Pattern Formation from Chemical Oscillators

A thesis submitted for the degree of Master of Philosophy by

Richard F. R. J. Denton

School of Chemistry
Faculty of Science and Engineering
University of Edinburgh
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Declaration

I hereby declare that this thesis has been entirely composed by myself and that the work described herein is my own except where clearly mentioned either in acknowledgement, reference or text. It has not been submitted in whole or in part, for any other degree, diploma or other qualification.

Richard F. R. J. Denton

October 2009
This thesis is dedicated

to my family.
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Abstract

Experimentation on, and analysis of biological reactions is inherently more difficult than performing similar reactions and studies with pure chemical systems. Therefore the Belousov-Zhabotinskii reaction, with its self oscillations provides an opportunity to work with a system which mimics the oscillatory nature of biological systems, and at the same time is more easily manipulated.

The BZ reaction has been performed in bulk solution with auto-initiation of the reaction, and its periodicity measured. Further to this a modified reaction has been utilised along with a membrane bound catalyst to allow initially generation of single wave fronts.

The modified BZ reaction and membrane bound catalyst have been utilised in a multi-electrode (currently 8) setup to generate patterns. Patterns are able to be generated by using a delay between the wave fronts which are initiated from each electrode, or by not utilising some of these electrodes.

Utilising silver wires in this reaction causes the deposition of silver bromide at the point of contact between the metal and the membrane, this result’s in the membrane becoming unusable. To overcome this problem, development of a system using a platinum wire as a replacement has taken place. The platinum is still able to remove bromide from the locality of the membrane however it will not result in the deposition of layer onto the membrane.

Additionally the usability of the membrane is also time limited due to “leaching” of the catalyst by the BZ reaction mixture. Currently we have prevented this from occurring by using maleonitriledithiolate as a ligand to create a neutral catalyst [Fe(bathophenanthroline)$_2$mnt].
Development of a TiO$_2$ substrate has begun using [Fe\(\text{bathophenanthroline}_{2}\text{mnt}\)] as the catalyst for this system. We hope that in the future this new system will provide a more robust platform for the catalyst, removing the problems caused by the "leaching" of the catalyst from the current polysulphone membrane. In addition this will result in catalytic surfaces which are no longer affected by modifications to their formulation unless we choose to.
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Introduction.
1.1 Oscillators

What is an Oscillator?

Oscillation is defined as a periodic and harmonic motion in one or more dimensions. An oscillator is a device or system which follows an oscillation, therefore it will periodically fluctuate between two or more different states. Two simple examples of oscillators are a spring and a swing.

In the case of a spring which is under ideal situations a simple harmonic oscillator, oscillations occur when the spring is used to suspend a mass\(^1\). This system can be moved out of equilibrium by pulling the mass downwards storing potential energy in it. Upon release, the potential energy is converted into kinetic energy and the spring rebounds past its equilibrium position. It will then proceed to fall back down to its initial position under the effects of gravity. These oscillations can be shown in a basic sine wave format to make the oscillatory nature more easily discernable.

Oscillators range from these simple mechanical oscillators to electrical devices, chemical systems (e.g. BZ reaction described below) and at the more complex end of the spectrum, biological systems (e.g. the neuron and heart which are both described later).

1.1.1 Biological Oscillators

Classically, biochemical reactions were thought of as being pathways where substrates A and B were converted directly into their end products Y and Z, with no option for true chemical dynamics (i.e. generation of intermediate products) to occur. Circadian rhythms along with electrical impulses were known for a long time
although the molecular basis which produced these observable facts were not known at these early stages in the field of biochemistry\textsuperscript{1,2}. However, new discoveries in the 1950's and 1960's proved that straight pathways from reactants to products were not always observed\textsuperscript{3,4}. The discovery of positive and negative feedback involved in the control of gene expression along with the periodical synthesis of enzymes in bacteria as well as oscillations in glycolysis in yeasts provided the evidence that biochemical reactions are able to follow oscillations\textsuperscript{4,16}.

With these discoveries, interest in understanding biological oscillations began to form with this interest initially being directed towards enzymes and their reactions\textsuperscript{4,9}. These were found to be often controlled by negative feedback in their synthesis at the gene transcription level\textsuperscript{5,13-16}. Following on from this, oscillations were recognised as being an important aspect of all genetic regulatory mechanisms\textsuperscript{4,9,13}.

A more modern way of thinking about biology would be that living cells are a type of digital computer in that they receive signals from their external environment as well as their internal state and respond to these signals via their own mechanisms\textsuperscript{4}. Once a signal is received then the cell proceeds to process the information via its internal signalling pathways which will result in a response occurring. This is much the same way as a computer processes information although in this case the information processing is via a series of silicon based switches and oscillators. In a cell these are replaced by biological varieties of these silicon components i.e. proteins and biochemical reactions which are often oscillators. The overall response to these signals will be gene expression and cell growth, movement or death.
1.1.1.1 Neurons and the action potential

Nature has produced many oscillating systems which are utilised in living organisms e.g. nerve impulses and the beating of a heart\(^1,^3\).

![Diagram of a standard neuron](image)

**Figure 1: Standard Neuron.**

The Neuron is the name given to the specialised cell which makes up nerves. Its basic structure is shown above (figure 1). Neurons transmit information from one area of a body to another by means of electrical impulses\(^{17,18,19}\). These impulses are generated electrochemically by the movement of sodium and potassium ions across the cell membrane generating the action potential\(^{20,21}\). The action potential is brought about by the ion channels found in the neuron’s membrane. At the resting potential both the potassium and sodium channels are closed, however the concentration of potassium ions both inside and outside the membrane is the same, whereas there is a higher concentration of sodium ions outside the membrane\(^{17,18,19,22}\). When the membrane potential increases by 20mV above the resting potential, the voltage-gated sodium channels open, allowing sodium ions to rush into the neuron\(^{22}\). This is a
positive feedback mechanism as the influx of sodium ions causes the localised membrane potential to increase thereby causing more neighbouring voltage gated sodium channels to open. This happens until the potential has increased to 40mV (an increase of around 110mV), then these channels close\textsuperscript{22}. The potassium channels now open, allowing potassium ions to move out of the cell with a small overshoot leading to a state called hyperpolarisation during which another action potential cannot be initiated\textsuperscript{17,20,21}. The neuronal membranes $\text{K}^+/\text{Na}^+$ antiporters then reset the initial membrane potential of -70mV and the internal cellular ion concentrations by utilising ATP\textsuperscript{17,19,22}. This is shown below in figure 2.
1.1.1.2 The Heart

The heart is the muscular organ which is found in all vertebrates and whose function is to pump blood around their bodies. The heart is comprised of a special type of muscle called myocardium and in the case of humans is subdivided into four sections: the right and left atria, and the right and left ventricles\textsuperscript{23,24}. The atria receive deoxygenated blood into the heart from the body in the case of the right atrium, and oxygenated blood from the lungs in the case of the left atrium\textsuperscript{23,24}. The ventricles are more muscular allowing them to perform their function of pumping blood out of
the heart, with the left ventricle pumping blood to the lungs and the right ventricle pumping blood to the rest of the body\textsuperscript{23,24}.

Contraction of the heart muscle is brought about through electrical impulses which originate in an area of the heart called the sino-atrial node (SA node) located in the right atrium\textsuperscript{24}. This impulse causes a wave of depolarisation to spread firstly across the atria, and, after it is transmitted through special fibres, to the ventricles\textsuperscript{24}. This causes the atria to contract first and thereby ensures the ventricles are full before they contract.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{heart_ecg_diagram.png}
\caption{Heart Electro-cardiogram.}
\end{figure}
The rhythmic beating of the heart is detected by an ECG (see electrocardiogram in figure 3)\textsuperscript{24,25}. On an ECG, each wave is labelled from the letters P to U. The SA node depolarisation is seen as the P wave, this is always followed by the QRS complex following normal contraction of the heart (also known as sinus rhythm)\textsuperscript{24,25}. The QRS complex is the result of the initiation of depolarisation in the myocardial cells, resulting in the contraction of the heart\textsuperscript{24,25}. The standard ECG (figure 3) shows the electrical activity detected during a typical heart contraction.

This oscillating electrical activity is the result of oscillations in the concentration of calcium ions in the myocardium. These calcium ions are stored in specialised structures within the myocardium called the sarcoplasmic reticulum\textsuperscript{24,25}. Release from this structure initiates the contraction of the muscle, after which a cellular pump (the sodium-calcium antiporter) removes the Ca$^{2+}$ ions from the sarcoplasm (cytoplasm found in muscle cells) back into the sarcoplasmic reticulum\textsuperscript{24,25}. This results in the muscle being reset, ready for the next contraction.

**Biological Summary**

As can be seen from the examples given above, oscillatory systems are essential to biological systems. The electrical oscillatory activity of the heart (figure 3) is essential for blood and the oxygen it carries to be pumped around bodies and the electrical activity of neurons (figure 2) is essential to the activities of the nervous systems of all organisms which contain nervous tissue. In both these cases, the electrical activities detected are the result of the movement of ions back and forth across cellular membranes.
1.1.2 Chemical Oscillators

Biological systems contain oscillations, however these are not always easy to study, hence the need for chemists to develop oscillating reactions for study. The reason for this is due to the complexity of biological systems, with many reactions numbering into the thousands taking place in each individual cell as well as the fact that cells interact with others\textsuperscript{1,3,4}.

Oscillating reactions have long fascinated chemists because they seem to challenge the general principles of chemical reactions. The majority of chemical reactions will go from the initial reactants to a mixture of products with or without some left over reactants i.e. to equilibrium\textsuperscript{26}. Oscillating reactions must, by the definition of oscillation, switch and run apparently in the opposite direction\textsuperscript{4,26}.

As previously mentioned, mechanical oscillators are some of the easiest oscillators to describe and visualise, and a pendulum is a good example because it oscillates from one side to the other and in doing so passes through its equilibrium position. Due to this it was often used as a comparison with the belief that a chemical oscillating reaction would also pass through its equilibrium position. The result of the reaction passing through its equilibrium position would be contrary to the 2\textsuperscript{nd} law of thermodynamics which states that entropy will increase over time till it reaches a maximum value at equilibrium.

In a chemically oscillating reaction the concentration of certain components in the reaction mixture are continuously altering so that in effect they pass through the same value multiple times\textsuperscript{4}. Energy is being utilised and lost from the reaction mixture, being transferred into the surroundings resulting in the free energy of the oscillating reaction decreasing with each cycle till it is all utilised. As such a much
improved model would be a grandfather clock rather than a pendulum. This is because the workings of a grandfather clock have internal weights which act as the energy store for the clock. They use up their energy store to cycle the hands of the clock face twice daily through a full rotation.

All chemical reactions are driven by a decrease in the free energy of the reaction mixture, including oscillating chemical reactions. However in the case of oscillating chemical reactions there has to be a feature of the reaction pathway which is out of the ordinary and allows the reaction to head towards its equilibrium whilst at the same time appearing to cycle between states. The reaction pathways which occur in oscillating reactions are complex and it is this complexity, coupled with the intricate changes in the reaction components, which allows these complex changes to occur.

**History of Chemical Oscillators.**

The first chemical oscillator to be described in the field of chemistry was that of an electrochemical cell which showed an oscillating current. This was by A.T. Fechner in 1828. The second chemical oscillator to be described was the periodically regular dissolving of chromium into an acidic solution. These two examples are of heterogeneous oscillating reactions. This means that the oscillation is occurring between two different states. In the case of the chromium it is an oscillation of the solubility within the solution, and for the electrochemical cell it is a chemical reaction, between an iron electrode and a weak acidic solution, which produces an electric current which oscillates. Due to this most scientists believed that these were the only form of oscillating reactions which existed. For many years it
was believed that homogenous oscillating reactions could not be found as they would break the laws of thermodynamics\textsuperscript{30}. This was because for an homogenous oscillator to occur the reaction would seem to need to flow in one direction and then to reverse itself and flow in the opposite, whereas chemical reactions always move towards an equilibrium or end state, and in so doing reduce the free energy of the reaction system\textsuperscript{27-30}.

\subsection*{1.1.1.2 The BZ Reaction}

\textbf{History of the BZ Reaction}

Discovered in the 1950's, the BZ reaction's full name is the Belousov-Zhabotinskii Reaction, being named after two scientists: Boris Pavlovich Belousov and Anatol Zhabotinskii. It is a reaction which self-oscillates, producing waves that propagate through the medium (if left unstirred), and under appropriate conditions can show chaos\textsuperscript{31-33}. This is an example of spatial oscillations, where the reaction front is seen to move. This reaction can also show temporal oscillations if the reaction mixture is stirred\textsuperscript{34}. In this case the total mixture will swap between the two catalytic states periodically, seen as a rapid change in the colour of the solution\textsuperscript{34}.

The reaction was initially discovered by Belousov whilst he was conducting research into the Krebs cycle, and at the same time trying to find an inorganic analogue of the main component of this cycle (citric acid). Due to this the initial mixture of reactants which he used was: citric acid, a source of bromate ions and Ceric ions. These chemicals will, when mixed together, produce an oscillating reaction which changes in colour from a very pale yellow to colourless (due to the change in state of
the ceric ions which are catalysing the reaction; Ce$^{4+}$ being pale yellow, and Ce$^{3+}$ being colourless). In 1951 Belousov wrote a paper describing his observations which was rejected by the scientific community on the grounds that homogenous, self-oscillating reactions could not happen as at the time they were believed to break the second law of thermodynamics$^{35}$. Six years later he attempted to publish another more thorough and descriptive paper which was again rejected, resulting in the reaction being ignored for a few years$^{30,35}$.

The next stage in the history of this reaction occurred a further four years later when Zhabotinskii rediscovered this oscillating reaction during his PhD$^{30,35}$. In his studies of the reaction he replaced the citric acid initially used by Belousov with malonic acid to remove the precipitation which can occur with the former. At the same time he added an indicator to the reaction to aid in the detection of the oscillations.

![Figure 4: Ferroin](image-url)
This indicator was ferroin \([\text{Fe(C}_{12}\text{H}_{36}\text{N}_{6})\text{]}^{2+}\) (shown in figure 4) which is blue when oxidised, and red when reduced (much more visible colour change than the pale yellow to colourless with the ceric ions). He also discovered that ferroin was able to catalyse the reaction by itself, so the ceric ions were removed from the mixture.

**Modern BZ Reaction**

The research of Zhabotinskii led to the standard mixture for a BZ reaction:

- Malonic Acid 0.2M
- Sodium Bromate 0.3M (it should be noted that potassium bromate may be used instead, but is not as soluble in higher concentrations).
- Sulphuric Acid 0.3M (this is to acidify the solution).
- Ferroin catalyst 0.005M (higher concentrations can be used to intensify the colour of the solution).

When mixed in these concentrations and stirred, the reaction mixture will periodically oscillate between the red (reduced), and blue (oxidised) form with a period of about 20s.

**Simplified Mechanism of the BZ reaction**

The full mechanism of the BZ reaction is believed to contain at least 80 separate chemical steps, so for ease of use different models have been developed that make assumptions allowing the reaction to be reduced to its key steps.
Three scientists (Field, Koros and Noyes) formulated a model showing the important steps involved in the BZ reaction, now known as the FKN mechanism. This reduced the multitude of steps into three essential processes:

- **Process A**: Inhibition – $\text{BrO}_3^-$ reacting with $\text{Br}^-$.
- **Process B**: Autocatalytic Step – $\text{Br}^-$ is now depleted, $\text{HBrO}_2$ has become the dominant reducing species for the $\text{BrO}_3^-$. This step is seen as a rapid colour change. The term autocatalytic is used as this process results in the generation of an increased amount of $\text{HBrO}_2$ by the reaction of itself with $\text{BrO}_3^-$.
- **Process C**: Re-reduction of the catalyst and regeneration of high concentrations of $\text{Br}^-$.

The mechanism for these three steps is as follows:

Process A which contains two steps:

1. $\text{BrO}_3^- + \text{Br}^- + 2\text{H}^+ \rightarrow \text{HBrO}_2 + \text{HOBr}$
2. $\text{HBrO}_2 + \text{Br}^- + \text{H}^+ \rightarrow 2\text{HOBr}$

Overall this gives the following reaction for Process A:

$\text{BrO}_3^- + 2\text{Br}^- + 3\text{H}^+ \rightarrow 3\text{HOBr}$

Provided the concentration of $\text{Br}^-$ is high, this is the dominant process and the ferroin catalyst remains unaltered in the reduced (Fe$^{2+}$) form.
Process B contains these two steps:\footnote{27,44}:

1. \( \text{BrO}_3^- + \text{HBrO}_2 + \text{H}^+ \rightarrow 2\text{BrO}_2^- + \text{H}_2\text{O} \)
2. \( \text{BrO}_2^- + \text{Fe}^{2+} + \text{H}^+ \rightarrow \text{HBrO}_2 + \text{Fe}^{3+} \)

Overall this gives the following for process B:

\( \text{BrO}_3^- + \text{HBrO}_2 + 2\text{Fe}^{2+} + 2\text{H}^+ \rightarrow 2\text{HBrO}_2 + 2\text{Fe}^{3+} + \text{H}_2\text{O} \)

This process becomes dominant once the \( \text{Br}^- \) concentration has dropped sufficiently low. It occurs abruptly due to the autocatalysis: 1 mole of \( \text{HBrO}_2 \) leads to the creation of 2 moles of \( \text{HBrO}_2 \) product.

Process C is the reaction of the malonic acid\footnote{27,44}:

\[ 2\text{Fe}^{3+} + \text{MA} + \text{BMA} \rightarrow f\text{Br}^- + 2\text{Fe}^{2+} + \text{products from breakdown of MA} \]

In the reaction above \( f \) is a stoichiometric factor which is included to provide an adjustable parameter allowing for the number of bromide ions to vary depending on the concentration of the MA, BrMA and HOBr.

It is currently believed that the BMA (bromo-malonic acid) is produced by a reaction of MA (malonic acid) with some of the HOBr (possibly through the release of \( \text{Br}_2 \) from this)\footnote{27,44}. This occurs during a preliminary stage following the mixing of the reagents.

Process C resets the system by regenerating a large concentration of \( \text{Br}^- \) thus favouring Process A and simultaneously inhibiting Process B.

In addition to these three main processes there are the following side reactions that occur\footnote{45}:

\[ \text{HOBr} + \text{HBrO}_2 \rightarrow \text{HBrO}_3 + \text{H}^+ + \text{Br}^- \]
and the disproportionation of $\text{HBrO}_2$:

$$2\text{HBrO}_2 \rightarrow \text{HOBr} + \text{BrO}_3^-$$

These side reactions are included as they will have some effect on the kinetics of the reaction.

*Figure 5: BZ Reaction Schematic.*

Figure 5 shows the essential steps needed to produce the colour changes in the oscillating BZ reaction, with it indicating that the $\text{Br}^-$ ions released from bromomalonic acid are used in the reaction of $\text{BrO}_3^-$, during which the ferroin/ferrin catalyst is converted in the opposite direction to that drive by the breakdown of malonic acid.

This schematic allows it to be more easily seen that of the three processes (A, B and C), process C forces the observed colour changes from the blue $\text{Fe}^{3+}$ to the red $\text{Fe}^{2+}$, and process B drives the opposite step.
Process A is the step which removes the bromide ions from the solution and allows the autocatalytic B step to take place.

**Oregonator Model**

The Oregonator model is a simplified numerical model of the BZ reaction whereby there are a reduced number of species to consider\(^{46,47}\). In a simplified BZ reaction there are 6 components to consider: the organic acid, which is oxidised (in the above example it is malonic acid), the catalyst (in the above example ferroin), bromate ions, HOBr, HBrO\(_2\) and Br\(^-\) ions\(^{44,46,47}\). The Oregonator model makes use of dimensionless variables to increase the speed, and reduce the complexity of the model being run\(^{44,47}\). There are two versions of this model, one with three variables and another with two.

For the three variable model, the dimensionless terms are produced from the concentration of the catalyst, the concentration of HBrO\(_2\) and the concentration of the bromide ions. Of these three dimensionless variables it can be said that the HBrO\(_2\) and bromide ions are in a dynamic steady state relative to each other\(^{44}\). This allows an approximation to be made, that the HBrO\(_2\) and Br\(^-\) are equivalent, reducing the number of dimensionless variables to two. This two variable model produces an accurate (although not 100%) correlation with wet experiments on the BZ reaction, whilst at the same time allowing the calculations which are needed to be performed within the model to be performable on a normal desktop computer\(^{44}\). In the case of both models the organic acid used and the bromate ions can be ignored as they are in excess from the initial reaction mixture so their concentrations will not alter until the reaction is nearing completion.
The use of dimensionless variables as mentioned above is normally done with the aid of FKN notation for process A, B and C described above. This was devised by Field and Noyes to increase the straightforwardness of performing these alterations.\(^\text{46}\)

The FKN notation results in the following five equations, along with their corresponding rates.

Where:

- \(A\) corresponds to \(\text{BrO}_3^-\)
- \(B\) corresponds to the organic species
- \(P\) corresponds to \(\text{HOBr}\)
- \(X\) corresponds to \(\text{HBrO}_2\)
- \(Y\) corresponds to bromide
- \(Z\) corresponds to the oxidised catalyst

From the two half equations for process A\(^\text{27,44}\):

- \(A + Y \rightarrow X + P\) \hspace{1cm} \text{Rate} = k_3 AY
- \(X + Y \rightarrow 2P\) \hspace{1cm} \text{Rate} = k_2 XY

From the equation for process B\(^\text{27,44}\):

- \(A + X \rightarrow 2X + 2Z\) \hspace{1cm} \text{Rate} = k_5 AX

From the disproportionation of \(\text{HBrO}_2\)\(^\text{27,44}\):

- \(2X \rightarrow A + P\) \hspace{1cm} \text{Rate} = k_4 X^2
From the equation for process C\textsuperscript{27,44}:

- \textbf{B} + \textbf{Z} \rightarrow 0.5\textit{fY} \quad \text{Rate} = \textit{k}_c\textit{BZ}

These can then be combined to produce the rate equations of \textit{X}, \textit{Y} and \textit{Z}. Once these have been obtained, the final step is to turn \textit{X}, \textit{Y} and \textit{Z} into the dimensionless variables by dividing throughout to remove all units from these terms. This is accomplished by replacing \textit{X}, \textit{Y} and \textit{Z} along with \textit{T} found in the equations below, for terms based on combinations of their reaction rate equation terms\textsuperscript{27,44}:

- \frac{d\textit{X}}{dt} = \textit{k}_3\textit{AY} - \textit{k}_2\textit{XY} + \textit{k}_5\textit{AX} - \textit{k}_4\textit{X}^2
- \frac{d\textit{Y}}{dt} = -\textit{k}_3\textit{AY} - \textit{k}_2\textit{XY} + 0.5\textit{f}\textit{k}_c\textit{BZ}
- \frac{d\textit{Z}}{dt} = 2\textit{k}_5\textit{AX} - \textit{k}_c\textit{BZ}

Therefore we get:

\[
x = 2 \frac{\textit{k}_4\textit{X}}{\textit{k}_5\textit{A}}, \quad y = \textit{k}_2\frac{\textit{Y}}{\textit{k}_5\textit{A}}, \quad z = \textit{k}_c \frac{\textit{k}_4\textit{BZ}}{(\textit{k}_5\textit{A})^2} \quad \tau = \textit{k}_c\textit{Bt}
\]

These terms can then be used with the rate equations above to give\textsuperscript{26,27}:

- \frac{\text{d}x}{\text{d}\tau} = \left[\textit{qy} - \textit{xy} + \textit{x}(1 - \textit{x})\right] / \varepsilon
- \frac{\text{d}y}{\text{d}\tau} = \left[-\textit{qy} - \textit{xy} + \textit{fz}\right] / \varepsilon'
- \frac{\text{d}z}{\text{d}\tau} = \textit{x} - \textit{z}

where the dimensionless parameters are\textsuperscript{26,27}:

\[
\varepsilon = \frac{\textit{k}_c\textit{B}}{\textit{k}_5\textit{A}}, \quad \varepsilon' = \frac{\textit{2k}_4\textit{k}_4\textit{B}}{\textit{k}_2\textit{k}_5\textit{A}}, \quad \textit{q} = \frac{\textit{2k}_3\textit{k}_4}{\textit{k}_2\textit{k}_5}
\]

This results in \textit{X}, \textit{Y}, \textit{Z} and \textit{T} being substituted for the terms \textit{x}, \textit{y}, \textit{z}, and \textit{\tau}, along with the introduction of the three dimensionless variables \varepsilon, \varepsilon' and \textit{q}\textsuperscript{26}.
BZ reaction summary

Oscillations in a bulk solution are a 3D effect, seen as the rapid alteration of the solutions colour from red to blue and back again (provided the catalyst is ferroin). These are temporal oscillations. If this mixture is poured into a thin layer then waves of colour change are seen. These redox waves are a pseudo-2D effect, also known as spatial oscillations$^{36,37}$.

Figure 5 shows us that we can control the oscillations seen within the BZ reaction by setting the reaction so that the ferroin is unable to react with the species present in the initial solution, whilst the ferrin form could. This is brought about by the addition of Br$^-$ to the initial mixture$^{36,37,43}$. The addition of potassium bromide inhibits the autocatalysis. With the autocatalysis inhibited, control of the oscillating reaction can now take place. If these additions were to be added to the bulk reaction mixture, then it would never change from its initial red colour to blue without some reaction occurring to remove the inhibition (Br$^-$ ions) and in so doing the temporal oscillations would be prevented. This allows the BZ reaction to show excitability$^{44}$.
Figure 6: Phase plane representation of BZ reaction where $X$ and $Z$ are concentrations from the FKN notation.

An excitable system is one which remains in a steady state, but a small perturbation of some type will disturb this state and the system will attempt to return to the steady state. In the case of the BZ reaction this occurs by the removal of $\text{Br}^-$ ions. Removal of a large enough amount of these $\text{Br}^-$ ions (above the critical threshold for the system) stimulates the reaction to fire off its redox wave. This can be best explained with the use of a phase plane diagram (figure 6). The steady state is indicated by the intersection of the $x$ and $z$-nullclines (point 1). Perturbations to the BZ system which fail to initiate a wave are indicated by the gap between point 1 and point 2. Point 2 indicated an alteration of the $\text{Br}^-$ ion concentration to the threshold at which time the system will oscillate, when this occurs the system will
snap across to point 3 on the diagram and follow the reaction pathway back to the steady state. In so doing an oscillation will be seen\textsuperscript{44}.

Temporal oscillation control requires another alteration to the reaction. In this case the reaction mixture needs to be in a thin layer to allow redox wave fronts to be seen. Whilst this can be seen in a thin layer of the BZ reaction mixture, it is not the best method of producing these waves as there is a very limited supply of the reactants and the reaction will stop very rapidly. Therefore the catalyst can be either embedded into a gel or bound onto a membrane\textsuperscript{36,37,43}. In doing this the catalyst is maintained in a thin layer whilst a large quantity of the rest of the reactants can be added into the surroundings so allowing spatial oscillations to occur for a longer period of time. In this case the modified reaction mixture stops the auto-initiation of redox waves in the membrane bound catalyst\textsuperscript{37}. Due to there being no auto-initiation the membrane bound catalyst can now be described as being excitable. With the BZ reaction setup in this state a small perturbation will result in the initiation of an oscillation. In this case silver wire could be used to remove the Br\textsuperscript{−} from the reaction through the generation of AgBr\textsuperscript{36,37}. Coupling this control mechanism with a thin layer of catalyst (either pouring this solution into a thin layer or using a membrane bound catalyst) will result in controlled initiation of redox waves (figure 7)\textsuperscript{36,37}. 
Using silver wire allows control of the BZ reaction to be performed through a chemical reaction whereby the silver reacts with the bromide to produce silver bromide reducing the concentration of bromide ions around the silver wire and thereby allowing a redox wave to initiate from that point\textsuperscript{36,37}. However it is not the only method of control with a further step being through the use of electrochemistry. This could be with a silver wire to control the chemical reaction (holding a silver wire at a very negative potential will prevent any bromide ions from reacting with the silver wire so stopping the reaction). Returning to a neutral or positive potential will allow the reaction to occur.

If the catalyst is altered so that it is susceptible to light then another control method is the use of photochemistry. An example of a light susceptible catalyst is
based on ruthenium ([Ru(bpy)$_3$]$^{2+}$). In this case instead of relying on a chemical reaction to initiate a change of state in the catalyst, light is used to convert the catalyst to its alternate state and thereby start off a BZ reaction wave.$^{43}$

1.1.2.2 Bray Reaction

The Bray reaction, also known as the Bray-Liebhafsky reaction, was the first documented example of a homogenous chemical oscillating reaction.$^{30,48}$ It was discovered in 1921 by W.C. Bray and Hermann Liebhafsky (a student working for him)$^{48}$. The Bray reaction consists of a solution of the following chemicals: sulphuric acid, hydrogen peroxide and potassium iodate.$^{48-50}$ These chemicals result in a mixture which can be seen to oscillate due to the periodic formation and removal of iodine along with the release of a gas.$^{48}$

The reaction has two main steps, the oxidation of hydrogen peroxide to oxygen gas which occurs simultaneously to the reduction of iodate to iodine.$^{50}$:

- $5\text{H}_2\text{O}_2 + 2\text{IO}_3^- + 2\text{H}^+ \rightarrow \text{I}_2 + 5\text{O}_2 + 6\text{H}_2\text{O}$

followed by the oxidation of the iodine produced above back to iodate by more of the hydrogen peroxide.$^{50}$:

- $5\text{H}_2\text{O}_2 + \text{I}_2 \rightarrow 2\text{IO}_3^- + 2\text{H}^+ + 4\text{H}_2\text{O}$

The overall reaction is therefore the catalytic disproportionation of hydrogen peroxide to release oxygen and water.$^{50}$:

- $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$
It is based on a mixture of sulphuric acid, iodate and hydrogen peroxide. When mixed these compounds react in a complex manner to release oxygen and I₂ in a periodic manner from the mixture.

1.1.2.3 Briggs-Rauscher Reaction

The Briggs-Rauscher reaction was first described in 1973 by two high school teachers, Thomas S. Briggs and Warren C. Rauscher. It is a chemical oscillating reaction which is the result of combining the Bray reaction which is described above, with the BZ reaction (described in 1.1.2.1). This combination results in an oscillating reaction which shows easily detectable colour changes more easily followed than those of the Bray reaction, thereby allowing visual demonstrations. The reaction is produced by using the following: potassium iodate, hydrogen peroxide, perchloric acid, or sulphuric acid, malonic acid, a source of Mn²⁺ ions, e.g. manganese II sulphate and starch.

These chemicals should be mixed initially into 3 separate solutions prior to final mixing:

- Potassium iodate (29g) dissolved in 400ml of water and 8.6ml of 6M sulphuric acid added to create the 1st solution
- Malonic acid (10.4g), manganese II sulphate (2.2g) and soluble starch (0.2g) dissolved in 400ml of water to create the 2nd solution
- The 3rd and final solution is just the hydrogen peroxide (3%)
An equal volume of the 1st and 2nd solutions can now be mixed together and stirred, and then the hydrogen peroxide is added to start the oscillations (a volume equal to the combined volumes of solutions 1 and 2).

The mixture above results in the following reaction which produces oscillations:

\[
\text{IO}_3^- + 2\text{H}_2\text{O}_2 + \text{CH}_2(\text{CO}_2\text{H})_2 + \text{H}^+ \rightarrow \text{ICH(}\text{CO}_2\text{H})_2 + 2\text{O}_2 + 3\text{H}_2\text{O}
\]

This reaction is accomplished in two component reactions:

1. \(\text{IO}_3^- + 2\text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{HOI} + 2\text{O}_2 + 2\text{H}_2\text{O}\)
2. \(\text{HOI} + \text{CH}_2(\text{CO}_2\text{H})_2 \rightarrow \text{ICH(}\text{CO}_2\text{H})_2 + \text{H}_2\text{O}\)

Reaction 2 is itself composed of the following 3 component reactions:

4. \(\text{IO}_3^- + \Gamma + 2\text{H}^+ \rightarrow \text{HIO}_2 + \text{HOI}\)
5. \(\text{HIO}_2 + \Gamma + \text{H}^+ \rightarrow 2\text{HOI}\)
6. \(\text{HOI} + \text{H}_2\text{O}_2 \rightarrow \Gamma + \text{O}_2 + \text{H}^+ + \text{H}_2\text{O}\)

Any of the HOI which is not utilised in reaction three will undergo a reduction to \(\Gamma\) by hydrogen peroxide as in reaction 6.

Once the iodide ions are depleted sufficiently another process takes over which involves the following five component reactions:

7. \(\text{IO}_3^- + \text{HIO}_2 + \text{H}^+ \rightarrow 2\text{IO}_2^- + \text{H}_2\text{O}\)
8. \(\text{IO}_2^- + \text{Mn}^{2+} + \text{H}_2\text{O} \rightarrow \text{HIO}_2 + \text{Mn(OH)}^{2+}\)
9. \(\text{Mn(OH)}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Mn}^{2+} + \text{H}_2\text{O} + \text{HO}_2\)
10. \(2\text{HO}_2' \rightarrow \text{H}_2\text{O}_2 + \text{O}_2\)
11. \(2\text{HIO}_2 \rightarrow \text{IO}_3^- + \text{HOI} + \text{H}^+\)

This gives the overall reaction shown in reaction 2. Reactions 7 and 8 are autocatalytic, producing 2 molecules of HIO2 for each one consumed.
The dramatic colour change which makes this oscillating reaction so useful in demonstrations occurs because reaction 3 takes places in the following two component reactions:\(^{52-55}\):

12. \( \Gamma + \text{HOI} + \text{H}^+ \rightarrow \text{I}_2 + \text{H}_2\text{O} \)

13. \( \text{I}_2 + \text{CH}_2(\text{COOH})_2 \rightarrow \text{ICH( COOH)}_2 + \text{H}^+ + \Gamma \)

The BR reaction releases oxygen during its oscillations, along with the oscillation of iodine and iodide ions\(^{52,53,56}\). The colour changes seen are from colourless to an amber colour, finally to a blue/black colour\(^{51,52}\). These colour changes can be attributed to the ions present in the solution at different stages. The starting solutions are colourless. The increase in hue to an amber colour is due to the formation of \( \text{I}_2 \) in the reaction mixture\(^34\). This occurs due to the reaction shown in 12, where iodide reacts with other intermediates to produce iodine\(^{53-55}\). As excess iodide is produced in reaction 6 its concentration will increase until it becomes greater than that of \( \text{HOI} \) which is reacting with hydrogen peroxide to produce it\(^{52}\). Once this point is reached, the iodide is able to complex with the iodine, which has produced the amber colouration, to form triiodide. This is able to form a complex with the starch solution which allows the mixture to suddenly change to a blue/black colour. The high concentration of iodine will next begin to decrease as it is utilised in reaction 3 resulting in the loss of the colour allowing the cycle to restart again\(^{53-55}\).
Oscillating reaction summary

Of these three reactions, the BZ reaction has found more favour for research than either the Bray reaction or the B-R reaction. The reasons for this can be determined if we compare these three reactions.

If we compare the BZ reaction and the Bray reaction, we can see that although the Bray reaction was the first of these to be discovered it has certain limitations. These are that it is comparatively difficult to detect the oscillations as they are seen as a mild colour change and effervescence at different stages whereas the BZ reaction has a definite and marked change from red to blue.

The B-R reaction does not suffer from the same problems as the Bray reaction since it was developed to be an easy demonstration. Due to this it shows marked colour changes. However it is a much more recent reaction and its mechanism is not as well analysed as that of the BZ reaction. This makes it far more difficult to analyse what is occurring during the different stages of the reaction.

1.1.2.4 Example applications of the BZ reaction

A novel use of a form of oscillating reactions, which is from the same grouping as the BZ reaction, was patented in August 1991. Currently water is disinfected by treatment with biocidal chemicals, normally halogens. Of these halogens, the most common choice for drinking water disinfection is chlorine as it has a very strong biocidal action. However side effects of utilising chlorine can result in noxious by-products. These by-products leave a nasty taste in the water as well as having reduced biocidal activity when compared to the chlorine.
Consequently a system of disinfecting water whilst reducing the production of these by-products would be useful. The easiest way of reducing the production of by-products would be to develop a method of generating the same level of disinfection with a reduced level of chlorine. There are two methods by which this could be achieved:

1. regenerating chlorine from its by-products
2. use of a second halogen, which when acting in combination with chlorine would be more effective than either on their own.

The patent filed in August 1991 utilised a combination of both of these. Iodine was included in the disinfecting mixture at low concentrations and an oscillating reaction was found to occur. This was due to the generation of iodate from the iodine, which when coupled with the chlorine already present allowed these oscillations to take place. The intermediate species generated by this oscillating reaction were found to have higher biocidal effect. Since the intermediate species are the ones producing this biocidal effect rather than just chlorine, then the concentration of chlorine needed is reduced to levels which are considered acceptable to public health concerns. With this reduction of chlorine concentration there is much reduced generation of by-products.

Another use for oscillating reactions utilises the propagation of waves through an excitable medium. In this case the waves in a BZ reaction can be utilised to determine the optimal path through a maze.

The current method of finding a path through a maze or between two points is by performing multiple searches (called an iterative search). An example would be a
computer algorithm trying to determine the best route between two points A and B. Every possible junction between A and B is called a node. The computer allocates each possible node which links to Point A, a value which is based on its likelihood of being the optimal path. The best node is picked, and this process repeated for the next stage of the path, however it is a time-consuming process.

Recently a Belgian research group created a theoretical model of the BZ reaction. Using this model, they were able to show that the path which the redox wave passes along will be the shortest available. This theoretical model has allowed research to be undertaken to produce a more efficient path finding method. In this case a maze was constructed and filled with a BZ reaction medium which is unable to spontaneously oscillate. This allows a wave to be initiated with a silver wire. The initiation of a wave is the result of a chemical reaction between the silver and excess bromide in this BZ reaction medium. This results in the removal of the bromide as silver bromide which resets the reaction mixture to that which can oscillate (but only in the locality of the silver wire). Frequent camera images of the maze can be taken which will allow an initiated wave to be followed as it travels through the maze. The main advantage of this model over the iterative search method is that one initiation sets off a wave which will travel along all paths, branching off at any bifurcations in the maze. This allows the optimal path to be determined far more rapidly and easily than with the conventional approach.

Chemical computing is a more recent addition to the uses of the BZ reaction. Also known as a reaction-diffusion computer, these are a form of unconventional
computing which relies on variation in the concentration of the oxidised and reduced forms of catalysts which are utilised in BZ reactions.

One form of chemical computer is the so called glooper computer developed by Adamatzky\textsuperscript{60}. Although this is termed a chemical computer it would not be recognised as one by most people because it is very limited in its function. In this case the computations are detected in the dish of BZ reaction chemicals as waves due to the catalyst changing state\textsuperscript{60}. Currently the research is focusing on developing a liquid brain based on these chemicals\textsuperscript{60}. To this end a mobile base for use with this reaction has been developed. In this case a dish containing the BZ reaction is placed onto a mobile base (much like a remote controlled car)\textsuperscript{60}. Attached to this setup, so that it can view the reaction mixture, is a camera\textsuperscript{60}. The BZ reaction used in this setup is a light catalysed reaction, which means that waves will be initiated from any source of light present\textsuperscript{43,60}.

The light catalysed BZ reaction is similar to the ferroin catalysed reaction except that it utilises a catalyst which changes between its reduced and oxidised form under the influence of light\textsuperscript{43}. The catalyst which is utilised for these light catalysed reactions is ruthenium which is in the form of $[\text{Ru(bpy)}_3]^{2+}$\textsuperscript{43}. In the absence of light no reaction waves are seen. Using this form of the BZ reaction has allowed the mobile base described above to be programmed to head towards the light source, by orientating itself so that the wave generation is at the front of the dish\textsuperscript{60}.

Another example involves global coupling which can be seen to occur when multiple oscillators are coupled to one another\textsuperscript{61}. This occurs frequently in biological systems, with a decent example being the synchronisation which can occur between amoebae in suspensions through the release of cyclic AMP (cAMP)\textsuperscript{61,62}. Under
favourable environmental conditions these amoebae (*Dictyostelium discoideum*) are free-ranging individual organisms\textsuperscript{61,62}. When the food supply these amoebae rely on is exhausted the enter a state where they produce and respond to cyclic AMP. After approximately 8 hours of starvation conditions the amoebae begin to aggregate in response to the cAMP, with this aggregation being towards randomly located pacemaker cells which are releasing the cAMP in periodic waves\textsuperscript{61,62}. In more advanced organisms this synchronisation can be seen occurring between the neurons which make up their nervous systems allowing interaction within networks of these cells\textsuperscript{61,63}. More recently research has been taking place detailing the behaviour of a global coupling which can occur in a spatially distributed BZ reaction with the use of light initiation. These reactions use cation-exchange beads as the substrate for the light sensitive BZ reaction catalyst, which can be packed into columns and surrounded by the reaction medium\textsuperscript{61}. Then initiation can occur at one point and the effect further along the column where no light exposure has occurred can be examined\textsuperscript{61}.

Another development in the field of chemical computing is the formation of logic gates within a BZ reaction. A possible method initiates the BZ reaction at two points, either of the points individually or not at all. This provides the input for the logic gate (generation of a wave being 1, no generation being 0)\textsuperscript{64,65}. After a predetermined time (when the two waves would have collided if both had been generated), the presence of And and Not gates can be detected\textsuperscript{64,65}. In this example an output of 1 is when the ferroin catalyst is in the blue state.

These developments might lead to the eventual production of more powerful computer though parallel computing. The major drawback in this is the slow speed of
propagation of the redox waves. However a more likely strategy would be to couple this parallel computing with the current silicon computers to obtain the best of both worlds.

Of all the possible uses of the BZ reaction to be attempted so far the only one which currently has the possibility of commercial success is its use in water purification. The other methods whilst novel have yet to be applied commercially. In the case of the path finding, the use is limited because of its reliance on passing waves through a liquid medium. This makes development of a portable and usable device unlikely in its current form. Similarly the chemical computer is not a true computer as its function is limited to movement towards a light source. A true chemical computer would be able to function more like current desktop systems showing versatility in its ability to run programs and analyse multiple different forms of data. To this end the next stage in evolving the BZ reaction towards a true chemical computer would be coupling it with modern silicon based systems to allow for speedier analysis of problems and data. As this would be a very complex system to jump straight to it is necessary to begin by initially developing more complex and more robust systems for wave initiation and propagation. Research and development on this has been detailed within the subsequent chapters of this thesis.
1.2 Electrochemistry

Introduction

A large number of the experiments in this thesis utilise electrochemical control for their operation. Electrochemistry is the study of reactions which are taking place in a solution and at the boundary between an electrode and the electrolyte. They are a form of redox reactions whereby the oxidation state of the species in solution is altered by the transfer of electrons with an electrode.

1.2.1 3 Electrode vs 2 Electrode

In an electrochemistry cell where there are only 2 electrodes, a working and counter electrode, there is a large potential drop over the distance between them. This potential drop is caused by a combination of the resistivity of the electrolyte, the distance between the two electrodes and also the size of the current between them.

The impact of this resistance can be significant on electrochemistry experiments, requiring overbiasing of the potential between two electrodes to achieve the required reaction. Due to the potential drop over the distance between the working and counter electrode, the actual voltage at the working electrode will be less than the total measured potential of the cell. To overcome this problem inherent in an electrochemical cell, a third electrode can be introduced, known as a reference electrode.

A reference electrode is an electrode which has a stable and well known electrode potential. When introduced into an electrochemical cell this acts as a standard against which the potential of the working electrode can be calculated. An example would be the silver/silver chloride electrode. With this third electrode
included in an electrochemical cell the measured potential drop will now occur all at the working electrode allowing for an accurate voltage to be applied there. This setup allows reactions to be performed where an accurate control of the potential is required at the working electrode, as well as the use of analytical electrochemical techniques\textsuperscript{66}.

1.2.2 Cyclic Voltammetry

Cyclic Voltammetry is an electrochemical technique which has become popular in a variety of chemical fields, as it allows the components of a solution to be analysed (provided they are susceptible to redox reactions)\textsuperscript{67}.

Cyclic voltammetry (CV) involves the use of an electrochemical cell, most usually with three electrodes.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{electrochemistry_cell.png}
\caption{3 electrode electrochemistry cell.}
\end{figure}

This setup (see figure 8) consists of a working electrode (often platinum), a reference electrode (e.g. silver/silver chloride) and a counter electrode (a platinum gauze)\textsuperscript{68,69}. The potential of the working electrode is referenced to the reference electrode which has a known potential, allowing the voltage drop between the working electrode and
the species in the solution to be accurately known\textsuperscript{66}. Solutions are usually degassed with an inert gas to remove any oxygen which is dissolved, and the inert gas is kept flowing into the cell during the experiment. This is to remove the redox trace of the oxygen from the cyclic voltammogram, prevent any oxygen from re-dissolving into the solution and prevent oxygen from reacting with any of the redox products\textsuperscript{68,69}.

As indicated above, the working electrode can be altered to suit the reactants being observed. Also it is essential to clean and polish the working electrode (in the case of a metal) to maintain standard conditions at this site\textsuperscript{66}.

Performing CV on a chemical involves the cycling of the potential which is being applied across the CV cell\textsuperscript{67}. This creates a trace of the oxidation and reduction of the active species.

\begin{center}
\begin{tikzpicture}
\begin{axis}[
    title={Figure 9: Example voltammogram.},
    xlabel={Potential (V)},
    ylabel={Current (A)}
]
\end{axis}
\end{tikzpicture}
\end{center}

In a voltammogram (figure 9), current is plotted against the voltage applied which results in the two peaks shown on the trace – the positive one for the oxidation and the negative one for the reduction.
In this thesis two types of electrochemical experiment were used. In the first, electrochemistry was coupled with a direct chemical reaction through the use of silver electrodes and their reaction with bromide ions in the excitable BZ reaction. The second is through the development and use of direct electrochemical control using platinum electrodes to perform the same function as the direct chemical reaction.
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Chapter 2:

Use of Fe(II) Complex Catalysts.
2.1 Introduction

The BZ reaction lends itself to research into oscillating systems due to the fact that it is well studied, with the reaction mechanisms being well defined, and at the same time is easy to follow due to the colour changes shown by its catalyst changing state (between red and blue for the iron based ferroin catalyst). Initial attempts at creating spatial oscillating systems have so far led to the immobilisation of a catalyst into either gels or more recently onto polysulphone membranes. These two forms of immobilisation have allowed redox waves to be generated and the initial analysis of them to occur. As previously mentioned, of these two immobilisation methods membranes have been found to be the easier to produce and use in a repeatable manner, but there are limitations to even this system.

2.1.1 Pattern Formation

Pattern formation is the science which describes mathematical orderly outcomes. Patterns are found throughout nature from spots on a leopard’s back, stripes on a zebra and many more animals, through to ripples found on a sandy beach. In addition to this it can be said that a major characteristic of all living organisms is the degree of orderliness within their component parts\(^1\text{-}^5\). This orderliness is seen through the patterns they generate during their development to produce special arrangements of cells often into tissues and organs\(^1\text{-}^5\).

Pattern formation during cellular development and differentiation is produced by several different mechanisms, with the major difference being between cell to cell interactions which are minimal and those where these interactions are the dominant factor.
If we take three different examples we can show these ranges:

1. In an egg there will not be cell to cell involvement but instead localisation of cytoplasm within the egg will determine the pattern which is initially formed\(^6\).

2. In nematode development cell to cell interactions are involved but are not the dominant effect. Cell division during development produces some asymmetry in the developing embryo and it is this which is the main determining factor in the pattern formation\(^7\).

3. Vertebrate embryos are an example where cell to cell interactions are the dominant mechanism for pattern formation. In these large sections can be removed or rearranged at early stages and yet development still occurs normally\(^8-12\).

This pattern formation from cell to cell interactions is due to chemical diffusion over short distances.

There are few if any random patterns seen during the development of biological organisms, with even areas where they could reasonably be expected showing ordered patterns to the extent that there is a minimum distance between the elements which make them up\(^13-16\). An example of this minimum distance pattern would be the spacing patterns found within the hairs on the surface of insects\(^1,13-16\). More complex spacing patterns which are at the same time frequently found in embryos are stripes. These stripes are a type of pattern which is considered isotropic and in addition to being found in embryos are also within the developed muscles of vertebrates in the form of muscle fibres\(^1,13-16\). Due to this plethora of patterns present in the biological world, methods are needed to study the formation of patterns\(^16\).
model chemical system would be ideal for this due to the comparative ease of setting up and following one when compared to attempting the same for even the most basic of biological systems.

This is where reaction-diffusion systems as famously described by Turing show their usefulness in allowing the easier development of an understanding of biological rhythms and patterns\textsuperscript{17}. Reaction-diffusion systems can be mathematical models which describe the changes in concentrations of one or more substances which are distributed in space\textsuperscript{17,18}. These models take into account several important changes of state including: position and velocity, stresses caused by any elasticities and motions present, the chemical reactions taking place, and the diffusion of the chemical substances taking part in these reactions\textsuperscript{17}. Under suitable choices of these parameters these models will show what occurs when a wave of concentration occurs\textsuperscript{17}. In addition there are chemical reaction-diffusion systems. In these chemical systems, stationary patterns can be formed similar to those patterns seen in chemical oscillating reactions. However in these cases the patterns are fixed rather than constantly changing. In these systems, as in chemical oscillating reactions, there must be a competing positive and negative feedback process allowing the systems to be far from their equilibrium. These are commonly called Turing patterns.

In addition to these classic Turing Patterns (static patterns) which can be generated by reaction-diffusion systems, oscillating reactions like the BZ reaction will produce non-static patterns\textsuperscript{18}. The BZ reaction is able to show spatially uniform steady states with waves propagating away from their sources only to be continually replaced by new waves generated behind the moving ones, as well as being able to show Turing patterns\textsuperscript{18}. As these waves are able to be continually generated and
refreshed they allow for a continuous and controlled pattern formation process to occur.

### 2.1.2 Complexity

Complexity is a term which is ubiquitous, having multiple different meanings dependent on the context in which it is used. This results in it being difficult to pin down an exact meaning. Usual definitions of complexity within science are based around a system e.g. in the field of chemistry a complex system would be one which is sensitive to the initial conditions of the system, and in which small perturbations of said system will result in a change in the evolution of the system. This will be due to the large number of possible interactions which are possible within the system. Complexity within physics deals with the dependence of molecular properties on structures and within chemical engineering complexity is often due to working with and optimising reactions containing dynamic structures. In biology and to a lesser extent chemistry, almost everything which is interesting and researched will be complex.

In any real biological system there will be a vast number of molecules taking part in a multitude of reactions. However in chemical systems the complexity is tailored to the reaction occurring. In order to develop an understanding of these complex systems, models needs to be developed which are able to show complexity and at the same time be understood. This will allow analogies to be determined which can then be applied to the more complex systems which are still not understood. The most common strategies used to consider complexity are to simplify the process being analysed, or use non-analytical methods (i.e. empirical
methodology). However more advanced development within the fields of genomics and proteomics mean a deeper understanding of complexity is now required.

The BZ reaction is well understood in terms of the reaction pathways which are occurring and also shows complexity within these interactions. Also at the macro level this reaction is able to generate patterns which will show complexity within their interactions. This will allow for analysis into complexity to take place should controllable systems which can be analysed be developed.

2.1.3 Similarity

Similarity is the quality or state of being similar through resemblance or likeness with another, in other words it is the degree of symmetry in either the resemblance or analogy between two or more concepts or objects.

Different fields have their own definitions of similarity which uses this general definition:

1. Chemical or molecular similarity is the similarity between chemical elements, molecules or compounds when compared to structural or functional qualities (its effect on a reaction pathway or on biological organisms). The biological effects are also quantified as a biological activity and therefore chemical similarity is important in predicting the effect of a new compound based on similarities to prior ones.

2. Mathematical similarity is usually thought of as the degree of similarity between geometrical objects e.g. both having similar shape in the case of two circular objects.
A similarity matrix is a matrix of scores which express the similarity between two pieces of data\textsuperscript{28-31}. They provide a useful method of showing similarity between objects in an easily identifiable manner. Most often these are used in biology to determine the similarity between proteins due to the complexity in their structures\textsuperscript{28-31}. Generally similarity matrices are utilised by biologists to compare the differences between multiple proteins, often taking the protein data bank file format as their inputs. This allows protein to then be grouped together based on this similarity data based on their structures (often the alpha helices and beta pleated sheet content)\textsuperscript{30,31}.

The Universal Similarity Matrix used in this chapter is a java program which takes two images as an input and provides a value of the similarity between them. It does this by comparing the image size i.e. width and height, along with a value for the number of bytes that make up the image. The program joins the two images left to right before performing these general comparisons. It then uses a compression algorithm to zip the images. This allows it to produce a value for the number of bytes present in each. This value will be characteristic to the content of the images and allows the comparison of number of bytes to define similarity.
2.1.4 Aims:

This chapter will describe the development of methods utilising a controlled BZ reaction for the purpose of pattern generation. This takes place through the use of a multi-electrode setup providing eight initiation sites, along with electrochemical control of the initiation process. This development is shown from the use of literature procedures, extension of this process through the addition of extra electrodes, development of methods to process and analyse the images produced and finally to the development of an improved catalyst to overcome limitations found within the literature procedures.
2.2 Experimental

2.2.1 BZ Reaction Recipes

**Uncontrolled Oscillation Mixture:**

The standard BZ reaction mixture consists of a mixture of all the components in the aqueous phase. There were 4 components of this mixture: sulphuric acid, sodium bromate, malonic acid and ferroin indicator. These were mixed to produce a final concentration in the bulk solution of 0.3M, 0.3M, 0.2M and at least 0.005M respectively.

**Controlled Oscillation Mixture:**

There were two levels of control which can be added to the basic BZ reaction allowing for the introduction of excitability to the system:

1. To add basic control to the oscillations required only the addition of a source of bromide ions to the standard BZ reaction mixture resulting in a solution consisting of the following: sulphuric acid, sodium bromat, malonic acid, potassium bromide and finally ferroin indicator. As described previously silver wire was then used to remove the bromide ions from a small locale within the solution, starting off the reaction.

2. An additional more advanced form of control was delivered through removing the catalyst from the reaction mixture and fixing it onto a surface. This resulted in a BZ reaction solution of: sulphuric acid, sodium bromate, malonic acid, potassium bromide and ammonium sulphate\(^{32}\). Once again silver wire was be used to initiate the reaction.
2.2.2 Membrane Preparation

For the more advanced form of BZ reaction control, derived from previous literature procedures, the catalyst was immobilised onto a polysulphone membrane resulting in a localisation of the iron catalyst\textsuperscript{32,33}. As the ferroin catalyst was not suitable for this process a new catalyst was created during the binding process which could attach to the membrane. This catalyst was:

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{membrane_catalyst.png}
\caption{Membrane catalyst: $[\text{Fe(bathophenanthroline)}_3]^{2+}$}
\end{figure}
The binding process required two different solutions to be created:

1. 5mg of bathophenanthroline dissolved in 5cm$^3$ of glacial acetic acid
2. A 20cm$^3$ mixture of 0.002M Fe(NH$_4$)$_2$(SO$_4$)$_2$ in 0.2M H$_2$SO$_4$ to which 5cm$^3$ of ethanol was added.

Polysulphone membranes were commercially available and purchased from Millipore (Express Plus Membranes, 47mm Diameter, pore size 0.22 μm).

The procedure for creating the membrane with catalyst immobilised onto it was as follows:

![Diagram of membrane preparation]

*Figure 11: Membrane preparation.*
The membrane was carefully dipped into solution 1 and then transferred to a dish containing solution 2 and submerged in this for 2 minutes (figure 11). During the transfer the membrane must be handled carefully to reduce the possibility of defects being introduced. These showed up as areas of greater colouration than was seen over the rest of the membrane surface. This process resulted in the membrane turning pink in colour indicating the catalyst has been formed. The polysulphone membrane with catalyst immobilised onto it was then washed with distilled water and stored under distilled water until it was used.

2.2.3 Electrode Setup and Control

Silver wire can be used to control the BZ reactions through a chemical reaction between bromide found within the BZ reaction mixture and the silver. This reaction took place as soon as the silver wire was brought into contact with the solution and can be used to initiate waves in a membrane bound catalyst. It however was limited in its applications as the wire must be removed from the solution after 30 seconds otherwise spiral wavefronts will be generated instead of a single wavefront.

Control of a single silver wire electrode can be brought about through the use of any electrochemical equipment which has the ability to set a fixed potential and alter this to a different one. In this case an AEW2 analytical electrochemical workstation was utilised.
Figure 12: Single electrode setup.

The reaction was setup with the membrane submerged in the reaction mixture and the silver electrode in contact with the membrane (figure 12)\textsuperscript{32,33}. The silver working electrode was held at a potential of -1.2V to prevent any reaction occurring between it and the bromide ions in solution. When a redox wave was required the potential was changed to +1.3V to allow the silver wire to react with the bromide ions and initiate the reaction. As this equipment was designed for use in cyclic voltammetry it can facilitate the addition of a reference electrode in addition to the working and counter electrode shown in the setup (figure 12).
Development of a multi-electrode setup was relatively simple in principle and moving to an 8 electrode setup required minor modifications to the apparatus, with the general setup being the same as that used for a single electrode (figure 13). As before a petri dish was used to hold the reaction mixture and membrane however the lid was also utilised to hold the 8 silver electrodes in position.

Figure 13: 8 electrode setup (side on).
Figure 14: Top down view of 8 electrode setup.

To facilitate this, 8 holes were drilled into the Petri dish lid in a circle of diameter 3cm. The prior mentioned AEW2 analytical electrochemical workstation could not be used for this setup as it was only designed to have a single working electrode. To overcome this limitation a control box was designed and built by Panagiotis Kapetanopoulos, University of Leeds.
This consisted of a metal control box with 9 ports in the front which allowed the 8 working electrodes and one counter electrode to be connected, along with a power connector and parallel port on the back to facilitate connection to a computer (Figure 15). Control was through the use of a program designed in labview which allowed for timing of the potential alterations to be changed and delays to be added prior to the electrodes being used by the software. The potential changes of holding the silver wire electrodes at -1.2V and switching to +1.3V to initiate were hardwired into the control box.

2.2.4 Image Capture

Image capture was achieved through the use of a Prosilica EC1350C. This was a high resolution 1.4 megapixel camera capable of resolutions up to 1360x1024
and fitted with a Sony ICX205AL CCD sensor which is a ½ inch sensor. It was capable of a frame rate of 18fps at the maximum resolution or 30.8fps at the more usual resolution of 640x480, allowing for enough images to be taken to produce useful videos of reactions. Connection to a computer for image capture was through firewire to provide high enough bandwidth. As this camera doesn’t include an integrated lens a Navitar Zoon 7000e was attached for this purpose. This lens provided a focal range of between 12.5mm and 75mm with an aperture f-number of 1.8.

Image acquisition was controlled through the labview package of software, allowing for a series of images to be saved at a defined time period. Images were acquired in the Portable Network Graphics (.PNG) format which employs lossless compression to provide images of a higher quality than other compression methods (e.g. jpeg) without taking up the same amount of space as a full bitmap image.

2.2.5 Data Processing

Images produced via the EC135OC and Navitar Zoom 7000E were converted into a usable form through the use of the open source program ImageMagick. This was a software suit which has been designed specifically for image manipulation with the ability to specifically create, edit and compose bitmap images. However in addition to this it was able to manipulate many other image file types including PNG, removing the need to interconvert between different compression methods with loss of image quality and data.
The initial command used during the image analysis process to provide edge enhancement was:

- convert -noise 3 -contrast -contrast -contrast -charcoal 3 1.PNG 1.PNG

The main command here was convert. This command allowed for a multitude of options including the conversion between different image formats, resize, blur, crop, despeckle, dither, draw on, flip, join, resample. However only three were important for creating a black and white edge enhanced image. The —noise option was an ability to add or reduce the noise in an image, in our case it was to reduce the noise. The -contrast option allowed the contrast found in an image to be either enhanced or reduced depending on need. These two in combination produced the edge enhancement. The –charcoal option was to simulate charcoal drawing on the image (i.e. turn the image into a high contrast black and white image to allowed for simpler analysis than if the original greyscale images were to be utilised).

Therefore what the above command did was take the image called 1.PNG, reduce the noise in it, run the enhancement of its contrast three times, turn it into a black and white image and resave it over the original image (the last 1.PNG indicated this last part).

The next stage was to remove the background from the image to reduce the amount of superfluous information left in the image. This was done once again with ImageMagick with the following command:

- composite -compose minus background.PNG 1.PNG 1.PNG

The composite command allowed for one image to be overlapped onto another, with the possibility of adding additional effects if chosen during the optional parameters. In our case only the –compose parameter was used. This sets an image composite.
operator, in our case minus was used which subtracted the image called background.PNG from the image 1.PNG and resaved it as 1.PNG. The end result of this was to reduce the effect of having electrodes visible in the images, and at the same time it removed any flaws present in the membrane which would adversely affect any further analysis.

Both of these processes were automated through using batch files to run one command after another on a folder of images.

2.2.6 Similarity Matrix

The universal similarity matrix values produced from the edge enhanced data images generated by the ImageMagick data processing were generated through the use of a program provided by Dr. Natalio Krasnogor from Nottingham University. The program provided was a java file which when run generated a numerical value of the similarity shown between two input images. This was tested out between the different images which were generated in 2.2.5, and against previously obtained pure white and pure black files. This information was placed into a spread sheet to allow for the differences to be shown.

2.2.7 [Fe(bathophenanthroline)$_2$mnt] Synthesis

Synthesis of the new neutral catalyst, [Fe(bathophenanthroline)$_2$mnt], required similar starting materials to the original catalyst.

Bathophenanthroline and Fe(NH$_4$)$_2$(SO$_4$)$_2$ were purchased from Sigma-Aldrich. Na$_2$(mnt) was supplied in house by Mr. Donald Robertson.
0.5g of the bathophenanthroline was added to a flask containing 300ml of acetone and stirred until dissolved. A solution of 0.2085g Fe(NH$_4$)$_2$(SO$_4$)$_2$ in 10ml H$_2$O was then added and left to stir for 30 minutes. At this point the solution turned a deep red colour. 0.23g of Na$_2$(mnt) was dissolved in 25ml of methanol and then added. The mixture was left stirring for a further hour after which the solution turned a much darker red. 200ml of distilled H$_2$O was then added and the resultant mixture left in the fridge for a day. Once a precipitate had formed it was filtered, washed with more distilled water and left to dry (figure 16).

![Neutral catalyst: [Fe(bathophenanthroline)$_2$mnt].](image-url)
The dry catalyst was then re-crystallised to purify it by dissolving in a minimum quantity of chloroform and adding isopropyl alcohol to precipitate out the purified catalyst.

Elemental analysis of [Fe(bathophenanthroline)₂mnt] was as follows:

<table>
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<th>%C</th>
<th>%H</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected Result</td>
<td>72.5</td>
<td>3.7</td>
<td>9.7</td>
</tr>
<tr>
<td>Actual Result</td>
<td>66.12</td>
<td>2.7</td>
<td>9.97</td>
</tr>
</tbody>
</table>

Mass spectrometry of the catalyst via positive electrospray ionisation resulted in peaks at a m/z of 860 corresponding to the catalyst as well as one at 1720 corresponding to two catalyst units. A major peak was present at a m/z ratio of 526 with a relative abundance of 100 which corresponded to the catalyst missing one of its bathophenanthroline subunits, with a much smaller peak at around a m/z of 333 with a relative abundance of 10 which corresponded to a bathophenanthroline subunit.

NMR was performed; however a paramagnetic species was shown to be present along with the [Fe(bathophenanthroline)₂mnt]. This resulted in the NMR only being useful to indicate that the catalyst had been prepared, with the effects of the paramagnetic side product dominating the resulting spectrum.
2.2.8 Membrane Preparation with [Fe(bathophenanthroline)$_2$mnt]

The [Fe(bathophenanthroline)$_2$mnt] catalyst was not prepared in situ as was the case for the [Fe(bathophenanthroline)$_3$]$^{2+}$ catalyst (section 2.2.2), therefore a new binding procedure was created.

10mg of [Fe(bathophenanthroline)$_2$mnt] was dissolved in 10ml of acetone. 100ml of methanol was added to a glass dish and the 10ml of catalyst dissolved in acetone was mixed with this. One of the polysulphone membranes was carefully submerged within this solution and left for 24 hours. The membrane containing bound catalyst was then removed from the solution, rinsed with distilled water and stored under more distilled water until used.

2.2.9 Protocol for confirming [Fe(bathophenanthroline)$_2$mnt] can replace [Fe(bathophenanthroline)$_3$]$^{2+}$

1. Testing Binding: A solution of the BZ reaction mixture as used in controlled wave generation, was made (described in 2.2.1). One membrane with [Fe(bathophenanthroline)$_2$mnt] bound was placed in a petri dish and the BZ reaction mixture poured over. The membrane was left overnight in this mixture and then placed into a fresh solution of the BZ reaction mixture the next morning, and left until the following morning to give a total time under the BZ reaction mixture of 48 hours. This was repeated with a membrane containing the [Fe(bathophenanthroline)$_3$]$^{2+}$ catalyst.
2. **Testing initiation:** A solution of BZ reaction mixture as used in part 1 above was made up and poured into a petri dish containing a membrane with [Fe(bathophenanthroline)$_2$mnt] bound. A silver wire was used to generate redox waves and compare with those from a membrane with [Fe(bathophenanthroline)$_3$]$^{2+}$ bound to it.

2.3 **Results and Discussion**

2.3.1 **[Fe(bathophenanthroline)$_3$]$^{2+}$ Single Initiation Site**

Single initiation can take place either through use of electrochemical control or manually. Manual control involves bringing a piece of silver wire into contact with the solution covered membrane for long enough to initiate a wave after which it is removed. In both cases subtraction of a background was not undertaken as it was not deemed to be necessary since the electrode could be moved out from under the camera once a single wave had been initiated.
The process of creating a catalyst which is bound to a polysulphone membrane is a valuable technique which has been previously used to provide a thin layer of catalyst allowing for the BZ reaction to generate spatial oscillations instead of the more readily generated temporal oscillations\(^{31,32}\). Under ideal conditions it is possible to create a membrane which has a high enough amount of catalyst bound so that waves initiated on it are clearly visible. An example of this can be seen where a single redox wave has been produced from one central initiation point (figure 17), and is emanating from the central locus where the electrode initially resided. In this idealised situation the membrane has no defects present and the silver wire used to initiate the redox wave has been removed from view before the image was captured. However, whilst the redox wave is visible the contrast is not enough for further analysis and so the contrast needs to be enhanced through the use of the edge enhancement command described in 2.2.5 (figure 18).
Figure 18: Single initiation after edge enhancement.

The resulting image after edge enhancement (figure 18) shows a greater degree of contrast at the wavefront than was visible previously (figure 17). This enhancement would allow for further analysis to be conducted by way of a universal similarity matrix provided a selection of images with different patterns has been produced. However no useful data pertaining to pattern generation can be generated from just use of a single initiation point. In addition artefacts are visible within this image due to defects from the catalyst binding process described in 2.2.2.

However there are problems with the processes used to get to this stage of image quality. The production of catalyst which is bound to membranes (described in 2.2.2) has multiple variables which affect the quality of the membranes and thereby affect the reproducibility of the experimental process. Once the membranes are initially submerged into solution 1 (figure 11) the edges rapidly curl together. If this is not prevented then the curling affects the ability of the catalyst to
be created properly since there will be a reduced amount of membrane surface in contact with the second solution. Therefore if this happens the membranes must be uncurled. However these membranes show an increase in amount of catalyst bound along the central curved sections even if they are allowed extra time in the initial solution. To prevent the curling from taking place then the membranes must be held flat under the first solution. This can also add defects to the evenness of the catalyst binding as the sections of the membrane which were used to hold it flat will either not be exposed to the first solution, or not be exposed for the same amount of time thereby resulting in a similar problem with areas containing a lack of catalyst. The only way to overcome these problems is to reduce the amount of time the membranes spend in the initial solution to 2 seconds, thereby reducing the chance of the membranes beginning to curl before they are transferred to and immersed in the second solution. However this reduces the amount of catalyst bound to the membrane and thereby reduces the contrast of images taken. This reduced contrast was initially tackled through the edge enhancement algorithm (figure 18) which showed great promise when used with single initiation points.

2.3.2 Multiple Initiation Sites with $[\text{Fe(bathophenanthroline)}_3]^{2+}$

Production of patterned images as described in 2.2.3 through the use of between 1 and 8 electrodes results in images which are more complex; however there are difficulties in using the data.
Figure 19: Multi-electrode image.

When a multi-electrode image was captured it was not possible to constantly remove and replace the electrodes so they are visible on the images (figure 19). Also the waves were not as easily detectable on these images. This is due in part to the difficulty in producing membranes with a high enough concentration of catalyst, but also due to the added layer of plastic between the camera lens and the membrane. This plastic is the lid used to hold the electrodes in fixed positions.

The difficulty of producing membranes with a high enough concentration of catalyst is compounded by the fact that these membranes do not always bind the same quantity of catalyst even when treated in the same way. This is due in no small part to the membranes being commercially available polysulphone membranes which are not designed specifically as a substrate for catalysts. This allows the company to slightly modify the batches without it affecting their primary purpose as filters and
yet shows up when utilised as catalyst binding substrates. In some cases the membranes manage to bind only a very small quantity of the catalyst, which is judged by the deepness of the colouration of the membranes. They can range from containing almost no colouration and therefore being of no use, to not having uniform coverage of catalyst. In the case of low concentration of catalyst bound it was initially hoped that edge enhancement would enhance the contrast to a great enough degree to provide a usable solution.

Figure 20: Edge enhancement of figure 19.

When edge enhanced the electrodes show up as more defined and interfere with any further analysis, along with the defects which are almost always present in the production of membranes by the original method (figure 20). Even though edge enhancement has been performed the wavefronts are not discernable. Therefore the
final step in increasing the contrast in these images is to subtract a background image from the one above.

A background image was taken when the membrane and electrodes were setup, but there had been no initiation of any waves (figure 21). Due to the fact that there are no waves on the background image membrane, only the waves should be left visible with any unwanted artefacts being removed when it is subtracted from the experimental image.

The original membrane bound catalyst setup using single initiation results in fairly high quality images that can be generated (figure 17) which can then be edge enhanced (figure 18). As previously stated, moving onto a multiple electrode setup results in degradation in the image quality (figure 19) resulting in the need to enhance the images further. This enhancement process is a double edged sword,
with the redox waves which are present will be enhanced, and at the same time so will the outlines of the electrodes, and any defects which are present on the membrane. To remove these, a background image is also edge enhanced (figure 21) and is subtracted from the data containing image which should result in just the waves being visible (figure 22).

Figure 22: Figure 20 after figure 21 has been subtracted.

A problem with subtracting background images is that much greater initial contrast is needed in the original image between the colours of the reduced and oxidised catalyst; otherwise it is difficult to observe the waves.

The contrast from the catalyst used is not great enough to be able to detect the waves to a high enough degree for any further analysis to occur (figure 22). In this image there are very faint waves which are only discernable to any reliable degree if the original membrane had been watched closely during the data collection. However
in the image after processing (figure 22), there are a multitude of slight changes in contrast which are not due to any waves being present.

Once a series of images similar to figure 21 have been produced they can be run through the universal similarity matrix program as described in section 2.2.6.

2.3.3 Similarity

In order to obtain a value denoting the degree of similarity between images produce by edge enhancement with background removal (an example being figure 20), the universal similarity matrix was run and the values collected were entered into a spreadsheet. As the inputs are of a binary nature, being only on or off we decided to name the different images based on this binary format with eight inputs of either 1 or 0.

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</tr>
</tbody>
</table>

Table 1: Values obtained from Universal Similarity Matrix.

In table 1 the images are notified by the number of electrodes firing and their firing order, therefore 1 indicates just the first electrode firing and each subsequent 1 indicates the next electrode in the sequence up to a total of 8 (noted as 11111111).

The universal similarity matrix program generates a number which can be used to indicate the similarity between images after edge enhancement and background
subtraction (table 1). The ones shown in red are where the universal similarity matrix was run on the same image twice, and this acts as a control to indicate what sort of value should be obtained for two identical images.

It was initially hoped that the universal similarity matrix would be able to overcome the shortcomings of membranes treated in this manner, and as such a java program was provided by Dr. Natalio Krasnogor from Nottingham University which calculates the universal similarity matrix values was used on a range of different runs. An example of these results is detailed above (table 1) and shows the trouble in even more detail. In this case the universal similarity matrix is unable to differentiate sufficiently between the different images, to the extent that the differences seen are very close to the values obtained when the same image is run against itself.

Another problem with the membranes comes from using commercially available polysulphone membranes. These are not designed as a structure to bind catalysts to but are instead used as filter discs. Due to this, different batches of membranes result in a multitude of problems when attempting to bind catalyst to them. These range from simply requiring longer times within each of the solutions during the binding process previously described (figure 11), to defective binding. The defective binding shows up most often as areas on the membranes where excessive amounts of catalyst have bound, creating a small area of highly concentrated colouration. These highly coloured areas are prone to generation of spirals instead of the controlled redox wavefronts which are needed for pattern generation. In addition these spirals end up taking over the whole surface of the membrane making it unusable until the reaction has been reset and begun anew.
In addition to the problems with getting the catalyst to bind to the membranes, and those defects found between different batches of polysulphone membranes it was also found that although the catalyst is initially bound to the membrane it slowly leached from the membrane during experiments. This happens within the lifetime of an experimental run and therefore also degrades the image contrast. As the catalyst slowly leaches out the colouration of the membranes begins to fade, resulting in the images collected at the start of a run having a higher contrast than those collected nearer to the end. Since the background images are collected at the beginning of an experiment this will also affect how the background removal works. If too much catalyst has leached out of the membrane near the end of the experimental run then the background image may end up being darker than the experimental image, resulting in even worse contrast.

To overcome these difficulties a new catalyst was needed; one which will bind to the same polysulphone membrane and have a greater contrast than the original catalyst. In addition it needs to be a neutral compound to reduce or remove the leaching effect resulting in a longer lifetime for the membranes. These needs have resulted in our development and synthesis of a new neutral catalyst using most of the same components as the original catalyst with the addition of a maleonitriledithiolate subunit to create the overall neutral effect (figure 16). Two bathophenanthroline units were used as these are already able to bind to the polysulphone membrane as shown by the fact that the original catalyst contains them (figure 10).
2.3.4 Characterisation of [Fe(bathophenanthroline)$_2$mnt]

Initial analysis to determine that [Fe(bathophenanthroline)$_2$mnt] had been synthesised was via mass spectrometry and elemental analysis (section 2.2.7). The elemental analysis indicated that the catalyst has been produced but with either a side-product, or some solvent still within the structure. Mass spectrometry shows the catalyst is present at its correct m/z ratio, along with a major peak for the catalyst with one bathophenanthroline subunit missing.

![Figure 23: UV/Vis of a 75μM sample of [Fe(bathophenanthroline)$_2$mnt] in acetone](image)

$\lambda_{max} = 532\text{nm}$, $\varepsilon = 11300\text{M}^{-1}\text{cm}^{-1}$. 
Figure 24: UV/Vis of a sample of [Fe(bathophenanthroline)_3]^2+.

Figure 25: CV of [Fe(bathophenanthroline)_2mnt] in acetonitrile with scan rates from 0.1 to 1.0 Vs⁻¹.
Figure 26: CV of $[\text{Fe(bathophenanthroline)}_3]^{2+}$ in a mixture of acetone, methanol and water with scan rates from 0.1 to 1.0 Vs$^{-1}$

Further characterisation of the compound via CV (figure 25) and uv/vis (figure 23) show that an active molecule has been produced, with similar characteristics to the original $[\text{Fe(bathophenanthroline)}_3]^{2+}$ catalyst (figure 24 and figure 26).

With $[\text{Fe(bathophenanthroline)}_2\text{mnt}]$ indicated to be a suitable compound to replace $[\text{Fe(bathophenanthroline)}_3]^{2+}$ the next major tests are binding of the catalyst to a membrane and testing its ability to stay bound to the membranes when covered in BZ reaction mixture, as well as the ability to initiate a redox wave with a silver wire.
Figure 27: Single initiation site \([Fe(bathophenanthroline)_{2}mnt]\)

As previously mentioned in 2.3.1 initiation of a redox wave can be brought about in membranes containing the \([Fe(bathophenanthroline)_{3}]^{2+}\) by bringing a piece of silver wire into contact with their surfaces for 30 seconds. Therefore the test needed to determine whether \([Fe(bathophenanthroline)_{2}mnt]\) will act as a replacement catalyst is to cause an initiation with this method. The results of these tests were positive with an example being shown in figure 27, with the waves produced from \([Fe(bathophenanthroline)_{3}mnt]\) progressing across the membrane at the same rate as those from \([Fe(bathophenanthroline)_{3}]^{2+}\).

Further to this the ability of \([Fe(bathophenanthroline)_{2}mnt]\) to remain bound to the membranes was also tested. This occurred over 48 hours, with the solution being replaced after 24 hours. The membranes were shown to still have a red colouration and after refreshing the solution were still able to be initiated in
comparison with the \([\text{Fe(bathophenanthroline)}_3]^2^+\) membranes which had no
colouration left after 12 hours use.

![Figure 28: Edge enhancement of figure 27.](image)

Edge enhancement of a single initiation with \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\)
shows similar improvements as are present when this is carried out with the
\([\text{Fe(bathophenanthroline)}_3]^2^+\) catalyst (figure 18). However as this new catalyst is far
easier to bind in a uniform way with a deeper colouration it lends itself to being a
much better-quality catalyst. Added to this is the fact that the
\([\text{Fe(bathophenanthroline)}_3]^2^+\) leeches out of the membranes readily when left under
the BZ reaction mixture whereas the \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) is much harder
to remove hence the new catalyst proved to be a superior replacement.
2.4 Conclusion

The work described in this chapter charts the development of a literature based method of controlling the BZ reaction from the single electrode format. This was extended to increase the system to eight electrodes which was done to allow the system to be utilised for research into pattern formation and complexity. This proved partially fruitless with the bound \([\text{Fe(bathophenanthroline)}_3]^{2+}\) providing too low a contrast for any analysis into these fields.

Further development of the system led to the synthesis of \([\text{Fe(bathophenanthroline)}_2\text{mnt}],\) a neutral catalytic species to replace the original literature based \([\text{Fe(bathophenanthroline)}_3]^{2+}\) and thereby improve the system to the point where it could be reliably used for further work.
2.5 References


Chapter 3:

[Fe(bathophenanthroline)$_2$mnt] and its Use in Pattern Generation and Analysis.
3.1 Introduction

As was mentioned in the introduction to Chapter 2, the BZ system is useful for research into oscillating systems due to the fact that it has been so well studied. However during the course of using and modifying the established system we have found the system to be lacking in certain areas which are needed. These limitations were the contrast of the images produced, and the lifetime of the membranes.

The new catalyst, [Fe(bathophenanthroline)\(_2\)mnt], characterised at the end of Chapter 2 mostly overcomes these two problems. When bound to membranes the [Fe(bathophenanthroline)\(_2\)mnt] has a deeper colour than the previously used catalyst, [Fe(bathophenanthroline)\(_3\)]\(^{2+}\), and therefore has improved the contrast of the images being collected. In addition as shown in the initial test of [Fe(bathophenanthroline)\(_2\)mnt] (Chapter 2, section 2.3.4), it remains bound within the membranes for a much longer duration than [Fe(bathophenanthroline)\(_3\)]\(^{2+}\).

With these two limitations vastly improved, a method is needed to convert the data generated by the Universal Similarity Matrix into an easier to understand visible format.

3.1.1 Clustering

Analysis of complex data sets relies on identifying underlying group structures within the data most commonly through a process known as clustering. Clustering is a method of assigning a set of observations into subsets such that the groupings show similarity. It is a method of statistical data analysis which is utilised in many fields including bioinformatics, data mining and image analysis.
Figure 28: The three main steps in data analysis through clustering.

The three main stages in analysing data through clustering are shown in figure 28. The initial step consists of pre-processing the data so that it is in a usable format that it can be separated into clusters of similarity. This stage refers to the use of a Universal Similarity Matrix in this thesis (Chapter 2, section 2.2.6), along with any further preparation of the data (image pre-processing detailed in Chapter 2, section 2.2.5). The resulting output of Stage 1 will be a list of numbers of the similarity between each pair of images. The second stage of feature extraction relates to running the data through a clustering algorithm to allow for any similarities within the data sets to be grouped together. The output from the 2nd stage will be a dendrogram. The final stage is to validate the results and check that they do relate to the original data. Most clustering algorithms have not been validated to any great degree resulting in a hit and miss situation for the end user when choosing the one
most suited to their task. This requires the end user of clustering servers to validate their own data after each run as the clustering servers will pick up on any miniscule difference in the input data whether it is statistically relevant or not. In our case this would be through finding the point of greatest spread of similarities.

Data clustering is often hierarchical with the algorithms being either agglomerative or divisive. Hierarchical clustering creates a hierarchy of the input clusters by generating a dendrogram, which is a similarity tree generated from multiple data sets. For a divisive dendrogram, the clustering algorithm starts with all the observations together and successively breaks off individual or groups of these observations to generate a tree which ends up with all the observations separated and linking back through the dendrogram branches to the points where there were degrees of similarity. So in the case of five different observations it would start with all five on the top branch, as at this point they are all similar, then it would proceed to separate each of the five one at a time to generate a tree which could be traced back to show their similarity. The opposite of this is called a combinatorial dendrogram which begins with each sample in a cluster of its own and proceeds to combine them together, working in the opposite way to the divisive dendrogram described previously. This generates hierarchical families of observations similar to how biologists classify organisms from domains all the way through to specific species.
3.2.1 Aims:

This chapter will present an example of a real chemical system which can be utilised for the purpose of pattern generation and analysis. Although the system is conceptually simple, the work for the first time demonstrates a methodology by which these patterns can be reproducibly generated and analysed through the use of the BZ reaction.
3.2 Experimental

3.2.1 [Fe(bathophenanthroline)$_2$mnt] Membrane Preparation

Detailed previously in Chapter 2 within section 2.2.8.

3.2.2 8 Electrode Setup for [Fe(bathophenanthroline)$_2$mnt] Membranes and Image Capture

Detailed previously in Chapter 2 within section 2.2.3 and 2.2.4 but using [Fe(bathophenanthroline)$_2$mnt]-treated membranes.

3.2.3 Universal Similarity Matrix and Clustering the data

The method of running a Universal Similarity Matrix is detailed previously in Chapter 2 within section 2.2.6.

An all ready developed clustering method was used to generate dendrograms from the similarity matrix data. This is the clustering server located at [http://www2.biology.ualberta.ca/jbrzusto/cluster.php](http://www2.biology.ualberta.ca/jbrzusto/cluster.php)

This clustering server uses only hierarchical and combinatorial methods. The one chosen for use was the unweighted arithmetic average, as this works on the average distances between the values for each cluster.
3.3 Results and Discussion

3.3.1 Multiple Initiation Sites

Figure 29: 1st out of the eight circularly arranged electrodes firing.

Figure 30: Edge enhancement of figure 29.

Figure 31: Background Image taken before the initiation shown in figure 29.
Figure 32: Background after edge enhancement of figure 31.

Figure 33: Figure 29 after edge enhancement and background subtraction.

Figure 34: Section of Figure 31 covering the centre of the membrane.
Chapter 2 described the process whereby initially \([\text{Fe(bathophenanthroline)}_3]^{2+}\) was bound to polysulphone membranes for use in image generation and analysis. These images were analysed by edge enhancing and removing their backgrounds but were found to not be usable due partly to problems associated with the binding affinity of this charged catalyst to the polysulphone membranes. Overcoming this occurred through the production of the new \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) catalyst which along with its improved binding affinity to the polysulphone membranes providing an improved contrast also reduced the problem of small areas of excessive binding found when using \([\text{Fe(bathophenanthroline)}_3]^{2+}\).

As can be seen in figure 29 a single initiation site is more easily visible with \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) using the eight electrode setup than was the case with the \([\text{Fe(bathophenanthroline)}_3]^{2+}\) (figure 19). However to further improve the image contrast the electrodes, along with the plastic petri dish lid used to hold them in position, have been removed from the area prior to image capture. The removal of these sections of the apparatus increased the time to run an experiment and also meant that the membranes are no longer fixed in an identical position between experimental runs. Edge enhancement was still needed even with these items being removed from the image area and the improvements were clearly visible when figure 29 was enhanced by this method, clearly showing the wave emanating from its initiation point where the silver electrode was present prior to its removal (figure 30). With the modifications to the setup causing the membrane to move slightly between runs background removal was now even more essential than with the previous setup. Blemishes present on the membrane were now more pronounced in any further
analysis as they were moving position therefore background removal was still needed, and this also serves to further enhance the contrast between the wave and the rest of the image.

To allow for background removal images were captured at the end of each experimental with an example being figure 31. These were then edge enhanced to produce the background image shown in figure 32 ready for background removal to take place. As mentioned previously, this process further enhanced the wave making it the most defined section of the image and at the same time reduce the visibility of the membrane edge making it much fainter (figure 33).

Due to the added step of removing the electrodes along with their supporting petri dish lid from the experimental setup each image no longer has the initiation points in an identical position. It was therefore necessary to crop the images generated in the experiment so that only the central section was used and hence produce images which could be compared. This was done by taking the initial 640x480 images and modifying it so that to the 220x240 pixels which corresponded to only the central section where the waves would congregate was left. This resulted in a much smaller image for further comparison (figure 34).
Figure 35: Spread of clustering data as time increases.

The experiments were run for 20 minutes to ensure that images were collected showing the wavefronts at all the different stages on the membrane, from initiation to the point where they reached the opposite edge of the polysulphone membrane. Due to this, a method of determining which time point of the experiment was the most useful for further analysis was needed. In order to determine this, the universal similarity matrix was run over the images at multiple different time points ranging from the start of the experiment to 800 seconds along with those occurring every 100 seconds. The data generated from this were run through the clustering server as detailed in section 3.2.3, and this clustering server produced a dendrogram.
for each of the time periods along with a value of the similarity shown between each branch. The spread of the similarities between the two furthest branches was then plotted against the time point at which it occurred and turned into figure 35.

The plot of this spread of similarities clearly shows a peak of the spread occurring at the 300 second time point, which corresponds with an increase in the separation of the clusters up until this point. This increase was due to the differences between the images occurring up until this time point increasing, owing to a larger occurrence of waves being present in the images at the 300 second time point. The greater occurrence of waves correlates with the progression of waves across the polysulphone membrane and their arrival at the central 220x240 pixel section chosen for analysis. The spread proceeds to fall off rapidly following this time point due to the waves progressing outside of this central section, or due to the destructive interference occurring when waves collide with each other. Therefore we can ascertain that the 300 second time point contained the most relevant images for further analysis. The greater spread of similarities at this time point indicates that there was the greatest difference between the most different images.
Comparison of figure 36 with figure 37 and figure 44 clearly indicates these points. These three images are for all eight of the electrodes firing at three progressively later time points. Figure 36 shows no waves present as this is taken only 10 seconds after initiation and therefore the waves have yet to travel far enough from their initiation points and reach the central section of the membrane. Figure 37 is from 200 seconds after initiation and clearly shows the eight waves have begun to arrive at the central section. Figure 44 is from 300 seconds after initiation and once again shows the waves visible in the image, however in this case they are now nearer to the centre, to the point where they are close to coming fully into contact with their opposites creating destructive interference and disappearing from the image. This therefore further indicates that the images from 300 seconds after initiation are the most useful for further analysis, and that the computational methods that have been used were able to pick up on these differences.
Figure 38: 11000000 after edge enhancement and background subtraction after 300 seconds.

Figure 39: 11100000 after edge enhancement and background subtraction after 300 seconds.

Figure 40: 11110000 after edge enhancement and background subtraction after 300 seconds.

Figure 41: 11111000 edge enhancement and background subtraction after 300 seconds.
Figure 42: 11111100 after edge enhancement and background subtraction after 300 seconds.

Figure 43: 11111110 after edge enhancement and background subtraction after 300 seconds.

Figure 44: 11111111 after edge enhancement and background subtraction after 300 seconds.

Figure 45: 10100000 after edge enhancement and background subtraction after 300 seconds.
Figure 46: 10010000 after edge enhancement and background subtraction after 300 seconds.

Figure 47: 10101000 after edge enhancement and background subtraction after 300 seconds.

Figure 48: 10101010 after edge enhancement and background subtraction after 300 seconds.

Figure 49: 10110000 after edge enhancement and background subtraction after 300 seconds.
Figure 50: 11101000 after edge enhancement and background subtraction after 300 seconds.

Figure 51: 11110100 after edge enhancement and background subtraction after 300 seconds.

Figure 52: 10101111 after edge enhancement and background subtraction after 300 seconds.

Figure 53: 11101010 after edge enhancement and background subtraction after 300 seconds.
Repeating the process used for figures 29-34 for the remainder of the electrode combinations generated the images shown in figure 37 through to figure 54. These images once again show the central section of the membrane after the waves have arrived, generating different images for analysis due to the different wavefronts meeting.

Figure 34 and figures 37 through to figure 44 show the images generated from increasing the number of electrodes firing from one through to all eight. The images shown in figure 45 through to figure 54 show the more complex combinations of electrodes firing where for example in figure 54 the 1st, 3rd, 5th and 6th electrodes are initiated. The different patterns generated by all these different combinations are clearly visible by looking at the images, and at the same time show a certain degree of similarity as all they consist of is a different number of whiter sections due to the different numbers of wavefronts which are visible and heading.
towards the central section. At the same time there are differences visible in that there are different numbers of the white wavefronts and they are in different sections of the image. These should be picked up in a computational methodology. All of the images shown from figure 29 through figure 34 and figure 38 to figure 54 are taken from a time of 300 seconds into the experiment.

The real test of similarity from a scientific stand point is a more statistical method which generates numbers which can be compared. This is where the Universal Similarity Matrix comes into use. As described in Chapter 2 section 2.2.6 the Universal Similarity Matrix is a program which allows two images to be compared, generating a number anywhere from 0 to 1 denoting the similarity between them. Running the images in figure 34 and figure 37 through to 54 through the Universal Similarity Matrix produced numbers showing above 90% similarity between all the images which is expected as they are all very similar, with the only differences due to an increasing number of wavefronts.

The data generated from the Universal Similarity Matrix was then put through the clustering server as detailed in 3.2.3 generating a hierarchical divisive dendrogram.
Figure 55: Hierarchical dendrogram for images at 300 seconds showing groupings of images based on similarity between them.
Figure 56: Hierarchical dendrogram for images at 200 seconds.
Figure 57: Hierarchical dendrogram for images at 10 seconds.

The dendrogram shown in figure 55 splits the images based on their similarity to one another for the previously shown images. The dendrograms shown in figure 56 and 57 were generated by the same computational method as figure 55 but at earlier time periods of the experiment (200 and 10 seconds respectively).

Figure 35 illustrates that the spread of similarities exposed in the data from figure 56 and figure 57 is similar, with the spread in figure 56 being slightly higher as indicated by the point on the graph corresponding to this. This can be seen in the dendrograms themselves with figure 57 showing the images forming two main sub-
branches from one another with a couple of images forming small sub-clusters. However the total spread in this dendrogram is only a fraction of a percent indicating that even though the clustering software is able to generate these groupings there is a great degree of similarity between the images. Figure 56's dendrogram shows a similar number of groupings being formed, with the image for 11000000 and 11111000 forming their own grouping at the start of the dendrogram and the image for 10000000 being split off next. The remaining images are all grouping off the same main branch indicating a greater degree of similarity between themselves than there is between this group and the earlier three, but at the same time showing limited groupings between themselves. Once again the total spread of similarities is very small, being only slightly similar to that shown in figure 57, and this can be seen on figure 35 as the spread for these two images are the initial and third points. This does indicate a limitation in the clustering software being used, that it will pick up on very small changes in the Universal Similarity Matrix values generated and generate clustering. This is why the spread is needed to ascertain if there is much of a degree of difference in the similarities between the two most extreme branches before it can be decided if the dendrogram contains any useful information.

Figure 55 however shows the dendrogram generated from the data with the largest degree of spread, and with it being approximately twice as great as the spread from the next highest point on the graph it should and does contain useful information. This dendrogram contains 5 main clusters of images. The first two are shown to the top right of figure 55, with images 11111110 and 10000000 being clustered together, and images 11111100 and 11000000 forming the second cluster. This means that the clustering server has decided that the images in these two groups
can be said to be similar, and at the same time the two groups are similar to each other since they link on close branches. However this does show a limitation with the technique as these images should on first impressions not be that similar. This limitation is most likely brought about by the clustering server putting those images which are least similar to the rest out of the way. The next main grouping is of the three images to the top left; 10100000, 11101000, and 11110000. The final two clusters of images are 1110101, 11111111, and 11110000 in one group linked by a branch to the final cluster consisting of the 5 images; 11110100, 10110000, 10101100, 10101000, and 10010000. The final two clusters of images are also linked off a single branch, indicating that they show a greater degree of similarity to each other than to those outside of the branch.

3.4 Conclusion

The work detailed in this chapter utilises the improvements developed in the membrane bound BZ reaction through the use of \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) as the catalyst to generate multiple images for analysis. Prior research with the BZ reaction and membrane bound catalyst has gone only as far as single electrode control. We have improved this system to the point where eight electrodes are now utilised for initiation. This expansion of the system to multiple inputs has allowed for the novel use of this system in the field of pattern generation, capture and the subsequent analysis of these patterns. Longevity has been greatly improved through the development of \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) to the point where the system can
now be run for extended periods as this catalyst remains bound for much longer (in excess of 48 hours compared to under half a day for \([\text{Fe(bathophenanthroline)}_3]^{2+}\)). These developments provide a possible method for the analysis of similarity in biological systems through producing a model system which will act in a similar way to the much more complex biological ones, along with the opportunity to attempt to reverse the system.
3.5 References


Chapter 4:

Platinum, Gold and TiO$_2$. 
4.1 Introduction

In Chapter 3 we detailed the use of [Fe(bathophenanthroline)₂mnt] as a method of improving the image contrast along with the lifetimes of the membranes. However the use of silver electrodes will result in AgBr deposition onto the membrane, below the silver wire electrodes resulting in a section of the membranes where initiation is unable to occur. This deposition slowly builds up with subsequent initiations. Moving to an inert electrode which does not rely on a direct chemical reaction should remove the problems produced by the depositing of AgBr, thereby increasing the lifetime of the reaction systems further than that already obtained with the development of the new catalyst in Chapter 3.

In addition to this the commercially purchased membranes still suffer from alteration between production batches which results in minor modifications of the binding protocol between them, as mentioned in Chapter 2. Therefore in this chapter we have detailed novel research into the use of TiO₂ as a replacement substrate for commercial membranes.

4.1.1 Alternate Substrates

The BZ reaction can be broadly split into four variants based on the substrate used for the catalyst.

1. The basic setup which utilised no substrate with the catalyst in solution along with the BZ reactants. This is a very limited setup for further analysis as the bulk solutions are difficult to control, mostly showing either bulk oscillations or chaotic spiral generation².
2. Gels can be utilised as a way of holding the BZ reaction's catalyst in a location and increasing the ability to generate patterns. Gels are often defined as crosslinked polymer networks which will swell when they absorb a large amount of solvent\(^3\). They will change volume reversibly when their environments are altered e.g. changing the solvent, temperature or pH\(^3\). Changes within gels can be cyclical in that changes in their volumes by environmental alterations are not permanent and will reverse when the conditions are reset\(^2,4\). This allows for their use in a multitude of areas including biosensors, cell cultures and substrates for catalysts\(^3,4\). Gels also bring additional benefits to the BZ reaction over utilising the standard systems which leave the catalyst in solution:

- Hydrodynamic convection is eliminated. In aqueous systems there is turbulence due to interactions at the surface of the solution with its environment due to evaporation\(^5,6\). This can result in spatially stationary structures which will bifurcate into much more complex patterns\(^6\).
- A gel matrix allows not only for the catalyst to be immobilised, but also for the immobilisation of the other chemical species utilised in the reaction\(^5\). In addition other substances can be introduced to provide additional affects, for example inhibitors.
- Concentration gradients can be setup in gel systems, along with the use of continuous flow stirred tank reactors allowing for the maintenance of the concentration of the starting species\(^7\). With the
catalyst fixed in a gel, these flow systems can be utilised without affecting the patterns being formed\textsuperscript{7}.

- Use of a gel substrate removes the possibility of bubbles forming during the reaction and adversely affecting the generated patterns\textsuperscript{12,13}. The bubbles can still form in the solution but will be unable to directly interact with the catalyst as this is held in the gel substrate and so protected\textsuperscript{12,13}.

3. Cation exchange beads are becoming a more popular substrate due to the ease of use over gel based systems, being readily available from multiple corporations negating the difficulties of in-house gel formation. These bead systems also allow for a setup which contains multiple catalyst bound substrates within the same experimental system. Due to this, use of these beads with a BZ catalyst bound allows for coupling of oscillations between a large group which can interact at their natural oscillating frequency\textsuperscript{14-19}. This is through the use of a light sensitive version of the BZ catalyst. A group of beads with catalyst bound to them can be placed into a petri dish and bathed in BZ reaction solution\textsuperscript{14-19}. Activation of a single or small group of the beads will result in a wave travelling through the population, with the neighbouring beads being coupled to each other by the diffusion of chemicals between them\textsuperscript{14-19}. This produces a system which begins to mimic the synchronisations found throughout many biological systems including that occurring between neurons in circadian rhythms and conditions such as Parkinson’s disease\textsuperscript{15,16}. Additionally these synchronisations will allow for models into disease of the
heart to be studied which are due to problems occurring in the Sino-Atrial Node of the heart, resulting in arrhythmias\textsuperscript{17}.

4. Polysulphone membranes are also utilised as a generic substrate for the BZ reaction catalysts. They provide a cheap and easily accessible substrate which can readily bind ferroin based BZ reaction catalysts, providing a thin pseudo two dimensional layer of catalyst for pattern generation\textsuperscript{20,21}.

4.1.2 Limitations of Membrane System

The benefits of a membrane system are that it is a cheap, off-the-shelf substrate which can be utilised with ferroin based BZ reaction catalysts\textsuperscript{20,21}. This made a perfect choice for beginning research into patterns generated by BZ wave propagation and annihilation. However as the membranes are produced by companies in batches there are minor differences between them. These are not vital to the original purpose of the membranes, in the case of the polysulphone membranes utilised in this thesis as filter discs, however it does affect the production of reproducible membrane bound catalysts for the purpose of pattern generation. These defects show themselves as either the general inability of the membrane to take up an adequate amount of the catalyst, shown by a very pale pink colouration instead of the deeper red usually generated, or more often as areas on the membranes which bind a very high concentration of catalyst when compared to the rest of that membrane. The production of pale membranes causes practical difficulty, but can be worked around by altering the times spent in the catalyst solution during the binding process.

The defects which cause areas to bind a large concentration of catalyst in a small section are more problematic. These areas will generate spirals frequently
which results in a membrane which is unusable for pattern generation as spirals take
over the membrane surface until the reaction is reset. Because of these problems, a
more user controlled catalyst substrate was required for further development of
pattern generation. It was hoped that TiO₂ bound onto glass slides would be a
suitable alternative as it can be produced in house and therefore the procedure was
standardised between each production run.

4.1.3 Aims:

This chapter will present developments to the BZ reaction system primarily
through new controlling electrodes to the stage where they are ready for use in
pattern generation.

Further work will be presented detailing the progress to date on a new
substrate to replace the problematic polysulphone membrane system with an in-
house version utilising TiO₂ paste on glass slides.
4.2 Experimental

4.2.1 Setup for using Alternative Electrodes

In Chapter 2 section 2.2.3 the setup of a single silver wire electrode was detailed (figure 12). Moving from a silver wire electrode required use of the same analytical electrochemical workstation as was used in this previous case, but also included the addition of a reference electrode to allow for more accurate control of the voltages and currents being applied (figure 58).

The reaction was setup with the membrane submerged in the reaction mixture with the inert electrode in contact with the membrane (figure 58). The two different inert electrodes used were platinum and gold. The apparatus was left idle till a wave was required, and swapped to a positive voltage resulting in a positive current for initiation. Initiation was brought about by applying a constant current of 1.2mA for a
duration of 30 seconds, with the apparatus being set to turn off after this length of time.

4.2.2 TiO$_2$ Glass Side Preparation

Production of TiO$_2$ bound to glass slides is a simple procedure with a few cleaning stages. The glass was initially cut into 6cm x 2.5cm sections and these sections were scored into 2.5cm x 1.5cm pieces without breaking. Then the 6cm x 2.5cm sections were cleaned by multiple 15 minute sonication stages; firstly in a 5% solution of decon, next in de-ionised water, then in ethanol followed by two times in isopropanol, and finally in ethanol once more. These cleaned and scored glass sections were then stored under ethanol till need for the application of TiO$_2$. 
The cleaned glass sections were dried and then a small amount of the TiO$_2$ paste (Dyesol, DSL-18NR-T) was applied and spread evenly over the central section of the glass resulting in a layer 12$\mu$m thick (figure 59). This glass section was then...
allowed to dry at room temperature for approximately 30 minutes after which the TiO$_2$ layer went transparent, followed by further drying at 150°C for another 30 minutes after which it turned brown. The final stage in preparation of the TiO$_2$ layer was to heat it for another 30 minutes in a furnace at 450°C to anneal the TiO$_2$ onto the glass sections.

The final step in creating the glass slides with TiO$_2$ bound was to apply pressure along the score lines previously added to the large section of glass, breaking them into slides of approximately 2.5cm x 1.5cm size (figure 60).
Annealed TiO$_2$

Score lines for 2.5cm x 1.5cm slides

Break along score lines

2.5cm x 1.5cm glass slides with TiO$_2$ bound

Figure 60: Creating the final glass slides.
4.2.3 Binding $[\text{Fe(bathophenanthroline)}_2\text{mnt}]$ to TiO$_2$

![Diagram of binding process](image)

**Figure 61: Binding $[\text{Fe(bathophenanthroline)}_2\text{mnt}]$ to TiO$_2$.**

To bind the $[\text{Fe(bathophenanthroline)}_2\text{mnt}]$ to the TiO$_2$, 10mg of the catalyst was initially dissolved in 10ml of acetone. This solution was then added to 100ml of methanol to create the final mixture for binding. The glass slides were heated in an oven for 30 minutes at 150°C to remove any residual solvent from the TiO$_2$, allowed to cool and submerged in the $[\text{Fe(Bathophenanthroline)}_2\text{mnt}]$ solution for 48 hours to allow binding to take place. The slides were washed with de-ionised water and stored under more de-ionised water till needed.

Previous research into TiO$_2$ systems with hexacyanoferrate(II) have indicated that binding with these ligands occurs through the cyano group via the formation of a cyanide bridge between the Iron and Titanium atoms$^{22}$. Therefore it was hoped that the $[\text{Fe(bathophenanthroline)}_2\text{mnt}]$ would utilise its cyano groups in binding to the TiO$_2$ glass slides.
4.2.4 Setup for Testing [Fe(bathophenanthroline)$_2$mnt] Bound to TiO$_2$

A solution of the BZ reaction mixture as used in controlled wave generation was made (described in Chapter 2, section 2.2.1). One membrane with [Fe(bathophenanthroline)$_2$mnt] bound was placed in a petri dish and the BZ reaction mixture poured over. A TiO$_2$ glass slide with [Fe(bathophenanthroline)$_2$mnt] bound to it (described in 4.2.3) was also placed into the same petri dish. The membrane and TiO$_2$ slide were left overnight with the BZ reaction solution being refreshed the next morning and left till the following morning. This refreshing was repeated once more to give a total duration of submersion under the mixture of three days. This process of replacing the BZ reaction solution with fresh was to imitate constant submersion under active BZ solution, with the membrane and TiO$_2$ slide placed under the same solution to give a comparison of how effective the binding was between the polyphone membrane substrate, and the new TiO$_2$. 
4.3 Results and Discussion

4.3.1 Alternative Electrodes

Throughout both Chapter 2 and Chapter 3 limitations to the BZ reaction were discussed and improved on by working with the easiest part of the problem, the catalyst. Developments here show the greatest improvements to the system by improving the longevity of the reaction, and at the same time allowed for improvements to the contrast of images collected to the point where they were finally usable in further analysis. However development of an improved catalyst only allowed the system to be improved so far, with the next stage of improvements requiring a reworking of the initiation system to remove the dependence on a direct chemical reaction and its related inconveniences. To that end we carried out work on two inert electrodes as possible replacements to the silver wire working electrode used throughout Chapter 2 and Chapter 3.

The two metals chosen for this development were platinum and gold, as these are easily purchasable metal wires and are inert with regards to the component chemicals of the BZ reaction mixture. The initial stage in development was to determine a voltage and duration which would allow for the initiation of the BZ reaction. This was through systematic trial and error using the setup shown in section 4.2.1. The applied voltage was increased until a wave was seen to be initiated, followed by further refinements in the combination of duration and size of the applied voltage. These refinements were carried out to prevent damage to the membranes from the new setup, as this was the reason for its development over the tried and tested silver working electrode.
4.3.1.1 Platinum Working Electrode

Figure 62: Single initiation with a Platinum wire electrode.

Figure 63: Figure 62 after edge enhancement.

Figure 64: Figure 62 after subtraction of the background image.

Single electrode initiation is possible with a platinum electrode, with figure 62 showing a wave originating from its initiation point near the edge of the membrane. This is similar to the image seen from a single initiation with a silver wire in Chapter 3 (figure 30). In both of these cases there is nothing between the camera and the petri dish containing the membrane. In the case of the platinum working electrode no petri dish lid was utilised as this was a preliminary testing
setup with the electrode being held in position by hand whilst the 2V, 1.2mA voltage current combination was applied and maintained for 30 seconds to cause initiation. Use of 2V, 1.2mA was found to provide the lowest point for initiation and thereby cause no lasting damage to the polysulphone membrane. Higher currents were tested but were found to often cause the membrane to become damaged, resulting in an area which could no longer initiate a wave.

As this setup was utilising the same membranes used in Chapter 3 with the \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) catalyst bound they were once again run through image enhancement as described in Chapter 2 section 2.2.5. This was to further show that the images generated by the new electrodes were a good replacement to the system used in Chapter 3. Running these enhancement methods improved the contrast of the image allowing the wave to be seen in greater detail with background blemishes also enhanced (figure 63), so once again a background needed to taken and also edge enhanced ready to remove these blemishes from the image. Background subtraction generates figure 64 which now shows the wave front emanating from its initiation point near the edge of the membrane. This can be contrasted with a single electrode initiation using a silver wire electrode (figure 33), showing a similar result with an enhanced wave visible in both of these images as well as the edge of the membrane being visible.
Figure 65 through to figure 67 show the progression of a wave generated from a platinum electrode, and one from a silver electrode. From these images it can be seen that the platinum initiated wave has propagated 1.8cm which was an identical distance to that travelled by the silver wire initiation, as well as thereby showing that the characteristics of the wave are not altered by changing to a platinum electrode. In addition the wavefront measures 1mm when initiated by either silver or platinum wire electrodes. Both the silver and platinum wire electrodes had the same dimension, being a piece of wire with a diameter of 0.5mm. Also it can be seen that
the waves are the same size, measuring 1mm for both electrodes, the only differences visible being due to the contrast shown after the edge enhancement has taken place. This measurement was repeated on subsequent initiations and found to be the same (1mm). Initiation with the platinum electrode could be repeated every 2 minutes without spiral formation, as is the case for silver wire initiation.

4.3.1.2 Gold Working Electrode

Figure 69: Single initiations with a Gold Wire Electrode.

Figure 70: Figure 69 after edge enhancement.
As with the platinum electrode initiation detailed in section 4.3.1.1, gold wire electrodes were also found to be able to initiate a wave on a membrane with [Fe(bathophenanthroline)$_2$mnt] bound to it. These gold wire electrodes were of the same dimensions (0.5mm diameter) as both the platinum and silver wires to allow for comparison of generated waves. Similarly the initiation occurred at the same settings as for the platinum electrode, namely a current of 1.2mA held for 30 seconds resulted in the wave being produced. The setup for the gold electrode was also identical to that for the platinum, with the gold working electrode being held in position by hand for the 30 second duration and removed from the area before images were taken. The resulting wave can be seen in figure 69 and once more required edge enhancement to improve the visibility of the wave (figure 70). Comparison of the gold wire wave after background removal (figure 70) with both the platinum wire version (figure 64), and the silver wire version (figure 33) show that the waves which are visible on all three images are similar in characteristics, with the waves on all three having a width
of 1mm. This shows further proof that the initiation of the waves by different electrodes of the same size does not affect the characteristics of the wave.

Figure 72: *Gold electrode initiation after 100 seconds.*

Figure 73: *Figure 72 after 300 seconds have passed.*

Figure 72 and figure 73 show the progression of a wave initiated by a gold electrode in 300 seconds. If this is compared with figure 65 through to figure 68 it can be see that the wave produced by a gold wire is once again the same as that produced by both the platinum electrode and the silver electrode. This speed of the wave is the same, with the wave having propagated 1.8cm across the images shown (all are taken with the same equipment and settings and resized by the same amount), showing conclusively that the waves generated in all three of these examples are identical. Repeating initiations confirmed this.
4.3.2 \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) bound to TiO\(_2\)

**Figure 74:**
\([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) bound to a TiO\(_2\) glass slide and membrane with no BZ reaction mixture present.

**Figure 75:** Figure 74 after submersion in a BZ reaction solution containing no source of bromide.

**Figure 76:** Figure 75 following addition of 2ml of 0.165M KBr.

**Figure 77:** Figure 75 following the addition of 10ml of 0.165M KBr.
Figure 78: Figure 75 following the addition of 25ml of 0.165M KBr.

Figure 79: Figure 78 after initiation with a silver wire.

The membrane bound catalytic systems used in the BZ reaction whilst improving the overall suitability of the reaction to image generation and analysis introduce inconveniences of their own. Of these inconveniences, the most essential to put right is that caused by the differences between batches of purchased membranes. To that end we have begun the development of a new substrate to replace the
polysulphone membranes which should improve reproducibility. The production of 
TiO$_2$ slides is an in-house procedure detailed in section 4.2.2, which removes the 
reliance on an outside company. Binding of the [Fe(bathophenanthroline)$_2$mnt] 
catalyst to the TiO$_2$ glass slides is a relatively simple procedure developed from the 
procedure used to bind this catalyst to the polysulphone membranes in Chapter 2 
section 2.2.8. This results in a glass slide with a deep red colouration on the area 
where the TiO$_2$ was affixed as can be seen in the example on the bottom left of figure 
74.

Demonstration of the effectiveness of the TiO$_2$ as a replacement for the 
polysulphone membranes has proceeded initially in a similar way to the original 
testing of the suitability of the [Fe(bathophenanthroline)$_2$mnt] as a replacement 
catalyst by initially testing that the catalyst remains bound when submerged under 
the controlled BZ reaction mixture (detailed section 4.2.4). This resulted in the 
catalyst remaining bound for three days without any noticeable changes in intensity 
of the red colour. As a comparison a polysulphone membrane was also submerged 
under the same solution and this maintained its colour for the same duration. 
A UV/VIS spectrum was recorded on the [Fe(bathophenanthroline)$_2$mnt] bound to 
TiO$_2$ (figure 80), showing a similar spectrum to that for the unbound catalyst (figure 
23).
With the binding test suitably passed the next stage was to check for initiation. This would lead onto the use of the TiO$_2$ in a similar series of experiments as already detailed over this thesis, and will be conducted in a separate project. Figures 74 through to figure 79 show the range of tests performed so far. A membrane and TiO$_2$ slide were initially photographed (figure 74) to provide a comparison for the end of the experiments. This TiO$_2$ slide and membrane were then submerged in a BZ reaction mixture which contained no source of bromide ions and left for 30 minutes. After this duration figure 75 was taken which clearly shows a change in the state of the catalyst to the pale blue colour, so pale that against the white background it seems as though there is almost nothing present on either the membrane or the TiO$_2$ slide, other than a very pale discolouration. From this stage
increasing amounts of a dilute solution of KBr (0.165M) was titrated into the BZ mixture to find the point at which initiation should become possible. The first change in state occurred after 2ml of this KBr solution had been added, with the TiO₂ slide and membrane showing a pale pink colouration (figure 76), however initiation was not possible at this stage when tested by bringing a silver wire into contact with either the membrane or the TiO₂ slide. After the addition of 10ml of KBr (0.165M), the colour visible on the membrane and the TiO₂ slide became darker (figure 77), however attempts at initiation with this concentration of KBr resulted in spiral formation on the membrane. This is an undesirable occurrence resulting in the membranes being unusable for data generation. Resetting the catalyst by soaking in distilled water and returning to the same BZ mixture (i.e. with 10ml of KBr (0.615M) added) resulted in a return to the situation shown in figure 77. Increasing the amount of KBr (0.165M) solution added to 25ml resulted in the return to the point of control seen in the experiments detailed throughout Chapter 2 and Chapter 3. Figure 78 shows that at this point the membrane and the TiO₂ glass slide have returned to the same colour as in the initial image (figure 74). At the same time the membrane is now at the point where it can be initiated with a silver wire, resulting in the wave shown in figure 79. Unfortunately it was not possible to initiate the [Fe(bathophenanthroline)₂mnt] which was bound to the TiO₂ glass slide. This could have been due to the electrodes making poor contact with the catalyst.
4.4 Conclusion

The work detailed in this chapter has established an important improvement on the controlled BZ reaction system. The success of both gold and platinum electrodes in generating waves provides a method of initiation which is no longer limited by the build up of deposits from a chemical reaction. This is the case with silver electrodes where silver bromide is produced as a product of the reaction between the silver wire and the bromide ions in solution which allows for the initiation to occur. Further work with either of these electrodes is needed with an eight electrode setup used in Chapter 3.

Development of TiO$_2$ as a replacement substrate has progressed although controlled initiation has not yet been successful. We have demonstrated the stability of the bound catalyst as well as its ability to exist in both states when bound to the TiO$_2$ and therefore further work in this area should yield successful initiation.
4.5 References


Chapter 5:

Conclusions and Future Work.
Throughout these four chapters the development of techniques for chemical pattern generation and analysis have been detailed. This ranged from the initial setup of a BZ reaction system through to its control by use of literature based methods and onto the development of a multi-electrode setup. Following on from this, further research went into the design of a much improved catalyst and its deployment with the multi-electrode system, to finally the development and testing of a gold and platinum based initiation technique and the initial stage in development of a new platform for the whole system. The four steps needed to create a system which is both reproducible and will run indefinitely are: to create a catalyst which will remain bound to its substrate, develop a system of initiation which does not result in any deposition onto or damage to the substrate, automation of the initiation system and finally develop a way to continuously replace the BZ reaction solution as it is used. Of these all but the final step have been developed and tested during this thesis. The only remaining step is to setup a continuous flow system which is also the easiest to implement, requiring only a pump to continuously replace the BZ reaction solution.

The initial use of a literature based controlled BZ reaction system has been detailed extensively in Chapter 2. This system utilised an in situ method of producing and binding the \([\text{Fe(bathophenanthroline)}_3]^{2+}\) catalyst onto commercially available polysulphone membranes. The method of producing and binding the catalyst produced the first of many inconveniences in the use of the membrane based BZ reaction system. In moving the membranes between solutions and agitating them to attempt to create a good covering of catalyst, we introduced unpredictability in the binding. The \([\text{Fe(bathophenanthroline)}_3]^{2+}\) would always bind to a degree, however the areas used to hold onto the membranes and transfer them between the different
solution were often partially damaged no matter how carefully the membranes were handled. This damage became apparent after the membranes were finally ready to use, showing up as areas with exceptionally high binding due to the damage cause by applying pressure to the membranes when moving them between solutions. Further to this, placing the membranes in the initial solution of acetic acid cause them to curl. Stopping this required the membranes be held at multiple points during this stage which resulted often in areas on the membrane which were not able to take up any of the bathophenanthroline dissolved in this acetic acid solution. These areas of damage made the affected membranes less useful as areas with excessive catalyst seemed to be much more prone to initiation of spirals instead of waves. These proceeded to take over the membranes rendering them useless for pattern generation and analysis.

Any useful membranes which were produced with \([\text{Fe(bathophenanthroline)}_3]^{2+}\) bound were found to have further faults. Use of these membranes over relatively short time periods showed that the \([\text{Fe(bathophenanthroline)}_3]^{2+}\) catalyst was slowly leached out of the membranes when they were submerged in the BZ reaction mixture, to the point that after three hours under a solution the membranes were too pale to be usable in pattern generation, and after being left overnight there was no catalyst visibly present. This spurred the need to develop an improved catalyst which would remain bound to the membranes for longer periods so that its usefulness was increased.

The improved catalyst developed for this purpose was \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) which it was hoped would be less likely to leach out of the membranes as it should be less water soluble since it is a neutral compound.
instead of being charged. Synthesis of this catalyst proved to be fairly straightforward, being basically a one pot reaction with some stirring involved, however purification proved to be troublesome with impurities persistently present to a small degree. These impurities proved to not be problematic, with the catalyst binding easily to the same polysulphone membranes used with the original [Fe(bathophenanthroline)₃]²⁺ catalyst. Initial testing of this new [Fe(bathophenanthroline)₂mnt] catalyst showed it to bind effectively to the membranes, showing a deeper red colour than was present with the original [Fe(bathophenanthroline)₃]²⁺, and thereby increasing the contrast available in images produced with this catalyst. This was in addition to being much easier to bind to the membranes. Not being created in situ meant that the membranes could be left to bind in a solution containing the [Fe(bathophenanthroline)₂mnt] without the need to transfer between solution creating additional defects in the polysulphone membranes. Initial testing of these membranes showed that they remained working for at least 48 hours under refreshed BZ reaction solutions instead of the three hours for the original.

Use of the [Fe(bathophenanthroline)₂mnt] membranes with the multi-electrode setup allowed investigation of pattern generation. With eight silver electrodes arranged in a circle around the centre of the membrane, the possible combination of these electrodes firing to create a range of patterns is substantially reduced when compared to the theoretical number of combinations which would be present from eight inputs without any symmetry. This is due to the symmetry inherent in a circular shape, detailed in Chapters 2 and 3. The range of images produced by this setup were further prepared before analysis could take place to
enhance the visibility of the waves, and remove the background and edge of the membranes. The results were finally converted into numerical values through the use of a Universal Similarity Matrix and used to generate a dendrogram which showed the similarity differentiation to be significant.

During the generation of these images further inconveniences were discovered in the system being used. The newly developed \([\text{Fe(bathophenanthroline)}_2\text{mnt}]\) increased the durability of the membranes, but the chemical reaction taking place to initiate waves produced an insoluble by-product of silver bromide which was deposited onto the electrodes and the membranes, resulting in areas on the membranes which were no longer able to be initiated. Development of a system which removed this limitation was decided as the best course, resulting in the testing of both gold and platinum as alternative electrodes for initiation. This new use of chemically inert electrodes was intended to allow initiation without the side effect of a deposition onto the membranes. This was successfully accomplished, with both the gold and platinum being viable alternatives to the silver electrode, only requiring a higher current to be applied for them to initiate.

The final problem in this system was the use of commercially available membranes. Whilst this was convenient initially it rapidly became apparent that control of the catalyst substrate was desirable. This occurred when it became evident that the formulation was being slightly altered between batches of the membranes, with each batch working slightly differently. Development of \(\text{TiO}_2\) affixed to glass slides was chosen as the best alternative as the group has prior experience with this substrate. The progression of this setup went as far as binding the
[Fe(bathophenanthroline)$_2$mnt] to TiO$_2$ and proving that it remains bound and can change state.

Further work required in this field is through the deployment of the gold and platinum electrodes in the eight electrode setup to provide for a long term pattern generation instrument. Along with this the TiO$_2$ substrate needs to be further developed to the point where it can be deployed as an alternative to the membrane based system.