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Research Article

Formulation and *In-vitro* Evaluation of Vinyl Ester Type Polymeric Prodrugs of Naproxen as Drug Delivery Systems

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ABSTRACT

This present research work describes synthesis and *in-vitro* evaluation of a serious of vinyl ester type polymeric systems linked to naproxen as materials for drug delivery. First, naproxen reacted with vinyl acetate in the presence of catalyst to obtain vinyl ester derivative of naproxen. The resulted material was then copolymerized with 2-hydroxyethyl methacrylate and methyl methacrylate by utilizing azoisobutyronitrile as an initiator at the temperature range of 65-70 ^{o}C to give drug-polymer conjugates. The obtained materials were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis techniques to confirm their structures. The hydrolysis of drug-polymer conjugates was carried out in cellophane membrane dialysis bags containing aqueous buffer solutions (pH 1, 7.4 and 10) at 37 °C for 24 h. Detection of hydrolysis solution by UV spectroscopy at selected intervals showed that naproxen can be released by hydrolysis of the ester bond between the drug and polymer backbone. The release profiles indicated that the hydrolytic behavior of polymeric prodrugs is strongly based on the polymer hydrophilicity and the pH value of the hydrolysis solution. The obtained results suggested that these polymeric prodrugs could be useful for release of naproxen in controlled release systems after *in-vivo* examinations.

INTRODUCTION

One field of application that has attracted polymer chemist's attention from the late 1960s onwards is the need for advanced drug delivery systems to improve drug efficacy. Polymer materials were 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 [1-3]. 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 polymeric drug delivery systems [4-6].

For the first time in 1975, Ringsdorf [7] proposed a rational model for pharmacologically active polymers. This proposed model consists mainly of five components: the polymeric backbone, the drug, the spacer, the targeting group and the solubilizing agent (Figure 1). This model, although still oversimplified, has been an important mark in the history of polymeric prodrugs.

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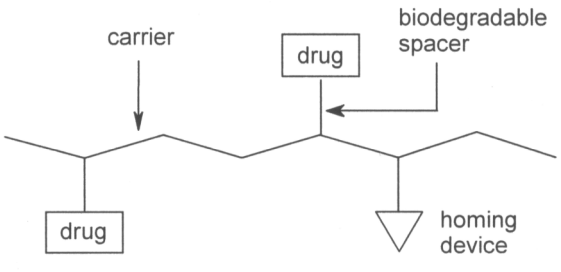


Figure 1: The proposed model by Ringsdorf for pharmacologically active polymers [7].

Naproxen, 2-(6-methoxy-2-naphtyl) propionic acid, is a non-steroidal anti-inflammatory drug (NSAID) associated with gastrointestinal side-effects, particularly stomach ulceration, bleeding and perforation [8]. The action of NSAIDs is thought to involve the inhibition of cyclooxygenases, responsible for prostaglandin synthesis, which controls pain and inflammation in rheumatic diseases. The main disadvantage of NSAIDs is a relatively short plasma half-life, which results in a short duration of activity, and a pronounced ulcerogenic potency. To reduce the gastrointestinal symptoms and prolong the drug activity, a recent approach has been the concept of retrometabolic drug design that incorporates targeting and metabolic considerations into the design processes [9, 10].

In the recent years, some of NSAIDs such as ibuprofen, naproxen, ketoprofen, diclofenac, and 5-aminosalicylic acid have been chemically bounded to various polymer backbones and their hydrolytic behavior has been studied by Babazadeh and co-workers [11-18].

This research work describes an efficient chemical method to design and evaluation of vinyl ester type polymeric prodrugs of naproxen. Vinyl- 2-(6-methoxy-2-naphtyl) propionate (VEN), as a vinyl ester type derivative of naproxen was first synthesized by reacting naproxen and vinyl acetate in the presence of mercuric acetate. The obtained VEN was then copolymerized with 2-hydroxyethyl methacrylate (HEMA) or methyl methacrylate (MMA) by free radical polymerization method. The release of naproxen 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 naproxen are discussed.

MATERIALS AND METHODS

Materials

Naproxen was purchased from Aldrich chemical company. Mercuric acetate, vinyl acetate, sodium acetate, HEMA and MMA were obtained from Merck chemical company and used as received. Azoisobutyronitrile (AIBN) was obtained from Fluka chemical company and recrystallized from methanol. *N*, *N*-dimethylformamide (DMF) was dried over anhydrous $MgSO_4$ for two days and distilled under reduced pressure. All other chemicals were reagent grade or purer.

Instrumental measurements

FT-IR spectra were recorded on a Shimadzu 4300 spectrophotometer. ^1H and ^{13}C-NMR spectra were recorded on Bruker 300 MHz spectrometer in $CDCl_3$ or DMSO-d_6 solution. The amount of released naproxen was determined by a 2100 Shimadzu UV spectrophotometer at the maximum adsorption of the free drug in aqueous buffered solutions (λ_{max}=272 nm) using a 1-cm quartz cell. The number and weight molecular weights (M_n and M_w) of polymers were determined with a Maxima 820 gel permeation chromatography (GPC) unit. (Mobile phase, DMF; run time, 50 min; column temperature, 50 ^oC). Well-characterized polyethylene oxide was used in the calibration within the range of M_w between "2600–885000". Elemental analyses were carried out with a Heareus CHN-ORAPID instrument.

Preparation of vinyl 2-(6-methoxy-2-naphtyl) propionate (VEN)

The amount of 2.9 g (12.6 mmol) of naproxen and 0.3 g of mercuric acetate were dissolved in 30 ml of vinyl acetate and stirred for 30 min at room temperature. Then, 0.2 ml of concentrated sulfuric acid was added into the solution and refluxed for about 3 h. After this time, the solution was cooled to room temperature and 1.0 g of sodium acetate was added to quench the catalyst. The solution was filtered, concentrated and the crude product was then purified by silica gel column chromatography by eluting with petroleum ether/ethyl acetate (30:1, v/v) to give 2.4 g (75%) of VEN as a white powder.

FT-IR (KBr, cm^{-1}) 3050 (C-H aromatic and vinylic), 2890 (C-H aliphatic), 1735 (C=O ester), 1600, 1480 (C=C). ^1H NMR ($CDCl_3$, ppm) 1.5 (d, 3H, -ArCH($C\underline{H}_3$)-), 3.75 (s, 3H, -O$C\underline{H}_3$), 3.9 (q, 1H, -ArC$\underline{H}$(CH_3)-), 4.5 (d, 1H, $C\underline{H}_2$=C), 4.9 (d, 1H, $C\underline{H}_2$=C), 6.9 (q, 1H, CH_2=C$\underline{H}$), 7.0-7.3 (m, 6H, aryl-$\underline{H}$), ^{13}C NMR ($CDCl_3$, ppm) 21 (1C, -CH-$\underline{C}H_3$), 45 (1C, -$\underline{C}$H-CH_3), 56 (1C, -O$\underline{C}H_3$), 106 (1C, $\underline{C}H_2$=CH-),

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

Sample	[M_1] (mmol/L)	[M_2] (mmol/L)	Non-solvent	Yield (%)
Poly(VEN-*co*-HEMA)	VEN (10)	HEMA (30)	Methanol	72.9
Poly(VEN-*co*-MMA)	VEN (10)	MMA (30)	Methanol	77.3

Table 2: Spectral characterization of the polymeric prodrugs

Sample	Functional group	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)	FT-IR (cm^{-1})
Poly(VEN-*co*-HEMA)	-COO-	-	173, 170	1735
	-OH	5.5	-	4200-3200
Poly(VEN-*co*-MMA)	-COO-	-	175, 171	1735
All polymers	$-OCH_3$	3.8	61	1100
	Ph	7.0-8.0	110-162	1600, 1480

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

Sample	C (%)	H (%)	M_n	M_w/M_n	VEN (%)	HEMA (%)	MMA (%)
Poly(VEN-*co*-HEMA)	64.34	7.08	34520	1.7	30	70	-
Poly(VEN-*co*-MMA)	66.17	7.25	32414	1.9	22	-	78

141 (1C, CH_2=CH-), 111-162 (10C, aromatic carbons), 172 (1C, C=O). Elemental analysis for $C_{16}H_{16}O_3$ (256 $gmol^{-1}$), calculated: C 75.0, H 6.25; found: C 74.83, H 6.31%.

Copolymerization of VEN with acrylic monomers

In two Pyrex glass ampoules, the mixtures of 2.56 g (10 mmol) of VEN, 0.16 g (1 mmol) of AIBN, 3.95 g (30 mmol) of HEMA or 3.0 g (30 mmol) of MMA were dissolved in 15 ml of dried DMF, respectively. The ampoules were then degassed, sealed under vacuum, maintained at 65-70 ºC in a water bath and shaken by a shaker machine for about 30 h. After this time, the obtained viscous solutions were separately poured 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 the resultant polymers are given in Table 1.

In-vitro hydrolysis

The polymer-drug conjugates were dried under vacuum at room temperature and sieved with a 200-mesh sieve. Each of dried polymer-drug conjugates (200 mg) was poured into 5 ml of aqueous buffered solution (pH 1, 7.4 and 10) at 37 ºC 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 ºC. 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 10) and maintained at 37 ºC. After 24 h, the hydrolysis solution was sampled, neutralized with 1 N HCl 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 naproxen.

RESULTS AND DISCUSSION

Synthetic route for preparation of VEN

Vinyl acetate has been used as acylating reagent in many successful resolution of alcohol. Since the alcohols freed from the reaction rapidly tautomerize to volatile acetaldehyde, making the process irreversible and simple for product isolation [19]. Yang [20] and Cai [21] have already reported a method for conversion of carboxylic acids to the related vinyl ester by using vinyl acetate as an acylating agent.

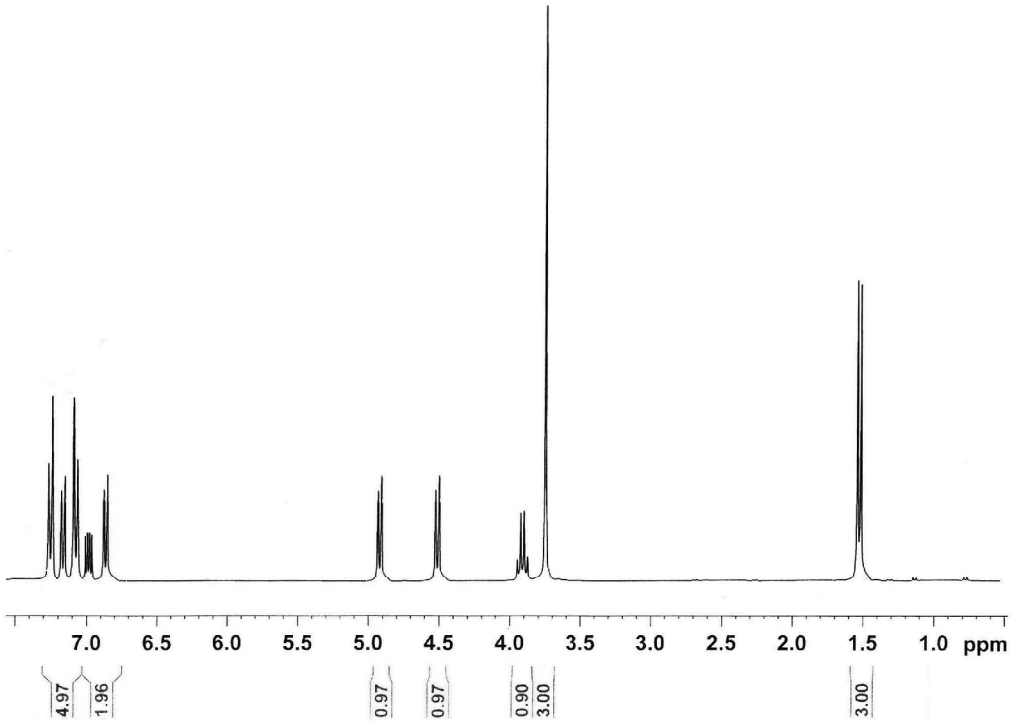


Figure 2: ^{1}H-NMR spectrum of VEN in $CDCl_3$.

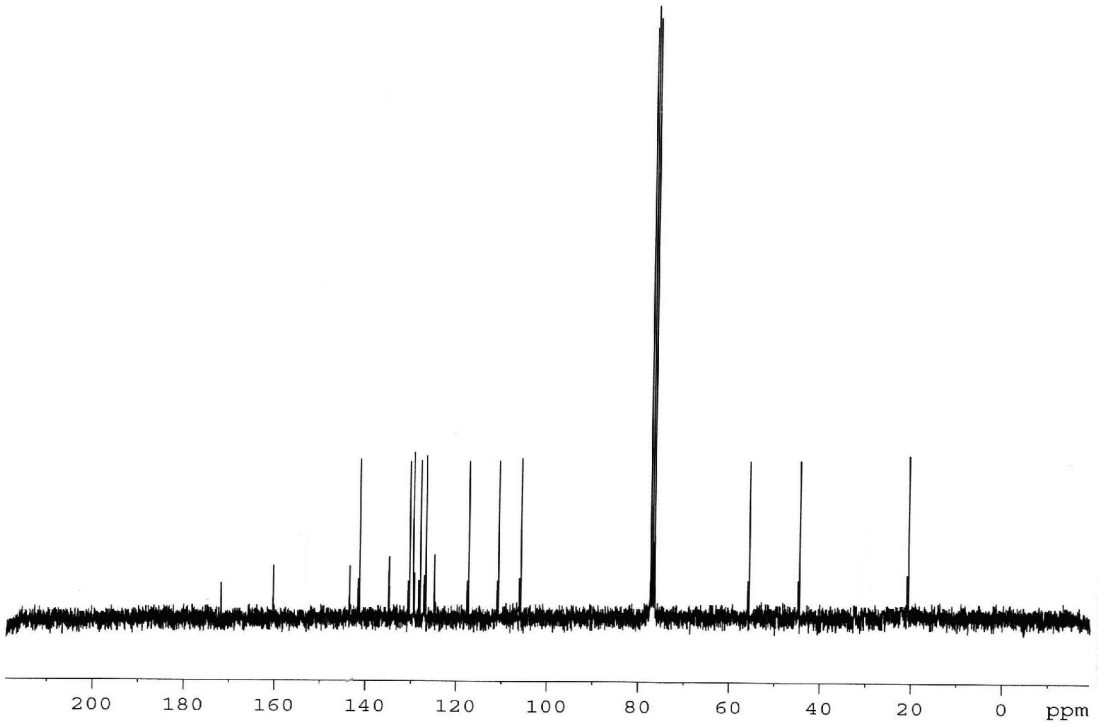
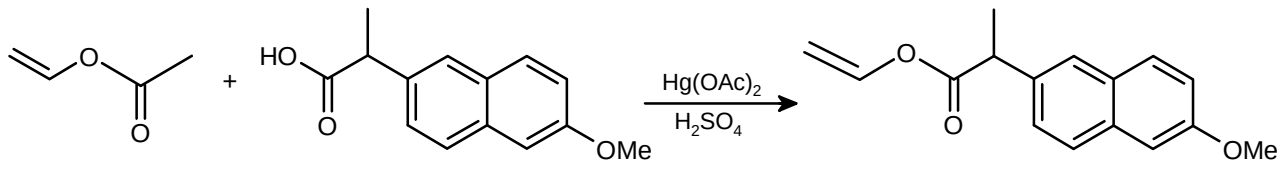
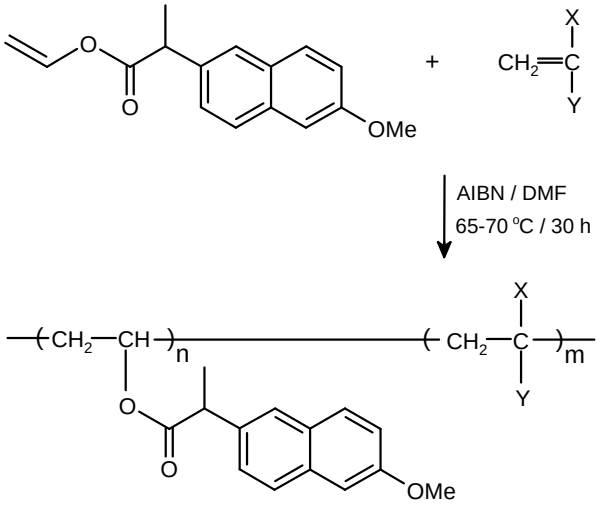


Figure 3: ^{13}C-NMR spectrum of VEN in $CDCl_3$.



Scheme 1: The synthesis route of vinyl ester type derivative of naproxen (VEN).



Poly(VEN-co-HEMA): X=Me, Y=$COOCH_2CH_2OH$
Poly(VEN-co-MMA): X=Me, Y=$COOCH_3$

Scheme 2: Copolymerization of VEN with HEMA or MMA to give polymeric prodrugs

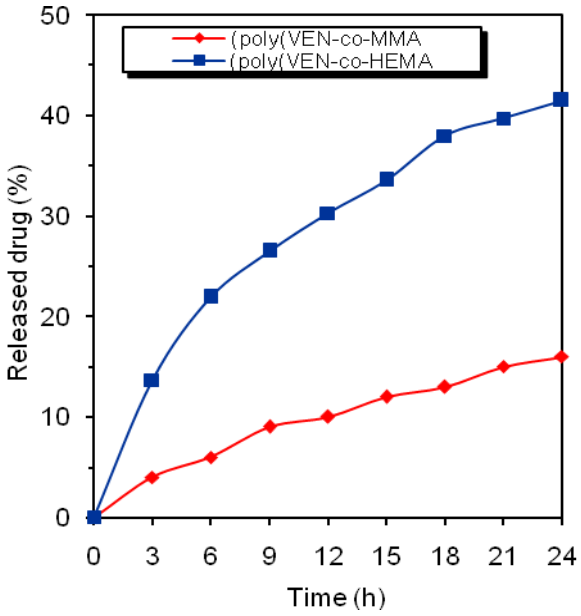


Figure 5: Percent of released naproxen from polymeric carriers as a function of time at phosphate buffer (pH 7.4) and 37°C

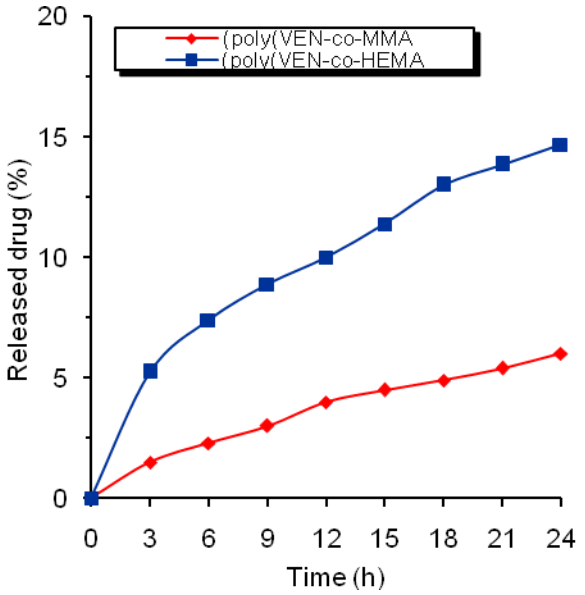


Figure 4: Percent of released naproxen from polymeric carriers as a function of time at hydrochloric acid buffer (pH 1) and 37°C

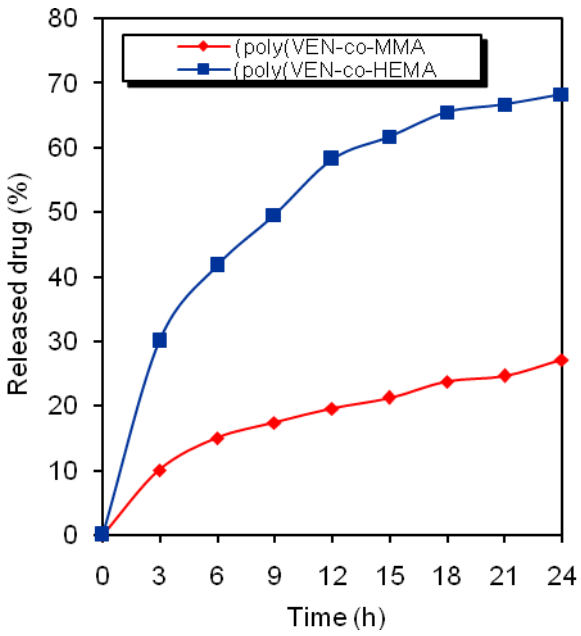
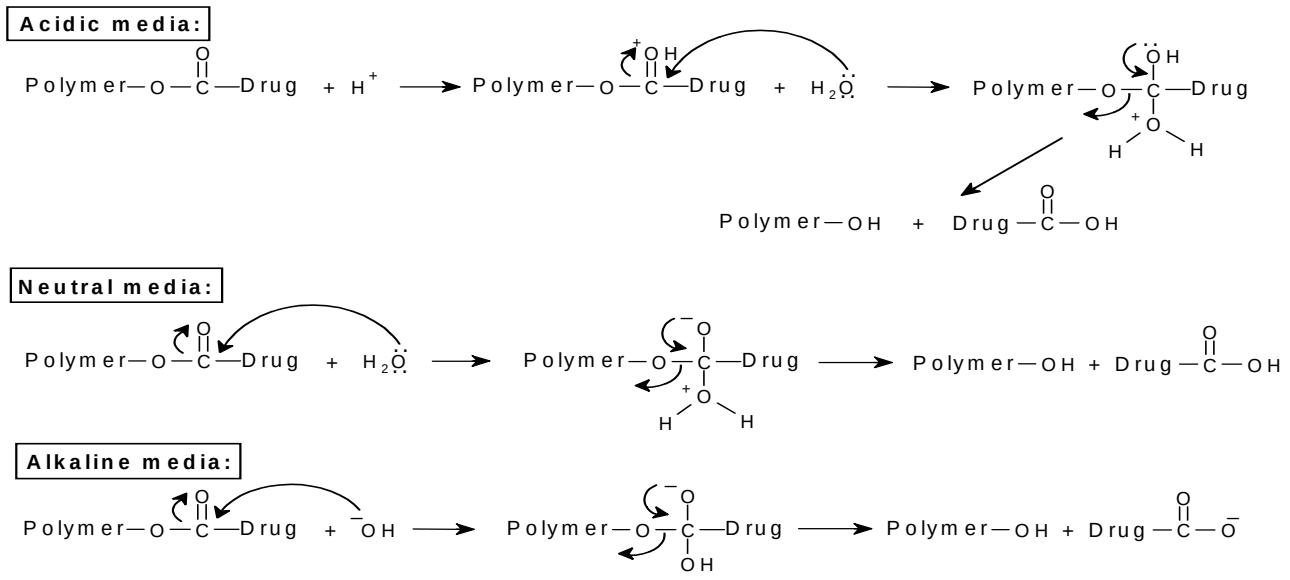


Figure 6: Percent of released naproxen from polymeric carriers as a function of time at phosphate buffer (pH 10) and 37°C

In this present work, naproxen reacted with vinyl acetate in the presence of mercuric acetate as a catalyst, and the related vinyl ester (VEN) was collected in high yield after purification by column chromatography (Scheme 1). The resultant FT-IR, ^{1}H-NMR, ^{13}C-NMR spectra and elemental analysis data confirmed the structure of VEN and its purity. The related ^{1}H and ^{13}C-NMR spectra of VEN are shown in Figures 2 and 3, respectively.

Synthesis and characterization of polymeric prodrugs

Drug-containing monomer, VEN, was easily copolymerized with HEMA and MMA in dried DMF solution, by free radical technique at the temperature range of 65–70°C using AIBN as initiator (Scheme 2). The resulted copolymers were colorless, amorphous and soluble in DMSO and DMF, but insoluble in water and alcohols.



Scheme 3: The hydrolysis mechanism of polymeric prodrugs in different pH media

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, ^{1}H and ^{13}C-NMR spectroscopy. The results confirmed the structure of the synthesized polymers. Spectral characteristics of functional groups of copolymers having naproxen substituent are given in Table 2. One parameter in characterization of polymeric prodrugs is determination of molecular weight distribution and the average molecular weights. The molecular weights of the synthesized polymeric prodrugs were estimated by GPC instrument and are shown in Table 3. Also, the mole composition of polymeric prodrugs was determined from the related elemental analyses data, and is presented in Table 3.

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 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 (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 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 determined by a UV spectrophotometer.

Figures 4-6 show the release of naproxen 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 (VEN-*co*-HEMA)>Poly (VEN-*co*-MMA). The release rate of naproxen 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. The hydrolysis mechanism of polymeric prodrugs in various pH media is shown in Scheme 3.

Different factors such as solubility of polymers and neighboring effect of side groups can affect the overall rate of hydrolysis. The hydrophilic copolymer containing naproxen was hydrolyzed in buffer solutions rather than hydrophobic

copolymer. As shown in Figures 4-6, poly(VEN-*co*-HEMA) was rapidly hydrolyzed because of higher hydrophilicity of HEMA units and poly(VEN-*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 naproxen.

CONCLUSIONS

In this work, VEN as a vinyl ester type derivative of naproxen was synthesized from reaction of vinyl acetate and naproxen in the presence of catalyst. Then, the polymeric prodrugs containing naproxen pendent groups were synthesized by the free radical polymerization of VEN with acrylic monomers such as HEMA or MMA. The structure of the synthesized VEN and polymeric prodrugs were characterized by various spectroscopy techniques. Hydrolysis of polymeric prodrugs was carried out similar to the physiological conditions and the results showed that the introduction of hydrophilic units along the polymer chain improves 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. 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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