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Michael addition reactions in macromolecular design for emerging technologies

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Abstract

The Michael addition reaction is a versatile synthetic methodology for the efficient coupling of electron poor olefins with a vast array of nucleophiles. This review outlines the role of the Michael addition reaction in polymer synthesis with attention to applications in emerging technologies including biomedical, pharmaceutical, optoelectronic, composites, adhesives, and coatings. Polymer architectures, which broadly range from linear thermoplastics to hyperbranched polymers and networks are achievable. The versatility of the Michael reaction in terms of monomer selection, solvent environment, and reaction temperature permits the synthesis of sophisticated macromolecular structures under conditions where other reaction processes will not operate. The utility of the Michael addition in many biological applications such as gene delivery, polymer drug conjugates, and tissue scaffolds is discussed in relation to macromolecular structure.

Keywords: Michael addition; Conjugate addition; Gene transfection; Drug delivery; Tissue scaffolds; Acetoacetates; Acrylates; Bioconjugates; Cysteine

Contents

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1. Motivation and scientific rationale

The Michael addition reaction, which is also commonly termed conjugate addition, has recently gained increased attention as a polymer synthesis strategy for tailored macromolecular architectures. The Michael addition, named for Arthur Michael, is a facile reaction between nucleophiles and activated olefins and alkynes in which the nucleophile adds across a carbon–carbon multiple bond [1]. The Michael addition benefits from mild reaction conditions, high functional group tolerance, a large host of polymerizable monomers and functional precursors as well as high conversions and favorable reaction rates [2]. The Michael reaction lends itself to both step growth [3] and chain growth polymerization [4] and has been employed in the synthesis of linear, graft, hyperbranched, dendritic and network polymers. Furthermore, post-polymerization modification [5] and coupling of biological and synthetic polymers are often facilitated by the Michael reaction [6]. These features make the Michael addition reaction well-suited to numerous emerging technologies including biomedical applications such as gene transfection [7], cell scaffolds [8] and tissue replacements [2].

The Michael addition reaction enables a wide range of polymers from diverse monomers, and corresponding polymers are prepared in environments in which other polymerization mechanisms will not operate. In biological applications such as protein derivitization the mild Michael addition reaction conditions are favorable since high temperatures, oxidizing radicals, and organic solvents are not feasible [9]. Furthermore, the Michael addition has recently found utility for the synthesis of crosslinked polymers such as hydrogels [10], thermoset resins [11], and coatings, where rapid cure and high conversions are necessary for performance. Few polymerizations offer sufficient rates to permit room temperature cure, and industrial coatings are often limited to toxic and environmentally hazardous isocyanate containing monomers. The Michael addition proceeds rapidly at room temperature, offers low cure times, and involves less toxic precursors. Non-linear optical materials were also realized using the Michael addition, which benefits from the absence volatile byproducts [12]. The Michael addition is also ubiquitous in classical polymer chemistry, such as the anionic polymerization of alkyl methacrylates and cyanoacrylates.

The Michael addition involves the addition of a nucleophile, also called a ‘Michael donor,’ to an activated electrophilic olefin, the ‘Michael acceptor’, resulting in a ‘Michael adduct’, as shown in Fig. 1b. Although, the Michael addition is generally considered

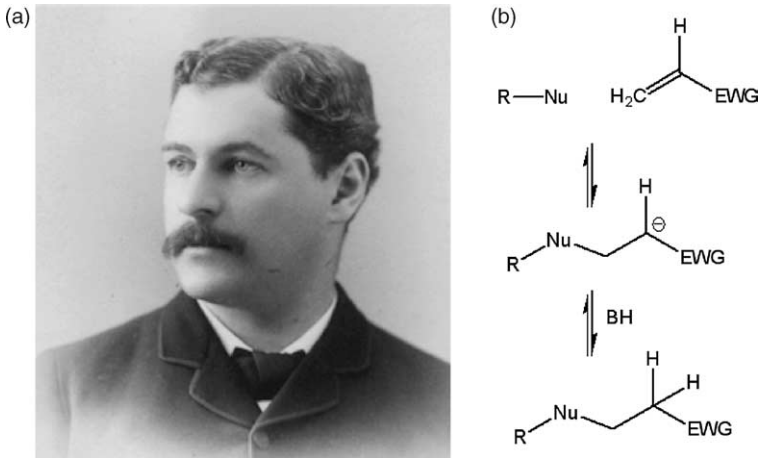


Fig. 1. (a) Arthur Michael (1855–1942), who discovered the Michael addition reaction. (b) Schematic depiction of the Michael addition reaction.

the addition of enolate nucleophiles to activated olefins, a wide range of functional groups possess sufficient nucleophilicity to perform as Michael donors. Reactions involving non-enolate nucleophiles such as amines, thiols, and phosphines are typically referred to as 'Michael-type additions'. In this review, we will refer to Michael-type addition reactions with all nucleophilic donors as Michael additions. The Michael acceptor possesses an electron withdrawing and resonance stabilizing activating group, which stabilizes the anionic intermediate. Michael addition acceptors are far more numerous and varied than donors, due to the plethora of electron withdrawing activating groups that enable the Michael addition to olefins and alkynes. Acrylate esters, acrylonitrile, acrylamides, maleimides, alkyl methacrylates, cyanoacrylates and vinyl sulfones serve as Michael acceptors and are commercially available. Less common, but equally important, vinyl ketones, nitro ethylenes, α,β-unsaturated aldehydes, vinyl phosphonates, acrylonitrile, vinyl pyridines, azo compounds and even β-keto acetylenes and acetylene esters also serve as Michael acceptors [13].

2. Introduction to the Michael addition reaction

The Michael reaction typically refers to the base-catalyzed addition of a nucleophile such as an enolate anion (Michael donor) to an activated α,β-unsaturated carbonyl-containing compound (Michael acceptor) as in Fig. 1b [14–18]. However, over the years, the scope of this reaction has increased dramatically to include a broad range of acceptors and the Michael-type additions of non-carbon donors. Due to the many types of Michael additions in the literature, we will focus here on investigating the mechanism and kinetics of only a few examples, namely the carbon–carbon bond forming Michael addition (referred to as the carbon-Michael addition), nitrogen (amine or aza) Michael additions, and the reaction of thiols with Michael acceptors.

2.1. The mechanism of the carbon Michael addition

One of the most well-known carbon-Michael transformations is the base-catalyzed addition of ethyl acetoacetate to methyl acrylate [19]. The mechanism of the reaction is fairly straightforward, with every step being in equilibrium and thermodynamically dependent on the relative strengths of the base and the type of acetoacetate. The acetoacetate is first deprotonated by the base, providing an enolate anion (Michael donor) in equilibrium (Fig. 2). The enolate anion then reacts in a 1,4-conjugate addition to the olefin of the acrylate (Michael acceptor). The carbonyl of the acrylate stabilizes the resulting anion until proton transfer occurs, regenerating the base. The overall driving force for the conjugate addition is the enthalpic change that accompanies replacement of a π-bond with a σ-bond. Thus, there is the preference for 1,4-addition over 1,2-addition. In some cases however, kinetically controlled reaction conditions can afford attack at the carbonyl carbon rather than at the β-carbon of the olefin [15,20].

From Fig. 2, one can observe that the rate determining step is the attack of the enolate anion on the activated olefin. The reaction rate is therefore second order overall and first order with respect to the enolate anion and the olefin acceptor. The concentration of the enolate is a function of the base strength and the K_{eq} of the deprotonation of the active methylene proton. It follows that the equilibrium constant is

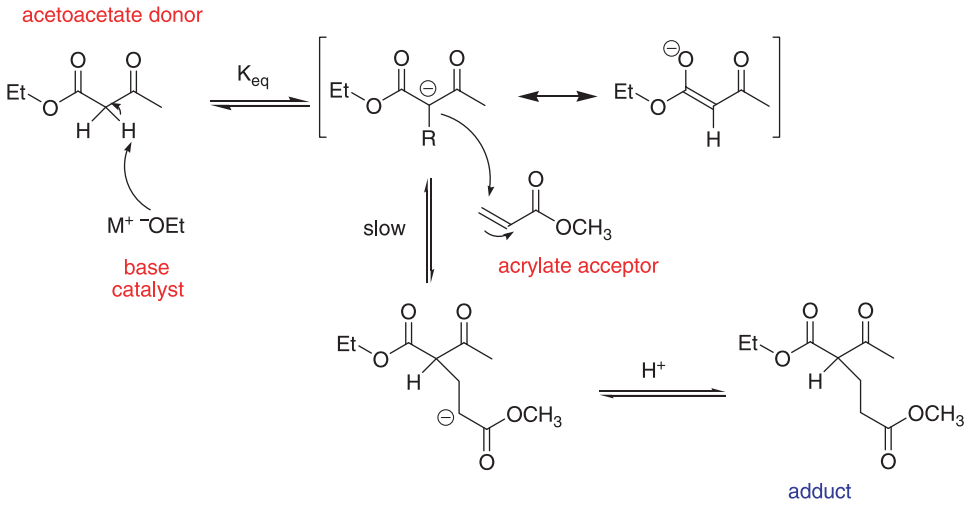


Fig. 2. General carbon-Michael reaction mechanistic scheme.

dependent on the relative strength of the base and the structure of the acetoacetate.

It is important to note that the product of the first Michael addition has a remaining active methylene hydrogen which can undergo a second addition to another acrylate (Fig. 3). Clemens et al. have documented that the second pK_a is expected to have a value of 13 (versus the pK_a of the initial active proton of 12) [21]. The second deprotonation therefore has a different equilibrium constant (K_{eq}) and it is assumed that the concentration of the first Michael adduct will be low. As a result, the concentration of the enolate at any rate (especially in the early stages of the reaction) is not strongly affected by this second reaction.

It follows that a rate law can be determined for the above reaction sequence. Writing the reactions in terms of the Michael addition, we arrive at the kinetic equations:

And corresponding rate equations:

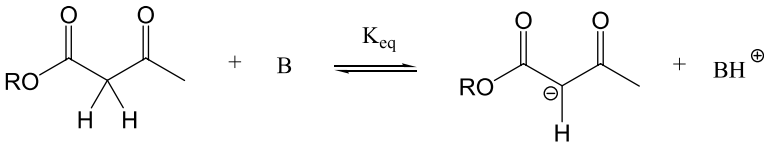
$$K_{eq} = [AcAc^-][BH^+]/[AcAc][B] \quad (3)$$

$$\text{Rate} = k_2[AcAc^-][\text{Acrylate}]$$

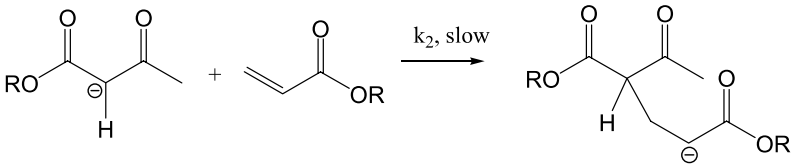
$$= k_2 k_{eq}([B]/[BH^+])[AcAc][\text{Acrylate}] \quad (4)$$

2.1.1. Effect of base strength

The choice of base catalyst has a tremendous effect on the reaction kinetics. As previously noted, the concentration of the enolate and therefore the value of K_{eq} is highly dependent on the relative base strength. For strong bases, K_{eq} will lie far to the right and the concentration of the enolate anion is then approximately equal to the concentration of the base (achieving a steady state concentration). In the resulting rate expression



(1)



(2)

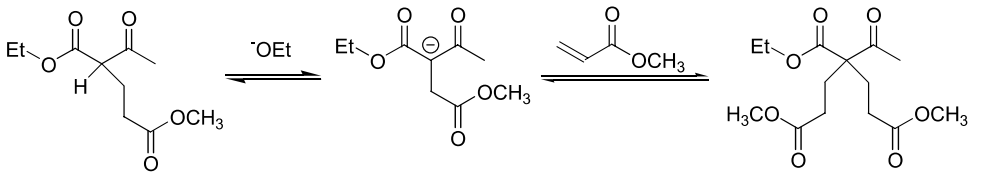


Fig. 3. Second Michael addition of acetoacetate group to methyl acrylate.

Eq. (5), there is a pseudo first-order dependence on acrylate concentration, where k_{obs} is a function of base concentration.

$$\text{Rate} = k_{obs}[\text{Acrylate}] \quad (5)$$

In the case of weaker base catalysts, K_{eq} has a moderate value, but is not large. This introduces the equilibrium constant into the rate law and results in second order behavior Eq. (4). Clemens et al. completed an extensive study on the effect of base strength on reaction kinetics and molecular weight when the Michael addition is used to prepare crosslinked acetoacetate resins for thermoset coatings [21]. In a model study, Clemens analyzed the ability of bases such as TMG (tetramethylguanidine), triethylamine, DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), and DBN (1,5-diazabicyclo[4.3.0]non-5-ene) and found that the rate of the reaction was dependent on catalyst concentration as indicated in Eq. (4). For strong bases such as hydroxide, a steady-state concentration of enolate anion is achieved and pseudo-first order kinetics were observed as in Eq. (5).

The pK_a of the Michael donor is important to the choice of base catalyst, which must have a pK_a for its conjugate acid in the same range as the Michael donor. Stronger bases such as tetramethylguanidine, with a pK_a of 13.6 in its protonated form, are needed in the case of the carbon Michael addition of enolates due to the high pK_a of the acetoacetate groups (pK_{a1} = 12, pK_{a2} = 13.6). Base catalysts are often unnecessary in the case of amines, because of the strong nucleophilicity of the nitrogen atom, whereas weak bases aid in deprotonation of thiols.

2.1.2. Effect of solvent

Typical solvents for the carbon-Michael reaction include methanol, ethanol, diethyl ether, tetrahydrofuran, benzene, xylene, dioxane and mixtures of these solvents. Initially, protic solvents were desirable in the carbon-Michael reaction to promote rapid proton transfer and to stabilize charged intermediates, however, Schlessinger's group has shown that high yielding reactions were also achieved using aprotic solvents [22–24]. The choice of solvent strongly depends on the solubility of the catalyst, donor, and acceptor as well as sensitivity to side reactions. For example, if the reactants or products are susceptible to alcoholysis (ester exchange or hydrolysis), self-condensation of the substrate or the 'retro-Michael' reaction, a non-hydroxylic solvent is desirable [14,25]. In some cases, reactions are carried out in the absence of solvent, especially for network or coating applications.

2.1.3. Effect of substrate

As mentioned before, the initial step of the carbon-Michael addition is deprotonation of an active methylene proton. Thus, donors must stabilize the resulting negative charge. Examples of functional groups, which stabilize enolate donors include ketones, aldehydes, nitriles, amides, nitrones, sulfones, malonates and acetoacetates. A great deal of thought must be directed to the selection of the proper donor and base, and knowledge of the pK_a of these various species is critical. Furthermore, strong bases such as hydroxide and methoxide may promote side reactions such as ester hydrolysis, so non-nucleophilic bases are often desirable.

Acceptors in the carbon-Michael addition are typically olefins that are activated for nucleophilic attack. The resulting anion from nucleophilic attack of the donor must also be stabilized for the reaction to occur. Thus, the use of α,β-unsaturated carbonyl compounds is prevalent in Michael addition chemistry. The reactivity of the acceptor will decrease if the substituent is electron rich, such as in the case of alkyl, aryl or carboethoxy substituted olefins such as styrene derivatives or vinyl

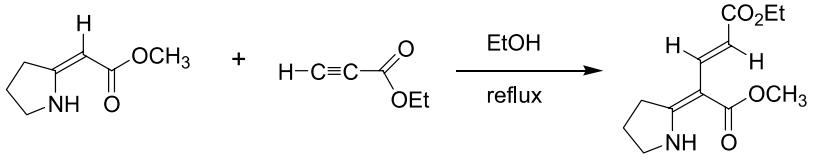


Fig. 4. Michael Addition involving an alkyne acceptor.

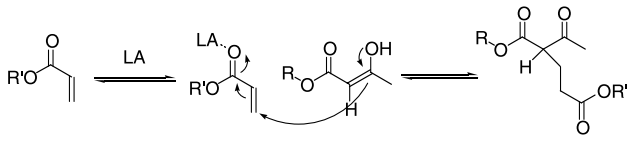


Fig. 5. Lewis-acid catalyzed Michael addition reaction.

ethers. Sterics are also an important factor. The larger the groups substituted at the α and β positions, the slower the reaction rate, although this can be offset by stronger polar effects [18,26]. Differences in reactivity between acceptors require differences in nucleophilicity of the Michael donors. For instance, alkyl methacrylates are relatively poor Michael acceptors. Thus, stronger nucleophiles such as carbanions are necessary for successful Michael addition to alkyl methacrylates. Better acceptors such as alkyl acrylate or acrylamide groups readily accept weaker amine and thiol nucleophiles.

Alkynes have also been utilized as Michael acceptors. In Fig. 4, electron-rich enamines react readily with activated alkynes in refluxing ethanol [27]. Products of Michael reactions with alkynes have the additional variables of stereochemistry of the unsaturated product, and regiochemistry of addition for disubstituted alkynes acceptors.

2.1.4. Other carbon-Michael catalysts

Although base-catalysis is most prominently used in the carbon-Michael addition, the reaction is also catalyzed with acids, particularly in the case of Lewis acids. Some of the earlier examples include the use of boron trifluoride, aluminum trichloride, and zinc chloride [28]. In these cases, the Lewis acid coordinates to the carbonyl of the acrylate to activate the olefin (Fig. 5). The coordinated complex will then react with the nucleophile to obtain the same adduct as in the base-catalyzed Michael addition. In the case where silyl enolates are donors, the reaction is often referred to as the Mukaiyama–Michael reaction.

Researchers have used Lewis acid complexes to their advantage over the years, especially in the area of enantioselective additions. For example, Heathcock's group has shown that silyl enolates will react enantioselectively with α,β-unsaturated ketones in the presence of $TiCl_4$ (Fig. 6) [29]. In the area of enantioselective carbon-Michael additions, the reader is directed to two recent reviews published on the subject [30,31].

Phosphines also catalyze the carbon-Michael reaction [13]. The reaction sequence begins with nucleophilic attack of the phosphine on the β-position of the olefin, generating a reactive phosphonium ylid (Fig. 7). The resulting anion can then either react as a nucleophile or as a base. If an acetoacetate is present in the reaction, the ylid deprotonates the acetoacetate first, which adds to the β-position of another olefin in a Michael fashion. Proton transfer then results in regeneration of the initial olefin and the phosphine catalyst.

2.2. Heteroatomic donors in the Michael reaction

Carbon-based nucleophiles represent only one class of donors used in Michael addition reactions. Heteroatomic nucleophiles involving nitrogen, sulfur, oxygen and phosphorus have also been used. In this section, two of these additions will be discussed: the aza-Michael reaction (amines) and the thiol Michael addition reaction. These are of primary importance due to the presence of sulfur and nitrogen nucleophiles in biological systems.

2.2.1. The aza-Michael reaction

The nitrogen-donor version of the Michael addition is often referred to as the aza-Michael reaction. Since only a few concepts will be touched upon here, the reader is encouraged to investigate several reviews for additional information [16,32–34]. Since amines can act as both nucleophiles and bases, no additional base is typically needed in these reactions. The reaction tends to follow second-order kinetics based on the concentration of the olefin acceptor and the amine (Fig. 8).

Primary amines can react with two equivalents of acceptor to form tertiary amines. In some cases, this

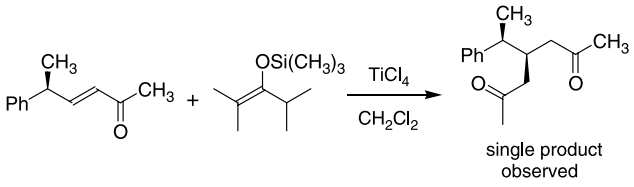


Fig. 6. Stereoselective Michael addition between enone and silyl enolate in the presence of $TiCl_4$.

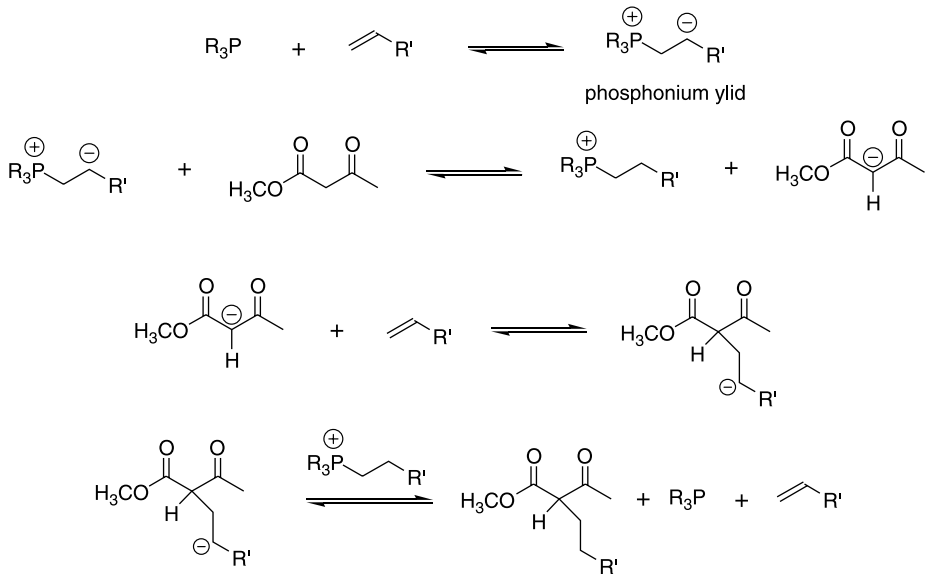


Fig. 7. Phosphine catalyzed Michael addition reaction.

second addition affects the observed kinetics, especially as the concentration of the secondary amine increases. An example is the reaction of methyl amine with ethyl acrylate, giving an excellent yield of the tertiary amine (Fig. 9) [35].

In the aza-Michael reaction, secondary amines are more nucleophilic than primary amines and are therefore more reactive. However, it is worth noting that this is highly dependent on the electronic and steric environment of the amine. For example, 1,4-butanediol diacrylate was allowed to react with 1-(2-aminoethyl)piperazine in an equimolar ratio (Fig. 10) [36]. During the initial part of the experiment, it was found that there was exclusive reaction with the secondary amine present in the piperazine ring. Only upon longer reaction times, did reaction occur with the primary amine, which led to polymerization.

Acid catalyzed aza-Michael additions have also been studied extensively. Spencer and coworkers have determined a general and efficient way of reacting carbamates (NHCOOR) with α,β-unsaturated carbonyl compounds (Fig. 11), providing precursors to β-amino acids [32].

Vedejs and Gringas have also shown that acids catalyze the aza-Michael addition [37]. In Fig. 12, a tertiary amine, as the donor, adds to the alkyne in the presence of catalytic *p*-toluenesulfonic acid. The resulting intermediate further undergoes a Claisen rearrangement and proton transfer to afford the final α,β-unsaturated amine.

Lewis acids have also been used to catalyze the aza-Michael reaction, as in Fig. 13. The mechanism is thought to occur in an analogous fashion to the

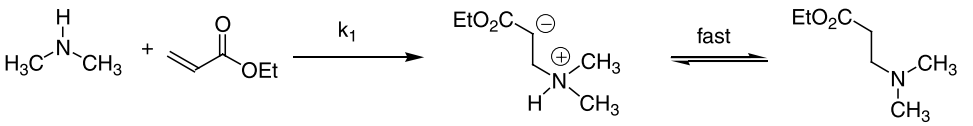


Fig. 8. Aza-Michael addition reaction of dimethylamine with ethyl acrylate.

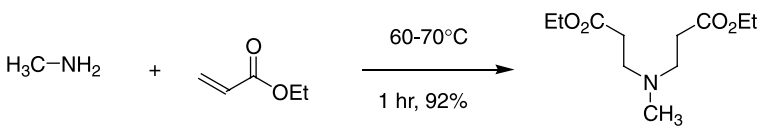


Fig. 9. Aza-Michael addition of methyl amine to two equivalents of ethyl acrylate.

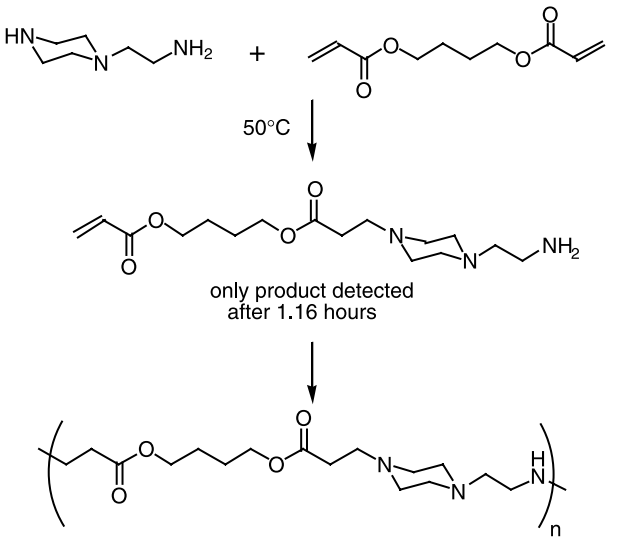


Fig. 10. Higher reactivity of secondary amines in aza-Michael addition reactions.

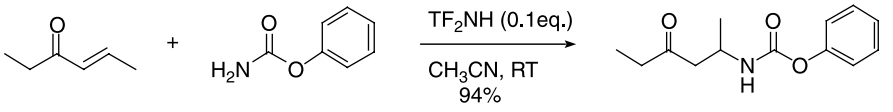


Fig. 11. Acid catalyzed aza-Michael addition.

carbon-Michael addition where the Lewis acid coordinates to the carbonyl of the α,β-unsaturated olefin. However, Spencer has reported that, in the case of weakly basic amine nucleophiles, Brønstead acids that are formed during the reaction may catalyze the reaction [32].

The ability to generate β-amino carbonyl compounds has become increasingly important to the natural product and pharmaceutical areas. The aza-Michael reaction is a key transformation which enables the preparation of such compounds. Jørgensen was the first to report the enantioselective conjugate amine additions (Fig. 14) [38]. The $TiCl_2$-BINOL Lewis acid catalyst coordinates with the *N*-acyloxazolidinone, creating a chiral environment in which the primary amine will preferentially attack from one face of the complex, resulting in the formation of one major diastereomer. The oxazolidinone ring is then removed to afford the β-amino acid. Although, the enantioselectivity is low (maximum enantiomeric excess of 42%), the proof of concept was accomplished. The area has grown significantly and Sibi has published two reviews which discuss stereoselective aza-Michael additions to generate β-amino acids [30,39].

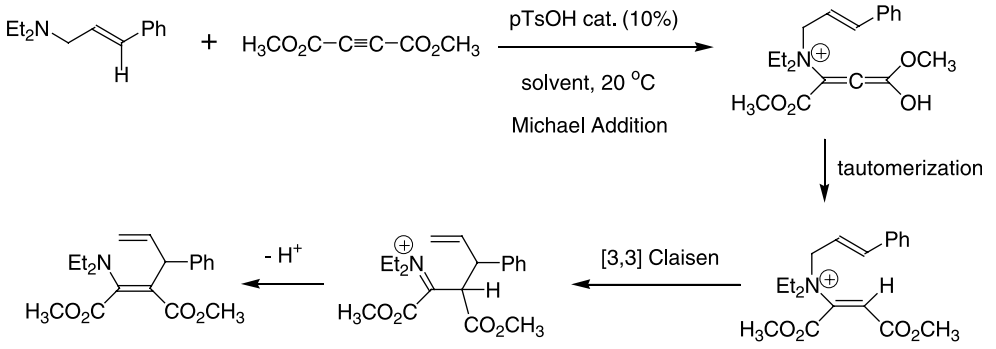


Fig. 12. Acid catalyzed aza-Michael addition to activated alkyne acceptors.

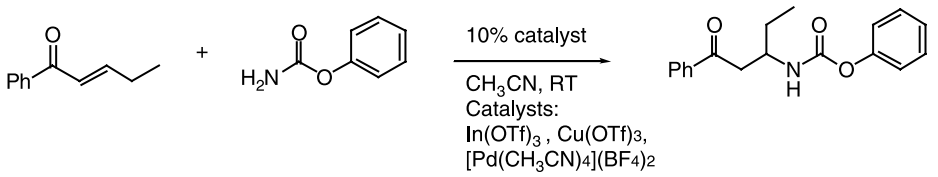


Fig. 13. Lewis acid catalyzed aza-Michael reaction.

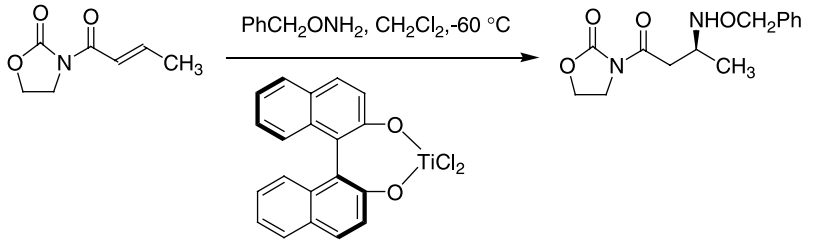


Fig. 14. Stereoselective aza-Michael additions.

2.2.2. Thiols as Michael donors

Thiols can also be used as heteroatomic donors in the Michael addition. Many of the same reaction scenarios that were described above can be directly applied to the addition of thiols to activated olefins. Thiols are generally more nucleophilic than amines, although bases are often used to deprotonate them due to their comparatively higher acidity. The thiolate anion is often the active species in Michael additions involving thiols. The thiol Michael addition reaction rates increase with pH due to the increased concentration of the thiolate anion [10]. The thiol group is a useful Michael donor in biological systems where thiols are present on proteins in cysteine residues. One primary side reaction in thiol Michael additions is disulfide bond formation, which often prompts the use of protecting group chemistry.

Regioselective Michael additions of thiols to asymmetrically substituted fumarate esters or amides was achieved using lithium salts as Lewis acids or base catalysts to alter the charge environment around the double bond through coordination of carbonyls or deprotonation of the thiol (Fig. 15a) [40]. Enantioselective Michael additions of thiols to methacrylates and other 1-alkyl acrylates was accomplished with chiral lanthanide catalysts to enantiomeric excesses as high as 90% ee [41]. Enzyme catalysis of the thiol addition to 2-butenal was shown with *Candida antarctica* lipase B [42]. The active site of the enzyme in this reaction is described in Fig. 15b. Suprisingly,

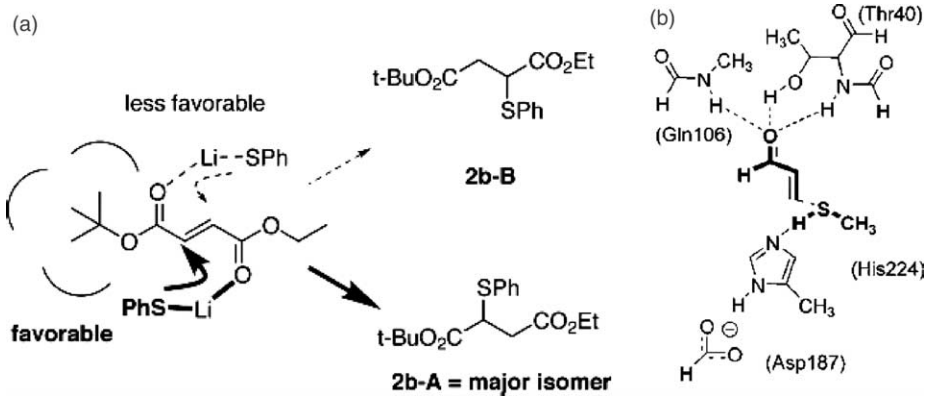


Fig. 15. (a) Regioselective Michael addition through preferential coordination of substrate carbonyls with lithium cations, reprinted from Tetrahedron, 59, Kamimura et al. 'On the regioselectivity for the Michael addition of thiols to unsymmetrical fumaric derivatives,' 9537–9546, Copyright 2003, with permission from Elsevier [40] (b). Michael addition in the active site of wild *Candida antarctica* lipase B, reproduced from 'Exploring the active-site of a rationally redesigned lipase for catalysis of Michael-type additions,' Carlqvist et al. Copyright 2006 Society of Chemical Industry with permission from John Wiley and Sons, Ltd on behalf of the SCI [42].

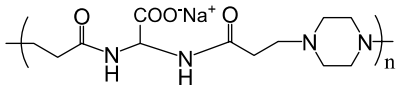
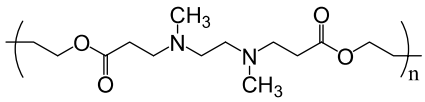
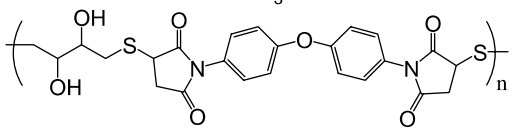
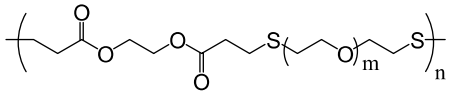
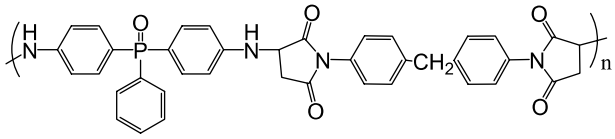
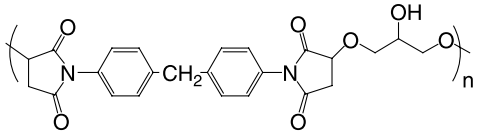
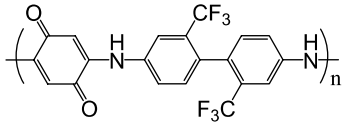
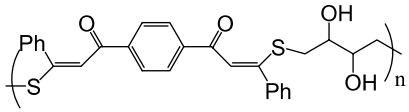
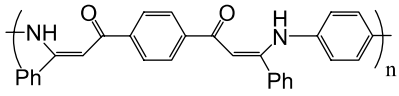
aqueous sodium dodecyl sulfate solutions perform as powerful catalysts of the Michael additions of thiols or amines to enones [43]. Firouzabadi et al. proposed that the accelerating effect of the sodium dodecyl sulfate related to the concentrating of the hydrophobic species into the micellar core.

3. Linear step growth Michael addition polymerizations

3.1. Overview

Significant earlier attention was devoted to linear step growth Michael addition polymerizations. Polymers that were produced via Michael addition polymerizations have ranged from segmented elastomers to high T_g engineering thermoplastics. Both rigid, amorphous, aromatic systems as well as semicrystalline aliphatic systems were pursued and a wide variety of monomers were employed. Numerous examples of step growth Michael addition polymerizations exist, however, a few main classifications exist, outlined in Table 1. Perhaps the earliest example is poly(amido amine)s, which were synthesized from bisacrylamides and diamines. A second class of step growth polymers are derived from bismaleimides and diamines, a type of poly(imido amine) referred to as poly(aspartamide)s in the literature. Thiol nucleophiles are involved in the synthesis of poly(imido sulfide)s, which are also based on bismaleimide monomers. Analogous poly(ester sulfide)s and poly(amino ester)s synthesized from diacrylates are potentially biodegradable due to the hydrolytic instability

Table 1
Step growth polymers derived from Michael addition polymerisation

		References
Poly(amido amine)		[53]
Poly(amino ester)		[61]
Poly(imido sulfide)		[68]
Poly(ester sulfide)		[69]
Poly(aspartamide)		[70]
Poly(imido ether)		[76]
Poly(amino quinone)		[3]
Poly(enone sulfide)		[68]
Poly(enamine ketone)		[81]

of the ester linkages. Poly(amino quinone)s are a unique class of polymers which possess redox activity [44] and serve as anticorrosion coatings on metals. α,β-unsaturated alkyne esters and ketones also serve as Michael acceptors in some cases and step growth polymerizations based on amine and thiol nucleophiles were pursued. Finally, hydrogen transfer polymerization allows the synthesis of polyamides and polyesters from AB-type Michael addition polymerizations. The high conversion of the Michael addition reaction and lack of side reactions are prerequisites for high molecular weight in step growth polymerizations, which is clearly obtained in numerous subsequent examples.

3.2. Poly(amido amine)s

Poly(amido amines) are synthesized through the reaction of secondary diamines with bisacrylamides at 15–60 °C in water for durations of hours to days. Also, primary amines, which have difunctionality in the Michael addition are employed in poly(amido amine) synthesis (Fig. 16). Ferruti et al. synthesized linear poly(amido amine)s from a range of monomers including piperazine bisacrylamide and *N*,*N*′-dimethyl-1,6-hexanediamine [45,46]. The poly(amido amine) polymerizations occurred rapidly at room temperature in protic media such as alcohols and water without catalyst. The rate of the Michael addition polymerization increased with protic solvents and was less dependent on solvent polarity or dielectric constant. Generally, these polymerizations do not proceed to high molecular weight in aprotic solvents. The rate of these polymerizations increased with the basicity of the secondary diamines, and with lower steric hindrance [47]. For instance, polymerization rates increased in the order: *N*,*N*′-diisopropylethylenediamine < *N*,*N*′-dimethylethylenediamine < 2-methylpiperazine < piperazine.

Numerous variations of poly(amido amine) polymerizations exist. Amino acids may be copolymerized with bisacrylamides, as long as triethylamine is added to deprotonate the amino acid zwitterion and an amino acid with low steric bulk is employed. Successful polymerization occurred with glycine, taurine and β-alanine. Peptide oligomers may also be used as comonomers as long as the amine terminus is a simple amino acid such as glycine. Monomers such as divinylsulfones and diacrylates [48] can replace bisacrylamides as Michael acceptors. Segmented poly(amido amine ether)s were derived from PEG bis(piperazine) monomers [49]. Other routes to poly(amido amine)s yield different ordering of amido and amino functionalities including homopolymerization of protected aminoacrylamides with in situ deprotection. Yet another route is the reaction of activated acrylic acid derivatives with secondary diamines through amidation and the also Michael addition [50]. Hydrazine and alkylated hydrazines were also employed in the synthesis of poly(amido amine)s, however, if excess bisacrylamide monomer is used, then crosslinking occurs with residual NH groups along the polymer backbone at long reaction times [47,51]. Thermal stability of aliphatic poly(amido amine)s is fairly low, and thermal decomposition occurs near 200 °C. Poly(amido phosphine)s are synthesized in a similar manner to poly(amido amine)s, with the exception that free radical inhibitors are employed to prevent radical catalyzed addition of the phosphines to the bisacrylamides. Poly(amido phosphine)s exhibit greater thermal stability than poly(amido amines), with decomposition temperatures near 360 °C.

Aliphatic poly(amido amines) often possess crystallinity and are water soluble due to the presence of tertiary amines in the backbone, which undergo partial protonation in water. Poly(amido amine)s exhibit a tendency toward amide hydrolysis. Ferruti et al.

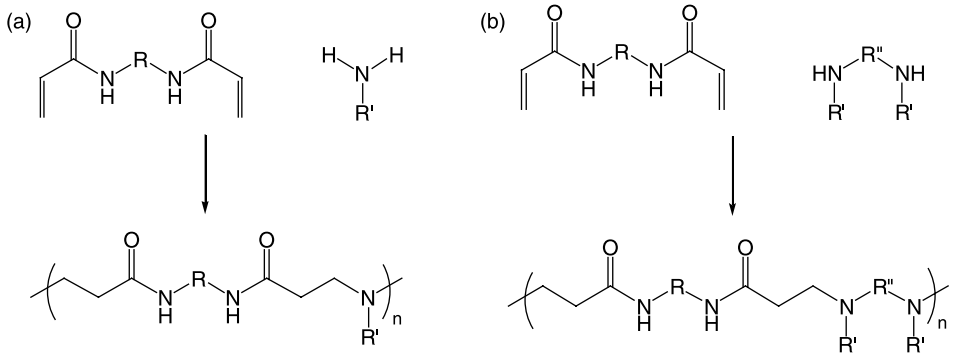


Fig. 16. Poly(amido amine) synthesis via (a) primary and (b) secondary amines.

demonstrated increased solution viscosity versus time during polymerizations at high temperatures (100 °C) in water, with a subsequent decrease in viscosity at later times. This attribute along with low toxicity of the amino acid degradation products enables poly(amido amine)s as degradable pro-drug conjugates [52]. Poly(amido amine)s based on hydrazine were found to decrease in molecular weight by a factor of four over 2 d in water at physiological conditions, and were also found to possess low cytotoxicity [51].

Linear and crosslinked poly(amido amine)s were found to complex with heparin, an anticoagulant in the human body [45]. In medical practice, when heparin levels are too high in the body, protamine sulfate is administered, which is also an anticoagulant. A solution to this problem is the use of linear or crosslinked poly(amido amine)s to remove heparin from the blood stream. Poly(amido amine)s selectively remove heparin over other plasma components and do not cause hemolysis or affect other properties of blood such as prothrombin time. The poly(amido amine)s were also recycled through removal of heparin upon treatment with alkaline solutions of pH 11. Heparin, when coated on non-physiological objects, reduces the thrombus formation response of the human body. Thus, poly (amido amine)s also find utility in creating non-thrombogenic surfaces through covalent attachment to surfaces. Ferruti et al. developed synthetic methods for surface attachment of poly(amido amine)s to Dacron™ and poly(vinyl chloride) (PVC) using Michael addition of terminal acrylamide groups with surface tethered amine groups [46]. These surfaces readily adsorbed heparin without heparin release.

Ferruti et al. reacted ethylene sulfide with piperazine to yield dithiol monomers, which were polymerized with bisacrylamides. The resultant polymers were quaternized with methyl iodide, yielding water soluble antimicrobial polyelectrolytes [52]. These polymers exhibited antimicrobial activity even against resistant strains such as *Pseudomonas aeruginosa*. The polymers also possessed surprisingly low rates of hemolysis relative to commercial antimicrobial polymers.

Ferruti et al. investigated further biomedical applications of poly(amido amine)s in creating platinum (II) complexes with antitumor activity [53]. Polymer–drug conjugates have improved the effectiveness of drugs through a combination of increased solubility and slow drug release. The net result is called the enhanced permeability and retention (EPR) effect. Linear poly(amido amine)s based on 2-methylpiperazine, carboxylated bisacrylamides, and amino-β-cyclodextrin moieties were complexed directly with cisplatin. The degree of cisplatin complexation and release rate of platinum increased for polymers containing the cyclodextrin moiety. Release was suppressed however, with the carboxylated bisacrylamide, and non-carboxylated bisacrylamides were also studied. Both in vivo and in vitro toxicity of most complexes were lower than pure cisplatin, and similar antitumor activities were observed. Earlier work on poly(amido amine)s revealed effectiveness in reducing tumor metastasis in Lewis lung tumors and Sarcoma 180 carcinoma implanted in mice [54]. The reduction in cancer metastasis was attributed to specific interactions of these charged polymers with cell membranes. Neuse et al. also studied poly(amido amine) complexes of cisplatin, using 1,2-dihydroxylated, 1,2-dicarboxylated or 1-hydroxy-2-carboxyl functional amine monomers that chelate to the platinum center [55].

Recently, Ferruti et al. devoted considerable effort to studying the delivery of genes and proteins via complexation with poly(amido amine)s. One advantage of poly(amido amine)s is rapid protonation and expansion at low pH inside tumor cells which releases the genes or proteins and a compact geometry at plasma pH 7.4 to effectively hold the desired agent prior to delivery. Small angle neutron scattering (SANS) studies revealed that decreasing the pH- of aqueous poly(amido amine)s solutions increases the radius of gyration from ~2 to 8 nm [56]. Furthermore, poly(-amido amine)s possess dramatically lower toxicity than conventional non-viral vectors such as poly(ethylene imine). In some cases, poly(amido amine)s were prepared that exhibited a 'stealth' behavior and maintained long circulatory lifetimes [57]. Duncan et al. recently investigated poly(amido amine)s that contained covalently bound endosomolytic protein melittin (a main component of bee venom) [58]. These conjugates were less toxic than pure melittin and were found to possess pH- selective hemolytic properties at pH 5.5 and not at pH 7.4 at low concentrations of the conjugates. Some of the conjugates were also effective in delivering the non-permeant toxin gelonin into cells. Segmented copolymers based on poly(amido amine)s containing carboxylic acid and hydroxyl functional blocks also showed promising endosomolytic and gelonin delivery activity [59]. Gene transfection studies of poly(amido amine)s have also demonstrated successful polyplex formation with λ Hind III DNA and transfection of β-galactosidase into HepG2 cells, with similar transfection efficiency as Lipofectin® and poly(ethylene imine) [7]. Ferruti et al. coupled bovine serum albumin and human serum albumin to poly(amido amine)s based on

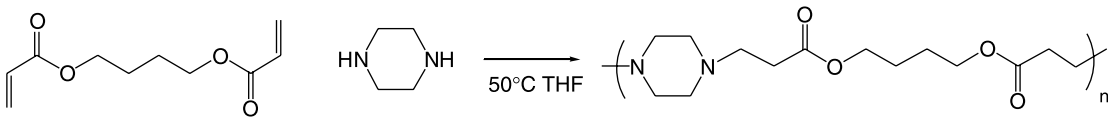


Fig. 17. Poly(amino ester) synthesis from butanediol diacrylate and piperazine.

piperazine and piperazine bisacrylamide through Michael addition of protein amine groups to terminal acrylamide groups on the poly(amido amine)s [60]. The reaction was conducted under mild conditions (pH 8–8.5, 25 °C) to high conversion and the coupled product was verified by size exclusion chromatography (SEC).

Poly(amino ester)s are similar to poly(amido amine)s, however, they utilize diacrylates instead of bisacrylamides. Langer et al. studied the use of Michael addition step growth linear poly(amino ester)s as gene transfection agents (Fig. 17) [61]. Like poly(amido amine)s, poly(amino ester)s have benefits over conventional gene transfection agents (such as poly(ethylene imine) or poly(L-lysine)) due to their low cytotoxicity as well as biodegradability into low toxicity byproducts. Furthermore, the structure and degradability of the poly(amino ester)s are tailored through the incorporation of a wide range of diamines and diacrylates. Langer et al. obtained high molecular weight poly(amino ester)s (32,000 g/mol) and performed successful complexation with plasmid DNA. The absence of a retro-Michael addition in these systems eliminates the possibility of carcinogenic diacrylate production during degradation. The degradability of these transfection agents is expected to aid in the release of the DNA inside the cell.

Non-biological applications of poly(amido amine)s also exist. Recently, Ferruti et al. examined the introduction of NLO chromophores into both linear and crosslinked poly(amido amine)s [12]. The Michael addition chemistry was well-suited to the primary amine containing chromophores and mild reaction conditions prevented chromophore degradation [62]. Initial studies showed that solution optical properties of the chromophores such as two photon pumped fluorescence were maintained in the polymer.

3.3. Poly(imido sulfide)s

Maleimides possess two carbonyl groups conjugated to the double bond, presenting a highly electron poor double bond susceptible to reaction with a range of nucleophiles. Poly(imido sulfide)s result from the step growth polymerization of dithiols with bismaleimide monomers. White and Scaia polymerized aromatic dithiols with aromatic bismaleimides in *m*-cresol with catalytic amounts of tri-*n*-butylamine at 80–100 °C [63]. The acidity of the *m*-cresol prevented the anionic homopolymerization of the maleimide groups upon reaction with thiolate anions, which would have resulted in crosslinking. The poly(imido sulfide)s containing aromatic thiol units were susceptible to degradation from bases such as diethylamine in aprotic solvents which was attributed to retro-Michael addition regeneration of the maleimide group. Aliphatic dithiol-containing polymers were less susceptible to depolymerization. Poly(imido sulfide)s with molecular weights ranging from 70,000 to 450,000 g/mol possessed glass transition temperatures from 185 to 212 °C. Semicrystalline poly(imido sulfide)s were produced through the reaction of aliphatic dithiols with aliphatic bismaleimides [64]. Melting temperatures of approximately 77 °C were observed. Semicrystalline poly(imido sulfide)s possessed stresses at break near 12 MPa and elongations of 120%. Thermal decomposition of the poly(imido sulfide)s occurred between 336–380 °C, and likely resulted from a retro-Michael addition depolymerization. Hydrogen sulfide serves as an effective dithiol in the synthesis of poly(imido sulfide)s and reacts very rapidly with bismaleimides in the presence of catalytic quantities of amines such as *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) or acids such as acetic acid (Fig. 18) [65]. Poly(imido sulfide)s were also obtained from biscitraconimides (methyl substituted maleimides),

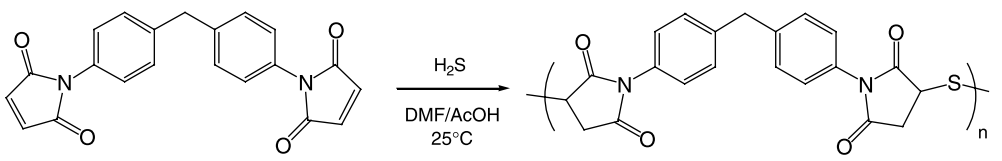


Fig. 18. Poly(imido sulfide) synthesis from a bismaleimide precursor and hydrogen sulfide gas.

which resulted in geminal substitution of the sulfide group on the maleimide ring at the same position as the methyl group [66].

Segmented polymers based on poly(imido sulfide) chemistry were developed by Crivello and Juliano [67]. These polymers were synthesized in an analogous fashion to polyurethanes, starting with an oligomeric 'soft block' consisting of a thiol terminated polysulfide (Thiokol™ resin) and reacting with an excess of aromatic bismaleimide monomer in cresol with tri-*n*-butylamine catalyst at room temperature. The reactive oligomer is chain extended through reaction with hydrogen sulfide to produce the poly(imido sulfide) 'hard block'. The poor thermal stability of the polysulfide oligomers limited the processing temperature of the polymers to less than 150 °C, but tensile strengths as high as 10 MPa and 500% elongation were obtained. The thermal instability limited the choice of bismaleimide monomer to maintain a T_g that was less than 100 °C. These segmented poly(imido sulfide)s exhibited two glass transition temperatures in dynamic mechanical analysis, indicating the presence of microphase separation. The rubbery plateau (service window) ranged from −30 to 130 °C. Increasing the hard segment content above 30 wt% led to higher tensile strengths with lower extensibilities and poor processability. The polysulfide containing segmented elastomers benefited from excellent solvent resistance.

Dix et al. showed that changing between protic and aprotic monomers in aprotic solvents influenced the topology of poly(imido sulfide)s (Fig. 19) [68]. Bismaleimides were reacted with oligomeric dithiols as well as low molar mass dithiols in the presence of catalytic quantities of triethylamine. Oligomeric bisthiols reacted with *N*′,*N*′-bismaleimido-4,4′-diphenylmethane to create crosslinked networks due to the reaction of the carbanion formed upon Michael addition with additional maleimide groups. The use of a protic small molecule dithiol monomer, dithiothreitol, resulted in linear polymers without crosslinking, due to protonation of the carbanions by hydroxyl groups on dithiothreitol. Triethylamine does not initiate maleimide polymerization, but deprotonates thiols, which are capable of polymerizing maleimides. For example, an oligomeric dithiol was reacted with excess monofunctional maleimide in the presence of triethylamine resulting in anionic polymerization of the maleimide from the chain ends of the oligomeric dithiol.

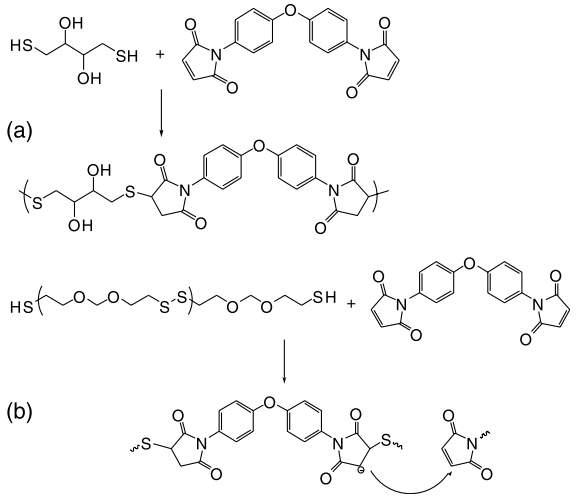


Fig. 19. (a) A typical poly(imido sulfide)s synthesis. (b) Possible crosslinking side reaction through maleimide homopolymerization observed for aprotic oligomeric dithiols.

The acrylic analog to poly(imido sulfide)s, poly (ester sulfide)s, were synthesized using PEG dithiol and butanediol diacrylate or PEG diacrylate using triethylamine base catalyst [69]. Varying the length of the PEG spacer enabled hydrophilicity control. The reaction kinetics were studied in a number of aprotic solvents and increased in the order benzene < dioxane ≪ chloroform with increasing dielectric constant. Weight average molecular weights ranged from 5000 to 28,000 g/mol and glass transition temperatures from −50 to −60 °C were observed. Crystallinity was observed in selected poly(ester sulfide)s.

3.4. Poly(aspartamide)s

Poly(aspartamide)s are a class of condensation polymers derived from bismaleimides and diamine monomers. Poly(aspartamide)s exhibit properties similar to poly(imide)s yet benefit from a facile, one-pot synthesis and increased solubility in organic solvents relative to aromatic poly(imide)s and poly(amide)s [70]. Poly(aspartamide) synthesis relies on a facile Michael addition of the amine functional group to the maleimide double bond and does not require a high temperature poly(amic acid) cyclization reaction as for poly(imide)s.

Crivello synthesized poly(aspartamide)s through the reaction of aromatic diamines with aromatic bismaleimides in cresol at 110 °C for 3 d with catalytic amounts of acetic acid [71]. The lower basicity of the aromatic amines allowed the use of acetic acid, which activated the maleimide group, through protonation [72]. Model reactions suggested that the polymerization mechanism involved charged intermediates due to increased rates in polar solvents. The poly(aspartamide)s exhibited glass transition temperatures near 210 °C and thermal

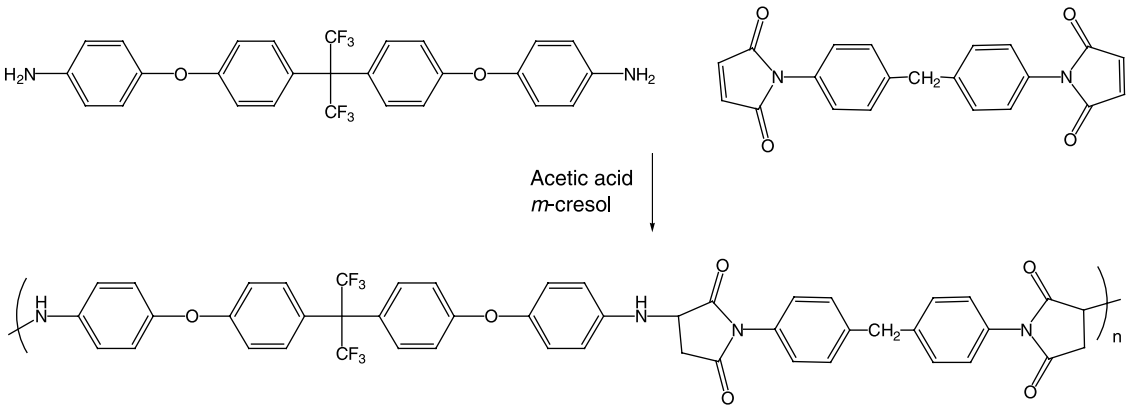


Fig. 20. Thermally stable poly(aspartamide)s synthesized from arylene ether diamines.

decomposition temperatures near 350 °C. This decomposition occurred at the same temperature (TGA) in both air and nitrogen atmospheres, suggesting that depolymerization occurred rather than oxidation. The poly(aspartamide)s possessed tensile strengths near 95 MPa and elongations of 5.5%. Flexural strengths and moduli as high as 140 MPa and 3.1 GPa, respectively, were reported.

Liaw studied poly(aspartamide)s based on arylene ether diamines with improved thermal stability and 10% weight loss temperatures near 400 °C (Fig. 20) [73]. The molecular weight of the poly(aspartamide)s increased with increasing monomer concentration, from $M_n = 7900$ at 0.38 M to $M_n = 12{,}800$ at 1.0 M. Weight average molecular weights ranged from 24,000 to 68,000 g/mol.

White and Scaia synthesized amorphous elastomeric poly(aspartamide)s via the polymerization of aliphatic secondary diamines and aliphatic bismaleimides [64]. Acetic acid was used in the polymerization of aromatic diamines and bismaleimides but not with aliphatic diamines due to the tendency to protonate the amine groups and prevent polymerization. The poly(aspartamide)s undergo slow crosslinking at ambient conditions likely due to Michael addition reaction of amine and maleimide end groups and subsequent homopolymerization of the maleimide group. Amorphous poly (aspartamide)s possessed elongations of 1700%, stresses at break near 9.3 MPa, and glass transition temperatures ranging from 0 to 86 °C.

Poly(aspartamide)s have potential application in second order non-linear optical (NLO) materials due to their high glass transition temperatures (~260 to 290 °C) which help maintain the critical orientation of the NLO chromophores. In addition, poly(aspartamide)s do not exhibit poling interferences due to water evolution as seen with polyimides during cyclization of the precursor poly(amic acid). Wu et al. used Michael addition to obtain an NLO-containing poly(aspartamide) pre-polymer with maleimide end groups that was thermally cured to obtain the final product [74]. At higher curing temperatures, the glass transition temperature of the cured poly(aspartamide)s appeared to increase, which demonstrated that curing continued to 250 °C.

Poly(amide aspartamide)s are similar to poly(aspartamide)s with the exception that polymerization occurs via both Michael addition of amines and maleimides and amidation reactions of amines and carboxylic acids [75]. Poly(amide aspartamide)s are synthesized through a step growth polymerization of an AA′ monomer containing a carboxylic acid and a maleimide functional group with a B_2 diamine monomer such as those shown in Fig. 21. The polymers are thus synthesized in a sequential process beginning with the Michael addition of the nucleophilic amine groups to

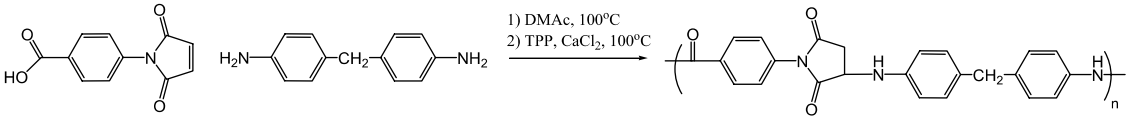


Fig. 21. Typical poly(amide aspartamide) polymerization. The first heating step produces the Michael adduct while the second step promotes amide bond formation.

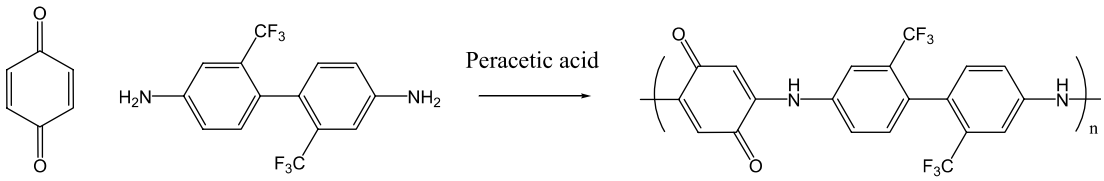


Fig. 22. Poly(amino quinone) synthesis involving peracetic acid as an oxidizing agent.

the maleimide group followed by the amidation at the carboxylic acid. These polymers are controllably branched through the addition of AB_2 monomers. One drawback of the poly(amide aspartamide)s is their relatively low thermal stability, with typical thermal degradation temperatures of 300–360 °C at 10% weight loss. The char yields of the polymers increased on end-capping the carboxylic acid end groups with aniline.

Oxygen nucleophiles are also effective in step growth polymerizations with bismaleimides. Hulubei and Rusu copolymerized glycerol and phenolphthalein with aliphatic bismaleimides resulting in poly(imido ethers) [76]. The reaction was performed in NMP using triethylamine or 2-mercaptobenzothiazole as catalysts at 85–115 °C for 2–3 d. In the case of glycerol, the difference in reactivity of the secondary and primary alcohols allowed synthesis of pendant hydroxyl polymers. Thermogravimetric analysis revealed initial weight losses ranging from 215 to 400 °C. Polymerization of bismaleimides with phenols was successful in solution (95–110 °C) and in the melt (180 °C), and fiber-glass reinforced composites were prepared [77].

3.5. Poly(amino quinone)s

Poly(amino quinone)s are synthesized from primary diamines and quinone, through Michael additions on both sides of the quinone (Fig. 22) [3]. The unsaturated nature of the quinone is maintained in the polymer, using either excess quinone or other oxidizing agents. Use of peracetic acid leads to improved yields and simplified purification. Poly(amino quinone)s have recently found application in anticorrosion coatings and moisture resistant adhesives. Numerous traditional adhesives suffer from delamination from solid surfaces in the presence of water. This is due to a stronger interaction of water with the surface. Poly(amino quinone)s, however, displace water on metal surfaces and bind preferentially. The strong interaction of these polymers with metal surfaces is thought to arise from the electron donating capabilities of the quinone group. Poly(amino quinone)s inhibit corrosion on stainless steel surfaces even in the presence of sulfuric acid, through the formation of a protective coating that forms spontaneously on the metal surface. These corrosion inhibiting coatings are used for ship hull protection as well as protection of magnetic particles on data storage media. Diamino quinone containing polyimides are used to improve the corrosion resistance of interlayer dielectrics in microelectronics [78]. Poly (amino quinone)s have also been introduced into polyolefins and polyurethanes.

Similar to poly(amino quinone)s are poly(thiophenylene)s formed from aromatic dithiols and

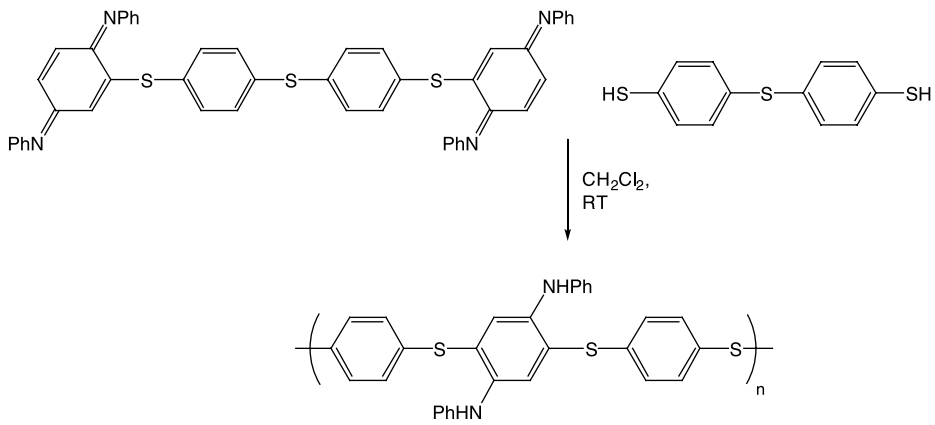


Fig. 23. Polymerization of poly(thiophenylene)s via Michael addition and oxidation.

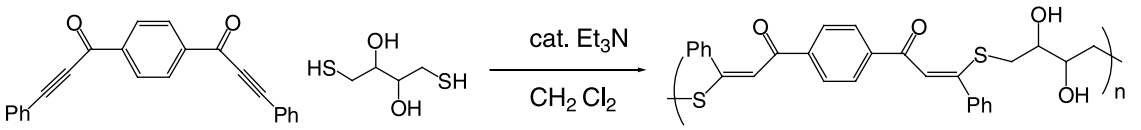


Fig. 24. Poly(enone sulfide) synthesis via Michael addition step growth.

bis(1,4-phenylenediimine)s, depicted in Fig. 23. The product of the Michael addition between the thiol groups and this nitrogen analog to quinone aromatizes through proton transfer to the imine nitrogens resulting in a poly(thiophenylene) with pendant amino groups [79]. The polymerization proceeds without a catalyst. These polymers exhibit electroresponsive behavior and are redox active under acidic conditions. The number average molecular weight of the polymer was 5400 g/mol and the glass transition temperature was 131 °C.

3.6. Step growth polymers derived from bisacetylene-containing monomers

Numerous step growth Michael addition polymers are synthesized from bisacetylenic precursors. The acetylene bond reacts electrophilically when conjugated to electron withdrawing groups, in a similar fashion to an olefin as noted earlier. Typical functional groups that serve as Michael receptors are conjugated acetylene ketones and conjugated acetylene esters. These carbon–carbon triple bond Michael acceptors react with oxygen, sulfur and amine nucleophiles and adducts possess the additional feature of stereochemistry at the double bond [80]. Michael addition polymers based on acetylenic precursors possess surprising thermal stability and produce remarkably tough thermoplastics.

Sinsky et al. studied poly(enamine ketone)s synthesized through the Michael addition reaction of aromatic diamines with aromatic bis(alkynone)s [81]. These nucleophilic additions to acetylenic compounds result primarily in *Z* isomer formation. The reactions were performed in *m*-cresol at 100 °C without catalyst. Glass transition temperatures between 198 and 235 °C were obtained and thermogravimetric decomposition temperatures were nearly 300 °C. The poly(enamine ketone)s were tough thermoplastics, with tensile strengths of 85 MPa (63 MPa at 93 °C), tensile moduli of 2.27 GPa, and elongations of 4%.

Bass et al. synthesized poly(enone sulfide)s, the sulfur analog to poly(enamine ketone)s through the reaction of aromatic dithiols (e.g. 1,3-benzenedithiol) with aromatic internal bis(alkynone)s in *m*-cresol at room temperature with *N*-methyl morpholine as a catalyst at temperatures from 25 to 40 °C [82]. The polymers had moderately high glass transition temperatures ($T_g \sim 107$–156 °C) and thermal decomposition temperatures near 330 °C. Tough, clear films were obtained from solution casting and exhibited tensile strengths of 78 MPa and tensile moduli near 3.2 GPa. Dix et al. also synthesized poly(enone sulfide)s from internal bis(alkynone)s and found a *Z:E* ratio of 60/40 (Fig. 24) [68]. Model reactions of thiols with phenylacetylene resulted in almost exclusive formation of the *Z* isomer [83].

3.7. Hydrogen transfer polymerization

Nylon-3 type polyamides are also synthesized via a Michael addition process. The base catalyzed polymerization of acrylamide to poly(β-alanine), as shown in Fig. 25, was originally proposed as an anionic chain growth process, which occurred with proton transfer of the amide NH_2 on the growing chain end to the enolate carbanion. This process was described as a 'hydrogen transfer polymerization'. However, it was later classified as a step growth polymerization due to proton transfer from the growing enolate carbanion to amide NH_2 groups on other monomers. Bush and Breslow demonstrated this via monitoring molecular weight as a function of monomer conversion [84]. At very short reaction times, high monomer conversion was achieved but high molecular weight product was not obtained suggesting initial dimerization and trimerization. Longer reaction times led to high molecular weight poly(β-alanine). These polymerizations were typically conducted at 80–120 °C in aprotic solvents such as DMF in the presence of radical inhibitors. Further work from Lim et al. demonstrated that branching occurred in these polymerizations in aprotic solvents due to propagation of both enolate and amide anions, which

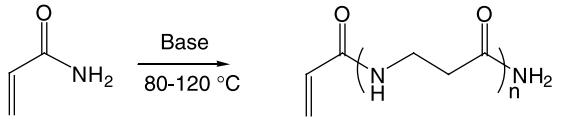


Fig. 25. Hydrogen transfer polymerization of acrylamide to poly(β-alanine).

are formed before and after hydrogen transfer, respectively [85]. Hydrogen transfer polymerization of acrylamide in the presence of carbon black and *n*-butyl lithium was effective in grafting of poly (β-alanine) onto the carbon black via polymerization from surface lithium alkoxides [86]. The grafted carbon black showed improved dispersability in water and organic solvents, and grafting ratios of 60–80% were achieved. Recently, the hydrogen transfer polymerization process was applied to *N*-acetylacrylamide and *N*-benzoylacrylamide and the proton transfer mechanism was preserved despite the steric hindrance and lower acidity of the amide NH groups [87]. Hydrogen transfer polymerization was also achieved with *N*-acryloyl-*N*′-*p*-tolylureas [88], *p*-styrenesulfonamides, heterocyclic base containing acrylamides [89], and maleimide.

In a similar nature to the hydrogen transfer polymerization of acrylamide, hydroxyalkyl acrylates are also polymerizable through Michael addition chemistry as AB monomers. The polymerization of hydroxyethyl acrylate was performed at temperatures from 85 to 150 °C in the presence of free radical inhibitors using bases such as sodium hydride and potassium *tert*-butoxide as initiators [90]. Molecular weights as high as 9000 g/mol were obtained and hydroxyethyl acrylate polymerized more effectively than hydroxyethyl methacrylate. The primary side reaction in these polymerizations is the disproportionation of hydroxyethyl acrylate to ethylene glycol diacrylate and ethylene glycol.

4. Linear chain growth Michael addition polymerizations

A classic example of Michael addition polymerization is the anionic chain-growth polymerization of methacrylate monomers. This review is not intended to comprehensively review anionic polymerization but rather to place the Michael addition in context for trends in polymer synthesis strategies. Anionic polymerization has been reviewed extensively in the literature [4,91,92]. The relatively low reactivity of the methacrylate, as noted earlier, towards Michael addition chemistry demands a stronger nucleophile, thus carbanionic species such as diphenylhexyllithium and nitrogen anions such as lithium diisopropyl amide(LDA) are suitable for the polymerization of alkyl methacrylates. One current limitation is the requirement for cryogenic temperatures to prevent potential nucleophilic attack of the enolate anion on ester groups. Group transfer polymerization, which was developed in the mid-1980s, enabled polymerization at ambient temperatures due to the use of a reversible silyl protecting group that was proposed to stabilize the propagating chain end. Anionic polymerization was demonstrated with acrylamides and acrylate monomers, later, and this may complicate step growth processes in some cases [93]. The polymerization of cyanoacrylates is another chain growth Michael addition process. Cyanoacrylates are initiated with very poor nucleophiles, such as water, due to the electron poor olefin and strong resonance contributions of the adjacent cyano and ester groups. Cyanoacrylate polymerizations are widely used in rapid cure adhesives, and adhesive products possess extremely high bond strengths.

4.1. Anionic polymerization of methacrylates

Anionic polymerization of alkyl methacrylates is an established chain growth polymerization technique, which relies upon Michael addition chemistry. Anionic polymerization is known for producing polymers of controlled molecular weights and narrow polydispersities. Anionic polymerization is particularly suited for well-defined block copolymers [94] and star-shaped polymers via chain-end coupling strategies. A further advantage of anionic polymerization is the potential for controlling tacticity, which was achieved for the sterically bulky *tert*-butyl methacrylate monomer in toluene by Long et al. [95]. Anionic polymerization is generally initiated using alkyllithium initiators with sufficient nucleophilicity to attack alkyl methacrylate monomers. However, due to the potential side reaction of the carbanionic chain end with ester carbonyls, the reaction must be conducted at low temperatures and with sterically hindered carbanions such as diphenylhexyllithium [94]. These bulky carbanions react selectively at the double bond, resulting in an enolate Michael adduct, as shown in Fig. 26.

The strong nucleophilicity of the anionic PMMA growing chain end was used in chain end functionalization strategies in numerous cases [92]. For example, addition of di-*tert*-butyl maleate at the end of the polymerization results in succinate ester end groups [96]. These end groups were then pyrolyzed to produce reactive anhydrides capable of imide coupling with amine functional polymers. Long et al. acheived end functionalization with hydrogen bonding groups via Michael addition of heterocyclic base pairs to acrylated polystyrenes (Fig. 27) [97]. Termination of the anionic polymerization of styrene with ethylene oxide created hydroxyl functionality which allowed esterification with acryloyl chloride. Michael addition of nucleotide

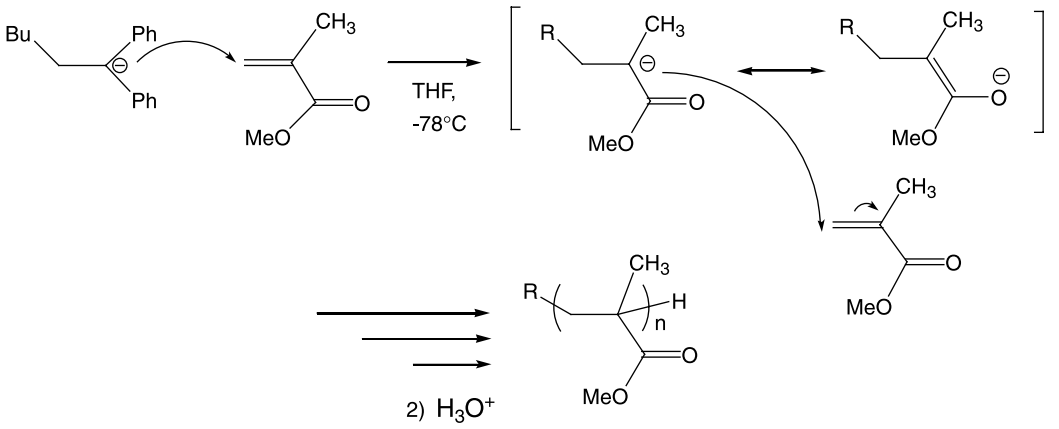


Fig. 26. Anionic polymerization of methyl methacrylate from diphenylhexyl lithium.

bases then allowed facile functionalization of the polystyrene chains, without detracting from hydrogen bonding properties. Adenine and thymine functionalized polystyrene demonstrated association in ^{1}H NMR spectroscopic investigations.

Long et al. [94,98] as well as Müller et al. [99] studied the synthesis of diene/acrylic, styrene/acrylic and all acrylic triblock copolymers containing *tert*-butyl methacrylate, which were later hydrolyzed and neutralized to obtain carboxylate block ionomers. The bulkier ester alkyl prohibits carbonyl ester attack at room temperature. Long et al. also established the utility of lithium diisopropylamide (LDA) as an initiator for methacrylate polymerizations in THF at −78 °C, despite the relatively low nucleophilicity of the hindered amide ion [100]. Anionic polymerization of acrylates, [93] maleimides, vinyl ketones [101] and acrylamides [102] are also known.

In some cases, anionically polymerized methacrylates have been designed for biologically related applications. For instance, Okamoto et al. synthesized macrocyclic ether containing acrylic monomers, which polymerized anionically to obtain highly isotactic polymers capable of functioning as synthetic ion channels in hexadecyl phosphate vesicles (Fig. 28) [103]. In contrast, radical polymerization of macrocyclic ether monomers yielded atactic polymer with dramatically lower ion transport.

4.2. Group transfer polymerization

Commonly, anionic polymerization of alkyl methacrylates is conducted at cryogenic temperatures. Such temperatures are generally not industrially feasible, except in the cationic polymerization of isobutylene, and significant research effort was directed towards a methodology that was amenable to higher temperatures. Group transfer polymerization (GTP) is a polymerization methodology that was developed in the mid-1980s by DuPont as a method of producing narrow polydispersity methacrylate and acrylate polymers and block polymers above room temperature

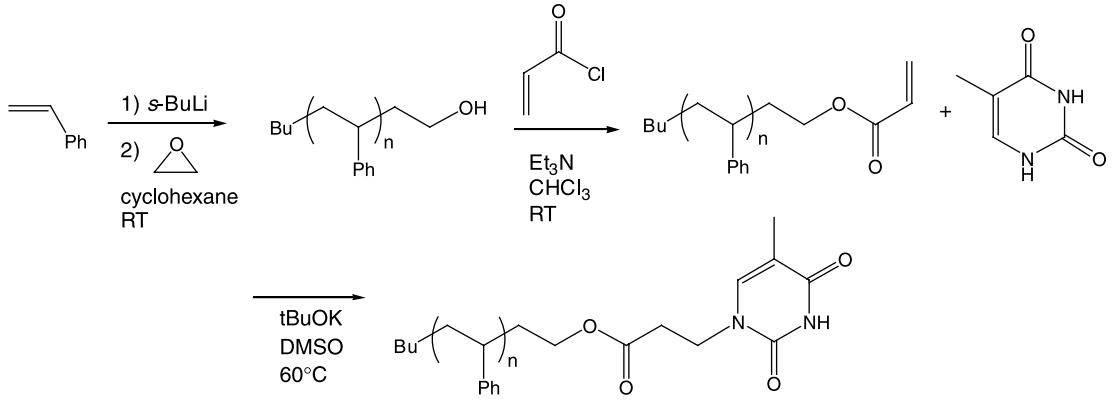


Fig. 27. Michael addition of heterocyclic nucleotide bases to acrylated anionic polystyrene.

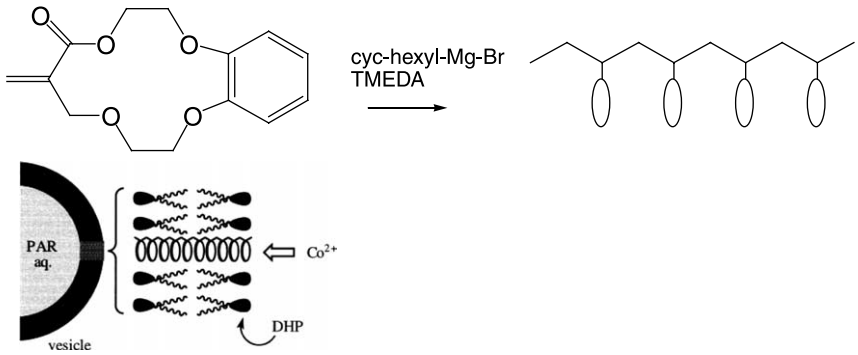


Fig. 28. Polymerization of macrocyclic ether acrylic monomers to form ion channels. Reprinted from Polymer, 43, Okamoto, et al. 'Stereospecific anionic polymerization of α-(hydroxymethyl)acrylate derivatives affording novel vinyl polymers with macrocyclic side chains', 3469–3474, Copyright 2002, with permission from Elsevier [103].

[104]. GTP is conveniently conducted at 80 °C using a silyl ketene acetal initiator in the presence of a nucleophilic anionic catalyst (Fig. 29). Initially, GTP was believed to proceed through a transfer of a silyl protecting group from the enolate oxygen on the chain end to monomers during the propagation step [105]. This initial mechanism has largely been disproven in favor of a dissociative anionic polymerization mechanism which involves a reversible dissociation of the silyl protecting group from the enolate oxygen, aided by anionic catalysts (HF_2^-, bibenzoate anion). The position of the equilibrium between the free enolate and the silyl protected enolate determines the rate of the polymerization. Similar to anionic polymerizations, the reaction is terminated through the addition of a protic species such as methanol. GTP was used by DuPont to produce acrylic block copolymers for pigment dispersing applications in automotive paints [104].

4.3. Anionic polymerization of α-cyanoacrylates

Cyanoacrylates are a class of monomers which undergo rapid anionic polymerization initiated by weak nucleophiles such as water, as shown in Fig. 30. These rapidly polymerizing monomers are used primarily in

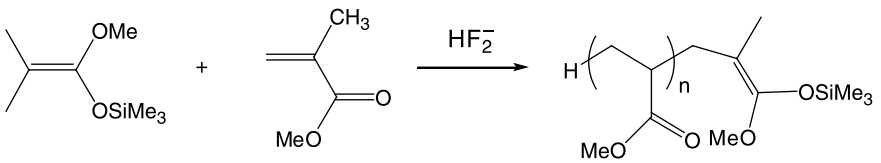


Fig. 29. Group transfer polymerization of methyl methacrylate.

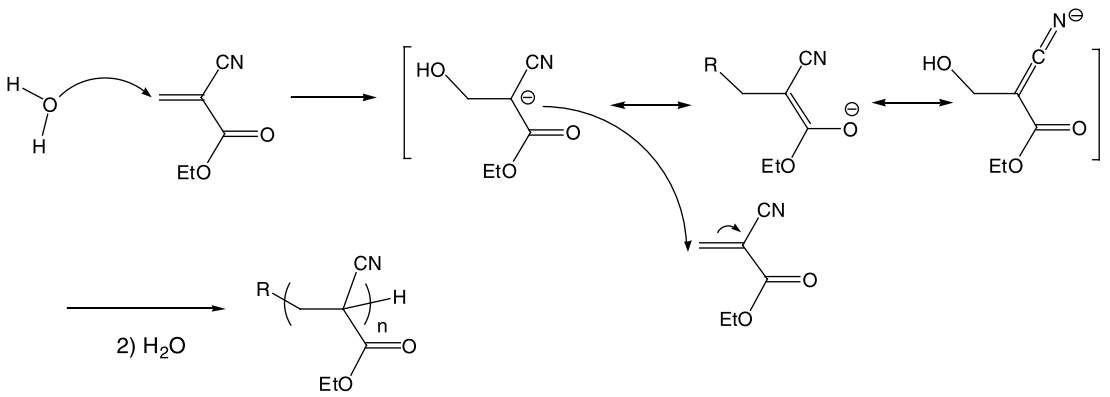


Fig. 30. Water-initiated polymerization of ethyl α-cyanoacrylate.

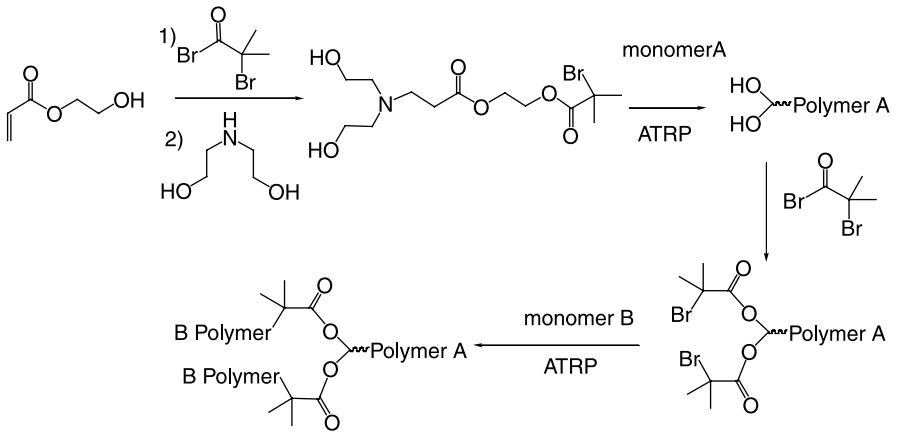


Fig. 31. Synthesis of a dihydroxyl functional ATRP initiator.

the adhesives industry as a high strength rapid setting adhesives [106]. The added electron withdrawing character of the nitrile group facilitates anionic polymerization of these monomers. Unlike conventional anionic polymerization, however, molecular weight control is absent. Klemarczyk studied the polymerization of ethyl cyanoacrylate using phosphine and amine initiators [107]. The initiated monomer species is zwitterionic, which makes it an effective nucleophile towards further monomer addition. Primary and secondary amines intiate more slowly than tertiary amines due to proton transfer after the first addition of monomer, which results in a neutral, less reactive adduct. In terms of current biomedical applications, octyl cyanoacrylate has recently found application as a tissue adhesive and gained FDA approval for surgical use [108]. This monomer polymerizes on contact with a wound and maintains flexibility due to the pendant octyl chain and furthermore possesses low water solubility.

4.4. Living radical polymerization

Living radical polymerization encompasses several techniques for producing well-defined homopolymers, block copolymers, and star polymers. Due to the radical nature of this polymerization methodology, a wider range of monomers are accessible compared to traditional anionic polymerization and less stringent purification techniques are required. Numerous living radical polymerization methods are currently known, including atom transfer radical polymerization (ATRP), which involves activated alkyl halides and metal catalysts [109], stable free radical polymerization (SFRP), which utilizes nitroxides [110], and reversible addition fragmentation chain transfer polymerization (RAFT), which typically utilizes dithioester or dithiocarbamate chain transfer reagents [111]. Michael addition reactions have recently proven useful in many of these living radical polymerization strategies.

Functional initiation is of interest for the synthesis of well-defined polymers with the potential for block copolymer synthesis, coupling with proteins or reaction with surfaces. Recently, Michael addition was used to synthesize an ATRP initiator containing two hydroxyl groups [112]. This novel dihydroxyl initiator allowed the synthesis of Y-shaped polymers via a combination of ATRP on the initial alkyl halide site and post polymerization conversion of the hydroxyl groups to alkyl halides which allowed polymerization of a second monomer (Fig. 31). Matyjaszewksi et al. recently studied the synthesis of ATRP ligands through the Michael addition reaction of tris(aminoethyl)amine with several acrylates, resulting in ligands that were suitable for the polymerization of methacrylic, acrylic and styrenic monomers in nonpolar media [113]. Matyjaszewski et al. also synthesized ATRP ligands containing dimethoxymethylsilylpropyl acrylate, which aided in post-polymerization catalyst removal via passage through silica gel and coupling of the complexes to the silica gel via sol–gel reactions. Shen et al. synthesized novel diaminopyridine (DAP) containing ATRP ligands through acrylation of DAP followed by Michael addition using bis(diethylaminoethyl)amine [114]. Silica gel modified with thymine residues created hydrogen bonded associations with these DAP ATRP ligands, which dissociated at reaction temperatures, allowing polymerization to occur. After cooling the reaction, re-association allowed removal and recycling of the catalyst.

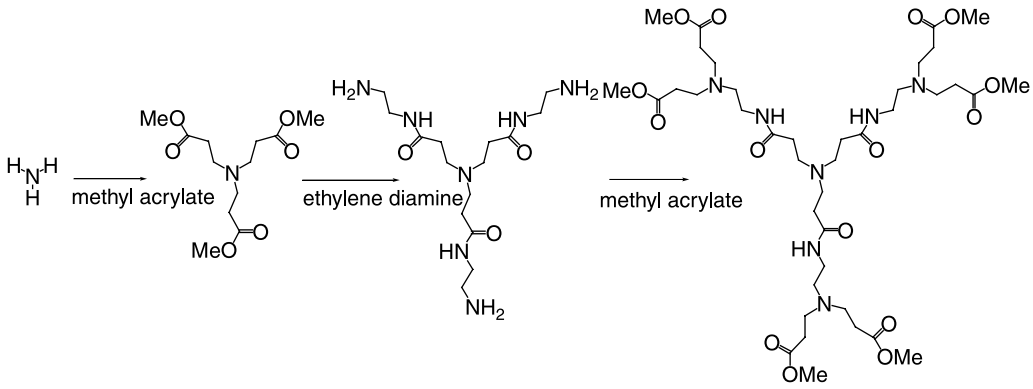


Fig. 32. Divergent PAMAM dendrimer synthesis via alternating Michael addition and amidation.

Synthesis of functional polymers is also achieved through post-polymerization strategies based on the Michael addition. For example, hydroxyl functionalized RAFT polymerized PMMA were synthesized using hydrolysis of the dithioester end groups followed by Michael addition with hydroxyethyl acrylate to the thiol group [115].

5. Synthesis of branched polymers via the Michael addition reaction

Branched polymers encompass a wide range of topologies, ranging from hyperbranched to graft and dendritic polymers. Michael addition polymerization mechanisms were broadly applied to each of these topological designs. Hyperbranched polymers have gained interest recently, due to a large number of functional termini and processing advantages in terms of lower melt viscosity relative to linear polymers of equivalent molecular weight. Michael addition provides the means to incorporate numerous types of terminal functionalities due to an inherent functional group tolerance and wide variety of monomers. Graft copolymers are also of increasing interest given the similarity to block copolymers and the ability to control the graft density and graft length. Graft copolymers often possess microphase separated morphologies and offer elastomeric properties. Dendrimers have traditionally been synthesized using Michael addition reactions, and in fact, Tomalia et al. synthesized the first dendrimer, poly(amido amine) (PAMAM) using Michael addition. Dendrimers and hyperbranched polymers, are interesting due to a plurality of functional groups. However, the absence of entanglements often limits the use of dendrimers to non-structural and mechanically non-demanding applications. Dendrimers based on Michael addition reactions are used in numerous applications, including biomedical applications and gene delivery [116,117].

5.1. Dendrimers

Michael addition reactions are ideal for dendrimer synthesis due to the low probability of side reactions and mild reaction conditions. In the first dendrimer synthesis, dendritic poly(amido amine)s were synthesized via the alternating Michael addition of methyl acrylate to amine substrates (ammonia, $f=3$; ethylenediamine, $f=4$) and aminolysis of the resultant esters with excess ethylene diamine [118]. As depicted in Fig. 32, the PAMAM dendrimer synthesis is divergent, beginning from a small molecule core and enlarging as further generations are added. The high conversion and facile nature of the Michael addition led to nearly monodisperse individual dendrimer molecules as revealed using electron microscopy [119]. The Michael addition was most efficient in methanol, while aprotic solvents led to incomplete alkylation of amine groups. Retro-Michael additions fragmented the dendrimer molecules at elevated temperature (80 °C) in solution [120].

PAMAM dendrimers are effective gene transfection agents due to the plurality of terminal amine groups available to complex with the negatively charged DNA phosphate backbone. In aqueous solution, protonation of the terminal amine groups produces a cationic charge that favors complexation with DNA. A DNA-polymer complex must have a net positive charge in order to pass through a cell membrane. The PAMAM dendrimers effectively transfected genes for β-galactosidase expression, luciferase expression, and also antisense genes that selectively suppress luciferase genes [116].

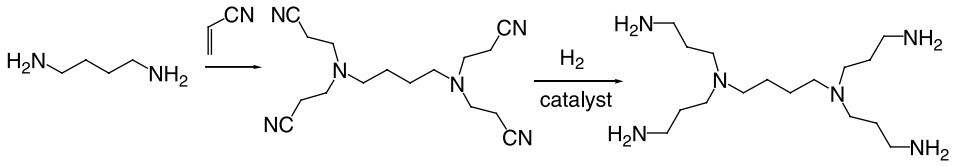


Fig. 33. Synthesis of PPI dendrimers via alternating Michael addition and hydrogenation.

PAMAM dendrimers received significant attention for effectiveness in gene transfection and numerous transfection studies were performed on diverse eukaryotic cell lines. Studies found increased transfection efficiency for higher generation dendrimers [117]. Higher generations were necessary to obtain efficient coverage of DNA, as higher generations possessed sizes close to the histone octamer. Heating the dendrimers in aqueous solution led to fragmentation, likely through retro-Michael additions and solvolysis, which significantly improved transfection. In fact, a commercial gene transfection agent, Superfectin™, is based on fragmented PAMAM dendrimers. PAMAM dendrimers of numerous sizes possess very low cytotoxicity and in vivo studies have also showed low toxicity.

Amine terminated PAMAM dendrimers were recently used to eliminate prion proteins from scrapie infected neuroblastoma cells [121]. Higher generation PAMAM dendrimers were more effective in removing the prion proteins and were most effective below pH 4, suggesting the importance of ammonium charge and activity in the lysosomal or endosomal regions. The importance of branching was demonstrated through the comparison of linear and branched poly(ethylene imine) controls. The ability to remove prions occurred at noncytotoxic dendrimer concentrations.

A second common dendrimer is poly(propylene imine) (PPI), marketed under the trade name Astramol™ [122]. PPI dendrimers are accessed through the Michael addition of primary diamines with acrylonitrile followed by hydrogenation of the nitrile groups to amines and repeated reaction with acrylonitrile (Fig. 33). First through fifth generation dendrimers are commercially produced in this manner. PPI dendrimers were recently synthesized with poly(propylene oxide) Jeffamine® cores, allowing greater flexibility and less steric congestion of the dendrimer molecules [123]. The terminal nitrile groups of poly(propylene imine) dendrimers are hydrolyzed under acidic conditions to obtain peripheral carboxylic acid functionality, but this often leads to degradation or discoloration of the polymer. Meijer et al. developed a route to carboxylic acid functional dendrimers via hydrogenation of the peripheral nitriles followed by the Michael addition of methyl acrylate and basic hydrolysis of the resultant ester functionalities [124]. In contrast to PAMAM dendrimers, PPI dendrimers have less potential for gene transfection and higher cytotoxicity [117]. Lower toxicity was achieved with PPI dendrimers containing ether and ester linkages, synthesized by the Michael addition of alcohols to acrylonitrile, reduction of the nitrile, Michael addition to methyl acrylate, and reduction back to alcohol terminal groups [125].

Majoral et al. developed novel dendrons containing Michael acceptors consisting of $CH_2{=}CH{-}P{=}N{-}P{=}S{-}(O{-}R)_2$ or $CH_2{=}CH{-}P{=}N{-}P{=}O{-}(O{-}R)_2$ groups at the dendrimer core. This allowed convergent synthesis of dendrimers via coupling of the dendrons through Michael addition with primary diamine donors (Fig. 34) [126,127].

Peripheral modification of dendrimers was also accomplished via Michael reactions with pendant Michael donors or acceptors. For instance, mesogenic cyanobiphenyl acrylates were introduced onto amine terminated poly(propylene imine) dendrimers resulting in liquid crystalline dendrimer molecules [128]. Michael addition of acryloxylethyl methacrylate to amine terminated dendrimers resulted in photocrosslinkable dendrimer methacrylates [129]. The Michael addition scheme was the only successful functionalization methodology as reaction with isocyanatoethyl methacrylate or acetoacetoxy methacrylate resulted in insoluble, crystalline products and reaction with methacrylic anhydride did not completely

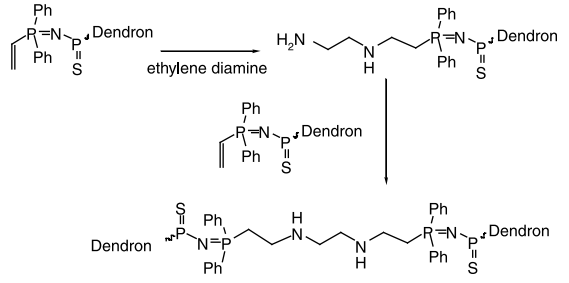


Fig. 34. Convergent dendrimer synthesis via dendron core-linking Michael addition reactions.

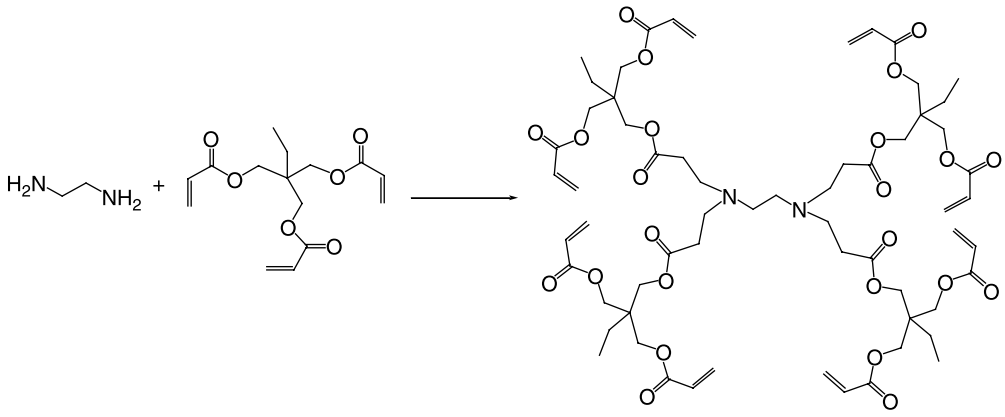


Fig. 35. Crosslinkable dendrimer synthesis using Michael addition reactions.

functionalize the terminal amines. The methacrylated dendrimers exhibited nearly quantitative cure reactions as determined through differential scanning calorimetry (DSC). In Fig. 35, dendritic acrylate oligomers were also synthesized through the Michael addition of trimethylolpropane triacrylate (TMPTA) with ethylene diamine in a 5:1 ratio [130,131]. The desired product possessed a TPMTA:EDA ratio of 4:1, however, network formation occurred under these conditions. The branched oligoacrylates resembled dendrimers with low viscosities and facile cure conditions using UV-photocuring techniques. Purification of these dendrimers was achieved primarily through precipitation.

5.2. Hyperbranched and highly branched polymers

Hyperbranched polymers were initially developed as inexpensive, one-pot substitutes for dendrimers, but have developed a unique character over the last several years. Hyperbranched polymers were traditionally approached from asymmetric monomer synthesis (AB_n monomers). Difficult monomer synthesis is a major disadvantage of the AB_n method due to a lack of commercial AB_n monomer sources. Recently, A_n+B_m approaches were used to synthesize hyperbranched polymers, enabling a much wider range of monomers and topologies. A further development is the use of oligomeric A_n or B_m monomers, resulting in 'highly branched polymers', which allows control of the molecular weight between branch points, an important parameter that affects degree of entanglement and has performance implications [132]. Michael addition reactions were successfully applied to both the AB_2 and A_n+B_m strategies for hyperbranched polymer synthesis.

In a conventional AB_2 hyperbranching polymerization, Endo et al. conducted acetoacetylation of hydroxyethyl acrylate through a reaction with diketene [133]. This produced an AB_2 monomer that was capable of producing hyperbranched Michael addition polymers in the presence of base catalyst (Fig. 36). Polymerization of this novel monomer in the presence of DBU resulted in number average molecular weights between 2000 and 12,000 g/mol with dispersities between 1.4 and 3.5 and degrees of branching between 43 and 83%. A high ratio of dendritic to linear units was observed, suggesting greater reactivity of the *mono*-Michael adduct towards the acrylate group compared to the reactivity of the original acetoacetate group.

Trumbo studied the step growth polymerization of low molar mass diacrylates (tripropylene glycol

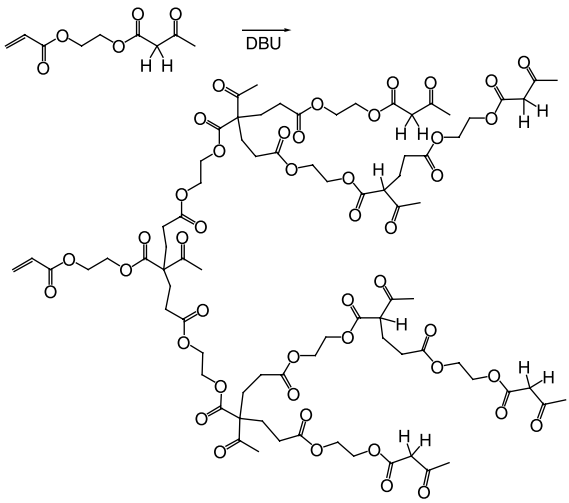


Fig. 36. Hyperbranched polymer synthesis via AB_2 Michael addition polymerization.

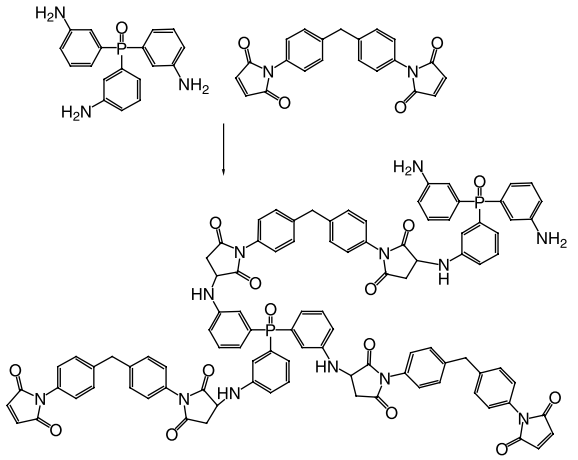


Fig. 37. Hyperbranched poly(aspartamide)s via A_2+B_3 polymerization.

diacrylate) with bisacetoacetates [134] and bisacetoacetamides [135]. These systems formed branched structures due to the difunctionality of the acetoacetate group. Gelation of the reaction mixture was avoided with an excess of bisacetoacetate monomer and through conducting the reaction in solution with a relatively mild base catalyst (DBU). Weight average molecular weights as high as 437,000 g/mol were observed, however broad dispersities ($M_w/M_n \sim 10$) were typical, which further suggested branching in this system. Decreases in molecular weight and dispersity with reaction time were observed during these polymerizations, suggesting the occurrence of a retro-Michael addition and subsequent equilibration.

Hyperbranched poly(aspartamide)s were synthesized from bismaleimides and aromatic triamines using the A_2+B_3 methodology [136]. The reaction stoichiometry was limiting in bismaleimide, thus controlling the extent of the reaction and favoring secondary amine terminated products. The hyperbranched polymers exhibited degrees of branching near 0.51 and 0.69, high glass transition temperatures ($\sim$210–250 °C) as well as equivalent thermal stability to analogous linear polymers. In Fig. 37, a tris (4-aminophenyl)phosphine oxide triamine was used to create polymers with higher thermal stability and also to allow degree of branching determination through ^{31}P NMR.

Feng et al. studied hyperbranched poly(amino ester)s that were synthesized from piperazine and trimethylolpropane triacrylate in molar ratios ranging from 1:1.08 to 1:2 [137]. These hyperbranched polymers formed aggregates in acetone/acidic water, which were proposed to contain a hydrophobic acrylate rich core and a hydrophilic, protonated amine periphery. The aggregates were crosslinked using UV light via reaction of residual acrylate groups. The size of the aggregates (DLS, TEM) decreased upon crosslinking and were smaller for higher molecular weight polymers, suggesting a packing efficiency limitation with the higher molecular weight, more disperse, polymers. Enhanced aggregation of similar poly(amino ester)s was achieved through reaction of peripheral amine groups with long chain acyl halides [138].

Wu et al. studied the effect of unequal functional group reactivity in a hyperbranching A_2+B_3 system with a butanediol diacrylate A_2 and a trifunctional amine B_3 containing both primary ($f=2$) and secondary ($f=1$) amines (Fig. 38) [36]. For an equimolar A_2:B_3 ratio, ^{1}H NMR studies indicated that secondary amines were consumed prior to primary amines. The secondary amine that was formed from reaction of the primary amine and the acrylate group was not consumed, and thus a predominantly linear product was obtained despite the functionality of the monomers. The reactivity of the amines was secondary $>$ primary $>>$ formed secondary. If the A_2:B_3 ratio was increased, participation of the formed secondary amine during the reaction of the primary amine occurred, thus resulting in a branched product. If the steric hindrance at the secondary amine position was increased, the primary amine was the most reactive and the reaction proceeded at the formed secondary amine during the reaction and again the product was branched. Thus, both the level of substitution of the amine groups and the stoichiometric ratio of the reactants controlled

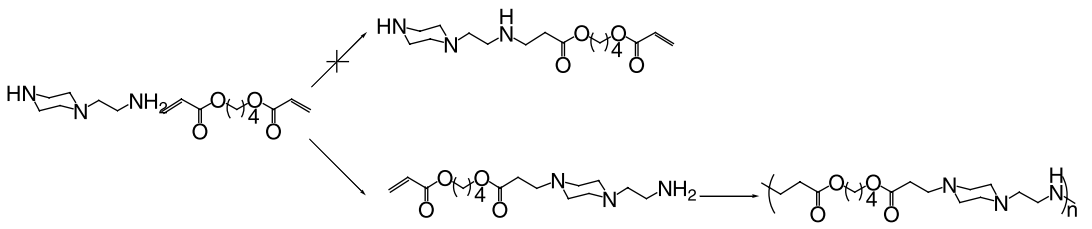


Fig. 38. Hyperbranched poly(ester amine)s from A_2+B_3 polymerization with unequal reactivity in the B_3 monomer functional groups.

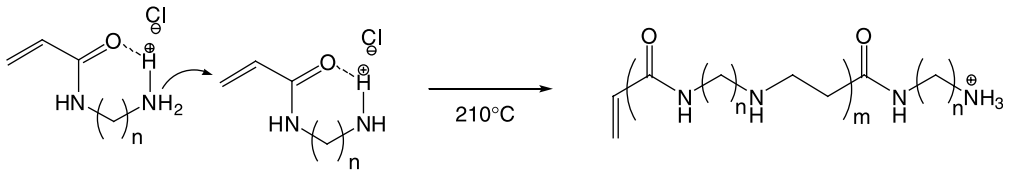


Fig. 39. Homopolymerization of an AB_2 poly(amido amine) monomer.

the degree of branching. Degrees of branching that ranged from 30 to 40% and weight average molecular weights as high as 23,500 g/mol were obtained.

Wu et al. also studied hyperbranched poly(amino ester)s that were synthesized using two equivalents of butanediol acrylate and one equivalent of 1-(2-aminoethyl)piperazine [139]. Due to the higher reactivity of the secondary cyclic amine, the Michael addition with butanediol diacrylate occurred preferentially. The primary amino group reacted second, resulting in the in-situ generation of an AB_2 intermediate that undergoes a branching at the secondary amine formed from the primary amine. The ratio of R_h/R_g of 1.0 as measured by SAXS and DLS demonstrated the hyperbranched nature of the polymers. The hyperbranched polymers had number average molecular weights of 29,000–38,000 g/mol and dispersities of 3.4–3.7.

In similar work, Gao et al. synthesized hyperbranched polymers from diethanolamine and methyl acrylate [140]. The reaction proceeded through an initial Michael addition reaction to form an ester diol AB_2 monomer in situ that self-condensed at elevated temperature (150 °C) through transesterification in the presence of a zinc acetate catalyst. Number average molecular weights as high as 268,000 g/mol were observed with a polydispersity of 2.4. Crosslinking occurred when the temperature in the second stage exceeded 160 °C, whereas lack of polycondensation occurred if the temperature was less than 120 °C. The degree of branching of these polymers was roughly 55%. These polymers degraded rapidly in water to form self-buffered pH 7 solutions, suggesting them as good candidates for drug delivery.

Hyperbranched poly(amido amine) hydrochloride polyelectrolytes were synthesized through the Michael addition polymerization of AB_2 monomers based on ammonium alkyl acrylamides, as shown in Fig. 39 [141]. The lack of nucleophilicity of the ammonium group toward the acrylamide functionality required the use of high temperatures (210 °C) in order to drive the polymerization. It was postulated that a small equilibrium concentration of free amine that exists at these high temperatures undergoes the Michael addition. The shorter alkylene spacer ($n=2$) between the acrylamide group and the ammonium group resulted in faster reaction, and an intramolecular deprotonation through a six membered cyclic transition state was proposed. Model reactions suggested that the ammonium group reacts twice with acrylamide functionalities, resulting in a highly branched product. ^{15}N NMR was used to characterize the degree of branching in these materials. Signals from linear units were not observed, corroborating the highly branched nature of the polymer. This methodology is a more facile route to hyperbranched polymers resembling PAMAM dendrimers.

Kadokawa et al. studied the triphenylphosphine initiated Michael addition polymerization of 2,2-bis(hydroxymethyl)propyl acrylate to yield phosphonium containing hyperbranched polymers of 1200–2700 g/mol, as shown in Fig. 40 [142]. This is one of the few examples of oxygen based nucleophiles involved in a Michael addition polymerization. Degrees of branching near 50% were obtained. Similar polymers were produced through initiation using sodium hydride. The poor quality of the oxygen nucleophile for Michael addition is evident in the reaction temperatures required (80–100 °C, with free radical inhibitors) [143].

Highly branched gene transfection agents were synthesized through the reaction of low molecular weight linear PEI with PEG diacrylate [144]. The polymers clearly did not represent linear topologies due to branching in the PEI as well as reaction at multiple amine sites along the polymer backbone. The presence of ester groups in the polymers facilitated degradation. The half-lives of the polymers in PBS at 37 °C were approximately 8 d. The polymers demonstrated complex formation with plasmid DNA and transfection studies were performed. Branched gene transfection

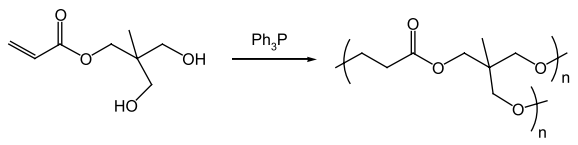


Fig. 40. Homopolymerization of an AB_2 poly(ester ether) monomer.

agents were also synthesized via reaction of trimethylolpropane triacrylate with primary amines such as *N*,*N*-dimethylethylenediamine [145]. Polyplex formation with plasmid DNA was verified with dynamic light scattering and transfection into various human and mouse cell lines exhibited transfection efficiencies similar to poly(ethylene imine). The branched nature of the poly(amino ester)s slowed polymer degradation and hence controlled the release of the plasmid DNA. The branched poly(amino ester)s showed little or no cytotoxicity.

5.3. Graft copolymers

Graft copolymers are synthesized through numerous routes including chain or step growth polymerization of macromonomers and post-polymerization grafting. Ferruti et al. examined graft copolymer synthesis using poly(amido amine)s [146]. PEG chains with weight average molecular weight of 750 g/mol were incorporated into poly(amido amine) backbones using monoamine terminated PEG as a comonomer. As a second route, PEG-NH_2 was first reacted with excess bisacrylamide monomer to obtain a PEG bisacrylamide, which was polymerized with piperazine. In both cases, vapor pressure osmometry indicated that the molecular weights obtained were quite low ($\sim$3000 g/mol). Ferruti concluded that the reactivity of the PEG-NH_2 in the polymerization was lower than typical small molecule amines. Ferruti et al. also studied poly(amido amine)s grafted to polyethylene via a post-polymerization strategy, although Michael addition was not involved in the coupling [147].

Jerome et al. utilized γ-acryloxy-ε-caprolactone as a graftable comonomer in copolymerizations with ε-caprolactone [148]. In Fig. 41, pendant acrylate groups were introduced into poly(ε-caprolactone), permitting grafting of thiol terminated oligomeric PEG (M_n = 900 g/mol). The Michael addition reaction of various thiols (mercaptoacetic acid and triphenylmethane thiol) with PEG-acrylate model compounds proceeded to completion. However, conversions of only 65 and 70% were obtained with PEG-thiol and mercaptoacetic acid, respectively, in grafting studies with poly(γ-acryloxy-ε-caprolactone-*co*-ε-caprolactone).

Much work was done in the area of 'grafting to' syntheses utilizing the Michael addition, especially in the case of biopolymers such as chitin and chitosan. These materials typically possess poor solubility, which is improved through grafting techniques. Modification of chitosan with poly(amido amine) dendrimers was

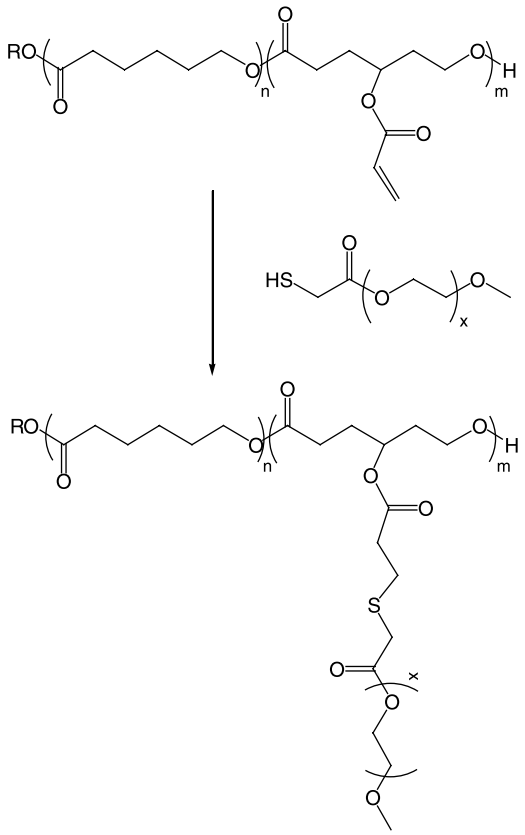


Fig. 41. Graft copolymerization via grafting of thiol terminated PEG to pendant acryloxy side groups on a poly(ε-caprolactone) derivative.

achieved by reacting chitosan surface amine groups with methyl acrylate followed by alternate reactions with ethylene diamine and further methyl acrylate [149]. The heterogeneity and steric hindrance of the backbone resulted in imperfect dendrimers. Grafting of living cationic poly(methyl oxazoline) and poly (isobutyl vinyl ether) onto the pendant amine groups of the grafted poly(amido amine) dendrimers was achieved successfully. Modification of chitin with small molecules was also achieved through Michael addition to pendant amine groups. Reaction with acrylonitrile yielded cyanoethylated chitin [5] and reaction with ethyl acrylate afforded ester-containing chitin [150] (Fig. 42).

6. Novel networks via the Michael addition reaction

Network polymers that are synthesized via Michael addition reactions offer applications in diverse areas such as drug delivery systems, high performance composites, and coatings. Networks are

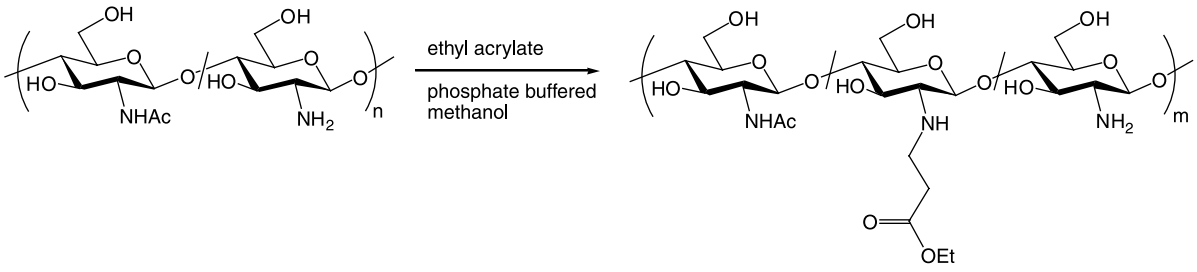


Fig. 42. Modification of chitin via Michael addition reaction with ethyl acrylate.

often synthesized through a combination of Michael addition step growth chemistry combined with chain growth polymerization using photoinitiated radical or anionic processes. The Michael addition is suited to the formation of well-defined networks due to the commercial availability or facile synthesis of narrow molecular weight distribution functional oligomers. Commercially available reactive oligomers include PEG diacrylate and numerous oligomeric diamines such as Jeffamine®. Several classes of Michael addition networks are currently of interest.

6.1. Bismaleimide networks and composite materials

Currently, bismaleimide based networks are used in high performance fiber-reinforced composite materials [151] and also in thermoset resins. These materials have applications in the aerospace industry due to their high temperature strength, mechanical robustness, and low density [152]. In the absence of other components, bismaleimides crosslink through chain growth type reactions at high temperature to form highly crosslinked, high T_g networks. Vitrification in these systems leads to incomplete conversion of maleimide groups. Brittleness is the primary drawback of these homopolymerized networks. Efforts to reduce the crosslink density and hence the brittleness of these thermosets have involved Michael addition chain extension reactions with primary or secondary diamines to consume some of the maleimide functional groups [70]. The chain extension process results in greater toughness and improved mechanical properties for composites. The introduction of diamines such as methylene dianiline (MDA) causes a low temperature Michael addition polymerization (<180 °C) during initial curing, followed on further heating (180–220 °C) with a crosslinking homopolymerization of the maleimide groups (Fig. 43) [152,153]. Under certain conditions, secondary amines formed during the initial Michael addition undergo further Michael addition crosslinking with maleimide groups. Hyperbranched amine terminated polyamides were also investigated as components of bismaleimide networks, successfully improving the toughness of these systems [154].

Kumar et al. incorporated bismaleimide crosslinking systems into silicon-containing epoxy resin networks [154]. The bismaleimides increased the flexural and tensile moduli, but decreased fracture toughness of the epoxy networks by introducing a more rigid, higher T_g component. This network reinforcement was due to the thermal cure and Michael addition reactions between the bismaleimide and the amines from the epoxy resin and the added diamine.

Bismaleimide networks are useful as NLO materials as well as composites due to their high glass transition temperatures and crosslink densities. These features are attractive for NLO materials for maintenance of the chromophore orientation. Furthermore, the absence of volatile by-products during cure allows poling during this process. Wu et al. synthesized a diamine NLO chromophore and incorporation into bismaleimide networks resulted in nonlinear optical coefficients as high as $d_{33}=60$ pm/V at 1064 nm [155]. Upon heating, the second harmonic generation (SHG) was stable to 120 °C, although the cure process was continued 250 °C in order to maximize crosslinking. Maintenance of the poling field during the cooling process maximized the NLO properties.

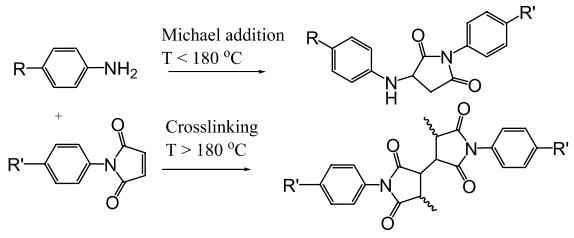


Fig. 43. Crosslinking of bismaleimide networks via a combination of Michael addition and maleimide homopolymerization.

6.2. Carbon Michael addition networks utilizing acetoacetate chemistry

Considerable interest in Michael addition networks from acetoacetate and acrylate precursors has recently emerged. These networks are interesting due to the relatively low toxicity of the components and their room temperature processing in the absence of UV radiation. Furthermore, functionalization of common polyols with the acetoacetate group is achieved through transesterification with *tert*-butylacetoacetate [156] or reaction with diketene precursors [157] providing a facile route to crosslinkable precursors from a wide variety of hydroxyl containing feedstocks.

Room temperature thermosetting coatings is one of the primary applications of Michael addition networks. The Michael addition mechanism is advantageous as neither UV radiation nor heat are required, both of which could be deleterious to the substrate and require additional processing equipment. The base catalyzed carbon Michael addition of acetoacetylated resins to acrylate acceptors, as depicted in Fig. 44, is an ideal route to crosslinked networks due to the difunctionality of the acetoacetate group and the absence of undesirable toxic amine and malodorous thiol nucleophiles in industrial processes. Acrylic monomers and oligomers are useful candidates for the formation of coatings and adhesives with highly reduced volatile organic compounds (VOCs) and improved material performance properties. Although UV curing is not necessary for network formation, the presence of photocrosslinkable groups in these systems allows tandem Michael and radical curing mechanisms.

Clemens and Rector studied the carbon Michael addition reaction between model compounds, and concluded that the reaction mechanism involved an equilibrium deprotonation of the acetoacetate and subsequent rate limiting addition of the enolate to the acrylate group [21]. Thus, the rate of the reaction was unaffected by the acetoacetate concentration but was directly proportional to the base catalyst concentration. The effect of various catalysts was investigated and as expected, higher basicity led to faster reaction kinetics. Typical base catalysts included DBU and TMG as well as hydroxide and methoxide bases. A striking difference, however, was observed in the degree to which bisadducts of acrylates with acetoacetates formed as a function of catalyst. Amidine and guanidine bases resulted in higher formation of bisadducts while monoadducts were favored by methoxide and hydroxide bases. Weaker bases such as triethylamine were not strong enough to deprotonate the acetoacetate groups and did not catalyze the Michael addition reaction effectively.

Clemens and Rector prepared crosslinked coatings consisting of randomly functionalized acetoacetylated precursors such as methyl methacrylate/acetoacetyloxy- ethyl methacrylate random copolymers with low molar mass multiacrylates such as trimethylolpropane triacrylate [21]. The level of functionality of the methacrylate copolymers strongly influenced cure times, with a doubling of the acetoacetate functionality resulting in a 75% reduction in cure time. Acetoacetylated cellulose was also investigated, and the more rigid character of the polymer coupled with the lack of a spacer between the backbone and the acetoacetate groups resulted in slower cure reactions. One disadvantage of the Michael crosslinked coatings was hydrolytic instability that was attributed to hydrolysis of the ester linkages in the presence of the base catalyst. Heat treatments reduced the hydrolytic instability of the network.

Marsh described the use of high solids acetoacetate-containing resins for coatings applications [158]. High

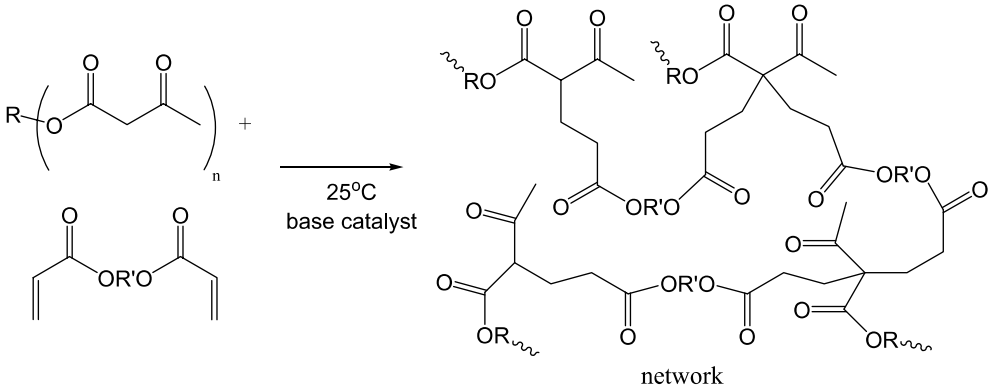


Fig. 44. Carbon-Michael addition polymerization of acetoacetates with acrylates catalyzed by bases for network synthesis.

solids polyester resins containing hydroxyl groups were synthesized and acetoacetylated to various levels. It was found that increasing levels of acetoacetyl groups lowered the T_g of the polyester resins significantly to yield solvent-free low viscosity resins. The crosslinking of the pendant acetoacetyl groups using Michael type reactions with electron deficient olefins and DBU base catalyst (pH 12.6) afforded formaldehyde- and isocyanate-free room temperature curable coatings.

Tung published a review on the performance of coatings made from multifunctional acrylate resins containing reactive diluents and acid blocked catalysts and compared them to two-package polyurethanes [159]. The use of a formic acid neutralized DBU catalyst yielded better pot life and humidity resistance of the coatings when compared to the highly basic DBU catalyst. It was found that incorporation of 10–15 mol% of a reactive diluent such as 2,2,4-trimethyl-1,3-pentane-bis(acetoacetate) resulted in low VOC coatings with desirable ultimate properties. Various multifunctional acrylate crosslinkers were investigated to determine the influence of crosslinker functionality on the resulting resin properties. A mixture of tri- and tetraacrylates produced the best hardness, flexibility, solvent resistance, humidity resistance, adhesion, and weatherability compared to the triacrylates or hexaacrylates.

6.3. Curing mechanisms involving Michael addition with photocrosslinking or sol–gel chemistry

Cure mechanisms using a combination of Michael addition and photoinitiated radical curing are currently of interest due to higher crosslink densities and functional group conversions. The use of multifunctional acrylate monomers in the carbon Michael addition networks is ideal for photocrosslinking. Residual acrylate dangling ends must be limited to maximize chemical and physical durability. Moszner and Rheinberger synthesized networks from multiacrylates such as PEG diacrylates, trimethylolpropane triacrylate, and pentaerythritol tetraacrylate with bisacetoacetates such as PEG bis(acetoacetate) and hexanediol bis(acetoacetate) using DBN base catalyst [160]. Isothermal DSC studies revealed less than 25% of the double bonds were consumed via Michael addition during cure. The conversion of acrylate groups increased for longer, more flexible, spacers to approximately 71%. Subsequent photocuring of the Michael addition networks using camphorquinone/*N*-(2-cyanoethyl)-*N*-methylaniline resulted in dramatically improved hardness and conversion of the acrylate groups. Moy et al. developed photocrosslinked networks from crosslinkable oligomers that were synthesized using Michael addition with an excess of multiacrylates relative to acetoacetate precursors [161]. The Michael addition of acetoacetoxyethyl methacrylate with multifunctional acrylates is another route to photocrosslinkable methacrylate oligomers. The ketone-containing oligomers were capable of self-initiation in UV light due to Norrish type cleavage reactions. Klee et al. conducted Michael addition using diamines and acryloxylethyl methacrylate to create crosslinkable branched multimethacrylate oligomers for dental composites with lower shrinkage upon photocrosslinking [162]. Dammann and coworkers demonstrated the formation of liquid oligomeric Michael adducts by reacting di-, tri- and tetraacrylates with ethyl acetoacetate in a specific ratio in the presence of catalytic amounts of epoxide and quaternary ammonium salt. These compositions were further crosslinked to obtain coatings, laminates, and adhesives [163].

Pavlinec and Moszner further studied crosslinked networks from poly(propylene glycol) bis(acetoacetate) or pentaerythritol tetrakis(acetoacetate) and pentaerythritol tetraacrylate cured with DBN [11]. In these Michael addition networks, a 2:1 ratio of acrylate to acetoacetate was studied. The residual acrylate groups in the networks were studied using FTIR, following the acrylate vinyl absorbance at 810 cm^{-1}. The concentration of residual unreacted acrylate groups was lowest for networks that were prepared using poly(propylene glycol) bis(acetoacetate)s due to the molecular mobility and long distance between crosslink points. Higher conversion of reactive functional groups for longer precursors was also observed in photocrosslinking studies of diacrylate oligomers [164]. Ambient temperature aging and thermal postcure treatments to 200 °C increased the conversion of acrylate groups from 88 to nearly 97%. The gel fractions that were obtained for the Michael addition networks ranged from 87 to 94%. The combination of Michael addition curing with free radical photocuring was also investigated with the addition of photoinitiators, excess pentaerythritol tetraacrylate, and PEG dimethacrylate monomers to selected compositions. The stability of the methacrylate against Michael addition prevented interference between the two cure mechanisms performed in a stepwise manner, beginning with a dark Michael cure and ending with the photocure. In the photocured systems, lower viscosity during the Michael addition stage was attributed to the additional PEG dimethacrylate monomer and excess acrylate

monomer. This was postulated to improve the cure process by improving molecular mobility and homogeneity.

Michael reactions between multifunctional acrylates and acetoacetates have found widespread applications in the formation of radiation curable coatings, printing ink formulations, and adhesives. UV curable ink formulations have gained importance as a substitute to solvent-based ink systems. Currently available UV curable ink formulations exhibit rapid curing under commercial line speeds, however, large quantities of photoinitiator such as benzophenone are required to ensure complete curing throughout the thickness of the film for printing applications. These photoinitiators are known to be toxic, expensive and also cause odor and color in the material. In order to overcome this issue, current studies have involved the use of liquid oligomeric Michael adducts formed from multifunctional acrylates by reaction with Michael donors such as acetoacetate compounds, which are amenable to further crosslinking under UV irradiation [165]. Through the incorporation of suitable additives such as acrylic monomers and oligomers, vinyl monomers, vinyl ethers, and primary and secondary amines, ink formulations were modified to obtain rheological and adhesion characteristics that were desirable for printing applications on many different kinds of substrates.

Recently Liu et al. described the formation of renewable and environmentally benign wood adhesives based on derivatized soy protein using the Michael addition crosslinking approach [166]. Soy proteins used as renewable wood adhesives suffer from low shear strength and moisture resistance. In order to improve the properties of the adhesive, the authors first maleated the soy protein isolates (SPI) using maleic anhydride, which was then reacted with poly(ethylene imine) via Michael addition. Wood composites that were bonded with adhesive exhibited shear strength of 7.0 MPa as compared to 2.0 MPa of those bonded using SPI. In addition the joints also gained water resistance.

In addition to the application of the Michael addition in the formation of crosslinked coatings, the formation of reactive intermediates through Michael additions which are subsequently crosslinked using other mechanisms yields useful coatings and adhesives. This strategy has often involved sol–gel chemistry of the Michael adducts to form networks. For example, Wilkes et al. have reported the formation of bis- and tris(maleimide) functionalized trialkoxysilanes by Michael addition reaction between 3-aminopropyl triethoxysilanes and corresponding maleimides [167]. The functionalized silanes were crosslinked via sol–gel methods and used as protective coatings on polycarbonate substrates exhibiting enhanced abrasion resistance. Tilley et al. recently described the use of the Michael addition reaction for the preparation of acrylate-functionalized silane coupling agents to introduce acrylate functionality to colloidal silica particles [168]. Such acrylate functionalized silica particles were used as toughness enhancers for UV-curable acrylic coatings. Incorporation of these functionalized silica particles into a wide variety of organic matrices such as polyesters, epoxies, and polyurethanes was demonstrated. Such inorganic oxide modified coatings exhibited much greater micro-hardness and abrasion

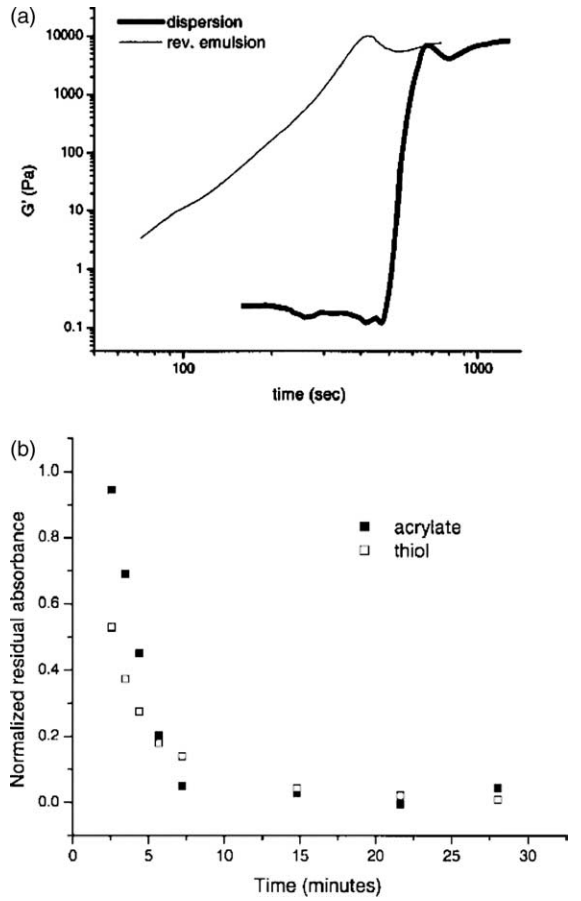


Fig. 45. (a) Rheological monitoring of gel formation in networks synthesized from multifunctional PEG acrylates with PEG dithiol in both reverse emulsion and dispersion form. The steep rise in G' with gel formation in the case of the dispersion network is an indication of a sudden percolation of crosslinked phases at later times relative to the continuous organic crosslinking phase in the reverse emulsion. (b) Depletion of functional groups with time during crosslinking. Reproduced from 'Water-borne, in situ crosslinked biomaterials from phase-segregated precursors,' Hubbell et al., Copyright 2003 Society of Chemical Industry with permission from John Wiley and Sons on behalf of SCI [2].

resistance compared to commercially prepared coatings. In a recent patent disclosure, Kobayashi et al. reported the synthesis of an acryloxy-functional silicone composition curable by high-energy radiation [169]. The silicones consisted of a mixture of multifunctional acrylates, amino-functional alkoxysilanes, organofunctional alkoxysilanes, and colloidal silica. The resultant coatings exhibited excellent storage stability, transparency, adhesiveness, water repellency, and abrasion resistance. Zhu et al. also demonstrated the formation of trimethoxysilane coupling agents containing *O*-butyrylchitosan via Michael addition of 3-acryloxypropyltrimethoxysilane with the amino groups of the butyrylchitosan [170]. Crosslinking of the modified silane derivatives resulted in colorless, flexible elastomeric coatings at 20 mol% incorporation of the modified silane. The resulting hydrogel films possessed good antithrombogenic properties as determined by blood-clotting and platelet adhesion.

6.4. Hydrogel networks for biomedical applications

Michael addition networks find application in areas beyond composites and coatings. Recently, numerous biomedical applications were investigated. Hubbell et al. studied in vivo gelling networks based on multifunctional thiols and multiacrylates as injectable tissue reinforcement [2]. Currently two mechanical ranges of in-vivo biomaterials exist: low modulus gels, which are used for drug delivery and high modulus bone cements. An intermediate strength material (0.5–50 MPa) is desirable for tissue reinforcement of invertebral disc annuli. Michael addition networks based on thiol and acrylate precursors are ideal due to the ability to control gelation with the addition of a basic catalyst, the absence of free thiols in the bloodstream, and the faster rate of the thiol-acrylate Michael addition in comparison to potential Michael reactions with amines in the bloodstream. Metters and Hubbell also recently demonstrated the biodegradability of these networks via slow hydrolysis of ester bonds adjacent to sulfide linkages, which leads to degradation on the order of days to weeks [171]. In a separate work, Hubbell et al. showed that the length of the alkyl spacer between the ester and the sulfide controlled the hydrolysis rate of these poly(ester sulfide)s, with higher degradation rates occurring for shorter alkyl spacers [172]. Both dispersion type and reverse emulsion type polymers were synthesized via mixing network components with basic PBS. Reverse emulsion type networks exhibited a slower change in shear storage modulus with time near the gel point and higher ultimate compressive strengths (6.7 vs. 1.8 MPa) due to greater continuity of the organic phase (Fig. 45). Dispersion networks exhibited rapid increases in shear storage modulus near the gel point, due to the percolation of dispersed crosslinking particles. Both materials exhibited maximum deformations near 37%. Modulus increased with increased amounts of tetra-acrylate precursors versus diacrylate precursors.

Rizzi and Hubbell [10] developed protein containing hydrogel networks for tissue repair applications. Cysteine-functionalized recombinant proteins containing sequences for integrin receptor ligation were

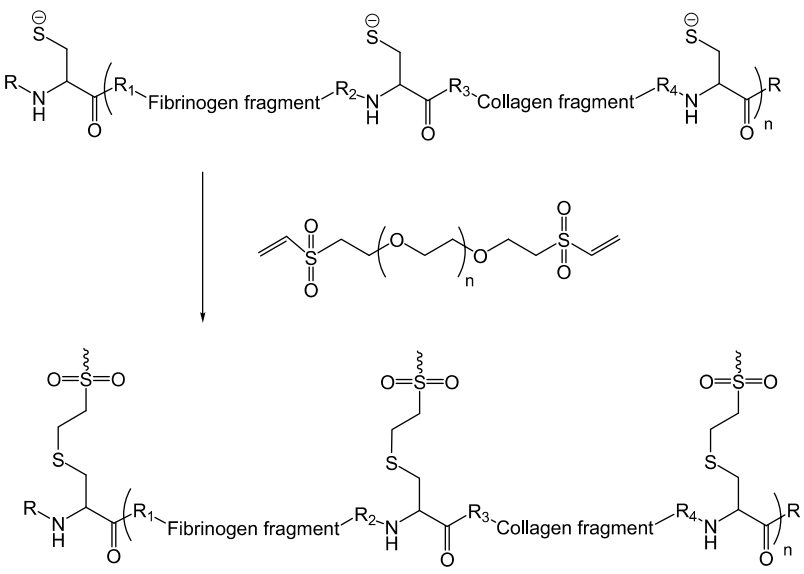


Fig. 46. Network formation of PEG bis(vinyl sulfone) and protein dithiols.

synthesized and crosslinked with PEG bis(vinyl sulfone) (Fig. 46). Integrin receptors are cellular receptors, which play important roles in tissue development processes. These networks were stable to aqueous solution and did not exhibit hydrolytic degradation. Thus, protease cleavable units were introduced into the proteins to enable biodegradability. Glutamic acid units were placed strategically near cysteine residues to inhibit disulfide bond formation prior to crosslinking, while glycine residues were used to prevent hindrance of the Michael addition. Previous work on cysteine containing peptides revealed that positively charged arginine groups adjacent to cysteine lower the pK_a of the cysteine group, accelerating Michael reaction by a factor of 2 and decreasing gel time by a factor of 2.5 [173]. Numerous charged residues were also used to enhance solubility of the proteins. The crosslinking kinetics were studied using rheological measurements, which showed increasing storage modulus with time during the crosslinking reaction. The gel time decreased with increasing pH, due to the fact that the thiolate ion is the active species in the Michael addition reaction with vinyl sulfone groups. A maxima in G′ and minimum in gel time was found for a ratio of thiol to vinyl sulfone (SH:VS) slightly greater than unity which differed with protein precursor. This non-stoichiometric maximum was likely due to disulfide bond formation during reaction. Higher storage modulus and lower swelling were generally observed with an increased molar ratio of thiol to vinyl sulfone (Fig. 47). A potential explanation for this phenomenon was decreased accessibility of reactive sites for crosslinking at lower SH:VS ratios. In similar work featuring non-degrading peptides with two cysteine residues, higher elastic modulus and lower swelling were observed for PEG bis(vinyl sulfone)s of higher functionality (3–8 arms) due to increased crosslink density [173]. Network elastic moduli in these systems reached a maximum at pH 8, likely because of slower kinetics at pH < 8 and greater disulfide bond formation at pH > 8. Furthermore, precursor concentration strongly influenced mechanical properties due to the propensity for cyclization reactions in dilute conditions.

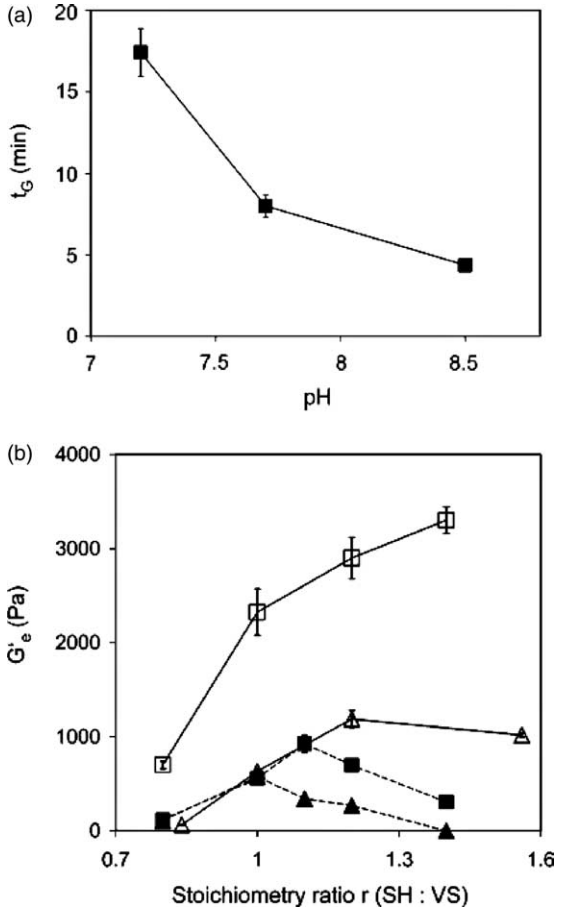


Fig. 47. (a) Effect of pH on gel time for PEG bis(vinyl sulfone). Gel time decreases at higher pH due to the greater thiolate anion concentration. (b) Effect of thiol to vinyl sulfone ratio (SH:VS) on the storage modulus of PEG-protein networks. The G′ maxima occurs at ratios slightly greater than unity suggesting disulfide formation. Reproduced from 'Recombinant protein-*co*-PEG networks as cell-adhesive and proteolytically degradable hydrogel matrixes. Part I: development and physicochemical characteristics,' Rizzi and Hubbell, Copyright 2005 with permission from the American Chemical Society [10].

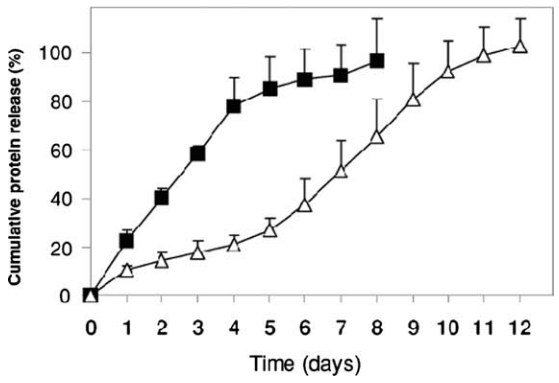


Fig. 48. Release of albumin from PEG networks based on PEG tetraacrylate (black squares) and PEG octaacrylate (white triangles). The lower functionality of the PEG tetraacrylate leads to lower crosslink density and more rapid release. Reprinted from J. Control Rel, 76, Hubbell et al. 'Protein delivery from materials formed by self-selective conjugate addition reactions,' 11–25, Copyright 2001, with permission from Elsevier [9].

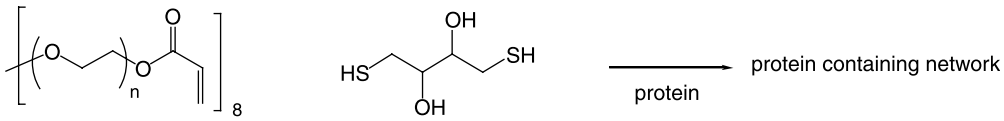


Fig. 49. Protein-containing PEG networks via Michael addition crosslinking of PEG octaacrylate with dithiothreitol. Human growth factor protein (hGH) was incorporated by mixing.

Michael addition networks were also developed for protein drug delivery applications. Protein drugs often suffer from rapid clearance from the bloodstream due to removal by the liver or kidneys. The use of drug delivery devices allows steady and long lasting delivery of protein drugs. Many current drug delivery materials such as poly(lactide)s can potentially denature the protein drug due to changes in pH associated with degradation byproducts. Another issue with current drug delivery materials is incorporating the protein into the material. Hubbell et al. studied Michael addition networks based on PEG multiacrylate and PEG dithiol that overcame many of these problems via incorporating bovine serum albumin protein during synthesis in aqueous media at physiological pH without denaturing the protein (Fig. 48) [9]. The crosslinking of these Michael addition networks required no basic catalyst or UV light that could potentially harm the protein as in photocuring of acrylate networks. Furthermore, the ester bonds in the PEG networks were degradable, allowing release of the protein as the network swells. The thiol-acrylate Michael addition reaction proceeded without significant side reactions with protein based amine nucleophiles, preserving the chemistry of the protein drugs. Furthermore, protein based free thiols are uncommon in extracellular proteins, lending greater selectivity to the approach. In cases where free thiols do exist, they are often confined to sterically hindered locations in the tertiary structure of the protein. Hubbell et al. introduced the protein as well-defined ~100 μm solid particles which were embedded into the Michael addition network during curing [9]. The release rate of the networks was tuned via the functionality of the PEG multiacrylate. PEG triacrylate networks resulted in rapid release of the protein. PEG tetraacrylate networks released protein over the course of 4 d at a linear rate of 20% per day. PEG octaacrylate networks exhibited a slower release with a release rate of 10–15% per day. Hydrolytic degradation was observed in the swelling kinetics of these networks. A decrease in gel time with increasing solution pH (up to pH 8.2) indicated that the thiolate anion is the active species in the Michael addition with the acrylate.

Hubbell et al. also studied protein drug delivery using Michael addition hydrogel networks from eight-arm PEG octaacrylate with dithiothreitol (Fig. 49) [174]. The protein drug human growth hormone (hGH), which suffers from rapid clearance was incorporated into these hydrogels without covalent attachment. Currently, daily administrations of this drug are required for treatment of the hormone deficiency as well as Turner's syndrome and renal failure. The

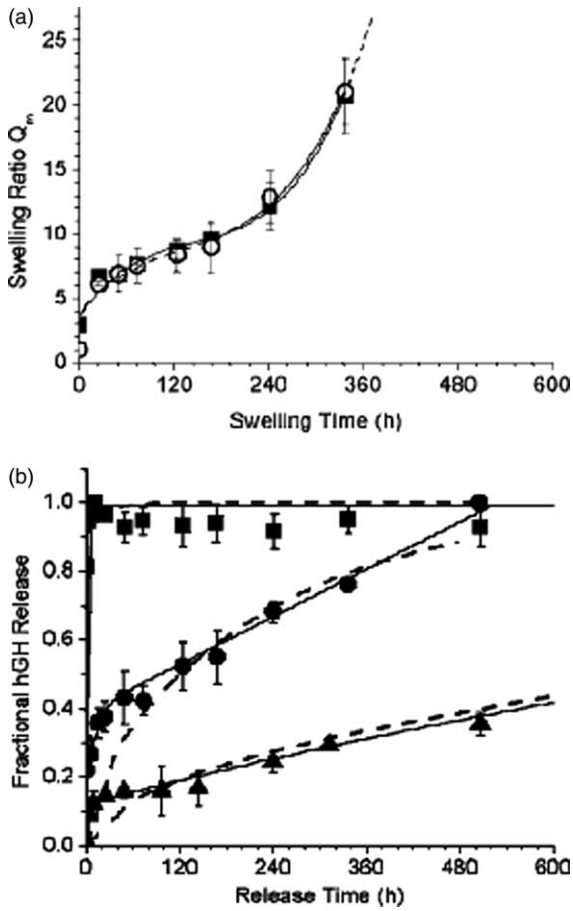


Fig. 50. (a) Swelling of PEG octaacrylate, dithiothreitol networks via initial solvent uptake followed by ester hydrolysis leading to degradation. (b) Release kinetics of hGH protein from networks as a function of PEG octaacrylate molecular weight (squares 10,000 g/mol, circles 10,000 g/mol mixed with 2000 g/mol (50:50), triangles 2000 g/mol). Reprinted from J Control Rel, 102, van de Wetering et al. 'The effect of the linker on the hydrolysis rate of drug-linked ester bonds,' 619–27, Copyright 2005, with permission from Elsevier [172].

crosslink density of the networks is controlled with the use of varying molecular weights of PEG octaacrylate, thereby influencing the drug diffusion. As shown in Fig. 50, the drug release rate was tuned to occur over periods from hours to months by changing the crosslink density of the networks. The ester sulfide linkages produced in the network degraded at a significantly faster rate than in free radically crosslinked acrylic networks, presumably due to neighboring group effects from the thioether group. Thus, the diffusion coefficient in these networks increases with time. The degradation rate of these networks varied from weeks to months and was directly related to the crosslink density.

Hubbell et al. also studied synthetic cell encapsulation networks [175]. These networks were synthesized from physically gelled four-arm Pluronics® (poly (ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide)) functionalized with acrylate groups and thiols. These hydrogels have potential applications in cell encapsulation. The propensity of the thiol groups to undergo disulfide bond formation prompted the use of the thioacetic ester protecting group which was deprotected during crosslinking. The reversible physical gelation process for Pluronics occurred in aqueous solution when a cold, slightly acidic medium (5 °C, pH 6.8) was increased both in temperature and pH (37 °C, pH 7.4). Once the physical gel formed, the covalent crosslinking via the Michael addition reaction produced gels, which were biodegradable due to the presence of ester groups. Degradation in PBS at physiological conditions occurred over periods of 1–2 weeks.

In order to mimic the extracellular matrix and guide the growth of cells, synthetic matrices should be biocompatible, resistant to non-specific adsorption of proteins, degradable via cell- secreted matrix metalloproteinases (MMPs), and further promote cell-adhesion. Extending on an earlier study, Hubbell et al. synthesized hydrogels comprising multiarm vinyl sulfone-terminated PEG, a monocysteine containing adhesion protein, and a bis(cysteine) metalloprotein substrate protein. The adhesion peptide was introduced to provide adhesion sites on the surface of the hydrogel matrix. The unreacted vinyl sulfone groups were reacted with the bis(cysteine) terminated MMP substrate peptide. Fig. 51 depicts the stepwise formation of the hydrogel containing the adhesion proteins and the MMP substrate proteins as well as the attack on the substrate protein by a metalloproteinase [176].

Analysis of the activity of an MMP to various substrates in the three-dimensional hydrogel indicated that the invasion of the cells was dependent on the enzymatic sensitivity of the substrate peptide and the rate of this process was dependent on the adhesion peptide concentration. Also the invasion of the cells decreased with an increase in crosslink density at lower PEG molecular weights. In order to demonstrate the

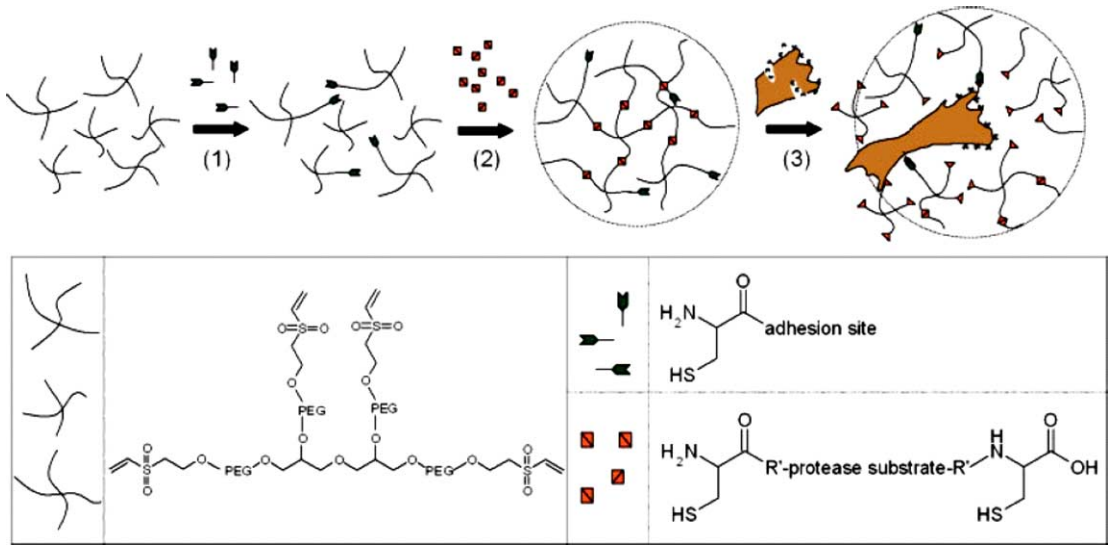


Fig. 51. Illustration of the stepwise synthesis of a PEG-based hydrogel by reacting multiarm vinyl sulfone-terminated PEG with small amounts of a monocysteine-containing adhesion peptide (1) followed by reaction of excess bis(cysteine) containing MMP substrate peptide (2). Attack of a cell-secreted MMP is also shown (3). Structures of the multiarm vinyl sulfone-terminated PEG (cross lines); monocysteine-containing adhesion peptide (green wedge shaped arrows), and bis(cysteine) containing MMP substrate peptide (orange divided squares) are shown in the legend. Reprinted from Proc Natl Acad Sci, 100, Lutolf et al., 'Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics.' 5413–5418, Copyright 2003, with permission from the National Academy of Sciences, USA [176].

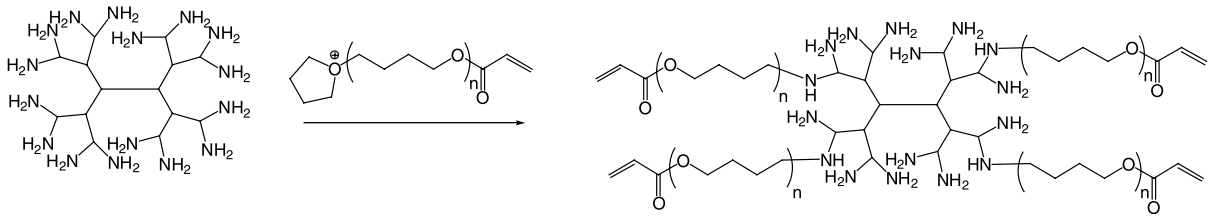


Fig. 52. Self-condensing dendrimer synthesized from PPI and acrylate initiated living PTMO.

capability of these hydrogels to promote tissue growth a bone protein containing hydrogel was placed in a rat cranial defect. Bone regeneration was observed after 4 weeks of hydrogel implantation and depended on the MMP sensitivity of the networks [176,177]. Enhanced bone regeneration was observed due to the additional affinity sites for the bone protein in the matrix [178]. Similarly, the covalent incorporation of a vascular endothelial growth factor in the hydrogel matrix alongside adhesion peptides and MMP substrate peptides aided the regeneration of human endothelial cells and formation of new vascularized tissue in place of the biomaterial [179].

Ferruti et al. studied tissue scaffolds consisting of crosslinked poly(amido amine) hydrogels based on 2,2-bisacrylamidoacetic acid, which was crosslinked using primary diamines [8]. The networks degraded completely within 10 d in Dulbecco's medium at physiological conditions, and cytotoxicity tests using direct contact with fibroblast cell lines demonstrated the non-cytotoxic nature of both networks as well as the degradation products. Bovine serum albumin was also incorporated into selected networks, through mixing with the network precursors prior to crosslinking. Ferruti et al. developed hydrogels containing agmatine, a guanidine functionalized amine precursor that resembles the RGD (arginine glycine aspartic acid) tripeptide, an agent for promoting cell adhesion [180]. The mechanical performance of these tissue scaffolds improved and degradation decreased through the use of a multi-amine functionalized crosslinker molecule, which was also synthesized through poly(amido amine) chemistry.

Goethals et al. reacted living cationic, acryloyloxybutyl triflate initiated poly(tetramethylene oxide) (PTMO) with Astramol™ dendrimers to produce acrylate functionalized dendrimers (Fig. 52) [181]. The presence of both amine and acrylate groups on the dendrimer molecules resulted in rapid gel formation with gel fractions of 88–99%. The pot life varied from 25 to 125 min with the molecular weight of the PTMO changing from 1000 to 4000 g/mol (Fig. 53). Higher degrees of swelling and higher tensile elongations were observed for higher molecular weight PTMO precursors. The pot life was also affected by the temperature of the reaction from 45 min at 25 °C to 4 min at 65 °C. The gel fraction increased with the incorporation of four PTMO arms as opposed to two arms.

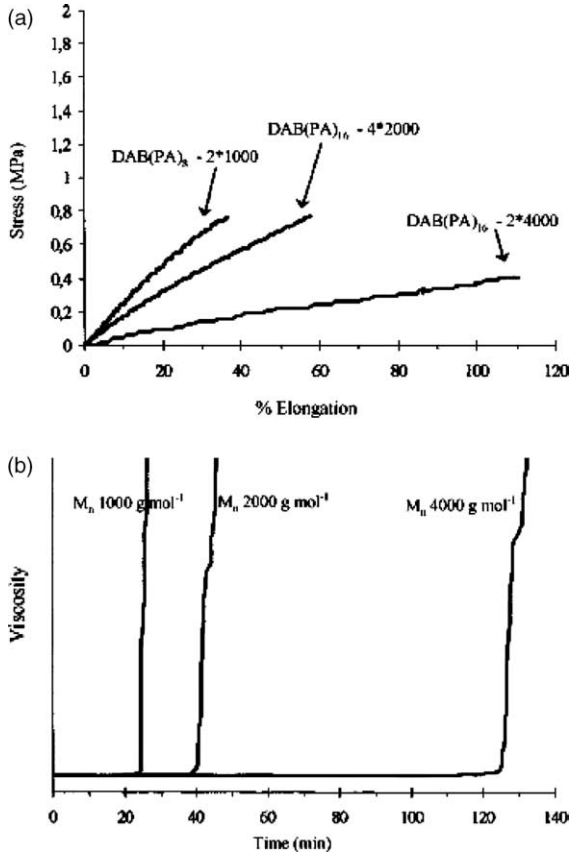


Fig. 53. (a) Effect of dendrimer generation and number of PTMO arms of various molecular weights on tensile strength. (b) Viscosity curves during crosslinking showed longer gel times at higher molecular weights. Reprinted from 'Segmented polymer networks containing amino-dendrimers,' Goethals et al., Copyright 2003 Society of Chemical Industry, with permission from John Wiley and Sons, Ltd on behalf of SCI [181].

7. Bioconjugates via the Michael addition

Strong interest in bioconjugates has developed in recent years. Bioconjugates consist of synthetic polymers covalently bound to biopolymers (proteins, polysaccharides, polynucleotides, antibodies) or bioactive species (pharmaceuticals). These materials possess special properties that make them useful in biomedical applications and fundamental studies. Coupling synthetic polymers to biomaterials is useful for numerous reasons. Polymer–protein bioconjugates may possess 'stealth' properties, resisting clearance from the bloodstream in terms of excretion from the liver or kidneys, thus increasing circulatory lifetimes of protein drugs. Furthermore, recognition properties may be imparted to synthetic polymers through the incorporation of receptor proteins. Recognition properties are important in artificial organs and implants as well as in biosensors. Polymer–protein and polymer–drug conjugates were used to improve solubility and circulation time within the body. In some cases, biomolecule linking using Michael addition chemistry was studied. Thus, Roy et al. used Michael addition to create sugar-protein conjugates from sialyloligosaccharides [182].

7.1. Polymer–protein and polymer-drug conjugates

Poly(ethylene glycol) (PEG) is the most common synthetic polymer for coupling with proteins. PEG is a non-biodegradable polymer ideal for coupling to proteins due to its biocompatibility and nonabsorbent properties with respect to proteins. Currently, numerous functional PEGs are commercially available for applications in protein pegylation [183]. Michael addition of protein-bound thiol groups to maleimide functionalized PEG is one of the most common methods of polymer–protein conjugate synthesis. A second route is through Michael addition of thiol groups to vinyl sulfone functionalized PEG, resulting in greater hydrolytic stability. PEG containing two maleimide functional groups was used for mimicking the heavy chain end of antibodies and is also useful for binding two proteins in close proximity [183]. The coupling of thiol groups on cysteine residues with polymeric Michael acceptors benefits from several advantages. The Michael addition chemistry occurs readily at physiological conditions and is selective to thiol-containing proteins over amine nucleophiles. The reaction also produces no potentially toxic byproducts [184]. Hubbell et al. studied the reactivity of protein-bound cysteine residues as a function of charge environment [185]. Positively charged residues placed adjacent to the cysteine resulted in a lower pK_a of the cysteine group and increased Michael addition to PEG diacrylates. Negatively charged residues produced the opposite effect. The specific order of the residues adjacent to the cysteine groups had no apparent effect on reaction kinetics. Vinyl sulfone functionalized PEG molecules reacted selectively with cysteine residues over lysine residues at pH 8 and possessed greater hydrolytic stability. Harris et al. coupled reduced ribonuclease with PEG vinyl sulfone [6]. Reaction with lysine residues required a higher pH of 9.3 but was still dramatically slower than the reaction with cysteine at comparable pH.

Lysine residues of proteins also serve as functional group handles for the Michael reaction of acrylamides. This strategy benefits from the lack of dimerization of lysine residues in comparison to cysteine residues. A large number of protein-modifying PEG reagents are directed toward reaction with the amine groups contained in lysine residues. Such reagents include activated acylating agents such as *N*-hydroxysuccinimidyl ester-PEG (NHS-PEG). However, attachment to lysine residues is disadvantageous when the lysine residues are critical to protein binding or enzymatic active sites. Examples of proteins that show diminished activity upon pegylation using lysine targeting modified PEGs are asparaginase, α-galactosidase, and CD4 [186]. The reaction with lysine groups is also less controlled and often results in multiple PEG attachments due to the presence of numerous lysine residues. The use of cysteine via Michael addition to PEG reagents is advantageous in these cases and further benefits from the lack of synthetic byproducts. Thus, Kogan synthesized a maleimide terminated PEG capable of reaction with free cysteine residues in proteins [186].

Hubbell et al. have performed a great deal of work in the area of polymer–protein conjugates, often favoring the method of cysteine Michael addition [187]. PEG bisacrylamide and PEG diacrylate were studied as PEG substrates for coupling with proteins via the Michael addition. Residual acrylamide or acrylate groups permitted free radical crosslinking of the resulting pegylated proteins to create hydrogels with embedded proteins that serve as tissue scaffolds. The reaction of the acrylamide groups with cysteines was more than an order of magnitude slower than the corresponding acrylate reaction, but the acrylamide group possesses superior hydrolytic stability. The more hydrolytically stable and more Michael reactive vinyl sulfone linkage could not be used due to its inability to polymerize free

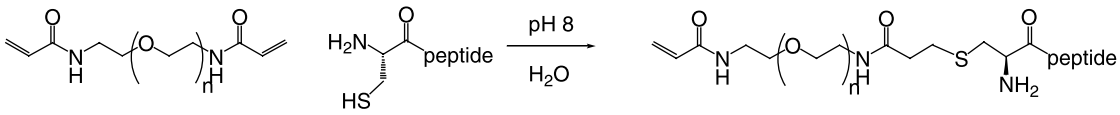


Fig. 54. Protein incorporation via Michael addition of thiol residues to PEG-acrylamide.

radically. The tissue scaffold environment is critical to inducing particular phenotypes to embedded cells. PEG is well known to possess low adhesion to proteins, which allows the study of specific cell-adhesion agents such as RGD protein. Thus, Hubbell et al. introduced RGD protein to PEG bisacrylamide and formed networks [187] (Fig. 54). These networks were treated with cells that spread on the surfaces of the network, clearly well-adhered. Furthermore, the mechanical properties of the crosslinked tissue scaffolds were sufficient to produce durable materials.

Polymer–drug conjugates are of special interest in anticancer therapy where numerous drugs have improved performance in comparison with the parent drug [188]. Conjugates of interest include poly(*N*-(2-hydroxypropyl)methacylamide) doxorubicin, poly(glutamic acid)-paclitaxel and PEG-camptothecin. Several reasons exist for the improved performance of these conjugates including improved solubility, lower toxicity, and longer retention in the bloodstream. Furthermore, some conjugates benefit from the enhanced vascular permeability and retention (EPR) effect, in which drugs are preferentially absorbed into tumor tissue and not released (Fig. 55). In fact, one reason for the lower toxicity of the polymer–drug conjugates is the absence of nonspecific uptake into nontargeted tissues and organs. Generally, polymer–drug conjugates must contain a hydrolysable or enzyme-cleavable linker to allow cellular uptake of the drug molecules at the tumor site. In some cases, linkers are introduced that cleave via lysosomic cysteine proteases. Generally, polymers used in these composites must either biodegrade or possess low enough molecular weight ($<30{,}000$ g/mol) to allow removal through the kidneys.

Goodson and Katre accomplished pegylation of recombinant interleukin-2 protein using PEG-maleimide [189]. Interleukin-2 is a glycosylated protein that serves important functions in the immune system and is useful as an anticancer drug. The recombinant

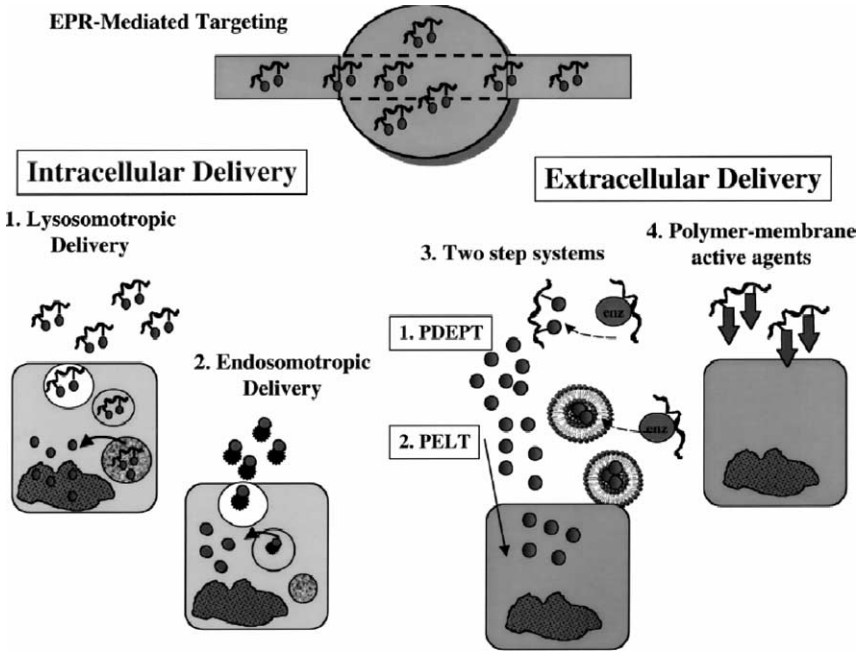


Fig. 55. Drug delivery via polymer–drug conjugates. Enhanced permeability and retention (EPR) mechanism of drug delivery relies on preferential permeation of the conjugate followed by drug release, without reverse permeation. Reprinted from J Control Rel, 74, Duncan et al., 'Polymer-drug conjugates, PDEPT and PELT: basic principles for design and transfer from the laboratory to clinic,' 135–146, Copyright 2001, with permission from Elsevier [188].

interleukin-2 was modified to include a cysteine site for Michael addition at the glycosidic linkage of the original protein, thereby preventing inhibition of the protein. The conjugation of recombinant interleukin-2 to PEG-maleimide resulted in an increase in hydrodynamic size and solubility, increasing systemic exposure four fold. An increase in hydrodynamic size results in less glomerular kidney filtration compared to the unmodified protein. Katre et al. showed a systematic decrease in clearance with increasing effective molecular weight up to 70,000 g/mol above which the rate of clearance was almost constant, possibly due to a different mechanism of clearance [190]. In previous work, modification of interleukin-2 via amine groups on lysine residues resulted in reduced bioactivity of the protein [191].

Jespers et al. studied recombinant staphlokinase pegylated at artificially introduced cysteine sites using PEG-maleimide [192]. Staphlokinase is an enzyme that is used to treat acute myocardial infarction, through thrombolysis of blood clots within blood vessels. Numerous sites on the enzyme were targeted for introduction of the cysteine residue. The circulatory lifetime of the staphlokinase was increased through pegylation while thrombolytic activity was maintained. The plasma clearance rates of the staphlokinase conjugate decreased with increasing PEG molecular weight. Clinical trials revealed successful restoration of circulation for patients suffering from acute myocardial infarction [193].

Currently, strong interest exists in functionalizing polymers with the RGD protein to promote cell adhesion for tissue scaffolds. The RGD protein selectively promotes cell adhesion, while avoiding nonspecific adsorption of proteins. Furthermore, cell spreading, a change in the cellular morphology, occurs in the presence of the RGD coating, indicating cell signaling has occurred. Hubbell et al. used a heterobifunctional PEG containing *N*-hydroxysuccinimide and vinyl sulfone linkages to couple the RGD containing peptides via cysteine linkages to the PEG and then to couple the PEG-RGD to poly(L-lysine) [194].

Surface grafting of biological molecules was achieved using the Michael addition reaction [184]. Model systems to achieve protein grafted surfaces were studied using PEG acrylated surfaces coupled to cysteine containing proteins such as portions of the Platelet factor 4 protein. A modification route beginning with thiol functionalized surfaces was also pursued, however, the surface grafted thiols were unreactive.

7.2. Coupling of biological molecules to Michael addition polymers

In addition to the Michael reaction as a strategy to couple synthetic polymers to biological molecules, it is also useful in the synthesis of polymers that will be coupled to biological molecules. Numerous Michael addition polymers are useful as precursors for bioconjugates due to their biocompatibility and biodegradability. Poly(amido amine)s are an excellent example of a biocompatible, biodegradable Michael addition polymer. Both linear poly(amido amine)s and dendritic poly(amido amine)s (PAMAM) proved useful in forming bioconjugates.

Ferruti et al. synthesized poly(amido amine) drug conjugates of the anticancer drug mitomycin C, which was bound to pendant hydroxyl groups on the poly(amido amine). In vivo studies showed similar antitumor activity for the conjugate compared to mitomycin C as well as lower toxicity values [45]. Poly(amido amine) dendrimers were also used to facilitate the release of the toxic anticancer drug 5-fluorouracil via covalent incorporation onto the terminal amines on the dendrimer structure [195]. Higher generation dendrimers released larger amounts of 5-fluorouracil, with complete release occurring over the period of about one week.

Glycodendrimers are dendrimers containing saccharide residues on the peripheral functional groups. Glycodendrimer interactions with proteins are often more pronounced than with individual saccharides due to increased ‘valency’. However, the density of functional groups at the dendrimer surface may potentially lead to steric hindrance of the sugar groups. Meijer et al. introduced peripheral sugar groups at various distances from the surface of PPI dendrimers using alkyl spacers of various lengths, thus reducing steric congestion [196].

8. Conclusions and future directions

This review outlined the current applications of the Michael addition to polymer synthesis. The Michael addition reaction serves important roles in polymer synthesis, allowing the development of novel polymers from diverse monomer feedstocks. Polymers of numerous different topologies including linear, dendritic, hyperbranched and network polymers are enabled. Both step growth and chain growth techniques are utilized. The flexibility of the Michael reaction in terms of monomer functionality, solvent environment, and conversion at ambient or near ambient temperature

allows the production of sophisticated macromolecular systems for technologically important applications. Other advantages include a general lack of sensitivity to oxygen and the absence of low molar mass byproducts, which require removal.

The applications of Michael addition polymers are as diverse as their compositions and topologies, ranging from networks for coatings and adhesives to linear, biodegradable polymers for gene transfection or drug delivery. Several Michael addition polymers such as poly(amido amine)s benefit from biocompatibility and non-toxic degradation products. Michael addition also serves as a synthetic tool for coupling polymers with biological systems such as proteins and enzymes. The ability to carry out the Michael addition under physiological conditions permits novel tissue replacements, cell scaffolds and direct application.

One aspect of the Michael addition largely unused in polymer science is the ability to carry out stereospecific Michael additions. The application of special catalysts to achieve stereospecific Michael addition may yield polymers with improved biological applications and potential crystallinity. Chirality is particularly important in biological systems including recognition processes with proteins. Another likely future direction for Michael addition polymerizations is the increased use of bio-based reactants. The number of bio-based precursors entering the market and the multitude of functionalities available for Michael addition chemistry are growing. Many of these materials are amenable to modification for introduction into Michael addition systems. Furthermore, the tolerance of the Michael reaction to protic impurities allows the use of less well-defined feed streams.

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