Novel Precursors for Polymer-Protein Conjugate Synthesis via Reversible Addition-Fragmentation Chain Transfer Polymerization

Dissertation

zur Erlangung des Doktorgrades
der Fakultät Biologie, Chemie und Geowissenschaften
der Universität Bayreuth

vorgelegt von

Christine Maria Schilli

geboren in Offenburg

Die vorliegende Arbeit wurde unter der Leitung von Herrn Prof. Dr. A.H.E. Müller in der Zeit von April 2000 bis April 2003 an der Universität Bayreuth durchgeführt.

Prüfungsausschuss:

Prof. Dr. A.H.E. Müller (Erstgutachter)
Prof. Dr. F.X. Schmid (Zweitgutachter)
Prof. Dr. H.-W. Schmidt (Vorsitzender)
Prof. Dr. H. Hoffmann
Prof. Dr. P. Strohriegl

Tag der Einreichung: 16.04.2003
Tag des wissenschaftlichen Kolloquiums: 11.07.2003

Vollständiger Abdruck der von der Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des Grades eines Doktor der Naturwissenschaften (Dr. rer. nat.).

Meiner Familie

Alles Wissen geht aus einem Zweifel hervor und endet in einem Glauben.

(M. v. Ebner-Eschenbach)

Table of contents

List of abbreviations .... **V**

1 Introduction **1**

1.1 Smart polymers and their bioconjugates **1**
1.1.1 Stimuli-responsive polymers 1
1.1.2 Smart polymer-protein conjugates 2

1.2 Polymer therapeutics in modern medicine **4**

1.3 Block copolymer micelles **8**

1.4 Synthesis of functionalized polymers via controlled radical polymerization **11**

2 Motivation **17**

3 Fundamentals of controlled radical polymerization **19**

3.1 Conventional radical polymerization **20**
3.1.1 Mechanism 20
3.1.2 Kinetics 20

3.2 Controlled/living polymerization **22**

3.3 RAFT polymerization **25**
3.3.1 Mechanism 26
3.3.2 Kinetics 28
3.3.3 Influence of chain transfer agent structure 30

4 Synthesis of homopolymers and block copolymers **37**

4.1 Synthesis of the chain transfer agents **37**
4.1.1 Substitution of halides with a dithiocarboxylic acid salt 37
4.1.2 Reaction of bis(thiocarbonyl) disulfides with azo compounds 38
4.1.3 Addition of dithiocarboxylic acids to unsaturated compounds 39
4.1.4 Other methods of chain transfer agent synthesis 40

4.2 Poly(acrylamide)s **42**
4.2.1 Poly(*N*-isopropylacrylamide) 42
4.2.1.1 Polymerization kinetics using in-situ FT-NIR spectroscopy 43
4.2.1.2 UV and MALDI-TOF MS analysis of the polymers 48
4.2.1.3 GPC analysis of the polymers 56
4.2.1.4 Determination of degree of polymerization 61
4.2.2 Poly(diacetone acrylamide) 63
4.2.3 Poly(2-vinyl-4,4-dimethyl-5-oxazolone) 65

4.3 Poly(acrylate)s 68
4.3.1 Poly(*tert*-butyl acrylate) 68
4.3.2 Poly(acrylic acid) 71
4.3.2.1 RAFT polymerization 72
4.3.2.2 GPC and MALDI-TOF MS characterization 73

4.4 Poly(methacrylate)s 74
4.4.1 Poly(*N*-hydroxysuccinimide methacrylate) 75
4.4.1.1 RAFT polymerization 75
4.4.1.2 GPC and MALDI-TOF MS characterization 76

4.5 Block copolymers 79
4.5.1 Poly(*N*-isopropylacrylamide)-*block*-poly(acrylic acid) 79
4.5.2 Poly(*N*-isopropylacrylamide)-*block*-poly(2-vinyl-4,4-dimethyl-5-oxazolone) 80

5 Properties of homopolymers and block copolymers 87

5.1 Poly(*N*-isopropylacrylamide) 87
5.1.1 LCST measurements 87

5.2 Poly(2-vinyl-4,4-dimethyl-5-oxazolone) 88

5.3 Poly(*N*-isopropylacrylamide)-*block*-poly(acrylic acid) 91
5.3.1 Behavior of PNIPAAm-*b*-PAA in solution 92
5.3.2 Temperature-sweep NMR 93
5.3.3 Investigation of micelle structure 94
5.3.3.1 Turbidimetry measurements 94
5.3.3.2 Dynamic light scattering 95
5.3.3.3 Raman and IR spectroscopy 97
5.3.3.4 Cryogenic transmission electron microscopy 99
5.3.4 Tensiometry 101
5.3.5 Potentiometric titrations 103
5.3.6 Differential scanning calorimetry 106

5.4 Poly(*N*-isopropylacrylamide)-*block*-poly(2-vinyl-4,4-dimethyl-5-oxazolone) 107

6 Novel route to polymer-protein conjugates 113

6.1 Bioconjugates 113
6.1.1 Functional targets 114
6.1.2 Streptavidin and avidin 115
6.1.3 Strategies for polymer-protein conjugation 118

6.2 Conjugation to model compounds 118
6.2.1 Amine-reactive polymer conjugates with a model peptide 118
6.2.2 Sulfhydryl conjugates with model compounds 122
6.2.2.1 Hydrolysis of the dithiocarbamate endgroup 122
6.2.2.2 Conjugation to model compounds 125

6.3 Conjugation to streptavidin 128
6.3.1 Conjugation to wild-type streptavidin 130
6.3.1.1 Conjugation of biotinylated PNIPAAm 130
6.3.1.2 Conjugation of biotinylated PNIPAAm-*b*-PAA 132
6.3.2 Conjugation of thiol-terminated PNIPAAm-*b*-PAA to streptavidin mutant S139C 134

6.4 Outlook 138

7 Experimental section 143

7.1 Synthesis of the chain transfer agents 143
7.1.1 Benzyl-1-pyrrolecarbodithioate 143
7.1.2 Cumyl-1-pyrrolecarbodithioate 145
7.1.3 1-Cyanoethyl-2-pyrrolidone-1-carbodithioate 147
7.1.4 Cyanoisopropyl-1-benzylcarbodithioate 149
7.1.5 1-Phenylethyl phenyldithioacetate 151

7.2 Homopolymer synthesis via RAFT 153
7.2.1 Poly(*N*-hydroxysuccinimide methacrylate) 153
7.2.1.1 Synthesis of *N*-hydroxysuccinimide methacrylate 153
7.2.1.2 RAFT polymerization 154
7.2.2 Poly(*tert*-butyl acrylate) 154
7.2.3 Poly(acrylic acid) 155
7.2.4 Poly(*N*-isopropylacrylamide) 155
7.2.4.1 RAFT polymerization 155
7.2.4.2 Hydrolysis of dithiocarbamate endgroups 156
7.2.4.2.1 Dioxane / 5 % aq. NaOH 156
7.2.4.2.2 Methanol / 5 % aq. NaOH 156
7.2.4.2.3 Methanol / ammonia 157
7.2.4.2.4 Methanol / 28 % aq. NaOH 157
7.2.4.3 Reaction of hydrolyzed PNIPAAm with 2-naphthoyl chloride 158
7.2.4.4 Reaction of hydrolyzed PNIPAAm with 5,5’-dithiobis-[2-nitrobenzoic acid] (DTNB) - Ellman test for thiol 158
7.2.4.5 Reduction of dithiocarbamate endgroups with Bu_3SnH 159
7.2.5 Poly(diacetone acrylamide) 159
7.2.6 Poly(2-vinyl-4,4-dimethyl-5-oxazolone) 160

7.3 Block copolymer synthesis via RAFT 160
7.3.1 PNIPAAm-*b*-PAA 160
7.3.2 PNIPAAm-*b*-PVO 161

7.4 Conjugation of polymers to proteins/peptides 161
7.4.1 Conjugation to PNIPAAm 161
7.4.1.1 Coupling to hydrolyzed PNIPAAm – model reactions 161
7.4.1.1.1 Coupling of 2-(trimethylsilyl)ethanethiol to PNIPAAm 161
7.4.1.1.2 Coupling to 1,1’-(methylenedi-4,1-phenylene)bismaleimide 162
7.4.1.1.3 Coupling to 2,2’-dithiobis(5-nitropyridine) 163
7.4.1.2 Addition of dithiocarbamate-terminated PNIPAAm to bismaleimide 163
7.4.1.3 Conjugation of biotinylated PNIPAAm to wild-type streptavidin 164
7.4.1.3.1 Biotinylation of hydrolyzed PNIPAAm with biotinBMCC 164
7.4.1.3.2 Conjugation of biotinylated PNIPAAm to wild-type streptavidin 165
7.4.2 Conjugation to PNIPAAm-*b*-PAA 165
7.4.2.1 Biotinylation with biotinBMCC 165

7.4.2.2 Conjugation to S139C via $BM[PEO]_3$ ... 166
7.4.2.3 Conjugation to S139C via direct disulfide ... 166
7.4.3 Conjugation to amine-reactive polymers ... 167
7.4.3.1 Conjugation of PNHSM to model peptide GlyLeu ... 167
7.4.3.2 Conjugation of PVO to model peptide GlyLeu ... 167
7.4.3.3 Conjugation of PDAA to model peptide GlyLeu ... 168

7.5 Characterization methods ... 169
7.5.1 MALDI-TOF mass spectrometry ... 169
7.5.2 In-situ FT-NIR spectroscopy ... 173
7.5.3 Dynamic light scattering ... 175
7.5.4 Tensiometry ... 177
7.5.5 Commonly used methods ... 179
7.5.5.1 NMR spectroscopy ... 179
7.5.5.2 UV spectroscopy ... 179
7.5.5.3 IR spectroscopy ... 179
7.5.5.4 Raman spectroscopy ... 179
7.5.5.5 Gel permeation chromatography ... 179
7.5.5.6 Cloud point measurements ... 180
7.5.5.7 Differential scanning calorimetry ... 180
7.5.5.8 Cryogenic transmission electron microscopy ... 180

8 Summary ... 185

9 Zusammenfassung ... 189

1 Introduction

1.1 Smart polymers and their bioconjugates

1.1.1 Stimuli-responsive polymers

Stimuli-responsive polymers are polymers that respond with large property changes to small physical or chemical changes in their environment. They are usually classified according to the stimuli they respond to as temperature-, pH-, ionic strength-, light-, electric- and magnetic field-sensitive. Some polymers respond to a combination of two or more stimuli.

Introduction of stimuli-responsive polymers into artificial materials or bioactive compounds allows for modulation of their structure that is induced by the respective external stimuli. Consequently, on/off switching of the corresponding functions may be achieved at a molecular level.[1]

Surfaces modified with stimuli-responsive polymers (SRPs) can dynamically change their physico-chemical properties in response to changes in their environmental conditions. These surfaces are frequently referred to as “smart” surfaces. The triggered control of interfacial properties that are imparted from immobilized SRPs at the solid-liquid interface has wide-spread application in the design of biomaterials, regenerable biosensors, and microfluidic bioanalytical devices. Nath et al. have created thermoresponsive surfaces by immobilization of an elastin-like polypeptide (ELP) on a glass surface. The authors succeeded in the reversible addressing of an ELP fusion protein to the surface, which enables a reversible modulation of protein binding at the solid-liquid interface.[2]

Much attention has been devoted to polymer gels whose degree of swelling changes considerably on variation of temperature, solvent, electric field, or pH.[3-6] Such materials could be useful as components of actuators that are able to convert chemical energy into mechanical energy, as absorbents for solvent extraction or as a part of drug delivery systems.[7,8] Kuckling et al. reported the synthesis of double-responsive graft copolymer hydrogels from poly(*N*-isopropylacrylamide), PNIPAAm, and poly(2-vinylpyridine), PVP, with temperature- and pH-dependent swelling properties. The swelling behavior was mostly dominated by PNIPAAm but at high PVP grafting densities, a cooperative effect on pH change was observed. Separation of the temperature- and pH-sensitive component led to a gel that could be swollen by either temperature or pH change.[9] Stile et al. have proposed peptide-modified PNIPAAm-*co*-poly(acrylic acid) hydrogels as model networks for the investigation of cell-material interactions in three dimensions and as potential injectable scaffolds for tissue engineering applications.[10]

1.1.2 Smart polymer-protein conjugates

Stimuli-responsive polymers can be physically mixed with or chemically conjugated to biomolecules to yield polymer-biomolecule systems that respond to biological as well as to physical and chemical stimuli. Conjugation of a synthetic polymer to a biomolecule yields a new, hybrid type of molecule that can synergistically combine the individual properties of the two constituents, leading to new and unusual properties. Based on their similarity with biopolymers, R. Dagani has introduced the expression "smart polymers" for stimuli-responsive polymers as they are able to mimic the non-linear response of biopolymers caused by cooperative interaction between monomers.[11] A.S. Hoffman et al. have synthesized and thoroughly investigated the conjugation of "smart" polymers to proteins. Conjugation was performed both randomly[12-14] and at specific sites of the protein.[15,16] Many other research groups have randomly conjugated smart polymers to proteins, especially for affinity separations and enzyme recovery,[17-19] but the Hoffman group seems to be the only one so far that has synthesized and studied *site-specific* smart polymer bioconjugates.

Random, smart polymer-protein conjugates are mainly used in phase separations for recovery of enzymes from complex solutions or in phase separation immunoassays. For example, thermally induced precipitation of PNIPAAm-protein conjugates from a complex solution will selectively remove only the protein conjugated to PNIPAAm from the solution, leaving the other components in solution.[13,14] Alternatively, if the conjugated protein forms a complex with another biomolecule, e.g. by affinity recognition, the complex will also be selectively precipitated from the solution, and the affinity receptor is detached by eluting with a displacer (Fig. 1.1).[20]

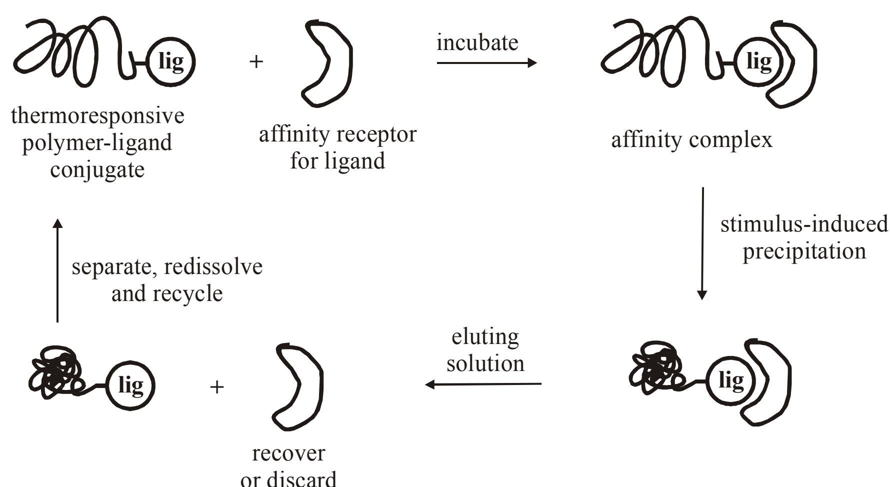


Fig. 1.1. Stimuli-induced phase separation of a conjugate of a smart polymer and a ligand that is complexed with a recognition protein.[20]

Conjugation of smart polymers to specific sites on proteins is performed by inserting a reactive amino acid at the selected site, such as cysteine that possesses a reactive thiol group. Such a functionalization of a protein is accomplished by genetically engineering a site-specific mutation into the DNA sequence of the protein and then cloning the mutant in cell culture. The specific site for polymer conjugation may be located far away from the active site to avoid interference with the biological function of the protein or nearby the active site to control the ligand-protein recognition process and the activity of the protein.[21,22]

Site-specific placement of a smart polymer near the active site of a protein may permit sensitive environmental control of the ligand/protein receptor recognition process, which controls all living systems. Small changes in environmental conditions can cause large changes in polymer conformation, leading to reversible "blocking" or "unblocking" of the protein active site and possibly to triggered release of a bound ligand from the protein binding site.[15,16] Hoffman et al. mainly used genetically engineered streptavidin, a tetrameric protein, in their studies of polymer-protein conjugates. Streptavidin is one of the most widely used proteins in affinity separations, analytical assays, and clinical diagnostics due to the high binding affinity of biotin to the four binding pockets of streptavidin. Ding et al. bound biotin to a conjugate composed of PNIPAAm and the streptavidin mutant E116C at temperatures below the lower critical solution temperature, LCST. Raising of the temperature to thermally induce polymer collapse triggered the release of some of the bound biotin molecules. Cycling of the temperature through LCST for several times led to the release of all of the bound biotin (Fig. 1.2).[16] The triggered release of bound ligands may be used to release therapeutics, for localized drug delivery within the body, or to release and recover affinity-bound ligands under eluate-free conditions. Size-selective blocking of biotinylated proteins was possible using a site-specific poly(*N*,*N*-diethylacrylamide)/streptavidin conjugate. Gating was found to be sensitive to the size of the protein, e.g. immuno-γ-globulin, IgG (150 kDa), was unable to bind below and above LCST, protein G (6.2 kDa) was found to bind at all temperatures but bovine serum albumin, BSA (67 kDa), bound only at temperatures above LCST, where the polymer is collapsed. In other words, below LCST, the polymer sterically interferes with the access to the adjacent binding site acting as a "polymer shield", whereas, above LCST, polymer collapse exposes the adjacent site.[23] Fig. 1.2 illustrates the concept of shielding by smart polymers.

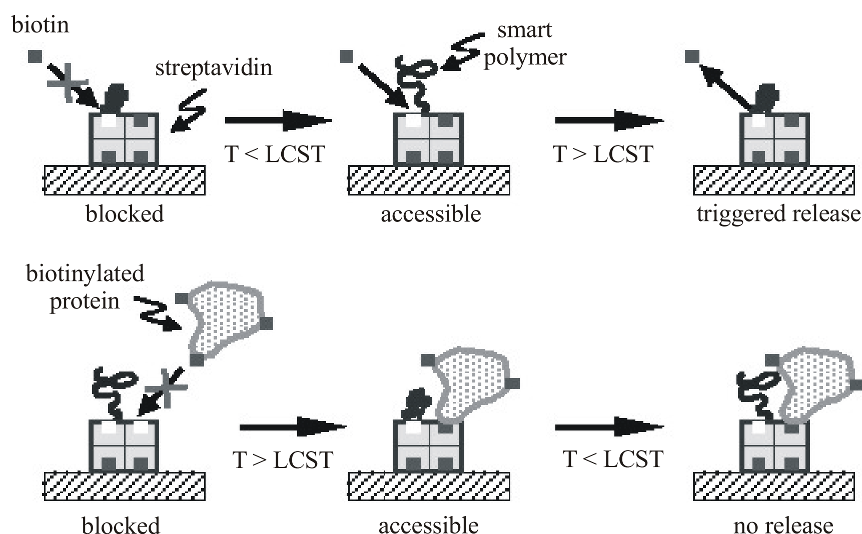


Fig. 1.2. Different shielding mechanisms; top: triggered release of bound biotin, bottom: blocking of biotinylated protein by expanded polymer at T < LCST and unblocking through polymer collapse at T > LCST.[23]

Other approaches for polymer-protein conjugation use polymers with binding sites for protein functionalities. For example, Uludag et al. have synthesized NIPAAm polymers that contain protein-reactive *N*-acryloxysuccinimide and LCST-altering, hydrophobic alkylmethacrylates to obtain thermoresponsive, protein-conjugating polymers. The thermosensitive polymers were capable of retaining a co-injected therapeutic protein at an application site where tissue regeneration was required and might therefore be applied for drug delivery.[24]

The above-mentioned investigations, along with those of many other researchers, are on the threshold of polymer therapeutics, which will be discussed in the following section.

1.2 Polymer therapeutics in modern medicine

Polymer therapeutics include polymers which are inherently biologically active,[25] polymer-drug conjugates, polymeric micelles,[26] polymer-protein conjugates,[27,28] and polymer-coated liposomes.[29,30] Fig. 1.3 shows an overview of these therapeutic agents.

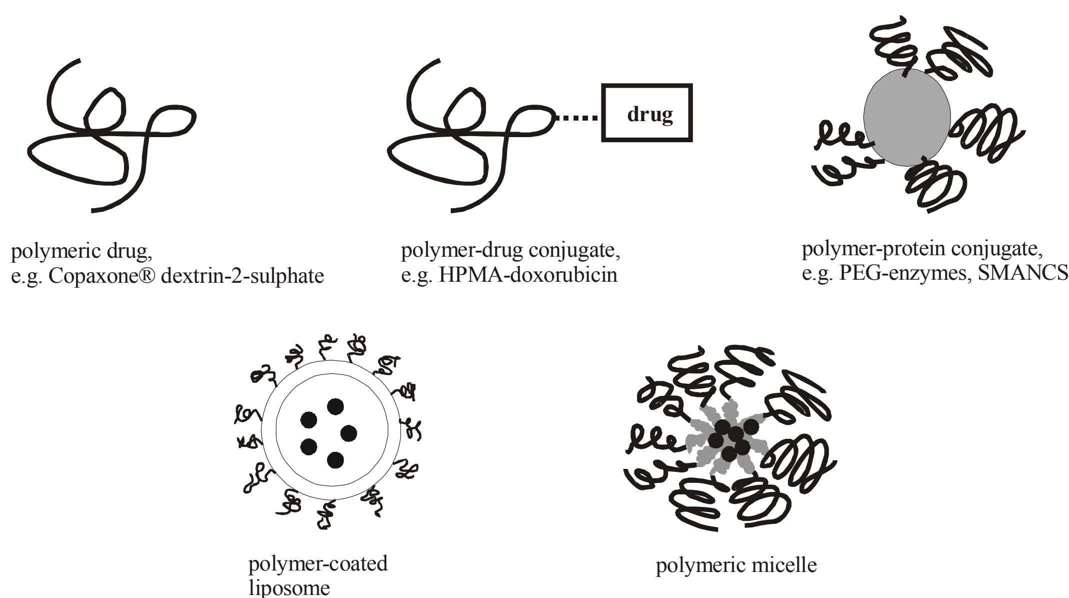


Fig. 1.3. Overview of different polymer therapeutics.[31]

Polymer-controlled drug delivery has evolved from the need for prolonged and better control of drug administration. Besides, toxic side effects involved with chemotherapy frequently limit the dosage levels. The different means for prolonging the permanence of substances in blood circulation include covalent conjugation of drugs to polymers, drug encapsulation in liposomes, and physical entrapment of drugs in particles, such as micelles or microspheres. The conjugation of anti-tumor agents to polymers yields a new class of anticancer agents that can mediate tumor-selective targeting and reduce toxicity. In conventional drug delivery, the drug concentration in the blood rises on administering, then peaks and declines. Controlled-release devices can maintain the drug in the desired therapeutic range with a single dose and localize delivery of the drug to a particular body compartment.

Most anti-tumor agents are low-molecular weight compounds that penetrate all tissues by passing across the cell membrane, whereas polymer conjugates can only gain entry to the cell by pinocytosis (uptake of material by a cell from the environment by folding inward and pinching off of the plasma membrane[32]). This process involves membrane internalization to form vesicles, which entrap the large polymer drug and deliver it to the cell's interior. The polymer drug circulates for a longer time in the body and accumulates more effectively in tumor tissue as compared to low-molecular weight components. This phenomenon has been termed "enhanced permeability and retention (EPR) effect" by Maeda et al.[33] and has been attributed to tumor vessels that are usually more "leaky" to macromolecules and to the lack of effective tumor lymphatic drainage so that macromolecules leaving the blood vessels are not returned to circulation very quickly. These factors allow conjugate concentration in tumor tissue to reach levels that are 10-

1000 times higher than normally found after administration of the free drug. Fig. 1.4 demonstrates the differences in cellular uptake of free and conjugated drugs.

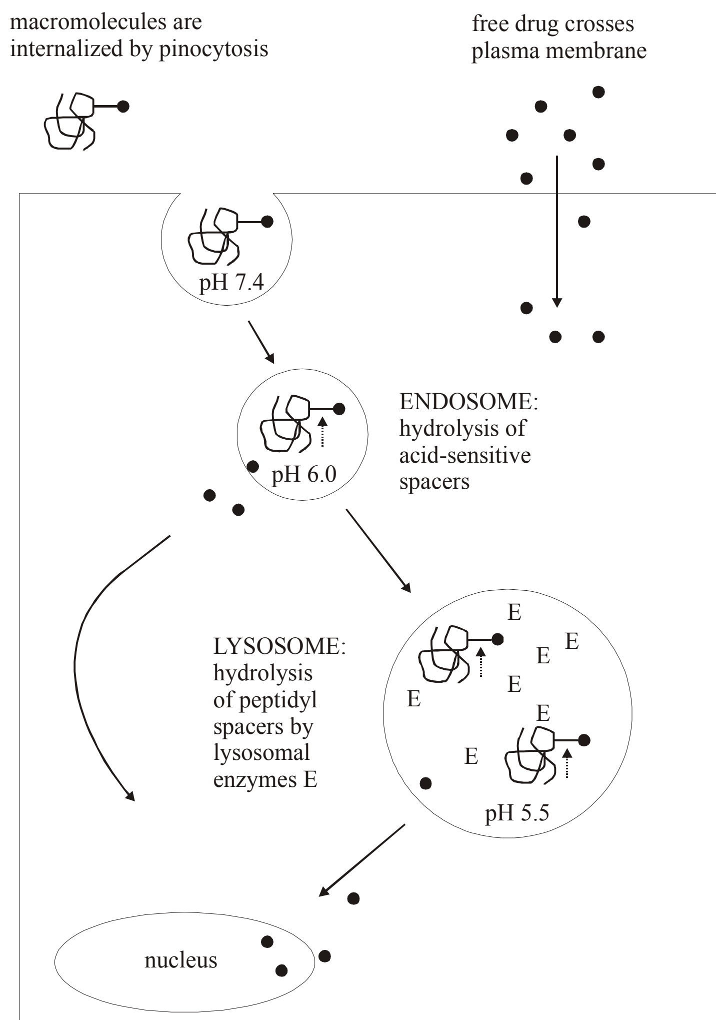


Fig. 1.4. Mechanisms of cellular uptake of low-molecular weight anti-tumor agents and polymer-drug conjugates.[31]

Molecular weight and stability are the key factors in the optimization of polymer conjugates: polymer backbone as well as polymer-drug linkages must be sufficiently stable and the molecular size must be small enough to ensure elimination from the body by the kidneys, i.e. renal excretion. Thus, polymer-drug conjugate not captured by tumor tissue can largely be removed and the harmful drug is directed away from potential sites of toxicity.

H. Ringsdorf was the first one to propose a model for polymer-drug conjugates, and he suggested using water-soluble polymers to which the drug could be bound covalently by a linkage that could be degraded at a desired rate in the target site. Furthermore, the inclusion of cell-specific targeting residues would enhance selective delivery further.[26] With the use

of synthetic polymers, potential carriers are at hand that can be tailor-made with all the desired features, such as targeting moieties, peptidyl spacers for enzymatic cleavage, pH-sensitive linkers, etc. Polymer carriers for drug conjugation have to meet some requirements that have to be considered in the design of polymer-drug conjugates, such as biocompatibility, lack of immunogenicity, biological inertia, and functional groups for covalent conjugation to drugs and targeting residues. Even though natural polymers, such as dextran or human serum albumin, are easily available and biocompatible, they exhibit high immunogenicity, which is a major drawback of these compounds.

N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer conjugates with anti-tumor agents have been most extensively studied. HPMA is water-soluble, biocompatible, non-immunogenic and non-toxic at the maximum administrable dose.[34] Generally, the drugs are bound to the polymer backbone using peptidyl spacers designed for cleavage by lysosomal thiol-dependent proteases. These enzymes are elevated in many human tumors.[35] There is also a number of cell-specific targeting groups that has been incorporated into the HPMA copolymer structure, e.g. galactose for targeting the liver. The first synthetic polymer-drug conjugate that was in clinical study is *N*-(2-hydroxypropyl)methacrylamide copolymer-doxorubicin (PK 1), in which the anti-tumor agent doxorubicin is bound to the polymer backbone by a Gly-Phe-Leu-Gly peptidyl side chain. The conjugate displays anti-tumor activity, is five to ten times less toxic than free doxorubicin and shows evidence of tumor-selective targeting.[36]

Beside HPMA copolymers, there are a number of other polymers suitable for drug conjugation. One of the most extensively studied polymers is poly(ethylene glycol), PEG, a linear polyether diol that is biocompatible, soluble in aqueous and organic media, non-toxic and exhibits very low immunogenicity.[37,38] Its polymer backbone is chemically inert, and the terminal hydroxyl groups are available for derivatization. Drug conjugates are usually prepared from monomethoxy-PEG, mPEG, which is generally activated first and then reacted with the target molecule.[39,40]

In recent years, the number of approved polymer-protein drugs as anti-tumor agents has grown and includes PEGylated L-asparaginase (Oncaspar®) for treatment of acute lymphocytic leukaemia in children[28,41] as well as a conjugate of poly(styrene-*co*-maleic anhydride) and the anti-tumor protein neocarzinostatin (SMANCS) for treatment of liver cancer.[27]

Beside the above-discussed polymer-drug and polymer-protein conjugates, polymers also play a vital role in the stabilization of drug-loaded micelles and liposomes. These areas are especially important where polymer-drug conjugation fails, e.g. lack of derivatizable groups in the drug, decrease or loss of activity after conjugation. Self-assembling micellar delivery systems are receiving increasing attention[42-44] and structure-

reactivity relationships of micellar structures formed from PEO as hydrophilic block and poly(L-amino acid) as hydrophobic block carrying doxorubicin are well documented in the literature. For example, poly(ethylene glycol)-*block*-poly(aspartate) doxorubicin conjugates form micelles that accumulate in solid tumors and exhibit anti-tumor activity.[45,46] An EPR effect is also found in the case of polymer micelles and maximizes tumor capture. The micelle may subsequently disassociate to give smaller block copolymer units that can be excreted.

1.3 Block copolymer micelles

One of the most prominent properties of amphiphilic block copolymers is their ability to form micelles in selective solvents. If a block copolymer is dissolved in a solvent that is a good solvent for one block but a poor solvent for the other, the formation of micelles is most likely. Provided that the block ratio is not too asymmetric, it is also possible to obtain inverse micelles from the same block copolymer by choosing appropriate solvents. Polymer micelles have a compact core constituted by collapsed insoluble parts and a diffuse corona composed of soluble chains.

Depending on the ratio of core radius, R_{core}, to corona diameter, d_{corona}, micelles are classified into crew-cut micelles ($R_{core} >> d_{corona}$) and star micelles ($R_{core} << d_{corona}$).[47] Consequently, star micelles are spherical with small cores and expanded coronas,[48] whereas crew-cut micelles possess large cores and short coronal “hair”.[49] For star micelles, the radius of the core seems to be independent of the length of the soluble block and scales as $N_B^{3/5}$, where N_B is the number of units in the insoluble block.[47] Beside the rather spherical shapes, there also exist other morphologies of block copolymer aggregates in solution, such as vesicles, wormlike micelles, etc.

Micelle formation requires the presence of two opposing forces, i.e. an attractive force between blocks leading to aggregation and a repulsive force that prevents the unlimited growth of micelles into a distinct macroscopic phase. Micellar growth is further limited by entropic factors due to a constraint in length that induces a negative entropy change owing to stretching of the chains. The micellization process is sufficiently cooperative to yield colloidal particles with narrow size distribution and high aggregation numbers.

The thermodynamic reasons for micelle formation are strong negative energy changes as a result of solvent incompatibility of the core block in conjunction with steric repulsion of the soluble, corona-forming polymeric chains and a combination of intermolecular forces, including hydrophobic interaction, electrostatic interaction, metal complexation, and hydrogen bonding of the constituent block copolymers.[50]

Critical phenomena play an important role in micelle formation; micelles exist only above a certain minimum concentration, i.e. the critical micelle concentration, cmc. The critical micelle concentration is defined as the concentration below which only single chains are present but above which single chains and micellar aggregates coexist. Similarly to a critical concentration for micellization, there is also a critical micelle temperature and, in the case of pH-responsive blocks, a critical micelle pH.[51,52] The block lengths of the copolymers have a considerable impact on the cmc, where the length of the insoluble block affects the cmc much more than that of the soluble block. Theories developed by Nagarajan et al.[53] and Whitmore et al.[54] suggest a scaling relation for aggregation numbers Z that is proportional to $N_A^{\alpha}N_B^{\beta}$, where N_A = length of insoluble block, N_B = length of soluble block, α and β = exponents of scaling relations. Typical exponent values are $\alpha = 0.73$ and $\beta = -0.17$ for polystyrene-*block*-polyisoprene in *n*-heptane or $\alpha = 0.7$ and $\beta = -0.08$ for poly(ethylene oxide)-*block*-poly(propylene oxide) in water.[53] Förster et al. have postulated a universal scaling relation $Z \propto N_A^2 N_B^{-0.8}$ for strongly segregated diblock and triblock copolymer systems that was derived from micellization experiments with polystyrene-*block*-poly(4-vinylpyridine) in toluene.[55]

The micellization process is believed to obey the scheme of “closed association”, which describes a dynamic equilibrium between micelles and molecularly dissolved block copolymer (unimers).[56,57] There is also a mechanism of “open association” that comprises a series of equilibria between unimers, dimers, trimers and so on. Micelles formed in selective solvents are dynamic if single block copolymer molecules are exchanged via a thermodynamic equilibrium. However, for a micelle with a glassy core, i.e. with a glass transition temperature of the core-constituting block that is sufficiently high, as is the case for polystyrene, the structure is “kinetically frozen” and may not represent the thermodynamic equilibrium.[58]

Micelles of block copolymers and low-molecular weight surfactants display different characteristics in terms of lability and exchange kinetics. For example, critical micelle concentrations for polymeric micelles are in the micromolar or nanomolar range, whereas those of low-molecular weight surfactants usually lie in the millimolar range.[59,60] Furthermore, polymer micelles display a smaller rate of dissociation as compared to surfactant micelles.

Ionic block copolymers possess hydrophilic blocks of ionic repeating units and hydrophobic blocks of nonionic units. Due to the high degree of incompatibility between the ionic and nonionic blocks, micelles formed from ionic block copolymers display extremely low critical micelle concentrations and high aggregate stabilities. Ionic block copolymers are usually divided into two categories, i.e. block polyelectrolytes and block ionomers, the difference being the polyelectrolyte forming either the micellar corona

(block polyelectrolyte) or the micellar core (block ionomer).[47] Block copolymers containing ionic groups in the corona have a much larger overall size despite their smaller aggregation numbers as compared to non-ionic polymers due to electrostatic repulsion. Non-ionic block copolymer micelles are constituted either of block copolymers containing two different hydrophobic segments or of amphiphilic block copolymers. The requirement for micelle formation in these systems is the use of a selective solvent for one of the blocks.[51]

Micellization conditions usually have to be found by trial and error and are mainly guided by the solubility properties of the individual blocks. Preparation of micelles is usually performed either by addition of a precipitating solvent (mixture) for one block or by direct dissolution in an appropriate solvent (mixture). Changing temperature, pH or ionic strength may result in selective solvent conditions, favoring the formation of micelles. For example, poly(vinyl pyrrolidone)-*block*-poly(ethylene oxide) forms micelles in aqueous solutions on titration from pH 1 to pH 10, and polystyrene-*block*-poly(methacrylic acid) forms micelles upon direct addition to a mixture of dioxane/water 80:20 (v:v) followed by stepwise dialysis to pure aqueous buffer.[61]

Armes et al. have investigated a plethora of micelles formed from ionic and non-ionic block copolymers, some of them displaying response to pH, temperature, and other stimuli. The term “schizophrenic” was coined, describing hydrophilic AB block copolymers that are able to form both conventional and inverse micelles in aqueous media.[62,63] Recently, Armes et al. have reported on the zwitterionic AB diblock copolymer poly(4-vinyl benzoic acid)-*block*-poly(2-(diethylamino)ethyl methacrylate), PVBA-*b*-PDEA, that undergoes spontaneous self-assembly in aqueous solution at 20 °C to form both micelles and inverse micelles simply by switching the solution pH.[62] At pH 2, PVBA-core micelles are found, whereas at pH 10, PDEA-core micelles form. Possible applications of this “schizophrenic” block copolymer are as a pigment dispersant or in the separation and purification of proteins.

Double-responsive behavior of block copolymer micelles has been reported by Laschewsky et al. who synthesized water-soluble block copolymers from *N*-isopropylacrylamide, NIPAAm, and the zwitterionic monomer 2-[*N*-(3-methacryl-amidopropyl)-*N*,*N*-dimethyl]ammoniopropane sulfonate, SPP. Double-thermoresponsive behavior is found due to the lower critical solution temperature (LCST) of PNIPAAm and the upper critical solution temperature (UCST) of PSPP displayed in aqueous media. The colloidal aggregates can switch reversibly with temperature, and the micellar domains are formed at low and high temperature from the block that is collapsed under the given conditions, i.e. PSPP at low temperatures and PNIPAAm at high temperatures.[64] The varying polarity of the micellar core – rather polar at low temperature with PSPP as core

and unpolar at high temperature with PNIPAAm as core – enables the solubilization of compounds simply by varying the temperature.

Possible applications of polymer micelles are manifold and range from biotechnology to nanoscience. Antonietti et al. used polymer micelles as "nanoreactors" to produce highly dispersed metal or semiconductor particles.[65,66] Spatz et al. performed a controlled mineralization of gold nanoparticles in micelles composed of polystyrene-*block*-poly(2-vinylpyridine).[67] Micelles that show a pH-dependent behavior suggest applications as sensors or pH-driven chemical or drug delivery systems. Polymer micelles as drug carriers were first envisioned by Ringsdorf et al.[68] The application as drug delivery systems arises from the micellar size that is typical of that of a virus, thereby avoiding filtration by the kidneys and reticuloendothelial system uptake (reticuloendothelial system = group of cells having the ability to take up and sequester inert particles and vital dyes[69]). Besides, polymer micelles used as carriers of tumor therapeutics circulate in the blood for a long period of time and eventually pass through the capillaries that are disrupted near tumor growth.[70]

1.4 Synthesis of functionalized polymers via controlled radical polymerization

Preparation of well-defined, functional polymers is a major concern in the development of polymer-protein and polymer-drug conjugates. Most of the polymers used so far for the synthesis of polymer therapeutics have relatively broad molecular weight distributions and compositions are not uniform. Especially in the light of a detailed investigation of the biodistribution of these conjugates, it is desirable to have well-defined polymers that allow for a detailed correlation of structure, molecular weight and solution properties with the biological profile. Very narrow molecular weight distributions ensure well-defined compositions and distinct retention times of the conjugates in the body.

The solution to this problem seems to be controlled/living polymerizations that yield polymers with low polydispersities and defined molecular weights. These polymerizations include anionic polymerization, atom-transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer (RAFT) polymerization.

Anionic polymerization requires the use of rather stringent reaction conditions, being very sensitive to impurities. Besides, a large number of monomers cannot be polymerized due to interaction with the reactive initiators (metal amides, alkoxides, or organometallic compounds). For example, anionic polymerization fails for monomers containing active hydrogen atoms, such as primary and secondary acrylamides, acrylic acid, etc. In order to

polymerize these monomers, protecting groups have to be introduced which necessitates deprotection of the functional groups after polymerization. Furthermore, polymerization of polar monomers in polar solvents is complicated by side reactions due to interaction of functional groups with the carbanion center.[71,72]

Atom-transfer radical polymerization generally uses transition metal ions complexed to nitrogen-containing ligands as catalysts. Even though requiring less severe polymerization conditions than anionic polymerization, ATRP suffers two major drawbacks. One is contamination of the polymers by the transition metal catalyst and the second is complexation of certain monomer functionalities by the metal ions. For the latter reason, polymerization of carboxyl-, amine-, or hydroxyl-containing monomers is only possible if the functionality is protected.[73,74] One exception to this rule has been the successful synthesis of *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers via ATRP in DMSO as solvent, leading to quite narrowly distributed molecular weights but displaying some difficulties in choice of monomer/solvent ratio due to possible competitive chelation of the transition metal ion by solvent.[75]

For the design of polymer-protein or polymer-drug conjugates from well-defined functional polymers, a polymerization technique is needed that does not require expensive reactors or other costly equipment but can be performed with means that are available in a standard, even non-polymeric, laboratory. No complicated purification of reactants should be necessary and protecting group chemistry should be evitable. The method of choice seems to be RAFT polymerization that can be applied to virtually all kinds of monomers without protection of functional groups using common solvents and initiators at temperatures ranging from 25 °C to 100 °C. For example, acrylic acid, that cannot be polymerized in a non-protected form via anionic or atom-transfer polymerization, can be RAFT polymerized without modification. The use of dithiocarbonyl compounds RS(C=S)Z as chain transfer agents results in end-functionalized polymers that can be further derivatized. The dithiocarbonyl-derived –S(C=S)Z chain ends are especially attractive for conjugation to proteins since hydrolysis yields thiol-terminated polymers that react selectively with thiol-reactive functionalities, such as cysteine residues, in the protein. If required, the R group of the chain transfer agent can be chosen in such a way that it contains a derivatizable functionality which is introduced to the other chain end in the RAFT process, giving rise to two functional groups at both ends of the polymer chain that may be modified further. Such a telomeric polymer might be interesting for attaching a protein to one end and a targeting moiety to the other, which transports the protein to the desired site in the body. Additionally, RAFT polymerization offers the possibility of synthesizing a vast range of different polymer architectures, including block, graft and star copolymers.[76-79]

References

(1) Hoffman, A. S.; Stayton, P. S.; Bulmus, V.; Chen, G.; Chen, J.; Cheung, C.; Chilkoti, A.; Ding, Z.; Dong, L.; Fong, R.; Lackey, C. A.; Long, C. J.; Miura, M.; Morris, J. E.; Murthy, N.; Nabeshima, Y.; Park, T. G.; Press, O. W.; Shimoboji, T.; Shoemaker, S.; Yang, H. J.; Monji, N.; Nowinski, R. C.; Cole, C. A.; Priest, J. H.; Harris, J. M.; Nakamae, K.; Nishino, T.; Miyata, T. *J. Biomed. Mater. Res.* **2000**, *52*, 577-586.

(2) Nath, N.; Chilkoti, A. *Adv. Mater.* **2002**, *14*, 1243-1247.

(3) Freitas, R. F. S.; Cussler, E. L. *Chem. Eng. Sci.* **1987**, *42*, 97.

(4) Tanaka, T.; Fillmore, D. J.; Sun, T.; Nishio, I.; Swislow, G.; Shah, A. *Phys. Rev. Lett.* **1980**, *45*, 1636.

(5) Osada, Y.; Kishi, R.; Hasebe, M. *J. Polym. Sci., Part C: Polym. Lett.* **1987**, *25*, 481.

(6) Brannon, L.; Peppas, N. A. *Chem. Eng. Sci.* **1991**, *46*, 715.

(7) Osada, Y. *Adv. Polym. Sci.* **1987**, *82*, 1.

(8) Lim, Y. H.; Kim, D.; Lee, D. S. *J. Appl. Polym. Sci.* **1997**, *64*, 2647-2655.

(9) Kuckling, D.; Wohlrab, S. *Polymer* **2001**, *43*, 1533-1536.

(10) Stile, R. A.; Healy, K. E. *Biomacromolecules* **2001**, *2*, 185-194.

(11) Dagani, R. *Chem. Eng. News* **1995**, *9*, 30-33.

(12) Cole, C.-A.; Schreiner, S. M.; Priest, J. H.; Monji, N.; Hoffman, A. S. *ACS Symp. Ser.* **1987**, *350 (Reversible Polym. Gels Relat. Syst.)*, 245-254.

(13) Chen, G.; Hoffman, A. S. *Bioconjugate Chem.* **1993**, *4*, 509-514.

(14) Ding, Z.; Chen, G.; Hoffman, A. S. *J. Biomed. Mater. Res.* **1998**, *39*, 498-505.

(15) Bulmus, V.; Ding, Z.; Long, C. J.; Stayton, P. S.; Hoffman, A. S. *Bioconjugate Chem.* **2000**, *11*, 78-83.

(16) Ding, Z.; Long, C. J.; Hayashi, Y.; Bulmus, E. V.; Hoffman, A. S.; Stayton, P. S. *Bioconjugate Chem.* **1999**, *10*, 395-400.

(17) Galaev, I. Y.; Mattiasson, B. *Biotechnol. Bioeng.* **1993**, *41*, 1101-1106.

(18) Nguyen, A. L.; Luong, J. H. T. *Biotechnol. Bioeng.* **1989**, *34*, 1186-1190.

(19) Gupta, M. N.; Mattiasson, B. *Highly Selective Separations in Biotechnology*; Blackie Academic & Professional: London, 1994.

(20) Chen, J. P.; Hoffman, A. S. *Biomaterials* **1990**, *11*, 631-634.

(21) Chilkoti, A.; Chen, G.; Stayton, P. S.; Hoffman, A. S. *Bioconjugate Chem.* **1994**, *5*, 504-507.

(22) Stayton, P. S.; Shimoboji, T.; Long, C.; Chilkoti, A.; Chen, G.; Harris, J. M.; Hoffman, A. S. *Nature* **1995**, *378*, 472-474.

(23) Ding, Z.; Fong, R. B.; Long, C. J.; Stayton, P. S.; Hoffman, A. S. *Nature* **2001**, *411*, 59-62.
(24) Uludag, H.; Norrie, B.; Kousinioris, N.; Gao, T. *Biotechnol. Bioeng.* **2001**, *73*, 510-521.
(25) Seymour, L. *J. Bioact. Compat. Polym.* **1991**, *6*, 178-216.
(26) Ringsdorf, H. *J. Polym. Sci. Polymer Symp.* **1975**, *51*, 135-153.
(27) Maeda, H. *Adv. Drug Delivery Rev.* **2001**, *46*, 169-185.
(28) Nucci, M. L.; Shorr, R.; Abuchowski, A. *Adv. Drug Delivery Rev.* **1991**, *6*, 133-151.
(29) Gregoriadis, G. *Liposome Technology*; CRC Press: Boca Raton, FL, 1992.
(30) Scheule, R. K.; Cheng, S. H. *Liposome delivery systems*; BIOS Scientific Publishers Inc.: Oxford, 1996.
(31) Duncan, R.; Dimitrijevic, S.; Evagorou, E. G. *S.T.P. Pharma Sciences* **1996**, *6*, 237-263.
(32) www.nlm.nih.gov/medlineplus/mplusdictionary.html. Merriam-Webster.
(33) Maeda, H.; Matsumura, Y. *CRC Crit. Rev. Ther. Drug Carrier Sys.* **1989**, *6*, 193-210.
(34) Rihova, B.; Kopecek, J.; Ulbrich, K.; Chytry, V. *Makromol. Chem. (Suppl.)* **1985**, *9*, 13-24.
(35) Duncan, R. *Pharmaceutical Science & Technology Today* **1999**, *2*, 441-449.
(36) Seymour, L. W. *Brit. J. Cancer* **1994**, *70*, 636-641.
(37) Powell, G. M. In *Handbook of Water Soluble Gums and Resins*; Davidson, R. L., Ed.; McGraw-Hill: New York, 1980; Vol. 18, pp 1-31.
(38) Dreborg, S.; Akerblom, E. B. *Crit. Rev. Ther. Drug Carrier Syst.* **1990**, *6*, 315-365.
(39) Zalipsky, S. *Bioconjugate Chem.* **1995**, *6*, 150-165.
(40) Herman, S.; Hooftman, G.; Schacht, E. *J. Bioact. Compat. Polym.* **1995**, *10*, 145-187.
(41) Fuertges, F.; Abuchowski, A. *J. Control. Release* **1990**, *11*, 139-148.
(42) Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. *J. Control. Release* **1994**, *32*, 269-277.
(43) Kabanov, A. V.; Alakhov, V. Y. *J. Control. Release* **1994**, *28*, 15-35.
(44) Calibrese, P.; Chabner, B. A. In *The Pharmacological Basis of Therapeutics*; Hardman, J. G.; Limbird, L. E., Eds.; McGraw-Hill: New York, 1996; pp 1225-1288.
(45) Kwon, G. S.; Kataoka, K. *Adv. Drug Delivery Rev.* **1995**, *16*, 295-309.
(46) Kataoka, K.; Kwon, G. S.; Yokoyama, M.; Okano, T.; Sakurai, Y. *J. Control. Release* **1993**, *24*, 119-132.

(47) Moffitt, M.; Khougaz, K.; Eisenberg, A. *Acc. Chem. Res.* **1996**, *29*, 95-102.
(48) Halperin, A. *Macromolecules* **1989**, *22*, 3806.
(49) Nagarajan, R.; Ganesh, C. *Macromolecules* **1989**, *22*, 4312.
(50) Tuzar, Z.; Kratochvil, P. In *Surface and colloid science*; Matijevic, E., Ed.; Plenum Press: New York, 1993; Vol. 15, p 1.
(51) Astafieva, I.; Zhong, X. F.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339-7352.
(52) Lee, A. S.; Bütün, V.; Vamvakaki, M.; Armes, S. P.; Pople, J. A.; Gast, A. P. *Macromolecules* **2002**, *35*, 8540-8551.
(53) Nagarajan, R.; Ganesh, K. *J. Phys. Chem.* **1989**, *90*, 5843.
(54) Whitmore, M. D.; Noolandi, J. *Macromolecules* **1985**, *18*, 657.
(55) Förster, S.; Zisensis, M.; Wenz, E.; Antonietti, M. *J. Chem. Phys.* **1996**, *104*, 9956-9970.
(56) Elias, H.-G. In *Light Scattering from Polymer Solutions*; Huglin, M. B., Ed.; Academic Press: London, 1972; pp 397-457.
(57) Elias, H.-G. *J. Macromol. Sci.* **1973**, *A7*, 601.
(58) Tuzar, Z. *Solvents and Self-Organization of Polymers*; Kluwer: Dordrecht, 1996; Vol. E 327.
(59) Wilhelm, M.; Zhao, C. L.; Wang, Y.; Xu, R.; Winnik, M. A. *Macromolecules* **1991**, *24*, 1033.
(60) Quintana, J. R.; Villacampa, M.; Katime, I. A. *Macromolecules* **1993**, *26*, 606.
(61) Webber, S. E. *J. Phys. Chem. B* **1998**, *102*, 2618-2626.
(62) Liu, S.; Armes, S. P. *Angew. Chem. Int. Ed.* **2002**, *41*, 1413-1416.
(63) Liu, S.; Billingham, N. C.; Armes, S. P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2328-2331.
(64) Arotcarena, M.; Heise, B.; Ishaya, S.; Laschewsky, A. *J. Am. Chem. Soc.* **2002**, *124*, 3787-3793.
(65) Antonietti, M.; Wenz, E.; Bronstein, L. M.; Seregina, M. V. *Adv. Mater.* **1995**, *7*, 1000.
(66) Antonietti, M.; Förster, S.; Hartmann, J.; Oesteich, S. *Macromolecules* **1996**, *29*, 3800.
(67) Spatz, J. P.; Mössmer, S.; Möller, M. *Chem. Eur. J.* **1996**, *2*, 1552-1555.
(68) Bader, H.; Ringsdorf, H.; Schmidt, B. *Angew. Makromol. Chem.* **1984**, *123/124*, 457-485.
(69) www.ndif.org/t-a.html. Nephrogenic Diabetes Insipidus (NDI) Foundation.
(70) Harada, A.; Kataoka, K. *Adv. Drug Delivery Rev.* **1995**, *16*, 295.

(71) Young, R. N.; Quirk, R. P.; Fetters, L. J.; Luston, J.; Vass, F. *Anionic Polymerization*; Springer-Verlag: Berlin, Heidelberg, New York, Toronto, 1984; Vol. 56.

(72) Hsieh, H. L.; Quirk, R. P. *Anionic Polymerization*; Marcel Dekker, Inc.: New York, Basel, Hong Kong, 1996.

(73) Wang, J. S.; Matyjaszewski, K. *Macromolecules* **1995**, *28*, 7901-7910.

(74) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921-2990.

(75) Godwin, A.; Hartenstein, M.; Müller, A. H. E.; Brocchini, S. *Angew. Chem. Int. Ed.* **2001**, *40*, 594.

(76) Le, T. P.; Moad, G.; Rizzardo, E.; Thang, S. H. In *WO 98/01478*; E.I. Du Pont De Nemours and Co., USA; Le, Tam Phuong; Moad, Graeme; Rizzardo, Ezio; Thang, San Hoa: Australia, 1998.

(77) Chiefari, J.; Mayadunne, R. T. A.; Moad, G.; Rizzardo, E.; Thang, S. H. In *WO 99/31144*; E.I. DuPont de Nemours and Company: Australia, 1999.

(78) Chong, Y. K.; Le, T. P. T.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1999**, *32*, 2071-2074.

(79) Rizzardo, E.; Chiefari, J.; Mayadunne, R. T. A. In *Controlled/Living Radical Polymerization - Progress in ATRP, NMP, and RAFT, ACS Symposium Series*; Matyjaszewski, K., Ed.; American Chemical Society: Washington, DC, 2000; Vol. 768, pp 278-295.

2 Motivation

The purpose of the present work was to create new routes for the synthesis of polymer-peptide / polymer-protein conjugates and eventually polymer-drug conjugates. As a means to achieve this, reversible addition-fragmentation chain transfer (RAFT) polymerization was employed, which is a novel, controlled radical polymerization technique that does not require protection group chemistry on functional monomers like other controlled polymerization methods and can be applied to virtually any radically polymerizable monomer. RAFT polymerization generally leads to well-defined polymers with narrow molecular weight distributions and chain end functionalization. The functionalized polymeric chain end can subsequently be modified for attachment of model compounds and proteins.

As the RAFT process tolerates virtually any monomer functionality, a great variety of polymers can be synthesized in a well-controlled manner.

Since RAFT is a relatively new polymerization method, it was of interest to perform this polymerization on a variety of monomers that are suited for the synthesis of bioconjugates and also to investigate the kinetic characteristics of the process as not many details were known at that point. Useful polymers include active esters, such as poly(*N*-hydroxysuccinimide methacrylate), or the stimuli-responsive poly(*N*-isopropylacrylamide) and poly(acrylic acid).

By the use of thiocarbonylthio compounds as chain transfer agents, these functionalities are incorporated into the polymeric structure. The thus obtained dithiocarbonyl-terminated polymers can be hydrolyzed to obtain thiol-terminated polymers. Thiol-terminated polymers provide ideal conjugation sites for the thiol groups of peptides and proteins that are relatively rare so that selective binding can be achieved.

Beside synthesizing endgroup-functionalized polymers, a variety of active ester monomers can be (co)polymerized to have binding sites for primary amino groups.

Characterization of the functionalized homopolymers and block copolymers in terms of endgroup functionality, solution behavior, and molecular weight (distribution) provides new insights into their potential use as drug carriers or as components of bioconjugates.

As an approach to the synthesis of bioconjugates, the conjugation of active ester polymers to model peptides and of stimuli-responsive polymers to thiol-functionalized proteins can be probed. The protein streptavidin was chosen for the synthesis of polymer-protein conjugates as this system had been investigated thoroughly and it is possible to

genetically engineer thiol groups at the desired sites. Characterization of the protein-polymer conjugates provides some new insights into the features of these conjugates, especially in terms of their ability to block/unblock binding of ligands to the protein's active site.

The synthesis of stimuli-responsive polymers, such as thermoresponsive poly(*N*-isopropylacrylamide) or pH-responsive poly(acrylic acid), and their incorporation into block copolymers with subsequent conjugation to proteins enables modulation of structure and binding properties by pH and/or temperature.

3 Fundamentals of controlled radical polymerization

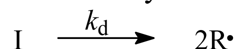
Controlled polymerization has become of vital importance since Szwarc[1,2] reported the "living" nature of the anionic polymerization of styrene and diene monomers in 1956. Living polymerization is defined as a polymerization that undergoes neither irreversible termination nor irreversible chain transfer. A plot of molecular weight versus conversion is therefore linear, and the first-order time-conversion plot results in a straight line in the absence of termination. If initiation and equilibration between active species are fast with respect to propagation, the polymer chains all grow at the same rate, thereby decreasing the polydispersity. Consequently, the molecular weight of the polymers produced in a living polymerization process is governed by the stoichiometry of the reaction and the degree of monomer conversion. The living nature of the propagating chains is the basis of the synthesis of block, graft, star, and hyperbranched copolymers.

Until recently, ionic polymerizations were the only "living" techniques available that controlled efficiently the architecture and structure of vinyl polymers. Due to the incompatibility of the propagating ionic polymer chains with a great number of functional groups and some monomer classes along with the rather drastic reaction conditions that require extremely pure solvents, complete absence of oxygen and mostly very low temperatures, more convenient polymerization methods were desired.[3] The answer to this problem was the control over radical polymerization which tolerates a much greater number of functional groups and offers moderate reaction conditions, such as a convenient temperature range and the tolerance of impurities. In a first attempt to control the radical polymerization of styrenes and methyl methacrylates, in 1955, Ferington and Tobolsky used dithiuram disulfides as initiators.[4] However, due to the high transfer constants involved, retardation of polymerization was observed. Considering the nature of ionic polymerizations, in order to establish a living radical polymerization process, it was reasonable to assume that initiation should be fast providing a constant concentration of growing chains and that the living process involves equilibration between propagating free radicals and dormant species. As these equilibria are shifted towards the dormant species, the concentration of free radicals decreases substantially and thereby suppresses any transfer and termination steps. Therefore, these polymerizations are usually denoted as controlled/living polymerizations rather than as true living polymerizations because termination and transfer cannot be avoided completely.

3.1 Conventional radical polymerization

3.1.1 Mechanism

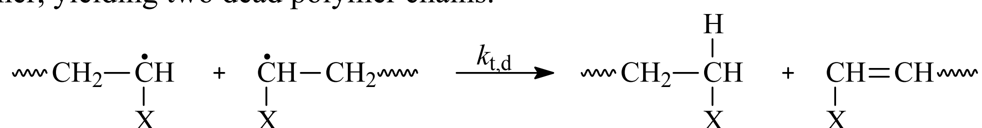
Radical chain polymerization may be considered as a process comprising three steps: initiation, propagation and termination.[5] The initiation reaction is the attack of the monomer by a primary radical originating from the initiator. This is generally achieved by homolytic cleavage of the initiator molecule to yield a pair of radicals:



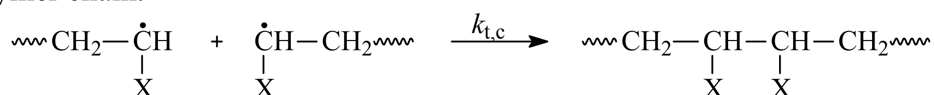
In the initiation step, radicals are usually generated by thermal decomposition of a particular species, such as an azo or peroxy compound (e.g. 1,1'-azobis(isobutyronitrile) AIBN and *tert*-butyl hydroxyperoxide TBHP). Alternatively, radicals can also be formed electrochemically or photochemically. The initiation process continues with the initiating species adding to a monomer molecule, yielding a propagating polymer chain.

In the propagation step, the polymer chain reacts with the unsaturated group in the monomer via radical addition to the double bond.

Once the propagating chain is established, the polymer chain continues to react with monomer until some sort of termination occurs. There are two main termination events: (i) disproportionation occurs when a hydrogen atom is transferred from one propagating chain to another, yielding two dead polymer chains.



(ii) Combination occurs when two propagating polymer chains combine to form one dead polymer chain.



3.1.2 Kinetics

The afore-mentioned steps can be translated into a kinetic scheme and a rate equation. Based on the assumption that the dissociation of the initiator is the rate-determining step in the initiation, the rate of initiation is given by:

$$R_i = 2 f k_d [I] \quad \text{Eq. 3.1}$$

where f is the efficiency of the initiation process, k_d is the rate constant of initiator decomposition and [I] is the initiator concentration. The overall rate of monomer consumption may be considered as the sum of the rate of initiation R_i and the rate of propagation R_p, i.e.

$$-\frac{d[M]}{dt} = R_i + R_p \quad \text{Eq. 3.2}$$

However, if the number of monomers consumed in the initiation step is much less than the number of monomers consumed in the propagation steps, which is the case for a process producing high-molecular weight polymers, then the equation simplifies to:

$$-\frac{d[M]}{dt} = R_p \quad \text{Eq. 3.3}$$

As the rate constant of propagation is principally independent of the chain length, R_p may be expressed as follows:

$$R_p = k_p [M][P_n^\bullet] \quad \text{Eq. 3.4}$$

where k_p is the rate constant of propagation, [M] and $[P_n^\bullet]$ are the concentrations of monomer and propagating radical chains, respectively. Based on the assumption that the number of radicals during the polymerization remains constant (steady-state approximation), the following relation between the rate of initiation R_i and the rate of termination R_t is obtained:

$$R_i = R_t \quad \text{Eq. 3.5}$$

Since termination processes are always bimolecular radical processes, the rate of termination is expressed as:

$$R_t = 2\, k_t\, [P_n^\bullet]^2 \quad \text{Eq. 3.6}$$

where k_t is the rate constant of termination. The value of k_t is composed of a disproportionation and a combination term. Inserting Eq. 3.6 into Eq. 3.1 and considering Eq. 3.5 yields Eq. 3.7:

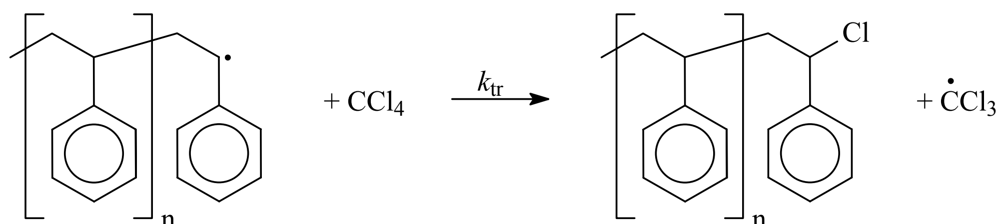
$$[P_n^\bullet] = \left(\frac{fk_d[I]}{k_t}\right)^{1/2} \quad \text{Eq. 3.7}$$

Eq. 3.7 can be substituted by Eq. 3.4, yielding the rate equation for free radical polymerization:

$$R_p = k_p[M]\left(\frac{fk_d[I]}{k_t}\right)^{1/2} \quad \text{Eq. 3.8}$$

Another side reaction of free radical polymerization is chain transfer. It occurs when the radical at the chain end is transferred to another species, resulting in the formation of dead polymer chains and a small radical. It is usually facilitated by the addition of a chain transfer agent, such as a halide or thiol. In the chain transfer process, an atom is transferred from the transfer agent to the growing polymer chain, thereby terminating its growth and giving rise to a new, shorter radical species. As a result, a lower-molecular weight polymer

is formed. An example of a chain transfer reaction is the chain transfer of a propagating styrene radical to carbon tetrachloride:



Chain transfer can be problematic in some systems since the propagating species might transfer quickly to initiator, monomer, polymer or solvent.

Chain transfer kinetics can be described by the Mayo equation:[6]

$$\frac{1}{\overline{P_n}} = \frac{1}{\left(\overline{P_n}\right)_0} + C_M + C_S \frac{[S]}{[M]} + C_I \frac{[I]}{[M]} \qquad \text{Eq. 3.9}$$

where $\overline{P_n}$ and $\left(\overline{P_n}\right)_0$ is the number-average degree of polymerization with and without chain transfer, respectively. $C_M = k_{tr,M}/k_p$ is the chain transfer constant of transfer to monomer, C_S is the chain transfer constant of transfer to solvent, C_I is the chain transfer constant of transfer to initiator, [M] is the monomer concentration, [S] is the chain transfer agent concentration, and [I] is the initiator concentration.[5]

In most cases, transfer to monomer and to initiator can be neglected, which simplifies Eq. 3.9 to Eq. 3.10:

$$\frac{1}{\overline{P_n}} = \frac{1}{\left(\overline{P_n}\right)_0} + C_S \frac{[S]}{[M]} \qquad \text{Eq. 3.10}$$

Degenerative chain transfer takes place when the polymer acts as a transfer agent itself, with chain transfer agent and chain transfer product having the same chemical structure. One example of such a transfer reaction is polymerization mediated by a polymeric iodide:

Under controlled conditions, degenerative transfer may be used for polymerization in a living manner. One example is reversible addition-fragmentation chain transfer (RAFT) polymerization, which will be discussed below.

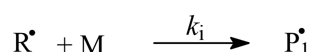
3.2 Controlled/living polymerization

The definition of the terms “controlled” and “living” has been the subject of much controversy and a uniform terminology has not yet been agreed on.[7] One definition of a living polymerization has been proposed by Webster:[8]

(a) the polymerization proceeds to complete conversion with further monomer addition leading to continuing polymerization
(b) the number-average molecular weight is directly proportional to conversion
(c) the number of polymer chains in the system remains constant throughout the polymerization process
(d) the molecular weight can be controlled via the reaction stoichiometry
(e) polymers with chain end functionality are obtained quantitatively

An ideal living polymerization process is characterized by the following reaction steps:

initiation:



propagation:

A polymerization is termed living if there are no irreversible transfer and termination reactions throughout the polymerization.[1,9] Considering a controlled living polymerization process, there is a fast initiation step and $k_i >> k_p$. The equilibration between different active centers will be faster than the polymerization process itself. This means that the number of active centers is always constant:

$$[P^\bullet] = \sum_i [P_i^\bullet] = const. \qquad \text{Eq. 3.11}$$

In this case, only the propagation reaction has to be taken into account. The polymerization rate R_p follows a pseudo-first order time law, where k_{app} can be defined as the "apparent" rate constant:

$$R_p = -\frac{d[M]}{dt} = k_p [P^\bullet][M] = k_{app}[M] \qquad \text{Eq. 3.12}$$

Integration of Equation 3.12 results in:

$$\ln \frac{[M]_0}{[M]_t} = k_p [P^\bullet] t = k_{app} t \qquad \text{Eq. 3.13}$$

In the absence of termination reactions, the first-order time-conversion plot is a straight line with slope $k_{app} = k_p [P^\bullet]$.

In living polymerizations, the number-average polymerization degree P_n increases linearly with monomer conversion x_p:

$$P_n = \frac{\text{concentration of reacted monomers}}{\text{concentration of polymer chains}} = \frac{[M]_0 \cdot x_p}{[P]} \qquad \text{Eq. 3.14}$$

where [P] is the total concentration of polymer chains (including those resulting from termination). A non-linearity of the relationship between number-average polymerization degree and monomer conversion is indicative of either a slow initiation or the occurrence of transfer reactions since the concentration of polymer chains increases with monomer conversion in both cases. The termination of polymer chains cannot be deduced from such a plot as only the concentration of active chains decreases, whereas the total concentration of all chains remains constant. If the number-average polymerization degree is found to be greater than the one calculated from Eq. 3.14, either initiator termination (initiator efficiency $f = [P^{\bullet}]/[I]_0 < 1$) or termination via recombination occurs.

In the case of living polymerization with fast initiation, the expected molecular-weight distribution should be identical with a Poisson distribution,[10] and the non-uniformity U or polydispersity index PDI, respectively, are given by:

$$U = PDI - 1 = \frac{M_w}{M_n} - 1 = \frac{P_{n-1}}{P_n^2} \approx \frac{1}{P_n} << 1 \qquad \text{Eq. 3.15}$$

Therefore, using living polymerization, it is basically possible to produce polymers with very narrow molecular-weight distributions. If, however, broad molecular-weight distributions should be observed in a controlled/living process, this might be ascribed to impurities of the reactants, slow initiation, co-existence of different active species or depolymerization.

In the last decade, three methods of controlled free radical polymerization have gained importance in the synthesis of well-defined polymers with controlled molecular weights and narrow molecular weight distributions. These recent methods include stable free-radical polymerization (SFRP) - best represented by nitroxide-mediated polymerization (NMP) – atom-transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer (RAFT) polymerization.

In nitroxide-mediated polymerization (NMP), nitroxides and *N*-alkoxyamines are used to deactivate the growing radical reversibly, thus reducing the overall concentration of the propagating radical chain end.[11-18] In the absence of other reactions resulting in the initiation of new polymer chains the probability of irreversible termination reactions is very low so that a high degree of control over the polymerization is obtained. Nevertheless, it has to be noted that NMP is successful for making homopolymers and block copolymers based on styrene and its derivatives, but fails mostly in other systems. The only exception known so far is the successful polymerization of acrylates in the presence of phosphonate-derivatized nitroxyl radicals that has been reported by Tordo et al.[16,19,20]

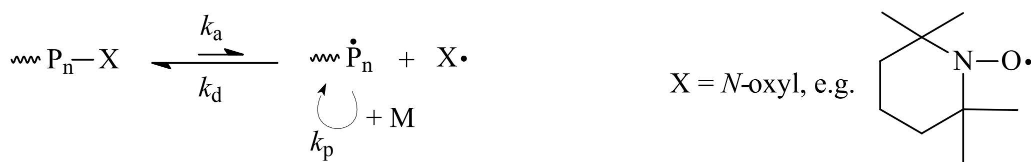
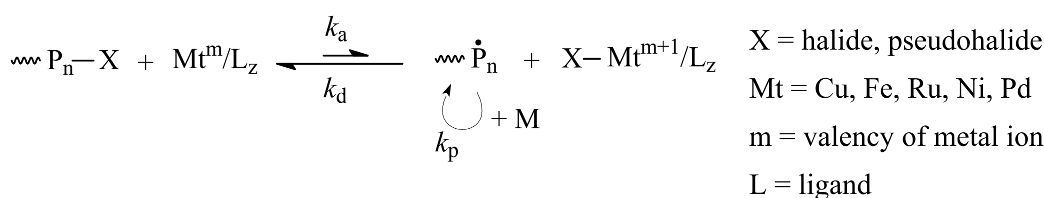


Fig. 3.1. Mechanism of nitroxide-mediated polymerization (NMP).

Atom-transfer radical polymerization (ATRP) makes use of a reversible transfer to a halogen atom between growing polymer chains and a redox-active transition metal catalyst.[21-30] In the key reaction step, macromolecular alkyl halides are activated by reduction to free radicals and the transition metal complexes are oxidized by coordinating the halogen atoms. A number of monomer classes have been polymerized successfully by ATRP, including styrenes, acrylates, methacrylates, and vinyl pyridine. The major drawbacks of the ATRP process are its incompatibility with a variety of monomers, such as acidic or highly polar monomers, due to interaction with the catalyst, and subsequent removal of the transition metal catalyst after polymerization.



X = halide, pseudohalide
Mt = Cu, Fe, Ru, Ni, Pd
m = valency of metal ion
L = ligand

Fig. 3.2. Mechanism of atom-transfer radical polymerization (ATRP).

Reversible addition-fragmentation chain transfer (RAFT) polymerization will be dealt with in the following section.

3.3 RAFT polymerization

Although the synthetic potential of the RAFT process is well documented, its mechanistic and kinetic understanding is still the subject of lively debate in the scientific community. Recently, the mechanism and kinetics of RAFT polymerization have been investigated by various research groups in order to determine rate coefficients and other kinetic parameters.[31-34] Despite the combined effort to elucidate the specifics of the RAFT process, there is some disagreement between different studies concerning kinetic and mechanistic details. It has to be noted, though, that even well-established controlled radical polymerization techniques, such as nitroxide-mediated or atom-transfer radical polymerization, are still being the subject of ongoing research in terms of their mechanism and kinetics.[18,25,35]

3.3.1 Mechanism

From a conceptual point of view, the “iniferter” (*ini*tiator – trans*fer* – *ter*minator) technique introduced by Otsu in 1982[36-38] is a predecessor to the controlled radical polymerization method known as reversible addition-fragmentation chain transfer (RAFT) polymerization.[39-41] In the iniferter case, disulfides R-S-S-R or *N*,*N*-diethyldithiocarbamoyl $Et_2N(C{=}S)SR$ compounds were proposed as photochemical initiators where cleavage occurs at the C-S bond to yield a carbon-based radical and the mediating thio radical (Fig. 3.3).

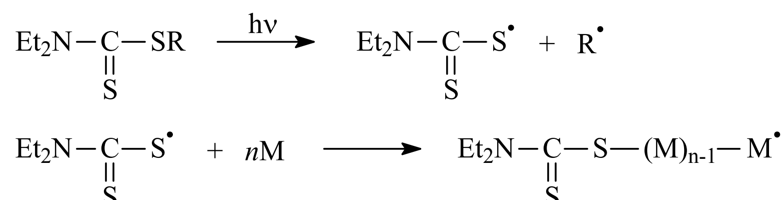


Fig. 3.3. Decomposition of dithiocarbamoyl compounds used in the iniferter technique.

Albeit the linear increase of molecular weight with conversion observed with this method, it fails to produce polymers with controlled molecular weights and low polydispersities as the thio radical can also initiate polymerization. With the introduction of a variety of (thiocarbonyl)sulfanyl derivatives of common structure Z-C(=S)-SR by Rizzardo et al., chain transfer agents became available that can be fragmented in a controlled manner in the presence of initiating species. The key to the living character of RAFT polymerization is the very high transfer constant associated with the thiocarbonylthio group and, consequently, the fast equilibration between active and dormant polymer chains. One of the major accomplishments of the RAFT method as compared to the iniferter technique is the use of dithiocarbamates that have the nonbonded electron pair of nitrogen incorporated into an aromatic system resulting in highly effective chain transfer agents in styrene and (meth)acrylate ester polymerization. In contrast, simple *N*,*N*-dialkyl dithiocarbamates are ineffective in RAFT polymerization.[42] Besides these dithiocarbamates, a great variety of dithioesters, trithiocarbonates, xanthates and similar compounds have been found to be effective chain transfer agents (cf. Fig. 3.4.).[43-45]

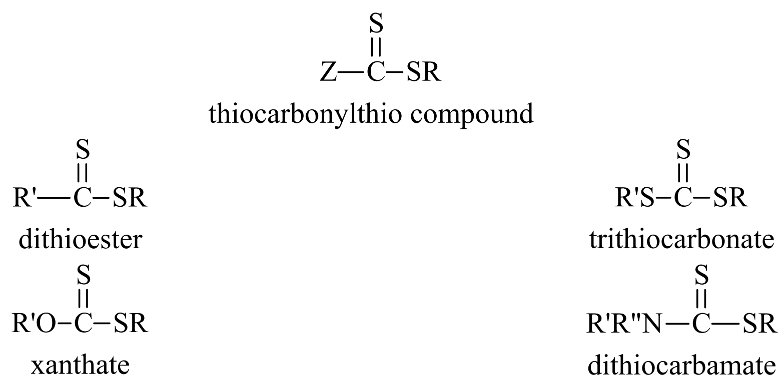


Fig. 3.4. General structures of chain transfer agents used in RAFT polymerization.

The experimental conditions employed in RAFT polymerization are those used for conventional free radical polymerizations. The polymerization can be performed in bulk, solution, emulsion or suspension. Common initiators, such as azo or peroxy compounds, are used and there are no particular limitations on solvent and reaction temperature. One of the major advantages of the RAFT process over other controlled/living radical polymerization processes is its compatibility with a wide range of monomers including functional monomers containing acid, acid salt, hydroxyl or amino groups.

The mechanism of the RAFT process is believed to involve a series of reversible addition-fragmentation steps. Addition of a propagating radical $P_n^\bullet$ to a thiocarbonylthio compound gives an adduct radical which fragments into a polymeric thiocarbonylthio compound and a new radical $R^\bullet$ (Fig. 3.5.). The radical $R^\bullet$ then reinitiates polymerization to give a new propagating radical $P_m^\bullet$. Subsequent addition-fragmentation steps set up an equilibrium between the propagating radicals $P_n^\bullet$ and $P_m^\bullet$ and the dormant polymeric thiocarbonylthio compounds by way of an intermediate radical. Equilibration of the growing chains gives rise to a narrow molecular weight distribution. Throughout the polymerization and at its end, the majority of the polymer chains are end capped by a thiocarbonylthio group.

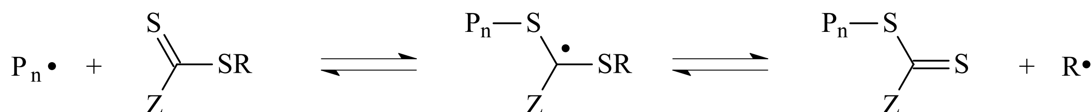


Fig. 3.5. Simplified mechanism of the RAFT process (addition-fragmentation step).

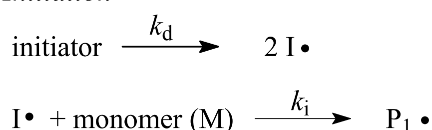
Evidence for this mechanism was found by direct ESR observation of the intermediate radical[46] and by end group analysis of the polymer products by NMR and UV-vis spectroscopy[41] as well as by MALDI-TOF mass spectrometry.[47-49]

3.3.2 Kinetics

Despite the growing number of publications in the area of RAFT polymerization, detailed kinetic data for RAFT systems are still rare but their investigation is one of the major subjects of recent research. Early estimates for some of the coefficients involved have been made by Fukuda et al.[32,34] and Monteiro et al.[50] Some research groups have investigated controlled/living processes using simulation,[51,52] with the most comprehensive studies performed by Fischer and Souaille,[53] Barner-Kowollik et al.,[33,54] and Vana et al.[55,56]

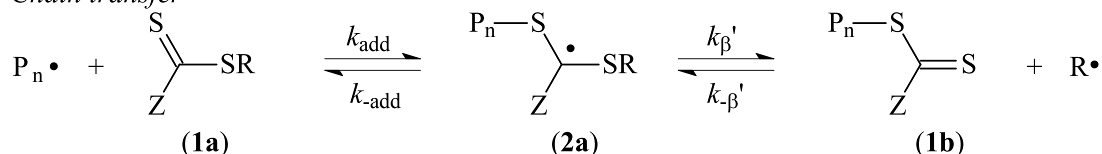
The overall mechanism of RAFT polymerization can be divided into five major steps: (1) initiation, (2) propagation, (3) chain transfer, (4) reinitiation, (5) chain equilibration, and (6) termination (Fig. 3.6).

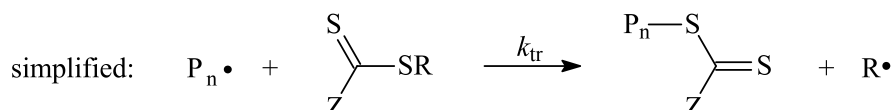
(1) Initiation



(2) Propagation

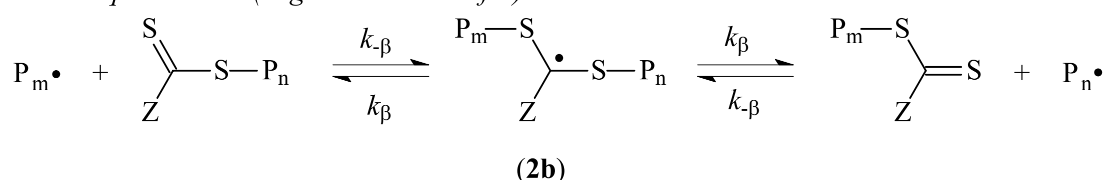
(3) Chain transfer





(4) Reinitiation

(5) Chain equilibration (degenerative transfer)



(6) Termination

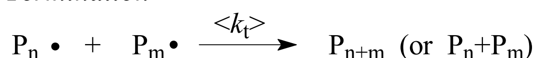


Fig. 3.6. Major steps of the RAFT process.

The decomposition of the initiator I proceeds with the effective rate coefficient $k_d = k_d^* \cdot f$, where k_d^* is the rate coefficient for initiator composition and f is the initiator efficiency. The reaction of an initiator-derived radical $I^\bullet$ with monomer is described by the rate coefficient of initiation k_i (step (1) in Fig. 3.6). The rate of addition of propagating radicals $P_n^\bullet$ to chain transfer agent is given by the rate coefficient of addition k_{add}, where the reverse reaction is described by the coefficient k_{-add} ((2) in Fig. 3.6). This RAFT preequilibrium can be considered as a transfer reaction in which the leaving group R is released as initiating free radical. The corresponding chain transfer coefficient k_{tr} is a composite of the rate coefficients governing the pre-equilibrium (simplified (2) in Fig. 3.6). Reinitiation of polymerization by the chain transfer agent leaving group $R^\bullet$ proceeds with the rate coefficient of initiation k_i' and propagation of the polymeric radicals is described by the rate coefficient of propagation k_p (step (3) in Fig. 3.6). The equilibrium between growing and dormant polymeric chains ((4) in Fig. 3.6) is the core of the RAFT process and is described by the equilibrium constant *K*, representing the quotient of the rate coefficient of addition k_β and the rate coefficient of fragmentation $k_{-\beta}$:

$$K = \frac{k_\beta}{k_{-\beta}} \qquad \text{Eq. 3.16}$$

In the addition step, k_β controls the bimolecular reaction between free polymeric radicals and polymeric chain transfer agent, which leads to the formation of macroRAFT radical (**2b**); $k_{-\beta}$ describes the inverse average lifetime of the intermediate macroRAFT radical.[55]

Bimolecular termination between growing chains to form "dead" polymer is described by the mean rate coefficient of termination $\langle k_t \rangle$ (step (5) in Fig. 3.6). Among the termination reactions not considered in the above mechanism are termination between free polymeric radicals and initiator-derived radicals $I^\bullet$ or initial chain transfer agent-derived radicals $R^\bullet$. These can usually be neglected.

The rate of polymerization R_p is similar to conventional free-radical polymerization:

$$R_p = k_p [M][P_n^\bullet] \qquad \text{Eq. 3.17}$$

The variation of $[P_n^\bullet]$ with time is quite different from that in free-radical polymerization and can be described as follows, assuming that [**2b**] << [**1b**]:

$$\frac{d[P_n^\bullet]}{dt} = 2f[I]_0 k_d e^{-k_d t} - k_t [P_n^\bullet]^2 - k_{add}[\mathbf{1a}][P_n^\bullet] + k_{-add}[\mathbf{2a}] \qquad \text{Eq. 3.18}$$

where [**1a**] and [**2a**] are the concentrations of chain transfer agent and intermediate radical, respectively (cf. Fig. 3.6).

The polymer chains that are able to propagate are divided among dormant CTA-capped chains, propagating chains $P_n^\bullet$, and intermediate radicals, leading to a reduced

concentration of propagating radicals and therefore to less termination reactions compared to free-radical polymerization.[57]

For the estimation of the chain transfer constants, the Mayo equation can only be used if consumption of chain transfer agent and monomer can be neglected. This is only the case for less active chain transfer agents in low-conversion polymerizations. The direct application of the Mayo method underestimates the transfer constant for more active chain transfer agents. For reversible chain transfer, the rate of consumption of chain transfer agent depends on two transfer constants, $C_{tr} = k_{tr}/k_p$ and $C_{-tr} = k_{-tr}/k_i$', which describe the reactivity of the propagating radical $P_n^\bullet$ and expelled radical $R^\bullet$, respectively:

$$-\frac{d[CTA]}{d[M]} \approx C_{tr}\frac{[CTA]}{[M]+C_{tr}[CTA]+C_{-tr}[macroCTA]} \qquad \text{Eq. 3.19}$$

Under the assumption that the adduct radical (**2a**) (Fig. 3.6) undergoes no reactions other than fragmentation, the rate constants for chain transfer are:[58]

$$k_{tr} = k_{add}\frac{k_\beta}{k_{-add}+k_\beta} \qquad \text{Eq. 3.20}$$

and

$$k_{-tr} = k_{-\beta}\frac{k_{-add}}{k_{-add}+k_\beta} \qquad \text{Eq. 3.21}$$

If the rate of the reverse reaction between $R^\bullet$ and macroCTA is negligible and the chains are long, Eq. 3.17 simplifies to that of conventional chain transfer:[59]

$$-\frac{d[CTA]}{d[M]} \approx C_{tr}\frac{[CTA]}{[M]}$$

$$C_{tr} = \frac{k_{tr}}{k_p} \approx \frac{[M]d[CTA]}{[CTA]d[M]} = \frac{d\ln[CTA]}{d\ln[M]} \qquad \text{Eq. 3.22}$$

As can be seen from Eq. 3.20, the slope of the plot of ln[M] versus ln[CTA] yields the transfer constant. If the rate of reactions of $R^\bullet$ with macroCTA is not negligible, the apparent transfer constant obtained from the plot is lower than the actual transfer constant. The transfer constants of various thiocarbonylthio compounds have been reported to extend over more than five orders of magnitude (< 0.01 to > 1000) depending on the R and Z groups of the CTA and the respective monomer.[60]

3.3.3 Influence of chain transfer agent structure

Different RAFT agents are required for monomers with different properties. Methyl methacrylate, for example, gives rise to radicals that are very good leaving groups and can only be polymerized effectively when the chain transfer agent has an at least equally good

leaving group. Furthermore, the thiocarbonyl group has to be activated toward radical addition. If the thiocarbonyl is not active enough, extensive propagation may occur before transfer. In the case of a highly active monomer such as vinyl acetate, the thiocarbonyl group can also be too active to radical addition and the intermediate radical formed will be too stable so that no chains will be available for propagation. In this case, the thiocarbonyl compound has to be deactivated toward radical addition.[61]

The selection of the transfer agent is crucial for the synthesis of low-polydispersity products. It does not only depend on the chain transfer constant but also on the structure of the transfer agent. The R moiety should be a good homolytic leaving group, and the formed $R^{\bullet}$ radical should be able to reinitiate the polymerization. Its leaving group ability is determined by both steric and stability factors. The R group can be either of alkyl or aryl nature. The most frequently used R groups in the RAFT polymerization of styrenes and (meth)acrylates are benzyl ($-CR'''_2Ph$) and cyanoalkyl ($-CR'''_2CN$) moieties. The capability of $R^{\bullet}$ as a leaving group is also determined by the nature of the propagating species formed in the course of polymerization. In order to avoid retardation, the R''' substituents should be chosen in a way that $R^{\bullet}$ easily adds to monomer. The ability of $R^{\bullet}$ to reinitiate polymerization will also depend on the nature of the monomers used in RAFT polymerization. It has been shown that the most effective R groups in RAFT polymerization of styrene and methacrylates are cyanoalkyl and benzyl derivatives, whereas benzyl derivatives are less effective in vinyl acetate polymerization due to the slow initiation which might result in retardation of the polymerization. For this reason, cyanoalkyl derivatives and their corresponding esters ($-CR'''_2CO_2$alkyl) are the R moieties of choice in vinyl acetate polymerization.[61]

The Z group should activate the C=S double bond toward radical addition in order to ensure a higher transfer constant. The Z moiety usually includes alkyl, aryl, or heterocyclic groups. In polymerization of (meth)acrylates and styrenes, dithiocarbamate chain transfer agents with conjugating or electron-withdrawing groups at the nitrogen atom are much more effective than dithiocarbamates with simple alkyl substituents. Consequently, the preferred Z groups are aromatic azacycles, such as pyrroles or imidazoles, or cyclic amides, such as lactams, imides or phthalimides. The reason for the higher effectiveness of the above-mentioned chain transfer agents seems to be correlated with the higher activity of the C=S double bond towards radical addition. This, in turn, is attributed to the conjugating or electron-withdrawing substituents that bestow greater double bond character upon the C=S double bond. In carbamates and amides, the N-CO link has partial double bond character as a result of the delocalisation of the non-bonded nitrogen lone pair with the p electrons of the carbonyl group.[62] As a result, the oxygen of the carbonyl group has a partial negative charge. Since sulfur has a higher electron affinity than oxygen, this effect

would be expected to be more pronounced in dithiocarbamates. If the nitrogen lone pair participates in an alternate π-system (e.g. the aromatic pyrrole ring), the lone pair will be less available for delocalization into the thiocarbonyl bond resulting in a greater double bond character for the C=S double bond and hence a greater reactivity of the chain transfer agent towards radicals (see Fig. 3.7.).

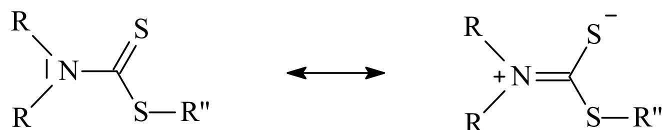


Fig. 3.7. Effect of nitrogen substituents R on double bond character of C=S bond in dithiocarbamates.

Similar considerations apply in the case of xanthate esters. The effectiveness of xanthate ester chain transfer agents in providing low polydispersity polymers in acrylate polymerization increases in the series where R’ is –OEt < $-OC_6H_5$ < OC_6F_5.

For the right choice of chain transfer agent, it has to be considered that the transfer constants of both xanthates and dithiocarbamates are strongly dependent on the type of monomer used. Dithiocarbamate and xanthate derivatives possess relatively low transfer constants in the polymerization of styrene and methacrylates. Nevertheless, in polymerization of vinyl acetate, vinyl butyrate, vinyl chloride and similar vinyl monomers, dithiocarbamates and xanthates show higher transfer constants, enabling the synthesis of polymers with narrow molecular-weight distributions.[61]

Tab. 3.1 summarizes the most effective CTA moieties used in the RAFT polymerization of different monomer classes.

Tab. 3.1. Overview of effective chain transfer agent moieties used for the RAFT polymerization of different monomer classes.[61]

	Z—C(=S)—SR	
monomer	Z	R
styrene	pyrrole, imidazole, lactams, imides, phthalimides	benzyl, 1-phenylethyl, 2-phenylethyl, 2-(alkoxycarbonyl)prop-2-yl, 2-cyanoprop-2-yl, 2-cyanobut-2-yl, 1-cyanocyclohexyl
methacrylates	phenyl, methylthio, pyrrole, imidazole, lactams, imides, phthalimides, OR’ (R’ = Et < C_6H_5 < C_6F_5)	2-phenylpropyl, 2-cyanoprop-2-yl, 2-cyanobut-2-yl, 1-cyanocyclohexyl
vinyl acetate*	*N*-aryl, *N*-alkyl, alkoxy	2-(alkoxycarbonyl)prop-2-yl, cyanomethyl, 2-cyanoprop-2-yl, 2-cyanobut-2-yl, 1-cyanocyclohexyl
acrylates/ acrylic acid	phenyl, pyrrole, methylthio, lactams	benzyl, 2-cyanoprop-2-yl
acrylamides	phenyl	2-phenylpropyl

*complete inhibition with dithioesters, trithiocarbonates and aromatic dithiocarbamates

References

(1) Szwarc, M. *Nature* **1956**, *178*, 1168.

(2) Szwarc, M.; Levy, M.; Milkovich, R. *J. Am. Chem. Soc.* **1958**, *78*, 2656.

(3) Quirk, R. P.; Kinning, D. J.; Fetters, L. J. **1989**, *7*.

(4) Ferington, T. E.; Tobolsky, A. V. *J. Am. Chem. Soc.* **1955**, *77*, 4510-4512.

(5) Odian, G. *Principles of Polymerization*, 3rd ed.; John Wiley & Sons, Inc.: New York, 1991.

(6) Mayo, F. R.; Gregg, R. A.; Matheson, M. S. *J. Am. Chem. Soc.* **1951**, *73*, 1691.

(7) Darling, T. R.; Davis, T. P.; Fryd, M.; Gridnev, A. A.; Haddleton, D. M.; Ittel, S. D.; Jr., R. R. M.; Moad, G.; Rizzardo, E. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 1709-1752.

(8) Webster, O. *Science* **1991**, *251*, 887.

(9) Matyjaszewski, K.; Müller, A. H. E. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1997**, *38*, 6.

(10) Flory, P. J. *J. Am. Chem. Soc.* **1940**, *62*, 1561.

(11) Georges, M. K.; Veregin, R. P. N.; Kazmaier, P. M.; Hamer, G. K. *Macromolecules* **1993**, *26*, 2987-2988.

(12) Hawker, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 11185-11186.

(13) Puts, R. D.; Sogah, D. Y. *J. Am. Chem. Soc.* **1996**, *29*, 3323 - 3325.

(14) Veregin, R. P. N.; Odell, P. G.; Michalak, L. M.; Georges, M. K. *Macromolecules* **1996**, *29*, 2746-2754.

(15) Burguière, C.; Dourges, M.-A.; Charleux, B.; Vairon, J.-P. *Macromolecules* **1999**, *32*, 3883 - 3890.

(16) Benoit, D.; Grimaldi, S.; Robin, S.; Finet, J.-P.; Tordo, P.; Gnanou, Y. *J. Am. Chem. Soc.* **2000**, *122*, 5929 - 5939.

(17) Harth, E.; Hawker, C. J.; Fan, W.; Waymouth, R. M. *Macromolecules* **2001**, *34*, 3856-3862.

(18) Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661-3688.

(19) Benoit, D.; Grimaldi, S.; Finet, J. P.; Tordo, P.; Fontanille, M.; Gnanou, Y. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1997**, *38*, 729-730.

(20) Benoit, D.; Grimaldi, S.; Finet, J. P.; Tordo, P.; Fontanille, M.; Gnanou, Y. In *Controlled Radical Polymerization, ACS Symposium Series*; Matyjaszewski, K., Ed.; American Chemical Society: Washington D.C., 1998; Vol. 685, pp 225-235.

(21) Kato, M.; Kamigaito, M.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1995**, *28*, 1721 - 1723.

(22) Wang, J. S.; Matyjaszewski, K. *Macromolecules* **1995**, *28*, 7901-7910.

(23) Wang, J. S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614-5615.
(24) Xia, J.; Matyjaszewski, K. *Macromolecules* **1997**, *30*, 7679-7700.
(25) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921-2990.
(26) Coessens, V.; Pintauer, T.; Matyjaszewski, K. *Prog. Polym. Sci.* **2001**, *26*, 337-377.
(27) Matyjaszewski, K. *Macromol. Symp.* **2000**, *161*, 1-9.
(28) Ando, T.; Kato, M.; Kamigaito, M.; Sawamoto, M. *Macromolecules* **1996**, *29*, 1070-1072.
(29) Kotani, Y.; Kato, M.; Kamigaito, M.; Sawamoto, M. *Macromolecules* **1996**, *29*, 6979-6982.
(30) Takahashi, H.; Ando, T.; Kamigaito, M.; Sawamoto, M. *Macromolecules* **1999**, *32*, 3820-3823.
(31) Monteiro, M. J.; Hodgson, M.; Brouwer, H. D. *J. Polym. Sci. Part A: Polym. Chem.* **2000**, *38*, 3864-3874.
(32) Goto, A.; Sato, K.; Tsujii, Y.; Fukuda, T.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **2001**, *34*, 402-408.
(33) Barner-Kowollik, C.; Quinn, J. F.; Morsley, D. R.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 1353-1365.
(34) Kwak, Y.; Goto, A.; Tsujii, Y.; Murata, Y.; Komatsu, K.; Fukuda, T. *Macromolecules* **2002**, *35*, 3026 -3029.
(35) Fischer, H. *Chem. Rev.* **2001**, *101*, 3581 - 3610.
(36) Otsu, T.; Yoshida, M. *Makromol. Chem. Rapid Commun.* **1982**, *3*, 127-132.
(37) Otsu, T. *J. Polym. Sci.: A Polym. Chem.* **2000**, *38*, 2121-2136.
(38) Otsu, T.; Matsumoto, A. *Adv. Polym. Sci.* **1998**, *136*, 75-137.
(39) Rizzardo, E.; Moad, G.; Thang, S. H.: Australia, 1998.
(40) Le, T. P.; Moad, G.; Rizzardo, E.; Thang, S. H. In *WO 98/01478*; E.I. Du Pont De Nemours and Co., USA; Le, Tam Phuong; Moad, Graeme; Rizzardo, Ezio; Thang, San Hoa: Australia, 1998.
(41) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559-5562.
(42) Mayadunne, R. T. A.; Rizzardo, E.; Chiefari, J.; Chong, Y. K.; Moad, G.; Thang, S. H. *Macromolecules* **1999**, *32*, 6977-6980.
(43) Thang, S. H.; Chong, B. Y. K.; Mayadunne, R. T. A. *Tetrahedr. Lett.* **1999**, *40*, 2435-2438.
(44) Laus, M.; Papa, R.; Sparnacci, K.; Alberti, A.; Benaglia, M.; Macciantelli, D. *Macromolecules* **2001**, *34*, 7269-7275.

(45) Donovan, M. S.; Lowe, A. B.; Sumerlin, B. S.; McCormick, C. L. *Macromolecules* **2002**, *35*, 4123-4132.

(46) Hawthorne, D. G.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1999**, *32*, 5457-5459.

(47) Ganachaud, F.; Monteiro, M. J.; Gilbert, R. G.; Dourges, M.-A.; Thang, S. H.; Rizzardo, E. *Macromolecules* **2000**, *33*, 6738-6745.

(48) Destarac, M.; Charmot, D.; Franck, X.; Zard, S. Z. *Macromol. Rapid. Commun.* **2000**, *21*, 1035-1039.

(49) Schilli, C.; Lanzendörfer, M. G.; Müller, A. H. E. *Macromolecules* **2002**, *35*, 6819-6827.

(50) Monteiro, M. J.; Brouwer, H. d. *Macromolecules* **2001**, *34(3)*, 349-352.

(51) Moad, G.; Anderson, A. G.; Ercole, F.; Johnson, C. H. J.; Krstina, J.; Moad, C. L.; Rizzardo, E.; Spurling, T. H.; Thang, S. H. In *Controlled Radical Polymerization, ACS Symposium Series*; Matyjaszewski, K., Ed.; American Chemical Society: Washington D.C., 1998; Vol. 685, pp 332-360.

(52) Shipp, D. A.; Matyjaszewski, K. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1999**, *40(2)*, 450-451.

(53) Souaille, M.; Fischer, H. *Macromolecules* **2000**, *33*, 7378-7394.

(54) Barner-Kowollik, C.; Quinn, J. F.; Nguyen, T. L. U.; Heuts, J. P. A.; Davis, T. P. *Macromolecules* **2001**, *34*, 7849-7857.

(55) Vana, P.; Quinn, J. F.; Davis, T. P.; Barner-Kowollik, C. *Aust. J. Chem.* **2002**, *55*, 425-431.

(56) Vana, P.; Davis, T. P.; Barner-Kowollik, C. *Macromol. Theory Simul.* **2002**, *11*, 823-835.

(57) Favier, A.; Charreyre, M.-T.; Chaumont, P.; Pichot, C. *Macromolecules* **2002**, *35*, 8271-8280.

(58) Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1996**, *29*, 7717-7726.

(59) Chong, B. Y. K.; Krstina, J.; Le, T. P. T.; Moad, G.; Postma, A.; Rizzardo, E.; Thang, S. H. *Macromolecules* **2003**, *in press*.

(60) Quinn, J. F. *Ph.D. thesis*; The University of New South Wales: Sydney, 2002.

(61) Chiefari, J.; Mayadunne, R. T. A.; Moad, G.; Rizzardo, E.; Thang, S. H. In *WO 99/31144*; E.I. DuPont de Nemours and Company: Australia, 1999.

(62) Deslongchamps, P. *Stereoelectronic effects in organic chemistry*, 1st ed.; Pergamon Press: New York, 1983; Vol. 1.