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Design of Novel Acrylic-Type Polymeric Prodrugs Containing 5-Aminosalicylic Acid as Colon Targeted Drug Delivery Systems

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ABSTRACT

The main purpose of this study was synthesize and *in vitro* evaluation of novel acrylic-type polymeric systems having degradable ester bonds linked to 5-aminosalicylic acid (5-ASA) as materials for application in drug delivery systems. 5-ASA was first linked to 2-hydroxypropyl methacrylat by esterification method to obtain methacryloyloxypropyl-5-aminosalicylate. The resulting new acrylic derivative of 5-ASA was then copolymerized with 2-hydroxyethyl methacrylat, methyl metacrylat and 2-ethylhexyl acrylate by free radical polymerization method in *N,N*-dimethyl formamid solution, utilizing azobisisobutyronitrile as an initiator at 70±2°C. After characterization of compounds by FT-IR, ^{1}H-NMR, elemental analyses, mass spectrum, themal analysis and gel permeation chromatography, the release studies of 5-ASA were performed into dialysis bags by hydrolysis buffered solutions (pH 1, 7, 8) at 37°C. Detection of hydrolysis by UV spectroscopy at selected interval showed that the drug can be released by selective hydrolysis of the ester bond at the side of drug moiety. The release profiles indicated that the hydrolytic behavior of polymeric prodrugs is strongly based on the hydrophobicity of polymers and the pH of the hydrolysis media. The results suggested that these polymeric prodrugs could be useful for releasing 5-ASA in controlled release systems.

Keywords: 5-aminoslicylic acid, polymeric prodrugs, 2-hydroxypropyl methacrylat, drug delivery, *in vitro* hydrolysis.

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INTRODUCTION

The colon specially the first part of the lower intestine, can be liable to numerous pathological conditions, such as constipation, Crohn's disease, ulcerative colitis, carcinomas and infections. Recommended treatments include the administration of anti-inflammatory drugs, chemotherapy drugs or antibiotics, which must be released in the colon [1]. The site-specific drug delivery to the colon has a number of important implications in the field of pharmacotherapy. Applications of colon-specific drug delivery include the local treatment of large intestine disorders, and the oral administration of protein and peptide drugs. Several diseases such as inflammatory bowel syndrome (IBS) can be treated more effectively by local delivery of anti-inflammatory agents to the large intestine. On the other hand, the large intestine may be optimal for peptide delivery because of high residence time and low digestive enzymatic activity [2]. Targeting drugs to the large intestine can be achieved by different routes; coating drugs with pH-sensitive polymers, coating drugs with bacterially degradable polymers, delivery of drugs through bacterially degradable matrices and hydrogels, and delivery of drugs as prodrugs [3].

5-aminosalicylic acid (5-ASA), or 5-amino-2-hydroxy benzoic acid, is an active ingredient of agents used for the long-term maintenance therapy to prevent relapses of Crohn's disease and ulcerative colitis [4]. It is very soluble above pH 5.5 and thus it is readily absorbed from the gastrointestinal tract as soon as it passes through the stomach. Systemic absorption of 5-ASA creates various physiological side effects. Hence research has focused on local (topical) delivery of 5-ASA at the diseased site (distal ileum and proximal colon) with intentionally minimized systemic absorption. The mechanism of action of 5-ASA is not fully understood, but it is suggested that it reduces inflammation by blocking cyclooxygenase and lipoxygenase in the arachidonic acid pathway and inhibits the production of prostaglandins and other inflammatory mediators in the intestine [5].

Polymeric prodrugs or polymer–drug conjugations are novel technique for drug delivery systems. These systems act as carriers for drugs and target them to the desired site in the body. The carriers then undergo enzymic hydrolysis, and the active drug is released, sometimes with sustained release profiles. The major attributes of polymeric prodrugs include the following: capacity to be stored in depots, unique pharmacokinetic profiles, potential body distribution and pharmacological efficacy [6]. Recently, in order to elimination of 5-ASA side-effects and its non-absorption from small intestine, 5-ASA has been linked to high molecular weight polymeric backbones and their *in vitro* hydrolysis investigated [7-9].

We have previously reported the preparation of acrylic formulation for non-steroidal anti-inflammatory drugs (NSAIDs), in which the drug was covalently linked to polymer backbone *via* hydrolysable bonds [10-15]. 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 reviewed by

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Dumitriu *et al.* [16]. These systems do not form toxic by-products during their biodegradation and which have tendency to swell, when they come in contact with biological environment.

In this article, the comparative study of the hydrolytic behavior of polymeric drugs based on 2-hydroxypropyl methacrylate bearing 5-ASA 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 *in vitro* at different pH values.

MATERIALS AND METHODS

Materials

5-ASA was purchased from Aldrich chemical company and recrystallized from water and ethanol, respectively. 2-Hydroxypropyl methacrylate (HPMA), methyl methacrylate (MMA), ethylhexyl acrylate (EHA), 2-hydroxyethyl methacrylate (HEMA) were obtained from Merck chemical company and purified by distillation under reduced pressure to remove inhibitors. *N,N*-dimethyl formamide (DMF) and 1,1-carbonyldiimidazole (CDI) were obtained from Merck chemical company and employed as received. Azobisisobutyronitrile (AIBN) was obtained from Fluka chemical company and recrystallized twice from methanol. All other chemicals were reagent and grade or purer.

Instrumental measurements

FT-IR spectra were recorded on a Shimadzu 4300 spectrophotometer. ^{1}H-NMR spectra were rocorded on Bruker 400 MHz spectrometer in DMSO-d_6 solution. The amount of released 5-ASA was determined by a 2100 Shimadzu UV spectrophotometer at the adsorption maximum of the free drug in aqueous buffered solutions (λ_{max}=300 nm for pH 1; λ_{max}=339 nm for pH 7 and 8) 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-ORAPID instrument. Mass spectrum was obtained with a Shimadzu Qp 100X spectrometer at 70 eV. Thermal analysis was performed on a STA 625 calorimeter at heating and cooling rates of 10°C/min under N_2.

Synthesis of methacryloyloxypropyl 5-amino salicylate (MOPAS)

One gram (6.5 mmol) of 5-ASA in 10 ml of 98% formic acid was refluxed for 30 min and 20 ml of cold distilled water was added. The precipitates were filtered, washed several times with cold water, and dried in vacuum. 5-Formylaminosalicylic acid (5-fASA) was obtained with 88% yield (m.p. 251°C). To the solution of 5-fASA (1 mmol) in 5 ml of DMF, CDI (1.5 mmol) was

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added slowly, and reacted for 1 h at room temperature. Then, HPMA (1 mmol) in 10 ml of DMF and triethylamine (0.8 ml) were added to the reaction mixture, and stirred for 24 h at room temperature. Addition of excess HCl (0.1 mol/l) produced precipitates of MOPAS. The precipitates were collected, washed with HCl for several times and dried under vacuum at room temperature to give 63% of MOPAS.

FT-IR (KBr, cm^{-1}) 3470 (O-H phenolic), 3423 (N-H stretching), 3060 (C-H aromatic), 3020 (C-H vinylic), 2950, 2860 (C-H aliphatic), 1725 (C=O ester), 1630 (C=C vinylic), 1600, 1490 (C=C aromatic). ^{1}H-NMR (DMSO-d_6, ppm) 1.30 (d, 3H, -OCH(C$\underline{H}_3$)-), 1.9 (s, 3H, =CCH_3), 4.2 (d, 2H, -OCH_2-), 4.4 (m, 1H, -OC$\underline{H}$(CH_3)-), 5.0 (s, 2H, -NH_2), 5.4 (d, 1H, CH_2=), 6.2 (d, 1H, CH_2=), 6.8-7.6 (m, 3H, aryl-H), 10.3 (s, 1H, -OH), *m/z* (EI): 279 (15%, M^+), 210 (12%, $[M-(CH_2C(CH_3)CO)]^+$), 136 (100%, $[M-HPMA]^+$), 143 (21%, $[M-5ASA]^+$). Elemental analysis for $C_{14}H_{17}NO_5$ (279 g/mol), calculated: C 60.2, H 6.1, N 5.0; found: C 59.9, H 5.9, N 5.3%.

Copolymerization of MOPAS with acrylic monomers

In three Pyrex glass ampoules, a mixture of 1.40 g (5 mmol) of MOPAS, 0.16 g (1 mmol) of AIBN, 1.95 g (15 mmol) of HEMA or 1.50 g (15 mmol) of MMA or 2.75 g (15 mmol) of EHA 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 48 h. After this time, the viscous solutions were separately poured from the ampoules into 150 ml of cooled methanol/water (1:1 v/v) mixture as non-solvent. The precipitates were collected, washed with non-solvent for several times and dried under vacuum at room temperature. The yields of the obtained polymers are shown in Table 1.

Method of hydrolysis

The polymer-drug conjugates were dried under vacuum at room temprature and sieved with a 200 mesh sieve. Each of dried polymer-drug conjugates (200 mg) was poured into 5 ml of a aqueous bufferd solution (pH 1, 7 and 8) 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 and determined from the calibration curve obtained previously under the same conditions.

Characterization of hydrolysis products

Twenty milligram of the polymer-drug conjugate was dispersed 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 5-

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ASA; m.p. 280°C (dec.), IR (KBr, cm^{-1}) 3400-2900 (O-H), 2950, 2870 (C-H aliphatic), 1730 (C=O), 1600, 1470 (C=C aromatic).

RESULTS AND DISCUSSION

Synthetic route for preparation of MOPAS

Two different synthetic method have been reported in the preparation of polymers that contain pendent drug substituents. In the 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 performed polymer backbones *via* degradable chemical bonds to produce polymeric prodrugs. As shown in Fig. 1, the synthesis of MOPAS involved two steps. The first step involved the conversion of 5-ASA into its formyl derivative (5-fASA) by using formic acid in order to make it susceptible for esterification reaction with HPMA. In the second step the HPMA was coupled to 5-fASA in the presence of DCI in DMF solution to get monomeric drug conjugate. After completing of reaction, the precipitate was seperated and the solvent was evaporated to give MOPAS as stable monomer. The elemental analysis, FT-IR, ^{1}H-NMR and mass spectroscopy confirmed the structure of MOPAS and its purity. ^{1}H-NMR spectrum of MOPAS is shown in Fig. 2.

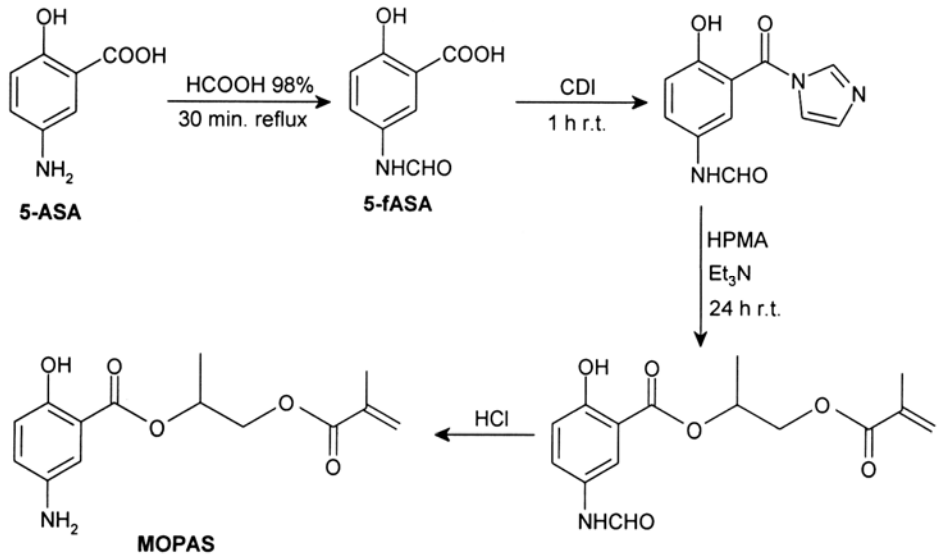

Fig. 1: The synthesis route of acrylic-type derivative of 5-ASA (MOPAS).

Synthesis and characterization of polymeric prodrugs

As shown in Fig. 3, the obtained MOPAS as a drug containig monomer was easily copolymerized with HEMA, MMA and EHA in dried DMF solution by free radical polymerization

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technique at 70±2°C using AIBN as initiator to obtain poly(MOPAS-co-HEMA), poly(MOPAS-co-MMA) and poly(MOPAS-co-EHA). 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 prepared prodrugs were characterized through a variety of techniques including FT-IR, ^{1}H-NMR spectroscopy. The results confirmed the structure of the synthesized polymers. In the all ^{1}H-NMR spectra, the proton signals of the aryl group were seen between 7 and 8 ppm. The resonance signals at 10 and 6 ppm were respectively attributed to hydroxyl and amine protons of drug in MOPAS units. Also the signals at 0.9–2.0 ppm were due to the methylene groups of backbone and α-methyl groups.

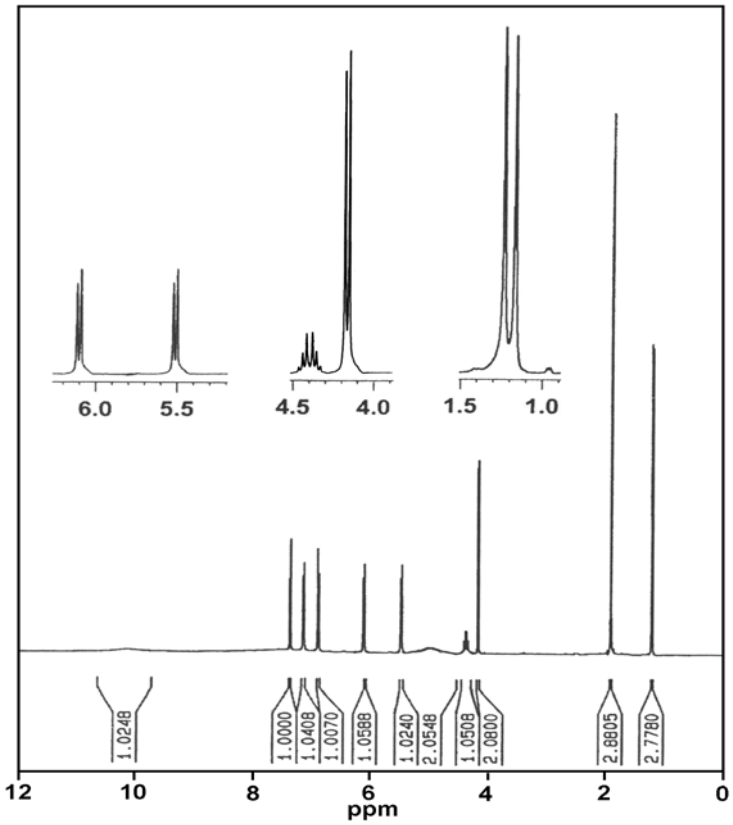

Fig. 2: ^{1}H-NMR spectrum of MOPAS in DMSO-d_6.

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. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) of the synthesized polymeric prodrugs were estimated by GPC instrument. The obtained values are shown in Table 1.

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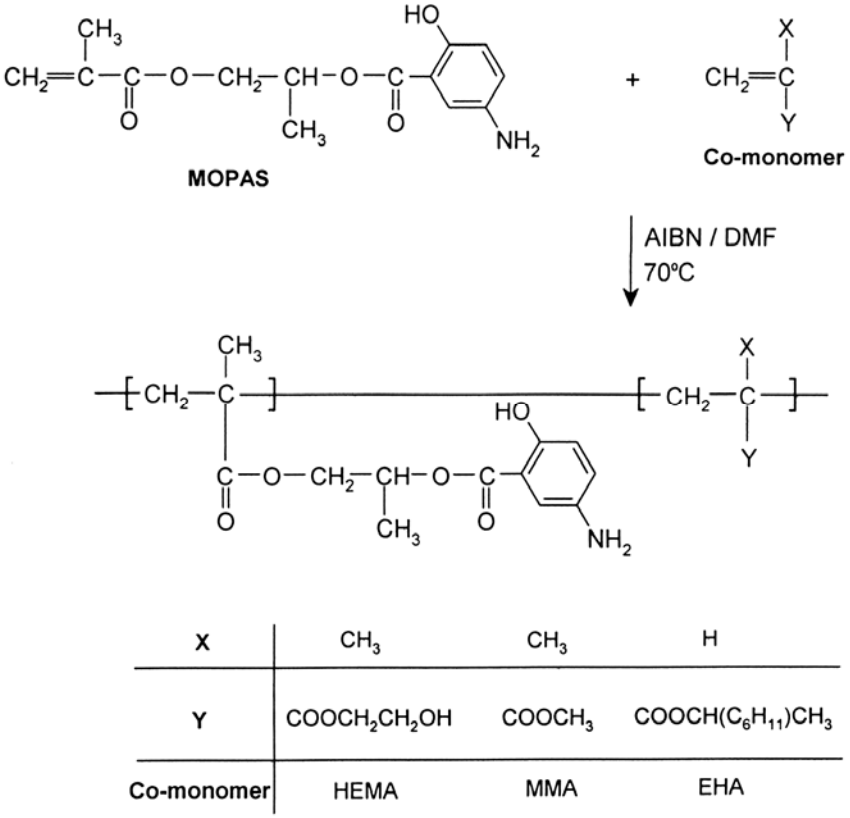

X	CH_3	CH_3	H
Y	$COOCH_2CH_2OH$	$COOCH_3$	$COOCH(C_6H_{11})CH_3$
Co-monomer	HEMA	MMA	EHA

Fig. 3: Copolymerization of MOPAS with HEMA, MMA and EHA to give polymeric prodrugs.

Thermal behavior of polymeric prodrugs

The thermal behavior of a polymer is important in relation to its properties for controlled release and its ability to be processed into suitable dosage form [9]. Differential scanning calorimetry (DSC) was used to determine the thermal properties of the polymeric prodrugs containing 5-ASA. The glass transition temperature (T_g) was determined from the DSC thermograms. The values are given in Table 1.

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 [17, 18]. 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 2. The results obtained from ^{1}H-NMR data and elemental analyses were relatively in good agreement.

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Table 1: The yields, molecular weights and glass transition temperatures of prodrugs

Sample	*Yield (%)*	M_n	M_w/M_n	T_g (°C)
Poly(MOPAS-*co*-HEMA)	76	45430	1.6	134
Poly(MOPAS-*co*-MMA)	72	33410	1.7	116
Poly(MOPAS-*co*-EHA)	70	44370	1.8	123

Table 2: Elemental analyses and mole compositions of polymeric prodrugs

Sample	*Elemental analyses*			*Mole compositions*	
	C (%)	*H (%)*	*N (%)*	*n (%)*	*m (%)*
Poly[$(MOPAS)_n$-*co*-$(HEMA)_m$]	57.6	6.9	2.4	24	76
Poly[$(MOPAS)_n$-*co*-$(MMA)_m$]	60.1	7.0	2.6	28	72
Poly[$(MOPAS)_n$-*co*-$(EHA)_m$]	67.2	9.0	1.9	30	70

Drug release by hydrolysis of polymeric prodrugs

It has been widely demonstrated that the side chain hydrolysis of drug pendent polymers depend on the strength and chemical nature of the drug polymer chemical bonds, the stucture of the polymer and the surrounding condition. The hydrolysis of a linkage ia also dependent on its distance from the polymer backbone. The length and hydrophilicity of the spacer unit between the drug and polymer chain can affetct the release rate [19, 20]. The *in vitro* hydrolysis behavior of polymeric prodrugs was studied in physiological conditions (aqueous phosphate or hydrochloric acid buffers, at 37°C). As the polymers were not soluble in water, they were dispersed in buffer solution and the hydrolysis was performed in a hetrogeneous 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 was 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 reation 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 dose not undergo hydrolysis under mild conditions. This can be related to the steric hindrance of bulk polymer chains which decrease the bond mobility.

Figs. 4-6 show the release of 5-ASA from polymeric prodrugs as a funtion of time under mild conditions in HCl buffer (pH 1) and KH_2PO_4-Na_2HPO_4 buffer (pH 7 and 8). The obtained results showed that the release rate of 5-ASA from polymeric prodrugs at alkaline medium was higher than the release rate of drug in acidic condition. The drug- release rate from polymeric

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prodrugs at acidic pH is very low. It seems that polymeric prodrugs have low degree of swelling in acidic medium and the drug is protected against hydrolysis. Also, at acidic media, the carboxyl group of hydrolyzed 5-ASA will be protonated and its aqueous solubility will be lower than in alkali media, where the acid group is deprotonated. Also, the hydrolysis of ester in acidic media is actually an equilibrium reaction, as ester formation is also catalysed by acid (Fig. 7). 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 liable bonds accessible to hydrolysis. The hydrolysis mechanism of polymers prodrugs in different pH conditions is shown in Fig. 7.

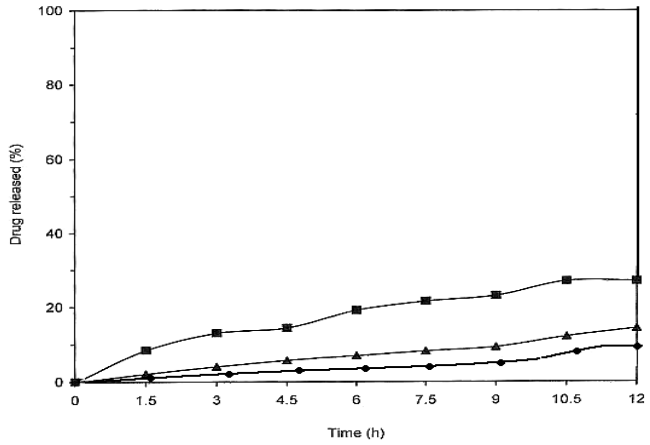

Fig. 4: Percent of 5-ASA released from polymeric carriers as a function of time at hydrochloric acid buffer (pH 1) and 37°C. ■ Poly(MOPS-*co*-HEMA); ▲Poly(MOPS-*co*-MMA); ●Poly(MOPS-*co*-EHA).

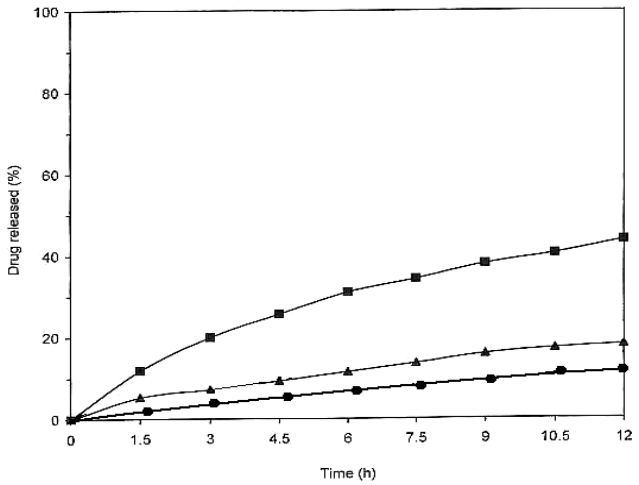

Fig. 5: Percent of 5-ASA released from polymeric carriers as a function of time at phosphate buffer (pH 7) and 37 °C. ■ Poly(MOPS-*co*-HEMA); ▲Poly(MOPS-*co*-MMA); ●Poly(MOPS-*co*-EHA).

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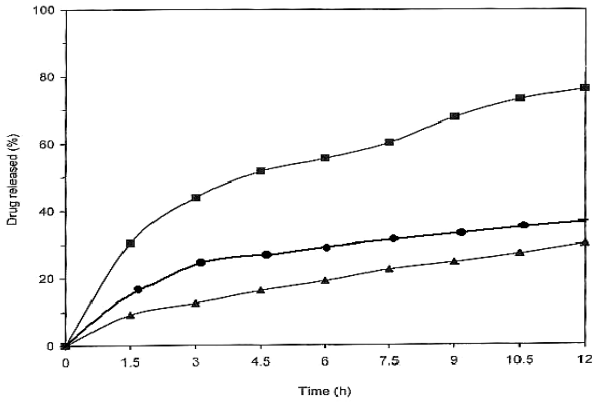

Fig. 6: Percent of 5-ASA released from polymeric carriers as a function of time at phosphate buffer (pH 8) and 37 °C. ■ Poly(MOPS-*co*-HEMA); ●Poly(MOPS-*co*-MMA); ▲Poly(MOPS-*co*-EHA).

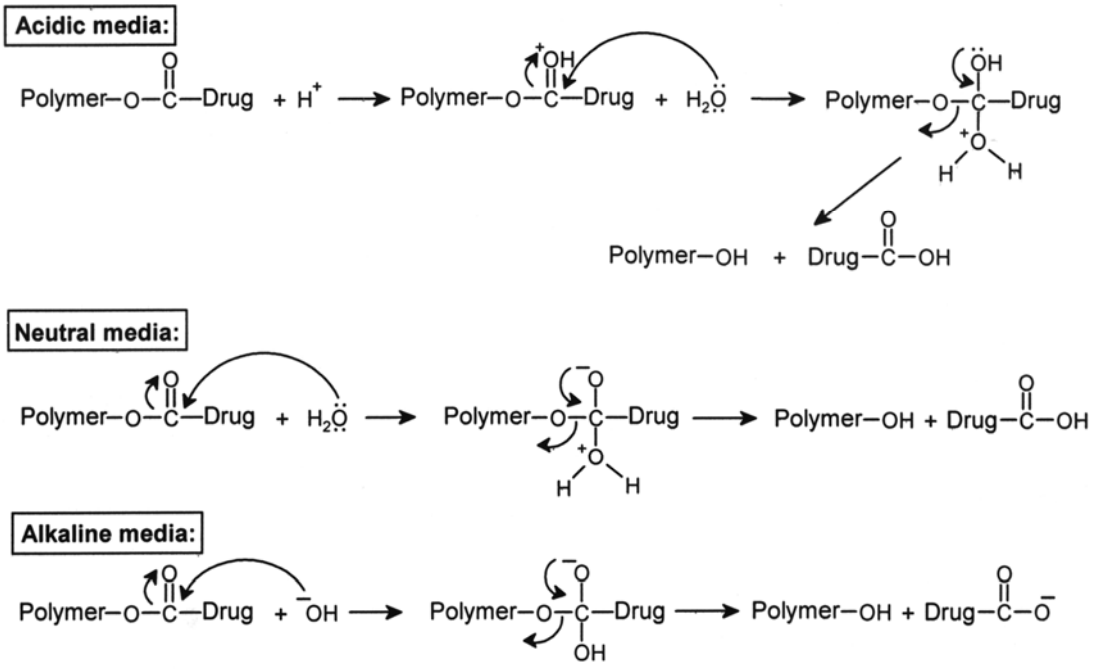

Fig. 7: The hydrolysis mechanism of polymeric prodrugs in different pH medias.

Also, the neighboring groups can affect the drug-release rate. As shown in Figs. 4-6, the hydrolysis rate of poly(MOPAS-*co*-HEMA) is higher than poly(MOPAS-*co*-MMA) and poly(MOPAS-*co*-EHA). It seems that the introduction hydrophilic units along the polymer chain improve the hydrolytic behavior. Poly(MOPAS-*co*-HEMA) has hydrophilic HEMA units and therefore, is rapidly hydrolyzed from other polymers containing hydrophobic MMA and EHA units.

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Finally, 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. As the main purpose of polymeric prodrugs is the achievement of controlled drug release or slow release, application of these polymers as a drug delivery system is expected after *in vivo* examinations.

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