INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Chemical modification of styrene based polymers with attaching antimicrobial 4-hydroxybenzoic acid group as a side chain

Mehrdad Mahkam

Department of Chemistry, Azarrbaijan University of Tarbiat Moallem, Tabriz, Iran.

ABSTRACT

Free radical copolymerization and cross-linking copolymerization of the 4-chloromethyl styrene (CMS) with styrene (in various mole ratios), were carried out using α,α-azobis(isobutyronitrile) AIBN as initiator and 1,4-divinylbenzene (DVB) as a crosslinking agent at 70 °C. The antimicrobial 4-hydroxybenzoic acid (4-HBA) was covalently attached to the obtained copolymers with replacement of all the chlorine atoms in CMS units. The copolymers, obtained in quantitative yields, were characterized by FT-IR and ^{1}H NMR spectroscopy, differential scanning calorimetry (DSC), and gel permeation chromatography (GPC). The composition of the cross-linked three-dimensional copolymer was determined by FT-IR spectroscopy. DSC showed that incorporation cyclic hydroxybenzoic acid substitute in the side chains of polymers increases their glass transition temperature. The new polymers were found to exhibit excellent bacteriocidal activities.

Keywords: 4-chloromethyl styrene, 4-hydroxybenzoic acid, pH-sensitive, antimicrobial polymer.

INTRODUCTION

Antimicrobial agent-bound polymers exhibit antimicrobial activities by slowly releasing active agents through hydrolysis, while some polymers are also antimicrobial by themselves[1]. Such polymers have certain advantages over low molecular weight active agents because they are more stable against volatilization, dissolution, and diffusion to the surface of the material to be protected.

Antimicrobial agents can be incorporated in linear or crosslinked carrier polymers via covalent bonds[2]. Linear polymers release the active agents faster than the crosslinked polymers because the duration period required for water penetration into the labile bonds and hydrolysis is normally shorter for linear polymers compared to crosslinked polymers[3]. As such linear polymers are more useful additives especially when blended with soluble polymers in organic solutions.

4-Chloromethylstyrene (CMS), also called p-vinyl benzyl chloride (VBC), is a monomer that can be reacted with a series of reagents to produce polymer with functional groups[4-7]. This monomer can polymerize or copolymerize before or after the functional groups reaction with benzyl chloride. The mobility of benzyl chloride bonds in poly(4-chloromethylstyren) (PCMS) and related copolymers allows their reaction with various nucleophilic reagents. Also functionalized PCMS and related copolymers have been widely used in different processes such as photosensitizers[8], solar energy storage[9], photoresist[10], nonlinear optics[11], and prodrugs in biochemical application[12].

One of the modifications of PCMS and related copolymers is achieved by nucleophilic substitutions of the chlorine atom, in this area is attaching suitable groups as a side chain.

4-Hydroxybenzoic acid (4-HBA), or p-hydroxybenzoic acid, is a phenolic derivative of benzoic acid. Substitution on either the carboxyl or phenolic hydroxyl groups may affect potency and toxicity, while placing the phenolic hydoxyl group meta- or para to the carboxyl group abolishes non-steroid anti-inflammatory drug (NSAID) activity[13]. Since metabolism affects pharmacological as well as toxicological effects of xenobiotics and drugs, the differentiation of structurally related metabolites is of biomedical interest[14]. 4-HBA and its esters[15] have also been reported to inhibit certain microbial and fungal growth.

In the current study, an intermediate copolymer was prepared, and its conversion into different polymers tested, including its antimicrobial activities. We first synthesized the copolymer and crosslinked copolymer of 4-chloromethyl styrene with styrene by radical polymerization. Then phenolic hydroxyl groups of 4-hydroxybenzoic acid (4-HBA) were attached to the resulting polymers by replacing the –Cl on the side-chain of chloromethyl styrene. The DSC analysis showed that the glass transition temperatures of polymers were higher than that of intermediate polymers due to hydrogen-bonding interactions.

EXPERIMENTAL

Materials

CMS (Aldrich, 90%), 1,4- divinylbenzene (Merck) and styrene (Merck) were distilled under reduced pressure to remove inhibitors, before use. The initiator of α,α-azobis (isobutyronitrile) (AIBN) (Merck) was purified by crystallization from methanol. 4-HBA was purchased from Aldrich (France).

Measurements

Infrared spectra were recorded with a 4600 Unicam FT-IR spectrophotometer as KBr pells. ^{1}HNMR spectra were run on a Bruker 400 MHz spectrometer at room temperature using $CDCl_3$ as a solvent. The molecular weights (M_W and M_n) were determined using a Waters 501-gel permeation chromatograph (GPC) fitted with 102 and 103 nm waters styragel columns. THF was used as an elution solvent at a flow rate of 1 mL/min, and polystyrene standard was employed for calibration. The differential scanning calorimetry (DSC) curves were obtained on a TGA/SDTA 851 calorimeter at heating and cooling rates of 10°C/min under N_2.

Copolymerization of 4-Chloromethylstyrene with Styrene: P-1 - P-6

For preparing of copolymers (P-1 - P-6), a mixture of 4-chloromethylstyrene with different amounts of styrene (as shown in Table 1), respectively, was dissolved in 15 mL of dioxan and was mixed with AIBN (1% molar) as a radical initiator, in a pyrex glass ampoule. The ampoule was degassed, sealed under vacuum, and maintained at 70 ± 1 ºC in a water bath, with stirring for about 48 h. Then the solutions were poured from ampoules into cooled methanol (about 4 ºC). The precipitates were collected and washed with methanol and dried under vacuum to yield (approximately 85%) of copolymers. For P-1 - P-6: ^{1}H NMR ($CDCl_3$, ppm) 1.1–2.1 (CH_2-CH), 4.54 (CH_2-Cl), 6.5–7.1 (Ar-H). FT-IR (KBr, cm^{-1}): 3081, 2921-2850, 1600-1492 (aromatic C=C).

Cross-linked Copolymerization: P-7

In Pyrex glass ampoule, a mixture consisting of 1 g (6 mmol) of 4-chloromethylstyrene, 0.68 g (6 mmol) of styrene and specific mol percent of CA (10%) was polymerized at 70 ^{o}C in a thermostatic water bath, using AIBN (1% molar) as initiator, and dried dioxane as solvent ([M]=1.0M). After the specified time (48 h), the precipitated network polymer was collected, washed with methanol and dried in vacuum. FT-IR (KBr, cm^{-1}): 3095, 2927-2875, 1600-1495 (aromatic C=C).

Attaching 4-HBA groups to copolymers: P-11-P-16

To a solution of copolymers (1 g) and pyridine in 25 mL anhydrous DMF was added excess amount of 4-HBA. The calculated molar ratio of copolymers, 4-HBA and pyridine was approximately 1:1.5:2. The reaction was heated at 90 ºC for 48 h under a nitrogen atmosphere, cooled to room temperature, and finally precipitated into ethyl ether. The precipitate was redissolved in DMF, and precipitated into ethyl ether, the precipitated was collected, washed with cold ether and water and dried in vacuum to yield (around 95%), (Scheme 1). ^{1}H NMR ($CDCl_3$, ppm): 1.3–2.2 (CH_2-CH), 4.3 (CH_2-O), 6.7–7.6 (Ar-H). FT-IR (KBr, cm^{-1}): 3500-2500, 1730, 1690, 1490–1600 (aromatic C=C).

Attaching 4-HBA groups to cross-linked copolymer: P-17

A mixture of 100 mg (0.7 mmol) 4-HBA and 81 mg (1.4 mmol) potassium hydroxide in 30 ml methanol was added with stirring to a suspension of P-7 (100 mg) in 10 ml methanol at room temperature. The reaction was heated at 60 ºC for 48 h and then the suspension is centrifuged and the yellow solid is washed several times with methanol before drying under vacuum. FT-IR (KBr, cm^{-1}): 3500-2500, 1690, 1490–1600 (aromatic C=C).

Amount of attaching of 4-HBA to cross-linked copolymer

The amount of 4-HBA attached in the P-17 was determined by an indirect method. After the finish reaction, the washings with methanol were collected and tested using UV-Vis spectroscopy. The different between the amount of 4-HBA initially employed and the 4-HBA content in the washings was taken as an indication of the amount of attaching (30 %).

Method of hydrolysis

The polymer-HBA conjugate (200 mg) was poured into 5 ml of aqueous buffered solution (pH 1) or SIF (pH 7.4) at 37 ºC and the mixture was conducted into a cellophane membrane dialysis bag. The bag was closed and transferred into a flask containing 25 ml of same buffer solution

maintained at 37 ºC. The external solution was continuously stirred and a 3-ml sample was removed at selected intervals and 3 ml of buffer was replaced. The quantity of released drug was analyzed by means of an UV spectrophotometer and determined from the calibration curve obtained previously under the same conditions.

Shake Flask Test

The biocidal activity test of the new copolymers by the contact method was performed for one type of bacteria species such as *E. coil* (KCTC 1682) [16]. A certain amount of pure DMF for a blank (control) sample or a solution (1.0 mL) of either 4-HBA or polymers in DMF (10 wt%) was transferred into each wide-mouth glass bottle. A bacterial culture suspension (5.0×10^3/mL) was added dropwise onto the above DMF solutions and DMF control, followed by the addition of 70 mL phosphate buffer solution (pH 7.4). After the bottles were incubated at 37° C for 24 h under shaking (150 rpm), 0.1 mL of each suspension was plated out on a Luria Broth (LB) agar. After incubation for 1 day, the number of the bacteria colony was counted. The percent reduction of the bacteria cells was calculated from the following formula:

$$\% \text{ reduction} = [(N_b - N_t)/N_b] \times 100$$

where N_t is the number of the bacterial cells recovered from the inoculated suspension which contains either 4-HBA or polymers in the bottle, and N_b is the number of bacteria recovered from the inoculated suspension which contains only DMF without the biocides in the bottle.

RESULTS AND DISCUSSION

For synthesis of new modified polymers, we used two methods: copolymerization and cross-linked copolymerization. In copolymerization, the 4-chloromethyl styrene (CMS) was copolymerized with styrene (in various mole ratios) in dioxane at 70 ^oC using AIBN as the radical polymerization initiator.

Because of the mobility of the benzyl chloride bonds in the resulted copolymers, nucleophilic substitutions were possible. Therefore, 4-HBA as a nucleophilic reagent was shown to be sufficiently reactive towards CMS units in copolymers with replacement of all the chlorine to give new copolymers with 4-HBA group attached to phenyl rings via methylene spacers. Due to ^{1}H NMR spectra data, with replacement of chlorine atoms with 4-HBA groups, the peak around 4.54 ppm corresponding to two methylene protons of benzyl chloride disappeared and new peak at 4.3 ppm corresponding to two methylene protons attached to 4-HBA group appeared.

The copolymer compositions were calculated from the ^{1}H-NMR spectra data. In the past few decades ^{1}H-NMR spectroscopic analysis has been established as a powerful tool for the determination of copolymer compositions because of its simplicity, rapidity and sensitivity [17]. Spectrum of copolymer P-1 in $CDCl_3$ is shown in Figure 1. The molar compositions of CMS and styrene in copolymer P-1 were calculated from the ratio integrated intensities of the peaks around 4.54 ppm, corresponding to two methylene protons of benzyl chloride in CMS units to the total area between 1.1-2.1 ppm, which were attributed to six protons marked by (#) in CMS and (*) in styrene. The molar compositions of CMS and styrene were calculated from Eqs. (1) and (2) where x and y were the mole fractions of CMS and styrene, respectively.

$$\frac{\text{Area at } 4.54}{\text{Area at } 1.1\text{-}2.1} = \frac{2x}{3x+3y} \qquad \text{Eqs. (1)}$$

$$x + y = 1 \qquad \text{Eqs. (2)}$$

A similar method was used to calculate the molar compositions of monomers in copolymers P-2 and P-3. The compositions of copolymers are presented in Table 1.

Due to the strongly hydrogen-bonded hydroxyl group, this band is spread over the range of 3500–2500 cm^{-1}. This absorption is one of the broadest absorptions in an infrared spectrum, and it is frequently more than 600 cm^{-1} wide, hence quite easy to recognize. The analysis of the IR spectra shows that with increase of pH from 2 to 8, the composite passes into the anion form, the band at 1730 cm^{-1} (the stretching vibrations of the carboxylic group) disappears, and in its place new absorption bands appear at 1595 and 1430 cm^{-1}, which are assigned to the stretching vibrations of the carboxylate anion COO^-.

Thermal Behaviour

We determined glass transition temperature (Tg) of all modified copolymers by the DSC analyses. The DSC scans showed that the presence of bulky 4-hydroxybenzoic acid group in the side chain increased the rigidity of copolymers and as a sample, lead to an increase in the Tg from 79 ºC for copolymer P-1 to 160 ºC for copolymer P-11 (Figure 2). On the other hand the introduction of a strongly polar carboxylic acid group can increase the Tg value because of the formation of internal hydrogen bonds between the polymer chains. Tg values of copolymers and modified by 4-HBA are shown in Tables 1 and 2, respectively.

As shown in Table 1 and 2, the higher Tg values for network copolymer probably related to the

introduction of cross-links, which would decrease the flexibility of the chains and the ability of the chains to undergo segmental motion, which would increase the Tg values [18]. The Tg value of the network copolymer with the 4-HBA (P-17) would be higher than Tg value of the P-7.

The solubility of copolymers

The resulting network polymer swells and become soft if it is exposed to solvents such as H_2O and most organic solvents without dissolving. The solubility behavior of the copolymers prepared in this study was determined for powdery samples in excess solvents at room temperature for 24 h and the results are listed in Table 3. It is shown that all the polymers only are soluble in polar organic solvents such as N-methyl pyrrolidone (NMP), N, N-dimethylformamide (DMF), N, N-dimethylacetamide (DMAC) and so on, the poor solubility of polymers in common solvents might be attributed to stiff units (Carboxylic acid) pendant group.

Swelling

Responsive polymers consisting of carboxylic acid is classic examples of pH-sensitive carriers that exhibit swelling transitions in response to changes in pH [19-21]. The composition of the polymer defines its nature as a neutral or ionic network and furthermore, its hydrophilic/hydrophobic characteristics. Ionic hydrogels, which could be cationic, containing basic functional groups or anionic, containing acidic functional groups, have been reported to be very sensitive to changes in the environmental pH. The swelling properties of the ionic hydrogels are unique due to the ionization of their pendent functional groups. Hydrogels containing basic functional groups is found increased swelling activity in acidic conditions and reduced in basic conditions but on the other hand pH sensitive anionic hydrogels shows low swelling activity in acidic medium but very high activity in basic medium.

In a typical test, the copolymers after having been soaked in various buffer solutions (pH 7.4 and pH 1) at 37 ºC for 8 hours were weighted after excess water was wiped off from the copolymers surface with filter paper. The swelling ratio (SR) of matrices was calculated using the following Eq. 3:

$$SW\ (\%) = [(W_s - W_d)/ W_d] \times 100 \qquad \text{Eq. 3}$$

Where, W_s and W_d represent the weight of swollen and dry samples, respectively. The swelling value of copolymers in pH 1 and pH 7.4 at 37 ºC are given in Table 2.

In these copolymers, an increase in the content of carboxylic acid in the copolymers resulted in less swelling in pH 1 but greater swelling in pH 7.4. This was because higher carboxylic acid content in the copolymer led to higher carboxylate anion concentration at high pH. In other words, the existence of hydrogen-bonding interactions between –COOH groups in the copolymer matrix results in a complex structure within the network, and so the movement of polymeric segments is restricted. This also accounts for minimum swelling of the copolymers in a medium of pH 1. However, when the sample is placed in a medium of pH 7.4, the almost complete ionization of –COOH groups present within the copolymer not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged $–COO^-$ groups. These two factors ultimately result in a greater increase in the water uptake.

As shown in Table 2, the swelling values of cross-linked polymers in both pH 1 and pH 7.4 are slower. In the network polymer with reticulated structure, diffusion of the water in the network's polymer is reduced and the swelling is slower.

4-HBA release from polymers

The chemical structure of the monomeric units along the polymer chain is an important factor in hydrolytic behavior of polymeric prodrugs. As shown in Table 2, the P-11-P-17 are pH-sensitive polymers. High different hydrolysis rate for these polymers at pHs 1 and 7.4 can be related to the number of carboxylic acid groups along the polymer chain. The number of carboxylic acid groups along the polymer chain response to decrease and increase hydrophilicity of polymer in pHs 1 and 7.4, respectively. In pH 7.4 solution with completed ionization and an increase in the hydrophilicity of the polymer, diffusion of the hydrolyzing agents on polymer increased and the hydrolysis rate increased. In P-17 with network structure diffusion of the hydrolyzing agents in the network's polymer is reduced and the hydrolysis rate is slower.

Antibacterial Test

The antibacterial activity of P-11- P-17 was investigated with the shake flask test method toward *E. coli* as a model. The number of bacterial cells in the bacterial culture suspension was 5.0×10^3/mL. After their contact with either bioactive agents (4-HBA) or polymers (P-11- P-16) in diluted phosphate buffer solution, the suspension were incubated at 37 ˚C for 24h, and the number of the bacteria colony was counted. The bacteria cells/mL was calculated by multiplying the number of colonies by the dilution factor. As shown in Table 4, the reduction in the specimens containing 4-HBA or polymers (P-11- P-16) was about 100%. This result indicates that the antibacterial activities of the polymers were similar to those of the

corresponding bioactive agents and excellent at least against *E. coli.*

As shown in Table 4, the P-17 with network structure, as it clearly shows that, is not a high antibacterial responsive. This is because diffusion of the hydrolyzing agents in the network's polymer is reduced and the hydrolysis rate is slower.

CONCLUSIONS

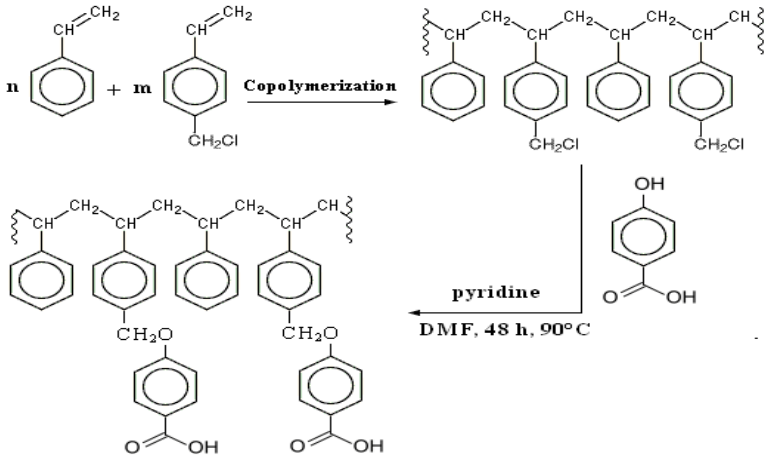
The copolymer and cross-linked copolymers were synthesized by free radical solution polymerization. The molar compositions of the obtained copolymers (P-1-P-6) were calculated by the ^{1}H NMR spectral method. Esters of 4-hydroxybenzoic acid such as methyl and ethyl, commonly known as parabens are widely used as preservatives. Founded on the esters of 4-hydroxybenzoic acid are widely used as preservatives, the 4-hydroxybenzoic acid group as side chains were linked to copolymers from the reaction between 4-HBA and benzyl chloride bonds to obtain the three new modified copolymers containing functional substituents. The ^{1}H NMR spectra data showed that 4-HBA groups were attached to phenyl rings via methylene spacers with replacement of all chlorine atoms. The existence of carboxylic acid in polymers not only increases the glass transition temperature (Tg) value but also give pH-sensitive properties to these polymers. In other hand, this preliminary result indicates that the new copolymers exhibited excellent bacteriocidal activities against *E. coli.* Due to the bactericidal activities of copolymers, these copolymers can be used as preservatives. Accordingly, this synthetic strategy can be conveniently used in synthesizing more bioactive polymers, as 4-chloromethyl styrene can react with any active agent containing hydroxyl group.

ACKNOWLEDGMENTS

The office of research vice chancellor Azarbaijan University of Tarbiat Moallem has supported this work.

Table 1: Molar composition and GPC data of copolymers

Copolymer	Molar composition of monomers in the feed (%)	Calculated from the ^{1}H-NMR		M_w	M_n	M_w/M_n	Tg(°C)
		(% mole) m	(% mole) n				
P-1: $(CMS)_m$ $(styrene)_n$	50: 50	49.3	50.7	16079	7308.6	2.2	79
P-2: $(CMS)_m$ $(styrene)_n$	43.5: 56.5	42	58	16490	8500	1.94	77
P-3: (CMS)m (styrene)n	30: 70	30	70	22344	11413	1.96	75
P-4: (CMS)m (styrene)n	25: 75	24.4	75.6	26122	16225	1.61	74
P-5: (CMS)m (styrene)n	16.7:83.3	16.1	83.9	22344	5923	3.76	73
P-6: (CMS)m (styrene)n	10:90	10.6	99.4	16225	7054.3	2.3	70
P-7 CMS)m (styrene)n	50: 50	----	----	----	----	----	165



Scheme 1: Preparation of copolymers and modification by 4-hydroxybenzoic acid

Table 2: Percent of swelling, 4-HBA release and glass transition temperature of modified copolymers

Polymers	maximum constant swelling (%)		maximum percent of 4-HBA release (6 day)		Tg (°C)
	pH 1	pH 7.4	pH 1	pH 7.4	
P-11	15	55	45	90	160
P-12	13	52	43	86	155
P-13	10	45	38	77	145
P-14	10	43	35	73	137
P-15	8	33	31	65	122
P-16	8	26	30	61	116
P-17	5	23	19	45	185

Table 3: The solubility of copolymer

Solvent	P-11	P-12	P-13	P-14	P-15	P-16	P-17
Water	-	-	-	-	-	-	-
THF	+	+	+	+	+	+	-
Acetone	±	±	+	+	+	+	-
DMF	++	++	++	++	++	++	-
NMP	++	++	++	++	++	++	-
DMAC	++	++	++	++	++	++	-

++ Soluble, + soluble on heating, ± partially soluble on heating, insoluble

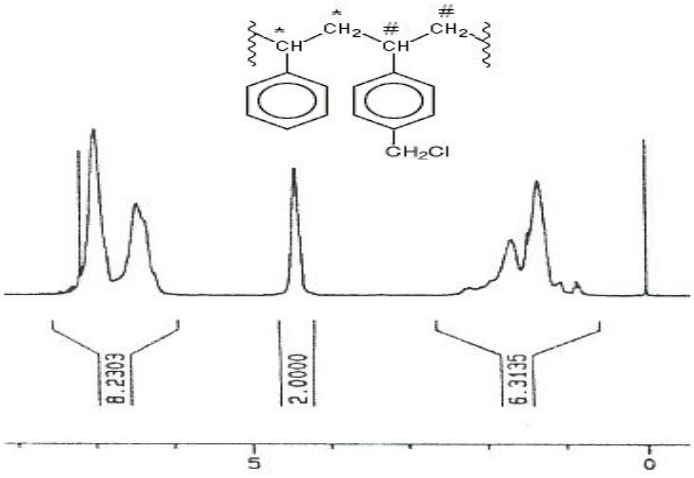


Fig. 1: ^{1}H NMR spectrum of P-1 in $CDCl_3$

Table 4: Biocidal activity of 4-HBA and polymers against *E. coli*, based on the shake test

Bacteria	Sample	Bacteria/mL (24 h contact)	Reduction (%)
E.coil	Blank	3.5×10^7	---
	4-HBA	0	100
	P-11	0	100
	P-12	0	100
	P-13	1.7×10^3	99.995
	P-14	3.3×10^3	99.99
	P-15	8.47×10^5	97.58
	P-16	2×10^6	94.29
	P-17	2.3×10^7	34.29

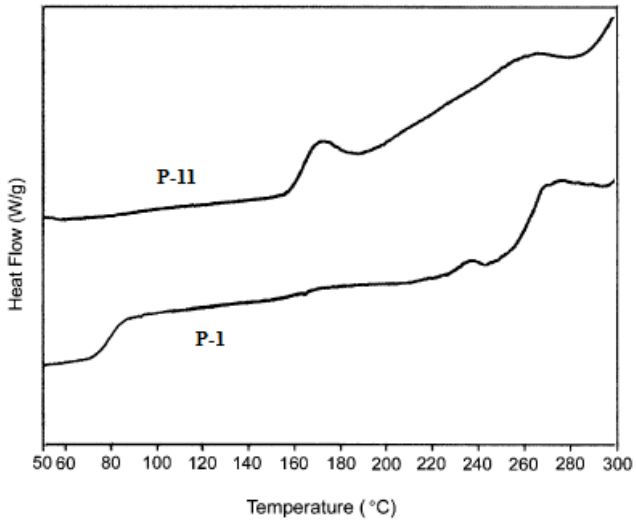


Fig. 2: DSC curves of copolymers P-1 and P-11

REFERENCES

1. Sauvet G, Dupond S, Kazmierski K, Chojnowski J, Biocidal polymers active by contact. V. Synthesis of polysiloxanes with biocidal activity, J. Appl. Polym. Sci. 2000; 75: 1005-1012.
2. Nayaka P and Lenka S.Polymeric Materials Encyclopedia, Salamone; J. C. CRC Press: New York, 1996.
3. Smith PK, Badger TJ, Antimicrobial and oxidative co-polymer. US Pat: 6921739, 2005.
4. Montheard JP, Jegat C, Camps M, Vinylbenzylchloride (chloromethylstyrene), polymers, and copolymers: recent reactions and applications, J. Macromol. Sci. Polym. Rev. 1999; 39(1): 135-174.
5. Yus M, Gomez C, Candela P, The first direct formation of an organolithium reagent on a soluble polymer by chlorine–lithium exchange: functionalised linear polystyrene, Tetrahedron Lett.2001; 42: 3977-3979.
6. Safa KD, Babazadeh M, Namazi H, Mahkam M, Asadi MG, Synthesis and characterization of new polymer systems containing very bulky tris(trimethylsilyl) methyl substituents as side chains, Eur. Polymer. J. 2004; 40: 459-466.
7. Assadi MG, Mahkam M, Tajrezaiy Z, Modification of styrene polymer with organosilicon groups, Heteroatom. Chem. 2007; 8(4): 414-420.
8. Suzuki M, et al. Synthesis, characterization and application of a novel polymer solid photosensitizer, Chem. Commun. 2000; 3: 213-214.
9. Taoda H, Hayakawa K, Kawasae K, Yamakita H, Photochemical conversion and storage of solar energy by azobenzene, J. Chem. Eng. Japan. 1987; 20: 265-270.
10. Cheng CM, et al. Synthesis of 1-(vinylbenzyl) thymine, a novel, versatile multi-functional monomer, J. Polym. Sci. Part A: Poly. Chem. 1995; 33: 2515-2519.
11. Noel C, Ching KC, Large M, Reyx D, Kajzar F, Synthesis and characterization of polymers containing 4-cyanobiphenyl-based side groups for nonlinear optical applications, Poly (*p*-chloromethylstyrene) derivatives, Macromol. Chem. Phys. 1997; 198: 1665-1678.
12. Tabrizi MH, Davaran S, Entezami AA, Synthesis of diclofenac polymeric prodrugs and their hydrolysis reactivity, Iran. Polym. J. 1996; 5: 243-249.
13. Taka MJ, Topić DV, FT-IR and NMR spectroscopic studies of salicylic acid derivatives. II. Comparison of 2-hydroxy- and 2,4- and 2,5-dihydroxy derivatives, Acta. Pharm. 2004; 54: 177-191.
14. Waksmundzka-Hajnos M, Chromatographic separations of aromatic carboxylic acids, J. Chromatogr. B. 1998; 717: 93-118.
15. Jeong JH, Byoun YS, Ko SB, Lee YS, Chemical modification of poly(styrene-alt-maleic anhydride) with antimicrobial 4-aminobenzoic acid and 4-hydroxybenzoic acid, J. Ind. Eng. Chem. 2001; 5: 310-315.
16. AATCC TECHNICAL MANUAL. 2004, 79, 1-459.
17. Mahkam M, Assadi MG, Mohammadzadeh R, Synthesis and characterization of crosslinked polyacrylates containing cubane and silyl groups, Macromol. Res. 2006; 14(1): 34-37.
18. Mahkam M, Sharifi N, Entezami AA, Regulation of conttolled releas of Ibuprofen from crosslinked polymer containing cubane as a new crosslinking agent, J. Bioact. Compat. Polym. 2000; 15, 396-405.
19. Lowman AM, Morishita M, Kajita M, Nagai T, Peppas NA, Oral delivery of insulin using pH-responsive complexation gels, J. Pharm. Sci. 1999; 88(9): 933-937.
20. Bartil T, Bounekhel M, Cedric C, Jerome R, Swelling behavior and release properties of pH-sensitive hydrogels based on methacrylic derivatives, Acta. Pharm. 2007; 57: 301-314.
21. Haglund BO, Kenneth RJ, Himmelstein J, An in situ gelling system for parenteral delivery, J. Control. Rel. 1996; 41: 229-235.