JOURNAL OF Applied Polymer SCIENCE

Synthesis of Amino Acid-Based Polymers Having Metronidazole Moiety and Study of Their Controlled Release *In Vitro*

Mohamed H. El-Newehy,[1,2,3*] Abeer S. Elsherbiny,[1,4] Hideharu Mori[2*]

[1]Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt

[2]Department of Polymer Science and Engineering, Graduate, School of Science and Engineering, Yamagata University, Jonan, Yonezawa 992-8510, Japan

[3]Petrochemical Research Chair, Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

[4]Department of Chemistry, Science and Art College, King Abdulaziz University, Rabigh Campus, Rabigh 21911, Kingdom of Saudi Arabia

*Permanent address: Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt

Correspondence to: M. H. El-Newehy (E-mail: mnewehy@yahoo.com) or H. Mori (E-mail: h.mori@yz.yamagata-u.ac.jp)

ABSTRACT: A new polymeric drug carrier system using amino acid-based polymers was developed. Amino acid-based polymers with controlled molar mass and narrow molecular weight distribution have been synthesized by reversible addition-fragmentation chain transfer polymerization of four amino acid-carrying monomers having different chirality and hydrophilicity. Metronidazole (MTZ) was immobilized onto the amino acid-based polymers, and the release profiles of the polymer-MTZ adducts were investigated in phosphate buffer solutions (pH = 2.0, 7.4, and 8.5). The model drug was released by the hydrolysis of the ester group, and the release rate and behavior of the polymeric prodrugs strongly depended on the configuration of the amino acid-based polymers-MTZ adducts and the pH of the release media. The release kinetics was determined using the Higuchi and Korsmeyer equations, which revealed the release mechanism of the polymeric prodrugs. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

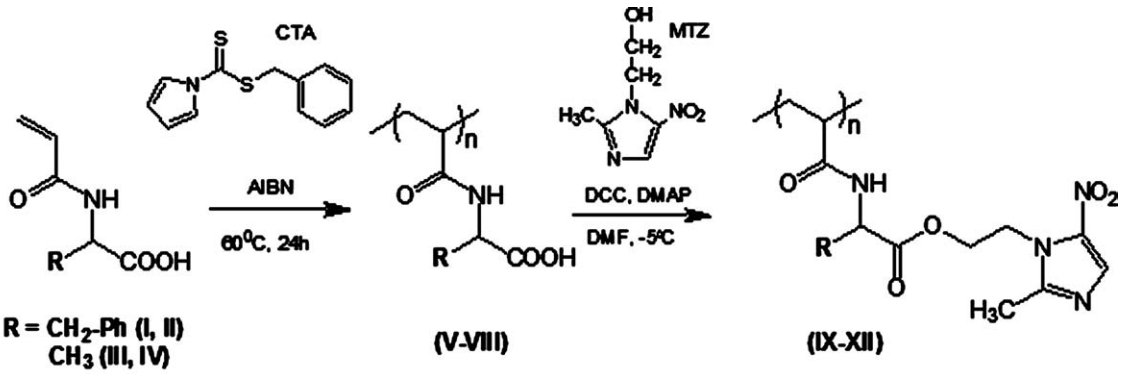
KEYWORDS: reversible addition-fragmentation chain transfer (RAFT); drug delivery systems; chiral; amino acid; metronidazole

Received 28 June 2011; accepted 24 February 2012; published online 00 Month 2012
DOI: 10.1002/app.37611

INTRODUCTION

In the past decades, considerable attention has been paid to the development of advanced drug delivery systems to improve drug efficacy using polymeric prodrugs or polymer-drug conjugates.[1–7] Polymeric drug delivery systems offer several advantages such as enhancement of drug bioavailability owing to an increase in water solubility of low soluble or insoluble drugs; protection of drugs from deactivation and preservation of its activity during circulation; reduction in the toxic side effects of the drugs; and achievement of selective transportation of drugs to targeted organs, tissues, and cells.[2] The rational design of polymer-based drug delivery systems is required to optimize the therapeutic properties of drugs and render them safer, more effective, and reliable.[3] A polymeric carrier can be either an inert or a biodegradable polymer, and the drug can be fixed directly or via a spacer group onto the polymer backbone.[1] The proper selection of this spacer opens the possibility of controlling the site and the rate of release of the active drug from conjugate by hydrolytic or enzymatic cleavage.

Synthetic polypeptides and amino acid-based polymers have attracted significant research interest, because of their potential applications as biodegradable and biomedical polymers, as well as their feasibility to create highly ordered hierarchical structures through noncovalent forces, such as hydrogen bonding. Amino acids are the constitutional components of peptides and proteins, which are able to produce highly ordered hierarchical structures scaling from nanometers to several microns. Incorporation of a high degree of amino acid functionality and chirality in polymer chains can enhance the potential to form secondary structures and higher ordered structures.[8–16] These synthetic polymers derived from amino acids can be useful as chiral recognition stationary phases,[17] metal ion absorbents,[18] drug delivery agents,[19,20] and biocompatible materials.[21] For example, Domb and coworkers[21] investigated the biological activity of various poly(*N*-acryl amino acid)s having different lipophilic characteristics and charged functional groups. They demonstrated that the heparin-like activities were significantly affected



Original amino acid	Monomer	Polymer	Prodrug
L-Phenylalanine (L-Phe-OH)	A-L-Phe-OH (I)	Poly(A-L-Phe-OH) (V)	Poly(A-L-Phe-OMTZ) (IX)
D-Phenylalanine (D-Phe-OH)	A-D-Phe-OH (II)	Poly(A-D-Phe-OH) (VI)	Poly(A-D-Phe-OMTZ) (X)
L-Alanine (L-Ala-OH)	A-L-Ala-OH (III)	Poly(A-L-Ala-OH) (VII)	Poly(A-L-Ala-OMTZ) (XI)
D-Alanine (D-Ala-OH)	A-D-Ala-OH (IV)	Poly(A-D-Ala-OH) (VIII)	Poly(A-D-Ala-OMTZ) (XII)

Scheme 1. Synthesis of amino acid-based polymers by RAFT polymerization and immobilization of metronidazole (MTZ).

by the structure of the amino acid moieties in the poly(*N*-acryl amino acid)s.

In this article, we report a new controlled drug-release system based on amino acid-based polymers having well-defined structures, which were prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization. The employment of amino acid-based polymers as a drug carrier offers the advantage of using amino acid as a spacer, on the basis of which the chirality, charge, amphiphilicity, configuration, and ordered structures of the prodrug can be manipulated arbitrarily. In such systems, the release rate and behavior are expected to be tuned by selecting the nature of the amino acid moiety used as the functional group for covalent linkage of a model drug to the polymer backbone, in addition to the structure and the property of the original polymers. Moreover, polymeric drugs prepared by controlled polymerization techniques should have predetermined molecular weights and low polydispersity, and the resulting well-defined polymer molecules should be free of either very small or very large molecules, which could exhibit nonuniform pharmacological properties.[22] Recently, we developed a novel synthetic method to produce various amino acid-based polymers having well-defined structures and characteristic chiroptical properties by RAFT polymerization of acrylamides that have amino acid moieties in the side chains.[8,9] In this study, four amino acid-based polymers synthesized by RAFT polymerization were employed for the preparation of well-defined polymeric prodrugs, as shown in Scheme 1. The release profiles were investigated in terms of the nature of the amino acids and the release conditions.

EXPERIMENTAL

Materials

Acryloyl chloride (99%) was purchased from Aldrich. 2,2′-Azobis(isobutyronitrile) (AIBN, Kanto Chemical, 97%) was purified by recrystallization from methanol. Metronidazole (2-methyl-5-nitroimidazole-1-ethanol, metronidazole [MTZ]) was purchased from TCI, Japan. *N, N′*-Dicyclohexylcarbodiimide (DCC, 99%) and 4-(dimethylamino)pyridine (DMAP) were purchased from Sigma-Aldrich. L-Phenylalanine (L-Phe-OH, Kanto Chemical, 99%), D-phenylalanine (D-Phe-OH, Kanto Chemical, 99%), L-alanine (L-Ala-OH, Kanto Chemical, >99%), and D-alanine (D-Ala-OH, Kanto Chemical, >99%) were used as received. Methanol (dehydrated MeOH, Kanto Chemical, 99.8%), *N, N*-dimethylformamide (dehydrated DMF, Kanto Chemical, 99.5%), and other materials were used as received. Benzyl 1-pyrrolecarbodithioate used as a chain transfer agent (CTA) was synthesized according to a previously reported procedure.[8] CTA was purified by column chromatography on silica with *n*-hexane as the eluent to afford the corresponding product as yellow oil.

Characterization

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM-ECX400. FTIR spectra were obtained with a JASCO FT/IR-210 spectrometer. The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) were estimated by size exclusion chromatography (SEC) using a Tosoh HPLC-8220 system equipped with refractive index and ultraviolet detectors at 40°C. The column set was as follows: four consecutive hydrophilic vinyl polymer-based gel columns

[TSK-GELs (pore size, exclusion limited molecular weight): α-M (13 μm, >1 × 10^7), α-4000 (10 μm, 4 × 10^5), α-3000 (7 μm, 9 × 10^4), α-2500 (7 μm, 5 × 10^3), 30 cm each] and a guard column [TSK-guard column α, 4.0 cm]. The system was operated at a flow rate of 1.0 mL/min, using DMF containing 10 m*M* LiBr as an eluent. Polystyrene standards were employed for calibration. UV spectra was recorded using a PerkinElmer Lambda 35 UV-vis spectrophotometer. Elemental analysis was carried out on a Perkin-Elmer 2400 II CHNS/O analyzer. The surface morphology of the final resultant materials was studied using JEOL GSM-6610LV scanning electron microscope.

Synthesis of Amino Acid-Based Polymers

The amino acid-carrying monomers, *N*-acryloyl-L-phenylalanine (A-L-Phe-OH, **I**), *N*-acryloyl-D-phenylalanine (A-D-Phe-OH, **II**), *N*-acryloyl-L-alanine (A-L-Ala-OH, **III**), and *N*-acryloyl-D-alanine (A-D-Ala-OH, **IV**) were prepared by the reaction of acryloyl chloride with the corresponding amino acids according to a method reported previously.[9,23] The reaction of the sodium salt of the amino acid with acryloyl chloride in aqueous solution, followed by acidification of the medium, produced acrylamide with the corresponding amino acid as a white solid. The product was purified by column chromatography on silica with ethyl acetate as the eluent to yield the amino acid-carrying monomer.

The amino acid-based polymers were synthesized by RAFT polymerization of the amino acid-carrying monomers using benzyl 1-pyrrolecarbodithioate as a CTA, according to a previously reported procedure.[9,23] All polymerizations were carried out with AIBN as the initiator in a degassed sealed tube. A representative example is as follows: the monomer, CTA, AIBN, and solvent (dehydrated MeOH for the polymerization of A-Ala-OH and dehydrated DMF for the polymerization of A-Phe-OH) were placed in a dry glass ampule equipped with a magnetic stirring bar, and then, the solution was degassed by three freeze-evacuate-thaw cycles. After the ampule was flame-sealed under vacuum, it was stirred at 60°C for 24 h. For the SEC measurement, the resulting polymer was converted into its methyl ester form by treating the carboxylic acid groups using trimethylsilyldiazomethane. In all cases, the SEC traces of the resulting products were unimodal with no evidence of high molecular weight species.

Immobilization of Metronidazole onto Polymers

Poly(*N*-acryloyl-L-phenylalanine-2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl ester), poly(A-L-Phe-OMTZ) **IX**, was prepared in the following manner.[24,25] To a solution of poly(*N*-acryloyl-L-phenylalanine) **V** (0.8 g, 3.65 mmol) and 4-(dimethylamino)pyridine (DMAP, 44.67 mg, 0.36 mmol) in 15 mL of DMF, a solution of DCC (0.75 g, 3.65 mmol) in 5 mL of DMF was added dropwise at −5°C. The mixture was kept at −5°C for 20 min, and then, a solution of MTZ (0.63 g, 3.65 mmol) in 5 mL of DMF was added dropwise within 30 min. The reaction mixture was stirred at −5°C for 3 h and slowly returned to room temperature and stirred for more than 20 h. The formed precipitate (dicyclohexyl urea) was removed by filtration. The solvent was concentrated to 2 mL using a rotavapor at 70°C. The product was precipitated by dropwise addition to water (100 mL), washed with water (100 mL), and dried at 55°C under vacuum overnight to afford 1.10 g (yield = 81.0%) of poly(A-L-Phe-OMTZ) **IX**. ^{1}H NMR (400 MHz, $CDCl_3$, ppm, from $Si(CH_3)_4$); δ 8.0 (1H, -CONH), 7.8 (1H, —N—CH=C—, imidazole), 7.5–6.7 (5H, Ar.), 4.5–4.0 (5H, >NHC*H*—, —OCH_2CH_2N—), 3.4 (2H, —CH_2Ph), 3.1–1.1 (6H, —CH_3, —CH_2CH-) ppm. FT-IR (KBr): 3328 (N—H, amide), 3029 (C—H, aromatic), 2930 (C—H, aliphatic), 2852 (—CH_3), 1671 (—C=C—N—), 1822 (C=O, ester), 1743 (C=O, amide), 1531 (NO_2, amide), 1428 (C—C), 1263–1188 (C—N), 1043 (C—O) cm^{-1}.

Poly(*N*-acryloyl-D-phenylalanine-2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl ester), poly(A-D-Phe-OMTZ) **X**, was prepared by the reaction of poly(*N*-acryloyl-D-phenylalanine) **VI** with MTZ using the same procedure described above for the preparation of poly(A-L-Phe-OMTZ) **IX**. The yield of poly(A-D-Phe-OMTZ) **X** = 85.3% (1.16 g). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, from $Si(CH_3)_4$): δ 8.1 (1H, —CONH), 7.9 (1H, —N—CH=C—, imidazole), 7.5–6.7 (5H, Ar.), 4.5–4.1 (5H, >NHC*H*—, —OCH_2CH_2N—), 3.5 (2H, —CH_2Ph), 3.1–1.0 (6H, —CH_3, —CH_2CH—) ppm. FTIR (KBr): 3327 (N—H, amide), 3029 (C—H, aromatic), 2929 (C—H, aliphatic), 2851 (—CH_3), 1627 (—C=C—N—), 1822 (C=O, ester), 1743 (C=O, amide), 1532 (NO_2, amide), 1455 (C—C), 1263–1189 (C—N), 1044 (C—O) cm^{-1}.

Poly(*N*-acryloyl-L-alanine-2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl ester), poly(A-L-Ala-OMTZ) **XI**, was prepared by the reaction of poly(*N*-acryloyl-L-alanine) **VII** with MTZ using he same procedure described above for the preparation of poly(A-L-Phe-OMTZ) IX to give 1.65 g of poly(A-L-Ala-OMTZ) (XI) as a yellowish white solid (yield = 94.5%). ^{1}H NMR (400 MHz, $CDCl_3$ ppm from $Si(CH_3)_4$): δ 8.2 (1H, —CONH), 7.9 (1H, —N—CH=C—, imidazole), 4.6–3.9 (5H, >NHC*H*—, —OCH_2CH_2N—), 2.5 (3H, —CH_3, imidazole), 2.4–1.0 (6H, —CH_3, —CH_2CH—) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, ppm, from $Si(CH_3)_4$): δ 175.2 (—*C*ONH—), 171.7 (—*C*OO—), 162.6 (—N—*C*=N—), 138.0 (—N—*C*=C—), 132.5 (—N—C=*C*—), 60.5 (—O*C*H_2CH_2N—), 47.5 (>NH*C*H<), 33.4 (—OCH$_2$*C*H_2N—), 24.3 (—CH_2*C*H—), 16.3 (—*C*H_3), 15.3 (—*C*H_2CH—), 13.8 (—CH_3, imidazole) ppm. FT-IR (KBr): 3327 (N—H, amide), 2929 (C—H, aliphatic), 2851 (—CH_3), 1627 (—C=C—N—), 1747 (C=O, ester), 1534 (C=O, amide), 1311 (NO_2, amide), 1455 (C—C), 1263–1188 (C—N), 1046 (C—O) cm^{-1}.

Poly(*N*-acryloyl-D-alanine-2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl ester), poly(A-D-Ala-OMTZ) **XII**, was prepared by the reaction of poly(*N*-acryloyl-D-alanine) **VIII** with MTZ using the same procedure described above for the preparation of poly(A-L-Phe-OMTZ) **IX**. The yield of poly(A-D-Ala-OMTZ) **XII** = 87.9% (1.45 g). ^{1}H-NMR (400 MHz, $CDCl_3$ ppm, from $Si(CH_3)_4$): δ 8.2 (1H, —CONH), 7.9 (1H, —N—CH=C—, imidazole), 4.6–4.1 (5H, >NHC*H*—, —OCH_2CH_2N—), 2.5 (3H, —CH_3, imidazole), 1.9–1.0 (6H, —CH_3, —CH_2CH—) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$ ppm, from $Si(CH_3)_4$): δ 176.7 (—*C*ONH—), 172.7 (—*C*OO—), 162.6 (—N—*C*=N—), 138.6 (—N—*C*=C—), 132.4 (—N—C=*C*—), 63.0 (—O*C*H_2CH_2N—), 48.4 (>NH*C*H<), 35.4 (—OCH$_2$*C*H_2N—), 26.3 (—CH_2*C*H—), 25.8 (—*C*H_3), 17.2 (—*C*H_2CH—), 14.3 (—CH_3, imidazole) ppm. FT-IR (KBr): 3328 (N—H, amide), 2930 (C—H, aliphatic), 2851 (—CH_3), 1627 (—C=C—N—), 1748 (C=O, ester), 1535 (C=O, amide), 1364 (NO_2, amide), 1458 (C—C), 1263-1188 (C—N), 1046 (C—O) cm^{-1}.

In Vitro Drug Release

The release of MTZ was followed spectrophotometrically by measuring the absorbance at $\lambda_{max} = 230$ nm as a function of time using a UV–vis spectrophotometer. The following procedure was used: 10 mg of the polymer-MTZ adduct was placed into bottles containing 10 mL phosphate solutions buffered at pH = 2.0, 7.4, and 8.5 at body temperature (37°C). At specified time intervals, 2.8 ml was collected for analysis, and this volume was returned to the release medium. The cumulative percentage of MTZ released was calculated using the standard calibrating curve for MTZ. All the experiments were carried out in triplicate.

Model Used for the Analysis of Drug Release Kinetics

The drug release kinetics was analyzed according to Higuchi[26] (Equation 1) and Korsmeyer et al.[27] (Equation 2):

$$M_t/M_\infty = kt^{1/2} \quad (1)$$

$$M_t/M_\infty = k't^n \quad (2)$$

where M_t/M_∞ is the fractional drug release into the dissolution medium at time t, k, and k' are kinetic constants related to the properties of the drug/polymer system, t is the release time, and n is the release exponent that depends on the release mechanism and the shape of the matrix tested.[28] When $n = 0.5$, the drug diffuses through and is released from the polymeric matrix with a quasi-Fickian diffusion mechanism. For $n > 0.5$, an anomalous, non-Fickian drug diffusion occurs. When $n = 1$, a non-Fickian, case II or zero-order release kinetics could be observed.[28]

RESULTS AND DISCUSSION

In this article, we developed a new controlled drug release system based on the use of well-defined amino acid-based polymers having characteristic hydrophilicity and chirality (Scheme 1). In the first step, amino acid-based polymers were synthesized by RAFT polymerization, which enables the synthesis of functional polymers with controlled molar mass, narrow molecular weight distribution, and well-defined architectures and functionalities.[8,9,23] Then, the immobilization of metronidazole (MTZ) onto the amino acid-based polymers was carried out via an esterification reaction in the presence of DCC as condensation agent and DMAP as a catalyst. Finally, we conducted an *in vitro* drug release study of the polymeric prodrugs.

Synthesis of Amino Acid-Based Polymers

Well-defined amino acid-based polymers with characteristic chiroptical properties were synthesized by RAFT polymerization of enantiomeric *N*-acryloyl amino acids (L and D forms) having different hydrophilicities (Scheme 1). Four amino acid-carrying monomers, *N*-acryloyl-L-phenylalanine (A-L-Phe-OH, **I**), *N*-acryloyl-D-phenylalanine (A-D-Phe-OH, **II**), *N*-acryloyl-L-alanine (A-L-Ala-OH, **III**), and *N*-acryloyl-D-alanine (A-D-Ala-OH, **IV**), were prepared by the reaction of acryloyl chloride with corresponding amino acids according to a previously reported method with a slight modification.[9,23] We selected A-Ala-OH (L and D forms) as enantiomeric amino acid-carrying monomers, because alanine is one of the simplest α-amino acids and should be more hydrophilic than other aliphatic and aromatic amino acids. Poly(A-Phe-OH)s obtained by RAFT polymerization of L and D form monomers (A-L-Phe-OH and A-D-Phe-OH) are employed as a hydrophilic segment with low hydrophilicity. In general, phenylalanine is classified as nonpolar because of the hydrophobic nature of the benzyl side chain. Because A-Ala-OH and A-Phe-OH have a carboxylic acid in the monomer unit, these polymers can be recognized as weak polyelectrolytes, in which the degree of ionization is governed by the pH and ionic strength of the aqueous solution. In this study, the carboxylic acid moiety in the amino acid-based polymers was used as sites to bind biorelated material.

Table I. Preparation of Amino Acid-Based Polymers by RAFT Polymerization Using Benzyl 1-Pyrrolecarbodithioate at 60°C for 24 h ([M]/[CTA]/[AIBN] = 150/2/1)

Monomer	Conv. %[a]	M_n (SEC)[b]	M_w/M_n (SEC)[b]	$M_{n,\ COOH}$[c]
A-L-Phe-OH (I)	99	20500	1.36	19200
A-D-Phe-OH (II)	97	21000	1.29	19600
A-L-Ala-OH (III)	97	18800	1.27	17100
A-D-Ala-OH (IV)	94	17100	1.41	15600

[a]Calculated by ^{1}H NMR in DMSO-d_6, [b]Methylated samples were measured by size exclusion chromatography (SEC) using polystyrene standards in *N, N*-dimethylformamide (DMF, 10mM LiBr), [c]The number-average molecular weights of the carboxylic acid forms were calculated from the values of the methylated samples determined by SEC.

To obtain well-defined polymers derived from various amino acids, we conducted a RAFT polymerization of the unprotected monomers using benzyl 1-pyrrolecarbodithioate, because the chain transfer agent (CTA) is known to be efficient in achieving controlled RAFT polymerization of monosubstituted acrylamides.[9,23] Direct RAFT polymerization of the amino acid-carrying monomers was conducted without any protection chemistry. The results are summarized in Table I. In all cases, almost full conversion (>90%, as determined by ^{1}H-NMR spectroscopy) was obtained at 60°C after 24 h, when polymerization was carried out at a $[M]_0/[CTA]_0/[AIBN]_0$ ratio of 150/2/1. The amino acid-based polymers having pre-determined molecular weights (M_n = 15000–20000 in the carboxylic forms) and low polydispersities (M_w/M_n = 1.27–1.41) were employed for the immobilization of MTZ.

Immobilization of Metronidazole onto Amino Acid-Based Polymers

MTZ was immobilized onto the amino acid-based polymers (**V–VIII**) via an esterification reaction in the presence of DCC as a condensation agent and DMAP as a catalyst at −5°C. In this study, we selected MTZ as the model drug, because it is an orally administered antimicrobial drug.[29] In addition, MTZ is widely used to treat a broad range of infections caused by anaerobic protists and bacteria, such as *Trichomonas vaginalis, Entamoeba histolytica*, and *Giardia lamblia*. Despite its beneficial activity, it has some adverse effects involving anaphylactic shock, hyperpyretic convulsion, and damage to DNA.[30]

Table II. Immobilization of Metronidazole onto Amino Acid-Based Polymers

Polymer-MTZ product	Amino acid-based polymer[a]	N content (%)[b]	Conversion (%)[c]	Yield (%)[d]
Poly(A-L-Phe-OMTZ) (IX)	V	12.46	83.3	81
Poly(A-D-Phe-OMTZ) (X)	VI	12.09	80.8	85
Poly(A-L-Ala-OMTZ) (XI)	VII	17.22	91.7	95
Poly(A-D-Ala-OMTZ) (XII)	VIII	16.07	85.6	88

[a]See Scheme 1, [b]Determined by elemental analysis, [c]Conversion of the immobilization (degree of the substitution) was calculated from N content, [d]Water-insoluble part.

Therefore, the immobilization of MTZ onto polymers via esterification can be used as a tool to improve its therapeutic effects.[29]

Esterification was carried out in DMF under cooling conditions to avoid racemization and *N*-acylurea formation[31] and to afford the polymer-MTZ adducts (**IX–XII**), as shown in Scheme 1. In all cases, the polymer-MTZ adducts were purified by precipitation from a concentrated DMF solution into a large excess of water. Table II summarizes the results of the immobilization of MTZ onto the amino acid-based polymers (**V–VIII**). In all cases, the yields of the products calculated on the basis of the amount of the water-insoluble part were more than 80%. The nitrogen analysis showed that the conversion was 83.3, 80.8, 91.7, and 85.6% for the polymer-MTZ adducts (**IX–XII**), respectively. In all cases, the ^{1}H-NMR spectra of the products showed a characteristic signal attributed to the imidazole group at around 7.9 ppm. Figure 1 shows ^{1}H-NMR spectrum of a representative polymer. The structures of the immobilized polymers were also confirmed by FTIR and ^{1}H- and ^{13}C-NMR measurements. These results indicate that the esterification reaction effectively immobilizes the model drug into the amino acid-based polymers to produce novel polymeric drug carriers with high conversion (degrees of the substitution) and recovery. SEM micrographs revealed that four different polymer-MTZ adducts possessed dense macroporous voids, as shown in Figure 2.

In Vitro Drug Release Study

The rate of MTZ released from the polymer-MTZ adducts was studied at various pH values. The rate of release is expected to depend on the configuration of the amino acid-based polymers, hydrophilicity, and bulkiness of the amino acid moiety near the ester linkage, as well as the pH of the release media. The effect of the configuration of the amino acid-based polymer on the release of MTZ from the polymer-MTZ adducts (**IX–XII**) was studied on the basis of the amino acid structure change from D- to L-configuration, as shown in Scheme 1. The drug release was investigated in phosphate buffer at different pH values.

The drug release profiles from the different polymer-MTZ adducts at pH 8.5 (alkaline pH) are illustrated in Figure 3. The release studies were performed over 48 h, and all experiments were carried out in triplicate. The polymer-MTZ adduct, poly(A-L-Phe-OMTZ) **IX**, showed 51% of its drug content after 48 h at 37°C, whereas poly(A-D-Phe-OMTZ) **X** released about 93% of its drug content under the same conditions. Similar results were obtained for the alanine-based adducts, poly(A-L-Ala-OMTZ) **XI** and poly(A-D-Ala-OMTZ) **XII**, which showed release of 50 and 89% of its drug content, respectively, under the same conditions. These results indicate that the polymer-MTZ adducts containing the D-configuration show higher drug release than the ones containing the L-configuration, which may be due to the difference in the hydrolysis rate of each enantiomer. In contrast, there is approximately no difference in the MTZ release if the amino acid changes from alanine to phenylalanine in the polymer-MTZ adducts, suggesting no significant effect of the bulkiness and the hydrophilicity of the amino acid moiety on the hydrolysis of the ester linkage in the side chain. In this system, the insoluble prodrugs would become water-soluble products with the release of MTZ, depending on the structure of the amino acid moiety and pH value. The poly(A-Ala-OH) was soluble in basic water (pH $>$ 10) and neutral water in the presence of a salt (e.g., 0.5 mg of NaCl/mL), while being insoluble in acidic water, suggesting that the degree of ionization and water solubility are affected by the pH value. In contrast, the poly(A-Phe-OH) with less hydrophilicity due to the presence of the phenyl group was soluble only in basic water (pH $>$ 11), while insoluble in neutral water (pH $\approx$ 7) and acidic water (pH $<$ 4). Hence, the solubility change of the prodrugs during the release experiments may affect the release rate only in the cases of poly(A-Ala-OMTZ), XI and XII, at pH = 8.5 and 7.4. However, no difference in the MTZ release when the amino acid changes from alanine to phenylalanine in the polymer-MTZ adducts is detected (Figure 3), suggesting the fact that the solubility change of the prodrugs has no significant effect on the release rate. Furthermore, the release rate is affected apparently by the chirality of the amino acid moiety, even if the effect of the solubility change of the prodrugs on the release rate should be independent on the chirality of the amino acid moiety. These results suggest that the solubility change of

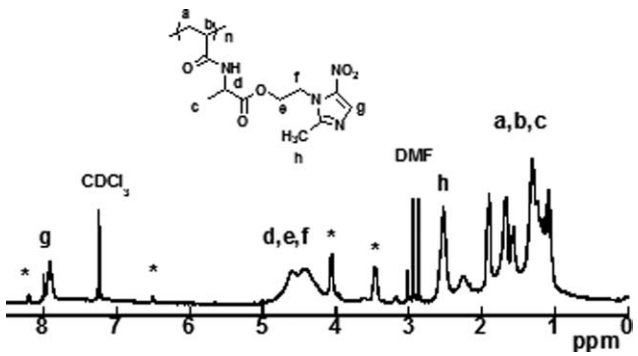


Figure 1. ^{1}H NMR ($CDCl_3$) spectrum of poly(*N*-acryloyl-D-alanine-2-(2-methyl-5-nitro-imidazol-1-yl) ethyl ester) (Poly(A-D-Ala-OMTZ)) (**XII**). The asterisk indicates the signals attributed to impurities involving DCC urea and DMAP.

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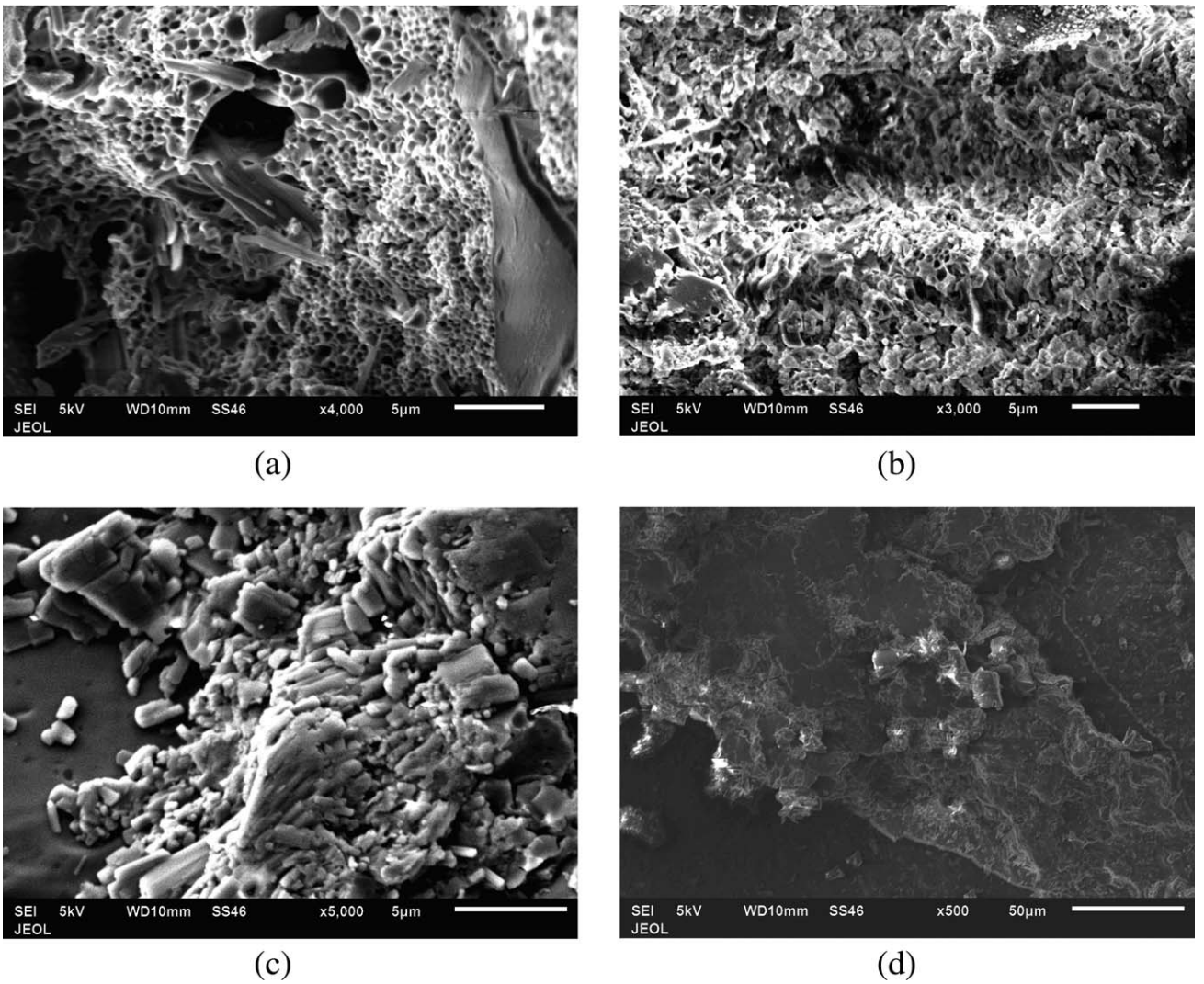

Figure 2. SEM micrographs of the polymer-adducts; (a) poly(A-L-Phe-OMTZ) (**IX**), (b) poly(A-D-Phe-OMTZ) (**X**), (c) poly(A-L-Ala-OMTZ) (**XI**), and (d) poly(A-D-Ala-OMTZ) (**XII**).

the prodrugs during the release experiments is not a crucial factor to determine the release rate and behavior

As mentioned earlier, the release rate analysis was carried out according to Higuchi[26] and Korsmeyer *et al.*[27] equations. The main parameter values are listed in Table III. The four matrices showed, in general, a good fit to the different equations. Matrices of the D-form (**X** and **XII**) were released from the polymer faster than those of the L-form. In addition, the D-form matrices have values of $n > 0.5$, which were obtained from the Korsmeyer equation. This means that the drug release mechanism can be controlled by an anomalous, non-Fickian drug diffusion. These results suggest that the drug release either from D- or L-form is govern by diffusion, which is attributed to the macroporous voids (open holes) in the polymer-MTZ adducts, as shown in SEM micrographs (Figure 2). In contrast, the n values from the Korsmeyer equation are equal to 0.52 for the L-form, suggesting that the drug release mechanism is mainly governed by quasi-Fickian drug diffusion. These results indicate that the

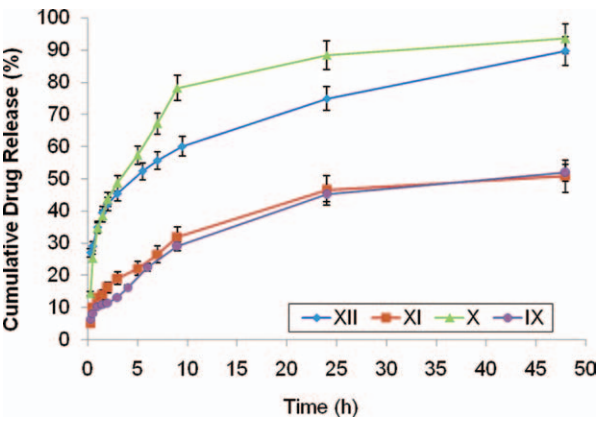

Figure 3. Effect of polymer microstructure on the cumulative release of MTZ from the polymer-MTZ adducts (**IX-XII**) in phosphate buffer of pH 8.5 at 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table III. Mathematical Modelling and Drug Release Kinetics of Metonidazole from the Polymer-MTZ Adducts at pH = 8.5

	Higuchi equation		Korsmeyer equation		
Polymer code	k ($min^{-0.5}$)	R^2	N	k' (min^{-n})	R^2
IX	0.016	0.9915	0.51	0.012	0.9885
X	0.021	0.9958	0.61	0.049	0.9978
XI	0.031	0.9872	0.52	0.025	0.9942
XII	0.040	0.9972	0.71	0.037	0.9756

k, Higuchi kinetic constant; n, release exponent; k', Korsmeyer kinetic constant.

configuration of the amino acid-based polymers affects not only the rate of the drug release but also the release mechanism, which is most probably related to the difference in the environment around the ester linkage in the buffer solution, resulting in the occurrence of the stereoselective hydrolysis. After the release of MTZ from the insoluble prodrugs, it would become water-soluble products having carboxylic acid moiety with increasing the release, in which the degree of ionization is governed by the pH and ionic strength of the aqueous solution. For example, the insoluble prodrug poly(A-Ala-OMTZ) was changed into poly(A-Ala-OMTZ-*co*-A-Ala-OH) by the hydrolysis, and the reacted polymer become water-soluble product if the content of the poly(A-Ala-OH) component is high enough at neutral pH range. In contrast, the reacted polymer, poly(A-Ala-OMTZ-*co*-A-Ala-OH), was insoluble in acidic water. In the cases of the poly(A-Phe-OMTZ), the reacted polymer, poly(A-Phe-OMTZ-*co*-A-Phe-OH), was insoluble in water, independent on the degree of the hydrolysis and pH value, because the poly(A-Phe-OH) was insoluble in neutral water and acidic water. It means that the pH value slightly affects the water-solubility of the reacted polymers in the buffer solution, which is related to the release rate, in addition to the actual hydrolysis rate of the ester bonds between the polymers and MTZ. In other words, the hydrolysis rate of the ester bonds, the diffusion rate, and the solubility of the reacted products may affect the release rate.

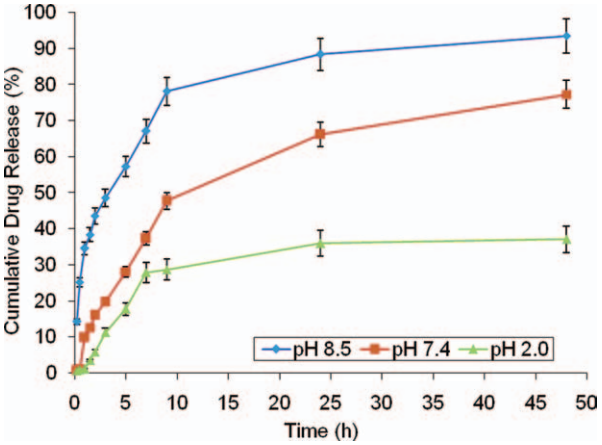

Figure 5. Effect of pH on the cumulative release of MTZ from poly(A-D-Phe-OMTZ) **X** in phosphate buffer with pH values of 2.0, 7.4, and 8.5 at 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The effect of pH on the release rate of MTZ was investigated at 37°C in specific pH media: pH 2.0 (stomach pH), pH 7.4 (physiological fluids pH), and pH 8.5 (colon pH). The percentage of the cumulative release of MTZ from the polymer-MTZ adduct (**IX**) in buffer solution at different pH values is shown in Figure 4. After 48 h, the polymer–MTZ adduct (**IX**) released about 18% of its drug content at pH 2.0, whereas about 41 and 52% of MTZ were released from the adduct (**IX**) at pH 7.4 and pH 8.5, respectively. The total amount of MTZ released from the polymer-MTZ adduct (**X**) was 37% of its drug content after 48 h at pH 2.0, compared to 77 and 93% at pH 7.4 and 8.5, respectively (Figure 5). Similar behavior was also observed in the alanine-based adducts (Figures 6 and 7), in which the percentages of the cumulative release were 25, 36, and 51% for the

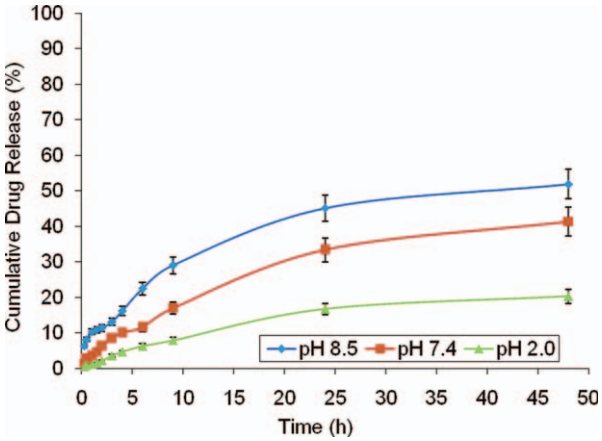

Figure 4. Effect of pH on the cumulative release of MTZ from poly(A-L-Phe-OMTZ) **IX** in phosphate buffer with pH values of 2.0, 7.4, and 8.5 at 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

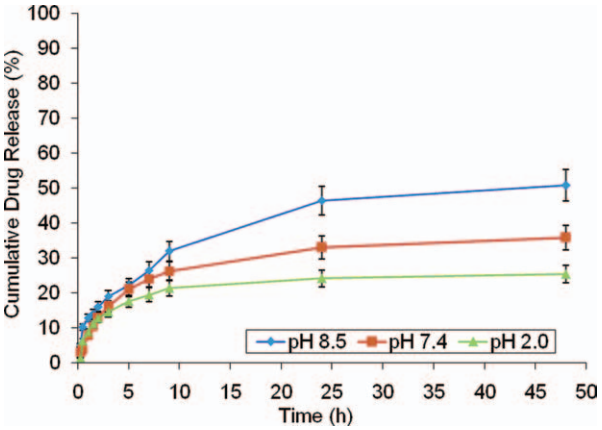

Figure 6. Effect of pH on the cumulative release of MTZ from poly(A-L-Ala-OMTZ) **XI** in phosphate buffer with pH values of 2.0, 7.4, and 8.5 at 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

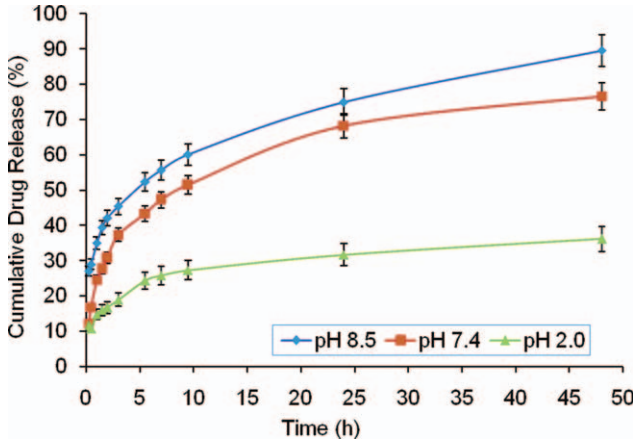

Figure 7. Effect of pH on the cumulative release of MTZ from poly(A-D-Ala-OMTZ) **XII** in phosphate buffer with pH values of 2.0, 7.4, and 8.5 at 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

L-form adduct (**XI**) and 36, 77, and 90% for the D-form adduct (**XII**). These results indicate that the release rate of MTZ from the polymer–MTZ adducts increases with the pH value, independent of the nature of the amino acids (L and D forms, alanine, and phenylalanine). This is an indication that polymer-MTZ adducts (**IX–XII**) show high hydrolytic stability in acidic medium (i.e., stomach pH), whereas the release rate of MTZ is the highest in an alkaline medium (i.e., colon pH). Similar tendency was observed in other systems.[3,24,31–33] Because the prepared prodrugs were adequately stable at acidic pH and more sensitive to the alkaline hydrolysis, these prodrugs seem to be suitable for the development of a colonic drug delivery system for MTZ.

CONCLUSIONS

We have demonstrated a new amino acid-based polymer system for drug delivery. Amino acid-based polymers synthesized using RAFT polymerization were employed to fix a model drug in the side chain via amino acid moiety as the spacer. The rate of metronidazole (MTZ) release from the polymeric products derived from four different amino acids, L-phenylalanine, D-phenylalanine, L-alanine, and D-alanine, was studied at various pH values. The release rate was found to mainly depend on the configuration of the amino acid-based polymer/MTZ adducts and the pH of the release media. The kinetic studies showed that the polymers containing the D-configuration were released faster than those containing the L-configuration, which was related to the difference in the release mechanism. In addition, the polymer-MTZ adducts showed high hydrolytic stability in acidic medium (i.e., stomach pH), whereas the release rate of MTZ was the highest in an alkaline medium (i.e., colon pH). These results suggest that amino acid-based polymers could be useful carriers in controlled release systems.

ACKNOWLEDGMENTS

Dr. Mohamed El-Newehy (Application form ID: 10430, Grant 0907 Cycle) would like to thank the Egyptian Ministry of Higher Education and State of Scientific Research (MHESR), Partnership and Ownership Agreement (ParOwn Grant) (www.mhesr-initiatives.org) for supporting him during his stay in Japan.

REFERENCES

1. Babazadeh, M. *Inter. J. Pharma.* **2008,** *356*, 167.
2. Hoste, K.; Winne, K. D.; Schacht, E. *Inter. J. Pharma.* **2004,** *277*, 119.
3. Kenawy, E. R.; Abdel-Hay, F.; El-Newehy, M.; Ottenbrite, R. M. *Polym. Inter.* **2008,** *57*, 85.
4. Nichifor, M.; Schacht, E.; Seymour, L. W. *J. Contr. Release* **1997,** *48*, 165.
5. Khandare, J.; Minko, T. *Prog. Polym. Sci.* **2006,** *31*, 359.
6. Babazadeh, M. *J. Appl. Polym. Sci.* **2007,** *104*, 2403.
7. Babazadeh. M.; Edjlali, L.; Rashidian, L. *J. Polym. Res.* **2007,** *14*, 207.
8. Mori, H.; Sutoh. K.; Endo, T. *Macromolecules* **2005,** *38*, 9055.
9. Mori, H.; Matsuyama, M.; Sutoh, K.; Endo, T. *Macromolecules* **2006,** *39*, 4351.
10. Klok, H. A.; Langenwalter, J. F.; Lecommandoux, S. *Macromolecules* **2000,** *33*, 7819.
11. Schlaad, H.; Kukula, H.; Smarsly, B.; Antonietti, M.; Pakula, T. *Polymer,* **2000,** *43*, 5321.
12. Floudas, G.; Papadopoulos, P.; Klok, H. A.; Vandermeulen, G.W. M.; Rodriguez-Hernandez, J. *Macromolecules* **2003,** *36*, 3673.
13. Li, B. S.; Cheuk, K.K. L.; Ling, L.; Chen, J.; Xiao, X.; Bai, C.; Tang, B. Z. *Macromolecules* **2003,** *36*, 77.
14. Klok, H. A. *J. Polym. Sci. Part A Polym. Chem.* **2005,** *43*, 1.
15. Hamley, I. W.; Ansari, A.; Castelletto, V.; Nuhn, H.; Rosler, A.; Klok, H. A. *Biomacromolecules* **2005,** *6*, 1310.
16. Cornelissen, J.J. L.M.; Rowan, A. E.; Nolte, R.J. M.; Sommerdijk, N. A. *J.M. Chem. Rev.* **2001,** *101*, 4039.
17. Oishi, T.; Lee, Y. K.; Nakagawa, A.; Onimura, K.; Tsutsumi, H. *J. Polym. Sci. Part A Polym. Chem.* **2002,** *40*, 1726.
18. Lekchiri, A.; Morcellet, J.; Morcellet, M. *Macromolecules* **1987,** *20*, 49.
19. Barbucci, R.; Casolaro, M.; Magnani, A. *J. Controlled Release* **1991,** *17*, 79.
20. Casolaro, M.; Barbucci, R. *Int. J. Artif. Organs.* **1991,** *14*, 732.
21. Bentolila, A.; Vlodavsky, I.; Ishai-Michaeli, R.; Kovalchuk, O.; Haloun, C.; Domb, A. J. *J. Med. Chem.* **2000,** *43*, 2591.
22. Frechet, J. M. *J. Prog. Polym. Sci.* **2005,** *30*, 844.
23. Mori, H.; Matsuyama, M.; Endo, T. *Macromol. Chem. Phys.* **2008,** *209*, 2100.
24. Chang, C. H.; Sheu, Y. M.; Hu, W. P.; Wang, L. F.; Chen, J. S. *J. Polym. Sci. Part A Polym. Chem.* **1998,** *36*, 1481.
25. Kenawy, E. R.; El-Newehy, M. H.; Abdel-Hay, F. I.; Ottenbrite, R. M. *Biomacromolecules* **2007,** *8*, 196.

26. Higuchi, T. *J. Pharm. Sci.* **1963,** *52*, 1145.
27. Korsmeyer, R. W.; Gurny, R.; Doelker, E.; Buri, P.; Peppas, N. A. *Int. J. Pharm.* **1983,** *15*, 25.
28. Ritger, P. L.; Peppas, N. A. *J. Control Release* **1987,** *5*, 23.
29. Martino, P. D.; Censi, R.; Malaj, L.; Capsoni, D.; Massarotti, V.; Martelli, S. *Cryst. Res. Technol.* **2007,** *42*, 800.
30. Wu, Q.; Wang, M.; Chen, Z. C.; Lu, D. S.; Lin, X. F. *Enzym. Microb. Tech.* **2006,** *39*, 1258.
31. Neilands, J. B. Struct Bonding (Berlin) **1966,** *1*, 59.
32. Roman, J.; Lucyna, H. *Polym. Bull.* **2010,** *64*, 459.
33. Gallardo, A.; Parejo, C.; San Roman, J. *J. Control Release* **2001,** *71*, 127.