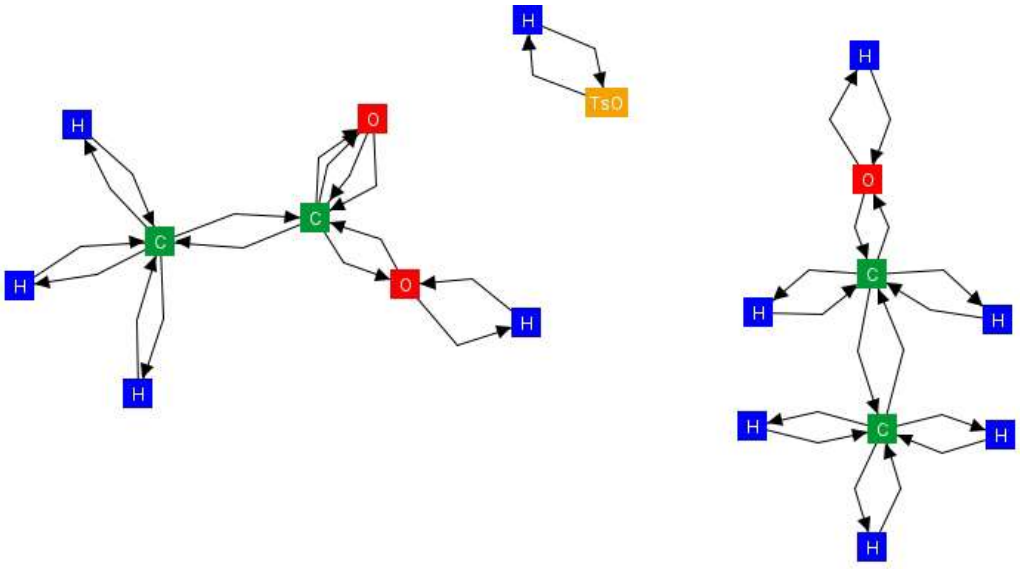


Figure 17 - esterification start graph, 1st attempt

The leftmost molecule is a carboxylic acid, ethanoic acid. Between the C on the right and the upper O, there are 4 edges in total, therefore 2 bonds. Under this type graph, this is how a double bond is represented. The small molecule is TsOH, an acid catalyst in the reaction. Here, we have encapsulated the complicated internal structure of *p*-Toluenesulfonic acid as a single TsO node, as this internal structure is not important to the reaction; only the attached H atom has any significance. The molecule on the right is the alcohol (ethanol) which reacts with the ethanoic acid to eventually form an ester.

As the minimum cardinalities are disabled for the graph, it is entirely possible that through the course of the reaction certain required conditions may become violated, in particular the fact that each bond must consist of two edges. This is particularly important for critical pair analysis, which we will discuss later, where we look for critical overlappings of nodes and edges between a pair of rules. When we specify rules, we can carefully control their outcome through the left and right hand sides. Critical overlappings however are extracted from all

possible permutations of an embedding of the two rules into one graph. To ensure all of these permutations conform to rules not specified in the type graph, we can use constraints. Constraints are specified for an entire graph grammar (rules and start graph). An atomic constraint consists of an atomic proposition, and one or more conclusions that must be true if the proposition is true. For example:

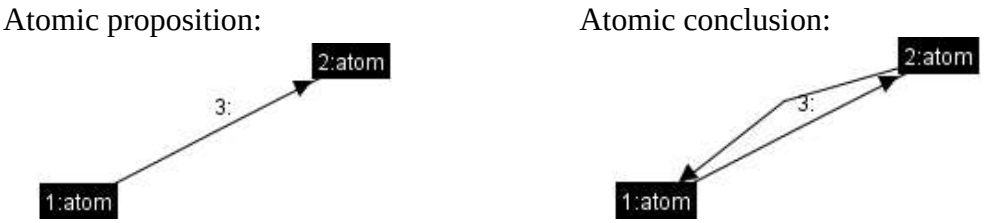


Figure 18 - atomic constraint for 1st attempt type graph

This atomic constraint states that whenever one atom is connected to another by an edge, there must be an accompanying edge in the opposite direction. A constraint can be formulated from many individual atomic constraints using Boolean logic. Constraints specifying the necessary presence of a situation can be expressed by leaving the atomic proposition empty and specifying an atomic conclusion. This means that the conclusion must always be true. To specify required absence, a constraint that falsifies such an atomic constraint must be defined.

While this was an extremely intuitive type graph, which could easily be understood by both chemists and computer scientists, there are some drawbacks because of its simplicity. Primarily, the dangling condition (described earlier) can easily be violated in the specification of a rule unless extended local context is given in that rule. For example, if a rule specified that one of the bonds of the C=O double bond in ethanoic acid (figure 17) should break to leave a single bond, with C becoming C^+, we would need to break the other bonds this C has and reform them with the C^+. If the rule only included the C which actually changes, and left out the local context, the rule would become inapplicable as the dangling condition would be violated when C changed to C^+. Generally, to specify reactivity that is as general and widely applicable as possible, rules should include only the functional groups that are affected by rules, and avoid local context. A way around this for this particular type graph, would be to split rules (as can be seen for rules entitled Step4a and Step4b, in file "esterification_attempt1.ggx"), but this adds unnecessary complication to the grammar.

2nd attempt

A second attempt looked at incorporating a hyperedge treatment. AGG does not allow the use of hyperedges. However, they can be simulated using bipartite graphs, as carried out in [5]. A hyperedge is an edge that connects more than two nodes. The nodes that a central hyperedge connects can be ordered thereby some semblance of 3-dimensional spatial configuration is preserved. This is an extremely useful property for the reactions of chiral molecules where only certain enantiomers (3-dimensional configurations) undergo reactions. For our case studies, chirality was not relevant. However, this representation also enables an easy way of limiting the number of bonds each atom is allowed, hence establishing valency maximums. CH_4 (methane) would have the following hyperedge representation:

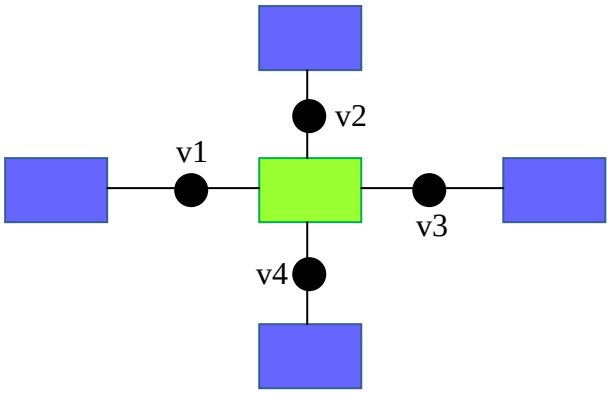


Figure 19 - hyperedge representation of CH_4

Contrary to the 1st model, atoms are now (hyper)edges, and bonds are nodes. The green hyperedge is the central carbon atom, and the blue hyperedges are the bonded hydrogen atoms. The black nodes represent a bond. Hyperedges of carbon type can connect four such nodes and hydrogen only one. This restriction information would be in the type graph for this hypergraph. The bipartite graph simulation of this would have the following form:

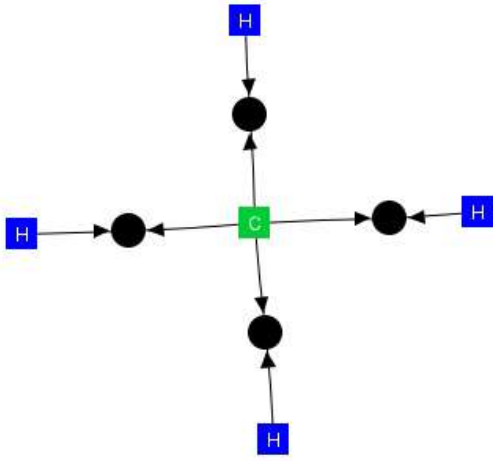


Figure 20 - bipartite graph representation of CH_4

The hyperedges are simply represented as a special type of node in AGG. The resulting form of the graph is very similar in appearance to the hyperedge approach.

The type graph for esterification in this model is given in figure 21. The corresponding starting materials (analogous to figure 17) are given in figure 22. The type graph in figure 21 is considerably simpler than the type graph in figure 16, for the previous model. However, there are again drawbacks with this model. While the number of bond nodes each atom node can connect to is appropriately limited, the opposite is not true. From the type graph it is clear that each bond node could have several atoms nodes attached, a maximum of 2 for C, 2 for O, 2 for O^+, 2 for H etc. To prevent this, a great number of constraints would need to be added to the grammar, ensuring every possible combination of atoms at a bond node is accounted for. This would increase the complexity of the grammar, and we can speculate that checking this many constraints could make critical pair analysis or rule applicability analysis perform much less efficiently. In addition, the generic bond nodes that are used throughout each molecule would increase potential inclusions to check for during critical pair analysis. Embedding one

rule into another, both of which use these nodes heavily, equates to far more possible embeddings to check through.

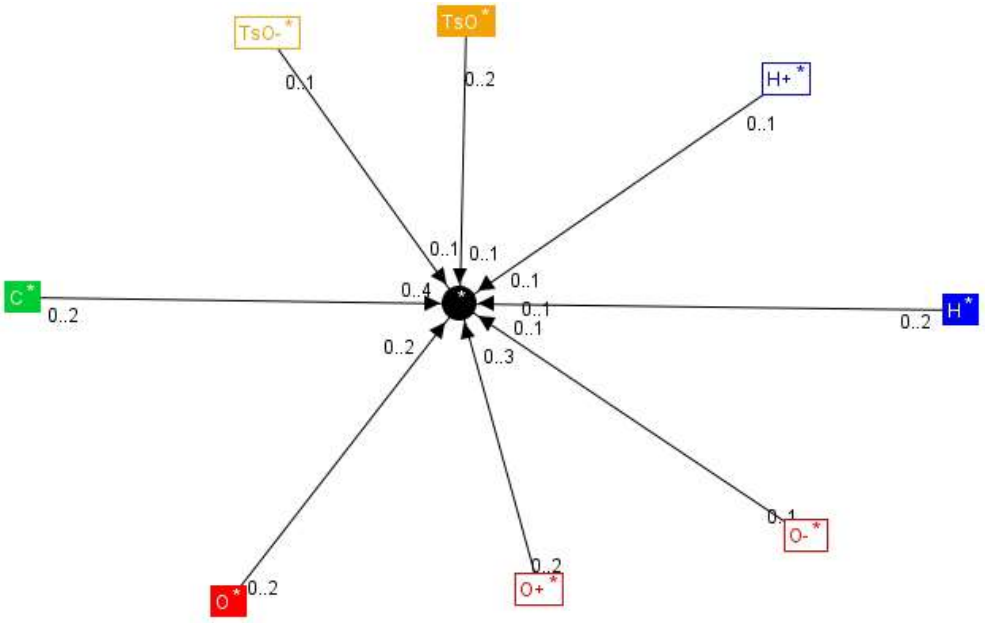


Figure 21 - esterification type graph, 2nd attempt

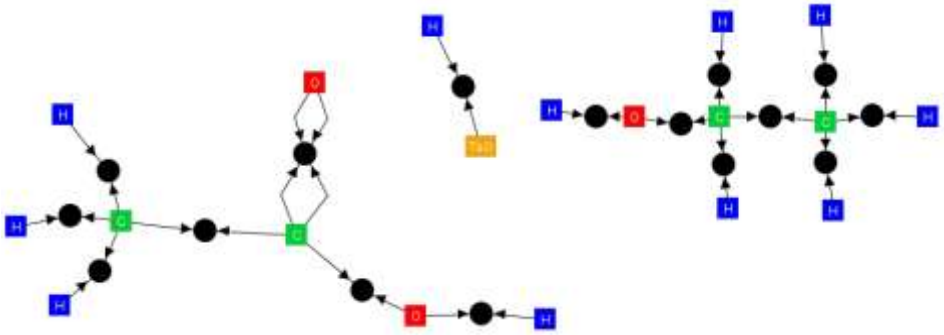


Figure 22 - esterification start graph, 2nd attempt

3rd attempt

After many experiments and modifications of the two type graphs given above, a final type graph was settled upon. This extends the bipartite graph idea, but solves the problem of spurious connections to each generic bond node, by introducing more detail to this node. Each generic bond node is now a pair of atom-specific bond nodes. Each atom node can have a certain no. of atom-specific bond nodes (thereby introducing valency in the same way as the bipartite graph system), and each atom-specific bond node can only be connected to one other atom-specific bond node. This is specified through a combination of the type graph and additional constraints. As edges are directed, there must be a simple way of determining the allowed direction between each possible pair of atom-specific bond nodes. It was decided that the relative electronegativities (the ability of an atom to draw electronic charge towards it) of each atom would determine this direction. As O is more electronegative than C, the direction of the edge would go from the C bond node to the O bond node. The final type graph used in

our case studies is given in figure 23. An instance of this, esterification starting materials as per the two previous examples, is given in figure 24.

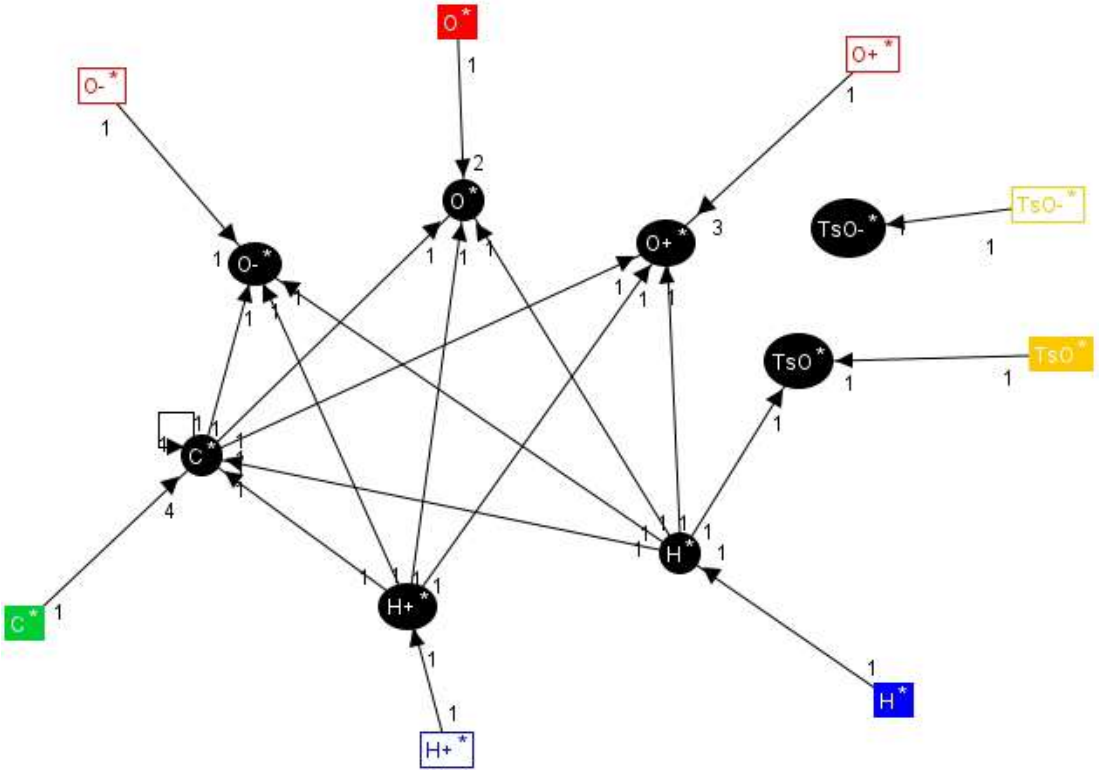


Figure 23 - esterification type graph, final version

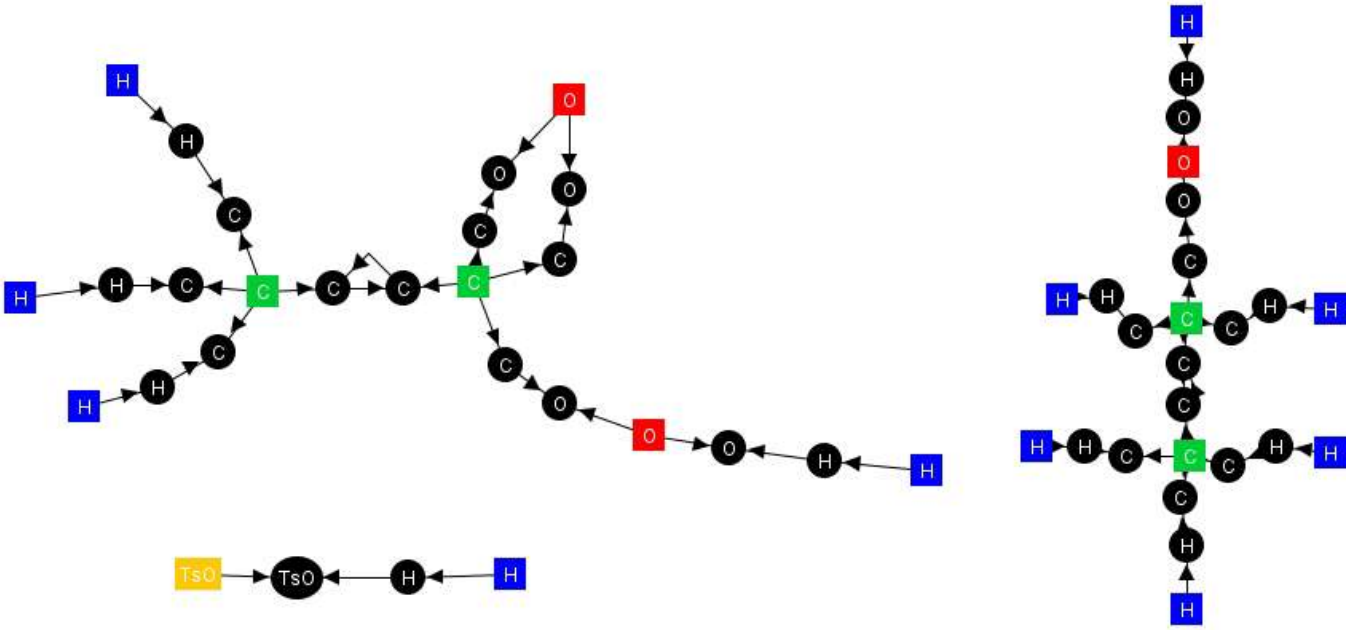


Figure 24 - esterification start graph, final version

Although the graph is much bigger, involving far more nodes, this representation solves many problems. As mentioned in the appraisal of the plan (see page 73), many changes to the representation came about through problems with the case study or implementation. This representation led to far fewer inclusions at the critical analysis stage (explained in "Methodology") due to the lack of generic or non-atom-specific nodes and was therefore selected for the project. A slight modification of this type graph was used for the second case

study, but this will be covered in more detail when describing that case study. Note that the C-C bond in both of the larger molecules has two edges (in opposite directions) between the bond nodes. This is because the C-C bond is symmetric in terms of electronegativity, so this added convention is useful. This can be enforced using a constraint in the same way that all bonds had to consist of two edges in the first attempt type graph (see figure 18).

As explained, additional constraints were also necessary to limit the number of edges each bond node could have. Each bond node should only have 2 connections (except for symmetric bonds). Atomic constraints took the following form and had to be specified for each permutation of 3 atoms, and then these had to be declared to be false in an overall constraint:

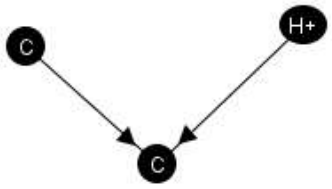
Atomic proposition:	Atomic conclusion:
(empty)	

Figure 25 - example bond node constraint for final type graph

As expected, this was very cumbersome and led to a total of over 40 such atomic conclusions. For a more complicated reaction system involving more varied elements these constraints would grow to be extremely difficult to define by hand. This was a major drawback of the 2nd attempt, and now this one. However, this drawback is fixed in the revised iteration of this type graph used for the second case study, using inheritance in the type graph and constraints. When the esterification type graph above was designed, after some discouraging results, it was unclear as to the effects of inheritance on AGG's implementation of critical pair analysis and was therefore avoided. Later, whilst working on the second case study, we speculated that inheritance should only cause problems with the critical pair analysis if generic supertypes were used in the definition of rules, and not in type graphs or constraints, as these are merely checking mechanisms for the validity of a graph. If inheritance is used in a rule, at critical pair analysis, the supertype node can be substituted for every subtype in the concrete overlapping graph, leading to a possible explosion in the number of inclusions to check. It is not even clear as to how inheritance in rules is treated by the critical pair analysis engine of AGG.

While the improved revision is discussed in more detail later, the type graph above gives the general idea of our approach, using two bond nodes for each bond. This is still a very intuitive molecular representation for chemists. The two bond nodes could even be visualized as the pair of electrons needed to form a directed bond between two atoms.

5. Methodology

A background on critical pairs was given in "Background – Graph Transformations". The use of critical pairs for deriving ODE's presented below is adapted from [9].

Use of critical pairs

The concept of critical pairs can be used to determine which molecules are consumed by which reactions within a network of reactions. To do this, first we need to establish the reactions as one set of graph transformation rules and the molecules (starting materials and intermediates in the reaction) as another set of identity (or identic) rules and check for critical pairings between members of these two sets. An identity rule is one in which the left hand side (LHS) of the rule and the right hand side (RHS) are identical. Hence, it can only be applied to a graph if the LHS of the rule is present within that graph, and its application does not change this graph. As such, identity rules are generally used to check for the presence of certain subgraphs within a graph.

Consider a reaction rule p_1 which defines a transformation that breaks bonds (i.e. in our graph representation of molecules, deletes edges and bond nodes) that are present in a molecule, m. Let us create an identity rule for m, called m_1. If we were to check for critical pairs between p_1 and m_1, all overlappings (or unions) of the LHS of rule p_1 and LHS of m_1 would need to be constructed. If in any of these overlappings, the nodes and edges that are deleted by p_1 are also in m_1, we have a critical pair as the application of p_1 at this particular match would mean m_1 could no longer be applied, since the molecule would be changed by p_1. Notice that we do not need to consider the gluing graph for m_1 as per the construction in figure 13 because for an identity rule, the LHS, gluing graph and RHS are all identical. Checking the LHS alone is sufficient. What the check actually equates to however, is the fact that the intersection of the LHS of m_1 and p_1 is not in the gluing graph of p_1, since p_1 deletes some of these nodes and edges. In summary then, a critical pair consisting of a particular reaction rule and molecular identity rule signifies that that reaction consumes that particular molecule.

In a similar fashion, the concept of sequential dependence (see figure 14) can be utilised to find which reactions produce molecules. If we have a reaction rule p_1, and a molecular identity rule, m_1, such that p_1 creates the molecule in the LHS of m_1 at some stage of the reaction mechanism, for some overlappings of R_1 (from p_1) and L_2 (from m_1), some nodes and edges in L_2 will not be in the gluing graph of p_1, since this reaction rule creates L_2. All nodes and edges in R_1 however will be in L_2, and therefore D_2 (since L_2, D_2 and R_2 are identical). In other words, for a reaction p_1 that produces a molecule in the LHS of m_1, p_1 can be applied before m_1, but m_1 cannot be applied before p_1. This is the condition for sequential dependence.

These two categories of critical pairs give us the crucial elements necessary to derive ODE's, since to do this, all we need is information regarding in which elementary reactions chemical species are produced or consumed. Parallel conflicts and sequential dependencies between reaction rules and molecular identity rules give us this information.

What follows is an account of how this theory is incorporated into an overall methodology in deriving ODE's. There are 10 steps, each of which is covered in detail. To test the methodology, these steps were implemented with the aid of the critical pair analysis tools of

AGG with some additional classes/modifications for added efficiency, automation, and the eventual output of ODE's. The details of how this was achieved are given in the next section, "Implementation".

Step 1: Specification of reaction rules and starting materials in AGG

> *Using AGG, and a suitable graph representation of molecules, specify all general reactivity as graph transformation rules, including all relevant reverse reactions. Define the starting reactants as the start graph for the grammar. Each starting chemical species should also be added as the LHS and RHS of identity rules.*

At this stage, the reaction rules must be as general as possible, possibly at the level of functional groups only. A functional group is the part of a molecule that undergoes a reaction. For example, in acid-catalysed esterification, the C=O double bond undergoes protonation at the start of the reaction. For a general reaction rule, we would only include the C=O part of the molecule in the LHS of the rule. The reason for this is so that the rule can be applied at a later stage in the reaction, for a possibly unknown intermediate. If the rule is too specific at this stage, the reactivity of this intermediate is missed, and the ODE's will not be accurate. This is particularly important for chaining reactions, where the intermediates are essentially too numerous to define or even count. The reactivity of each of these needs to be considered in an automated way.

Reaction rules should be labelled Step1, Step2 etc. while molecular identity rules should be named by the molecule they represent in the textual form of the structural formula of the molecule. For example, ethanoic acid with the following form, would be CH_3COOH:

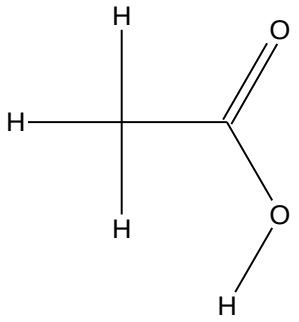


Figure 26 - structural representation of ethanoic acid

This is a convention that is necessary for the extraction of ODE's in recognized chemical forms at a later stage.

Step 2: Application of reaction rules to the start graph to obtain intermediates

> *With the graph grammar defined in step 1, attempt to apply all general reaction transformations to the start graph. For each resulting graph, add any new chemical species as new identity rules. Repeat for the resulting graphs until all rules have been applied to all possible graphs in the reaction mechanism.*

This step attempts to find all possible reaction intermediates so that they can be included in the critical pair analysis in the next stage. If, however, not all intermediates are captured at this stage, the critical pair analysis in the next stage should achieve the same results. However, running the critical pair analysis and examining the results to find all possible intermediates is less efficient than completing this step, especially as we are aided by AGG's GUI here.

For larger reaction networks, this step would be difficult to accomplish in its entirety. As such, for added efficiency, this iterative application of reaction rules and subsequent definition of identity rules should be automated if possible. This is discussed further in the "Critical Appraisal".

Step 3: Execution of first pass critical analysis

> *Using AGG's critical pair analysis engine, conduct a first pass critical pair analysis between the set of general reaction rules and set of identity rules.*

This step gives an indication of which general reaction rules are applicable to which molecules. Both, parallel conflict analysis (to determine which reactions consume which molecules) and sequential dependence analysis (to determine which reactions produce which molecules) should be conducted.

Step 4: Removal of structurally equivalent overlappings

> *Reduce the number of critical overlappings found for each pairing of reaction rule and identity rule, so that only chemically different overlappings remain.*

This stage is not compulsory, but makes the next step (which requires manual interaction) much easier. Due to the nature of our molecular representation, some of the critical overlappings returned from step 3 may be spurious overlappings. Consider the example of two critical overlappings for the same rule pair obtained from the critical pair analysis of the SN1 reaction, given in figure 27. Structurally, these two overlappings are identical. There are six such similar overlappings in total, arising from the nature of our molecular representation, which requires two atom-specific bond nodes and an edge between them to represent a bond. In the two overlappings shown, the identities of the H bond nodes (17, 19 and 14) and C-H bond node edges (20, 23 and 25) connected to the central C bond nodes (3, 4 and 5) are rotated around. Six such overlappings arise because there are 6 configurations these identities can be in. In real chemistry, however, these configurations have no significance to the selection of a reaction or to the outcome of the reaction. As no configuration is unique, they should all be treated as one. This step therefore removes spurious overlappings such that only one unique overlapping remains for each set of chemically equivalent overlappings.

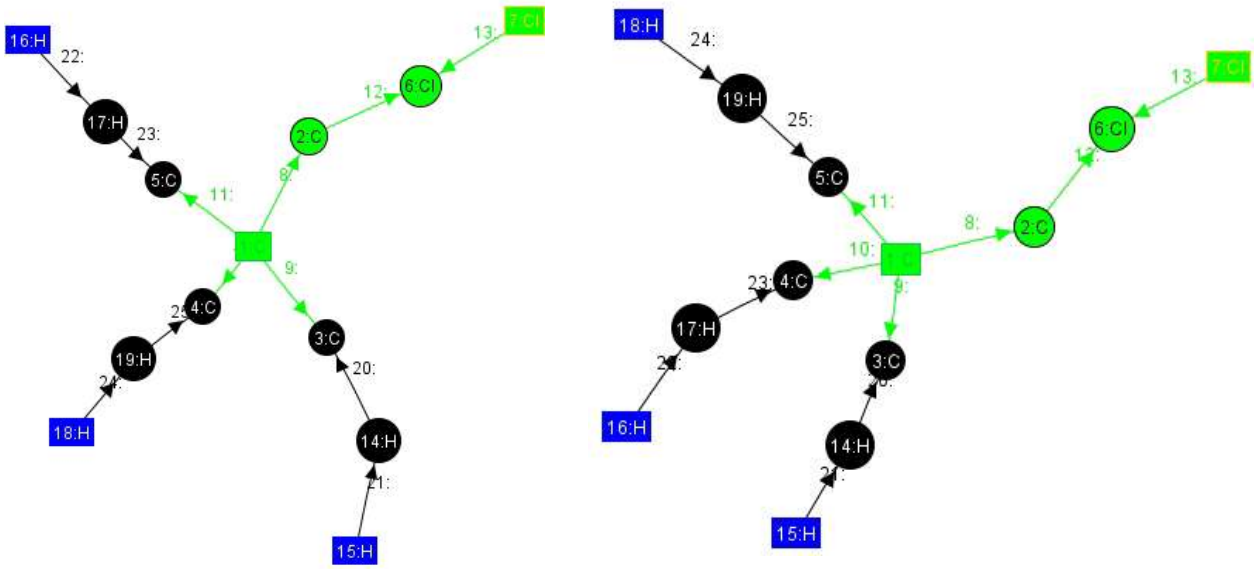


Figure 27 - example of chemically equivalent structural overlappings

Step 5: Manual observation of results and instantiation of rules

> *View the critical overlappings resulting from Steps 3 and 4, and instantiate the general rules to concrete rules to make their application specific to one match within one molecule. These rules are then added to the graph grammar.*

At this stage, we derive instantiated rules from the general ones by limiting the application of each instantiated rule to one particular situation i.e. one part of one molecule. Further details about the instantiation of rules are given in [7] and [16]. We do this because the application of a general rule to two different molecules, or even two different parts of the same molecule will have a different rate constant, and should be considered a different elementary reaction in the overall mechanism. Small factors such as local context (even the presence of an attached carbon rather than a hydrogen) can have an effect on the reactivity of a site, and hence the rate constant.

For each unique critical overlapping, to gain the LHS of the instantiated rule, the LHS of the reaction rule is embedded within the LHS of the molecular identity rule, using the match given by the critical overlapping. To gain the RHS of the instantiated rule, we complete the morphism by copying the RHS of the general reaction rule to the embedded LHS, after deleting the unpreserved nodes and edges. Essentially, we have applied the general reaction rule to the molecule graph and taken the result as the RHS of a new rule. The result (i.e. RHS of the newly instantiated rule) should also now be added as a new molecular identity rule if it does not already exist. Figure 28 gives a construction that summarises the process. L_{mol} is the LHS of the molecular identity rule. The instantiated rule, $p_{instantiated}$ is constructed from L_{mol}, D_{ins} and H_{ins}, after commutation of L_{mol} with the LHS (L_{gen}), gluing graph (K_{gen}) and RHS (R_{gen}) of the general rule, $p_{general}$.

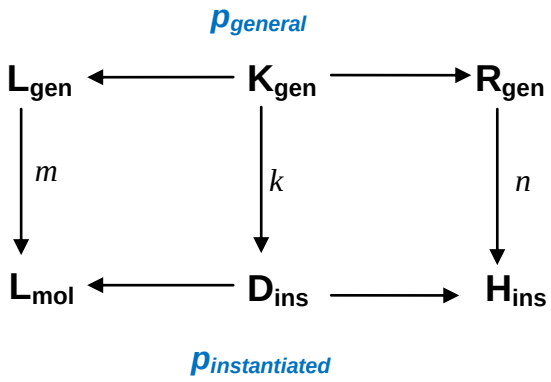


Figure 28 - double pushout construction depicting instantiation of rule

If upon examining the results of the critical pair analysis, it is clear that each general rule is only applicable to one situation, there is no need to instantiate a new rule and the unmodified general rule can be used.

If new molecular identity rules do arise at this stage, we must repeat steps 3 to 5, as the newly discovered intermediates may also undergo general reactions such that even more intermediates can be determined. Only when no new intermediates arise can we deem that the reaction network is fully defined in terms of the chemical species possible (based on our definition of the general reactivity allowed in the system).

Step 6: Disabling of original general rules, and renaming of all rules

> *For general rules that have been instantiated, the general rules they were derived from should be disabled. All rules should now be renamed with the rate constant to which the elementary reaction should be associated.*

This is to ensure results are not duplicated for instantiated rules, since the reactivity proposed by the general rule should now be assumed by a set of more specific instantiated rules. As rules are now specific to a set context, all rules (instantiated ones and general ones that did not need to be instantiated) should be renamed as a rate constant, e.g. k_1, k_2 etc., preferably with the numbering representing the order that reactions take place in the reaction mechanism. Reverse reactions should be labelled with a negative number, corresponding to the forward reaction it reverses e.g. if a rule is the reverse reaction of k_1, it should be renamed k_{-1}. This convention ensures ODE's that include the rate constant at step 9.

Step 7: Execution of critical pair analysis with fully instantiated rules

> *Run the critical pair analysis once more, between the set of instantiated rules (that now have rate constants associated to them) and the complete set of molecular identity rules.*

This will finally yield a stoichiometric matrix informing us of the elementary reactions that produce or consume each chemical species. One major drawback of the current methodology, which originates with this step however, is that reactions that consume or produce more than

one of a certain molecule cannot yet be ascertained. This is discussed in more detail in the "Critical Appraisal" section. It is a flaw that should be solvable given more time.

If we view the resulting ".cpx" file in AGG, we are presented with a table that already closely resembles the stoichiometric matrix that we need to derive ODE's. The following is the conflicts analysis summary table for the SN1 reaction (after rule instantiation):

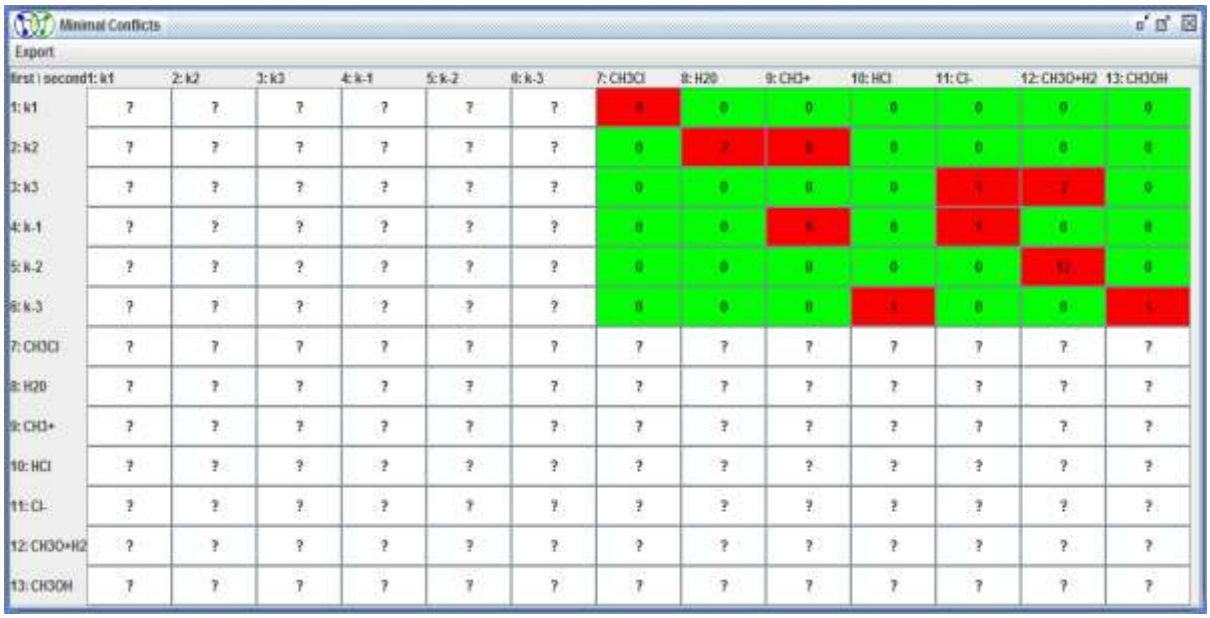


Figure 29 - AGG critical pair conflicts summary example

Only the upper right corner of the table is useful since the other rule pairings are not necessary for kinetic analysis.

Step 8: Removal of structurally equivalent overlappings for instantiated rules

> *The critical overlappings obtained from Step 7 are passed through the Structural Equivalence analysis program to remove spurious pairs so that only unique overlappings remain.*

The logic behind this step was presented in step 4. After performing this step, we are finally presented with the stoichiometric matrix needed for the derivation of ODE's. The table in figure 29 is reduced to that in figure 30.

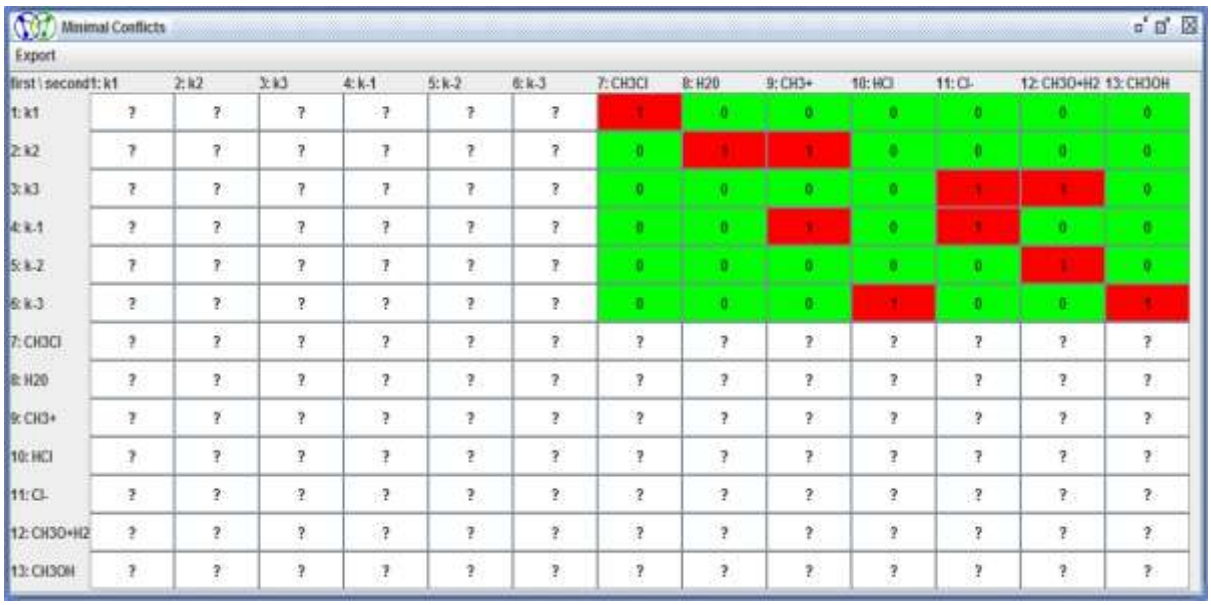


Figure 30 - AGG critical pair conflicts summary example after structural equivalence reduction

Step 9: Execution of ODE extraction program

Run the ODE extraction program with the output ".cpx" file from Step 8 as input. This will generate the ODE's from the stoichiometric matrix and output them in string format in a text file.

This stage uses the theory set out in "A Chemistry Background" of the "Relevant Background Information" section. The output currently is in string format. However, the output can be modified to any format necessary (e.g. XML). Integration with step 10 requires knowledge of the specific math solver to be used and its input format. As step 10 was not fully realised in the timescale of the project, it was decided that the output would simply be in string format, to demonstrate that output is possible. Currently, it has the following format:

```
d[CH3+]/dt =  -k-1[CH3+][Cl-] +k-2[CH3O+H2] +k1[CH3Cl] -k2[CH3+][H20]

d[CH3Cl]/dt =  +k-1[CH3+][Cl-] -k1[CH3Cl]
```

There is one such line for each chemical species in the reaction network.

Step 10: Solving ODE's using a 3rd party math solver

Pass the ODE's extracted in Step 9 into a suitable math solver software. A single equation defining the progress of the reaction should be returned, in terms of the change in concentration of one interesting species with time.

Although this step was not completed for the project, it is included for completion. In the final stage, a 3rd party math solver allows us to use the results of the kinetic analysis to generate quantitative data and a graph representing the kinetic profile of the reaction. For this, the values of all of the rate constants for the reaction need to be known. These can be derived by numerous small experiments or gathered from existing sources. While these sources have

not been investigated, further research should reveal libraries (perhaps online) where such information on reactions is maintained.

The results of this final stage can be used to validate our reaction mechanism. If the extracted graph does not have the same form as established data for a particular reaction, it is clear that the reaction rules were not complete or perhaps implemented incorrectly.

6. Implementation

The implementation of our methodology involved three distinct newly constructed components. Each of these was implemented as a self-contained Java module and had the following functions:

1. Critical pair analysis
2. Structural equivalence testing
3. ODE extraction

These were combined into a simple command line user interface (using shell and batch scripting) which we shall call the kinetic analysis suite. This section will first cover how this suite, along with AGG, can be used to apply our methodology to a concrete example, specifically which steps in the methodology each tool/module relates to. A description of the Java coding that was needed to implement each of the three modules is then given, along with an account of the developed batch/shell script.

Tools that implement the methodology

Steps 1 and 2: AGG

To input rules, start graph and type graph AGG is used:

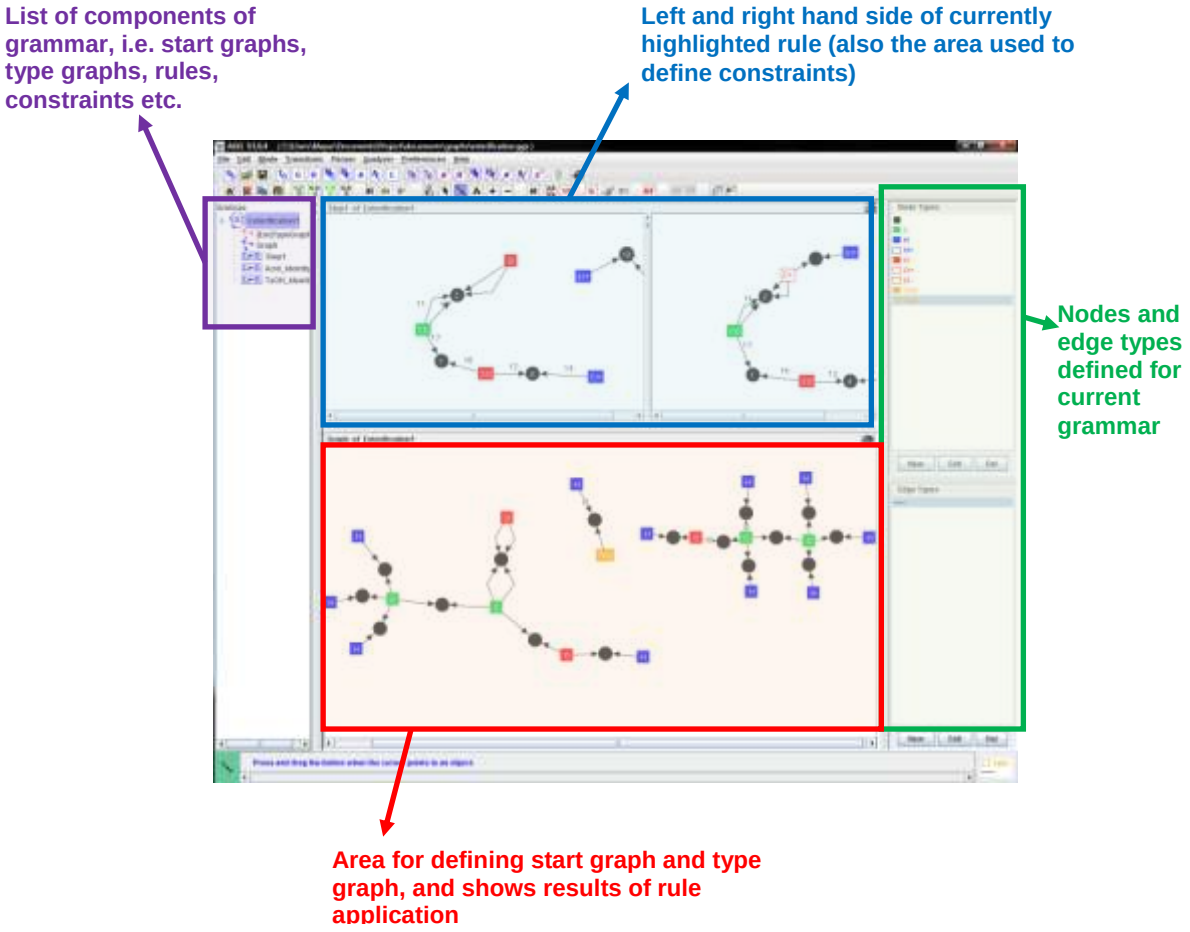


Figure 31 - main layout of AGG

The red area can be used in step 2 to see which new chemical species occur as the result of applying rules to previous graphs. Further documentation on the use of AGG can be found at [19].

Steps 3 and 4: Kinetic Analysis Script

Here the prepared shell scripts need to be run. Initially the user is presented with an option of whether they wish to run the full suite or component programs. For steps 2 and 3, the user should choose "no". Next, they are presented with an option for which program they wish to run. For step 2, option 1 ("Critical Pair Analysis") should be chosen. For step 3, when the user has started the program for a second time, option 2 ("Structural Equivalence Analysis of Pairs") should be chosen. In both cases, they will be asked to input the location of the input file.

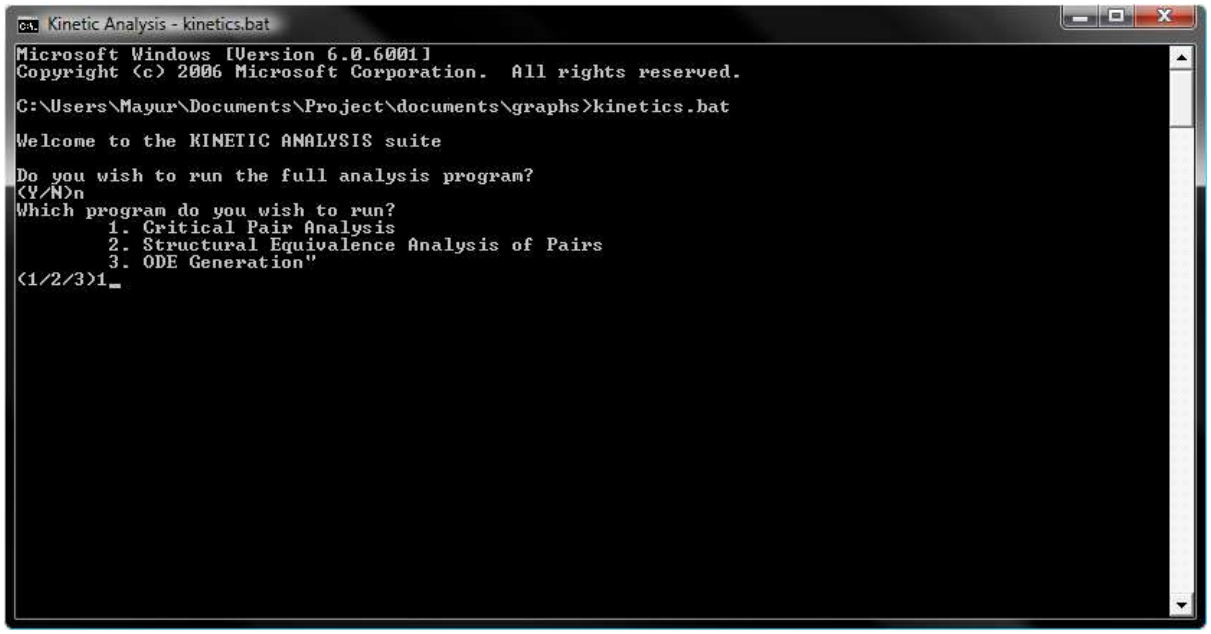


Figure 32 - kinetic analysis program, steps 2 and 3

Step 5: AGG Critical Pair GUI

Critical pairs can be examined using AGG's critical pair GUI (figure 33). This is accessed from the main AGG window by selecting "Analyzer > Critical Pair Analysis > Load > In This Window" from the menu bar.

A summary table of the total conflicts and dependencies is given initially. By clicking a square in this table the actually critical overlappings along with the 2 conflicting rules can be examined for a rule pair. The top half of figure 33 shows the two conflicting rules. The graph in the bottom right corner shows the critical overlapping. The nodes highlighted in green show the graph objects that are responsible for the conflict. Mapping identities allow the user to relate these to the same nodes and edges in the rules.

For this step, each critical overlapping should be examined to see if this rule needs to be instantiated (i.e. more context introduced to the rule to make it more specific). If it does, the main AGG window should be returned to, and the new rule added. The rule should then be applied to the molecule for which the critical overlapping was found, and the new molecule added as a new identity rule.

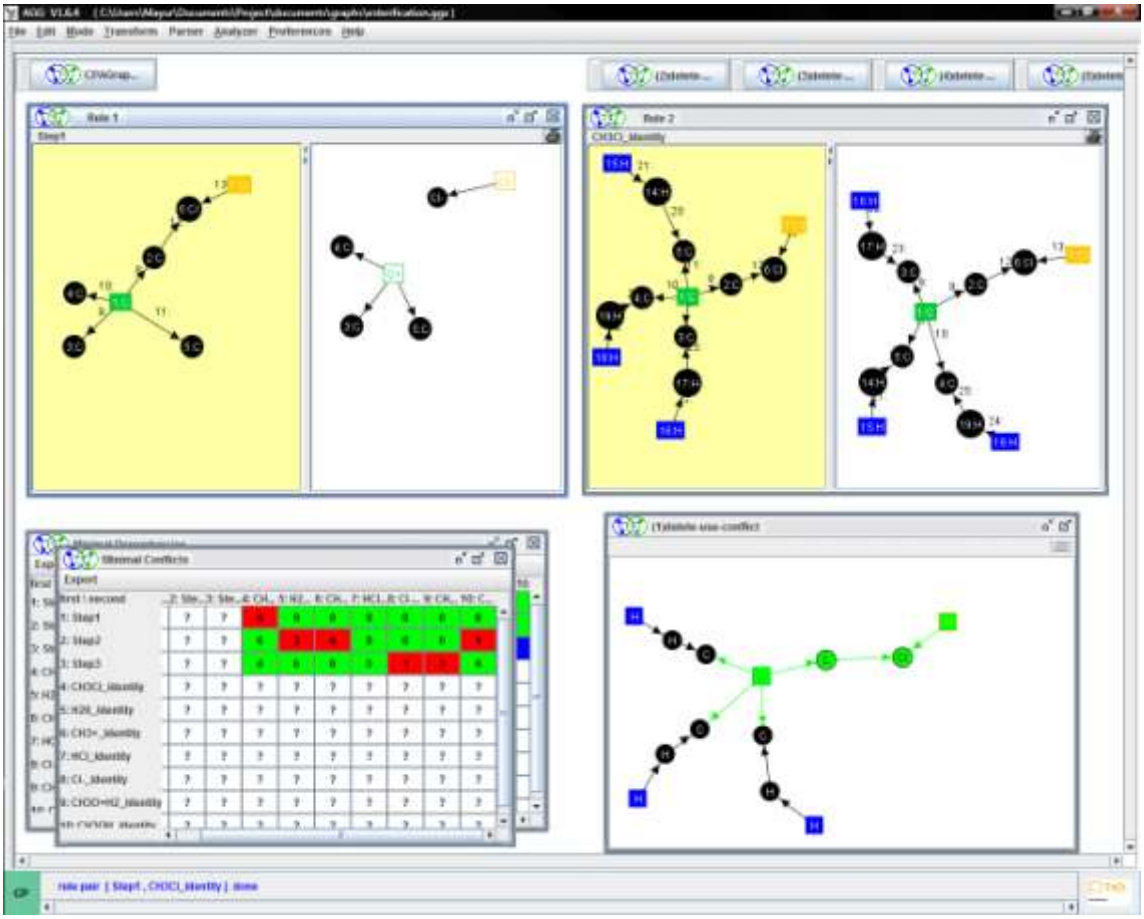


Figure 33 - critical pair analysis GUI module of AGG

Step 6: AGG

This step requires AGG once more. Once the rules have been instantiated, the old rules should be disabled, by right clicking them in the grammar components list and selecting "disabled".

Steps 7, 8 and 9: Kinetic Analysis Script

The script should be run once more. This time, the full analysis suite option should be chosen. Once the analysis is complete, the user is presented with the message in figure 34, which tells them the filename which contains the generated ODE's. These are also printed to the system output during the analysis.

Step 10: Third Party Algebra Solver

This part was not completed in time, partially due to the very simple nature of the case study used, which did not require quantitative verification of results. Several commercial applications were researched however. The majority of these were not freeware, especially the most suitable for solving differential equations.

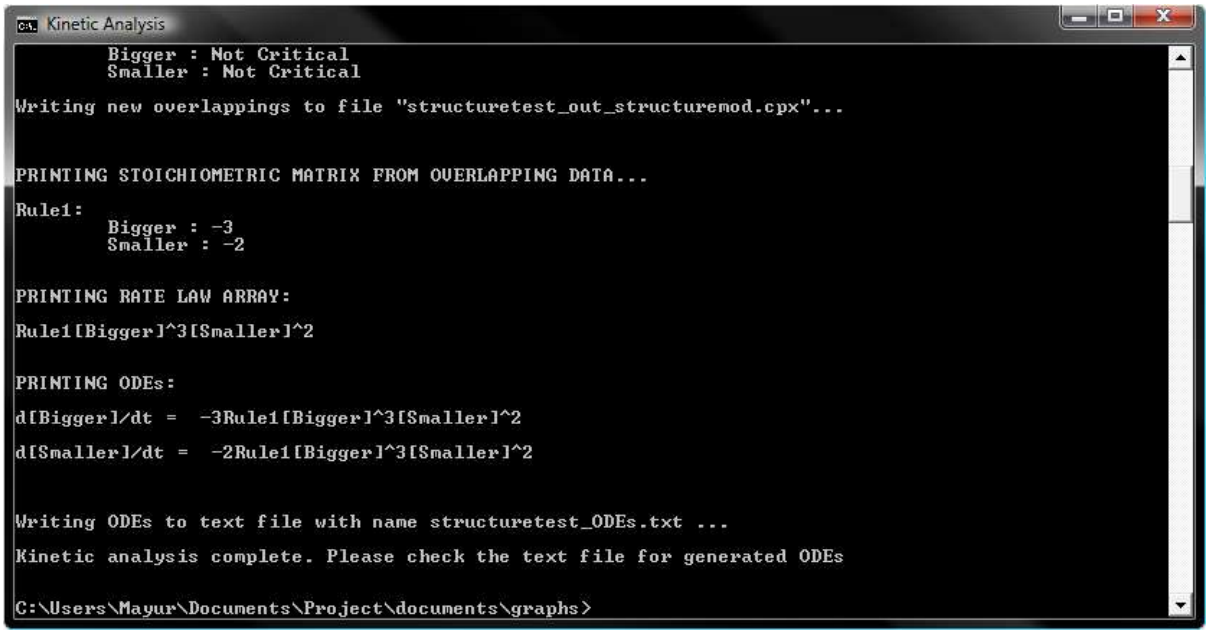


Figure 34 - result of running complete kinetic analysis suite

The AGG API

This API (for version 1.6.4) is included on the software CD for the project. Details of how to access it are included on the readme file. AGG is an open source graph transformation tool that is available at [19]. It is widely used to implement graph grammars and contains a depth of functionality that can be accessed through its API, allowing low-level access to and the opportunity to extend what is presented in its GUI. Because of the ease with which rules, type graphs, and graphs can be implemented in AGG, it was a natural choice for the implementation of the project. There are very few free open source graph transformation tools like it. More information on its development and evolution can be found at [19].

In order to adapt what the software does to our needs, several classes needed to be extended, and others were required for low-level functionality. At times, this proved extremely difficult. The software itself has some depth and not all classes have complete Javadoc comments. This is perfectly understandable for a non-commercial piece of software. Fortunately, the source code was available which allowed us to deduce each method's purpose from its code and also included some non-Javadoc comments. This was partially written in German, but use of online translators helped translate parts of this. Nevertheless, the complexity of understanding the API was a great technical challenge. With perseverance, and many hours of investigation, however, much of the functionality required was attained. When it was felt that progress was not being made at all (see the section on structural equivalence testing), help was available directly from a member of the AGG development team.

The critical pair analysis part of AGG was the bottleneck in the methodology as it was extremely memory intensive and time-consuming. Certain grammars actually had to be abandoned (e.g. the esterification case study) because the Java virtual machine eventually ran out of heap space. This is a limitation of the software that members of the AGG staff are currently looking into. For future work, it would be extremely helpful if this problem was resolved. In the meantime, alternative software that can carry out critical pair analysis for graph transformation systems should be researched.

Critical Pair Analysis

The classes needed for this part are arranged in the following package structure:

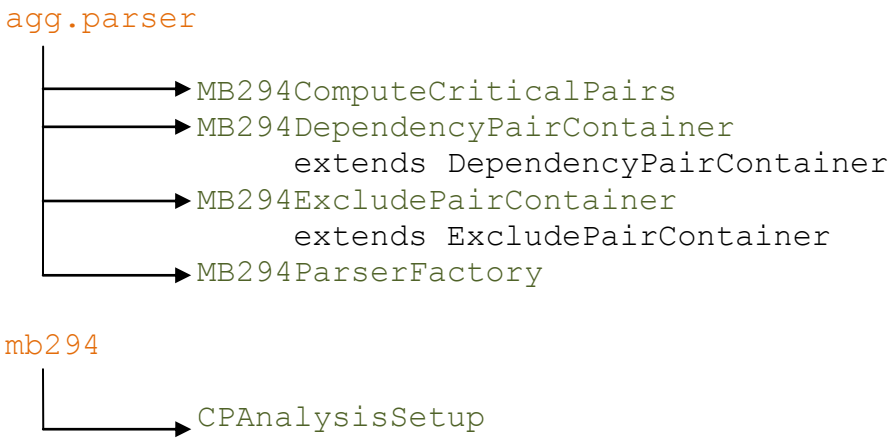


Figure 35 - critical pair analysis package structure

As can be seen from this package structure, many of the classes are extensions of existing AGG classes. MB294ComputeCriticalPairs is an almost exact copy of ComputeCriticalPairs, with minor changes. This class could not be extended as certain methods that needed to be overridden in the subclass required copying code from the superclass, and this contained some private data members.

The main purpose of the modification to AGG's own critical pair analysis program is that this conducts an analysis of critical pairs between every possible pairing of rules. Kinetic analysis only requires the analysis of pairs where one member is a reaction rule and the other is a molecular identity rule. CPAnalysisSetup loads and analyses a grammar, separating the rules into two lists, one for reaction rules and the other for molecular identity rules. The crucial code segment is given below:

```
054   private void setUpRules() {
055     System.out.print("Sorting rules...");
056     long start = System.currentTimeMillis();
057     List<Rule> allRules = gragra.getListOfEnabledRules();
058     ListIterator<Rule> itr = allRules.listIterator();
059     while (itr.hasNext()) {
060       Rule tmp = itr.next();
061       if (tmp.getLeft().compareTo(tmp.getRight())) {
062         identityRules.add(tmp);
063       } else {
064         reactionRules.add(tmp);
065       }
066     }
067     long elapsedTimeMillis = System.currentTimeMillis() - start;
068     System.out.println("Finished sorting rules in " + elapsedTimeMillis
069         + " milliseconds");
070   }
```

Code 1 - CPAnalysisSetup, setUpRules method

This method extracts all of the enabled rules from its associated grammar as a list. Then using a list iterator, checks each rule. If the LHS and RHS of the rule are identical, it is an identity

rule and is added to this object's identity rules list data member, else it is assumed to be a reaction rule and is added to this object's reaction rules list.

The scheduling of rule pairs for critical pair analysis takes place in AGG's ExcludePairContainer class for conflicts. MB294ExcludePairContainer extends this class and overrides the fillContainers method (responsible for scheduling pair analysis). Its constructor creates a new CPAnalysisSetup object, passing in as a parameter the graph grammar for which it is being used. MB294ExcludePairContainer then has two extra data members – a list of identity rules and a list of reaction rules.

```
40  @Override
41  protected void fillContainers() {
42
43    if (useHostGraph && grammar != null) {
44      grammar.getApplicableRules(testGraph, strategy);
45    }
46
47    if (!useHostGraph) {
48      isComputed = false;
49    }
50
51    ListIterator<Rule> itr1 = reactionRules.listIterator();
52
53    while (itr1.hasNext()) {
54      Rule r1 = itr1.next();
55
56      ListIterator<Rule> itr2 = identityRules.listIterator();
57      while (itr2.hasNext()) {
58        Rule r2 = itr2.next();
59        this.scheduleForComputing(r1, r2);
60      }
61
62    }
63
64    if (!useHostGraph) {
65      isComputed = true;
66    }
67  }
```

Code 2 - MB294ExcludePairContainer, fillContainers method

Some of this code is copied from the superclass' method. Lines 51-62 are the crucial part which carries out the scheduling. For every reaction rule, every member of the identity rules list is paired with it and scheduled for computing critical overlappings (line 59). scheduleForComputing is a method of the superclass.

Similarly, for DependencyPairContainer, which computes sequentially dependent pairs, MB294DependencyPairContainer extends this and overrides the following crucial method.

```
34  @Override
35  protected synchronized void computeCritical(Rule r1, Rule r2){
36    if (reactionRules.contains(r1) && identityRules.contains(r2)){
37      super.computeCritical(r1, r2);
38    }
39    else {
40      //do nothing
41    }
42  }
```

Code 3 - MB294DependencyPairContainer, computeCritical method

In the original superclass, all rule pairs are automatically scheduled for computing. In order to restrict the pairs that are actually computed, we override the computeCritical method and check whether the rule 1 parameter is a reaction rule and rule 2 is an identity rule. If they are, the superclass' original computeCritical method is called. Otherwise, the method does nothing, thereby successfully bypassing the computation of unnecessary pairs.

In the original ComputeCriticalPairs class, the analysis engine retrieves and uses ExcludePairContainer and DependencyPairContainer objects (used to calculate and store critical pairs) from a ParserFactory object. MB294ParserFactory is a copy of this class, which instead returns MB294ExcludePairContainer and MB294DependencyPairContainer objects (see lines 37, 47 and 51 of the source code). MB294ComputeCriticalPairs then simply retrieves the new customised PairContainer objects using MB294ParserFactory wherever ParserFactory was originally used, as in the following code segement:

```
:
:
357       if (excludePairContainer == null)
358         excludePairContainer = MB294ParserFactory
359             .createEmptyCriticalPairs(gragra,
360                 CriticalPairOption.EXCLUDEONLY, cpOption
361                     .layeredEnabled());
362
:
:
```

Code 4 - MB294ComputeCriticalPairs, use of MB294ParserFactory

This ensures that this program only calculates the pairs needed, and is a first attempt at a more efficient and optimized analysis.

Structural Equivalence Testing

To reduce structurally equivalent overlappings (in terms of Chemistry) a program was developed that could accept the ".cpx" XML file output from the critical pair analysis, perform the reduction, and save the new pairs in a new file (with the suffix "_structuremod.cpx"). The package structure for this program is as follows:

Code 5 - structural equivalence testing package structure

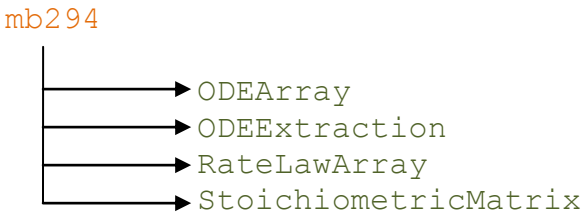
The source code for this class is heavily commented so only a summary of our method is presented here. The constructor uses API methods to load critical pair information from a file (identified by filename supplied through the command line). The method reduceStructurallyEquivalentOverlappings then extracts all the overlapping information and performs the reduction. For each rule pair, all overlappings are tested for uniqueness. It was decided that the best way to do this would be to apply the reaction rule to the molecular graph represented by the LHS of the identity rule, using the match provided by the critical overlapping of the two rules. The result of the application should be checked for isomorphism

with all other unique overlappings for this rule pair. If it is isomorphic to any of them, it should be discarded, else it should be added as a unique overlapping. Applying the rule is a sufficient check for structural uniqueness.

The help of Olga Runge (an AGG developer) should be greatly acknowledged here. Although the method presented above was recognised as the best way to test for structural uniqueness, a way to apply the rule using the match information from the critical pair could not be deduced from the API without affecting the original XML file. A method was designed, but it led to nodes and edges in the critical overlapping being deleted when written back to file. Also, the method of reversing a rule and applying it (necessary for checking for uniqueness in dependency pair overlappings, see the construction in figure 14) could not be deduced. Olga Runge's help and advice at this stage through numerous emails was invaluable and very greatly appreciated. Code that she helped with is noted in comments within the source code. The makeStep method in its entirety was provided by Olga.

ODE Extraction

The classes needed for this part are arranged in the following package structure:



Code 6 - ODE extraction package structure

ODEExtraction is the program with a main method that produces the ODE's. It has an object of each of the other 3 classes listed above as data members. All of these classes are heavily commented so only a summary of their main functions are presented here.

ODEArray contains a Hashtable that stores these ODE's. For each identity rule key in the Hashtable (i.e. chemical species), there is a string representation of its corresponding ODE as the object. StoichiometricMatrix takes the overlappings data from the XML output file of the structural equivalence component and discards superfluous information making it easier for other methods to process. The stoichiometric matrix data is stored as a Hashtable, with reaction rules as keys, and another Hashtable as objects. This second Hashtable has the identity rules as keys and Integers as objects. These integers represent the entry in the stoichiometric matrix for each reaction-identity rule pairing. The RateLawArray object is created using information from a StoichiometricMatrix object (as described in "Relevant Background Information"). The rate laws for each elementary reaction are stored as an array of pairs. Each pair in the array consists of a reaction rule, and a string representing its rate law.

ODEExtraction loads overlappings data in the same way that the structural equivalence module does, using API methods. Once loaded, this class instantiates an ExcludePairContainer and a DependencyPairContainer object to store conflict and dependency overlapping information respectively. This occurs in the constructor. The main method calls this constructor and then calls the following method:

```
100   public void outputODEs() throws Exception {
101     System.out.println("\n");
102     populateStoichiometricMatrix();
103     getStoichiometricMatrix().printStoichiometricMatrixInfo();
104     populateRateLawMatrixFromStoichiometricMatrix();
105     getRateLawArray().printRateLawArray();
106     generateODEs();
107
108     String output = getODEArray().odeEquationsToString();
109     System.out.println("\n\nPRINTING ODEs:\n" + output + "\n");
110     System.out.println("Writing ODEs to text file with name " + newfilename
111         + " ...");
112     // write the ODEs string to file
113     BufferedWriter out = new BufferedWriter(new FileWriter(newfilename));
114     out.write(output);
115     out.flush();
116     out.close();
117     // System.out.println("Done.");
118   }
```

Code 7 - ODEExtraction, outputODEs method

The first task is population of the StoichiometricMatrix object (line 102). The method that does the work within the private populateStroichiometricMatrix method is the fillMatrix method of StoichiometricMatrix, which takes the no. of critical conflict overlappings for each reaction-identity rule pair (the no. of molecules consumed by a reaction) and minuses it from the no. of dependency overlappings for the same pair (the no. of molecules produced by the reaction). This gives the overall effect a reaction has on a particular chemical species. This number is added to the stoichiometric matrix for this reaction-identity rule pair.

When the stoichiometric matrix is filled, the rate law array is extracted from it (line 104). This is done by the fillArray method in the RateLawArray class. For each elementary reaction, we look for negative entries in the stoichiometric matrix. By assuming that negative entries mean involvement in initiation of a reaction, we can extract the rate law for that reaction.

generateODEs (line 106) then calls the generateODEArray method of ODEArray. This simulates the matrix multiplication of the rate law array by the stoichiometric matrix and stores the results. This class also contains a method that returns all the ODE's as a String object. Lines 108 to 118 in code 7 show how the ODE's are output to console and to a text file using this String.

Running the Kinetic Analysis

The classes described above were all compressed into a JAR file entitled "kinetic_analysis_in_agg.jar" and placed in the agg root directory, where the JAR files that hold AGG's other classes are also located. A batch file (for Windows) and a shell script (for Linux) were created labelled "kinetics.bat" and "kinetics.sh" respectively. These scripts are the GUI through which many steps in the methodology can be performed, guiding the user through input and output of the 3 programs described above. There are some variables which must be changed in these files to allow them to run on a specific machine (such as JAVA_HOME). A "maxheap" variable also specifies the maximum heap memory allowed by the Java virtual machine. As mentioned previously, the critical pair analysis is extremely memory intensive and can fail if not supplied with enough heap space. This variable should be set as large as possible. An additional argument is supplied to the Java virtual machine,

namely “XX:-UseGCOverheadLimit”. This ensures that OutOfMemory errors are not thrown if the virtual machine spends too much time in garbage collection. This was necessary for larger grammars which led to many such errors. However, Sun Microsystems deem this an unstable option, and it should possibly be removed in future iterations when AGG’s critical pair analysis engine is improved for efficiency (see http://java.sun.com/javase/technologies/hotspot/vmoptions.jsp for more details).

The scripts enable the user to run all three programs described above to run consecutively without any need for user interaction. In order to prevent subsequent programs from running when an exception occurs in an earlier program, system exit codes were utilised. For the structural equivalence analysis and ODE extraction, if an exception occurred, the main method returns a -1 exit code, else 10 is returned (highlighted in red):

```
379   public static void main(String[] args) {
380
381     try {
382       StructuralEquivalenceAnalysis sea = new StructuralEquivalenceAnalysis(
383           args[0]);
384       sea.processFileForStructuralEquivalence();
385       System.exit(10);
386     } catch (ArrayIndexOutOfBoundsException e) {
387       System.err
388           .println("There was an error loading your specified file - please try
again!");
389       e.printStackTrace();
390       System.exit(-1);
391     }
392
393   }// end of main method
```

Code 8 - StructuralEquivalenceAnalysis, main method demonstrating use of exit codes

This is not possible for the main method of MB294CriticalPairAnalysis however.

```
413   public static void main(String[] args) {
414     try {
415       MB294ComputeCriticalPairs mcp = new MB294ComputeCriticalPairs();
416       mcp.run(args);
417     }catch (Exception e){
418       System.err.println("An error occurred during critical pair analysis!");
419       e.printStackTrace();
420       System.exit(-1);
421     }
422   }
```

Code 9 - MB294CriticalPairAnalysis, main method

Line 416 initiates a thread. While this thread is running the main method continues to execute its next line in its own thread of execution. Any system exit code would immediately cause this program to end. Therefore, for this program, we use the default system exit code (of value 0) which is automatically returned when all threads are finished. In the event of an exception however, -1 can still be returned.

The Windows script uses these codes as shown in code 10. If an error is returned the control is forwarded to the printing out of an error message and then the end of the script. Otherwise, the next program is started with the output file of the last program as input.

```
:section1command
        java -Xmx%MAXHEAP%m -XX:-UseGCOverheadLimit agg.parser.MB294ComputeCriticalPairs %file%
        IF ERRORLEVEL 0 goto :section1-1
        IF ERRORLEVEL -1 goto :section1-1error

:section1-1
        set nextfile=%file:.ggx=_out.cpx%
        java -Xmx1000m mb294.StructuralEquivalenceAnalysis %nextfile%
        IF ERRORLEVEL 1000 (goto :section1-2) ELSE (goto :section1-2error)

:section1-2
        set lastfile=%nextfile:_out.cpx=_out_structuremod.cpx%
        java -Xmx1000m mb294.ODEExtraction %lastfile%
        IF ERRORLEVEL 1000 (goto :section1complete) ELSE (goto :section1-3error)
:
:
:
:section1-1error
        echo There was a problem running the critical pair analysis.
        goto :eof
```

Code 10 - Windows batch script demonstrating use of Java system exit codes

7. Case Study 1 – Esterification

As mentioned previously, this case study was intended as a simple example of a finite and closed reaction network, which would allow the testing of our methodology. Unfortunately, due to the bottleneck in the implementation created by the AGG critical pair analysis module, and its associated memory errors, we could not proceed past step 3 for this reaction. However, it is still useful to document steps 1 and 2, and also discover for which rule pair the memory problem occurs. The grammar for this case study is on the software CD in the graphs folder as "esterification_general.ggx".

Esterification is the reaction of an alcohol and a carboxylic acid to create an ester and give off water. The reaction can be catalysed by acid or alkali. The acid catalysed reaction was chosen as it made the example more complex and interesting. The mechanism for the reaction was verified with that found at [3].

Step 1

The first step is to define a type graph, which includes all the elements possible in the reaction. This was presented in figure 23 (see "Molecular Representation Using Graphs"). The next step is to define the starting materials as a graph typed over this type graph. This is given in figure 24. Here, we have the carboxylic acid, alcohol and acid catalyst.

Then we define as much general reactivity as possible. This can be done by examining the reaction mechanism and determining what happens to functional groups in the molecules throughout the reaction. To illustrate this, the chemical representations of the five forward steps in the reaction are presented along with their corresponding general graph rewriting rules.

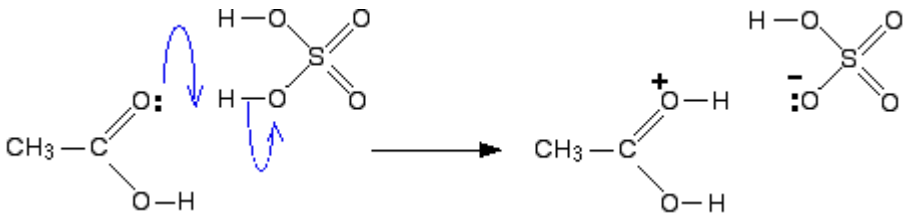


Figure 36 - step 1 of esterification reaction [3]

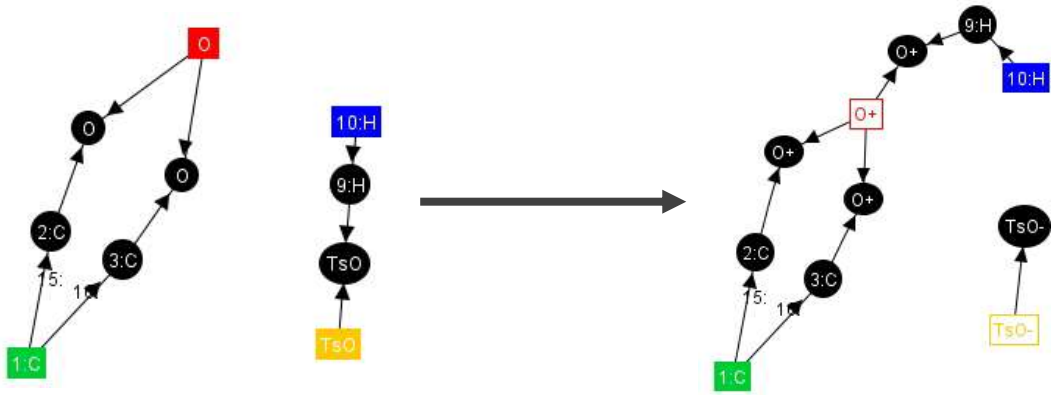


Figure 37 - step 1 of esterification reaction, GT rule

As can be seen in figure 37, the whole molecule is not presented in the rule, only the part that undergoes reaction. This makes the rule applicable to any molecule that might have this functional group, which is exactly what is required. This reaction is reversible, so the C=O double bond can be deprotonated once it is protonated. This is represented in the grammar by a rule labelled step-1, where the LHS and RHS of step1 are simply switched.

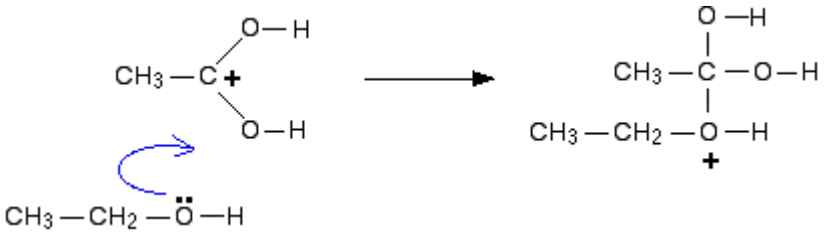


Figure 38 - step 2 of esterification reaction [3]

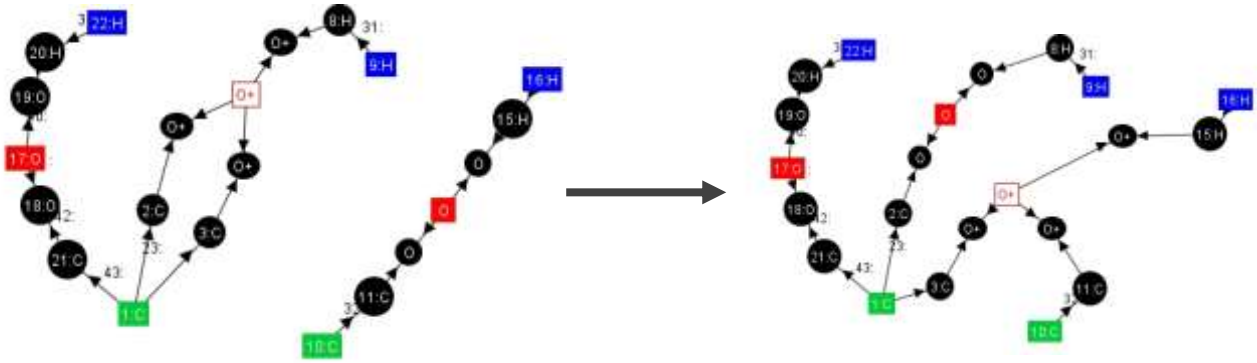


Figure 39 - step 2 of esterification reaction, GT rule

Step 2 of the reaction is the attack of the negatively charged oxygen in the alcohol on the now positively charged (due to the protonation in step 1) central carbon atom in the carboxylic acid. This results in the joining of the two large molecules into one. This step is also reversible and is represented by step-2 in the grammar.

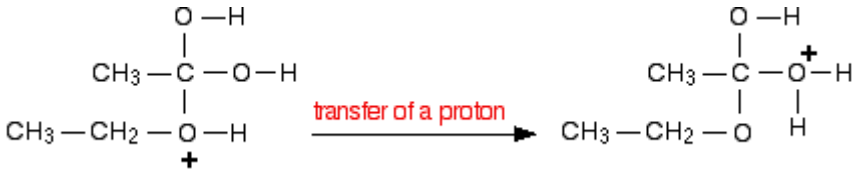


Figure 40 - step 3 of esterification reaction [3]

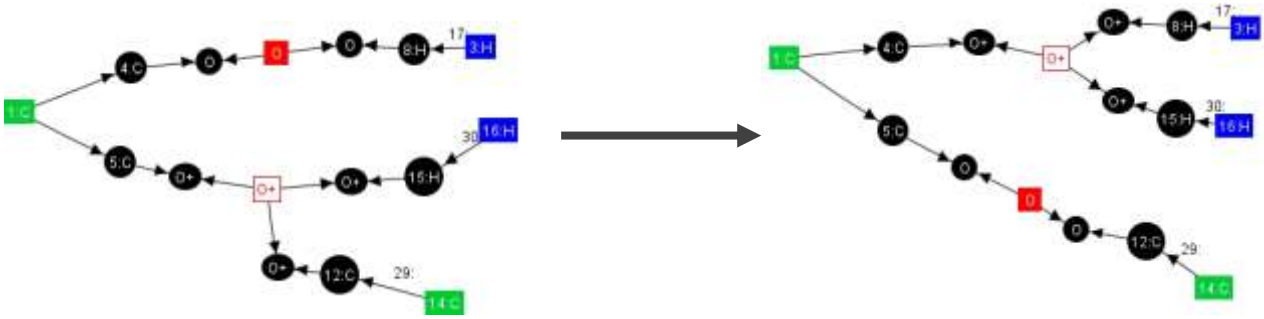


Figure 41 - step 3 of esterification reaction, GT rule

This reaction is the simple change in the location of a proton (H atom) within the newly formed large molecule. The reverse of this reaction is labelled step-3 in the grammar.

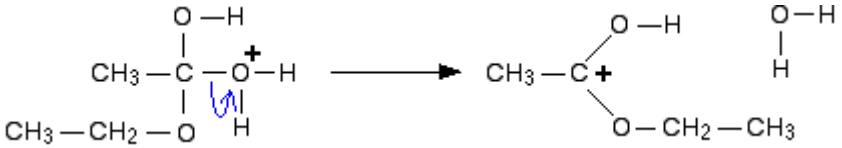


Figure 42 - step 4 of esterification reaction [3]

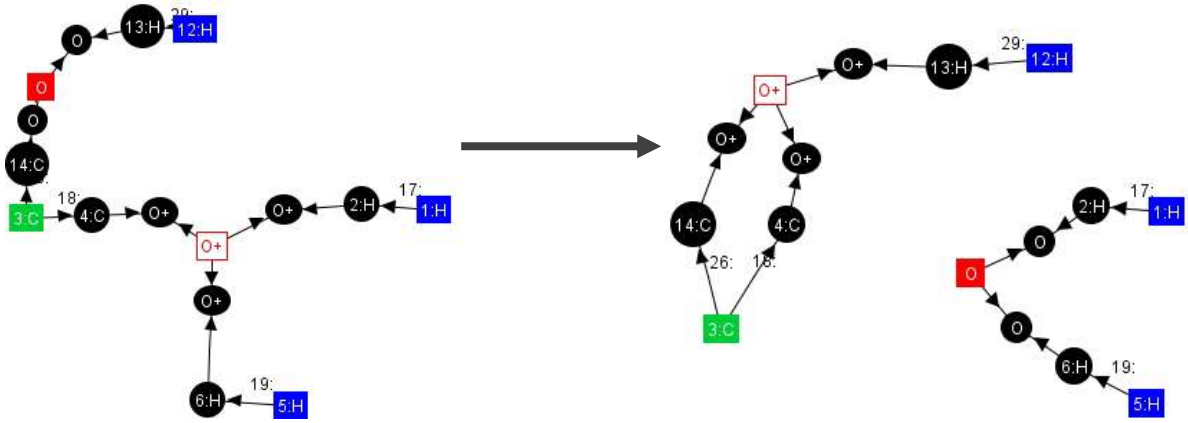


Figure 43 - step 4 of esterification reaction, GT rule

Here, the protonated OH group leaves the larger molecule as water, allowing the C=O double bond to reform. The reverse of this step is labelled step-4. The representation of the positively charged $C=O^+$ in the RHS of the graph transformation rule is identical to that presented in figure 42. Here, the electrons in the $O\text{-}C=O^+$ structure are delocalised over all 3 bonds, and the positive charge can also be spread over all three atoms.

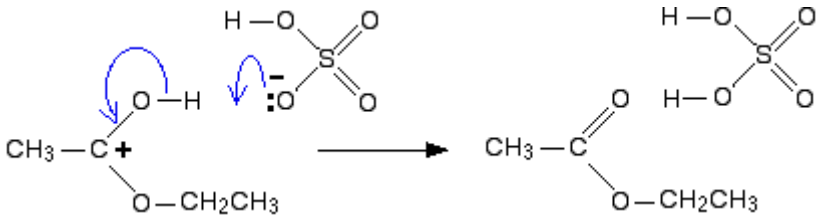


Figure 44 - step 5 of esterification reaction [3]

The final step in the reaction (also reversible) is deprotonation of the reformed C=O double bond. However, this was not added as a general rule, as this reactivity is already captured by step-1. Step 5 and 6 of the methodology would see two instantiations of the step-1 reaction rule, one for the initial deprotonation (as a reverse of step1) and one for the final deprotonation. Each would be assigned their own rate constant.

Step 2

Step 3 was carried out before step 2 to check that the critical pair analysis gave correct results for the consumption of the starting materials. As step 3 failed, it was felt there was little point in returning to and completing step 2. Therefore, the complete set of intermediates in the reaction is not present as molecular identity rules in the grammar.

Step 3

As already mentioned, step 3 failed due to OutOfMemory errors from the Java virtual machine. The critical pair analysis reached the testing of the step2 reaction rule against Acid_Identity when it could progress no further. The LHS of the rule for step2 is a rather large graph as it describes the coming together of the two large starting materials. The alcohol molecule is itself quite large. The number of possible overlappings for which to calculate critical overlappings (i.e. possible unions of the two graphs) grows as the size of the graphs in the LHS of both rules grows. Therefore, this particular pairing exceeded the calculable limit. In fact, the number of possible overlappings was so large that the program did not even get as far as outputting exactly how many overlappings there were, but instead froze at this point for many hours until an OutOfMemory error occurred.

To give an indication of the scalability problem, the following table shows how many total overlappings and eventual critical overlappings occurred for each rule pair. Those rule pairs that came after the step2, acid-identity pair have been omitted since the program did not progress past this point.

	Acid_Identity		Alcohol_Identity		TsOH_Identity	
	Total	Critical	Total	Critical	Total	Critical
step1	1558	2	1558	0	30	1
step-1	-	0	-	0	-	0
step2	Not calculable					

Table 1 - delete-use overlapping information for esterification - conflicts

8. Case Study 2 – SN1 Reaction

SN1 stands for unimolecular nucleophilic substitution. It involves the replacement of a good leaving group on a carbon atom (e.g. a chlorine atom) with a group that is less able to support negative charge (e.g. a hydroxyl, OH, group). Unimolecular refers to the fact that the rate determining step involves only one molecule, the halocarbon. The reaction occurs once the Cl^- group leaves the molecule. There is also an SN2 reaction which is bimolecular in its rate determining step. The substituting group and the halocarbon must collide for reaction to occur. A transition state where the new group and leaving group are both partially bonded to the central carbon atom occurs, before the leaving group leaves. There is one less step in the SN2 reaction, so SN1 was chosen to make the example more testing of the methodology.

This reaction is very simple and its selection was prompted by the memory problems that occurred in the esterification example. The reactant molecules are quite small, so the number of possible overlappings for each reaction rule during critical pair analysis is reduced. Despite this, the analysis still took up to 80 minutes on some occasions for the instantiated grammar using 2.6GB of heap memory on a Linux machine.

The general grammar can be found in the graphs folder of the software CD as "SN1_general.ggx". The instantiated grammar has filename "SN1_instantiated.ggx".

Step 1

The type graph for this reaction is given below:

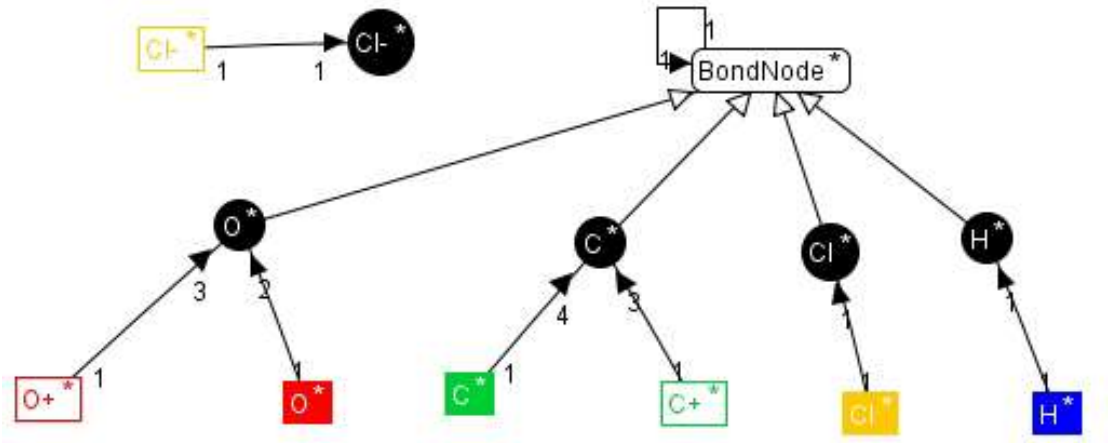


Figure 45 - SN1 type graph

The individual connections between different types of bond nodes present in the esterification type graph are replaced with an inheritance relationship. To ensure every bond node is only connected to one other bond node, the supertype BondNode has a 1 to 1 relation to itself. It is now much easier to solve the problem described in "Molecular Representation Using Graphs" where numerous constraints were needed to restrict multiple bond node connections. These numerous constraints can be replaced by just one atomic constraint which must always be false:

Atomic proposition: Atomic conclusion:

(empty)

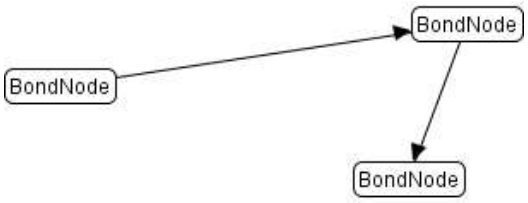


Figure 46 - constraint limiting number of bonds allowed

However, as the connections between each type of bond node are no longer explicit, this type graph has lost bond direction conventions. For example, there is no indication that if a C bond node is connected to an O bond node, the edge should go from the C to the O. To solve this, additional bond direction atomic constraints are added to the grammar. For the C-O example, we have the following atomic constraint, which is developed into a graph constraint where this atomic constraint is designated as always being false:

Atomic proposition: Atomic conclusion:

(empty)

Figure 47 - constraint designating direction of edges in bonds

In this type graph, O^+ and C^+ no longer have their own bonding nodes. This was an experiment to determine whether limiting the types of nodes made the grammar any more efficient. Unfortunately, the results were inconclusive, as each time the grammar was run, the time taken fluctuated greatly without any pattern. Nevertheless, the simpler type graph was kept. This does however necessitate two further constraints that prevent both a C and C^+ connecting to a single C bond node (and similarly for O and O^+). The atomic constraint is given below, and again this is developed into a graph constraint where it must always be false:

Atomic proposition: Atomic conclusion:

(empty)

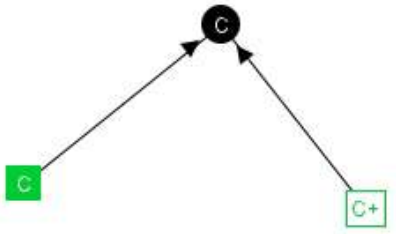


Figure 48 - constraint limiting C and C^+ connection to same bond node

The starting materials are defined as follows:

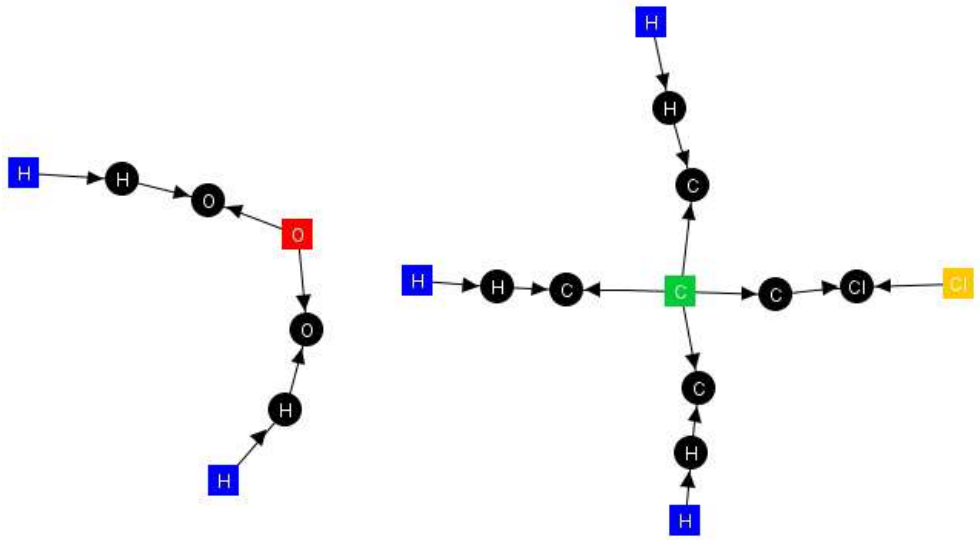


Figure 49 - SN1 starting materials

The molecule on the left is water. The OH group of this is what will attack the positively charged central carbon atom of the molecule on the right, chloromethane (or methyl chloride).

There are three steps in the reaction which we can assume are reversible. Whether these reactions are actually viable at all are determined by the rate constant that will eventually be assigned to their instantiated forms. As it turns out, these reactions are not very reversible at all particularly for the size of the hydrocarbon molecule we are considering (only one carbon). For the time being, however, they are included as possible reactions. The reverse of the reactions below are not given explicitly, but can be viewed in the grammar file on the software CD. The reverse rule just switches the LHS and RHS of the forward rule.

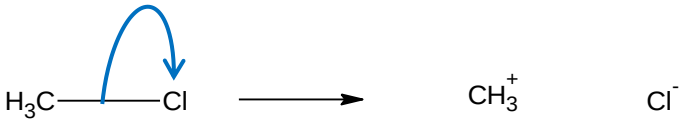


Figure 50 - step 1 of SN1 reaction

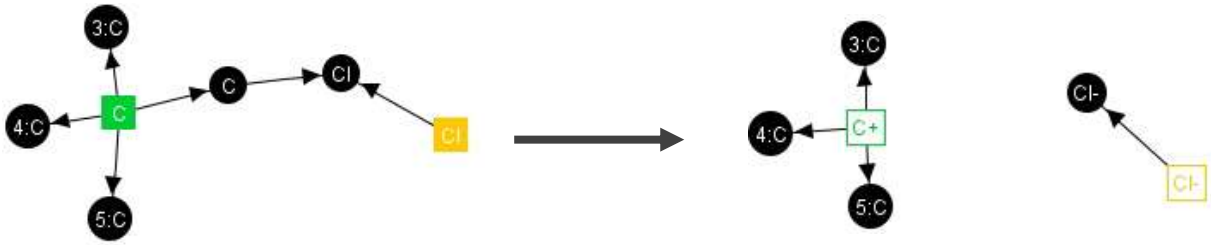


Figure 51 - step 1 of SN1 reaction, GT rule

This is the initial step of the reaction where the chlorine leaves the hydrocarbon. The central carbon is left positively charged and therefore unstable. An atom with lone pairs of electrons (electrons not involved in bonding and in the outer electronic orbital of the atom) can attack this carbon centre to form a new bond. This can be chlorine (i.e. the reverse of the reaction is possible) or the oxygen of a water molecule as in figure 52. Note that the C bond nodes must

be included in the definition of the general rule in figure 51. This is because the central C atom changes to a C^+ atom. This is a deletion of the C followed by creation of the C^+. During the deletion, if the C bond nodes are not explicitly shown in the rule, a violation of the dangling rule occurs (see "Molecular Representation Using Graphs").

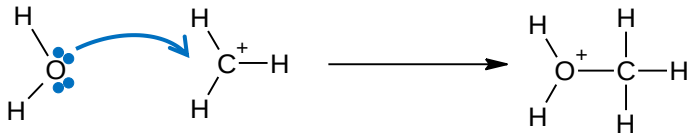


Figure 52 - step 2 of SN1 reaction

Figure 53 - step 2 of SN1 reaction, GT rule

The final step of the reaction is deprotonation of the positively charged O to leave the alcohol (methanol in this case). The Cl^- generated from step 1 co-ordinates with the H^+ that is released, to give hydrochloric acid (HCl).

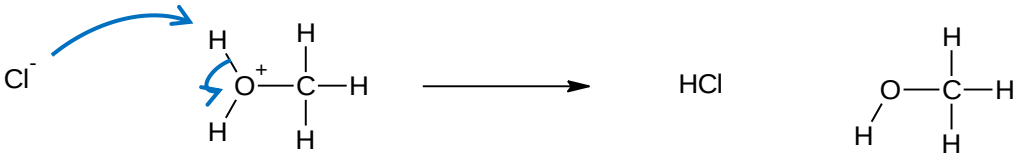


Figure 54 - step 3 of SN1 reaction

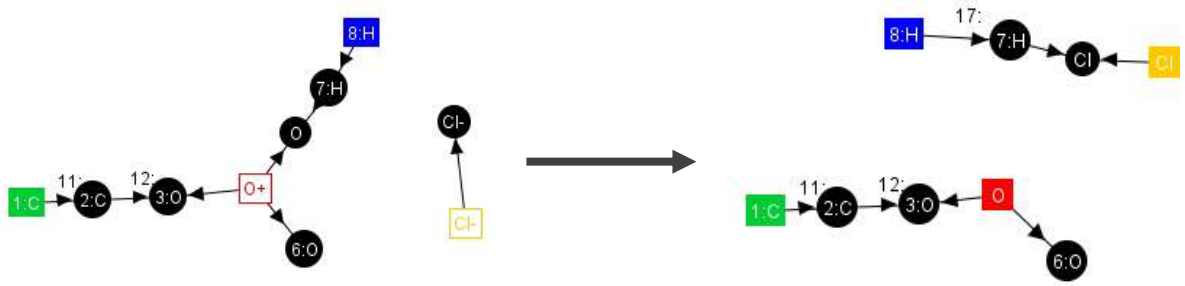
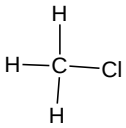
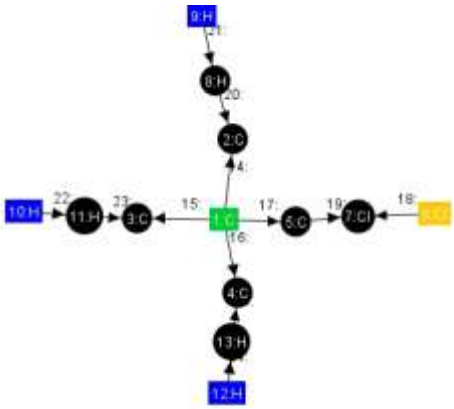
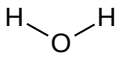
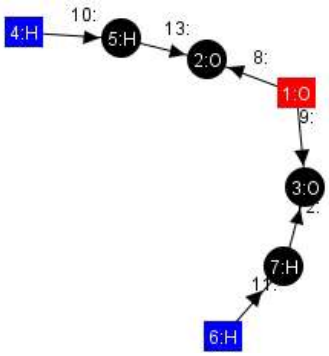
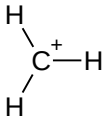
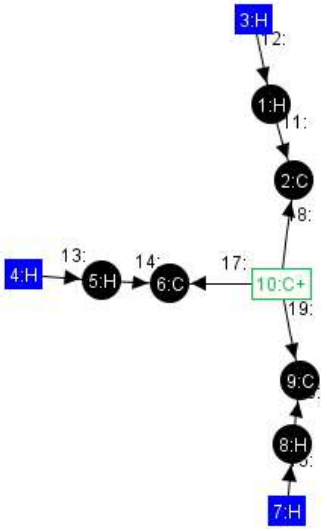


Figure 55 - step 3 of SN1 reaction, GT rule

Step 2

Applying the rules to the start graph non-deterministically yielded the following preliminary intermediates and products. Each one was added as a molecular identity rule. The molecules in the start graph (CH_3Cl and H_2O in figure 49) were also added as identity rules.

Chemical Formula	Structural Formula	Graph Representation
CH_3Cl		
H_2O		
CH_3^+		

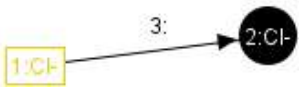
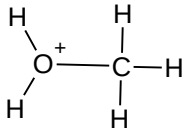
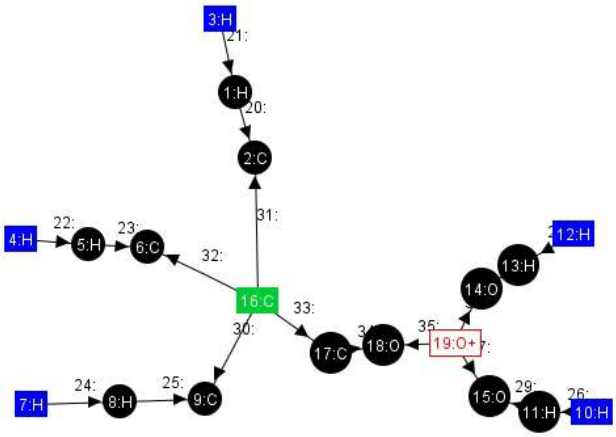
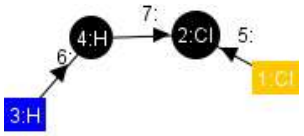
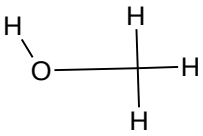
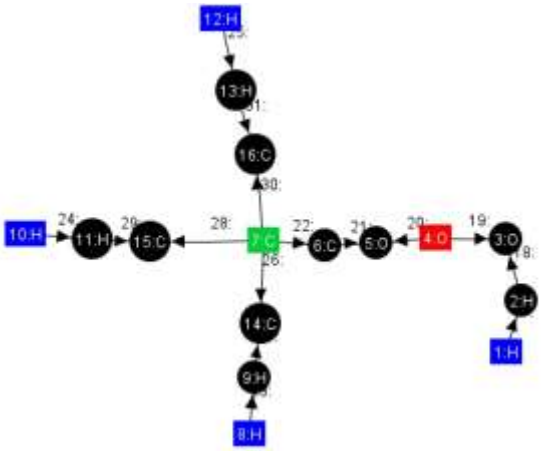
Cl-	Cl^-	
$CH_3O^+H_2$		
HCl	Cl—H	
CH_3OH		

Table 2 - preliminary intermediates in SN1 reaction

Step 3

The AGG screenshot below shows the exact results obtained from the first pass critical pair analysis.

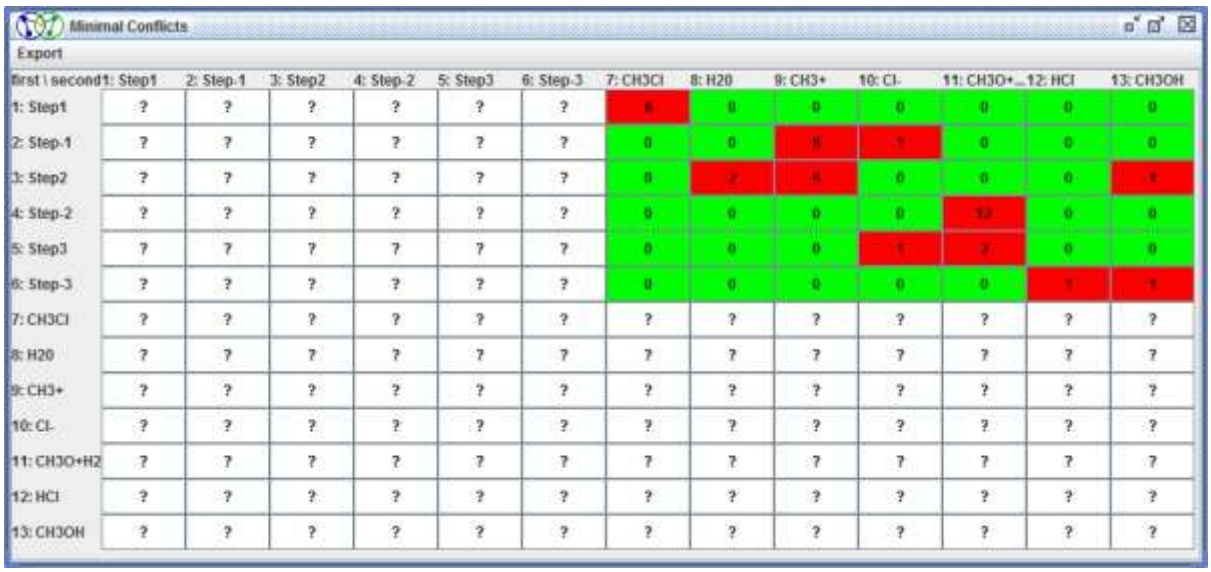


Table 3 - summary of conflict overlappings from critical pair analysis (first pass)

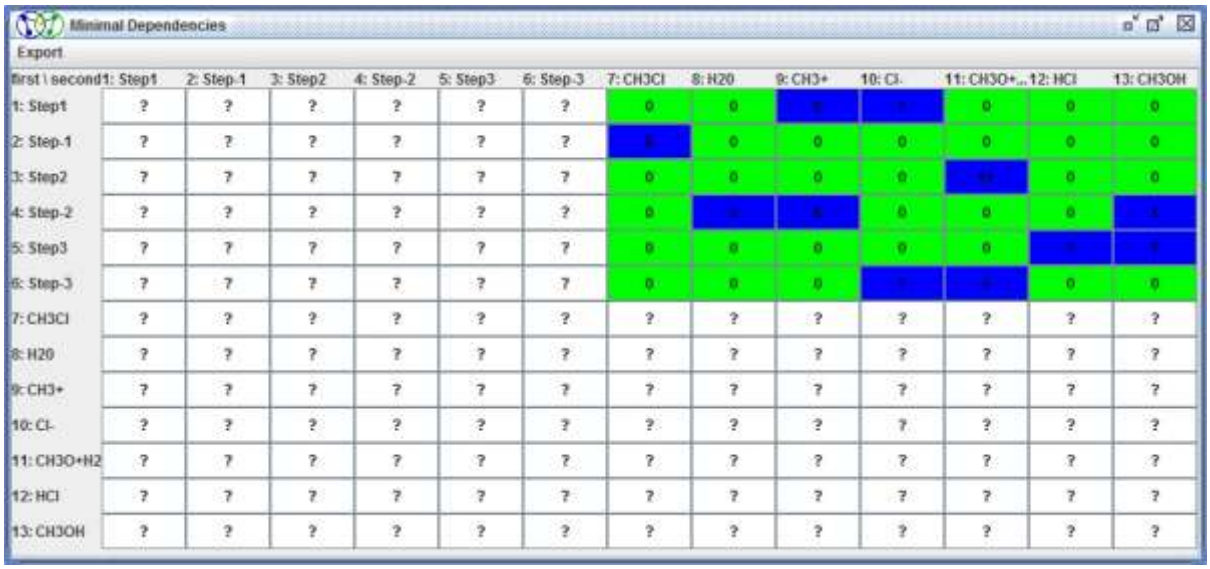


Table 4 - summary of dependency overlappings from critical pair analysis (first pass)

To give an indication of the scalability of the critical pair analysis, the following tables show how many total overlappings and eventual critical overlappings occurred for each rule pair at this stage (as shown for the esterification example).

As can be seen from this, a total of over 32,500 inclusions need to be checked for certain rule pairs. These correspond to the combination of the reaction rules consisting of most nodes in the LHS graph, and identity rules describing the largest molecules, namely step2 and CH3O+H2. Adding even one more node to the LHS of the rule takes the no. of overlappings to check to over 100,000. This is a serious limitation of the AGG tool to handle more complex molecular structures and reactions, particularly if a reaction rule requires slightly more local context than that represented in the reaction rules of this case study.

	CH3Cl		H20		CH3+		Cl-		CH3O+H2		CH3OH		HCl	
	Total	Critical	Total	Critical	Total	Critical	Total	Critical	Total	Critical	Total	Critical	Total	Critical
step1	663	6	-	0	8	0	-	0	89	0	89	0	4	0
step-1	-	0	-	0	27	6	4	1	-	0	-	0	-	0
step2	-	0	63	2	135	6	-	0	-	0	-	0	504	1
step-2	445	0	26	0	40	0	-	0	32593	12	6448	0	-	0
step3	-	0	32	0	-	0	4	1	1531	2	208	0	-	0
step-3	100	0	45	0	-	0	-	0	-	0	315	1	29	1

Table 5 - delete-use overlapping information for SN1 - conflicts

	CH3Cl		H20		CH3+		Cl-		CH3O+H2		CH3OH		HCl	
	Total	Critical	Total	Critical	Total	Critical	Total	Critical	Total	Critical	Total	Critical	Total	Critical
step1	-	0	-	0	27	6	4	1	-	0	-	0	-	0
step-1	663	6	-	0	8	0	-	0	89	0	89	0	4	0
step2	445	0	26	0	40	0	-	0	32593	12	6448	0	-	0
step-2	-	0	63	2	135	6	-	0	-	0	504	1	-	0
step3	100	0	-	0	-	0	-	0	-	0	315	1	29	1
step-3	-	0	32	0	-	0	4	1	1531	2	208	0	-	0

Table 6 - delete-use overlapping information for SN1 - dependencies

Step 4

Step 4 (structural equivalence testing of results of step 3) was bypassed for this case study as the overlappings were quite easy to study without doing this step. For more complex mechanisms though with larger molecules (and hence more critical overlappings with the same structure) or more rules, this step would make manual analysis in step 5 far easier.

Step 5

Examination of the results from step 3 yields a necessary instantiation of the general rule, step2. Step2 consumes H_2O and CH_3^+ (deduced from the conflicts table), but also seems to consume CH_3OH. This suggests that the following reaction is also possible:

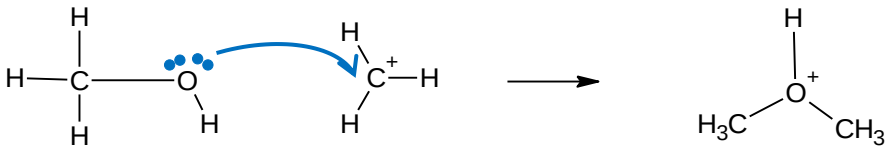


Figure 56 - reaction of CH_3OH with CH_3^+

In other words, the alcohol product, as well as water, can react with the carbocation. In fact, this may even be a stronger reaction, as the CH_3 group attached to the oxygen is very slightly electron donating, meaning the push of the lone pair to the carbocation is made easier. This intermediate would have been discovered at stage 2 of the methodology had the product alcohol molecule been added to the start graph as a possible reactant.

The general rule, step2, therefore needs to be instantiated twice; once for the reaction with water and once for the reaction with methanol. These new rules are shown in figures 57 and 58.

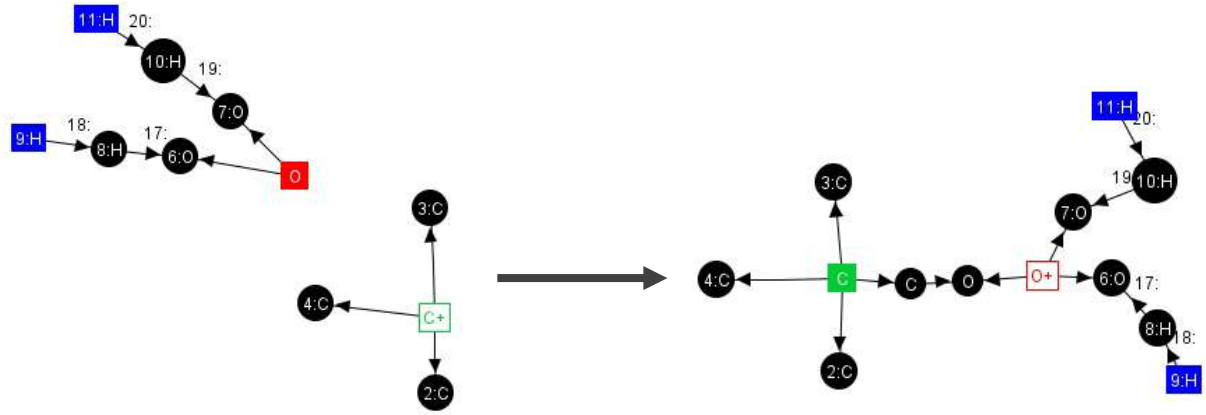


Figure 57 - instantiation of step2 for reaction with water

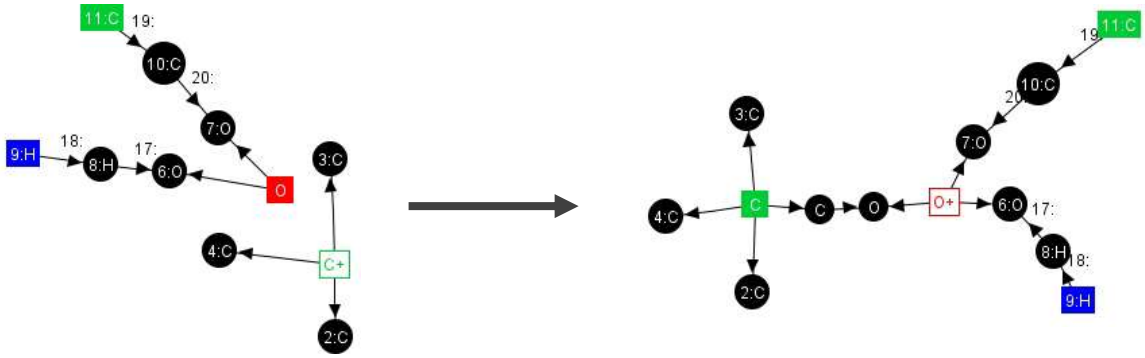


Figure 58 - instantiation of step2 for reaction with methanol

Notice that it is sufficient to replace one of the hydrogen atoms in the attacking molecule in figure 57 with a single carbon. This introduces enough local context to make this rule apply only to the product methanol, and not water. This is preferable to adding the entire molecule as this increases the time taken for critical pair analysis, because more nodes equates to more overlappings to check through. The reverse rules were also instantiated here.

At this stage, the new intermediates and instantiated rules were added to the grammar. With the general rules still in place (in case they are applicable to the new intermediates) a second critical pair analysis pass was tried. This led to OutOfMemory problems however. It seemed that the new large molecule created by the reaction in figure 56 passed the threshold for allowed analysis. As this was quite late in the project, a decision was made to ignore the possibility that this reaction occurs, disabling the instantiated rule that represents it, and any molecular identity rules of intermediates that arise from it. The verification of overall results and extracted ODE's would take this omission into account.

After doing this, the critical pair analysis was reattempted. Once again an OutOfMemory error occurred which prevented the analysis from completing. This was now due to the instantiated rule set up for water (figure 57). It seemed that even two nodes and one edge more than the number present in the general rule is enough to prevent the analysis engine from completing successfully. Another strategy was needed that would use the general rule as the instantiated rule for water then, but disallow the reaction with methanol. A negative application condition (NAC) was employed to achieve this. NAC's are preconditions to rules. They constitute a graph which if it occurs in the match for the LHS of the rule, prevents the application of the rule for that match. The following NAC was added to the general rule for step 2 (rule shown in figure 53).

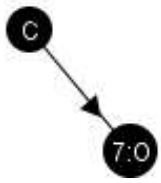


Figure 59 - NAC for step2 general rule to create instantiated rule

The NAC specifies that a carbon bond node must not be connected to the oxygen bond node with mapping identity 7, therefore disallowing the reaction of methanol with the carbocation. Water is still able to react.

This simpler grammar that ignores the full reactivity of the system can be found on the software CD in the graphs folder, with name "SN1_instantiated_simpler.ggx". This grammar was used for all subsequent steps in the methodology.

Step 6

Once the rules are instantiated and no further intermediates are found the general rules are usually disabled. In our case, the instantiated rules which allowed the reaction with methanol had to be disabled. In the end, the original general rules were used (one with an added NAC). All reaction rules were renamed as rate constants. The "step" prefix in the name of every general rule was replaced with a "k". Hence, step1 became k1 and step-1 became k-1 etc.

Step 7

The critical pair analysis was run once again, yielding the following results:

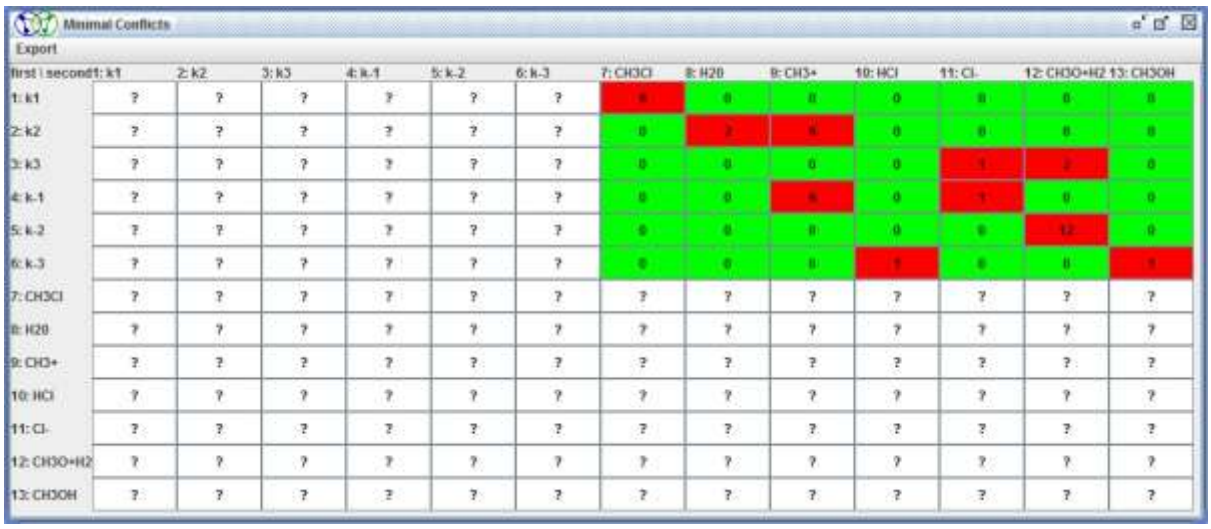


Table 7 - summary of conflict overlappings from critical pair analysis (final pass)

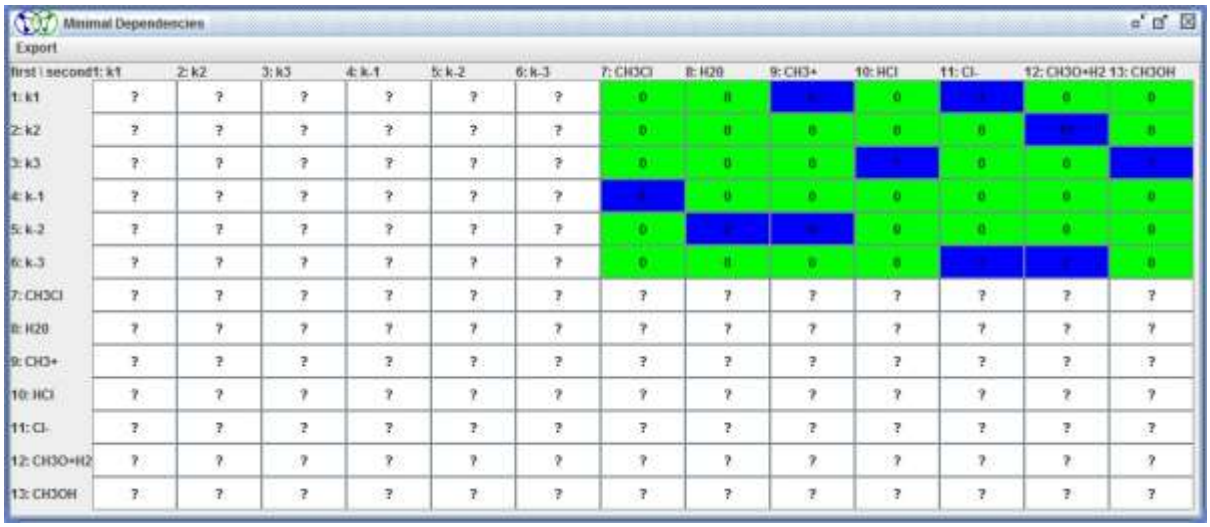


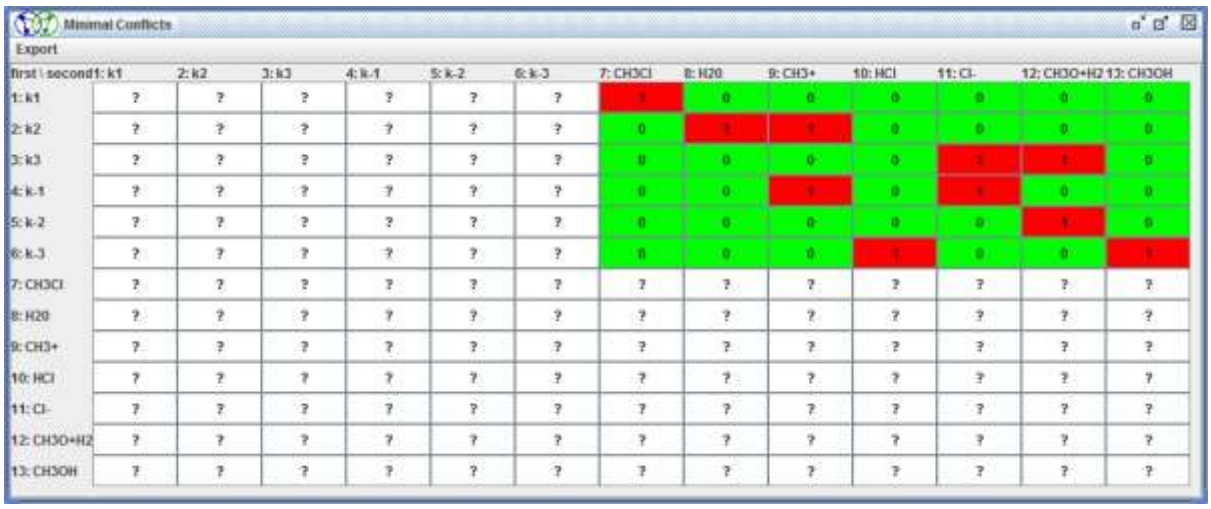
Table 8 - summary of dependency overlappings from critical pair analysis (final pass)

As can be seen from table 4, the reaction of step2 (now k2) no longer consumes the methanol product (CH3OH). These results are found in the file "SN1_instantiated_simpler_out.cpx" in the results folder on the software CD.

Step 8

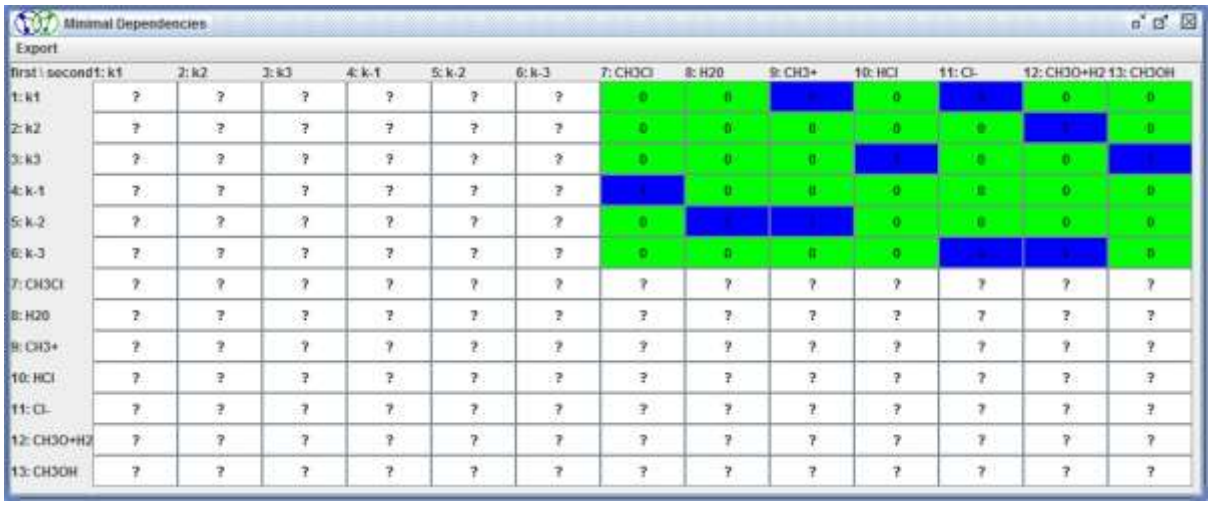
Running the structural equivalence analysis reduced the number of critical overlappings in tables 4 and 5 to those in tables 6 and 7. All of the entries were reduced to 1, indicating that all of the original overlappings were the same. This was verified to be the case by manually examining the overlapping information produced in step 7.

These results are found in the file "SN1_instantiated_simpler_out_structuremod.cpx" in the results folder on the software CD.



first \ second	1: k1	2: k2	3: k3	4: k-1	5: k-2	6: k-3	7: CH3Cl	8: H20	9: CH3+	10: HCl	11: Cl-	12: CH3O+H2	13: CH3OH
1: k1	?	?	?	?	?	?	1	0	0	0	0	0	0
2: k2	?	?	?	?	?	?	0	1	1	0	0	0	0
3: k3	?	?	?	?	?	?	0	0	0	0	1	1	0
4: k-1	?	?	?	?	?	?	0	0	1	0	1	0	0
5: k-2	?	?	?	?	?	?	0	0	0	0	0	1	0
6: k-3	?	?	?	?	?	?	0	0	0	1	0	0	1
7: CH3Cl	?	?	?	?	?	?	?	?	?	?	?	?	?
8: H20	?	?	?	?	?	?	?	?	?	?	?	?	?
9: CH3+	?	?	?	?	?	?	?	?	?	?	?	?	?
10: HCl	?	?	?	?	?	?	?	?	?	?	?	?	?
11: Cl-	?	?	?	?	?	?	?	?	?	?	?	?	?
12: CH3O+H2	?	?	?	?	?	?	?	?	?	?	?	?	?
13: CH3OH	?	?	?	?	?	?	?	?	?	?	?	?	?

Table 9 - summary of conflict overlappings after structural equivalence analysis



first \ second	1: k1	2: k2	3: k3	4: k-1	5: k-2	6: k-3	7: CH3Cl	8: H20	9: CH3+	10: HCl	11: Cl-	12: CH3O+H2	13: CH3OH
1: k1	?	?	?	?	?	?	0	0	1	0	1	0	0
2: k2	?	?	?	?	?	?	0	0	0	0	0	1	0
3: k3	?	?	?	?	?	?	0	0	0	1	0	0	1
4: k-1	?	?	?	?	?	?	1	0	0	0	0	0	0
5: k-2	?	?	?	?	?	?	0	1	1	0	0	0	0
6: k-3	?	?	?	?	?	?	0	0	0	0	1	1	0
7: CH3Cl	?	?	?	?	?	?	?	?	?	?	?	?	?
8: H20	?	?	?	?	?	?	?	?	?	?	?	?	?
9: CH3+	?	?	?	?	?	?	?	?	?	?	?	?	?
10: HCl	?	?	?	?	?	?	?	?	?	?	?	?	?
11: Cl-	?	?	?	?	?	?	?	?	?	?	?	?	?
12: CH3O+H2	?	?	?	?	?	?	?	?	?	?	?	?	?
13: CH3OH	?	?	?	?	?	?	?	?	?	?	?	?	?

Table 10 - - summary of dependency overlappings after structural equivalence analysis

Step 9

Finally, running the ODE extraction program yields the following ODE's:

```
d[CH3+]/dt =  -k-1[CH3+][Cl-] +k-2[CH3O+H2] +k1[CH3Cl] -k2[CH3+][H20]

d[CH3Cl]/dt =  +k-1[CH3+][Cl-] -k1[CH3Cl]

d[CH3O+H2]/dt =  -k-2[CH3O+H2] +k-3[CH3OH][HCl] +k2[CH3+][H20] -k3[CH3O+H2][Cl-]

d[CH3OH]/dt =  -k-3[CH3OH][HCl] +k3[CH3O+H2][Cl-]

d[Cl-]/dt =  -k-1[CH3+][Cl-] +k-3[CH3OH][HCl] +k1[CH3Cl] -k3[CH3O+H2][Cl-]

d[H20]/dt =  +k-2[CH3O+H2] -k2[CH3+][H20]

d[HCl]/dt =  -k-3[CH3OH][HCl] +k3[CH3O+H2][Cl-]
```

Brief Analysis of results

The ODE's derived in step 9 do in fact agree with the ODE's derived by hand for the SN1 reaction, taking into account the omission of certain reactivity at steps 5 and 6. This is a positive result that shows the methodology (and its implementation) are capable of deriving ODE's for simple reactions. While the wider applicability of this methodology is limited (as discussed earlier in "Methodology"), this is a substantial first step in developing a more universally applicable method.

The SN1 reaction in reality is a very simple one and is often quoted as having a rate of:

$$\frac{d[CH_3Cl]}{dt} = -k[CH_3Cl]$$

This is because the reaction k_1 in our grammar, the departure of the Cl^- group from CH_3Cl, is considered to be the rate determining step i.e. the only step of any significance to the rate. Subsequent reactions occur very quickly once this step occurs. The equation above can actually be derived from our ODE's from step 9. If all other elementary reactions do not occur at all or are assumed to be very rare (particularly the reverse ones) k_1 is the only rate constant that doesn't have negligible value. For reactions that occur extremely quickly once the rate determining step does, intermediates are immediately consumed so their concentration throughout the reaction can be approximated to zero. If these approximations are taken into account, the differential reaction for the rate of change of concentration of CH_3Cl approximates to the equation given above. Once concrete rate constants are found, any arbitrary system of reactions will be reduced down in this way so that only significant reactions remain. Our methodology ensures all possible reactivity is taken into account before this simplification occurs.

9. Planning and Timescales

Tasks

The following is a preliminary account of the expected tasks of the project, as presented in the Project Plan document. As predicted, these underwent significant changes as the project progressed, new understanding came to light and new problems or challenges occurred. Due to the open nature of the project, it would have been unwise to continually update and modify the plan. This would have been time-consuming. It was discovered that any attempt to adhere to a concrete plan limited further exploration in a particular area of interest and failed to incorporate significant changes to the direction of the project. Therefore, the plan is presented here unaltered, and a discussion of the deviations that occurred follow it.

1. *Further background research*

    1.1 Stochastic graph transformation theory

        This is fundamental to the project and therefore must be understood clearly. In addition to what has already been read, other sources will be investigated. Papers [10] and [11] will be thoroughly revised to ensure complete familiarity with the process of creating stochastic graph transformation systems.

    1.2 Example stochastic graph transformation system

        To ensure understanding, a simple example will be constructed in AGG, with supporting tools for stochastic simulation. The major challenge here may be tool support, as PRISM and GROOVE have not been encountered yet. However, this stage will also allow us to gain familiarity with these tools and AGG.

    1.3 Research into CCS and π-calculus

        In order to understand and learn from the methodology in [2], a quick study of [15] will be undertaken. Some lecture notes from the department's course on Communicating and Concurrent processes will also be reviewed. [15] covers an extensive and technically involved subject. The challenge here will be not to dwell on an area of computer science that won't be used extensively in the final project. It will be important to restrict the time given to this task.

2. *Developing a Methodology*

    2.2 Deriving ordinary differential equations

        This is the most important and possibly time-consuming part of the project, and has been scheduled accordingly. This part will involve formulating the methodology behind defining a stochastic graph

transformation from reactant molecules and how to assign rates to transitions between graphs. This part of the project cannot be broken down any further at this stage because of its theoretical nature – the particular details of how the derivation will occur are not known at this time. This stage will draw from all of the research conducted in part 1 of the project, as well as additional chemistry research needed at this stage, such as kinetics, thermodynamics, reaction mechanisms, and molecular orbital theories. As already mentioned this is a highly theoretical and challenging part of the project. There is substantial risk here if a suitably accurate methodology cannot be formed. In this case, an iterative process of simpler models (incorporating less and less real chemistry) will be adopted in order to get some kind of methodology. Additionally, the level of automation in the process of assigning rates can be lowered if necessary. If finding the rates by checking the change in the molecular orbital makeup of a molecule presents itself as too difficult and time-consuming, the rate constant assignment can be reverted to a manual process. Simplifying our case study subjects may also be an option if the eventual methodology is deemed unsuitable for the existing ones. Technical help will be requested from members of the Chemistry department and from Prof. Heckel and Dr. Fer-Jan de Vries if necessary.

3. Tool Investigation

3.1 Tool investigation

AGG cannot be used for stochastic simulation despite being extremely good for testing the application of transformation rules. At this stage other tools such as PRISM, GROOVE and FERN should be investigated to see how they can aid the project. Although these tools have not been used before, [6], [10] and [11] outline their use in stochastic simulation. Prof. Heckel has used [10] and [11] before and may be able to help at this stage in case of difficulties.

3.2 Tool development

This step may not be necessary if existing tools are readily available. However, if work needs to be done to develop a stochastic simulation tool or integration with AGG, some time will be reserved for this. AGG is implemented in Java and is supplied with an API for just such an integration task. FERN may prove to be an ideal candidate for the stochastic part of the overall tool chain, as it is also developed in Java and designed to be easily integrated.

4 Case Study 1- esterification

4.1 Background research

The reaction mechanism will be investigated using standard chemistry textbooks, journals and websites. Peculiarities of the reaction will be

noted. Empirical data on the reaction rate and form of the rate law will be gathered using textbooks, journal and online databases. The challenge here may be access to this empirical data. If empirical data is lacking, the Chemistry department will be contacted for help, and possibly to set up an actual experiment.

4.2 Rule generation

The reaction mechanism found in 4.1 will be implemented in AGG as a start graph and transformation rules. This step should not prove challenging.

4.3 Rate calculations

Using the methodology developed in part 2, probabilities of graph transformations will be applied to the mechanism input in 4.2. Again, once the methodology is in place, this step should be fairly simple.

4.4 Simulation

Using the tool decided upon in part 3, the complete case study will be implemented and the stochastic simulation will be run to gain the ordinary differential equation for the reaction. The challenges for this section are limited to the challenges in part 3, unless there are some particular peculiarities in the reaction mechanism which are not implementable. We do not foresee this happening.

5 Case Study 2 – condensation and hydration of glucose

The subtasks will follow the same format as part 4. More time will be dedicated to part 5 however, as the reaction mechanism is more extensive (making 5.1 and 5.2 more time-consuming). The polymerisation aspect of the case study also makes the implementation of automatic rule calculation (5.3) a little more complex. These two factors combined may make 5.4 a lengthier process.

6 Review

6.1 Verification of results

The differential equations from the empirical data gained in 4.1 and 5.1 will be compared to the derived differential equations gained in 4.4 and 5.4 respectively.

6.2 Refinements and amendments

This will be a necessary step in critically evaluating the methodology designed in the project. Depending on the results of 6.1 and the availability of time, the methodology may be changed to incorporate improvements outlined in this part of the project. The project may then

undergo an iterative process whereby part 5 and 6 are repeated until there is not enough time, or satisfactory results are obtained.

7 Final Deliverables

7.1 Final deliverables

The final part of the project will include the write-up of the final report, as well as preparation for the viva. Due to the theoretical nature of the project the final report document may be quite large and there may not be sufficient time at the end of the project to complete it. Therefore, preparation of this should occur incrementally throughout the project, with the time allocated at the end for refinement and review. Some tools/plugins which allow the easy input of stoichiometric equations, chemical structural formulae and graphs should be found early on in the project, as without them the preparation of this document could be slower than necessary.

Challenges and Risks

As outlined above, the two main challenges we can envision with the project are the theoretical nature of the project and adequate tools to implement the methodology. Measures to reduce the effects of risk from these two areas have been outlined in the "Tasks" section above.

If however, success in deriving a methodology is not forthcoming by the end of January 2009, the goals of the project may be reassessed. A meeting with Dr. Fer-Jan de Vries will be scheduled to monitor progress and the likelihood of success.

Another challenge is the scope of the project. It may appear that the project is not viable due to the amount of work needed and time constraints, particularly for a 30 credit module. This term in particular is restrictive in that only 10 credits have been assigned to the project, with 55 other credits for 3 other modules (including a management module that requires extensive reading and an involved piece of coursework). As such, fruitful results this term may be limited. However, time will be reserved over the holidays for project work and a weekly meeting will be scheduled with available supervisors to keep the input into the project flowing. There is more time next term with only one computer science and one management module other than the project. In anticipation of time needed for the project, some of the background reading for the management module was conducted over the summer. Time for the project over the summer was limited due to a 3 month fulltime industrial placement. Some time during the winter vacation will be lost due to Christmas & New Year, January examinations and a 3-day computer science conference.

Bugs within AGG also need to be considered. [19] will be reviewed regularly and bug fixes will be downloaded frequently to avoid any serious risk to the implementation. In case a particular bug does affect the project, the AGG team can be emailed directly with suggestions. The source code can also be scrutinised ourselves to attempt a bug fix. In the event that AGG becomes unusable for our needs, other tools such as PRISM will be kept in reserve. Other tools should also be searched between now and the implementation stage.

Deliverables

The deliverables for the project are outlined below. These are marked on the Gantt chart which follows, but time is not specifically allocated to their preparation. This must be undertaken in a timely and responsible manner, and incorporated into the overall structure of the project naturally.

- Weekly SVN uploads:
  - 30th November
  - 14th December
  - 1st February
  - 22nd February
  - 15th March
  - 3rd May

- Project plan presentation
  - 1st – 5th December

- 1st Interview
  - 2nd – 6th February

- 2nd Interview
  - 17th March

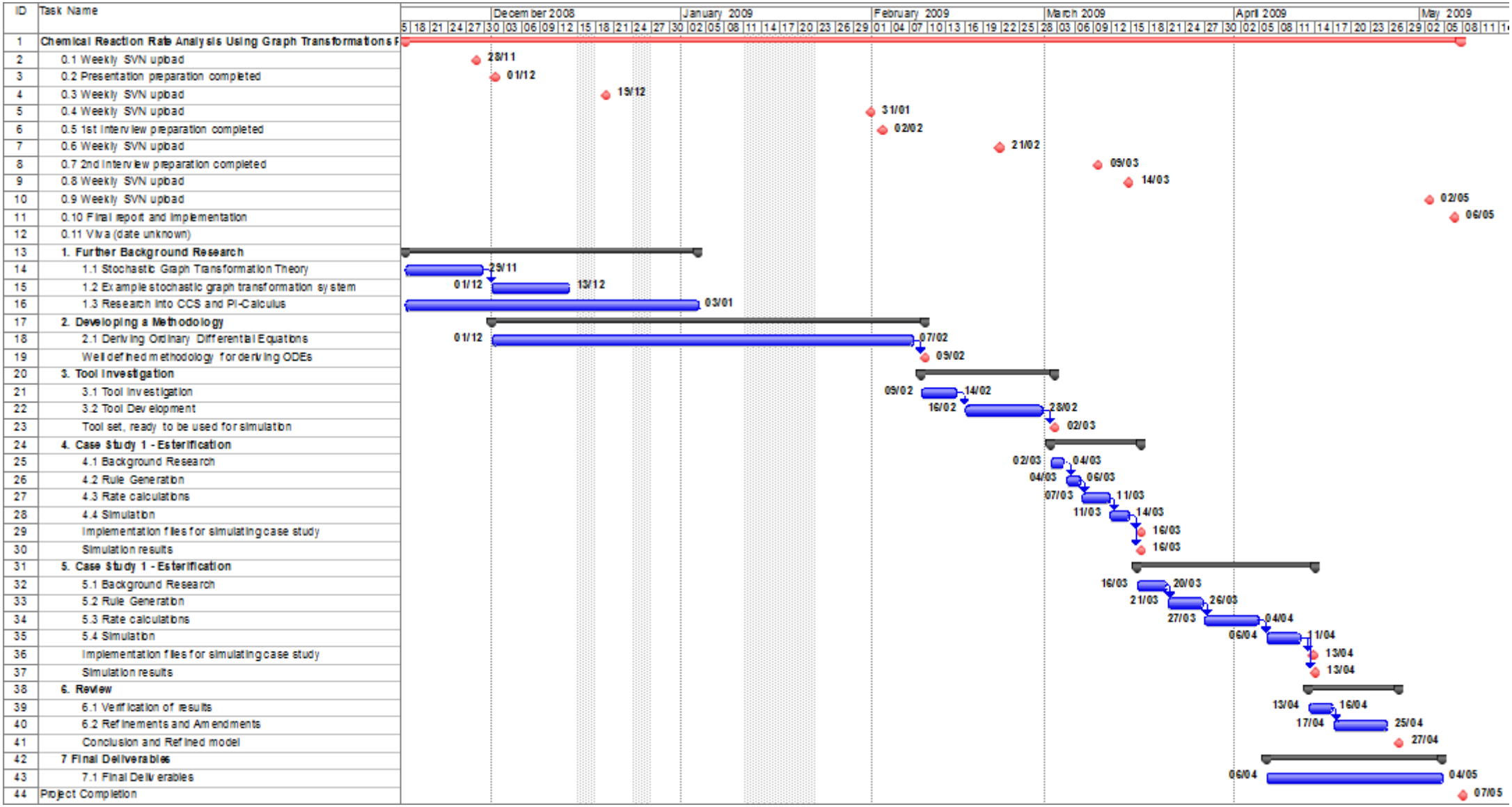
- Final report and Implementation
  - 14th May

- Viva
  - Date Unknown

Gantt Chart

The approximate allocation of time for each task is given in the following Gantt chart. Again, these timings are approximate and are subject to changes as the project progresses.

Dates for assessed deliverables are given at the top of the chart as milestones. Other intermediate milestones are interspersed with the tasks described above. Weekly meetings with supervisors and the weekly discussion group session with PhD students have been omitted for the sake of simplicity.


ID
Task Name
December 2008
January 2009
February 2009
March 2009
April 2009
May 2009
1 Chemical Reaction Rate Analysis Using Graph Transformations P
2 0.1 Weekly SVN upload
3 0.2 Presentation preparation completed
4 0.3 Weekly SVN upload
5 0.4 Weekly SVN upload
6 0.5 1st Interview preparation completed
7 0.6 Weekly SVN upload
8 0.7 2nd Interview preparation completed
9 0.8 Weekly SVN upload
10 0.9 Weekly SVN upload
11 0.10 Final report and implementation
12 0.11 Viva (date unknown)
13 1. Further Background Research
14 1.1 Stochastic Graph Transformation Theory
15 1.2 Example stochastic graph transformation system
16 1.3 Research into CCS and PI-Calculus
17 2. Developing a Methodology
18 2.1 Deriving Ordinary Differential Equations
19 Well defined methodology for deriving ODEs
20 3. Tool Investigation
21 3.1 Tool Investigation
22 3.2 Tool Development
23 Tool set, ready to be used for simulation
24 4. Case Study 1 - Esterification
25 4.1 Background Research
26 4.2 Rule Generation
27 4.3 Rate calculations
28 4.4 Simulation
29 Implementation files for simulating case study
30 Simulation results
31 5. Case Study 1 - Esterification
32 5.1 Background Research
33 5.2 Rule Generation
34 5.3 Rate calculations
35 5.4 Simulation
36 Implementation files for simulating case study
37 Simulation results
38 6. Review
39 6.1 Verification of results
40 6.2 Refinements and Amendments
41 Conclusion and Refined model
42 7 Final Deliverables
43 7.1 Final Deliverables
44 Project Completion

Appraisal of Plan

Section 1 of the plan, entitled "Further Background Research" provided a very useful background in the existing use of graph transformation theory in determining reaction rates. However, as it turned out, the stochastic simulation elements of the theory would not be as prominent in our methodology as initially thought. As much of the first semester was spent researching and understanding this, there was less time in the second term to concentrate on our eventual methodology. It would probably have been wiser to restrict the time spent on this research. Due to the open nature of the project however, the changing aims, and unfamiliar theoretical territory this was largely unavoidable and the appreciation of stochastic techniques should not be considered wasteful.

Due to exam and coursework pressure, 1.2 (the implementation of an example stochastic system) and 1.3 (background research in CCS and π-calculus) could not be completed. Not spending time on 1.2 was in fact a benefit as this would not have been useful to the project in the end. Completing 1.3 however would have been useful, but was perhaps an unrealistic goal due to the scope of the subject matter and the necessary time to understand such an involved field, particularly when it was scheduled during a time when there were other heavy academic obligations.

The time allocated to developing a methodology was also unrealistic. Again, because of the open, challenging nature of the project and the unfamiliar territory more time should have been afforded to this part. Initially several weeks over the vacation were allocated to this, but many of these were spent on Christmas holidays and examination revision. A major shortcoming of the proposed plan was the assumption that there would be any time at all over the vacation to focus on the project. Preparation for three exams made this largely untenable. As it happened, the methodology was continuously evolving as case studies were tried and tools were developed.

The first task of the tool investigation stage was research into the integration of AGG with other tools for stochastic simulation. As stochastic simulation was no longer an aim of the project, this part of the project instead focused primarily on studying and understanding AGG's supplied API. This would be vital when adapting AGG's capabilities to our specific application domain. Stages 2, 3 and 4 in fact ran concurrently and iteratively rather than linearly. This was a necessity as limitations in AGG's capabilities, or the complexity of the case study implementation highlighted defects or inefficiencies in the methodology and vice versa.

A major change to the outcome of the project was the change in case studies midway through the project. Esterification was originally planned as a simple introductory test case. However, memory limitations (discussed in detail later) rendered this case study unsolvable. It was therefore decided to try a simpler chemical reaction with fewer steps and smaller intermediate molecules, hence the introduction of the SN1 (the symbolic representation for unimolecular nucleophilic substitution) reaction. This was chosen as a simple example to test the methodology and is in fact a trivial one as discussed earlier. However, this also incurred some severe Java virtual machine memory limitations which limited the speed at which results could be processed. The glucose condensation reaction had to unfortunately be abandoned due to a lack of time. This is a complex reaction which requires a different methodology to the simple closed SN1 reaction network, and would have been interesting to study. As the whole glucose monomer is modelled as one node, the analysis would most probably have had

fewer memory problems associated with it (which we believe to be more likely as the number of nodes in the rules and graph representations of intermediate molecules increases). However, it was important to first understand and validate the methodology for the more fundamental closed system before progressing to this, so this reaction had to be sidelined.

Subsection 4 of the case study (stochastic simulation) was naturally discarded. Instead, a suite of Java programs was developed that produced the ODE's in text format.

In the end, the project involved a substantial amount of work and time was used effectively and efficiently, but is in no way complete in producing a universally applicable methodology. The methodology developed works well for the simple SN1 reaction, but has some limitations that make it potentially useless for other types of reactions without further work. This is discussed in the "Critical Appraisal" section.

10. Critical Appraisal

Summary of completed work

The following has been achieved during the 3rd year project:

- Extensive research into graph transformation theory and stochastic modelling, as well as a revision of some basic chemical kinetics. If the career plan in appendix 1 is adhered to, this will prove to be an invaluable foundation for future work. The mathematics required to understand many of the graph transformation papers and critical pair theory also helped to fill gaps in academic knowledge (e.g. set theory that is not covered in the "with Management" degree).

- A suitable molecular representation necessary to specify molecules and their reactions using graph transformations. Several variations were experimented with. Finally, a type graph, and constraint system, were decided upon, which incorporated the necessary abstraction level and remained intuitive enough for chemists to understand.

- A prototype 10-step methodology for deriving ordinary differential equations. While this is currently limited to simple, finite reactions, it is a good basis for researching infinite systems such as the glucose polymerisation reaction. Also, there are limitations in that the consumption or production of more than one of a particular type of molecule by an elementary reaction cannot be ascertained by this method, since each critical pair between a reaction rule and molecular identity rule only signifies one molecule of that type being consumed in that reaction. Slight modifications to the methodology would be necessary for more universal applicability, where the involvement of multiple molecules in a reaction can be ascertained. This could involve a convention whereby the molecular identity rule is duplicated, or additional steps in the methodology for testing the occurrence of such a reaction could be designed.

- A prototype implementation to test the methodology described above. The SN1 case study showed that our methodology can be realised using a combination of existing software (AGG and its critical pair analysis engine) and new Java classes.

- A simple case study testing both the methodology and the implementation, namely the SN1 reaction. This allowed a complete run through of the methodology and revealed limitations to the implementation, in particular, the critical pair analysis engine.

Self-assessment

While the project may not have yielded complete results, considerable progress has been made in the direction of this goal. There are a number of shortcomings of the methodology and implementation, many of which have been discussed already throughout this report. A summary of these is given here.

- The methodology is not universal, in that in can only be applied to simple finite systems where each elementary reaction only produces or consumes at most one of each molecule. While this is disappointing, it does not render the methodology useless. Rather, a modification or enhancement to the existing idea is most likely required,

rather than a complete overhaul. Solving the universal applicability problem would have exceeded the time deadline for the project.

- The implementation can only be tested for very simple systems, and even then some elementary reactions had to be disabled. This is a problem with AGG's memory intensive critical pair analysis engine. It might be avoided by altering our type graph, but currently it has not been discovered how to achieve this. In order to reduce the number of possible overlappings for each rule pair, the idea of linking and therefore fixing the nodes peripheral to a central carbon atom was tested, but this did not solve the problem. Perhaps more research should have been done into alternatives to AGG.

- The problems with the critical pair analysis rendered both of our initially planned case studies untenable. This was disappointing as the glucose condensation and hydration example in particular would have been interesting to study. Also, esterification, which has a fairly complex reaction network, was replaced with the SN1 reaction which in reality is not very complex. Complexity was artificially introduced to make the example more interesting. Finding rate constant data for all of the unviable elementary reactions in SN1 would therefore be quite difficult, so this may have been a poor choice of simple reaction.

- Integration with an algebra solver (step 10 of the methodology) was not completed, partly due to a lack of importance to the core of the project, and partly because the algebra solver was not really needed for the SN1 reaction, since the generally quoted rate law for this reaction is extremely simple. However, for completion's sake, this integration should have been scheduled in. A suitable piece of freeware could have been found time-allowing and this would have helped possible future iterations of the project.

- The current GUI to the system (non AGG stages) is a simple command line one. While not of central importance, a more aesthetically pleasing GUI developed in Java would have been more satisfying. The possibility of integrating the AGG critical pair analysis GUI (for checking the critical overlappings) and our programs into an overall program is also appealing, allowing the user to complete the kinetic analysis through one window. The possibility of human interaction in such a GUI to manually alter entries in the stoichiometric matrix is a preliminary suggestion to solve the universal applicability problem (although not an ideal one).

Most of these shortcomings came at the implementation phase of the project. While the implementation part of the project seems quite simple with only a few classes written entirely from scratch, navigation of the AGG API was very time-consuming. Therefore the implementation was far from easy. Furthermore, due to the theoretical nature of the project, the focus (particularly at early stages of the project last term) was on the methodology rather than the software. As the theory required the majority of the time available, the opportunity to deal with all of the shortcomings in the implementation was sparse.

In terms of organisation and time management, there were weekly PhD discussion groups, weekly one hour meetings with Prof. Heckel (on occasion twice a week), and occasional meetings with the project supervisor. In retrospect, perhaps more meetings with the project supervisor should have been scheduled. However, with two or three other hours of meetings a week, this would have put pressure on time to do work. More work should have been

completed in the first term, in particular research into critical pairs and their use in the project. The realisation that stochastic simulation was not needed to derive ODE's came perhaps a little late. Understanding stochasticity was useful, but a greater focus on critical pairs and AGG may have left more time to solve some of the shortcomings described above.

Overall, however, it is felt that a sufficient amount of work was done. The disappointment in the implementation is tempered by the fact that a working case study was developed. There were many learning outcomes from the project too. An introduction to graph transformation theory, certain categories of mathematics, stochasticity and a revision of chemistry were all important by-products of the project. Finally, a deeper understanding of the research process and how to progress with open projects was one of the main outcomes, specifically learning to limit goals, look for alternatives, private research, use of contacts and presentation skills.

Suggestions for further work

1. Solving the shortcomings described above in "Self-assessment", both in terms of methodology and implementation, would be the first stage of a further iteration of the project.

2. The development of a GUI that is attractive and easy to use for chemists so that the methodology derived here has some actual usability. Integration of all steps of the methodology, and finally with a library of rate constant data, would make this kinetic analysis suite a useful tool in the lab for understanding reaction mechanisms.

3. Automation in the derivation of the molecular identity rules. Currently, rules must be applied or several rounds of critical pair analysis must be performed, followed by manual addition of discovered intermediates as identity rules. With an extra step in the methodology, and use of API methods (to apply rules), automation of this step should be possible. This would be useful for larger, or open-ended systems where the no. of intermediates is very large, or unbounded.

4. Investigation of AGG's critical pair analysis engine to see if it can be made more efficient, and if the OutOfMemory problem can be solved. This would go hand in hand with modifications of the type graph used to study reactions (since the analysis engine seems to handle other large grammars from other application domains well). The source code may need to redesigned. Alternatively, other software packages that can carry out critical pair analysis should be researched. These exist for term rewriting, but if the molecules are represented as terms rather than graphs, we would lose much of the rich information in graphs, such as bond order, valency, isomerism and structural complexity. Such a representation would be less intuitive and appealing to chemists.

5. Investigation of open ended, unbounded systems such as the polymerisation of glucose and free radical reactions.

6. Research into how local context affects the rate constant, and to see if the value of the rate constant can be derived automatically. This may require a lower level representation of the molecule as molecular orbitals that store electrons. The energy of these orbitals determines how easy it is to make or break bonds and this in turn determines the rate constant. The molecular orbitals may not have to be represented, but could be inferred from the atomic elements present. Other physical considerations

on reactivity could also be investigated, such as the importance orientation and size of a molecule have when it reacts. In an SN2 reaction for example, nucleophilic attack must always occur from the side opposite to that of a potential leaving group. If bulky groups are on this side (e.g. methyl, CH_3, groups instead of H atoms) the reaction is less likely, therefore lowering the rate constant for the reaction. At first glance, the derivation of rate constants involves a large number of variables and its difficulty placed it outside the scope of the project.

7. The effect of temperature on rate constants and reactivity could be investigated. Each elementary reaction gives out or takes in energy from the reaction medium. Many such reactions would lead to a noticeable change in temperature on the macro scale. This would affect the rate constant since it has an exponential temperature dependence.

8. Finally, a comparison of the results obtained from this project (derivation of ODE's) and the results of stochastic modelling should be carried out to see how closely the two approaches agree if at all.

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Appendix 1 – Career Plan

1. Where do I want to go after graduation?

A large portion of the Leicester Award, which I undertook in the second year involved career plan formulation. During this period, I performed extensive research into my skills, the kinds of tasks I enjoy and the kind of company I would like to work for. As a result of this, by the beginning of this academic year, I had already mapped out a career plan to become a graduate software developer, and eventually an analyst/developer for a small to medium sized enterprise. With this in mind, I organised a three month internship over summer to work as a Java developer for a London company called RI3K. Due to the enjoyable and fulfilling nature of the work, I decided this would be an ideal career for me.

As the year progressed, I had many talks with my supervisor about postgraduate studies. Due to my age, a lack of funds and a desire to progress to industry, to which I thought I would be more suited, I was initially quite resistant to the idea. However, as the project progressed, I came into contact with PhD students, and began to very much enjoy aspects of the project, particularly brainstorming sessions with Prof. Heckel. As the economic crisis also fully came to light, it appeared there were very few satisfactory positions in the job market for developers, in terms of salary, type of company and level of responsibility.

I believe postgraduate studies would give me an excellent chance to develop a more in depth knowledge of a subject I greatly enjoy, be it a Masters of PhD. I believe this will appease my desire to gain a much better, and more theoretically sound knowledge of the subject. While this would serve my future career prospects well, it is primarily out of a personal interest in bettering myself that I wish to pursue this. I do not want to feel that I have wasted my talents and interest in the subject.

2. What will I do this academic year to get there?

I decided to submit some postgraduate applications to keep this route open to me. I was accepted into a Masters course at Oxford University which I have provisionally accepted. I have also submitted an application for the University of Leicester "University of the Year" PhD scholarship. I hope to hear the outcome of this by the end of April. If I am accepted for the PhD I will decline the Oxford offer. I have also kept the option to progress to industry open, in case I am unable to acquire funding for postgraduate study. In preparation for this route, I have prepared an updated CV and had it checked by the Careers Office. I have also stayed in touch with the company I interned with over summer, and used Milkround and contacts to learn more about various companies that I might want to work for. In my spare time, I have been undertaking web design projects that use new technology, as a learning opportunity and showcase for potential employers. On the surface, this may appear to be a lack of commitment to one path, but I feel by cultivating both possibilities fully (study or full-time work), I can easily decide to switch my plan to incorporate the other without having to suddenly do missing groundwork.

To prepare myself for postgraduate study, I have attended Prof. Heckel's PhD group discussion meetings every week this year. This has given me a great insight into the type of research that others are doing, how to present work and the level of depth expected. For direct entry into a PhD, my lack of a Masters may be brought into question. As such, I have tried

my best to understand highly mathematical papers and also had a look at some of the modules I missed in the first year due to management modules.

3. How does my project contribute to my career?

My PhD project proposal would be a direct continuation of this year's project. As such, I will have hopefully developed much of the background information necessary. The open, research-style nature of the project will also prepare me for the self-disciplined approach to a PhD. My project required the reading of many journal articles too, the understanding of which I believe is a skill in its own right, particularly as a gap in knowledge identified by reading one journal may prompt the reading of further articles. This kind of selective knowledge search is very useful for self-teaching.

In addition the project has helped me to understand limitations to open projects, particularly at the implementation stage, and how to get around these either by modifying software or by altering ideas. I have had much contact with other academics, particularly Olga Runge of AGG, which has improved my communication skills and methods of explanation and cooperation. In presenting my project to PhD students and lecturers, I have also learnt important lessons about appropriate levels of abstraction. In addition, time-keeping and organisation skills were enhanced through the project, both of which are important for PhD work.

If I decide instead to progress to industry, the open nature of the project has taught me organisation and project management, how to focus on deliverables, how to be realistic about outcomes, scaling back project depth due to lack of resources or time, and most importantly, more complex problem solving than I have been used to before. It has also taught me how to communicate and absorb ideas from others, as well as how to approach completely new theoretical domains. Dealing with the complex and sometimes perplexing API used in the implementation stage of the project is an added skill. In industry many legacy or off-the-shelf components are often necessary for an application and knowledge of how to integrate them is very useful.

Finally, the project has taught me diligence and the need for a great deal of hard work to succeed in any project. While this is something everyone knows, the extremely challenging nature of this particular project made it clear just how much dedication is needed to ensure success in difficult undertakings.

Appendix 2 – Weekly Diaries

The following is a complete set of weekly diaries for the duration of the project, signed and dated by the project supervisor, Dr. Fer-jan de Vries.