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Microwave-Assisted Synthesis and Characterization of Poly(1-Azabicyclo[4.2.0]octane) and Its Water-Soluble Derivatives

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Abstract

In this study we offer a new method to synthesize positively charged poly(1-Azabicyclo[4.2.0]octane)-common name is polyconidine- and its water-soluble polyampholyte derivative synthesized with bromoacetic acid using microwave irradiation as a model carrier system for biological macromolecules like peptides, proteins and etc.

1-Azabicyclo[4.2.0]octane containing unsubstituted four-membered azetidine ring was synthesized using microwave-assisted synthesis method and used as monomer in cationic ring-opening polymerization. Different alkyl halide derivatives were used as initiators to start polymerization. Microwave-assisted synthesis of polyconidine derivatives was carried out by bromoacetic acid using microwave irradiation. Characterization of polyconidine and its derivatives was achieved by size-exclusion chromatography (SEC) with on line quadruple detection system, ATR FT-IR and UV Spectroscopy.

Key Words: Polyconidine, microwave-assisted polymer synthesis, cationic ring-opening polymerization.

INTRODUCTION

In recent years there have been made so many research which are intended for developing functional biopolymer systems. In most of the studies until now, it is emphasized on the synthesis, characterization and the diversity of biologically featured polyelectrolytes [1]. Since polyelectrolytes have positive and/or negative charges, they are intended to use in biological systems. The synthesis and modification of a biodegradable polyelectrolyte, poly(1-Azabicyclo[4.2.0]octane) (PC) which has an heteroatom in its structure was explained.

A polymeric tertiary amine is available by cationic ring-opening polymerization of 1-Azabicyclo[4.2.0] octane (C). The polymerization of bicyclic monomer containing azetidine ring, C, proceeds without appreciable transfer and/or termination. The synthesis and homopolymerization of this monomer was first published in 1960 [1]. 2-(2-Hydroxyethyl) piperidin was used as starting compound instead of

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2-(β-Hydroxyethyl) pyridine. Several authors studied the mechanism and kinetics of the polymerizaton [1, 2]. More recently the NMR spectra and some details about the structure and properties of this polymer were published [3]. In all of these studies the synthesis of C and its polymers were carried out by conventional organic synthesis methods. The synthetic application of azetidine polymerization is limited, however, due to the difficulties with monomer synthesis. The closure of 4-membered ring is much more difficult than the closure of 3-,5-, or higher member rings [4-6]. So after a long-term work scientists have obtained only a small amount of final product conidine [7-10].

More recently microwave-assisted organic synthesis is a new developed method to obtain more efficient compounds in a short reaction time period. Today we offer a novel synthesis route for synthesis both C and PC by using microwave irradiation to increase the amount of C and PC. As the second goal of our study we have obtained water-soluble derivatives of PC's which have several molecular weights with quaternization of tertiary nitrogen atom in the polymer chains. PC is considered to use in biological systems. So it was modified with bromoacetic acid which gives polyampholyte derivative of PC. Positive and negative charges are on the same polymer chains.

Thereafter, polymers and their derivatives having different molecular weights were synthesized and the characterization of these polymers were done by SEC with on line quadruple detection system (light-scattering, UV, refractivity index and viscosity), ATR FT-IR and UV spectroscopy [11].

It is determined that molecular weights of the polymers were affected by the change in the amount and the type of initiators used and also from the polymerization media [12-13].

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were obtained from commercial sources. 2-(2-hydroxyethyl) piperidine (C_7H_5NO) was from Acros Organics and Calcium oxide (CaO), Calcium chloride ($CaCl_2$), Ethyl bromide (C_2H_5Br), Trimethylsilane bromide $(CH_3)_3SiBr$, t-buthyl bromide, benzoyl chloride and methyl iodide were purchased from Sigma. All solvents used were from Merck.

Solvent Preparation

All solvents and starting materials should be dried before using. 100 g of CaO was remained in an oven at 600 ºC for 6 hours for dried ethanol. Hot CaO was added to 500 mL of ethanol. Ethanol was distillated for 6 hours and dry ethanol was obtained.

200 g $CaCl_2$ was added to 600 mL of chloroform. It was retained during one night. $CaCl_2$ was filtered and 150 g $CaSO_4$ was added to chloroform. Suspension mixture was refluxed for three hours. Mixture was distillated for 3 hours and dry chloroform was obtained.

Although 2-(2-hydroxyethyl) piperidine was from a commercial source, it should be purified with vacuum distillation. This step is very important for obtaining more purified reaction conditions. 60 g of it was distillated with vacuum distillation.

The synthesis of 2-(2-chloroethyl) piperidine hydrochloride salt

Fresh distilled 2-(2-hydroxyethyl) piperidine was reacted with $SOCl_2$ to obtain 2-(2-chloroethyl) piperidine hydrochloride salt. 60 g of 2-(2-hydroxyethyl) piperidine dissolved in 90 mL of $CHCl_3$ was added to 78 mL of $SOCl_2$. Due to the formation of HCl this reaction should be carried out slowly and the temperature should be adjusted to 0ºC. After reaction completed temperature was

increased to 25ºC and mixture was stirred for 2 hours at this temperature. While mixture was stirring continuously 70 mL of ethanol was added to the reaction vessel drop by drop. Mixture was irradiated with 250 Watt microwave energy for half an hour and maximun temperature was 160ºC. During microwave synthesis all precipitate dissolved. After this reaction mixture was refluxed for 2 hours. Temperature was decreased to the room temperature. After one night obtained chloride salt was cleaned with the mixture of ethanol and diethyl ether. Off white 2-(2-chloroethyl) piperidine hydrochloride precipitate was dried under vacuum. ATR FT-IR spectrum of 2-(2-chloroethyl) piperidine hydrochloride is given in Figure 3 (Shimadzu IRPrestige-21 Spectrometer).

The synthesis of C

36.4 g of 2-(2-chloroethyl) piperidine hydrochloride was dissolved in 1.6 L distillated water and 16.8 g of NaOH was added to the solution. Solution was refluxed for 2 hours at 65 ºC. 129.76 g of KOH was added to the mixture by stirring until all KOH was dissolved (pH of the mixture was 13). Mixture was irradiated with 250 Watt microwave energy for an hour and maximun temperature was 185 ºC. Mixture was distillated by water steam distillation and eight distillate each one is 800 mL was collected. Distillation was carried out until pH came to the 7. 40 g of KOH was added to every distillates and dissolved. Distillate was extracted with 100 mL of diethyl ether for six times. Ether phases of extraction were collected and distillated by fractionated distillation. Ether was fully removed and C was obtained. ATR FT-IR spectrum of C is given in Figure 4 (Shimadzu IRPrestige-21 Spectrometer). 6.8 g of C was obtained after this steps and the reaction output was 19%.

Polymerization of C

Four similar types of initiators (R-X) was chosen for cationic ring-opening polymerization of C. All reactions were carried out by microwave energy. 250 Watt of microwave energy was used for an hour. Temperature was 100 ºC. Mole ratios of initiator/monomer was 1/1000 for all reactions. All polymers were precipitated by water. PC's having different molecular weights and physicochemical characteristics were obtained. Table 1 indicates these parameters for four different system.

The synthesis of water-soluble polyampholyte derivative of PC

PC is a positively charged polyelectrolyte and it is not soluble in water. We decided to make a modification of PC to obtain a water-soluble polymer derivative. We decided to use a reagent having hydrophilic groups in its structure. Bromoacetic acid was used as a carboxy group carrier reagent. Modification of PC was also carried out by our microwave-assisted synthesis method. All polymers were reacted with extreme amount of bromoacetic acid and modified polymers were precipitated with cold ethyl acetate.

Characterization of monomer and obtained polymers

All synthesis stages of monomer were checked by ATR FT-IR Spectroscopy. Figures from 1 to 5 indicate all intermediates. UV spectrums of PC synthesized with four different initiators are shown in Figure 1. Physicochemical characteristics of polymers will be discussed on the basis of the experimental results obtained by the size-exclusion chromatography (SEC) with on line quadruple detection system: UV absorption (UV), refractive index (RI), right angle light scattering (LS) and viscosity (VIS) detectors. Viscotek TDA 302 detector system with refractive index (660 nm), right angle light scattering (670 nm) and four-capillary differential viscometer detectors were used for on-line SEC signal detection. A separate UV detector obtained from Viscotek was connected to this detector system and the detectors were in the

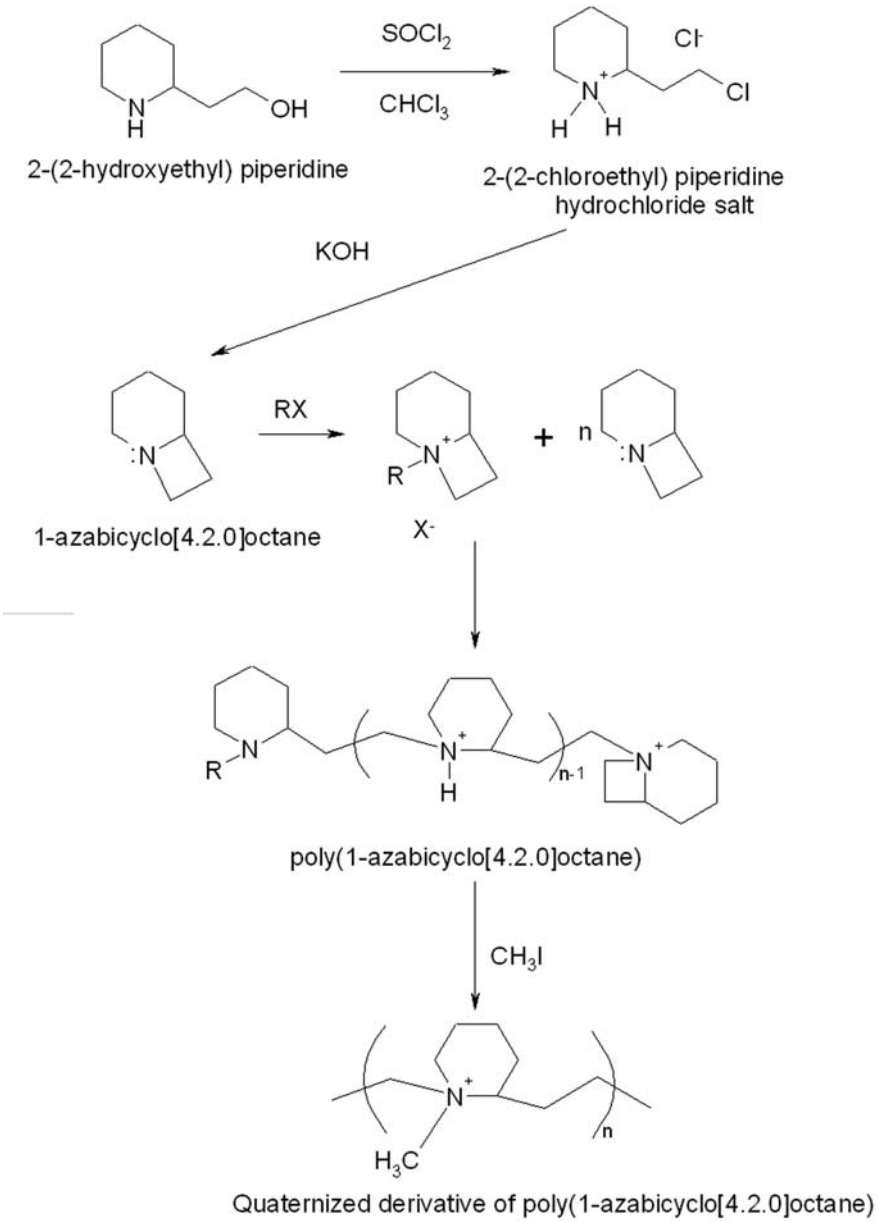

Figure 1. All synthesis reactions from 2-(2-hydroxyethyl) piperidine to PC.

following order: UV–LS–RI–VIS. 0.2 µm nylon pre-filter was used between the column and detectors. HPLC pump, degasser and autosampler with 100 µL injection loop were built-in Viscotek GPCmax VE 2001 pump system, which is connected to the detectors. OmniSEC 4.1 software was used for the acquisition and analysis of SEC data.

Microwave-enhanced reactions were carried out by MicroSYNTH of Milestone.

RESULTS AND DISCUSSION

PC and its bromoacetic acid derivative are considered to be a new developed synthetic polyelectrolytes for using in liver organisms. PC was synthesized in 1960's by using conventional organic synthesis methods. Investigations have showed that PC is not a toxic polymer system. In this study we have developed a new synthesis method using microwave energy. Microwave-assisted organic synthesis is a newly developed method. After 1990's this method has been started to be used for organic synthesis and polymer synthesis. Quaternization of PC with bromoacetic acid makes its water-soluble

polyampholyte derivative because of its carboxy groups. This charge equilibrium is also very important for polymer characteristic. Charges in the polymer chains determine polymer attitude in physiological fluids. The number of carboxy groups makes stick conformation of polymer chains.

The cationic polymerization of cyclic amines which are known to provide living systems with small anions like F^-, Cl^- or Br^-. Because of this, it was decided to use initiators presented in Table 1. M_W, polydispersity values, Mark-Houwink-Kuhn constants and hydrodynamic radius values obtained from SEC with on line quadruple detection systems which are initiated with four different initiators were shown in Table 1. All of the initiator/monomer rates were 1/1000. Since the counter ions can stop the chain growth the amount of initiators were released very small. Polydispersity values of each polymers were close one another. This condition is appropriate for living type polymerization because polydispersity values of living type polymers are closer to one.

It is known that the values of exponent ''*a*'' of the Mark–Kuhn–Houwink equation, $[\eta]=MK^a$, suggests a shape model of soluble polymer particles. The quantitative determination of polymer chain conformation relies on the measurement of Mark–Houwink exponent ''*a*'' in this equation. Because [η] and M_W values in SEC-LS–VIS are measured directly from detector signals, the precision of determining the Mark–Houwink *a* value by quadruple detector SEC is significantly better than any other technique. Mark-Houwink constants (*a*) of four different polymers are between 0.556 and 0.650. It is demostrated that polymer chains have various conformation between coil type and stick type. This conformations are important for interactions between side chain groups of modified polymers (carbonyl groups) and biological macromolecules like peptides and proteins which have free amine groups.

Hydrodynamic radiuses (R_h) of four polymers initiated by different initiators have defined that when the weight average molecular weights of polymers were increased R_h values of these polymers were

Table 1. Physicochemical values (M_n, M_W, M_W/M_n intrinsic viscosity, R_h, a, Mark-Houwink logK) obtained from size-exclusion chromatography (SEC) with on line quadruple detection system.

Initiator	CH_3I	Benzoyl chloride	TMS-Br	t-butyl bromide
Peak RV (ml)	10.523	11.192	10,523	11,367
Mn (Daltons)	20279	9831	20953	6304
Mw	29556	12626	31802	8032
Mz	39436	16114	43694	10086
Mp	26914	10713	28578	6916
Mw/Mn	1457	1,284	1,518	1,274
IV (dl/g)	0.4747	0,3024	0,4300	0,1713
Rh (nm)	5.876	3,756	5,808	2,736
Mark-Houwink a	0.607	0,556	0,651	0,613
Mark-Houwink logK	-3,029	-2,856	-3,287	-3,151
Initiator/monomer rate	1:1000	1:1000	1:1000	1:1000

also increased. Since polymer chains become more longer after the polymerization, this condition is quite normal.

As all initiator/monomer ratios are same for all polymers the weight average molecular weights of polymers are different from one another. This condition is related to typical characteristic of cationic ring-opening polymerization and living polymers. The type of initiator and also counter ions existed in the polymerization media are important for cationic ring-opening polymerization. Alkyl halides (R-X) were used for the polymerization of conidine. Partial positively charged alkyl group of initiator is bound to nitrogen atom of monomer and quarternary ammonia cation is obtained. This is the first active center of polymer chain. There is two possible nucleophylic attack to this quaternary ammonia cation. One is monomer which is quite nucleophylic and the other one is counter ion originate from initiator. There is a nucleophylic competition between monomer and counter ion. When counter ion is more nucleophylic than monomer the ionic polymerization stops in a short

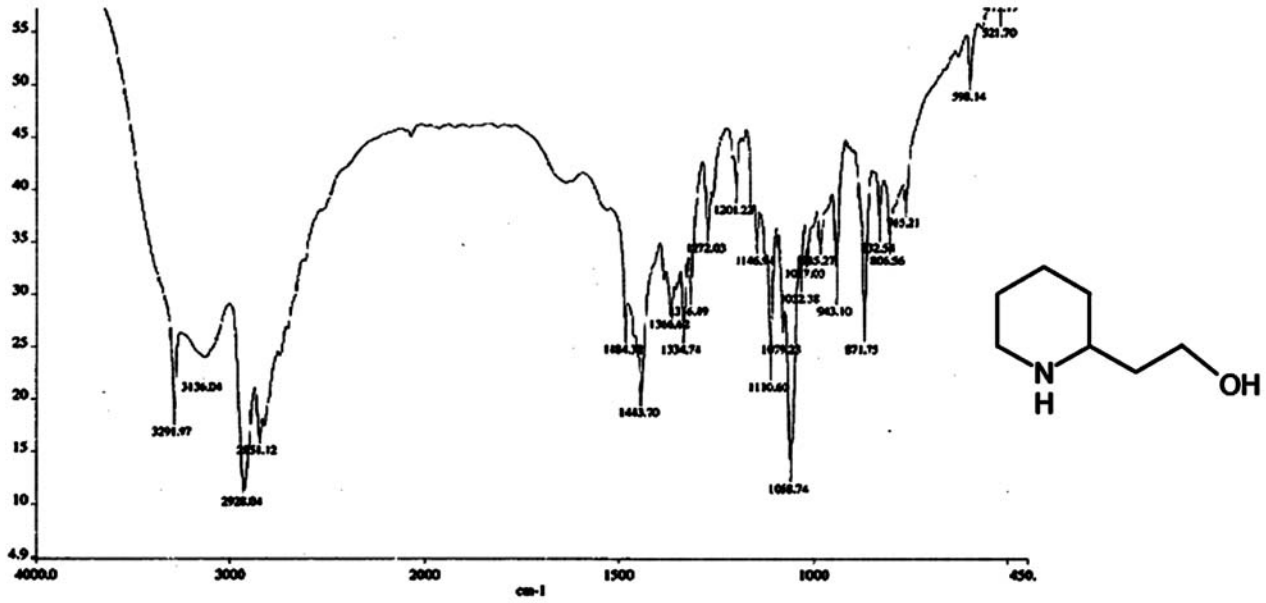

Figure 2. ATR FT-IR spectrum of starting compound 2-(2-hydroxyethyl) piperidine.

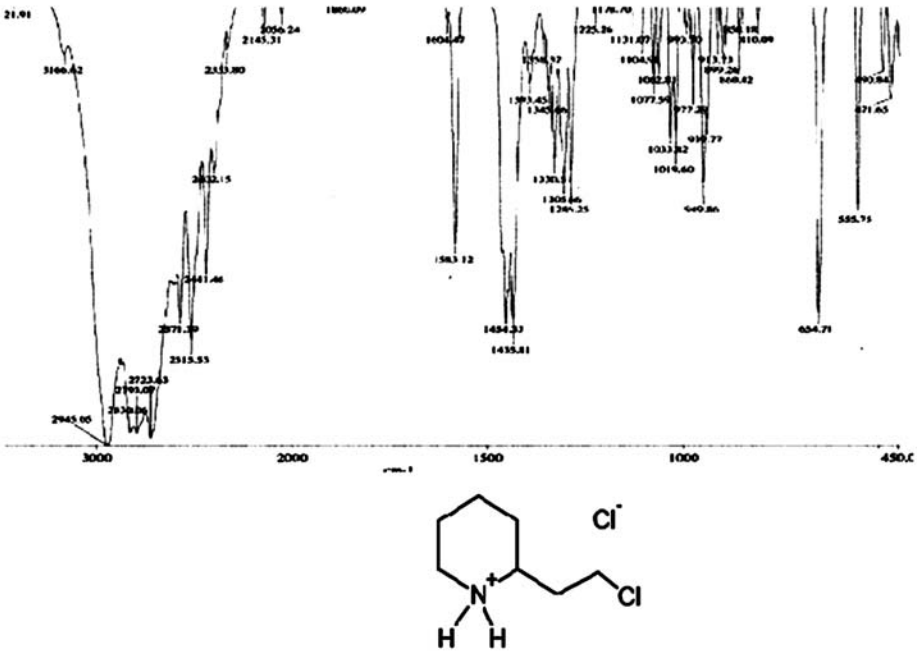

Figure 3. ATR FT-IR spectrum of 2-(2-chloroethyl) piperidine hydrochloride salt.

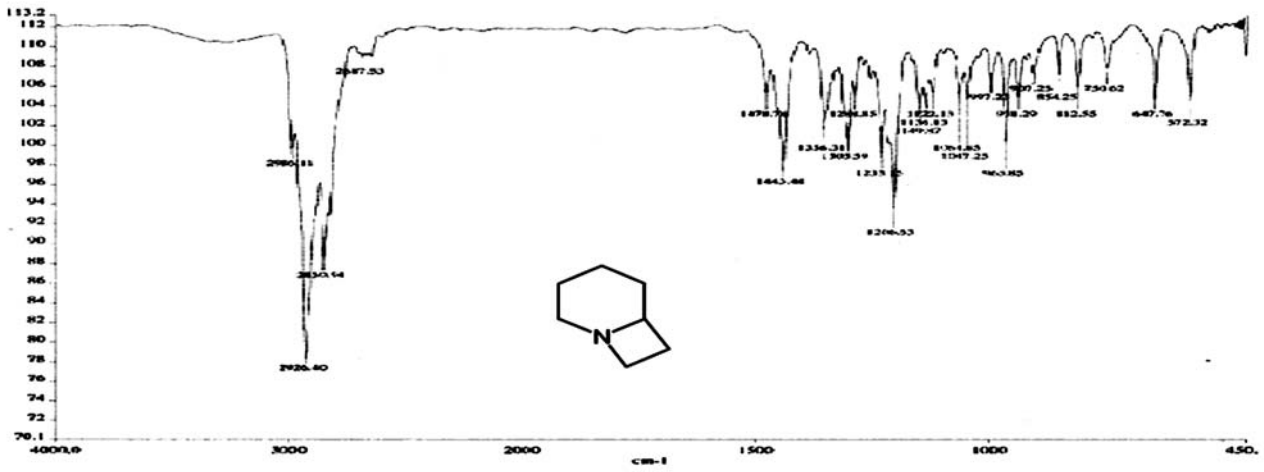

Figure 4. ATR FT-IR spectrum of C.

time period. In this state polymer chains are not often grown and so average molecular weight of polymers are quite reduced. However nucleophylic characteristic of counter ion is crucial for the propagation of polymer chains. The M_W values strongly indicated that PC's initiated with more stronger nucleophyl.

ATR FT-IR spectrums are useful results for determination of the synthesis route. Figure 2 and Figure 3 indicate the differences between starting compound and chloride salt of it. A C-Cl band at 654 cm^{-1} indicates that chloride is bounded to alkyl group of compound. FT-IR spectrum of monomer is given in Figure 4. This spectrum is quite simple according to starting compound and chloride salt of it. This condition was considered normal. Because closing of azetidine ring increased the number of methylene groups. As a result of this 2900 cm^{-1} band is more strong. Also band at 654 cm^{-1} was disappeared after ring closing reaction. After all these investigations we offer a positively charged polymer (PC). This polymer may be used as a carrier for negatively charged macromolecules. Also it can be a candidate for DNA targeting applications to the cell. Our investigations carry on for these kind of studies in our laboratuaries.

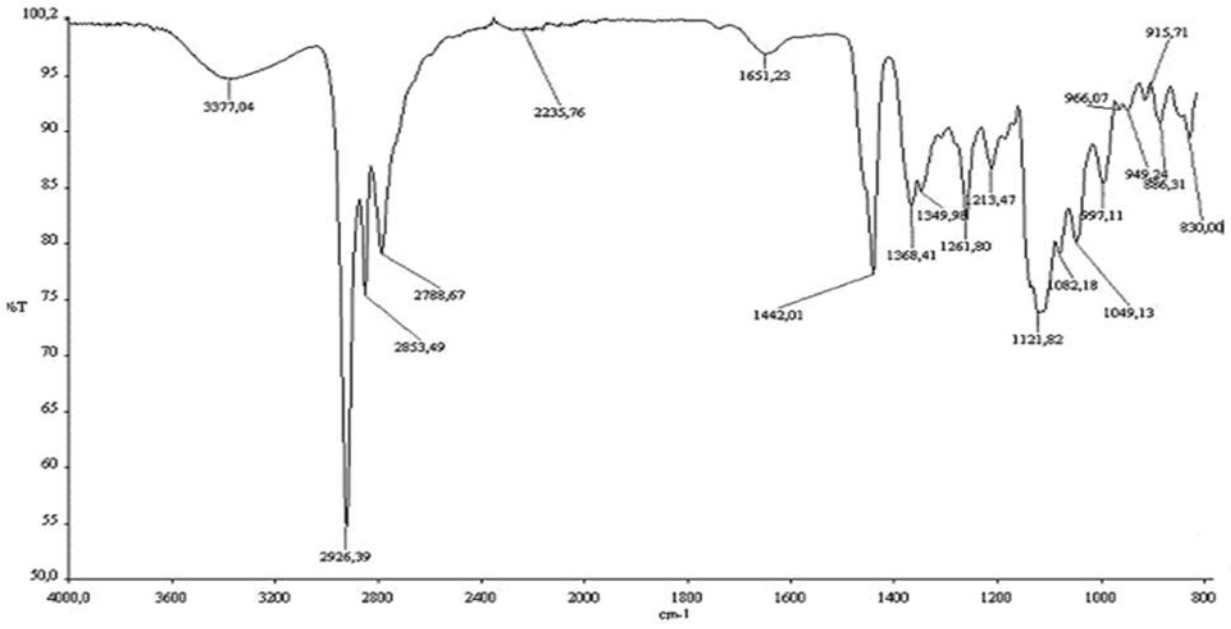

Figure 5. ATR FT-IR spectrum of PC initiated with CH_3I.

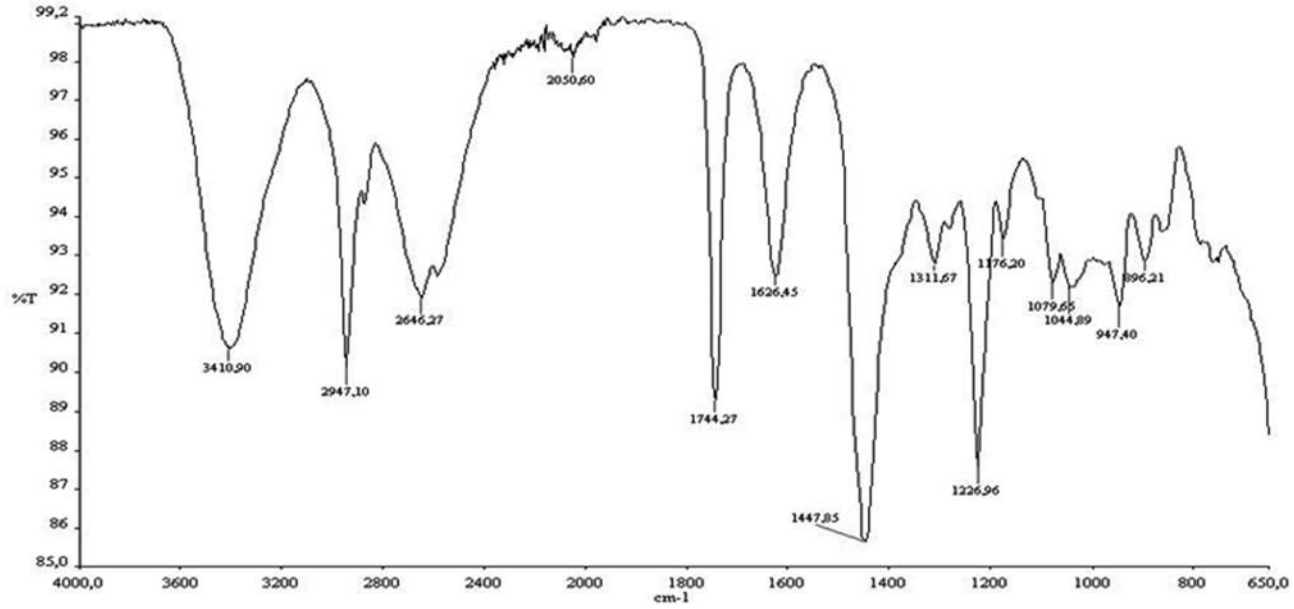

Figure 6. ATR FT-IR spectrum of PC modified by bromoacetic acid with microwave-assisted organic synthesis.

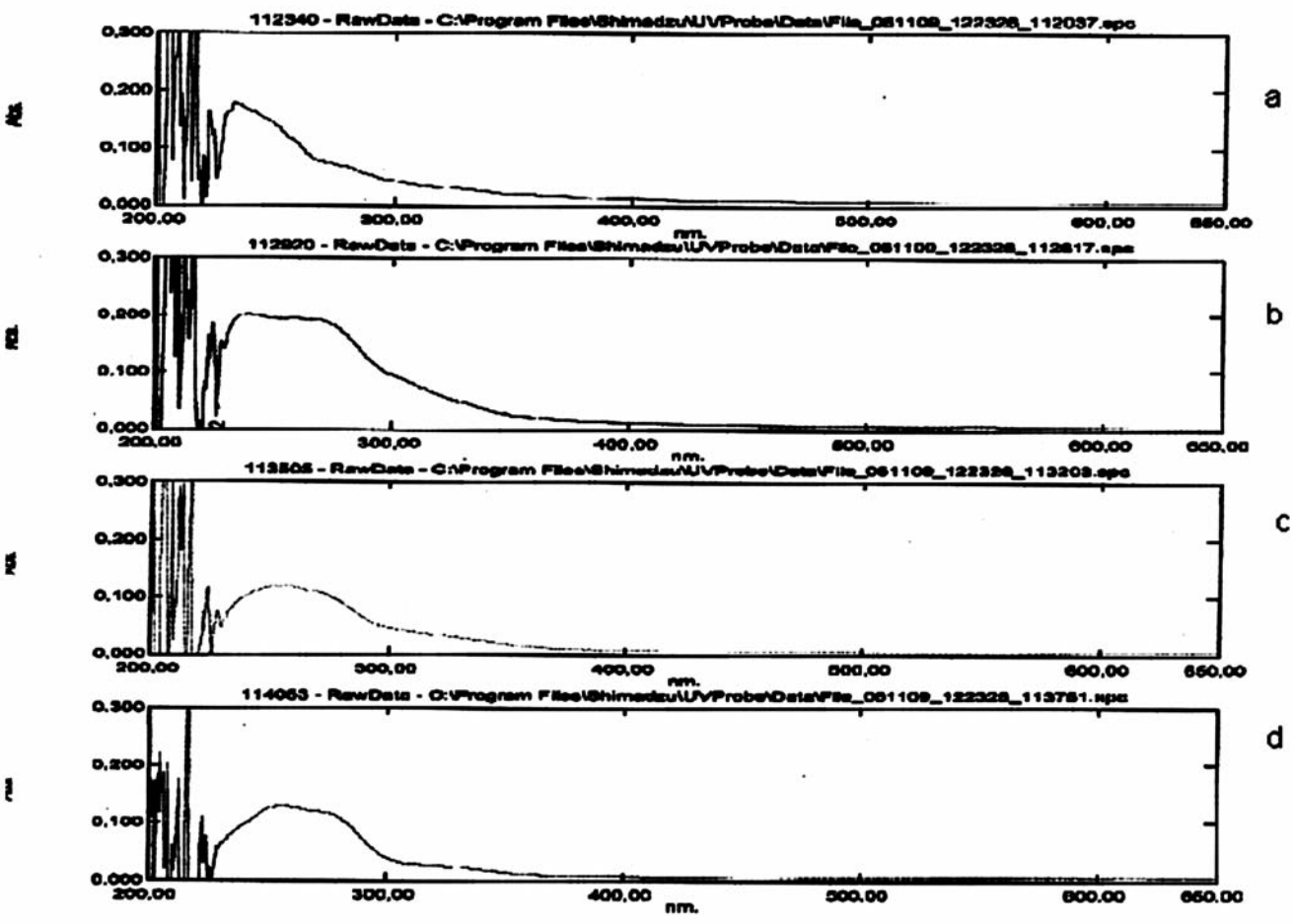

Figure 7. UV spectra of PC initiated with four different initiators. (a) CH_3I, (b) benzoyl chloride, (c) trimethylsilane bromide and (d) t-butyl bromide. (mole ratios of initiator/monomer were 1/1000).

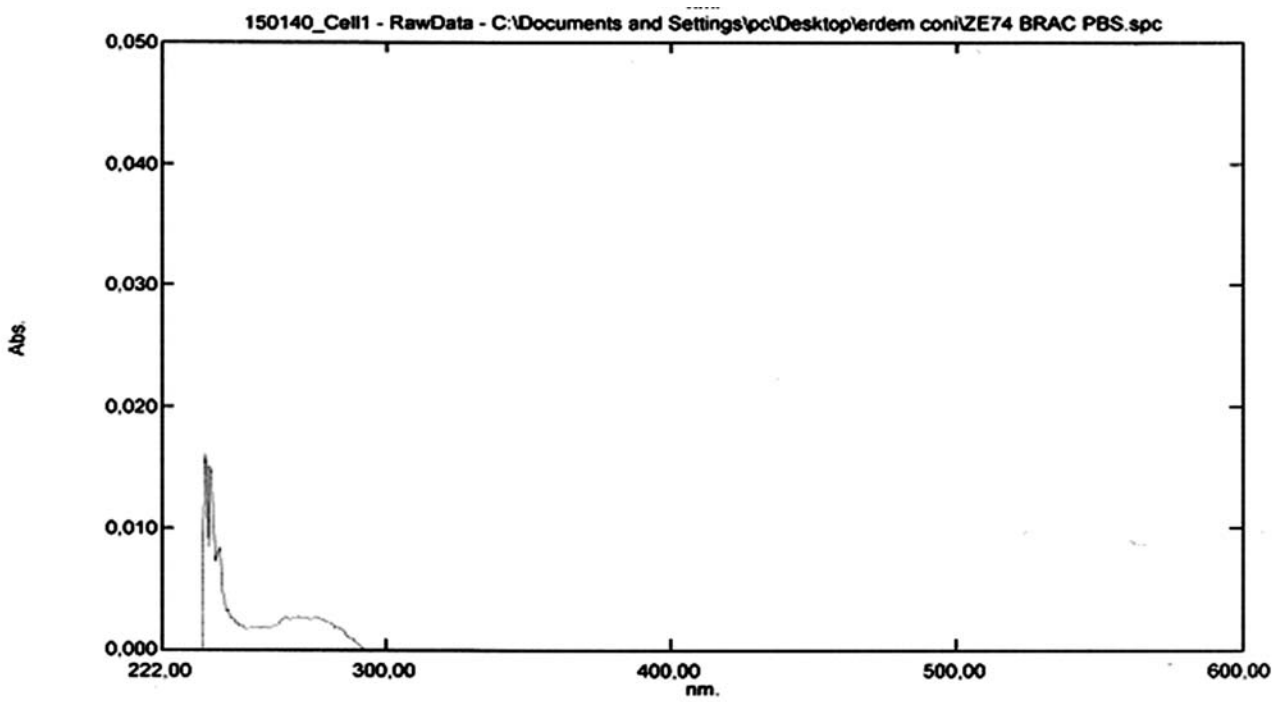

Figure 8. UV spectrum of bromoacetic acid derivative of PC.

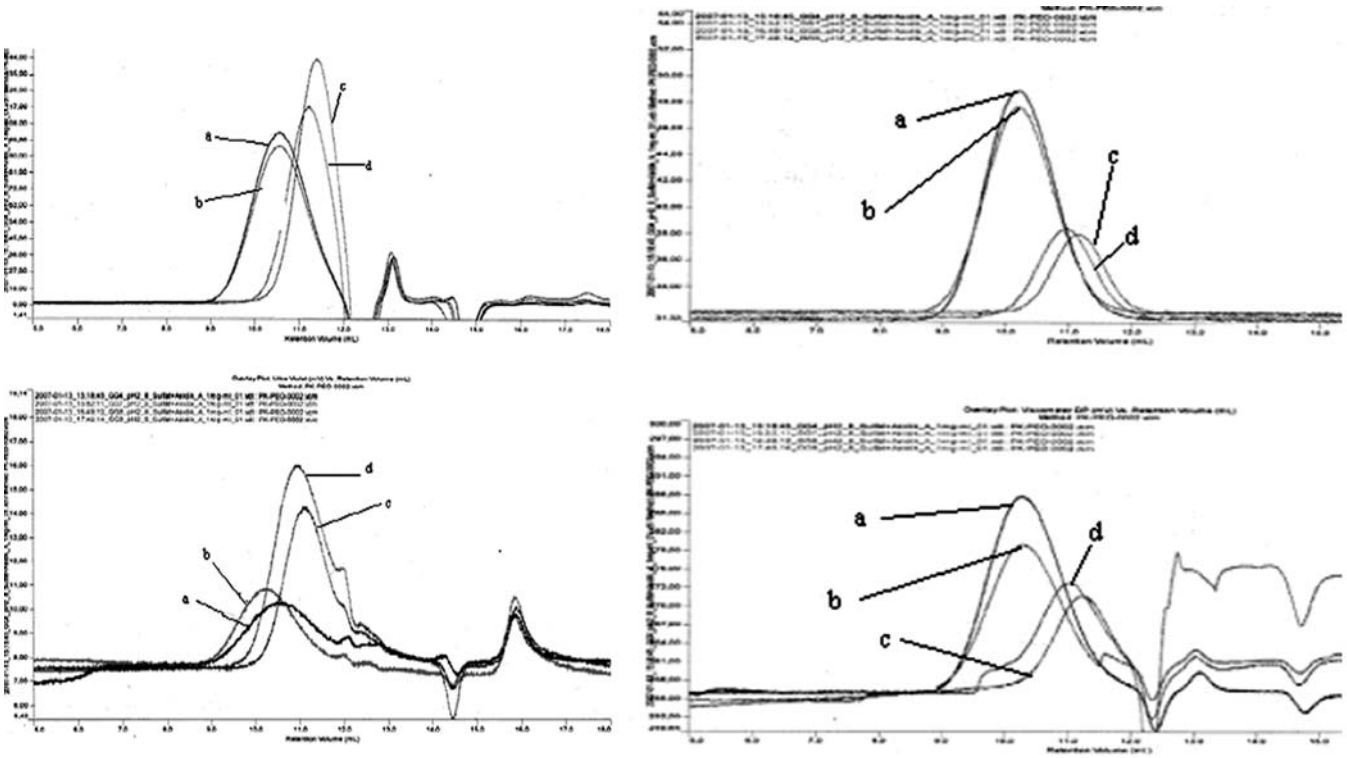

Figure 9. Refractive index, UV, light scattering and viscosity chromatograms of polyconidine samples which started with different initiators (mole ratios are 1/1000 of initiator and monomer). Methyl iodide (a), trimethysilane bromide (b), t-buthyl bromide (c), benzoil chloride (d).

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