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Indomethacin macromolecular prodrugs: Synthesis, characterization and *in vitro* evaluation

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ABSTRACT

The present research work describes the synthesis and in vitro evaluation of novel acrylic-type polymeric systems having degradable ester bonds linked to indomethacin as materials for drug delivery systems. First, indomethacin was linked to 2-hydroxyethyl methacrylate by an activated ester methodology in a one-pot procedure with a high yield. The resulted polymerizable monomer was then copolymerized with 2-hydroxyethyl methacrylate or methyl methacrylate (in 1:3 mole ratios) by the free radical polymerization method, utilizing benzoyl peroxide at 70±2°C. The characterization of the obtained polymer-drug conjugates by FT-IR, ^{1}H-NMR, elemental analysis, and gel permeation chromatography techniques confirmed their structure successfully. Indomethacin release from the obtained polymers was preliminarily evaluated at different buffered solutions (pH 1, 7.4, and 10) into dialysis bags to show the capacity of prodrugs to release the indomethacin under hydrolytic conditions. Detection of hydrolysis by UV spectroscopy at the wavelength of maximum absorption of the free indomethacin in selected intervals showed that the drug can be released by selective hydrolysis of the ester bond at the side of the drug moiety. The release profiles indicated that the degree of hydrolysis increase as the polymeric prodrug passes from acidic to alkali medium. In alkali pH, the polymeric prodrugs reach degree of swelling that makes the liable bonds accessible to hydrolysis.

Keywords: Indomethacin; Macromolecular prodrugs; Acrylic-type polymers; *In vitro* evaluation; Polymerization.

INTRODUCTION

Indomethacin, or 2-(1-[(4-chlorophenyl) carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl) acetic acid, is a non-steroidal anti-inflammatory drug (NSAID) commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling (Figure 1). It works by inhibiting the production of prostaglandins, molecules known to cause these symptoms. Indomethacin is a nonselective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate in prostaglandin synthesis from arachidonic acid. Prostaglandins are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation[1, 2].

The design and application of polymeric prodrugs are interesting fields that are expanded and developed continuously because of the intrinsic advantages offered by specific macromolecular systems in new and risk therapies. A conjugation of a drug with a polymer forms so-called ‘polymeric prodrug’. The polymeric carrier can be either an inert or a biodegradable polymer. The drug can be fixed directly or via a spacer group onto the polymer backbone. The proper selection of this spacer opens the possibility of controlling the site and the rate of release of the active drug from the conjugate by hydrolytic or enzymatic cleavage [3, 4]. Polymer-drug conjugates may offer

many advantages compared to other drug-delivery systems, such as increased drug solubility, prolonged drug release, increased stability and decreased toxicity. Thus, binding of drugs to polymer carriers could provide sustained release and activity of lower doses [5, 6].

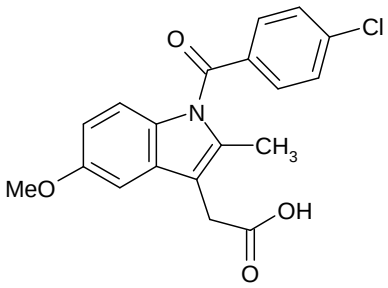

Figure 1. The structure of indomethacin

In the recent years, some of NSAIDs such as ibuprofen, ketoprofen, naproxen, diclofena and 5-aminosalicylic acid have been linked to various polymer backbons *via* hydrolysable chemical bonds and their *in vitro* evaluation has been investigated by Babazadeh *et al* [7-14]. It was found that the hydrolysis behaviors of these polymeric prodrugs are strongly based on the hydrophilicity of polymer and the pH of the hydrolysis solution.

Acrylic-type polymers are an important class of used macromolecules in drug delivery systems. The advantages of acrylic based macromolecular prodrugs have been reviwed by Dumitriu *et al* [15]. 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.

The main purpose of this study, is synthesise and *in vitro* evaluation of acrylic-type polymeric systems having degradable ester bonds linked to indomethacin as materials for application in drug delivery systems. The comparative study of the hydrolytic behavior of polymeric prodrugs based on 2-hydroxyethyl methacrylate bearing indomethacin is reported. 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.

MATERIALS AND METHODS

Materials

Indomethacin was purchased from Merck and used as received. 2-Hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) were obtained from Merck and purified by distillation under reduced pressure to remove inhibitors. Benzoyl peroxide (BPO) was obtained from Fluka and recrystallized twice from the methanol/chloroform mixture 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.

Instrumental measurments

FT-IR spectra were recorded on a Shimadzu 4300 spectrophotometer. ^{1}H-NMR spectra were rocorded on Bruker 400 MHz spectrometer in $CDCl_3$ solution. The amount of released indomethacin was determined by a 2100 Shimadzu UV spectrophotometer at the adsorption maximum of the free drug in aqueous buffered solutions (λ_{max}=318 nm) using a 1-cm quartz cell. The values of number-average molecular weight (M_n), weight-average molecular weight (M_w) and the polydispersity index of polymers were determined with a Maxima 820 gel permeation chromatography (GPC) instrument consisted of two GPC columns (Ultrastyragel 10^4 Å and 10^3 Å) connected in series (Mobile phase: DMF, run time: 50 min, column temperature: 50°C, detector: refractive index model 410). Well-characterized polyethylene oxide was used in the calibration within the range of M_w between "2600-885000". Elemental analyses were caried out with a Heareus CHN instrument. Melting point was determined on a 9100 Electrothermal apparatus.

Synthesis of methacryloyloxyethyl derivative of indomethacin (MOEIN)

In a two-necked flask, 7.2 g (20 mmol) of indomethacin and 0.25 g (2 mmol) of dimethylaminopyridine (DMAP) were dissolved in 70 ml of DMF. The flask was cooled until -20°C and a solution of 4.1 g (20 mmol) of DCC dissolved in 40 ml of DMF was added dropwise into flask solution at this temperature. Then, 2.6 g (20 mmol) of HEMA was dissolved in 10 ml of DMF and added to the flask mixture at the mentioned temperature. The reaction mixture was vigorously stirred at -20°C for 1 h and returned slowly to room temperature. The mixture was stirred at room temperature about 24 h and filtered for remove of white precipitation of *N*,*N*-dicyclohexylurea (DCU). Then, DMF was evaporated in vacuum and the obtained solid was recrystallized from methanol to give 6.7 g (72%) of MOEIN.

FT-IR (KBr, cm^{-1}) 3050 (C-H aromatic), 3030 (C-H vinylic), 2950, 2850 (C-H aliphatic), 1735, 1710 (C=O ester), 1675 (C=O amide), 1600, 1480 (C=C aromatic). ^{1}H-NMR ($CDCl_3$, ppm), 2.1 (s, 3H, $=CCH_3$), 2.3 (s, 3H, Ar-C$\underline{H}_3$), 3.7 (s, 3H, Ar-OC$\underline{H}_3$), 4.1 (s, 2H, Ar-C$\underline{H}_2$COO-), 4.2 (t, 2H, -C$\underline{H}_2$OCOCH_2-), 4.4 (t, 2H, -CH_2OCOC=), 5.1 (d, 1H, CH_2=C), 5.7 (d, 1H, CH_2=C), 6.7-7.3 (m, 7H, aryl-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%.

Copolymerization of MOEIN with acrylic monomers (general procedure)

In two Pyrex glass ampoules, a mixture of 2.35 g (5 mmol) of MOEIN, 0.25 g (1 mmol) of BPO, 1.95 g (15 mmol) of HEMA or 1.5 g (15 mmol) of MMA was dissolved in 10 ml of dried DMF, respectively. The ampoules were then degassed, sealed under vacuum, maintained at 70±2°C in a water bath and shaken by a shaker machine for about 24 h. After this time, the viscous solutions were separately poured from the ampoules into 150 ml of cooled methanol as non-solvent. The precipitates were collected, washed with non-solvent for several times and dried under vacuum at room temperature. The yields of polymers are given in Table 1.

In vitro drug release study

Each of dried polymer-drug conjugates (20 mg) was poured into 5 ml of a aqueous bufferd solution (pH 1, 7.4 and 10) at 37°C and 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 sample was removed at selected intervals and 3 ml of buffer was replaced. The quantity of released drug was analyzed by means of an UV spectrophotometer at λ_{max} (318 nm) and determined from the calibration curve 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.

Characterization of hydrolysis products

Twenty milligram of the polymer-drug conjugate was dispered into 20 ml of buffered solution (pH 8) and maintained at 37°C. After 24 h, the hydrolysis solution was sampled, neutralized with HCl (1 N) and the solvent was removed in vacuum. The resulting crude product was treated with 10 ml of acetone and heated. the suspension was then filtered and the acetone solution was evaporated under reduced pressure. The residue was characterized by melting point measurement and IR spectroscopy and showed that the hydrolysis product is indomethacin; m.p. 159°C, IR (KBr, cm^{-1}) 3450 (O-H acid), 2970, 2820 (C-H aliphatic), 1750, 1670 (C=O carboxylic acid and amide), 1640 (C=C aromatic).

RESULTS AND DISCUSSION

Synthetic route for preparation of MOEIN

Two different synthetic methods have been reported in the preparation of polymers that contain pendent drug substituents. In first method, the drug is converted to a polymerizable monomer by consecutive aminolysis or transesterification procedure, and then polymerized or copolymerized with a wide range of suitable monomers to produce polymer-drug combinations. This method covers a wide range of nucleophiles such as primary, secondary and aromatic amines and alcohols. In other method, the drug agent is attached to preformed polymer backbones *via* degradable chemical bonds to produce polymeric prodrugs [16, 17]. As shown in Figure 2, MOEIN was easily prepared by direct esterification of indomethacin with HEMA in the presence of DCC in DMF solution. The hydroxyl group of HEMA reacted with carboxyl group of indomethacin 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 to give MOEIN as stable monomer.

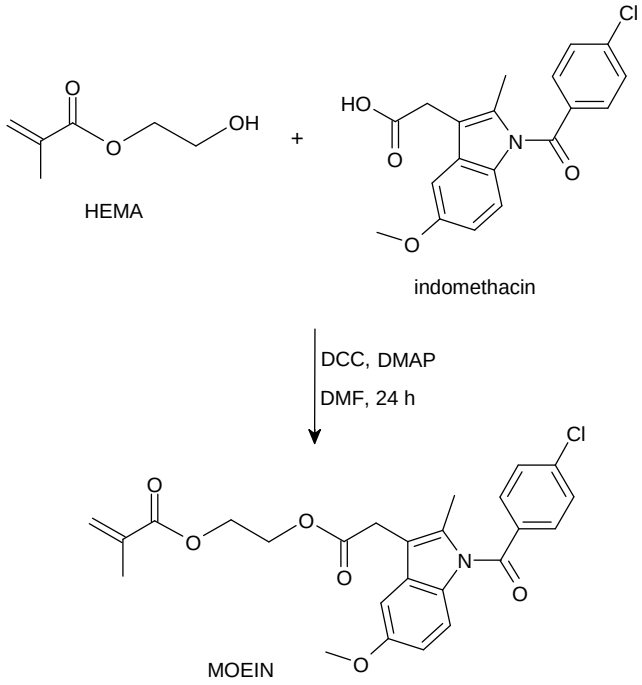

Figure 2. The synthesis route of MOEIN

The resultant FT-IR, ^{1}H-NMR, and elemental analysis data confirmed the structure of MOEIN and its purity. The related ^{1}H-NMR spectrum of MOEIN is shown in Figure 3.

Table 1. The preparation conditions and yields of the polymeric prodrugs

Sample	*[M_1] (mmol/L)*	*[M_2] (mmol/L)*	*Non-solvent*	*Yield (%)*
Poly(MOEIN-*co*-HEMA)	MOEIN (10)	HEMA (30)	Methanol	67.0
Poly(MOEIN-*co*-MMA)	MOEIN (10)	MMA (30)	Methanol	63.0

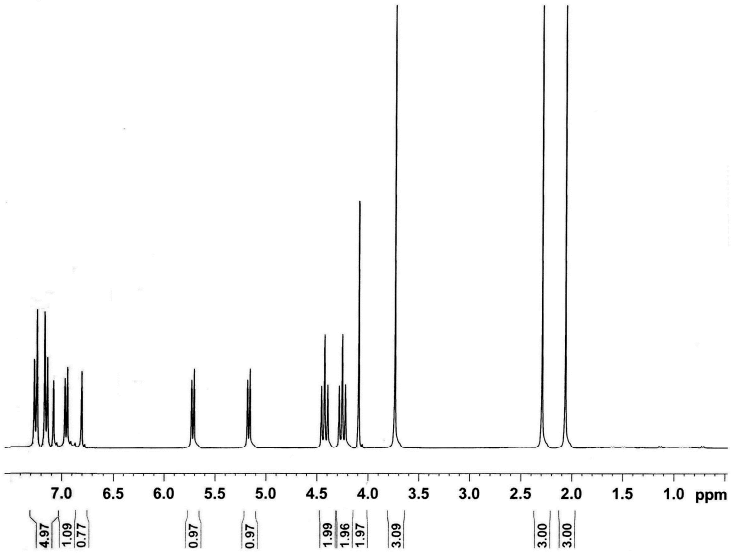

Figure 3. ^{1}H-NMR spectrum of MOEIN in $CDCl_3$ solvent

Synthesis and characterization of polymeric prodrugs

As shown in Figure 4, the obtained MOEIN as a drug containig monomer was easily copolymerized with HEMA and MMA in dried DMF solution by free radical polymerization technique at 70±2°C using BPO as initiator to obtain poly(MOEIN-*co*-HEMA) and poly(MOEIN-*co*-MMA). The resulted copolymers were colorless, amorphous and soluble in DMSO and DMF, but insoluble in water. The conversions of monomers to the related copolymers were determined gravimetrically after exhaustive drying of the isolated copolymer samples. The preparation conditions and yields of copolymers are shown in Table 1. The prepared prodrugs were characterized through a variety of techniques including FT-IR and ^{1}H-NMR spectroscopy. Spectral characteristics of functional groups of copolymers having indomethacin substituents are given in Table 2.

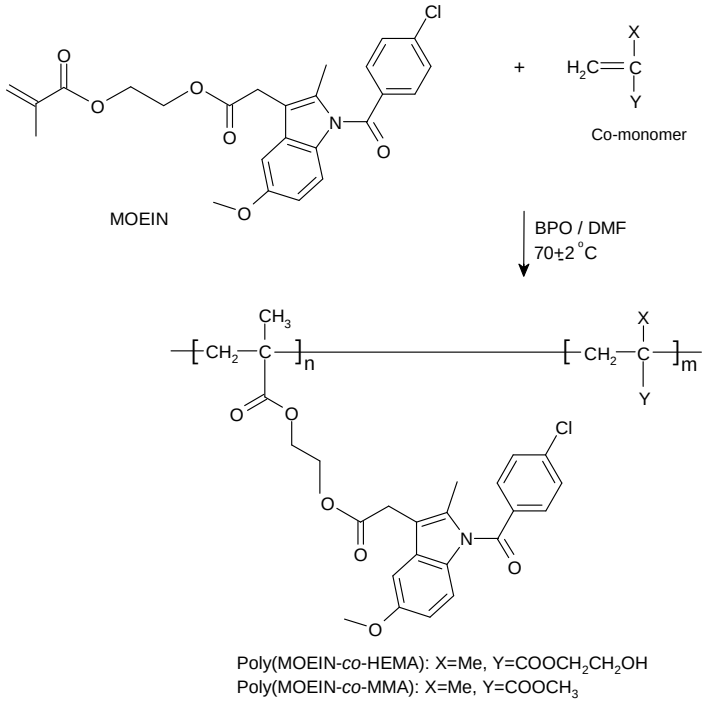

Figure 4. Copolymerization of MOEIN with HEMA and MMA to give polymeric prodrugs

Table 2. Spectral characterization of the polymeric prodrugs

Sample	*Functional group*	*^{1}H-NMR (ppm)*	*FT-IR (cm^{-1})*
Poly(MOEIN-*co*-HEMA)	COO	-	1735
	OH	5.5	4200-3200
Poly(MOEIN-*co*-MMA)	COO	-	1735
All polymers	CH_2O	4.2	1100
	Aryl	7.0-8.0	1600, 1450

Molecular weights of polymeric prodrugs

One parameter used to characterize polymeric prodrugs is the determination of molecular weight. In relation to the polymeric prodrugs, the rate of hydrolysis in the heterogeneous system can be controlled by the structure of the polymer substrates and their molecular weight. The rate of hydrolysis is lowered as the molecular weight increases [18]. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) of the synthesized polymeric prodrugs were estimated by gel permeation chromatography (GPC) instrument. The obtained values are shown in Table 3.

Mole compositions of polymeric prodrugs

^{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 [19, 20]. Therefore, copolymer compositions were determined from ^{1}H-NMR spectroscopic data and elemental analysis of prodrugs. The calculated compositions of polymeric prodrugs are presented in Table 3. The results obtained from ^{1}H-NMR data and elemental analyses were relatively in good agreement.

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

Sample	*C (%)*	*H (%)*	*N(%)*	*M_n ($\times 10^{-3}$)*	*M_w/M_n*	*n^a (%)*	*m^b (%)*
Poly(MOEIN-*co*-HEMA)	60.0	6.2	1.6	35.6	2.1	24	76
Poly(MOEIN-*co*-MMA)	62.3	6.3	1.8	27.7	1.8	29	71

a; the mole composition of MOEIN in polymeric prodrugs.
b; the mole composition of co-monomer in polymeric prodrugs.

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. The *in vitro* hydrolysis behavior of polymeric prodrugs was studied in physiological conditions at 37°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. 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 IR spectroscopic data and melting point measurements of the residue corresponded to the free drug. 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.

Figures 5-7 show the release of indomethacin from polymeric prodrugs as a function of time under mild conditions in HCl buffer (pH 1) and KH_2PO_4-Na_2HPO_4 buffer (pH 7.4 and 10). The order of hydrolysis is: poly(MOEIN-*co*-HEMA) > poly(MOEIN-*co*-MMA). The release rate of indomethacin 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 acidic 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. Different factors such as solubility of polymers and neighbouring effect of side groups can affect the overall rate of hydrolysis. The hydrophilic copolymer containing indomethacin was hydrolyzed in buffer solutions rather than hydrophobic copolymer. As shown in Figures 5-7, poly(MOEIN-*co*-HEMA) was rapidly hydrolyzed because of higher hydrophilicity of HEMA units and poly(MOEIN-*co*-MMA) was

slowly hydrolyzed because of hydrophobicity of MMA units in the copolymer structure. The results show that with passing polymeric prodrugs from acidic media to slightly alkaline pH, the labile bonds are better accessible to hydrolysis. Therefore, in alkaline pH value, the polymers are easily degraded to release of indomethacin.

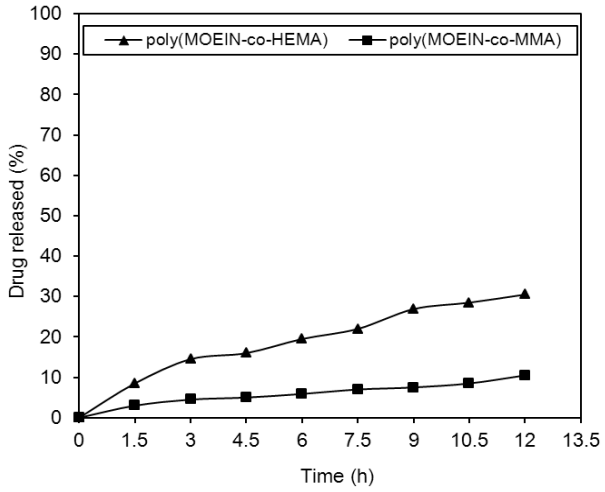

Figure 5. Percent of indomethacin released from polymeric carriers as a function of time at hydrochloric acid buffer (pH 1) and 37°C

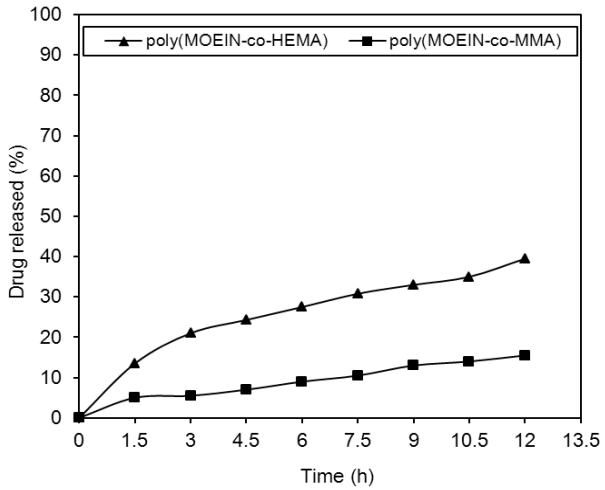

Figure 6. Percent of indomethacin released from polymeric carriers as a function of time at phosphate buffer (pH 7.4) and 37°C

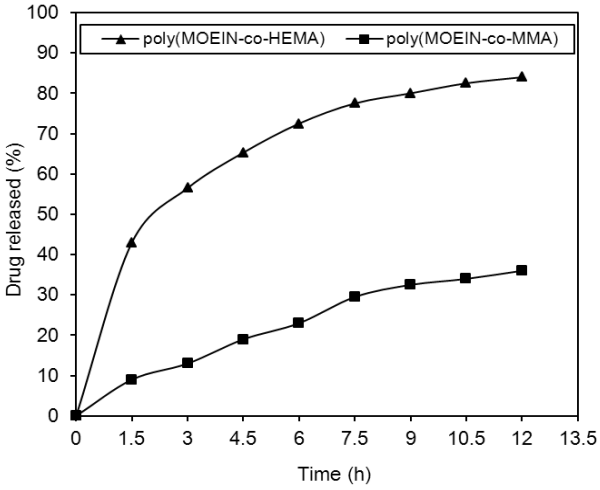

Figure 7. Percent of indomethacin released from polymeric carriers as a function of time at phosphate buffer (pH 10) and 37°C

CONCLUSION

In this work, MOEIN as an acrylic-type polymerizable derivative of indomethacin was synthesized from reaction between HEMA and indomethacin by esterification method. Then, the polymeric prodrugs containing indomethacin pendent groups were synthesized by the free radical polymerization of MOEIN with HEMA or MMA. The structure of the synthesized MOEIN and polymeric prodrugs were characterized by spectroscopy techniques. Hydrolysis of polymeric prodrugs was carried out similar to the physiological conditions and the results showed that the introduction hydrophilic units along the polymer chain improve the hydrolytic behavior. Also, the resultant release profiles of drug from prodrugs showed that the synthesized polymeric prodrugs were pH-sensitive polymers. Therefore, the studied polymers in the present investigation can be used in prolongation of transit time and are useful as drug carriers for development of pH-sensitive polymeric prodrugs.

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