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Chiroptical Properties of Homopolymers and Block Copolymers Synthesized from the Enantiomeric Monomers *N*-Acryloyl-L-Alanine and *N*-Acryloyl-D-Alanine Using Aqueous RAFT Polymerization*

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Chiral homo- and block copolymers based on the enantiomeric monomers *N*-acryloyl-L-alanine (ALAL) and *N*-acryloyl-D-alanine (ADAL) were prepared directly in water using controlled reversible addition–fragmentation chain transfer (RAFT) polymerization. The polymerization of the chiral monomers proceeded in a controlled fashion producing the respective homopolymers, block copolymers, and a statistical copolymer with targeted molecular weights and narrow molecular weight distributions. The chiroptical activity of these biomimetic polymers and their analogous model compounds was investigated using circular dichroism (CD). P(ALAL) and P(ADAL) were shown to be optically active exhibiting mirror image CD spectra. In addition, statistical and enantiomeric block copolymers prepared at 1:1 stochiometric ratios exhibited virtually no optical activity.

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Introduction

Optically active synthetic polymers have been the subject of a large number of scientific investigations,[1–10] some beginning as early as the 1950s because chiral structures which mimic attributes of naturally occurring polypetides, nucleic acids, and polysaccharides have many potential applications including chiral amplification, molecular recognition, liquid crystalline formation, supermolecular assembly, and polymeric catalysis at asymmetric sites. Chiral polyolefins generally fall into two major catergories—those in which asymmetry is induced due to tacticity during the polymerization and those with inherent asymmetry resulting from incorporation of monomers with chiral side groups. Control over the polymer tacticity is typically achieved through Ziegler–Natta polymerization or the anionic polymerization of bulky substituents with chiral initiators. Some degree of stereoregularity can also be achieved using coordinating species such as Lewis acids in conventional or controlled free radical polymerization techniques.[11–14] Polymers with chiral side chains are usually synthesized from vinyl monomers with pendant chiral groups. Recent advances in amino acid synthesis and isolation have increased the availability of optically pure amino acid enantiomers for synthesis of chiral monomers.[5,15–18] Polymers containing amino acid side chains are of interest because their hydrogen bonding ability can lead to the formation of higher ordered structures, for example α-helices and β-sheets.

The advent of controlled/'living' radical polymerization (CRP) techniques such as nitroxide mediated polymerization (NMP),[15] atom transfer radical polymerization (ATRP),[19] and reversible addition–fragmentation chain transfer (RAFT)[20] polymerization have allowed for the synthesis of polymers with controlled molecular weights, low molecular weight distributions, and complex architectures. Due to these attributes, CRP techniques have become quite attractive for the preparation of well defined optically active polymers. Recent reports of the preparation of optically active polymers using acyclic diene metathesis (ADMET),[5] ATRP,[11,21–25] and RAFT[11,12] have shown the synthesis of well controlled optically active polymers with chiral side chains to be possible. All controlled or controlled/'living' free radical polymerizations of optically active monomers reported to date have been carried out in organic solvents. To the best of our knowledge, the polymerization of such chiral monomers and the formation of enantiomeric block copolymers directly in water have not been previously reported. Our group has a long-standing interest in the aqueous RAFT polymerization of hydrophilic (meth)acrylamido monomers[26] and

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the self-assembly behavior of block copolymers in aqueous solutions.[27,28] Herein we report the RAFT polymerization of the enantiomers *N*-acryloyl-L-alanine (ALAL) and *N*-acryloyl-D-alanine (ADAL) directly in water without the necessity of protecting groups. As well, we compare the chiroptical properties of the homopolymers and the 1:1 statistical and 1:1 block copolymers with those of the respective model compounds.

Experimental

Materials

All reagents were purchased from Aldrich at the highest purity available and used as received unless otherwise stated. 2-Ethylsulfanylthiocarbonylsulfonyl-2-methyl-propionic acid (EMP) was synthesized according to literature procedures.[29] 4,4′-Azobis(4-cyanopentanoic acid) (V-501) was donated by Wako Chemicals and was recrystallized twice from methanol before use. ALAL and ADAL were synthesized by the drop-wise addition of acryloyl chloride to an aqueous solution of L-alanine and D-alanine and recrystallized from water before use. mp 162–164°C. δ_H 6.33 (m, CH_2CHCO), 5.71 (d, CH_2CHCO), 4.45 (m, $HNCH(COOH)CH_3$), 2.17 (d, $HNCH(COOH)CH_3$). Model compounds *N*-acetyl-L-alanine (mp 124–125°C) and *N*-acetyl-D-alanine (mp 124–125°C) were purchased from Aldrich.

General Procedure for Aqueous RAFT Polymerization

RAFT-mediated polymerizations of ALAL and ADAL were conducted at 70°C, employing V-501 as the primary radical source and EMP or P(ALAL) as the RAFT CTA and macroCTA, respectively. Polymerizations were performed directly in water (pH 6.5) with an initial monomer concentration ($[M]_0$) of 1.0 M under a nitrogen atmosphere in round-bottomed flasks equipped with magnetic stir bars and sealed with rubber septa. The initial monomer to CTA ratios ($[M]_0/[CTA]_0$) were varied between 140:1 and 225:1 while the initial CTA to initiator ratio ($[CTA]_0/[I]_0$) was held at a constant ratio of 5:1. Absolute molecular weights were determined from aliquots (0.5 mL) taken at pre-determined time intervals and subsequently quenched via rapid cooling and exposure to oxygen. The products were purified by dialysis against deionized water and isolated by lyophilization.

Aqueous Size Exclusion Chromatography

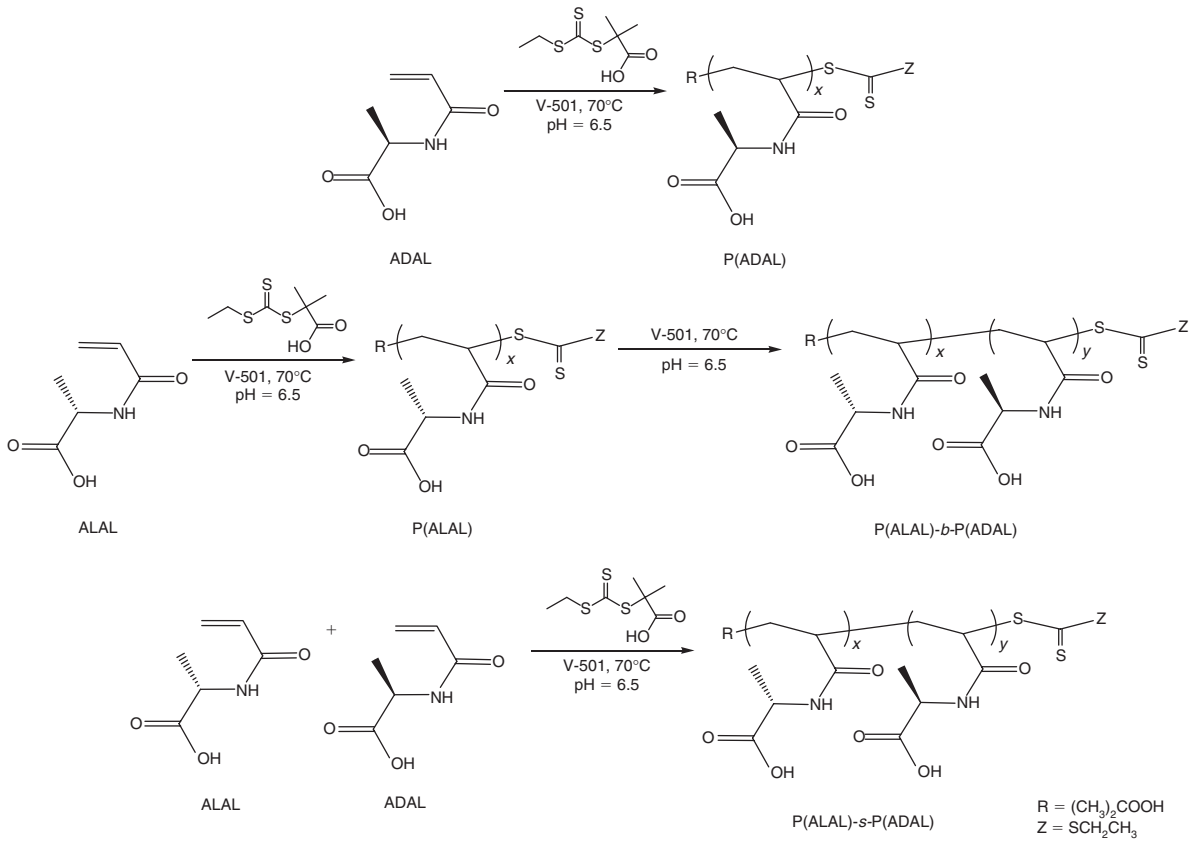
Polymers were analyzed directly by aqueous size exclusion chromatography (ASEC) using an aqueous eluent of 20%/80% acetonitrile/0.5 M Na_2SO_4. A flow rate of 0.5 mL min^{-1} at 25°C, TOSOH Biosciences TSK-GEL columns (G3000 PWXL, <50000 g mol^{-1}, 200 Å) and G4000 PWXL (2000–300000 g mol^{-1}, 500 Å), a Polymer Laboratories LC 1200 UV/vis, Wyatt Optilab DSP interferometric refractometer, and a Wyatt DAWN EOS multiangle laser light scattering detector (690 nm) were employed in our analysis.

Nuclear Magnetic Resonance Spectroscopy

^{13}C NMR spectra were recorded with a temperature controlled Mercury Innova 500 MHz spectrometer. Samples were prepared as 20% w/w solutions in D_2O (HOD internal standard). The tacticity for the methine peak (δ_C 44–47 ppm) was determined based on triad assignments from a previous literature report for acrylamide.[13]

Circular Dichroism Spectroscopy

Samples were prepared by directly dissolving the dried sample in HPLC grade water (pH ≈ 6.5) at concentrations of 50 and 1.5 μM for the monomers and polymers, respectively, such that an absorbance value of 0.5 was obtained at 210 nm using a HP 8453 diode array spectrophotometer in a 1 cm quartz cuvette. CD spectra were collected at room temperature using a Jasco J-815 spectropolarimeter in a low volume rectangular 0.5 cm path length cell. An 'ozone free' lamp was used to



Scheme 1. Pathway for aqueous RAFT synthesis of acryloyl-D- and L-alanine homopolymers, block copolymers, and a statistical copolymer.

limit exposure of the optical bench to ozone generated by far ultraviolet (UV) radiation, eliminating the need to purge the optical bench. The response time used was 16 s with a data pitch of 0.1 nm and a scan speed of 10 nm min^{-1}.

Results and Discussion

The enantiomeric monomers ALAL and ADAL were selected for this study based on their facile synthesis from readily available and optically pure amino acid precursors. Additionally, the amphoteric nature of the monomers allows polymerization, purification, and characterization directly in aqueous solution. In order to prepare the precisely defined polymers for circular dichroism studies, we employed a recently communicated[29] aqueous RAFT polymerization method using the chain transfer agent 2-ethylsulfanylthiocarbonylsulfonyl-2-methyl-propionic acid (EMP) and the initiator 4,4′-azobis(4-cyanopentanoic acid) (V-501) at 70°C. In the current study, we have prepared homopolymers of ALAL and ADAL, as well as the 1:1 statistical and 1:1 and 1:2 block copolymers of these two enantiomers (Scheme 1).

By controlling the [monomer]/[EMP] ratio and the conversion, molecular weights of the homopolymers were targeted for ∼34000 g mol^{-1}. Table 1 lists the number average molecular weights and molecular weight distributions as determined by aqueous size exclusion chromatography (ASEC) using mutli-angle laser light scattering (MALLS) and refractive index (RI) detectors.

Shown in Fig. 1a are the normalized ASEC chromatograms and Fig. 1b cumulative weight fractions for the P(ALAL) macroCTA and the resultant $P(ALAL)_{118}$-*b*-$P(ADAL)_{119}$ copolymer. The efficient blocking (percentage of macroCTA converted to block copolymer) expected when using the trithiocarbonate EMP[29] is confirmed by the shift in the ASEC trace (RI detector) to higher elution volume and the shift of the near-monodisperse distribution to higher molecular weight.

Circular dichroism (CD) spectroscopy was used to assess the optical activity of each of the homo- and copolymers (Fig. 2) and the respective enantiomorphic model compounds (Figs 3a and 3b). The CD spectra of the homopolymers P(ALAL) and (PADAL) shown over the 240–190 nm range are near mirror images with respective molar elipticity values of about 5000 and −5000 Θ at 220 nm decreasing to zero at the crossover of 211 nm and rapidly increasing in magnitude with opposite sign at increasingly lower wavelengths. Maximum negative and positive values of molar ellipticity of approximately −35000 and +40000 Θ are reached in the 190 nm range for P(ALAL) and P(ADAL), respectively. The statistical 1:1 copolymer synthesized with equal molar amounts of the D- and L-monomers in the feed shows no optical activity and the block copolymer prepared by sequential addition of the D-monomer to the preformed poly(ALAL) block shows only slight activity, presumably from the slightly larger block (two or three units) of the D-enantiomer. The block copolymer containing a roughly 2:1 D to L ratio exhibits intermediate behavior as might be expected. Examination of the CD spectra for the respective L- and D-homopolymers and their small molecule counterparts reveals that the amplitude of the CD maxima for each homopolymer is around two to four times that of its constitutive monomer. Since the molar concentrations of model compounds and repeating units of the homopolymers are equivalent in our experiments, the optical activity of the homopolymers is greater than that of the summation of the amino acid repeat units alone; therefore, additional interactions appear to contribute in a synergistic fashion to the optical activity.[4,30]

Table 1. Experimental data for homo- and copolymer[A] synthesis based on the chiral monomers ADAL and ALAL

	t [min]	Conv	M_n[B] [g mol^{-1}]	$M_{n,th}$ [g mol^{-1}]	M_w/M_n[B]	Tacticity (*rr*/*mr*/*mm*)[C]
$P(ALAL)_{118}$ macroCTA	30	0.75	16900	15000	1.09	42/42/16
$P(ALAL)_{215}$	45	0.91	30800	27500	1.03	40/48/12
$P(ADAL)_{201}$	45	0.87	28700	26300	1.05	37/44/19
$P(ALAL)_{118}$-*b*-$P(ADAL)_{119}$	45	0.93	33900	32500	1.04	43/43/13
$P(ALAL)_{118}$-*b*-$P(ADAL)_{217}$	45	0.89	47900	44400	1.05	—
$P(ALAL)_{121}$-*s*-$P(ADAL)_{121}$	45	0.91	34600	29300	1.07	—

[A] Abbreviations: P poly, *b* block, *s* statistical. [B] As determined using aqueous size exclusion chromatography, see Experimental. [C] Measured using 500 MHz ^{13}C NMR. Triad assignments based on ref. [20].

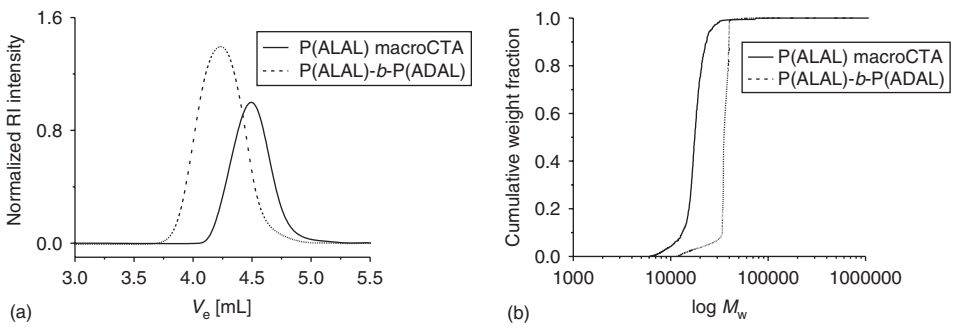


Fig. 1. (a) RI traces and (b) cumulative weight fractions for the P(ALAL) macroCTA (M_n 16900 g mol^{-1}, PDI 1.09) and P(ALAL)-*b*-P(ADAL) (M_n 33900 g mol^{-1}, PDI 1.04).

As early as 1961, Kulkarni and Morawetz,[4] in a seminal contribution, explored the possible reasons for optical activity enhancements of the amphoteric polymer formed from classical free radical polymerization of *N*-acryloyl glutamic acid as compared to its low molecular weight analog. They observed substantial changes in both the magnitude and sign of the specific optical activity as a function of pH of the polymerization medium. Since the CD behavior of molecules containing asymmetric centers is due to statistical averaging of all conformations, Morawetz reasoned that the steric restrictions of the side chains of the polymer as compared to the low molecular weight analogue 'should result in a corresponding change in the contribution of the asymmetric center to optical activity'. He discussed optical activity as a function of conformational restrictions dependent on solvent quality, hydrogen bonding, and possibly by helix formation.

In our work and that reported by Morawetz, water-soluble polymers with chiral, amino acid-based pendant groups were obtained by free radical polymerization techniques. Since these were neither conducted at low temperature with constrained monomers nor with catalysts or coordinating species, highly stereoregular polymers would not be predicted. However, considering the somewhat bulky nature of the enantiomorphic monomers, one might expect the energetically more favorable syndiotactic propagation which results in long sequences of *trans* and occasional pairs of *gauche* conformations as compared to isotactic propagation yielding alternating *trans* and *gauche* conformations.[4,10] Indeed, evaluation of the triad content of the D- and L-homopolymers and the 1:1 block using ^{13}C NMR using resonance assignments reported in the literature revealed a syndiotactic tendency of our RAFT polymerizations conducted in water at 70°C. The backbone methine resonances located between 44 and 48 ppm (Fig. 4) yield *rr*/*rm*/*mm* ratios that are shown in Table 1. Syndiotactic propagation is favored for both hompolymers and the block copolymer over isotactic propagation. Interestingly, the statistical copolymer displays a single broad peak which cannot be resolved, likely indicating a larger number of conformational interactions of the methine carbon and possibly a completely atactic structure.

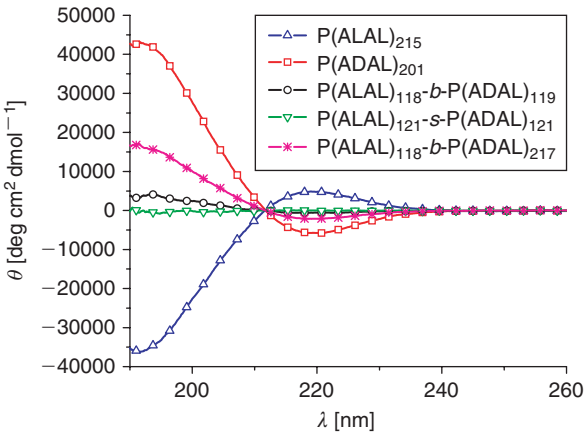


Fig. 2. CD spectra of $P(ALAL)_{215}$, $P(ADAL)_{201}$, $P(ALAL)_{118}$-*b*-$P(ADAL)_{119}$, $P(ALAL)_{118}$-*b*-$P(ADAL)_{217}$, and $P(ALAL)_{121}$-*s*-$P(ADAL)_{121}$. See Table 1 for structural data and designations.

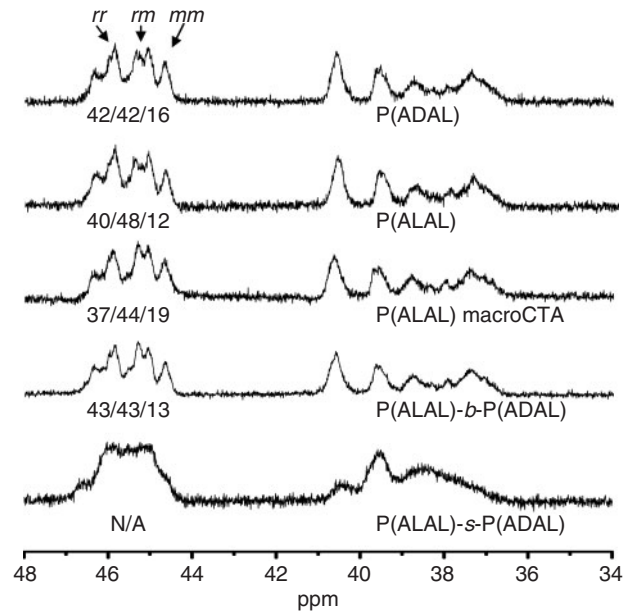


Fig. 4. ^{13}C NMR spectra for methylene (34–40 ppm) and methine (44–47 ppm) backbone carbons for P(ADAL), P(ALAL), P(ADAL) macroCTA, P(ALAL)-*b*-P(ADAL), and P(ALAL)-*s*-P(ADAL). See Table 1 for structural designations.

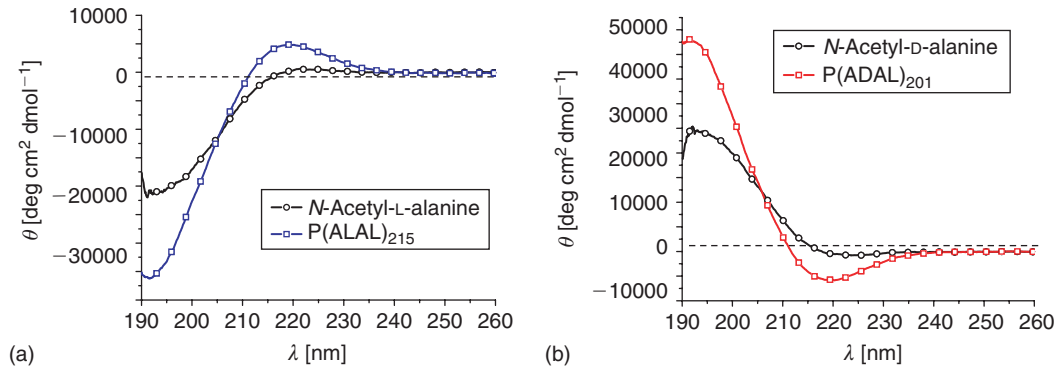


Fig. 3. CD spectra for (a) $P(ALAL)_{215}$ and the model compound *N*-acetyl-L-alanine and (b) $P(ADAL)_{201}$ and the model compound *N*-acetyl-D-alanine.

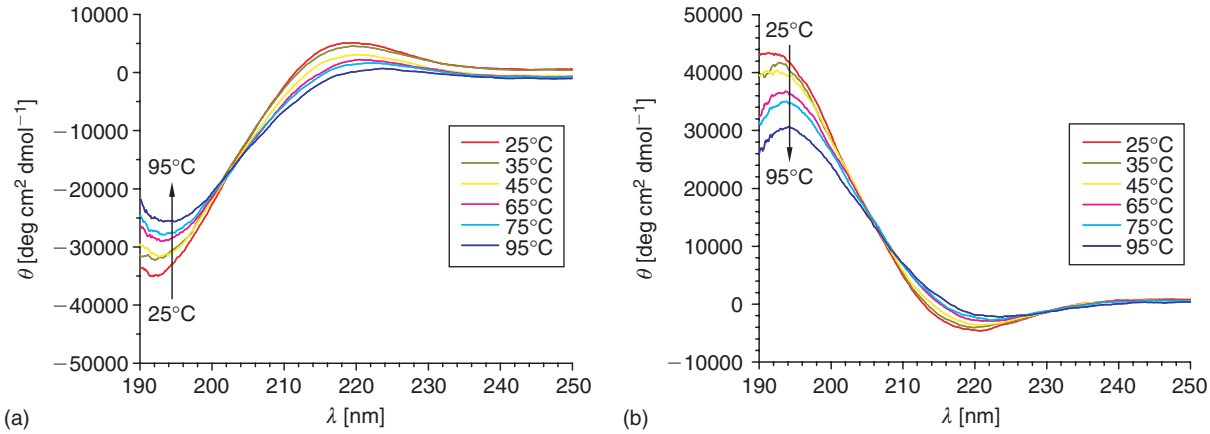


Fig. 5. CD spectra at selected temperatures for (a) $P(ALAL)_{215}$ and (b) $P(ADAL)_{201}$.

Whether the apparently favored syndiotactic structure in concert with the chiral side chains is totally responsible for the enhanced optical behavior of the homopolymers is not clear. The mirror image CD spectra of the respective D- and L-homopolymers, the loss of optical activity with the statistical copolymer and the near cancellation of amplitude with the nearly balanced block copolymer (Fig. 2) suggest similar, but opposite 'sense' interactions. However, since the amphoteric chiral side chains are capable of multiple hydrogen bonding interactions, it is possible that these interactions might also play a role in inducing additional order either during or after polymerization. For example, hydrogen bonding influenced by solvent, pH, and temperature can affect orientation and local dielectric constants. In preliminary studies, we find significant changes in the CD spectra with increasing temperature of the respective homopolymers P(ALAL) and P(ADAL) (Fig. 5). The molar elipticity values decrease steadily with increasing temperature over the 25 to 95°C range examined. Also changes are observed in CD spectra with changes in pH (data not shown). However, a residual enhancement in CD activity over that predicted by simple summation of chiral units appears to remain in both cases.

Conclusions

In this paper we present a facile synthetic route for the formation of optically active, enantiomeric (co)polymers based on L- and D-alanine directly in water, requiring no protecting group chemistry. Homo- and copolymers with targeted molecular weights and low molecular weight distributions have been synthesized via aqueous RAFT polymerization. The resulting P(ALAL) and P(ADAL) homopolymers were shown to be optically active and exhibit mirror image CD spectra. ^{13}C NMR studies indicate chain end control slightly favoring syndiotactic placement as elucidated by the measured ratios of *rr*/*rm*/*mm* sequences. In addition, 1:1 statistical and block copolymers were synthesized and showed little optical activity attributed to CD amplitude cancelling by the respective enantiomeric repeat units. Whether the slightly favored syndiotactic placement is responsible for the 'enhanced' elipticity values or whether other factors such as hydrogen bonding are operable are yet to be determined, but it is clear that temperature and pH affect chiroptical behavior.

We believe the technological importance of the work reported here lies in the possibility of employing RAFT polymerization directly in water using non-protected chiral amino acid-based monomers to yield block copolymers with 'tunable' optical activity. This technique will allow extension of the early work of Kulkarni and Morawetz[4] which demonstrated the pH-dependent optical response for homo- and random copolymers but which was limited by the lack of structural and molecular weight control inherent to classical free radical polymerization. Now, copolymers with precise amphoteric, enantiomeric content can easily be tailored by a simple, facile polymerization technique. Additionally, it is anticipated that issues of conformational and configurational effects on structural organization of chiral centers in aqueous media can now be addressed directly. In turn, such studies of optical activity can then be compared to those reported for protected (or substituted) amino acid derivates polymerized in organic media.

Acknowledgements

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