Degradable copolymers with incorporated ester groups by radical ring-opening polymerization using atom transfer radical polymerization

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This paper is dedicated to memory of an outstanding scientist and a great friend and collaborator Prof. Andrzej Duda – on the occasion of his premature passing away.

Abstract: Preparation of degradable materials using reversible deactivation radical polymerizations (RDRP) is of particular interest for biomedical applications. In this paper we report preparation of degradable copolymers of 2-methylene-4-phenyl-1,3-dioxolane (MPDL), monomer which undergoes ring-opening reaction and forms ester bond upon radical polymerization, with hydrophobic and hydrophilic methacrylate monomers using atom transfer radical polymerization (ATRP). Copolymers composition and degradation were evaluated upon varied temperature and monomer type.

Keywords: degradable materials, reversible deactivation radical polymerizations, copolymers of 2-methylene-4-phenyl-1,3-dioxolane, atom transfer radical polymerization.

Degradowalne kopolimery zawierające wiązania estrowe otrzymywane metodą polimeryzacji rodnikowej z otwarciem pierścienia w polimeryzacji rodnikowej z przeniesieniem atomu

Streszczenie: Otrzymywanie materiałów degradowalnych metodą polimeryzacji rodnikowej z odwracalną dezaktywacją (RDRP) ma szczególne znaczenie w zastosowaniach biomedycznych. W artykule opisano otrzymywanie degradowalnych kopolimerów 2-metyleno-4-fenylo-1,3-dioksolanu (MPDL). Monomer ten ulega reakcji otwarcia pierścienia, a następnie tworzy wiązania estrowe z hydrofobowymi i hydrofilowymi monomerami metakrylanowymi w polimeryzacji rodnikowej z przeniesieniem atomu (ATRP). Zbadano wpływ rodzaju monomeru i temperatury polimeryzacji na skład oraz degradację powstających kopolimerów.

Słowa kluczowe: materiały degradowalne, polimeryzacja rodnikowa z odwracalną dezaktywacją, kopolimery 2-metyleno-4-fenylo-1,3-dioksolanu, polimeryzacja rodnikowa z przeniesieniem atomu.

Degradability is one of the most important requirements for materials targeting biomedical applications [1–7], including degradable sutures, drug delivery systems, hydrogels, wound dressings and cell growing platforms [1–3, 8–11]. Indeed, designed degradable polymers have become the material of choice for drug/biomolecule delivery due to their initially large hydrodynamic size, solubility, stealth properties, and stimuli responsiveness [5–7, 12–15]. These degradable materials can be applied for delivery of hydrophobic drugs, which have very limited solubility in aqueous environment [16–19] or biomolecules which would degrade or cause an immune response if added to a living entity on their own [13, 20–23]. A larger hydrodynamic radius provides longer circulation time, and also helps targeting cancer cells due to enhanced permeability and retention effect [20, 21, 23, 24]. However, robust drug delivery systems can accumulate in organs, such as liver and kidneys, during their circulation, and without timely excretion can cause immune response and inflammation [1, 4, 25]. Thus for the drug delivery applications, where the delivery material is targeted to circulate inside a human body, polymer degradability is especially important. This is why degradable synthetic polymers such as polycaprolactone, poly(lactic acid) or natural polymers such as chitosan are often utilized in this field [3, 4, 9, 26].

Reversible deactivation radical polymerization (RDRP) methods allow incorporation of various functionalities during the synthesis of polymers with diverse compositions and architectures [27]. However, if only vinyl mono-

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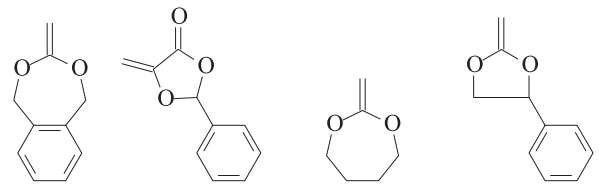
mers are incorporated into the polymers, the resulting materials consist solely of carbon-carbon bonds that have very limited degradability under physiological conditions [4]. Consequently, generating polymers by RDRP methods with appropriate degradation profiles remains a subject of high interest. There are several degradable linkages that are commonly utilized in synthetic delivery systems such as esters, acetals and disulfide bonds [2, 4, 10, 28, 29]. Acetals and esters can be hydrolytically degraded, while disulfide bonds are redox sensitive [2, 4, 28]. There are several approaches to incorporate degradable functionalities into copolymers synthesized by atom transfer radical polymerization (ATRP) [30]. Linear polymers can be grown from a degradable dual functional initiator, which would allow splitting polymer in half upon degradation [31–34]. For a star polymer synthesis one can either use multifunctional degradable initiators, or star cores prepared with a degradable crosslinker to dissociate the star copolymer into its arms [35–37]. Degradable crosslinkers or inimers can also be utilized in the synthesis of degradable hydrogels and nanogels [10, 38]. It is also possible to prepare degradable polymers containing heteroatoms by other techniques (ring opening, polycondensation) and extend them by ATRP [39–49]. However, some of these approaches can result in preparation of materials, which degrade into chains with broad molecular weight distributions (*MWD*s), and one has to consider the upper limits for molecular weight (*MW*) of the degraded components.

In order to incorporate several degradable groups along a polymer chains made from (meth)acrylates or (meth)acrylamides (comprised of only C-C bonds in a backbone) one can use cyclic comonomers with double bonds and incorporated degradable units such as cyclic ketene acetals (CKA), which will undergo ring opening once reacted with a radical, and the degradable moiety will be subsequently incorporated into the backbone of the copolymer [50–54]. Once such monomeric units undergo radical ring-opening polymerization (RROP) and are incorporated into the main C-C chain, the final product would contain ester bonds distributed along the backbone, which would provide desirable degradable properties under physiological conditions.

To date several CKAs have been examined as comonomers for RDRP procedures [Formulas (I)–(IV)].



BMDO (I) MPDO (II) MDO (III) MPDL (IV)

Copolymers with both water-soluble and hydrophobic monomers and CKA monomers, such as 5,6-benzo-2-methylene-1,3-dioxepane (BMDO), were synthesized by reversible addition–fragmentation chain transfer (RAFT), atom transfer radical polymerization (ATRP), and nitroxide-mediated radical polymerization (NMP) [29, 50, 51, 55–63]. Polymerizations were characterized by controlled/"living" behavior, yielding degradable copolymers. Among other CKAs polymerizable by RDRP were 5-methylene-2-phenyl-1,3-dioxolan-4-one (MPDO) [64, 65], 2-methylene-1,3-dioxepane (MDO), and 2-methylene-4-phenyl-1,3-dioxolane (MPDL) [29, 50, 61, 66]. Recently it was reported that NMP copolymerization of MPDL and a water-soluble methacrylate yielded polymers with the higher level of the incorporated CKA comonomer, compared to other tested CKAs like MDO and BMDO [29, 50, 61]. There was one report on homopolymerization of MPDL by ATRP [67], but copolymerization was not investigated. Therefore, it was of interest to investigate copolymerization of MPDL with various types of monomers, typically polymerizable by ATRP, for degradable polymers for potential biomedical applications.

This paper reports the results of a series of studies on the synthesis of copolymers of MPDL with hydrophobic and hydrophilic monomers. *n*-Butyl acrylate was chosen as a hydrophobic monomer. Methacrylates with either oligo(ethylene oxide) (8–9 units) or poly(ethylene oxide) (45 units) as a side chain were chosen as hydrophilic monomers. This type of water-soluble monomers form biocompatible polymers with comb structures due to their longer side chains. They are commonly used in biomaterials preparation, and it would be beneficial to develop their hydrolytically degradable equivalents. The level of MPDL incorporation, ring-opening efficiency and degradation behavior of the synthesized copolymers were studied.

EXPERIMENTAL PART

Materials

– Butyl acrylate (BA, 99 %, Sigma Aldrich), oligo(ethylene oxide) methyl ether acrylate ($OEOA_{480}$, 99 %, number average molecular weight $\overline{M}_n$ = 480, Sigma Aldrich), oligo(ethylene oxide) methyl ether methacrylate ($OEOMA_{500}$, 99 %, $\overline{M}_n$ = 475, Aldrich) were passed over a column of basic alumina (Fisher Scientific) prior to use.

– Poly(ethylene oxide) methyl ether acrylate ($PEOMA_{2k}$, 50 % aqueous solution, $\overline{M}_n$ = 2000, Sigma Aldrich) was extracted by dichloromethane and precipitated into hexane prior to use.

– Copper(II) bromide (99.999 %, Sigma Aldrich), *N*,*N*-dimethylformamide (DMF, ACS grade, Fisher Scientific), dichloromethane (DCM, HPLC grade, Fisher Scientific), ethyl ether (ACS grade, Fisher Scientific), chloroform-d (Cambridge Isotope Laboratories), acetonitrile-d3 (Cambridge Isotope Laboratories), tris[2-(dimethylamino)ethyl]amine (Me_6TREN, 97 %, Sigma Aldrich), ethyl-2-bromo-2-methylpropionate (EBiB, 98 %, Sigma Aldrich), were used as received.

– Radical thermal initiators: 2,2′-azobis(2-methylpropionitrile) (AIBN, Sigma Aldrich), 1,1′-azobis(cyclohexanecarbonitrile) (V40, Sigma Aldrich), 2,2′-azobis(*N*-butyl-2-methylpropionamide) (Vam110, Wako) were used as received.

– Chloroacetaldehyde dimethyl acetal (97 %), styrene glycol (97 %), Dowex 50WX8 hydrogen form and potassium *tert*-butoxide (KO-*tert*-Bu, 98 %) were purchased from Acros.

– 2-methylene-4-phenyl-1,3-dioxolane (MPDL) was synthesized according to previous procedure [69].

Methods of testing

^{1}H NMR (300 and 500 MHz) spectra were recorded on a Bruker Avance 300/500 spectrometer. The conversion of acrylates and methacrylates were determined using near infrared spectroscopy. Molecular weights and distributions were determined by THF, DMF and aqueous GPC. The THF GPC system was based on Polymer Standards Services (PSS) columns (Styrogel 10^2, 10^3, 10^5 Å) with, respectively, tetrahydrofuran (THF) as the eluent at a flow rate of 1 cm^3/min at 35 °C. DMF GPC utilized dimethylformamide (DMF) containing 50 mM LiBr as the eluent at a flow rate of 1 cm^3/min at 50 °C. The differential refractive index (RI) detector (Waters, 2414) and multi-angle laser light scattering detector (MALLS) (Wyatt TREOS) were used. The apparent molecular weights and dispersity ($\overline{M}_w/\overline{M}_n$) were determined with a calibration based on linear poly(methyl methacrylate) standards using for THF GPC. The aqueous GPC system (model Alliance 2695) was based on an Ultrahydrogel linear column (7.8––300 mm, Waters) with phosphate buffered saline (PBS) as the eluent at a flow rate of 1 cm^3/min at room temperature and differential RI detector (Waters, 2414). The apparent molecular weights and dispersity ($\overline{M}_w/\overline{M}_n$) were determined with a calibration based on linear PEG standards.

Synthesis of the copolymers with incorporated ester groups by radical ring-opening polymerization using atom transfer radical polymerization (ICAR ATRP)

ICAR ATRP of BA with MPDL

BA (2.4 g, 18.7 mmol), MPDL (1.5 g, 9.4 mmol) were mixed with 0.375 cm^3 of radical initiator stock solution (25 mM), 0.375 cm^3 of $CuBr_2$/Me_6TREN stock solution (1/2, 7.5 mM of $CuBr_2$), 0.375 cm^3 of EBiB stock solution (250 mM). Reaction mixture was placed in Schlenk flask, sealed and purged with nitrogen for 30 min. Polymerization was started by immersing reaction mixture in a heated oil bath set at either 65 °C, 90 °C, or 120 °C.

ICAR ATRP of $OEOA_{480}$ with MPDL

$OEOA_{480}$ (2.4 g, 5 mmol), MPDL (0.4 g, 2.5 mmol) were mixed with 0.1 cm^3 of radical initiator stock solution (25 mM), 0.1 cm^3 of $CuBr_2$/Me_6TREN stock solution (1/2, 7.5 mM of $CuBr_2$), 0.1 cm^3 of EBiB stock solution (250 mM), and 2.2 cm^3 of DMF. Reaction mixture was placed in Schlenk flask, sealed and purged with nitrogen for 30 min. Polymerization was started by immersing reaction mixture in a heated oil bath set at 90 °C.

ICAR ATRP of $OEOMA_{500}$ with MPDL

$OEOMA_{500}$ (2.5 g, 5 mmol), MPDL (0.4 g, 2.5 mmol) were mixed with 0.05 cm^3 of radical initiator V40 stock solution (25 mM), 0.1 cm^3 of $CuBr_2$/Me_6TREN stock solution (1/2, 7.5 mM of $CuBr_2$), 0.1 cm^3 of EBiB stock solution (50 mM), and 2.2 cm^3 of DMF. Reaction mixture was placed in Schlenk flask, sealed and purged with nitrogen for 30 min. Polymerization was started by immersing reaction mixture in a heated oil bath set at 90 °C.

ICAR ATRP of $PEOMA_{2k}$ with MPDL

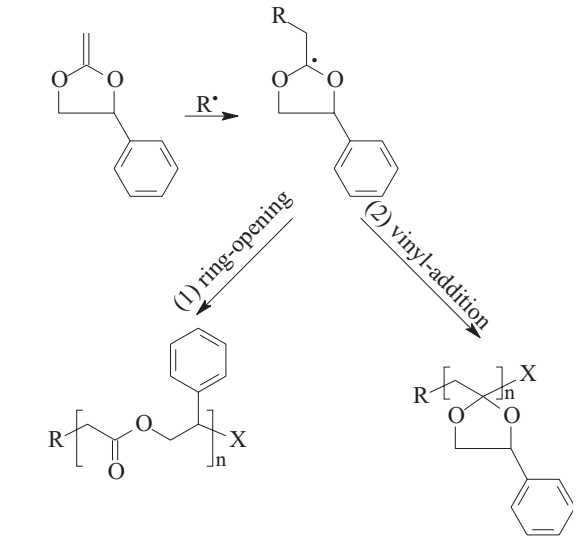
$PEOMA_{2k}$ (3 g, 1.5 mmol) was dissolved in 4.5 cm^3 of DMF. After that MPDL (0.4 g, 2.5 mmol) were mixed with 0.04 cm^3 of radical initiator V40 stock solution (25 mM), 0.04 cm^3 of $CuBr_2$/Me_6TREN stock solution (1/2, 7.5 mM of $CuBr_2$), 0.1 cm^3 of EBiB stock solution (50 mM) and added to the dissolved $PEOMA_{2k}$. Reaction mixture was placed in Schlenk flask, sealed and purged with nitrogen for 30 min. Polymerization was started by immersing reaction mixture in a heated oil bath set at 90 °C.

Hydrolytic degradation

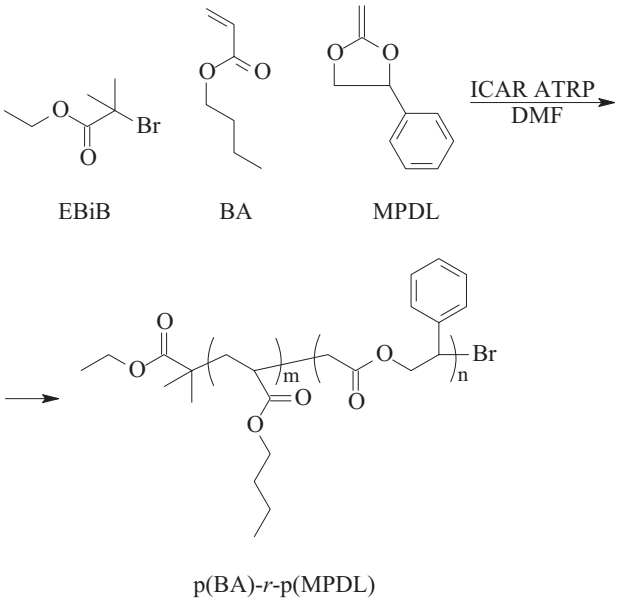
Poly(BA)-*r*-poly(MPDL) copolymers were degraded in 5 % KOH solution in mixture of THF/MeOH with a ratio 1/1. Degradation products were neutralized with HCl and precipitated into hexane prior to analysis. Water-soluble polymers were degraded in aqueous 5 % KOH. Samples were dissolved in PBS prior to analysis. Polymers were typically dissolved at 10 mg/cm^3 concentration.

RESULTS AND DISCUSSION

There are several factors which can influence ring-opening efficiency during RROP. It was reported that the presence of high ring strain in the monomer, the formation of a thermodynamically stable functional group, presence of a radical stabilizing group, and elevated temperatures, all favor a ring-opening reaction during a radical polymerization [69]. It was also reported that MPDL can be copolymerized by free radical polymerization (FRP) with 100 % ring-opening at temperatures between 60 °C–120 °C [Scheme A, reaction (1)] [36, 37]. However, in the ATRP homopolymerization of MPDL the efficiency of the ring-opening reaction strongly depended on temperature. The ring-opening became prevalent over vinyl-addition [Scheme A, reaction (2)] only at higher temperatures, above 120 °C [67].



Scheme A



Scheme B

Therefore, the first set of experiments was designed to investigate ring-opening efficiency during copolymerization of MPDL with BA at different temperatures and monomer concentrations (Scheme B, Table 1).

Polymerization analysis of the initial reaction conducted at 65 °C (Table 1, entry 1) indicated a well-controlled polymerization (Fig. 1), according to kinetic studies.

Copolymerization conditions: [BA]:[MPDL]:[EBiB]:[$CuBr_2$]:[Me_6TREN]:[AIBN] = 100:50:1:0.015:0.03:0.1, reaction solvent – DMF, 65 °C, [BA] = 1 M, [MPDL] = 0.5 M. *MW* and GPC traces were obtained by THF GPC with PMMA calibration standards. Linear first-order kinetics plots were obtained for both comonomers, with MPDL being incorporated into the copolymer at a rate a little faster than BA, at the given monomer feed ratio, BA/MPDL = 2/1. At low monomer conversions, *MW* increased linearly with conversion, but started to deviate toward lower *MW* when conversion increased to > 20 % (Fig. 1b). $\overline{M}_w/\overline{M}_n$ values also increased with conversion. According to GPC traces, last two samples were characterized by shift towards higher *MW*, but low *MW* tailing was detected (Fig. 1b). Such results suggested some loss of chain-end functionality. Nevertheless, the final copolymer still had a relatively low $\overline{M}_w/\overline{M}_n$, and thus it was isolated and further characterized to determine its composition.

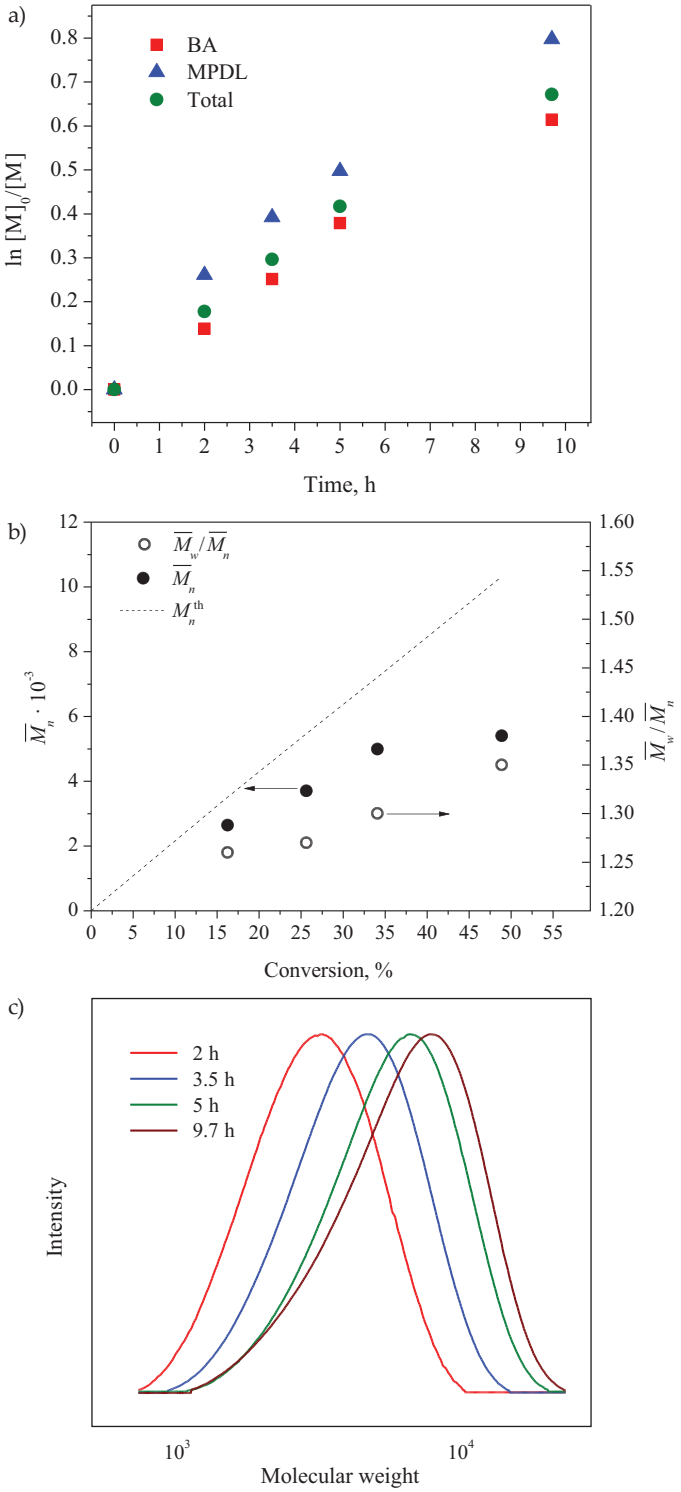


Fig. 1. Copolymerization of BA with MPDL by ICAR ATRP: a) first-order kinetic plots, b) evolution of $\overline{M}_n$ and $\overline{M}_w/\overline{M}_n$ with conversion, c) GPC traces for ATRP of p(BA)-*r*-p(MPDL)

The purified copolymer was further characterized by ^{1}H NMR to determine the mode of incorporation of MPDL,

T a b l e 1. Copolymerization of BA with MPDL by ICAR ATRP

Entry	$M_1/M_2/I/CuBr_2/L/RI$	*T*, °C	Conv., %	Time, h	M_n^{th}	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	f_{MPDL}, %	RO, %
1	100/50/1/0.015/0.03/0.1	65	55	9.7	10 320	5 400	1.35	29.9	35
2	100/50/1/0.015/0.03/0.1	88	52	6	11 030	4 820	1.39	23.4	46
3	100/50/1/0.015/0.03/0.1	110	46	2	9 800	4 360	1.41	26.4	55

$[M_1]$ = [BA] = 1 M, $[M_2]$ = [MPDL] = 0.5 M, [I] = [EBiB] = 10 mM, 10 ml total; L – Me_6TREN; reaction solvent – DMF; RI – radical initiator: entry 1 – AIBN ($T_{t1/2=10h}$ = 65 °C), entry 2 – V40 ($T_{t1/2=10h}$ = 88 °C), entry 3 – Vam110 ($T_{t1/2=10h}$ = 110 °C); RO – % of MPDL monomer in ring-opened form to ring-closed form; M_n^{th} – theoretical mass, f_{MPDL} – fraction of MPDL incorporated into the p(BA) backbone, monomer conversion was measured by ^{1}H NMR; $\overline{M}_n$ and $\overline{M}_w$ was obtained by THF GPC with PMMA calibration standards.

i.e., determine what fraction of incorporated monomer exhibited ring-opening *vs.* vinyl addition. The composition of the p(BA)-*r*-p(MPDL) copolymer was determined from the ratio of aromatic protons (P_{1-3}) present in MPDL to the protons from butyl acrylate side chain (B_1) (Fig. 2).

According to this calculation, MPDL incorporation was 29.9 %. The ring-opening efficiency was calculated from ^{1}H NMR spectra, where the signal at ~5.05 ppm corresponded to the methine proton (M_2) on the carbon between the acetal oxygen and the phenyl group (Fig. 2). The difference between the integration of methine proton and phenyl proton provided a value of the percentage of MPDL which underwent the ring-opening reaction. According to the values calculated for copolymerization of BA with MPDL at 65 °C 35 % of incorporated MPDL was in its ring-opened form. ^{13}C NMR was also used to confirm the presence of an acetal carbon (Fig. 3), detected at δ = 110 ppm.

The next two copolymerizations of BA with MPDL were performed at higher temperatures (Table 1, entries 2–3). Different free radical initiators were selected for each reaction: the initially used radical initiator (RI) AIBN was replaced by RIs with higher decomposition temperatures, V40 $T_{t1/2=10h}$ = 88 °C (where *t*1/2=10h is the 10 h half lifetime of the initiator), and Vam110 with $T_{t1/2=10h}$ = 110 °C for the highest temperature reaction. Polymerizations at 90 °C and 110 °C were characterized by faster rate, but were also less controlled, yielding polymers with higher $\overline{M}_w/\overline{M}_n$. However, the final copolymers were characterized by higher percentage of incorporated MPDL, which underwent ring-opening instead of vinyl addition. According to ^{1}H NMR analysis the peak due to the methine proton present in MPDL (M_2), which represents incorporated MPDL that underwent vinyl addition, decreased for the polymers synthesized at the elevated temperatures (Fig. 4).

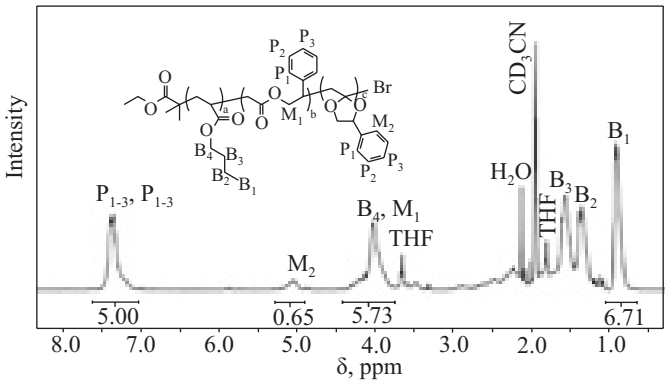


Fig. 2. ^{1}H NMR of purified copolymer p(BA)-*r*-p(MPDL) synthesized at 65 °C (300 MHz, CD_3CN)

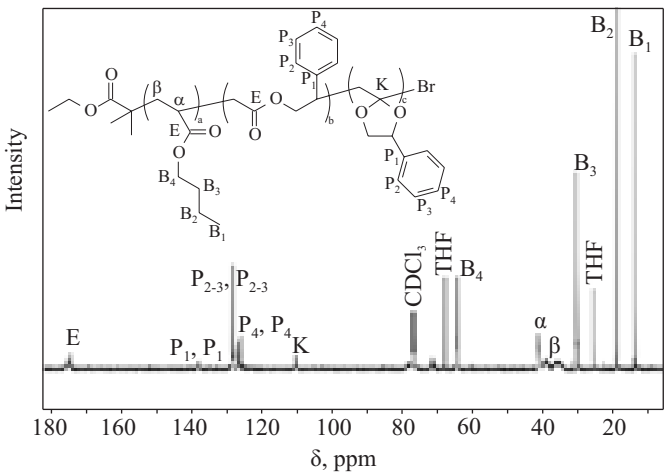


Fig. 3. ^{13}C NMR spectra of purified copolymer p(BA)-*r*-p(MPDL) synthesized at 65 °C (500 MHz, $CDCl_3$)

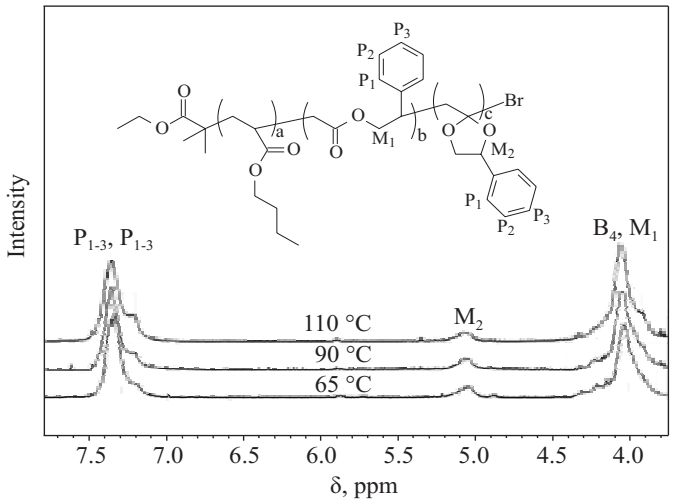


Fig. 4. ^{1}H NMR of purified copolymers p(BA)-*r*-p(MPDL) synthesized at different temperatures (300 MHz, CD_3CN); spectra were normalized to phenyl protons in each sample; signal at 5.05 ppm corresponds to methine proton (M_2) in the polymer unit structure