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REVIEW

pH Responsive Polymers with Amino Acids in the Side Chains and Their Potential Applications

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ABSTRACT: Design and synthesis of pH responsive polymeric materials has become an important subject in academia as well as in industrial field in recent years due to their applications in diverse field including controlled drug delivery, biomedical applications, membrane science, sensors and actuators, oil recovery, colloid stabilization, etc. Efforts have been made to incorporate stimuli-responsive biomolecules in synthetic polymers to develop pH responsive "smart" non-biological hybrid macromolecules with high water solubility, enhanced biocompatibility, bio-mimetic structure and properties. This review is focused on the recent advances in side-chain amino acid-based pH responsive polymers synthesis and potential application aspects of these macromolecular architectures in drug and gene delivery, and other fields. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41084.

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INTRODUCTION

pH responsive polymers are materials whose solubility, volume, and conformation can reversibly be manipulated in aqueous solution by changing the environmental pH.[1] pH responsive polymers usually consist of ionizable functional group in the backbone or as side groups which can accept or release protons depending upon surrounding pH and regulate the electrostatic interactions between the polymer chains that can expand or collapse in aqueous solution. This class of polymers find applications in various fields such as controlled drug delivery,[2] biological and membrane science,[3] sensor and actuator,[4,5] viscosity modification,[6] oil recovery,[7] colloid stabilization,[8] water remediation,[9] etc. Depending upon their surface charge, pH responsive polymers can be classified into anionic and cationic polymers. Commonly used anionic pH responsive polymers are polyacids, e.g., poly(acrylic acid) (PAA), poly(methacrylic acid) (PMA), and poly(sulphonamides).[10,11] Whereas, cationic polymers are polybases such as poly(ethyleneimine) (PEI), poly(dimethylaminoethyl methacrylate) (PDMAEMA), poly(2-(diisopropylamino)ethyl methacrylate), poly(4-vinyl pyridine), etc.[12] Interestingly, pH profile of our normal physiological system, extracellular tumor and cancer cells, diseased and inflammatory tissues advocated the great deal of interest to exploit the existing pH responsive polymers, and design and synthesis of new class of pH responsive polymers toward pH sensitive drug and gene delivery devices.

Recently, tremendous effort has been made for the incorporation of stimuli-responsive bio-molecules in synthetic polymers that may create a new class of non-biological hybrid macromolecules with bio-mimetic structure and property.[13] In particular, incorporation of naturally occurring amino acid, which is the building block of proteins and peptides, into the synthetic polymers have drawn considerable attention due to its water solubility, biocompatibility, and possibility of forming higher order hierarchical structure through intra- and inter-chain association via non-covalent interactions such as H-bonding, hydrophobic staking, electrostatic interactions, etc.[14] Several approaches have been made for the introduction of amino acid moiety into the polymers; as end group,[15] in the main chain,[16] or as side-chain pendants.[17] In the first approach, amino acid moiety has been introduced in the polymer chain-end by using amino acid-based initiator or chain transfer agent during the polymerization of vinyl monomers. In the second approach, amino acid-based main chain polymers (polypeptides) have been synthesized via ring opening polymerization of amino acid-based cyclic monomers, *N*-carboxy anhydrides (NCA). Lastly, amino acid is first converted into vinyl monomer via –COOH or $–NH_2$ functional group modification followed by conventional free radical polymerization (FRP) or controlled radical polymerization (CRP) to form synthetic polymers with saturated carbon backbone with amino acid side-chain pendants. The free –COOH or $–NH_2$ functional groups in the amino acid moiety enhance the solubility and allow the post polymerization

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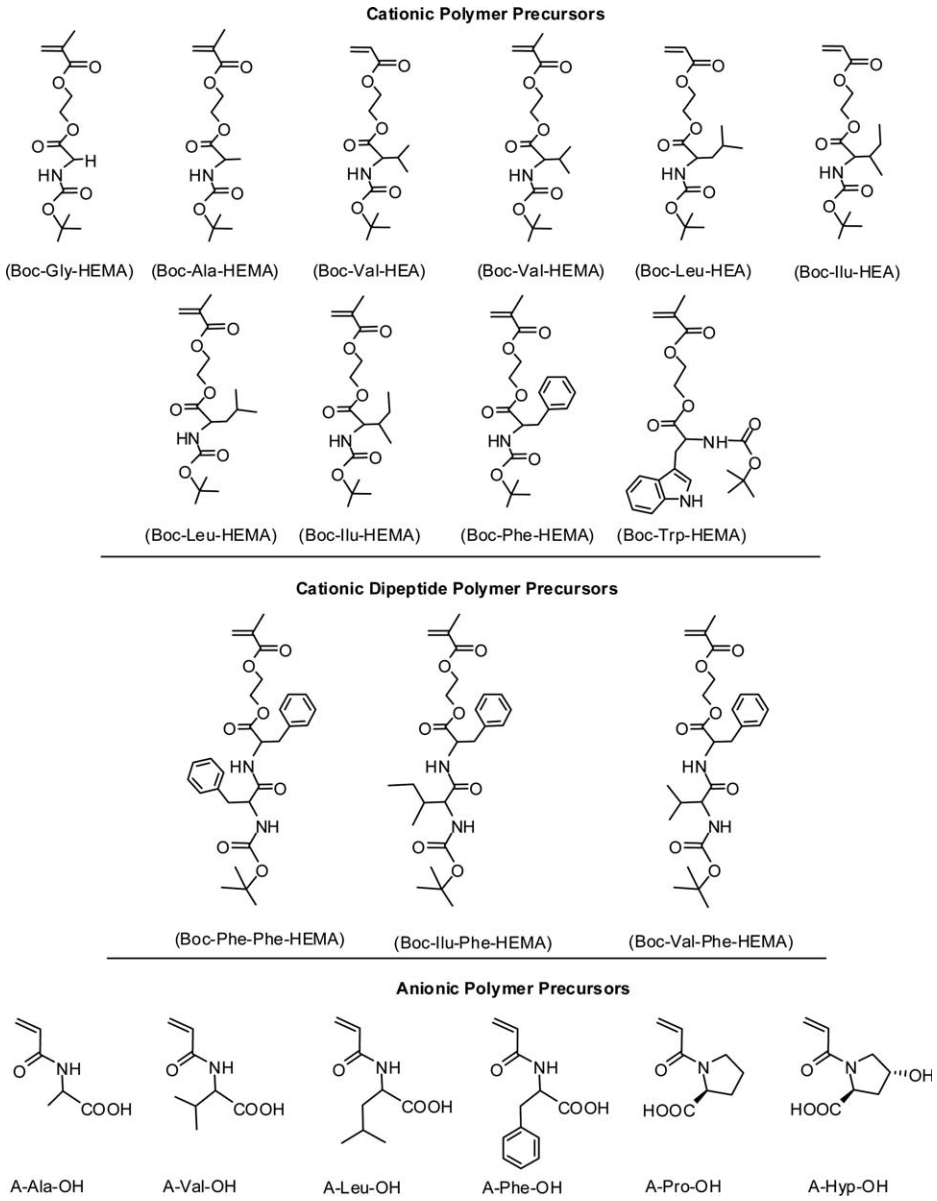
modifications of polymer chains to form various other chain architectures. Furthermore, block copolymerizations through CRP techniques offer the opportunity to construct various other polymeric architectures and nanostructures.[18] Also, introduction of amino acid units as side chain into the polymers provide them stimuli responsive (thermo-, pH- and salt-) property and thus make these polymers important candidates for applications in various field, e.g., polyelectrolytes,[19] photochromatic materials,[20] chiral recognition,[21] biologically active materials,[22] etc.[23]

Side chain amino acid containing synthetic polymers can be prepared from amino acid-based vinyl monomers via FRP or CRP techniques, namely, nitroxide-mediated polymerization (NMP),[24] atom transfer radical polymerization (ATRP),[25] and reversible addition-fragmentation chain transfer (RAFT) polymerization.[26] O'Reilly discussed about the synthesis of functional polymers containing amino acid moieties using CRP technique,[14] whereas Mori and Endo reviewed the synthesis of amino acid-based thermo- and pH-responsive block copolymers by RAFT polymerization technique including their self-assembly behavior, tuneable chiroptical properties, catalytic, and optoelectronic characteristics.[27] This review highlights recent progress in the development of side-chain amino acid-based synthetic pH responsive polymers and their possible applications. The focus is on the synthesis of two different types (cationic and anionic) of side-chain amino acid-based pH responsive polymers followed by discussion on the particular aspect of this field. Quantitative comparison of the material properties will be discussed as to demonstrate the benefit for perspective applications. However, this article does not include amino acid derived thermo-responsive polymers, and synthetic polymers such as polypeptide or poly(amino acids) obtained by ring opening polymerization of NCA monomers.

SIDE-CHAIN AMINO ACID-BASED MONOMERS AND THEIR CORRESPONDING POLYMERS

Since amino acids have –COOH and $–NH_2$ functionalities, their corresponding vinyl monomers could be synthesized by C-terminus or N-terminus modification. N-Terminus modified monomers such as methacryloyl-D-alanine (MA-Ala-OH), methacryloyl-L-glutamic acid (MA-Glu-OH), and acryloyl-L-glutamic acid (A-Glu-OH) were first reported by Kulkarni and Morawetz in 1961, via coupling reaction by exploiting the amine group of D-alanine and L-glutamic acid with acryloyl or methacryloyl chloride.[28] This process was further successfully demonstrated for the wide range of amino acids including leucine, phenylalanine, tyrosine, methionine, tryptophan, and histidine.[29] Whereas, amino acid-based cationic vinyl monomers were first introduced by Sun and Gao via C-terminus modification of Boc protected amino acids; Boc-L-phenylalanine, Boc-glycine, Boc-L-alanine, Boc-L-valine, and Boc-L-lysine through coupling reaction of –COOH group of amino acids with 2-hydroxyethyl methacrylate (HEMA) in the presence of *N*,N′-dicyclohexylcarbodiimide (DCC) as coupling agent and 4-dimethylaminopyridine (DMAP) as catalyst.[30] Our group recently reported (meth)acrylate containing side-chain amino acid-based chiral Boc protected monomers from L- and D-tryptophan,[31] L- and D-leucine,[32] L-isoleucine,[33] and side-chain Boc protected dipeptide monomers[34] (Scheme 1).

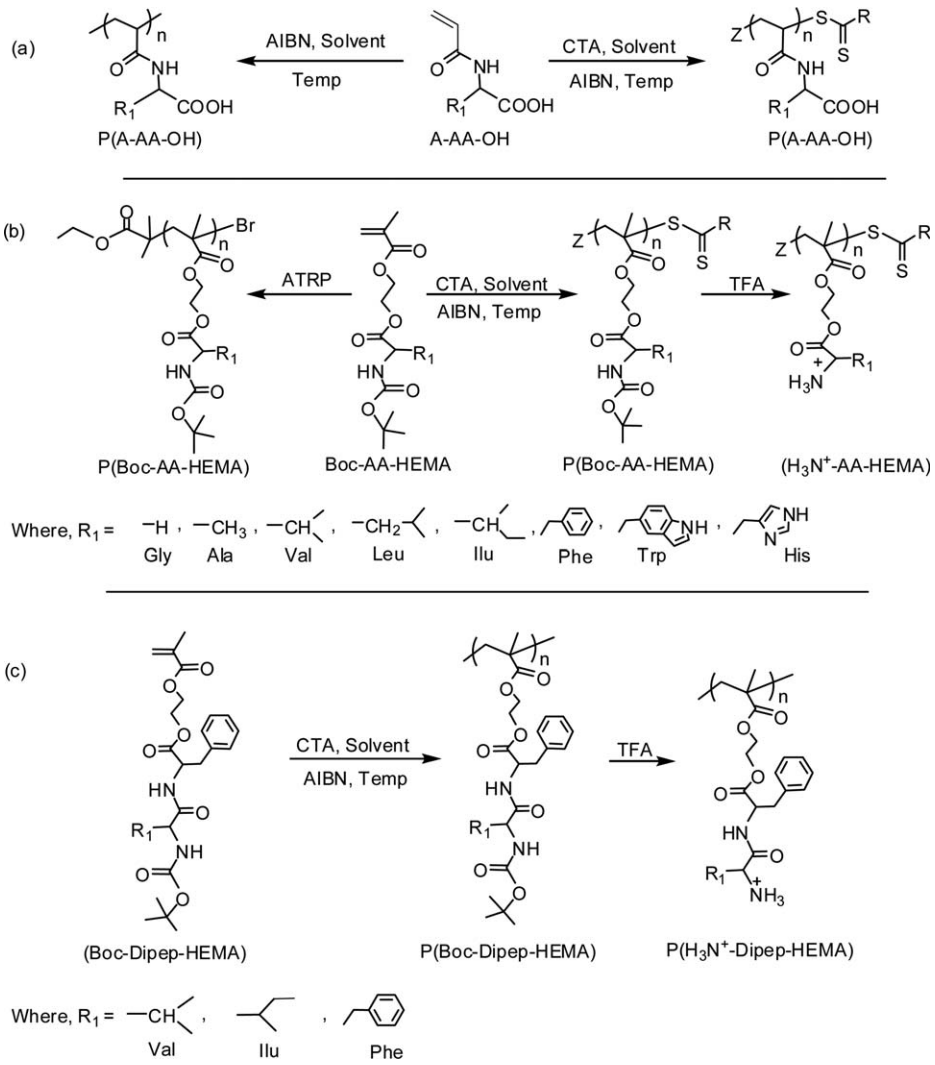
The MA-Ala-OH, MA-Glu-OH, and A-Glu-OH were first polymerized via FRP to their respective chiral homo- and co-polymers with free –COOH groups [Scheme 2(a)].[28] Morcellet and coworkers also synthesized copolymers of P(MA-Ala-OH)[35] and studied their properties.[36,37] Later, Casolaro extensively investigated conventional homo- and co-polymerization of *N*-acryloyl-L-valine (A-Val-OH),[38] *N*-methacryloyl-L-valine (MA-Val-OH), and *N*-acryloyl-L-leucine (A-Leu-OH).[39,40] Casolaro et al. also prepared homo- and co-polymer gels of *N*-acryloyl-L-histidine (A-His-OH) and *N*-acryloyl-L-phenylalanine (A-Phe-OH) by employing FRP technique to study the pH-induced swelling behavior and drug release property of polymer gels.[41,42] However, polymers prepared via FRP technique are ill defined in nature due to uncontrolled molecular weight (M_n) and polydispersity (PDI). Therefore, recently considerable attention has been paid for the development of new technique of



Scheme 1. Selected side-chain amino acid containing monomers for the synthesis of pH responsive polymers via FRP and CRP techniques.

polymerization to prepare the polymers with targeted molecular weight, narrow PDI, predetermined compositions, and chain-end functionality. Among various CRP techniques, RAFT is employed successfully to acrylamides or methacrylamides, and acrylates or methacrylates, and the key feature of successful RAFT polymerization is the selection of suitable chain transfer agent (CTA), solvent, and other reaction conditions. In 2005, Endo and coworkers first introduced successful RAFT polymerization of A-Phe-OH using benzyl 1-pyrrolecarbodithioate as CTA, without any protecting chemistry, to obtain corresponding polymer with controlled M_n and narrow PDI.[43] Afterwards, they effectively extended their effort to other monomers such as A-Ala-OH,[18] *N*-acryloyl-L-proline (A-Pro-OH), and *N*-acryloyl-4-*trans*-hydroxy-L-proline (A-Hyp-OH) [Scheme 2(a)].[44] McCormick and coworkers introduced the aqueous RAFT polymerization of *N*-acryloylalanine using water soluble CTAs to prepare ABC triblock and ABCBA pentablock copolymers for the facile preparation of crosslinkable thermally responsive polyelectrolyte micelles.[45]

In 2010, Sun and Gao introduced C-terminus modified side-chain amino acid-based biocompatible multi-amino cationic polymers via ATRP method followed by Boc deprotection [Scheme 2(b)].[30] In 2012, our group employed the RAFT polymerization technique to synthesize poly(Boc-L-alanine/phenylalanine methacryloyloxyethyl ester) P(Boc-Ala/Phe-HEMA) from Boc-Ala/Phe-HEMA monomers using 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid (CDP) as CTA with controlled M_ns and narrow PDIs, where Boc group removal produced polymers with pH responsiveness at pH 6.4 and 5.4



Scheme 2. Synthesis of side-chain amino acid-based anionic (a), cationic (b), and (c) dipeptide-based cationic polymers via FRP, ATRP, and RAFT polymerization techniques.

for alanine and phenylalanine side-chain polymers, respectively.[46] As a part of our constant endeavor, RAFT polymerization was successfully employed to prepare a series of pH responsive cationic polymers from Boc-L-leucine/isolucine acryloyloxyethyl/methacryloyloxyethyl ester (Boc-L-Leu/Ilu-HEA/HEMA),[33] Boc-L/D-tryptophanmethacryloyloxyethyl ester (Boc-L/D-Trp-HEMA) [Scheme 2(b)].[31] We have also extended our work to make pH responsive side-chain dipeptide-based cationic polymers [Scheme 2(c)].[34] Therefore, from the above studied amino acid-based cationic and anionic polyelectrolytes were obtained, where transition pH of the polymer aqueous solution depends on the *–R* group substitution of the side-chain amino acid residue.

IMPORTANT ASPECTS OF SIDE CHAIN AMINO ACID-BASED pH RESPONSIVE POLYMERS

Physio-chemical properties such as solubility, conformation, configuration, phase transitions of responsive polymers can be manipulated by regulating the environmental stimuli or the polymer's chemical structure in aqueous solution. For example, pH responsive block copolymers can undergo self-assembly into different morphology by adjusting the pH of the solutions.[47] The free –COOH/–NH_2 functionalities in the side-chains amino acids can be protonated or deprotonated by changing the pH of their aqueous solution. The presence of naturally occurring amino acid precursors, expected to induce biocompatibility, makes them interesting candidate for various biological applications, which we will discuss in this section.

pH Responsive Homo- and Block-Copolymers and Their Applications

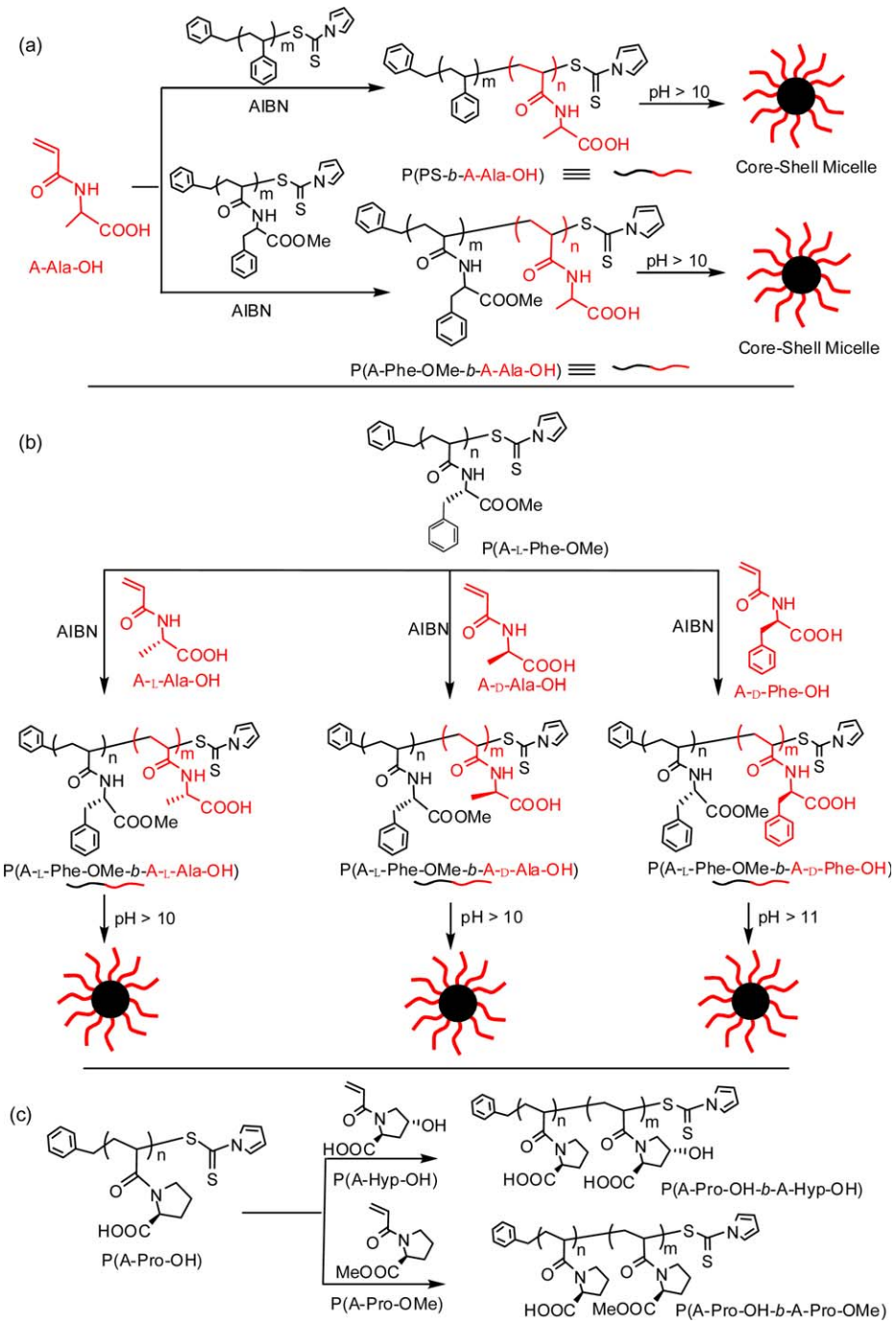
Kulkarni and Morawetz first synthesized side-chain amino acid-based homo- and co-polymers from MA-Ala-OH, MA-Glu-OH, and A-Glu-OH with free –COOH groups via FRP technique [Scheme 2(a)].[28] This work mainly focused on the change of optical activity of homo- and co-polymers in aqueous media with the change of polymerization solvent, might be due to the difference in the degree of stereo-regulation and degree of ionization in water, the pH of the aqueous media. However, the detail

investigation on the pH responsive property of homo- and co-polymers was not included in this work. Afterwards, Morcellet and coworkers carried out the extensive investigation on the conformational behavior and chiroptical properties of P(MA-Ala-OH) and a series of copolymers of MA-Ala-OH with *N*-phenylmethacrylamide.[48] The homopolymers are soluble in water and present as random coil conformation. The statistical copolymers behave like normal polyeletrolytes in aqueous media when the mole fraction of hydrophobic achiral units is less than 15%. Beyond this value, polymers exist as compact conformations and can undergo transformation to coil conformation by increasing the pH of the solution.[36] They also carried out the detail investigation about the effect of pH and methanol-induced conformational change on the chiroptical property of copolymers.[49] Barbucci et al. studied the pH dependent thermodynamic protonation behavior of poly(*N*-methacryloylglycine) and P(*N*-methacryloyl-β-alanine).[50] Thermodynamics of protonation of pH responsive polyelectrolytes P(A-Val-OH) and P(A-Leu-OH) in 0.1*M* NaCl at different temperatures revealed that basicity constant (log *K*) of P(A-Val-OH) decreased in a wider range in relation to degree of protonation (α) of –COOH up to the critical α value compared to P(A-Leu-OH).[38] Calorimetric study revealed sharp change in extended coil to collapsed coil conformation by changing α value as the hydrophobic interaction between the alkyl groups outweighing the electrostatic repulsive force. Comparative protonation and phase separation behavior of a series of homopolymers prepared from A-Val-OH and MA-Val-OH, and their copolymers with *N*-isopropyl acrylamide (NIPAM) were investigated with respect to the pH and temperature.[39] Aqueous solution behaviors of P(A-Lue-OH) and its copolymers with NIPAM, and their complexation characteristics with Cu(II) ions were also investigated.[40] Solution properties of temperature and pH responsive NIPAM-*co*-MA-Leu-OH copolymers with various molar ratios were studied in wide range of pH (3–11) and sharp cloud point transitions (T_{cp}) were observed at pH 4.0–5.0 and increased linearly with the increase of pH of the solutions. It was also noted that T_{cp} of copolymers increased with the increase of ionic content of polymers.[51] Conformational transition of thermo and pH responsive P(NIPAM-*co*-MA-Leu-OH) copolymer with 90.9% NIPAM content in aqueous solution showed the distinct sigmoidal increase in the circular dichroism (CD) signal at 220 nm with the increase of temperature at pH = 4.0–5.5, which diminished with the increase of pH, and at pH 6.0 or higher no such phenomenon was observed.[52] Chiroptical property and polymer chain conformation of P(MA-Leu-OH) were also investigated with respect to pH, where intense cotton effect in ethanol at 220 nm due to the secondary interaction (H-bonding) was noticed and in alkaline medium cotton effect is reduced due to the destruction of ordered structure to coil structure.[53] These homopolymers also showed the pH-induced swelling and deswelling characteristics in aqueous solutions. Reversible nanoscale structures are formed in solutions from pH-sensitive hydrophobic polyelectrolytes, poly(*N*-methacryloyl-L-valine) or poly(*N*-methacryloyl-L-phenylalanine) and surfactant. Presence of cavity was observed in the formed nanoparticles, which is not common in typical micelles and the authors pointed out that such particles could be potential candidate for drug delivery with a high sensitivity.[54]

Synthesis of stimuli-responsive ampholytic terpolymers of A-Val-OH, acrylamide, and (3-acrylamidopropyl)trimethylammonium via CRP method and their solution properties as a function of solution pH, ionic strength, and polymer concentration in solution have been investigated by McCormick and coworkers, where the pK_a of terpolymers increased with the increasing valine content and charge balanced terpolymers displayed polyampholyte behavior at pH $\geq$ 6.5. Polyampholyte terpolymers transformed into cationic polyeletrolytes with the decreasing pH due to the –COOH groups of valine units.[55] They extended their work to a series of low-charge-density terpolymers and amphoteric copolymers with different amino acid units, such as A-Ala-OH and A-Asp-OH,[56] where conversion of polyampholytes to polyeletrolytes or vice versa could simply be obtained by changing the pH of the solution or concentration of electrolyte. Therefore, this kind of polyampholyte terpolymers with variable viscosity under specified conditions can be exploited as potential viscosity modifier and should allow designing the system for enhanced petroleum recovery.[57]

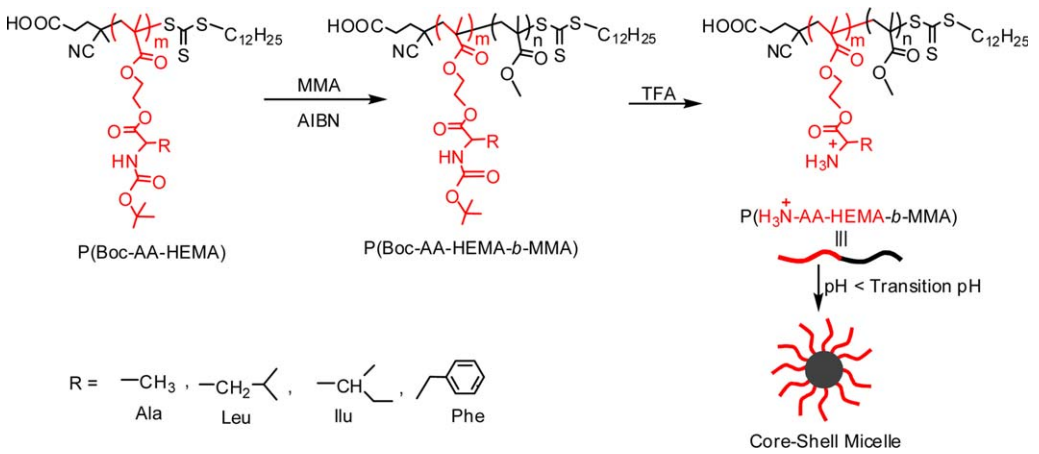
In 2005, Mays and coworkers first reported the CRP method for the synthesis of side chain amino acid polymers with –COOH groups from A-β-Ala-OH using ATRP technique [Scheme 2(b)] and polymers are proposed to be biocompatible in nature and able to mimic some characteristic of polypeptides.[58] Endo and coworkers demonstrated the successful RAFT polymerization of A-Phe-OH with free –COOH groups with controlled molecular weight and low PDI values [Scheme 2(b)], where P(A-Phe-OH) was insoluble in neutral water but became soluble in alkaline solution at pH 11.[43] They also demonstrated the synthesis of amphiphilic block copolymers involving P(A-Ala-OH) using the dithiocarbamate-terminated polystyrene or poly(*N*-acryloyl-L-phenylalanine methyl ester) (P(A-Phe-OMe)) as a macro-CTA [Scheme 3(a)], where block copolymers showed characteristic chiroptical property and pH-induced solution properties. Since alanine is much less hydrophobic than phenylalanine, amphiphilic block copolymers involving P(A-Ala-OH) with polystyrene or P(A-Phe-OMe) segments showed pH-induced self-assemble organization in aqueous media.[18] Double hydrophilic and amphiphilic block copolymers with characteristic chiroptical properties (L-L and L-D) were synthesized from A-L-Phe-OMe, A-L-Phe-OH, A-L-Ala-OH, and their D-forms (A-D-Phe-OH and A-D-Ala-OH), using hydrophilic P(A-L-Phe-OH) and hydrophobic P(A-L-Phe-OMe) macro-CTAs [Scheme 3(b)]. These block copolymers also showed pH-induced aggregation in aqueous media with core-shell like micelle having different chitoptical properties.[59] The phenylalanine containing 4 and 6 arms amphiphilic star polymers were reported via RAFT technique from the polymerization of (A-L-Phe-OMe) and tetrahydropyranyl acrylate.[60]

Extensive investigations were carried out to design and synthesize thermo-responsive homo- and co-polymers with pendant L-proline moiety, an important amino acid due to its key functionality and acts as turn inducer in polypeptide.[61,62] Degree of ionization and water solubility of poly(*N*-acryloyl-L-proline) (P(A-Pro-OH)) can be manipulated by regulating the pH of the solution as it is soluble in basic water at pH 10 but insoluble in neutral or acidic water. Whereas, poly(*N*-acryloyl-4-*trans*-L-



Scheme 3. Synthesis of amphiphilic and double hydrophilic pH responsive block copolymers with pH responsive side-chain amino acid-based anionic block. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

proline) (P(A-Hyp-OH)) is nicely soluble in water and solubility is independent of pH.[44] Proline-based dual thermo- and pH responsive block and random copolymers conspiring of thermo-responsive segment poly(*N*-acryloyl-L-proline methyl ester) (P(A-Pro-OMe)) and pH responsive segment P(A-Pro-OH) was also carried out via RAFT method [Scheme 3(c)] to manipulate the transition temperature and pH. Self-assemble organization of P(A-Pro-OH-*b*-A-Pro-OMe) polymers at pH 10 showed the formation of stable core-shell type micelle at 20°C, where P(A-Pro-OMe) formed hydrophobic core and P(A-Pro-OH) segment was on the hydrophilic shell.[63] O'Reilly's group prepared a range of well-defined copolymers of styrene and L-proline functionalized styrene via RAFT method and explored their use in supported catalysis for the aldol condensation. They reported higher activity and selectivity at low loadings when compared to unsupported L-proline and the polymer support could be recycled a number of times while maintaining activity and selectivity.[64,65]

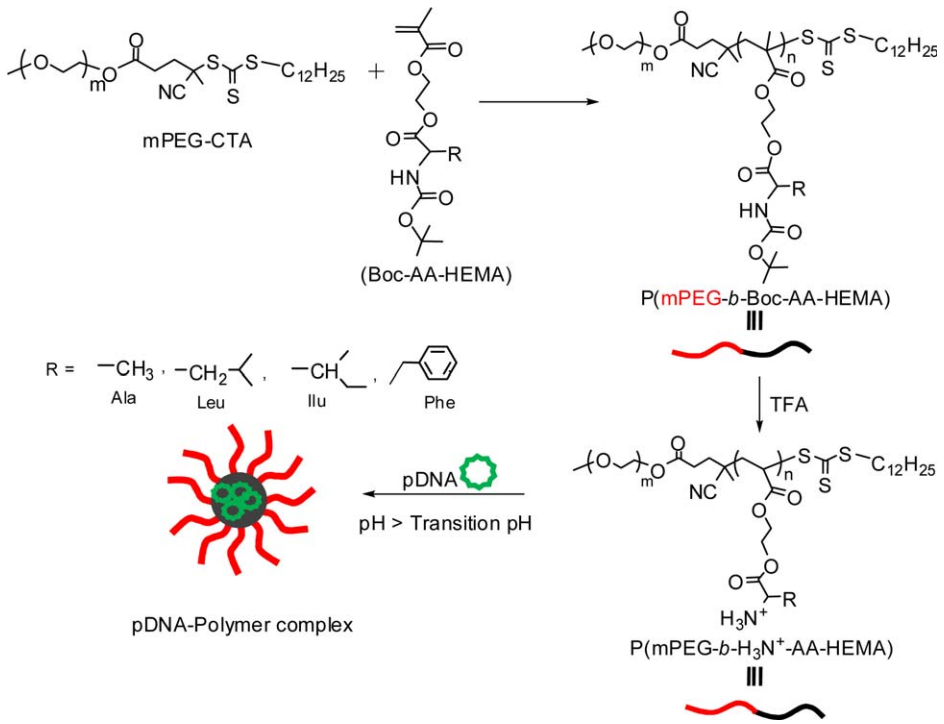


Scheme 4. Synthesis of amphiphilic pH responsive cationic block copolymers with pendant amino acid units via RAFT technique. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Shell "locked" nanoassembles from interpolyelectrolyte complexation reaction of poly(*N*,*N*-dimethylacrylamide)-*b*-(*N*-acryloylalanine)-*b*-(*N*-isopropylacrylamide)[66] (P(DMA-*b*-A-Ala-OH-*b*-NIPAM)) with the homopolymer poly(ar-vinylbenzyl)trimethylammonium chloride (PVBTAC) with varying size 34–78 nm were prepared in water at pH = 9.0 and 50°C. Temperature-induced micellization of P(DMA-*b*-A-ala-OH-*b*-NIPAM) at pH = 6.8 showed net negative surface charge −30 mV which reached to zero upon equivalent mixing of P(DMA-*b*-A-Ala-OH-*b*-NIPAM) and PVBTAC due to interpolyeletrolyte complexation between $-COO^-$ group of A-Ala-OH with $-N(CH_3)_3^+Cl^-$ of PVBTAC. These kind of shell "locked" nanostructures are considered to be the perspective candidate for targeted delivery and controlled release of active agents.[45]

Apart from pH responsive anionic polymers, side chain amino acid-based cationic polymers with free $-NH_2$ groups also showed characteristic pH responsive property due to protonation/deprotonation of $-NH_2$ groups depending upon the pH of the solution. Boc group deprotection from polymers (poly(Boc-amino acid-HEMA)) having Boc-L-phenylalanine, Boc-glycine, Boc-L-alanine, Boc-L-valine, and Boc-L-lysine side chains gave multiple side amine groups. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) based assay showed that cell viability rate in the presence of these multiamino polymers were more than 90% and cytotoxicity of these polymers were much lower compared to linear PEI. These polymers showed positive surface charge and binding property with DNA at a suitable concentration to form granulated structure as studied by atomic force microscopy.[30] Therefore, these kinds of polymers are promising materials for gene delivery.

At present, our group is working extensively in this area for the construction of a variety of pH responsive polymeric architectures including pendant amino acid-based or short peptide-based homopolymers, amphiphilic and double hydrophilic block copolymers, covalent or dynamic covalent crosslinked gels, hyperbranched, and star polymers. The Boc-Ala/Phe-HEMA monomers were successfully polymerized via RAFT to get corresponding homopolymers, which were further employed for the synthesis of block copolymers with methyl methacrylate (MMA). Subsequent Boc deprotections from homo- and block co-polymers with trifluoroacetic acid (TFA) produced their respective water soluble pH responsive homo- and block co-polymers with positive surface charge (Scheme 4). Block copolymers showed self-assemble aggregation behavior with hydrophobic PMMA core and Boc deprotected amino acid hydrophilic segment as shell.[46] Further, double hydrophilic cationic pH responsive di-block copolymers, P($mPEG_{2k/5k}$-*b*-H_3N^+−Phe/Ala-HEMA), were synthesized using $mPEG_{2k/5k}$-macroCTAs (Scheme 5). These polymers are biocompatible in nature as determined by MTT assay and they are non-toxic up to the polymer concentration of 200 μg/mL. Since these polymers were cationic in nature, they showed excellent pDNA binding capability with polymer/DNA weight ratios >1 as determined by gel electrophoresis study.[67] Similarly, P(Boc-Leu/Ilu-HEMA/HEA) polymers were prepared from their respective vinyl monomers in a controlled fashion and chain extension with MMA gave block copolymers, P(Boc-Leu/Ilu-HEMA/HEA-*b*-MMA). Subsequent Boc deprotection in the presence of TFA produced corresponding pH responsive homo- and block copolymers having free $-NH_3^+$ pendants. Amphiphilic block copolymers self-assembled in aqueous solution to form core-shell like micelle bellow their transition pH (Scheme 4).[33] Further, Boc-L/D-Leu-HEMA were copolymerized with 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) with varied monomers compositions to prepare a series of statistical copolymers with chiroptical properties which on Boc deprotection showed dual pH and thermo-responsive characteristics. Transition pH of copolymers increased from 6.6 to 7.4 as the mole fraction of MEO_2MA increased from 20 to 80% in the copolymer. The lower critical solution temperature (LCST) of copolymers can be manipulated with respect to pH of the solution. These copolymers showed zeta potential (ζ) values ranging from +26 to +36 mV. They were employed for chiral recognition of 1,1′-bi-2-naphthol (*rac*-BINOL).[32] The interactions of side-chain phenylalanine and tryptophan containing monomers and polymers were studied in the presence of *rac*-BINOL.[68] The rational synchronization of living carbocationic and RAFT polymerization has successfully enabled the synthesis of P(Boc-L-Ala/Leu-HEMA)-*b*-PIB diblock chiral copolymers, which showed higher order helical secondary



Scheme 5. Synthesis of mPEG-CTA-derived amino acid-based cationic, pH responsive block copolymers via RAFT polymerization and their complexation with pDNA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

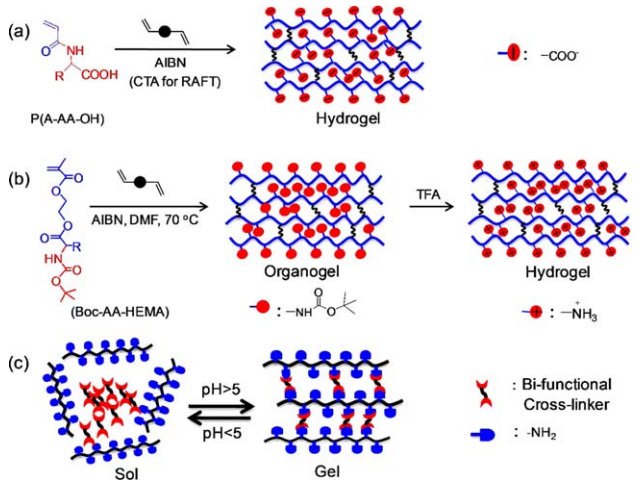
structures in protected and Boc-deprotected forms in organic solvent and water, respectively.[69]

Due to the presence of intrinsic florescence property, tryptophan is considered to be important protein fluorophore and its photophysical property was studied extensively.[70] We synthesized P(Boc-L/D-Trp-HEMA) from their respective vinyl monomers, followed by Boc group expulsion in TFA produced polymers with smart pH responsiveness and pH dependent florescence property. The *in vitro* cytotoxicity study with MTT assay established the 100% viability of HeLa cells in the presence of homopolymers up to 250 mg mL^{-1}.[31] The pH responsive property, surface charge, and fluorescence intensity were further tuned by copolymerization of Boc-Trp-HEMA with MEO_2MA (pH from 5.2 to 5.8 and ξ from +25.4 to −2.7 mV) and DMAEMA (pH from 5.2 to 7.8 and ξ from +5.8 to +7.8).[71] The amphiphilic block copolymers of *N*-acryloyl-L-tryptophan (A-Trp-OH) prepared from DMA, polyethylene glycolmethacrylate, and A-Hyp-OH showed chiroptical properties, fluoride ion (F^-) sensing ability and interesting core-shell like self-assembly in aqueous solution, where P(A-Trp-OH) acts as micelle core at pH = 7. Optoelectronics property of block copolymers were affected after micellization and enhanced in F^- probed micelle.[72] A catalytic thermo-responsive nanoreactor for the asymmetric aldol reaction in water without the need of additional organic solvents has been prepared by integrating L-proline moieties within the hydrophobic core.[73] Therefore, these polymeric architectures with pendant amino acids are potential candidates for chiral recognitions, sensing, and therapeutic drug/gene delivery.

Recently, our group reported biocompatible dipeptide pendant homo-polymers from three new dipeptide vinyl monomers Boc-Phe-Phe-HEMA, Boc-Ilu-Phe-HEMA, and Boc-Val-Phe-HEMA, and their respective block copolymers with mPEG to afford mPEG-*b*-P(Boc-dipep-HEMA) [Scheme 2(c)].[34] Boc group expulsion of homo- and block copolymers introduced pH responsiveness with cationic surface charge. These polymers showed minimum cytotoxicity to HeLa cells and application of these polymers toward inhibition for fibril formation by Aβ to treat Alzheimer's disease is under investigation.

Amino Acid Containing Gels and Their Applications

pH responsive gels are 3-dimensional crosslinked polymeric networks with ionic pendant groups which ionize and develop fixed charge at a particular pH and ionic strength, and electrostatic repulsive force is responsible for pH dependent swelling/deswelling of gels. Most commonly studied pH responsive polymers for gel synthesis are PAA, PMA, PDMAEMA,[74] and their gels have been exploited in the field of artificial mussels, tissue engineering, protein recycling, and drug delivery.[75] The thermo- and pH-responsive copolymer gel with A-Pro-OEt/MA-Gly-OH (40/60 mol % design) showed threshold of swelling at pH 5 and maximum swelling at pH = 7.5 at 37°C. Therefore, drug loaded system showed cumulative release of ketoprofen drug above pH = 5 and 100% release was observed at pH 7.5.[76] Hydrogel platform based on L-valine with pH responsive property has been developed to support and improve the release of *cis*-platin for chemotherapy of solid tumor.[77] Histidine-based polyeletrolyte hydrogels prepared via FRP method [Scheme 6(a)] showed pH-induced thermodynamic protonation and swelling behaviors at different degree of crosslinking.[41] The L-proline functionalized nanogels with a range of catalyst functionalization (0.5–15 wt %) and crosslinking densities (0–50 wt %) were prepared via emulsion polymerization. The catalyst



Scheme 6. Synthesis of side-chain amino acid-based pH responsive (a) anionic and (b) cationic gel networks, and (c) reversible gels by employing dynamic covalent chemistry. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

efficiency in aqueous medium[78] was studied and an unprecedented reduction in catalyst loading, whilst maintaining high catalytic activity was reported for a model asymmetric aldol reaction.[79]

A series of Boc protected organogels (swells in organic solvent) were synthesized via FRP and RAFT method from Boc-L/D-Ala-HEMA [Scheme 6(b)] with varied crosslinking ratio, which showed superabsorbency in volatile organic solvents. Organogels are interesting material due to their application for the recovery of volatile organic compound (VOC) and oil spill in water.[80] Subsequent Boc group expulsion under acidic condition resulted hydrogel and exhibited superabsorbency in water with pH-induced swelling behavior.[81] Presently, design and synthesis of different Boc-Amino Acid-HEMA polymer gels are in progress to study the effect of -*R* groups on pH and ionic strength-induced swelling property of hydrogels. These pH responsive cationic hydrogels are expected to be biocompatible and are considered to be prospective candidates for biological and medicinal applications.

Boc deprotected amino acid polymers with free $-NH_2$ can also form dynamic imine bond (C═N) with –CHO groups and therefore were employed to prepare pH-reversible covalently cross-linked polymeric gels.[82] Boc deprotected side-chain containing homopolymer of tryptophan and copolymers of tryptophan with MEO_2MA and DMAEMA were reacted with 4-formylphenyl 4′-formylbenzoate to form florescent gels at pH > 5 through the formation reversible C═N bonds in the gel network and gels showed pH-dependent reversible sol–gel transitions [Scheme 6(c)].[71] These covalently crosslinked stable reversible gels are promising materials which could be reshaped and regenerated after use.

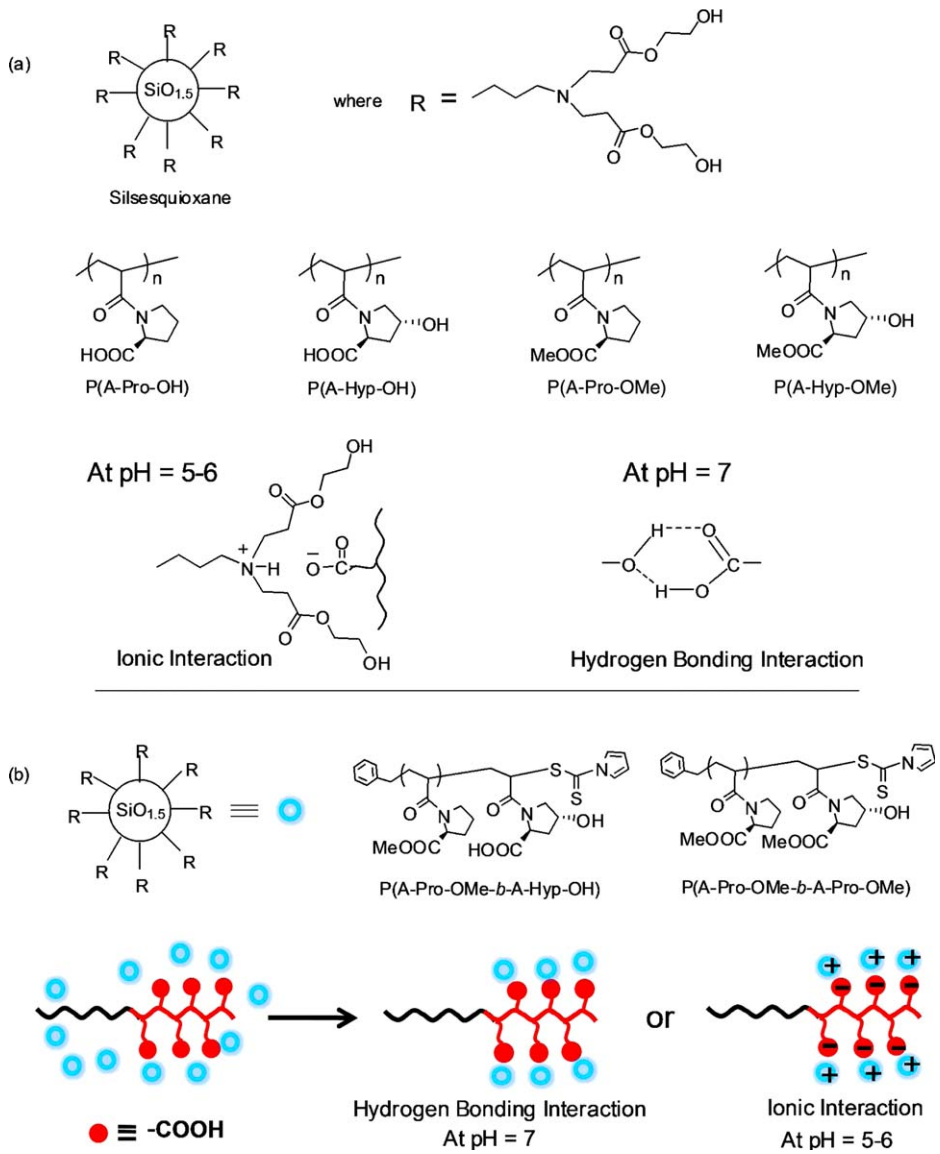
pH Responsive Organic–Inorganic Hybrid Materials and Their Applications

Stimuli responsive organic–inorganic hybrid materials are important because incorporation of inorganic materials in organic molecules might develop a new nanosized material.[83,84] Conjugation of biomolecules like protein, peptide, and amino acid with inorganic material developed a new class of hybrid materials, which are extremely relevant in bio-interface for bio-sensing and biotechnology applications.[85,86] The silsesquioxane/biomolecule hybrids were prepared using noncovalent interactions between water-soluble silsesquioxane nanoparticles containing tertiary amine moieties and amino acid-based polymeric weak polyelectrolytes such as P(A-Hyp-OH) and P(A-Pro-OH).[87] Straightforward mixing of aqueous solutions of P(A-Hyp-OH) and the silsesquioxane nanoparticles led to formation of pH responsive hybrids [Scheme 7(a)]. Furthermore, thermoresponsive properties and transition temperatures of the hybrids were manipulated by the selective complexation via non-covalent interactions between water-soluble silsesquioxane nanoparticles and amino acid-based block copolymers [Scheme 7(b)].[88]

Amphiphilic pyrene-poly(Boc-Phe/Ala-HEMA-*b*-di(ethylene glycol) methyl ethermethacrylate) (py-P(Boc-Phe/Ala-HEMA-*b*-DEGMA)) block comopolymers were synthesized via RAFT using py-P(Boc-Phe/Ala-HEMA) macroCTA, which produced double hydrophilic cationic block copolymers upon Boc deprotection and showed pH and thermo-responsive self-organization behavior in aqueous media with characteristic florescence property. These α-pyrene terminated polymers were also employed for solubilization of carbon nanotube through noncovalent π–π interaction.[89]

PROSPECTIVE APPLICATIONS

Due to the presence of above discussed important features, side-chain amino acid-based polymers could be exploited for applications in diverse fields. The side-chain groups are capable of selective metal binding and thus will allow the preparation of selective membrane for metal ion separation and catalysis in materials science.[90] The pH responsive property makes these polymers for construction of "chemical valve" by grafting these polymers from some porous materials or can be explored as viscosity modifier and in enhanced petroleum recovery. Several studies revealed that these pH responsive polymers are biocompatible in nature which makes them potentials candidate for drug delivery applications due to their pH-induced self-assembled aggregates (micelle or vesicle) and positive surface charge of cationic polymers make them prospective aspirant for gene delivery vector. Side chain amino acid-based crosslinked polymer gels showed characteristic superabsorbent properties for VOCs and can be exploited in recovery of VOCs or oil spill on water and can save the marine life from its catastrophic effect. The pH dependent volume phase transition of hydrogels indicates their probable utility for "artificial mussel," tissue engineering and drug delivery applications. The pH responsive superabsorbent hydrogels can also be exploited in preparation of baby diapers and feminine hygiene products. The pH reversible sol–gel transition of dynamic covalently crosslinked nanogels is promising for injectable drug delivery applications. Besides, chiral polymers can also be applicable in chiral recognition and as supported catalyst for aldol condensation and other synthetic processes. Side chain amino acid-based monomers can be exploited for the construction of cationic hyperbranched and star architectures for the application as alternative gene delivery matrix compared to branched PEIs which are widely used as



Scheme 7. Formation of side-chain amino acid-based pH-induced organic–inorganic hybrid (a) from amino acid-based homopolymers and silsesquioxane nanoparticles, and (b) amino acid-based block copolymers and silsesquioxane nanoparticles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

nonviral gene delivery vector with high transfection efficiency. However, the major drawback associated with high cytotoxicity of PEIs is due to the presence of primary, secondary, and tertiary amine group in the polymer backbone. Synthesis and characterization of organic–inorganic hybrid molecules from side chain amino acid-based cationic pH responsive polymer and polyhedral oligomeric silsesquioxane are in progress in our laboratory, which are expected to show characteristic properties that can be exploited for biological, biomedical, or industrial applications.

CONCLUSIONS

In this review, we briefly discussed various aspects of side-chain amino acid-based synthetic polymers and their perspective applications in various field. The presence of amino acid moiety in the polymer structure improves some physical properties of polymer in terms of solubility and biocompatibility. Throughout the discussion in this review, medicinal applications of this class of polymers are confined in *in vitro* study in laboratory environment. There are very few reports on the application of these polymers in the real world and the future investigation will be expected to be in the direction of *in vivo* applications. In addition, there are still a lot of polymeric architectures including hyperbranched, star, organic–inorganic hybrids, and so on based on pH responsive polymers with amino acid pendants to be developed and improved.

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REFERENCES

1. Dai, S.; Ravi, P.; Tam, K. C. *Soft Matter* **2008**, *4*, 435.
2. Schmaljohann, D. *Adv. Drug Deliv. Rev.* **2006**, *58*, 1655.
3. Nunes, S. P.; Behzad, A. R.; Hooghan, B.; Sougrat, R.; Karunakaran, M.; Pradeep, N.; Vainio, U.; Peinemann, K.-V. *ACS Nano* **2011**, *5*, 3516.
4. Adhikari, B.; Majumdar, S. *Prog. Polym. Sci.* **2004**, *29*, 699.
5. Abu-Lail, N. I.; Kaholek, M.; LaMattina, B.; Clark, R. L.; Zauscher, S. *Sens. Actuators B* **2006**, *114*, 371.
6. Chari, K.; Hsu, R.; Bhargava, P.; Figura, B.; Yang, W.; Park, J. H.; Clifford, T.; Kadir, M. *Langmuir* **2013**, *29*, 15521.
7. Al-Wahaibi, Y. M.; Al-Wahaibi, T. K.; Abdel-Goad, M. A. *J. Energ. Source, Part A* **2011**, *33*, 1048.
8. Jaquet, B.; Wei, D.; Reck, B.; Reinhold, F.; Zhang, X.; Wu, H.; Morbidelli, M. *Colloid Polym. Sci.* **2013**, *291*, 1659.
9. Aelion, C. M.; Davis, H. T.; Flora, J. R. V.; Kirtland, B. C.; Amidon, M. B. *Environ. Pollut.* **2009**, *157*, 186.
10. Schilli, C. M.; Zhang, M.; Rizzardo, E.; Thang, S. H.; Chong, Y. K.; Edwards, K.; Karlsson, G.; Müller, A. H. E. *Macromolecules* **2004**, *37*, 7861.
11. Kang, S., II; Bae, Y. H. *J. Controlled Release* **2002**, *80*, 145.
12. Zhu, L.; Smith, P. P.; Boyes, S. G. *J. Polym. Sci. Part B: Polym. Phys.* **2013**, *51*, 1062.
13. Chen, C.-L.; Qi, J.; Zuckermann, R. N.; DeYoreo, J. J. *J. Am. Chem. Soc.* **2011**, *133*, 5214.
14. O'Reilly, R. K. *Polym. Int.* **2010**, *59*, 568.
15. Venkataraman, S.; Wooley, K. L. *Macromolecules* **2006**, *39*, 9661.
16. Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Sakellariou, G. *Chem. Rev.* **2009**, *109*, 5528.
17. Bentolila, A.; Vlodavsky, I.; Ishai-Michaeli, R.; Kovalchuk, O.; Haloun, C.; Domb, A. J. *J. Med. Chem.* **2000**, *43*, 2591.
18. Mori, H.; Matsuyama, M.; Endo, T. *Macromol. Chem. Phys.* **2008**, *209*, 2100.
19. Jin, Y.; Ye, F.; Wu, C.; Chan Y.-H.; Chiu, D. T. *Chem. Commun.* **2012**, *48*, 3161.
20. Sogawa, H.; Terada, K.; Masuda, T.; Sanda, F. *Polym. Bull.* **2009**, *63*, 803.
21. Takeuchi, T.; Haginaka, J. *J. Chromatogr. B, Biomed. Sci. Appl.* **1999**, *728*, 1.
22. Kakizawa, Y.; Harada, A.; Kataoka, K. *Biomacromolecules* **2001**, *2*, 491.
23. Skey, J.; O'Reilly, R. K. *J. Polym. Sci. Part A: Polym. Chem.* **2008**, *46*, 3690.
24. Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661.
25. Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921.
26. Moad, G.; Rizzardo, E.; Thang, S. H. *Polymer* **2008**, *49*, 1079.
27. Mori, H.; Endo, T. *Macromol. Rapid Commun.* **2012**, *33*, 1090.
28. Kulkarni, R. K.; Morawetz, H. *J. Polym. Sci.* **1961**, *54*, 491.
29. Sanda, F.; Abe, T.; Endo, T. *J. Polym. Sci.Part A: Polym. Chem.* **1997**, *35*, 2619.
30. Sun, H.; Gao, C. *Biomacromolecules* **2010**, *11*, 3609.
31. Roy, S. G.; Acharya, R.; Chatterji, U.; De, P. *Polym. Chem.* **2013**, *4*, 1141.
32. Bauri, K.; Pant, S.; Roy, S. G.; De, P. *Polym. Chem.* **2013**, *4*, 4052.
33. Bauri, K.; Roy, S. G.; Pant, S.; De, P. *Langmuir* **2013**, *29*, 2764.
34. Kumar, S.; Acharya, R.; Chatterji, U.; De, P. *J. Mater. Chem. B* **2013**, *1*, 946.
35. Morcellet-Sauvage, J.; Morcellet, M.; Loucheux, C. *Die Makromol. Chem.* **1981**, *182*, 949.
36. Morcellet-Sauvage, J.; Morcellet, M.; Loucheux, C. *Macromolecules* **1983**, *16*, 1564.
37. Morcellet-Sauvage, J.; Morcellet, M.; Loucheux, C. *Polym. Bull.* **1983**, *10*, 473.
38. Casolaro, M. *React. Polym.* **1994**, *23*, 71.
39. Casolaro, M. *Macromolecules* **1995**, *28*, 2351.
40. Casolaro, M. *Polymer* **1997**, *38*, 4215.
41. Casolaro, M.; Bottari, S.; Cappelli, A.; Mendichi, R.; Ito, Y. *Biomacromolecules* **2004**, *5*, 1325.
42. Casolaro, M.; Cini, R.; Bello, B. D.; Ferrali, M.; Maellaro, E. *Biomacromolecules* **2009**, *10*, 944.
43. Mori, H.; Matsuyama, M.; Sutoh, K.; Endo, T. *Macromolecules* **2006**, *39*, 4351.
44. Mori, H.; Kato, I.; Matsuyama, M.; Endo, T. *Macromolecules* **2008**, *41*, 5604.
45. Lokitz, B. S.; Convertine, A. J.; Ezell, R. G.; Heidenreich, A.; Li, Y.; McCormick, C. L. *Macromolecules* **2006**, *39*, 8594.
46. Kumar, S.; Roy, S. G.; De, P. *Polym. Chem.* **2012**, *3*, 1239.
47. Felber, A. E.; Dufresne, M.-H.; Leroux, J.-C. *Adv. Drug Deliv. Rev.* **2012**, *64*, 979.
48. Morcellet-Sauvage, J.; Morcellet, M.; Loucheux, C. *Makromol. Chem.* **1982**, *183*, 821.
49. Morcellet-Sauvage, J.; Morcellet, M.; Loucheux, C. *Macromolecules* **1984**, *17*, 452.
50. Barbucci, R.; Casolaro, M.; Magnani, A.; Roncolini, C. *Macromolecules* **1991**, *24*, 1249.
51. Bignotti, F.; Penco, M.; Sartore, L.; Peroni, I.; Mendichi, R.; Casolaro, M.; D'Amore, A. *Polymer* **2000**, *41*, 8247.
52. Lebon, F.; Bignotti, F.; Penco, M.; Gangemi, R.; Longhi, G.; Abbate, S. *Chirality* **2003**, *15*, 251.
53. Bag, D. S; Dutta, D.; Shami, T. C.; Rao, K. U. B. *J. Polym. Sci. Part A: Polym. Chem.* **2009**, *47*, 2228.
54. Filippov, S.; Hruby, M.; Koňák, Č.; Macková, H.; Špírková, M.; Štěpánek, P. *Langmuir* **2008**, *24*, 9295.
55. Ezell, R. G.; Gorman, I.; Lokitz, B.; Ayres, N.; McCormick, C. L. *J. Polym. Sci. Part A: Polym. Chem.* **2006**, *44*, 3125.
56. Ezell, R. G.; Gorman, I.; Lokitz, B.; Treat, N.; McConaughy, S.; McCormick, C. L. *J. Polym. Sci. Part A: Polym. Chem.* **2006**, *44*, 4479.

57. Ezell, R. G.; McCormick, C. L. *J. Appl. Polym. Sci.* **2007**, *104*, 2812.
58. Chung, D., II; Britt, P.; Xie, D.; Harth, E.; Mays, J. *Chem. Commun.* **2005**, 1046.
59. Mori, H.; Matsuyama, M.; Endo, T. *Macromol. Chem. Phys.* **2009**, *210*, 217.
60. Skey, J.; Willcock, H.; Lammens, M.; Du Prez, F.; O'Reilly, R. K. *Macromolecules* **2010**, *43*, 5949.
61. Mori, H.; Iwaya, H.; Nagai, A.; Endo, T. *Chem. Commun.* **2005**, 4872.
62. Mori, H.; Iwaya, H.; Endo, T. *React. Funct. Polym.* **2007**, *67*, 916.
63. Mori, H.; Kato, I.; Endo, T. *Macromolecules* **2009**, *42*, 4985.
64. Lu, A.; Smart, T. P; Epps, T. H., III; Longbottom, D. A.; O'Reilly, R. K. *Macromolecules* **2011**, *44*, 7233.
65. Evans, A. C.; Lu, A.; Ondeck, C.; Longbottom, D. A.; O'Reilly, R. K. *Macromolecules* **2010**, *43*, 6374.
66. Lokitz, B. S.; Stempka, J. E.; York, A. W.; Li, Y.; Goel, H. K.; Bishop, G. R.; McCormick, C. L. *Aust. J. Chem.* **2006**, *59*, 749.
67. Kumar, S.; Acharya, R.; Chatterji, U.; De, P. *Langmuir* **2013**, *29*, 15375.
68. Moore, B. L.; O'Reilly, R. K. *J. Polym. Sci. Part A: Polym. Chem.* **2012**, *50*, 3567.
69. Bauri, K.; De, P.; Shah, P. N.; Li, R.; Faust, R. *Macromolecules* **2013**, *46*, 5861.
70. Shen, X.; Knutson, J. R. *J. Phys. Chem. B* **2001**, *105*, 6260.
71. Roy, S. G.; Bauri, K.; Pal, S.; De, P. *Polym. Chem.* **2014**, *5*, 3624.
72. Mori, H.; Takahashi, E.; Ishizuki, A.; Nakabayashi, K. *Macromolecules* **2013**, *46*, 6451.
73. Zayas, H. A.; Lu, A.; Valade, D.; Amir, F.; Jia, Z.; O'Reilly, R. K.; Monterio, M. *J. ACS Macro Lett.* **2013**, *2*, 327.
74. Gupta, P.; Vermani, K.; Garg, S. *Drug Discov. Today* **2002**, *7*, 569.
75. Hoffman, A. S. *Adv. Drug Deliv. Rev.* **2002**, *54*, 3.
76. Yoshida, M.; Asano, M.; Suwa, T.; Katakai, R. *Radiat. Phys. Chem.* **1999**, *55*, 677.
77. Marioa, C.; Barbara, D. B.; Emilia, M. *Colloids Surf. B* **2011**, *88*, 389.
78. Lu, A.; Cotanda, P.; Patterson, J. P.; Longbottom, D. A.; O'Reilly, R. K. *Chem. Commun.* **2012**, *48*, 9699.
79. Lu, A.; Moatsou, D.; Longbottom, D. A.; O'Reilly, R. K. *Chem. Sci.* **2013**, *4*, 965.
80. Basak, S.; Nanda, J.; Banerjee, A. *J. Mater. Chem.* **2012**, *22*, 11658.
81. Roy, S. G.; Haldar, U.; De, P. *ACS Appl. Mater. Interfaces* **2014**, *6*, 4233.
82. Jackson, A. W.; Fulton, D. A. *Chem. Commun.* **2011**, *47*, 6807.
83. Förester, S.; Abetz, V.; Müller, A. H. E. *Adv. Polym. Sci.* **2004**, *166*, 173.
84. Moughton, A. O.; O'Reilly, R. K. *Macromol. Rapid Commun.* **2010**, *31*, 37.
85. Kaneshiro, T. L.; Lu, Z.-R. *Biomaterials* **2009**, *30*, 5660.
86. Kuo, S.-W.; Lee, H.-F.; Huang, W.-J.; Jeong, K.-U.; Chang, F.-C. *Macromolecules* **2009**, *42*, 1619.
87. Mori, H.; Saito, S. *React. Funct. Polym.* **2011**, *71*, 1023.
88. Mori, H.; Saito, S.; Shoji, K. *Macromol. Chem. Phys.* **2011**, *212*, 2558.
89. Kumar, S.; De, P. *Polymer* **2014**, *55*, 824.
90. Shimazaki, Y.; Takani, M.; Yamauchi, O. *Dalton Trans.* **2009**, 7854.