SYNTHETIC AND KINETIC INVESTIGATIONS INTO LIVING FREE-RADICAL POLYMERISATION USED IN THE PREPARATION OF POLYMER THERAPEUTICS

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Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

“It is what we think we know already that often prevents us from learning.”

Claude Bernard, French Physiologist (1813 – 1878)

CONTENTS

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Abstract

The aim of this work was to successfully prepare polymers of *N*-(2-hydroxypropyl)methacrylamide, (PHPMA) using controlled/"living" free-radical polymerisation technique.

For this purpose, atom transfer radical polymerisation (ATRP) and reversible addition-fragmentation (chain) transfer (RAFT) polymerisation were used in preparation of a number of base polymers with the intention of quantitatively converting them into PHPMA. Both methods were applied under varying polymerisation conditions, and the kinetics of the systems investigated. Various rate constants were measured, while computer modelling of the experimental data allowed estimation of other kinetic parameters of interest.

Investigations into solvent and ligand effects on the kinetics of ATRP of the activated ester methacryloyloxy succinimide (MAOS) and one of the archetypal methacrylate monomers, methyl methacrylate (MMA) were carried out. The equilibrium constant K_{eq} was estimated for these monomer systems. In the case of MMA the dependence of K_{eq} on the monomer content in the solution was established. The kinetic modelling was based on the 'persistent radical effect' and gave excellent agreement with experimental data in most cases.

Modelling was also used in studies of the effect of common radical inhibitors in ATRP. Two commonly used radical-trapping compounds that were used as additives in ATRP of MMA, produced a somewhat unexpected result. It was found that no significant rate retardation was observed. Modelling confirmed that a more profound effect on the kinetics of the ATRP of MMA should be observed.

The method of RAFT was also employed in polymerisation of MAOS and a number of other monomers in the hope of finding the best synthetic precursor of PHPMA. Polymers of methacryloyl chloride (MAC) and *p*-nitrophenyl methacrylate (NPMA) were prepared, as well as the polymers of HPMA itself and *N*-isopropyl methacrylamide. Polymerisation of MMA by RAFT was also attempted in view of adding to current knowledge on the monomer's behaviour and the kinetic characteristics of its RAFT polymerisation.

So-called ‘hybrid’ behaviour of HPMA was achieved under RAFT conditions and the conditions were used in copolymerisation of HPMA and *p*-nitrophenyl ester of *N*-methacryloylglycylglycine, with the latter introducing chemical functionality that potentially allows further functionalisation of the copolymer.

Two kinetic models of RAFT were used to analyse data: the traditional Mayo approach and the method of moments. The modelling was used for estimating the chain transfer constant value, C_{tr}, for the systems of interest.

Preparation of PHPMA from PMAOS, PMAC and PNPMA was attempted. Successful preparation of PHPMA from the polymer of the acid chloride was achieved under mild reaction conditions, while displacement of *N*-hydroxysuccinimide groups of PMAOS resulted in unexpected modification of the polymer under the conditions used. Conversion of PNPMA into PHPMA was not achieved. At this stage these results suggest inadequacy of both PMAOS and PNPMA as reactive polymeric precursors.

Chapter One. Introduction[1]

1.1 Living Free-Radical Polymerisation

It was famously remarked at the end of the 19th century that there was nothing left to discover in physics. In recent times many chemists have had the same dismissive attitude towards free-radical polymerisation (FRP). Just as with the physics forecast, so this attitude towards FRP is proving to be highly mistaken, in two major ways. Firstly, it confuses invention with scientific discovery. For example, commercial production of polystyrene began a full eight years before people recognised that the process had a free-radical mechanism. Even today the underlying science behind many market products of free-radical polymerisation is not well understood. So even about conventional free-radical polymerisation there remains much fundamental science to be unearthed. Secondly, and still more importantly, far from FRP being a completely explored landscape, much new and inventive FRP chemistry has emerged over the last two decades.[1,2] In particular, living free-radical polymerisation (LFRP) has been developed,[1-3] and it promises to revolutionise polymer production. The following outlines the story of LFRP to date.

1.1.1 Background

Today many commercial polymers are prepared by conventional free-radical polymerisation (CFRP). The method's popularity is high as a wide range of monomers can be used under mild reaction conditions. For these advantages one sacrifices a large degree of control over the polymer product. This can be explained using Figure 1.1, which shows a conceptual outline of CFRP. Radicals are continuously formed from initiator, and as a radical forms it quickly adds to monomer, a process which is repeated many times until at

[1] The majority of this chapter has been published as Adash, U.; Russell, G. T. *Chemistry in New Zealand* **2005**, *69*, 8.

some stage a (macro)radical is converted into a *'dead'* polymer chain by participating in either combination, disproportionation (together called *termination*) or chain transfer.

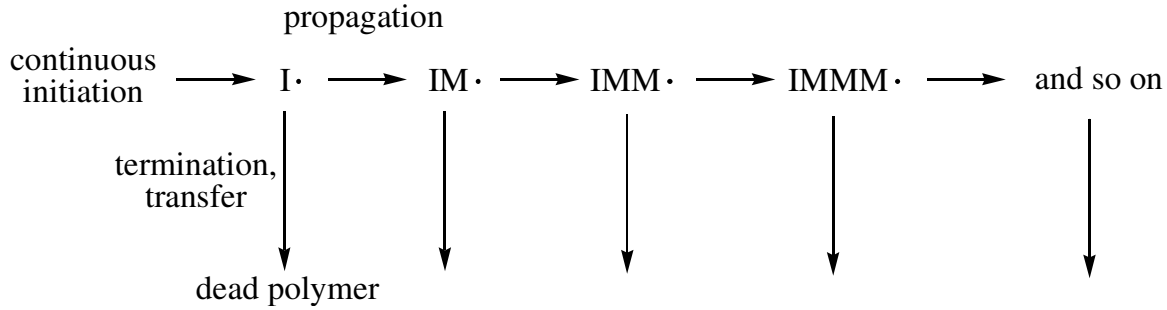


Figure 1.1 Conceptual representation of the process of conventional free-radical polymerisation, where I denotes the initiating species, M monomer, and the arrows a reaction (as indicated).

Human populations are a good analogy for this: babies are born at all times (akin to initiation), people inexorably age (propagation), and at some time they die (termination and transfer). Just as human death can occur at any age, resulting in a *distribution* of ages at death, so too the dead-chain-forming reactions of FRP can occur at any stage of a radical's life. For CFRP carried out over constant conditions, one typically obtains an exponential-like distribution of molecular weights, as shown in Figure 1.2.

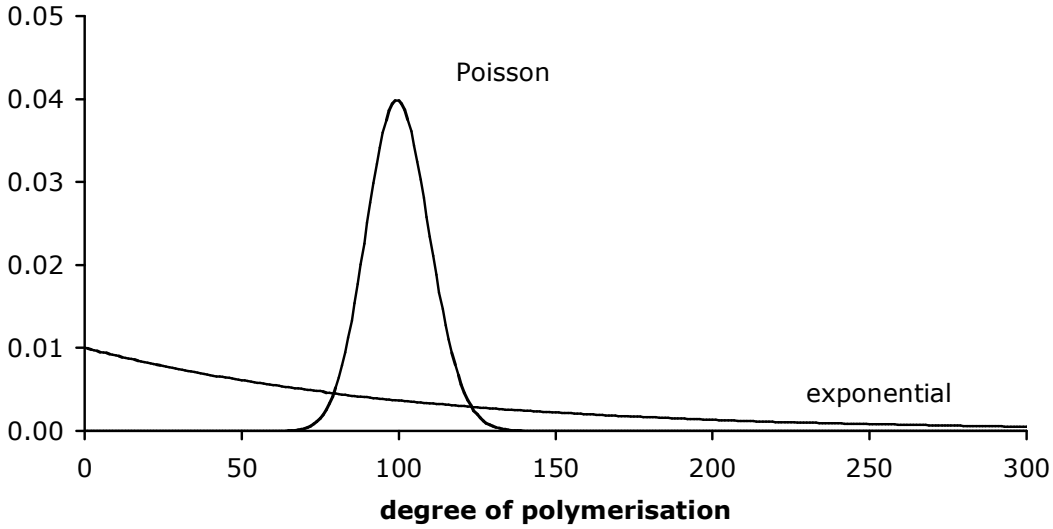


Figure 1.2 An exponential distribution of chain sizes, as typically obtained from conventional free-radical polymerisation carried out over constant conditions, and a Poisson distribution of chain sizes, as obtained from ideal living polymerisation. Both the shown distributions are normalised and have number-average degree of polymerisation of 100.

Although *average* size can be controlled through choice of reaction conditions, nothing can be done to eradicate the *polydisperse* nature of the polymer product, i.e., one must accept that the dead chains have a wide variety of sizes, even if they are otherwise chemically identical.

Implicit in Figure 1.1 is an even more important way in which CFRP is lacking: once a dead chain has been formed, there is no easy way for its growth to be reinitiated. Thus, for example, there is no easy way of forming *block copolymers* by CFRP, i.e., polymers that consist of a long block of residues of one monomer followed by a long block of residues of another monomer. Such polymers are highly prized, because they possess properties of both the corresponding homopolymers. For example, poly(styrene-*block*-isoprene-*block*-styrene) is a so-called 'thermoplastic elastomer' because it behaves like both a plastic (due to the styrene) and a rubber (due to the isoprene). However there is no easy way of making such polymers by CFRP. Hence they must be made by other, more temperamental means, and so they are expensive.

With the above in mind one can grasp the impetus for the development of *'living polymerisation*' (LP). This term was defined in the 1950s to describe a chain-growth process that proceeds in the absence of irreversible chain-termination and chain-transfer steps:[4] there are no dead chains, and thus all chains are *living*. So once initiation occurs, chains grow in a continuous manner until the supply of monomer is depleted, as is conceptually illustrated in Figure 1.3.

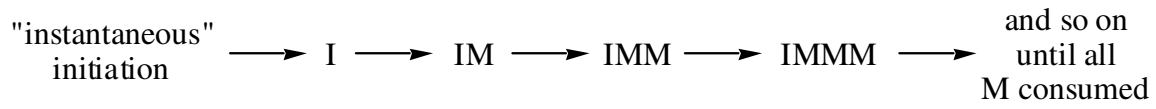


Figure 1.3 Conceptual representation of the process of (ideal) living polymerisation, where I denotes the initiating species, M monomer, and the arrows a reaction.

If initiation is rapid on the timescale of monomer consumption, then all chains are (approximately) the same size. To use once again the analogy of human populations, this is like a multitude of babies being born at the same time: forever after they will be the same age. With living polymerisation the situation is not exactly the same, because the stochastic nature of chemical kinetics means that some chains undergo more propagation events than others. However the distribution of sizes is still relatively narrow. In fact, all going ideally the resulting molecular weight distribution is a Poisson distribution,[5] as

shown in Figure 1.2. Both the distributions shown in Figure 1.2 have a number-average degree of polymerisation of $\overline{DP}_n = 100$. This makes it clear just how much more *monodisperse* is the product polymer of LP.

A further characteristic of living polymerisation is that even after monomer supply is exhausted, chains remain active (unless a terminating agent is introduced). Thus one may synthesize a block copolymer simply by introducing a second monomer after polymerisation of the first monomer is complete. This exemplifies how LP also offers greater control over microstructure and architecture than does CFRP.

Living polymerisation is most commonly realised by *anionic polymerisation*. However it is synthetically demanding in that it is prey to trace quantities of impurities. Thus all reactants and solvents must be rigorously purified and polymerisation must be carried out under inert conditions in scrupulously-clean, sealed apparatus.[5] For this reason the process is very expensive to carry out commercially. Polar monomers undergo side reactions, leading to loss of control. Thus anionic polymerisation is applicable only to a small number of monomers.

For the polymer chemist the Holy Grail is facile polymer synthesis by a process affording a high degree of control of the product polymer. While a narrow molecular weight distribution (MWD) is not always desirable in terms of product properties, in general it is, and certainly it is always desirable to have control of microstructure and architecture. Thus one needs to marry the best features of CFRP (synthetically easy, widely applicable) with the best of LP (narrow MWD, control of composition and topology). For a long time this Holy Grail seemed just that: there was no way of using free radicals, which react easily and multitudinously, to mediate LP. But then in the 1980s living free-radical polymerisation emerged.

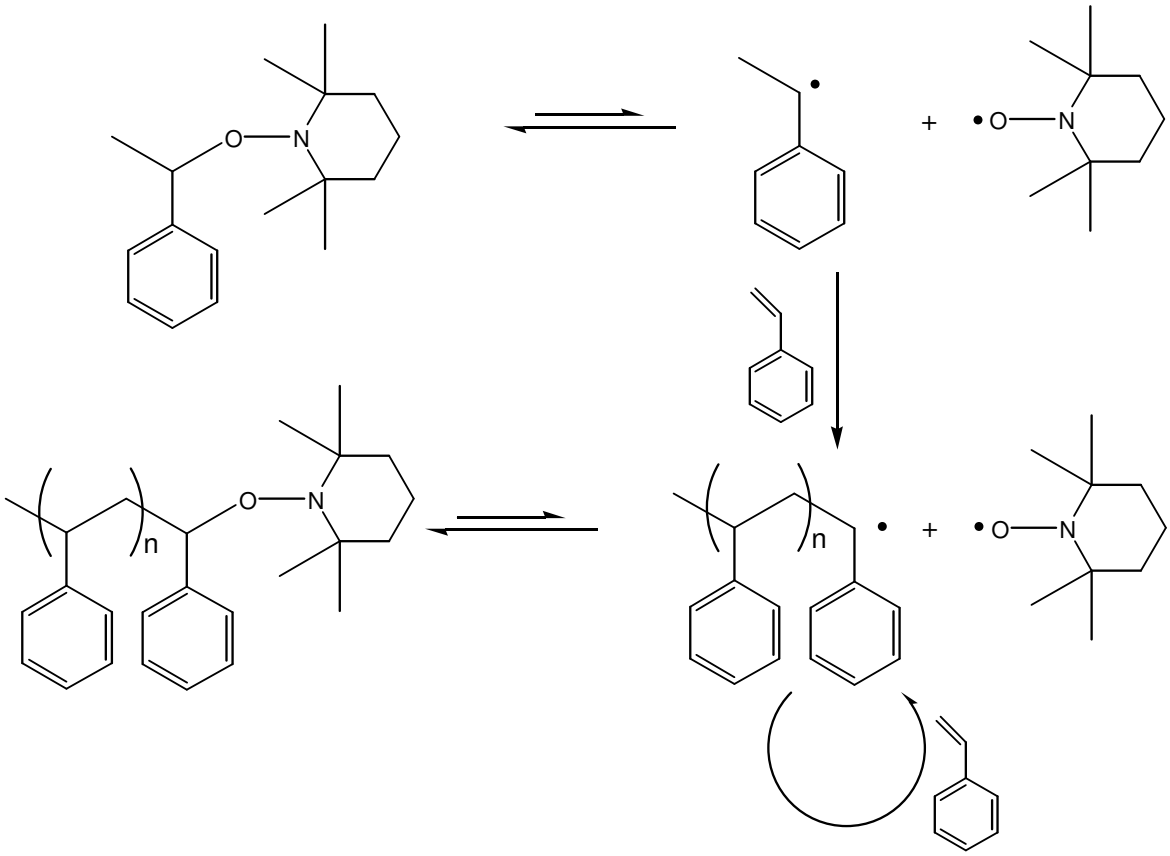
1.1.2 The Paradigm of Living Free-Radical Polymerisation

The key to living polymerisation is elimination of termination, the process that leads to production of dead polymer chains of all sizes. Of course it is impossible to prevent free radicals from reacting with each other. However, because propagation is first order in radical concentration, [R$^\bullet$], while termination is second order, it follows that one can promote propagation over termination by lowering the radical concentration. One way of

accomplishing this is to include a reagent that can *reversibly deactivate* a radical. Both words here are pivotal: the reagent must *deactivate* the radical in order to protect it from termination, but the process must be *reversible* so that the radical can sporadically spring back to life and grow a bit more, before going back into hibernation (so to speak). After many such deactivation/activation cycles, a radical will have grown to polymeric size, and it will be capable of further growth as long as monomer is present.

Various reagents that more or less achieved the above paradigm were experimented with in the early 1980s.[2] However it is fair to say that it was not until the employment of alkoxyamines[2] by Rizzardo and co-workers in the mid-1980s that people became fully cognisant of what they were doing, and thus that 'living free-radical polymerisation' (LFRP) was born.

Scheme 1.1 illustrates the principles involved by showing how a TEMPO adduct achieves LFRP of styrene.



Scheme 1.1 Use of 1-phenylethyl TEMPO to effect living free-radical polymerisation of styrene.

As is indicated, the activation/deactivation equilibria lie toward the deactivated ('*dormant*') species. This is because the reaction between TEMPO and a carbon-centred radical is fast – it is close to diffusion-controlled – whereas obviously the reverse reaction, involving bond scission, is much slower (even if the bond involved is labile). Thus the radical concentration is maintained low, and so termination is suppressed. At the same time the activation reaction is still fast enough that polymers of large chain lengths can be obtained on a comfortable timescale, viz. hours.

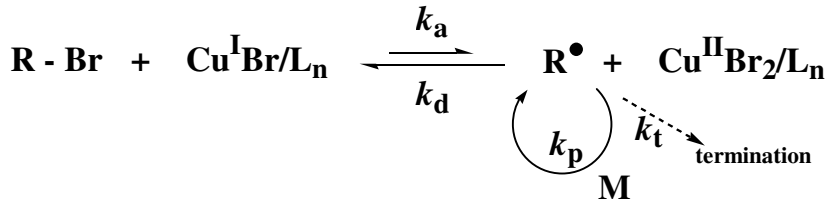
So-called '*nitroxide mediated polymerisation*' (NMP) was the first form of LFRP to find widespread use. As is implicit in Scheme 1.1, a key to its successful use is that nitroxide radicals do not self-react, i.e., they are stable free radicals. This point will be returned to in the section on Kinetics and Molecular Weights. On the other hand, propagating radicals do react with each other, even if they are only present in very low concentration. Thus the occurrence of conventional radical-radical termination is unavoidable in LFRP, which emphasizes that the process can never function as an ideal living polymerisation. Nevertheless by now it has been established in (literally) thousands of experimental studies that in a successful LFRP one obtains polymer with a MWD almost as narrow as the Poisson distribution of Figure 1.2.

Although he did not discover NMP, Hawker has been its main champion,[6] and he has invested much effort into developing an alkoxyamine that is a 'universal initiator',[7] i.e., one that may be successfully employed for a large number of monomers over a wide variety of conditions. However Hawker was never destined to succeed on this crusade, because other forms of LFRP have emerged which are superior to NMP except for polymerisation of styrenic monomers. Specifically, NMP paved the way for the development of so-called 'atom transfer radical polymerisation' (ATRP) and 'reversible addition-fragmentation (chain) transfer' (RAFT) polymerisation.

1.1.3 ATRP – Atom Transfer Radical Polymerisation

Sawamoto *et al.*[8] were the first to recognise that the activation/deactivation equilibria of Scheme 1.1 can also be effected by transition metal complexes, using the Ru(II)/Ru(III) couple to demonstrate this. Their idea was almost instantaneously seized upon by Matyjaszewski, who without delay showed that Cu(I)/Cu(II) systems seem to do an even better job.[9,10] The chemistry involved is shown in Scheme 1.2: active radicals (**R$^\bullet$**) are

generated when a copper(I) complex ($\mathbf{Cu^{I}Br/L_n}$) undergoes a one-electron oxidation to a copper(II) complex ($\mathbf{Cu^{II}Br_2/L_n}$) with simultaneous extraction of a halogen atom (bromine) from an initiator (**R-Br**). The reverse of this process is extremely fast, meaning that the radical only has a small amount of time to react with monomer before it is converted back into an alkyl halide. But this cycle may occur over and over again, meaning that one has LFRP.



Scheme 1.2 General mechanism of copper-based atom transfer radical polymerisation.

Because the process of Scheme 1.2 is just the application to polymerising systems of the well-known organic chemistry process of 'atom transfer radical addition', it has become known as 'atom transfer radical polymerisation' (ATRP).

As just mentioned, (copper-based) ATRP is extremely versatile. This is because there are many components that may be varied in striving for optimum results,[11] as discussed below.

1.1.3.1 Initiator

An alkyl halide is usually used as an initiator. Halogenated alkanes (e.g. 1-bromoethyl benzene, Chart 1.1), α-bromoesters (e.g. ethyl 2-bromoisobutyrate, Chart 1.1) and α-chloroesters are examples of compounds employed as initiator species.

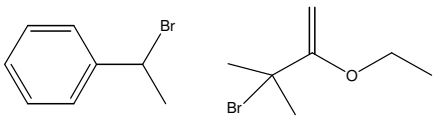


Chart 1.1 Typical initiators for (copper-based) ATRP.

1.1.3.2 Monomer

It is generally held that ATRP is superior for LFRP of methacrylates. The method has also been used successfully for the controlled polymerisation of styrenes, acrylates and many

other monomers,[11] a selection of which are shown in Chart 1.2. Certainly ATRP gives good results for a greater number of monomers than does NMP.

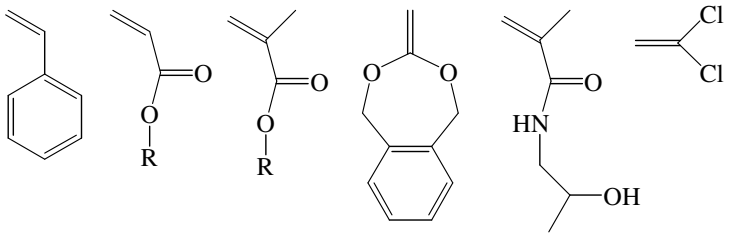


Chart 1.2 Some monomers that have been successfully polymerised by ATRP.

1.1.3.3 Ligand (L)

As transition metals are often insoluble in organic solvents, addition of a suitable ligand to the ATRP mixture improves the solubility of the metal catalyst by forming a complex with the latter. It is the solubility of the complex that will determine the actual concentration of the catalyst in the reaction mixture, therefore affecting the position of the equilibrium of Scheme 1.2.

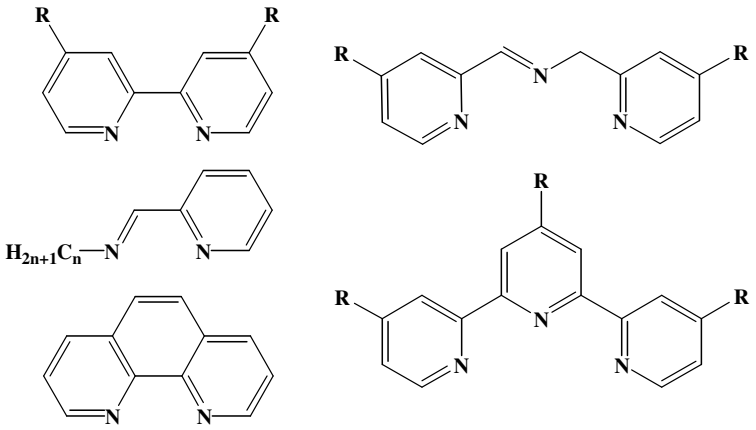


Chart 1.3 A selection of ligands that have been used for copper-based ATRP.

This in turn influences the overall kinetics of the polymerisation as well as the MWD of the produced polymer. Nitrogen-based ligands (Chart 1.3) have been successfully used in copper-mediated ATRP. A variety of bidentate and multidentate ligands have been used in polymerisation of various monomers, producing polymer with narrow MWD. The key appears to be that ligands should form a strong complex with the metal centre. This is thought to be because in a highly labile complex there will be displacement of ligands by solvent or monomer molecules, which obviously will compromise the transfer of the halogen counter ion, resulting in uncontrolled polymerisation.

1.1.3.4 Solvent

Use of different solvent systems also affects the rate of polymerisation by altering the homogeneity of the catalyst and shifting the position of the ATRP equilibrium. That in turn could affect the polydispersity of the final product. Solvents such as acetonitrile, benzonitrile and DMF combine high polarity with the ability to act as a ligand, and the overall effect of these solvents on the kinetics of polymerisation will depend on which of these properties dominates. The solvent must also be able to dissolve the formed polymer.

1.1.4 Kinetics and Molecular Weights

While the idea behind LFRP may seem "obvious" as presented above, a conundrum soon emerges: if the process is started by a reaction that generates radicals and stable species (nitroxide radical, Cu(II) complex, etc.) in equal number, and if cross-reaction of these products occurs essentially equally quickly as self-reaction of radicals (both processes are essentially diffusion controlled), why is it that that the former reaction is so heavily favoured over the latter? In other words, how is it that LFRP works? In a series of brilliant articles, Fischer developed the answer.[12-14]

In summary, it is that the extreme selectivity at the heart of LFRP – that radical-radical reaction is suppressed almost to the point of non-occurrence while cross-reaction between radicals and stable species occurs almost exclusively – is a *concentration* effect rather than a reactivity effect. What happens is that in the early stages of LFRP, conventional radical-radical termination does occur, and this process is indispensable in that it depletes the radical concentration while the stable species, not being able to self-react, rises and rises in concentration. Thus an extreme imbalance in concentration develops, and as long as this happens relatively quickly on the timescale of polymerisation, LFRP will subsequently take place. Because the situation just described relies on production of a stable species, it has been named the *'persistent radical effect'*.[12-14]

For ideal living polymerisation one has that $\overline{DP}_n = x\left(\frac{[M]_0}{[\text{Initiator}]_0}\right)$ and $PDI = 1 + 1/\overline{DP}_n$. Here M denotes monomer, x is the fractional conversion of monomer into polymer and *PDI* stands for *polydispersity index*, the quantity with which polymer scientists characterise the broadness of a MWD. For example, *PDI* = 2 for the exponential distribution of Figure 1.2, *PDI* = 1.01 for the Poisson distribution of Figure 1.2, and *PDI* =

1 when all polymer molecules are exactly the same size. Fischer was able to show that to reasonable approximation the two just given expressions hold also for LFRP.[13,14] Therefore they are graphed in Figure 1.4.

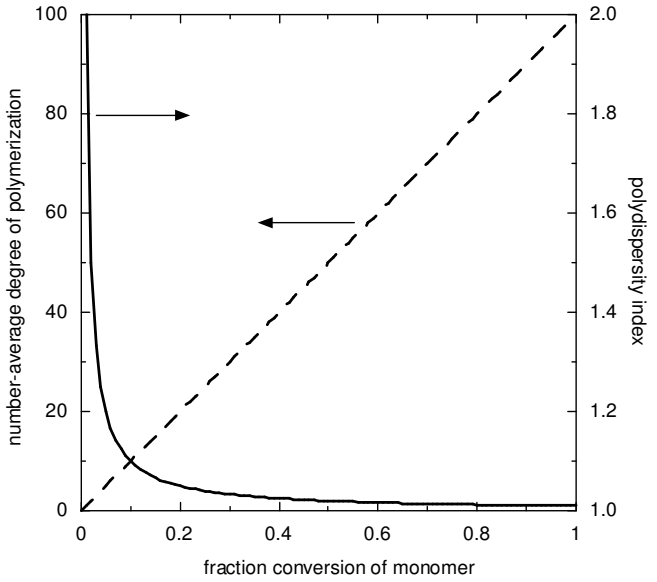


Figure 1.4 Evolution of the number-average degree of polymerisation ($\overline{DP}_n$, broken line) and polydispersity index (*PDI*, solid line) with fraction conversion of monomer (x) for ideal living polymerisation in which $[M]_0/[I]_0 = 100$.

The displayed behaviours are considered the hallmark of '*successful*' LFRP:

There is a linear increase of average polymer size ($\overline{DP}_n$) as the reaction proceeds, with the final value simply being equal to the starting ratio of monomer to initiator. It is obvious how this affords easy control of polymer size.

PDI is low and decreases slightly during the polymerisation. Of course in practice it is not possible to achieve *PDI* as low as in Figure 1.4, but *PDI* = 1.1–1.2 is routinely obtained with LFRP.

Fischer also derived[12-14] that in LFRP there is an unusual dependence of monomer consumption with time: $\ln([M]_0/[M]) \sim t^{2/3}$. I have verified this prediction, as shown in Figure 1.5.[15]

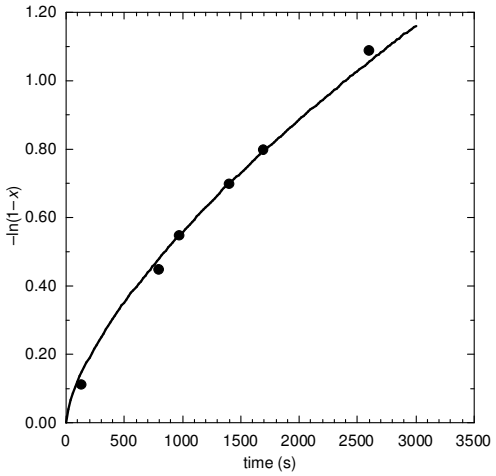


Figure 1.5 Showing the kinetics of LFRP: values of fraction conversion of monomer into polymer, x, plotted as $-\ln(1-x)$, versus time. Points: experimental measurements from an ATRP of methyl methacrylate;[15] curve: theory[12-14].

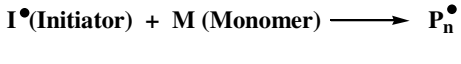
One of the fascinating aspects of Fischer's recent review[14] is the history he gives of the persistent radical effect. He shows that this concept is present in organic and inorganic chemistry stretching back to the 1930s. Of course workers were only intuitively aware (at best) of why they were obtaining such unusual preference for their particular unsymmetrical coupling reaction. Now that physical chemists have given the concept a sound theoretical backing and polymer chemists have demonstrated just how potent an idea it is, one wonders if it might find wider use in organic and inorganic chemistry.

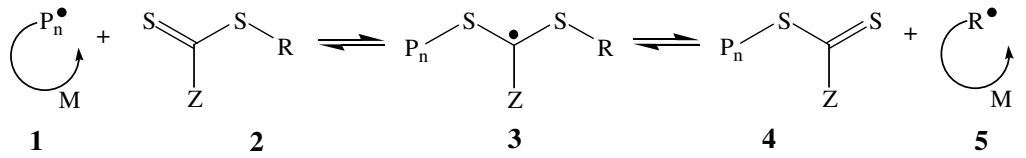
1.1.5 RAFT – Reversible Addition-Fragmentation (Chain) Transfer Polymerisation

Both NMP and ATRP involve reversible *termination*. However so-called '*transfer agents*', for example thiols, are routinely employed in conventional FRP in order to bring about dead chain formation (in preference to termination). It therefore follows that LFRP might equally be brought about by reversible transfer. The first truly successful demonstration of this idea was by Rizzardo *et al.* in the late 1990s, using dithioesters.[16,17] The chemistry is shown in Scheme 1.3. As is immediately evident, it involves reversible addition-fragmentation (chain) transfer: hence the name RAFT polymerisation.

First of all Scheme 1.3 shows the generation of free radicals by a conventional free-radical initiator (as opposed to the alkyl halide and alkoxyamine 'initiators' of the preceding

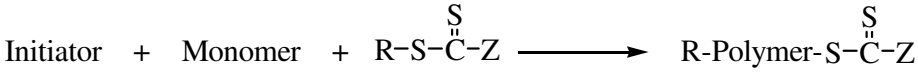
sections). The addition of the resulting propagating radical $\mathbf{P_n}$ (**1**) to the thiocarbonyl compound or dithioester (**2**) (also termed RAFT agent) results in formation of the adduct radical **3**.





Scheme 1.3 General mechanism of reversible addition-fragmentation (chain) transfer polymerisation, as mediated by a dithioester. Note that 'Initiator' here refers to a conventional free-radical initiator.

This addition is followed by a fragmentation of 3 to form a polymeric thiocarbonylthio compound 4 and a new propagating radical R (5). As indicated, all these reactions are reversible. Equilibrium between the propagating radicals **1** and **5** and the dormant (polymeric) thiocarbonylthio species **2** and **4** is established. An excess of the dithioester (relative to initiator) is used so that at any instant the majority of the polymer chains are capped by a thiocarbonylthio group and thus are dormant.



Scheme 1.4 Overall representation of (dithioester-mediated) RAFT polymerisation.

While all the above may seem complicated, the overall representation of Scheme 1.4 reveals a simpler and highly elegant picture. Indeed, it shows that a RAFT polymerisation is nothing more than a conventional free-radical polymerisation to which is added a transfer agent with the special property of being able to react reversibly with radicals.

Various components – e.g. polymerisation conditions and monomer – determine the effectiveness of a RAFT polymerisation, but the choice of the RAFT agent is believed to be the most important. Its effectiveness depends on the nature of its leaving (**R**) and stabilising (**Z**) groups (see Chart 1.4). In a successful RAFT process there should be a rapid transfer between free radical (**1** and **5)** and intermediate (**3**) species, i.e. the rates of addition and fragmentation should be high. The rate of addition of radicals to RAFT agents **2** and **4** depends on the nature of the substituent **Z** of the RAFT agent. It is believed

that radical stabilising groups enhance the rate of addition. The rate of fragmentation is affected by the nature of the leaving group **R,** and for a successful initial fragmentation to occur, **R** has to be a better leaving group than the polymeric chain $\mathbf{P_n}$. A number of commonly used RAFT agents, including those used by me,[18] are shown in Chart 1.4.[19] Through choosing an appropriate RAFT agent, successful LFRP of a variety of meth(acrylates), styrenes, meth(acrylamides) and even of troublesome vinyl acetate has by now been carried out.[19]

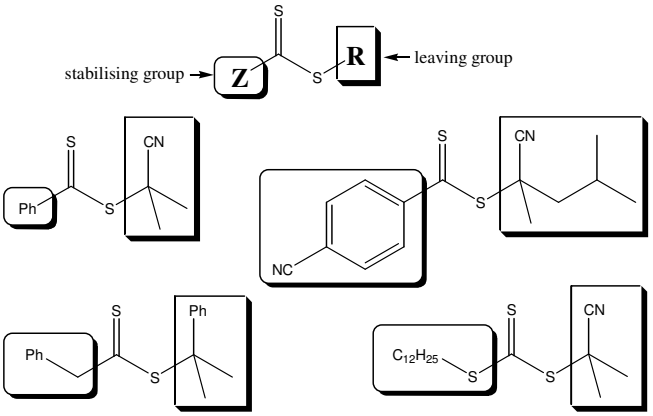


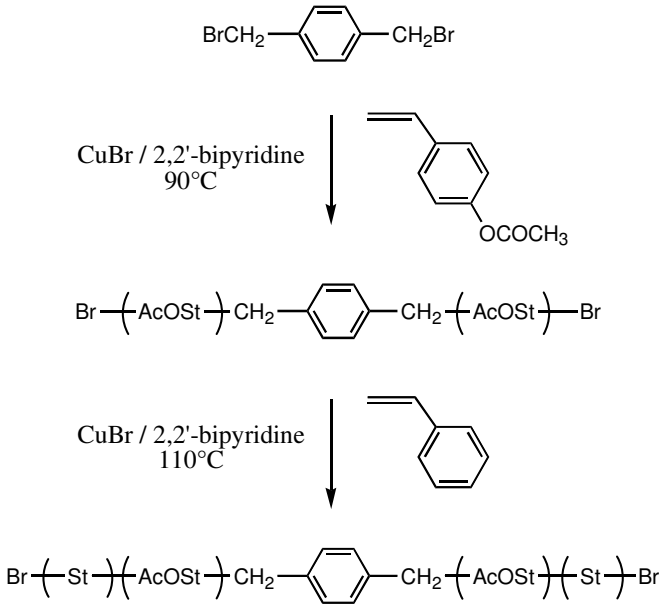
Chart 1.4 General representation of a RAFT agent and some of the RAFT agents currently used in polymerisation of methacrylates and methacrylic acid.

In addition to dithioesters, there are other types of compounds that are reversible chain transfer agents. For example, *xanthates* and *dithiocarbamates* have been used to carry out LFRP successfully.[19] In fact the xanthate promulgators refer to their system as 'MADIX', short for 'macromolecular design via interchange of xanthates', as if the paradigm involved were distinct. Of course it is not, for they are just carrying out xanthate-mediated RAFT. But this does give a feeling for the extraordinary versatility of RAFT.

1.1.6 Macromolecular Design

So far it has been emphasized how LFRP gives a narrow distribution of polymer sizes and great control of the average size (Figure 1.4). However LFRP also affords unprecedented command over other aspects of macromolecular design. This is well illustrated by Scheme 1.5, which shows the use of ATRP to synthesize a *triblock* copolymer.[11,20] From this example one can readily envisage how easy it is to use LFRP to do things like synthesize *graft copolymers* (e.g. carry out ATRP from halogen atoms incorporated along a polymer), *star polymers* (e.g. carry out ATRP using an initiator with 3 or more halogen atoms), *functional polymers* (use an initiator that also contains a desired functional group), and so

on. Of course all this is accomplished also with control over size (block size, graft size, arm size, etc.).



Scheme 1.5 Synthesis of poly(styrene-block-4-acetoxystyrene-block-styrene) using ATRP.[11,20]

This newfound capacity to tailor macromolecular design is leading to many novel polymeric materials and thus promises an age of 'smart' polymers (e.g. see the following section). A vast literature on this exciting aspect of LFRP has arisen, and the reader is referred to notable reviews for many spectacular examples of macromolecular architectures that are now possible.[1,3,6,11]

Because of the above, LFRP is also sometimes called 'controlled free-radical polymerisation'.[3,9,10] While there is no disputing that LFRP opens many new doors in this regard, the term 'controlled FRP' is misleading in that (1) complete control of macromolecular design is not offered, and (2) it implies that conventional FRP lacks control. In fact the latter is not the case: with conventional FRP one can control, for example, average molecular weight and average copolymer composition, one can incorporate functionality (e.g. through an initiator or chain transfer agent), and one can create different architectures. What LFRP offers is the capacity to do these things even better.

1.2 Polymer Therapeutics

Of many possible applications of LFRP, I will discuss just the one here: *polymer therapeutics* is an area in which work has been occurring at the University of Canterbury.[18] Ringsdorf's seminal idea[21] was that by attaching a drug molecule to a polymer, the efficacy of the drug could be increased, through what has become known as the 'EPR effect', standing for 'enhanced permeability and retention'.[22] While quite a few 'polymer therapeutics' are already in, or progressing towards, clinical development,[23] future progress in the field would certainly be promoted by an ability to synthesize polymer components of uniform size.

All manufactured drugs should be composed of a well-defined species. However polymer samples synthesised by conventional means contain macromolecules of different size, i.e. chains of different length. This would lead to polydisperse polymer therapeutic pharmaceuticals, a situation frowned upon by regulatory authorities. Until now this difficulty has been overcome by exhaustive fractionation of samples. Obviously it would be preferable if monodisperse polymer could be synthesised in the first place, hence the hope that LFRP might play a major part in the further development of polymer therapeutics. Not only this, but it is highly desirable to be able to make polymer therapeutics of a highly specific target size, as will be discussed in the following section. This too is something that LFRP can do. In summary, because LFRP can deliver polymer of close-to-uniform and controlled size, it has the potential to lead to better polymer therapeutics, and hence superior treatments for cancer and other diseases.

In using LFRP with the above in mind, I and others have experienced that ATRP and RAFT are not the panaceas that their advocates make them out to be: poly(*N*-(2-hydroxypropyl)methacrylamide) or PHPMA, the drug-carrying polymer of choice, of uniform, controlled size has not been immediately obtained by either LFRP method, as will be detailed in this thesis. However, better recipes are being developed with time. For what it is worth, my feeling is that RAFT is the more user-friendly and versatile synthetic method and is therefore perhaps the one of greater long-term potential.

Ideally we want to be able to directly apply a method of controlled polymerisation to the synthesis of a polymer of HPMA from its monomer, *N*-(2-hydroxypropyl)methacrylamide. Success of such synthesis will mark a further step in development and production of anti-

cancer agents that prove to show lower toxicity and improved targeting properties when compared with low molecular weight drugs. The high toxicity of low molecular weight drugs is proving to be the main hurdle in successful treatment of cancer.

1.2.1 Cancer

1.2.1.1 History and nature of the disease

The oldest description of cancer was discovered in Egypt and dates back to approximately 1600 BC. The writing says about the disease: "There is no treatment." Since then there has been staggering growth in knowledge of cancer biology, cancer treatment and prevention. Despite this, more people in the UK died of cancer in 1998 than any other disease,[24] and this year more than 500,000 people are expected to die from cancer in the United States alone.[25]

The term 'cancer' in fact refers to a group of diseases in which cells grow and spread unrestrained throughout the body.[26] In normal tissues, the rates of new cell growth and old cell death are kept in balance. In cancer, this balance is disrupted. This disruption can result from uncontrolled cell growth or loss of a cell's ability to undergo 'apoptosis'. Apoptosis, or 'cell suicide', is the mechanism by which old or damaged cells normally self-destruct. When this 'suicide trigger' fails, it results in a gradual increase in the number of dividing cells. It is believed that DNA-damaging carcinogens cause mutations in genes that are responsible for cell division. Such damaged cells increase in number and create a growing mass of tissue called a 'tumour'. Tumours increase in size rapidly because new cells are being produced in greater numbers than needed. Cancer cells migrate and penetrate into neighbouring tissues, causing the spread of cancer to other organs. Eventually normal tissue organisation becomes disrupted; if as a result an organ becomes unable to function, then it will fail, which often can be life threatening.

Current methods of cancer treatment include surgical removal of tumours, radiation therapy, and chemotherapy that targets and kills cancer cells.

1.2.1.2 Current methods of treatment

Early in the twentieth century, the only curable cancers were those that were small and localised enough to be completely removed by surgery. Later, radiation was used after

surgery to control small tumour growths that were not removed by the surgery. Finally, chemotherapy – the use of drugs that kill tumour cells – was introduced, so as to destroy small tumour growths that had spread beyond the reach of the surgeon and radiotherapist. More recently it was found that multiple chemotherapeutic agents – known as combination chemotherapy – are much more efficient than single agents.

Drugs have various modes of action, and chemotherapy employs various drugs depending on the type of cancer being treated. However, the main effect of use of the drugs is retardation in the growth rate of cancer cells. Cells decrease in number and as a result of this tumours stop growing and start shrinking.

One of the major disadvantages of any chemotherapy treatment is that while it is expected to improve a patient's condition, it is also a source of serious side effects. Since most drugs target and disrupt the process of cell growth, they affect mostly fast growing tissues such as hair and bone marrow. For instance, the side effects of chemotherapy include temporary or permanent loss of hair, skin irritation, temporary change in skin colour, and tiredness. Most chemotherapeutic agents are toxic to healthy cells, as well as to cancer cells. As noted by Duncan,[22] effective chemotherapy is urgently required to treat those common solid tumours, e.g. breast, prostate, lung and colon, that contribute most to the increasing incidence of cancer-related mortality.[27]

1.2.1.3 Polymer therapeutics

Ideally, an anti-tumour drug would seek out and selectively destroy tumour tissue whilst minimising non-specific toxicity towards healthy normal tissue. Current strategies for such targeting of tumour cells include drug, antibody and gene-therapy based approaches. A recent development in this area is the introduction of synthetic polymer-drug conjugates.

A polymer-drug conjugate consists of polymer chain, linker and a conjugated drug, as exemplified in Figure 1.6. Recently, this polymer therapeutic was used in a clinical trial and found to display considerably reduced toxicity compared with the free drug, with the evidence of selective targeting of the tumour.[28] The reduction in toxicity of the drug is due to the effect of enhanced permeability and retention (EPR) of the polymer-drug conjugate in cancer tissues. The first aspect of this is that due to its large size, the polymer

therapeutic cannot permeate into healthy tissues via blood vessels, and thus circulates in the blood system until it reaches tumour tissue. This it may enter, as now explained.

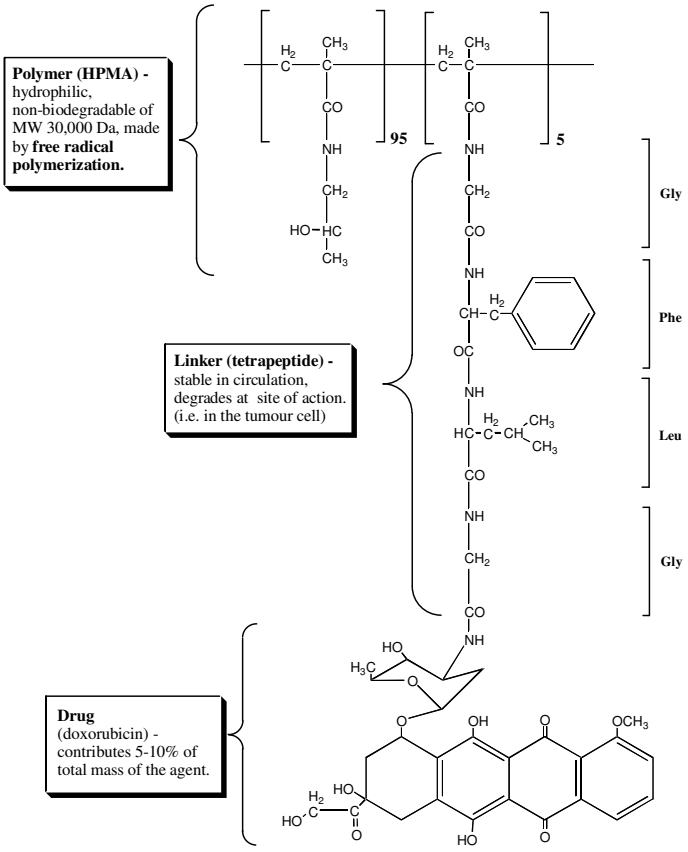


Figure 1.6 The prototypal polymer therapeutic PK1, consisting of HPMA (*N*-(2-hydroxypropyl)methacrylamide) copolymer with doxorubicin conjugate.

The phenomenon of increased vascular permeability in tumours has been known for a long time. The effect was utilised in clinical radiology, in particular in gallium scintigraphy. In this method radioactive gallium binds to an iron-binding protein transferrin. The gallium-transferrin complex enters the tumour site and remains there for some time, thereby allowing visualisation of the tumour location and size.[29] This mechanism was identified and the retention of macromolecular drugs is indeed the key mechanism for the EPR effect in tumour tissue.

Because tumours grow very fast, they tend to have a very poor blood supply. As a result, auxiliary blood vessels are formed. Due to their rapid growth and development, newly formed capillaries have very thin, leaky walls. It is believed that tumour blood vessels lack smooth muscle cells that surround the endothelial cells and the endothelial intercellular junctions are opened. This results in so-called ‘leakiness’ of tumour vasculature, which

allows the escape of the polymer-drug conjugate from the blood vessel into the tumour cells. Here there is consequent degradation of the linker molecule inside the tumour cell, and thus there is accumulation of the free drug in the tumour, as shown in Figure 1.7: this is the *retention* aspect of the EPR effect. The toxicity of the drug then results in the death of the cell. The EPR effect is also attributed to suppressed or poor lymphatic drainage of the tumour growth.

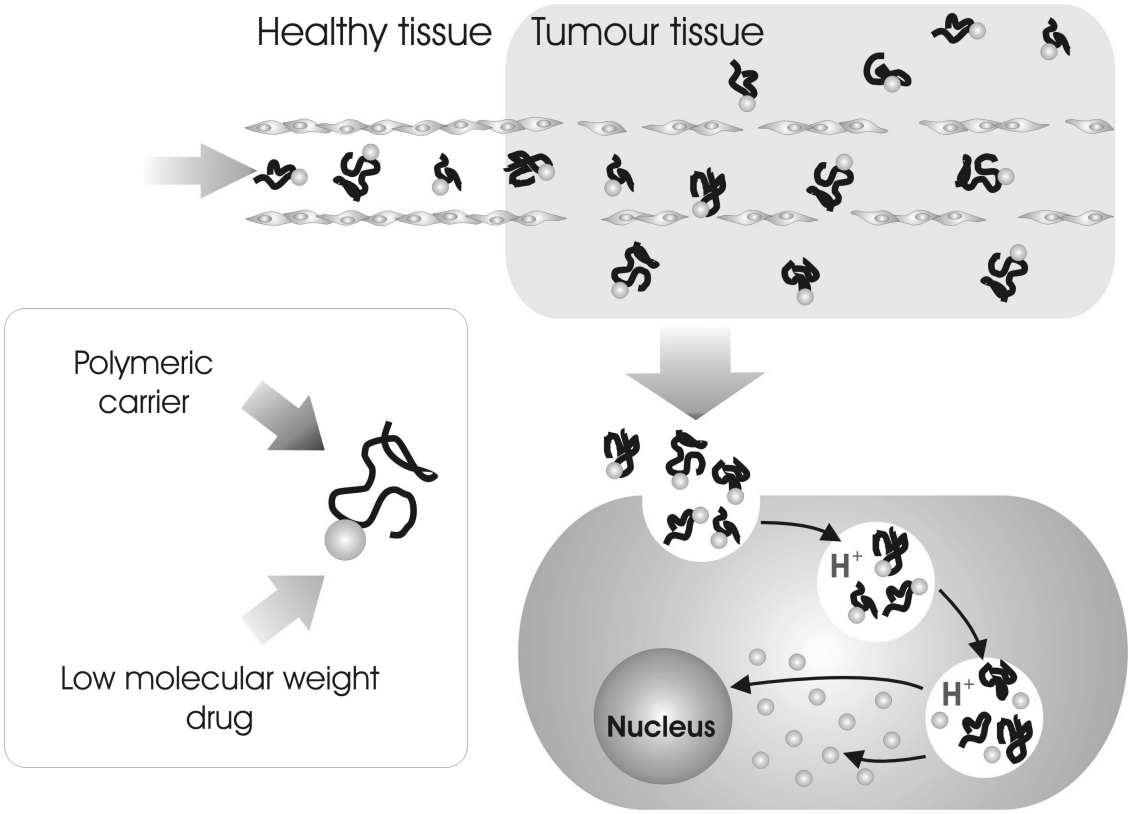


Figure 1.7 The EPR effect in tumour tissue.

Low molecular drugs released from a polymer-drug conjugate on their own would not selectively enter a tumour site, as their small size allows their escape from healthy blood vessels, as exemplified in Figure 1.8. Thus one sees the potential advantage of a polymeric carrier of the drug, i.e., of a polymer therapeutic.

The Marine Group of the Chemistry Department at the University of Canterbury, lead by Prof. Munro and Prof. Blunt, has isolated a number of bioactive products that have the potential to be employed as anti-cancer agents. However, because of solubility issues and because of their high potency, which endangers also healthy cells, it is of interest to incorporate these bioactives into polymer therapeutics. Synthesis of a suitable carrier molecule (i.e., the polymer component) became the ultimate aim of this work.

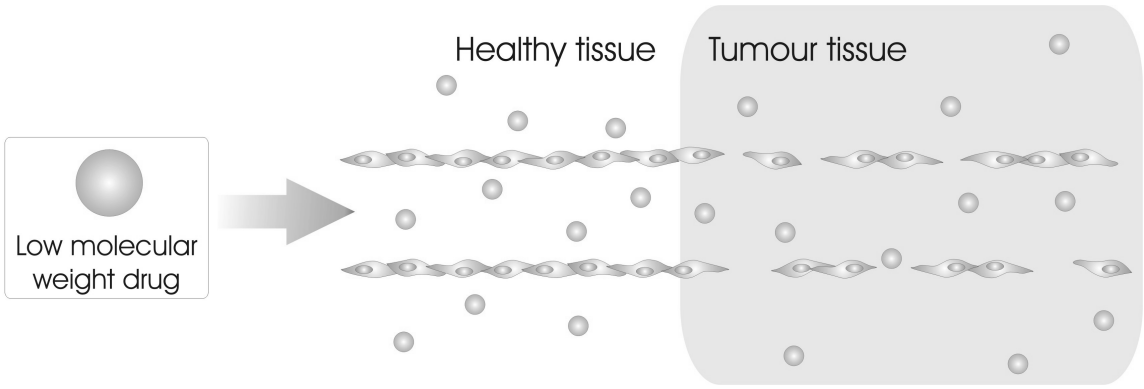


Figure 1.8 Distribution of low molecular weight drugs in healthy and tumour tissue.

The ideal polymeric carrier should be hydrophilic and must contain functional groups that allow covalent linkage of the drug. For this purpose a so-called 'linker' is used, e.g. the tetrapeptide of Figure 1.6. The linker should be stable in general circulation (i.e., in the bloodstream) but be able to degrade in the lysosome, thereby allowing intracellular drug release. Taking into account the renal threshold of 25,000-30,000 g/mol (i.e., human kidneys have difficulty expelling molecules larger than this) and the slow rate of cellular intake of smaller molecules, it has been suggested that a polymeric carrier of relatively uniform size of 25–30 kDa would be optimal for providing successful targeted delivery of the conjugate. This optimum size of the macromolecular vector has been established as a result of extensive research on drug accumulation in tumour tissue. The ability of LFRP to produce a target size like this with uniform chain length is one of the reasons LFRP is felt to be of promise for the better development of polymer therapeutics.

To illustrate the preceding point, Figure 1.9 shows the dependence of rate of tumour uptake and renal clearance of polymeric drugs on the size of the latter. Low-molecular weight drugs get cleared from the tumour by diffusion and return to the main blood stream, from which they get cleared very rapidly.[30] However, it is also evident that such small polymers have a low rate of tumour uptake, which is undesirable. At the other extreme, macromolecular drugs of large size experience good uptake but poor renal clearance, the latter being undesirable as the polymer will accumulate in the body. It is clear that an optimum size of about 20–30 kDa is highly advantageous for *all* conjugates, because it delivers both reasonable uptake and reasonable renal clearance.[22]

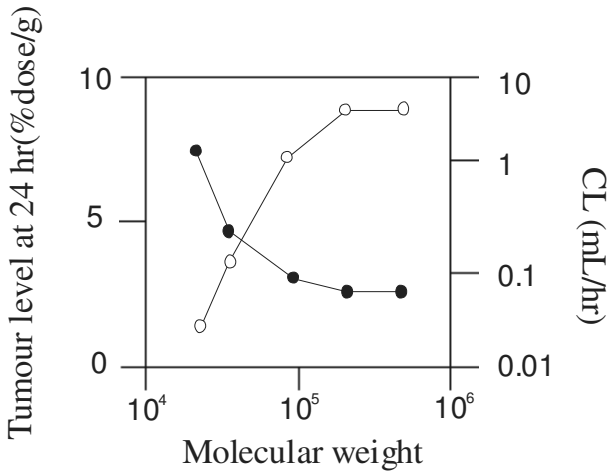


Figure 1.9 Schematic representation of relationships for molecular weight and tumour uptake and clearance of polymeric drugs; • CL, renal clearance over 72 hrs; o, tumour uptake over 24 hrs.[29]

Currently, the synthesis of a copolymer like PK1 (Figure 1.6) involves copolymerisation of HPMA and a functionalised linker by conventional free-radical polymerisation.[31] Fractionation of the resulting copolymer is performed by semi-preparative chromatography, using the same gel permeation chromatography (GPC) columns that are used in the analysis of the copolymer. In this highly laborious and wasteful fashion, isolation of a (relatively monodisperse) fraction containing the copolymer of required molecular weight is achieved.

While the procedure described above results in production of the copolymer of required molecular weight and size, it is clear that in order to produce a significant amount of the product, a large amount of the copolymer species will be wasted. Such an approach cannot be considered as an efficient one, especially for large scale production of the copolymer such as by a pharmaceutical company. Also, the process is time consuming and laborious. Clearly these problems could be overcome by successful LFRP synthesis of the polymer: no polymer would be wasted, and fractionation would not be necessary.

1.3 This thesis

1.3.1 Aims and rationale

The overall aim of this research was to identify the best way of preparing a polymeric precursor of PK1 by LFRP. Ideally, the method should allow successful copolymerisation

of HPMA and a linker to give a copolymer of required molecular weight. The technique should also assure incorporation of a desired amount of linker in the polymer backbone. An even distribution of the linker along the polymeric carrier is believed to result in a more efficacious drug distribution in the polymer therapeutic (as opposed, say, to if a block copolymer was formed, and the entire drug was located in one portion of the polymer). LFR copolymerization should achieve such a random distribution.

In view of the general principle that one should try to walk before running, obviously it was considered sensible for investigations to begin with homopolymerisation of HPMA rather than copolymerization with a linker. Thus here an attempt was made to prepare the polymer of HPMA of target molecular weight by the RAFT polymerisation method. Because HPMA is believed to spoil ATRP catalysts,[32,33] it was not attempted to polymerise this monomer this way.

An alternative approach to preparation of PHPMA has been suggested by Godwin *et al.*[34] The authors suggested synthesis of a polymer of activated ester, with subsequent quantitative conversion of this polymer into PHMPA. If controlled polymerisation of a monomer is successful and allows the preparation of the polymer of required number-average molecular weight and low polydispersity, then such polymer can serve as a precursor for preparation of a library of functionalised polymers with the same molecular weight characteristics.

With the above in mind, the use of methacryloyloxy succinimide (MAOS) was suggested and its successful polymerisation under the conditions of controlled polymerisation became a major aim of this research. The monomer was polymerised using the ATRP and RAFT methods.

Once appraised of the idea of using an ‘activated’ monomer, it becomes evident that there are other alternatives to MAOS. For example, the polymerization of both *p*-nitrophenyl methacrylate (NPMA) and methacryloyl chloride (MAC) should in principle produce a polymer that can easily be converted into PHPMA by reaction with 1-amino-2-propanol. All this is illustrated in Figure 1.10. Therefore this thesis also pursues the LFRP of NPMA and MAC, both by the RAFT polymerisation method (as with HPMA, MAC is believed to kill an ATRP catalyst).

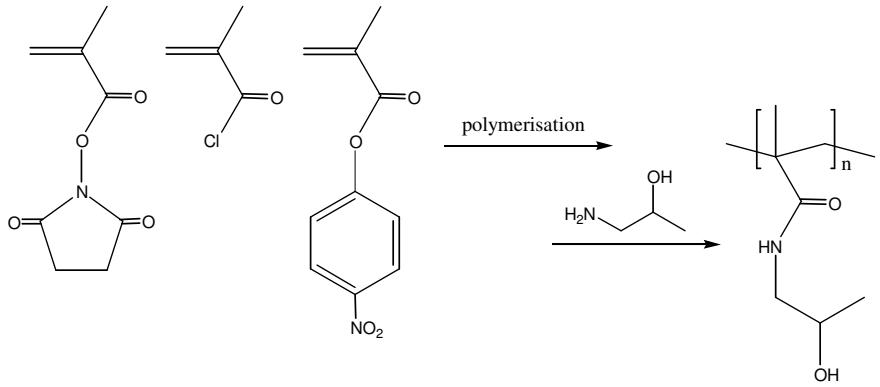


Figure 1.10 Proposed preparation of PHPMA from its polymeric precursors.

Inevitably in trying to carry out a specific research task, other questions arise. So it was in this work. This resulted in further investigations into LFRP, some of which are reported in this thesis.

Specifically, the following two should be noted:

(1) Because methyl methacrylate (MMA) is the archetypal ATRP monomer, preparatory ATRP experiments were carried out with MMA. It was observed that the rate of polymerization was highly dependent on the ATRP conditions. Although there have been some investigations of this in the literature, they were not as complete as would have been expected, and therefore many ATRP rate experiments were carried out with MMA in this work.

(2) While the success of RAFT is undeniable, it was a surprise to find that it has not been overly successful for effecting LFRP of monomers with an α-methyl group. Thus some RAFT experiments with MMA and *N*-isopropylmethacrylamide (NIPMAM) were also carried out. The latter was also employed as a non-hydroxylated analogue of HPMA, because the suggestion arose that the hydroxyl group of HPMA might be interfering with RAFT chemistry.

While this thesis has as its primary concern the effecting of LFRP of various monomers, at the same time there was also some interest in the kinetics of these processes, as is implicit in the paragraph above. Therefore quite a detailed kinetic analysis is reported herein.

Although the majority of this thesis is about homopolymerisation, the ultimate aim was to produce a polymer therapeutic. Some work in this regard was carried out, in the form of

attempts to convert PMAOS, PNPMA and PMAC into PHPMA, and also the LFR copolymerization of HPMA with a linker monomer.

1.3.2 Outline

Chapter 2 of this thesis describes general details of experimental procedures involved in this research. It contains details of syntheses of compounds used and characterisation data for the latter.

Details of experimental investigations into atom transfer radical polymerisation of methyl methacrylate (MMA) and methacryloyloxy succinimide (MAOS) are presented in Chapter 3. The chapter contains experimental details of the ATRP of the monomers, results and discussion of kinetic analyses, and modelling of kinetic data.

Chapter 4 discusses results of the RAFT polymerisation of a number of monomers with various RAFT agents were. Molecular weight and kinetic analyses of the polymerisation systems are presented and discussed. The chapter also contains results of modelling of kinetic data and evaluation of transfer constants.

In Chapter 5 a strategy for preparing a polymer of HPMA from various polymeric precursors is discussed and experimental results of that work are presented.

Some concluding thoughts are presented in Chapter 6.

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Chapter Two. Experimental details

2.1 General experimental

Commercially obtained chemicals were used as received unless otherwise stated.

Solvents were purified according to well established procedures.[1] All polymerisations were carried out using standard Schlenk line techniques under constant flow of argon.[1]

Methyl methacrylate (Mitsubishi Rayon stabilised with 4-methoxyphenol inhibitor) was passed through a column of basic alumina, distilled under reduced pressure, stored at 4 °C and used within a week. *N*-Isopropylmethacrylamide (Aldrich, 97%) was used as received. Other monomers used in this work were synthesised according to the procedures described in this chapter.

Acetonitrile, benzene, toluene and triethylamine were distilled from calcium hydride before use. Anisole was passed through basic alumina, dried on magnesium sulfate and distilled from sodium. Benzonitrile was distilled from calcium chloride. *N,N*-Dimethylformamide and dimethyl sulfoxide were dried over 4 Å molecular sieves for two periods of 24 hours and stored on 4 Å molecular sieves under argon. Dioxane was freshly distilled from sodium/benzophenone. Ethyl acetate was distilled. Methanol was distilled of magnesium and stored over 4 Å molecular sieves under argon. Tetrahydrofuran was freshly distilled from sodium/benzophenone. Xylene was distilled from sodium. Petroleum ether refers to the fraction with boiling point 50–70 °C. Sodium methoxide solution was prepared by reacting dry methanol with Na.

Removal of solvents under reduced pressure was done on a rotary evaporator.

The silica used in flash column chromatography was Kieselgel-60 (Merck) and the general guidelines for the procedure were followed.[1] Analytical TLC was conducted using aluminium-backed Merck Kieselgel silica plates.

Melting points were determined using an electrothermal melting point apparatus.

2.2 Polymerisation

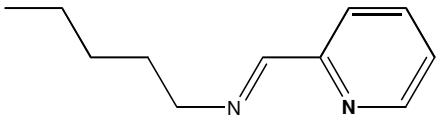
The following general polymerisation procedure was used in all polymerisation experiments.

All polymerisation components were added to a two-neck round-bottomed flask with magnetic stir bar. The mixture was placed under argon, and subjected to three "freeze-pump-thaw" cycles to minimise the presence of oxygen. The flask was placed in an oil bath at constant temperature to start polymerisation.

Samples were extracted from the polymerisation mixture using a degassed syringe and needle, and injected dropwise into a pre-weighed flask containing an appropriate non-solvent. These mixtures were stirring vigorously. The weight of samples was recorded. The precipitated polymer was filtered and placed in a dry, pre-weighed vial. The samples were dried under high vacuum overnight and re-weighed, allowing determination of the weight of formed polymer, and therefore conversion of monomer to polymer.

2.3 Syntheses

2.3.1 *N*-(n-Pentyl)-2-pyridylmethanimine (pen)



N-(n-Pentyl)-2-pyridylmethanimine is a ligand used in atom transfer radical polymerisation (ATRP) (see Chapter) and was prepared according to the procedure reported by Haddleton *et al.*[2]

Amylamine (6.20 g, 71 mmol) was added dropwise to pyridine-2-carboxaldehyde (7.59 g, 71 mmol) under vigorous stirring in an ice bath. After the addition was completed, magnesium sulfate (1.5 g) was added to the mixture and the reaction was left stirring for another 2 h. The solution was filtered and distilled under vacuum (0.1 Torr, °C) allowed isolation of the product as a clear yellow oil.

Yield: 3.8 g, 45%

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 0.88 (t, *J* = 6.3 Hz, 3H), 1.31 (m, 4H), 1.70 (m, 2H), 3.64 (t, *J* = 6.8 Hz, 2H), 7.26 (t, *J* = 5.9 Hz, 1H), 7.70 (t, *J* = 7.8 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 8.34 (s, 1H), 8.61 (d, *J* = 4.9 Hz, 1H)

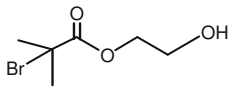
2.3.2 Copper (I) bromide

Copper (I) bromide is a catalyst used in ATRP (see Chapter), it was synthesised according to the following procedure.[3]

Copper (II) sulfate pentahydrate (7.50 g, 30 mmol) and sodium bromide (4.44 g, 43 mmol) were dissolved in 25 mL of warm distilled water. A solution of sodium sulfite (2 g, 16 mmol) in water (20 mL) was added dropwise to the mixture. A precipitate was formed. The mixture was poured into 1 L of water containing concentrated HCl (2 mL) and sodium sulfite (1 g). After 30 minutes of stirring, the mixture was filtered and the precipitate was collected. It was washed with water (100 mL), glacial acetic acid (100 mL), ethanol (90 mL) and anhydrous ether (90 mL). The precipitate was dried under high vacuum overnight and isolated as a white solid.

Yield: 2.27 g, 53%

2.3.3 Hydroxyethyl 2-bromoisobutyrate (HEBisoB)



Hydroxyethyl 2-bromoisobutyrate it an initiator used in ATRP (see Chapter) and was synthesised according to the following procedure.[4]

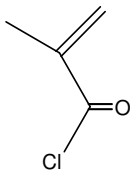
Bromine (4.5 mL, 88 mmol) was added dropwise over the period of 5 h to a mixture of isobutyric acid (3.5 g, 40 mmol) and phosphorus (0.12 g, 4 mmol). The resulting solution was heated to 100 °C over the period of 6 h. Residual bromine and formed HBr were removed under reduced pressure on a rotary evaporator. The crude 2-bromoisobutyryl bromide was added dropwise to a solution of ethylene glycol (9 mL, 160 mmol) in dichloromethane (DCM) (20 mL) with vigorous stirring. The mixture was refluxed for 4 h, filtered and added to distilled water (500 mL). The product was extracted into the organic phase by washing the aqueous solution with DCM three times. The organic phase was washed with water and saturated $NaHCO_3$ solution, and dried over $MgSO_4$. DCM was

removed under reduced pressure. The final product was isolated by vacuum distillation at 65 °C and 0.1 Torr and was obtained as a clear colourless liquid.

Yield: 3.8 g, 45%

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 1.96 (s, 6H), 3.86 - 3.88 (m, 2H), 4.30 – 4.32 (m, 2H)

2.3.4 Methacryloyl chloride (MAC)



Methacryloyl chloride is a monomer under investigation (see Chapter , Chapter Four and Chapter Five) and was prepared according to the reported procedure.[5]

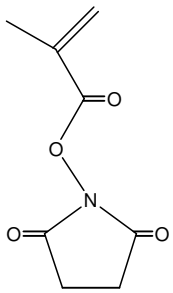
A standard distillation apparatus was set up and the distilling flask was charged with methacrylic acid (20 mL, 0.236 mol), benzoyl chloride (50 mL, 0.431 mol) and butylated hydroxytoluene (50 mg). The mixture was heated to reflux at 95 °C for 1 hour and heated further until condensation of product was observed. The fraction boiling at 95-105°C was collected into a receiving flask containing a small amount of butylated hydroxytoluene (BHT) under nitrogen atmosphere. The collected fraction was immediately re-distilled. Methacryloyl chloride was collected as a clear colourless liquid with strong acidic odour.

Yield: 13.3 g, 53%

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 2.01 (s, 3H), 6.03 (s, 1H), 6.50 (s, 1H)

2.3.5 Methacryloyloxy succinimide (MAOS)

Methacryloyloxy succinimide is a monomer under investigation (see Chapter , Chapter Four and Chapter Five) and was synthesised according to the procedure reported by Tirelli *et al.*[6]



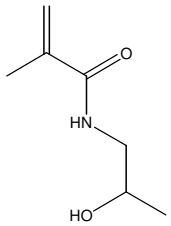
A solution of *N*-hydroxysuccinimide (11.5 g, 0.10 mol), triethylamine (28 mL, 0.20 mol) and butylated hydroxytoluene (10 mg) in dry dioxane (100 mL). The mixture was cooled to 0 °C under a dry argon atmosphere. A solution of methacryloyl chloride (14.5 mL, 0.15 mol) in dry dioxane (20 mL) was added to the mixture dropwise with vigorous stirring; the mixture was left stirring at 0 °C for an hour, then allowed to warm to room temperature and left overnight under constant stirring. The solvent was removed under reduced pressure and the solid was re-dissolved in DCM (1 L). The solution was washed with 5% aqueous HCl solution, 10% aqueous $NaHCO_3$ solution and water till the organic phase was neutral. The solution was dried with Na_2SO_4 and solvent was removed under reduced pressure. The product was recrystallised from ethanol and obtained as white crystals.

Yield: 13.25 g, 72%

Mp: 104 - 105 °C

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 2.05 (s, 3H), 2.86 (s, 4H), 5.88 (s, 1H), 6.41 (s, 1H)

2.3.6 *N*-(2-Hydroxypropyl)methacrylamide (HPMA)



N-(2-Hydroxypropyl)methacrylamide is a monomer under investigation (see Chapter Four and Chapter Five) and was prepared by following the literature procedure.[7]

A mixture of 1-amino-2-propanol (15.0 g, 0.20 mol) and sodium bicarbonate (19.3 g, 0.23 mol) in DCM (70 mL) was cooled to 0 °C under an argon atmosphere. A mixture of methacryloyl chloride (19.87 g, 0.19 mol) and BHT (one spatula) in DCM (30 mL) was

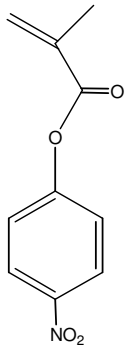
added dropwise over 1 h. After 30 minutes of vigorous stirring at room temperature, sodium sulfate (10 g) was added, and the mixture was left stirring overnight. The suspension was filtered; the volume was reduced down to half the original volume by means of removing the solvent under reduced pressure without heating. The remaining solution was placed in the freezer for 16 h. The product crystallised out from the mixture, it was filtered off and isolated as white crystals.

Yield: 21.2 g, 74%

Mp: 66-67 °C

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 1.20 (d, J = 6.3 Hz, 3H), 1.97 (s, 3H), 3.17 – 3.50 (m, 2H), 3.95 (m, 1H), 5.35 (s, 1H), 5.73 (s, 1H), 6.37 (broad s, 1H)

2.3.7 *p*-Nitrophenyl methacrylate (NPMA)



p-Nitrophenyl methacrylate is a monomer under investigation (see Chapter Four and Chapter Five) and was prepared following the following procedure.[8,9]

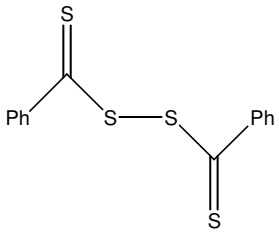
Dicyclohexylcarbodiimide (DCC) (33.01 g, 0.16 mol) in ethyl acetate (70 mL) was added dropwise to a solution of methacrylic acid (9 g, 0.11 mol) and *p*-nitrophenol (21.82 g, 0.16 mol) in ethyl acetate (100 mL) at 0 °C. The mixture was stirred overnight at room temperature. The resulting suspension was filtered; the solvent was removed from the filtrate under reduced pressure, reducing the volume to 50 mL. The solution was cooled to minus 50 °C, allowing formation of precipitate. The precipitate was filtered, recrystallised from ethanol and obtained as white crystals.

Yield: 18.32 g, 80%

Mp: 94 - 95 °C

^{1}H NMR (300 MHz, DMSO) δ_{ppm} 2.10 (s, 3H), 5.95 (s, 1H), 6.30 (s, 1H), 7.50 (d, J = 9.0 Hz, 2H), 8.32 (d, J = 9.15 Hz, 2H)

2.3.8 Bis(dithiobenzoyl) Disulfide



Bis(dithiobenzoyl) disulfide was used as starting material in preparation of dithioesters (see sections 2.3.9, 2.3.11 and 2.3.12). Preparation of bis(dithiobenzoyl) disulfide was based on the method previously described by Perrier *et al.*[10]

Elemental sulfur (3.2 g, 0.1 mol), 25% sodium methoxide solution in methanol (20 g) and methanol (20 g) were placed in a round bottom flask. Benzyl chloride (6.32 g, 0.05 mol) was added dropwise, over a period of 1 h, to the solution. Upon the completion of the addition, the solution had changed colour from clear to brown. It was heated to 80 °C and left to reflux overnight. The mixture was allowed to cool to room temperature and the mixture was filtered to remove sodium chloride. The solvent was removed under reduced pressure.

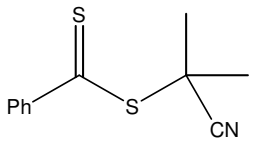
The brown solid was re-dissolved in distilled water (50 mL), and the solution was washed with diethyl ether (3 x 20 mL). A further portion of diethyl ether (25 mL) was added to the solution and the two-phase mixture was acidified with 32% aqueous HCl until the bottom (aqueous) layer lost the brown colour and the top (ether) layer became deep purple. The organic phase containing dithiobenzoic acid was isolated and distilled water (60 mL) and 1M NaOH solution (120 mL) were added. The organic phase was extracted two more times with the NaOH solution. The aqueous phases, containing the sodium dithiobenzoate, were combined. A solution of potassium ferricyanide (III) (17 g, 0.05 mol) in distilled water (250 mL) was added dropwise to the freshly prepared solution of sodium dithiobenzoate under constant vigorous stirring. A red precipitate was formed, which was isolated by filtration. The precipitate was washed with distilled water until the washings became colourless. The product was purified by column chromatography using ethyl

acetate/petroleum ether (1:1) as the eluent to yield the product as a purple solid. The compound was dried under high vacuum overnight.

Yield: 11.54 g, 75%

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.40 (t, *J* = 7.8 Hz, 2H), 7.61 (t, *J* = 6.2 Hz,1H), 8.08 (d, *J* = 7.3 Hz, 2H)

2.3.9 2-(2-Cyanopropyl)dithiobenzoate



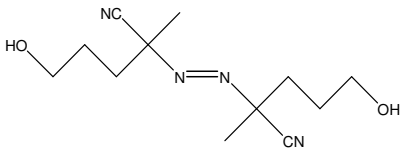
2-(2-Cyanopropyl)dithiobenzoate is a RAFT agent used in polymerisation of a number of monomers (see Chapter Four) and was synthesised according to the following method.[10]

A solution of AIBN (0.56 g, 3.4 mmol) and bis(dithiobenzoyl) disulfide (0.7 g, 2.3 mmol) in ethyl acetate (5 mL) was allowed to reflux for 18 h. Volatiles were removed under reduced pressure, and the residue was purified by column chromatography using 2% ethyl acetate/petroleum ether. The product was isolated as red oil at room temperature.

Yield: 0.51 g, 67%

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 1.95 (s, 6H), 7.40 (t, *J* = 7.8 Hz, 2H), 7.57 (t, *J* = 6.4 Hz,1H), 7.91 (d, *J* = 7.9 Hz, 2H)

2.3.10 4,4'-Azobis(cyanopentanol)



4,4'-Azobis(cyanopentanol) was used as starting material in preparation of dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester (see section 2.3.11) and was synthesised according to the procedure reported by Reed.[11]

Under constant stirring a solution of sodium cyanide (1.08 g, 22 mmol) was dissolved water (10 mL) was added to a mixture of hydrazine sulfate (1.43 g, 11 mmol) and 5-

hydroxy-2-pentanone (2.25 g, 22 mmol) in water (15 mL). The mixture was stirred overnight, then cooled in an ice bath. The solution was acidified to pH 5 by addition of 15% aqueous HCl solution. Bromine (3.5 g, 22 mmol) was added dropwise to the mixture over a period of 5 h. The yellow coloration of remaining bromine was removed by addition of saturated solution of $NaHSO_3$. The mixture was left overnight. The solution was washed with DCM/acetone (2:1) allowing extraction of the product into the organic phase. The solvents were removed under reduced pressure and the resulting brown, oily residue was cooled in an ice bath and left in a refrigerator overnight. The azocompound precipitated from the mixture and collected by filtration. The product was recrystallised from $CHCl_3$/petroleum ether as a white solid.

Yield: 3.2 g, 58%

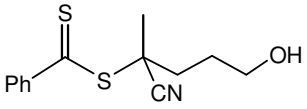
Mp: 81 - 96 °C

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 1.47-1.83 (m, 2H), 1.76 (s, 3H), 2.06-2.32 (m, 2H), 3.66-3.72 (m, 2H)

^{13}C NMR (75 MHz, CD_3CN) δ_{ppm} 22.2, 26.4, 33.6, 59.6, 71.5, 117.6

HRMS: Calculated for $C_{12}H_{20}N_4O_2$ (M^+) 253.1620, found 253.1620

2.3.11 Dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester



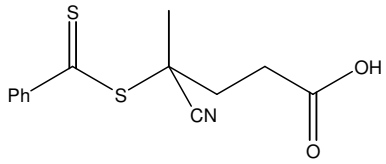
Dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester is a RAFT agent used in polymerisation of a number of monomers (see Chapter Four) and was prepared as described below.

A solution of 4,4'-azobis(cyanopentanol) (0.86 g, 3.4 mmol) and bis(dithiobenzoyl) disulfide (0.7 g, 2.3 mmol) in ethyl acetate (6 mL) was degassed and then heated to reflux at 70 °C for 24 h. The volatiles were removed under reduced pressure and the residual red solution was purified by column chromatography using acetonitrile/ethyl acetate/petroleum ether (0.5 : 3 : 7) as the eluent. The product was recrystallised from benzene and isolated as a bright pink solid.

Yield: 0.42 g, 46%

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 1.99 (s, 3H), 2.46 - 2.52 (m, 2H), 2.65 – 2.70 (m, 2H), 3.73 (m, 2H), 7.44 (t, *J* = 7.5 Hz, 2H), 7.61 (t, *J* = 7.5 Hz,1H), 7.95 (dd, *J* = 1.2, 8.3 Hz, 2H)

2.3.12 4-Cyanopentanoic acid dithiobenzoate



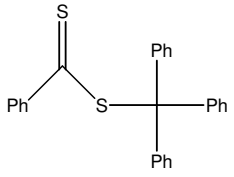
4-Cyanopentanoic acid dithiobenzoate is a RAFT agent used in polymerisation of a number of monomers (see Chapter Four) and was prepared according to the literature procedure.[12]

A solution of 4,4'-azobis(cyanovaleric acid) (0.75 g, 2.7 mmol) and bis(dithiobenzoyl) disulfide (0.51 g, 1.7 mmol) in ethyl acetate (5 mL) was degassed and then heated to reflux at 70 °C for 24 h. The volatiles were removed under reduced pressure and the residual red solution was purified by column chromatography using acetonitrile/ethyl acetate/petroleum ether (0.2 : 3.9 : 5.9) as the eluent. The product was isolated as a bright pink solid.

Yield: 0.53 g, 70%

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 1.99 (s, 3H), 2.46 – 2.70 (m, 2H), 2.74 – 2.81 (m, 2H), 7.44 (t, *J* = 7.5 Hz, 2H), 7.61 (t, *J* = 7.13 Hz, 1H), 7.95 (dd, *J* = 1.2, 8.1 Hz, 2H)

2.3.13 Triphenylmethyl dithiobenzoate



Triphenylmethyl dithiobenzoate is a RAFT agent used in polymerisation of a number of monomers (see Chapter Four) and was synthesised according to the method reported by Alberti *et al.*[13]

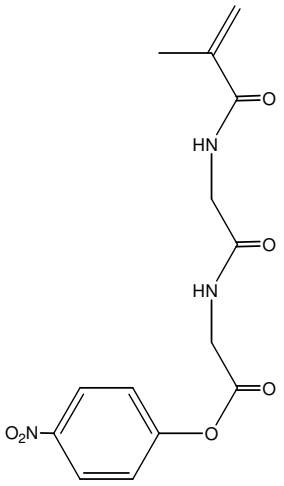
A solution of bromobenzene (0.14 mL, 1.27 mmol) in dry THF (20 mL) was added dropwise to a mixture of Mg (0.03 g) and a crystal of I_2 in THF (10 mL). After the reaction was left stirring for 2 h, it was cooled to 0°C and CS_2 (0.1 g, 1.3 mmol) was added. After the addition the solution was allowed to reach room temperature. The solution was left stirring at room temperature for another 3 h. After that a solution of trityl chloride (0.35 g, 1.27 mmol) in THF (1.5 mL) was added dropwise. After stirring overnight, CH_2Cl_2 (20 mL) was added to the reaction and the solution was filtered. The solvent was removed under reduced pressure and the product was purified by column chromatography using CH_2Cl_2/pentane (1 : 4) as the eluent. The product was obtained as a purple solid.

Yield: 0.34 g, 67%

Mp: 42 - 43 °C

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.251 (m, 12H), 7.536 (d, *J* = 8 Hz, 6H), 8.018 (d, *J* = 8 Hz, 2H)

2.3.14 *N*-Methacryloylglycylglycine p-nitrophenyl ester (MA-Gly-Gly-ONp)



N-Methacryloylglycylglycine-*p*-nitrophenyl ester is a model linker that was copolymerised with HPMA (see Chapter Five) and was prepared according to the following procedure.[14]

NaOH (0.40 g, 0.01 mol) was added to a solution of glycylglycine (Gly-Gly) (1.32 g, 0.01 mol) in water (3 mL). On cooling to 0 °C, methacryloyl chloride (1.05 g, 0.01 mol) and NaOH (0.40 g, 0.01 mol) in water (3 mL) were added dropwise and simultaneously. The reaction mixture was stirred at room temperature for an hour and acidified with concentrated HCl to pH 2. The crystals of MA-Gly-Gly-OH precipitated from the acidified solution, were filtered, recrystallised from ethanol/water mixture (50%) and dried under high vacuum overnight.

Yield: 1.39 g, 70%

Mp: 196 - 199 °C

^{1}H NMR (300 MHz, DMSO) δ_{ppm} 1.962 (s, 3H), 3.829 (t, *J* = 6 MHz, 4H), 5.465 (s, 1H), 5.841 (s, 1H), 8.223 (t, *J* = 5 MHz, 1H), 8.341 (t, *J* = 5 MHz, 1H)

To MA-Gly-Gly-OH (0.5 g, 2.5 mmol) and *p*-nitrophenol (0.42 g, 3.0 mmol) in DMF (20 mL) was added DCC (0.62 g, 3.0 mmol) in DMF (1 mL). The reaction mixture was allowed to stir at -10 °C for 3 hours and then left to stand at room temperature overnight.[15] N,N'-Dicyclohexylurea precipitated out of the mixture and was removed by filtration. The solution was concentrated by removing DMF under reduced pressure. The product crystallised out of the remaining solution upon cooling of the latter and was collected as white solid. It was washed with diethyl ether 4 times and dried under high vacuum overnight.

Yield: 0.43 g, 53%

Mp: 155 - 162 °C

^{1}H NMR (500 MHz, DMSO) δ_{ppm} 1.902 (s, 3H), 3.732 (s, 2H), 4.196 (s, 2H), 5.408 (s, 1H), 5.776 (s, 1H), 7.457 (s, 2H), 8.332 (s, 1H)

^{13}C NMR (75 MHz, acetone) δ_{ppm} 18.6, 41.2, 42.2, 120.0, 123.0, 125.4, 139.4, 145.1, 155.2, 167.8, 168.3, 170.0

2.4 Instrumental

2.4.1 Gel permeation chromatography

2.4.1.1 Ian Wark Laboratories, CSIRO, Melbourne

Molecular weights of THF-soluble polymer samples were characterized by gel permeation chromatography (GPC) performed in tetrahydrofuran (THF, 1.0 mL/min) at 25°C using a Waters GPC instrument, with a Waters 2414 Refractive Index Detector, a series of four Polymer Laboratories PLGel columns (3 × 5 μm Mixed-C and 1 × 3 μm Mixed-E), and Millennium Software. The GPC was calibrated with narrow polydispersity polystyrene standards (Polymer Laboratories EasiCal, MW: 264 – 256000 g mol^{-1}), and molecular weights are reported as polystyrene equivalents.

DMF-soluble samples were analysed using a series of four Waters Styragel columns HT2, HT3, HT4 and HT5, and an oven temperature of 80 °C. The solvent was DMF + 0.05 M LiBr at a flow rate of 1.0 mL/min. A Dawn EOS light scattering detector with Optilab DSP interferometer (both set at 690 nm) was used.

2.4.1.2 Key Centre for Polymer Colloids, University of Sydney

Molecular weights of THF-soluble polymer samples were characterized using a Shimadzu system fitted with a series of Waters columns (HR4, HR3, and HR2) using THF as an eluent (1.0 mL/min). Molecular weight was determined from refractive index data analyzed with Polymer Laboratories Cirrus software, with all molecular weights being relative to poly(methyl methacrylate) standards (PSS Readycal-Kit, MW: 500 – 3000000 g mol^{-1}).

Analyses of PMAOS samples were carried out using a Shimadzu system fitted with a series of two Polymer Standards Service (PSS) GPC columns (PSS SDV 5 μ linear XL and PSS SUPREMA linear XL 10 μ) at 80 °C. DMF/0.5% LiBr was used as eluent. Molecular weight was determined from refractive index data analyzed with Polymer Laboratories Cirrus software, with all molecular weights being relative to poly(methyl methacrylate) standards (PSS Readycal-Kit, MW: 500 – 3000000 g mol^{-1}), i.e., for non-PMMA samples, universal calibration was not carried out.

2.4.1.3 Polymer Institute of the Slovak Academy of Sciences, Bratislava

Analyses of PHPMA samples were carried out using the following setup: Waters degasser, Waters 515 pump, 7725i Rheodyne injector, DRI Waters 2410 and WinGPC 7.20 software. The system was fitted with a series of PSS Mainz columns (10 mm GRAM – guard 8x50 mm + three 8x300mm 100, 1000 and 3000 Å) at 50 °C. DMF/0.01 M LiBr/0.05 M CH_3COOH was used as eluent at a flow rate of 0.8 mL/min.

Universal calibration to PEO and PEG standards (Polymer Laboratories) using MHS parameters from Mendichi *et al.*[16,17] determined for the eluent composition at 50 ºC:

PHPMA: a = 0.690, K = 0.0124 mL g^{-1}

PEO: a = 0.709, K = 0.02538 mL g^{-1}

PEG: a = 0.594, K = 0.07895 mL g^{-1}

Calibration curve was made combining PEG standards (MW: 2010 - 12000 g mol^{-1}) and PEO standards (MW: 21000 – 570000 g mol^{-1}). Both sets of standards satisfactorily fit the 3rd polynomial and provided a calibration curve for analysis of polymers.

2.4.2 Nuclear magnetic resonance spectroscopy (NMR)

^1H NMR spectra were recorded at the University of Canterbury on either a Varian Unity 300 or Varian Inova 500 instrument. ^{13}C NMR spectra were recorded on a Varian Unity 300 instrument. All chemical shifts were reported relative to solvent residual peaks as follows: $CDCl_3$ (7.26 ppm), $(CD_3)_2SO$ (2.50 ppm), CD_3CN (1.94 ppm), D_2O (4.79 ppm) for ^1H and CD_3CN (118.26 ppm) for ^{13}C NMR spectra.[18]

2.4.3 High resolution mass spectrometry

High resolution mass spectrometry (HRMS) was performed at the University of Canterbury on a micromass LCT TOF mass spectrometer in electrospray ionisation (ESI) mode with a probe voltage of 3200 V, temperature of 150 °C and a source temperature of 80 °C.

2.5 References

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Chapter Three. Atom transfer radical polymerisation – ATRP

3.1 Introduction

Today many commercial polymers are prepared by free radical polymerisation. While a wide range of monomers can be polymerised under mild reaction conditions, the major drawback of conventional free radical polymerisation is that the resulting polymers tend to have a relatively broad molecular weight distribution. Synthesis of polymers with controlled molecular weight and well-defined architecture is becoming a major goal of polymer chemistry. Recently, new polymerisation methods that allow the synthesis of polymer chains with required degree of polymerisation $\overline{DP}_n$ from a range of monomers have been developed.[1-3]

The key step in a fundamental mechanism of those methods is establishment of a rapid equilibrium between active and dormant radical species. The established equilibrium should favour formation of dormant species, therefore lowering the concentration of active radicals in the system. The amount of reactive radicals should remain at such levels that the extent of termination reactions, which still occur under the conditions of controlled polymerisation, is negligible.

In *atom transfer radical polymerisation* or ATRP the concentration of active species is kept very low by the presence of a catalytic transition metal complex, which reversibly deactivates growing polymer chains via a halogen atom transfer. As long as active radicals are present in a system, termination reactions i.e., radical coupling and disproportionation, cannot be suppressed fully. However, it is believed that in a well-controlled ATRP system only up to 5% of the polymer chains terminate, allowing a good level of molecular weight control of produced chains.[4]

In addition to offering control over molecular weight of produced polymer, ATRP also affords unprecedented command over other aspects of macromolecular design. Where ATRP is employed, functionality of a polymer can be predetermined by use of

functionalised monomers[5] or monomer derivatives if the desired monomer itself cannot be polymerised using the method.[6] Initiators with various functionalities allow synthesis of chains with specific end-groups.[7,8] Through strategically placing certain functionality on polymer chain-ends, it is possible to perform various chemical transformations of the polymer. Chains produced by the method of ATRP are normally capped by a halogen. This halogen can be displaced via a number of reactions, yielding polymer chains with new termini.[9] Block and gradient polymers can be synthesised by this method.[10] Even monomers that could not be homopolymerised via ATRP were incorporated into block copolymers where the first block was prepared by ATRP.[11] This newfound capacity to tailor macromolecular design often serves as starting point for synthesis and is leading to many novel polymeric materials and thus promises an age of 'smart' polymers.

Of many possible applications of ATRP, this research has been concentrated on design and synthesis of *polymer therapeutics*. Ringsdorf's seminal idea[12] was that by attaching a drug molecule to a polymer, the efficacy of the drug could be increased, through what has become known as the 'EPR effect', standing for 'enhanced permeability and retention'.[13] While quite a few 'polymer therapeutics' are already in, or progressing towards, clinical development,[14] future progress in the field would certainly be promoted by an ability to synthesise polymer components of uniform size.

There is hope that new methods of polymerisation will allow synthesis of polymeric materials with such narrow polydispersity, as regulatory authorities frown on pharmaceuticals that consist of molecules of a variety of sizes. Because ATRP can deliver polymer of close-to-uniform size, it has the potential to lead to better polymer therapeutics, and hence superior treatments for cancer and other diseases.

Ideally, one wants to be able to directly apply a method of controlled polymerisation to synthesis of a polymer of HPMA from its monomer, i.e. *N*-(2-hydroxypropyl)methacrylamide. Success of such synthesis will mark a further step in development and production of anti-cancer agents that prove to show lower toxicity and improved targeting properties when compared to low molecular weight drugs. The high toxicity of the latter is proving to be a major hurdle in successful treatment of cancer.

In this work the system of ATRP of methyl methacrylate (MMA) and its kinetics have been investigated. Although this is not of direct importance in terms of the stated aim, it

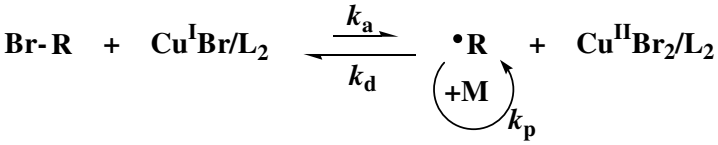
is of interest in its own right and is of indirect importance in that MMA is an excellent monomer for understanding the process of ATRP in general, and by doing this the capacity to use ATRP for all monomers - e.g., HPMA, the one of direct interest – is enhanced. The method was also applied in synthesis of polymer of activated ester methacryloyloxy succinimide (MAOS) that is believed to have a potential to give rise to a library of functionalised polymers, including a polymer of HPMA.

3.1.1 Mechanism

Before proceeding to the discussion of results of this work, it is imperative that the reader should be acquainted with the mechanism of the ATRP process and have a clear understanding of the role each component plays in the polymerisation process.

As a multicomponent system, a typical ATRP system consists of a monomer, an ATRP initiator and a transition metal species in a complex with a ligand (L).

As with any other free-radical polymerisation, ATRP starts with generation of free radicals. Free radicals (R) are generated when a halogen atom (Br) is abstracted from the initiator e.g., R-Br, by the transition metal e.g., $Cu^{I}Br/L_2$ complex. Upon the halogen atom transfer, $Cu^{I}Br/L_2$ complex is converted into a corresponding $Cu^{II}Br_2/L_2$ complex, Scheme 3.1.



Scheme 3.1 Reaction scheme for copper-mediated ATRP.

Active radical chains initiate and grow by reacting with monomer (M) with the rate constant of propagation k_p. The described process would result in a polymerisation which would not be different from a conventional free-radical process if it was not for a *deactivation reaction*, in which the halogen atom is abstracted from the deactivator i.e., $Cu^{II}Br_2/L_2$ complex, by the growing radical species. Now the growing radicals are capped by the halogen atom and are in their dormant or unreactive state, therefore unable to propagate further unless the halogen cap comes off again. As a result of deactivation, Cu^{II} gets oxidised back into Cu^{I}.

3.1.1.1 Metal – Ligand Catalyst

The transition metal species utilised in the ATRP process is often termed *a catalyst*. It is the most important component of ATRP as its properties determine the position of the equilibrium that governs concentration of active and dormant radical species.

A catalyst must satisfy a number of requirements in order to keep the polymerisation process under control:

(1) The metal centre must have two oxidation states, interchange between which should be achieved by a single electron transfer via abstraction and addition of a halogen atom.

(2) As transition metals are often insoluble in organic solvents, addition of a suitable ligand to the ATRP mixture improves the solubility of the metal catalyst by forming a complex with the latter.[4] Solubility of the complex will determine the actual concentration of the catalyst in the reaction mixture, therefore affecting the position of the equilibrium, the overall kinetics of the polymerisation and the molecular weight distribution of produced polymer chains.

(3) When the complex abstracts the halogen atom from the initiator, the coordination sphere of the complex must expand, and thus the metal centre should have a coordination site available for the addition to occur. It was found that for copper-mediated ATRP four coordination sites must be filled by ligands, leaving one site available for the abstraction of the halogen atom. In the case of bidentate ligands, the kinetically optimal ratio of the ligand species to the $Cu^{I}Br$ was found to be 2:1.[4]

(4) Ligands should form a strong complex with the metal centre, as high lability of the complex will result in displacement of the ligands by solvent or monomer molecules. That might result in formation of a new strong complex, in which all coordination sites of the metal will be filled, therefore preventing transfer of the halogen counter ion and resulting in uncontrolled polymerisation.

As the transition metal undergoes a one-electron oxidation, it abstracts the halogen atom from the initiator by cleaving the carbon-halogen bond, which results in formation of a carbon-centred radical. Polymer chains start growing by the addition of newly-formed radicals to monomer. The key to a controlled polymerisation is suppression of

termination reactions. In ATRP this is achieved by keeping the concentration of reacting radicals at a low level. This is done by creating and maintaining an equilibrium at which formation of dormant species is favoured, i.e., K_{eq} (= k_a/k_d) is low. It is evident that if the value of K_{eq} is very low, polymerisation would occur at a very slow rate. A high value of K_{eq} will result in faster polymerisation rates, however, due to the increase in concentration of actively growing chains the extent of termination reactions would also increase, resulting in a formation of dead-polymer chains throughout the process. Much desired control of molecular weight and molecular weight distribution of produced chains would be lost. It is thus clear that a successful ATRP involves a balancing act between the need for a practicable rate (high K_{eq}) and controlled character (low K_{eq}).

Activation and deactivation reactions identified in Scheme 3.1 occur with the rate constants k_a and k_d respectively. The rate constants depend on the structure of monomer, on the halogen and the transition metal complex. As indicated in the scheme by the arrows, the equilibrium must lie heavily toward the reactant side to assure that the majority of polymer chains are capped and in a dormant state. The position of the equilibrium is also determined by relative concentrations of activating Cu^{I} and deactivating Cu^{II} species. These concentrations will depend on the initial amount and the stability of ligands that form the complexes with the metals. Higher solubility of the catalytic species results in higher concentration of the latter in the reaction mixture. It is evident that higher relative concentration of deactivating species Cu^{II} will ensure production of polymer chains with reduced polydispersity.

3.1.1.2 Solvent

ATRP polymerisation can be carried out in bulk and in solution. While catalyst solubility will be determined by type and amount of ligand used, polarity of polymerisation medium will also affect homogeneity of the transition metal species in solution. Solvent interaction with the metal centre can result in formation of a different catalyst complex. Therefore it is important to note that the notation used for describing the catalyst complexes should serve as an indication of a stoichiometric ratio of ligand to metal species only, rather than a description of the catalyst's structure in solution. Previously work has been done on investigating the effect that reaction medium has on kinetic parameters of ATRP, where possible change in catalyst structure was used as an

explanation for unexpected rate acceleration when ATRP of *n*-butyl acrylate was conducted in ethylene carbonate.[15]

3.1.1.3 This work

An enormous amount of research has been carried out on the effect of components on ATRP, most notably by Matyjaszewski[4] and with Haddleton[16] also to the fore. These studies have largely been of a qualitative nature but have also included some good quantitative work. This research is to add to this work with a deeper investigation of ligand, solvent and temperature effect on the kinetics of ATRP of methacrylates, in particular methyl methacrylate.

3.2 Experimental

3.2.1 Chemicals

Commercially obtained chemicals were used as received unless otherwise stated.

Details of syntheses involved are described in Chapter Two of this thesis.

Methyl methacrylate (Mitsubishi Rayon stabilised with 4-methoxyphenol inhibitor) was passed through a column of basic alumina, distilled under reduced pressure, stored at 4 °C and used within a week. Solvents were purified according to well established procedures.[17] Copper (I) bromide was synthesised according to a well established method.[18] *N*-(*n*-Pentyl)-2-pyridylmethanimine was prepared according to the procedure reported by Haddleton *et al.*[19] Methacryloyloxy succinimide[20] and hydroxyethyl 2-bromoisobutyrate were synthesised,[21] Ethyl 2-bromoisobutyrate (Lancaster Synthesis, 98+%) was distilled prior to use. Butylated hydroxytoluene (Aldrich, 99+%) was recrystallised from methanol. 4-Methoxyphenol (Aldrich, 99%) was distilled under reduced pressure using Kugelrohr ball-tube distillation apparatus. 2,2'-Bipyridine (Aldrich, 99+%) was used as received.

3.2.2 Methyl methacrylate (MMA) polymerisation procedure

MMA (4.00 g, 0.04 mol), ligand (8.00×10^{-4} mol), $Cu^{I}Br$ (57.4 mg, 4.00×10^{-4} mol) and initiator (4.00×10^{-4} mol) were dissolved in appropriate amount of solvent in a 25 mL

two-neck round bottom flask and placed under argon. The mixture was degassed by three "freeze-pump-thaw" cycles and placed in an oil bath at the appropriate temperature.

3.2.2.1 PMMA Analysis methods

Samples were periodically withdrawn from the mixture with a degassed syringe and placed in pre-weighed aluminium plates. Samples were dried in an oven at 60 °C overnight, their weight was determined, from which the monomer conversion was established. Samples were analysed by ^{1}H NMR.

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 0.79–1.42 (br m, 3H, CH_3 in backbone), 1.78–2.03 (br m, 2H, CH_2 in backbone), 3.55 (br s, 3H)

For molecular weight analysis, samples were re-dissolved in THF and passed through the column of basic alumina to remove catalyst. Samples were precipitated in methanol, dried and re-dissolved in THF and filtered prior to GPC analysis. GPC analyses were carried out at Key Centre for Polymer Colloids (KCPC) at the University of Sydney using a Shimadzu system fitted with a series of Waters columns (HR4, HR3, and HR2) using THF as an eluent. Molecular weight was determined from refractive index data analyzed with Polymer Laboratories Cirrus software, with all molecular weights being relative to poly(methyl methacrylate) standards (PSS Readycal-Kit, MW: 500 – 3000000 g mol^{-1}).

3.2.3 Methacryloyloxy succinimide (MAOS) polymerisation procedure

MAOS (4.00 g, 2.20×10^{-2} mol), ligand (4.37×10^{-4} mol), $Cu^{I}Br$ (31.4 mg, 2.20×10^{-4} mol) and initiator (2.20×10^{-4} mol) were dissolved in DMF (4.00 g) in a 25 mL two-neck round bottom flask and placed under argon. The mixture was degassed by three "freeze-pump-thaw" cycles and placed in an oil bath at the appropriate temperature.

3.2.3.1 PMAOS Analysis methods

Samples were withdrawn from the mixture with degassed syringe and for isolation of the polymer were precipitated in a pre-weighted flask with acetone under vigorous stirring.

Polymer was filtered and dried overnight under vacuum. Samples were analysed by ^{1}H NMR.

^{1}H NMR (300 MHz, DMSO) δ_{ppm} 1.3 (br s, 5H, CH_3 and CH_2 in backbone), 2.82 (br s, 2H)

For the purpose of GPC analysis, polymer samples were re-dissolved in DMF and filtered prior to the analysis. Analyses of PMAOS samples were carried out at Key Centre for Polymer Colloids (KCPC) at the University of Sydney using a Shimadzu system fitted with a series of two Polymer Standards GPC columns (PSS SDV, 5 μ, linear XL and PSS SUPREMA linear XL 10 μ) at 80 °C. DMF/0.5% LiBr was used as eluent. Molecular weight was determined from refractive index data analyzed with Polymer Laboratories Cirrus software, with all molecular weights being relative to poly(methyl methacrylate) standards (PSS Readycal-Kit, MW: 500 – 3000000 g mol^{-1}).

Theoretical molecular weights were calculated based on measured monomer conversion using the following formula:

$$M_{n,theory} = MW_{monomer}\, x \left(\frac{[M]_0}{[I]_0}\right) \qquad (3.1)$$

where x is monomer fractional conversion, $MW_{monomer}$ is monomer molecular weight, $[M]_0$ and $[I]_0$ are monomer and initiator initial concentrations respectively.

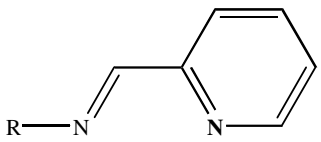
3.3 ATRP of MMA

The ATRP of MMA was chosen to be a model system for this research. Methyl methacrylate (MMA) is a common monomer in many types of polymerisation and its behaviour has been well studied. Indeed, ATRP of MMA has been carried out by other research groups and there are many publications related to it.[4,22] It is held to be an archetypal monomer for ATRP and here results for ligand, solvent and temperature effects on kinetics of ATRP of MMA are presented.

3.3.1 Ligand effect

As transition metals are often insoluble in organic solvents, addition of a suitable ligand to the ATRP mixture improves the solubility of the metal catalyst by forming a complex with the latter.[4] Solubility of the complex will determine the actual concentration of the catalyst in the reaction mixture, therefore affecting the position of the equilibrium, and the overall kinetics of the polymerisation as well as molecular weight distribution of produced polymer chains. A number of different ligands have been used in various ATRP systems in an attempt to achieve the maximum control over molecular weights of products.[23] It was found that in copper and iron-mediated ATRP systems, nitrogen-based ligands produced the best results.[24] It was also found that while polydentate macrocyclic ligands can provide a well-defined environment around the metal centre in solution, they can also bind Cu^{I} quite strongly, which will restrict the flexibility of geometry around the metal core.[25] This lack of flexibility might compromise the conformational changes the copper species will have to undergo in order to accommodate a halogen atom abstracted from the initiating species.[4] This and commercial availability of simpler bidentate ligands are the reasons why bidentate ligands are widely used in ATRP.

Haddleton uses Schiff base *N*-(*n*-alkyl)-2-pyridylmethanimine ligands (Figure 3.1), which in copper-mediated ATRP proved to be fairly effective in polymerisation of methacrylates, particularly where non-polar solvents were used.[26]



R = C_3H_7 *N*-(*n*-Propyl)-2-pyridylmethanimine
C_5H_{11} *N*-(*n*-Pentyl)-2-pyridylmethanimine
C_8H_{17} *N*-(*n*-Octyl)-2-pyridylmethanimine

Figure 3.1 Schiff base *N*-(*n*-alkyl)-2-pyridylmethanimine ligands.

In this work the effect of two different types of nitrogen-containing ligand on the rate of ATRP of MMA was investigated. The ligands used were *N*-(*n*-pentyl)-2-pyridylmethanimine (*pen*) and 2,2'-bipyridine (*bpy*), structures of which are presented in Figure 3.2.

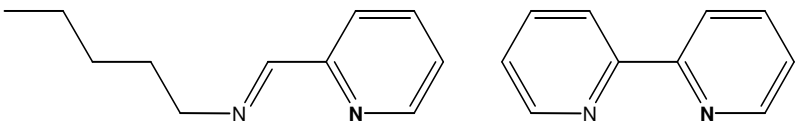


Figure 3.2 Ligands used in ATRP of MMA, *N*-(*n*-pentyl)-2-pyridylmethanimine and 2,2'-bipyridine.

While 2,2'-bipyridine is widely used in various research groups, its use does not always produce a homogeneous reaction mixture, therefore raising the question if such a heterogeneous ATRP system is a living system. In order to compare results of such heterogeneous ATRP with the ATRP system with improved solubility of copper species, it was decided to use one of the Schiff base ligands. Haddleton notes that the catalytic species of copper complexed by *N*-(*n*-propyl)-2-pyridylmethanimine in some systems shows poor solubility. As the length of the alkyl chain is increasing, solubility of the complex in non-polar solvents increases. While it was suggested that *N*-(*n*-propyl)-2-pyridylmethanimine is a ligand of choice in polymerisation of MMA,[27] in this work a more non-polar *N*-(*n*-pentyl)-2-pyridylmethanimine was used.

3.3.1.1 Rate dependence on the type of ligand used

It is well known that the choice of ligand affects the properties of the catalyst used in ATRP and thus also the polymerisation rate.[28] These properties determine the position of the equilibrium and the kinetics of exchange between active and dormant radical species.[29] It is believed the equilibrium is mainly affected by the steric effects and the electronic interaction of the ligands with the transition metal centre. An excessive steric hindrance around the metal centre would reduce the activity of the catalyst by making the addition of a halogen atom more difficult or simply impossible. Ligands bearing strong electron-withdrawing groups are known to stabilise the lower oxidation state of a transition metal, therefore favouring the production of dormant radical species by shifting the position of the equilibrium towards the reactants.

The overall rate of polymerisation is determined by the relative solubilities of the activating catalyst species Cu^{I} and a deactivating catalyst species Cu^{II}, as they determine the value of K_{eq}.[29] Solubility normally depends on the polarity of the medium and its temperature. For a comparison study, a series of experiments was performed at 90 °C and 60 °C in solution and in bulk. Polymerisation of MMA was carried out under argon, using ethyl 2-bromoisobutyrate (EBisoB) (Figure 3.3) as initiator.

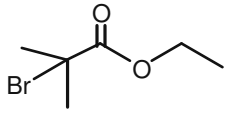


Figure 3.3 The ATRP initiator ethyl 2-bromoisobutyrate (EBisoB).

The molar ratios of reactants $[MMA]_0$:$[I]_0$:$[Cu^IBr]_0$:$[Ligand]_0$ were 100:1:1:2. For every (successful) experiment the conversion measurements were plotted as a first-order rate graph and the apparent polymerisation rate constant k_{app} was determined. Typical results are shown in Figure 3.4.

k_{app} can be determined from the slope of the plot of $\ln\left(\frac{[M]_0}{[M]}\right)$ vs. time, where $[M]_0$ is the starting monomer concentration. Thus k_{app} is the pseudo-first order rate coefficient for polymerisation, hence it follows that $k_{app} = k_p[R]$, where [R] is the total free-radical concentration.

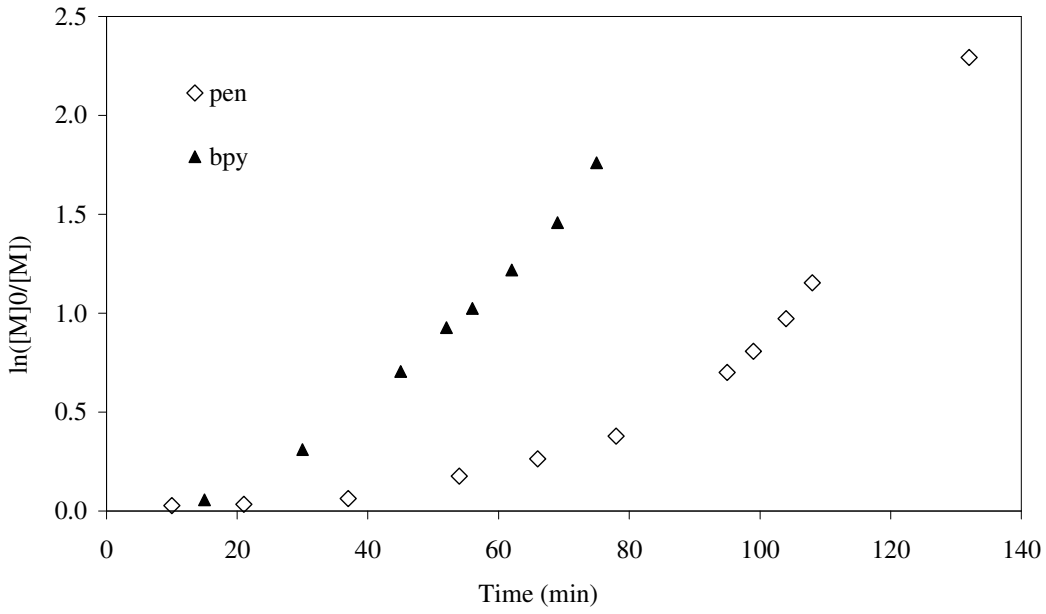


Figure 3.4 First order kinetic plots for ATRP of MMA in 50 wt.-% toluene at 90 °C catalysed by $Cu^IBr/(bpy)_2$ and $Cu^IBr/(pen)_2$.

At 90 °C both systems show a short induction time but the use of *pen* ligand results in formation of a complex of higher solubility compared to that of $Cu^IBr/(bpy)_2$. In addition to that, $Cu^IBr/(pen)_2$ catalyst complex has an interesting effect on the rate of polymerisation, i.e., it causes a reasonably long retardation time where polymerisation is occurring but at a very slow rate. Then, there is an unexpected acceleration in the rate of the reaction, and the same rate as with $Cu^IBr/(bpy)_2$ is attained after about 80 min. The same effect was observed by Haddleton *et al.*[30,31] It is suspected that the presence of residual oxygen in the system could be the origin of the short induction period,[32] while Haddleton[31] speculates it is due to the formation of the active initiator *in situ*. It is

possible that generation of active free-radical from the reaction of $Cu^{I}Br$ and the initiator might be slow therefore causing the retardation observed by Haddleton *et al.* in all reactions involving the use of Schiff base ligands as complexing agents.[31]

Non-polar toluene was used in ATRP of MMA. As expected, $Cu^{I}Br$ complexed by 2 equivalents of *N*-(*n*-pentyl)-2-pyridylmethanimine was found to have a higher solubility in toluene and MMA than a $Cu^{I}Br$ complex with 2,2'-bipyridine under the same conditions. As anticipated, polymerisation rates of the ATRP of MMA catalyzed by $Cu^{I}Br/pen_2$ were consistently lower than the rates obtained when $Cu^{I}Br$ was complexed by *bpy* ligand. This is discussed below.

Matyjaszewski *et al.* have observed the opposite affect of catalyst homogeneity on the rate of ATRP. In comparing the rate of polymerisation of *n*-butyl acrylate in benzene obtained with $Cu^{I}Br$/4,4'-di(5-nonyl)-2,2'-bipyridine (dN*bpy*) and $Cu^{I}Br/bpy$ as catalyst, it was found that use of $Cu^{I}Br$/dN*bpy* results in production of a homogeneous polymerisation mixture with polymerisation rate 40% higher than that in heterogeneous polymerisation mixture where *bpy* was used as a ligand.[15] However, this study involved a different monomer to the present study, and another difference was that the different solvent was employed: both could explain that different results were found.

According to the equation 3.2, the rate of polymerisation will be affected by the difference in relative solubilities of Cu^{I} and Cu^{II} species, as these will determine the effective concentration of either species.

$$R_p = k_p \frac{k_a}{k_d} [M] [I]_0 \frac{[Cu(I)]}{[Cu(II)]} \qquad (3.2)$$

where R_p – rate of polymerisation, k_p – propagation rate constant, $[I]_0$ – an ATRP initiator initial concentration.

While in a homogeneous ATRP the concentration of the catalyst species in the reaction mixture is higher when compared to a heterogeneous ATRP, in case where the relative concentrations of activating and deactivating species are affected to the same extent, it cannot be expected that a homogeneous ATRP system should exhibit changed polymerisation rate. The results of Figure 3.4 suggest that in fact [Cu(I)]/[Cu(II)] is lower in the more homogeneous system (*i.e.*, with *pen*). However, homogeneous ATRP is

expected to provide better molecular weight control by keeping the concentration of deactivator $Cu^{II}Br$ high as is evident from equation 3.3.

$$\frac{M_w}{M_n} = 1 + \left(\frac{k_p[I]_0}{k_d\,[Cu(II)]}\right)\left(\frac{2}{x} - 1\right) \qquad (3.3)$$

where x – fractional conversion.

As it is possible that not all of the catalyst is present in a solution, in a heterogeneous ATRP lower concentration of the catalyst might increase the overall rate of polymerisation, however, according to equation 3.3, obtained polydispersity values will be higher as concentration of Cu^{II} in such solution will be lower.[33] Indeed this was the case as molecular weight of PMMA samples prepared by ATRP in presence of Cu^{II}/*bpy* complex was consistently higher than predicted values, while polydispersity also increased in the course of the reaction. Comparison of theoretical values of the molecular weights and experimentally determined values is shown in Figure 3.5. Theoretical weights were calculated in the standard way based on experimentally determined monomer conversion and initial concentrations of reacting species, i.e., according to equation 3.1.

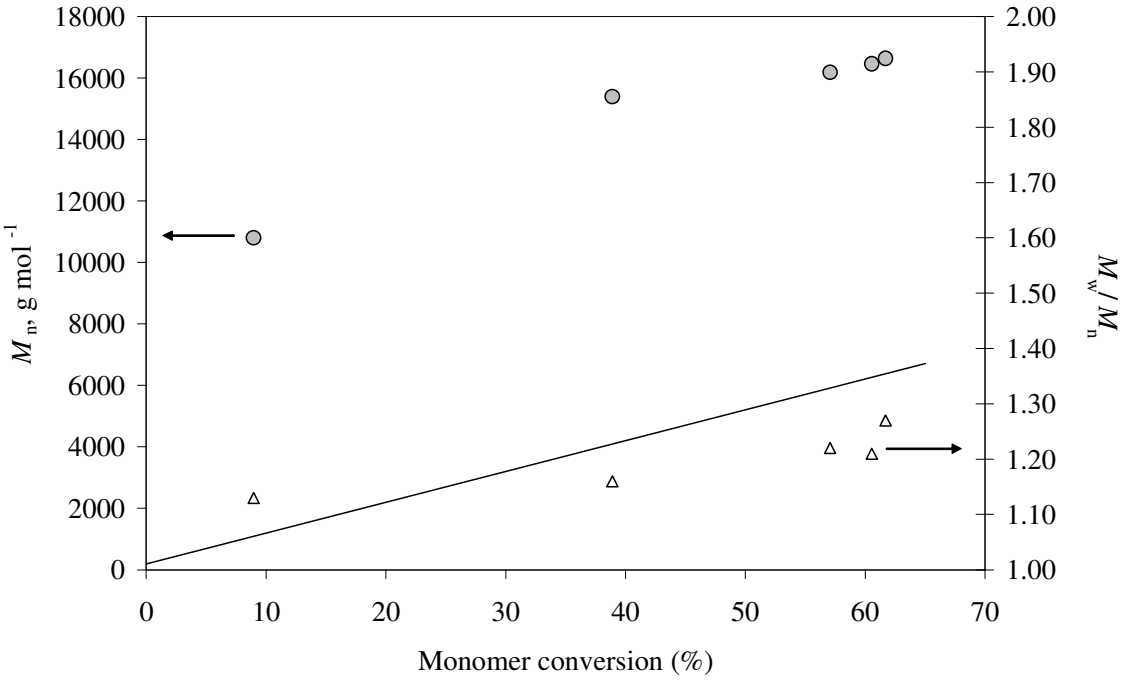


Figure 3.5 Dependence of M_n and M_w/M_n on monomer consumption in the ATRP of MMA in 50 wt.-% toluene at 90 °C with $Cu^{I}Br/(bpy)_2$. Line is theoretical M_n calculated based on measured monomer conversion using equation 3.1.

While such lack of control can be attributed to poor solubility of $Cu^{I}Br$/*bpy* species alone, ATR polymerisation of styrene under similar conditions yielded well-defined polymers with fairly low polydispersity values.[15] However, this was attributed to a low value of propagation rate constant k_p for styrene which according to equations 3.2 and 3.3 will lower the rate of polymerisation while also keeping polydispersity of polymer low. This serves as another indication that ATRP is not a simple process and a minor change in kinetics can be attributed to a number of factors and they should be considered in entirety rather than separately. Note that Haddleton *et al.* have shown that under the present conditions, *pen* provides similar M_w/M_n as found here for *bpy*, however their M_n is much closer to theoretical expectation.[26] Hence there was no need to reproduce these results here.

In summary: even though *pen* gives a more homogeneous ATRP system, it has a lower rate than *bpy*; however, consistent with equation 3.3 *pen* gives better MW control.

3.3.1.2 Rate dependence on the amount of ligand used

It is the coordination chemistry of a transition metal used in ATRP that will determine activity of the catalyst and therefore will affect the rate of polymerisation. It has been established that activity of nitrogen-based ligands in ATRP increases with increasing number of coordinating sites and a number of various multidentate ligands were developed for use in copper-based ATRP process.[4] The exact structure of a catalyst in polymerisation mixture is not yet established. Various studies such as UV-vis studies of Cu^{I} and Cu^{II} species and electron paramagnetic studies (EPR) of Cu^{II} in polymerisation medium indicated presence of very complex structures.[34] There is also evidence that ligands on both Cu^{I} and Cu^{II} species are labile in solution and there is fast exchange between ligands coordinating the metal and the free ligands in solution.[35]

A systematic study of rate dependence on the ratio of ligand to copper in ATRP revealed that the maximum rate is obtained when two equivalents of ligand species were used for every mole of copper present.[36,37] In this work ATRP of MMA systems with varied ligand to copper (I) ratio were investigated; both *bpy* and *pen* ligands were employed in this investigation.

While normally 2 equivalents of the ligand molecule are used for every mole of $Cu^{I}Br$, increasing the ratio did not result in any decrease in the rates observed. In other words, it is not found here that using 2 equivalents of the ligand gives maximum rate. In fact, if anything, the rate is slightly higher with 3 equivalents for both ligands, which is in contrary to what others have found.[26,38] Haddleton reported that the optimum ratio of ligand to copper for MMA was approximately 2:1, with the rate of polymerisation increasing significantly as the ratio is raised to this value with no further increase in the rate as the concentration of ligand was increased.[26] However, some results provided by the authors also showed an increase in the rate of polymerisation when 3 equivalents of ligand were used. Quite possibly for that very reason in some of his work Haddleton reported using the 3:1 ratio of ligand to metal.[16,31]

Figure 3.6 shows the variation in the rate of the polymerisation with increasing amount of the ligand species used.

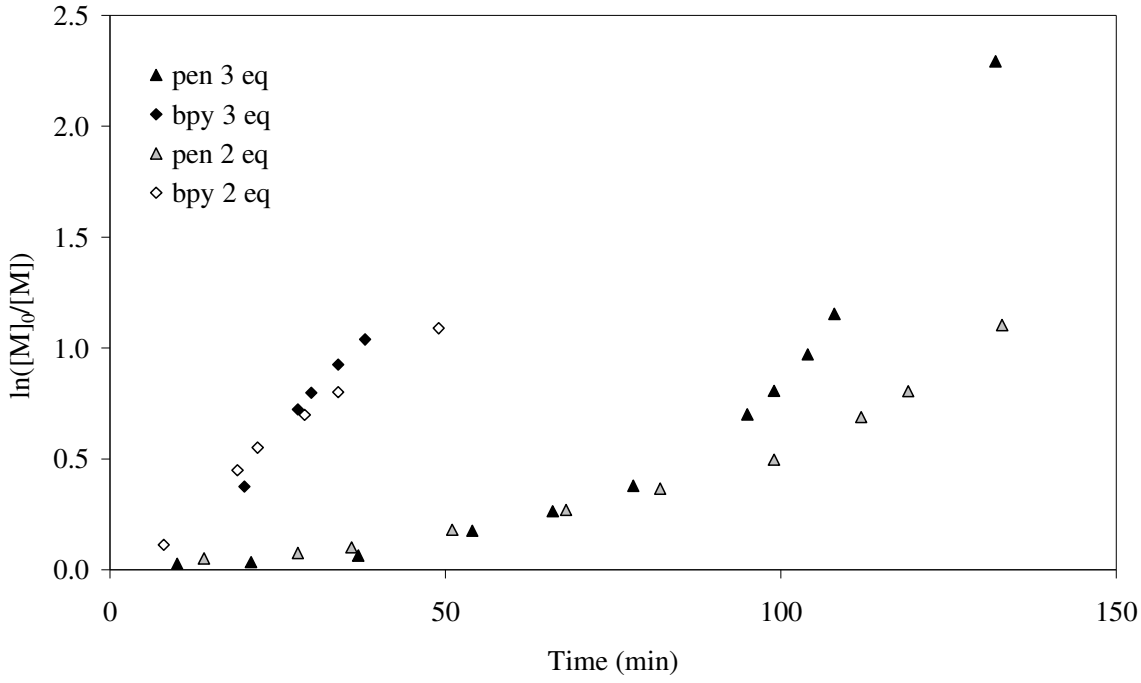


Figure 3.6 The dependence of the rate of polymerisation on the amount of ligand used in ATRP of MMA.

It is interesting that in the experiments where *pen* was used, the retardation period seems to be shorter with 3 equivalents of *pen* as ligand. If the origin of the retardation period is indeed in formation of the active initiator *in situ*,[31] then the increase in ligand concentration should slow down the process of abstraction even further by creating more

steric hindrance around the metal core of the catalyst. The opposite phenomenon was observed, and it is evident that the origin of the retardation is not strongly determined by the amount of ligand.

The results discussed indicate yet again that the structure of the catalyst complex has an effect on the kinetics of ATRP process. While addition of ligands is necessary for solubilising the transition metal, interaction of the latter with solvent and monomer molecules might result in formation of a different catalyst complex. This change will be manifested in kinetic changes and quite possibly also in production of polymer with different molecular weight distribution as a consequence. The following section contains details of a study of solvent and monomer concentration effect on polymerisation of MMA under ATRP conditions.

3.3.2 Solvent effect

Generally, ATRP is carried out in bulk, but the presence of solvent would be required when forming a polymer that is not soluble in the monomer. That means that the selection of a proper solvent appropriate for the reaction under study is of great importance for the success of a chemical process carried out in solution. When choosing a solvent it is important to consider the possible effect it might have on the course of polymerisation and therefore its kinetic characteristics. While it is crucial to use solvents with low chain transfer constant, it is also important to take in consideration possible interaction of the solvent with the catalyst as the structure of the latter will change in different media.[15] In particular, Munakata *et al.* suggested that the structure of the catalyst complex will be altered depending on the polarity of the medium.[39]

In this part of the research various solvent were used in ATR polymerisation of MMA. The monomer content was also varied, and polymerisations were conducted at different temperatures with both *bpy* and *pen* ligands.

3.3.2.1 Monomer concentration effect

The apparent polymerisation rate constant k_{app} is determined from the slope of the plot of $\ln\left(\frac{[M]_0}{[M]}\right)$ vs. time. It is the pseudo-first order rate coefficient and it is equal to $k_p[R]$. As it is evident from the expression, the observed rate will change with the concentration of

free radicals [R]. To assess the dependence of k_{app} on solvent fraction, experiments were carried out in different amounts of toluene. A summary of results is presented in Table 3.1 and it shows increase in k_{app} with increasing MMA fraction at both 60 and 90 °C.

$$[R] = ([Initiator]_0[Cu^IBr]_0)^{1/3} \left(\frac{k_a}{3k_d 2k_t}\right)^{1/3} t^{-1/3} \quad (3.4)$$

The experiments were run using both *bpy* and *pen* ligands. Figure 3.7 shows the variation of k_{app} with MMA fraction in both systems. It is clear that the variations are very similar, despite a difference in solubility of copper species due to the use of different ligands. The variation can be understood using equation 3.4, which describes the concentration of active radicals in an ATRP system.[40,41]

Table 3.1 ATRP of MMA with *bpy* in toluene under various conditions.[A]

Temperature	MMA, wt.-%	Time, hours	Conv., %	k_{app} ($\times 10^5$), s^{-1}
90 °C	25	4.2	90	17.3
	50	1.3	83	46.7
	80	0.7	99	67
	90	0.5	75	154
	100	0.5	95	322
60 °C	25	5.8	54	4.33
	50	3.5	78	13.2
	90	1.0	78	49.7
	100	0.7	80	105

[A] $[MMA]_0:[bpy]_0:[Cu^IBr]_0:[Initiator]_0 = 100:2:1:1$

From the expression it is evident that a change in initial concentration of initiator and copper (I) species will lead to a change in concentration of active radicals produced in the system. Therefore, the observed variation is not unexpected as concentration of all reactants was varied with fraction of MMA in reaction mixture in order to maintain a constant target degree of polymerisation of 100. However, it is clear that these changes in starting concentration do not explain quantitatively the variation of k_{app}. For example, starting concentrations double in going from 50 to 100 wt.-% MMA. According to equation 3.4 this leads to an increase of $(2 \times 2)^{1/3}$ in k_{app} which is significantly weaker than the observed increase.

Table 3.2 ATRP of MMA with *pen* in toluene under various conditions.[A]

Temperature	MMA, wt.-%	Time, hours	Conv., %	k_{app} ($\times 10^5$), s^{-1}
90 °C	25	4.2	90	13.2
	50	1.3	83	48.4
	75	0.7	99	84.8
	100	0.5	95	160

[A] $[MMA]_0:[pen]_0:[Cu^IBr]_0:[Initiator]_0 = 100:2:1:1$

A number of factors can affect the concentration of the radicals in the system. One of them is the initial concentration of Cu^IBr which can be greatly affected by polarity of the reaction medium. There is also a possibility that changes in concentrations of activating Cu^I and deactivating Cu^{II} species caused by change in a content of monomer in solution can cause an equilibrium shift or change in $K_{eq} = k_a/k_d$. This again will lead to change in concentration of active radicals in ATRP.

This issue will be addressed in the forthcoming section of this chapter containing the details of the computer modelling of the data and estimation of kinetic parameters governing the process of ATRP.

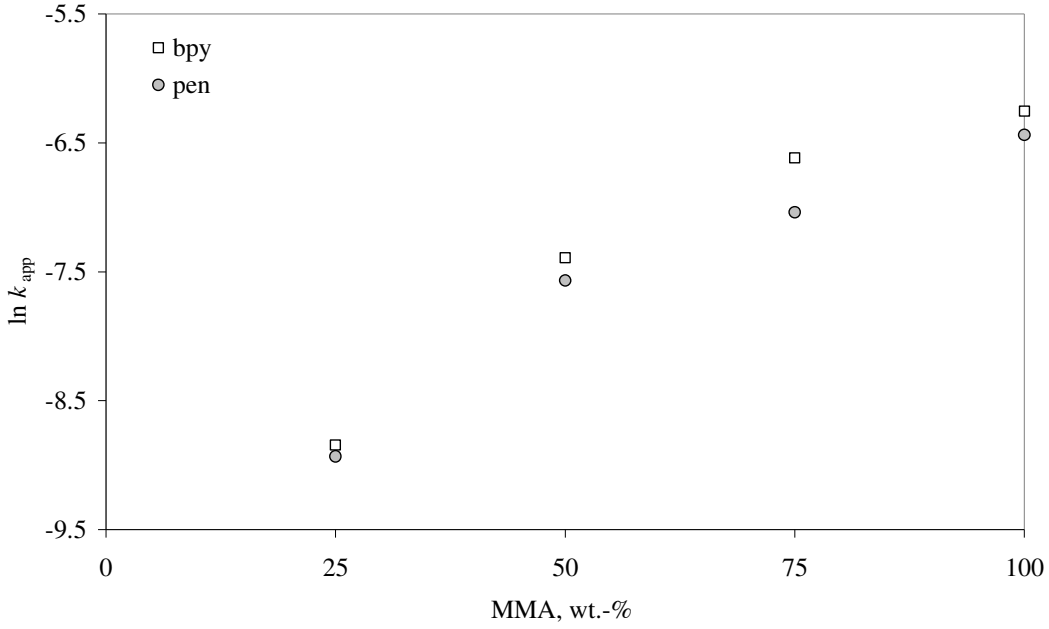


Figure 3.7 Variation of k_{app} with starting monomer concentration in toluene; $[MMA]_0:[bpy]_0:[Cu^IBr]_0:[Initiator]_0 = 100:2:1:1$.

3.3.2.2 Solvent polarity effect

The previous section contained results of the ATRP of MMA experiments where content of the monomer was varied. This has shown to have a slightly unexpected effect on the apparent rate constant k_{app}. The results allowed us to conclude that the change in the polymerisation medium due to the varied amount of solvent used probably causes a shift in a position of the equilibrium due to different solubilities of copper species at given conditions. It is common knowledge that in ATRP the solvent affects the homogeneity of the reaction mixture, the rate of polymerisation and possibly even the polydispersity of final product.[42]

The influence of solvent polarity on the kinetics of ATRP of MMA has been discussed in the literature;[43] it was suggested that lowering the polarity of the reaction mixture results in a lower concentration of Cu^{II} in solution, resulting in faster polymerisation rate.[43,44] However, the effect of solvent coordinating ability should be also taken into account as saturation of the coordination sphere of the copper complex by the solvent molecules would result in a decrease the reaction rate.[45]

Chemists often discuss solvent effect in terms of *solvent polarity*. Solvent polarity was defined as the "overall solvation capability (or solvation power) for educts and products, which influences chemical equilibria and reactants and activated complexes (''transition states''), which determines reaction rates".[46] A solvent polarity scale derived from negatively solvatochromic pyridinium N-phenolate betaine dyes, called the $E_T(30)$ scale, was introduced as a measure of solvent polarity.[47] Solvatochromism is the distinct change in position and sometimes intensity of an electronic absorption or emission band accompanying a change in the polarity of the medium.[48] The $E_T(30)$ values are defined as the molar transition energies (in kcal mol^{-1}) of the betaine dye[49], measured in solvents of different polarity at room temperature (25 °C) and ambient pressure (1 bar).[48,49] The $E_T(30)$ scale ranges from 63.1 kcal mol^{-1} for water, the most polar solvent, to 30.7 kcal mol^{-1} for tetramethylsilane (TMS), the least polar solvent. In 1983 the dimensionless normalized E_T^N scale was introduced, using water (E_T^N = 1.00) and TMS (E_T^N = 0.00) as reference solvents to fix the scale, according to equation 3.5.[50]

$$E_T^N = \frac{[E_T(\text{solvent}) - E_T(\text{TMS})]}{[E_T(\text{water}) - E_T(\text{TMS})]}$$

$$= \frac{[E_T \text{ (solvent)} - 30.7]}{32.4} \qquad (3.5)$$

Today E_T^N and $E_T(30)$ values are known for over 360 solvents, and these values are commonly used as a measure of a solvent's polarity or its overall solvation capability.[51] This section of the ATRP work presents and discusses findings on the effect of increasing solvent polarity on the rate of polymerisation. For the purpose of this investigation a number of solvents with different E_T^N were used in the ATRP of MMA.

Solvents with very different E_T^N values were employed in polymerisation of MMA under ATRP conditions with DMSO representing a solvent of highest polarity (E_T^N = 0.444) and xylene being the solvent with the lowest value of E_T^N in the range (E_T^N = 0.074). Experiments were run at 50 wt.-% MMA at 90 °C. Apparent rate constant was determined for the experiments and Table 3.3 demonstrates the solvent influence on rate parameters reflected in variation of the latter for ATRP systems that employ both *bpy* and *pen* as complexing agents for the copper catalyst.

Table 3.3 Polymerisation of MMA 50 wt.-% in various solvents[A], ξ – dielectric constant value of solvent and E_T^N is the normalised solvent polarity parameter at 25 °C.

Solvent	ξ	E_T^N	$k_{app}(\times 10^5)$, s^{-1}	
			bpy	pen
Acetonitrile	36.2	0.460	14.1	4.82
Anisole	4.3	0.198	75.1	31.4
Benzene	2.27	0.111	52.9	19.8
Benzonitrile	25.2	0.333	12.8	27.9
DMF	36.7	0.386	22.8	6.72
DMSO	49	0.444	121	12.5
Ethyl Acetate	6.02	0.228	21.9	18.3
MMA	2.7	0.222	192	164
THF	18.5	0.207	39.4	23.8
Toluene	2.38	0.099	73.1	33.2
Xylene	2.3	0.074	156	27.6

[A] At 90 °C; $[MMA]_0:[Ligand]_0:[Cu^IBr]_0:[EBisoB]_0$=100:2:1:1.

It needs to be remembered that the quoted E_T^N value is not strictly exact in that it ignores the role of the 50 wt.-% MMA in each system. While E_T^N value if available for MMA, no attempt to account for it was made. Polar behaviour of binary mixtures can be described quantitatively using a widely applicable two-parameter equation; however, required parameters are not available.[52] Clearly taking the value of E_T^N for MMA in account will compress the E_T^N variation to a narrower range; however it will not change the values relative to each other. Thus there is no compromise of the purely qualitative relationship that is sought by this study.

In most cases the use of the *pen* ligand resulted in formation of soluble catalyst complex, while Cu^IBr/*bpy* complex was only fully soluble in acetonitrile, DMF and DMSO. The k_{app} values for the ATRP of MMA, catalyzed by Cu^IBr/*pen* complex, are consistently lower than those of ATRP of MMA experiments where *bpy* ligand was used. This is consistent with earlier results.

To assess the relationship between the rate parameters and the polarity of the solvent, k_{app} values were plotted against E_T^N. Figure 3.8 and Figure 3.9 show the similar trends in variation in k_{app} in either system.

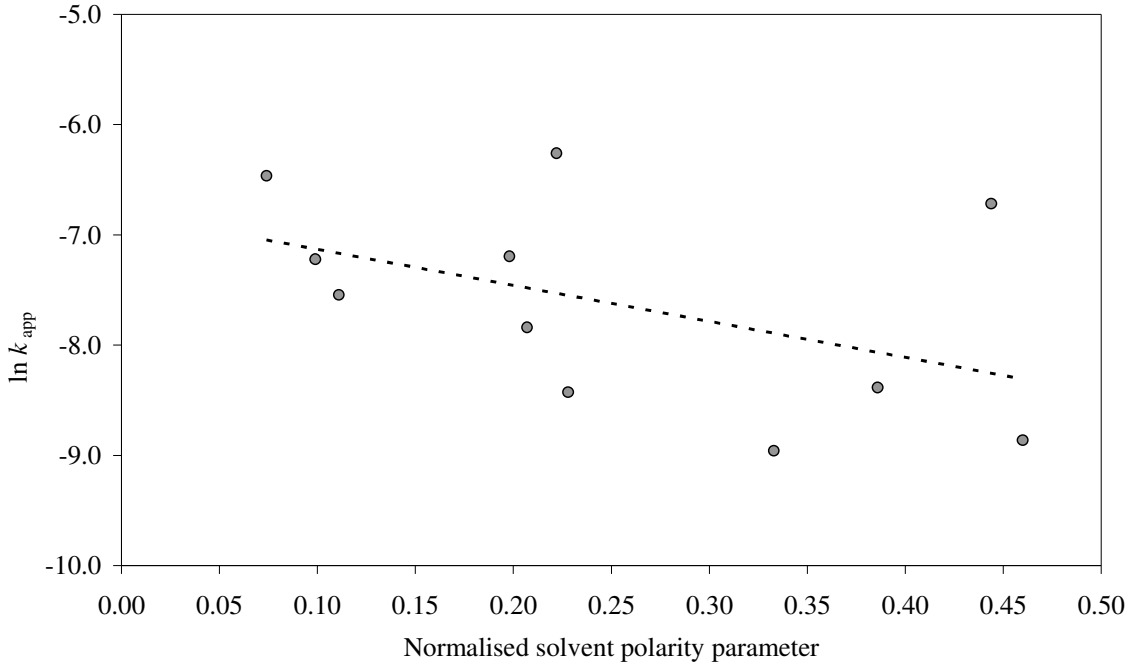


Figure 3.8 Variation of k_{app} with polarity of solvent for the ATRP of MMA in 50 wt.-% solvent at 90 °C with $Cu^IBr/(bpy)_2$.

It is clear that k_{app} generally decreases with the polarity of the reaction mixture.

Since k_{app} is defined as $kapp = kp[R]$, variation in k_{app} can be attributed to change either in the value of k_p or in the concentration of active radicals in the reaction mixture. According to equation 3.4, concentration of the free radicals in ATRP is predetermined by the initial concentration of initiating and copper (I) species in the solution. It might be safe to assume that the initial concentration of initiator does not vary depending on the solvent used, however, the choice of solvent has a far greater impact on a catalyst's structure, which in turn may alter concentration of the latter in the reaction mixture.

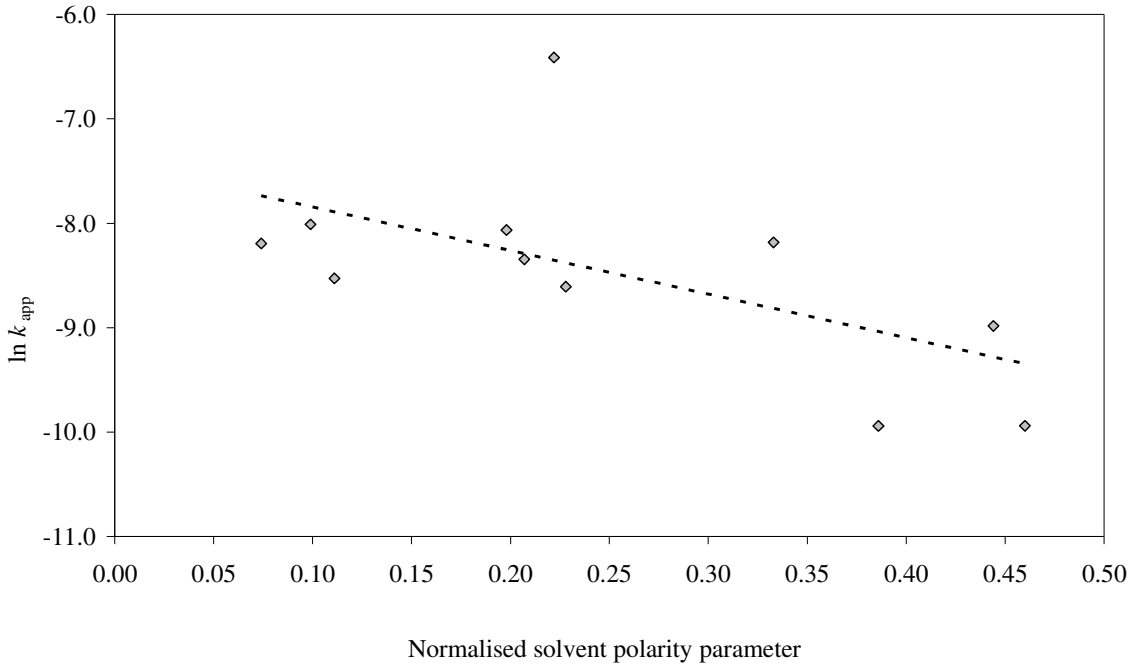
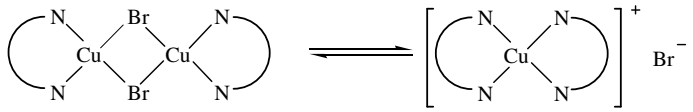


Figure 3.9 Variation of k_{app} with polarity of solvent for the ATRP of MMA in 50 wt.-% solvent at 90 °C with $Cu^{I}Br/(pen)_2$.

Possible interaction of solvents of different type with the ATRP metal catalyst has been discussed in the literature. The various model studies suggest that copper catalyst complexed by *bpy* exists in two forms, a tetrahedral $Cu^{I}(bpy)_2$ which upon abstraction of bromine from the initiator changes its conformation to a trigonal bipyramidal Br-$Cu^{I}(bpy)_2$.[53,54]

However, it is believed that the catalyst might in fact have a number of possible structures and it is solvent polarity that will have a profound effect on the structure the catalyst will adopt in a given solution.[55] Figure 3.10 represents two possible structures

copper (I) species can adopt in solution; the bridged dimer **A** could exist in non-polar solvents while the monomeric form **B** will predominantly exist in solvents of higher polarity.[4]



A **B**

Figure 3.10 Possible structures of copper (I) species in solution.

Some solvents such as acetonitrile, benzonitrile and DMF combine high polarity with the ability to act as a ligand and the overall effect of these solvents on the kinetics of polymerisation would depend on which of these properties dominates. In the case of ATRP of MMA using $Cu^{I}Br$/*bpy*, it was observed that the rate of polymerisation decreased when these solvents were used.

This is an indication that the solvent is interacting with the copper complex, which is possibly hindering the halogen abstraction by Cu^{I} species and thus reducing the concentration of active radicals. As the use of these solvents resulted in improved solubility of the copper complex, decreased rates might be also explained by higher solubility of Cu^{II} species compared to Cu^{I}. Higher effective concentration of deactivator would slow the rate down. Matyjaszewski *et al.* also report the similar observation that the highest values of k_{app} are observed in non-polar solvent mixture.[56]

Another hypothesis is such that in polar solvents, the Cu^{II}-Br bond becomes very labile, therefore allowing water molecules to replace Br on the copper. This newly formed species will not act as deactivator, therefore allowing the concentration of active radicals to increase, which will lead to an increase in the rate of polymerisation, which agrees with our observation reported above.[57] Another possible change in structure of the catalyst might also be caused by coordination of one or more monomer molecules.

As noted earlier, variation in k_{app} with solvent might also be a reflection of variation in k_p. However, k_p is believed to be almost independent of the type of solvent used in polymerisation,[58] and despite slight variation of k_p in MMA homopolymerisation in various solvents, the results do not show any correlation of k_p with the dielectric constant

of the solvent.[59] Also, where variation of MMA k_{app} with solvent has been observed, it is much smaller in magnitude than the k_{app} variation observed here.

Additional results are summarised in Table 3.4.

Table 3.4 Atom transfer radical polymerisation of 50 wt.-% MMA.[A]

Ligand	Solvent	Time, min	Conv., %	$M_{n,\,theory}$[B]	$M_{n,\,GPC}$[C]	PDI
pen	Anisole	140	84	8400	11599	1.20
	Benzene	203	90	9000	11988	1.17
	BN	181	80	8000	14665	1.22
bpy	Anisole	92	68	6800	13831	1.22
	DMF	110	51	5100	9046	1.20
	DMSO	262	52	5200	7043	1.82
	Xylene	85	83	8300	18545	1.28

[A] At 90 °C; $[MMA]_0:[Ligand]_0:[Cu^IBr]_0:[EBisoB]_0=100:2:1:1$.

[B] $M_{n,\,theory}$ was calculated based on measured conversion using equation 3.1.

[C] Molecular weight and *PDI* values were established by GPC; the analysis was performed at the KCPC, University of Sydney, Australia.

Gel-permeation chromatography (GPC) analysis of the polymer samples indicates a slight disagreement between predicted and experimentally determined molecular weights for all ATRP systems where both *bpy* and *pen* ligands were employed. Nevertheless it is clear that *pen* gives M_n closer to the predicted value, as already discussed earlier. It is worth noting that the polymer of MMA obtained in all but one experiment is of narrow polydispersity which is indicative of a reasonable degree of control maintained throughout the polymerisation process.

3.3.3 Additives effect – radical inhibitors

Results obtained in this work were analysed based on the understanding that the mechanism of ATRP can be described by Scheme 3.1. Although the radical pathway has been proposed in most ATRP systems, there is still some mystery about the radical nature of the mechanism. Direct detection and analysis of growing radicals in ATRP mixture is often impossible due to the presence of transition metals.[4] However, free-radicals can be "trapped" by reacting them with various radical inhibitors. This will reduce concentration of growing radicals, reducing the rate of polymerisation

dramatically. Such change in the kinetics of ATRP can serve as a confirmation of free-radical nature of the polymerisation process.

While phenols are generally used as radical inhibitors, their addition to an ATRP of MMA mixture gave unexpected results.[4,19,60,61] Haddleton *et al.* found that addition of methyl hydroquinone and phenol (Figure 3.11) to the ATRP of MMA increased the rate of polymerisation at lower temperatures while the control over molecular weight and molecular weight distribution was maintained.[19] Taking into account the fact that methyl hydroquinone is often used as an inhibitor for MMA, its clearly opposite effect on the rate of polymerisation of MMA under ATRP conditions is surprising.

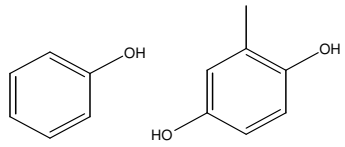


Figure 3.11 Phenol and methyl hydroquinone (MeHQ).

In this work the effect of the phenol derivatives 4-methoxyphenol (hydroquinone, HQ) and 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT) on the kinetics of ATRP of MMA was investigated. The reason for using these rather than those of the Haddleton investigation was to add to the study.

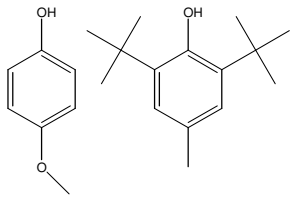


Figure 3.12 Phenol derivatives 4-methoxyphenol (hydroquinone, HQ) and 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT), commonly used as radical traps.

3.3.3.1 2,6-Di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT)

Butylated hydroxytoluene is widely used in the food industry as an antioxidant.[62] Its antioxidant activity is based on its ability to quench reactive radical species: one molecule of BHT can react with two radicals forming stable species that cannot react further.[63]

Addition of a 10 molar excess (with respect to the amount of the initiator) of BHT (Figure 3.12) to an ATRP of MMA resulted in no change of the inhibition period and polymerisation still occurred readily. The kinetic plot is shown in Figure 3.13. It clearly indicates no significant change in rate of polymerisation. When the same excess of the radical inhibitor was added to a conventional free radical polymerisation of 50 wt.-% MMA in toluene at 90 °C with 0.08 wt.-% AIBN, such addition inhibited the reaction and no polymer formation was observed after 48 hours.

3.3.3.2 4-Methoxyphenol (hydroquinone, HQ)

4-Methoxyphenol (Figure 3.12) is commercially used as an inhibitor of vinyl and acrylic monomers and as an antioxidant. It is used as a stabiliser to inhibit peroxide formation in ethers, chlorinated hydrocarbons and ethyl cellulose. The effect of this radical inhibitor on ATRP of MMA was also tested.

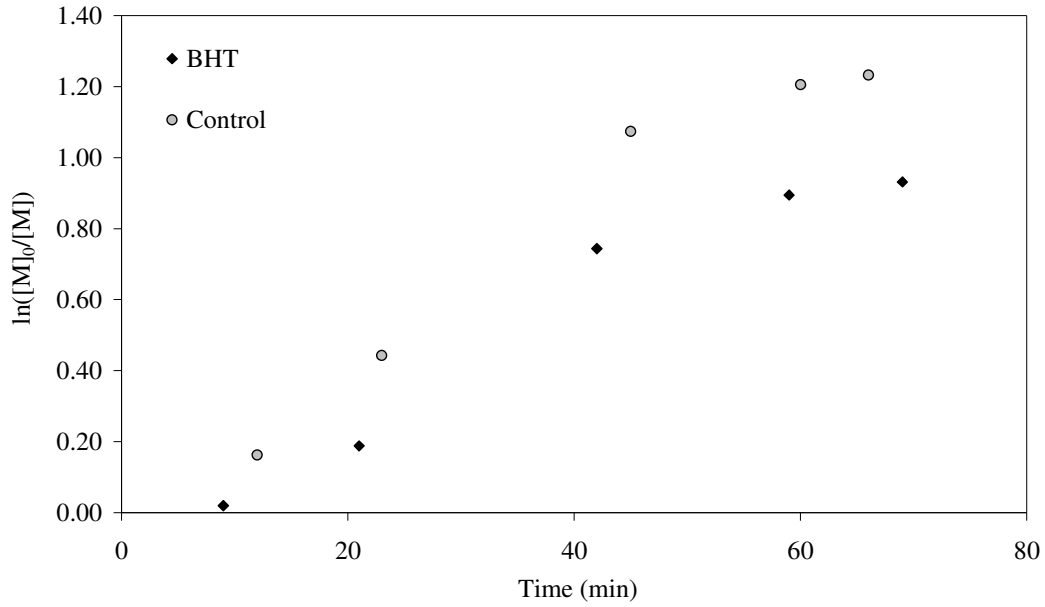


Figure 3.13 Comparison of kinetic plots of ATRP of MMA with BHT added and with no BHT (control). Reaction conditions for control ATRP - $[MMA]_0:[Cu^IBr]_0:[bpy]_0:[EBisoB]_0$ = 100:1:2.5:1. While keeping the same ratio of the components, a 10 molar excess of BHT with respect to concentration of EBisoB was added to the second reaction.

When an excess of 4-methoxyphenol was added to an ATRP of MMA mixture, there was no significant induction period although the rate of polymerisation dropped slightly. At this stage it was important to establish whether addition of HQ will have a profound

effect on kinetics of conventional free-radical polymerisation of MMA. Upon the addition of 4-methoxyphenol to a conventional free-radical polymerisation mixture of 50 wt.-% MMA in toluene at 90 °C with 0.08 wt.-% AIBN, a small decrease in the rate of the polymerisation was observed, $k_{app\ (control)} = 2.26 \times 10^{-4}\ s^{-1}$, $k_{app\ (methoxyphenol)} = 1.88 \times 10^{-4}\ s^{-1}$. Results are summarized in Figure 3.14 and it is clear that addition of either radical inhibitor caused no significant change in rates of polymerisation.

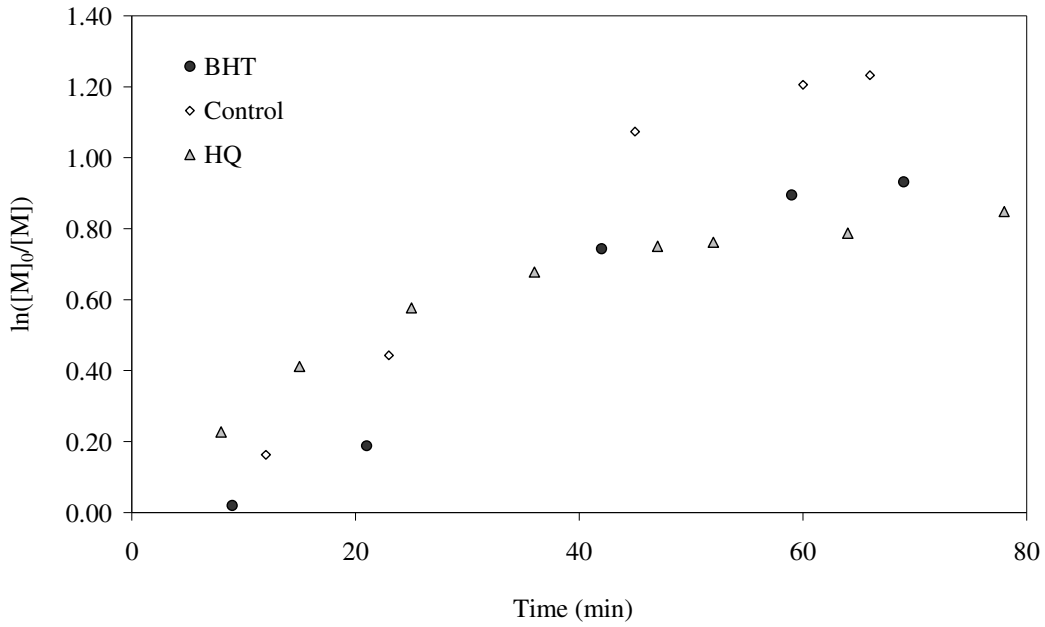


Figure 3.14 Kinetic plots of ATRP of MMA catalysed by $Cu^{I}Br/(bpy)_2$ with various phenol additives; $[MMA]_0:[Cu^{I}Br]_0:[bpy]_0:[EBisoB]_0:[Inhibitor]_0 = 100:1:2.5:1:10$.

Because 4-methoxyphenol has negligible effect on conventional FRP, its lack of effect on ATRP of MMA does not mean anything. However, BHT is a good inhibitor of FRP, possibly because the resulting oxygen-centred radical is highly shielded by the neighbouring *tert*-butyl groups, hence making addition to monomer very difficult. But as BHT does not affect the course of ATRP, it has been interpreted that this process does not occur via free-radical mechanism.[16,60] However, it is worth noting that the key to a living polymerisation is suppression of the termination reaction by keeping the concentration of free radicals very low. It may be that the low radical concentration simply results in a very low rate of reaction with a radical trapping species. However, the kinetic simulations of these systems carried out by Greg Smith (results presented later in this chapter) do not support this hypothesis.

Earlier studies also indicated an insignificant effect of phenols on radical polymerisation of (meth)acrylate where only 1% retardation was observed for MMA polymerisation in the presence of phenol, while addition of 4-methoxyphenol actually increased the polymerisation rate.[61,64] The observed phenomena could be attributed to possible interaction of phenols with the catalyst, resulting in an equilibrium shift and an increased rate. Phenoxy groups acting like ligands can coordinate to Cu species, altering the structure of the catalytic complex and therefore altering the position of the equilibrium, which sees the development of a catalyst with a higher equilibrium constant, resulting in rate acceleration.

It is believed that in the presence of oxygen, BHT and HQ function by reacting with alkyl peroxy radicals rather than alkyl radicals.[63] In reported results addition of BHT and HQ to conventional free-radical polymerisation of MMA was shown to cause significant retardation, however, it is worth noting that the experiments were conducted under slightly different conditions than were used in this work. In particular, BHT was added to polymerisation of MMA in the presence of oxygen[65] while HQ was used in benzylperoxide (BPO) initiated polymerisation of MMA.[66] It remains unclear why addition of HQ did not result in inhibition here.

In a forthcoming section, simulation of various polymerisation systems will be used in an attempt to establish why these common inhibitors of free-radical processes have no effect on ATRP of MMA.

3.4 ATRP of MAOS

Upon establishing appropriate experimental techniques and acquiring necessary basic skills for successful ATRP work, further investigations moved onto application of the method in synthesis of a polymeric precursor of *N*–(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin (PK1).[67]

The monomer of interest is HPMA, a polymer of which is the backbone of the above polymer therapeutic. The polymer is water-soluble and non-toxic, its use for medicinal applications has been previously investigated.[68,69]

The idea of designing a macromolecular carrier for a drug first appeared in the literature in the 1970s, when Ringsdorf suggested use of polymer drugs.[12] Such compounds were termed *smart polymer vehicles* and it was expected that their use will improve selectivity and targeting of drugs that they were carrying.[70] A number of various macromolecular agents are being investigated, their properties are being assessed in view of their possible application for improved targeting and delivery of low molecular weight chemotherapeutic agents.[71-74]

This idea was picked up by a number of research groups and it was and still is a subject of their investigations. Prof Duncan is currently a head of a school of polymer therapeutics in Cardiff, UK, and her research dates back to the 1980s.[75]

When polymer is used for medicinal purposes, ability to control molecular weight of the polymer is absolutely crucial (see the detailed explanation and reasoning given in Chapter 1). With that in mind, a number of research groups are currently working on finding the best approach to synthesise the polymer of HPMA. While direct synthesis of the polymer from the monomer is highly desirable, the task might not be as simple if methods of controlled polymerisation are to be used in such synthesis.

A lot of effort has been put into design and synthesis of a functionalised polymer such that it could be converted into the polymer of interest as a result of chemical manipulations and modifications.[76] A polymer of an activated ester, methacryloyloxy succinimide (MAOS), is believed to be an ideal precursor to PHPMA and in this research ATRP of MAOS has been investigated in the hope that successful polymerisation of the monomer will allow for more efficient synthesis of PK1.

3.4.1 Initiator effect

Figure 3.15 contains details of the ATRP of MAOS procedure used by Brocchini *et al*. in their work.[77] The monomer is polymerised via copper-mediated ATR polymerisation in DMSO at 130 °C.

While hydroxyethyl 2-bromoisobutyrate (HEBisoB) was used in LSP work, here initial experiments were run using ethyl 2-bromoisobutyrate (EBisoB) as an initiator (Figure 3.16).

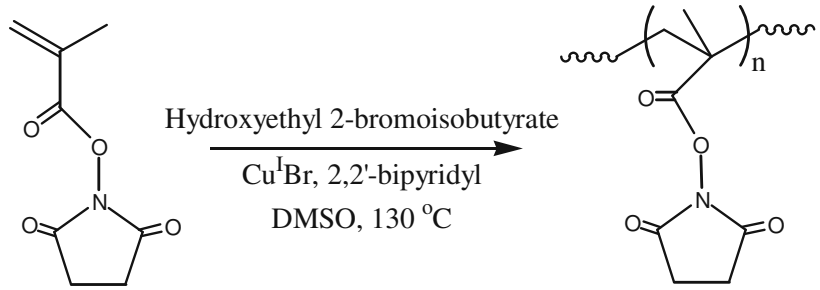


Figure 3.15 Proposed polymerisation of *N*-methacryloyloxy succinimide by ATRP.

A number of experiments were done in DMSO at 130 °C giving no product. It was suspected that EBisoB had a lower boiling point compared to that of HEBisoB and at 130 °C was in a form of vapour, which prevented it from reacting with the copper complex. That would mean that there was no initiation and thus no formation of polymer.

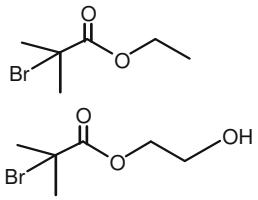


Figure 3.16 Initiators used in ATRP of MAOS: ethyl 2-bromoisobutyrate (EBisoB) and hydroxyethyl 2-bromoisobutyrate (HEBisoB).

To test the hypothesis, HEBisoB was used in the ATRP of MAOS at 130 °C; HEBisoB was prepared by reacting 2-bromoisobutyryl bromide with ethylene glycol at room temperature. The polymerisation resulted in formation of a polymer but while monitoring the kinetics of the reaction, an unexplained decrease in monomer conversion with time was observed.

The decrease in monomer conversion indicates the occurrence of depolymerisation, which under normal reaction conditions does not take place. The only explanation for the peculiar results is the inefficiency of the sampling technique that involves withdrawing the sample from the reaction mixture, weighing it, precipitating the polymer and drying it. After the final weight of the dry polymer product is determined, the monomer conversion is calculated. As this was one of the very first experiments carried out in this work, this problem is perhaps understandable. It was subsequently rectified. It would be reasonable to interpret this early result as indicating that the ATRP reached a “dead end” at intermediate conversion.

ATRP of MAOS experiment was repeated, this time using EBisoB as the initiator, employing DMF as a solvent and lowering the reaction temperature to 90 °C. The experiment was successful, showing an increase in the monomer conversion with time, and the overall yield was approximately 56% after 2 hours.

3.4.2 Ligand effect

The ligand effect on polymerisation kinetics of MMA was discussed in a previous section of this chapter. Similar investigation was conducted for ATRP of MAOS. Results of the two experiments are presented in Figure 3.17.

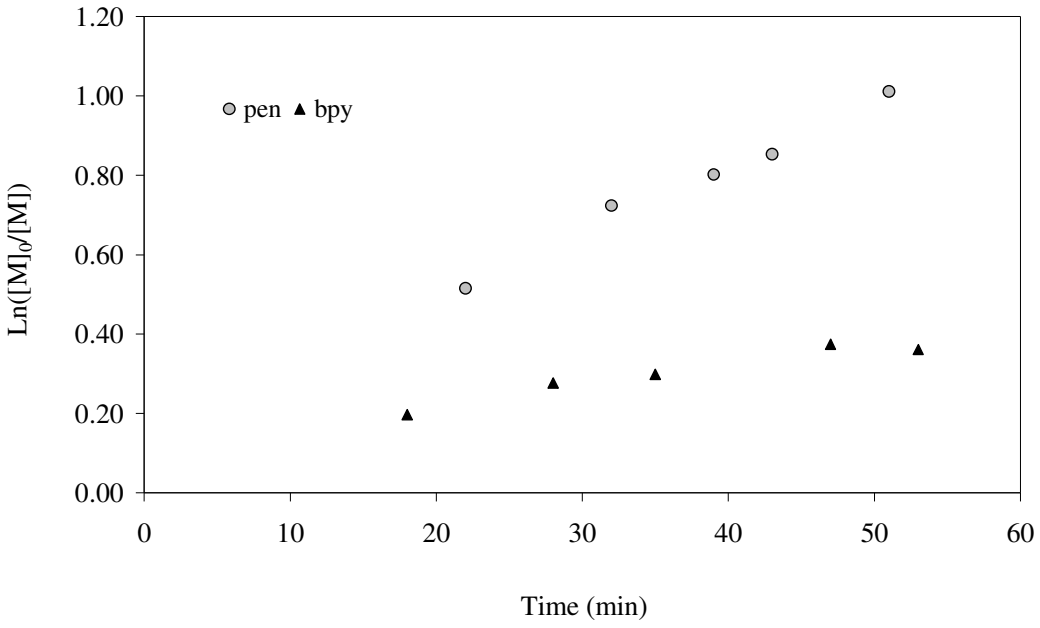


Figure 3.17 Kinetic plots for ATRP of 50 wt.-% MAOS in DMF at 90 °C;
$[MAOS]_0:[Ligand]_0:[Cu^IBr]_0:[EBisoB]_0 = 100:2:1:1$.

These results show a noticeable increase in k_{app} when *bpy* ligand is substituted by *pen*. That once again confirms that the rate of ATRP is affected by the choice of the ligand, although now the effect is the opposite of that with MMA: *pen* increases the rate with MAOS. However, it is important to realise that DMF which was used as a solvent in the polymerisation experiments could act as a ligand itself. Coordination of DMF molecules to the copper might cause displacement of the ligands used and therefore formation of a new complex. This might result in a loss of control in the polymerisation, causing production of chains with broad MWD. Analysis of molecular weights of the samples allows establishing a degree of control in those systems and such analysis was done for

the purpose of establishing whether there was a linear relationship between molecular weight of the product and monomer conversion.

3.4.3 Molecular weight and polydispersity

As was mentioned earlier, in a controlled/"living" system there is a linear relationship between molecular weight of polymer and monomer conversion. The relationship is described by equation 3.6 where x – fractional conversion.

$$\overline{DP}_{\mathrm{n,theory}} = x\left(\frac{[\mathrm{M}]_0}{[\mathrm{Initiator}]_0}\right) \tag{3.6}$$

Therefore, to establish the nature of a polymerisation system, the molecular weight of polymer at measured conversion should be determined to see if there is an evolution of MW with increasing conversion. GPC is the standard method for doing this and was used here.

GPC is used in determination of MW and MWD of different types of polymers.[78] In GPC, the polymer is dissolved in a solvent and the sample is passed through a gel-permeation column. These columns are packed with porous beads. As the molecular size of polymer decreases, the chains start penetrating into the pores and accordingly elute at a later time.

It has been established that when samples are passed through a GPC column, their elution time depends on their hydrodynamic volume rather than on their MW. However, there is a relationship between the two, which is governed by the choice of solvent and MW of the sample. Therefore, as with many other analytical methods, GPC needs to be calibrated with polymer standards of known molecular weight and narrow MWD so the relationship between MW and the retention time can be established. For this purpose poly(methyl methacrylate) (PMMA) standards with narrow MWD were used. Elution times were plotted against vendor-quoted molecular weights of the standards, giving a calibration curve that was later used in analysis of PMAOS samples.

Firstly, obtained calibration data was used in determination of molecular weight of PMMA samples that were prepared by the ATRP method. Theoretical MW for each sample was calculated and compared with the results obtained by GPC analysis. The results are presented in Figure 3.18.

While there is always awareness of the possibility that GPC results might differ from the predicted values, the magnitude and the consistency of the observed difference was rather surprising because it is well established that these ATRP systems are controlled/"living" in nature.

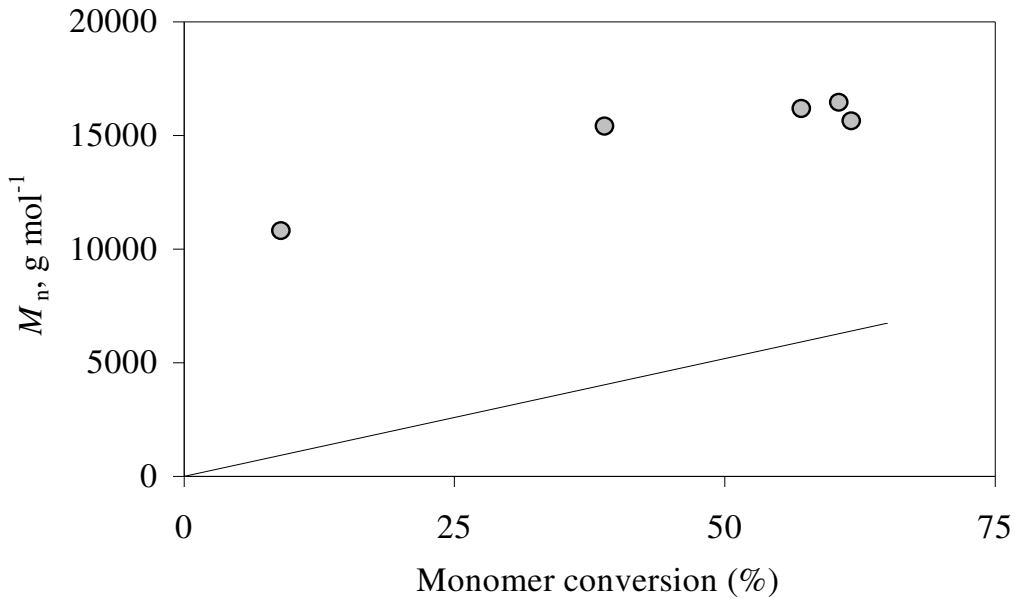


Figure 3.18 Evolution of the molecular weight with conversion for ATRP polymerisation of 50 wt.-% MMA in toluene at 90 °C; $[MMA]_0$:$[bpy]_0$:$[Cu^IBr]_0$:$[EBisoB]_0$ = 100:2:1:1. Line is theoretical M_n calculated based on measured monomer conversion using equation 3.1.

The results suggested the existence of some systematical error, most likely related to the calibration of the equipment and the choice of solvent. For calibration here PMMA standards and DMF solvent to which 0.1 wt.-% LiBr was added were used. Normally, pure DMF would be used in this calibration but DMF/LiBr mixture is used in GPC of PMAOS later and in order to assess MW of PMAOS samples correctly, we need to obtain calibration data for the solvent mixture used. The addition of the Li salt is a necessary measure as it eliminates the interactions between the PMAOS sample molecules cause distortions and the appearance of an additional peak at higher MW.[79-81] It is suggested that some polar chains repel each other in solution and therefore, by taking up more volume, elute at earlier time causing the appearance of an additional peak. The addition of counter-ions lessens the extent of repulsion and eliminates that peak.

When analysing GPC results for PMMA samples, it is worth remembering that PMMA is a relatively non-polar molecule and being dissolved in polar medium such as DMF/LiBr

would affect its hydrodynamic volume. When non-polar chains are placed in a polar medium, they tend to coil up tighter and therefore take less volume than they normally would in a non-polar medium. As a consequence of that, the sample would elute at later time and a whole calibration plot would shift. If this is the case, it perhaps explains the consistent nature of the disagreement between calculated and obtained values. Nevertheless this effect should exist for both standards and samples, and so it is still something of a mystery that the calibration procedure should not work.

When PMAOS samples were analysed, there was no linear relationship between calculated weights and measured conversion for any of the experiments. Final results are presented in Figure 3.19, Figure 3.20 and accompanying Table 3.5.

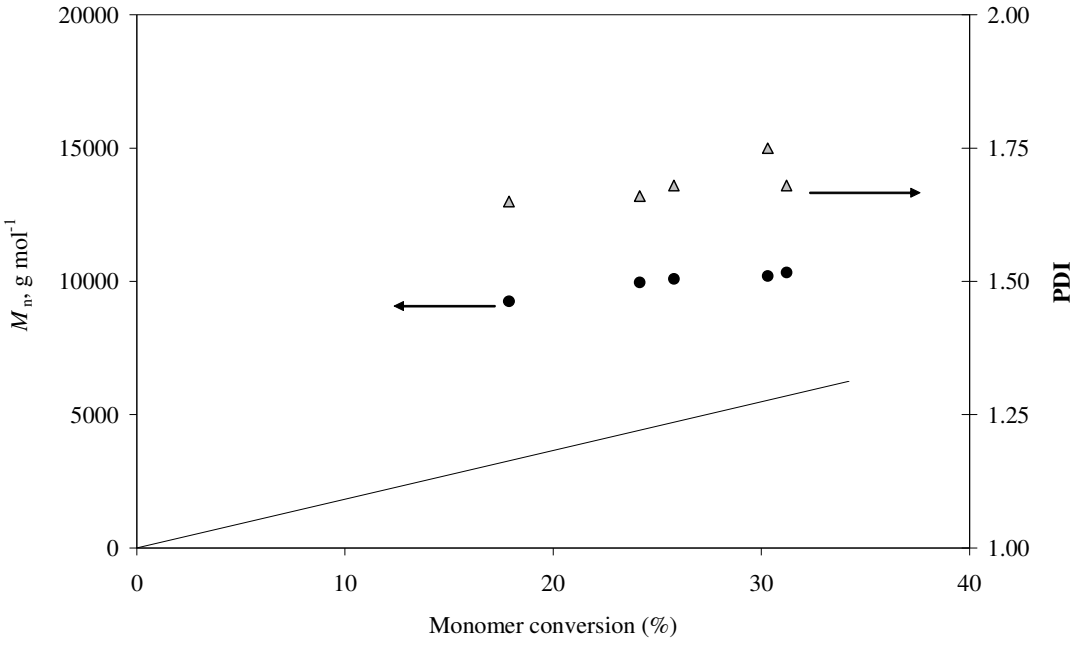


Figure 3.19 Evolution of the molecular weight and polydispersity index with monomer conversion for ATRP polymerisation of 50 wt.-% MAOS in DMF at 90 °C; $[MAOS]_0:[bpy]_0:[Cu^IBr]_0:[EBisoB]_0 = 100:2:1:1$. Line is theoretical M_n calculated based on measured monomer conversion using equation 3.1.

Again, the disparity between calculations and observed M_n values is observed and, in addition to that, the high polydispersity values suggest non-living behaviour of the polymerisation systems. However, it must be stressed that all the above GPC results were obtained using DMF/0.1 wt.-% LiBr as eluent, and there are suggestions that this may exaggerate the broadness of the molecular weight distribution. All in all the above results

are a start and are challenging, especially as various research groups have commented on similar problems in similar systems.[82,83]

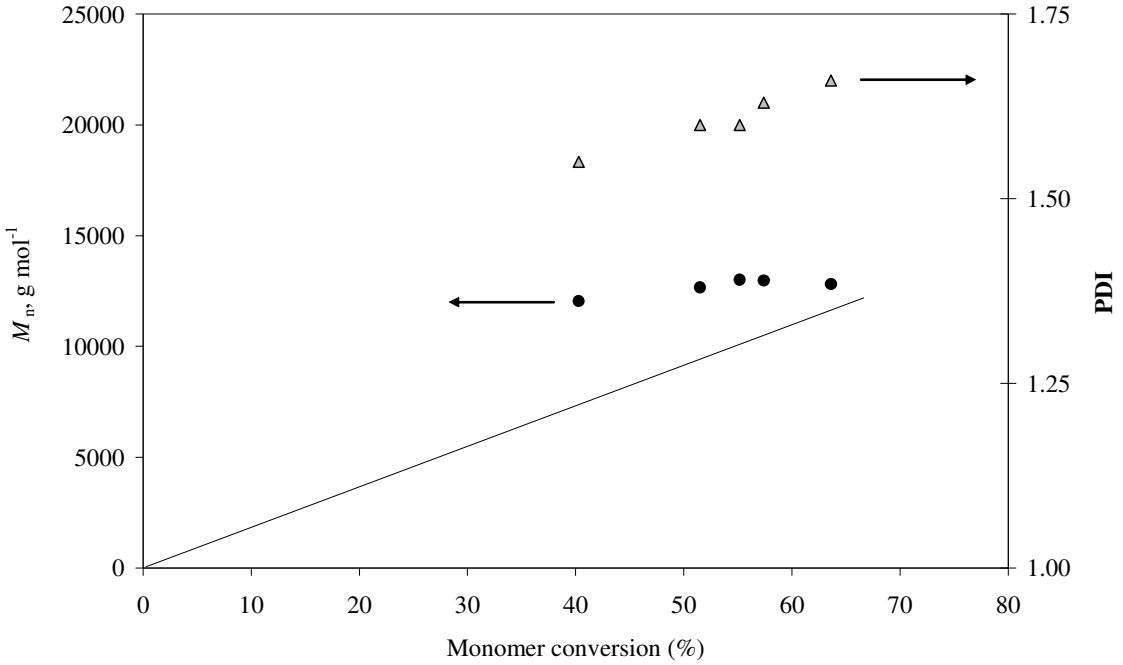


Figure 3.20 Evolution of the molecular weight and polydispersity index with monomer conversion for ATRP polymerisation of 50 wt.-% MAOS in DMF at 90 °C; $[MAOS]_0$:$[pen]_0$:$[Cu^IBr]_0$:$[EBisoB]_0$ = 100:2:1:1. Line is theoretical M_n calculated based on measured monomer conversion using equation 3.1.

One should remember that the quoted $M_{n,\ GPC}$ values are not absolute, because Mark-Houwink parameters for PMAOS are not available, and so universal calibration could not be carried out. Thus the quoted $M_{n,\ GPC}$ values are relative to PMMA standards, and the absolute values could be closer to the theoretical values. That said, *PDI* and the apparent variation of $M_{n,\ GPC}$ with conversion should still be quite accurate and both suggest non-living behaviour. Having said all above, it is interesting that *pen* ligand appears to give M_n closer to target value.

Analogous results were obtained by other research groups.[77,84,85] While both groups used copper-mediated ATRP system, they have chosen different ligands: Brocchini employed 2,2'-bipyridine[84] and Haddleton used *N*-(*n*-propyl)-2-pyridylmethanimine (Figure 3.1).[85] In his work Brocchini targeted molecular weight of 10000 g mol^{-1} and thus at measured conversion of 96% was expecting to obtain a polymer of about 9500 g mol^{-1}. However, the measured molecular weight of the product was 25320 g mol^{-1} which is reasonably

higher than the expected value. The molecular weight was measured by GPC with DMF/0.1 wt.-% LiCl and PMMA polymer standards. Obtained polydispersity value of 1.39 indicated a low level of control, which was supported by a large discrepancy between calculated and measured molecular weight.[84] These results are quite similar in nature to those here.

Table 3.5 Conversion and M_n data for polymerisation of 50 wt.-% MAOS in DMF.[A]

Ligand	Time, min	Conv., %	$M_{n,\,theory}$[B]	$M_{n,GPC}$[C]	PDI
pen	22	40	7371	12047	1.55
	32	52	9425	12665	1.60
	39	55	10094	13010	1.60
	43	57	10504	12976	1.63
	51	64	11644	12819	1.66
bpy	18	18	3272	9255	1.65
	28	24	4422	9963	1.66
	35	26	4721	10091	1.68
	47	31	5712	10336	1.68
	53	30	5546	10197	1.75

[A] At 90 °C, $[MAOS]_0:[Cu^IBr]_0:[Ligand]_0:[EBisoB]_0=100:1:2:1$.

[B] $M_{n,\,theory}$ was calculated based on measured conversion using equation 3.1.

[C] Molecular weight and *PDI* values were established by GPC; the analysis was performed at the CSIRO Ian Wark Laboratories, Clayton, Australia.

Haddleton's group has achieved a better result by producing polymers with polydispersity index of 1.15-1.18. However, there was a disagreement between targeted molecular weight and measured values similar to the discrepancy observed by us and Brocchini. GPC analysis yielded M_n values 4-5 times higher than expected. ^{1}H NMR was used as an alternative to establish the absolute molecular weight of the polymers by utilising peak intensity ratios, and it has produced figures very close to calculated values.[85] However, one cannot see the results as the ultimate proof of superiority of the NMR method over the GPC method in determination of molecular weight of PMAOS samples.

A series of experiments where molecular weights of polymer standards with narrow polydispersity are determined by both GPC and ^{1}H NMR might be required in order to

establish which method produces correct results rather than results that are close to targeted values.

What are the reasons for the lack of control in polymerisation of methacryloyloxy succinimide? Production of chains of high molecular weights is an indication of high concentration of active radicals in the system. The key to suppression of the termination reaction in a living system such as ATRP is keeping the concentration of growing radicals very small by creating equilibrium where production of dormant species is favoured. If for any reason the position of such equilibrium is shifted, the deactivation process might be retarded, leading to higher concentration of active radicals. These radicals can now undergo termination reaction and overall control over molecular weight of resulting chains is lost.

Rademacher *et al*. and Matyjaszewski *et al*. mention slow deactivation step when they offer several reasons explaining ill-controlled ATR polymerisation of (meth)acrylamides. [86,87] Both research groups suggested a possibility of Cu species forming a complex with amide group of the chain end which results in stabilisation of radical of poly(*N,N*-dimethylacrylamide). They claim that such complexation would result in slow deactivation step which in turn results in production of large amount of free-radicals and loss of control over termination reaction.[87]

It has been shown that the presence of Lewis acid promotes formation of free-radical from corresponding alkyl bromide.[88] Rademacher *et al*. refer to the claim suggesting Cu complex acts as a Lewis acid, promoting the removal of bromine by complexation of Cu to amide moiety on polymer chains.[86] It has also been suggested that a terminal halogen atom could be removed through displacement of the latter by an amide group. In end-group analysis, mass spectrometry has confirmed such removal.[86] It is also possible that coordination of Cu species to succinimide groups is causing retardation of the deactivation step, which results in termination and production of polymer chains of such high molecular weight.

While there is a distinct possibility that some type of side reaction involving the catalyst is a cause of lack of control in ATRP of MAOS, the effect should manifest itself in adjusted kinetic parameters that govern the position of the equilibrium. For that purpose

experimental data obtained was used in kinetic simulations in an attempt to extract the activation (k_a) and deactivation (k_d) constants.

3.5 Modelling of data

3.5.1 Background

According to the general mechanism of ATRP depicted in Scheme 3.1, the rate of polymerisation is given by the following equation:

$$R_p = k_p \frac{k_a}{k_d} [M] [I]_0 \frac{[Cu^I]}{[Cu^{II}]} \quad (3.2)$$

According to this equation the rate of polymerisation should be first-order with respect to monomer [M], initiator [I] and activating Cu^I species and therefore should produce linear time-dependence. However, Fischer has shown that ATRP is a subject to a *persistent radical effect* (PRE), which predicts the evolution of deactivating species throughout the polymerisation as a result of which the first-order kinetic plot of monomer consumption is not perfectly linear.[89] Indeed, the majority of first-order kinetic plots of consumption of MMA in the course of ATRP based on the results of this work show a slight curvature, as will be seen.

Dissociation of the initiator R-Br yields free radicals and Cu^{II} in the form of $Cu^{II}Br_2/L_2$ complex. At this stage concentration of free radicals equals concentration of Cu^{II} in the mixture. Some free radicals will be undergoing irreversible bimolecular termination reaction, therefore the concentration of free radicals will be reduced, while Cu^{II} will undergo no such reaction. During this time, concentration of free radicals will reach its maximum and will decrease thereafter, while concentration of Cu^{II} will increase steadily and will lead to the accumulation of Cu^{II} in the mixture. Due to the low concentration of free radicals, the radicals will be now predominantly reacting with Cu^{II} which is present in excess. The majority of chains will now be capped by Br which will prevent them from terminating. At this instance $Cu^{II}Br_2/L_2$ complex acts as a *persistent (stable) radical species*, causing a PRE.[41] Coupled with a high deactivation rate parameter, high concentration of the persistent radical species will ensure fast rate of deactivation, and therefore a living character of the ATRP system. This finding led to the widely used

practice of adding a small amount of deactivating Cu^{II} species to an ATRP mixture. Such addition causes reduction in the proportion of terminated chains due to the generation of sufficient concentration of stable radical species early in the course of the polymerisation process.[90]

Fischer has shown that in the ideal LFRP system the monomer consumption can be described using equation 3.7,[40,41] which in the case of chain length independent termination will lead to non-linear dependence of the logarithmic monomer conversion on time.

$$\ln\left(\frac{[M]_0}{[M]}\right) = \frac{3}{2}k_p\,([\text{Initiator}]_0[Cu^IBr]_0)^{1/3}\left(\frac{k_a}{3k_d 2k_t}\right)^{1/3} t^{2/3} \qquad (3.7)$$

The concentration of active radicals and deactivator Cu^{II} in an ATRP system is described by the equations 3.4 and 3.8 respectively.[40,41]

$$[R] = ([\text{Initiator}]_0[Cu^IBr]_0)^{1/3}\left(\frac{k_a}{3k_d 2k_t}\right)^{1/3} t^{-1/3} \qquad (3.4)$$

$$[Cu^{II}] = ([\text{Initiator}]_0[Cu^IBr]_0)^{2/3}\left(\frac{3k_a{}^2 2k_t}{k_d{}^2}\right)^{1/3} t^{1/3} \qquad (3.8)$$

From the equation 3.4 it is evident that k_{app} (= k_p[R]) will increase as starting initiator and Cu^I concentrations are increased, as was the case in the experiments discussed in the earlier section on *Monomer concentration effect*. These equations were used in calculations, results of which are described in the following subsections.

3.5.2 ATRP of MMA

In this work modelling of data was expected to produce explanations for two sets of results obtained, i.e., if observed increase of k_{app} with wt.-% MMA could be explained by the increasing initiator and Cu^I concentrations alone and why commonly used inhibitors have no consistent effect on ATRP of MMA.

3.5.2.1 Radical inhibitor effect

The results of the investigation of the effect of the 4-methoxyphenol (hydroquinone, HQ) and 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT) on kinetics of

ATRP of MMA were discussed in an earlier section of this chapter. It was found that both inhibiting species caused no significant change in rate of ATR polymerisation. The aim of this modelling is to determine whether the observed lack of inhibition could be predicted. In other words is the radical concentration in ATRP simply too low for inhibitors to work efficiently? The most significant effect is that of the inhibitor BHT, which effectively stops conventional polymerisation, but has no consistent effect on ATR polymerisation. A simulation program was written and kinetic parameters were used in the modelling of data. Greg Smith is acknowledged for writing the program.

The following reaction scheme was used:

$$\textit{Activation / deactivation}: \mathrm{RX + ML} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \mathrm{R + XML} \qquad (3.9)$$

Where RX is an initiator, ML is a metal-ligand complex, R is generated free radical and XML is a halogenated metal-ligand complex.

$$\textit{Chemical initiation}: \mathrm{AIBN} \xrightarrow{f\,k_{decomp}} \mathrm{R} \qquad (3.10)$$

$$\textit{Spontaneous initiation}: \mathrm{M} \xrightarrow{R_{init}} \mathrm{R} \qquad (3.11)$$

A constant rate of spontaneous initiation from monomer is assumed. Although this certainly does not apply for styrene polymerisation and probably does not apply for the present MMA system for which less is known about spontaneous initiation, it is only a minor effect and certainly does not alter the obtained conclusions.

$$\textit{Propagation}: \mathrm{R + M} \xrightarrow{k_p} \mathrm{R} \qquad (3.12)$$

$$\textit{Termination}: \mathrm{R + R} \xrightarrow{k_t} \mathrm{D \text{ or } D + D} \qquad (3.13)$$

$$\textit{Inhibition/ Quenching}: \mathrm{R + Q} \xrightarrow{k_q} \mathrm{D + Q'} \qquad (3.14)$$

M is a monomer, D is a dead chain formed as a result of a termination event, Q' is a stable radical.

The following parameters are used in simulations: $[Q]_o$, $[I]_o$, f, k_d, k_p, k_a, k_d k_t, k_q. Using the model and the experimental concentration of initiator and an inhibitor and literature values of f, k_{decomp}, k_p and k_a values for k_t, k_q, k_d were defined.

Simulations of inhibitor-free ATRP systems produced values for k_t and k_d which subsequently were used in model calculations for systems with introduced inhibitor species.

3.5.2.1.1 Parameters used in the modelling

A number of kinetic parameters were used in the modelling of the experimental data (Table 3.7). Initial concentrations of reactants are reported in Table 3.6.

Table 3.6 Initial concentrations of ATRP reactants used in modelling of experimental data.

Reactant	Concentration, M
$[AIBN]_0$	4×10^{-3}
$[HQ]_0$ or $[BHT]_0$	4×10^{-1}
$[Cu^IBr]_0$	4×10^{-2}
$[EBisoB]_0$	4×10^{-2}

Table 3.7 Kinetic parameters used in the modelling of experimental data of ATRP of MMA.

Rate parameter	Value
Propagation rate coefficient for MMA,[91]	$k_p = 1624$ L mol^{-1} s^{-1}
Initiation rate,[92]	$fk_{decomp} = 2.44 \times 10^{-4}$ s^{-1}
[a]Initiator efficiency,[93]	$f = 0.77$
[b]Rate of spontaneous initiation,[94]	$R_{init,thermal} = 1.35 \times 10^{-15}$ mol L^{-1} s^{-1}
[c]Activation rate coefficient,[53,95,96]	$k_a = 0.19$ L mol^{-1} s^{-1}
[d]Deactivation rate coefficient,[95,96]	k_d was fitted to data

[a]Buback *et al.* measured $f = 0.72$ for AIBN in styrene at 90 °C and 1000 bar. It was found that f increases by approximately 0.05 for every 100 bar reduction of pressure. Therefore, the value of $f = 0.77$ was estimated and used in the modelling. This enables k_{decomp} to be determined, which is necessary for initiator consumption.

[b]The value was calculated using the following expression for bulk polymerisation of MMA at 90 °C: $R_{init,thermal} = 3.71 \times 10^{-17}$ mol L^{-1} s^{-1} $10^{-5670\ K/T}$.

[c]The value was calculated using the Arrhenius expression for activation rate coefficient, $k_a = 2.2 \times 10^5$ L mol^{-1} s^{-1} exp(-42.1 kJ mol^{-1}/ RT).[53] This value would be for polystyryl bromide with 4,4'-diheptyl-2,2'-*bpy* while the value for PMMA-Br with *bpy* is required for the modelling. It is evident that there is a large variation in k_a for MMA with different ligands and a value of k_a with a substituted *bpy* is not necessarily the same as the value for *bpy* itself. Indeed, there are literature values of k_a calculated for PMMA/Cu^{I}Br/*bpy* with ethyl 2-bromoisobutyrate (EBisoB) at 35 °C in polar media of acetonitrile and acetonitrile/chlorobenzene mixture.[97,98] However, the experiments used in the modelling were done in 50/50 wt.-% MMA/toluene, a different solvent environment to that of the experimental data above and it has been established the choice of solvent influences activation rate parameter. The above expression gave $k_a = 0.19$ L mol^{-1} s^{-1} at 90 °C and the value was used in the modelling.

[d]The literature data suggested that the value of k_d of order 10^7 L mol^{-1} s^{-1} is what modelling of experimental data should yield.

k_{app} is determined by a standard method - it is the slope of a linear fit of -ln(1-x) *vs* t (where applicable, high conversion data has been omitted from the fit). Induction times for simulations are estimated from the t intercept of the same linear fit. The list of k_{app} values obtained experimentally is in Table 3.8.

Table 3.8 k_{app} values determined experimentally.

k_{app}	Value, L mol^{-1} s^{-1}
$k_{app,\ AIBN}$	2.26×10^4
$k_{app,\ AIBN,\ HQ}$	1.88×10^4
$k_{app,\ AIBN,\ BHT}$	0.00
$k_{app,\ ATRP}$	4.42×10^4
$k_{app,\ ATRP,\ HQ}$	4.78×10^4
$k_{app,\ ATRP,\ BHT}$	3.67×10^4
$k_{app,\ ATRP,\ BHT}$	5.63×10^4

3.5.2.1.2 Results of the modelling

The best fit of the experimental data for polymerisation of MMA in 50 wt.-% toluene at 90 °C using AIBN was obtained with $k_t = 3.5 \times 10^7$ L mol^{-1} s^{-1}, see Figure 3.21.

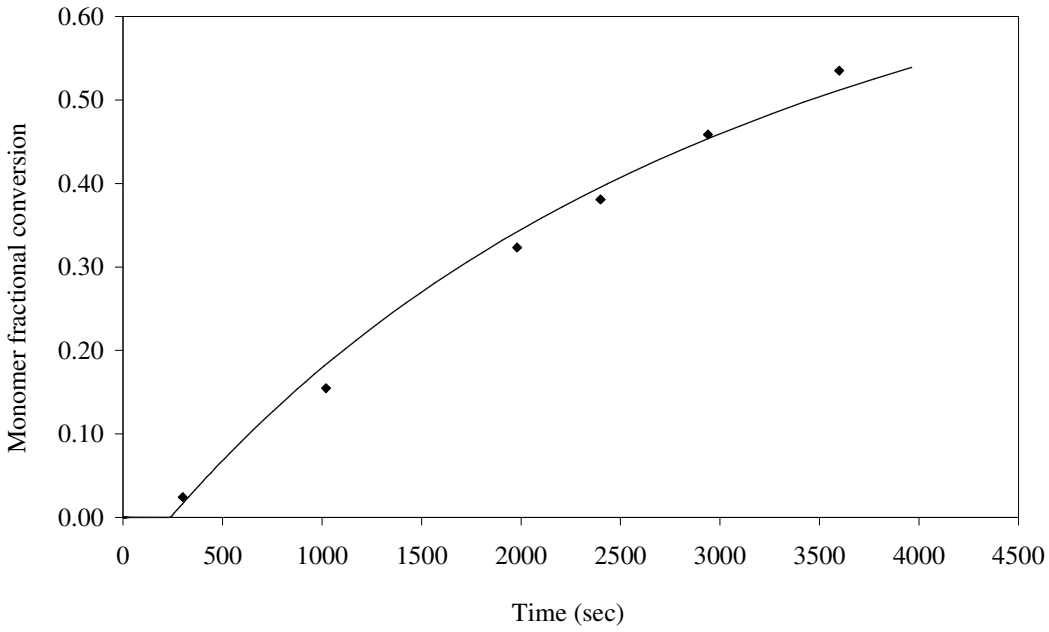


Figure 3.21 The simulation of the experimental data of FRP of MMA in 50 wt.-% toluene at 90 °C with AIBN.

The obtained value was subsequently used in simulation of data for the similar polymerisation of MMA in 50 wt.-% toluene using AIBN to which a 10 molar excess (with respect to the amount of the initiator) of HQ was added.

As already mentioned above, HQ proved to be quite ineffective as an inhibitor of a free-radical polymerisation of MMA. Thus the simulation of data yielded the very low value of $k_{q,HQ}$ = 3.0 L mol^{-1} s^{-1}, see Figure 3.22. This is an upper bound for the effective value (any value lower than this will give no effect). Of course, the actual value must be considerably higher than this, but the point is that this value of k_q represents the rate of removal of radicals by inhibitor. It would seem that reaction of radicals and HQ generally does not remove radicals from the system.

In the similar system of free-radical polymerisation of MMA in 50 wt.-% toluene, initiated by AIBN, no polymerisation was detectable after 2 days upon the addition of a 10 molar excess of BHT. Therefore, the lowest value of $k_{q,\,BHT}$ consistent with this result had to be determined. After 6 hours, 10 half-lives of the initiator have passed, and the likelihood of further polymerisation is low. The mass of sample that could potentially be converted to polymer is typically 0.2 g. Assuming an instrumental uncertainty for the mass balance of 10^{-4} g, the lowest measurable conversion is 0.05%. By simulation, it was

found that $k_{q,\ BHT} = 2.5 \times 10^4$ L mol^{-1} s^{-1} gives approximately 0.05% conversion after 6 hours, and therefore represents a lower bound for $k_{q,\ BHT}$. This is low for an inhibitor but the actual value could be many orders of magnitude higher – that is still consistent with the data.

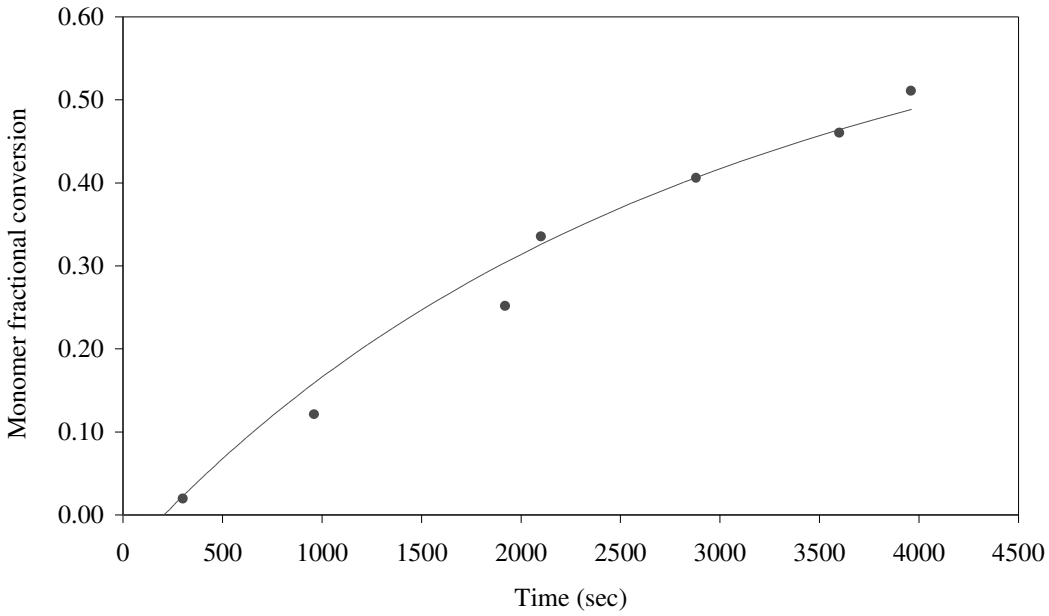


Figure 3.22 The simulation of the experimental data of FRP of MMA in 50 wt.-% toluene at 90 °C with AIBN with 10 molar excess of HQ.

Inhibitor-free ATRP data was modelled to find k_d. The literature indicates a relatively broad range of acceptable values for k_a, and so a lower bound (0.19 L mol^{-1} s^{-1}) and an upper bound (10 L mol^{-1} s^{-1}) of the value were used. The remaining unknown parameter, k_d, was then fitted to the data. Figure 3.23 shows the data for this system, and a simulation with $k_a = 0.19$ L mol^{-1} s^{-1}, $k_d = 1.5 \times 10^5$ L mol^{-1} s^{-1}.

For the second simulation with $k_a = 10$ L mol^{-1} s^{-1}, it was decided to keep the ratio k_a/k_d (= K_{eq}) constant, giving $k_d = 7.5 \times 10^6$ L mol^{-1} s^{-1}, Figure 3.24.

Clearly the two simulations are very similar, suggesting that the individual rate constants are of secondary importance to the equilibrium constant (at least over this time range). Of the two, the second simulation seems more reasonable as we expect k_d of the order of 10^7 L mol^{-1} s^{-1}. Accordingly, the second set of parameters will be used in subsequent simulations.

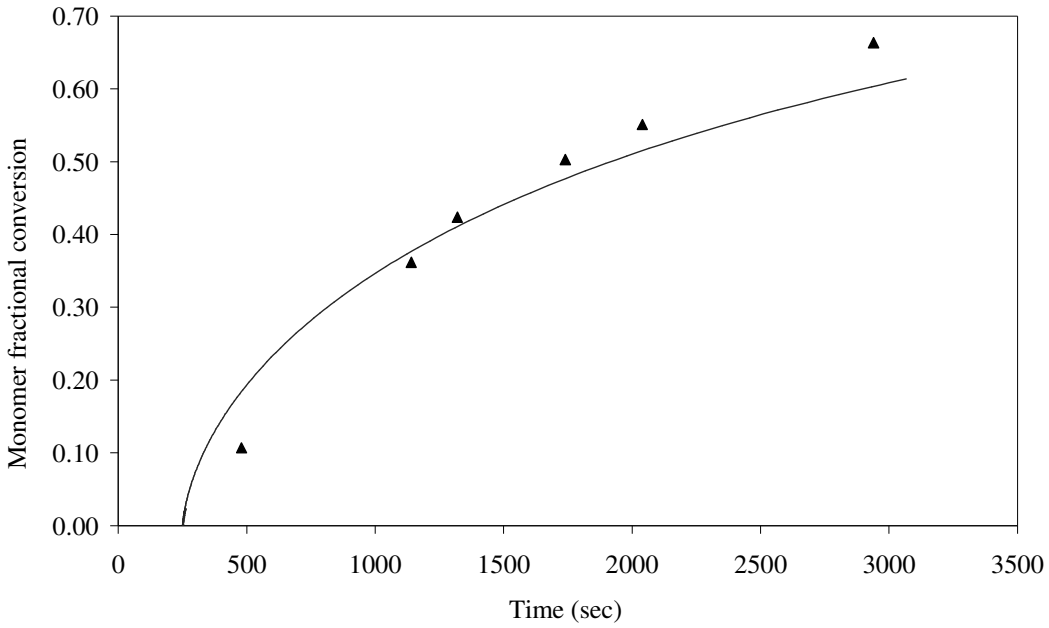


Figure 3.23 The simulation of the experimental data of ATRP of MMA in 50 wt.-% toluene at 90 °C with k_a = 0.19 L mol^{-1} s^{-1}, k_d = 1.5 × 10^5 L mol^{-1} s^{-1}; [MMA]$_0$:[*bpy*]$_0$:[Cu^{I}Br]$_0$:[EBisoB]$_0$ = 100:2:1:1.

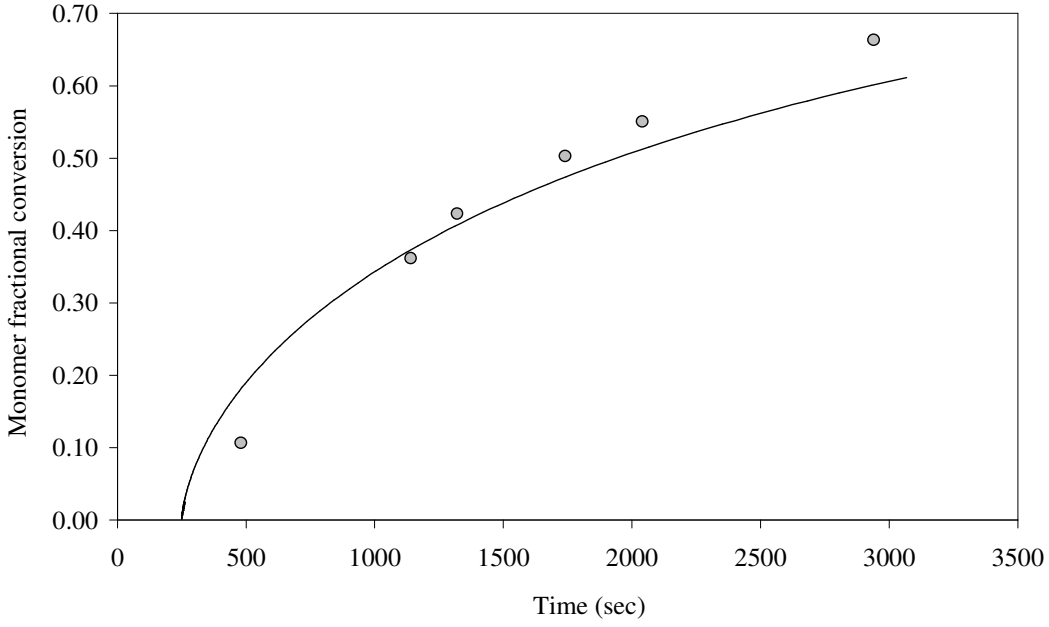


Figure 3.24 The simulation of the experimental data of ATRP of MMA in 50 wt.-% toluene at 90 °C with k_a = 10 L mol^{-1} s^{-1}, k_d = 7.5 × 10^6 L mol^{-1} s^{-1}; [MMA]$_0$:[*bpy*]$_0$:[Cu^{I}Br]$_0$:[EBisoB]$_0$ = 100:2:1:1.

The value of the rate of thermal initiation used here is so low that thermal initiation has a negligible effect on the outcome of the simulation - the rate of termination is about 8 ×

10^{-7} mol L^{-1} s^{-1} at 1 hour, which is about 7 orders of magnitude higher than the rate of thermal initiation. In a slower polymerisation, the effect of thermal initiation would be to eventually balance the rate of termination (once the radical concentration becomes low enough), leading to a steady state in radical concentration. For this system that situation never arises at low conversions.

The calculated parameters were required for the simulation of the ATRP/HQ and ATRP/BHT systems, which could now be carried out a priori.

Clearly the fit in Figure 3.25 is not particularly good as the perfect agreement between the experimental data and the simulations could not be obtained. Possibly a value of k_q for HQ could be found that simultaneously fits this data and the CRP data, however. This was not attempted as it must be conceded that the negligible effect of HQ on both CFRP and ATRP kinetics makes such a not particularly meaningful exercise.

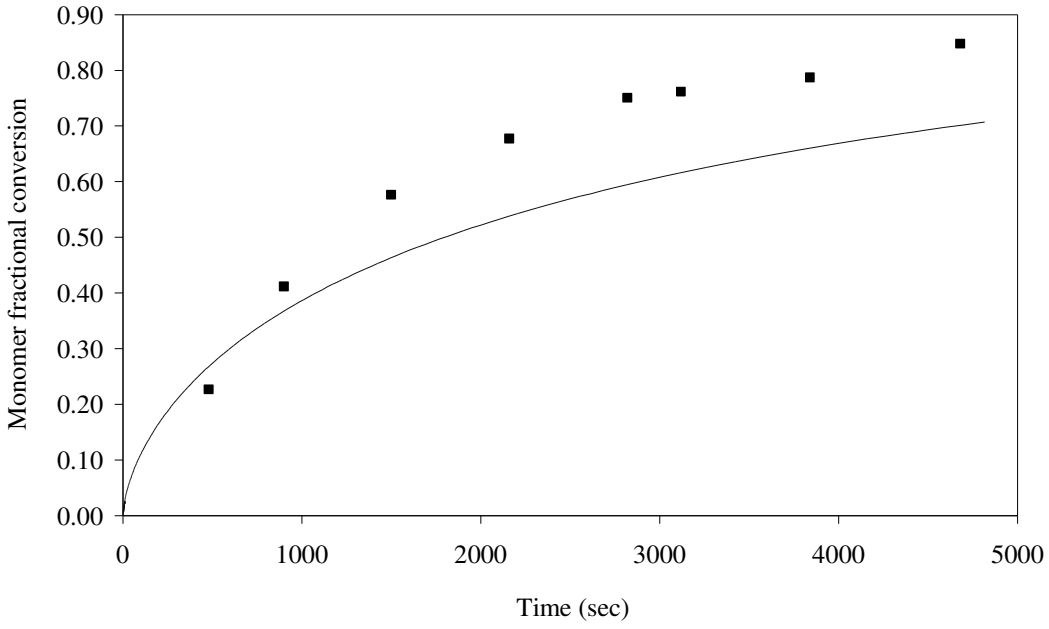


Figure 3.25 The simulation of the experimental data of ATRP of MMA in 50 wt.-% toluene at 90 °C with 10 molar excess of HQ; $[MMA]_0:[bpy]_0:[Cu^IBr]_0:[EBisoB]_0:[HQ]_0 = 100:2:1:1:10$.

The ATRP/BHT data predicts a much lower rate of polymerisation than is observed. The graph in Figure 3.26 shows both data sets. Figure 3.27 shows that the radical concentration is too low for polymerisation to occur. It is interesting to compare radical concentration in the presence and absence of BHT. This is also done in Figure 3.27. The hallmark t^1 and $t^{-1/3}$ regimes are evident in the absence of BHT. Also, radical

concentration is higher and thus one sees inhibitor should still significantly suppress rate in ATRP. But it does not thus proven as not being due to PRE. Rather it must be an effect of some unexplained chemistry. To establish this was the point of the present modelling work.

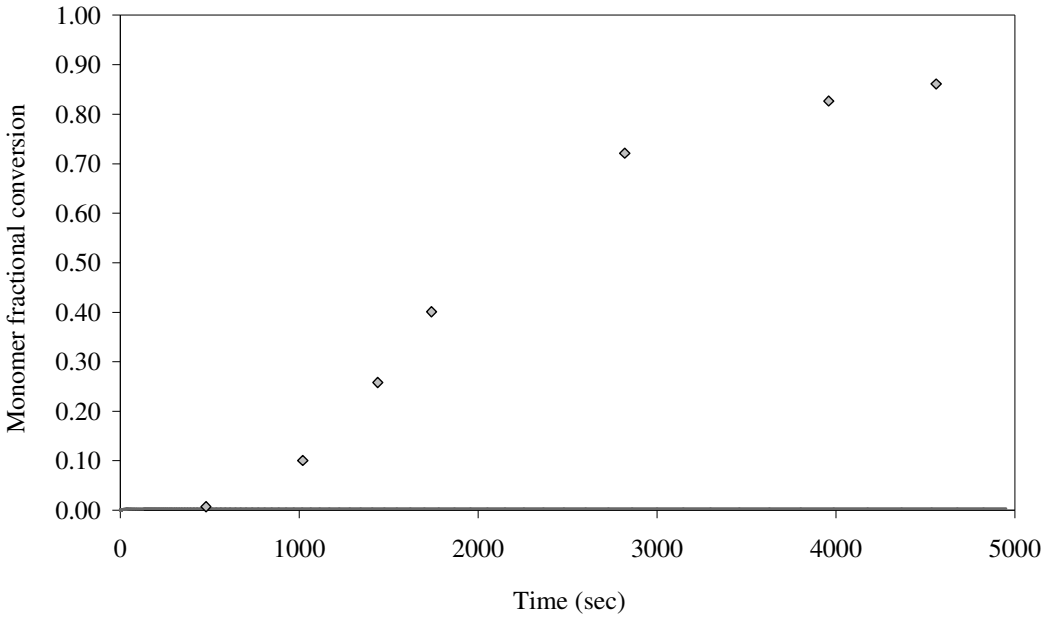


Figure 3.26 The simulation of the experimental data of ATRP of MMA in 50 wt.-% toluene at 90 °C with 10 molar excess of BHT; $[MMA]_0$:$[bpy]_0$:$[Cu^IBr]_0$:$[EBisoB]_0$:$[BHT]_0$ = 100:2:1:1:10.

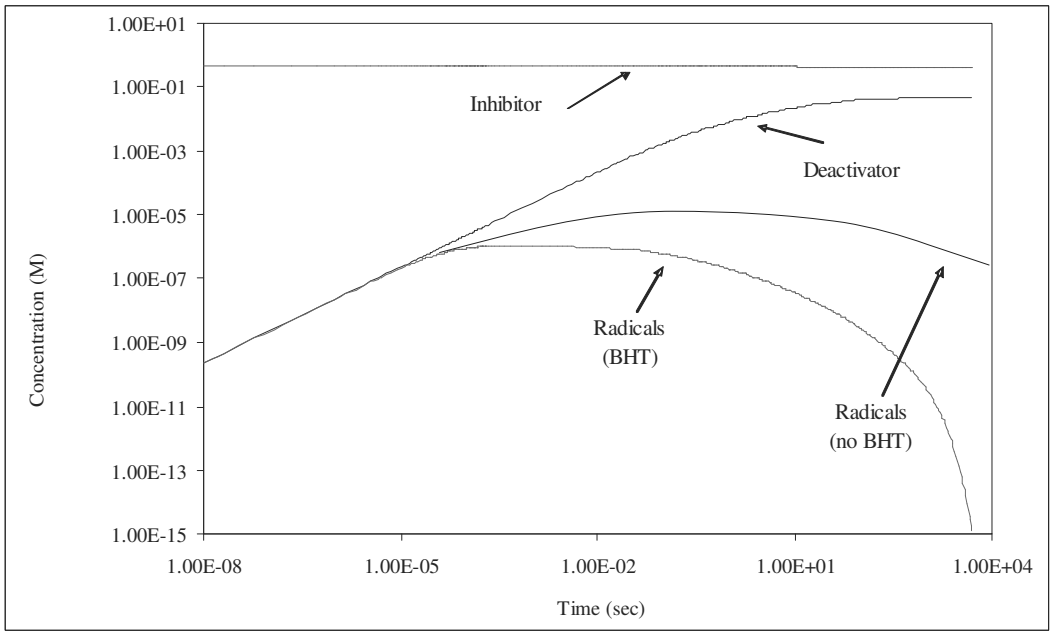


Figure 3.27 Evolution of the concentrations of various species in the course of the ATRP in the presence of radical inhibitor.

3.5.2.2 Simulation of ATRP systems of MMA with various K_{eq}

Equation 3.7 was used in the calculations, where experimental values of initiator and copper catalyst concentrations were used, along with measured conversion and time.

$$\ln\left(\frac{[M]_0}{[M]}\right) = \frac{3}{2}k_p\,([\text{Initiator}]_0[Cu^IBr]_0)^{1/3}\left(\frac{k_a}{3k_d 2k_t}\right)^{1/3} t^{2/3} \qquad (3.7)$$

When initial concentrations of initiating and Cu^I species were used in calculations, the modelled data revealed that the observed increase of k_{app} with wt.-% MMA could not be explained by the increasing initiator and Cu^I concentrations alone. Therefore, the change must be due to shift in the equilibrium, i.e., $K_{eq}= k_a/k_d$ must change as solvent amount and thus the reaction medium changes.

The termination rate constant k_t of value of 3.5×10^7 L mol^{-1} s^{-1} was used in calculations. This was the value found to describe an analogous conventional free radical polymerisation of 50 wt.-% MMA in toluene at 90 °C using AIBN which was carried out in this work. The best fit of the experimental data for the polymerisation was observed with $k_t = 3.5 \times 10^7$ L mol^{-1} s^{-1}, see Figure 3.21.

Value of the propagation rate constant was calculated according to the equation 3.15 which at the reaction temperature of 363 K produced the k_p value of 1592 L mol^{-1} s^{-1}.[91]

$$\ln[k_p\ (\text{L mol}^{-1}\ \text{s}^{-1})] = 14.685 - \frac{22190\ \text{J mol}^{-1}}{R\,T} \qquad (3.15)$$

A complete list of parameters used including literature values of various rate constants is in Table 3.9. From further modelling of the experimental data, the value of the only unknown parameter $K_{eq}= k_a/k_d$ was obtained, producing for example the value of 3.00×10^{-6} for the ATRP of 50 wt.-% MMA. Further values as well as the fitted sets of data are shown below (Figure 3.28, Figure 3.29, Figure 3.30, Figure 3.31 and Figure 3.32 and accompanying Table 3.9).

Table 3.9 reveals that a constant k_t was not assumed. Strictly speaking this is false: there will be some variation as the monomer/toluene ratio changes. However toluene is a "normal" solvent for MMA, so it will exert no strong effect on k_t, approximately a factor of 2 at most when going from 25 to 100 wt.-% MMA. This is far smaller than the

observed variation in K_{eq}. Therefore this assumption about k_t introduces only a small error (equation 3.7 reveals that uncertainty in k_t results in the same uncertainty in K_{eq}). Also assumed here is that k_t from conventional FRP applies also in ATRP. Strictly, this cannot be true because of chain-length-dependant termination, but in the absence of any specific information about this, it is justified to proceed in this way.

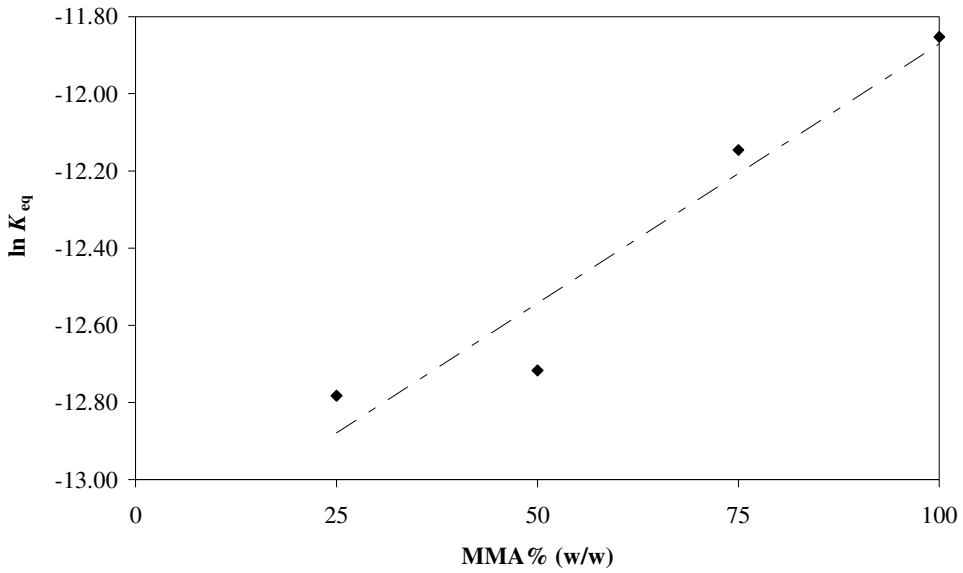


Figure 3.28 Variation in K_{eq} with monomer content in ATRP of MMA mediated by *bpy*.

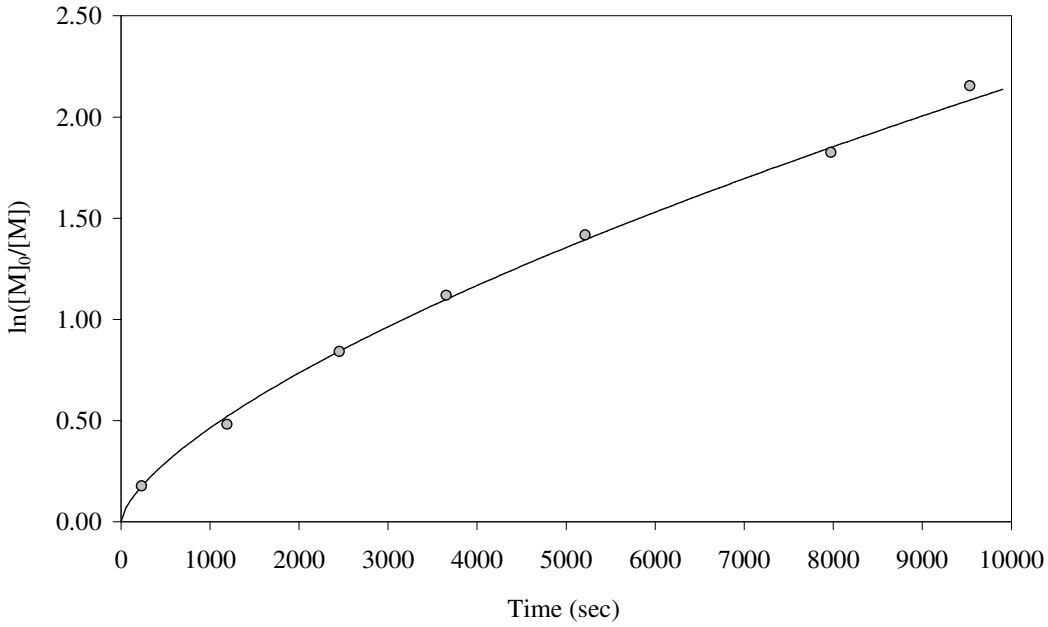


Figure 3.29 Modelling of the ATRP of 25 wt.-% MMA using equation 3.7; $[MMA]_0:[EBisoB]_0:[Cu^IBr]_0:[bpy]_0$=100:1:1:2 at 90 °C in toluene.

Figure 3.29 - Figure 3.32 show how well Fischer's equation fits the data, especially given that all parameter values are very much as expected. The modelling confirms that k_a/k_d increases significantly as MMA amount increases. This means that the equilibrium increasingly favours the Cu^{II} complex as the solvent environment is changed from toluene to MMA.

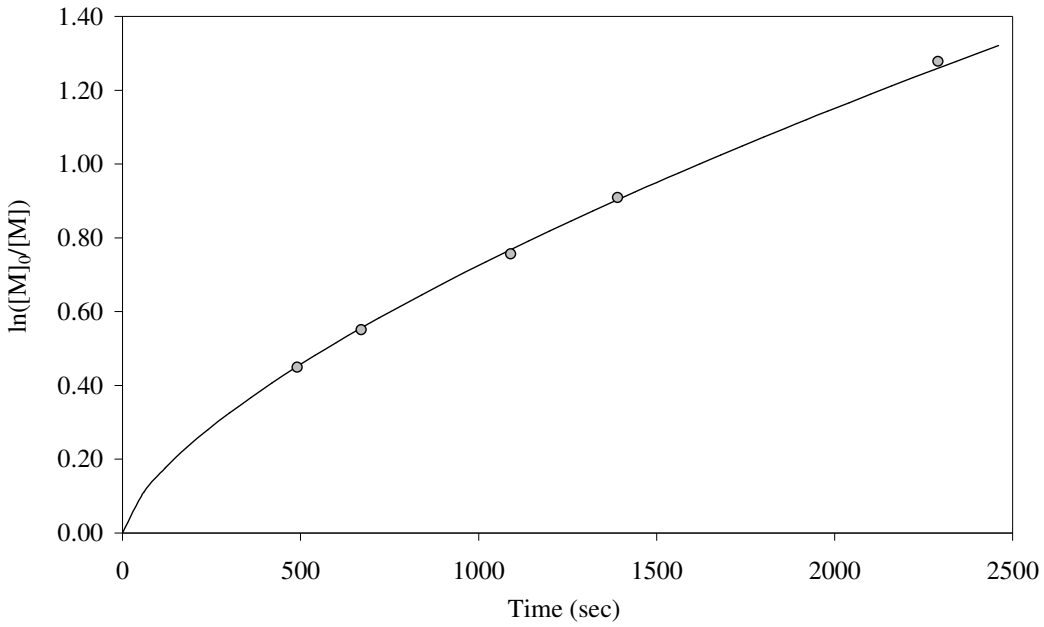


Figure 3.30 Modelling of the ATRP of 50 wt.-% MMA using equation 3.7; $[MMA]_0$:$[EBisoB]_0$:$[Cu^IBr]_0$:$[bpy]_0$=100:1:1:2 at 90 °C in toluene.

The contrast between this work and that recently published by Shipp[99] is noted. Both works (carried out without any knowledge of each other) are in essence the same in that that fit equation 3.7 to conversion-time data. However, Shipp assumed the value of K_{eq} and thus fitted to obtain k_t, whereas in this work k_t was estimated for an independent experiment and then used in fitting data to obtain K_{eq}. It has been commented that Shipp's modus operandi is a convoluted way of obtaining a parameter (k_t) that may be more easily and better determined by other methods, as is in this work, Further, this work uses the ATRP data to obtain an ATRP-specific parameter, K_{eq}, and thus may probe variations of this parameter, something Shipp could not do.

The effect of solvent polarity on activation rate parameters has been discussed in literature, indicating the increase in k_a values with an increase in solvent polarity.[100] For example, in the ATRP of methyl acrylate in toluene k_a decreases by a factor of 5 when compared to the results obtained in acetonitrile.[100] Change from toluene to MMA

environment is not as dramatic as both are solvents of low polarity, however presence of MMA could facilitate a formation of a catalytic complex through coordination of the monomer units to the Cu centre which might change the solubility of the copper species.

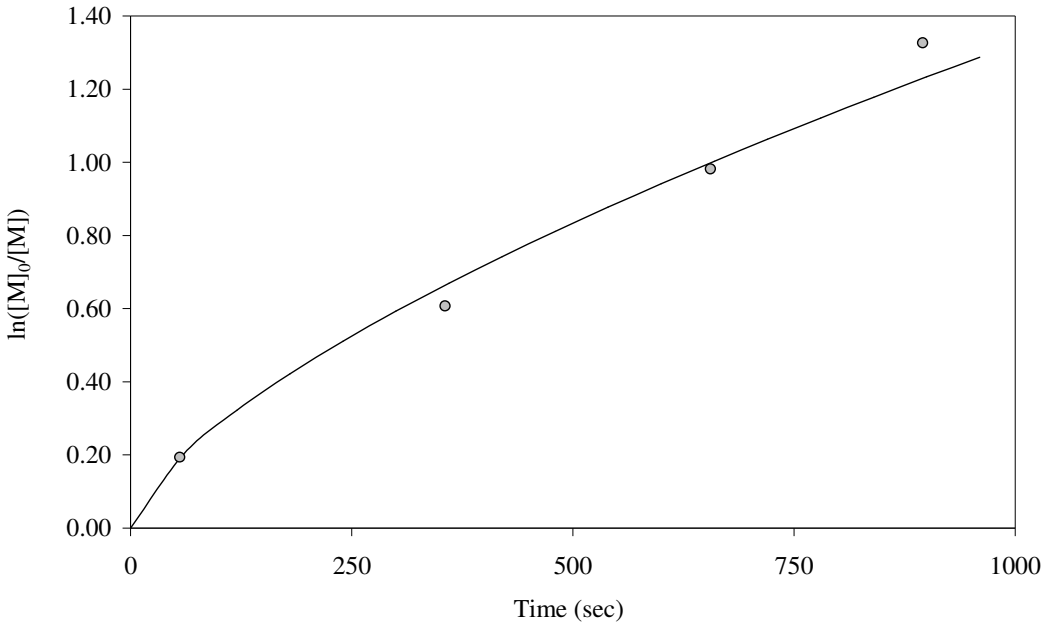


Figure 3.31 Modelling of the ATRP of 75 wt.-% MMA using equation 3.7; $[MMA]_0:[EBisoB]_0:[Cu^IBr]_0:[bpy]_0=100:1:1:2$ at 90 °C in toluene.

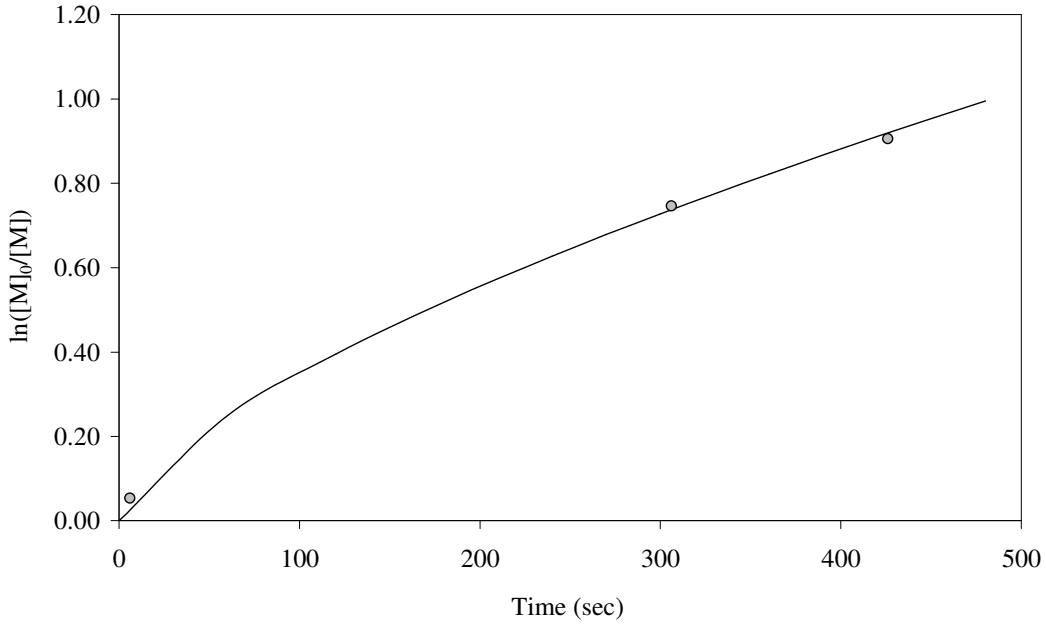


Figure 3.32 Modelling of the ATRP of 100 wt.-% MMA using equation 3.7; $[MMA]_0:[EBisoB]_0:[Cu^IBr]_0:[bpy]_0=100:1:1:2$ at 90 °C in toluene.

Table 3.9 Concentrations and various kinetic parameters used in ATRP/*bpy* simulations.

bpy	MMA, wt.-%			
	25	50	75	100
$[EBisoB]_0$, M	2.20×10^{-2}	4.49×10^{-2}	9.13×10^{-2}	1.83×10^{-1}
$[Cu^IBr]_0$, M	2.49×10^{-2}	4.91×10^{-2}	7.38×10^{-2}	1.48×10^{-1}
[A] k_t, L mol^{-1} s^{-1}	3.50×10^7			
[B] k_p, L mol^{-1} s^{-1}	1.59×10^3			
[C] t_{ind}, sec	847	692	545	294
[D] k_a, L mol^{-1} s^{-1}	0.18	0.19	0.34	0.45
[E] k_d, L mol^{-1} s^{-1}	6.34×10^4			
$K_{eq}(= k_a/k_d)$	2.81×10^{-6}	3.00×10^{-6}	5.31×10^{-6}	7.12×10^{-6}

[A] The value was found by simulation of experimental data for polymerisation of MMA in 50 wt.-% toluene at 90 °C with $[MMA]_0:[EBisoB]_0:[Cu^IBr]_0:[bpy]_0$=100:1:1:2

[B] See reference 91

[C] t_{ind} estimated induction time calculated in Excel.

[D] See references 53 and 95

[E] The value was found by simulation of experimental data for ATRP of MMA in 50 wt.-% toluene at 90 °C at k_a = 0.19 L mol^{-1} s^{-1}.

Similar modelling of data for the ATRP of MMA system where *pen* was employed presented an opportunity to estimate upper and lower boundaries of K_{eq} value.

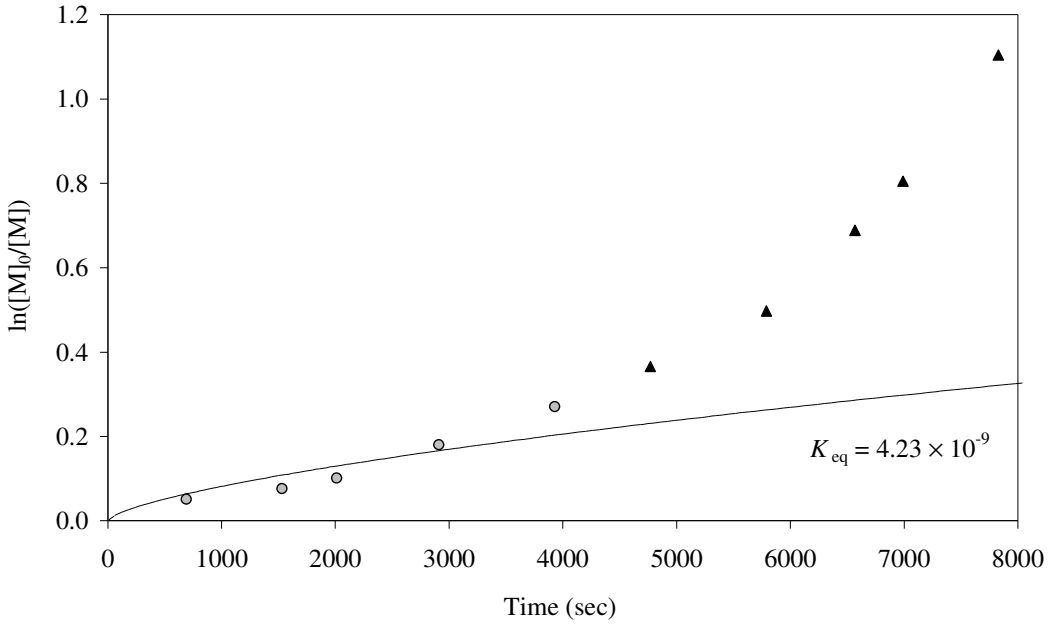


Figure 3.33 Modelling of the ATRP of 50 wt.-% MMA at lower conversion; $[MMA]_0:[EBisoB]_0:[Cu^IBr]_0:[pen]_0$=100:1:1:2 at 90 °C in toluene.

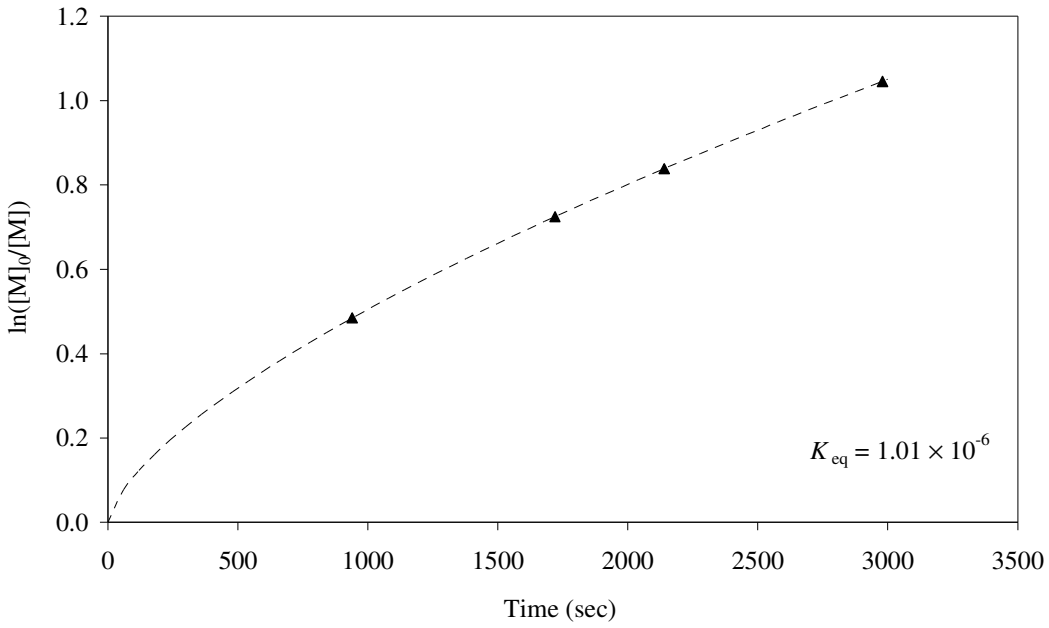


Figure 3.34 Modelling of the ATRP of 50 wt.-% MMA at higher conversion; $[MMA]_0$:$[EBisoB]_0$:$[Cu^IBr]_0$:$[pen]_0$=100:1:1:2 at 90 °C in toluene.

Table 3.10 Concentrations and various kinetic parameters used in ATRP/*pen* simulations.

pen	MMA, wt.-%			
	25	50	75	100
$[EBisoB]_0$, M	2.22×10^{-2}	4.50×10^{-2}	6.86×10^{-2}	9.33×10^{-2}
$[Cu^IBr]_0$, M	2.42×10^{-2}	4.95×10^{-2}	7.38×10^{-2}	1.01×10^{-1}
[A]k_t, L mol^{-1} s^{-1}	3.50×10^7			
[B]k_p, L mol^{-1} s^{-1}	1.53×10^3			
[C]lower t_{ind}, sec	500	150	0	0
upper t_{ind}, sec	7000	5000	950	800
lower k_a, L mol^{-1} s^{-1}	0.06	0.04	0.02	0.02
upper k_a, L mol^{-1} s^{-1}	13.9	10.1	11.2	71.6
[D]k_d, L mol^{-1} s^{-1}	1.00×10^7			
lower K_{eq}(= k_a/k_d)	5.54×10^{-9}	4.23×10^{-9}	2.19×10^{-9}	2.27×10^{-9}
upper K_{eq}(= k_a/k_d)	1.39×10^{-6}	1.01×10^{-6}	1.23×10^{-6}	7.16×10^{-6}

[A] The value was found by simulation of experimental data for polymerisation of MMA in 50 wt.-% toluene at 90 °C with $[MMA]_0$:$[EBisoB]_0$:$[Cu^IBr]_0$:$[pen]_0$=100:1:1:2

[B] See reference 91

[C] t_{ind} estimated induction time calculated in Excel.

[D] The literature data suggested the value of k_d of order 10^7 L mol^{-1} s^{-1}. See references 95 and 96.

3.5.3 ATRP of MAOS

Results of ATRP of MAOS experiments discussed earlier in this chapter were a clear indication that the system is not of an ideal "living" behaviour. Two different types of ligand were used for the purpose of improved solubility of the copper catalyst in the polymerisations and neither system resulted in formation of product with narrow molecular weight distribution. The resulting polymers had molecular weights higher than targeted values, suggesting the lack of control in the system. However, the relatively small M_n values suggest that one does not have uncontrolled, conventional FRP. As a purely modelling approach and in the absence of any better way to proceed it therefore seems reasonable to use Fischer's equations for ideal ATRP kinetics even though it is recognised that they cannot be exactly applicable.

It is the position of the equilibrium (Scheme 3.1) that governs the degree of polymerisation of the product by coupling high deactivating rate parameter (k_d) with low concentrations of active radicals, therefore assuring the majority of chains remain in their dormant state, unable to propagate and terminate. Thus, the aim of the modelling of the kinetic data from the abovementioned experiments is to evaluate K_{eq} in ATRP system of MAOS. Such evaluation will provide an estimated value of the equilibrium constant and might offer an explanation on why a better control has not been achieved in ATRP of MAOS.

3.5.3.1 Simulation of ATRP system of MAOS with various K_{eq}

For the simulation of the data we used the approach used in evaluation of K_{eq} values in ATRP of MMA discussed in section 3.5.2.2, where equation 3.7 was used in the calculations.

$$\ln\left(\frac{[M]_0}{[M]}\right) = \frac{3}{2}k_p\,([\text{Initiator}]_0[Cu^IBr]_0)^{1/3}\left(\frac{k_a}{3k_d 2k_t}\right)^{1/3} t^{2/3} \qquad (3.7)$$

While the purpose of the modelling was evaluation of $\frac{k_a}{k_d} = K_{eq}$, it was evident that values of propagation rate constant , k_p, and termination rate constant, k_t, were necessary for carrying out the calculations. Despite the importance of these constants, their values for

MAOS were not measured, thus such values had to be estimated for consequent use in the calculations.

For a number of alkyl methacrylates, propagation rate constant, k_p, have been measured.[101,102] It was stated that for the alkyl methacrylates under the investigation, there was an increase in k_p value with an increase in size of the ester group, an example of such change being an increase of the rate constant by a factor of 1.5 when going from methyl methacrylate to dodecyl methacrylate.[101] Values are even higher still for monomers with a cyclic ester groups for which following equation has been reported by Beuermann *et al.*[102]

$$\ln[k_p \text{ (L mol}^{-1} \text{ s}^{-1})] = 15.26 - \frac{2634}{T} \qquad (3.16)$$

One would expect this equation to provide the best available estimate of k_p for MAOS. Using equation 3.16 the value of k_p of 2992 L mol^{-1} s^{-1} was produced and used in the calculations as a lower limit, while k_p = 4500 L mol^{-1} s^{-1} was used as the higher limit. The upper limit is a rough estimation based on the propagation rate constant k_p for relativley polar monomer 2-hydroxyethyl methacrylate (HEMA) being 2.5 times higher than the value for cyclohexyl methacrylate (CHMA) and glycidyl methacrylate (GMA). Value of k_p for HEMA is expressed by the equation 3.17 which at 90 °C yields the value of 6272 L mol^{-1} s^{-1}.[103]

$$\ln[k_p \text{ (L mol}^{-1} \text{ s}^{-1})] = 16.0 - \frac{2634}{T} \qquad (3.17)$$

The relatively high value of k_p seems to agree with the initial observation of a reasonable rate of polymerisation of MAOS under conditions of ATRP. However, it was decided not to use the value of 6272 L mol^{-1} s^{-1}, as MAOS is unlikely to be as reactive. Nevertheless it is possible that MAOS being an “activated ester”, has k_p higher than the value of 2992 L mol^{-1} s^{-1}, hence approach adopted here.

The termination rate constant k_t of value of 3.5×10^7 L mol^{-1} s^{-1} was used in calculations conducted for ATRP of MMA system. However, such a high value of the constant should not be used in this modelling as the fast polymerisation rates suggest a much lower rate of termination reaction. In the absence of any specific information, three values of k_t

were used in these calculations; 0.50×10^7 L mol^{-1} s^{-1} as a lower boundary, and value of 1.75×10^7 L mol^{-1} s^{-1} as an upper boundary. The complete list of parameters used in the calculations is in Table 3.11.

Table 3.11 Estimated values of K_{eq} parameter in ATRP of MAOS.[A]

k_t, L mol^{-1} s^{-1}	k_p, L mol^{-1} s^{-1}	K_{eq}	
		bpy[B]($\times 10^{10}$)	***pen***[C]($\times 10^9$)
0.50×10^7	2992	1.62	2.53
	4500	0.49	0.74
1.00×10^7	2992	3.24	5.03
	4500	0.95	1.48
1.75×10^7	2992	5.67	8.81
	4500	1.67	2.59

[A]At 90 °C in 50 wt.-% DMF with $[MAOS]_0:[Cu^IBr]_0:[Ligand]_0:[EbisoB]_0$=100:1:2:1
[B]$[EBisoB]_0 = 5.79 \times 10^{-2}$ M, $[Cu^IBr]_0 = 5.61 \times 10^{-2}$ M
[C]$[EBisoB]_0 = 5.81 \times 10^{-2}$ M, $[Cu^IBr]_0 = 5.44 \times 10^{-2}$ M

Table 3.11 contains estimated values of the unknown parameter $K_{eq} = k_a/k_d$ for each set of results. Examples of the data fitted to the calculated values for the ATRP systems catalysed by Cu^IBr/*bpy* and Cu^IBr/*pen* are presented in Figure 3.35 and Figure 3.36 respectively.

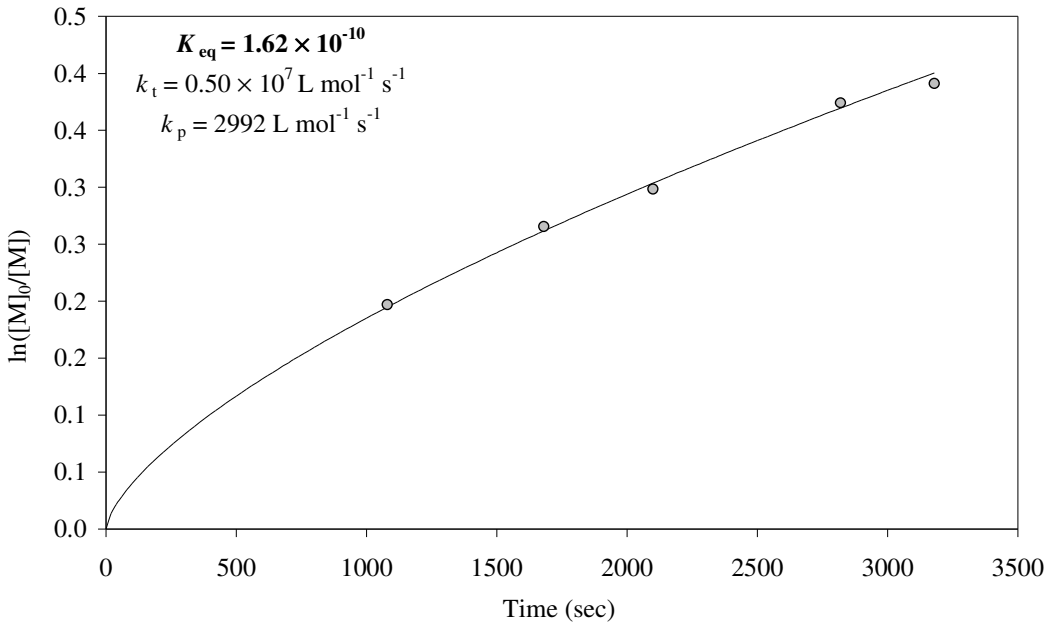


Figure 3.35 Modelling of ATRP of MAOS in 50 wt.-% DMF;
$[MAOS]_0:[Cu^IBr]_0:[bpy]_0:[EbisoB]_0$ = 100:1:2:1.

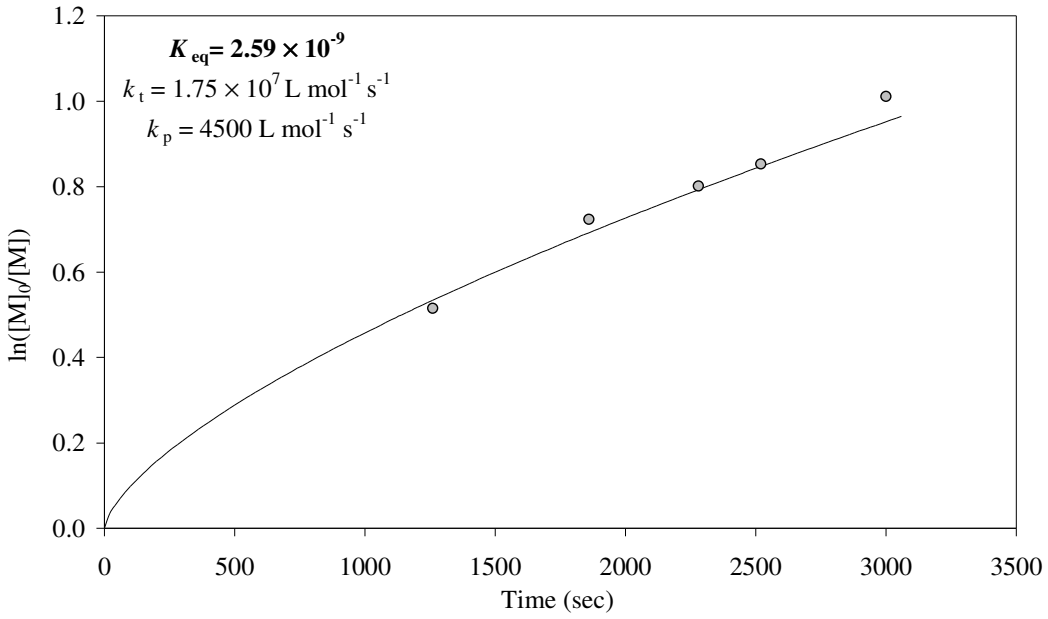


Figure 3.36 Modelling of ATRP of MAOS in 50 wt.-% DMF; $[MAOS]_0:[Cu^IBr]_0:[pen]_0:[EbisoB]_0$ = 100:1:2:1.

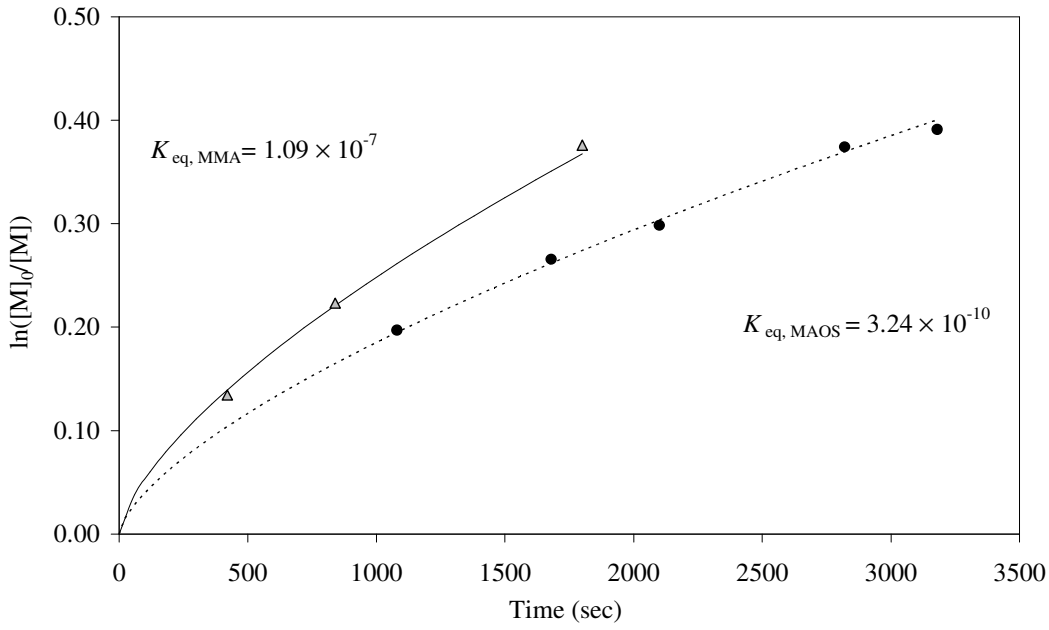


Figure 3.37 Estimated $K_{eq,MMA} = 1.09 \times 10^{-7}$ (k_p = 1529 L mol^{-1} s^{-1} , $k_t = 3.50 \times 10^7$ L mol^{-1} s^{-1}) and $K_{eq,MAOS} = 3.24 \times 10^{-10}$ (k_p = 2992 L mol^{-1} s^{-1} , $k_t = 1.00 \times 10^7$ L mol^{-1} s^{-1}) in 50 wt.-% DMF at 90 °C; $[Monomer]_0:[Cu_IBr]_0:[bpy]_0:[EBisoB]_0$ = 100:1:2:1.

When obtained values were compared to the equilibrium constants estimated for the ATRP of MMA systems, the values of K_{eq} for ATRP of MAOS seem to be fairly low.

That observation might be interpreted as an indication of the shift in the position of the equilibrium towards dormant species. A further comparison was made by modelling the experimental data for ATRP of MMA experiment conducted in 50 wt.-% DMF at 90 °C, the conditions matching that of the standard ATRP/MAOS experiment. The graph in Figure 3.37 contains the two sets of data.

Such a large difference in estimated values of K_{eq} was unexpected, however one has to recognise that other contributing kinetic parameters used in the calculations were adjusted to achieve a correct description of the systems. It is quite clear however, that the lower rate of polymerisation of MAOS can be attributed to either high value of the deactivation rate constant, k_d or to low value of the activation rate constant, k_a, or to both. While measurement of these individual constants is crucial for better understanding of the mechanism of the ATRP, such measurements are not trivial and pose a real challenge for interested parties.[104,105]

Such a large difference between the estimated values of $K_{eq,MMA}$ and $K_{eq,MAOS}$ (of almost three orders of magnitude) when the kinetics for the polymerisations look very similar, is surprising and could be partially attributed to the use of the different values of k_p and k_t in the calculations. It is also important to note the difference in starting concentrations of initiating and catalytic species used in the polymerisations.

The value of estimated $K_{eq,MMA(DMF)} = 1.09 \times 10^{-7}$ in 50 wt.-% DMF was compared to another estimated value of $K_{eq,MMA(tol)} = 3.00 \times 10^{-6}$ which was determined for the ATRP/MMA system in 50 wt.-% toluene. The difference between the two values is in agreement with the earlier evaluation of the apparent rate constants for the two polymerisation systems. The values of $k_{app,MMA(DMF)} = 22.8 \times 10^{-5}$ and $k_{app,MMA(toluene)} = 73.1 \times 10^{-5}$ are quoted in Table 3.3. One can see that according to Fischer's equations (3.4 and 3.7) difference in observed rates can be used attributed to a shift in the position of the equilibrium. The rate of ATRP of MMA in toluene is 3 times higher than that in DMF, which is in agreement with the fact that the value of $K_{eq,MMA(tol)}$ is 27 or 3^3 times higher than $K_{eq,MMA(DMF)}$. This result once again supports the hypothesis that polarity of reaction medium has a significant effect on the kinetics of the ATR polymerisation.

3.6 Conclusion

ATRP is a powerful tool in synthesis of polymer chains of required molecular weight and architecture. While it is applicable to many monomers that could be polymerised under mild conditions of ATRP, there is still some mystery as to what the true mechanism of the polymerisation is.

The widely accepted mechanism that involves an establishment of the equilibrium governed by the rate constants k_d and k_a is believed to be of free-radical nature. The amount of active free radicals in the reaction mixture is determined by the position of the equilibrium, the position of which depends on the nature of the catalyst complex.

In this research the ATRP system of MMA was investigated. Various parameters and additives were varied and results of those experiments were scrutinised and experimental data was manipulated allowing extraction and evaluation of the kinetic rate constants. Such evaluations lead to a conclusion that reaction medium and its polarity in particular has profound effect on structure and solubility of the copper species used. Use of solvents of different polarity and variation in MMA concentration lead to changes in the apparent rate constant k_{app}. Those changes could not be fully justified by changes in initial concentration of reactants and therefore must be due to the shift in the equilibrium.

In this work two widely used in ATRP nitrogen-containing ligands were employed in a controlled polymerisation of MMA, initiated by EBisoB and catalyzed by $Cu^{I}Br$. It was found the catalyst complex formed through a coordination of *pen* to the Cu is of higher solubility in MMA/toluene medium when compared to the similar complex of Cu with *bpy*, producing polymerisation rates consistently lower than the rates obtained for the ATRP with $Cu^{I}Br$/*bpy* complex. Further investigation of this counter-intuitive effect included study of the effect of addition of substituted phenols on the behaviour of the ATRP/MMA/$Cu^{I}Br$/*bpy* system. It was found that commonly used radical inhibitors HQ and BHT had very little or no effect on the kinetics of the ATRP, while modelling of the data predicted significant inhibition. The values of k_{app} increased with MMA content, it is suspected the equilibrium is shifting towards the formation of deactivating species Cu^{II}, favouring the higher concentration of propagating radicals. Higher concentration of MMA could result in a formation of a different catalyst complex via coordination of MMA to the copper metal centre. The modelling of the data yielded K_{eq} values which

increase with the MMA wt.-%. An outstanding finding of this work is that Fischer's equations for ideal ATRP system provide a superb description of the kinetics of these real systems.[106]

The method was also used in preparation of PMAOS, an activated ester polymer which can be converted into a polymer of HPMA. However, in using ATRP, we and others have experienced that ATRP is not the panacea that their advocates make it out to be: poly(*N*-(2-hydroxypropyl)methacrylamide) or PHPMA, the drug-carrying polymer of choice, of uniform, controlled size has not been immediately obtained by this method. Polymeric MAOS was synthesised by ATRP using both 2,2'-bipyridine and *N*-(*n*-pentyl)-2-pyridylmethanimine, however, the resulting polymer was not of targeted molecular weight and polydispersity index was high.

Computer modelling allowed estimation of the equilibrium rate constant values for the ATRP of MAOS systems that employ both *pen* and *bpy* ligand. Obtained values of $K_{eq,MAOS}$ ranging from 5.67×10^{-10} to 0.49×10^{-10} (ATRP/MAOS/*bpy*) and from 8.81×10^{-9} to 0.74×10^{-9} (ATRP/MAOS/*pen*) are reasonably low when compared to the value obtained for an ATRP/MMA experiment under the same reaction conditions. This once again indicates the complexity of an ATRP system and the necessity of finding appropriate ways and tools for determining correct rate parameters. This in turn will allow for a better understanding of the mechanism of the polymerisation, and the roles each individual component plays. Such understanding will provide researchers with information required for design of better functioning ATRP systems. Better recipes will be developed with time and design of new ligands and catalytic complexes might lead to successful synthesis of the PHPMA by the method of atom transfer radical polymerisation in the near future.

3.7 References

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Chapter Four. Reversible addition-fragmentation (chain) transfer polymerisation – RAFT

4.1 Introduction

This chapter presents results of the investigation of another method of *living* free-radical polymerisation that allows for the preparation of polymer chains with narrow molecular weight distribution.

This method functions by reversible addition-fragmentation (chain) transfer (RAFT) and employs thiocarbonylthio compounds acting as chain transfer agents. The acronym RAFT is now widely used as a name for the process that was first introduced in 1998.[1] The development of the method was promoted by the growing need for a truly *living* polymerisation system that will offer all benefits of a controlled polymerisation without having serious disadvantages. An ideal method should be applicable to a variety of monomers of different functionalities; it should not involve use of an expensive reagent or a reagent that is difficult to remove from the product. The "livingness" of the system should not be compromised by the choice of solvent or the acidity of the monomer. As it is believed that RAFT meets these criteria better than other methods, it is now being used successfully by an ever-growing number of research groups around the world. This could serve as a very convincing proof that RAFT has become one of the most versatile polymerisation methods, in particular where control over the architecture and molecular weight of polymer product are priorities. All this makes RAFT polymerisation an ideal method for synthesis of polymeric biomaterials, which due to strict regulations imposed on all pharmaceuticals and other health related products have to be of well-controlled molecular weight and other properties.

The living character of the RAFT process allows synthesis of chains with targeted degree of polymerisation and narrow polydispersity. Polymerisations under the RAFT conditions could be carried out in bulk, solution and emulsion, and a range of solvents could be

employed for polymerisation of various monomers at various temperatures.[1] This method of polymerisation offers a choice of conditions and monomers that can be used in an attempt to synthesise a copolymer of *N*-(2-hydroxypropyl)methacrylamide (HPMA) that is expected to serve as a backbone of a polymer carrying a conjugated drug.

In this work RAFT polymerisation of methyl methacrylate (MMA), methacryloyloxy succinimide (MAOS), methacryloyl chloride (MAC), *p*-nitrophenyl methacrylate (NPMA), *N*-isopropylmethacrylamide (NIPMAM) and HPMA itself under varying conditions was investigated. The first four monomers were polymerised in view of their possible use as precursors of poly(HPMA) or other possible polymers of interest. In this work a number of chain transfer agents were used under various polymerisation conditions in the hope of obtaining a final product of the required molecular weight. However, despite the method's popularity, the results of this work have clearly shown that the method has some limitations with regard to the level of control over the molecular weight distribution of methacrylate derivatives. This chapter presents results of these investigations.

4.1.1 Mechanism

An understanding of basic principles and reactions of a conventional free radical polymerisation is important as such knowledge will allow a reader to appreciate the nature and mechanism of processes involved in RAFT polymerisation.

In a conventional free radical polymerisation chains are initiated by radicals formed from an initiator and grow by means of radical addition to monomer. Chain termination events such as combination and disproportionation result in formation of inactive or "dead" chains and such events occur at any stage of the growth process. Such termination events are unavoidable in any system where active radicals are present and inability to control them is a major drawback of a conventional polymerisation process as usually it causes formation of chains with broad molecular weight distribution.

In *living* free radical polymerisation most radicals are kept in a so-called dormant form. While the average concentration of active radical species in such polymerisation systems is similar to that of a conventional system, the average time an individual radical spends in its active form is low. This is achieved by establishing an equilibrium between growing

radicals and a reversible radical trap (a dormant species). In the case of RAFT the underlying process for such exchange is reversible addition-fragmentation chain transfer. This chain transfer process is induced by an introduction of an organic agent, such as a thiocarbonylthio compound or dithioester (ZC(=S)SR), into what otherwise is a conventional polymerisation mixture. General structure of a RAFT agent is shown in Figure 4.1. Group R is a good leaving group, while Z is a stabilising group, variation of which will determine the value of C_{tr} of the system, where C_{tr} is the so-called transfer constant.

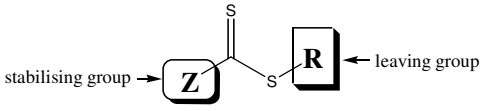
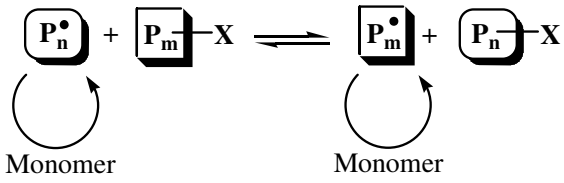


Figure 4.1 General structure of RAFT agent.

This process allows control and moderation of molecular weight of polymers. The general scheme of the process is depicted in Scheme 4.1.



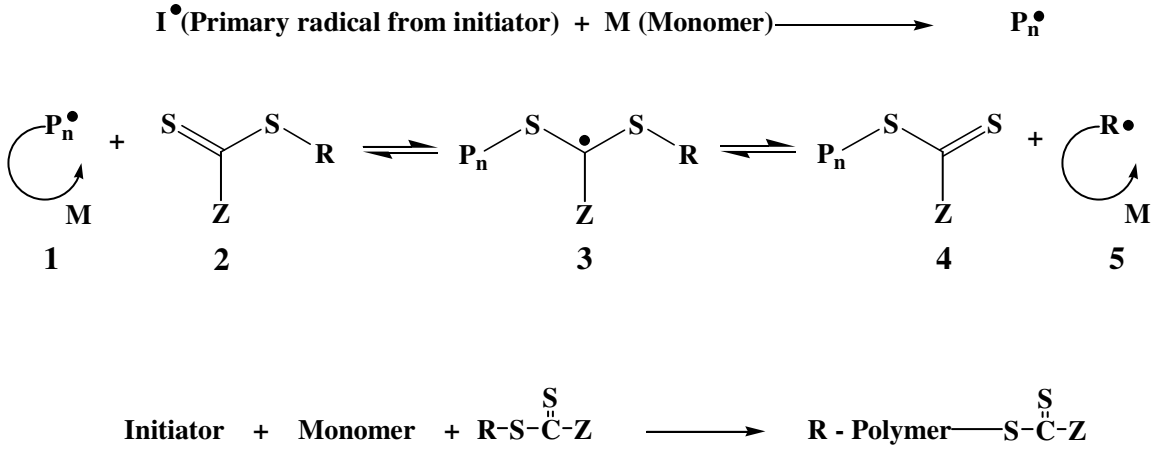
Scheme 4.1 General scheme of reversible transfer.

The scheme shows propagating radicals $\mathbf{P_n}$ reacting with a chain-transfer agent (CTA), $\mathbf{P_m}$-**X**, and forming dormant species, $\mathbf{P_n}$-**X**. This addition is reversible, allowing the fragmentation of the free radical, $\mathbf{P_m}$ that can react with the monomer to form a propagating chain. Fast equilibration between dormant and active forms of radical species will ensure an overall chance of growing that is much the same for every chain. This phenomenon ensures that the extent of irreversible chain termination events occurring in the system can now be considered to be low.

A general mechanism for RAFT polymerisation is shown in Scheme 4.2. It shows a RAFT polymerisation system normally consists of the following components: a free-radical initiator, a monomer and a chain-transfer species **2**.

The presence of initiator, **I**, in the mixture ensures generation of primary radicals that can add to the carbon-carbon double bond of a monomer, **M,** and form polymerising radicals,

P_n. The RAFT process normally uses an azo compound or peroxide as a radical initiator. In this work 2,2'-azobisisobutyronitrile (AIBN) was used predominantly. Decomposition of AIBN is accompanied by loss of nitrogen and leads to generation of two tertiary cyanoisopropyl radicals, as shown in Figure 4.2.



Scheme 4.2 Overall mechanism for RAFT polymerisation.

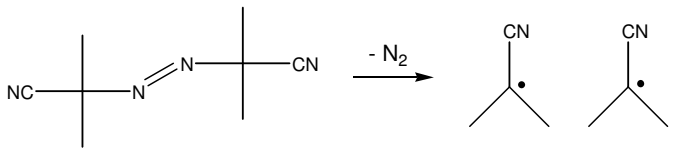


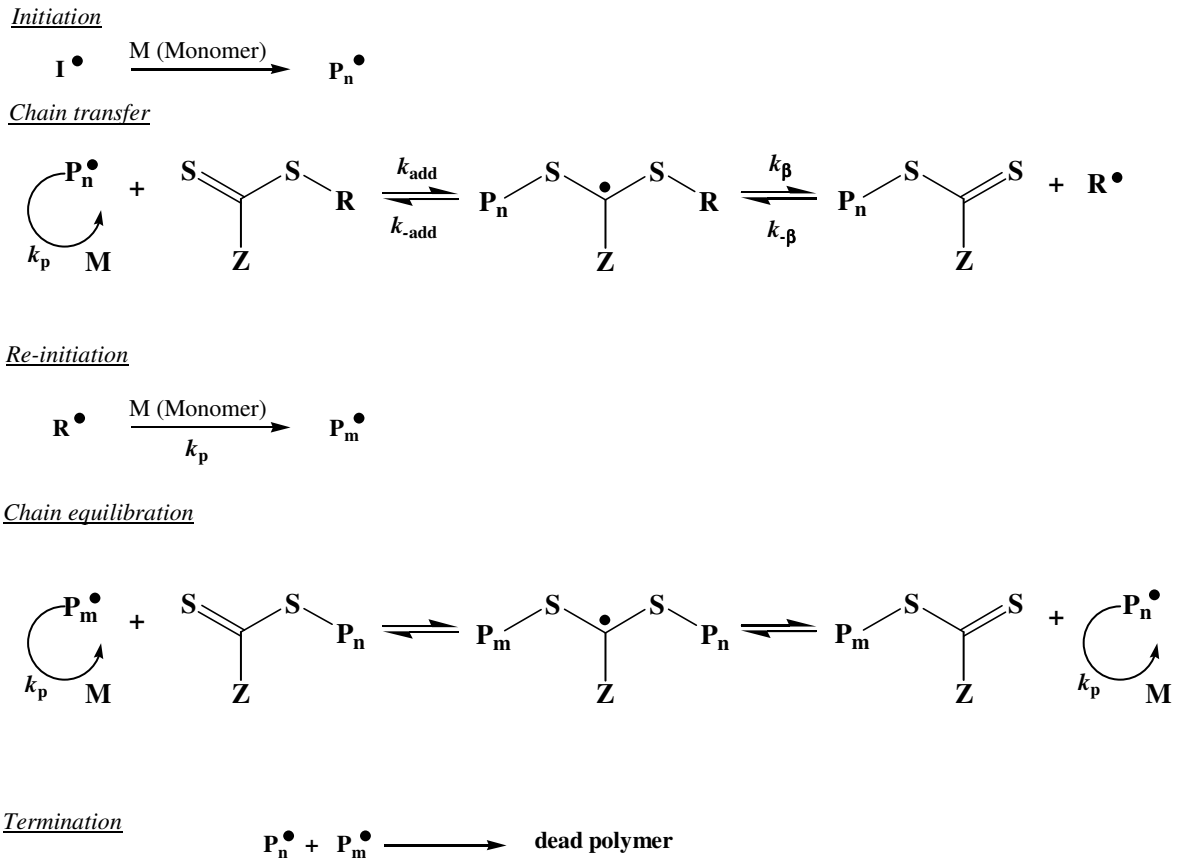
Figure 4.2 Decomposition of 2,2'-azobisisobutyronitrile (AIBN).

In a conventional free-radical polymerisation the radicals are allowed to grow until the termination event occurs. The same termination event is unavoidable in a RAFT polymerisation, however here the propagating radicals $\mathbf{P_n}$ **(1)** can react with the sulphur-carbon double bond of a RAFT agent **2**. Such addition is followed by a fragmentation of a new radical **R (5)** that can now undergo propagation reaction and form polymer chains. The mechanism of the process shows that both addition and fragmentation steps are reversible and propagating radicals **1** and **5** will be in rapid equilibrium with dormant species **4**.

It is clear that in an effective RAFT process the chain transfer must compete with propagation, i.e., rates of addition and fragmentation steps must be high compared to the rate constant of propagation. For that reason, it is important that the **C=S** double bond of the RAFT agent is reactive towards the propagating free radical. A weak single bond between **S** and the leaving group **R** will ensure fast fragmentation of an intermediate

radical. This expelled radical must quickly react with a monomer, thereby re-initiating the polymerisation process.

The mechanism of the RAFT process is presented in more detail in Scheme 4.3. It indicates that the equilibrium between propagating and dormant species is governed by various rate parameters. Here the nature and kinetic parameters of a chain transfer (or in this case RAFT) agent is of great importance as the choice of the RAFT agent will predetermine the effectiveness of the polymerisation process with regard to obtaining a polymer of required molecular weight.



Scheme 4.3 Detailed mechanism of RAFT polymerisation.

In an ideal RAFT polymerisation, a high value of the rate constant of addition, k_{add}, ensured by reactive nature of the S=C double bond of the RAFT agent, will guarantee fast radical addition of propagating radical to the agent and formation of intermediate radical species. In case of a high value of the fragmentation rate constant, k_{β}, the leaving group of the RAFT agent will fragment off quickly, resulting in formation of a new dormant thiocarbonylthio compound and a radical **R**. The newly formed radical should be efficient

in re-initiating the polymerisation process by means of its propagation reaction with the monomer, as is shown in the re-initiation step. It is also clear that in a successful RAFT process the rate constant for fragmentation of the leaving group **R** should be higher than that of the newly added radical $\mathbf{P_n}$, or in other words $k_\beta \geq k_{\text{-add}}$.

The chain transfer activity of the CTA is characterised by the chain transfer constant C_{tr} which is the ratio of the overall rate constant for chain transfer k_{tr} to the propagation rate constant k_p, i.e. $C_{tr} = \frac{k_{tr}}{k_p}$. While the leaving group of the RAFT agent **R** and the stabilising group **Z** determine the value of C_{tr} of the system, the C_{tr} will also depend on polymerisation conditions and the choice of monomer, as these will affect the value of k_p. In case of relatively high value of C_{tr}, the major chain terminating reaction will involve chain transfer to CTA, resulting in production of chains with CTA end-group functionality.

Since the overall activity of a RAFT agent in a polymerisation system is characterised by the chain transfer constant C_{tr}, it is imperative to be able to estimate the value of C_{tr}. As was mentioned above, for conventional chain transfer the value of C_{tr} is given by the ratio of k_{tr} to k_p. In a RAFT system k_{tr} however becomes a composite term which depends on a number of parameters.[2] In RAFT polymerisation k_{tr} depends on the rate constant for addition of propagating radical to RAFT agent, k_{add} (Scheme 4.3) and the rates of fragmentation of the intermediate radical species, $k_{\text{-add}}$ and k_β, as shown in equation 4.1.

$$k_{tr} = k_{add} \frac{k_\beta}{k_{\text{-add}} + k_\beta} \qquad (4.1)$$

In order to obtain the highest degree of livingness in RAFT polymerisation, it is crucial to minimise the average lifetime of active species and it is impossible to achieve when the rate of transfer is much lower than the rate of propagation, i.e., C_{tr} is too low. In the case of low C_{tr}, deactivation of radicals will not be fast, therefore allowing active species to remain in the active form for a long time and therefore participate in propagation and termination reactions.

A number of parameters define the rate constants for addition of radicals to a double bond; their values depend on steric hindrance, electronic and polar effects.[3] When designing optimum conditions for RAFT polymerisation, it is important to assess the transfer

constant of both original RAFT agent and a polymeric agent formed during the polymerisation reaction. Due to the fact that the transfer constant C_{tr} will depend on the groups R and Z and the monomer, it has been shown that in some instances C_{tr} of a RAFT agent changes dramatically upon the addition of the first monomer unit, changes further with chain lengths from dimer to tetramer and remains constant for higher oligomers. That might be due to the remoteness of the structural differences between chains of different length from the double bond, allowing the reactivity of the latter to remain unaltered.[4] It has been established that in a successful RAFT polymerisation its value should be higher than 2.[5]

In this work a number of RAFT agents were used in polymerisation of various monomers of interest. The main purpose of this section of research was to find a RAFT system that will allow for successful synthesis of a polymeric material of targeted molecular weight and narrow polydispersity. The prepared polymers were analysed by gel permeation chromatography and the values of the transfer constant C_{tr} for polymerisation systems under the investigation were established by means of modelling experimentally determined monomer conversion and molecular weight data.

4.2 Experimental details

4.2.1 Materials

Commercially obtained chemicals were used as received unless otherwise stated.

Details of syntheses involved are described in Chapter Two of this thesis.

Methyl methacrylate (Mitsubishi Rayon stabilised with 4-methoxyphenol inhibitor) was passed through a column of basic alumina, distilled under reduced pressure, stored at 4 °C and used within a week. Solvents were purified according to well established procedures.[6]

4.2.2 RAFT polymerisation procedures.

The following general polymerisation procedure was used in all experiments.

All polymerisation components were added to a two-neck round-bottomed flask with magnetic stir bar. The mixture was placed under argon, and subjected to three “freeze-

pump-thaw" cycles to assure minimal presence of oxygen. The flask was placed in an oil bath at constant temperature to start polymerisation.

4.2.2.1 Analysis methods

Samples were extracted from the polymerisation mixture using a degassed syringe (purged with argon) and needle. Samples were injected dropwise into a pre-weighed flask containing an appropriate solvent with vigorous stirring. The weight of samples was recorded. The precipitated polymer was filtered and placed in a dry, pre-weighed vial. The samples were dried under high vacuum overnight and re-weighed, allowing determination of the weight of formed polymer. Details are presented in Table 4.1. Isolated polymer was characterised by ^{1}H NMR and by GPC where available.

Theoretical molecular weights were calculated based on measured monomer conversion using the following formula:

$$M_{n,theory} = MW_{monomer}\, x \left(\frac{[M]_0}{[RAFT]_0} \right) \qquad (4.2)$$

where x is monomer fractional conversion, $MW_{monomer}$ is monomer molecular weight, $[M]_0$ and $[RAFT]_0$ are monomer and RAFT agent initial concentrations respectively.

4.2.2.1.1 METHYL METHACRYLATE (MMA)

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 0.79–1.42 (br m, 3H, CH_3 in backbone), 1.78–2.03 (br m, 2H, CH_2 in backbone), 3.55 (br s, 3H)

4.2.2.1.2 METHACRYLOYLOXY SUCCINIMIDE (MAOS)

^{1}H NMR (300 MHz, DMSO) δ_{ppm} 1.3 (br s, 5H, CH_3 and CH_2 in backbone), 2.82 (br s, 2H)

Table 4.1 Summary of the RAFT polymerisation conditions used in this study.

Monomer	Initiator	Solvent		Temperature (°C)	GPC solvent (location)
		for polymerisation	for precipitation		
MMA	AIBN	toluene	methanol	80 90	THF (KCPC, CSIRO)
MAOS	AIBN	DMF	acetone	80 90	DMF/LiBr (KCPC, CSIRO)
MAC	AIBN	toluene	pentane	90 92	THF (CSIRO)
NPMA	AIBN	DMF	diethyl ether	90	n/a
HPMA	AIBN V - 501	DMF aq. acetic buffer	acetone	90 100	DMF (Polymer Institute, Slovakia)
NIPMAM	AIBN	DMF	ethanol	90	THF (CSIRO)

4.2.2.1.3 METHACRYLOYL CHLORIDE (MAC)

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 0.80–1.28 (br m, 3H, CH_3 in backbone), 1.50–2.05 (br m, 2H, CH_2 in backbone)

For GPC analysis the PMAC samples were converted into PMMA using the following procedure.[7] PMAC (0.22 g, 2.1 mmol) and pyridine (0.8 mL) were dissolved in dry THF (35 mL) and the solution was placed in a two-neck round bottom flask charged with a magnetic stirrer, condenser and argon inlet. To the mixture a solution of dry methanol (0.12 mL, 3 mmol) in dry THF (5 mL) was added. The reaction mixture was vigorously stirred for 3 days at reflux. The polymer was isolated by precipitation of the reaction mixture into methanol (200 mL), the product was filtered and dried under high vacuum overnight to afford white solid, 0.17 g (80% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 0.79–1.42 (br m, 3H, CH_3 in backbone), 1.78–2.03 (br m, 2H, CH_2 in backbone), 3.55 (br s, 3H)

4.2.2.1.4 *P*-NITROPHENYL METHACRYLATE (NPMA)

^{1}H NMR (500 MHz, DMSO) δ_{ppm} 1.42 (br s, 3H, CH_3 in backbone), 2.50 (br s, 2H, CH_2 in backbone), 7.40 (br s, 2H), 8.22 (br s, 2H)

4.2.2.1.5 *N*-(2-HYDROXYPROPYL)METHACRYLAMIDE (HPMA)

^{1}H NMR (300 MHz, D_2O) δ_{ppm} 0.9 (br s, 3H, CH_3), 1.07 (br s, 3H, CH_3 in backbone), 1.75 (br s, 2H, CH_2 in backbone), 3.0 (br s, 2H), 3.75 (br s, 1H), 4.9 (br s, 1H, OH), 7.3 (br s, 1H, NH)[8]

4.2.2.1.6 *N*-ISOPROPYLMETHACRYLAMIDE (NIPMAM)

^{1}H NMR (500 MHz, DMSO) δ_{ppm} 0.91 (br s, 3H, CH_3 in backbone), 1.10 (br s, 6H), 1.70 (br s, 2H, CH_2 in backbone), 3.46 (br s, 1H), 6.91 (br s, 1H, NH)

4.3 Results and discussions

4.3.1 RAFT of Methyl methacrylate

RAFT polymerisation was a new method of polymerisation that has not been attempted at the University of Canterbury prior to this research and required preliminary investigation and mastering of the technique. For that reason the use of RAFT was pioneered at the University of Canterbury by investigating the kinetics of a model RAFT system of methyl methacrylate (MMA) with AIBN as an initiator and cumyl phenyldithioacetate (CPDA) (Figure 4.3) as a RAFT agent.

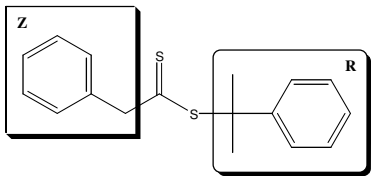


Figure 4.3 Cumyl phenyldithioacetate (CPDA), employed in RAFT polymerisation.

Cumyl phenyldithioacetate that was used in this work was kindly provided by Dr San H Thang of CSIRO, Melbourne. The polymerisations were done in THF solution at varying weight content of the monomer.

Successful preliminary experiments showed linear increase in monomer conversion with time. The dependence of the rate of polymerisation on temperature and concentration of monomer is shown in Figure 4.4 and Figure 4.5, and accompanying Table 4.2.

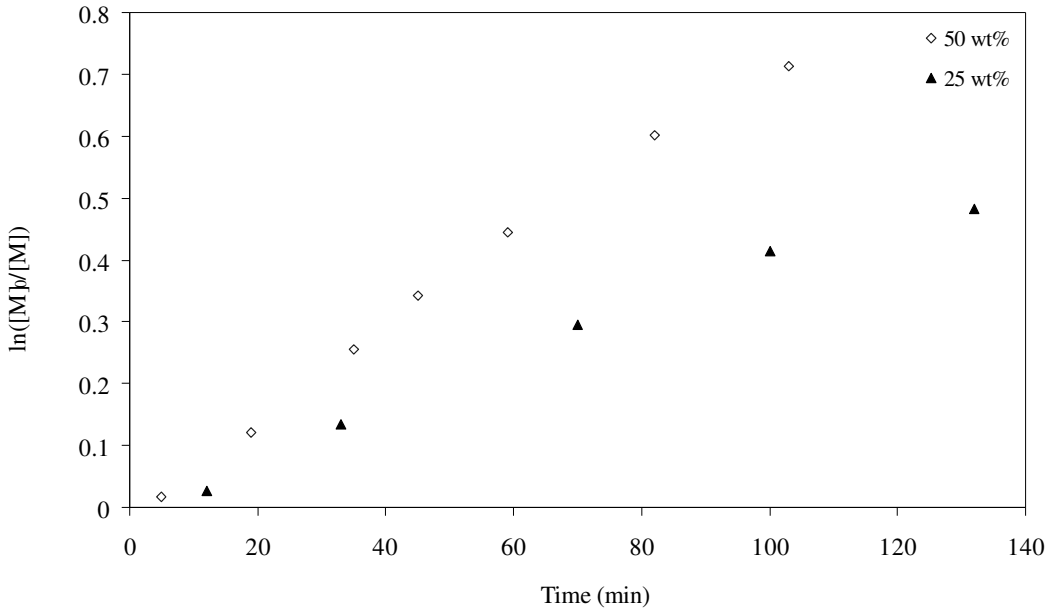


Figure 4.4 Pseudo first-order rate plots for polymerisation of MMA in THF at 90 °C in presence of cumyl phenyldithioacetate; $[MMA]_0$:$[AIBN]_0$:$[CPDA]_0$ = 1400:1:2.

Cumyl phenyldithioacetate (CPDA) is known to provide poor control in RAFT polymerisation of MMA. The system shows uncontrolled behaviour at low temperatures and hybrid behaviour at high temperature.[9] Use of cyanopentanoic dithiobenzoate (CPAD) however has been proved successful in a number of systems including the RAFT polymerisation of dimethylaminoethyl methacrylate (DMAEMA)[1], sodium 2-acrylamido-2-methylpropanesulfonate (AMPS) and sodium 3-acrylamido-3-methylbutanoate (AMBA).[10]

Table 4.2 Conversion data for polymers formed by polymerisation of MMA in the presence of cumyl phenyldithioacetate.[A]

Temperature, °C	MMA, wt.-%	Time, min	Conversion, %
90	25	132	38.3
	50	59	39.9
80	25	245	29.9
	50	70	18.7

[A] $[MMA]_0$:$[AIBN]_0$:$[CPDA]_0$ = 1400:1:2.

As the results of this work show, the use of CPDA in the RAFT polymerisation of MMA produces the system that shows the linear increase in the molecular weights of the samples with monomer conversion shown in Figure 4.6. The increase is a clear indication of a *living* character of the polymerisation system.

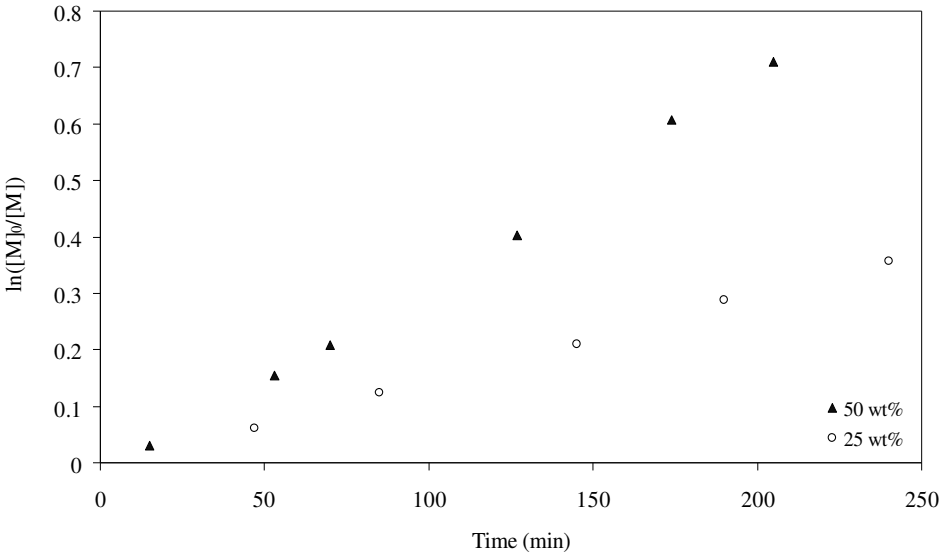


Figure 4.5 Pseudo first-order rate plots for polymerisation of MMA in THF at 80 °C in presence of cumyl phenyldithioacetate; $[MMA]_0$:$[AIBN]_0$:$[CPDA]_0$ = 1400:1:2

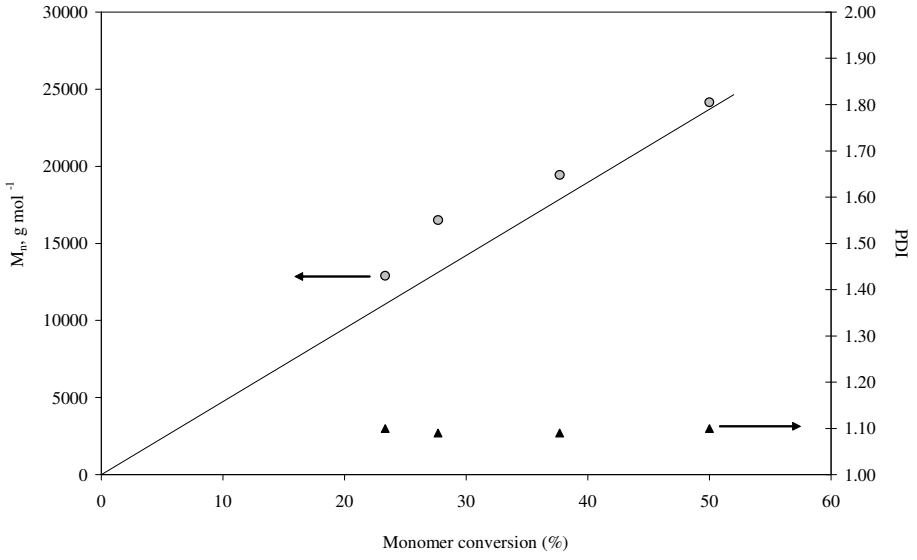


Figure 4.6 Molecular weight and polydispersity evolution with monomer conversion during RAFT polymerisation of MMA in bulk; $[MMA]_0$=9.36 M, $[MMA]_0$:$[AIBN]_0$:$[CPAD]_0$ = 1000:1:2 at 90 °C. Line is theoretical M_n calculated based on measured monomer conversion using equation 4.2.

Later in the research, the use of a different RAFT agent in polymerisation of MMA was attempted. Addition of 4-cyanopentanoic acid dithiobenzoate (CPAD) (Figure 4.11) to MMA and AIBN reaction mixture resulted in a fast rate of polymerisation and isolation of pink-coloured polymer of MMA. The samples were analysed via GPC.

4.3.2 RAFT of Methacryloyloxy succinimide

When this project started in July 2001, the use of the RAFT technique in polymerisation of MAOS had not been reported in literature. Thus the work has been concentrated on applying the method of RAFT in polymerisation of MAOS. MAOS is a solid monomer which rules out the possibility of bulk polymerisation. However, the polymer of MAOS is insoluble in most commonly used solvents, therefore restricting the choice to DMSO and DMF only. All polymerisations were conducted in DMF.

All attempts to polymerise MAOS using the RAFT method resulted in formation of polymer. With the solution containing 50 wt.-% MAOS in DMF at 90 °C it was impossible to monitor the kinetics of the polymerisation due to the surprisingly fast product formation: the monomer fully polymerised within 30 minutes. This is undesirable because the variation of molecular weight with conversion if needed in order to establish if living polymerisation is occurring. Also one would likely carry out proper kinetic analysis of the reaction. For these reasons the reaction conditions were altered to slow the rate so that proper monitoring of the kinetics was possible.

All further experiments were done with < 20 wt.-% MAOS in DMF at 80 °C. Lowering the amount of AIBN and increasing the amount of RAFT agent also slowed down the rate quite noticeably, allowing measurements of the monomer conversion and calculation of the expected molecular weight values. Some of the results obtained are presented in Table 4.3.

Once the optimal reaction conditions were established, a series of experiments were conducted in order to obtain a series of PMAOS samples for a GPC analysis. The analysis was to indicate whether RAFT of MAOS was of a *living* character. For comparative studies the control polymerisation was done, to which no RAFT agent was added while initial concentration of initiating and monomer species were kept unchanged. The results of experiments are presented in Table 4.4.

Table 4.3 Conversion data for the RAFT polymerisation of MAOS in DMF.

MAOS, wt.-%	Time, min	Conversion, %
50[A]	<30	N/A
33[B]	55	77
17[C]	120	50

[A]At 90 °C with $[MAOS]_0$=5.78 M, $[MAOS]_0$:$[AIBN]_0$:$[CPDA]_0$=780:1:5.
[B]At 90 °C with $[MAOS]_0$=2.80 M, $[MAOS]_0$:$[AIBN]_0$:$[CPDA]_0$=780:1:5.
[C]At 80 °C with $[MAOS]_0$=1.09 M, $[MAOS]_0$:$[AIBN]_0$:$[CPDA]_0$=2000:1:10.

Figure 4.7 shows results of an experiment in which the degree of polymerisation of 113 (at complete conversion of the monomer) was targeted. The determined molecular weights are in disagreement with calculated values and produced polymers are of a consistently high polydispersity, an indication of complete lack of control over the chain length and polydispersity of produced polymer.

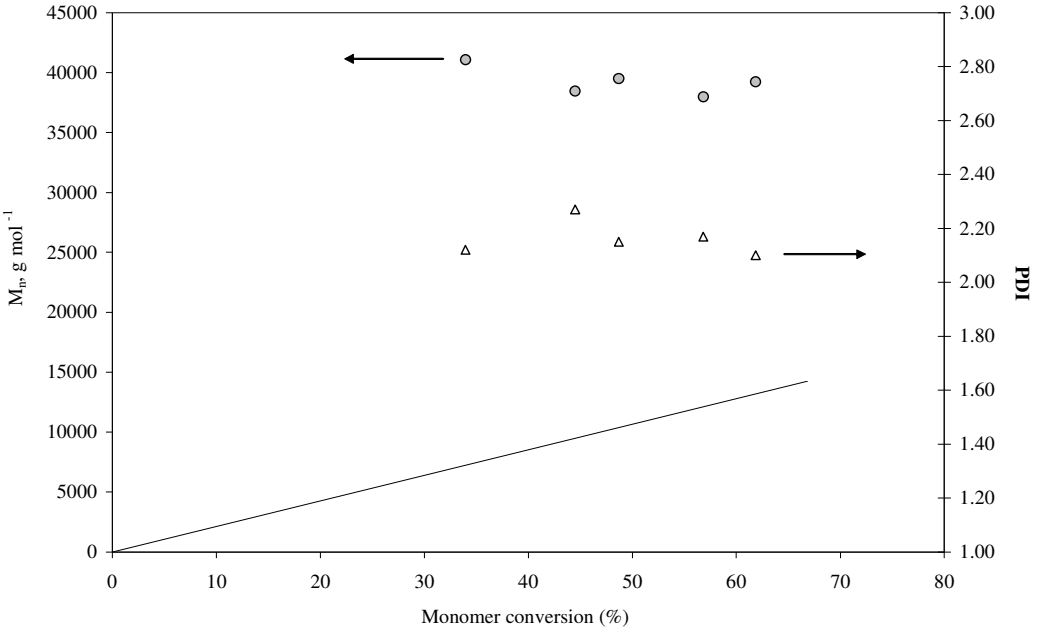


Figure 4.7 Molecular weight and monomer conversion data for the polymerisation of MAOS (1.09 M) in DMF at 80 °C in the presence of cumyl phenyldithioacetate (5.53×10^{-3} M) (experiment **C** in Table 4.3). The line represents predicted molecular weight values calculated based on experimentally determined monomer conversion values using equation 4.2, dark circles represent molecular weights measured by the GPC.

A control experiment, containing no RAFT agent, resulted in synthesis of polymer product of a molecular weight value close to that obtained in the RAFT polymerisation. Similar

results were obtained from another experiment, where targeted molecular weights were much lower than experimentally determined values.

The obtained data are summarised in Table 4.4. The GPC results yet again showed no sign of linear increase in molecular weight of PMAOS with conversion and fairly high *PDI* values. The results were very similar to the result of a conventional free-radical polymerisation used as a control experiment. Observed lack of control clearly illustrates unsuccessful choice of a RAFT agent for polymerisation of MAOS.

Table 4.4 Molecular weight and conversion data for polymerisation of MAOS in the presence of cumyl phenyldithioacetate.[A]

Time, min	$[RAFT]_0$, M	$[AIBN]_0$, M	$[MAOS]_0$, M	Conv., %[B]	$M_{n,\,GPC}$[C]	*PDI*	$M_{n,\,theory}$[D]
60	0	7.59×10^{-4}	1.1047	74	39226	2.10	N/A
95				34	40012	2.20	7055
132				45	41065	2.12	9246
150	9.73×10^{-3}	7.59×10^{-4}	1.1047	49	38439	2.27	10117
180				57	39498	2.15	11807
195				62	37988	2.17	12851
60	0	5.17×10^{-4}	1.0316	63	31265	2.32	N/A
88				39	38833	2.23	14207
119				50	35902	2.5	18099
159				55	33688	2.37	19786
174	5.22×10^{-3}	5.17×10^{-4}	1.0316	57	33051	2.47	20549
204				61	33405	2.27	21951
219				63	31180	2.4	22666
249				65	30827	2.31	23322

[A]At 80 °C with 17 wt.-% MAOS in DMF.

[B]Conversion was measured by precipitating samples into acetone and drying the polymer under vacuum overnight.

[C]Molecular weight and *PDI* values were established by GPC; the analysis was performed at the KCPC, University of Sydney, Australia.

[D]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2

As the popularity of the method has grown, more novel RAFT agents have been synthesised and used for polymerisation of various monomers. In an attempt to find a suitable agent for the synthesis of the polymer of MAOS, further experiments with

different RAFT agents were conducted. Structures of the RAFT agents used are shown in Figure 4.8.

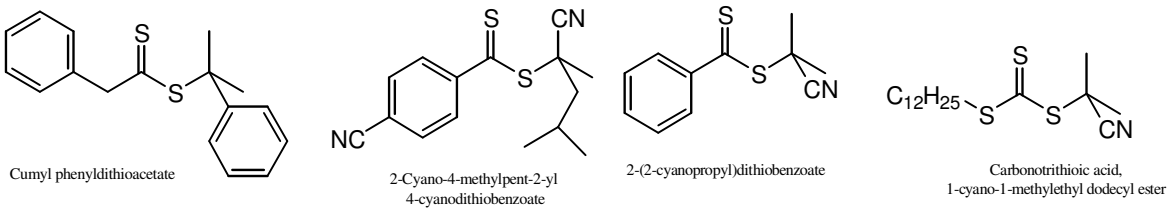


Figure 4.8 Structures of RAFT agents used in polymerisation of MAOS (see Table 4.5).

While cumyl phenyldithioacetate is known to provide good control in polymerisation of styrene[9] and *n*-vinyl pyrrolidone[11], its use in polymerisation of MMA has produced very little control at low temperatures and a hybrid behaviour at higher temperatures[9]. An attempt to employ the agent in polymerisation of MAOS resulted in formation of chains of high molecular weight and polydispersity values, therefore raising a question of its suitability for polymerisation of methacrylates.

The structures of other RAFT agents used are shown in Figure 4.8 and the summary of the results is presented in Table 4.5.

Table 4.5 Experimental data for polymerisation of MAOS.[A]

RAFT agent	Conv., %[B]	$M_{n,\,GPC}$[C]	*PDI*	$M_{n,\,theory}$[D]
cumyl phenyldithioacetate	67	30320	2.288	2465
2-(2-cyanopropyl)dithiobenzoate	77	21440	1.386	2816
2-cyano-4-methylpent-2-yl 4-cyanodithiobenzoate	51	20770	1.323	1865
1-cyano-1-methylethyl dodecyl ester of carbonotrithioic acid	82	44430	1.828	3000

[A]At 60 °C in DMF with $[MAOS]_0$=5.14 M; $[MAOS]_0$:$[AIBN]_0$:$[RAFT]_0$ = 100:1:5.

[B]Conversion was measured by precipitating samples into acetone and drying the polymer under vacuum overnight.

[C]Molecular weight and *PDI* values were established by GPC; the analysis was performed at the CSIRO Ian Wark Laboratories, Clayton, Australia.

[D]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2

2-(2-Cyanopropyl)dithiobenzoate (CPDB) has been used by a number of research groups, owing its popularity to its ability to provide consistently good control over the molecular weights for most monomer systems. It was employed in polymerisation of monomers such as vinyl benzoate,[12] styrene,[13] MMA[14], methacrylic acid[15,16] and 2-vinyl-4,4-dimethyl-5-oxazolone.[17] However, its use in preparation of the polymer of MAOS produced results that indicated no linear relationship between molecular weight and conversion for any of the experiments. Similar results were obtained in polymerisations where 2-cyano-4-methylpent-2-yl 4-cyanodithiobenzoate and 1-cyano-1-methylethyl dodecyl ester of carbonotrithioic acid were used.

Part of the discrepancy between measured and calculated molecular weights was at first attributed to differences in hydrodynamic volumes of polymer samples and polymer standards used in calibration of the GPC equipment. In the GPC analysis and the calibration where PMMA standards of narrow molecular weight were used, DMF/0.1 wt.-% LiBr eluent system was employed. The addition of the Li salt was necessary as it eliminated an additional high molecular weight peak which was observed due to repulsion of PMAOS chains in the solution. Addition of LiBr lessens the extent of such repulsions between chains; therefore eliminating the additional peak of higher molecular weight.[18-20] Due to the consistency of the observed discrepancy between measured and calculated molecular weights of PMAOS samples, the assumption was made that use of highly polar DMF/0.1 wt.-% LiBr eluent solution could affect the calibration results since PMMA standards might behave differently in solution of such polarity. However, further work and literature results indicated that the difference in molecular weights was genuine and was not due to solvent-solute interactions between the eluent mixture and the polymer standards employed.

In parallel with this work, another research group used the RAFT method in polymerisation of MAOS. The chain transfer agents used in their work are presented in Figure 4.9 and include cumyl phenyldithioacetate that was used in this work.[17] Schilli *et al.* report production of polymer chains of molecular weights consistently higher than the values that were calculated based on measured monomer conversion, the result obtained agreeing with results of this work. Polydispersity values reported by Schilli were also high and again indicative of lack of control in the polymerisation experiments.[21]

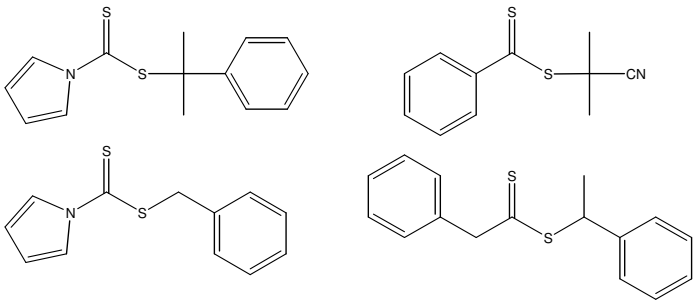


Figure 4.9 Some of the chain transfer agents used in RAFT polymerisation of MAOS by Schilli *et al.*[17]

Another group has reported use of cumyl dithiobenzoate (CDB) (Figure 4.10) in polymerisation of MAOS.[22] The attempt to employ this RAFT agent in polymerisation of MAOS yet again yielded unsatisfactory results: formation of chains with average degree of polymerisation of 75 while targeting the $\overline{DP}_n$ value of 10.[22]

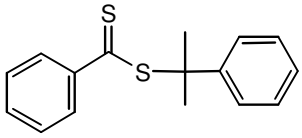


Figure 4.10 Cumyl dithiobenzoate (CDB) - RAFT agent used by Hwang *et al.*[22]

Use of CDB by a number of research groups has proved to yield adequate results in polymerisation of styrene[23], MMA[24] and MA.[1] Reasonable control in polymerisation of *N,N*-dimethylacrylamide was also achieved.[25] However, its use in polymerisation of *N*-vinyl pyrrolidone did not produce satisfactory results.[26]

From the results of the work done in this research, along with results reported by other research groups, it has become clear that to date polymerisation of MAOS under RAFT polymerisation conditions has failed to provide any control over molecular weight and molecular weight distribution of produced polymer. Despite reports of production of PMAOS samples with low polydispersity values which is often a sign of a *living* character of a polymerisation system, in all reported cases calculated number average molecular weight values are consistently lower than experimentally determined molecular weights.

Attempts to polymerise MAOS in controlled manner proved to be more challenging than anticipated, especially in view of the fact that its acrylate derivative acryloyloxy

succinimide has been successfully polymerised by RAFT.[27,28] An investigation was required as to establish possible causes of such lack of control.

Firstly, it was important to establish whether the RAFT agents participated in the polymerisation process and whether the lack of their participation in the process was due to a possible disruption of their structural integrity under the reaction conditions. The following analysis was done in order to establish that.

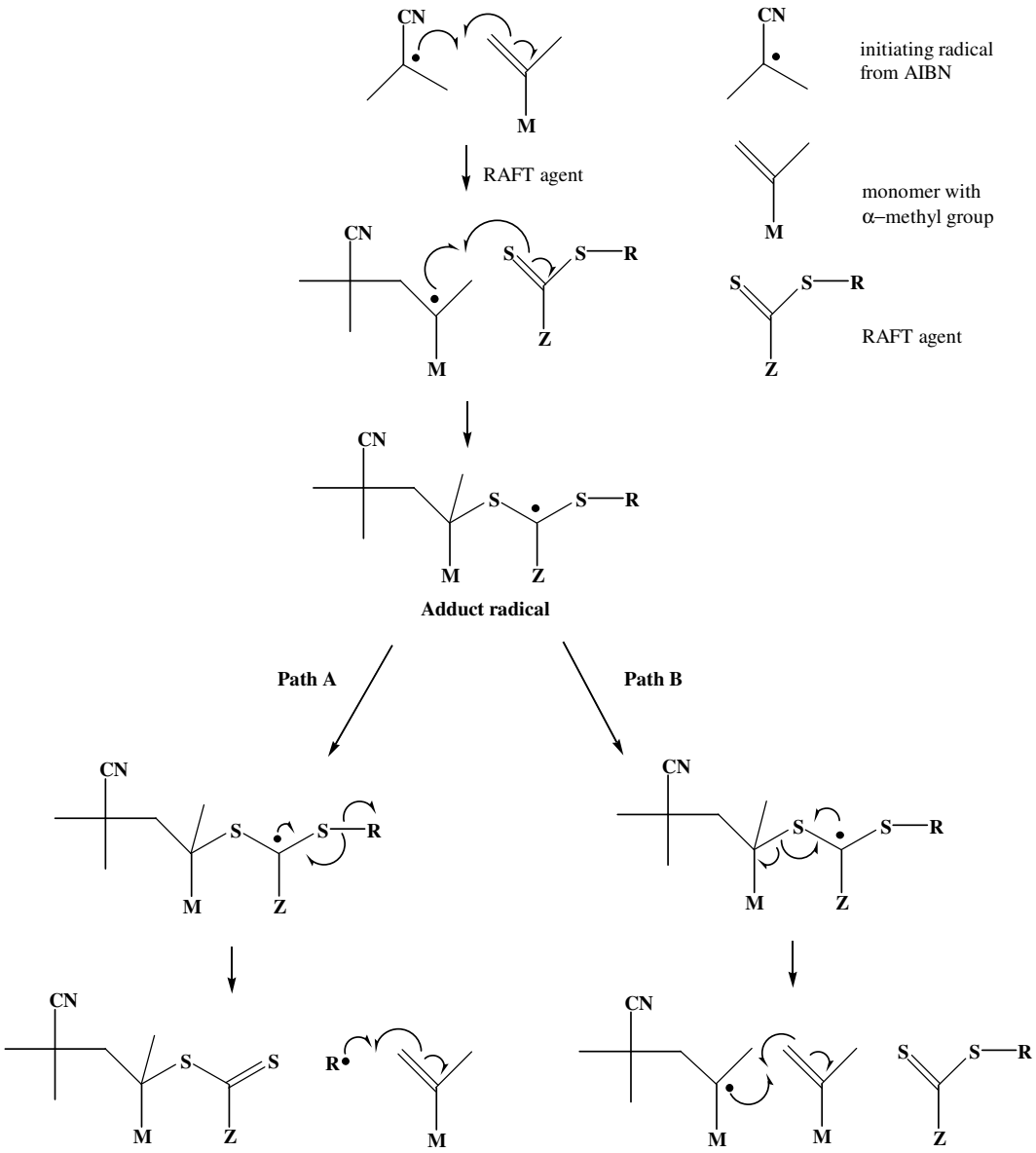
Normally, depending on the colour of the RAFT agent used, successful RAFT polymerisation results in formation of coloured polymers, indicating incorporation of the chain transfer agent into the polymer chain. A polymer of MAOS was isolated by precipitating the reaction mixture into acetone. On all occasions the recovered polymer was colourless while the mother liquor was coloured indicating the presence of RAFT agent in it. ^{1}H NMR analysis of the polymer indicated no presence of RAFT end-group which would be expected in the case of RAFT agent participating in the polymerisation process. The mother liquor was analysed by ^{1}H NMR, the analysis revealed presence of a high content of unreacted and intact RAFT agent, whereas in a successful RAFT polymerisation almost all RAFT agent will become a polymer end-group. These results lead to a conclusion that chosen RAFT agents did not take part in the process and therefore were highly ineffective in controlling polymerisation of MAOS. Further evidence for this is that obtained molecular weights are much the same whether or not RAFT agent is present (see Table 4.4).

Further investigations were necessary so as to establish what might be the cause for such profound inactivity of the agents used. The role of the free-radical leaving group **R** of a RAFT agent in controlling the polymerisation process has been discussed in a number of publications, with a summary outlined by Chong *et al.*[29] The authors have established that in polymerisation of methyl methacrylate the effect of the R group is much greater than it is in polymerisation of styrene and butyl acrylate. It was found that a number of RAFT agents successfully used for controlling polymerisation of styrene and butyl acrylate were very ineffective in controlling polymerisation of MMA due to very low values of C_{tr} (transfer constants) in RAFT/MMA systems.

If styrene and MMA are to be compared, it is clear that while there are a few differences between the monomers, the main difference is in stability of radicals they produce. While

styrene and butyl acrylate produce secondary radicals, the propagating radical of MMA is a tertiary radical, with stability much higher than that of a secondary radical species. Having identified this property as one of the main characteristics of the monomer of interest, i.e. MAOS, it was concluded that a tertiary radical of the activated ester will also be very stable under RAFT polymerisation conditions.

Scheme 4.4 shows two alternative pathways of fragmentation that an intermediate (or an adduct) radical can undergo.



Scheme 4.4 Two possible fragmentation routes in RAFT polymerisation of MAOS.

This is where properties of fragmented radicals become crucial as they determine the pathway and the fate of the reaction. The scheme shows an addition of the initiating

radical to a monomer, which results in a formation of a new radical. If a RAFT agent is present in the mixture, depending on the value of the rate constant governing the addition process, the propagating radical may react with the S=C double bond of the agent, forming an adduct radical. At this point two pathways exist.

Route **A** shows fragmentation of the leaving group **R**, which results in formation of radical **R**, that starts propagating by adding to the monomer and forming a new radical **R-M**. Meanwhile, the former monomeric radical is bound to the RAFT moiety and being "capped" and inactive it cannot undergo any further reactions. With high rate of addition, it will not be long before the propagating radical **R-M** will react with the S=C double bond of the RAFT agent, again resulting in formation of another adduct radical. At this time either radical might be fragmented, allowing for propagation reaction to continue. Due to the high addition and fragmentation rate constants radicals will be spending very short time as active species which will restrict either from participating in radical-radical termination events. In other words, this pathway is indicating what is happening in a *living* polymerisation system, where radicals spend most of their time "capped" by RAFT agent moiety and therefore polymer chains with incorporated RAFT agent functionality are obtained at the end of the process.

However, the analysis indicated that the above was not the case in polymerisation of MAOS under the RAFT conditions. It is possible that the attacking monomeric radicals did not follow the route A of the scheme. Route B indicates another fragmentation process where the **R** group of the RAFT agent was poorly chosen and is not a good leaving group. If a monomeric/polymeric radical is a better leaving group, then it is most likely to preferentially fragment off resulting in regeneration of an original RAFT agent and the original monomeric propagating radical. This polymeric radical will now undergo further propagation by adding to a monomer, while the RAFT agent will remain effectively inactive and will not participate in the polymerisation process.

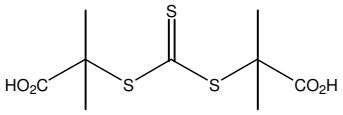
Scheme 4.3 clearly indicates that in an effective RAFT polymerisation in the first chain transfer step the adduct radical should fragment in favour of products. The requirement could also be expressed in terms of the corresponding rate parameters: $k_{\beta} \geq k_{\text{-add}}$. The rate of the fragmentation of the leaving group will depend on the presence of electron-withdrawing groups and radical stabilising groups on **R**. There is a distinct possibility that RAFT polymerisation of MAOS follows the pathway **B**. This will be the case if the

attacking radical is a better leaving group than the R group of the RAFT agent. Then the stable tertiary radical of MAOS either will preferentially undergo fragmentation reaction soon after its addition to the S=C double bond of a RAFT agent, or simply never undergo the addition reaction due to its stability. The uncapped radicals generated in such way will propagate and terminate in an uncontrolled manner.

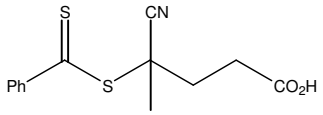
This hypothesis was supported by other researchers, suggesting a RAFT agent with an equally effective leaving group should be employed in the polymerisation of MAOS.[17,30] Similarly poor results obtained in RAFT polymerisations of MMA[29] and α-methylstyrene[31] suggest that none of the employed agents had a leaving group **R** of suitable stability for its successful fragmentation to take place. Attempts to use a monomer analogous radical as the leaving group **R** on a RAFT agent proved to be unsuccessful possibly due to penultimate unit effect.[4,29,32] This suggests that a low k_{add} is part of the problem. It is understandable that regardless of the stability of the monomeric radical, in most cases it will be less stable than the propagating polymeric radical with expected difference being due to steric effects.[30]

An extensive literature research was done in order to identify RAFT agents most suitable for polymerisation of the monomer of interest. According to the literature a number of RAFT agents were used in polymerisation of methacrylates, producing polymerisation systems with characteristics indicating *living* character. Selected dithiobenzoates (4-cyanopentanoic acid dithiobenzoate[1,10], dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester[1,4] and 2-(2-cyanopropyl)dithiobenzoate)[1,16,21,24,33,34] were synthesised and their use was attempted in further polymerisation reactions of MAOS. Structures of those agents are presented in Figure 4.11.

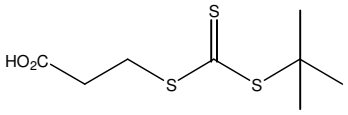
The series of dithiobenzoate derivatives that has been used in RAFT polymerisation of MAOS allowed the examination of the effect of the R group on polymerisation kinetics. All polymerisations resulted in formation of polymer, however yet again the polymer was white and mother liquor was coloured which indicated presence of RAFT agent in the latter, rather than its incorporation into polymer chains. Monomer conversion results and calculated molecular weights are summarised in Table 4.6.



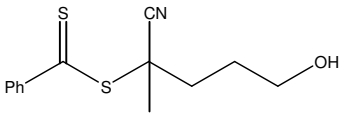
2,2'-[(thioxomethylene)di(sulfanyl)]bis(2-methylpropanoic acid)



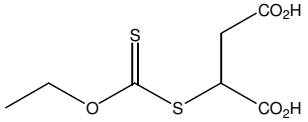
4-Cyanopentanoic acid dithiobenzoate



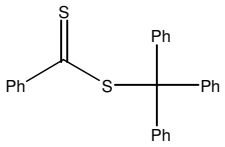
3-{[(tert-butylsulfanyl)carbonothioyl] sulfanyl}propanoic acid



Dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester



2-Ethoxythiocarbonylsulfanyl-succinic acid



Triphenylmethyl dithiobenzoate

Figure 4.11 Dithiobenzoate derivatives used in further RAFT polymerisation of MAOS.

Table 4.6 RAFT polymerisation of MAOS.[A]

RAFT agent	Time, min	Conv., %[B]	$M_{n,\ theory}$[C]
none	140	83	n/a
2-(2-cyanopropyl)dithiobenzoate	228	62	20221
2,2'-[(thioxomethylene)di(sulfanyl)]bis(2-methylpropanoic acid)	240	85	29000
2-ethoxythiocarbonylsulfanyl-succinic acid	240	83	27238
3-([(tert-butylsulfanyl)carbonothioyl] sulfanyl)propanoic acid	231	85	28238
4-cyanopentanoic acid dithiobenzoate	234	61	21279
dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester	234	25	8265
triphenylmethyl dithiobenzoate	263	6	1800

[A]At 80 °C in DMF with $[MAOS]_0$=1.36 M; $[MAOS]_0$:$[AIBN]_0$:$[RAFT]_0$ = 400:1:2.

[B]Conversion was measured by precipitating samples into acetone and drying the polymer under vacuum overnight.

[C]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2.

While GPC analysis was required to provide values of molecular weight of the samples, due to financial constrains it was impossible to perform such analysis. Therefore, there are no data available to confirm whether control of polymerisation of MAOS under the conditions of RAFT polymerisation was achieved. However, due to the presence of unreacted RAFT agents in mother liquor, it is clear that there could not have been complete participation of the agents in the polymerisation process, and thus that polymerisation was not controlled.

While formation of PMAOS was observed in all polymerisations, kinetic parameters varied producing different values of the rate of polymerisation. High values of the monomer conversion were obtained within 4 hours of polymerisation in most systems, however, where triphenylmethyl dithiobenzoate was used; polymerisation was very slow, achieving only 6% monomer conversion. This result has proved once again that the choice of the R group is essential for successful polymerisation; in addition to being a good leaving group, the group has to be a good initiating radical species in order for re-initiation and further propagation to take place.

The triphenylmethyl radical, which is the leaving group of the triphenylmethyl dithiobenzoate, is a poor re-initiating species and its use therefore results in retardation of the polymerisation.[30] The significant drop in observed rate of polymerisation where the agent was used confirms that.

The effectiveness of a RAFT agent is often determined quantitatively by evaluation of the transfer coefficient of the agent. This has been done for some RAFT agents used in polymerisation of MAOS. The calculations are discussed in a forthcoming section of this chapter, along with the results obtained.

4.3.3 RAFT of Methacryloyl chloride

A primary aim of this work was to prepare a family of functionalised polymers using the homopolymer of methacryloyloxy succinimide as a precursor. So far controlled polymerisation of the active ester has not yielded satisfactory results, having resulted in production of polymer chains of molecular weights higher than targeted values and with broad molecular weight distribution.

An alternative to the use of PMAOS is to find another monomer that can be polymerised in a controlled manner and can be converted into PHPMA for consequent drug attachment. If the polymer can also be converted into another well characterised polymer such as PMMA, the MWD characterisation using GPC will be more reliable as there will be no difference in hydrodynamic volumes of analysed samples and PMMA standards used in calibration of the equipment. With all that in mind, attention was switched to methacryloyl chloride (MAC). Methacryloyl chloride (MAC) is used in the synthesis of MAOS[35] and its conventional free-radical polymerisation has been reported in literature.[7,36] While MAC is also a precursor in HPMA synthesis[37], Yamaguchi *et al.* also report quantitative conversion of the polymer of the acid chloride into the polymer of MMA by reacting PMAC with excess of dry methanol in presence of pyridine, as shown in Figure 4.12.[7]

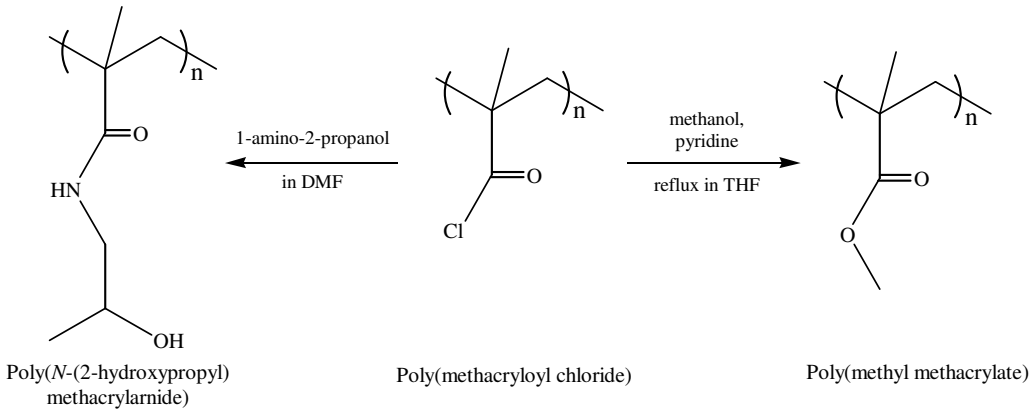


Figure 4.12 Preparation of PHPMA and PMMA from PMAC precursor.

Quite possibly the use PMAC will allow for preparation of families of functionalised polymers, after all acid chlorides are used extensively in organic synthesis. While synthesis of PHPMA is the ultimate goal, the ability to convert the polymer of MAC into a polymer of MMA will help to eliminate a possible discrepancy between calculated and experimentally determined molecular weight values. Normally, calibration of GPC equipment is done by using polymer standards with narrow molecular weight distribution. Often, these are commercially prepared and while there are a variety of polymer standards that could be used, not always the chosen ones are suitable for characterisation of a polymer. In this work PMMA standards were used. In addition to the possible difference in hydrodynamic volumes of PMAC samples and PMMA standards that might lead to wrong estimation of the molecular weights of PMAC, it is not clear how the highly

reactive acid chloride will behave on a GPC column. Presence of water in eluent might lead to hydrolysis of the PMAC samples, which will result in formation of hydrochloric acid, presence of which can disturb the integrity of the packing on the GPC column and compromise the results. Therefore, all samples of PMAC to be analysed by GPC were quantitatively converted into PMMA prior to the analysis (see section 4.2.2.1.3).

To date there have been no reports in the literature on use of RAFT in polymerisation of MAC. In this work the first attempt to employ RAFT polymerisation in preparation of the polymer of the acid chloride did result in formation of the polymer in 74% yield. Cumyl phenyldithioacetate (structure shown in Figure 4.3) was used as a chain transfer agent. Samples were withdrawn from the RAFT experiment to monitor the kinetics of the polymerisation. In addition to that, a control polymerisation of MAC was done with same concentration of reacting species but no RAFT agent. The samples of the polymer of MAC were converted into PMMA and were analysed by GPC at CSIRO, Clayton. The results of the polymerisations and the analysis of the polymer samples are presented in Table 4.7.

Table 4.7 Experimental data for polymerisation of MAC.

Method	Conversion, %[C]	$M_{n,\,theory}$[D]	$M_{n,\,GPC}$[E]	*PDI*
RAFT[A]	36.0	6331	9377	1.56
	59.1	10410	11068	1.55
	74.0	13032	15272	1.73
CFRP[B]	40.0	n/a	14283	2.89
	47.8	n/a	15335	4.10

[A] At 90 °C in toluene with $[MAC]_0$=4.60 M; $[MAC]_0$:$[AIBN]_0$:$[CPDA]_0$ = 350:1:2.

[B] At 90 °C in toluene with $[MAC]_0$=4.60 M; $[MAC]_0$:$[AIBN]_0$ = 350:1.

[C] Conversion was measured by precipitating samples into ether and drying the polymer under vacuum overnight.

[D] Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2.

[E] Molecular weight and *PDI* values were established by GPC; the analysis was performed at the CSIRO Ian Wark Laboratories, Clayton, Australia.

Clearly there is a living character, even if it is not ideal. The molecular weight values are reasonably close to theoretical prediction, while polydispersity values are clearly lower when compared with control experiments. Thus results look promising and clearly there is a possibility that the use of an appropriately chosen RAFT agent will result in a well

controlled polymerisation of MAC. A radical of methacryloyl chloride is a tertiary species; therefore a RAFT agent with a good leaving group is required for polymerisation to be controlled.

RAFT agents used in polymerisation of MAOS (Figure 4.11) have been used again here. All polymerisation reactions resulted in formation of PMAC, however, a change in the initial colour of reaction mixture (from pink to orange) was observed during the course of the reactions. To avoid possible hydrolysis of reactive monomeric and polymeric species of MAC during polymerisation, reactions were run in thoroughly dried solvents. Dried polymer samples were stored under argon. Monomer conversion was measured by gravimetry and for PMAC samples were converted into PMMA for GPC analysis using the literature procedure.[7]

The colour of the reaction mixture is due to the use of coloured RAFT agents and therefore the change in the colour could signal that the structural integrity of the agents has been disrupted under the reaction conditions. To determine if any chemistry is occurring between the acid chloride and RAFT agents, various mixtures of the monomer and RAFT agents were stirred at 92°C for 4 hours and subjected to proton NMR analysis prior and after the reaction. The change in the colour was observed, however the change was not as profound as observed during the polymerisation.

Despite the change in colour, the NMR analysis did not show any changes in either reactant. No polymerisation of MAC has also occurred. This does not explain the change in the colour of the reaction mixture however it could serve as a sign that RAFT agents are becoming successfully incorporated into polymer chains as polymer end group, which might cause the change in colour observed.

A summary of results is presented in Table 4.8. The results show reasonable monomer conversion values obtained in the polymerisations; however, the use of triphenylmethyl dithiobenzoate yet again produces RAFT polymerisation system with low rate of polymerisation. This could be due to the poor properties of the triphenylmethyl radicals as an initiator of polymerisation as discussed in the earlier section of this chapter.

Table 4.8 RAFT polymerisation of MAC.[A]

RAFT agent	Time, min	Conv., %[B]	$M_{n,\ theory}$[C]
none	190	28	n/a
2-(2-cyanopropyl)dithiobenzoate	312	24	4422
2,2'-[(thioxomethylene)di(sulfanyl)]bis(2-methylpropanoic acid)	332	58	13000
2-ethoxythiocarbonylsulfanyl-succinic acid	305	51	12239
3-([(tert-butylsulfanyl)carbonothioyl] sulfanyl)propanoic acid	305	53	12838
4-cyanopentanoic acid dithiobenzoate	320	43	8280
dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester	417	25	7265
triphenylmethyl dithiobenzoate	325	17	3500

[A] At 92 °C in toluene with $[MAC]_0$=6.35 M; $[MAC]_0$:$[AIBN]_0$:[RAFT agent]$_0$ = 400:1:2.
[B]Conversion was measured by precipitating samples into pentane and drying the polymer under vacuum overnight.
[C]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2.

Some PMAC samples were converted into PMMA by means of reaction of the polymer of the acid chloride with dry methanol. Obtained PMMA samples were analysed by GPC and obtained results are summarised in Table 4.9 and Figure 4.13.

GPC results did not produce clear proof of a *living* character of the RAFT/MAC system; neither did they rule out the possibility of some degree of control occurring. They showed that experimentally determined molecular weight values did not agree with calculated values, but polydispersity indexes were lower than that for a control system where no RAFT agent was added to the reaction mixture while concentration of other reactants was kept the same. *PDI* values also showed decrease in their values with monomer conversion.

Overall, results of RAFT polymerisation of MAC look encouraging. It is our belief that the possibility of synthesis of a polymer of acid chloride under controlled conditions will provide immense flexibility in design of libraries of functionalised polymers. While the reactivity of a polymer does not normally match the reactivity of its monomer species due to introduced steric hindrance, the polymer of acid chloride still retains a great degree of

reactivity and therefore allows for introduction of different functional groups into the polymer backbone or a complete conversion of the latter into another homopolymer of interest.

Table 4.9 Experimental data for MAC polymerisation under RAFT conditions.[A]

RAFT agent	Time,min	Conv., %[B]	$M_{n,\,GPC}$[C]	*PDI*	$M_{n,\,theory}$[D]
None	65	74	39226	2.10	N/A
2-(2-Cyanopropyl dithiobenzoate) (CPDB), Figure 4.13	135	33	11700	1.69	6570
	270	40	11717	1.80	8083
	320	44	11750	1.46	8801
	375	50	12778	1.50	9980
	400	52	10697	1.49	10385
4-Cyanopentanoic acid dithiobenzoate (CPAD), Figure 4.14	120	24	9867	2.16	4806
	223	34	8430	1.45	6673
	313	38	10815	1.52	7544
	355	40	10255	1.31	7917
	383	41	8942	1.42	8139
Dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester (DTBA), Figure 4.15	120	30	10704	1.40	5900
	223	45	11651	1.55	8849
	313	61	14894	1.54	11945
	355	65	13723	1.50	12770
	383	69	13304	1.55	13593

[A]At 92 °C in toluene. $[MAC]_0$ = 5.75 M; $[MAC]_0$:$[AIBN]_0$:$[RAFT\ agent]_0$ = 400:1:2.

[B]Conversion was measured by precipitating samples into ether and drying the polymer under vacuum overnight. Samples were converted into PMMA for GPC analysis.

[C]Molecular weight and *PDI* values were established by GPC; the analysis was performed at the CSIRO Ian Wark Laboratories, Clayton, Australia.

[D]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2.

Whether the use of such unconventional monomer is fully justified will depend on the success in both controlling the polymerisation process and the possibility of using the polymer as a precursor for polymers of desired functionalities. While the ability to control molecular weight of PMAC under conditions of RAFT is still under question, it is important to recognise however that the failure to obtain a polymer of desired molecular

weight under conditions used is only an indication that more careful research of possible use of other RAFT agents is required.

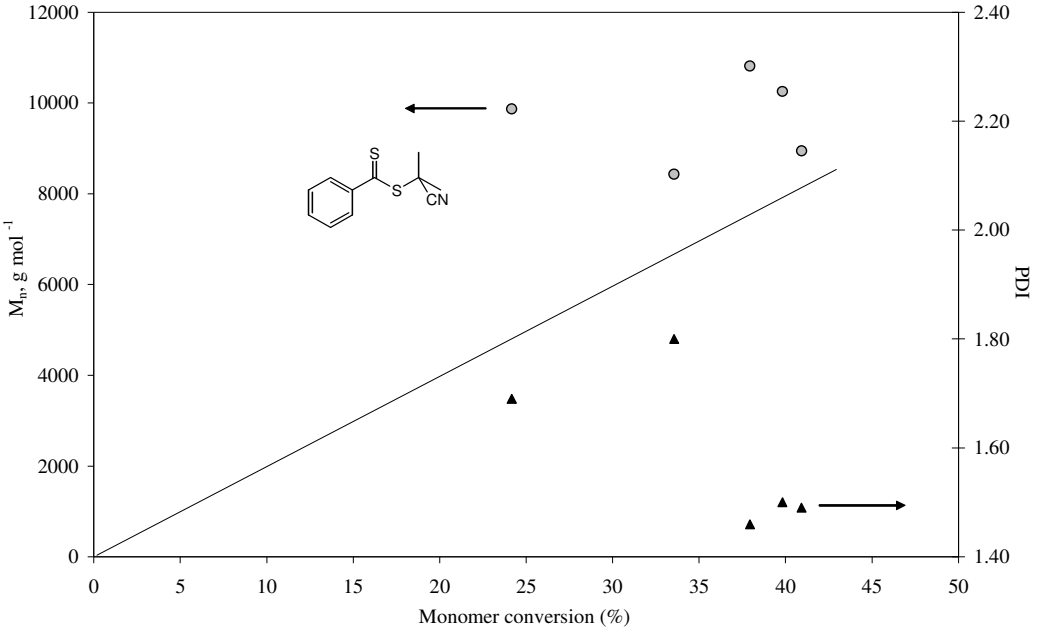


Figure 4.13 Evolution of molecular weight, M_n, and polydispersity index, *PDI*, with monomer conversion in RAFT polymerisation of MAC at 92 °C in toluene with $[MAC]_0 = 5.75$ M; $[MAC]_0:[AIBN]_0:[CPDB]_0 = 400:1:2$.

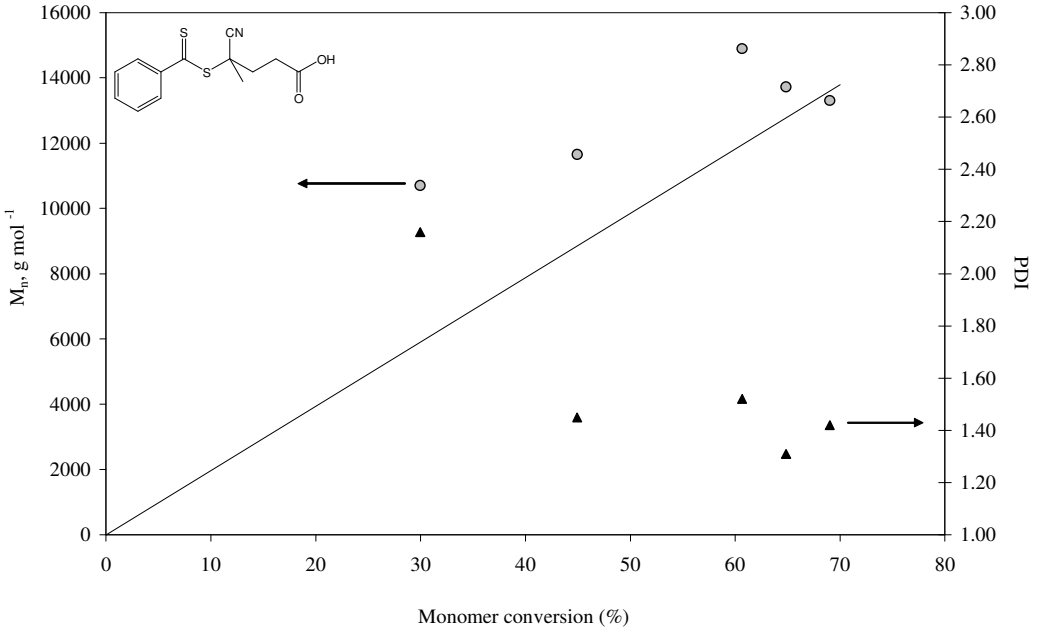


Figure 4.14 Evolution of molecular weight, M_n, and polydispersity index, *PDI*, with monomer conversion in RAFT polymerisation of MAC at 92 °C in toluene with $[MAC]_0 = 5.74$ M; $[MAC]_0:[AIBN]_0:[CPAD]_0 = 400:1:2$.

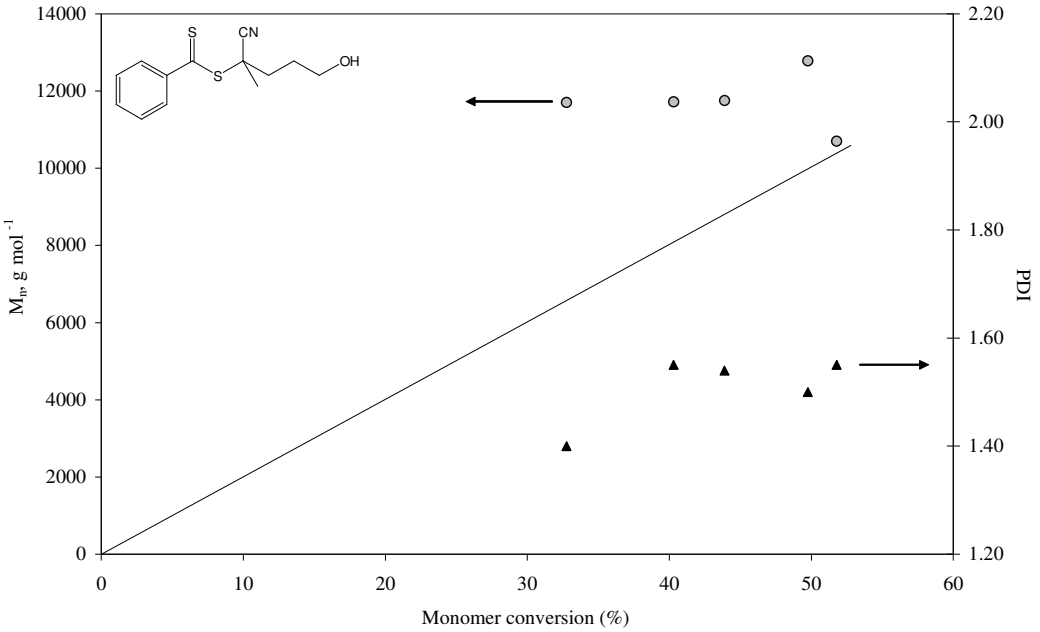


Figure 4.15 Evolution of molecular weight, M_n, and polydispersity index, *PDI*, with monomer conversion in RAFT polymerisation of MAC at 92 °C in toluene with $[MAC]_0 = 5.75$ M; $[MAC]_0:[AIBN]_0:[DTBA]_0 = 400:1:2$.

4.3.4 RAFT of *p*-Nitrophenyl methacrylate

While a direct synthesis of PHPMA from its monomer is highly desirable, it is important to recognise ways of obtaining this polymer of interest. As has been discussed in the previous sections of this chapter, there is an attempt to synthesise a polymeric precursor first, which will enable creation of a library of functionalised polymers from it.

There are two important characteristics that one has to take into account when selecting a monomer for synthesis of a polymer precursor; it is crucial that the polymer is reactive allowing its conversion into another polymer species. Another equally important property to consider is whether the monomer could be polymerised under *living* polymerisation conditions yielding polymer chains of targeted molecular weight with narrow molecular weight distribution.

Earlier in this work the possibility of using the activated ester methacryloyloxy succinimide (MAOS) and its precursor methacryloyl chloride (MAC) has been investigated. As the attempts to employ MAOS and MAC monomers in synthesis of the precursor had not produced desirable results, the use of another monomer became of interest. The monomer of *p*-nitrophenyl methacrylate (NPMA) (Figure 4.16) has not been

used extensively in polymer chemistry, with only a handful of publications relating to its polymerisation.

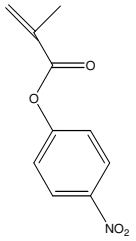


Figure 4.16 *p*-Nitrophenyl methacrylate (NPMA).

While NPMA has been polymerised by ATRP[38], no studies of its behaviour under conditions of RAFT polymerisation have been reported in the literature. Five different RAFT agents were used in polymerisation of NPMA. Monomer conversion values obtained in the experiments are summarised in Table 4.10. PNPMA samples were isolated as solid lightly coloured depending on the colour of a RAFT agent used. The mother liquor was not coloured, the fact suggesting that RAFT agent was incorporated in polymer chains.

Table 4.10 RAFT polymerisation of NPMA.[A]

RAFT agent	Time, min	Conv., %[B]	$M_{n,\,theory}$[C]
none	140	79	n/a
2-(2-cyanopropyl)dithiobenzoate	142	72	13316
2-ethoxythiocarbonylsulfanyl-succinic acid	104	79	17805
3-([(tert-butylsulfanyl)carbonothioyl] sulfanyl)propanoic acid	102	80	14746
4-cyanopentanoic acid dithiobenzoate	345	71	14723
dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester	340	63	11507

[A]At 90 °C in DMF, $[NPMA]_0$ = 1.48 M. $[NPMA]_0$:$[AIBN]_0$:$[RAFT]_0$ = 200:1:2.

[B]Conversion was measured by precipitating samples into diethyl ether and drying the polymer under vacuum overnight.

[C]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2.

The polymer of *p*-nitrophenyl methacrylate was found to be insoluble in THF which is a solvent most commonly used in GPC analysis. Due to time and financial constrains the only GPC equipment available at the time was the set-up where THF was used as GPC eluent. Therefore no attempts were made to evaluate molecular weights of the produced chains of THF-insoluble PNPMA.

^{1}H NMR analysis was used to estimate the average degree of polymerisation of the chains by comparing the areas under integrated peaks due to CH_2 groups of a backbone and peaks due to protons in the RAFT-end groups on polymer chains. However, in cases where RAFT agent contained an aromatic moiety, such analysis was impossible due to an overlap of signals arising from protons on the phenyl ring of the monomer units with the signals due to protons of the end-group.

PNPMA samples were however used in further work serving as a precursor from which a synthesis of polymer of HPMA was attempted. This will be discussed in chapter five of this thesis.

4.3.5 RAFT of *N*-(2-Hydroxypropyl)methacrylamide

The overall aim of this work is to be able to synthesise a polymer of *N*-(2-hydroxypropyl)methacrylamide (HPMA) of certain molecular weight. While synthesis of PHPMA from HPMA by means of conventional free-radical polymerisation has been utilised by a number of research groups, the main problem of uncontrolled molecular weights remained unsolved. Preparation of PHPMA co-polymer of required molecular weight is often achieved by preparation of the copolymer via conventional methods of polymerisation, followed by fractionation of the product and isolation of a fraction containing chains with required $\overline{DP}_n$.[39-41] There are two ways of employing a method of controlled polymerisation in preparation of a polymer of HPMA. The polymer could be synthesised directly from its monomer under the conditions of controlled polymerisation. Alternatively it can be prepared from its polymeric precursor that was prepared under the conditions of controlled polymerisation.

PHPMA is a water-soluble poly(methacrylamide). Such polymers have many potential medicinal applications such as a potential of being employed as drug vectors. For that reason there have been a number of reports on polymerisation of acrylamides by the

method of RAFT.[24,27,42,43] As the method of RAFT promises controlled synthesis of polymer chains of required length, it was applied in polymerisation of *N*-isopropylacrylamide (NIPAM). NIPAM is known to mimic the molecular structure of amino acids[44] and its polymer has been prepared via the RAFT polymerisation procedure.[17,42,43]

The reported use of cumyl dithiobenzoate and benzyl dithiobenzoate resulted in a production of relatively monodisperse polymer chains at low monomer conversions in particular. However, at higher monomer conversion the discrepancy observed between calculated and measured molecular weights became fairly significant.[43] The use of benzyl 1-pyrrolecarbodithioate and cumyl 1-pyrrolecarbodithioate as RAFT agents in polymerisation of NIPAM resulted in production of polymer chains with low polydispersity index. However, the index increased with the monomer conversion and measured molecular weights were slightly lower than calculated values.[17] Copolymerisation of NIPAM with MAOS under RAFT conditions with 2,2'-cyanoisopropyl dithiobenzoate as a chain transfer agent has produced copolymer chains with *PDI* of 1.11 and molecular weights in agreement with theoretically predicted values.[42] The method of RAFT was also employed in copolymerisation of *N,N*-dimethylacrylamide (DMA) and *N*-acryloyloxy succinimide (NAS).[27] The obtained copolymer chains were of low polydispersity (*PDI* < 1.1) and a homopolymer of DMA had a *PDI* of 1.17.

These examples have been described to allow the reader to appreciate the variety of results obtained. While some groups report promising and encouraging results, other results provide more questions requiring further investigations.

Despite the fast growing popularity of the method, at the stage at which this research began there were no publications related to use of RAFT in polymerisation of HPMA.

In this research in the first attempt to use a RAFT chain transfer agent in preparation of PHPMA, 2-ethoxythiocarbonylsulfanyl-succinic acid (Figure 4.17) was used. A control polymerisation was conducted in parallel where the RAFT agent was not added to the polymerisation mixture.

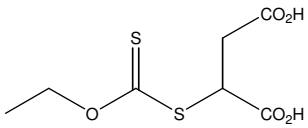


Figure 4.17 2-Ethoxythiocarbonylsulfanyl-succinic acid.

The control experiment resulted in complete conversion in less than 45 minutes, while the RAFT experiment showed an unexpected result. In the course of the RAFT polymerisation a steady increase in monomer conversion was disrupted by some retardation in the rate of polymerisation once the conversion reached 25%. The conversion did not increase significantly after that and this part of the kinetic plot was termed a *conversion plateau*, as is exemplified in Figure 4.18.

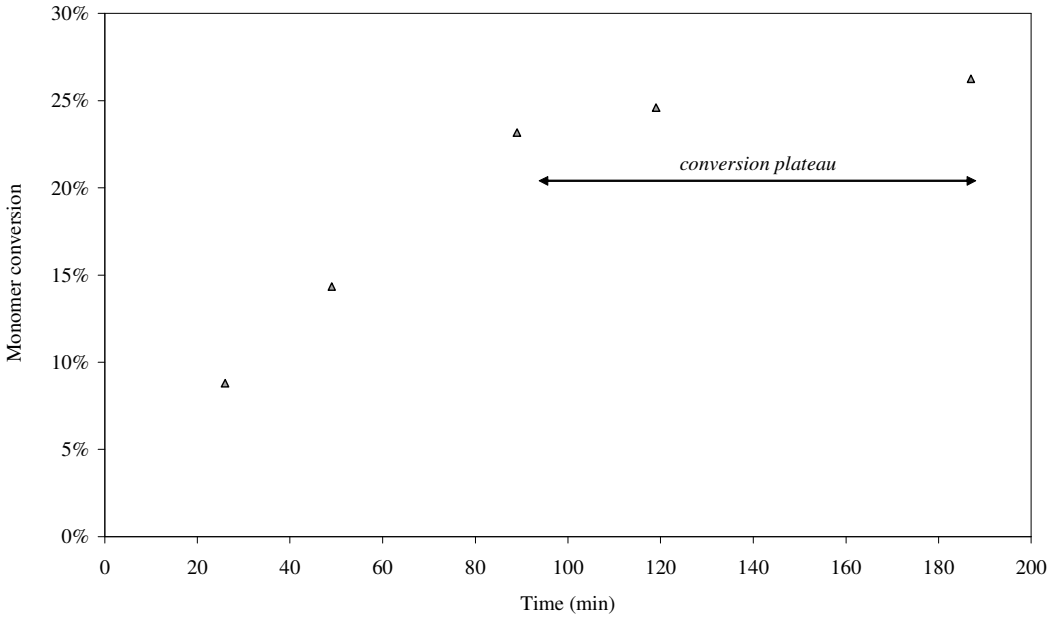


Figure 4.18 Polymerisation of HPMA (2.19 M) with RAFT agent 2-ethoxythiocarbonylsulfanyl-succinic acid in DMF at 90 °C; $[HPMA]_0$:$[AIBN]_0$:$[RAFT\ agent]_0$ = 200:1:2.

Three other RAFT agents have been employed in polymerisation of HPMA under the same conditions. Figure 4.19 shows that in all three experiments the initial period of inhibition was followed by acceleration of the rate which then dramatically slowed down and reached a conversion plateau after approximately 100 minutes of reaction time.

Samples were normally extracted from the reaction mixture with a degassed syringe therefore a possibility of contamination of reaction vessels with oxygen as a result of sampling was addressed. The experiments were repeated at the same conditions with no

sampling at all so as to eliminate the possibility of oxygen contamination. However, the experiments reproduced results obtained earlier and the hypothesis was rejected.

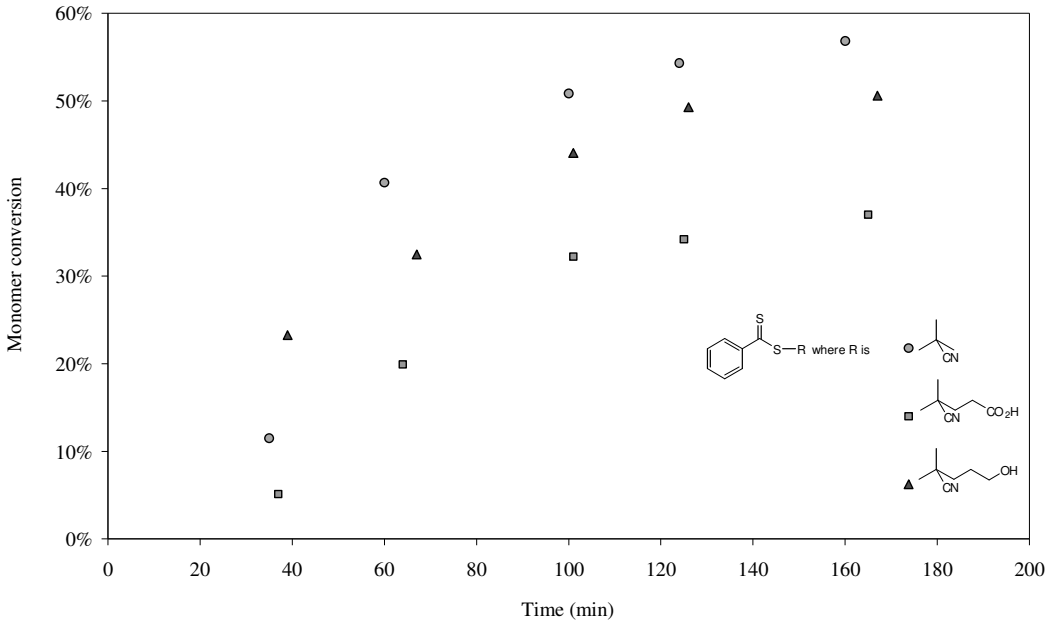


Figure 4.19 Conversion data for RAFT polymerisation of HPMA (6.60 M) at 90 °C in DMF; $[HPMA]_0$:$[AIBN]_0$:$[RAFT\ agent]_0$ = 200:1:2.

A possibility of some residual oxygen remaining in the reaction mixture after "freeze-pump-thaw" cycles was also considered. Reactions were repeated in ampoules which were sealed under vacuum after being subjected to three "freeze-pump-thaw" cycles. Yet again the measured conversions produced results very similar to the ones obtained in all previous experiments.

The experimental data is summarised in Table 4.11. The monomer conversion data indicated that despite long reaction time a complete monomer conversion was not achieved in any of the RAFT polymerisation experiments. Molecular weights measured by GPC at Polymer Institute of the Slovak Academy of Sciences, Bratislava, were found to be consistently higher than theoretically predicted values as shown in the figures.

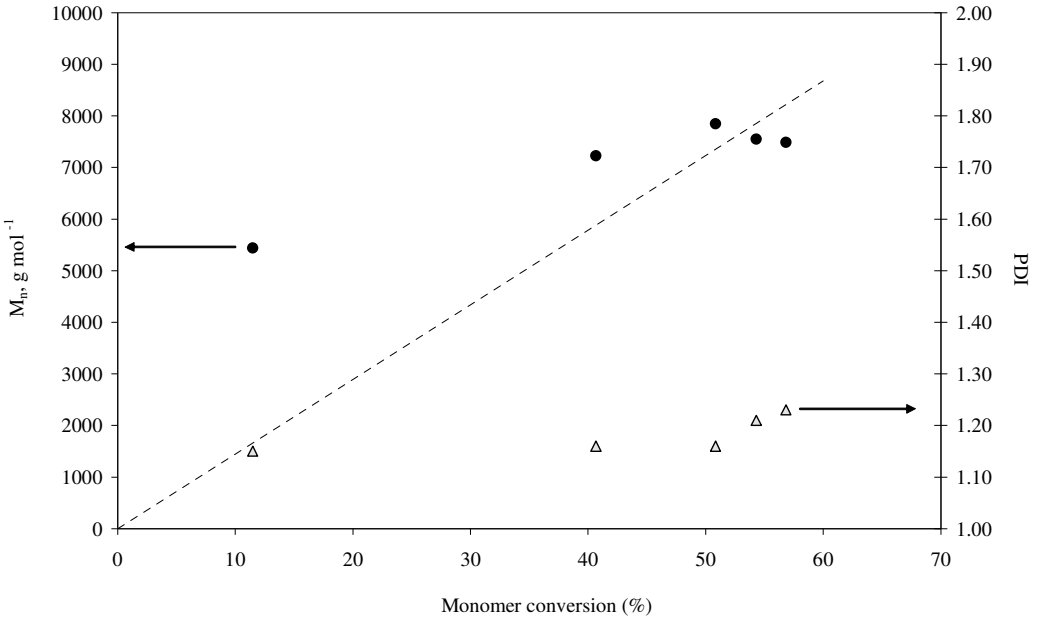


Figure 4.20 Evolution of molecular weight, M_n, and polydispersity index, *PDI*, with monomer conversion in RAFT polymerisation of HPMA in DMF at 90 °C with $[HPMA]_0$=6.60 M, $[HPMA]_0$:$[AIBN]_0$:$[CPDB]_0$ = 200:1:2.

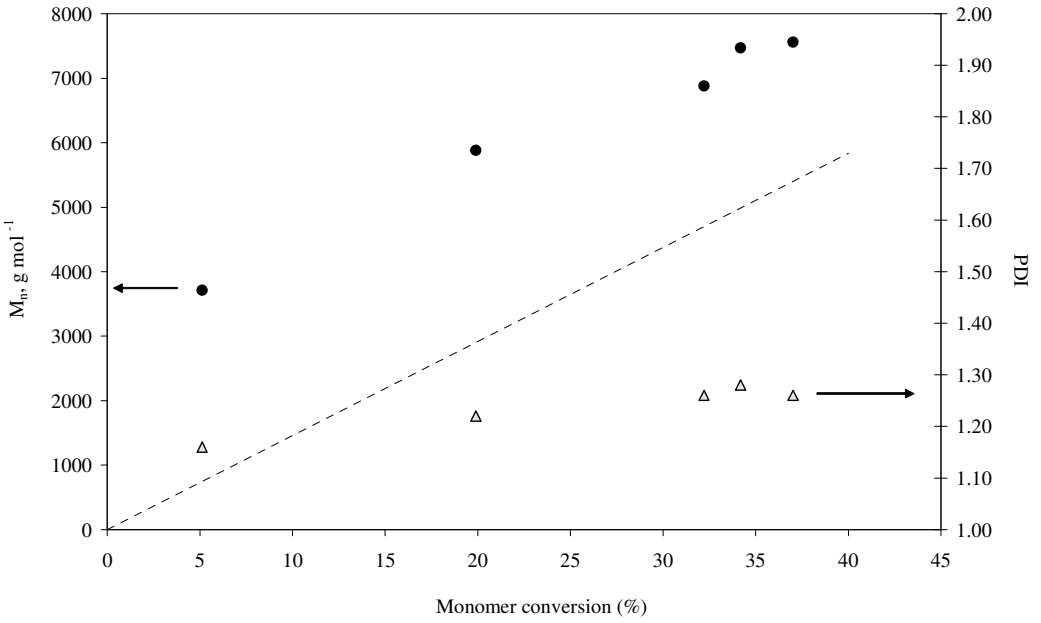


Figure 4.21 Evolution of molecular weight, M_n, and polydispersity index, *PDI*, with monomer conversion in RAFT polymerisation of HPMA in DMF at 90 °C with $[HPMA]_0$=6.60 M, $[HPMA]_0$:$[AIBN]_0$:$[CPAD]_0$ = 200:1:2.

Table 4.11 RAFT polymerisation of HPMA mediated by various RAFT agents.[A]

RAFT agent	Time, min	Conv., %[C]	$M_{n,\,GPC}$[D]	*PDI*	$M_{n,\,theory}$[E]
None	120	~100	58100	2.20	N/A
2-(2-cyanopropyl)dithiobenzoate (Figure 4.20)	35	11	5440	1.15	1834
	60	41	7230	1.16	5932
	100	51	7850	1.16	7359
	124	54	7550	1.21	7847
	160	57	7490	1.23	8200
2-(2-cyanopropyl)dithiobenzoate[B] (Figure 4.23)	45	29	6210	1.28	4379
	115	31	4730	1.26	4699
	225	39	5930	1.34	5823
	295	40	5140	1.24	5978
	375	42	5440	1.19	6294
4-cyanopentanoic acid dithiobenzoate (Figure 4.21)	37	5	3710	1.16	983
	64	20	5880	1.22	3015
	101	32	6880	1.26	4709
	125	34	7470	1.28	4980
	165	37	7560	1.26	5370
dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester (Figure 4.22)	39	23	5230	1.19	3531
	67	32	6740	1.27	4820
	101	44	8560	1.35	6446
	126	49	8270	1.35	7181
	167	51	8420	1.41	7367

[A]At 90 °C in DMF; $[HPMA]_0$=6.60 M, $[HPMA]_0$:$[AIBN]_0$:$[RAFT]_0$ = 200:1:2.

[B]Polymerisation was done in sealed ampoules.

[C]Conversion was measured by precipitating samples into acetone and drying the polymer under vacuum overnight.

[D]Molecular weight and *PDI* values were established by GPC; the analysis was performed at Polymer Institute of the Slovak Academy of Sciences, Bratislava.

[E]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2.

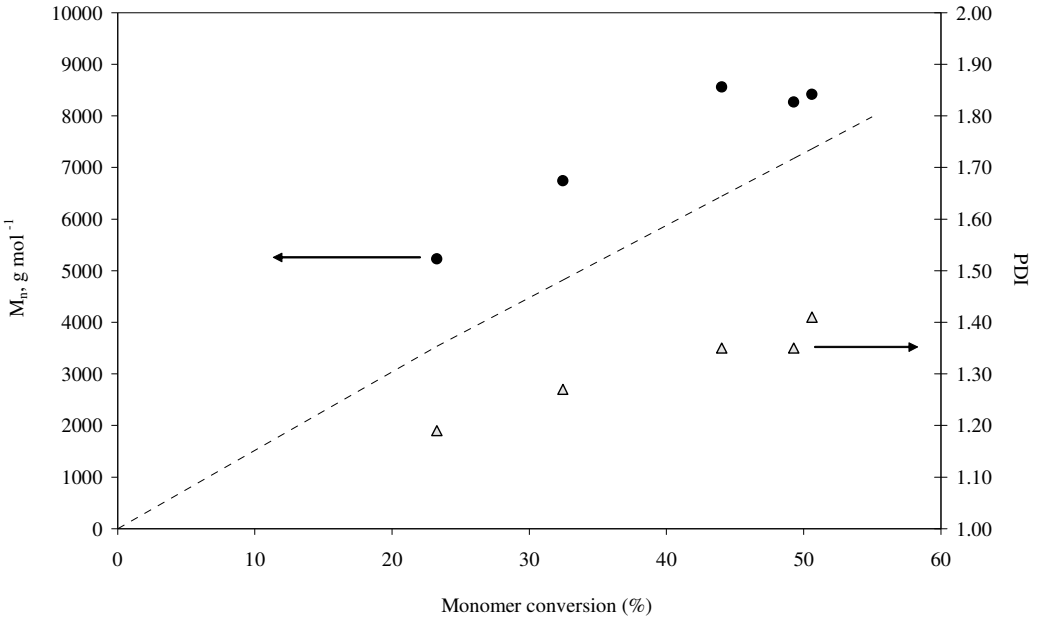


Figure 4.22 Evolution of molecular weight, M_n, and polydispersity index, *PDI*, with monomer conversion in RAFT polymerisation of HPMA in DMF at 90 °C with $[HPMA]_0$=6.59 M, $[HPMA]_0$:$[AIBN]_0$:$[DTBA]_0$ = 200:1:2.

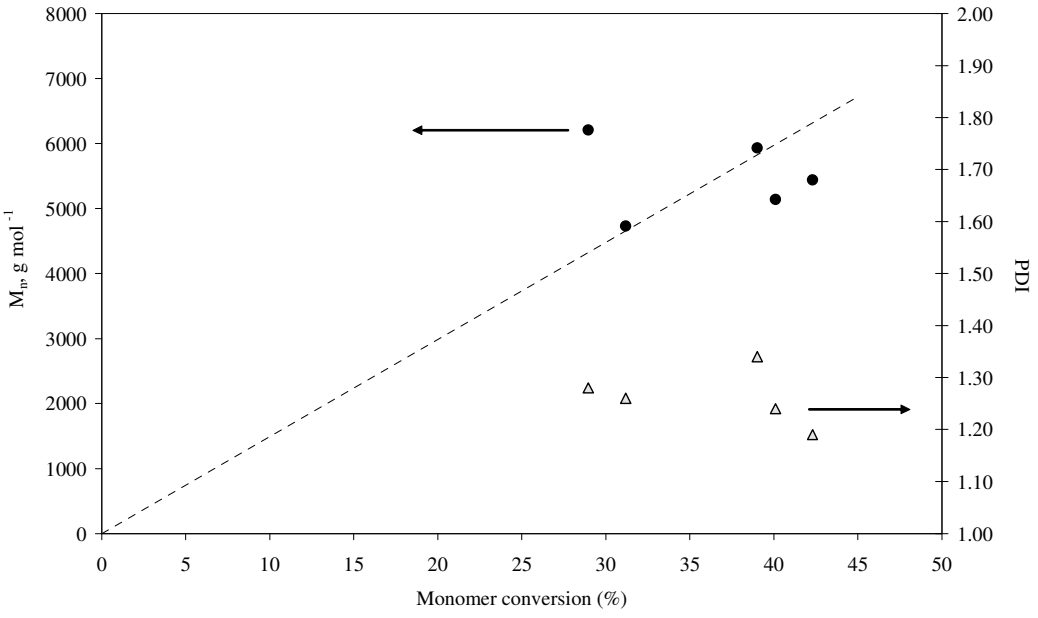


Figure 4.23 Evolution of molecular weight, M_n, and polydispersity index, *PDI*, with monomer conversion in RAFT polymerisation of HPMA in DMF at 90 °C conducted in sealed ampoules; $[HPMA]_0$=6.60 M, $[HPMA]_0$:$[AIBN]_0$:$[CPDB]_0$ = 200:1:2.

Similar kinetic data was obtained by other researchers in RAFT polymerisation of methyl α-hydroxy methacrylate[45] and *N,N*-dimethylacrylamide[27] (Figure 4.24).

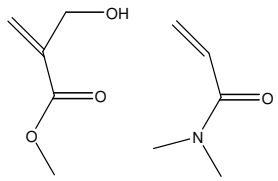


Figure 4.24 Methyl α-hydroxy methacrylate (MHM) and *N,N*-dimethylacrylamide (DMA).

It was postulated that significant depletion of initiating species at high reaction temperatures was the cause of a dramatic reduction of rate of polymerisation and inability to reach anywhere new complete conversion.[27,45] To test the hypothesis two separate experiments were conducted.

In one experiment the total added concentration of AIBN in the polymerisation of 50 wt.-% HPMA in DMF at 90 °C ($[HPMA]_0$:$[AIBN]_0$:$[CPDB]_0$ = 200:1:2) was doubled after an hour of reaction time by means of adding degassed stock solution containing required amount of the initiator to the mixture. After the addition was completed, the reaction was allowed to proceed, samples were withdrawn from the mixture for analyses as per usual however no significant change in rate was observed.

In the second experiment (RAFT polymerisation of 50 wt.-% HPMA in DMF at 90 °C at $[HPMA]_0$:$[AIBN]_0$:$[CPDB]_0$ = 200:1:2) the initial amount of AIBN was doubled, however, after a steady increase in monomer conversion the kinetic plot hit the plateau region. These results proved that the existence of the conversion plateau in these polymerisations cannot be exclusively blamed on the depletion of the initiator. Another explanation of the phenomenon must exist.

At this stage results of polymerisation of HPMA by the method of RAFT were published by Scales *et al.*[46] The researchers reported use of 4-cyanopentanoic acid dithiobenzoate as the chain transfer agent and 4,4'-azobis(4-cyanopentanoic acid) or V-501 (Figure 4.25) as the initiator in polymerisation of HPMA in aqueous media.

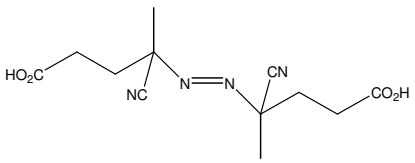


Figure 4.25 4,4'-Azobis(4-cyanopentanoic acid) (V-501).

The reported RAFT polymerisation was performed in an aqueous acetic buffer at 70 °C and resulted in formation of the polymer chains with narrow polydispersity. Overall a steady increase in molecular weights with the monomer conversion was observed despite some discrepancies between measured and calculated molecular weights predominantly in the region of lower monomer conversion. The measured weights were consistently higher than predicted values that were calculated based on the monomer conversion values determined by comparing the area of the UV signal of the monomer at the beginning of the polymerisation to that at the time of sampling.

In this work for comparison the initiator was changed from AIBN to V-501 in the further experiments. The new initiator was used in both a control polymerisation of HPMA and a RAFT experiment at 60 °C. After 21 hours of reaction time the monomer conversion in a control system reached only 19% while the parallel RAFT experiment which was done with 4-cyanopentanoic acid dithiobenzoate as a RAFT agent did not result in polymer formation.

These experiments were conducted at 60 °C rather than 90 °C. It is possible that such dramatic change in polymerisation temperature will result in significant reduction in the rate of polymerisation regardless of what initiating species is used. For comparative studies kinetics of the RAFT polymerisation of HPMA at 60 °C with AIBN was also evaluated. Two experiments were conducted in parallel using the $[HPMA]_0$:$[AIBN]_0$:$[CPDB]_0$ = 200:1:2 and $[HPMA]_0$:$[V\text{-}501]_0$:$[CPDB]_0$ = 200:1:2 ratios. Both polymerisations were left at 60 °C overnight, both resulted in formation of no polymer. Since previous experiments using AIBN at 90 °C were successful, it was assumed that the reaction temperature of 60 °C results in a very slow polymerisation of HPMA.

In an attempt to reproduce the results published by Scales *et al*., HPMA was polymerised under the reported conditions. Scales reports using the $[HPMA]_0$:$[V\text{-}501]_0$:$[RAFT]_0$ = 2340:1:3 and 3900:1:5 ratios. In this work the ratios were kept at $[HPMA]_0$:$[V\text{-}501]_0$:$[RAFT]_0$ = 2340:1:3. After 24 hours of polymerisation reaction a small amount of the polymer was isolated, with the overall monomer conversion reaching less than 5% which was a much lower value than the one reported by Scales *et al*.[46] Scales reports steady increase in monomer conversion with the final value of 88% after 24 hours.

Table 4.12 Conversion and molecular weights data for aqueous RAFT polymerisation of HPMA.[A]

RAFT agent	Time, min	Conv., %[B]	$M_{n,\ GPC}$[C]	*PDI*	$M_{n,\ theory}$[D]
4-cyanopentanoic acid dithiobenzoate (CPAD)	60	42	7230	1.10	12069
	120	63	12790	1.07	17901
	180	76	15340	1.06	21531
	240	84	15840	1.06	23694
	360	88	15720	1.07	25010

[A]At 70 °C in acetate buffer; $[HPMA]_0$=1.06 M, $[HPMA]_0$:$[V\text{-}501]_0$:$[RAFT]_0$ = 400:1:2.
[B]Conversion was measured by precipitating samples into acetone and drying the polymer under vacuum overnight.
[C]Molecular weight and *PDI* values were established by GPC; the analysis was performed at Polymer Institute of the Slovak Academy of Sciences, Bratislava.
[D]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2.

Here the polymerisation of HPMA in acetate buffer solution was repeated, this time the ratio of reactants used was changed to $[HPMA]_0$:$[V\text{-}501]_0$:$[RAFT]_0$ = 400:1:2. This time the reaction resulted in formation of the polymer, the product was isolated as pink hygroscopic solid by precipitation of a sample into acetone. Results of the experiment including monomer conversion and molecular weight data are summarised in Table 4.12.

Figure 4.26 shows evolution of the molecular weights and the polydispersity values with increasing monomer conversion. The shape of the curve in Figure 4.26 suggests linear increase in the molecular weight of the chains with conversion at the lower conversion values in particular. Low *PDI* values were indicative of the polymerisation process proceeding in a controlled fashion; however, the produced chains were found to be consistently shorter than predicted. The observed discrepancy between experimentally determined and calculated molecular weight values could be considered to be somewhat unusual as in most polymerisation processes produced chains are of higher molecular weight than predicted based on the measured monomer conversion.

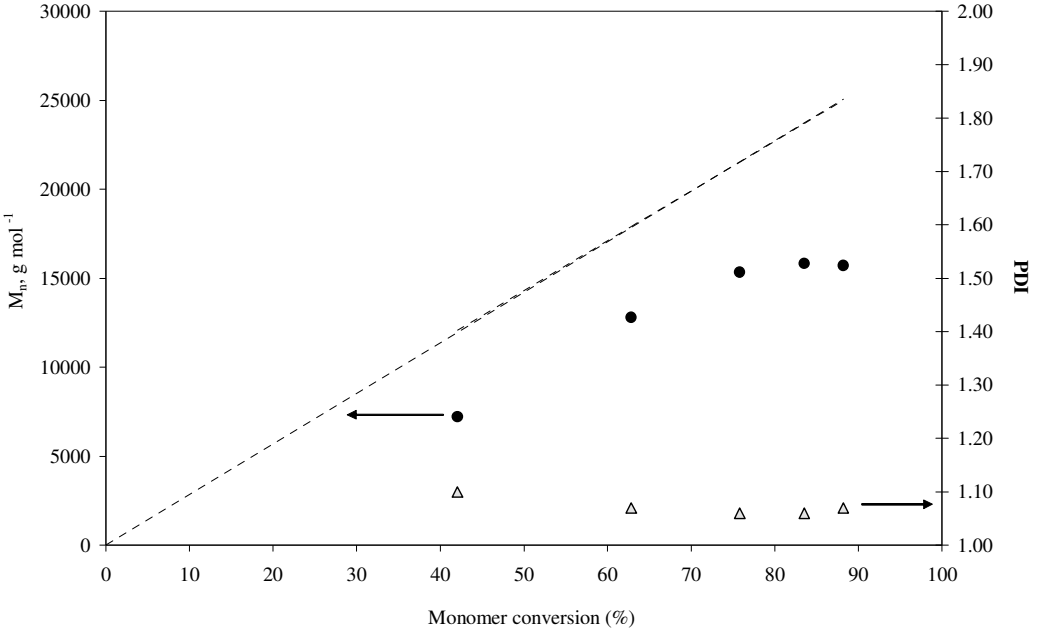


Figure 4.26 Molecular weight, M_n, and polydispersity index, *PDI,* development with the monomer conversion in RAFT polymerisation of HPMA in acetic buffer at 70 °C; $[HPMA]_0$=1.06 M, $[HPMA]_0$:$[V\text{-}501]_0$:$[CPAD]_0$ = 400:1:2.

Further, the discrepancy here is relatively small. GPC calibration could be contributing to it. Also, the theoretical $\overline{DP}_n$ is an overestimate as it does not include chains from V-501, which should properly be included.

The overall result of the RAFT polymerisation of HPMA in acetate buffer was considered to be promising, as it clearly showed there is a possibility of controlling the polymerisation process of HPMA under RAFT conditions. The key breakthrough seems to be the employment of acetate buffer. Scales *et al*. suggest that the use of acetate buffer in RAFT polymerisation of α-methyl substituted monomer such as HPMA allows fast monomer conversion relative to hydrolysis of the dithioester chain-ends.[46]

4.3.6 RAFT of *N*-Isopropylmethacrylamide

From the work discussed in the previous section it became evident that while the use of acetate buffer in the polymerisation of HPMA allows the polymerisation to proceed to high monomer conversion, the use of organic solvent such as DMF results in significant retardation in the rate of polymerisation. As it was mentioned previously, similar results were obtained by other research groups when RAFT polymerisation of methyl α-hydroxy

methacrylate (MHM)[45] and *N,N*-dimethylacrylamide (DMA)[27] were attempted. When the results of both this research and the published work[27,45] were considered, the fact that both HPMA and MHM contain hydroxyl group suggested that this functional group might affect the course of RAFT polymerisation and in fact might be responsible for the observed rate retardation.

This hypothesis could be tested by either protecting the group prior to polymerisation of the monomer or using a monomer analogous to HPMA but without a hydroxyl group. The second approach was investigated and the monomer of choice was *N*-isopropylmethacrylamide (NIPMAM), Figure 4.27.

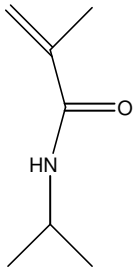


Figure 4.27 *N*-Isopropylmethacrylamide (NIPMAM).

Of course this is not exactly analogous to HPMA but to all intents and purpose it is. It is stressed that this work was conceived of and carried out before being aware of the work of Scales *et al*.[46]

A control polymerisation (no RAFT agent) and the polymerisation of NIPMAM under RAFT conditions using 2-(2-cyanopropyl)dithiobenzoate as a chain transfer agent produced fairly similar kinetic plots, achieving approximately 50% monomer conversion after 6 hours of the reaction time. The plots are shown in Figure 4.28 and also indicate significant induction period of 70-80 minutes.

Two experiments were left running overnight under similar conditions to access whether a complete monomer conversion could be achieved at longer reaction times. However, both RAFT and a control polymerisation resulted in only 48 and 58% monomer conversion respectively despite the reaction time being over 24 hours.

Conventional free-radical polymerisation of NIPMAM was reported in the literature.[47,48] The polymerisation of 0.25 (v/v) NIPMAM was done in an ethanol/water mixture with V-

501 as the initiator. However, no conversion was reported. Thereby it is not clear whether CFRP of NIPMAM giving a limiting conversion in 80 wt.-% DMF observed in this work, is also known for NIPMAM in the literature.

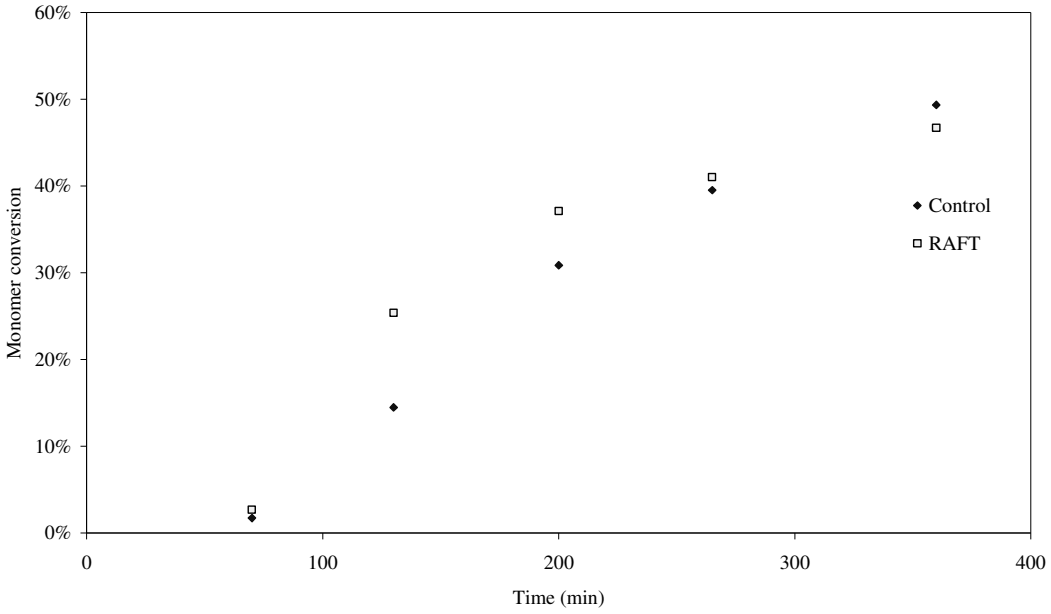


Figure 4.28 Control and RAFT polymerisation (mediated by 2-(2-cyanopropyl)dithiobenzoate) of NIPMAM in DMF at 90 °C; $[NIPMAM]_0 = 2.2$ M, $[NIPMAM]_0:[AIBN]_0:[CPDB]_0 = 200:1:2$.

To identify whether a depletion of the initiator was responsible for the occurring phenomenon, the amount of AIBN in the RAFT polymerisation mixture was doubled by means of adding a stock solution to the reaction mixture after 80 minutes of reaction time. This however did not result in a significant rate acceleration, therefore rejecting the hypothesis.

CFRP of NIPMAM was repeated at the higher monomer concentration (i.e., in 50 wt.-% DMF), the polymerisation resulted in complete conversion of NIPMAM after 80 minutes. For that reason the RAFT polymerisation of NIPMAM mediated by CPDB was also attempted at 50 wt.-% monomer; however, a complete monomer conversion was not achieved once again.

Further RAFT experiments were done with 4-cyanopentanoic acid dithiobenzoate and dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester as RAFT agents at 20 wt.-% NIPMAM. Here the observed inhibition period was less significant; however, a complete monomer conversion was not achieved once again, as shown in Figure 4.29.

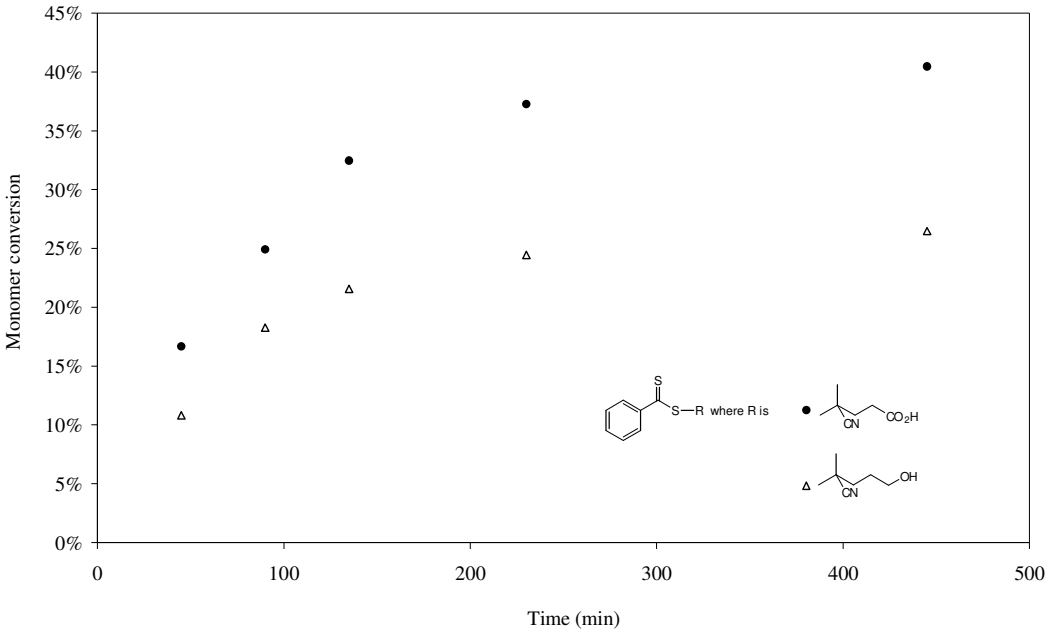


Figure 4.29 Monomer conversion data for RAFT polymerisation of NIPMAM in DMF at 90 °C; $[NIPMAM]_0 = 2.22$ M, $[NIPMAM]_0$:$[AIBN]_0$:[RAFT agent]$_0$ = 200:1:2.

Molecular weight and polydispersity values were measured by GPC and the results of the analysis are summarised in Table 4.13. Polydispersity values were low (< 1.3) and could be indicative of the *living* character of the polymerisations. The chains with the lowest *PDI* value were obtained in the experiment where the maximum degree of polymerisation of 50 was targeted. Here the chains were of molecular weight slightly higher than the theoretical value and were fairly monodisperse, i.e., *PDI* = 1.07.

In the rest of the cases measured molecular weight was found to be consistently lower than calculated values, in most cases indicating the maximum degree of polymerisation of 10. However, no increase in molecular weights with the monomer conversion is observed. In the observed monomer conversion plateau region this could be explained by the fact that the monomer conversion remains the same, suggesting that the growth of polymer chains in the region is not significant. However, it is not clear why the measured M_n values remain unchanged from the very beginning of the polymerisation process or why they are so low. GPC analysis was also used in estimation of the molecular weight of the control polymer sample which was prepared by conventional free-radical polymerisation. The obtained results indicated the product chains to be of a degree of polymerisation of 10 and of very narrow polydispersity. This result suggested unsuccessful GPC analysis producing unreliable measurements of the poly(methacrylamide) samples.

Table 4.13 Molecular weights and polydispersities for PNIPMAM prepared under RAFT polymerisation conditions.[A]

RAFT agent	Time, min	Conv., %[E]	$M_{n,GPC}$[F]	*PDI*	$M_{n,theory}$[G]
None	360	50	1359	1.07	N/A
2-(2-Cyanopropyl dithiobenzoate) (CPDB)	130[B]	25	1307	1.24	3340
	200	37	1330	1.20	4780
	265	41	1349	1.24	5260
	360	47	1389	1.26	5955
	45[C]	19	1594	1.07	1173
	130[D]	28	1354	1.23	5563
4-Cyanopentanoic acid dithiobenzoate (CPAD)	90	25	1130	1.24	3542
	135	32	1146	1.25	4530
	230	37	1156	1.26	5158
	445	40	1157	1.26	5578
Dithiobenzoic acid 1-cyano-4-hydroxy-1-methyl-butyl ester (DTBA)	90	18	1139	1.22	2541
	135	22	1181	1.24	2950
	230	24	1188	1.23	3310
	445	26	1243	1.23	3561

[A]At 90 °C in DMF, $[NIPMAM]_0$ = 2.22 M

[B]$[NIPMAM]_0$:$[AIBN]_0$:$[RAFT]_0$ = 200:1:2

[C]$[NIPMAM]_0$:$[AIBN]_0$:$[RAFT]_0$ = 100:1:2

[D]$[NIPMAM]_0$:$[AIBN]_0$:$[RAFT]_0$ = 300:1:2

[E]Conversion was measured by precipitating samples into ether and drying the polymer under vacuum overnight.

[F]Molecular weight and *PDI* values were established by GPC; the analysis was performed at the CSIRO Ian Wark Laboratories, Clayton, Australia.

[G]Theoretical molecular weights were calculated based on measured monomer conversion using equation 4.2.

Polymer of *N*-isopropylacrylamide (PNIPAM) has been widely investigated for drug delivery and while its successful synthesis via RAFT has been reported in the literature, a number of publications refer to problems associated with the GPC analysis of the polymer.[49,50] Ganachaud *et al*. report that for higher molecular weights, GPC of PNIPAM gives lower apparent molecular weights than those obtained by other methods. The problems associated with the GPC analysis are believed to arise from irreversible chain aggregation after complete drying of the polymer samples.[43] Schilli *et al*. report that the

GPC analysis with pure THF as eluent produced no analysable results, while the addition of tetrabutylammonium bromide to the THF solution produced good results.[50]

Even if one disregards the GPC results, it is clear from the conversion versus time measurements that RAFT polymerisation of NIPMAM is not successful. Thus the hypothesis that the OH group of HPMA and MAM is responsible for a conversion plateau in RAFT is disproved. In highlight this also follows from the work of Scales *et al*. that results in successful RAFT polymerisation of HPMA achieved in acetic buffer as a result of reduction in the extent of hydrolysis of dithioester end-groups.[46] Following this, an obvious suggestion was to attempt the RAFT polymerisation of NIPMAM in acetic buffer. However, the control and the RAFT experiments conducted in acetic buffer failed as the polymer of NIPMAM precipitated out of the solution upon its formation.

4.4 Modelling of data

4.4.1 Modelling of chain transfer constants

It has been stressed that only RAFT agents with high transfer coefficient C_{tr} will ensure a reasonable level of control in polymerisation process.[29] The observed discrepancy between calculated and experimentally determined molecular weights in RAFT polymerisation of the monomers discussed above might be indicative of low values of chain transfer coefficient of the RAFT agents used in the systems.

Traditionally chain transfer constants are evaluated using the Mayo approach.[51] The approach uses the number-average degree of polymerisation. For cases where "hybrid" RAFT behaviour is observed, the chain transfer constant, $C_{tr} = \frac{k_{tr}}{k_p}$ can be estimated using the following equation 4.3.[52]

$$C_{tr} = \frac{[M]_0}{(DP_n^{inst} - 1) \cdot [RAFT]_0} \qquad (4.3)$$

DP_n^{inst} is the degree of polymerisation of the polymer formed instantaneously, which is estimated by the extrapolation of the experimentally determined molecular weights to zero conversion, and $[M]_0$ and $[RAFT]_0$ are initial concentrations of the monomer and the

RAFT agent. The idea is that at 0% conversion radicals behave as if in a conventional transfer system, and it is only after that that living behaviour starts taking place.

In reality equation 4.3 is a variation of the Mayo equation (equation 4.4), it allows the estimation of the chain transfer value C_{tr} by plotting the inverse of the number-average degree of polymerisation versus the concentration ratio of the RAFT agent to the monomer.[9]

$$\frac{1}{DP_n^{inst}} = \frac{1}{DP_{n,0}^{inst}} + C_{tr}\frac{[RAFT]_0}{[M]_0} \qquad (4.4)$$

Here $DP_{n,0}^{inst}$ is the zero-conversion value of $\overline{DP}_n$ in the absence of any transfer agent.

The two equations were used in the estimation of values of C_{tr} for a number of RAFT systems investigated in this research. Because only one value of $[RAFT]_0$ was used in this work, C_{tr} could not be determined as the slope of a graph, only as a single point estimate.

The results are summarised in Table 4.14.

Table 4.14 Estimation of the chain transfer constant C_{tr}.

Monomer	$DP_{n,0}^{inst}$	RAFT agent	DP_n^{inst}	$\frac{[M]_0}{[RAFT]_0}$	C_{tr} (Eq 4.3)	C_{tr} (Eq 4.4)
HPMA[A] (Figure 4.30)	406	CPDB	35	98	2.89	2.56
		CPAD	23	96	4.37	3.94
		DTBA	19	98	5.46	4.92
NIPMAM[B] (Figure 4.31)	11	CPDB	10	97	11.36	1.10
		CPAD	7	103	16.51	4.60
		DTBA	9	98	13.00	2.32
MAC[C] (Figure 4.32)	377	CPDB	115	193	0.88	0.60
		CPAD	91	191	1.11	0.83
		DTBA	78	189	1.30	1.02

[A] At 90 °C in DMF, $[HPMA]_0$ = 6.60 M; $[HPMA]_0$:$[AIBN]_0$:$[RAFT]_0$=200:1:2.

[B] At 90 °C in DMF, $[NIPMAM]_0$ = 2.22 M; $[NIPMAM]_0$:$[AIBN]_0$:$[RAFT]_0$=200:1:2.

[C] At 92 °C in toluene, $[MAC]_0$ = 5.75 M; $[MAC]_0$:$[AIBN]_0$:$[RAFT]_0$=400:1:2.

C_{tr} values estimated by using the full Mayo equation (4.4) are always slightly lower than the values obtained using the simplified version of the same equation. This is because the

full equation more correctly allows for a small contribution by termination. It is worth noting that both sets of values are low, which is an indication of a relatively slow transfer process that is occurring in the RAFT polymerisations of the monomers under investigation.

The calculations were not done for the experimentally measured molecular weights of PMAOS samples as they actually decreased slightly with conversion meaning that DP_n^{inst} would have been higher than the measured values. Thus there is no evidence of any hybrid behaviour in these systems, and so the analysis method is invalid here.

The unreliability of the GPC analysis results could demand the exclusion of the PNIPMAM results from the calculations, however, the estimation of the chain transfer constants in RAFT polymerisation of this monomer was nevertheless attempted. But probably only HPMA and MAC results here are meaningful. In fact Figure 4.30, for HPMA, is clearly reminiscent of "hybrid" behaviour, suggesting that there was some genuine living character in these systems.

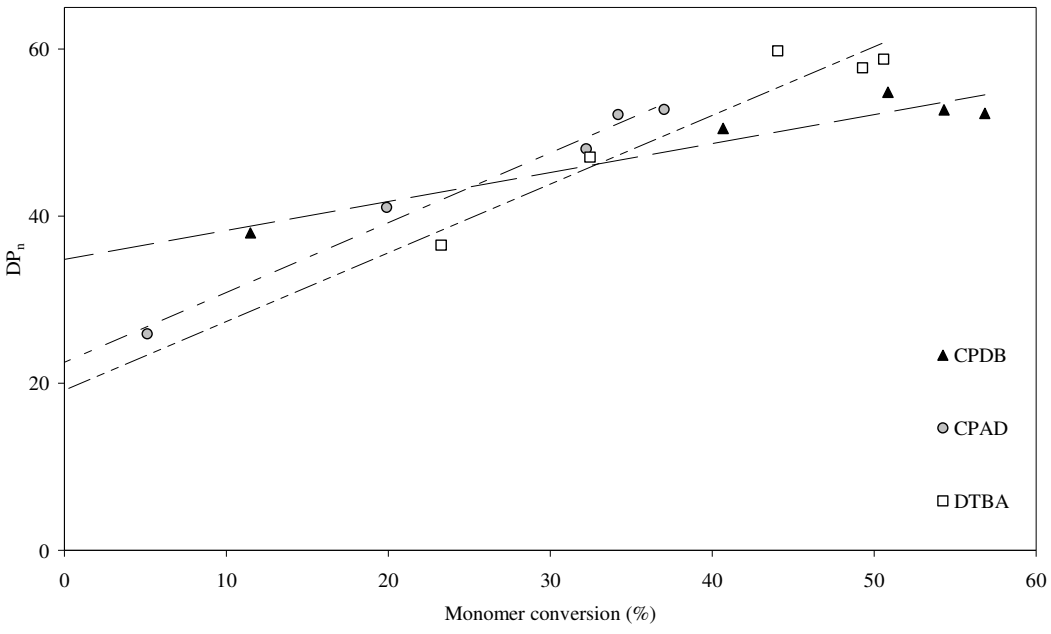


Figure 4.30 DP_n^{inst} estimation for RAFT polymerisations of HPMA at 90 °C in DMF, $[HPMA]_0$=6.60 M; $[HPMA]_0$:$[AIBN]_0$:$[RAFT]_0$ = 200:1:2.

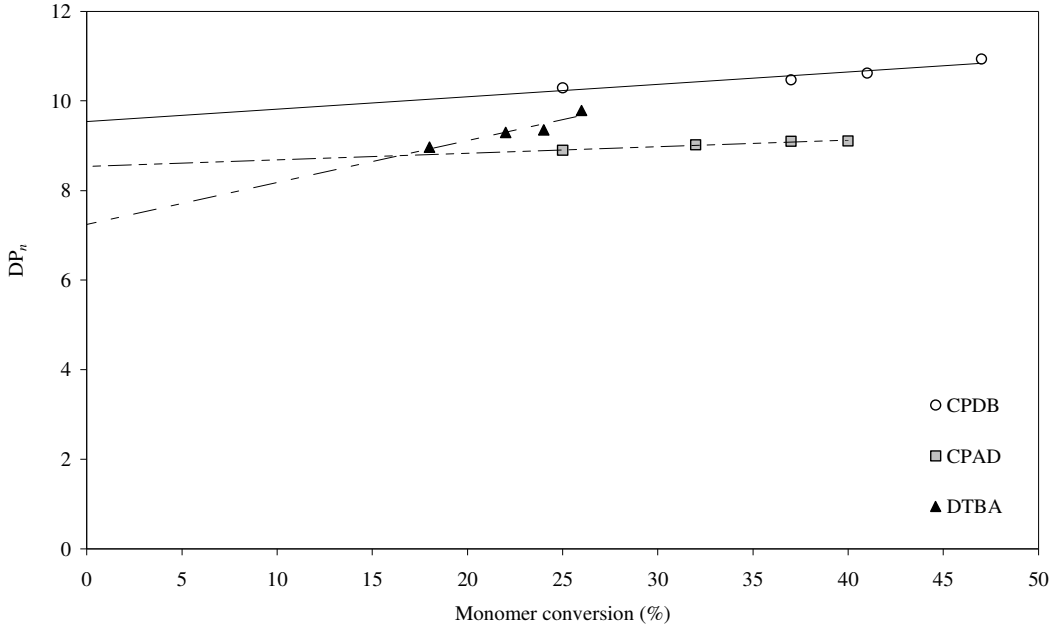


Figure 4.31 DP_n^{inst} estimation for RAFT polymerisations of NIPMAM at 90 °C in DMF, $[NIPMAM]_0$=2.22 M; $[NIPMAM]_0$:$[AIBN]_0$:$[RAFT]_0$ = 200:1:2.

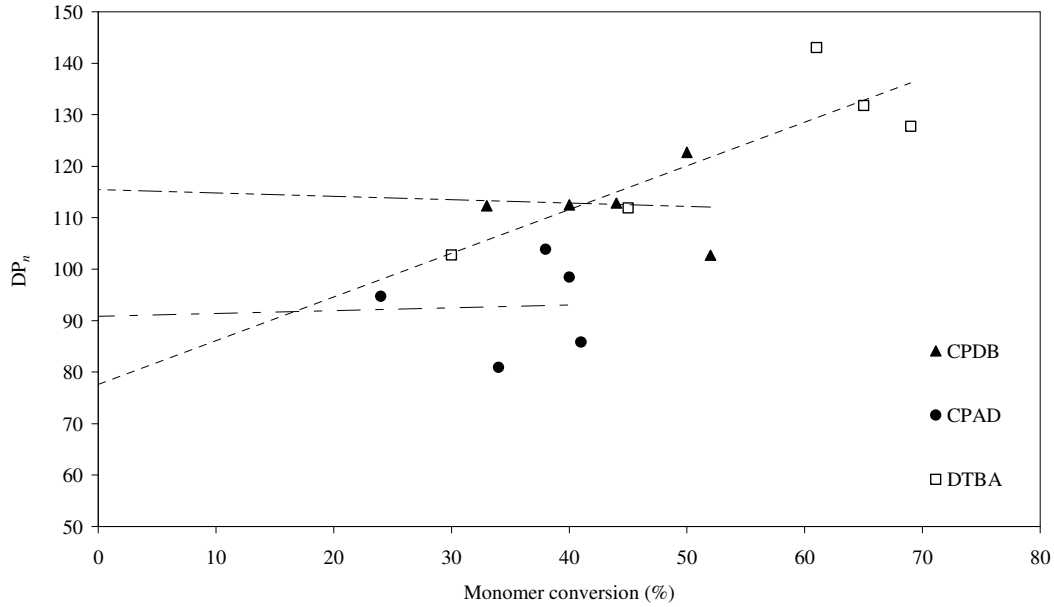


Figure 4.32 DP_n^{inst} estimation for RAFT polymerisations of MAC at 92 °C in toluene, $[MAC]_0$=5.75 M; $[MAC]_0$:$[AIBN]_0$:$[RAFT]_0$ = 400:1:2.

The transfer constants of various thiocarbonylthio compounds have been found to span more than five orders of magnitude. Depending on the groups R and Z and the monomer, the values range from 0.01 to 1000.[2,53] Theory suggests the C_{tr} value for a system should

be greater than two if a product with narrow polydispersity is required.[5,54,55] For example, an estimate of C_{tr} for a successful RAFT polymerisation of styrene was found to be 6000, while the value of C_{tr} measured for the RAFT of MMA system was estimated to be 140.[56] Moad *et al*. report the C_{tr} values calculated for various RAFT polymerisation systems ranging from 26 to 0.03.[2]

In this work the obtained values of chain transfer constant are fairly low, supporting the speculation that the polymerisation systems are not functioning in an ideal *living* manner. In fact even MMA is not regarded as an ideal monomer for RAFT, and yet the quoted value of C_{tr} for it is about two orders of magnitude higher than what has been found here. In fact from the HPMA results found here one would have to say that even the suggested 'minimum value' of 2 for C_{tr} gives only moderate hybrid behaviour. This supports once again the statement that for a successful living polymerisation a higher value of transfer constant is required.[5,57] Nevertheless it is interesting that the values of C_{tr} found here for MAC are all less than 2 and are lower than the values found for HPMA (slightly greater than 2). The analysis thus confirms the impression that MAC is no good RAFT system at all but at least HPMA gives hybrid RAFT behaviour. Thus there is the need for development of better RAFT agents for both monomers and for MAC in particular.

4.4.2 Modelling the RAFT process using the method of moments

A model for simulating the RAFT polymerisation process was developed by Wang *et al*. and is based on the method of moments.[58] The detailed description of the model that includes definition of various chain moments and the relationship between the latter and number- and weight-average chain lengths is reported in literature.[59] The model utilises a number of differential equations of moments, a simultaneous solution of which for given initial conditions produces the number- and weight-average molecular weights as characteristic of the molecular weight distribution. The model was developed based on the elementary reactions involved in RAFT polymerisation that are listed in Table 4.15.

Various chains moments and the relationship between adduct radical chain moments are identified in Table 4.16; the number-average and weight-average chain lengths were calculated from the moments that are identified in Table 4.17.

Table 4.15 Elementary reactions involved in RAFT polymerisation.

Reaction step	Scheme
Initiation:	$I \xrightarrow{f k_d} 2P_n$
Propagation:	$P_n + M \xrightarrow{k_p} P_{n+1}$
Addition-fragmentation:	$P_n + \text{RAFT-}P_m \underset{k_\beta}{\overset{k_{add}}{\rightleftharpoons}} P_n\text{-RAFT-}P_m \underset{k_{add}}{\overset{k_\beta}{\rightleftharpoons}} P_n\text{-RAFT} + P_m$
Termination:	$P_n + P_m \xrightarrow{k_{td}} D_n + D_m$
	$P_n + P_m \xrightarrow{k_{tc}} D_{n+m}$
	$P_n + P_m\text{-RAFT-}P_o \xrightarrow{k_{ct}} D_{n+m+o}$

where k_d – rate constant for initiator decomposition;
f – initiator efficiency;
k_p – rate constant for monomer propagation;
k_{add} – rate constant for propagating radical addition;
k_β – rate constant for adduct radical fragmentation;
k_{td} – rate constant for propagating radical termination by disproportionation;
k_{tc} – rate constant for propagating radical termination by recombination;
k_{ct} – rate constant for propagating and adduct radical cross-termination;
I – initiator,
P_n and P_{n+1} – propagating radical chains;
M – monomer;
RAFT-P_m – transfer (RAFT) agent;
P_n – RAFT – P_m – adduct radical chain;
P_m – leaving group on a RAFT agent;
D – dead chain;
P_n – RAFT – dormant chain.

In this research the model of Wang *et al.* was used to model the experimentally obtained molecular weights and polydispersity index and monomer conversion for some monomer systems. The differential equations of moments were solved simultaneously[58] for the three monomer systems while varying the initial rate parameters. Greg Smith is acknowledged for writing the program to do this.

There are a number of individual parameters considered in the RAFT process. It is important to realise that while for a specific polymerisation system one ideally should use the system-specific values, often such values are not available.

Table 4.16 Various chains moments and the relationship between adduct radical chain moments.

Type of chain	Definition of moment
Propagating radical chains:	$Y_i = \sum_{n=0}^{\infty} n^i \ [\mathrm{P}_n]$
Adduct radical chain:[A]	$Y_i^T = \frac{1}{2} \sum_{n=0}^{\infty} n^i \sum_{m=0}^{n} [\mathrm{P}_{n-m} - \mathrm{RAFT} - \mathrm{P}_m]$ $Y_{ij}^T = \sum_{n=0}^{\infty} \sum_{m=0}^{\infty} n^i m^j [\mathrm{P}_n - \mathrm{RAFT} - \mathrm{P}_m]$
Dormant chain:	$Q_i^T = \sum_{n=0}^{\infty} n^i \ [\mathrm{P_n} - \mathrm{RAFT}]$
Dead chain:	$Q_i = \sum_{n=0}^{\infty} n^i \ [\mathrm{D}_n]$

[A]Relationship between Y_i^T and Y_{ij}^T: $Y_0^T = \frac{1}{2} Y_{0\,0}^T$; $Y_1^T = Y_{1\,0}^T$; $Y_2^T = Y_{2\,0}^T + Y_{1\,1}^T$

The lack of reliable kinetic parameters that are required to be employed in the modelling is a difficulty that researchers face when modelling the RAFT process. In cases when the values are reported in the literature there is often no agreement between the values reported by different research groups. One example of such discrepancy is a difference of six orders of magnitude between k_β values measured for the RAFT polymerisation of styrene; Barner-Kowollik *et al.*[60] reported the value of 3×10^{-2} s^{-1}, while Kwak *et al.*[61] produced a value of = 7×10^4 s^{-1}.

Therefore it was decided to limit the modelling to the use of the kinetic parameters "typical" of radical polymerisation in their orders of magnitude.[62] To obtain a good agreement between modelled data and experimental measurements, k_d[59] and f[59] values were kept unchanged while the remaining values[58,59,62] – k_{td}, k_{tc}, k_{ct}, k_{add}, k_β, k_p – were changed one at a time. For obvious reasons the values of k_{td} and k_{tc} were always kept the same and of the order of magnitude one would expect for a termination rate coefficient. Generally k_{ct} was also set equal to this value, because it is also the rate coefficient of a termination reaction. However, because the coupling of the RAFT adduct and a radical is not exactly the same as radical-radical termination, some variation of k_{ct} was allowed.

To start off with all modelling used a value of order 10^3–10^4 L mol^{-1}s^{-1} for k_p.[63] This is of order what one would expect for functional methacrylates at the temperatures of interest here. Of course none of NIPMAM, MAC and HPMA are methacrylates, even if there is a resemblance, so there is considerable uncertainty in the value of k_p. For this reason it was treated as a fully adjustable parameter.

Table 4.17 Chain moments and number- and weight-average chain lengths.

Type of chain	Number-average chain length	Weight-average chain length
Propagating radical chain	$\overline{n_N} = Y_1/Y_0$	$\overline{n_N} = Y_2/Y_1$
Adduct radical chain	$\overline{n_N^T} = Y_1^T/Y_0^T$	$\overline{n_N^T} = Y_2^T/Y_1^T$
Dormant chain	$\overline{n_N^T} = Q_1^T/Q_0^T$	$\overline{n_N^T} = Q_2^T/Q_1^T$
Dead chain	$\overline{n_N} = Q_1/Q_0$	$\overline{n_N} = Q_2/Q_1$
Total chain	$\overline{\overline{n_{Ntot}}} = \frac{Y_1 + Y_1^T + Q_1^T + Q_1}{Y_0 + Y_0^T + Q_0^T + Q_0}$	$\overline{\overline{n_{Ntot}}} = \frac{Y_2 + Y_2^T + Q_2^T + Q_2}{Y_1 + Y_1^T + Q_1^T + Q_1}$

In some cases the reported values from modelling are unlikely (too low or too high), but these should not be taken too seriously: the primary purpose of the modelling was to see how well the experimental data could be reproduced, and it is likely that multiple sets of parameter values could achieve this if it is found that one can. Besides, it is perhaps worth remembering that 'world record' values of for k_p have been reported for acrylic acid (higher than 10^5 L mol^{-1}s^{-1})[64,65], so extremely high values of k_p should not immediately be dismissed: the present monomers are not too different.

Finally, of primary interest in the modelling are the values of k_{add} and k_β. It should be remembered that with so many variable parameters, it was impossible to find k_{add} and k_β precisely.

Rather than attempting 'global' modelling of all data, the approach used was to first of all reproduce the conversion-time data, and see how well this parameter set fared in predicting the corresponding MWD data. Having done this, the opposite was carried out, i.e., the molecular weight data was modelled as well as possible, and the corresponding conversion-time estimates scrutinized. This was done for all systems.

4.4.2.1 RAFT of NIPMAM

Figure 4.33 shows the results of the modelling of the monomer conversion data and comparison of the result to the experimentally measured conversion of NIPMAM in a course of RAFT polymerisation of the latter. While the use of the kinetic parameters listed in Table 4.18 produced a reasonably good agreement between experimental and calculated monomer conversion values, the predicted molecular weight values did not agree with the GPC measured ones.

Possibly this is because the GPC values are not accurate, as discussed earlier. Equally possible is that it is because whatever is responsible for the phenomenon of a conversion plateau, which is observed for NIPMAM, is not in the model. Note though that the model should still be capable of reproducing "hybrid" RAFT behaviour, because such kinetics are in the model.

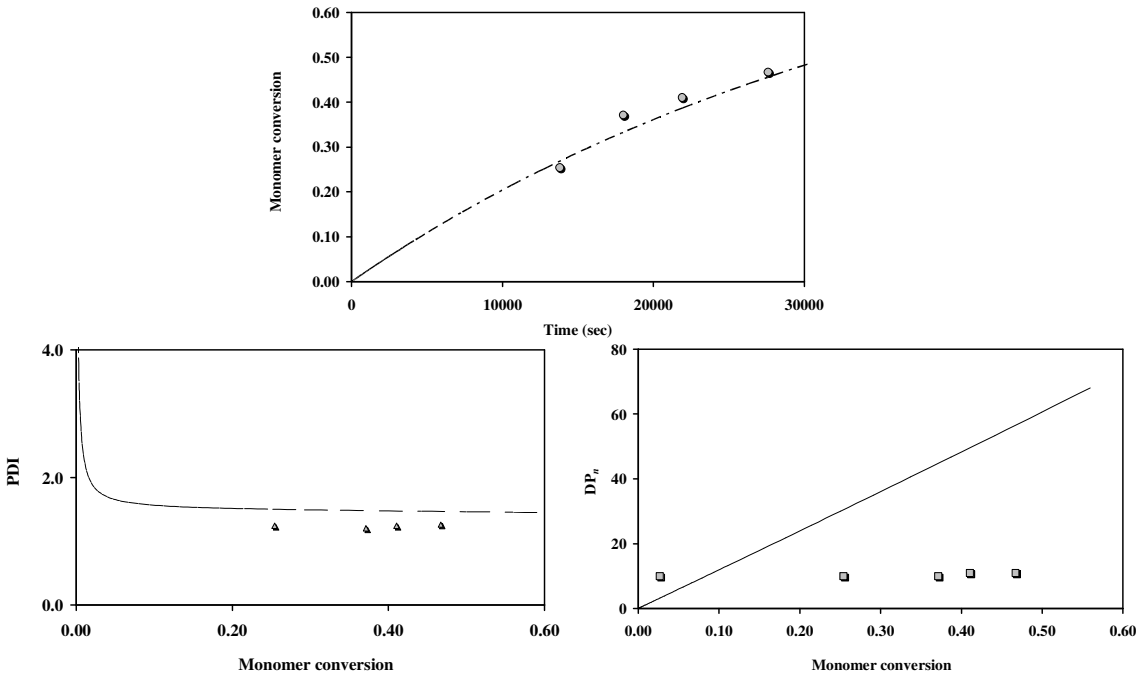


Figure 4.33 Simulation of conversion versus time, number-average chain length, $\overline{DP}_n$, and polydispersity index, *PDI*, versus monomer conversion for the RAFT polymerisation of NIPMAM (2.22 M) in DMF at 90 °C. $[NIPMAM]_0:[AIBN]_0:[CPDB]_0 = 200:1:2$. Parameters used are listed in Table 4.18.

Table 4.18 Parameters used in modelling of the monomer conversion data in the RAFT polymerisation of NIPMAM mediated by CPDB.[A]

Rate parameter	Value
k_d	1.00×10^{-5} s^{-1}
f	0.75
k_p	1.40×10^{3} L mol^{-1} s^{-1}
k_{td}	8.00×10^{7} L mol^{-1} s^{-1}
k_{tc}	8.00×10^{7} L mol^{-1} s^{-1}
k_{ct}	8.00×10^{7} L mol^{-1} s^{-1}
k_{add}	8.00×10^{6} L mol^{-1} s^{-1}
k_β	6.00×10^{4} s^{-1}
C_{tr}	2857

[A]At 90 °C in DMF with $[NIPMAM]_0$=2.22 M; $[NIPMAM]_0$:$[AIBN]_0$:$[CPDB]_0$ = 200:1:2

4.4.2.2 RAFT of MAC

The method of moments was used in simulations of the data for RAFT polymerisation of MAC. Figure 4.34 shows the results of the modelling which are in good agreement with experimentally measured monomer conversion. However, the use of the same parameters (listed in Table 4.19) did not produce similarly good agreement between the predicted and measured molecular weight values. For that reason, the kinetic parameters were varied to obtain a better agreement in molecular weights modelling. The adjusted parameters are listed in Table 4.20 with the results of the modelling shown in Figure 4.35.

The use of the second set of kinetic parameters in the modelling produced a better agreement between calculated and observed molecular weight development. However, according to the calculations, such a system should be characterised by a much slower monomer conversion rate than was observed.

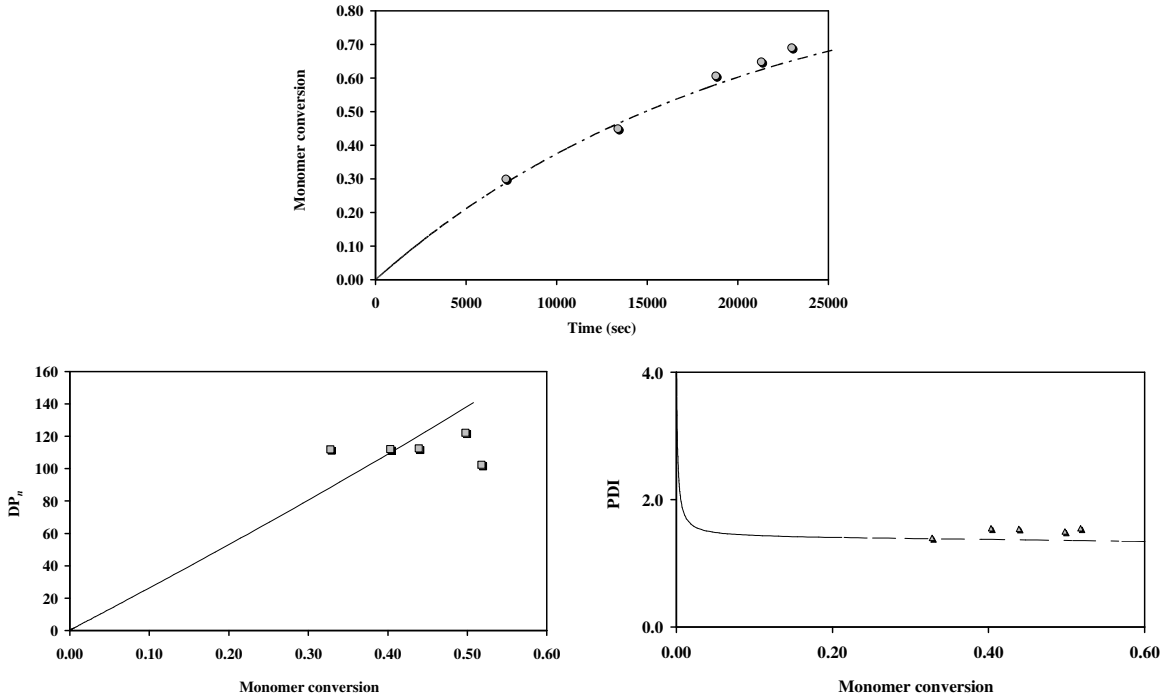


Figure 4.34 Simulation of conversion versus time, number-average chain length, $\overline{DP}_n$, and polydispersity index, *PDI*, versus monomer conversion for the RAFT polymerisation of MAC (5.55 M) in toluene at 92 °C. $[MAC]_0$:$[AIBN]_0$:$[DTBA]_0$ = 400:1:2. Parameters used are listed in Table 4.19.

Table 4.19 Parameters used in modelling of the monomer conversion data in the RAFT polymerisation of MAC mediated by DTBA.[A]

Rate parameter	Value
k_d	1.00×10^{-5} s^{-1}
f	0.75
k_p	3.80×10^{3} L mol^{-1} s^{-1}
k_{td}	8.00×10^{7} L mol^{-1} s^{-1}
k_{tc}	8.00×10^{7} L mol^{-1} s^{-1}
k_{ct}	8.00×10^{7} L mol^{-1} s^{-1}
k_{add}	8.00×10^{6} L mol^{-1} s^{-1}
k_β	3.00×10^{4} s^{-1}
C_{tr}	1053

[A]At 92 °C in toluene with $[MAC]_0$=5.55 M; $[MAC]_0$:$[AIBN]_0$:$[DTBA]_0$ = 400:1:2

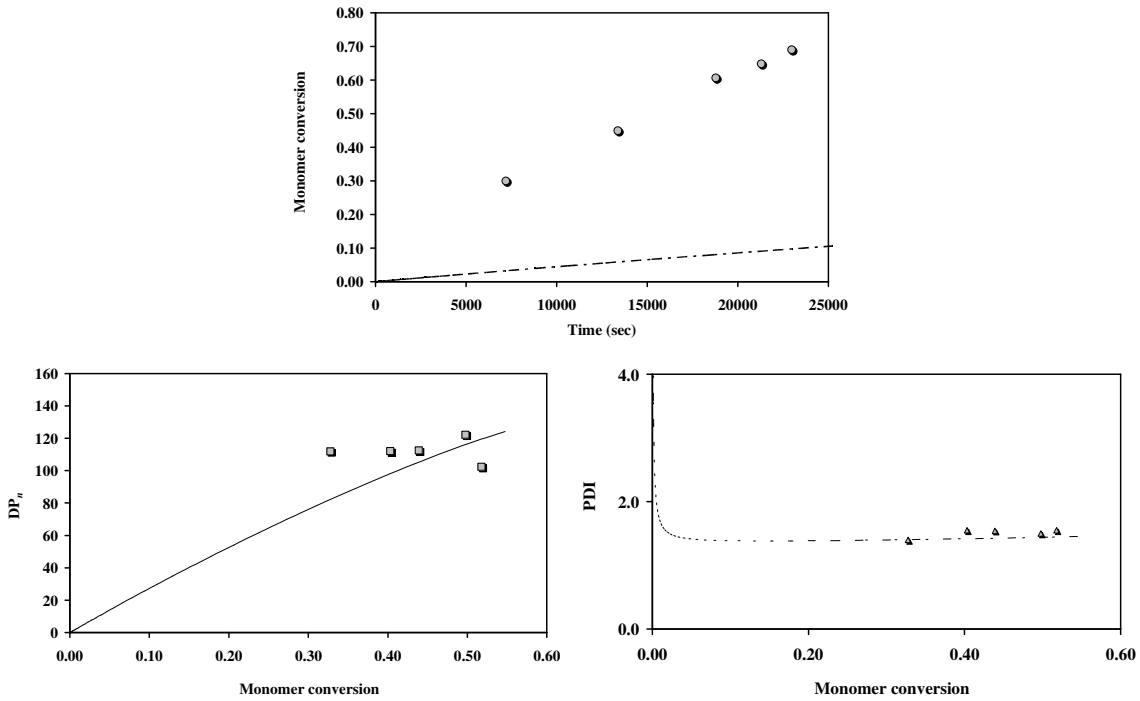


Figure 4.35 Simulation of conversion versus time, number-average chain length, $\overline{DP}_n$, and polydispersity index, *PDI*, versus monomer conversion for the RAFT polymerisation of MAC (5.55 M) in toluene at 92 °C. $[MAC]_0:[AIBN]_0:[DTBA]_0 = 400:1:2$. Parameters used are listed in Table 4.20.

Table 4.20 Second set of parameters used in modelling of the molecular weight data in the RAFT polymerisation of MAC mediated by DTBA.[A]

Rate parameter	Value
k_d	1.00×10^{-5} s^{-1}
f	0.75
k_p	3.00×10^{3} L mol^{-1} s^{-1}
k_{td}	8.00×10^{7} L mol^{-1} s^{-1}
k_{tc}	8.00×10^{7} L mol^{-1} s^{-1}
k_{ct}	8.00×10^{7} L mol^{-1} s^{-1}
k_{add}	1.00×10^{8} L mol^{-1} s^{-1}
k_β	5.00×10^{3} s^{-1}
C_{tr}	1667

[A] At 92 °C in toluene with $[MAC]_0$=5.55 M; $[MAC]_0:[AIBN]_0:[DTBA]_0 = 400:1:2$

That can be explained by examining which rate constants were varied. In the first simulation the faster rate of polymerisation is explained by higher values of the rate constant of propagation, k_p, and the rate constant of fragmentation, k_β.

Increase in either of those values will favour faster conversion rate. In the second set of simulations those values were lowered while the value of the rate constant of addition was increased. An increase in k_{add} normally slows down the rate of polymerisation because it is a radical removing reaction.

4.4.2.3 RAFT of HPMA

In a similar fashion the monomer conversion and molecular weight data was modelled for two of the polymerisation systems of HPMA conducted under the conditions of RAFT.

4.4.2.3.7 RAFT of HPMA in aqueous acetate buffer

Table 4.21 contains kinetic parameters used in simulating experimental data from the polymerisation of HPMA conducted in aqueous acetate buffer solution. The modelled data shows good agreement with experimentally measured monomer conversion, shown in Figure 4.36, and agreement between molecular weights is also reasonable, even if not perfect.

Adjustment of certain kinetic parameters allowed production of simulated data that fitted the molecular weight profile of the experiment. A significant drop in the rate of propagation resulted in slower rate of polymerisation, allowing better agreement between calculated and measured values of chain lengths. However here the kinetics are poorly reproduced and the overall agreement of all data is poorer than in Figure 4.36.

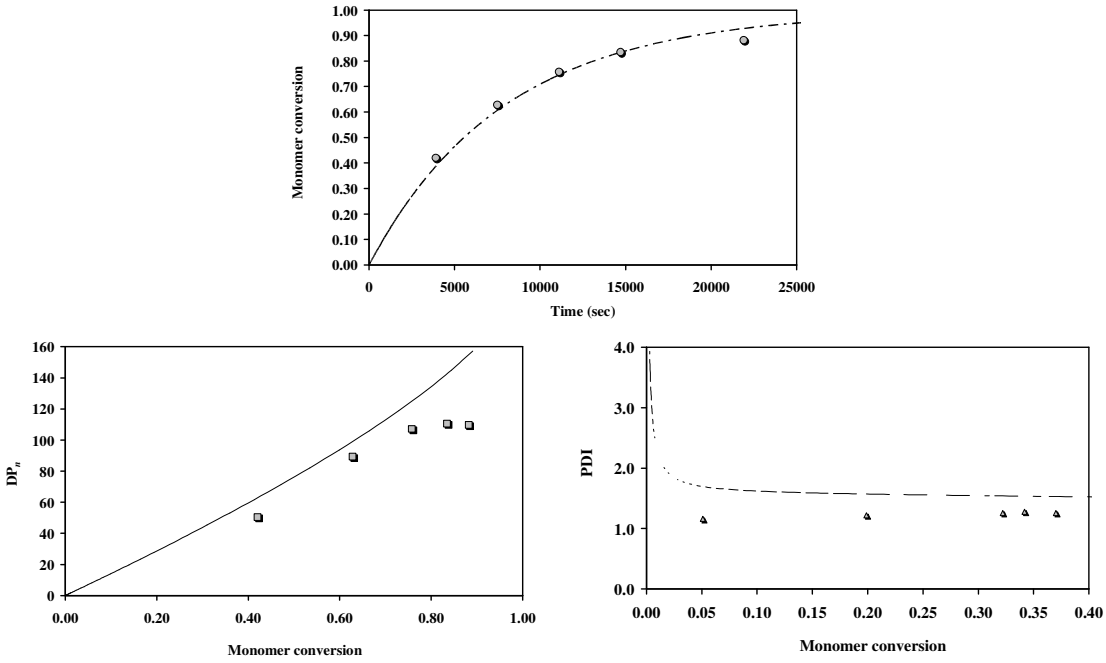


Figure 4.36 Simulation of conversion versus time, number-average chain length, $\overline{DP}_n$, and polydispersity index, *PDI*, versus monomer conversion for the RAFT polymerisation of HPMA (1.06 M) in aqueous acetic buffer at 70 °C. $[HPMA]_0$:$[ACP]_0$:$[CPAD]_0$ = 200:1:2. Parameters used are listed in Table 4.21.

Table 4.21 Parameters used in modelling of the monomer conversion data in the RAFT polymerisation of HPMA mediated by CPAD in aqueous acetic buffer solution.[A]

Rate parameter	Value
k_d	1.00×10^{-5} s^{-1}
f	0.75
k_p	3.00×10^{3} L mol^{-1} s^{-1}
k_{td}	1.00×10^{7} L mol^{-1} s^{-1}
k_{tc}	1.00×10^{7} L mol^{-1} s^{-1}
k_{ct}	1.50×10^{8} L mol^{-1} s^{-1}
k_{add}	1.00×10^{8} L mol^{-1} s^{-1}
k_β	1.00×10^{8} s^{-1}
C_{tr}	16667

[A]At 70 °C in aqueous acetic buffer with $[HPMA]_0$=1.06 M; $[HPMA]_0$:$[ACP]_0$:$[CPAD]_0$=200:1:2

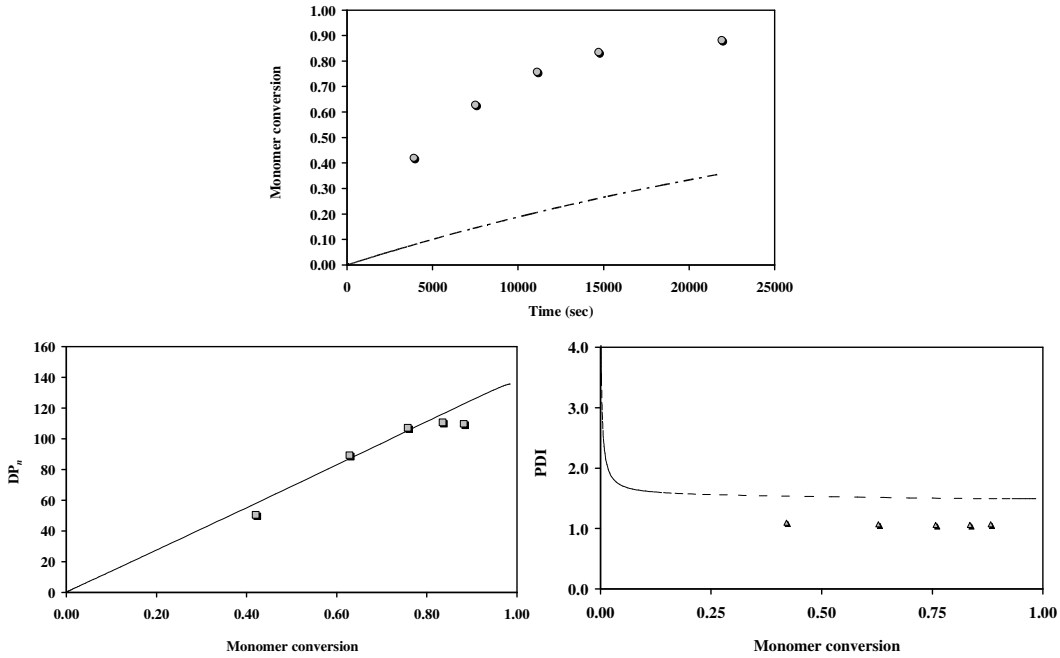


Figure 4.37 Simulation of conversion versus time, number-average chain length, $\overline{DP}_n$, and polydispersity index, *PDI*, versus monomer conversion for the RAFT polymerisation of HPMA (1.06 M) in aqueous acetic buffer at 70 °C. $[HPMA]_0$:$[ACP]_0$:$[CPAD]_0$ = 200:1:2. Parameters used are listed in Table 4.22.

Table 4.22 Second set of parameters used in modelling of the molecular weight data in the RAFT polymerisation of HPMA mediated by CPAD in aqueous acetate buffer solution.[A]

Rate parameter	Value
k_d	1.00×10^{-5} s^{-1}
f	0.75
k_p	5.00×10^{2} L mol^{-1} s^{-1}
k_{td}	1.00×10^{7} L mol^{-1} s^{-1}
k_{tc}	1.00×10^{7} L mol^{-1} s^{-1}
k_{ct}	1.00×10^{8} L mol^{-1} s^{-1}
k_{add}	1.00×10^{6} L mol^{-1} s^{-1}
k_β	1.00×10^{6} s^{-1}
C_{tr}	1000

[A]At 70 °C in aqueous acetic buffer with $[HPMA]_0$=1.06 M; $[HPMA]_0$:$[ACP]_0$:$[CPAD]_0$ = 200:1:2

4.4.2.3.8 RAFT of HPMA in DMF

Modelling of the monomer conversion experimental data obtained in polymerisation of HPMA in DMF produced best overall agreement so far even if the unexplained retardation in rate of polymerisation could not be predicted. The data are shown in Figure 4.38 with corresponding kinetic parameters listed in Table 4.23. What is especially interesting is the satisfactory reproduction of the "hybrid" behaviour in $\overline{DP}_n$ versus monomer conversion modelling. Overall it would have to be said that all data is satisfactorily reproduced here, which gives confidence to the verdict that this HPMA system displays moderate living behaviour.

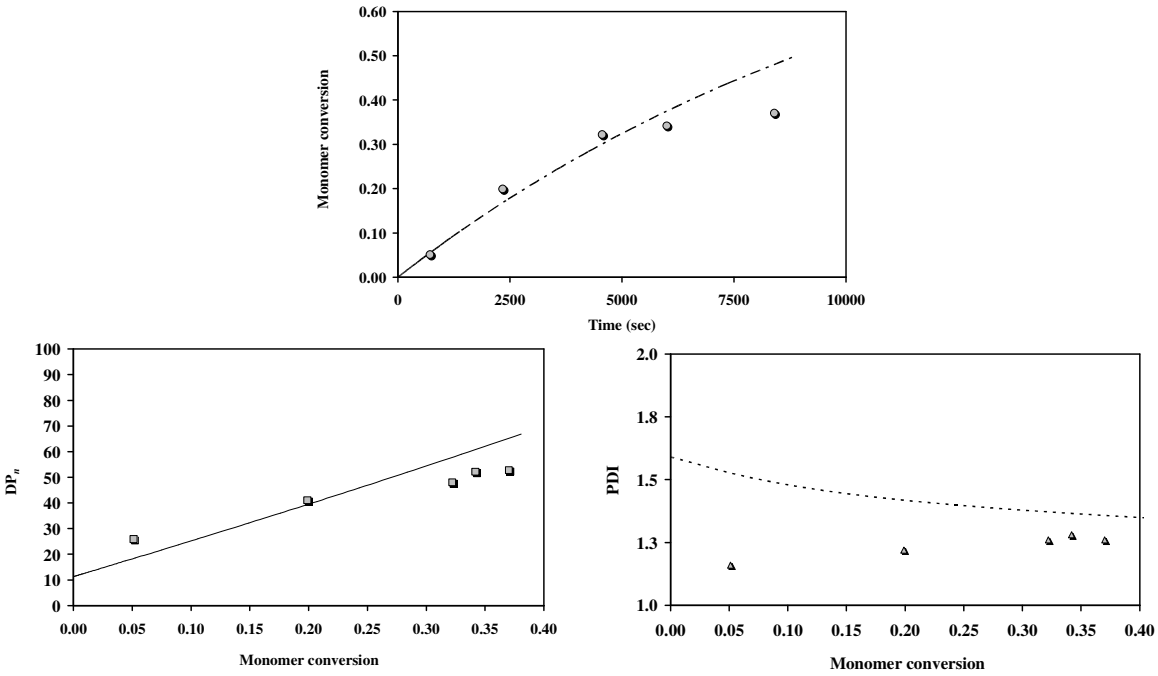


Figure 4.38 Simulation of conversion versus time, number-average chain length, $\overline{DP}_n$, and polydispersity index, *PDI*, versus monomer conversion for the RAFT polymerisation of HPMA (6.6 M) in DMF at 90 °C. $[HPMA]_0:[AIBN]_0:[CPAD]_0 = 200:1:2$. Parameters used are listed in Table 4.23.

Table 4.23 Parameters used in modelling of the monomer conversion in the RAFT polymerisation of HPMA mediated by CPAD in DMF.[A]

Rate parameter	Value
k_d	1.00×10^{-5} s^{-1}
f	0.75
k_p	2.95×10^{4} L mol^{-1} s^{-1}
k_{td}	1.00×10^{8} L mol^{-1} s^{-1}
k_{tc}	1.00×10^{8} L mol^{-1} s^{-1}
k_{ct}	5.50×10^{8} L mol^{-1} s^{-1}
k_{add}	1.00×10^{6} L mol^{-1} s^{-1}
k_β	1.00×10^{3} s^{-1}
C_{tr}	17

[A]At 90 °C in DMF with $[HPMA]_0$=6.6 M; $[HPMA]_0$:$[AIBN]_0$:$[CPAD]_0$ = 200:1:2

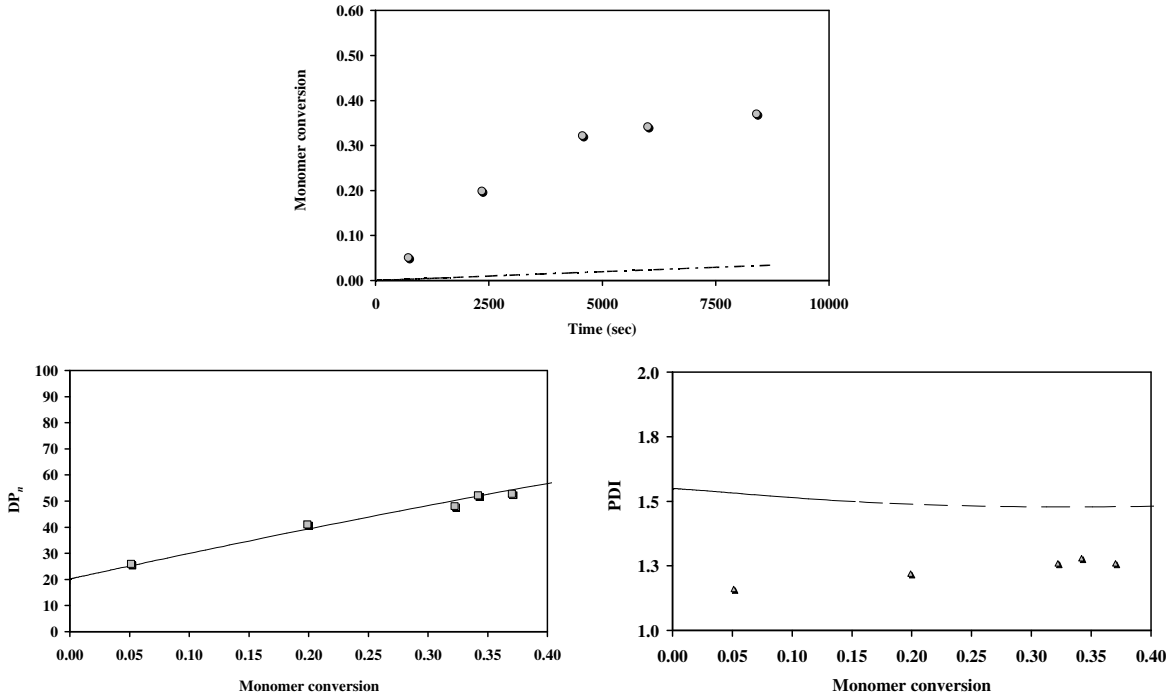


Figure 4.39 Simulation of conversion versus time, number-average chain length, $\overline{DP}_n$, and polydispersity index, *PDI*, versus monomer conversion for the RAFT polymerisation of HPMA (6.6 M) in DMF at 90 °C. $[HPMA]_0$:$[AIBN]_0$:$[CPAD]_0$ = 200:1:2. Parameters used are listed in Table 4.24.

Table 4.24 Parameters used in modelling of the molecular weight data in the RAFT polymerisation of HPMA mediated by CPAD in DMF.[A]

Rate parameter	Value
k_d	1.00×10^{-5} s^{-1}
f	0.75
k_p	6.20×10^{5} L mol^{-1} s^{-1}
k_{td}	1.00×10^{8} L mol^{-1} s^{-1}
k_{tc}	1.00×10^{8} L mol^{-1} s^{-1}
k_{ct}	1.00×10^{10} L mol^{-1} s^{-1}
k_{add}	1.00×10^{7} L mol^{-1} s^{-1}
k_β	1.00 s^{-1}
C_{tr}	8

[A]At 90 °C in DMF with $[HPMA]_0$=6.6 M;
$[HPMA]_0$:$[AIBN]_0$:$[CPAD]_0$ = 200:1:2

In further modelling of the number-average chain length of the polymer of HPMA a very good agreement between calculated and experimental values was obtained. However, a large discrepancy is now observed in monomer conversion versus time plot, as shown in Figure 4.39. Given that experimental error is greater in $\overline{DP}_n$ than in conversion measurements, this gives a good reason to prefer the modelling of Figure 4.38 in addition to the fact that it is overall of better quality in reproducing the data.

4.4.3 Comparison of values from different modelling methods

It is possible to compare parameter values from both the modelling methods that have been used here. Values of the transfer constant, $C_{tr} = \frac{k_{tr}}{k_p}$, from the method-of-moments modelling can be calculated using the following equation:

$$k_{tr} = k_{add} \frac{k_\beta}{k_{-\,add} + k_\beta} \qquad (4.1)$$

It is important to appreciate the fact that the Wang and Zhu model used here does not distinguish between k_{add} and $k_{-\beta}$ or between k_β and k_{-add}, as is shown in Scheme 4.3. Therefore, assumption is made that $k_\beta = k_{-add}$. Certainly for anything other than small radicals this assumption should be accurate. It leads to the following expression:

$$k_{tr} = k_{add} \frac{k_\beta}{k_\beta + k_\beta} = \frac{1}{2} k_{add} \quad (4.5)$$

Therefore C_{tr} values were calculated according to the equation 4.6.

$$C_{tr} = \frac{k_{add}}{2\, k_p} \quad (4.6)$$

Paradoxically, values of C_{tr} thus do not depend on k_β. Obtained values from method-of-moments modelling are listed in Table 4.25 alongside the values reported from the Mayo-equation modelling (i.e. from section 4.4.1).

The transfer constant values, C_{tr}, obtained from method-of-moments modelling are considerably larger when compared to the values estimated by using the Mayo approach that uses the number-average degree of polymerisation.[51] In principle one would expect the method of moments to give better estimates of parameter values, because it uses a wider variety of data taken from throughout the polymerisation, whereas the Mayo method uses only an average degree of polymerisation from zero conversion. However, one sees from Table 4.25 that the method of moments resulted in high values of C_{tr} that would suggest a high degree of living behaviour, contrary to what was observed.

Table 4.25 Comparison of the transfer constant, C_{tr}, values calculated based on the monomer conversion[A] and degree of polymerisation[B] data with the values reported in section 4.4.1.

Monomer	C_{tr}		
	Equation 4.6[58]	Equation 4.3[9]	Equation 4.4[51]
NIPMAM[C]	2857[A]	11.36	1.10
MAC[D]	1053[A] 16667[B]	1.30	1.02
HPMA[F]	16.95[A] 8.06[B]	4.37	3.94

[C]At 90 °C in DMF with $[NIPMAM]_0$=2.22 M; $[NIPMAM]_0$:$[AIBN]_0$:$[CPDB]_0$ = 200:1:2
[D]At 92 °C in toluene with $[MAC]_0$=5.55 M; $[MAC]_0$:$[AIBN]_0$:$[DTBA]_0$ = 400:1:2
[F]At 90 °C in DMF with $[HPMA]_0$=6.60 M; $[HPMA]_0$:$[AIBN]_0$:$[CPAD]_0$ = 200:1:2

So probably the Mayo values should be regarded as better estimates. This is not to dismiss the method of moments, because, as has already been discussed, it was difficult to use in that so many parameter values could be varied. The results reported here are not from an

optimised search, but rather are just parameter sets giving a good level of agreement. With the benefit of hindsight one could now go back to the computer and, using lower values of k_{add} as dictated by equation 4.6 and the Mayo estimates of C_{tr}, attempt more refined modelling.

Having said the above, it is important to remember that the Mayo method gives the value of C_{tr} at time zero, and thus it is the value for the RAFT agent itself. On the other hand, the modelling with the method of moments is over the entire course of polymerisation, for most of which one has macroRAFT agent rather than the starting RAFT agent. Thus this method should give C_{tr} for a macroRAFT agent. Given that the leaving group R of a RAFT agent will usually be quite different to the polymeric leaving group of a macroRAFT agent, one should actually not expect these two estimates of C_{tr} to be the same. That said, one should not expect the different to be too large. Given this, the HPMA values above look to be in very acceptable agreement, which yet again endorses the feeling that some RAFT behaviour was obtained in these systems.

Another point is that the Mayo estimates of C_{tr} were obtained from molecular weight data, and thus they should be compared with the method-of-moments values from fitting of MWD data, i.e., the 'B' values above (as explained, the 'A' values are from fitting of conversion-time data). Thus the agreement between HPMA values becomes even more acceptable.

On the other hand, the above two points do nothing to rescue the situation with regard to the MAC and NIPMAM results. Thus it would seem that these systems were probably far from RAFT-like in behaviour. The kinetic analyses have at least been useful in establishing that this is most likely the case.

To conclude this section one can say that it has been seen that kinetic analyses play a useful role in understanding RAFT polymerisations better.

4.5 Conclusion

RAFT polymerisation is gaining its popularity as its versatile nature allows its application in preparation of polymers of various functionalities and properties. The method promises

good control over the molecular weight of polymers and can be used at various reaction conditions.

In this work the use of the RAFT process in preparation of a polymeric precursor of a polymeric drug carrier, a copolymer of *N*-(2-hydroxypropyl)methacrylamide (HPMA), was attempted. The method was used in polymerisation of a number of monomers, such as methyl methacrylate (MMA), methacryloyloxy succinimide (MAOS), methacryloyl chloride (MAC), *p*-nitrophenyl methacrylate (NPMA), *N*-(2-hydroxypropyl)methacrylamide (HPMA) and *N*-isopropylmethacrylamide (NIPMAM). A number of RAFT agents were used in the synthesis in the hope that good control over the molecular weight of polymer chains could be achieved. However, most results obtained were not positive as the systems showed very little or no control. The modelling of the kinetic data supports the conclusion that for all the monomers under this investigation a higher degree of control is required.

One of the most important results of this work however, comes from the measurement of number-average molecular weight of PMAOS prepared by conventional free-radical polymerisation. Here MAOS is polymerised with AIBN alone, producing polymer with M_n of 39226. This result suggests that despite all efforts, the M_n of 39226 will be an upper limit for living polymerisation of MAOS under like conditions.

Two kinetic models were used in modelling the RAFT process. Discrepancy observed between values of chain transfer constant, C_{tr}, obtained by different methods based on the models, point at the necessity of careful consideration of major assumptions that are present in the kinetic models. As there is an ongoing debate in scientific community about the true nature of kinetic processes involved in the RAFT polymerisation, the results here once again show that a better understanding of the processes involved is required if polymerisation systems with better control of molecular weights are to be designed. Such knowledge is crucial when new transfer agents are being designed as improved RAFT agents should have appropriate leaving groups which will allow the preferential fragmentation of the group and its radical's ability to propagate by reacting with a monomer.

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Chapter Five. Preparation of a polymeric precursor of a *polymer therapeutic*

5.1 Introduction

The overall aim of this research was to identify the best way of preparing a polymeric precursor of PK1 of required molecular weight. PK1 (*N*-(2-hydroxypropyl)methacrylamide) (HPMA) copolymer doxorubicin (PK1, FCE28068)[1] (Figure 5.1) is an example of a *polymer therapeutic*. The use of water-soluble macromolecules as drug carriers has been shown to improve tumour targeting and result in better accumulation of drugs in tumour tissues.[2] Eleven different polymer-drug conjugates have entered phase I and II clinical trials in the last decade.[3] The promising results of their clinical applications stimulated further research in the area of the development of polymer therapeutics.[4] The term polymer therapeutics today is used to describe a range of polymer-based therapeutics such as polymeric drugs[5], polymer-drug conjugates[6], polymeric micelles[7] and polymer-protein conjugates[8]. Some of the examples of polymer therapeutics are the abovementioned PK1, HPMA copolymer-paclitaxel (PNU 166945) and HPMA copolymer doxorubicin-galactosamine (PK2, FCE28069).[4]

To meet strict clinical and pharmaceutical requirements for the new therapeutics, the polymeric component of a drug must be well-characterised and have well-established properties. For a polymer to meet such stringent specifications, it has to be of a certain molecular weight. However, the reality is such that it is virtually impossible to prepare polymeric chains of uniform size.

Current methods of the synthesis of the copolymer involve copolymerisation of HPMA and a functionalised linker under conditions of conventional free-radical polymerisation. In 1970s precipitation fractionation used to be carried out in an attempt to isolate relatively monodisperse copolymer fractions of desired molecular weight.[9] Today fractionation of the resulting copolymer is performed by semi-preparative chromatography, using the same GPC columns that are used in the analysis of the copolymer.[10] The polymeric product is fractionated according to molar mass, which

allows the isolation of a relatively monodisperse fraction containing the copolymer of required molecular weight.

While the procedure described above results in production of the copolymer of the required molecular weight and size, it is clear that in order to produce significant amount of the product, large amounts of the residual copolymer species of various size will not find their use and will be discarded. Further, this process also wastes time and energy in that it involves fractionation steps that could be done away with if monodisperse polymer could be directly synthesised. So the current approach cannot be labelled as an efficient one, especially on a large scale production of the copolymer such as in a pharmaceutical industry. Hence, the aim of this research was to find an efficient way of preparing a copolymer of HPMA of the required molecular weight with a possibility of the method being successfully upscaled.

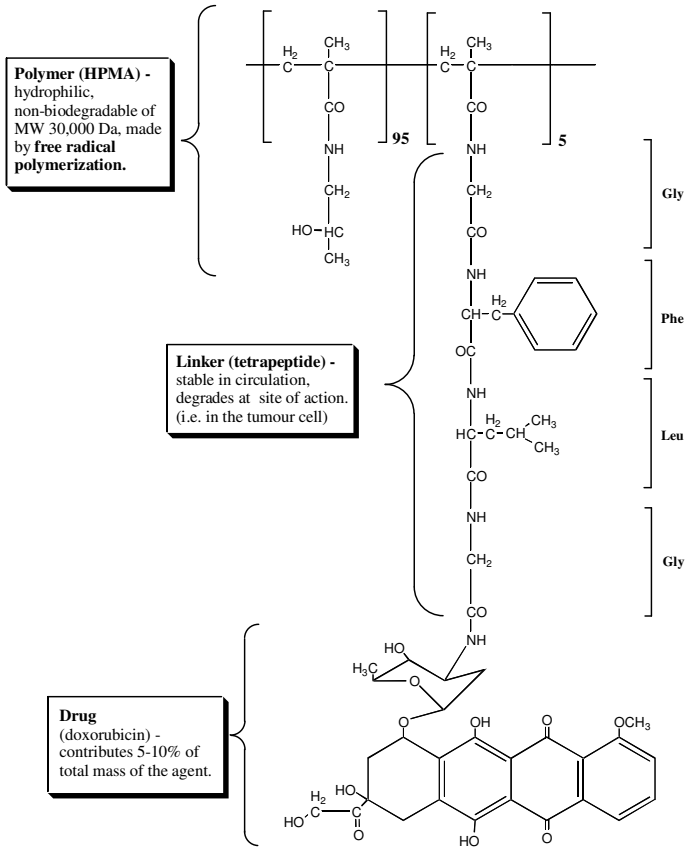

Figure 5.1 The prototypal polymer therapeutic, PK1, consisting of HPMA (*N*-(2-hydroxypropyl)methacrylamide) copolymer with doxorubicin conjugate.

Theoretically there are a number of ways in which such copolymer can be prepared.

(1) One way is to synthesise the copolymer directly from the monomers by means of controlled/"living" free-radical polymerisation in order to obtain the product of the required size. However, the success of the earlier attempts to employ the method of atom transfer radical polymerisation (ATRP) in preparation of poly(methacrylamides) proved to be somewhat doubtful. While the use of a novel initiating system allowed achievement of the linear increase of molecular weight with conversion, ATRP of HPMA in ethanol resulted in incomplete conversion of the monomer and production of polymer chains of degree of polymerisation higher than predicted.[11] The authors saw the limited monomer conversion as a result of the inactivation of the catalyst that can occur in ATRP.

(2) An alternative approach to preparation of PHPMA was suggested by Godwin *et al.*[12] The authors suggested synthesis of a polymer of an activated ester, methacryloyloxy succinimide (MAOS), with the consequent quantitative conversion of the resulting polymer into the polymer of HPMA. If controlled polymerisation of a monomer is successful and allows the preparation of the polymer of the required number-average molecular weight and low polydispersity, then such a polymer can serve as a precursor for preparation of a library of functionalised polymers with the same molecular weight characteristics.

With the second approach in mind, the use of methacryloyloxy succinimide was suggested and its successful polymerisation under the conditions of controlled polymerisation became a major aim of this research. Other types of monomer were also considered such as *p*-nitrophenyl methacrylate (NPMA) and methacryloyl chloride (MAC). Their polymers were prepared using the reversible addition-fragmentation (chain) transfer polymerisation method. The resulting polymers were then reacted with 1-amino-2-propanol (1A2P) in an attempt to prepare a homopolymer of HPMA, as exemplified in Figure 5.2.

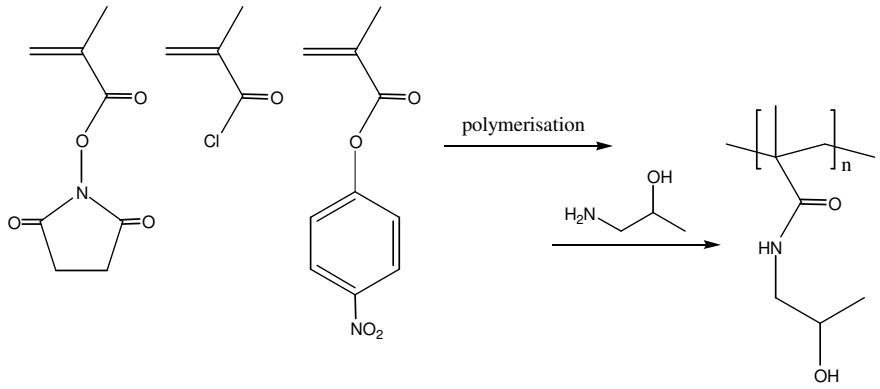

Figure 5.2 Proposed synthesis of PHPMA from polymer of MAOS, MAC and NPMA.

Preparation of random copolymers of HPMA with *N*-methacryloylglycylglycine *p*-nitrophenyl ester (MA-Gly-Gly-ONp) was reported in the literature and was done by radical precipitation polymerisation in acetone.[13] Copolymers were characterised by UV-vis spectrophotometry by calculating the content of *p*-nitrophenyl groups in the chain based on absorbance measurements conducted in DMSO.

In this work an attempt to employ RAFT polymerisation in direct synthesis of PHPMA[14] (discussed in Chapter Four) gave promising results. This success argued against the use of a functionalised intermediate and instead the direct use of HPMA with the established reaction conditions in preparation of the HPMA-model linker copolymer was attempted. For this purpose the copolymer of HPMA with MA-Gly-Gly-ONp was prepared by the RAFT polymerisation at 70 °C in aqueous acetic buffer. The resulting product was analysed by UV-vis in DMF and DMSO.

The results of this work are presented and discussed in this chapter.

5.2 Experimental details

5.2.1 Synthesis of HPMA homopolymer by conjugation of 1-amino-2-propanol to the polymeric precursors

The reaction was attempted at various conditions, i.e. three different temperatures and three different amounts of the amine were used in the aminolysis of the polymers.

5.2.1.1 From PMAOS

Polymer of MAOS was prepared by the method of RAFT polymerisation of MAOS mediated by CPDB at 80 °C in DMF; full experimental procedure is described in section 4.2.2.1.2. The aminolysis procedure is based on that in reference 12 but is different in that the reported reaction was conducted at 50 °C for 16 hours. The conditions used in this work are listed in Table 5.2.

To polymer of MAOS (1.64 mmol of reactive groups, 0.30 g) in DMF (3 mL) was added 1-amino-2-propanol (1.1 eq, 2 eq or 5 eq) dropwise under vigorous stirring at 0 °C. The reaction mixture was allowed to reach room temperature and then was heated to the required temperature. The reaction mixture was then cooled to room temperature and precipitated in acetone (25 mL). The product was isolated as colourless solid.

5.2.1.2 From PMAC

Polymer of MAC was prepared by conventional free-radical polymerisation of MAC at 90 °C in toluene; experimental procedure is described in section **2.2** The aminolysis procedure is based on that in reference 12, the reactions were conducted at 20, 30 and 70 °C for 5 days, 2 days and 4 hours respectively. The detailed conditions used in this work are listed in Table 5.3.

To a mixture containing polymer of MAC (1.64 mmol of reactive groups, 0.17 g) and pyridine (1.5 eq, 0.20 mL, 2.46 mmol) in DMF (3 mL) was added 1-amino-2-propanol (1.1 eq, 2 eq or 5 eq) dropwise under vigorous stirring at 0 °C. The reaction mixture was allowed to reach room temperature and then was heated to the required temperature. After the completion of the reaction the reaction mixture was then cooled to room temperature, filtered and the residue was precipitated in acetone (25 mL).The product was isolated as colourless hygroscopic solid.

5.2.1.3 From PNPMA

Polymer of NPMA was prepared by conventional free-radical polymerisation of NPMA at 90 °C in toluene; experimental procedure is described in section **2.2**. The aminolysis

procedure is based on that in reference 12, the reactions were conducted at 20, 30 and 70 °C for 5 days, 2 days and 4 hours respectively.

To polymer of NPMA (1.64 mmol of reactive groups, 0.34 g) in DMF (3 mL) was added 1-amino-2-propanol (1.1 eq, 2 eq or 5 eq) dropwise under vigorous stirring at 0 °C. The reaction mixture was allowed to reach room temperature and then was heated to the required temperature. The reaction mixture was cooled to room temperature and precipitated in acetone (25 mL). No product was isolated; ^{1}H NMR analysis confirmed presence of unreacted PNPMA.

5.2.2 Copolymerisation of HPMA and MA-Gly-Gly-ONp under RAFT conditions

N-Methacryloylglycylglycine *p*-nitrophenyl ester (MA-Gly-Gly-ONp) was used as a model linker in an attempt to prepare HPMA copolymer under conditions of the RAFT process. The linker was prepared according to the reference [15], the details of the synthesis can be found in 2.3.14 of this thesis.

While MA-Gly-Gly-ONp and HPMA have been copolymerised under conditions of conventional free-radical polymerisation before,[13] there are no reports on the use of the method of RAFT in the literature. Polymerisation procedure used here is based on that reported by Scales *et al.*[14] but is different in that the initial concentration of the initiator was increased and the reaction involved copolymerisation of two monomers.

HPMA (0.21 g, 1.47 mmol), MA-Gly-Gly-ONp (8.0 mg, 25 μmol), 4,4'-azobis(cyanovaleric acid) (V-501) (2.2 mg, 7.8 μmol) and 4-cyanopentanoic acid dithiobenzoate (CAPD) (4.3 mg, 15 μmol) were dissolved in aqueous acetic buffer (1 mL, 0.27 mol L^{-1} acetic acid and 0.73 mol L^{-1} sodium acetate) and the solution was placed in a two-neck round-bottomed flask with magnetic stir bar. The mixture was placed under argon, and subjected to three "freeze-pump-thaw" cycles to assure minimal presence of oxygen. The flask was placed in an oil bath at constant temperature of 70 °C to start polymerisation.

After 24 hours the product was precipitated with acetone, filtered and placed in a dry, pre-weighed vial. The product was dried under high vacuum overnight and re-weighed, allowing determination of the weight of formed copolymer.

Yield: 22.5 mg, 10%

5.3 Results and discussion

5.3.1 Preparation of PHPMA from polymeric precursors by aminolysis

5.3.1.1 From PMAOS

Preparation of PHPMA from PMAOS was reported by Godwin *et al.*[16] The authors reported successful conjugation of 1-amino-2-propanol to PMAOS at 50 °C, claiming only trace levels of competitive hydrolysis were observed. It was also stated that possible competitive imide formation can be avoided at the reaction conditions used. More recent work on conjugation of 1-amino-2-propanol to PMAOS suggested little or no imide formation when amine conjugation was conducted at reaction temperatures of 50-60 °C.[17] However, in much earlier work Ringsdorf claimed observing formation of insoluble cross-linked polymers when the reaction was conducted at temperatures above 40 °C.[18]

Table 5.1 lists the aminolysis reaction conditions used by various research groups in an attempt to prepare poly(amide)(s) from PMAOS by means of aminolysis reaction.

Table 5.1 Reported reaction conditions used in aminolysis of PMAOS prepared by ATRP.

Temp., °C	Amine (equivalents)	Reaction time	Reference	Solvent	Method of analysis
50-60 followed by ambient *T*	Trimethylammonium, Dimethylamine (4 eq)	~ 16 h	Pedone *et al.*[17]	DMSO	FT-IR, ^{1}H NMR
50	Benzylamine (2 eq)	5 h	Monge *et al.*[19]	DMSO	FT-IR, ^{1}H NMR
50	1-Amino-2-propanol (2 eq)	1.25 h	Godwin *et al.*[16]	DMSO	FT-IR, ^{1}H NMR

In an attempt to clarify the ambiguity, a series of experiments were conducted with 1.1 equivalents of 1-amino-2-propanol used at different temperatures. The experiments resulted in isolation of colourless hygroscopic solid which was analysed by ^{1}H NMR. However, the ^{1}H NMR analysis of the isolated polymeric products showed unexpected

signals, including the one at 2.5 ppm (see Figure 5.3). The signals did not belong to either starting material or to the expected product. Similar results were obtained by other researchers working with the polymer of methacryloyloxy succinimide.[20]

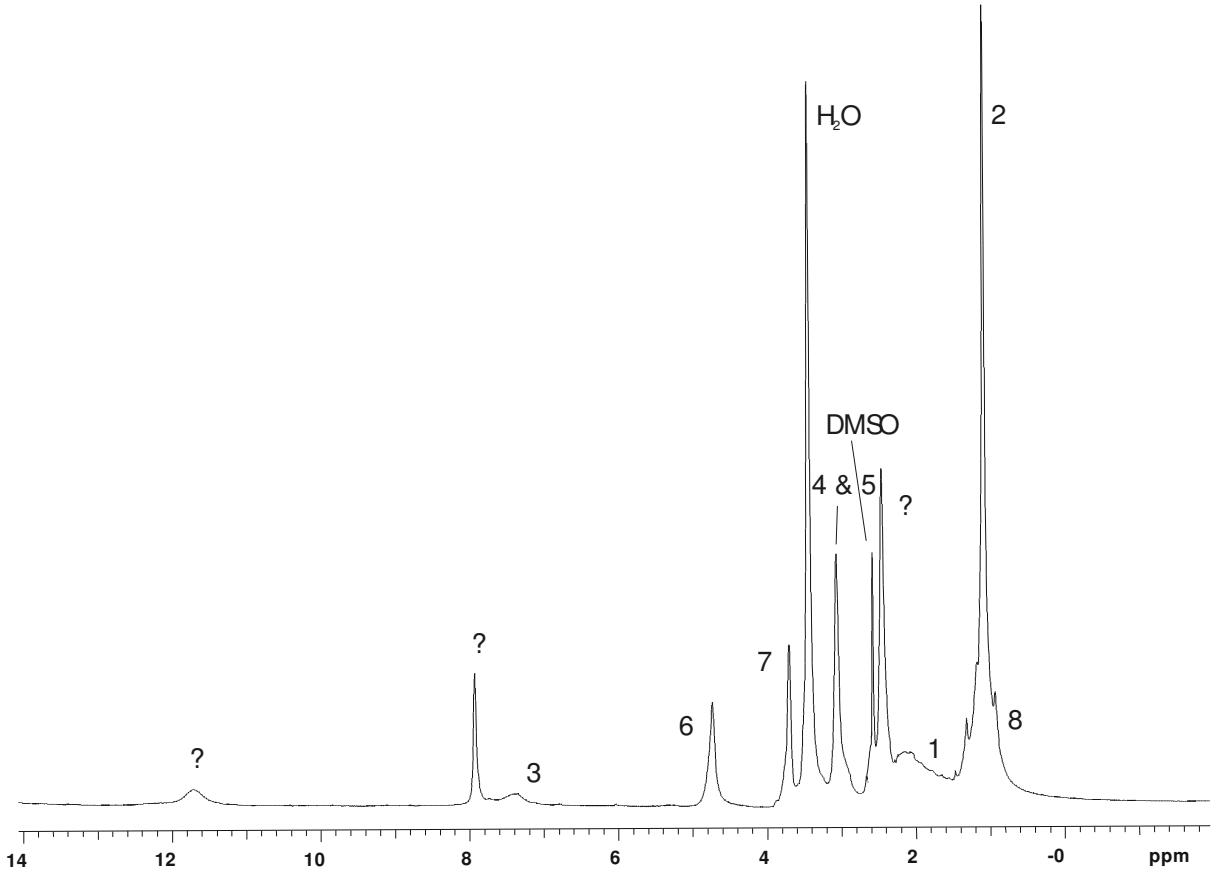

Figure 5.3 ^{1}H NMR spectrum of the polymeric product (d_6-DMSO) obtained in aminolysis reaction of PMAOS with 1A2P (5 eq) at 30 °C.

In an extensive investigation conducted by Devenish *et al*. the authors established that the reaction of the PMAOS with 1-amino-2-propanol resulted in only partial displacement of *N*-hydroxysuccinimide moiety, with the rest of the rings being opened as a result of attack by 1-amino-2-propanol at an imide carbonyl of the *N*-hydroxysuccinimide. The proposed structure of the resulting copolymer is shown in Figure 5.4 and the signal at 2.5 ppm (Figure 5.3) is believed to correspond to protons in the two CH_2 groups indicated in the scheme by arrows.[21] All remaining signals shown in the 1H NMR spectrum in Figure 5.3 were also assigned.

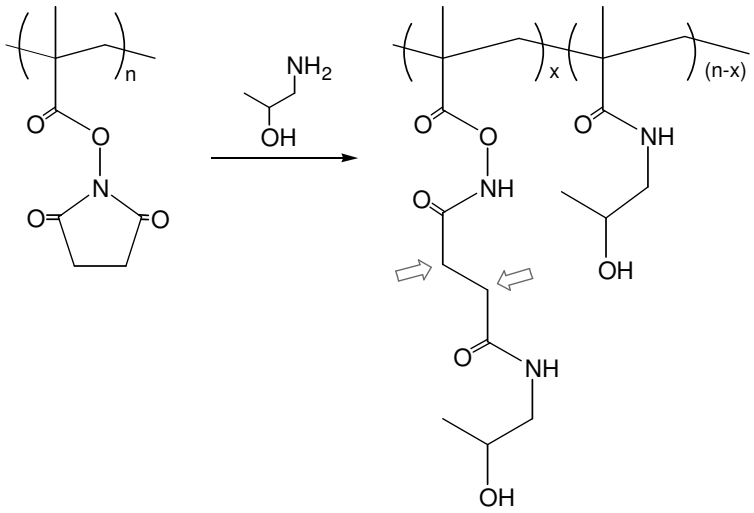

Figure 5.4 Proposed structure of the product of aminolysis of PMAOS.

In an attempt to establish optimal reaction conditions which will ensure quantitative aminolyses of PMAOS and production of PHPMA, the experiments were repeated at various ratios of the amine to the polymer and different temperatures. The reaction conditions are summarised in Table 5.2.

Table 5.2 Conditions of the attempted synthesis of PHPMA from PMAOS

Temperature, °C	1A2P equivalents	Reaction time	Product yield, %
22	1.1	5 days	74
	2.0		69
	5.0		75
30	1.1	2 days	71
	2.0		77
	5.0		64
70	1.1	4 hours	82
	2.0		75
	5.0		75

Despite the use of lowered temperatures and higher amounts of the amine, which are believed to favour higher conversion into PHMA, quantitative yield of this desired final product was not obtained in any of the experiments: ^{1}H NMR analysis of the water-soluble products isolated always showed presence of the extra signal that is believed to be an indication of the opened ring of *N*-hydroxysuccinimide. Thus in this work there is no

evidence that lower temperature and larger amounts of 1A2P promote the formation of PHPMA, however the non-quantitative nature of the present work means that these possible effects cannot be excluded.

In their work Hill *et al* have shown that a complete displacement of the *N*-hydroxysuccinimide groups is possible however under the reported conditions another side reaction took place, resulting in formation water-insoluble polymer rather than pure HPMA homopolymer.[20,21]

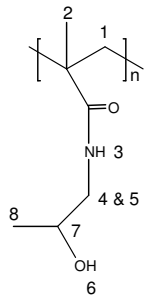

Figure 5.5 Structure of PHPMA, the expected polymer from aminolysis of PMAOS, with numbers corresponding to the signals of Figure 5.3.

Direct synthesis of poly(amides) under conditions of the ATRP has been labelled not suitable as the amide group is capable of inactivating the copper catalyst by complexing the copper.[22,23] Published results suggested that aminolysis of the polymer of MAOS of narrow polydispersity (prepared by the ATRP) is a way of producing PHPMA of targeted molecular weight. The results of this work however suggest that the proposed conversion of the polymer of MAOS into PHPMA is not as straightforward as it might seem.

It appears that the use of similar to the literature reaction conditions resulted in production of unexpected polymeric product every time aminolysis of PMAOS with 1A2P was attempted. In this work ^{1}H NMR analysis was used in identifying product of the aminolysis. It is worth noting that Pedone *et al*. also used ^{1}H NMR, however, the authors state that the analysis only confirmed the absence of any *N*-hydroxysuccinimide (NHS) moieties, while it remains unclear whether the same analysis confirmed formation of the desired product, i.e. PHPMA.[17] FT-IR was also employed in an attempt to establish the presence of any remaining NHS groups bound to the polymer. Again, that analysis only confirmed the absence of NHS groups, rather than formation of PHPMA.[17]

Monge *et al*. appear to have used 2 equivalents of benzylamine in their attempt to prepare polyamide from PMAOS prepared by the ATRP.[19] They operated at 50 °C for 5 hours (see Table 5.1). Again ^{1}H NMR was used in analysis of product of the aminolysis reaction, while FT-IR was used to monitor the progress of the reaction. FT-IR has shown disappearance of the signal due to the carbonyl group of the activated ester during the reaction. This would occur regardless of the reaction pathways at issue, so it tells nothing to discriminate between the two. ^{1}H NMR was used in the analysis of the product and suggested formation of the expected polymer of *N*-benzyl methacrylamide, although that has been questioned.[20] When comparing these results to the ones obtained in this work, it is important to remember however, that Monge *et al*. have employed a different type of amine for their aminolysis reaction – this would appear to be the only point of difference to the present work.[19]

Quite possibly, the nature of amine, its amount and the temperature are crucial factors in achieving successful aminolyses of PMAOS. And while theoretically it is possible to find reaction conditions under which successful aminolyses of PMAOS can be achieved, it is not clear whether the conditions will be appropriate for their use on large scale if the method is ever to be employed in the pharmaceutical industry. In addition to that, it is worth noting that despite all the effort put into trying to obtain the polymer of the required molecular weight and narrow polydispersity, the ideally controlled polymerisation of MAOS has not yet been achieved.

It is clear however, that in order to understand the mechanism and establish optimal reaction conditions more thorough work is required in investigating the aminolysis of PMAOS reaction. It might be wise to carefully monitor the reaction in order to identify any possible reaction intermediates that could be forming under the reaction conditions. It is important to consider timescale on which the reaction should be performed in the hope of obtaining the desired product – longer reaction times may be necessary. ^{1}H NMR can serve as a powerful tool here since it can be used to easily identify signals that are due to the ring-opened product. By adjusting the reaction conditions and monitoring the reaction by ^{1}H NMR, one might find optimal conditions and achieve the minimal formation of the unwanted product and therefore meet the final aim of preparing the polymer of HPMA from its polymeric precursor with the narrow molecular weight distribution.

5.3.1.2 From PMAC

Methacryloyl chloride (MAC) is a synthetic precursor of MAOS and HPMA.[24] In this work it was polymerised under conditions of controlled polymerisation in the hope of obtaining a monodisperse polymer material that can serve as a polymeric precursor for a library of functionalised polymers. It was assumed that the polymer will retain some of its monomer's reactivity which will allow an easy displacement of chloride and therefore synthesis of another polymer.

Table 5.3 Conditions of the synthesis of PHPMA from PMAC.

Temperature, °C	1A2P equivalents	Reaction time	Product yield, %
22	1.1	5 days	64
	2.0		70
	5.0		67
30	1.1	2 days	71
	2.0		81
	5.0		80
70	1.1	4 hours	69
	2.0		68
	5.0		54

In this work synthesis of PHPMA from PMAC was attempted at a number of different reaction conditions (Table 5.3). All experiments resulted in formation of PHPMA, as was confirmed by ^{1}H NMR analysis of the isolated solids.

It is clear that higher reactivity of PMAC when compared with that of PMAOS can be responsible for such drastic difference in the results obtained in an attempt to use the polymers as polymeric precursors. In addition to that, one has to recognise the more complex structure of the activated ester, the functionality of which provides more room for unwanted side-reactions, i.e. ring opening as a result of the amine addition that was discussed in section 5.3.1.1.

If successful preparation of PMAC under conditions of controlled free-radical polymerisation can be achieved, then the polymer can be quantitatively converted into a

polymer of interest under relatively simple and cost-effective conditions, i.e. low reaction temperature and low concentration of reacting amine or other nucleophile.

In this work it was shown that while ideal living behaviour was not displayed in RAFT of MAC systems, there was an obvious indication of some living character (see section 4.3.3), which suggests that work towards improving the control should be definitely carried out in the future.

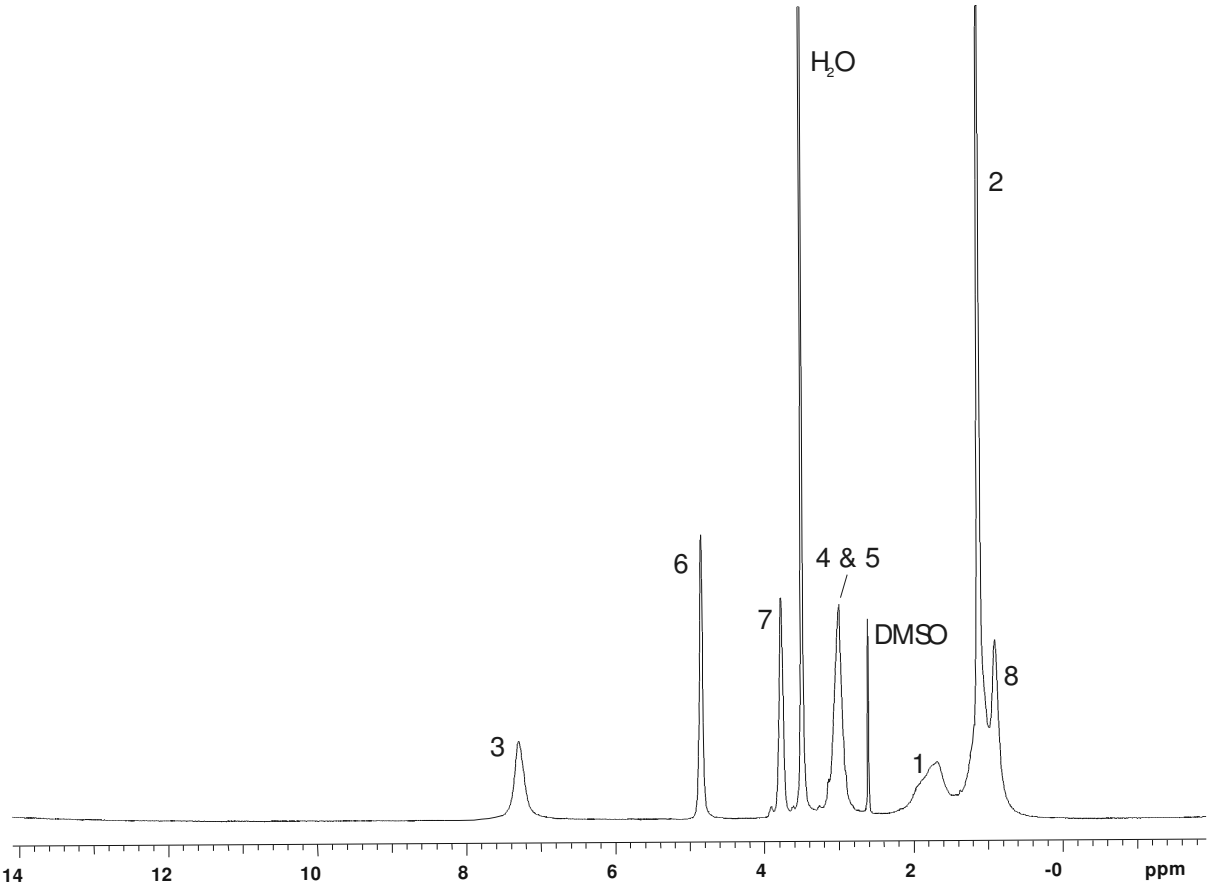

Figure 5.6 ^{1}H NMR spectrum of the polymer of HPMA prepared from the polymer of MAC (d_6-DMSO).

The successful outcome of the aminolysis work discussed in this section suggests that the use of PMAC as a polymeric precursor has a potential to become a starting point in creating a variety of functionalised polymers, based on the polymer of MAC of the same molecular weight.

This result is extremely important as it gives hope that synthesis of polymeric drug carriers of the required molecular weight is possible indeed. However, one has to recognise the unconventional nature of MAC as monomer, and carefully consider all pros

and cons of using it on large scale. MAC itself and its polymer are prone to hydrolysis upon their exposure to air, and therefore precautions are to be taken when storing and preparing both the monomer and the polymer to minimise their contact with water.

5.3.1.3 From PNPMA

Preparation of polymer of *p*-nitrophenyl methacrylate (NPMA) was reported in the literature.[25] In the published work the kinetics of aminolysis of the polymer was also investigated.[25] However, it was unreasonable to use the reported conditions in this research as it required the use of 100 eq of amine relative to the amount of NPMA used in the reaction. The use of such a large amount of the amine would be considered to be highly unpractical on an industrial scale. For the purpose of a systematic investigation the reaction conditions (Table 5.2) that were used in aminolysis of PMAOS and PMAC were employed in aminolysis of PNPMA. However, none of the experiments resulted in formation of PHPMA. As was confirmed by ^{1}H NMR the reaction mixtures contained unreacted PNPMA.

Rejmanová *et al*. reported results of their investigation into the kinetics of aminolysis reaction of polymeric *p*-nitrophenyl esters of *N*-methacryloylamino acids.[25] The aim of that work was to evaluate the effect of the structure of the ester, amine, reaction medium and polymer backbone on the kinetic parameters governing the process. A number of *p*-nitrophenyl esters of *N*-methacryloylamino acids were prepared, polymerised and subjected to aminolysis reaction in DMSO at 25 °C. The progress of the reaction was monitored using UV-vis spectrophotometry by measuring the absorbance of the reaction mixture that was due to the *p*-nitrophenyl moiety present on polymer. The authors concluded that while the amine component shows strong influence on the reaction rate[26], in the aminolysis of PNPMA the hindering effect of polymer backbone became a deciding factor. It was reported that in PNPMA, due to the relatively small distance between the active ONp group and the actual polymer backbone, the latter causes some sort of hindering effect and hence slows down the aminolysis of the polymer. Such low rate of aminolysis was observed specifically for PNPMA when ammonia and *tert*-butylamine were used in the reaction.[25]

Taking this into consideration, it is possible to suggest that the aminolysis of PNPMA with 1A2P does occur, however, it might be relatively slow even with 5 eq of 1A2P at 70 °C.

Rejmanová *et al*. conducted their experiments with much higher amount of amines and still reported observation of unusually slow rate of aminolysis that was specific to the polymer of NPMA only.[25]

Therefore, it is reasonable to suggest that future attempts to prepare PHPMA from PNPMA are to be conducted at much higher ratio of amine to the ester. However, even in a case of success, one again has to consider whether such approach is justified and cost-effective on a larger scale.

5.3.1.4 Summary

When comparing the aminolysis results obtained for the three different polymers, it is easy to see that PMAC is quite possibly the most promising of all due to its reactivity, which allows successful preparation of PHPMA under mild conditions. At this stage it is not clear whether reasonable rate could be achieved in the aminolysis of PNPMA; it is possible that a much higher concentration of amine will be required in order to achieve reasonable conversion of the polymer with time. Conversion of the polymer of MAOS into PHPMA has also proved to be a challenge rather than a trivial laboratory exercise. The rates of the aminolysis obtained at various conditions were reasonable, however, no desired product was obtained under the conditions employed, suggesting that fairly reactive PMAOS is undergoing unexpected ring-opening reaction rather than anticipated aminolysis.

Overall, these results show that successful conversion of these polymers into PHPMA is possible; however reaction conditions should be carefully adjusted for each polymer in order for the reactions to produce the desired product.

5.3.2 Copolymerisation of HPMA with a model linker

5.3.2.1 Background

PK1 contains a drug incorporated onto a polymer of HPMA. The drug is conjugated to the polymer via tetrapeptide linkers distributed along the polymer. Once the macromolecule is taken up by a cell, the linker is expected to be cleaved, releasing the drug.[13] The loading of the drug onto the polymer can be controlled via adjusting the amount of the linker incorporated into the chain.

There are a number of reasons why incorporation of a linker is necessary.

Firstly, presence of linker allows introduction of reactive groups into the polymer, which then can be relatively easily displaced when a drug is being attached to the polymer. For example, in the case of using PHPMA as the polymer backbone, one has to recognise that PHPMA contains hydroxyl groups which generally display weak reactivity.[25] Thus it is important to modify the structure of the polymer by introducing reactive groups into its structure. *p*-Nitrophenyl ester group (ONp) is believed to be fairly reactive and for that reason it is used in synthesis of peptides.[26,27] For the same reason its use in preparation of a PK1 precursor was suggested and utilised.[13] Presence of the ONp groups in the polymer structure is expected to make the chemistry of drug attachment easier.

Loading of a drug onto a polymer is quite an important issue as the amount of the drug will determine the overall potency of the polymer-drug conjugate. By introducing a certain amount of a linker into the basic polymer backbone, one can achieve selective placement of the drug onto the polymer specifically via reactive group of the linker. Therefore, ability to control the amount of the linker being incorporated into the polymer chain is crucial here.

Such control can be achieved in two ways: a monomer and a linker can be copolymerised in required stoichiometric ratio or homopolymerisation of a monomer can be followed by quantitative linker attachment. Both ways should result in preparation of a copolymer that consists of unreactive HPMA and reactive linker units, with the latter used for drug attachment. In this work it was decided to use the first approach and attempt the copolymerisation of HPMA and Ma-Gly-Gly-ONp. It is noted that Godwin *et al*. used the second approach to attach a different Gly-Gly dipeptide, with quantitative FT-IR apparently confirming quantitative attachment to PMAOS.[16]

Ma-Gly-Gly-ONp was chosen as a model linker, its copolymerisation with HPMA was reported in the literature.[13] Etrych *et al* reported successful reaction of the resulting copolymer with excess of hydrazine monohydrate (relative to ONp), as a result of which they created the hydrazone linkage for conjugating a drug.

In this work reactivity of ONp groups in their polymeric form was tested when a polymer of *p*-nitrophenyl methacrylate (NPMA) was subjected to aminolysis reaction under various conditions (section 5.2.1.3). The attempts were unsuccessful as no formation of

poly(amide) was observed and only the starting polymer was recovered from the reaction mixture. This result could have made the claims about reasonable reactivity of employed ONp groups sound questionable, as a reason for using them is to be able to displace them under mild conditions and functionalise the polymer. The results of this work did not show that was the case for PNPMA. However, it was found that the longer the distance between the ONp group and the polymer backbone, the easier it is for the displacement to occur. Rejmanová *et al*. showed that aminolysis of the polymer of Ma-Gly-Gly-ONp was fast and quantitative, suggesting that use of Ma-Gly-Gly-ONp as a linker in a polymer therapeutic will allow successful attachment of a drug as is desired. Further, reaction involving ONp in order to attach a drug is not the same as aminolysis of PNPMA, so it is obviously possible that the former reaction might proceed but the latter not.

Statistical copolymers of HPMA and the linker have been prepared in the literature by radical precipitation polymerisation in acetone with 0.6 wt.-% AIBN at 50 °C for 24 hours.[13] Copolymers were analysed by UV-vis spectrophotometry and GPC and fractions containing copolymers of the targeted molecular weight were utilised in further work.

In an attempt to utilise a new method of "living" polymerisation, copolymerisation of HPMA and MA-Gly-Gly-ONp was done under conditions of the RAFT polymerisation.

Our previous attempts to polymerise HPMA under RAFT conditions were unsuccessful in that a complete monomer conversion was never achieved when the polymerisation was done in DMF. Scales *et al*. reported successful RAFT polymerisation of HPMA conducted in aqueous acetic buffer solution.[14] Thus in this work RAFT polymerisation of HPMA was carried out using reported aqueous acetic buffer solution as the polymerisation medium instead of previously used DMF. The results of this work show increase in molecular weight with monomer conversion and they are discussed in full detail in chapter four of this thesis (see section 4.3.5).

Here the reaction conditions reported by Scales *et al.*[14] were adjusted, i.e. initial concentration of initiator in the reaction mixture was increased and used in copolymerisation of HPMA and Ma-Gly-Gly-ONp.

5.3.2.2 Present investigation

Since suggested use of aqueous acetic buffer solution as polymerisation medium proved to enhance the living character of the RAFT polymerisation of HPMA (as has been shown by Scales *et al.*[14] and by this research), it was decided to employ the solution as the copolymerisation medium as well.

Copolymerisation was done in aqueous acetic buffer solution (1 mL of 0.27 mol L^{-1} acetic acid and 0.73 mol L^{-1} sodium acetate)[14] at 70 °C. The following ratio of reactants was used, $[HPMA]_0:[MA\text{-}Gly\text{-}Gly\text{-}ONp]_0:[V\text{-}501]_0:[CPAD]_0$ = 200:3:1:2. The polymerisation (exemplified in Figure 5.7) was allowed to proceed for 25 hours, then acetone was dropwise added to the mixture and the precipitated product was isolated as pink solid.

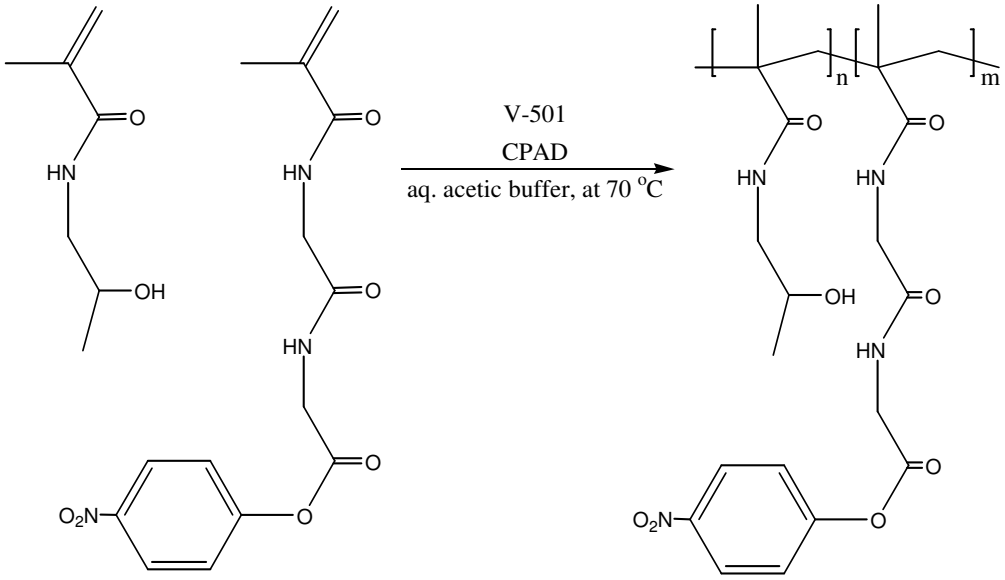

Figure 5.7 Copolymerisation of HPMA and MA-Gly-Gly-ONp under RAFT conditions; $[HPMA]_0:[MA\text{-}Gly\text{-}Gly\text{-}ONp]_0:[V\text{-}501]_0:[CPAD]_0$ = 200:3:1:2.

Despite the long reaction time, the product of the copolymerisation was isolated in a yield of 10%. Other research groups possibly have achieved higher yields when the copolymer was prepared by the conventional method.[13] Huang *et al.*[28] report the copolymer yield of 66.6%, which suggests that conditions of the RAFT copolymerisation attempted here could be optimised. However, due to time constrains the conditions were not optimised, hence seeking the RAFT copolymerisation conditions giving higher yield of the copolymer will be an important part of future work.

The prepared copolymer was characterised by UV spectrophotometry in DMF and DMSO. The content of *p*-nitroxyphenol-terminated dipeptide linker (MA-Gly-Gly-ONp) was measured at λ = 318 nm in DMF and at λ = 274 nm in DMSO.

Figure 5.8 shows UV-vis absorption spectrum of the copolymer in DMSO.

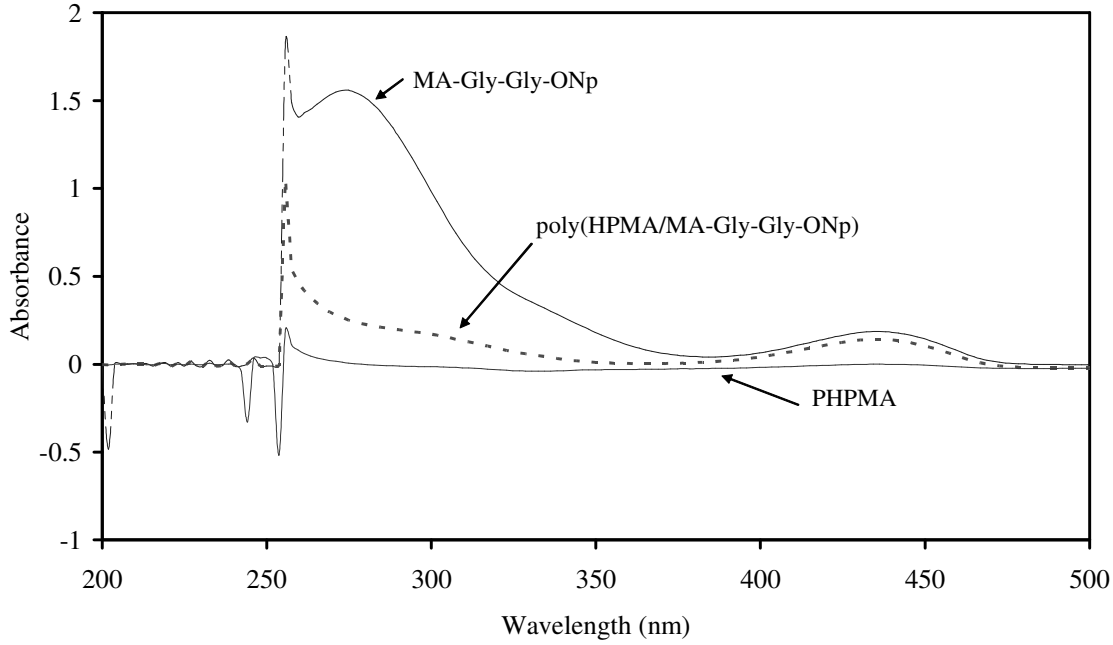

Figure 5.8 UV-vis spectra of MA-Gly-Gly-ONp, PHPMA and the copolymer measured in DMSO at λ = 274 nm.

UV-vis absorbance of MA-Gly-Gly-ONp of known concentration was measured at λ = 274 nm, from that the molar absorbtivity value of MA-Gly-Gly-ONp in DMSO, ε (L mol^{-1} cm^{-1}), was calculated using Beer's Law:

$$A = \varepsilon\, c\, l \qquad (5.7)$$

where A is absorbance, c is the concentration of the compound in solution, expressed in mol L^{-1}, and l is the path length of the sample, that is, the path length of the cell in which the sample is contained, normally expressed in centimetres. Calculated values are listed in Table 5.4.

A homopolymer of HPMA was prepared under the same RAFT polymerisation conditions that were used in preparation of the copolymer, UV-vis absorption spectrum of the polymer was also recorded and it showed no absorption at the wavelength of 274 nm.

When the spectrum of the copolymer was recorded, the assumption was made that the absorbance measured at λ = 274 nm was due to the presence of the incorporated *p*-nitroxyphenol-terminated dipeptide linker. Concentration of the nitrophenol groups in the sample was calculated, allowing estimation of the mole percentage of the dipeptide linker in the copolymer. Figure 5.9 contains the UV-vis absorption spectra of MA-Gly-Gly-ONp, PHPMA and the copolymer dissolved in DMF.

Table 5.4 Calculated ε values for UV-vis absorbance of MA-Gly-Gly-ONp in DMSO and in DMF.

Parameter	For measurements done in	
	DMSO	DMF
Concentration, M	2.86×10^{-4}	4.86×10^{-5}
A	1.56	0.40
λ, nm	274[13]	318[29]
ε, L mol^{-1} cm^{-1} (calculated)	5445	8222

Absorbance measurements in DMSO and DMF were conducted at different wavelengths. The molar absorbtivity values of MA-Gly-Gly-ONp in DMSO and DMF, ε (L mol^{-1} cm^{-1}), were determined by measuring the absorbance of the solution of the linker of known concentration at λ = 274 nm[13] and λ = 318 nm[29] respectively. These values were used in further calculations.

Table 5.5 The UV-vis absorption data for poly-HPMA-MA-Gly-Gly-ONp.

Parameter	For measurements done in	
	DMSO	DMF
A	0.25	0.04
λ, nm	274[13]	318[29]
[MA-Gly-Gly-ONp] in copolymer, M	4.59×10^{-5}	5.7×10^{-6}
Mass [MA-Gly-Gly-ONp] in copolymer, g	7.38×10^{-5}	8.5×10^{-6}
mol% [MA-Gly-Gly-ONp] in copolymer	0.62%	0.07%
mol% [MA-Gly-Gly-ONp] in copolymer, expected	1.50%	

Results obtained are summarised in Table 5.5. The calculated mole percentage of *p*-nitroxyphenol-terminated dipeptide linker in the product of the copolymerisation based on the measurements done in DMSO and DMF was 0.62 and 0.07% respectively while the molar ratio of components in the polymerisation mixtures was aimed to achieve 1.50%

mole percentage of the linker (assuming ideal copolymerisation, i.e. both reactivity ratios are 1).

The absorbance measurements of the same polymeric product in two different solvents produced two different results when molar percentage of the MA-Gly-Gly-ONp in the product was estimated based on the measurements. The measurements conducted in DMSO gave value of 0.62 mol% of the linker, while the same measurements done in DMF, produced value of 0.07 mol%.

It is not clear why there is such drastic difference between the two values. Had the measurements been done in DMSO alone, one could suggest successful incorporation of the linker in the polymer of HPMA. Had the measurements been done in DMF only, the value of 0.07 mol% compared with the expected 1.50 mol% would have suggested the failure of the copolymerisation.

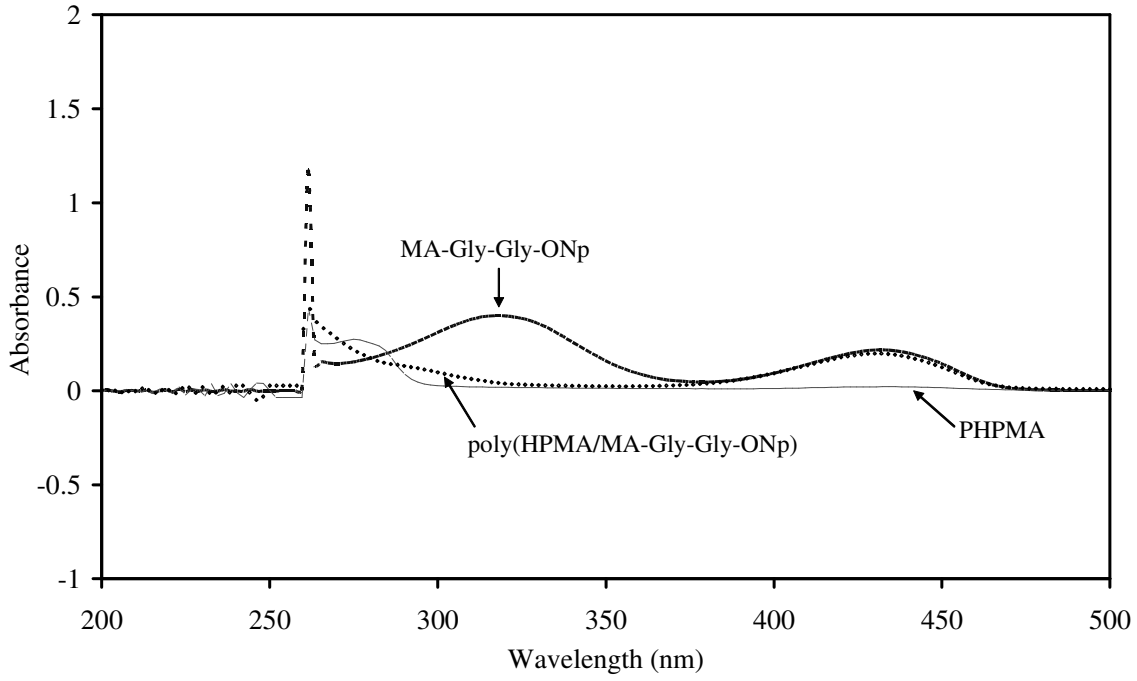

Figure 5.9 UV-vis spectra of MA-Gly-Gly-ONp, PHPMA and the copolymer measured in DMF at λ = 318 nm.

Similar measurements reported in literature were normally done in DMSO.[13,28] And while it is tempting to assume that the result of this work obtained in DMSO is the correct one, as it is indicative of successful copolymerisation, one has to remain sceptical and

recognise the fact that further investigations are required before any definite conclusion can be made.

In future work the measurements should also be attempted in other solvents. Copolymers with varying content of the linker should be prepared and analysed, as results obtained there would show if there is any relationship between the measured mol% of the linker and the initial concentration of the latter in copolymerisation mixture. One could also think of using NMR or combustion analysis, the two most commonly used techniques for measuring copolymer composition (what is being done here).

5.4 Conclusion

Use of anticancer agents is known to cause a number of side effects due to the toxicity associated with the drugs. There is an attempt to utilise polymer therapeutics as one of the solutions to the problem. The polymer drug conjugate offers the advantages of improved solubility, increased half-life and improved tumour targeting. It has been shown that HPMA copolymers can adequately perform as drug vectors and currently various polymer therapeutics based on HPMA are being evaluated.[30]

Synthetic polymers have great potential as theoretically their properties and size can be tailored. In this work preparation of HPMA polymer from three different polymeric precursors was attempted. The precursors PMAOS, PMAC and PNPMA were prepared by means of controlled free radical polymerisation and reacted with 1-amino-2-propanol (1A2P) in the hope of producing polymer of HPMA. However, the reaction of PMAOS with 1A2P resulted in occurrence of unforeseen reaction as a result of which a polymer with ring-opened succinimide moiety was isolated. Reaction of PNPMA with the amine did not produce the desired result either as in every attempt only starting material was recovered. However, the reaction of PMAC with 1A2P did yield a polymer of HPMA under mild reaction conditions with the minimal amount of the amine and the product was confirmed by ^{1}H NMR.

Copolymerisation of HPMA and MA-Gly-Gly-ONp under conditions of RAFT polymerisation resulted in isolation of polymeric product. The product was analysed by UV-vis spectrophotometry and the content of *p*-nitroxyphenol-terminated dipeptide linker (MA-Gly-Gly-ONp) was measured in DMF and DMSO as 0.07 and 0.62% respectively

while the target value was 1.50% molar percentage of the linker. The success of this effort is thus uncertain. It should thus be treated as a promising start in the use of RAFT polymerisation in preparation of HPMA copolymers which can find use in various medicinal applications.

5.5 References

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Chapter Six. Conclusion

The overall aim of this research was to identify the best way of preparing a polymeric precursor of PK1 by LFRP. Ideally, the method should allow successful copolymerisation of HPMA and a linker to give a copolymer of required molecular weight. The technique should also assure incorporation of a desired amount of linker in the polymer backbone. An even distribution of the linker alongside the polymeric carrier is believed to allow a better drug distribution in the polymer therapeutic.

Thus here an attempt was made to prepare a polymer of HPMA of target molecular weight by the RAFT polymerisation method. The RAFT method was also employed in polymerisation of *N*-isopropylmethacrylamide (NIPMAM), which was used as a non-hydroxyl-functionalized analogue of HPMA. Promising results were obtained in employing RAFT polymerisation in direct synthesis of PHPMA.

This prompted the use of the established RAFT conditions in preparation of an HPMA-model linker (MA-Gly-Gly-ONp) copolymer. This resulted in isolation of polymeric product. The product was analysed by UV-vis spectrophotometry and the content of *p*-nitroxyphenol-terminated dipeptide linker (MA-Gly-Gly-ONp) was measured in DMF and DMSO as 0.07 and 0.62% respectively while the target value was 1.50% molar percentage of the linker. Thus this result was ambiguous. It should therefore be treated as a promising start in the use of RAFT polymerisation in preparation of HPMA copolymers that will find their use in various medicinal applications. A logical area of further work is obviously the optimisation of copolymerisations such as these.

An alternative approach for the preparation of PHPMA has been suggested by Godwin *et al.*[1] The authors suggested synthesis of a polymer of activated ester, with subsequent quantitative conversion of this polymer into PHMPA. If controlled polymerisation of a monomer is successful and allows the preparation of the polymer of required number-average molecular weight and low polydispersity, then such

polymer can serve as a precursor for preparation of a library of functionalised polymers with the same molecular weight characteristics.

With that in mind, the use of a number of monomers such as methacryloyloxy succinimide (MAOS) (the suggestion of Godwin *et al.*[1]), *p*-nitrophenyl methacrylate (NPMA) and methacryloyl chloride (MAC) were tried in this work. Investigations into polymerisation of these monomers under the conditions of controlled polymerisation became an aim of this research.

The monomers were polymerised via ATRP (MAOS only) and RAFT polymerisation. The resulting polymers were then reacted with 1-amino-2-propanol in an attempt to prepare a homopolymer of HPMA. However, the reaction of PMAOS with 1-amino-2-propanol resulted in occurrence of an unforeseen reaction, as a result of which a polymer with ring-opened succinimide moiety was isolated. Reaction of PNPMA with the amine did not produce the desired result either – in every attempt only starting material was recovered. However, the reaction of PMAC with the amine did yield a polymer of HPMA, which was confirmed by ^{1}H NMR. Both the non-reaction of PNPMA and the undesired reaction of PMAOS are somewhat at odds with some literature reports. Thus the aminolysis of these polymers is an area where further work might be valuable.

In this work RAFT polymerisation of the monomers under varying conditions was investigated. A number of chain transfer agents were used here in the hope of obtaining a system with close-to-ideal living behaviour. However, despite the method's popularity, the results of this work have clearly shown that the method has some limitations with regard to the level of control over the molecular weight distribution of monomers with an α-methyl group, especially when the monomer also contains an amide group. The modelling of the kinetic data supports the conclusion that for all the monomers under this investigation a higher degree of control is required.

Two kinetic models were used in modelling the RAFT process. Discrepancies were observed between values of the chain transfer constant, C_{tr}, obtained by the different methods. These point at the necessity of careful consideration of the major assumptions that are present in the kinetic models. As there is an ongoing debate in

the scientific community about the true nature of the kinetic processes involved in RAFT polymerisation, the results here once again show that a better understanding of the processes involved is required if polymerisation systems with better control of molecular weights are to be designed. Such knowledge is crucial when new transfer agents are being designed, as improved RAFT agents should have appropriate leaving groups which will allow the preferential fragmentation of the group and its radical's ability to propagate by reacting with a monomer.

In this work the ATRP of methyl methacrylate (MMA) and its kinetics also have been investigated. Although this was not of direct importance in terms of the stated aim, it was of indirect importance in that MMA is an excellent monomer for understanding the process of ATRP in general. By doing this the capacity to use ATRP for all monomers is enhanced. Various parameters and additives were varied and results of those experiments were scrutinised. Experimental data were manipulated, thereby allowing extraction and evaluation of kinetic rate constants. Such evaluations lead to the conclusion that reaction medium, in particular its polarity, has a profound effect on structure and solubility of the copper species used.

No doubt today ATRP is a powerful tool in synthesis of polymer chains of required molecular weight and architecture. While it is applicable to many monomers that could be polymerised under mild conditions of ATRP, there is still some mystery as to what the true mechanism of the polymerisation is. The widely accepted mechanism, which involves an establishment of the equilibrium governed by the rate constants activation and deactivation, is believed to be of free-radical nature. The amount of active free radicals in the reaction mixture is determined by the position of the equilibrium, the position of which also depends on the nature of the catalyst complex.

Two widely-used-in-ATRP-nitrogen-containing ligands were employed in this research. In both cases the value of the apparent rate constant increased with MMA content. It is suspected that this results from the equilibrium shifting towards the formation of deactivating species Cu^{II}, thereby favouring the higher concentration of propagating radicals. The modelling of the data yielded the equilibrium constant values which increased with the MMA content in the reaction mixture. An outstanding finding of this work is that Fischer's equations for ideal ATRP systems provide a superb description of the kinetics of these real systems. However, no tale is

complete with a mystery: in further studies it was found, as also previously by Haddleton, that commonly used radical inhibitors had very little or no effect on the kinetics of the ATRP, while modelling of the data predicted significant inhibition. An area of further work is to understand whether this reflects a genuine non-radical nature to the ATRP process, which should thus instead be called ATP, or whether, more likely, it is simply the result of the inhibitor somehow being 'deactivated' by the reagents that are present.

The method of ATRP was also used in preparation of PMAOS, however, the resulting polymer was not of targeted molecular weight and the polydispersity index was high. Again, this is somewhat at odds with literature reports. At the very least it suggests that ATRP of MAOS is far from routine to carry out successfully.

Computer modelling allowed estimation of the equilibrium rate constant values for the ATRP of MAOS systems. The obtained values were found to be reasonably low when compared to the value obtained for an ATRP/MMA system that was done under the same reaction conditions. This once again indicates the complexity of an ATRP system and the necessity of finding appropriate ways and tools for determining correct rate parameters. This in turn will allow for a better understanding of the mechanism of the polymerisation, and the roles each individual component plays. Such understanding will provide researchers with information required for design of better functioning ATRP systems. Better recipes will be developed with time and design of new ligands and catalytic complexes might lead to successful synthesis of PHPMA by the method of atom transfer radical polymerisation in the near future.

While the concept of polymer therapeutics has been introduced in the 1970s, there are only eleven different polymer-drug conjugates in current clinical trials.[2] A number of conjugates have failed due to toxicity of the polymeric carrier and this could serve as evidence that further and more thorough research is required. However the potential of the basic concept is undeniable, for example PEG-ylated hepatitis C vaccine has given a quantum leap in treatment of this disease.[3] This will not be the last chemistry thesis that has polymer therapeutics in mind!

Interferon is a type of protein produced by the body's cells in response to viral hepatitis and other infections. Interferon stimulates the body's immune system to fight

viral infections and affects the ability of viruses to divide in liver cells. In PEG-ylation, one or more chains of PEG are bonded to an interferon molecule. Whereas three injections per week are normally required with regular interferon treatment, only one injection of PEG-ylated interferon is required per week. PEG-ylated interferon retained ~50% of the activity of the unmodified protein and had significantly improved pharmacokinetic properties following intravenous administration in rats. The elimination half-life of the PEG-ylated protein was ~13-fold greater than for the unmodified protein, which means greater exposure of damaged tissues to the drug.[3]

What the interferon story suggests is that PEG-ylation is the way to go in polymer therapeutics, and indeed, apparently pharmaceutical executives are currently enamoured of this approach. One may therefore wonder at the work of this thesis, i.e., why bother investigating the living free radical polymerisation of monomers like HPMA if the future lies with conjugating drugs to PEG? The answer is that PEG is just one avenue, and other polymers will surely find niches as drug carriers. Further, PEG is not made by free-radical polymerisation, but the history of polymer science is generally (not always!) that polymerisation is best carried out by FRP if such is possible. Thus the studies of this work have been useful in that they investigate potential FRP routes to polymer therapeutics. Haddleton's current work nicely brings together all these strands of thought: he polymerises PEG-ylated methacrylates by ATRP, so that he gains the advantages of FRP while at the same time giving the pharmaceutical executives their security blanket of PEG!

With regard to the long-term potential of polymer therapeutics, what perhaps we are learning is that they are not a panacea. This should not be surprising as a lesson from the history of medicinal chemistry is that there is no magic bullet for all diseases, and all diseases should be treated on a case-by-case basis. Equally, a lesson of the history of polymer chemistry is that with time polymers for any application can be developed. So there should be no doubt that with time polymer therapeutics will come to play a major part in the treatment of various major diseases. But it will take time.

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