Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (2):40-48
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Targeted and controlled release of indomethacin from polyacrylic carrier systems

Farnaz Esmaeili and Mirzaagha Babazadeh*

Department of Chemistry, Tabriz Branch, Islamic Azad University, Tabriz, Iran

---

ABSTRACT

In this research work, a serious of prodrugs of poly(2-hydroxyethyl methacrylate) grafted with indomethacin were moddly synthesized and their chemical structures were characterized by FT-IR and ^{1}H-NMR spectroscopy. The results showed that indomethacin was conjugated with polymer backbones through the ester bond. For in vitro experiment, the simulated medium for stomach, small intestine and colon was a chloric acid solution (pH 1.2), and a sodium phosphate buffer solution (pH 7.4 and 8.5). Detection of hydrolysis by UV spectroscopy at the wavelength of maximum absorption of the free indomethacin in the selected intervals showed that the drug was hardly released in the simulated stomach medium, but could be controlled release by hydrolysis of the polymeric prodrugs in the simulated small intestine and colonic conditions. Hydrophilic properties of polymeric prodrugs, as well as the reactivity of ester side groups used as weak links between the drug and the polyacrylic matrix, are considered on the basis of results obtained from in-vitro evaluation at different pH values.

Keywords: Indomethacin; Polyacrylic prodrugs; Controlled release systems; *In-vitro* evaluation; Polymerization.

INTRODUCTION

Indomethacin (IND), known as a typical model drug in non-steroidal anti-inflammatory drugs (NSAIDs), is widely used for the treatments of rheumatoid arthrites, spondylitis, and osteoarthritis (Figure 1). It works by inhibiting the production of prostaglandins, molecules known to cause these symptoms. But IND usually generates gasterointestinal side effects clinically, such as gastric ulcers and gastric perforation [1]. Thus it is important to realize the intestine targeted and controlled release of IND in order to decrease the gastric damage and achieve sustained medication clinically, espicially for alleviating the pain during sleeping at night [2,3]. Drug design in recent years has attempted to use a prodrug strategy as a chemical/biochemical approach to overcome various barriers which hinder drug delivery, such as the damage of tissue and mucous membrane, poor water solubility, and too rapid absorption or too rapid excretion [4,5].

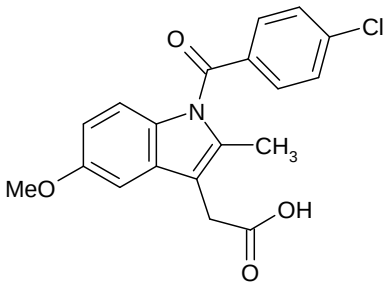

Figure 1. The structure of indomethacin.

It is well known that polymeric prodrug or polymer-drug conjugate is an effective and fast growing technique for improved use of drugs for therapeutic applications. Polymer conjugated drugs generally exhibit prolonged half-life, higher stability, water solubility, lower immunogenicity and antigenicity and specific targeting to tissues or cells. Polymers are used as carriers in polymeric prodrugs for the delivery of drugs, proteins, targeting moieties, and imaging agents [6]. The polymeric prodrug can be regarded as drug delivery systems that exhibit their therapeutic activities by means of releasing smaller therapeutic drug molecules from a polymer chain molecule for a prolonged period of time which results in enhanced pharmacokinetic behaviour by increasing the $t_{1/2}$, bioavailability, and hence prolonged pharmacological action. The potential of the polymer-drug conjugates have already been proved by success of many products in the market for the treatment of different diseases [7].

Ringsdorf [8] developed a rational model of polymeric prodrug for the first time in 1975. He was the first to recognize the immense potential of polymeric prodrugs, if only polymer chemists and biologists would work together in the field [9]. The proposed model consists mainly of five components: the polymeric backbone, the drug, the spacer, the targeting group and the solubilising agent (Figure 2). The role of spacer is to control the site and the rate of release of the active drug from the conjugate by hydrolytic or enzymatic cleavage [10,11]. The drug must be covalently bonded to the polymer and must remain attached to it until the macromolecule reaches the desired site of action.

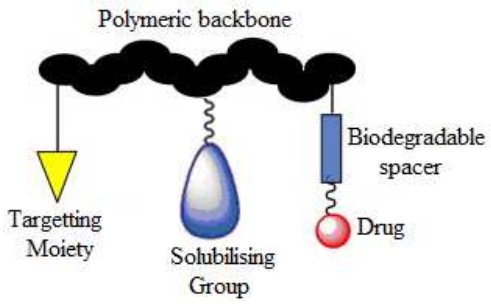

Figure 2: Ringsdorf's model of polymeric prodrug.

The choice of drug for use in this system is based on three criteria. First, only potent drug can be used because there is restriction on the amount of drug that can be administered. Second, the drug should have a functional group by which it can bind with the polymer backbone directly or by means of spacer molecule. Third, the drug must be sufficiently stable and should not be excreted in this conjugate form until it is released at the desired site [12].

During the past two decades, scientists have focused their attention to formulation and *in-vitro* or *in-vivo* evaluation of polymeric prodrugs of NSAIDs such as ibuprofen [13-15], ketoprofen [16,17], naproxen [18,19], fenoprofen [20], mefnamic acid [21], diclofenac [22,23], 5-aminosalicylic acid [24-26] and etc linked to synthetic or natural polymer backbons directly or by means of spacer molecules.

In this work, the release of IND from a prodrugs of polyacrylic grafted with IND was investigated. Acrylic-type polymers are an important class of used macromolecules in drug delivery systems. These system do not form toxic by-products during their biodegradation and which have tendency to swell, when they come in contact with

biological environment [27]. Hydrolysis of the obtained polymeric prodrugs was carried out similar to the physiological conditions and the results showed that the synthesized polymeric prodrugs were pH-sensitive polymers, and introduction hydrophilic units along the polymer chain improve the hydrolytic behavior.

MATERIALS AND METHODS

Materials

2-Hydroxyethyl methacrylate (HEMA), 2-hydroxypropyl methacrylate (HPMA), ethyl methacrylate (EMA), *N*,*N*´-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), and IND were bought from Merck. Azobisisobutyronitrile (AIBN) was obtained from Fluka and recrystallized twice from methanol and dried in a desiccator. *N*,*N*´-dimethyl formamide (DMF) was dried over anhydrous $MgSO_4$ for two days and later with phosphoric anhydride overnight. After drying, DMF was distilled under reduced pressure. All other reagents were used without further purification.

Instrumental measurments

The values of number average molecular weight (M_n), average molecular weight (M_w) and the polydispersity index (M_w/M_n) of polymers were measured by a gel permeation chromatography (GPC) instrument equipped with refractive-index detector (Waters 2410) and Waters Styragel GPC columns. The GPC columns were standardized with narrow dispersity polystyrene in molecular weights ranging from 4.7×10^6 to 2000. The mobile phase was DMF at a flow rate of 1.5 ml min^{-1}. FT-IR spectra were recorded on a Shimadzu 4300 spectrophotometer in KBr pellets. ^{1}H-NMR spectra were rocorded on Bruker 400 MHz spectrometer in DMSO-d_6 and $CDCl_3$ solution for determination of polymeric prodrugs and monomer, respectively. The released IND content was determined by a 2100 Shimadzu double beam UV-Visible spectrophotometer at the adsorption maximum of the free IND in aqueous buffered solutions (λ_{max}=318 nm) using a 1-cm quartz cell. Elemental analyses were caried out with a Heareus CHN instrument.

Synthesis and characterization of polymerizable monomer of IND-HEMA

IND drug (3.6 g, 10 mmol) was dissolved in 50 ml of dried DMF and cooled until -20°C. Then, DMAP (0.2 g), and DCC (2 g, 10 mmol) was dissolved in 20 ml of DMF and added dropwise. HEMA (1.3 g, 10 mmol) was dissolved in 10 ml of DMF and added into the mixture at -20°C. The reaction mixture was vigorously stirred at -20°C for 1 h and returned slowly to room temperature. After the reaction was stirred at room temperature about 24 h, the reaction mixture was filtered to remove of white precipitate of *N*,*N*´-dicyclohexylurea (DCU). DMF was evaporated and the obtained precipitate was recrystallized from methanol and dried under vacuum for 24 h to give 3.4 g (72%) of IND-HEMA monomer. The chemical structure of IND-HEMA was characterized FT-IR, ^{1}H-NMR, and elemental analysis as follows:

FT-IR (KBr, cm^{-1}) 3050 (C-H aromatic), 3030 (C-H vinylic), 2950, 2850 (C-H aliphatic), 1735, 1710, 1680 (C=O ester), 1600, 1480 (C=C aromatic), 1155 (C-O).

^{1}H-NMR ($CDCl_3$, ppm), 2.1 (s, 3H, =CC$\underline{H}_3$), 2.3 (s, 3H, Ar-C$\underline{H}_3$), 3.7 (s, 3H, Ar-OC$\underline{H}_3$), 4.1 (s, 2H, Ar-CH_2COO-), 4.2 (t, 2H, -C$\underline{H}_2$OCOCH_2-), 4.4 (t, 2H, -C$\underline{H}_2$OCOC=), 5.1 (d, 1H, CH_2=C), 5.7 (d, 1H, CH_2=C), 6.7-7.3 (m, 7H, aryl-$\underline{H}$).

Elemental analysis for $C_{25}H_{24}O_6NCl$ (469.5 $gmol^{-1}$), calculated: C 63.82, H 5.11, N 2.98; found: C 63.51, H 5.34, and N 3.12%.

Solubility test of IND-HEMA

One milligram of IND-HEMA monomer was placed in 1 mL of organic solvents. Solubility was observed with the naked eye.

Synthesis of polymeric prodrugs of IND (general procedure)

IND-HEMA was separately copolymerized with HPMA and EMA as follows and the obtained polymeric prodrugs were designated as PD1 and PD2, respectively. A mixture of IND-HEMA (2.35 g, 5 mmol), AIBN (0.2 g), HPMA (2.15 g, 15 mmol) was dissolved in 10 ml of dried DMF in a Pyrex glass ampoule. Then the ampoule was degassed, sealed under vacuum and maintained at 70±2ºC in a water bath and shaken by a shaker for about 30 h. The obtained viscous solution was poured into 150 ml of cooled methanol as non-solvent. The precipitate was collected and

washed with non-solvent for several times and dried under vacuum at room temperature to give 3.0 g (67%) of PD1. Also, a mixture of IND-HEMA (2.35 g, 5 mmol), AIBN (0.2 g), EMA (1.7 g, 15 mmol) in 10 ml of dried DMF was copolymerized as above method and gave 2.8 g (69%) of PD2 after drying under vacuum.

Method of hydrolysis

Each of powdered polymeric prodrugs (20 mg) was poured in 5 ml of a aqueous bufferd solution (pH 1.2, 7.4 and 8.5) at 37°C. The mixture was conducted into a cellophane membrane dialysis bag. The bag was closed and transferred into a flask containing 25 ml of same buffer solution maintained at 37°C. The external solution was continuously stirred and a 3 ml samples were removed at selected intervals and 3 ml of buffer was replaced. The quantity of hydrolyzed IND was analyzed by means of UV spectrophotometer at λ_{max} of IND (318 nm) and determined from the calibration curves obtained previously under the same conditions. In each concentration measurement, an equal volume of fresh buffer is added into hydrolysis solution and the dilution of hydrolysis solution occurs during hydrolysis process. Therefore, for calculation of the mean concentration of released drug, the each concentration measurement was corrected according to equation (1):

$$C_n = C_{n.meas} + \frac{\Delta V}{V_{total}} \sum_{i=1}^{i=n-1} C_{i.meas} \qquad (1)$$

where, n indicates the n^{th} concentration measurement, V_{total} is the total volume of hydrolysis solution (25 ml), ΔV is the withdrawn volume at each measurement (3 ml), $C_{n.meas}$ is the obtained drug concentration at the n^{th} measurement, and C_n is the corrected drug concentration in the hydrolysis solution due to introduction of a volume ΔV of buffer.

RESULTS AND DISCUSSION

Synthesis of IND-HEMA monomer

IND-HEMA was easily synthesized by direct esterification of IND with HEMA in the presence of DCC in DMF solution. The hydroxyl group of HEMA reacted with carboxyl group of IND and the resulted water was absorbed by DCC to produce DCU as a white precipitate. After completing of reaction, the white precipitate was isolated and the solvent was evaporated under vacuum to obtain IND-HEMA as stable polymerizable monomer (Figure 3). The solubility of this monomer was tested by dissolving 1 mg of monomer in 1 ml of solvent and listed in Table 1. The test will help us to find the non-solvents in copolymerization.

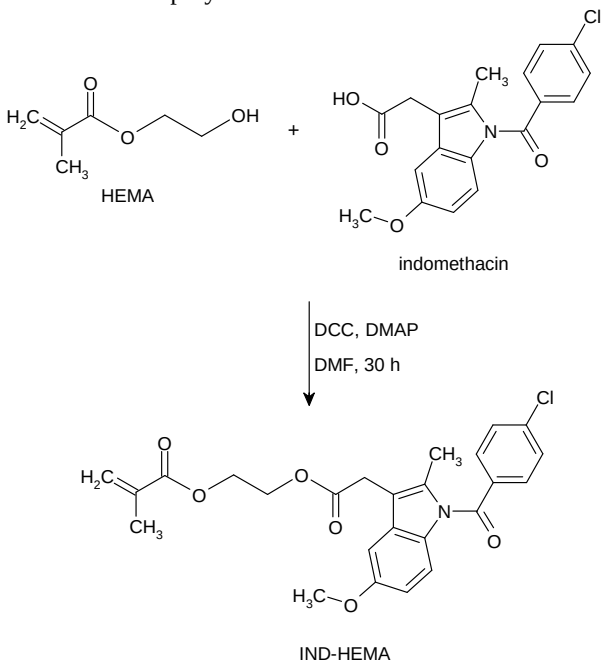

Figure 3. The synthesis route of IND-HEMA monomer

Table 1. Solubility test of the synthesized IND-HEMA monomer

Sample	*Methanol*	*Ethyl acetate*	*Hexan*	*Chloroform*	*DMF*	*Water*
IND-HEMA	Soluble (hot)	soluble	partial soluble	soluble	soluble	insoluble

The resultant FT-IR, ^{1}H-NMR, and elemental analysis data confirmed the structure of IND-HEMA and its purity. The related FT-IR and ^{1}H-NMR spectra of IND-HEMA are shown in Figures 4 and 5.

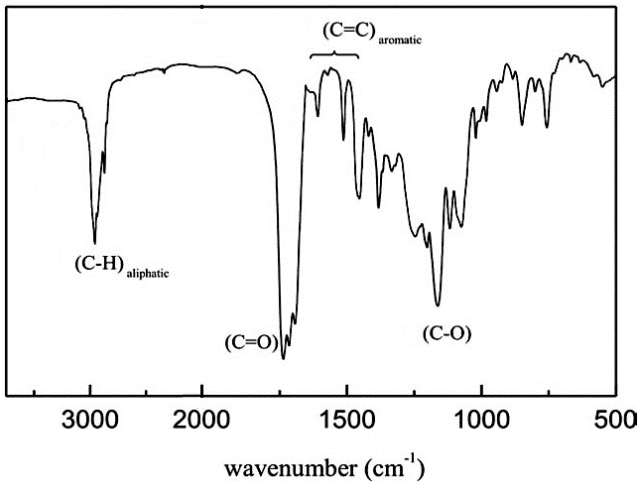

Figure 4. FT-IR spectrum of IND-HEMA in KBr pellet

Synthesis and characterization of polymeric prodrugs PD1 and PD2

IND-HEMA was easily copolymerized with HPMA and EMA in dried DMF solution by free radical polymerization technique at 70±2°C using AIBN as initiator to obtain PD1 and PD2 containing IND substituents (Figure 6).

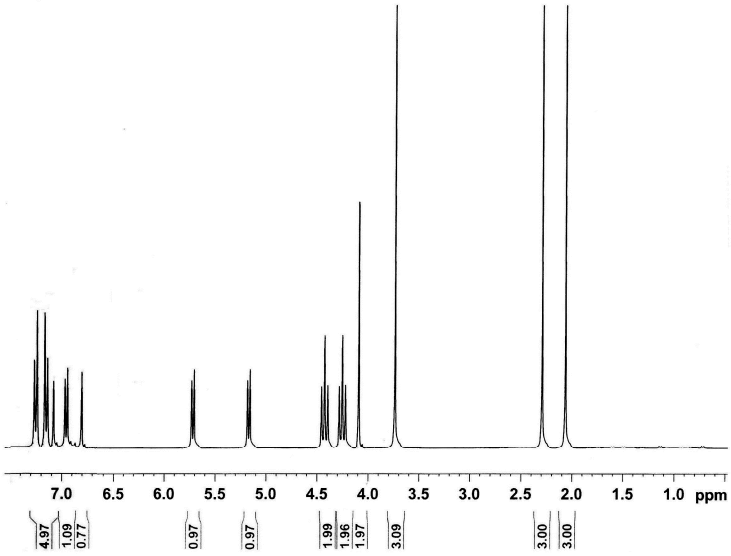

Figure 5. ^{1}H-NMR spectrum of IND-HEMA in $CDCl_3$ solvent

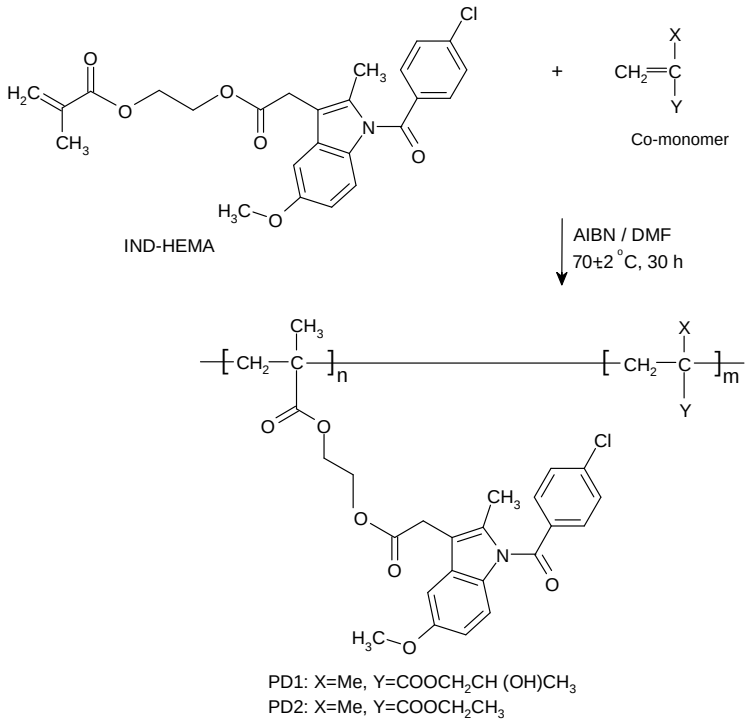

Figure 6. Copolymerization of IND-HEMA with HPMA and EMA to give polymeric prodrugs

The resulted polymeric prodrugs PD1 and PD2 were colorless, amorphous and soluble in DMSO and DMF, but insoluble in water and alcohols. The preparation conditions and yield of polymeric prodrugs are shown in Table 2. The prepared prodrugs were characterized through a variety of techniques including FT-IR and ^{1}H-NMR spectroscopy. The values of M_n and polydispersity index (M_w/M_n) of the synthesized polymeric prodrugs were estimated by GPC instrument and shown in Table 2.

Table 2. The preparation conditions, yields and molecular weights of the polymeric prodrugs

Sample	*[M1] (mmol/L)*	*[M2] (mmol/L)*	*Non-solvent*	*Yield (%)*	*M_n (×10^{-3})*	*M_w/M_n*
PD1	IND-HEMA (10)	HPMA (30)	Methanol	53.0	39.7	1.8
PD2	IND-HEMA (10)	EMA (30)	Methanol	65.0	33.5	2.1

The copolymer compositions of PD1 and PD2 were determined from ^{1}H-NMR spectroscopic data and elemental analysis of prodrugs. The calculated compositions of polymeric prodrugs are presented in Table 3. ^{1}H-NMR spectroscopic analysis and elemental analysis data are powerful tools for the determination of copolymer compositions because of their simplicity, rapidity and sensitivity [28, 29]. The results obtained from ^{1}H-NMR data and elemental analyses were relatively in good agreement.

Table 3. Elemental analyses, and mole compositions of the polymeric prodrugs

Sample	*C (%)*	*H (%)*	*N(%)*	*IND-HEMA (%)*	*HPMA (%)*	*EMA (%)*
PD1	61.2	6.6	1.5	24.6	75.4	-
PD2	62.4	6.4	1.7	28.5	-	71.5

Drug release by hydrolysis of polymeric prodrugs

It has been widely demonstrated that the side chain hydrolysis of drug pendent polymers depends on the strength and chemical nature of the drug polymer chemical bonds, the structure of the polymer and the surrounding condition. The hydrolysis of a linkage is also dependent on its distance from the polymer backbone. The length and hydrophilicity of the spacer unit between the drug and polymer chain can affect the release rate. We have studied the hydrolysis behavior of polymeric prodrugs in physiological conditions (aqueous hydrochloric acid or phosphate buffers, at 37^{o}C). As the polymers were not soluble in water, they were dispersed in buffer solution and the

hydrolysis was performed in a heterogeneous system. The hydrolysis was carried out in cellophane membrane bags permeable to low molecular weight compounds. The released drug passed through the high molecular weight polymers into the external buffer solution and determined by a UV spectrophotometer. Figures 7-9 show the release of IND from polymeric prodrugs as a function of time under mild conditions. The order of hydrolysis was as follows: PD1>PD2.

Two hydrolysable ester bonds are present in polymers. Detection of the hydrolyzing solution by UV spectrophotometer showed that only the ester bond between drug moiety and methylene group is hydrolyzed during the reaction time. The direct ester linkage between the main chain of polymer and methylene group does not undergo hydrolysis under mild conditions. This can be related to the steric hindrance of bulk polymer chains, which decrease the bond mobility [30]. The release rate of IND from polymeric prodrugs at alkaline medium was higher than the release rate of drug in acidic condition. It seems that polymeric prodrugs have a low degree of swelling in the stomach medium and the drug is protected against hydrolysis. The degree of hydrolysis increases as the polymer passes from acidic to alkali medium. In alkali pH, the polymers have reached a degree of swelling that makes the labile bonds accessible to hydrolysis.

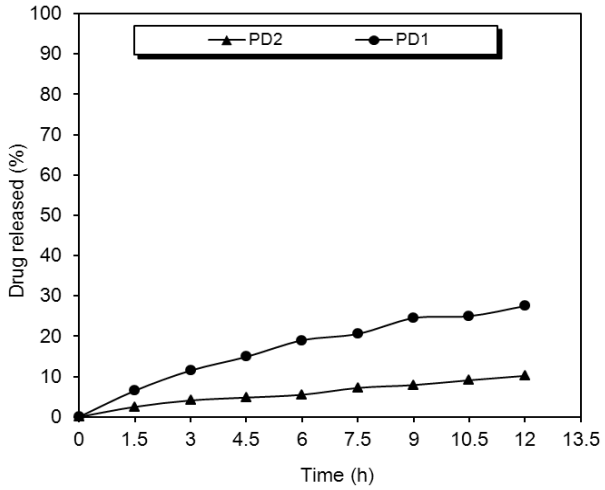

Figure 7. Release of IND from polymeric carriers as a function of time at pH 1.2 in 37°C

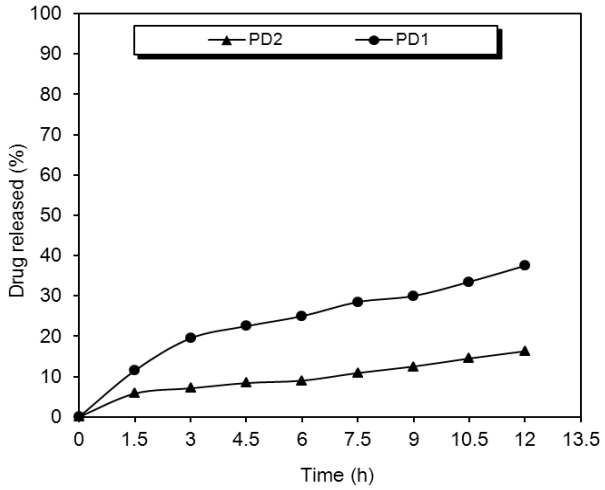

Figure 8. Release of IND from polymeric carriers as a function of time at pH 7.4 in 37°C

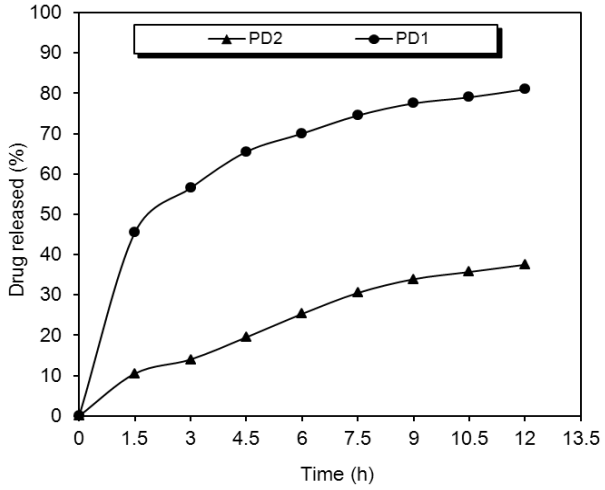

Figure 9. Release of IND from polymeric carriers as a function of time at pH 8.5 in 37°C

Solubility of polymers and neighbouring effect of side groups can affect the overall rate of hydrolysis. The hydrophilic copolymer containing IND was hydrolyzed in buffer solutions rather than hydrophobic copolymer. PD1 was rapidly hydrolyzed because of higher hydrophilicity of HPMA units and PD2 was slowly hydrolyzed because of hydrophobicity of EMA units. The results show that with passing polymeric prodrugs from stomach media to colonic pH, the labile bonds are better accessible to hydrolysis. Therefore, in alkaline pH value, the polymers are easily degraded to release of IND drug.

CONCLUSION

In this research work, IND-HEMA as a new acrylic monomer with an IND pendant group was synthesized in a one-step process by reacting HEMA and IND using esterification methodology. This polymerizable monomer was then polymerized with HPMA and EMA by free radical polymerization technique to obtain PD1 and PD2 as polymeric prodrugs of IND. The structure of the synthesized monomer and polymeric prodrugs were characterized and confirmed by spectroscopy techniques. Hydrolysis of polymeric prodrugs in the mild physiological conditions showed that introducing hydrophilic units along the polymer chain improve the hydrolytic behavior. The obtained release profiles showed that the synthesized polymeric prodrugs are pH-sensitive polymers and in alkaline pH value, the prodrugs are easily degraded to release of drug. *In-vitro* release experiments showed that 2-hydroxyethyl metacrylate-based conjugates with IND could exhibit a sustained drug release behavior in colonic condition and were stable in the simulated media of the stomach and small intestine. Therefore, they are promising candidates for future applications in colon-specific drug delivery.

REFERENCES

[1] JG Hardman; AG Gilman; LE Limbird. The Pharmaceutical Basis of Therapeutics, McGraw-Hill, New York, **1996**.
[2] Y Zhang; CC Chu. *J Biomed Mater Res*, **2002**, 59, 318.
[3] S Ye; C Wang; X Liu; Z Tong. *J Control Release*, **2005**, 106, 319.
[4] J Quan; C Wu; GR Williams; CJ Branford-White; H Nie; L Zhu. *J Appl Polym Sci*, **2013**, 130, 1570.
[5] V Delplace; P Couvreur; J Nicolas. *Polym Chem*, **2014**, 5, 1529.
[6] Rohini; A Neeraj; J Anupam; M Alok. *J Antivir Antiretrovir*, **2013**, S15.
[7] A Rasheed; U Krishna; PS Reddy; A Mishra. *Ars Pharm*, **2011**, 52, 5.
[8] H Ringsdorf. *J Polym Sci Polym Symp*, **1975**, 51, 135.
[9] K Hoste; K Winne; E Schacht. *Int J Pharm*, **2004**, 277, 119.
[10] T Etrych; P Chytil; M Jelinkova; B Rihova; K Ulbrich. *Macromol Biosci*, **2002**, 2, 43.
[11] M Babazadeh; T Mosanejhad. *Iran Polym J*, **2009**, 18, 179.
[12] M Babazadeh; M Sheidaei; S Abbaspour; L Edjlali. *Sci Pharm*, **2013**, 81, 281.

[13] M Babazadeh. *Int J Pharm*, **2006**, 316, 68.
[14] R Paris; JM Garcia; I Quijada-Garrido. *J Appl Polym Sci*, **2010**, 117, 3271.
[15] S Davaran; AA Entezami. *J Control Release*, **1997**, 47, 41.
[16] M Babazadeh. *Int. J. Pharm.*, **2008**, 356, 167.
[17] LF Wang; HN Chiang; PC Wu. *J Biomater Sci Polym Ed*, **2002**, 13, 287.
[18] M Babazadeh. *Indian J Novel Drug Deliv*, **2014**, 6, 43.
[19] X Cai; N Wang; X Lin. *Polymer*, **2006**, 47, 6491.
[20] TVD Merwe; B Boneschans; B Zorc; J Breytenbach; M Zovko. *Int J Pharm*, **2002**, 241, 223.
[21] DP Kemisetti; S Manda; J Aukunuru; KM Chinnala; NK Rapaka. *Int J Pharm Pharm Sci*, **2014**, 6, 437.
[22] M Babazadeh. *J Appl Polym Sci*, **2007**, 104, 2403.
[23] D Kemisetti; S Manda; J Aukunuru; KM Chinnala; NK Rapaka. *Int J Chem Tech Res*, **2014**, 6, 2637.
[24] M Babazadeh; L Edjlali; L Rashidian. *J Polym Res*, **2007**, 14, 207.
[25] M Babazadeh. *Der Pharma Chem*, **2014**, 6, 411.
[26] S Davaran; MR Rashidi; J Hanaee; A Khani; M Mahkam; M Hashemi. *J Bioact Compat Polym*, **2006**, 21, 315.
[27] S Dumitriu; M Popa; M Dumitriu. *J Bioact Compat Polym*, **1989**, 4, 151.
[28] CH Chang; YM Sheu; WP Hu; LF Wang; JS Chen. *J Polym Sci Polym Chem*, **1998**, 36, 1481.
[29] H Namazi; M Babazadeh; A Sarabi; A Entezami. *J Polym Mater*, **2001**, 18, 301.
[30] M Babazadeh; L Edjlali; Z Hajizeynalabedini. *J Iran Chem Res*, **2008**, 1, 41.