Amino Acids
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ORIGINAL ARTICLE

Controlled radical polymerization of an acrylamide containing L-alanine moiety via ATRP

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Abstract Homopolymerization of an optically active acrylamide having an amino acid moiety in the side chain, *N*-acryloyl-L-alanine (AAla) was carried out via atom transfer radical polymerization (ATRP) at room temperature using 2-hydroxyethyl-2′-methyl-2′-bromopropionate (HMB) or sodium-4-(bromomethyl)benzoate (SBB) as initiator in pure water, methanol/water mixture and pure methanol solvents. The polymerization reaction resulted in the optically active biocompatible amino acid-based homopolymer in good yield with narrow molecular weight distribution. The number average molecular weight increased with conversion and polydispersity was low. The structure and molecular weight of synthesized polymer were characterized by ^{1}H NMR, FT-IR spectroscopic techniques and size-exclusion chromatography.

Keywords Atom transfer radical polymerization · Optically active · Homopolymerization

Abbreviations

AAla	*N*-Acryloyl-L-alanine
ATRP	Atom transfer radical polymerization
bpy	2,2′-Bipyridine
HMB	2-Hydroxyethyl-2′-methyl-2′-bromopropionate
1H-NMR	Proton nuclear magnetic resonance
L	Ligand
Mt	Metal ion
PS	Polystyrene
RX	Alkyl halide
SBB	Sodium-4-(bromomethyl)benzoate
SEC	Size exclusion chromatography
z	Oxidation state

Introduction

The synthesis and application of optically active polymers have received extensive interest owing to their chiral structure which is common to naturally occurring polymers (Okamoto and Nakano 1994; Pu 1998; Zhi et al. 2009; Sogawa et al. 2013; Huang et al. 2015; Song et al. 2013). Most of the naturally occurring polymers are optically active and some of them indicate characteristic functionalities such as molecular recognition ability and catalytic activity, due to their specific chiral structure as denoted by genes and proteins. In synthetic polymer chemistry, it seems that one of the most challenging tasks is to make functional polymeric systems that will be as effective as those in living systems (Han et al. 2013; Mallakpour and Soltanian 2012; Takeoka et al. 2013; Sogawa et al. 2011; Mallakpour and Rafiee 2008, 2011; Mallakpour and Rafiee 2009; Miyake et al. 2010; Tsuji et al. 2010; Kolitz et al. 2009; Raus et al. 2014).

Amino acids are the constitutional units of peptides and proteins, which are able to produce highly ordered hierarchical structures, organized on a length scale ranging from a few nanometers to several microns. Their secondary structures and higher order structures are highly packed through intra- and interchain associations by noncovalent forces, such as hydrogen bonding, hydrophobic stacking, electrostatic, and dipolar interactions. The primary structures of the biomacromolecules are created by covalent bond of the building blocks, and the

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molecular information such as amino acid sequence, chain chirality, and amphiphilicity encoded in the primary structures determines their highly ordered structures. Incorporation of amino acid residues into synthetic polymers is of attention because these combinations may produce nonbiological macromolecules with biomimetic structures and properties (Sanda and Endo 1999; Fox et al. 2015; Rafiee and Zare 2015).

Atom transfer radical polymerization (ATRP) is a controlled radical polymerization technique that is used for the synthesis of functional macromolecules with controlled and complex architectures because of its versatility and compatibility with a range of monomers (Matyjaszewski and Xia 2001; Tsarevsky and Matyjaszewski 2007; Ran et al. 2014; Shi et al. 2014; Ran et al. 2014; Cho et al. 2013; Maria et al. 2005).

This method is as an effective synthetic way to produce functional polymers with controlled molar mass, narrow molecular weight distribution, and well-defined architectures and functionalities. ATRP is based on the reversible reaction of a low oxidation state metal complex, Mt^zL_m (Mt^z represents the metal ion in oxidation state z, and L is a ligand), with an alkyl halide (RX). This reaction produces radicals and the corresponding high oxidation state metal complex with a coordinated halide ligand, $XMt^{z+1}L_m$. ATRP involves the reactivation of the alkyl halide adduct of the unsaturated compound (monomer) and the further reaction of the formed radical with the monomer (propagation). The livingness of this polymerization process can be determined from a linear first-order kinetic plot, accompanied by a linear increase in polymer molecular weights with conversion, with the value of the number-average degree of polymerization determined by the ratio of reacted monomer to initially introduced initiator.

In this study, ATRP of a monosubstituted acrylamide with an amino acid moiety, *N*-acryloyl-L-alanine (AAla) was investigated in the presence of two initiators, 2-hydroxyethyl-2′-methyl-2′-bromopropionate (HMB) and sodium-4-(bromomethyl)benzoate (SBB), separately at room temperature.

Experimental

Materials

All chemicals were purchased from Fluka Chemical Co. (Buchs, Switzerland), Aldrich Chemical Co. (Milwaukee, WI), Riedel–deHaen AG (Seelze, Germany) and Merck Chemical Co. L-Alanine, acryloyl chloride, 2,2′-bipyridine (bpy), 2-bromo-2-methylpropanoyl bromide, ethylene glycol and SBB were purchased and used as received without further purification. CuBr was purified as reported in the literature (Keller and Wycoff 1946). It was stored under nitrogen and weighed in the open air prior to polymerization.

Techniques

Number-average molecular weight (Mn) and molecular weight distribution (Mw/Mn) were estimated by size-exclusion chromatography (SEC), the elution rate was 1 mL/min and standard polystyrene (PS) was used for calibration. Proton nuclear magnetic resonance (^{1}H-NMR, 500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in DMSO-d_6 solution using a Bruker (Germany) Avance 500 instrument. FT-IR spectra were recorded on (Jasco-680, Japan) spectrophotometer. The spectra of solids were obtained using KBr pellets. Inherent viscosities were measured using a Cannon–Fenske Routine Viscometer (Germany) at concentration of 0.5 g dL^{-1} at 25 °C. Specific rotations were measured by a Jasco Polarimeter (Japan).

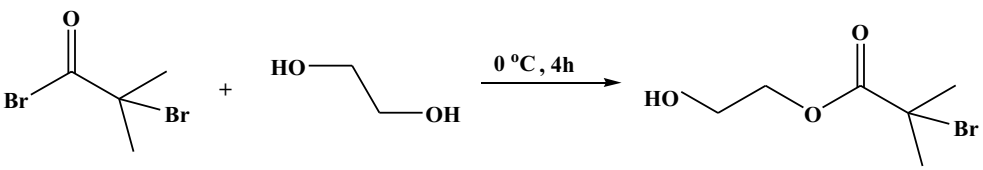
Synthesis of initiator

2-Hydroxyethyl-2′-methyl-2′-bromopropionate (HMB) was synthesized as described previously (Scheme 1) (Yin et al. 2001; Haddelton et al. 1997). Bromoisobutyryl bromide was reacted with cold anhydrous ethylene glycol (excess) under stirring for 4 h. The molar ratio of ethylene glycol and bromobutyryl bromide was 25 to avoid coupling reaction. The reaction mixture was added to deionized water, and the reaction product was extracted by dichloromethane. The organic solution was washed with water and sodium carbonate and dried over anhydrous magnesium sulfate. The final product was isolated as a colorless liquid upon removal of the solvent, and it was vacuum-distilled. The chemical structure and purity of HMB were proven with FT-IR and ^{1}H-NMR spectroscopic techniques. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 4.4 (t, 2H), 3.83 (t, 2H), 3.2 (s, 1H); 1.96 (s, 6H).

Synthesis of monomer

The vinyl monomer *N*-acryloyl-L-alanine (AAla) was synthesized by the general techniques previously reported (Bueno et al. 1991). As shown in Scheme 2, the acylation

Scheme 1 Synthesis of 2-hydroxyl-2′-ethyl-2′-bromopropionate (HMB)



Scheme 2 Synthesis of poly(*N*-acryloyl-L-alanine) [poly(AAla)] via ATRP using two initiators

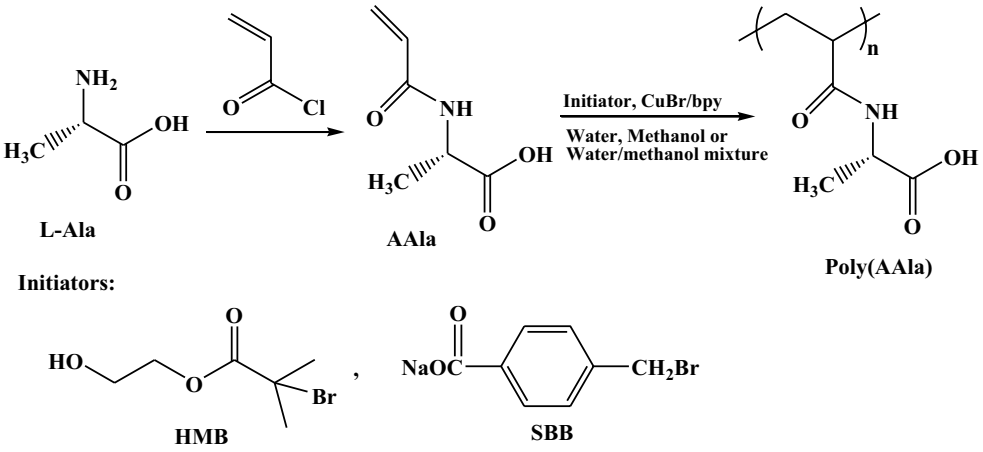
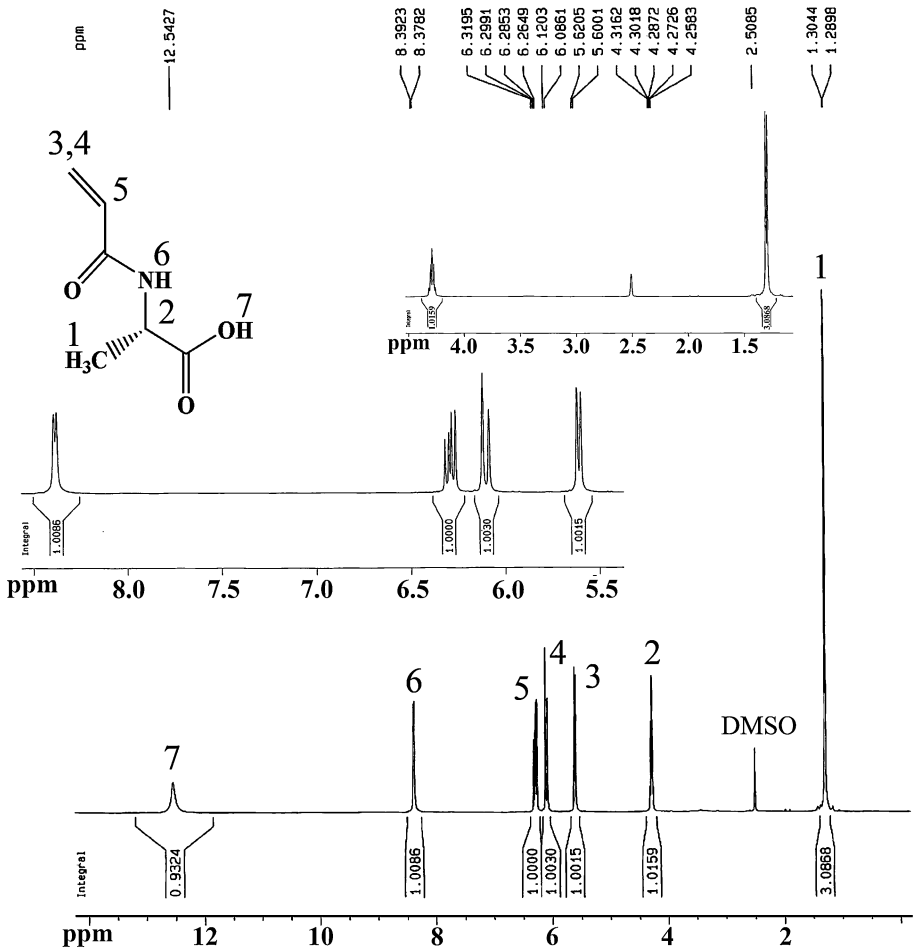


Fig. 1 ^{1}H-NMR (500 MHz) spectrum of AAla in DMSO-d_6 at room temperature



reaction of L-alanine with acryloyl chloride was performed in the presence of alkali at 0 °C. Treatment with concentrated hydrochloric acid provided a white solid. Recrystallization from ethanol gave white crystals with good yield and purity. The chemical structure and purity of the optically active monomer were proven with FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopic techniques (Figs. 1, 2). $[\alpha]_D^{25} = +21.48$ (0.05 g in 10 mL ethanol).

FT-IR (KBr, cm^{-1}): 3422–2760 (COOH stretch), 3323 (N–H stretch), 3029 (=C–H stretch), 2928 (–C–H stretch), 1729 and 1647 (C=O stretch), 1210 (C–O stretch). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 1.29–1.30 (d, J = 7.3 Hz, 3H), 4.26–4.32 (m, 1H), 5.60–5.62 (d, J = 10.2 Hz, 1H), 6.09–6.12 (d, J = 17.1 Hz, 1H), 6.26–6.32 (dd, J_1 = 10.2 Hz, J_2 = 17.1 Hz, 1H), 8.38-8.39 (d, NH, J = 7.0 Hz, 1H), 12.54 (s, COOH, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ

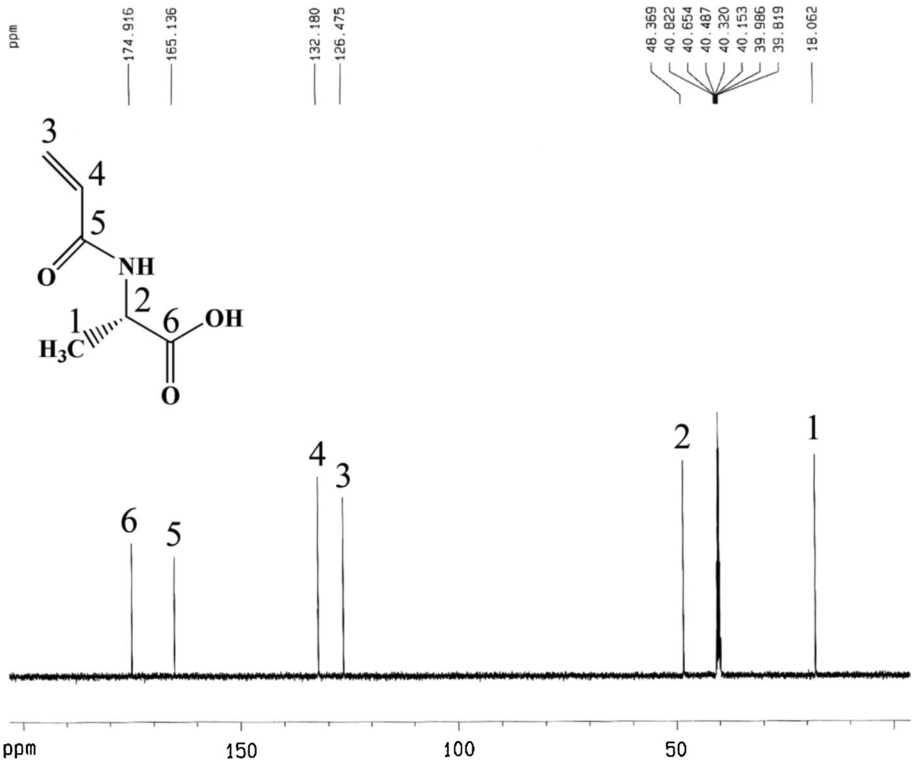


Fig. 2 ^{13}C-NMR (125 MHz) spectrum of AAla in DMSO-d_6 at room temperature

18.06 ($\underline{C}H_3$), 48.37 ($\underline{C}H$ chiral center), 126.47 ($=\underline{C}H_2$), 132.18 ($=\underline{C}H$), 165.14 ($\underline{C}=O$ amide), 174.92 ($\underline{C}=O$ acidic). Anal. Calcd for $C_6H_9NO_3$: C, 50.35; H, 6.34; N, 9.78. Found: C, 50.30; H, 6.57; N, 9.90.

ATRP

General procedure, mixture of HMB (0.018 g, 0.087 mmol), AAla monomer (1.00 g; 6.99 mmol) were dissolved in 2 mL of a 50:50 v/v % methanol/double-distilled water mixture at room temperature, then 5 % NaOH was added to achieve pH = 8. This solution was degassed by passing dry nitrogen gas, then 2,2′-bipyridine (bpy) ligand (0.033 g; 0.21 mmol) and $Cu^{I}Br$ catalyst (0.012 g; 0.087 mmol) was added rapidly. Upon addition of these reagents a dark brown solution was formed. An exothermic polymerization reaction started immediately leading to an increase in viscosity of the mixture and it was observed by sensing the vessel of reaction. After certain period of reaction time (Table 1) the mixture was exposed to air. The dark brown reaction solution turned blue, indicating aerial oxidation of Cu(I) to Cu(II). The polymer solution was diluted with 5 mL of water and was mixed with about 1 g of silica gel and was stirred for 1 h. Then it was filtered off and was acidified with a solution of concentrated HCl (12 M) to provide white solid polymers. The resulting polymers were washed with cold THF to remove traces of residual AAla monomer and/or unreacted initiator, affording AAla homopolymer in high yield. The monomer conversion was determined gravimetrically.

Result and discussions

Controlled radical polymerization of amino acid-containing monomers is promising for producing tailored polymers and copolymers for biomaterials and medical applications. The amino acid-based polymers are expected to be non-toxic, as an extensive variety of polymers bearing amino acid moieties either in the main chains or in side chains, such as poly(glutamic acid) and polyleucine, have been prepared, and they showed biocompatibility and biodegradability similar to polypeptides (Sanda and Endo 1999). The well-defined poly(AAla) was prepared via ATRP using CuBr/bpy catalyst system and HMB or sodium-4-(bromomethyl)benzoate (SBB), separately as an initiator in pH = 8 at room temperature. Bpy was chosen as ligand since it is commercially available and readily forms a water-soluble complex with Cu^{I}. The route of the synthesis of poly(AAla) is outlined in Scheme 2. The water-soluble initiator, HMB was synthesized from the reaction of 2-bromoisobutyryl bromide with ethylene glycol. The ^{1}H-NMR spectrum of poly(AAla) is shown in Fig. 3. The ^{1}H-NMR of this polymer shows the characteristic resonance of acidic and amidic protons at 12.68 and 8.08 ppm, C–H chiral center at 4.27 ppm. The disappearance of vinyl

Table 1 Summary of conversion, molecular weight and polydispersity data for homopolymerization of AAla using two initiators in various ratios of water and methanol at room temperature

Initiator	Solvent H_2O:MeOH	Time (h)	Conversion[a] (%)	Molecular weight			M_w/M_n	$[\alpha]_{Na,589}^{25,c}$
				M_n^b (theory)	M_n (SEC)	M_n (titration)		
SBB	50:50	1	84.5	–	–	–	–	+21.78
SBB	50:50	2	99.1	11,630	12,489	12,317	1.1	+26.18
SBB	50:50	4	99.2	–	–	–	–	+27.14
SBB	50:50	6	99.2	–	–	–	–	+27.64
SBB	50:50	8	99.4	–	–	–	–	+27.57
SBB	50:50	10	99.5	–	–	–	–	+26.94
SBB	50:50	12	100.0	11,734	12,834	12,711	1.2	+26.83
SBB	100:0	2	98.9	–	–	–	–	+22.77
SBB	0:100	2	84.7	–	–	–	–	+23.06
HMB	50:50	1	84.9	–	–	–	–	+23.17
HMB	50:50	2	86.8	–	–	–	–	+24.02
HMB	50:50	4	87.1	–	–	–	–	+21.69
HMB	50:50	6	87.9	–	–	–	–	+22.53
HMB	50:50	8	89.7	–	–	–	–	+25.68
HMB	50:50	10	90.5	–	–	–	–	+25.94
HMB	50:50	12	92.3	–	–	–	–	+24.47

$[AAla]_o = 3.495$ M and the molar ratio of initiator:CuBr:bpy was 2:2:5

[a] Calculated from: $[W_p/(W_i + W_m)] \times 100$ %, where W_p, W_i, and W_m were the weights of the polymer produced and the initial weights of the related initiator and monomer, respectively

[b] M_n (theory) $= 143.1([AAla]_o/[initiator]) \times$ %Conversion/100 $+ M_w$(initiator)

[c] Measured at a concentration of 0.5 g/dL in DMF at 25 °C

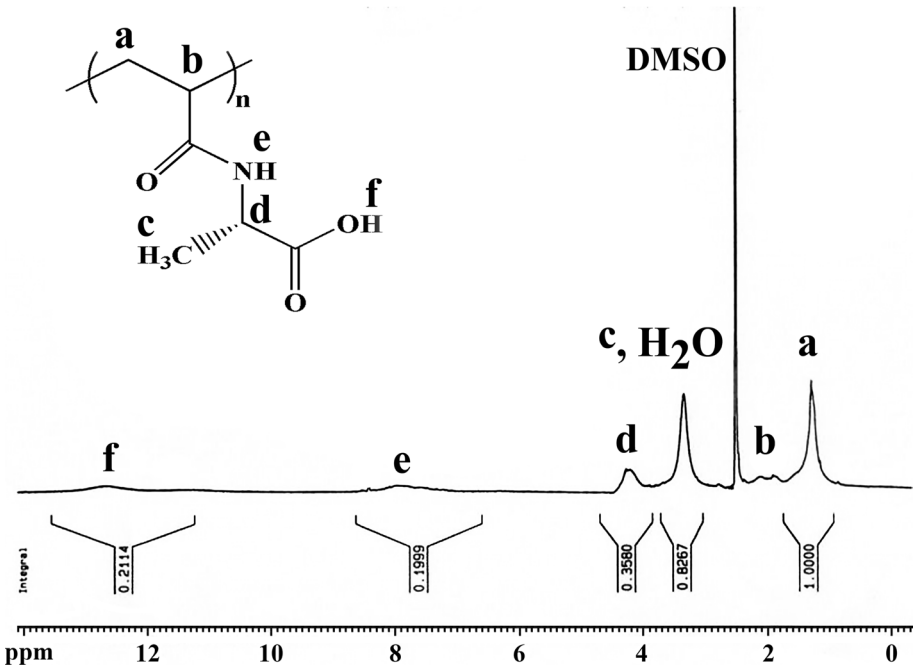


Fig. 3 ^{1}H-NMR (500 MHz) spectrum of poly(*N*-acryloyl-L-alanine) [poly(AAla)] [prepared via the polymerization of *N*-acryloyl-L-alanine (AAla) in the presence of sodium-4-(bromomethyl)benzoate (SBB) as an initiator in 50/50 methanol/water mixture as a solvent at room temperature at pH = 8] in DMSO-d_6 at room temperature. Monomer concentration = 3.495 M and the molar ratio of initiator:CuBr:bpy was 2:2:5

peaks at 5.60–5.62, 6.09–6.12 and 6.26–6.32 ppm confirmed complete conversion of monomer to poly(AAla) after 2 h reaction time. In the first step, we examined the homopolymerization of AAla in double-distilled, deionized water at room temperature under a nitrogen atmosphere. Exceptional rate acceleration in polymerization was

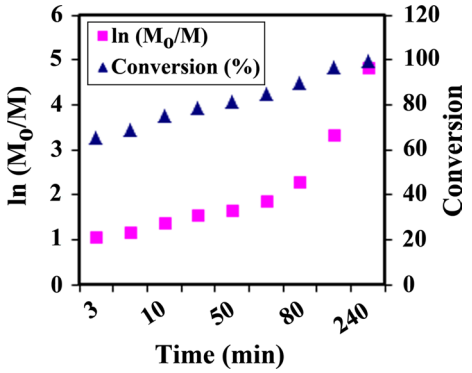


Fig. 4 Kinetic plot of ATRP of AAla in 50/50 methanol/water mixture as a solvent at room temperature at pH = 8. Monomer concentration = 3.495 M and the molar ratio of initiator:CuBr:bpy was 2:2:5

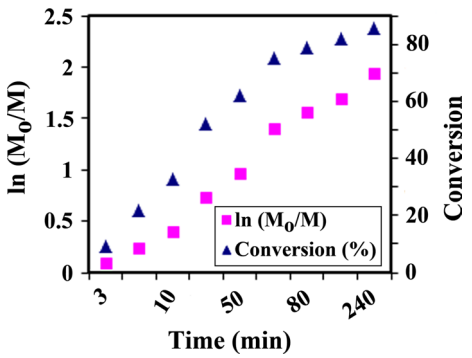


Fig. 5 Kinetic plot of ATRP of AAla in methanol as a solvent at room temperature at pH = 8. Monomer concentration = 3.495 M and the molar ratio of initiator:CuBr:bpy was 2:2:5

observed, so high conversion was obtained in short period of time. The same experiments were also conducted in the presence of 50:50 methanol/water mixture or pure methanol as a solvent. Rates of polymerization were noticeably slower in methanol compared to 50:50 methanol/water, probably owing to the more active nature of the Cu(I) catalyst in the presence of water. The results of a particular set of experiments are summarized in Table 1. As predictable for a living polymerization, the molecular weight of the two homopolymers enhanced with conversion. Figure 4 represents typical kinetic data for the homopolymerization of AAla in 50:50 methanol/water mixture at room temperature. Conversion of more than 96 % is attained within 100 min and the plot of $\ln([M]_o/[M])$ versus time was linear up to 89.8 % conversion, but after this time a deviation from first-order kinetics is observed. Figure 5 depicts kinetic data for the homopolymerization of AAla in pure methanol. Under these conditions, the polymerization is rather slower than in the presence of water and 50:50 methanol/water mixture, so conversion of more than 96 % is achieved within 12 h. In this case, the polymerization is first order with respect to AAla monomer up to around 85.6 % of conversion. Chiral poly(AAla)s with M_n = 12,489 $gmol^{-1}$ (M_w/M_n = 1.1) and M_n = 12,834 $gmol^{-1}$ (M_w/M_n = 1.2) were obtained, so molecular weight distribution value remained low (M_w/M_n = 1.2). Molecular weight of poly(AAla)s was also determined by titration, and it gave a result which is close to one that was determined by SEC (Table 1). Since the reactions with SBB initiator gave lower molecular weight distribution compared to HMB initiator, this initiator was chosen to be more suitable for the synthesis of these homopolymers. But it is important to note that HMB initiator from environmental point of view is friendlier since it is biocompatible.

Summary

The ATRP of AAla was successfully carried out using CuCl/bpy catalyst with HMB and SBB water-soluble initiators in pure water, 50/50 methanol/water mixture or pure methanol. The controlled character of the polymerization was confirmed by the formation of narrow polydispersity products and the linear relationship between the molecular weight and conversion. Since the resulting homopolymer has amino acid moieties in the backbone; it exhibits biocompatibility and biodegradability. In this work, well-defined chiral poly(AAla) having amino acid L-alanine in the side chain was obtained directly by ATRP, without requiring protection chemistry for the carboxylic acid residues, under mild conditions.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest with regard to this work.

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