Synthesis, Characterization, and *In Vitro* Drug-Release Properties of 2-Hydroxyethyl Methacrylate Copolymers

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ABSTRACT: 2-Hydroxyethyl methacrylate was copolymerized with acrylamide, *N*-vinyl-2-pyrrolidone, and *n*-butyl methacrylate by free-radical solution polymerization with α,α′-azobisisobutyronitrile as an initiator at 70 ± 1°C. The average molecular weights and molar compositions of the resultant copolymers were determined with gel permeation chromatography and ^{1}H-NMR spectroscopy data, respectively. Diclofenac or 2-[(2,6-dichlorophenyl)amino]-benzene acetic acid, a nonsteroidal anti-inflammatory drug, was chemically attached to the copolymers by transesterification reaction in the presence of *N*,*N*′-dicyclohexylcarbodiimide to give macromolecular prodrugs. All the synthesized polymers were characterized with Fourier transform infrared, ^{1}H-, and ^{13}C-NMR spectroscopy techniques. The polymer–drug conjugates were hydrolyzed in cellophane member dialysis bags containing aqueous buffered solutions (pH 8) at 37°C, and the hydrolysis solutions were detected by UV spectrophotometer at selected intervals. The results showed that the drug could be released by selective hydrolysis of the ester bond from the side chain of the drug moiety. The release profiles of the drug indicated that the hydrolytic behavior of polymeric prodrugs strongly depends on the hydrophilicity of the polymer. The results suggest that the synthesized copolymers could be useful carriers for the release of diclofenac in controlled-release systems. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 2403–2409, 2007

Key words: diclofenac; 2-hydroxyethyl methacrylate; polymeric prodrugs; drug delivery; hydrolysis

INTRODUCTION

In the last decades, polymer chemists have been actively involved in designing polymer materials for biomedical applications. One field of application that has attracted polymer chemists' attention since the late 1960s is advanced drug-delivery systems, which are needed to improve drug efficacy. Polymer materials have been designed and proposed as matrices or depot systems for injectable or implantable systems or devices. One particular approach towards an improved use of drugs for therapeutic applications is the design of polymeric prodrugs or polymer–drug conjugates.[1,2]

It was already early in the 1950s and 1960s when polymer chemists started to link drugs to polymers to improve their efficiency. At that time, however, they were mainly concentrating on the chemistry itself, and almost any class of polymers was covalently combined with any class of drugs. The biological aspects for the design of polymeric prodrugs were hardly taken into account.[3]

For the first time in 1975, a rational model for pharmacologically active polymers was proposed. Ringsdorf[4] was the first to recognize the immense potential of polymeric prodrugs, if only polymer chemists and biologists would work together in the field. The proposed model consists mainly of five components: the polymeric backbone, the drug, the spacer, the targeting group, and the solubilizing agent (Fig. 1). This model, though still oversimplified, is an important milestone in the history of polymeric prodrug design.

The polymeric carrier can be either an inert polymer 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.[1,2,5]

Diclofenac (Scheme 1), a nonsteroidal anti-inflammatory drug (NSAID), is therapeutically used for inflammatory and painful diseases of rheumatic and nonrheumatic origins. The therapeutic use of NSAIDs is often restricted by the necessity of delivering the drug to specific sites of the target organ or tissue. Also, the use of NSAIDs is limited by their irritant side effects on the gastroenteric mucous and by their frequently poor water solubility. Therefore, to minimize the NSAIDs side effects and to increase their therapeutic efficiency, the delivery of NSAIDs by polymeric carriers has been developed.[6–11]

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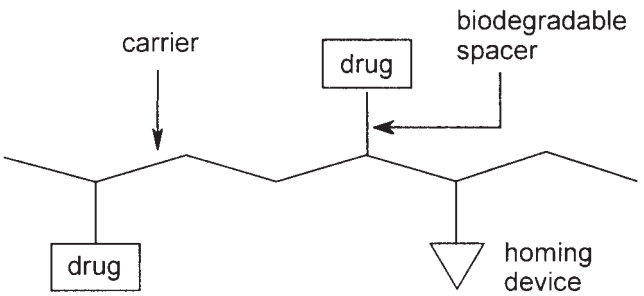


Figure 1 Ringsdorf-proposed model for polymeric prodrugs.[4]

In recently years, polymer–drug conjugates of NSAIDs such as ibuprofen,[12–15] indomethacin,[16–18] naproxen,[19,20] ketoprofen,[14,19] and fenoprofen[21,22] have been synthesized, and their hydrolysis properties have been studied.

This article reports the synthesis and hydrolytic behavior of acrylic-type polymeric prodrugs of diclofenac. Copolymers of 2-hydroxyethyl methacrylate (HEMA) with acrylamide (AAm), *N*-vinyl-2-pyrrolidone (NVP), and *n*-butyl methacrylate (*n*-BMA) were synthesized by free-radical polymerization. Then, diclofenac was attached to the resultant copolymers by hydrolyzable ester bonds. The hydrolysis of the polymer–drug adducts was studied under physiological conditions in aqueous phosphate buffer solutions (pH 8). The pendent diclofenac group was hydrolyzed under mild conditions, and the quantity of the released drug was detected by UV spectroscopy. Also, the influence of neighboring groups with various hydrophilic effects on the release of the drug from the polymeric carriers was studied.

EXPERIMENTAL

Materials and instruments

Diclofenac sodium was purchased from Sigma Chemical Co. (St Louis, MO). It was dissolved in distilled water and converted to diclofenac by the addition of HCl (0.1*N*). The precipitated diclofenac was collected and dried *in vacuo*. HEMA, NVP, and BMA were obtained from Merck (Darmstadt, Germany) and distilled under reduced pressure to remove inhibitors before use. *N*,*N*′-Dicyclohexylcarbodiimide (DCC) and AAm were purchased from Merck and used as received. α,α′-Azobisisobutyronitrile (AIBN) was obtained from Fluka (Buchs, Switzerland) and recrystallized twice from methanol. *N*,*N*-Dimethylformamide (DMF; Merck) was dried over anhydrous $MgSO_4$ for 2 days and distilled under reduced pressure. All other chemicals were reagent-grade or purer.

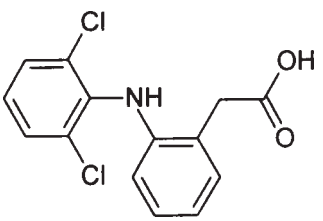
Fourier transform infrared (FTIR) spectra were recorded with KBr pellets on a Shimadzu 4300 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 400-MHz spectrometer in a dimethyl sulfoxide-d_6 solution. The amount of the released drug was determined with a Shimadzu 2100 UV spectrophotometer at the adsorption maximum of diclofenac (275 nm) in an aqueous buffered solution with a 1-cm quartz cell. The molecular weights of the polymers were determined with a Maxima 820 gel permeation chromatography (GPC) analysis instrument (mobile phase, DMF; run time, 50 min; column temperature, 50°C). Well-characterized poly(ethylene oxide) was used in the calibration within the weight-average molecular weight range of 2600–885,000.

Preparation of the copolymers: General polymerization procedure

Copolymerization reactions were carried out in dried DMF solutions at 70 ± 1°C in Pyrex glass ampules sealed off *in vacuo*. AIBN was used as an initiator ([HEMA] + [Comonomer] = 40 mmol, [AIBN] = 0.4 mmol). The sealed ampules were vigorously shaken by a shaker machine and immersed in a water bath held at the aforementioned temperature of polymerization for about 20 h. After the reaction time, the ampules were removed from the bath, and at once the contents were poured into a large excess of a cooled proper nonsolvent. Poly(2-hydroxyethyl methacrylate-*co*-acrylamide) [poly(HEMA-*co*-AAm)] and poly (2-hydroxyethyl methacrylate-*co*-*n*-butyl methacrylate) [poly(HEMA-*co*-BMA)] were separately precipitated in cooled ethanol. Also, poly(2-hydroxyethyl methacrylate-*co*-*N*-vinyl-2-pyrrolidone) [poly(HEMA-*co*-NVP)] was precipitated in cooled *n*-hexane. The precipitated samples were washed with the same precipitant and dried *in vacuo*.

Attaching diclofenac to the synthesized copolymers: Esterification procedure

In a two-necked flask containing two dropping funnels, 1.0 g (3.4 mmol) of diclofenac was dissolved in 5 mL of dried DMF, and the flask was cooled to 0–5°C with an ice–water bath. Then, 0.72 g (3.5 mmol) of DCC was dissolved in 5 mL of dried DMF and added dropwise to the solution of the flask through the first



Scheme 1 Structure of diclofenac.

dropping funnel. The resultant solution was stirred at 0–5°C for 10 min. In another dropping funnel, 1.0 g of poly(HEMA-*co*-AAm) or poly(HEMA-*co*-NVP) or poly(HEMA-*co*-BMA) was dissolved in 5 mL of dried DMF and added dropwise with stirring to the solution of the flask. The mixture was vigorously stirred in room temperature for 12 h, and the produced white precipitate was filtered. The remaining solution was poured into an excess of cooled ethanol for poly (HEMA-*co*-AAm)–drug or poly(HEMA-*co*-BMA)–drug and into cooled *n*-hexane for poly(HEMA-*co*-NVP)–drug. The precipitated polymer–drug conjugates were collected, washed several times with the same precipitant, and dried *in vacuo* at room temperature.

Method of hydrolysis

The polymer–drug conjugates were dried *in vacuo* at room temperature and sieved with a 200-mesh sieve. The powdered polymer–drug adduct (200 mg) was poured into 5 mL of an aqueous buffered solution (phosphate buffer, pH 8) at 37°C, and the mixture was placed in a cellophane membrane dialysis bag. The bag was closed and transferred into a flask containing 25 mL of the same buffer solution maintained at 37°C. The external solution was continuously stirred, a 3-mL sample was removed at selected intervals, and 3 mL of the buffer was replaced. The quantity of the released drug was detected with a UV spectrophotometer and determined from the calibration curve obtained previously under the same conditions.

Characterization of the hydrolysis products

Twenty milligrams of the polymer–drug adduct was dispersed in 20 mL of a buffered solution (pH 8), and the reaction mixture was maintained at 37°C. After 24 h, the hydrolysis solution was sampled and neutralized with HCl (1 N), and the solvent was removed *in vacuo*. 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 FTIR spectroscopy and melting-point measurements. The results showed that the hydrolysis product was diclofenac.

RESULTS AND DISCUSSION

Two different synthetic methods have been reported for 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 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 the other method, the drug agent is attached to preformed polymer backbones via degradable chemical bonds to produce polymeric prodrugs.[5,23,24]

Diclofenac has a secondary amine group in its structure. When diclofenac is converted to a suitable polymerizable monomer, the obtained monomer is not polymerized by free-radical polymerization because the amine group in the structure of diclofenac acts as an inhibitor and prevents radical polymerization of the monomer. Therefore, for the preparation of polymeric prodrugs, diclofenac must be bound to preformed polymers by chemically links.[25]

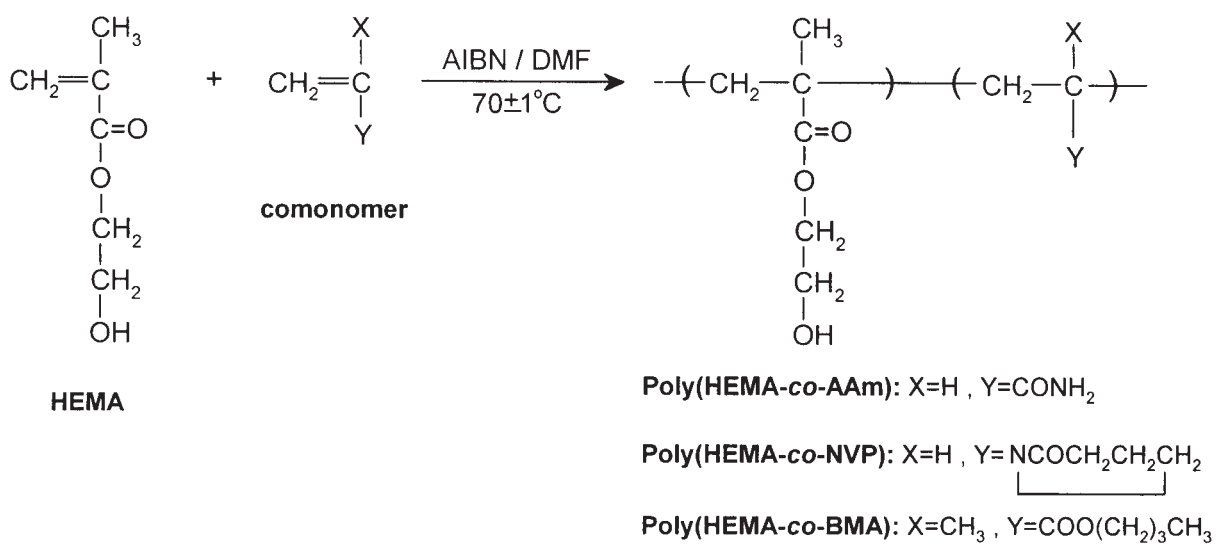


Figure 2 Copolymerization of HEMA with AAm, NVP, and *n*-BMA.

TABLE I
Preparation Conditions of the HEMA Copolymers

Copolymer	Monomer 1	Monomer 2	[1] (mmol/L)	[2] (mmol/L)	Nonsolvent	Conversion (%)
Poly(HEMA-*co*-AAm)	HEMA	AAm	10	30	Ethanol	62
Poly(HEMA-*co*-NVP)	HEMA	NVP	10	30	*n*-Hexane	55
Poly(HEMA-*co*-BMA)	HEMA	*n*-BMA	10	30	Ethanol	68

Synthesis and characterization of the HEMA copolymers

HEMA was copolymerized with AAm, NVP, and *n*-BMA in dried DMF solutions at 70 ± 1°C with AIBN as a free-radical initiator (Fig. 2). The preparation conditions for the copolymers are shown in Table I.

All the FTIR spectra of the synthesized copolymers showed two peaks at 3400 and 1735 cm^{-1} due to hydroxyl and ester carbonyl stretching vibrations of the HEMA units, respectively. In poly(HEMA-*co*-AAm), the N—H and carbonyl stretching vibrations of the amide group in the AAm units were observed at 3300 and 1680 cm^{-1}, respectively. In poly(HEMA-*co*-NVP), the carbonyl stretching vibration of NVP could be seen at 1720 cm^{-1}. Also, the ester carbonyl stretching vibration of *n*-BMA in poly(HEMA-*co*-BMA) appeared at 1735 cm^{-1}.

In the ^{1}H-NMR spectra of all the copolymers, one proton of the hydroxyl group and two methylene protons of —COOCH_2— appeared at 5.0 and 4.2 ppm, respectively. The proton signals of —CONH_2 in poly(HEMA-*co*-AAm) could be seen at 9.9 ppm. The resonance signal at 3.1 ppm was attributed to two methylene protons of —CONCH_2— in poly (HEMA-*co*-NVP). Also, in the ^{1}H-NMR spectra of all the copolymers, the broad signal at 0.9–2.6 ppm was due to the methylene protons of the backbone and other alkyl protons.

In the ^{13}C-NMR spectra of all the copolymers, the resonance signal at 174.5 ppm was due to the ester carbonyl carbon. The methylene carbon of —CH_2OH and the attached methylene carbon to the oxygen of ester, —CH_2OCO—, gave signals around 53.0 and 59 ppm, respectively. The carbon of the amide group in poly(HEMA-*co*-AAm) and poly(HEMA-*co*-NVP) appeared at 169.5 and 176.5 ppm, respectively. The backbone carbon signals were observed at 40–45 ppm. A series of resonance peaks between 12.5 and 20 ppm was due to the α-methyl group and other alkyl groups.

The number- and weight-average molecular weights of the synthesized copolymers were determined by GPC and are presented in Table II.

The copolymer compositions were calculated from ^{1}H-NMR spectroscopy data. In the past few decades, ^{1}H-NMR spectroscopy analysis has been established as a powerful tool for the determination of copolymer compositions because of its simplicity, rapidity, and sensitivity.[14,26–28] The assignment of the resonance peaks in the ^{1}H-NMR spectrum leads to the accurate evaluation of the content of each kind of monomeric unit incorporated into the copolymer chains. Thus, the molar fraction of HEMA in the copolymer chains was calculated from the measurements of the integrated peak area of the hydroxyl proton of the HEMA unit and the total integrated peaks of the aliphatic protons of HEMA and the comonomer. The following expression was used to determine the composition of poly(HEMA-*co*-BMA). Let m_1 be the molar fraction of HEMA and $1-m_1$ be the molar fraction of *n*-MBA. HEMA contains one hydroxyl proton and nine aliphatic protons, and *n*-MBA contains 14 aliphatic protons:

$$B = \frac{\text{Integrated peak area of hydroxyl proton}}{\text{Integrated peak area of aliphatic protons}} = \frac{m_1}{9m_1 + 14(1 - m_1)} \quad (1)$$

Upon simplification, this gives

$$m_1 = \frac{14B}{5B + 1} \quad (2)$$

Therefore, the molar fraction of HEMA in poly (HEMA-*co*-BMA) was determined with eq. (2). A similar method was used to calculate of the molar compositions of the other copolymers. Table III gives the values of B and the corresponding molar fractions of HEMA and the comonomer in the synthesized copolymers.

TABLE II
Molecular Weights of the HEMA Copolymers[a]

Copolymer	$M_w \times 10^{-3}$	$M_n \times 10^{-3}$	M_w/M_n
Poly(HEMA-*co*-AAm)	34.0	19.5	1.9
Poly(HEMA-*co*-NVP)	32.3	14.7	2.1
Poly(HEMA-*co*-BMA)	31.1	18.4	1.7

M_w = weight-average molecular weight; M_n = number-average molecular weight.

Attaching diclofenac to the synthesized copolymers

Diclofenac was attached to the synthesized copolymers by transesterification. Esterification reactions

TABLE III
Calculation of the Molar Compositions of the HEMA Copolymers

Copolymer	I_{OH}[a]	I_{Ali}[b]	$B = I_{OH}/I_{Ali}$	m_1[c]	HEMA (mol %)	Comonomer (mol %)
Poly(HEMA-*co*-AAm)	1.23	18.56	0.066	$3B/(1 - 6B) = 0.33$	33	67
Poly(HEMA-*co*-NVP)	1.15	32.85	0.035	$9B = 0.32$	32	68
Poly(HEMA-*co*-BMA)	1.29	56.52	0.023	$14B/(5B + 1) = 0.29$	29	71

[a] Integrated peak area of the hydroxyl protons in the copolymer.
[b] Integrated peak area of the aliphatic protons in the copolymer.
[c] Molar fraction of HEMA in the copolymer.

were carried out in the presence of DCC as a water absorber. The hydroxyl group from HEMA units in the copolymers reacted with the carboxyl group from diclofenac to give a new ester bond between the drug and copolymers (Fig. 3). In these reactions, the obtained water was absorbed by DCC and produced *N,N′*-dicyclohexylurea as a white precipitate. After the completion of the reactions, the white precipitate was isolated, and each solution was poured into the proper nonsolvent. The copolymers containing diclofenac were dried and collected in high yields (between 75 and 80%).

The ^{1}H-NMR spectra of the drug-linked copolymers showed that all the hydroxyl groups in HEMA units had been converted to ester groups. These spectra showed that with the formation of a new ester bond, the peak at 5 ppm corresponding to one proton of the hydroxyl group disappeared, and the integration peak at 4.2 ppm due to methylene protons of —COOCH_2— was increased. Also, a new signal related to one amine proton of the drug appeared at 10.3 ppm. The proton signals of the aryl groups in diclofenac could be seen between 6.5 and 7.7 ppm.

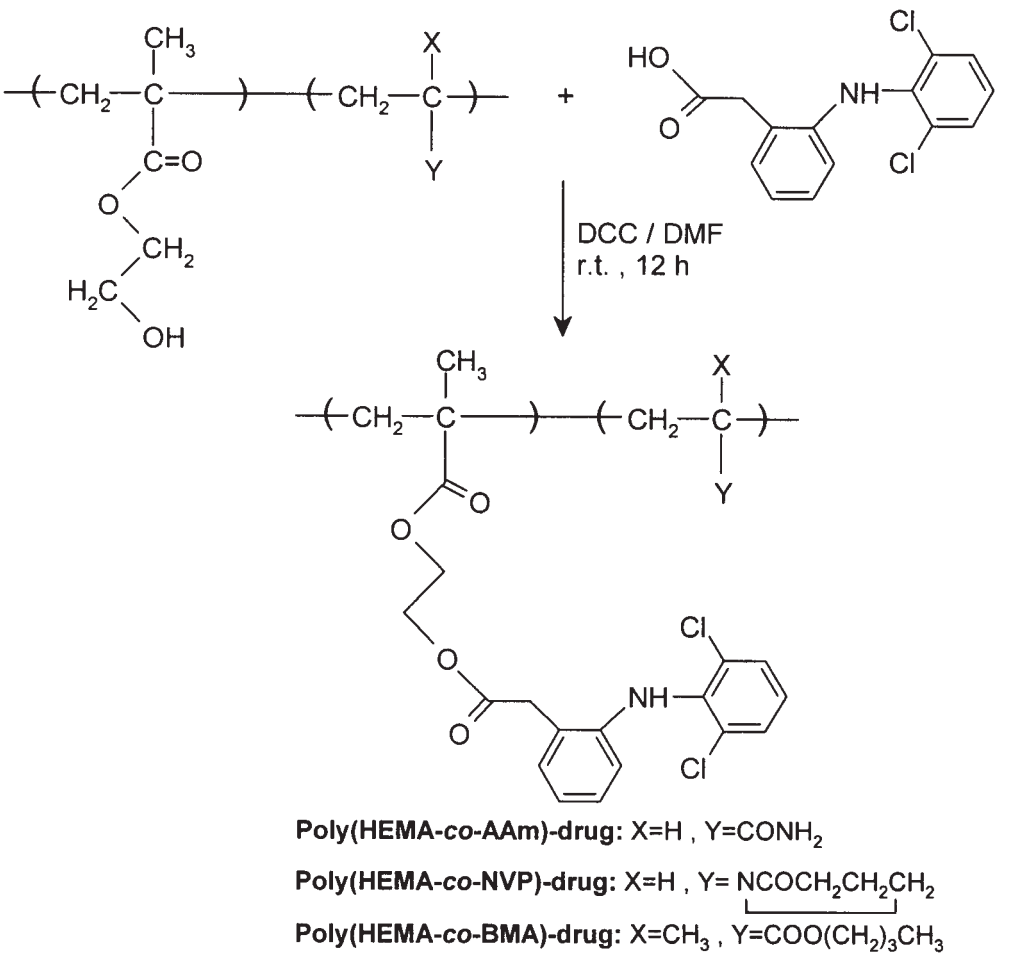


Figure 3 Attaching diclofenac to HEMA copolymers.

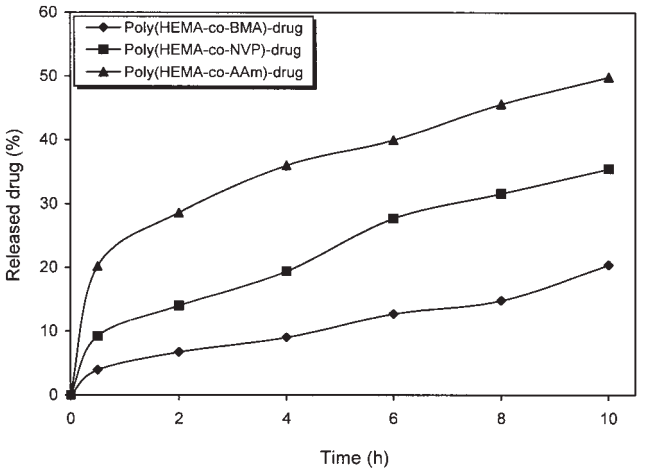


Figure 4 Percentage of diclofenac released from polymeric carriers as a function of time in a phosphate buffer (pH 8) at 37°C.

In the FTIR spectra of the polymeric prodrugs, the peak due to the O—H stretching vibration of HEMA at 3400 cm^{-1} disappeared, and a new peak at 3250 cm^{-1} due to the N—H stretching vibration of the drug amine group appeared. The C—H and C=C stretching vibrations of the aromatic rings were observed at 3030 and 1600 cm^{-1}, respectively. The peaks at 2990 and 2950 cm^{-1} were attributed to the asymmetrical and symmetrical C—H stretching of methylene and methyl groups. The ester carbonyl stretching was observed at about 1730 cm^{-1}. The asymmetrical and symmetrical bending vibrations of the methyl groups could be seen at 1453 and 1380 cm^{-1}, respectively. The peaks at 1270 and 1150 cm^{-1} were due to C—O stretching.

In the ^{13}C-NMR spectra of the drug-linked copolymers, the resonance of the carbonyl carbon of the new ester bond between the copolymer and drug was observed at 176 ppm. Also, the aromatic carbons gave signals at 115–145 ppm.

Drug release by the hydrolysis of the polymeric prodrugs

To study potential applications of diclofenac pendent copolymers as pharmaceutically active compounds, the *in vitro* hydrolysis behavior of the polymeric prodrugs was studied in an aqueous phosphate buffer at 37°C. 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.[29] The polymer–drug conjugates were dispersed in buffer solutions, and their 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 was detected by a UV spectrophotometer at 275 nm. Two hydrolyzable ester bonds were present in the copolymers. The detection of the hydrolysis solution by UV spectroscopy showed that the polymer–drug conjugates released the drug gradually under mild conditions in a KH_2PO_4–Na_2HPO_4 buffer (pH 8) by the hydrolysis of the ester bond between the drug and side chain of the polymer during the reaction time (10 h). The direct ester linkage to the main chain of the polymer was less susceptible towards hydrolysis. This could be related to the steric hindrance of bulk polymer chains and the decrease in the bond mobility.[30] Figure 4 shows the degree of hydrolysis of the polymeric prodrugs as a function of time. The order of hydrolysis was as follows: poly (HEMA-*co*-AAm)–drug > poly(HEMA-*co*-NVP)–drug > poly (HEMA-*co*-BMA)–drug.

Different factors such as the solubility of the polymers and neighboring effects of side groups can affect the overall rate of hydrolysis. The hydrophilic polymer–drug adducts were hydrolyzed in buffer solutions rather than the hydrophobic systems. As shown in Figure 4, poly(HEMA-*co*-AAm)–drug was hydrolyzed rather than poly(HEMA-*co*-NVP)–drug and poly(HEMA-*co*-BMA)–drug, and this was related to the high hydrophilicity of the AAm units. The obtained results suggest that these systems could be useful for the preparation of a controlled-release formulation of diclofenac.

CONCLUSIONS

We prepared polymeric prodrugs containing diclofenac pendent groups. The structure of the polymers was characterized by spectroscopy techniques, and their compositions were calculated with ^{1}H-NMR spectroscopy data. Hydrolysis studies were carried out under the same physiological conditions. The results showed that the introduction of hydrophilic units along the polymer chain improved the hydrolytic behavior. However, the development of such systems into a drug product will require a truly multidisciplinary approach. As the main purpose of polymeric prodrugs is the achievement of controlled drug release or slow release, the application of these polymers as drug-delivery systems is expected after *in vivo* examinations.

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