Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs

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Abstract: The aim of this work was to investigate the possibility of employing semi-covalent molecularly imprinted polymers (MIPs) as a controlled release device for ibuprofen and naproxen in biological fluids, especially gastrointestinal ones, compared to non imprinted polymers (NIPs). The carboxyl groups of ibuprofen and naproxen were converted to vinyl ester group by reacting ibuprofen and vinyl acetate as an acylating agent in the presence of catalyst. The semi-covalent molecularly imprinted polymers (MIPs) were synthesized by free radical polymerization of vinyl esters derivatives of ibuprofen and naproxen in the presence of methacrylic acid and ethyleneglycol dimethacrylate (EGDMA) as the functional monomer and cross-linker, respectively. The composition of the cross-linked three-dimensional polymers was determined by FTIR spectroscopy. The hydrolysis of drug polymer conjugates was carried out in cellophane membrane dialysis bags and the in vitro release profiles were established separately in enzyme-free simulated gastric and intestinal fluids (SGF, pH 1 and SIF, pH 7.4). Detection of hydrolysis solution by UV spectroscopy at selected intervals showed that the drug can be released by hydrolysis of the ester bond between the drug and polymer backbone in low rate.
[Mehrdad Mahkam, Nahid Poorgholy. **Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs.** Nature and Science 2011;9(5):163-168]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: molecularly imprinted polymer, pH-sensitive, anti-inflammatory drugs, sustained release.

1. Introduction

Molecular imprinting is an efficient technique for the introduction of regions with highly specific molecular arrangements into polymeric matrices [1, 2]. The first example of a molecularly imprinted polymer (MIP) was reported half a century ago, however it is only in the last decade that the use of molecular imprinting as a practical tool became established [3, 4]. MIPs were used as chromatographic stationary phases [5], for enantiomeric separation [6] and for Solid-Phase Extraction (SPE) [7] and also as receptors [8], antibody [9] and enzyme mimic [10]. In addition, in the last years, MIPs have been reported to be suitable as drug delivery systems (DDS) [11-15], as base excipients for controlled release devices of drugs with a narrow therapeutic index.

There are three different approaches to prepare MIPs: covalent (pre-organized approach), non-covalent (self-assembly approach) and semi-covalent approach. The covalent or pre-organized approach, involves the formation of reversible covalent bonds between the template and monomers before polymerization. Then the template is removed by cleavage of the covalent bonds, which will be re-formed upon rebinding of the target molecule. The semi-covalent approach is an intermediate option, where the template is covalently bound to a functional monomer, but the rebinding is based on non-covalent interactions. The non-covalent or self-assembly approach is based on the formation of relatively weak non-covalent interactions (e.g. hydrogen bonding, electrostatic interaction, hydrophobic interaction, Vander Waals forces and dipole-dipole bonds) between the template molecule and functional monomers before polymerization. The association and disassociation of the imprint occurs by plain diffusion in and out of the sites [16].

The ibuprofen and naproxen is non-steroid anti-inflammatory drug (NSAIDs) and are widely used for the treatment of rheumatoid arthritis. But, the use of NSAIDs is also limited by their irritant side effects on the gastro-enteric mucous and by their frequent poor water solubility. These problems can be solved by the preparation of polymeric prodrug backbones *via* hydrolyzable bonds. Polymer-drug conjugates of NSAIDs have been developed in order to minimize delivery problems and reduce gastrointestinal side effects by controlling the rate, duration, and site of release. These polymeric prodrugs have been designed for localized and prolonged duration of drug action by parental administration, or as dermal prodrugs [17].

In this work a new potential polymeric device, based on semi-covalent MIPs, for the sustained release of anti-inflammatory drugs are described. The vinyl ester type derivative of ibuprofen (VIP) and naproxen (VIN) was first synthesized by reacting ibuprofen and naproxen with vinyl acetate in the presence of mercuric acetate. The semi-covalent molecularly imprinted polymers (MIPs) were synthesized by free radical polymerization of VIP and VIN in the presence of methacrylic acid and ethyleneglycol dimethacrylate (EGDMA) as the functional monomer and cross-linker, respectively. The release of drugs from the obtained drug delivery vehicles 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. Considerable differences in the capacity of the polymers to recognize and to bind the template selectively between imprinted and non imprinted polymers (NIPs) have been observed.

2. Experimental

2.1 Materials

The vinyl ester derivative of ibuprofen (VIP) and naproxen (VIN) were prepared by the method described in the literature [18]. Ibuprofen, naproxen and ethyleneglycol dimethacrylate (EGDMA) were purchased from Aldrich chemical company. Mercuric acetate, vinyl acetate, sodium acetate and MMA were obtained from Merck chemical company and were purified by distillation under vacuum. Azoisobutyronitrile (AIBN) was obtained from Fluka chemical company and recrystallized from methanol. Enzyme-free SGF (pH 1) or SIF (pH 7.4) were prepared according to the method described in the US Pharmacopeia [19].

2.2 Instrumental measurements

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker 400 AC spectrometer in $CDCl_3$. The IR spectra were recorded on a Shimadzu FT IR-408 spectrophotometer. The amount of released ibuprofen and naproxen was determined by a Philips PU 8620 UV spectrophotometer at the maximum adsorption of the free drug in aqueous buffered solutions using a 1-cm quartz cell.

2.3 Preparation of vinyl ester derivative of ibuprofen (VIP) and naproxen (VIN)

The amount of (12.6 mmol) of drugs 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 about (85%) of VIP and VIN as a colorless liquid (Figure 1).

For VIP:

FT-IR (KBr, cm^{-1}) 3050 (C-H aromatic and vinylic), 2890 (C-H aliphatic), 1740 (C=O ester), 1600, 1480 (C=C).

^{1}H NMR ($CDCl_3$, ppm) 0.8 (d, 6H, $-CH(CH_3)_2$), 1.55 (d, 3H, $-ArCHCH_3$), 1.9 (m, 1H, $-CHMe_2$), 2.5 (d, 2H, Ar-CH_2-), 3.8 (q,1H, Ar-CH-), 4.5 (dd, 1H, CH_2=C), 4.9 (dd, 1H, CH_2=C), 7.0-7.27 (q, 4H, aryl-H), 7.3-7.34 (q, 1H, CH_2=CH).

^{13}C NMR ($CDCl_3$, ppm) 20 (1C, $-CH-CH_3$), 21 (2C, $-CH(CH_3)_2$), 22 (1C, $-CHMe_2$), 30 (1C, Ar-CH_2-), 45 (1C, $-CH-CH_3$), 125 (1C, CH_2=CH-), 155 (1C, CH_2=CH-), 126, 129, 138, 140 (6C, aromatic carbons), 172 (1C, C=O).

For VIN:

FT-IR (KBr, cm^{-1}) 3050 (C-H aromatic and vinylic), 2890 (C-H aliphatic), 1750 (C=O ester), 1644, 1480 (C=C).

^{1}H NMR ($CDCl_3$, ppm) 1.64 (3H, d, CH_3), 2.11 (1H, m, C_6H_4CH), 3.94 (3H, t, CH_3O), 4.58 (1H, dd, CH_2=C), 4.88 (1H, dd, CH_2=C), 7.29 (1H, dd, CH_2=CH), 7.38–7.15 (6H, m, ArH).

^{13}C NMR ($CDCl_3$, ppm) 18.8, 45.6, 55.7 (aliphatic carbons), 126.5 (1C, CH_2=CH-), 158.1 (1C, CH_2=CH-), 98.3, 106, 119.5, 127.7, 129.3, 129.7, 134.3, 135.2, 141.8 (aromatic carbons), 172.1 (1C, C=O).

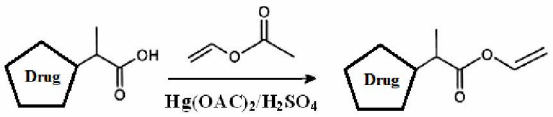

Figure 1. The synthesis route of vinyl ester type derivatives of ibuprofen and naproxen.

2.4 Synthesis of ibuprofen and naproxen molecularly imprinted polymer: (MIPs 1-4)

Methacrylic acid was used as the functional monomer to prepare the MIP by the semi-covalent imprinting method. Briefly, templates, methacrylic acid, EGDMA and AIBN with a variable feed ratio as shown in Table 1 were dissolved in acetonitrile (20 mL) in a thick-walled glass tube. The obtained solution was purged with nitrogen and sonicated for 10 min. The mixture was then incubated under a nitrogen atmosphere at 70 °C for 48 h. The resultant bulk rigid polymer systems were crushed, grounded into powder and sieved through a 63 µm stainless steel sieve. Reference NIPs matrices (acting as a control) were prepared under the same conditions without using the template. FTIR (KBr): 3380-2500 (broadened, -COOH group), 1730, 1705, 1245, 1225 cm^{-1}.

Table 1. Composition of molecular imprinted polymers and percentage of particles adhered onto rat intestine

MIPs	Molar composition of monomers in the feed				Percentage adherence
	VIP	NIP	MAA	CA	
MIP-1	1	--	6	30	61
MIP-2	1	--	10	30	71
MIP-3	--	1	6	30	64
MIP-4	--	1	10	30	69

2.5 Extraction of the templates from the polymer matrix: (MIP′s 1-4)

The resultant MIP materials were extracted with 100 mL mixtures of CH_3OH: NaOH (1:3, 1N) solution at 70 °C for at least 20 day and the solution were changed every
24 hours in order to remove any template. The washed MIPs (MIP′s) materials were checked to be free of drugs and any other compound by HPLC analysis. The extracted MIP′ materials were dried overnight in an oven at 60 °C.

2.6 Binding experiments

The binding experiments were performed, at room temperature in aqueous media (water solution pH 7). The sieved MIP′ and NIP particles (50 mg) were placed in 10 mL of ibuprofen or naproxen (1 mg.ml-1) for 240 min. Then the mixture was centrifuged for 10 min and the drug concentration in the liquid phase was measured by UV–VIS spectroscopy. The difference between the amount of drug initially employed and the drug content in the liquid phase is taken as an indication of the amount of drug entrapped. The amount of drugs bound to the polymer matrix was obtained by gentle washing of MIP′ and NIP for remove of any surface absorbance drug and comparing the drug concentration in the MIP′ samples and in the NIP ones. Experiments were repeated five times. The amounts of binding experiments are given in Table 2.

Table 2. Percentage of bound drugs by the imprinted and non-imprinted polymers after 240 min, in aqueous media (pH 7)

MIPs′	SA	DB	NIPs	SA	DB
MIP′-1	21	79	NIP-1	39	61
MIP′-2	16	84	NIP-2	66	34
MIP′-3	13	87	NIP-3	45.5	54.5
MIP′-4	30	70	NIP-4	40	60

Surface absorbance: SA (%)
Drugs bound: DB (%)

2.7 Drug Loading by the Soaking Procedure

The MIP′ and NIP particles (2.0 g) was immersed in a drug solution in water (20 mL, 5.5 mM) and soaked for 1 day at room temperature. During this time, the mixture was continuously stirred and then the solvent was removed by filtration. Finally the powder was dried under vacuum overnight at 40°C.

2.8 In vitro release studies

The MIP, MIP′ and NIP (50 mg) were poured into 3 mL of aqueous buffer solution (SGF: pH 1 or SIF: pH 7.4). The mixture was introduced into a cellophane membrane dialysis bag. The bag was closed and transferred to a flask containing 20 mL of the same solution maintained at 37° C. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The volume removed was replaced with SGF or SIF. Triplicate samples were used. The sample of hydrolyzate was analyzed by UV spectrophotometer (ibuprofen: (λmax=264 nm) and naproxen: (λmax=315 nm)), and the quantity of drugs was determined using a standard calibration curve obtained under the same conditions.

2.9 In situ Bioadhesivity Studies

Bioadhesivity testing was done by a novel in situ method as described by Ranga Rao and Buri [20]. A freshly cut 5-6 cm long piece of small intestine of rat was obtained and cleaned by washing with isotonic saline. The piece was cut open and the mucosal surface was exposed. Known weights of MIPs were added evenly on the mucosal surface. The intestinal piece was maintained at 80% relative humidity for 30 mts in a desiccator. The piece was taken out and phosphate buffer pH 6 was allowed to flow over the intestinal piece for about 2 mts at a rate of 20 ml/min. The perfusate was collected and dried to get the particles not adhered. The percent of bioadhesion was estimated by the ratio of amount applied to adhere MIPs. The values are given in Table 1.

3. Results and discussion

Yang et al. [21] and Cai et al. [22] have already reported a method for conversion of carboxylic acids to the related vinyl ester by using vinyl acetate as an acylating agent. In this present work, ibuprofen and naproxen reacted with vinyl acetate in the presence of mercuric acetate as a catalyst, and the related vinyl esters were collected in high yield after purification by column chromatography. The resultant FT-IR and ^{1}HNMR spectra confirmed the structure of vinyl esters and its purity.

Analysis of the MIPs by IR spectra shows that with increase of pH from 2 to 8, the composite passes into the anion form, the band at 1705 cm^{-1} (the stretching vibrations of the carboxylic group) disappears, and in its place new absorption bands appear at 1560 and 1420 cm^{-1}, which are assigned to the stretching vibrations of the carboxylate anion COO^-.

4. Drug release in vitro

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 hydrolysis of a linkage is also dependent on its distance from the polymer backbone, but the length and hydrophilicity of the spacer unit between the drug and the polymer chain can

also affect the release rate.

Release studies were carried out for semi-covalent MIPs (MIP 1-4) and drug loading matrices (MIP′ 1-4). For drug loading matrices were supposed to have a better ability in controlling drug release in comparison to NIP. The data obtained from the experiments clearly show that drug release from NIP was remarkably faster than that observed when MIP was used. In particular, it is possible to note that while in the first case the drug is completely released within five hours, for MIP samples even after 8 hours the release is not yet complete. Under these conditions the non-imprinted polymers do not have specific binding cavities in which the drug is bound with semi-covalent interactions, whereas MIP, due to its specific network structure, still retains a significant percentage of drugs. Such behavior is in accordance with results obtained from the binding experiments (Table 2). This observation supports a model of retention mechanism which assumes that the selective sites have stronger interaction with the drug than the non-selective sites [23].

It appears that the degree of hydrolysis of network polymers depends on the amount of the MAA units in copolymer and reticulated degree of cross-linking. In MIP systems as drug delivery vehicles, because high reticulated degree of the polymer, diffusion of the hydrolyzing agents in the network's polymer is reduced and the hydrolysis rate in the basis is slower.

As shown in this Figures 2 and 3, the drug release proceeds more efficiently at a higher pH (SIF). As the content of MAA in the feed monomers increased, hydrolysis rate decreased at pH 1 but increased at pH 7.4. This was because a higher MAA content in the polymer networks led to higher carboxylate anion concentration at high pH. In other words, the existence of hydrogen-bonding interactions between –COOH groups in the polymer matrix results in a complex structure within the network, and so the movement of polymeric segments is restricted. This also accounts for minimum hydrolyzing of the gel in a medium of pH 1. However, when the sample is placed in a medium of pH 7.4, the almost complete ionization of –COOH groups present within the polymer network not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged $–COO^-$ groups. These two factors ultimately result in a greater increase in the water uptake. In pH 7.4 with completed ionization and an increase in the hydrophilicity of the polymer, diffusion of the hydrolyzing agents on polymer is increased and the hydrolysis rate increased [24].

Therefore, in alkaline pH value, the polymers are easily degraded to release of drug.

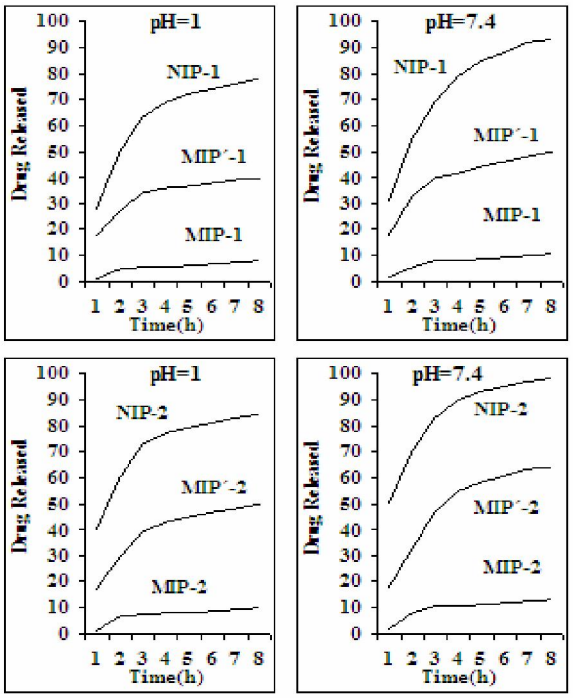

Figure 2. Release of ibuprofen from MIP, MIP′ and NIP as a function of time at 37 °C.

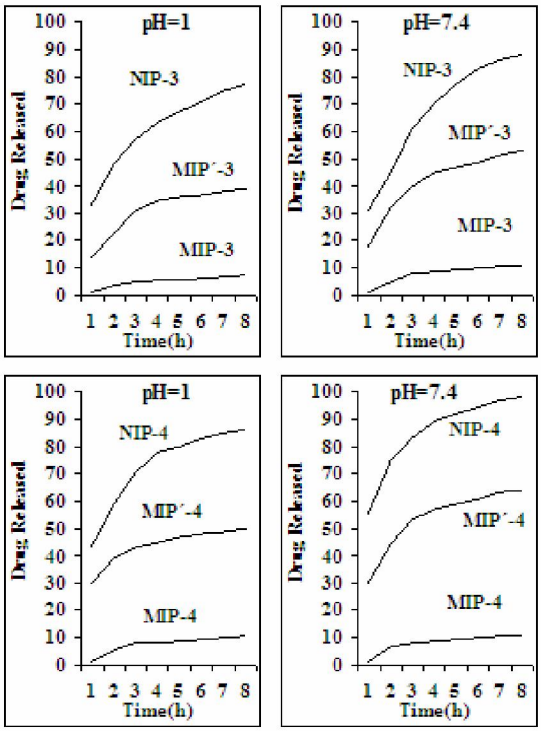

Figure 3. Release of naproxen from MIP, MIP′ and NIP as a function of time at 37 °C.

5. Conclusions

The starting point of this work was the preparation of a specific delivery system for ibuprofen and naproxen based on semi-covalent molecularly imprinted polymers synthesized using MAA as a functional monomer and EGDMA as a crossilnker in the presence of vinyl esters derivatives of ibuprofen and naproxen as templates. The particles are able to selectively re-bind the bioactive agent in aqueous media, under acidic conditions as well as under neutral conditions. The percentages of drug bound by the imprinted matrices were significantly higher than those obtained when the non imprinted ones were used. Despite, the imprinted polymers bound much more drugs than the corresponding non-imprinted ones and showed a controlled/sustained drug release, with MIPs release rate being indeed much more sustained than that obtained from NIPs.

The results obtained from the in vitro release studies indicated that these polymeric matrices are also suitable for a controlled/sustained delivery of the tested anti-inflammatory agent in biological fluids, both in gastrointestinal and in intestinal fluids. The release using the imprinted polymers cannot be easily classified according to the usual mechanisms of delivery because every matrix is highly specific for the drug used as a template; in fact, in order to obtain a matrix suitable for another drug it is necessary to synthesize a different imprinted polymer. Finally, because of their selective binding properties, the new polymeric networks reported in this paper represent a promising device for the preparation of novel controlled release dosage forms.

Acknowledgments

The office of research vice chancellor Azarbaijan University of Tarbiat Moallem has supported.

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4/21/2011