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Full Paper

Molecularly Imprinted Polymers for 5-Fluorouracil Release in Biological Fluids

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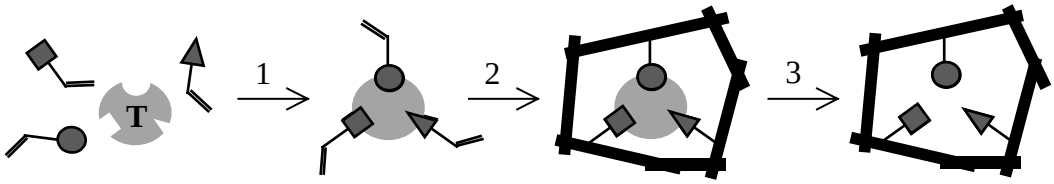
Abstract: The aim of this work was to investigate the possibility of employing Molecularly Imprinted Polymers (MIPs) as a controlled release device for 5-fluorouracil (5-FU) in biological fluids, especially gastrointestinal ones, compared to Non Imprinted Polymers (NIPs). MIPs were synthesized using methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EGDMA) as crosslinking agent. The capacity of the polymer to recognize and to bind the template selectively in both organic and aqueous media was evaluated. An *in vitro* release study was performed both in gastrointestinal and in plasma simulating fluids. The imprinted polymers bound much more 5-Fu 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. These polymers represent a potential valid system for drug delivery and this study indicates that the selective binding characteristic of molecularly imprinted polymers is promising for the preparation of novel controlled release drug dosage form.

Keywords: 5-Fluorouracil, Drug Delivery System, Molecular Imprinting, Radical Polymerization, Sustained Release.

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 mimics [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. The technique to produce MIPs, using the non-covalent approach, involves the arrangement of functional monomers around a templating ligand. This ligand is the selected target substance and it should form a prepolymerization complex with the functional monomer by non-covalent interactions such as hydrogen bonding, ionic or hydrophobic interactions [16]. The formed complex is subsequently copolymerized, *via* a radical reaction, with a suitable crosslinker. After copolymerization, the template is removed to obtain binding sites specific for the original template (Figure 1).

Figure 1. Schematic representation of the molecular imprinting process (T = Template; 1 = Assembly; 2 = Polymerization; 3 = Template Extraction).



In this work a new potential polymeric device, based on MIPs, for the sustained release of 5-fluorouracil (5-FU) (Figure 2a) is described. This drug is an anticancer agent widely used in the clinical treatment of several solid cancers such as breast, colorectal, liver and brain cancer [17].

Figure 2. a) 5-FU, b) uracil.

5-FU is quickly metabolized in the body, therefore the maintenance of high serum concentrations of this drug to improve its therapeutic activity is needed. The maintenance of these serum concentrations requires continuous administrations, but 5-FU shows severe toxic effects; and, of course, reaching and/or exceedng the toxic concentration must be avoided [18]. In the literature, numerous studies report devices for the controlled release of 5-FU. These devices are based on

polypeptides and polysaccharides and often improve the performance of this drug [19]. On the other side, no such drug delivery systems for 5-FU were obtained starting from molecularly imprinted polymers.

The only MIP prepared using 5-FU as a template was proposed for analytical studies [20]; furthermore, it must be pointed out that the functional monomer and the crosslinker used for that studied device are not compatible with physiological conditions, thus it was not suitable for pharmaceutical applications. The purpose of this study was to investigate the possibility of employing MIPs as devices for the controlled release of 5-FU in biological fluids. The delivery of this drug by molecularly imprinted polymers offers the possibility of maximising its efficacy and safety and to provide a suitable rate of delivery of the therapeutic dose, at the most appropriate site in the body, in order to prolong the duration of the pharmacological activity, to reduce the side effects and to minimize the administration frequency, thus enhancing patient compliance [21]. In this paper the preparation of the MIP is described; the matrix affinity for 5-FU and its selectivity, using uracil (Figure 2b) as an analogue, were tested. The target sites of 5-FU are all the organs of the human body [22], especially the gastrointestinal tract, therefore the release profile of this drug was evaluated both in gastrointestinal and in plasma simulating fluids. Considerable differences in the release characteristics between imprinted and non imprinted polymers (NIPs) have been observed.

Results and Discussion

Rationale of MIP preparation and choice

Three kinds of MIPs using different molecular ratios among template, functional monomer and crosslinker were synthesized (Table 1).

Table 1. Polymer preparation.

Polymer	5 – FU (mmol)	MAA (mmol)	EGDMA (mmol)	AIBN (mg)
MIP-1	2.0	8.0	32.0	103
MIP-2	2.0	8.0	40.0	103
MIP-3	2.0	16.0	20.0	103

The choice of using DMF as porogen was dictated by the low solubility of 5-FU in organic solvents such as acetonitrile, chloroform and methanol. By increasing the solubility and the amount of the template in the pre-polymerization mixture it should be possible to obtain a material with more binding sites and consequently to have a better performance in the recognition profile of the matrices.

As reported in Table 1, MIP-1 was prepared using the typical molar ratio (1:4) of the usual molecularly imprinted matrices [15]. Different molar ratios were used for the other two matrices: for MIP-2 the MAA/EGDMA ratio was 1:5 while, in order to maximise the binding sites into the matrix, the MAA amount was increased for MIP-3, leading to a ratio of 4:5 (Table 1). After the grinding, sieving and suspending processes, the obtained materials were characterized by a dimensional size in the range of 20-63 μm.

MIP and NIP recognition properties

Evaluation of the capacity of the matrices to recognize and bind the template was performed in acetonitrile (organic medium), in water at pH = 1.0 and in a buffered water solution at pH = 7.4. In Tables 2 and 3, the percentage of 5-FU bound by each matrix after 24 hours is reported.

Table 2. Percentage of bound 5-FU by the imprinted and non-imprinted polymers after 24 hours in organic medium (i.e., acetonitrile).

Matrix	5-CFU bound	Matrix	5-CFU bound
MIP-1	5 ± 3 %	**NIP-1**	5 ± 3%
MIP-2	10 ± 3%	**NIP-2**	7 ± 3%
MIP-3	30 ± 3%	**NIP-3**	10 ± 3%

Table 3. Percentage of bound 5-FU by the imprinted and non-imprinted polymers after 24 hours in aqueous media (i.e., pH=1.0 and 7.4)

Matrix	5-CFU bound pH = 1.0	5-CFU bound pH = 7.4	Matrix	5-CFU bound pH = 1.0	5-CFU bound pH = 7.4
MIP-1	15 ± 3 %	10 ± 3 %	**NIP-1**	15 ± 3 %	10 ± 3 %
MIP-2	19 ± 3 %	12 ± 3 %	**NIP-2**	20 ± 3 %	8 ± 3 %
MIP-3	35 ± 3 %	27 ± 3 %	**NIP-3**	6 ± 3 %	9 ± 3 %

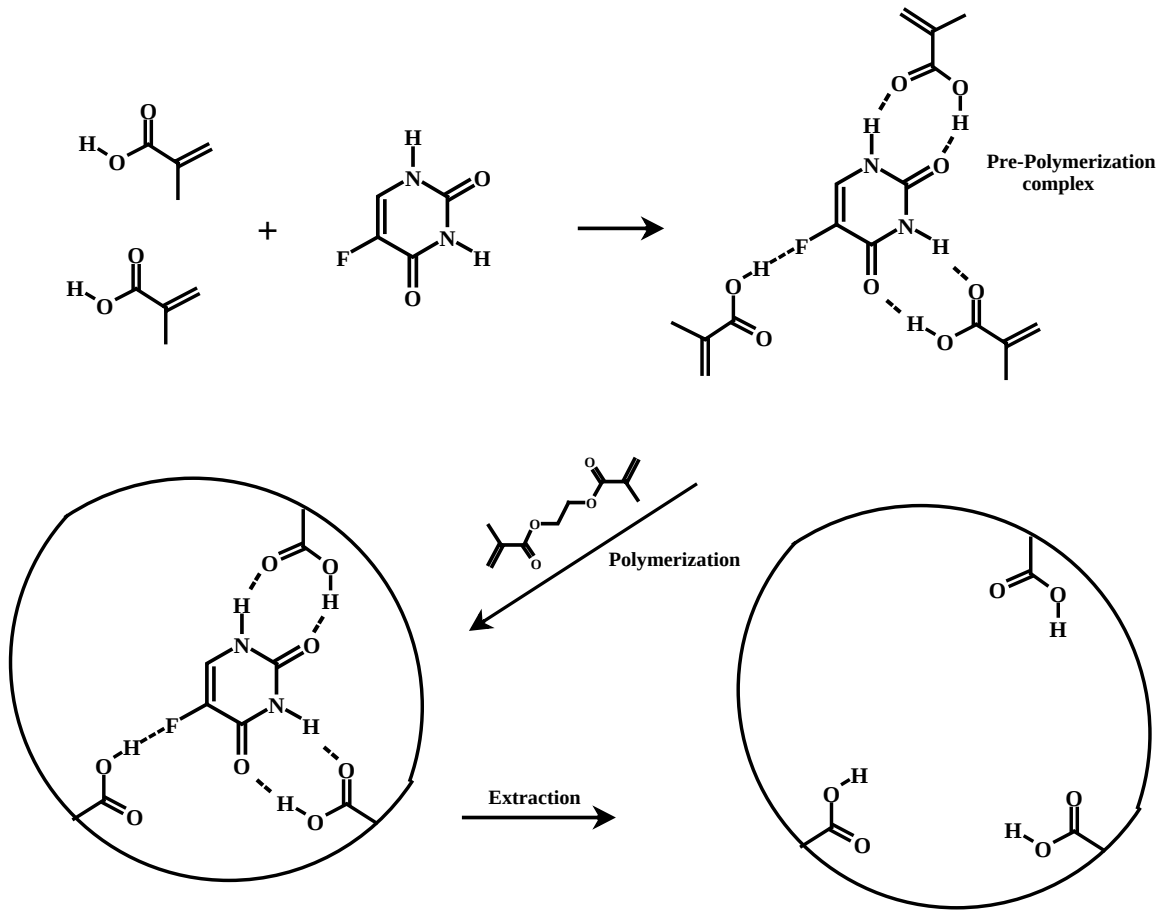
As it may be noted, the most powerful polymeric network was the MIP-3 one, and the binding ability was comparable both in organic and in aqueous media, at pH 1.0 as well as at pH 7.4. The differences can be related to the different interactions of the template with the solvents and to the ionization of carboxylic groups, respectively. In order to evaluate the imprinting effect, the binding selectivity of MIP-3 was tested by performing the same experiments using a molecule quite similar to 5-FU. For this purpose uracil, which that differs from 5-FU only in substituent at position 5 of the ring (Figures 2a-b) was used. The binding percentages of uracil by MIP-3 and NIP-3 are reported in Table 4: as it is possible to note that the polymers practically do not interact with this molecule, and the obtained data are quite similar in the different environmental conditions.

Table 4. Percentage of bound Uracil by the imprinted and non-imprinted polymers after 24 hours in organic (i.e., acetonitrile) aqueous media (i.e., pH=1.0 and 7.4).

Matrix	Uracil bound (organic solvent)	Uracil bound pH = 1.0	Uracil bound pH = 7.4
MIP-3	16 ± 3 %	11% ± 3 %	9 ± 3 %
NIP-3	15 ± 3 %	10 ± 3 %	10 ± 3 %

Anyhow, binding is always significantly lower than that obtained with 5-FU under the various conditions tested. In 5-FU, indeed, fluorine plays an important role in the formation of the binding sites because of its interaction with the functional monomer (Figure 3). This is one of the most important factors which lead to the selective interaction between the polymeric network and 5-FU.

Figure 3. Schematic representation of 5-FU imprinting process.



Drug release in vitro

MIP-3 matrices, which are the most effective in template recognition, were tested *in vitro* as devices for 5-FU delivery and their possible targeting ability to colon and plasma. Release studies were carried out in two parallel experiments, both at 37 °C. The first one was performed at pH 1.0 (simulated gastric fluid) for two hours (gastric tract) and then at pH 6.8 (simulated intestinal fluid) using the pH change method. In the second experiment, the release profile was evaluated at pH 7.4 (simulated biological fluids). MIP-3 were supposed to have a better ability in controlling drug (5-FU) release in comparison to NIP-3. The release profile of 5-FU from MIP-3 confirmed this hypothesis, both in gastrointestinal and in physiological media (Figures 4 and 5).

Figure 4. Release profile of 5-FU from MIP-3 and NIP-3 in gastrointestinal simulating fluids.

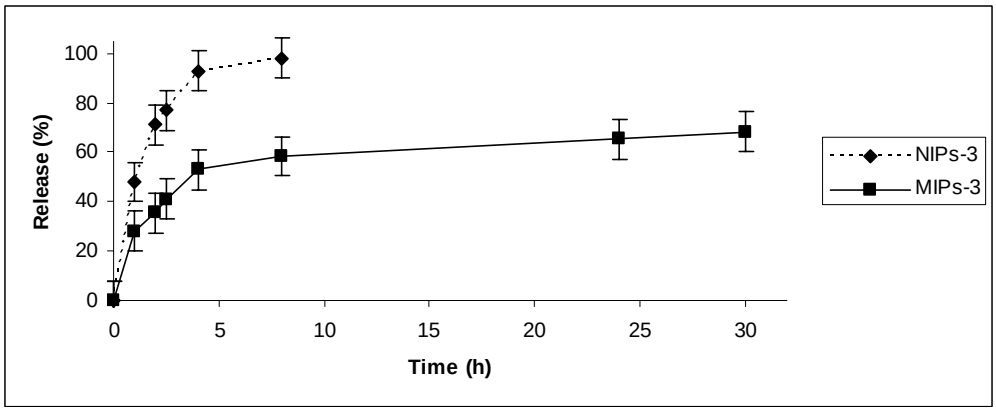
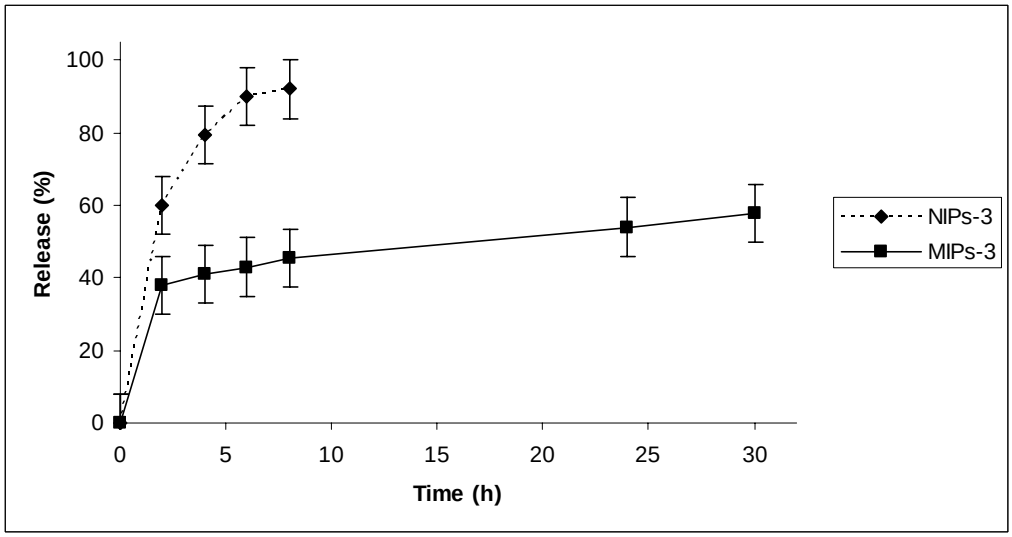


Figure 5: Release profile of 5-FU from MIP-3 and NIP-3 in plasma simulating fluids.



The data obtained from the experiments simulating the gastrointestinal fluids (Figure 4) clearly show that drug release from NIP-3 was remarkably faster than that observed when MIP-3 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-3 samples even after 30 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 non-covalent interactions, whereas MIP-3, due to its specific network structure, still retains a significant percentage of drug. Such behaviour is in accordance with results obtained from the binding experiments (Table 3). 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].

The experimental data at pH 7.4 also revealed a better controlled release of drug from the MIP-3 sample than that obtained from NIP-3. The explanation of the drug retention is the same of that proposed for the experiments carried out in simulating gastrointestinal fluids, and the complete release in MIP-3 is again not complete even after 30 hours. In order to evaluate selectivity of MIP-3, Uracil release experiments were also performed. The data obtained from the experiments simulating both the

gastrointestinal and the physiological fluids showed that uracil release from MIP-3 was remarkably faster than that obtained when 5-FU was used; in fact, uracil was completely released in 2 hours (data not shown). The release profile of uracil, moreover, was quite similar when the model drug was entrapped in MIP-3 and in NIP-3, thus confirming the non-specific interactions between the polymeric matrices and uracil. The binding experiments performed with uracil are in accordance with the release data that clearly show how MIP-3 is very selective in binding 5-FU and controlling its release in biological fluids.

Conclusions

The starting point of this work was the preparation of a specific delivery system for 5-FU based on molecularly imprinted polymers synthesized using MAA as a functional monomer and EGDMA as a crossilnker in the presence of 5-fluorouracil as a template. The particles are able to selectively re-bind the bioactive agent both in organic and in aqueous media, under acidic conditions as well as under neutral conditions. The percentages of 5-FU bound by the imprinted matrices were significantly higher than those obtained when the non imprinted ones were used. Furthermore, MIPs pratically do not interact with uracil, a molecule quite similar to 5-FU that was used to evaluate the specificity of the recognition properties of the matrices. The results obtained form the *in vitro* release studies indicated that these polymeric matrices are also suitable for a controlled/sustained delivery of the tested anticancer agent in biological fluids, both in gastrointestinal and in plasma simulating 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.

Experimental

General

Ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), 2,2'-azoisobutyronitrile (AIBN) and 5-FU and uracil were obtained from Aldrich. All solvents were reagent grade or HPLC-grade and used without further purification and they were provided by Fluka Chemie.

Synthesis of 5-FU molecularly imprinted polymer

The MIP stationary phase was prepared by bulk polymerization. Methacrylic acid was used as the functional monomer to prepare the MIP by the non-covalent imprinting method. Briefly, template 5-FU, methacrylic acid, EGDMA and AIBN were dissolved in dimethylformamide (DMF, 4 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 68 °C for 24 h. The resultant bulk rigid polymer systems were crushed, grounded into powder and sieved through a 63 μm stainless steel sieve.

The fine particles were removed by repeated precipitation from acetone (5 x 30 min) [24]. The resultant MIP materials were Soxhlet extracted with an acetic acid-methanol mixture (1:1, 200 mL) for at least 48 h, followed by methanol (200 mL) for another 48 h. The extracted MIP materials were dried overnight in an oven at 60 °C. The washed MIP materials were checked to be free of 5-FU and any other compound by HPLC analysis. Reference NIPs matrices (acting as a control) were prepared under the same conditions without using the template. The formulations used for the preparation of the different matrices (MIP-1, MIP-2, MIP-3) are shown in Table 1.

Binding experiments

The binding experiments were performed, at room temperature, both in organic (acetonitrile) and in aqueous media (water solution pH 1.0 and phosphate buffer pH 7.4). the sieved MIP and NIP particles (50 mg) were mixed with 5-FU solutions (0.3 mM, 5 mL) in a 10 mL conical centrifugation tube and sealed. The tubes were oscillated by a wrist action shaker (Burrell Scientific) in a water bath for 24 h. Then the mixture was centrifuged for 10 min and the 5-FU concentration in the liquid phase was measured by HPLC. The amount of 5-FU bound to the polymer matrix was obtained by comparing the drug concentration in the MIP samples and in the NIP ones. The same experiments were performed using uracil solutions. Experiments were repeated five times.

Drug Loading by the Soaking Procedure

Polymeric matrix (2.0 g) was immersed in a 5-FU solution in acetonitrile (20 mL, 5.5 mM) and soaked for 3 days 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. The same experiments were performed using uracil solution.

In vitro release studies

Release studies were carried out using the dissolution method described in the USP XXIV (apparatus 1-basket stirring element). Two parallel experiments for each matrix were performed. In the first one, MIP and NIP particles (30 mg) loaded with 5-FU were dispersed in flasks containing 10 mM phosphate buffer solution (pH 7.4 simulating the biological fluids, 10 mL), while in the second one, the samples were dispersed in flasks containing 0.1 N HCl (pH 1.0, simulating the gastric fluid, 10 mL) and maintained at 37 ± 0.5 °C in a water bath for 2 h under magnetic stirring (50 rpm). Disodium hydrogen phosphate (0.4 M, 5 mL) was then added to the samples to adjust the pH value to 6.8 (simulated intestinal fluid). These conditions were maintained throughout the experiment. Samples (2 mL) were drawn from the dissolution medium at appropriate time intervals to determine the amount of drug released. A HPLC method was employed. The amount of 5-FU released from six samples of each formulation was used to characterize drug release. The same experiments were performed using particles loaded with uracil.Experiments were repeated five times.

HPLC Analysis

A Jasco BIP-I pump and Jasco UVDEC-100-V detector set at 266 nm [19] were used. A 250 × 4 mmC-18 Hibar® column, 10 mm particle size (Merck, Darmstadt, Germany) was employed. The mobile phase was methanol/phosphate buffer 5mM, pH 6.8 (9/1, v/v) and the flow rate was 0.5 mL/min.

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Sample Availability: Contact the authors.