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Research Paper

Molecularly imprinted polymers as drug delivery systems for the sustained release of glycyrrhizic acid

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Abstract

Objectives The aim was to synthesize molecularly imprinted polymers (MIPs) with high recognition properties towards glycyrrhizic acid and to evaluate the performance of these materials to act as base excipients in glycyrrhizic acid sustained release in gastrointestinal simulating fluids.

Methods MIPs were synthesized using methacrylic acid (MAA) as acidic, 2-(dimethylamino)ethyl methacrylate (DMAEMA) as basic, and 2-hydroxyethylmethacrylate (HEMA) as neutral functional monomers, while ethylene glycol dimethacrylate (EGDMA) was chosen as a crosslinking agent. The imprinting effect was evaluated by binding experiments using glycyrrhizic acid and glycyrrhetic acid (analogue molecule) solutions and in-vitro release studies were performed in gastrointestinal simulating fluids.

Key findings Good recognition and selectivity properties were found in all the synthesized materials in both ethanol and ethanol–water mixture. The release from non-imprinted polymers was indeed higher at acidic pH, while a slower release was observed in MIPs' case, because of the presence of imprinted cavities in the polymeric structure. The stronger capacity of MAA to interact by hydrogen bonds with the template makes MAA-containing MIPs the most effective materials in both rebinding and release experiments.

Conclusions The release tests confirm the applicability of imprinted polymer for glycyrrhizic acid sustained release in gastrointestinal simulating fluids.

Keywords drug delivery; glycyrrhizic acid; molecularly imprinted polymers

Introduction

In recent years, several studies have focused on the development of drug delivery systems (DDS) based on polymeric materials and the importance of this field of research is growing as ever more complex drugs and biopharmaceuticals are being developed, many of which cannot be administered without a controlled dosage system. An ideal DDS is required to ensure the drug release occurs at the right site, in the right dose and for the required time.[1]

Among the different kind of polymeric materials applied in this research field (erodible devices, implants, etc.),[2] particular attention has been devoted to molecularly imprinted polymers (MIPs).[3] MIPs are extensively crosslinked polymeric materials especially designed to offer valuable molecular recognition properties towards analytes of interest.[4] These properties are related to specific recognition sites within the polymer matrix, which are complementary to the analyte molecule regarding the shape and positioning of functional groups.[5]

This selectivity arises from the synthetic procedure followed to prepare the MIP. In this procedure, a template molecule is linked, by covalent or non-covalent forces, to monomers containing specific functional groups.[6] These interactions are responsible for the subsequent formation of specific binding sites in the MIP structure. Once the polymer is obtained, in fact, the template is removed from the matrix; consequently, the template leaves its imprint in the polymer structure.[7]

This molecular recognition characteristic has proved very attractive in many different fields, such as sensors,[8] capillary electrochromatography,[9] enantiomeric separation,[10] organic synthesis,[11] solid-phase extraction (SPE),[12] catalysis[13] and enzyme mimics,[14] and as base excipients for controlled release devices for several drugs.[15]

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In this work, the applicability of MIPs as a drug delivery device was proved by using glycyrrhizic acid as model template.

Glycyrrhizic acid, also known as (3β,20β)-20-carboxy-11-oxo-30-norlean-12-en-3-yl-2-*O*-β-17-glucopyranuronosyl-α-D-glucopyranosiduronic acid, is a triterpenoid saponin extracted from the plant *Glycyrrhiza glabra*.[16] This natural compound has been demonstrated to possess antiviral, immunomodulatory, anti-inflammatory and anti-hepatotoxic effects and is currently suggested as a possible chemopreventive drug.[17] It acts by inhibition of 11β-hydroxysteroid dehydrogenase which catalyses the reversible conversion of cortisol to cortisone. Inhibition of 11β-hydroxysteroid dehydrogenase leads to increased cortisol levels in the kidney and in other mineralocorticoid-selective tissues.[18]

A controlled drug delivery system offers the possibility of maximizing a drug's efficacy and safety and providing 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.[15,19]

Glycyrrhizic acid imprinted polymers were synthesized by the non-covalent imprinting approach, and a screening of the functional monomers (acidic, neutral or basic) was performed to select the best materials to be tested as releasing device for this biological active compound. All the synthesized polymers were characterized in terms of imprinting efficiency, and the release studies were carried out in gastrointestinal simulating fluids.

Materials and Methods

Reagents and standards

Ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), 2-hydroxyethylmethacrylate (HEMA), 2,2′-azoisobutyronitrile (AIBN), methacrylic acid (MAA), glycyrrhizic acid and glycyrrhetic acid were obtained from Sigma-Aldrich (Sigma Chemical Co., St Louis, USA). All solvents were reagent grade or HPLC grade and used without further purification and were provided by Fluka Chemika-Biochemika (Buchs, Switzerland). Monomers were purified before use by distillation under reduced pressure.

Synthesis of glycyrrhizic acid imprinted polymers

Three different glycyrrhizic acid imprinted polymers (MIPs) were prepared using MAA, DMAEMA or HEMA as functional monomers. Briefly, 0.5 mmol of template glycyrrhizic acid and 16.0 mmol of functional monomers were dissolved in 5 ml of anhydrous dimethyl formamide in a thick-walled glass tube, and then 25.0 mmol of EGDMA and 0.100 g of AIBN were added. The tube was sparged with nitrogen, sonicated for 10 min and thermo-polymerized under a nitrogen atmosphere for 24 h at 60°C. The resultant bulk rigid polymers were crushed, ground into powder and sieved through a 63-nm stainless steel sieve. The sieved MIP materials were collected and the very fine powder, suspended in the supernatant solution (acetone), was discarded. The resultant MIP materials were soxhlet-extracted with 200 ml ethanol–acetic acid (8 : 2 v/v) for at least 48 h, followed by 200 ml of ethanol for another 48 h. The extracted MIP materials were dried in an oven at 60°C overnight. The washed MIP materials were checked to be free of glycyrrhizic acid and any other compound by HPLC analysis of the washing media.

Table 1 Polymer compositions

Polymer	Glycyrrhizic acid (mmol)	MAA (mmol)	DMAEMA (mmol)	HEMA (mmol)	EGDMA (mmol)
MIP1	0.5	16.0	0	0	25.0
NIP1	0	16.0	0	0	25.0
MIP2	0.5	0	16.0	0	25.0
NIP2	0	0	16.0	0	25.0
MIP3	0.5	0	0	16.0	25.0
NIP3	0	0	0	16.0	25.0

Non-imprinted polymers (NIPs; to act as a control) were also prepared whereby polymerization was carried out in the absence of glycyrrhizic acid.

The formulations used for the preparation of the different matrices are shown in Table 1.

Binding experiments

The binding efficiency of polymeric matrices towards glycyrrhizic acid was evaluated by performing rebinding experiments in ethanol and in ethanol–water (6 : 4 v/v). Briefly, 50 mg of polymer particles were mixed with 1 ml glycyrrhizic acid solution (0.2 mM) in a 1 ml Eppendorf tube and sealed. The Eppendorf tube was oscillated by a wrist action shaker (Burrell Scientific) in a water bath at 37 ± 0.5°C for 24 h. Then the mixture was centrifuged for 10 min (10 000 rev/min) in an ALC microcentrifugette 4214 and the glycyrrhizic acid concentration in the liquid phase was measured by HPLC. The amount of glycyrrhizic acid bound to the polymer was obtained by comparing its concentration in the MIP samples to the NIP samples.

The same experiments were performed using glycyrrhetic acid solutions.

Specific imprinting parameters α and ε were determined. The α value corresponds to the ratio between the amount (%) of analyte (glycyrrhizic acid or glycyrrhetic acid) bound by the MIP and NIP, while ε is the ratio between the amount (%) of glycyrrhizic acid and glycyrrhetic acid bound by the MIP.

Drug loading by the soaking procedure

Polymeric matrix (2.0 g) was immersed in a glycyrrhizic acid solution in ethanol (40 ml, 0.2 mM) and soaked for 24 h at room temperature. During this time, the mixture was continuously stirred and then the solvent was removed under vacuum. Finally the powder was dried under vacuum overnight at 40°C. The same experiments were performed using glycyrrhetic acid solution (glycyrrhizic acid analogue molecule).

In-vitro release studies

Release studies were carried out using the dissolution method described in the USP XXIV (apparatus 1 basket stirring

element). To mimic the pH in the digestive tract, 0.1 N HCl (pH 1.0) was used as a stimulated gastric fluid, and after 2 h disodium hydrogen phosphate (0.4 M) was added to adjust the pH value to 6.8 to simulate intestinal fluid.

The experiments were performed as follows: 30 mg of MIP and NIP particles loaded with glycyrrhizic acid were dispersed in flasks containing 10 ml of 0.1 N HCl and maintained at 37 ± 0.5°C in a water bath for 2 h under magnetic stirring (50 rev/min). Disodium hydrogen phosphate (0.4 M, 5 ml) was then added to the samples. These conditions were maintained throughout the experiment. To characterize the drug release, 2-ml samples were drawn from the dissolution medium at designated time intervals and the same volume of simulated fluid was supplemented. Glycyrrhizic acid was determined by HPLC analysis and the amount of glycyrrhizic acid released from five samples of each formulation was used to characterize the drug release profile. The percentage of glycyrrhizic acid released was calculated considering 100% to be the glycyrrhizic acid content in polymeric samples after the drying procedure.

HPLC analysis

The liquid chromatography was performed using a Jasco Model (Tokyo, Japan) consisting of a Jasco BIP-I pump and Jasco UVDEC-100-V detector set at 254 nm. A Tracer Excel 120 ODS-A column particle size 5 μm, 15 × 0.4 cm (Barcelona, Spain) was employed. The mobile phase consisted of methanol–acetonitrile–water–glacial acetic acid (35 : 35 : 30 : 1, by volume).[20]

Statistical analysis

All of the experiments were done in triplicate and data expressed as means ± SD. One-way analysis of variance was performed to assess the significance of the differences among data in Table 2. Tukey–Kramer post-test was used to compare the means of different treatment data. $P < 0.05$ was considered statistically significant.

Results and Discussion

Synthesis and characterization of imprinted polymers

For the synthesis of glycyrrhizic acid imprinted polymers, we chose the non-covalent approach[21] because of the simplicity of the process and because of the wide range of functional monomers, acidic, basic or neutral, that can be used[22]. In this approach, non-covalent forces, such as H-bonding, ion-pairing and dipole–dipole interactions, are involved in both the pre-polymerization process and the rebinding step.[23] This method is the most widely used to synthesize MIPs because of the fast kinetics of binding and the absence of toxic reaction products.[24,25]

This approach is preferred over the covalent one because in physiological application, especially in the field of drug delivery, the latter approach is not the most appropriate as there are only a limited number of readily reversible covalent linkages that are known. In addition, the cleavage of the monomer template complex can lead to the formation of toxic species with possible serious safety issues.[26,27]

The first parameter to be considered in the synthesis of MIPs is the choice of a suitable functional monomer, and for this, in our study, a screening of different functional monomers was performed. Three different MIPs were synthesized employing MAA (MIP1 and NIP1) as acidic, DMAEMA (MIP2 and NIP2) as basic and HEMA (MIP3 and NIP3) as neutral functional monomers (Table 1). To evaluate the effect of the different monomers on the recognition properties of the resulting materials, all the polymeric matrices were prepared using the same molar ratio between template, functional monomer and crosslinker, which is the ratio commonly used in research work involving MIPs.[12]

The imprinting effect of the synthesized materials was evaluated by binding experiments in which amounts of polymeric particles were incubated with a 0.2 mM glycyrrhizic acid solution for 24 h in water and in ethanol–water (6 : 4 v/v). The obtained results are shown in Table 2, and a good imprinting efficiency was observed for all the matrices as shown by the higher amount of template bound by the imprinted cavities in the MIP structure. NIPs, because of the absence of these cavities with high chemical and spatial complementarity to the template, show lower interactions with the template. To evaluate the cross-reactivity of imprinted polymers towards glycyrrhizic acid analogue molecules, the same binding experiments were performed using glycyrrhetic acid, which differs from glycyrrhizic acid by the absence of the two sugar moieties (Figure 1).

The imprinting effect was characterized by the introduction of two specific parameters, α and ε, which are reported

Table 2 Percentage of bound analytes after 24 h incubation in ethanol and ethanol–water (6 : 4 v/v) mixture with glycyrrhizic acid and glycyrrhetic acid concentration 0.2 mM

Polymer	% Bound glycyrrhizic acid		% Bound glycyrrhetic acid		α_{GL}		α_{GA}		ε	
	EtOH	EtOH–H_2O	EtOH	EtOH–H_2O	EtOH	EtOH–H_2O	EtOH	EtOH–H_2O	EtOH	EtOH–H_2O
MIP1	40 ± 1.3	38 ± 0.9	18 ± 1.1	32 ± 0.9	3.07	6.33	0.94	0.97	2.22	1.25
NIP1	13 ± 0.6	6 ± 0.7	19 ± 0.6	33 ± 0.6						
MIP2	73 ± 0.8	90 ± 1.1	1 ± 0.5	16 ± 1.4	4.86	1.50	1.00	1.13	73.00	4.56
NIP2	15 ± 1.0	60 ± 1.3	1 ± 0.8	14 ± 1.2						
MIP3	27 ± 1.4	17 ± 1.2	20 ± 1.2	25 ± 0.9	2.45	5.66	1.05	1.04	1.35	1.08
NIP3	11 ± 0.8	3 ± 0.9	19 ± 0.9	24 ± 0.4						

Data are shown as means ± SD.

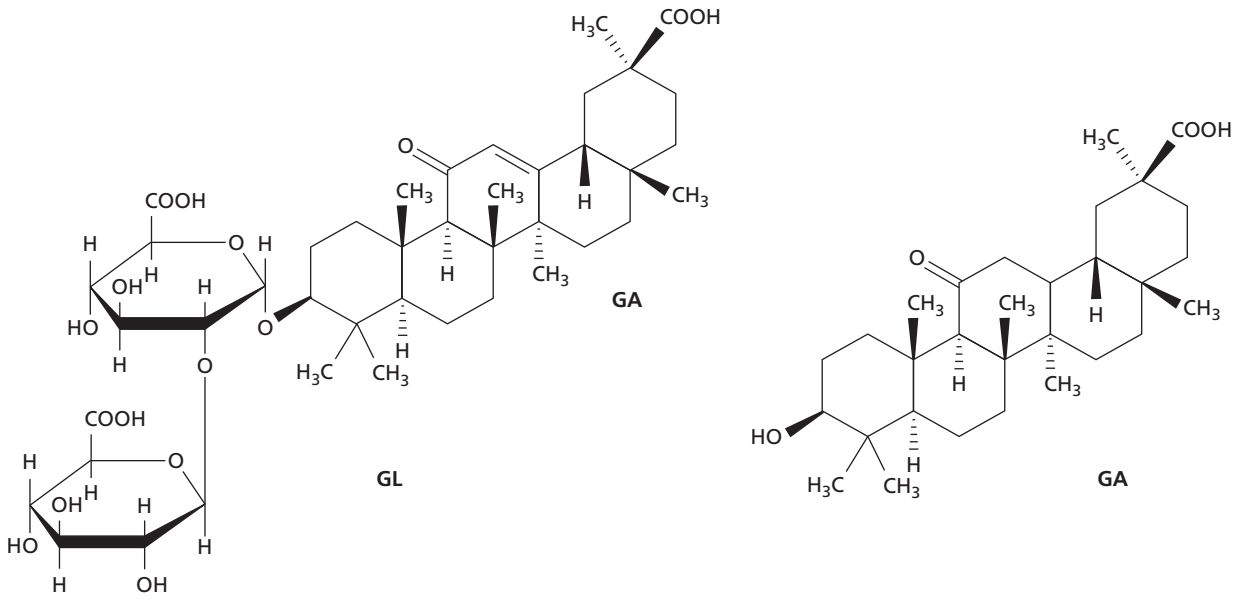

Figure 1 Chemical structures of glycyrrhizic acid (GL) and glycyrrhetic acid (GA)

in Table 2. The α value is a quantitative determination of the recognition properties of the synthesized material and is determined as the ratio between the amount (%) of analyte (glycyrrhizic acid or glycyrrhetic acid) bound by MIP and NIP,[28] while the ε value, which is a quantitative measure of the imparted selectivity within the imprinted microparticles, is the ratio between the amount (%) of glycyrrhizic acid and glycyrrhetic acid bound by MIP.

In this work, for all the polymers, high α values for glycyrrhizic acid (α_{GL}) were recorded in all the tested environments, proving the specificity of the interaction between the template and the functional groups on the polymeric microparticles. This specificity was confirmed by the recorded values for glycyrrhetic acid (α_{GA}), which were lower than the corresponding α_{GL} values.

In addition, all the obtained ε values were higher than 1.00, confirming that the imprinted cavities are characterized by stronger interaction with the template around which they are formed and lower affinity for the analogue.

The three synthesized polymers showed different behaviour in ethanol and in ethanol–water mixture. Polymers from series 1 and 3, which are characterized by acidic and neutral functional monomer, respectively, bound more analytes in ethanol than in ethanol–water, while the opposite behaviour was observed with polymers from series 2. This effect is ascribable to the presence of a basic functional monomer in MIP2 and NIP2, which strongly interacts with the carboxylic functionalities on glycyrrhizic acid and glycyrrhetic acid in water media, as proved by the higher amount of glycyrrhizic acid and glycyrrhetic acid (%) bound by these polymers. In this case, high non-specific hydrophobic interaction between polymeric matrices and analytes was observed (Table 2). Comparing MIP1 and MIP3, a higher imprinting effect was recorded from MIP1, as a result of the higher capacity of MAA to form hydrogen bonds compared with HEMA.

In-vitro release studies

After the characterization of the synthesized MIPs in terms of imprinting effect, their applicability as devices for the controlled release of glycyrrhizic acid in gastrointestinal simulated fluids was tested. Our hypothesis was that MIPs are better able to control drug release than the corresponding NIPs because of the presence of specific imprinted cavities in the polymeric structure, which are able to slowly release the template.

For the development of such a type of drug delivery system, the most promising polymer is MIP1, as shown by the highest α_{GL} value recorded in water media; MIP3 was also employed for the development of the release studies. MIP2 was not tested further because of the high non-specific hydrophobically driven interaction between polymeric matrices and template, as shown by the higher value of bound glycyrrhizic acid (%) by NIP2 and by the low α_{GL} value recorded in water media (only 1.50, while the other α_{GL} values were 6.33 and 5.00 for MIP1 and MIP3, respectively).

In-vitro release studies were performed by immersing samples of the microparticles loaded with glycyrrhizic acid at pH 1.0 (simulated gastric fluid) for 2 h and then at pH 6.8 (simulated intestinal fluid) using the pH change method.[28]

For both the tested polymers, the experimental data confirmed that MIPs are more effective in controlling the template release because of the presence of the specific functional groups in the imprinted cavities which strongly interact with glycyrrhizic acid.

By comparing the release profile (Figures 2 and 3 for MIP1 and MIP3, respectively), a better control in the drug release was observed with MIP1 because, as reported in the characterization of the imprinting efficiency, MAA is much better able to form interactions with the template than HEMA.

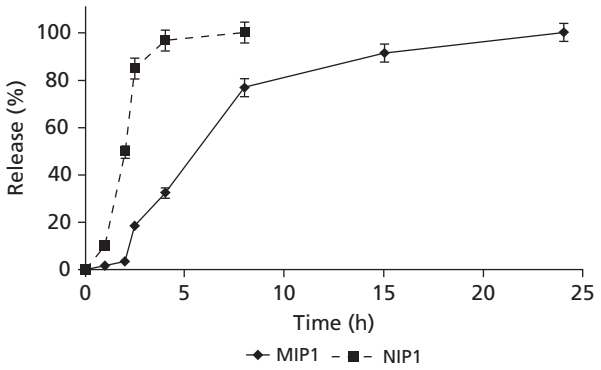

Figure 2 Gastrointestinal release profile of glycyrrhizic acid from MIP1 and NIP1

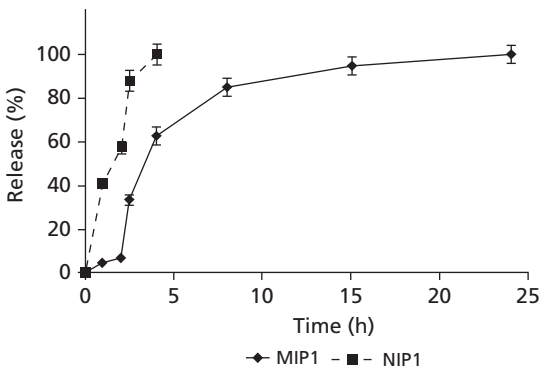

Figure 3 Gastrointestinal release profile of glycyrrhizic acid from MIP3 and NIP3

As shown by the release profiles at acidic pH, the drug was highly released within 2 h for both NIP1 and NIP3 (the amount of glycyrrhizic acid released was 50% and 58%, respectively), because the interaction between polymeric matrices and glycyrrhizic acid is ascribable to non-specific interaction, while at this time, only 19% and 34% of glycyrrhizic acid was released from MIP1 and MIP3 samples, respectively (Figures 2 and 3).

After the pH change, glycyrrhizic acid release from MIPs continued and the release was completed in almost 15 h for both MIP1 and MIP3, but the release was slower for MIP1. The acid groups of the selective sites have, indeed, stronger interaction with the template than the alcoholic ones.

Considering the release from polymers of series 1, at low pH (1.0) values the carboxylic groups are not ionized and so there is a good interaction with the template. These results might help to understand also the behaviour of these matrices when the pH increases to simulate the intestinal fluid. At pH 6.8 the diffusion rate of the buffer, on the polymeric surface of NIP is fast; the carboxylic groups of both the polymer and the template are ionized, and the drug is rapidly released. Instead, with MIPs, the diffusion rate of the buffer into specific cavities of imprinted polymers is slower and the functional groups are ionized more slowly, obtaining a good control of release.

In polymers of series 3, the same effect was observed but the ionization process involves only the functionalities on the template molecule, and the faster release after the pH change is ascribable to the lower efficiency of the alcoholic functionalities of HEMA in interacting with the template.

These observations show that MIP1 can be considered as a very useful polymeric device to be applied for the selective release of glycyrrhizic acid in gastrointestinal simulating fluids.

Conclusions

Three different MIPs for the selective recognition of glycyrrhizic acid were synthesized by employing acidic, neutral and basic functional monomers. All the synthesized polymers were characterized by the evaluation of the imprinting efficiency; a good imprinting effect was recorded for all the materials, even if the DMAEMA-containing matrices were characterized by a higher non-specific interaction with the template, which reduces their imprinting efficiency. The most promising matrix to be applied as a glycyrrhizic acid controlled delivery device gastrointestinally was found to be the MAA-containing MIP.

In-vitro release studies were carried out using the glycyrrhizic-acid-loaded MIPs, and the results show that MIP1 were the most effective material in controlling the release of glycyrrhizic acid over time. The release from non-imprinted polymers was almost completed in acidic pH conditions, while the release from both MIP1 and MIP3 was completed in 15 h with different kinetics of ionization referable to the stronger interaction between MAA and glycyrrhizic acid compared with HEMA.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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