Original Research Article

Novel Polymeric Prodrugs of Valproic Acid as Anti-Epilepsy Drugs: Synthesis, Characterization and *In-vitro* Evaluation

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Abstract

Purpose: To synthesize and evaluate, in-vitro, novel polymeric prodrugs of valproic acid (VPA) for anti-epileptic activity.
Methods: Homopolymer of 4-chloromethyl styrene (CMS) and its copolymers with various acrylic-type monomers such as 2-hydroxyethyl methacrylate and methyl methacrylate were prepared by free radical polymerization method. VPA was then covalently linked to the obtained polymers by treating CMS polymers with sodium valproate. All the compounds were characterized by Fourier transform infrared (FT-IR), nuclear magnetic resonance (^{1}H and ^{13}C-NMR), elemental analyses, and gel permeation chromatography (GPC). The release of VPA from polymeric prodrugs was studied using cellophane membrane dialysis bags containing aqueous buffer solutions (pH 1, 7 and 10) at 37 ^{o}C. The quantity of released drug was detected by ultraviolet (UV) spectroscopy.
Results: ^{1}H-NMR and elemental analyses data for calculating mole composition of CMS polymers were relatively in good agreement. FT-IR and NMR data for the polymeric prodrugs showed attachment of drug substituents to phenyl rings of CMS units via methylene spacer. The drug-release profiles indicated that selective hydrolysis of ester bond between the drug and the polymer backbone is strongly dependent on polymer hydrophilicity and the pH of the hydrolysis solution.
Conclusion: The synthesized VPA polymeric prodrugs may be cost-effective compounds for release of VPA in vivo when formulated as controlled release systems.

Keywords: 4-Chloromethyl styrene, Valproic acid, Polymeric prodrugs, Controlled release, Hydrolysis

INTRODUCTION

Polymeric prodrugs or polymer-drug conjugations are novel technique for drug delivery systems. In these systems, macromolecular chains act as carriers for drugs in a physically bound state or by true chemical linkages as side groups. These latter systems, in which drugs are delivered by chemically or biologically induced cleavage of the covalent bonds, increase water solubility, stability, and therapeutic efficiency of drugs whit reducing the required doses, side effects, and toxicity of drugs by controlling the rate, duration, and site of release [1-3].

Valproic acid (VPA), so called (2-propylpentanoic acid), is a branched short-chain fatty acid derived from naturally occurring valeric acid. VPA is used in the treatment of epilepsy and seizures but also migraine, bipolar, mood, anxiety and psychiatric disorders [4-7]. VPA has been used in combination with sodium valproate. These

formulations show smaller differences in the pharmacokinetics and accessibility in market [8]. VPA single or in combination with sodium valproate is available in different dosage forms; capsule, tablet, enteric-coated tablet, sprinkle, liquid, intravenous, suppository and controlled-release formulations [9]. Valproate is believed to affect the function of the neurotransmitter γ-aminobutyric acid (GABA) in the human brain. VPA inhibits GABA transaminase by binding to it and thus enhances the neurotransmission of GABA in brain [10,11]. VPA also blocks the voltage-gated sodium channels and T-type calcium channels [12]. These mechanisms make VPA a broad-spectrum anticonvulsant drug.

4-Chloromethyl styrene (CMS) is a dual functional monomer that can polymerize or copolymerize before or after functional reactions with the benzyl chloride group. Polychloromethyl styrene (PCMS) and related copolymers are able to react with various nucleophilic reagents at fairly high yields [13,14]. Functionalized PCMS and its copolymers have been widely used in different processes as photo-sensitizers [15], solar energy storage [16], photo-resists [17], non-linear optics [18], cholesterol trapping of human serum [19], and prodrugs in biomedical applications [20].

In previous works, we reported the synthesis and *in-vitro* evaluation of some acrylic-type polymeric prodrugs of non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, naproxen, ketoprofen, diclofenac, and 5-aminosalicylic acid [21-28].

In this research work, the synthesis and comparative study of the hydrolytic behavior of polymeric drugs based on CMS bearing VPA is reported. CMS was homopolymerized and copolymerized with 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) by free radical polymerization method. VPA was then linked to the resulted polymers by nucleophilic substitution reaction. The *in-vitro* hydrolysis behavior of the polymeric prodrugs was studied in physiological conditions at 37 $^{\circ}$C. Hydrophilic properties of polymeric prodrugs and pH values of hydrolysis media are affected on the basis of results obtained *in-vitro* evaluation.

EXPERIMENTAL

Materials

Sodium valproate was purchased from Sigma. HEMA and MMA were obtained from Merck and

purified by distillation under reduced pressure to remove inhibitors. CMS was purchase from Acros Organics and used as received. Azobisisobutyronitrile (AIBN) was obtained from Fluka and recrystallized twice from methanol. All other chemicals were reagent grade or purer.

Instrumental measurements

FT - IR spectra were recorded on a Shimadzu 4300 spectrophotometer. 1H - NMR spectra were recorded on Bruker 400 MHz spectrometer in CDCl3 solution. The amount of released VPA was determined by a 2100 Shimadzu UV spectrophotometer at the adsorption maximum of the free VPA in aqueous buffered solutions (213 nm) using a 1 - cm quartz cell. The values of number - average molecular weight (Mn), weight average molecular weight (Mw) and the polydispersity index of polymers were determined with a Maxima 820 gel permeation chromatography (GPC) instrument. Well - characterized polyethylene oxide was used in the calibration within the range of Mw between "2600– 885000". Elemental analyses were carried out with a Heareus CHN - ORAPID instrument.

Synthesis of PCMS (P1)

In a quickfit Pyrex polymerization tube, 0.032 g (0.2 mmol) of AIBN and 3.0 g (20 mmol) of CMS were dissolved in 10 ml of toluene, and the solution flushed gently with argon for about 20 min. Then the solution was shaken vigorously in a thermostatic water bath at 70 ± 1 $^{\circ}$C under argon for 12 h. After this time, the resulted yellowish viscous solution was poured into 120 ml of cooled methanol as a non-solvent. The precipitated PCMS was collected and dissolved in 15 ml of toluene and poured into 100 ml of cooled methanol. The obtained white precipitate was washed with methanol and dried in vacuum at room temperature to give 2.6 g of **P1**. FT-IR (KBr): 3030 (aromatic C-H), 2910, 2860 (aliphatic C-H), 1590, 1480 cm^{-1} (aromatic C=C). ^{1}H-NMR (CDCl$_3$): δ = 1.4 (2H, CH$_2$-CH), 1.9 (1H, CH$_2$-CH), 4.5 (2H, CH$_2$-Cl), 6.3 - 7.2 (4H, Ar-H). ^{13}C-NMR (CDCl$_3$): δ = 40 (1C, CH$_2$-CH), 41 (1C, CH$_2$-CH), 46.2 (1C, CH$_2$-Cl), 125, 128.9, 135 and 145.5 (aromatic carbons).

Copolymerization of CMS with acrylic-type monomers: general procedure

Poly(CMS-co-HEMA) (**P2**) and poly(CMS-co-MMA) (**P3**) were synthesized as follows.

A mixture of 0.5 g (3.2 mmol) of CMS, 0.02 g

(0.13 mmol) of AIBN and 1.25 g (9.6 mmol) of HEMA or 0.96 g (9.6 mmol) of MMA was dissolved in 10 ml of dried DMF in a Pyrex glass ampoule. Then the ampoule was degassed, sealed under vacuum and maintained at 70 ± 1 ^{o}C in a water bath and shaken for about 30 h. Then the viscous solution of ampoule was poured into 150 ml of cooled ethanol. The precipitate was collected and washed with non-solvent for several times and dried under vacuum at room temperature to give 1.3 g of **P2** or 1.1 g of **P3**.

Synthesis of polymeric prodrugs containing VPA

In a 100 ml two-necked flask equipped with a dropping funnel and a reflux condenser, 1 g of **P1** or **P2** or **P3** was dissolved in 10 ml of DMF. A gas-inlet is attached to the top of the dropping funnel and the system is maintained under a slight pressure of argon. A solution of 2 g (12 mmol) of sodium valproate in DMF (30 ml) was added dropwise from dropping funnel with stirring to solution of polymers into flask at room temperature. The reaction mixture was heated at 80 ^{o}C for about 3 h, and stirred for 12 h at room temperature. Then, the solution was poured into an excess cooled methanol and the precipitate filtered and washed with water for several times to removing NaCl and unreacted drug. The obtained polymers containing VPA substituents (P1-VPA, P2-VPA or P3-VPA) was collected and dried under vacuum in high yield (73-85 %).

Method of hydrolysis

Each of dried polymer - drug conjugates (200 mg) was poured into 5 ml of an aqueous buffered solution (pH 1, 7 and 10) at 37 ^{o}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 ^{o}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

The polymer-drug adduct (20 mg) was dispersed into 20 ml of buffered solution (pH 10) and maintained at 37 ^{o}C. 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 as a clear colorless liquid with boiling point 220 °C showed that the hydrolysis product is VPA.

RESULTS

Characteristics of polymers P1-P3

The CMS was easily hompolymerized and copolymerized with HEMA and MMA by free radical polymerization technique at 70 ± 1 ^{o}C using AIBN to obtain amorphous **P1-P3**. The resulted polymers were soluble in DMSO and DMF, but insoluble in alcohols and water. The prepared polymers were characterized by FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The FT-IR spectra of polymers **P1-P3** showed a peak at 3035 cm^{-1} due to C-H stretching of the aromatic ring. The peaks at 2996 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 1735 cm^{-1}. The ring stretching vibrations of the aromatic nuclei were observed at 1600 and 1485 cm^{-1}. The hydroxyl stretching vibration of **P2** was observed at 3400 cm^{-1}. The asymmetrical and symmetrical bending vibrations of methyl groups are seen at 1453 and 1380 cm^{-1}, respectively. The peaks at 1273 and 1160 cm^{-1} were due to C-O stretching. The elemental analyses data for the synthesized **P1-P3** are presented in Table 1.

In the ^{1}H-NMR spectra of **P1-P3**, two methylene protons of benzyl chloride appeared at 4.6 ppm. The proton signals of the aryl group were seen
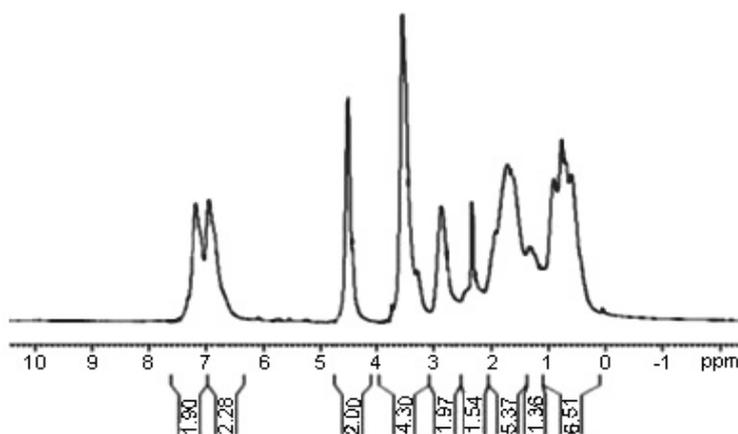
Table 1: Molecular weights, calculation of integrated peak area of protons, mole compositions, and elemental analysis of **P1-P3**

Sample	M_n (×10^{-3})	M_w (×10^{-3})	M_w/M_n	I_{Ar} [a]	I_{Ali} [b]	B	CMS (%)	Comonomer (%)	C (%)	H (%)
P1	35.2	63.4	1.8	-	-	-	100	-	70.8	5.9
P2	23.2	48.7	2.1	3.9	25.6	0.15	32	68	60.9	7.0
P3	27.5	46.7	1.7	4.1	25.1	0.16	29	71	63.8	7.2

[a] Integrated peak area of aromatic protons in copolymer; [b] integrated peak area of aliphatic protons in copolymer

Figure 1: ^{1}H-NMR spectrum of copolymer **P3** in CDCl₃

between 6.7 and 7.2 ppm. The resonance signal at 4 ppm was attributed to two methylene protons of -COOCH₂ in copolymer **P2**, and three methyl protons of -COOCH₃ in copolymer **P3** appeared at 3.6 ppm. Also, one proton of hydroxyl group of **P2** appeared at 5.0 ppm. The broad signal at 0.7-2.9 ppm was due to the methylene groups of backbone and other alkyl groups. A typically ^{1}H-NMR spectrum of the copolymer **P3** is shown in Figure 1.

In the ^{13}C-NMR spectra of **P1-P3**, the resonance signal at 176.5 ppm was due to the ester carbonyl carbon in copolymers. The aromatic carbons gave signals at 128.2, 128.7, 135.3 and 145.5 ppm. The signal at 46 ppm was attributed to the methylene carbon of benzyl chloride. The methyl and methylene carbons attached to the oxygen of ester appeared at 51.6 and 59.6 ppm, respectively. The backbone carbon signals were observed at 40-45 ppm. The signal at 12.5 ppm was due to α–methyl group.

The number and weight-average molecular weights of the synthesized polymers **P1-P3** were determined by GPC and are presented in Table 1. The average composition of the copolymer samples was determined from the corresponding ^{1}H-NMR spectra and elemental analysis. In the past few decades ^{1}H-NMR spectroscopic analysis and elemental analysis have been established as a powerful tool for the determination of copolymer compositions because of its simplicity, rapidity and sensitivity [29,30]. 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 mole fraction of CMS in the copolymer chain was calculated from measuring

the integrated peak areas of aromatic protons of the CMS unit and aliphatic protons of the CMS and other monomer units. The following expression is used to determine the composition of copolymer **P3**. Let m be the mole fraction of CMS and 1-m or n is that of MMA monomer. The CMS contains four aromatic protons and five aliphatic protons and MMA contains eight aliphatic protons:

$$B = I_{Ar}/I_{Ali} = 4m/[5m+8(1-m)] \dots\dots\dots\dots \text{(1)}$$

Where I_{Ar} is the integrated peak area of aromatic protons and I_{Ali} is the integrated peak area of aliphatic protons.

On simplification, equation 1 becomes

$$m = 8B/(3B+4) \dots\dots\dots\dots\dots\dots \text{(2)}$$

Therefore, the mole fraction of CMS in the copolymer **P3** was determined from Eq. 2. A similar method was used to calculate of the mole compositions of **P2**. Table 1 gives the values of B and the corresponding mole fractions of CMS and related co-monomer in the copolymers. The results obtained from ^{1}H-NMR data and elemental analyses were relatively in good agreement.

Synthesis of polymeric prodrugs containing VPA

Because of the mobility of the benzyl chloride bonds in the resulted polymers **P1-P3**, nucleophilic substitutions were possible and easy. Therefore, a solution of sodium valproate as a nucleophilic reagent reacted with benzyl chloride bonds in polymers with replacement of the chlorine atoms to give new polymeric

prodrugs and VPA substituents attached to phenyl rings via methylene spacer. These new polymeric prodrugs P1-VPA, P2-VPA and P3-VPA were isolated in the related non-solvent in high yields.

The obtained polymeric prodrugs containing VPA were soluble in the same solvents that copolymers **P1-P3** are dissolved. The dissolving time for copolymers containing VPA groups was longer in comparison with copolymers without VPA groups. Since, with incorporating of VPA in polymer structure, the free volume of the polymer decreases. Therefore the solvent entering time between polymer chains is long and mild heat for dissolving is occasionally required.

The replacement of chlorine atom by VPA group is easily followed by NMR technique. The obtained ^{1}H-NMR spectra for polymeric prodrugs showed that VPA groups replace all the chlorine atoms. With replacement of chlorine atoms by VPA substituents, the peak at 4.6 ppm corresponding to two methylene protons of benzyl chloride disappears and new signals at 0.7-3.0 ppm due to fifteen aliphatic protons of VPA group appear. The other chemical shift assignments for polymeric prodrugs are the same observed chemical shifts for polymers **P1-P3**.

In the ^{13}C-NMR spectra of polymeric prodrugs, carbon absorption at 46 ppm corresponding to one methylene carbon of benzyl chloride disappears and a new peak at 65 ppm appears. Also, a new signal at 179 ppm corresponding to new ester carbonyl carbon and four signals at 50, 34, 21 and 15 ppm corresponding to aliphatic carbons of VPA observes, respectively.

Drug release from polymeric prodrugs

The *in-vitro* hydrolysis behavior of polymeric prodrugs was studied in physiological conditions (aqueous phosphate or hydrochloric acid buffers, at 37 ^{o}C). As the polymers were not soluble in water, they were dispersed in buffer solution and the hydrolysis was performed in a heterogeneous system. The hydrolysis was carried out in cellophane membrane bags permeable to low molecular weight compounds. The released drug passed through the high molecular weight polymers into the external buffer solution and determined by a UV spectrophotometer.

Figures 2 and 3, show the release of VPA from polymeric prodrugs as a function of time under mild conditions in HCl buffer (pH 1) and KH$_2$PO$_4$-

Na$_2$HPO$_4$ buffer (pH 7 and 10). The order of hydrolysis is: P2-VPA > P1-VPA > P3-VPA.

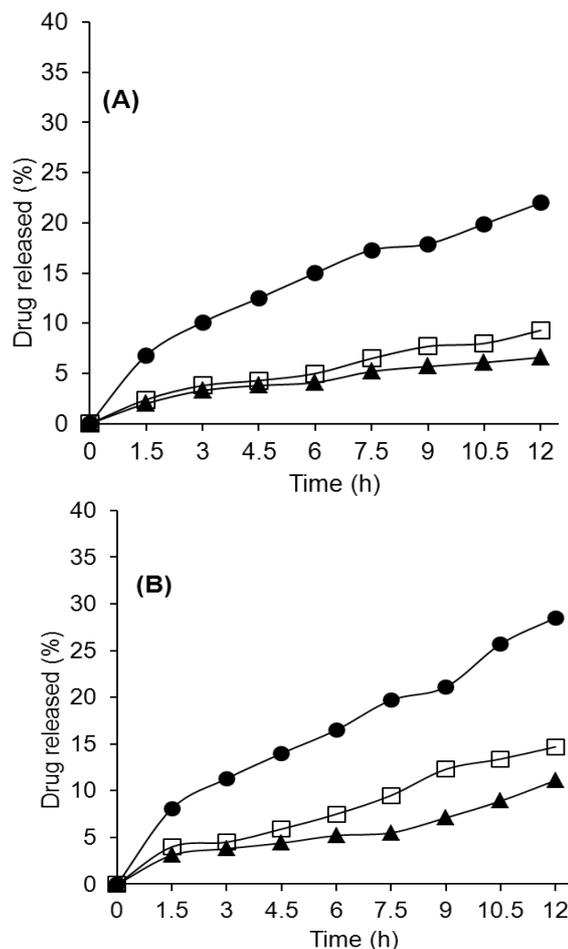**Figure 2:** VPA release from polymeric carriers as a function of time in (A) hydrochloric acid buffer (pH 1) and (B) phosphate buffer (pH 7) at 37 ^{o}C. ▲P3-VPA, ❑ P1-VPA, and ● P2-VPA.
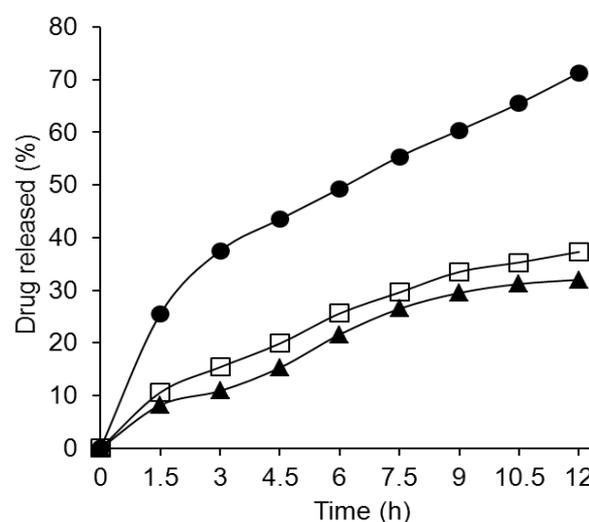

Figure 3: VPA release from polymeric carriers as a function of time in phosphate buffer (pH 10) at 37 ^{o}C. ▲P3-VPA, ❑ P1-VPA, and ● P2-VPA

DISCUSSION

It has been widely demonstrated that the side chain hydrolysis of drug pendent polymers depends on the strength and chemical nature of the drug polymer chemical bonds, the structure of the polymer and the surrounding condition. The length and hydrophilicity of the spacer unit between the drug and polymer chain can affect the release rate. Two hydrolysable ester bonds were present in the synthesized prodrugs. Detection of the hydrolyzing solution by UV spectrophotometer showed that only the ester bond between drug moiety and methylene group was hydrolyzed during the reaction time. This matter was evidenced from the IR and melting point measurement of the residual compound after hydrolysis, which corresponded to the free drug. The direct ester linkage between the main chain of polymer and methylene group was not undergo hydrolysis under mild conditions. This can be related to the steric hindrance of bulk polymer chains, which decrease the bond mobility.

As shown in Figures 2 and 3, the release rate of VPA from polymeric prodrugs at alkaline medium was higher than the release rate of drug in acidic condition. 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 VPA 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 catalyzed by acid. The degree of hydrolysis increases as the polymer passes from acidic to alkali medium. In alkali pH, the polymers have reached a degree of swelling that makes the labile bonds accessible to hydrolysis.

Different factors such as solubility of polymers and neighbouring effect of side groups can affect the overall rate of hydrolysis. The hydrophilic copolymer containing VPA was hydrolyzed in buffer solutions rather than hydrophobic copolymer. As shown in Figures 2 and 3, P2-VPA was rapidly hydrolyzed because of higher hydrophilicity of HEMA units and P3-VPA 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 VPA.

CONCLUSION

A series of novel polymeric prodrugs containing VPA pendent groups has been successfully synthesized as materials for drug delivery systems. Introducing hydrophilic units along the polymer chains improves hydrolytic behaviour. The developed systems would be useful for the development of pH-sensitive polymeric prodrugs for controlled release systems. As the main purpose of polymeric prodrugs is the achievement of controlled drug release. However, *in-vivo* studies are required to determine their suitability in clinical applications.

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