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Preparation and Utilization of Microporous Molecularly Imprinted Polymer for Sustained Release of Tetracycline

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ABSTRACT: A polyacrylate tetracycline (TC) selective microporous molecularly imprinted polymer was prepared in three different porogenic solvents (chloroform, acetonitrile, and methanol) via precipitation polymerization, using methacrylic acid monomer, ethylene glycol dimethacrylate crosslinker, and TC as template. In all three solvents this method produced microporous particles in the scale range (200–400 nm), simply, quickly, cleanly, and in good yield. The effect of polarity of porogenic solvents on binding capacity was investigated. The imprinted polymer prepared in chloroform gave much higher binding capacity ($K_D = 198.6$) for TC than the polymers prepared in acetonitrile ($K_D = 133.2$) or methanol ($K_D = 104.7$). The selectivity of imprinted polymers was evaluated by rebinding other structurally similar compounds. The results clearly indicated that the imprinted acrylate polymer exhibits an excellent selectivity toward TC, and has better ability to control the release of TC than the non-imprinted polymer.© 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Tetracycline (TC) is a yellow, odorless, crystalline powder that is stable in air but exposure to strong sunlight causes it to darken. Its potency is affected in solutions of pH below 2 and is rapidly destroyed by alkali hydroxide solutions.[1] Because of many usages, TC is a good and powerful drug; therefore delivery of sufficient amount of TC in active form is so important.

Molecular imprinting is a rapidly developing technique for preparing polymeric materials that are capable of high molecular recognition. During the last years, application of these materials as affinity phase in solid-phase extraction, recognition element in sensors, stationary phase for preparative purification or separation of enantiomers, catalysts, and adsorbents for biochemical and pharmaceuticals is being actively pursued.[2–9]

Generally, when a template is mixed with a functional monomer and a crosslinker in an organic solvent, a complex is formed between the template and the functional monomer through polar interactions. Subsequent polymerization with the crosslinker fixes positions of polar groups of the functional monomer. Finally, washing the template away leaves recognition sites that are specific for the template molecules. The key point in this development is identification and optimization of main factors affecting the polymer structure and molecular recognition properties. These factors are property and concentration of functional monomer and crosslinking monomer, polymerization temperature, pressure, and solvent characteristics.[10]

It is known that the best shape for imprinted polymer particles in many applications including drug delivery is the spherical and microporous shape, and the techniques utilized are emulsion polymerization, multi-step swelling, and precipitation polymerization.

Most molecularly imprinted polymers (MIPs) have been prepared by bulk polymerization, where the polymer is ground and sieved to obtain granules. This widely used procedure generates irregularly shaped imprinted particles, and typically less than 50% of the ground polymer is recovered. In addition, during the grinding process some recognition sites are always destroyed. Precipitation polymerization is a novel straightforward technique that have been used to prepare spherical and microporous MIPs. In this method, no grinding and sieving are required. Moreover, in contrast to emulsion and multi-step swelling polymerization, no surfactant or stabilizer is used. Therefore the particles are clean and free of surfactants.[11]

Table I. The Chemical Composition Used to Prepare Tetracycline Acrylate Polymer in Different Porogenic Solvent and Different Amount of MAA, EGDMA, and Ttetracycline

Polymer	Tetracycline (mmol)	MAA (mmol)	EGDMA (mmol)	Porogenic solvent	Partition coefficient (K_D) (binding capacity)
TIP_1	0.255(1)	0.911(3.57)	3.64(14.27)	Chloroform	198.6
TIP_2				Acetonitrile	133.2
TIP_3				Methanol	104.7
TIP_4	0.456(1)	0.46(1)	2.3(5)	Chloroform	131.3
TIP_5				Acetonitrile	102.4
TIP_6				Methanol	94.2
TIP_7	0.309(1)	0.984(4.18)	1.22(3.95)	Chloroform	145.6
TIP_8				Acetonitrile	113.4
TIP_9				Methanol	89.7

The choice of solvent is critical for achieving good imprints and for successful rebinding isotherms. It would be better to use a solvent that dissolves all of the components of the prepolymerization mixture, allows for optimal template–monomer interaction, contributes to good porosity characteristics in the final MIP,[12] and not participate in polymer structure, so it must be a porogenic inert solvent. More specifically, use of a thermodynamically good porogenic solvent tends to lead to polymers with well developed pore structures and high specific surface areas. Increasing the volume of porogenic solvent increases the pore volume.[13] Furthermore, a few studies have been done to evaluate the structure of the polymer with changing polarity of the solvent.

In a previous work, Cai and Gupta[14] prepared a TC imprinted polymer by bulk polymerization method and pure methanol as solvents. But neither selectivity of the synthesized polymer toward TC compared to the other structurally similar compounds nor different polarities of solvents were investigated.

In a recent study, Jing et al.[15] prepared some polymer by precipitation polymerization. The most important were that the particles prepared in pure methanol were irregular and aggregated, and with decreasing polarity of solvent via changing the percentage of methanol from 100% to 46% by adding acetonitrile, caused particles to change from irregular to regular. But further decreasing of solvent polarity was not done.

According to these results, the aim of this work was set to the better understanding of the solvent effect on the shape and selectivity of TC imprinted polymers.

Our result firmly confirmed previous work. Further decreasing of solvent polarity, by changing from methanol-acetonitrile to pure chloroform, significantly produced polymers with much higher binding capacity and selectivity for TC.

In this study, we also evaluated rebinding two structurally similar compounds (deoxycycline and cephalexin) in comparison to TC.[16]

EXPERIMENTAL

Materials and Instruments

Methacrylic acid (MAA) from Merck (Germany) was distilled in vacuum prior to its usage, in order to remove the stabilizers. Ethylene glycol dimethacrylate (EGDMA) and 2,2-azobis isobutyronitrile (AIBN) from Merck (Germany) were of reagent grade and were used without any further purification. TC was purchased from Sigma (Switzerland). Other chemicals were of analytical grade.

A Shimadzu UV-240 double-beam spectrophotometer and a scanning electron microscopy (SEM) Joel JSMT 300 A were used.

The FTIR spectra of the polymers samples were obtained using a Shimadzu 8400S FTIR spectrometer. A total of 2% (wt/wt) of sample was mixed with dry potassium bromide (KBr), the mixture was ground into a fine powder, before being compressed to form KBr disc. Each KBr disc was scanned at 4 mm/s at a resolution of 4 cm^{-1} between 400 and 4000 cm^{-1}.

Pore size distribution and specific surface area analysis of polymer particles were measured by nitrogen adsorption–desorption using a BELSORPMini II (Japan) at 196°C before the measurement, 150 mg of the polymer particles were heated at 100°C for 2 h under vacuum. The specific surface area, pore volume, and average pore diameter of polymer particles were obtained by Brunauer–Emmett–Teller (BET) method using BELSORP analysis software.

Synthesis of MIPs

The chemical compositions used for the synthesis of tetracycline imprinted polymer (TIM) are shown in Table I. The polymerization procedure for producing the MIPs and the non-imprinted polymers (NIPs) were similar.

The principle of precipitation polymerization is based on the use of an excess of organic solvent relative to the amount of monomers present. Typically, a 2% wt/vol ratio of monomer/solvent is used, where monomer refers to both functional monomer and crosslinker.[11] The MIPs were prepared in the presence of TC as a template. The synthetic procedure for the preparation of the standard polymer TIP_1 was as follows: 0.255 mmol of template and 0.911 mmol of MAA were dissolved in 35 mL of chloroform in a 100 mL thick walled glass tube, then 3.64 mmol of EGDMA and 0.0825 mmol AIBN were added. The polymerization mixture purged with nitrogen for 10 min in

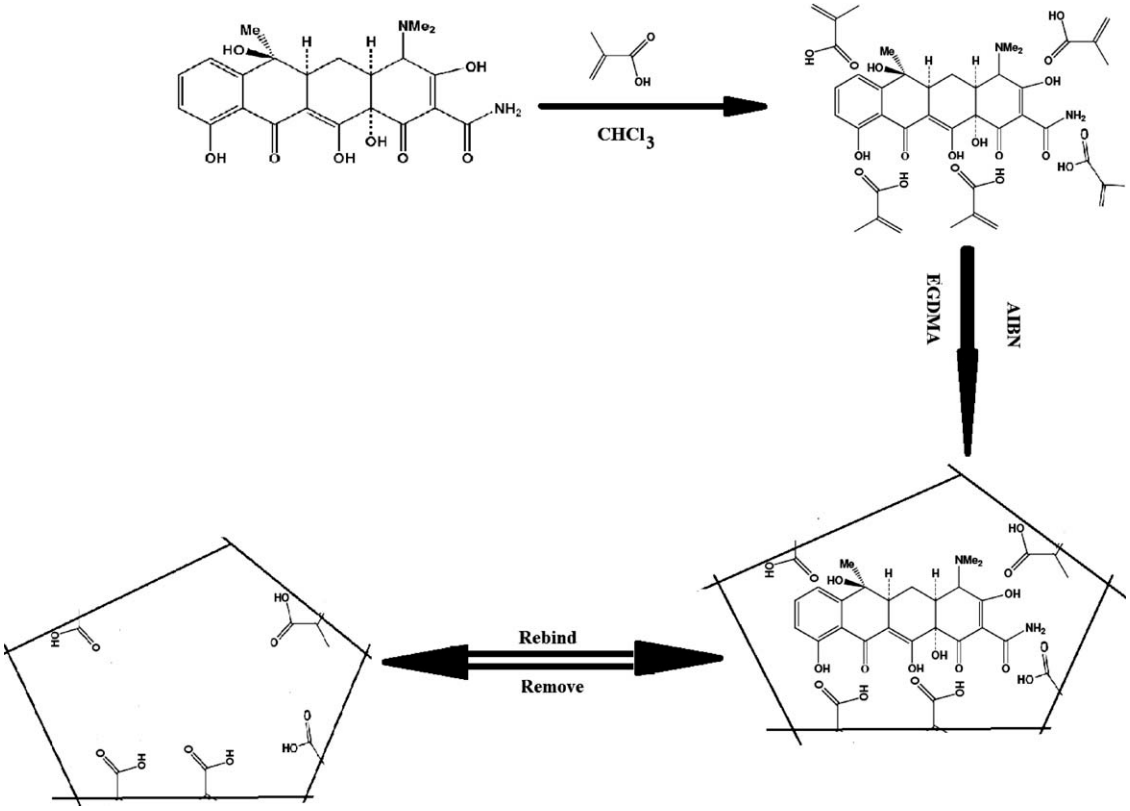

Figure 1. Schematic of representation of the MIP synthesis for TC.

a sonication water bath, maintaining a flow of nitrogen; the reaction flask was removed from the sonicating bath, sealed and placed inside a water bath at 60°C to begin the reaction. After 24 h, the polymerized particles collected by centrifugation at 15,000 rpm for 10 min. TC was extracted by washing repeatedly with methanol containing 10% acetic acid (v/v) (15 mL) (5× 1 h), followed by a final wash with the same volume of acetone. These successive centrifugation and decanting steps extracted the print molecules from the polymer. The microspheres and microporous particles of TIPs were finally dried in vacuum. The reference NIP prepared and treated in exactly the same way, except that no print molecule was used in the polymerization stage. The schematic representation of the imprinting and the removal of TC, from the imprinted polymer, are shown in Figure 1.

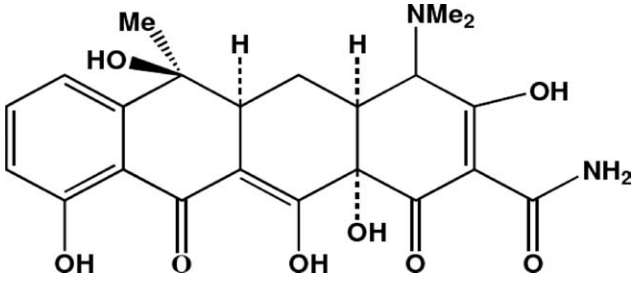

Figure 2. Chemical structure of tetracycline.

Binding Experiments

The washed polymer particles (40.0 mg) were placed in a 50 mL conical flask, and mixed with 15 mL of known concentration of TC in water. The conical flask was oscillated in a constant temperature at 25°C for 24 h. The mixture was transferred into a centrifuge tube, and centrifuged for 10 min. Concentration of the free TC in the solution was determined using a spectrophotometer at 364 nm wavelength. The amount of TC bound to the MIP and NIP were determined by the equilibrium binding method.[15] The partition coefficient (binding capacity) K_D is defined as:

$$K_D = C_p/C_s \quad (1)$$

where C_P = concentration of substrate on the polymer (in μmol/g) and C_s = concentration of substrate in the solution (in μmol/mL).

RESULT AND DISCUSSION

Polymerization Mechanism

Radical polymerization can be initiated by using thermal decomposition of radical initiators. Typically, AIBN is used. The initiation radicals formed by the decomposition attack the monomer, producing the propagating radicals. The reactions are very simple and economical. However, it is important to remove molecular oxygen from polymerization mixtures, since it traps the radical and retards (or even stops) the polymerization. In order to remove oxygen, degassing with nitrogen or argon, as

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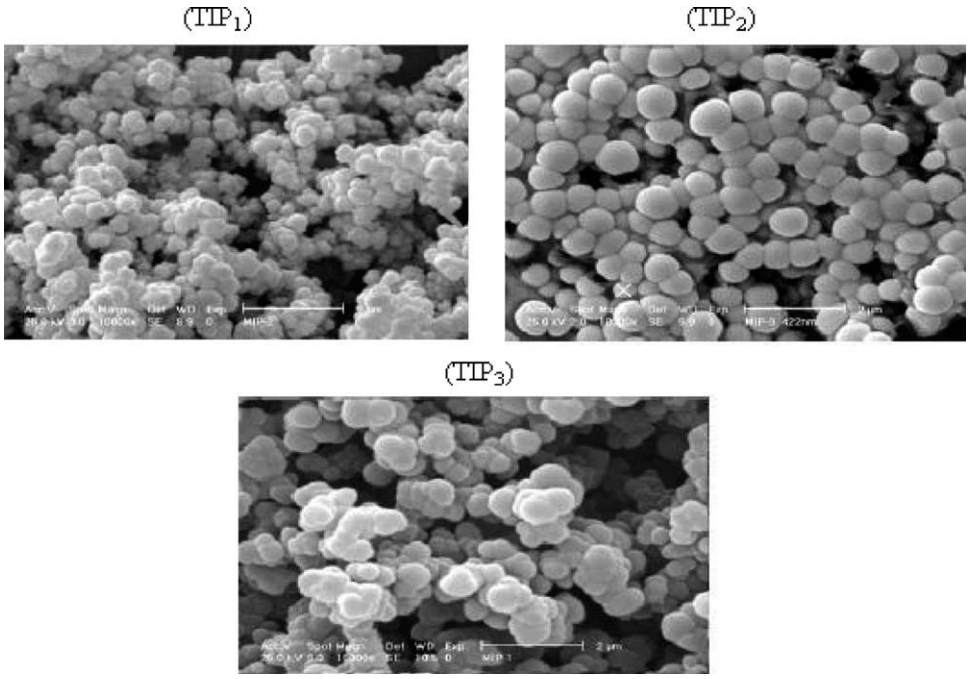

Figure 3. Photomicrographies of MIPs, TIP_1, TIP_2, and TIP_3.

well as freeze-and-thaw cycles under reduced pressure is effective.

In some cases, non-covalent adducts between functional monomer and template is too unstable to be used at higher temperatures, and the polymerization must be carried out at lower temperatures. Under these conditions, the thermal decomposition of initiator cannot be used to initiate the polymerization, and the initiators are decomposed with UV-light irradiation (photoinitiation never requires high temperatures). If the monomers themselves absorb UV light sufficiently, the polymerization is initiated even in the absence of any radical initiators.

Morphologies of MIP

Three different ratios of monomer, crosslinker, and template were selected for the synthesis of TIP in different porogenic solvents which is represented in Table I.

SEM images were obtained to characterize the structure morphologies of MIP in three different porogenic solvents (Figure 3). The surface image of the MIP in chloroform is presented in Figure 3(a), which shows smaller particle size rather than other MIPs. According to the SEM images, the solvent plays an important role in the morphology of MIP. With changing polarity of the solvent, we can prepare smaller particle. In addition, changing the polarity of solvent is related to particle size.

However, the SEM images confirm the BET measurement.

Characterization

The FTIR spectra of the NIP, and the unleached and leached MIP samples are shown in Figure 4. In the FTIR spectra, similar characteristic peaks are observed for NIP, and the unleached and leached MIP represents the similarity in the backbone structure of the different polymers. The spectra of the unleached MIP shows three bands at 1710, 3457, and 1388 cm^{-1} which are correspond to —COOH group of MAA, the C=O stretching, the OH stretching, and the bending vibrations, respectively.[16] These peaks are also present in the leached MIP spectra; however, they were shifted to 1722, 3473, and 1394 cm^{-1}, accompanied by appearance of new sharp band at 1522 cm^{-1}, with low relative intensity. In addition, one band with high relative intensity at 2965 cm^{-1} that appeared at 1538 and 2940 cm^{-1} corresponds to unleached MIP, respectively.

Additionally, other peaks of MIP are matched together, as well as NIP: 1252, 1129 cm^{-1} (symmetric and asymmetric ester C—O stretch bands), 1636 cm^{-1} (stretching vibration of residual vinylic C=C bonds), and 985 cm^{-1} (out-of-plane bending vibration of vinylic C—H bond). Moreover, the imprinted and non-imprinted polymers after the extraction are similar; indicating that the template was completely removed from the imprinted polymer. This observation can be attributed to the

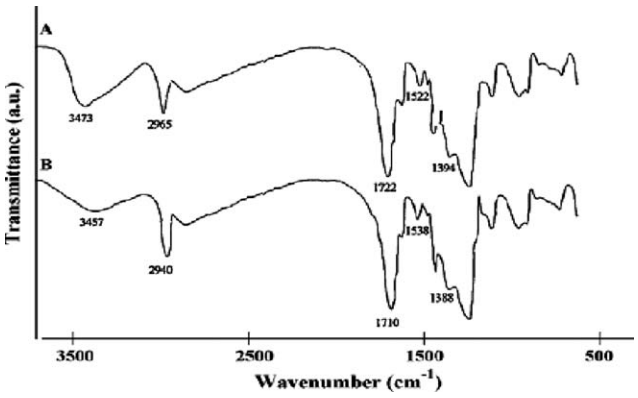

Figure 4. Infrared plots of the leached (A) and unleached (B) MIP particles.

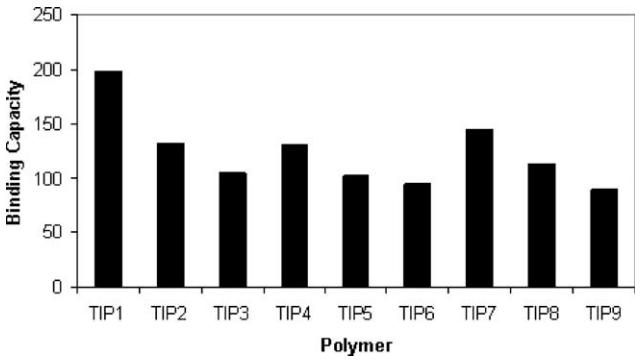

Figure 5. The binding amounts of TC imprinted acrylate polymer to TC in different porogenic solvent and different amount of compound (MAA, EGDMA, and TC).

fact that the drug did not interfere with the synthesis and recognition of the imprinted polymer, and was removed from the imprinted polymer after the extraction.

Effect of Polarity of the Porogenic Solvent on the Binding Capacity

According to Table I, binding capacities for MIPs were estimated by equilibrium binding method,[12] and are shown in Figure 4. The binding capacity of MIP changes effectively by properties of solvents such as polarity, dielectric constant, and hydrogen bond parameter (HBP). In order to increase the binding capacity of MIP, an organic solvent with low polarity is required.[17]

Because highly polar aprotic solvents tend to interfere with the ionic and Van der waals (VDW) forces between the template and the functional monomer, and protic solvents interfere with hydrogen bonding interactions, and therefore, the efficiency of template–monomer interaction decreases. Another important solvent property is the dielectric constant. Electrostatic forces between two charged entities are effectively influenced by polarity of the surrounding media. Therefore, binding strength between the TC template and MAA increases as dielectric constant of the solvent decreases. The third parameter that is vital in the choice of an optimal solvent system is the HBP. It denotes the ability of the solvent to form hydrogen bonds, either with molecules of the same kind or with solutes. A solvent with a HBP will easily dissolve polar molecules which are able to create hydrogen bonds. But it will consequently also interfere with the hydrogen bonds that are formed between functional monomer and template and may thereby lead to MIPs with lower selectivity.[11] As expected, the TIPs synthesized in chloroform, acetonitrile, and methanol showed satisfactory imprinting effects that were in high degree of consistency with the previously reported similar studies.

The partition coefficient values (binding capacity) for the polymers TIP_1, TIP_2, and TIP_3 in chloroform, acetonitrile, and methanol were $K_D = 198.6$, $K_D = 133.2$, and $K_D = 104.7$, respectively. Again indicated that TIPs synthesized in chloroform have the highest binding capacity and chloroform with the least polarity is the most suitable solvent for synthesizing TIPs (Figure 5).

The specific surface area, pore volume, and average pore diameter of polymer particles were obtained by BET method using BELSORP analysis software. Most solids of high surface area are to some extent porous. The texture of such materials is defined by the detailed geometry of the void and pore space. Porosity is a concept related to texture and refers to the pore space in a material. An open pore is a cavity or channel communicating with the surface of a particle, as opposed to a closed pore. Void is the space or interstice between particles. In the context of adsorption and fluid penetration, powder porosity is the ratio of the volume of voids plus the volume of open pores to the total volume occupied by the powder. Similarly, particle porosity is the ratio of the volume of open pores to the total volume of the particle. It should be noted that these definitions place the emphasis on the accessibility of pore space to the adsorptive. The adsorption isotherm obtained has been analyzed using BET equation and BJH algorithm, the obtained results for specific surface area (m^2/g), specific pore volume (cm^3/g) were 250 and 1.2, respectively. As a result, the specific surface areas, the specific pore volumes, and average pore diameters have shown that TIP which were prepared in two polar solvents not only consist of uniform bead with narrow connected particle size that have symmetric porous surface with good pore volume but also TIP_1 were good in binding and selectivity specification. TIP_3 which were prepared in mixture of two solvents differs in polarity, including bulk and shapeless particles with wild range and unconnected particle size, have asymmetric porous surface with less pore volume than TIP_1 and slowly saturates. Also TIP_3 binding and selectivity specification properties are weaker than TIP_1.

All mentions above could be interpreted as following: when we choose precipitation polymerization method by two solvents system the second solvent, which is different in polar properties, has major effect on all MIPs attribute.

Substrate Selectivity

The substrate selectivity of MIPs and NIPs was carried out using a series of structurally related antibiotics, deoxycycline, and cephalexin as substrates in aqueous media. The amount of substrates bound to MIP and NIP were determined by the equilibrium binding method.[16] The prepared TIP exhibited the highest selectivity for TC compared to the other substrates, and K_D values for deoxycycline was higher than that for cephalexin. It revealed that TC-based MIP owns better affinity to the template molecule because of hydrogen bonding interaction, between functional groups possessed by all drugs, and carboxylic groups in the MIP (Table II). This can be easily explained by their close similarity to TC, in the arrangement of the functional groups, and in the size of the three-dimensional

Table II. K_D of Tested Substrates on MIP and NIP under Equilibrium Binding Conditions

Substrates	MIP	NIP
Tetracycline	198.6	39.3
Deoxycycline	44.2	31.9
Cephalexin	29.86	16.67

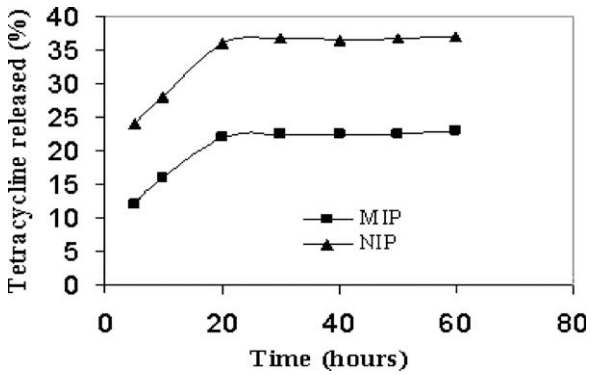

Figure 6. Release profile of TC from MIP and NIP in aqueous media.

structure. In addition, obvious difference of selectivity binding between MIP and NIP mainly results from the carboxyl functional groups within the microcavities created in the MIP matrix by imprinting. Although NIP has the same chemical composition as MIP, it does not have the recognition sites complementary in both shape and functional groups to those of TC, and the arrangement of carboxyl groups in NIP are random. Therefore, it would only relatively and poorly bind the test substrates by weak non-specific adsorption, and does not show any selectivity for them. Thus, these evidences indicated that the imprinting method creates a micro-environment based on shape selection and position of functional groups that recognizes TC template molecule better than other substrates which is better than the previous work.[14]

Drug Release

TC imprinted polymers have a better ability to control the release of TC in an aqueous media compared to the NIP. Figure 6 shows the release profile of TC when TC-saturated MIP and NIP are placed in water. TC leaches out from both TIP and NIP matrices. The TC release from MIP is slower than from NIP. In the beginning, release from NIP is almost twice faster than from MIP due to attractive binding of TC with MIP. This slow release can be utilized in the controlled release of pharmaceuticals.

CONCLUSIONS

In this study, a polyacrylate TC selective microporous molecularly imprinted polymer was prepared in three different porogenic solvents (chloroform, acetonitrile, and methanol) via precipitation polymerization, using MAA monomer, EGDMA cross-linker, and TC as template. In all three solvents this method produced microporous particles in the micron scale range (200–400 nm), simply, quickly, cleanly, and in good yield. The MIP has higher affinity to TC in water than NIP.

The effect of polarity of porogenic solvents on binding capacity was investigated. The binding ability of MIP is maximum in chloroform porogenic solvent, and with increasing polarity of porogenic solvents (methanol>acetonitrile>chloroform), the TC affinity to the MIP decreases. Furthermore, the synthesized MIPs exhibited a slow release of TC in aqueous media. The selectivity of imprinted polymers was evaluated by rebinding with other structures that are similar to TC. The results clearly indicated that the imprinted acrylate polymer exhibits an excellent selectivity toward TC, and has better ability to control the release of TC than the NIP. This kind of MIPs, which are operational in aqueous solution, may open new applications in the fields of life sciences. In addition, the results of this work and practical use of inhibitors will be reported in detail in forthcoming articles.

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