Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(3):411-419
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis and *in vitro* evaluation of acrylate-based macromolecular prodrugs containing mesalazine for colon-specific drug delivery

Mirzaagha Babazadeh*

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

---

ABSTRACT

This work, describes synthesis and in vitro evaluation of new acrylic-type polymeric prodrugs containing mesalazine, as colon targeted drug delivery systems. First, mesalazine reacted with formic acid, and the obtained material was reacted with 2-hydroxypropyl methacrylat in the presence of 1,1-carbonyldiimidazole to produce methacryloyloxypropyl 5-amino salicylate. The resulted acrylic-derivative of mesalazine was then polymerized with various acrylic monomers by free radical solution polymerization, utlizing azobisisobutyronitrile as an initiator at 70±2°C. The obtained polymer-drug conjugates were characterized by FT-IR, ^{1}H-NMR, elemental analysis, gel permeation chromatography, and differential scanning calorimetery techniques. The release studies were performed into dialysis bags by hydrolysis buffered solutions at 37°C. Detection of hydrolysis by UV spectrophotometer at free drug λ_{max} (300 nm for pH 1; 339 nm for pH 7 and 8) in selected intervals showed that the mesalazine can be released by selective hydrolysis of the ester bond at the side of drug moiety. The release profiles indicated that the hydrophobicity of polymers and the pH value of the hydrolysis media have strongly effects on the hydrolytic behavior of the polymeric prodrugs.

Keywords: Mesalazine; Polymeric prodrugs; Acrylic-type polymers; *In vitro* evaluation; Polymerization.

INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by chronic inflammation in the mucosal membrane of the large intestine. Although many treatments have been recommended for IBD, they do not treat the cause but are effective only in reducing the inflammation and accompanying symptoms in up to 80% of patients. The primary goal of drug therapy is to reduce inflammation in the colon that requires frequent intake of anti-inflammatory drugs at higher doses. Mesalazine, or 5-aminosalisylic acid (5-ASA), is an active ingredient of agents used for the long-term maintenance therapy to prevent relapses of Crohn's disease and ulcerative colitis [1]. It is very soluble above pH 5.5 and thus it is readily absorbed from the gastrointestinal (GI) tract as soon as it passes through the stomach. Systemic absorption of mesalazine creates various physiological side effects. Hence research has focused on local (topical) delivery of mesalazine at the diseased site (distal ileum and proximal colon) with intentionally minimized systemic absorption. The mechanism of action of mesalazine 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 [2].

Colon targeted drug delivery systems have attracted many researchers due to distinct advantages such as near neutral pH, longer transit time and reduced enzymatic activity. Colon specific drug delivery not only increases the

bioavailability of the drug at the target site, but also reduces the side effects [3]. In the recent studies, colon targeted drug delivery systems are gaining importance to treat local pathologies of the colon (Crohn's disease, IBD and colonic cancer) and also for the systemic delivery of protein and peptide drugs. 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 hydrogels, and delivery of drugs as prodrugs [4, 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.

It was for the first time in 1975 that a rational model for pharmacologically active polymers was proposed. Ringsdorf was the first to recognize the immense potential of polymeric prodrugs, if only polymer chemists and biologists would work together in the field [6]. The proposed model consists mainly of five components: the polymeric backbone, the drug, the spacer, the targeting group and the solubilising agent. This model, although still oversimplified, has been an important mark in the history of polymeric prodrug design.

Polymeric prodrug is a conjugation of a drug with a polymer which has several advantages. The main advantages include: (a) an increase in water solubility of low soluble or insoluble drugs, and therefore, enhancement of drug bioavailability; (b) protection of drug from deactivation and preservation of its activity during circulation, transport to targeted organ or tissue and intracellular trafficking; (c) an improvement in pharmacokinetics; (d) a reduction in antigenic activity of the drug leading to a less pronounced immunological body response; (e) the ability to provide passive or active targeting of the drug specifically to the site of its action; (f) the possibility to form an advanced complex drug delivery system, which, in addition to drug and polymer carrier, may include several other active components that enhance the specific activity of the main drug. Due to these advantages over to free form of a drug, the polymeric prodrug conjugates has lead into a new era of drug delivery systems [7-10].

Literature studies showed that various research works about synthesis and *in vitro* evaluation of polymeric prodrugs containing mesalazine linked to the polymer backbones have been recently reported [11-15]. In these researches, the linkages between mesalazine and the polymer backbone, including azo, ester and amide bonds, were susceptible to enzymatic attack in the large intestine for release of mesalazine at this site.

Previously, we reported the preparation of acrylic formulation for non-steroidal anti-inflammatory drugs (NSAIDs), in which the drug was covalently linked to polymer backbone *via* hydrolyzable bonds [16-23]. It was found that the hydrolysis behavior of these polymeric prodrugs is 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 Dumitriu *et al.* [24]. 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.

The objective of the present work is synthesis and studying *in vitro* release behaviour of polymeric prodrugs containing mesalazine for sustained and site-specific delivery. Therefore, a polymerizable acrylic-type derivative of mesalazine, namely methacryloyloxypropyl 5-amino salicylate (MOPAS), was successfully synthesized and copolymerized with 2-hydroxyethyl methacrylate, methyl methacrylate or ethylhexyl acrylate by free radical polymerization technique. The release of mesalazine from the obtained polymeric prodrugs was carried out *in vitro* by hydrolysis in buffered solutions at various pH values and the quantity of the released drug detected by UV spectroscopy. The effects of neighboring groups and pH values on release of mesalazine are discussed.

MATERIALS AND METHODS

Materials

Mesalazine (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) and 2-hydroxyethyl methacrylate (HEMA) were obtained from Merck chemical company and purified by distillation under reduced pressure to remove inhibitors. Azobisisobutyronitrile (AIBN) was obtained from Fluka chemical company and recrystallized twice from methanol. *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 DMSO-d_6 solution. The amount of released mesalazine 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. Melting points were determined on a 9100 Electrothermal apparatus.

Synthesis of methacryloyloxypropyl 5-amino salicylate (MOPAS)

One gram (6.5 mmol) of mesalazine 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 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 brown precipitates were collected, washed with HCl for several times and dried under vacuum at room temperature to give 63% of MOPAS with melting point of 318°C.

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<u>H</u>$_3$)-), 1.9 (s, 3H, =CCH_3), 4.2 (d, 2H, -OCH_2-), 4.4 (m, 1H, -OC<u>H</u>(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 (general procedure)

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 24 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 light brown precipitates were collected, washed with non-solvent for several times and dried under vacuum at room temperature to constant weight.

In vitro drug release study

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 aques 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 at λ_{max} (300 nm for pH 1; 339 nm for pH 7 and 8) 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 mesalazine; 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

As shown in Figure 1, the synthesis of MOPAS involved two steps. The first step involved the conversion of mesalazine 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.

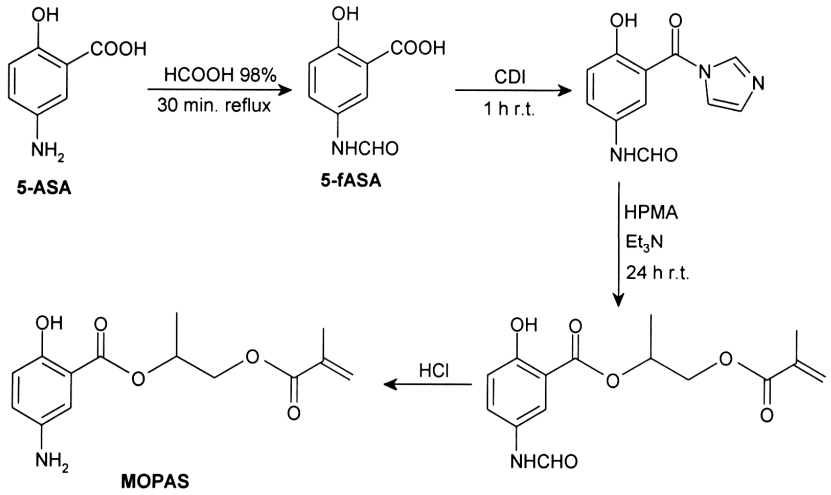

Figure 1. The synthesis route of MOPAS.

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 Figure 2.

Synthesis and characterization of polymeric prodrugs

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

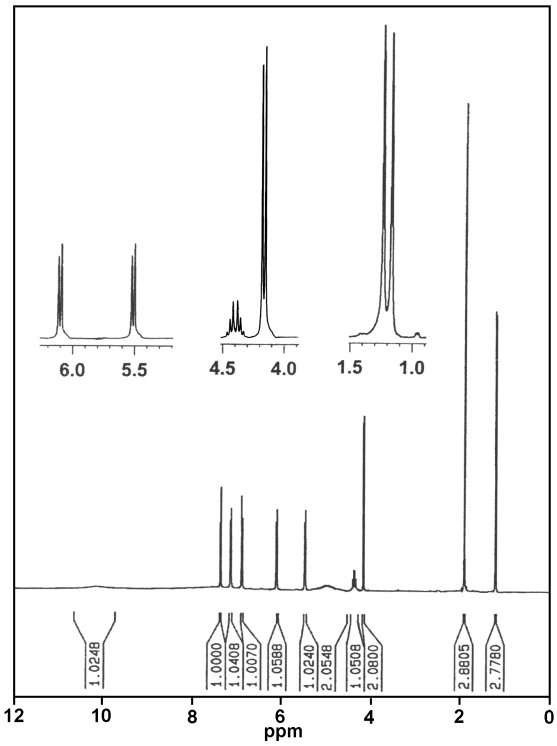

Figure 2. ^{1}H-NMR spectrum of MOPAS in DMSO-d_6 solvent.

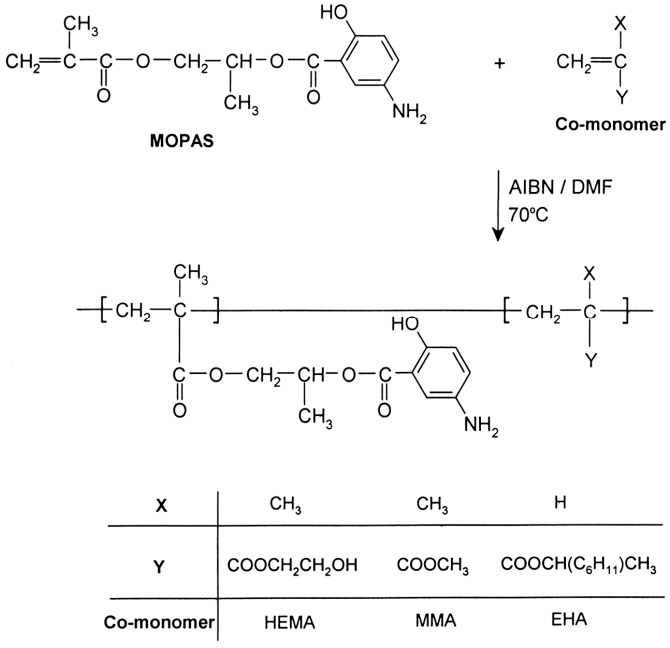

Figure 3. Copolymerization of MOPAS with HEMA, MMA and EHA to give polymeric prodrugs.

The prepared polymeric prodrugs were characterized through a variety of techniques including FT-IR, ^{1}H-NMR spectroscopy. The results confirmed the structure of the synthesized polymers. A typically ^{1}H-NMR spectrum of poly(MOPAS-*co*-MMA) is shown in Figure 4. In this ^{1}H-NMR spectrum, the proton signals of the aryl group were

seen between 7 and 8 ppm. The resonance signals at 9.9 and 6.2 ppm were respectively attributed to hydroxyl and amine protons of drug in MOPAS units. Also the methyl protons of -$COOCH_3$ in MMA units and protons of -$COOCH_2CHOOC$- in MOPAS units were observed at 3.8 and 4.2 ppm, respectively. The signals at 0.9–2.0 ppm were due to the methylene groups of backbone and α-methyl groups.

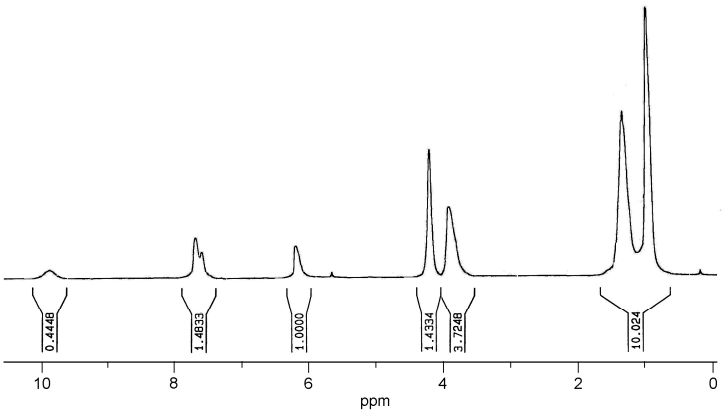

Figure 4. ^{1}H-NMR spectrum of poly(MOPAS-*co*-MMA) in DMSO-d_6.

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.

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 [13]. Differential scanning calorimetry (DSC) was used to determine the thermal properties of the polymeric prodrugs containing mesalazine drug. The value of the glass transition temperatures (T_g's) determined from the DSC thermodiagrams is given in Table 1.

Table 1. The yields, molecular weights and glass transition temperatures of the synthesized 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

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 [25, 26]. Therefore, copolymer compositions were determined from ^{1}H-NMR spectroscopic and elemental analysis data of the polymeric prodrugs. The calculated compositions of the polymeric prodrugs from elemental analysis are presented in Table 2. The obtained results from elemental analysis were relatively in good agreement with ^{1}H-NMR data. For example, the following expression was used to determine of the molar composition of poly(MOPAS-*co*-MMA) from Figure 4. Let "*m*" be the mole fraction of MMA and "1-*m*" be the mole fraction of MOPAS. Unit of MOPAS contains three aromatic protons around 7-8 ppm and MMA unit contains three protons of -$COOCH_3$ around 3.8 ppm. Therefore, the mole fraction of MMA in poly(MOPAS-*co*-MMA) was determined from the equation (2):

$$\frac{\text{integrated peak area of 7-8 ppm}}{\text{integrated peak area of 3.8 ppm}} = \frac{3(1-m)}{3m} \qquad (2)$$

The value of "*m*" as mole fraction of MMA was obtained 71.5% which is in good agreement with the obtained value from elemental analysis (72%).

Table 2. Elemental analysis and mole compositions of the polymeric prodrugs

Sample	*Elemental analysis*			*Mole composition*	
	C (%)	*H (%)*	*N (%)*	*MOPAS (%)*	*co-monomer (%)*
Poly(MOPAS-*co*-HEMA)	57.6	6.9	2.4	24	76
Poly(MOPAS-*co*-MMA)	60.1	7.0	2.6	28	72
Poly(MOPAS-*co*-EHA)	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 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 [16, 18]. The *in vitro* hydrolysis behaviour 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 membraine 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.

There are two hydrolysable ester groups, one adjacent to the polymeric backbone and the other relegated to the pendant chain by a spacer group. It is obvious that hydrolysis of the latter ester group is much more facile than the one adjacent to the polymeric backbone because of steric hindrance reason which decrease the bond mobility.

Figures 5-7 show the release of mesalazine 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 mesalazine from polymeric prodrugs at alkaline medium was higher than the release rate of drug in acidic condition. The drug- release rate from polymeric 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 mesalazine 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. The degree of hydrolysis increase 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.

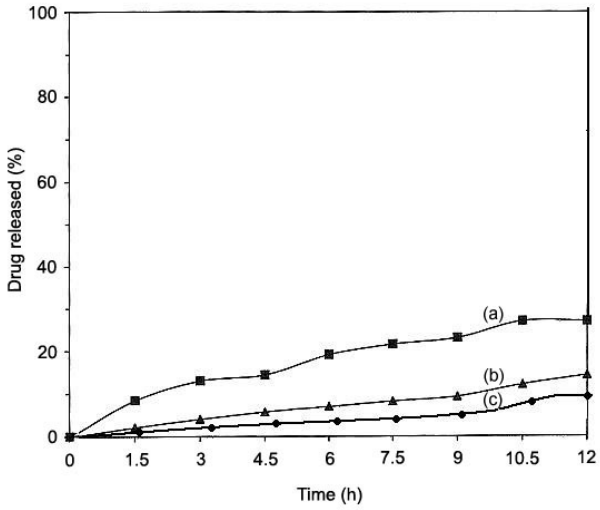

Figure 5. Percent of mesalazine released from polymeric carriers as a function of time at hydrochloric acid buffer (pH 1) and 37°C. (a) poly(MOPAS-co-HEMA); (b) poly(MOPAS-co-MMA); (c) poly(MOPAS-co-EHA).

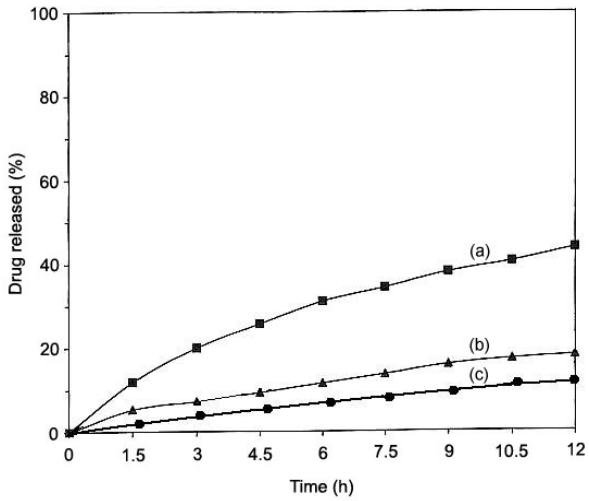

Figure 6. Percent of mesalazine released from polymeric carriers as a function of time at phosphate buffer (pH 7) and 37 °C. (a) poly(MOPAS-*co*-HEMA); (b) poly(MOPAS-*co*-MMA); (c) poly(MOPAS-*co*-EHA).

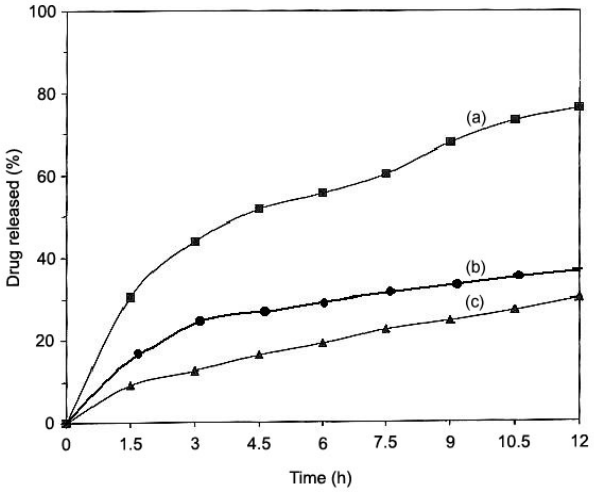

Figure 7. Percent of mesalazine released from polymeric carriers as a function of time at phosphate buffer (pH 8) and 37 °C. (a) poly(MOPAS-*co*-HEMA); (b) poly(MOPAS-*co*-MMA); (c) poly(MOPAS-*co*-EHA).

In the alkaline environment of the lower GI tract, however, hydrolysis is mainly take place by microfloral enzymes. Where esterase enzyme released by microbes is expected to hydrolyze the ester linkage and releasing the free drug. Even allowing for a certain amount of hydrolysis of the prodrug in the acidic environment of the upper GI tract, the amount of drug released here should be much less because the residence time in the upper GI tract (stomach and duodenum) is less than two hours. As well as a certain amount of hydrolysis of the prodrug taken place even in absence of microfloral enzymes due to a nucleophilic attack of the hydroxyl group on the electron deficient carbonyl carbon in the lower GI tract, but the most of drug release takes place predominantly in the lower GI tract only in presence of microfloral enzymes especially esterase which is expected to hydrolyze the ester linkage and releasing the free drug thus allowing for site-specific delivery [1]. Also, the neighboring groups can affect the drug-release rate. As shown in Figures 5-7, 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 behaviour. Poly(MOPAS-*co*-HEMA) has hydrophilic HEMA units and therefore, is rapidly hydrolyzed from other polymers containing hydrophobic MMA and EHA units.

CONCLUSION

In this work, HPMA-based polymeric prodrugs containing mesalazine pendent groups were synthesized by the free radical polymerization of MOPAS with various acrylic-monomers. The structure of the obtained polymers was characterized by spectroscopy techniques and their compositions calculated by the ^{1}H-NMR spectra and elemental analysis data. Hydrolysis of the polymeric prodrugs was carried out similar to the physiological conditions and the results showed that with introducing hydrophilic units along the polymer chain, the release percentage of mesalazine is increased. Also, the release profiles of mesalazine from prodrugs showed that the synthesized polymeric prodrugs were pH-sensitive polymers. However, a certain amount of mesalazine can be released by hydrolysis of the polymeric produgs in small intestine (pH 5-7), but the amount of the released mesalazine in colon (pH 8) is very high. 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. The molecular weight of the prodrugs after hydrolysis, toxicity of the hydrolyzed products and clinical studies will be reported in next works.

REFERENCES

[1] R. Yadav, O.P. Mahatma, *J. Pharm. Sci. & Res.*, **2011**, 3, 966.
[2] S. Ahmad, R.F. Tester, A. Corbett, J. Karkalas**,** *Carbohydr Res.*, **2006**, 341, 2694.
[3] M. Ojhal, N.V.S. Madhav, A. Singh, *Int. Curr. Pharm. J.*, **2012**, 1, 209.
[4] S. Dhaneshwar, M. Kandpal, G. Vadnerkar, *J. Drug Del. Sci. Tech.*, **2009**, 19, 67.
[5] R. Wiwattanapatapee, L. Lomlim, K. Saramunee, *J. Control. Release,* **2003**, 88, 1.
[6] H. Ringsdorf, *J. Polym. Sci. Symp.*, **1975**, 51, 135.
[7] J. Sanchis, F. Canal, R. Lucas, M.J. Vicent, *Nanomedicine,* **2010**, 5, 915.
[8] K. Hoste, K. De Winne, E. Schacht, *Int. J. Pharm.,* **2004**, 277, 119.
[9] S. Davaran, M.R. Rashidi, A. Khani, *Drug Dev. Ind. Pharm.*, **2007**, 33, 881.
[10] S. Davaran, M.R. Rashidi, J. Hanaee, A. Khani, M. Mahkam, M. Hashemi, *J. Bioact. Compat. Polym.*, **2006**, 21, 315.
[11] S. Kumar, Y.S. Negi, *J. Pharm. Sci. & Res.*, **2012**, 4, 1995.
[12] J. Lai, W. Li, H. Wang, K. Tu, L. Wang, *Acta Poly. Sin.*, **2007**, 1, 8.
[13] M. Mahkam, M.G. Assadi, R. Zahedifar, M. Ramesh, S. Davaran, *J. Bioact. Compat. Polym.*, **2004**, 19, 45.
[14] A. Garjani, S. Davaran, M. Rashidi, N. Maleki, *Daru,* **2004**, 12, 24.
[15] E. Schacht, A. Gevaert, E.R. Kenawy, K. Molly, W. Verstraete, P. Adriaensens, R. Carleer, J. Gelan, *J. Control. Release*, **1996**, 39, 327.
[16] M. Babazadeh, M. Sheidaei, S. Abbaspour, L. Edjlali, *Sci. Pharm.*, **2013**, 81, 281.
[17] M. Babazadeh, L. Edjlali, L. Rashidian, *J. Polym. Res.*, **2007**, 14, 207.
[18] M. Babazadeh, T. Mosanejhad, *Iran. Polym. J.*, **2009**, 18, 179.
[19] M. Babazadeh, *Int. J. Pharm.*, **2008**, 356, 167.
[20] M. Babazadeh, *J. Appl. Polym. Sci.*, **2007**, 104, 2403.
[21] M. Babazadeh, L. Edjlali, Z. Hajizeynalabedini, *J. Iran. Chem. Res.*, **2008**, 1, 41.
[22] M. Babazadeh, *Int. J. Pharm.*, **2006**, 316, 68.
[23] H. Namazi, M. Babazadeh, A. Sarabi, A. Entezami, *J. Polym. Mater.*, **2001**, 18, 301.
[24] S. Dumitriu, M. Popa, M. Dumitriu, *J. Bioact. Compat. Polym.*, **1989**, 4, 151.
[25] C.H. Chang, Y.M. Sheu, W.P. Hu, L.F. Wang, J.S. Chen, *J. Polym. Sci. Polym. Chem.*, **1998**, 36, 1481.
[26] M. Babazadeh, *Polym. Degrad. Stab.*, **2006**, 91, 3245.