Scientific paper

Synthesis and Characterization of Novel Polymer-Drug Conjugates Based on the Poly(Styrene–*alt*–Maleic Anhydride) as a Potential Method for Drug Release

Ardeshir Khazaei,[1,*] Shahnaz Saednia,[1,*] Javad Saien,[1] Maryam Kiani Borazjani,[2] Sadegh Rahmati,[3,†] Ali Hashempour-Zaviye[2,†] and Fatemeh Abbasi[1,†]

[1] *Faculty of Chemistry, Bu-Ali Sina University, Hamedan, PO Box 651783868, Iran*

[2] *Department of Chemistry, Payame Noor University, P.O. Box 19395-3697, Tehran, Irann*

[3] *Young Researchers & Elites Club, Hamedan Branch, Islamic Azad University, Hamedan, Iran*

[†] *These authors contributed equally to this work.*

** Corresponding author: E-mail: Khazaei_1326@yahoo.com (AK); Tel.: +98-811-825-2807; fax: +98-811-838-0709*
ssaednia@gmail.com (SS); Tel.: +98-811-825-7407; fax: +98-811-838-0709

Received: 10-12-2012

Abstract

Six well known drugs, captopril, metformin·HCl, metroniazole, nortriptyline·HCl, fluoxetine·HCl and betahistin·HCl, were grafted to poly(styrene–*alt*–maleic anhydride) (PSMA). Grafting was attained by combining of anhydride groups in the PSMA with therapeutic agents containing NH, OH or SH groups. The covalently grafted drugs were identified by infrared, ^{1}H NMR and UV-Vis spectroscopy. The drug release data at different times fit well to the Korsmeyer-Peppas equation. The analysis of the exponent *n* of this model revealed a dominant Fickian diffusion mechanism under the in vitro conditions. Furthermore, mean dissolution time values (45.9 to 86.7 h) indicate a high resistance against drugs transport, the highest being obtained for betahistin·HCl.

Keywords: Drug release, polymer-drug conjugate, PSMA, kinetics

1. Introduction

Chemically controlled drug delivery systems can be used for (1) sustained constant concentration of therapeutically active compounds in the blood with minimum fluctuation; (2) protection of bioactive compounds having a very short half-life; (3) elimination of side-effects, waste of drug and frequent dosing; (4) optimized therapy and better patient compliance.[1]

In these systems, therapeutic agent is chemically linked to the backbone of polymer with degradable chemical bonds and the drug is released by hydrolytic or enzymatic cleavage. The rate of drug release is controlled by the rate of hydrolysis. This approach provides an opportunity to target the drug to a particular cell type or tissue. Chemically controlled systems have two choices; pendant–chain system and biodegradable system. In a pendent–chain system drug is chemically bonded to a polymer backbone. In the body, in the presence of enzymes and biological fluids, chemical hydrolysis or enzymatic cleavage, occurs with concomitant release of the drug at a controlled rate.[2] In the biodegradable system, the controlled release of the drug involves polymers that gradually decompose. The drug dispersed uniformly throughout the polymer and is slowly released as the polymer disintegrates.[3]

Poly(styrene-*alt*-maleic anhydride) (PSMA) belongs to a group of vinylic polymers and has been used for the preparation of chemically and diffusionally controlled

polymeric prodrugs of dopamine,[4] ampicillin,[5] arciflavine,[6] 4-hydroxybenzoic acid,[7] 4-aminophenol,[8] fenoprofen and gemfibrozil.[9] The most successful example is attachment of the neocarzinostatin to PSMA by amide linkage. Maeda and coworkers,[10] used low molecular weight PSMA copolymers clinically to deliver the antitumor protein neocarzinostatin.

In this work, we report novel types of polymer-drug conjugates, combining PSMA with medically important compounds such as nortriptyline (treatment of depression),[11] fluoxetine (antidepressant),[12] betahistine (antivertigo drug),[13] metformin (antidiabetic drug),[14] metronidazole (antibiotic),[15] and captopril (treatment of hypertension).[16] Drugs with active group (NH, SH or OH) are linked to PSMA by amide, thioester or ester bond formation. The hydrolysis reactions are carried out in buffer solution of pH = 1.3 at 37 °C. The kinetics and mean duration time of drug release are also investigated.

2. Experimental Section

2. 1. Materials

Styrene, maleic anhydride, anhydrous dimethylformamide (DMF), tetrahydrofuran (THF), and benzoyl peroxide were purchased from Merck. Drugs, such as metformin·HCl, betahistin·HCl, nortriptyline·HCl, fluoxetine·HCl, metronidazole and captopril were purchased from Shenyang Antibiotic Manufacturer. All chemicals were used without further purification.

2. 2. Characterization

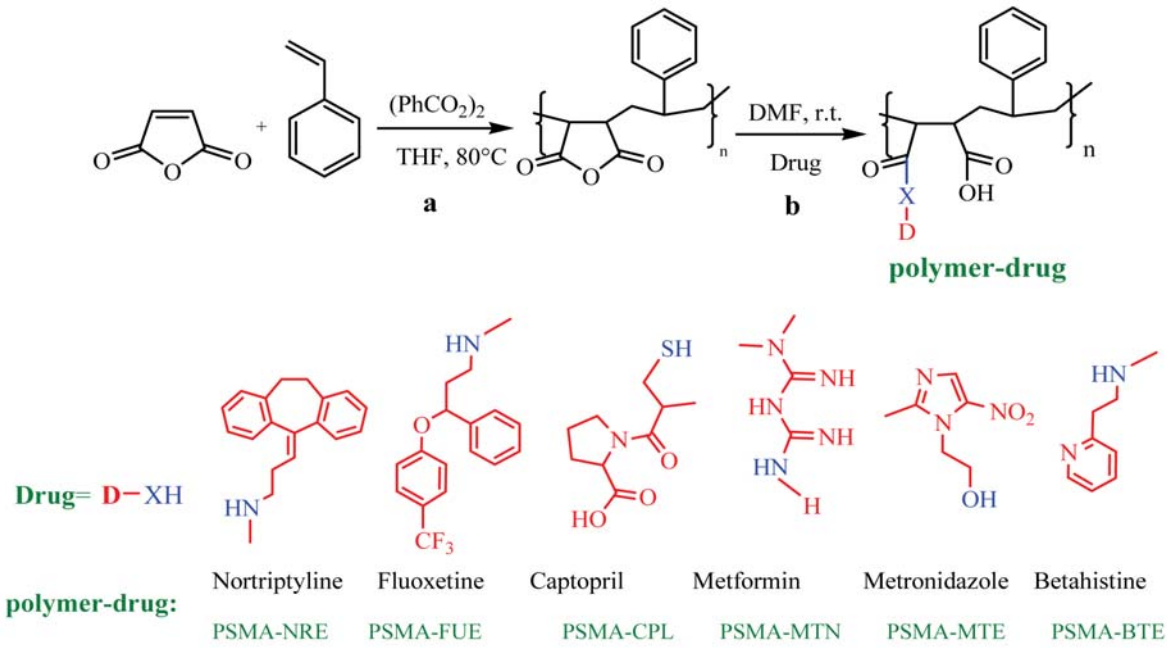
IR spectra were taken on a Shimadzu 435-U-04 spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer using $(CD_3)_2SO$ as solvent. Molecular weight of PSMA copolymer was determined with a Waters 150 GPC analysis instrument (mobile phase: THF; flow: 1.0 mL min^{-1} and column temperature: 30 °C).

2. 3. Synthesis of PSMA Copolymer

Poly(styrene-*alt*-maleic anhydride) (PSMA) was prepared through thermally initiated free-radical polymerization of styrene and maleic anhydride (Scheme **1a**): A mixture of the equimolar amounts (0.043 mol) of styrene (5.0 mL) and maleic anhydride (4.23 g), in addition to 4.3 mmol of benzoyl peroxide (1.04 g), as an initiator, in 25 mL of THF were placed in a flask equipped with a reflux condenser and a magnetic stirrer. The reaction was carried out at 80 °C under nitrogen atmosphere for 7 h. The viscous liquid of PSMA was purified and precipitated by cold methanol. The precipitated pure PSMA copolymer was collected and dried under vacuum at room temperature.

2. 4. General Procedure for Drug–Loading on PSMA

The loading of drugs having free NH, OH, or SH groups to PSMA was carried out by following method: 1g



Scheme 1. General synthetic scheme: (a) synthesis of PSMA alternating copolymer, and (b) grafting of drugs (metformin, betahistin, nortriptyline, fluoxetine, metronidazole, and captopril) to the PSMA.

of PSMA dissolved in dry DMF (25 mL) with a magnetic stirrer for 15 min, and then 5 mmol of selected drug was added into solution. In the reaction of grafted betahistine HCl to PSMA 5 mmol of triethylamine was also added to the solution for dissolving betahistine HCl in DMF. Reaction was carried out at room temperature under nitrogen atmosphere for 12 h. Resulting products were purified by distilled water and dried under vacuum at room temperature (Scheme **1b**).

2. 5. In Vitro Drug Release Study

In vitro drug release studies of grafted drugs have been achieved by placing dried samples in defined volume of buffer pH 1.3 at 37 °C. The standard calibration curve of drugs after suitable dilution was first prepared using a UV-Vis spectrophotometer (Shimadzu UV-265) for the absorbance measurements at λ_{max} in buffer solution, and then quantity of hydrolyzed drugs were determined. The λ_{max} of the used drugs in buffer solution are given in Table **1**. Buffer solution of pH 1.3 was prepared by adding 25 mL of KCl (0.2 mol L^{-1}) to the 33.6 mL of HCl (0.2 mol L^{-1}) in volumetric flask and diluting to 100 mL with distilled water.[17]

Table 2. Polymer characteristics of the PSMA copolymer used in this study

polymer	M_w (g mol^{-1})	M_n (g mol^{-1})	PDI
PSMA	3995	1973	2.024

ne respectively. Methine protons of maleic anhydride appear between δ = 3.3–3.5 ppm. Molecular weight of PSMA was determined by gel permeation chromatography (GPC). Table **2** shows the average molecular weight (M_w), number average molecular weight (M_n) and polydispersity (PDI = M_w·Mn^{-1}) of PSMA.

3. 2. Characterization of Drugs Grafted to PSMA Copolymer

The loading of a drug onto PSMA copolymer was performed by formation of chemical bond to a polymer backbone (Scheme 1). Characterization of functional groups of drugs covalently bonded to PSMA was performed by IR and UV-Vis spectroscopic methods. Amide, ester and thioester groups are created by reaction of OH, NH or SH groups of drugs with anhydride groups in PS-

Table 1. Maximum absorbance wavelength of used pure drugs in buffer solution of pH 1.3.

drug	nortriptyline·HCl	fluoxetine·HCl	betahistine·HCl	metformin·HCl	metronidazole	captopril
λmax (nm)	239	210	260	207	278	207

3. Results and Discussion

3. 1. Characterization of PSMA Copolymer

IR spectrum of PSMA copolymer shows that the band corresponding to the anhydride groups is at 1856 cm^{-1}, 1799 cm^{-1} (cyclic anhydride C=O) and 1224 cm^{-1} (cyclic C-O-C). In ^{1}H NMR spectrum of PSMA copolymer broad overlapping absorptions between δ = 1.1–2.8 ppm and absorptions between δ = 6–7.9 ppm are due to methylene/methane and aromatic ring hydrogens of styrene. Table **3** summarizes the main observed bands in IR spectrum of polymer-drug conjugates. By integration of appropriate absorptions area in ^{1}H NMR spectra, the molar ratio of attached drug per unit of monomer was calculated separately for each PSMA-drug copolymer sample. The evaluated percentages of attached drug per unit of monomer were about 70.8, 33.8, 73.3, 38.6, 78.2 and 22.4% for captopril, metformin, metroniazole, nortriptyline, fluoxetine and betahistin, respectively.

The solubility characteristics of PSMA-drug conjugates and PSMA in different solvents were also inve-

Table 3. IR absorption bands for the selective drugs grafted to PSMA solid samples.

Compound	Region (υ, cm^{-1})	Band assignments
PSMA-NRE, PSMA-FUE, PSMA-BTE, PSMA-MTN, PSMA-MTE	1779, 1779, 1780, 1778, 1778	Anhydride C=O stretch vibration
PSMA-NRE, PSMA-FUE, PSMA-BTE, PSMA-MTN, PSMA-MTE	1855, 1856, 1857, 1856, 1855	Anhydride C=O stretch vibration
PSMA-NRE, PSMA-FUE, PSMA-BTE, PSMA-MTN, PSMA-CPL	1651, 1615, 1647, 1648, 1647	Amide C=O stretch vibration
PSMA-NRE, PSMA-FUE, PSMA-BTE, PSMA-MTN, PSMA-MTE, PSMA-CPL	1712, 1721, 1726, 1720, 1724, 1728	Carbonyl C=O stretch vibration
PSMA-NRE, PSMA-FUE, PSMA-BTE, PSMA-MTN, PSMA-MTE, PSMA-CPL	3442, 3440, 3504, 3443, 3448, 3438	Carboxylic acid O-H stretch vibration

Table 4. Solubility of various polymer-drug conjugates in different solvents.[a]

Solvent	PSMA-NRE	PSMA-FUE	PSMA-BTE	PSMA-MTN	PSMA-MTE	PSMA-CPL	PSMA
DMSO	+	+	+	+	+	+	+
DMF	+	+	+	+	+	+	+
Acetone	+	+	+	+	+	+	–
EtOH	–	–	–	–	–	+	–
$CHCl_3$	–	×	–	–	–	–	–
Acetonitrile	×	+	–	+	+	–	–
Ethyl acetate	–	–	–	–	×	–	–
CH_2Cl_2	–	–	–	–	–	–	–
n-Hexane	–	–	–	–	–	–	–

[a] Soluble (+), insoluble (–), and dispersible (×).

stigated. The results showed that an increase in the solubility in organic solvents was resulted from binding of the selected drugs on PSMA copolymer, and the highest solubility was attained in acetone. Table 4 shows the solubility of various polymer-drug conjugates in different solvents.

3. 3. In Vitro Release of Drug From Drug-Loaded PSMA System

All drugs used in this research are oral. Therefore, in vitro hydrolysis investigations of drug-loaded PSMAs were performed according to the simulated gastric juice condition of human body[18] at acidic pH values (range of 1.1–1.5). In such acidic medium, drug-loaded PSMAs hydrolysis were completed gradually as shown in Figure 1. In this way drug release is more controlled and permanent. The percentage of released drug was measured by following the absorbance of the solution at λ_{max}, corresponding to the pure selected drugs (see Table **1**). Samples were taken at different intervals (Figure **1**) and the rate of drug release is rather fast at the beginning of the process (first 50 hours).

In the buffer solution of pH 1.3, hydronium ion in the presence of water catalyzes the hydrolysis reaction of the drug from the polymer. It is notable that, due to the basicity of amines, hydrochloride salts are formed in the buffer solution of pH 1.3 from the amino drugs (Scheme **2**).

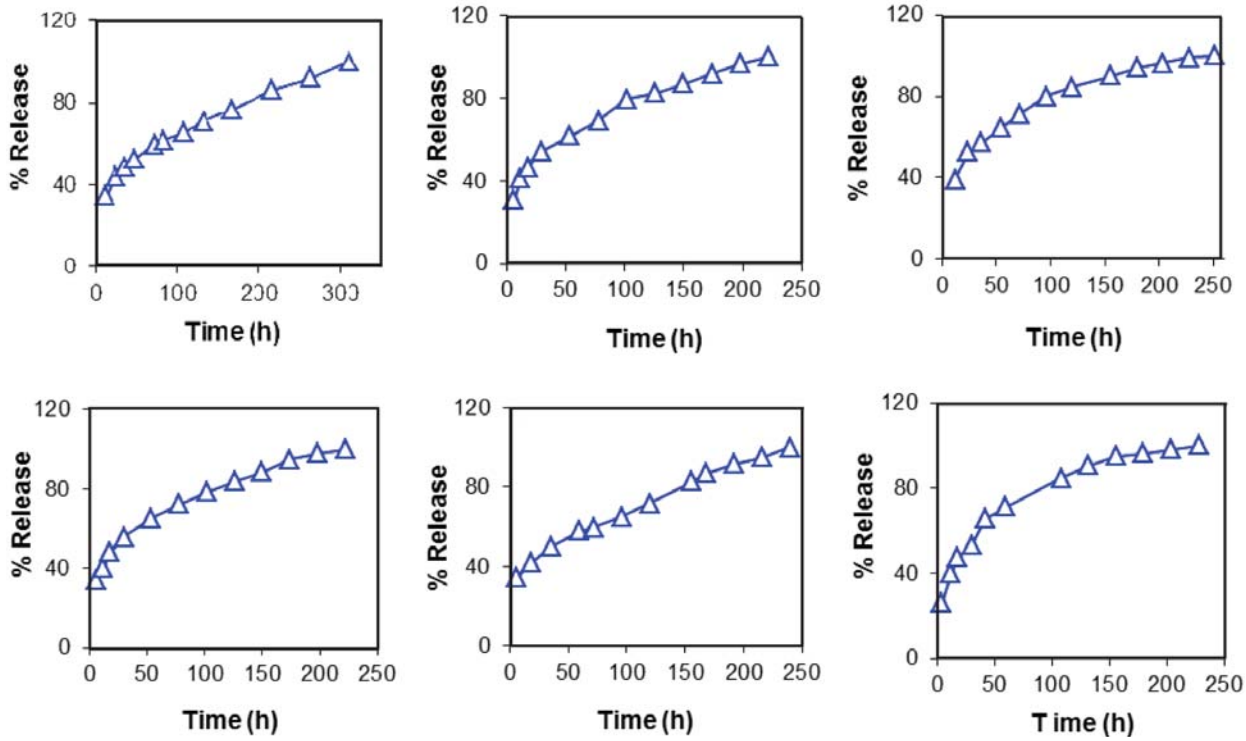
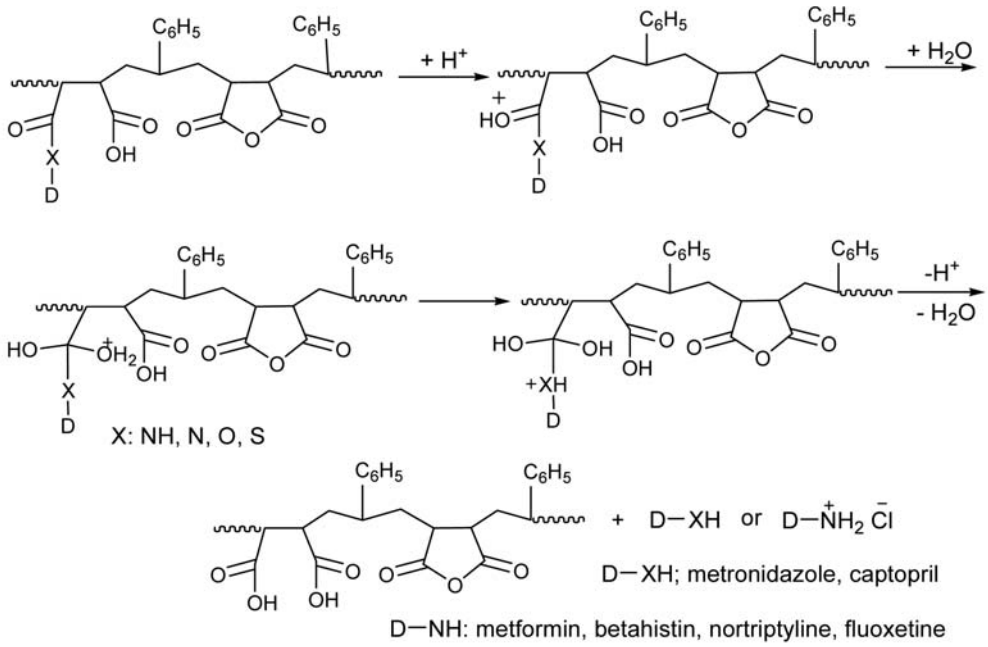


Figure 1. Drug Release profiles (pH =1.3, T =37 °C): (a) betahistin·HCl (PSMA-BTE), (b) metronidazole (PSMA-MTE), (c) metformin·HCl (PSMA-MTN), (d) captopril (PSMA-CPL), (e) nortriptyline·HCl (PSMA-NRE).and (e) fluoxetine·HCl (PSMA-FUE).



Scheme 2. The proposed mechanism of hydrolysis of PSMA-drug conjugates.

Different expressions can be used for describing the kinetics of drug release from polymeric systems. The kinetics of drugs release from PSMA was determined by searching the best proportion of the provided data with the well-known models. In this work, first-order (eq. 1),[19] Higuchi (eq. 2)[20] and Korsmeyer-Peppas (eq. 3)[21] models were examined for describing drug release from drug-loaded PSMA systems.

$$C_t = C_\infty (1 - e^{-k_1 t}) \quad (1)$$

$$C_t = k_H \sqrt[2]{t} \quad (2)$$

$$\frac{C_t}{C_\infty} = k_{KP} t^n \quad (3)$$

where C_t is the amount of drug released at time t, C_∞ is the maximum amount of released drug measured experimentally after long times (more than 200 h), k_1 is the first order drug releasing constant, k_H is the Higuchi rate constant, k_{KP} is the Korsmeyer-Peppas rate constant and n is the reaction order.

To study the release kinetics, data obtained from in vitro drug release studies were plotted as (a) – ln[1 – (C_t/C_∞)] versus time in first order model, (b) amount of drug released versus square root of time in Higuchi model, and (c) log (C_t/C_∞) versus log t in Korsmeyer-Peppas equation.

Figure **2** demonstrates results of these three used models. The kinetic parameters were obtained from slope and intercepts, and the results, together with the correlation coefficient r^2 are listed in Table **5**. The analysis of results shows that the best kinetic model for describing of the release of selected drugs from drug-loaded PSMA is Korsmeyer-Peppas equation. Results also reveals that first order model indicates better coherence than Higuchi model for all the drugs under investigation.

Table 5. Kinetic parameters and correlation coefficient (r^2) for release of selected drugs from drug-loaded PSMA.

model	parameter	drug					
		nortriptyline HCl	fluoxetine HCl	betahistine HCl	metformin HCl	metronidazole	coaptopril
first-order	k_1 (h^{-1})	0.0126	0.0192	0.0097	0.0168	0.0155	0.017
	r^2	0.9434	0.9765	0.9033	0.9513	0.9174	0.9318
Higuchi	k_H(ppm $h^{-1/2}$)	1.9628	4.2641	1.9172	1.4816	5.162	4.4035
	r^2	0.9296	0.8762	0.9001	0.8657	0.8095	0.8744
	k_{KP} (h^{-n})	0.1809	0.1758	0.1558	0.1878	0.1825	0.1978
Korsmeyer	n	0.2983	0.3317	0.3153	0.3088	0.3135	0.3002
-Peppas	r^2	0.9624	0.9897	0.9934	0.9912	0.9949	0.9981

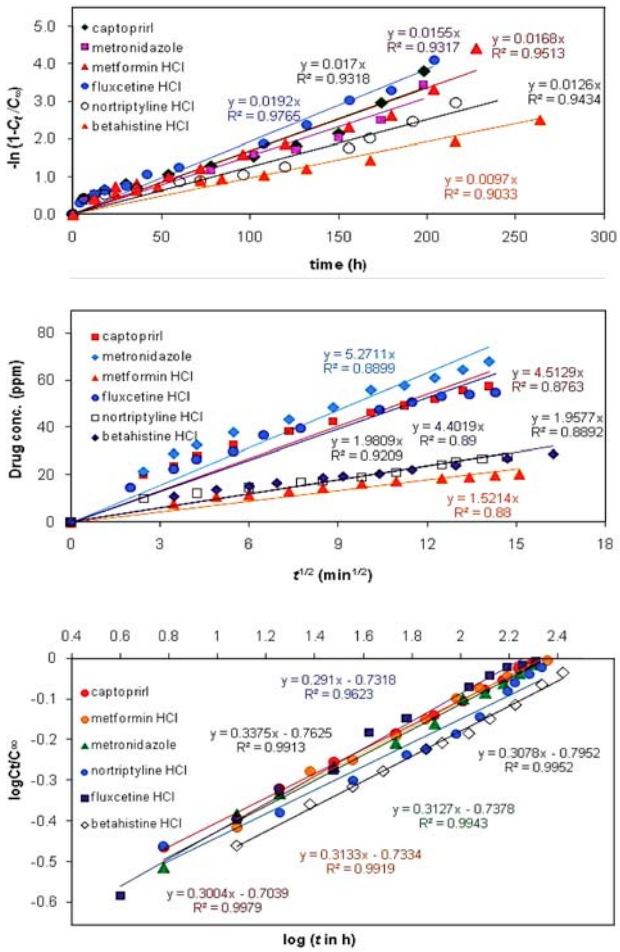


Figure 2. Drug release Kinetic plots for drug release obtained by fitting experimental data to (a) first order release model, (b) Higuchi release model and (c) Korsmeyer-Peppas kinetic model.

3. 4. Water Uptake

The water diffusion into polymer-drug conjugates is based on Fickian diffusion that supposes proportionality between the flux and concentration gradient.[22] Water uptake of the PSMA-drug conjugates (S_w) was obtained gravimetrically in distilled water at room temperature overnight (W_s). Before each measurement, the dry weight of polymer (W_d) which was dried under vacuum overnight, was also determined. The excess amount of water was gradually removed with filter paper. The water uptake was calculated by using the following equation:[22]

$$S_W(\%) = \frac{W_s - W_d}{W_d} \times 100 \quad (4)$$

Obtained swelling percentages were 30, 67, 73, 59, 50, 48, and 62% for PSMA, PSMA-CPL, PSMA-MTE, PSMA-MTN, PSMA-NRE, PSMA-FUE, and PSMA-BTE, respectively. These results indicate that drug-loaded PSMA systems have greater water uptake potential compared to the original polymer. Due to the possible hydrogen bonding of residual carboxylic acid groups in skeleton of drug-loaded polymeric chains, the swelling degree was increased.

3. 5. Mechanism of Drug Release

To evaluate the mechanism of drug release, the Korsmeyer-Peppas model[21c, 21d] can be used (eq. 5). This model is a semi-empirical power law model and to gain some insight into the drug release mechanism can be given in the form:

$$\log\left(\frac{C_t}{C_\infty}\right) = \log k_{KP} + n \log t \quad (5)$$

where (C_t/C_∞) is the fraction of drug released at time t and n is the exponent which depends on the release mechanism and is thus used to characterize it.[23] If the exponent n is 0.45 or less the release mechanism is purely Fickian diffusion (case-I), however, higher values $0.45 < n < 0.89$ for mass transfer follow indicate a non-Fickian model or anomalous transport (coupled diffusion/relaxation).[24] An exponent n value of 0.89 is indicative for a typical zero-order release (case-II transport),[21b] and $n > 0.89$ indicates a polymer relaxation or swelling-controlled mechanism (super case-II transport).[25]

As presented in Table **5**, for all the formulations under study, the n value vary between 0.29 and 0.33, i.e., is smaller than 0.45 and indicating that in our study the release mechanism is Fickian diffusion. This sort of release and transport of a drug through a polymeric controlled device is an ordinary molecular diffusion. Therefore, drug delivery devices can be designed that other mechanisms control the release rate, such as polymer relaxation or polymer swelling.

The mean dissolution time (t_m) was also calculated from kinetic measurements, according to the equation below (eq. 6), which has already been used in summation form by Vueba et al.[23b]

$$t_m = \frac{\int_0^{C\infty} t dC_t}{C_\infty} \quad (6)$$

This parameter reflects the level of drug-release retarding by the polymer matrix and the in vitro media. The t_m calculated values of the investigated drugs are presented in Figure **3**. Mean dissolution times are within 45.9 to 86.7 h. Among drugs, betahistin·HCl and fluoxetine·HCl show the highest and lowest dissolution times. This can be mainly attributed to the electronic interaction between nitrogen of pyridine group in betahistine·HCl and carbonyl groups of PSMA, causing the hydrolysis to be slow. Ester bond breaks faster than amide bond, however, the presence of electron-withdrawing CF_3group accelerate the hydrolysis of fluoxetine·HCl.

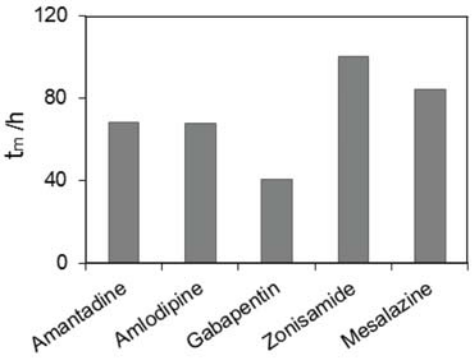


Figure 3. Mean dissolution time (t_m) for the drugs under study.

4. Conclusion

The covalent grafting of some known drugs with different pharmaceutical activities to PSMA was achieved by formation of amide, thioester and ester bond between drugs having NH, SH or OH groups and the anhydride group of PSMA. The study of in vitro release of drugs from grafted PSMA shows that drug release can occur from covalent PSMA-drug conjugate during long times, and can be considered as a useful method for drug release. The kinetic parameters, together with the mean dissolution time, indicate that a Fickian diffusion controls the release of drugs under study, and among them betahistin·HCl and fluoxetine·HCl exhibit the slowest and the fastest rate of release from drug-loaded PSMA, respectively.

5. Acknowledgments

The authors acknowledge to Bu-Ali Sina University Research Council and Center of Excellence in Development of Chemical Methods (CEDCM) for support of this work.

6. References

1. a) A. Gallardo, J. S. Roman, *Polymer* **1993**, *34*, 394–400. b) A. K. Bajpai, S. K. Shukla, S. Bhanu, S. Kankane, *Prog. Polym. Sci.* **2008**, *33*, 1088–1118.
2. a) H. Ringsdorf, *J. Polym. Sci. Symp*. **1975**, *51*, 135–145. b) S. S. Shah, J. Wertheim, C. T. Wang, C. G. Pitt, *J. Control. Release* **1997**, *45*, 95–101. c) H. Patel, D. A. Raval, D. Madamwar, *Ind. J. Pharma. Sci.* **1997***, 59*, 153–157. d) N. J. Lee, J. Koo, S. S. Ju, S. B. Moon, W. J. Cho, I. C. Jeong, *Polym. Int.* **2002***, 51*, 569–576.
3. a) C. Elvira, J. F. Mano, J. S. Roman, R. L. Reis, *Biomaterials* **2002**, *23*, 1955–1966. b) S. Lu, K. S. Anseth, *Macromolecules* **2000***, 33*, 2509–2515. c) H. S. Choi, T. Ooya, S. Sasaki, N. Yui, O. Yuichi, T. Nakai, *Macromolecules* **2003**, *36*, 9313–9318. d) N. J. Einerson, K. R. Stevens, W. J. Kao, *Biomaterials* **2002***, 24*, 504–523. d) N. B. Graham, In: *Biotechnical and biomedical application.* J. M. Harris, Ed., Plenum Press: New York**,** 1992, pp. 1–13.
4. I. Kalčić, B. Zorc, I. Butula, *Int. J. Pharm.* **1996**, *136*, 31–36.
5. J. S. Patel, S. V. Patel, N. P. Talpada, H. A. Patel, *Angew. Makromol. Chem.* **1999**, *271*, 24–27.
6. H. A. Patel, D. A. Raval, D. Mandamwar, S. P. Patel, *Angew. Makromol. Chem.* **1998**, *263*, 25–30.
7. J. H. Jeong, Y. S. Byoun, Y. S. Lee, *J. Ind. Eng. Chem.* **2001**, *7*, 310–315.
8. J. H. Jeong, Y. S. Byoun, Y. S. Lee, *React. Funct. Polym.* **2002**, *50*, 257–263.
9. M. Zovko, M. Barbarić, B. Zorc, A. Hafner, Filipović-Grčić, *J. Acta Pharm.* **2005**, *55*, 169–176.
10. a) H. Maeda, M. Ueda, T. Morinaga, T. Matsumoto, *J. Med. Chem.* **1985**, *28*, 455–461. b) H. Maeda, *Adv. Drug Delivery Rev*. **2001**, *46*, 169–185. c) H. Maeda, T. Sawa, T. Konno, *J. Control. Release* **2001**, *74*, 47–61. d) S. M. Henry, M. E. El-Sayed, C. M. Pirie, A. S. Hoffman, P. S Stayton, *Biomacromolecules* **2006**, *7*, 2407–2414.
11. a) J. C. Nelson, J. S. Kennedy, B. G. Pollock, F. Laghrissi-Thode, M. Narayan, M. S. Nobler, D. W. Robin, I. Gergel, J. Mc Cafferty, S. Roose, *Am. J. Psychiatry* **1999**, *156*, 1024–1028. b) W. Bondareff, M. Alpert, A. J. Friedhoff, E. M. Richter, C. M. Clary, E. Batzar, *Am. J. Psychiatry* **2000**, *157*, 729–736.
12. a) A. Coppen, J. Bailey, *J. Affect. Disord.* **2000**, *60*(2), 121–30. b) E. Padilla, J. Shumake, D. W. Barrett, E. C. Sheridan, F. Gonzalez-Lima, *Brain Res.* **2011**, *1387*, 71–84.
13. a) C. D. Pepa, G. Guidetti, M. Eandi, *Acta Otorhinolaryngol. Ital.* **2006**, *26*, 208–215. b) J. J. Wang, M. B. Dutia, *Exp. Brain Res.* **1995**, *105*, 18–24.
14. a) E. Liepinsh, E. Skapare, B. Svalbe, M. Makrecka, H. Cirule, M. Dambrova, *Eur. J. Pharmacol.* **2011**, *658*, 277–283. b) J. B. Majithiya, R. Balaraman, *Life Sci.* **2006**, *78*, 2615–2624. c) J. Oleksyszyn, *Med. Hypotheses* **2011**, *77*, 171–173.
15. a) J. M. V. Cerdá, M. A. Almiñana, J. N. Guinot, V. M. Sanjuán, J. L. S. Sanchís, *Farm. Hosp.* **2008**, *32*(2), 77–82. b) P. F. Lanzky, B. Haning-Snrensen, *Chemosphere* **1997**, *35*(11), 2553–2561.
16. a) I. Ghfir, F. Z. Berehou, N. Ben Ra¿s, *Medecine Nucleaire,* **2007**, *31*, 388–391. b) C. C. Lin, Y. C. Shiau, T. C. Li, A. Kao, C. C. Lee, *J. Diabetes. Complicat.* **2002**, *16*, 344–346. c) E. Susalit, N. Agus, I. Effendi, R. R. Tjandrawinata, D. Nofiarny, T. Perrinjaquet-Moccetti, M. Verbruggen, *Phytomedicine*, **2011**, *18*, 251–258.
17. a) R. A. Robinson, R. H. Stokes, *Electrolyte solutions, the measurement and interpretation of conductance, chemical potential, and diffusion in solutions of simple electrolytes,* 2nd rev. ed., London, Butterworths, 1968. b) CRC Handbook of Chemistry and Physics. R. C. Weast, Ed., 63rd edition, CRC Press.
18. L. Yang, R. Zeng, C. Li, G. Li, R. Qiao, L. Hu, Z. Li, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2566–2569.

19. a) D. W. Bourne, In: *Modern pharmaceutics.* G. S. Banker, C. T. Rhodes, Eds., 4th ed., New York: Marcel Dekker Inc, 2002, pp. 67–92. b) A. S. Bravo, C. M. Lamas, J. C. Salomon, *J. Pharm. Pharm. Sci.* **2002***, 5*, 213–219. c) B. Narashimhan, S. K. Mallapragada, N. A. Peppas, In: *Encyclopedia of controlled drug delivery,* E. Mathiowitz, Ed.; John Wiley & Sons, Inc: New York 1999, pp. 921–935.
20. a) T. Higuchi, *J. Pharm. Sci*. **1961**, *50*, 874–875. b) T. Higuchi, *J. Pharm. Sci.***1963**, *52*, 1145–1149.
21. a) R. W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, N. A. Peppas, *Int. J. Pharm.* **1983**, *15*, 25–35. b) J. Siepmann, N. A. Peppas, *Adv. Drug Delivery Rev.* **2001**, *48*, 139–157. c) R. W. Korsmeyer, N. A. Peppas, *J. Membr. Sci.* **1981**, *9*, 211–227. d) R. W. Korsmeyer, N. A. Peppas, In: *Controlled Release Delivery Systems,* T. J. Roseman, S. Z. Mansdorf, Eds., Marcel Dekker Inc: New York, 1983, pp. 77–90.
22. A. M. Zalfen, D. Nizet, C. Jérôme, R. Jérôme, F. Frankenne, J. M. Foidart, V. Maquet, F. Lecomte, P. Hubert, B. Evrard, *Acta Biomater.* **2008**, *4*, 1788–1796.
23. a) N. A. Peppas, *Pharm. Acta Helv.* **1985**, *60*, 110–111. b) M. L. Vueba, L. A. E. Batista de Carvalho, F. Veiga, J. J. Sousa, M. E. Pina, *Eur. J. Pharm. Biopharm.* **2004**, *58*, 51–59.
24. M. Efentakis, I. Pagoni, M. Vlachou, K. Avgoustakis, *Int. J. Pharm.* **2007**, *339*, 66–75.
25. C. Ferrero, D. Massuelle, E. Doelker, *J. Control. Release* **2010**, *141*, 223–233.

Povzetek

Avtorji poročajo o vgradnji šestih zdravilnih učinkovin (kaptopril, metformin·HCl, metroniazol, nortriptilin·HCl, fluoksetin·HCl in betahistin·HCl) na poli(stiren–*alt*–maleinski anhidrid) (PSMA). Kovalentno vezavo so dosegli z reakcijo anhidridnih skupin PMSA z NH, OH in SH funkcionalnimi skupinami zdravilnih učinkovin ter jih tudi identificirali s pomočjo IR, ^{1}H NMR in UV-Vis spektroskopije. Študirali so tudi časovno sproščanje učinkovin s polimernega nosilca in ugotovili, da se dobro ujema s Korsmeyer-Peppasovo modelno enačbo. Analiza eksponentne vrednosti *n* iz tega modela kaže na pretežno Fickijev difuzijski mehanizem pod in vitro pogoji. Povprečni čas sproščanja učinkovin (45.9 do 86.7 ur) pa kaže na veliko rezistenco pri transportu učinkovin; največjo pri betahistinu·HCl.