International Journal of Pharmaceutics 424 (2012) 67–75

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Synthesis, characterization and evaluation of computationally designed nanoparticles of molecular imprinted polymers as drug delivery systems

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ARTICLE INFO

Article history:
Received 10 September 2011
Received in revised form
20 November 2011
Accepted 27 December 2011
Available online 2 January 2012

Keywords:
Molecularly imprinted polymers (MIP)
Density functional theory (DFT)
Drug delivery systems
Naltrexone

ABSTRACT

The aim of the present study was to prepare nanoparticles of molecular imprinted polymers (MIPs) with high loading capacity for naltrexone as template drug. To achieve this goal, a computational protocol was employed to select the most appropriate monomer for MIP preparation. Density functional theory (DFT) method at the B3LYP level of theory in conjugate with the 6-31+G(d) basis set was used to evaluate the extent of interaction between naltrexone and a small library of frequently used vinylic monomers. The results revealed that acrylic acid (AA) and methacrylic acid (MAA) can be considered as suitable monomers. To select the best monomer, two MIPs with AA and MAA monomer were synthesized and their loading capacity, selectivity and release profile were evaluated. The experimental results showed that the MIPs synthesized using AA (MIP–AA) exhibited a surprisingly high loading capacity to naltrexone (75 mg of drug/g of MIP) compared to MIP–MAA (34 mg of drug/g of MIP). In vitro release dynamics of the drug from MIPs was also investigated and modeled. It was found that non-Fickian-type diffusion mechanism was responsible for drug release. The results can lead to the conclusion that MIPs designed by computational approach can be considered as promising candidates for drug delivery systems.

1. Introduction

Naltrexone is an efficient narcotic antagonist used mainly in the treatment of narcotic addiction. It is currently given orally as tablets or capsules in a daily dose of 50 mg. There are a number of issues associated with administration of naltrexone hydrochloride including bioavailability and patient compliance. On the other hand, for naltrexone treatment to be effective, it is necessary to provide a sufficient level of the drug concentration for a long period of time. To overcome these problems, there is a need for development of novel drug delivery systems capable of delivering such drugs more effectively and efficiently. Therefore, substitution of common drug delivery systems has attracted significant attention of the scientists working in the pharmaceutical fields (Asadi et al., 2011a,b; Dong and Feng, 2004). Molecular imprinting polymers (MIPs) which possess great potential in a number of applications in the life sciences represent a promising approach as drug carriers (Yin et al., 2006).

MIPs are known as smart materials with a pre-determined selectivity. To prepare MIPs, a functional monomer and a crosslinker are polymerized in the presence of a template molecule. The template is then extracted, leaving sites which are complementary in both chemical functionality and shape to those of the template.

The application of MIPs in different drug delivery systems has dramatically expanded in the past decade (Piletsky et al., 2006; Zhao et al., 2009; Singh and Chauhan, 2008; Cirillo et al., 2009; Chianella et al., 2006). Alvarez-Lorenzo et al. (2006) have imprinted norfloxacin into soft contact lenses prepared from poly(hydroxyethylmethacrylate)-based hydrogels. The hydrogels showed controlled release of norfloxacin for more than 24 h.

The effectiveness of MIPs can be improved by increasing their loading capacity and selectivity. Since an optimal template–functional monomer interaction has a determining impact on successful imprinting, the choice of appropriate monomers play a critical rule in successful imprinting process (Hiratania and Alvarez-Lorenzo, 2004). The best functional monomers generally are selected by trial and error method which is expensive and time consuming. Nowadays, with the appreciation of the advent of computational chemistry, computer-aided study of MIPs has been suggested as a rational and fast technique to search for optimal imprinting conditions (Gholivand et al., 2010; Nicholls et al., 2009). Li et al. (2009) used molecular dynamics simulations and computational screening to identify functional monomers capable of strong interaction with sulfadimidine as template. The combination of molecular dynamics simulations and quantum mechanics calculations were also used to select the optimum monomer and progen

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doi:10.1016/j.ijpharm.2011.12.054

solvent (Dong et al., 2009; Chianella, 2006). Chianella et al. (2006) have demonstrated that with the aid of computational design, it is possible to prepare MIPs with high binding capacity for Abacavir, which is a HIV-1 reverse transcriptase inhibitor. Leapfrogk algorithm was used to rational design of MIPs to be employed as carriers for controlled release of simazine into water (Piletska et al., 2005). It was found that the speed of herbicide release was correlated with the calculated binding characteristics.

The present research was focused on the development of a fast and reliable screening method to aid the design and synthesis of nanoparticles of MIP with high loading capacity and selectivity for naltrexone. Nanoparticles properties as drug delivery vehicles were also evaluated.

2. Experimental

2.1. Materials and methods

Azobisisobutyronitrile (AIBN) was supplied by Kemikalieimport and all other chemicals were purchased from Merck (Germany) and used as received. Naltrexone hydrochloride was supplied by Sun Pharmaceuticals Ltd. (India).

Due to solubility problems in most organic solvents, naltrexone hydrochloride was converted into the free base by diethyl ether extraction of an aqueous solution after addition of Na_2CO_3. The resulting naltrexone free base was then used as template for the synthesis of MIPs.

The computer used to simulate monomer–template interactions was a Pentium IV 1.2 GHz, 256 MB of RAM, running a Windows 2007 operative system.

2.2. Computational modeling

The aim of calculations was to compare some monomers interaction with naltrexone in order to search for the optimal naltrexone–monomer complex for MIP synthesis. To achieve this goal, three methods were considered: the first was geometrical parameters of complex and its shift in comparison with the isolated naltrexone and monomers. The second was the relative stability of complex and the third was the atom in molecules analysis (AIM).

2.3. Synthesis of molecular imprinted PAA (MIP–AA) and PMAA (MIP–MAA) nanoparticles

Particles of MIPs were prepared using precipitation polymerization. Briefly, to prepare MIP–AA, naltrexone (0.0341 g), AA monomer (0.041 ml), and ethylene glycol dimethacrylate (EGDMA) as cross linker (molar ratio of functional monomers to cross-linker was 1:5.4) were dissolved in 3 ml of THF in three neck flask. The solution was stirred slightly for 4 h at 2 rpm to allow monomers and template molecules to form complex, and then AIBN (0.041 g) as initiator was added. The solution finally syringed to 25 ml water. The mixture was saturated with dry nitrogen and polymerization was carried out by placing the flask in an oil bath for 24 h at 90 °C. The particles were collected using a centrifuge at 15,000 rpm for 20 min and the process was followed by a final rinsing step with water and acetone, respectively. The nanoparticles were then dried and loaded naltrexone was extracted from MIPs through soxhelt method using a mixture of acetic acid and methanol (1:1) as solvent for 24 h to ensure that the drug is nearly completely removed from the polymer matrix. Synthesis of MAA based MIPs (MIP–MAA) was carried out using the same procedure stated above with MAA monomer (0.049 ml).

As a reference, non-imprinted particles (NIPs) of each monomer were also prepared without naltrexone by using the same synthetic protocol.

2.4. MIPs characterization

The structure of MIPs was characterized using FT-IR spectroscopy (Bruker, Tensor 27, Germany). The hydrodynamic diameter and the zeta potential (ζ) of nanoparticles were determined in phosphate-buffer saline (PBS, pH 7.4) by dynamic light scattering using a NanoZS apparatus (Malvern Instruments, Worcestershire, UK) equipped with a 633 nm laser at a fixed scattering angle of 173°. The temperature of the cell was kept constant at 25 °C.

2.5. Evaluation of loading capacity of MIP–AA and MIP–MAA

The loading capacity of MIP–AA and MIP–MAA were assayed through analysis of naltrexone concentration in the soxhelt solution by UV spectrophotometer at $\lambda = 280$ nm. The loading capacity of MIPs (Q, mg/g_{MIP}) was calculated based on the concentration of naltrexone in the soxhelt solution (c, mg ml^{-1}), the volume of soxhelt solution (v, ml) and the weight of the MIPs (w, g) according to the following Eq. (1):

$$Q = \frac{CV}{W} \quad (1)$$

In order to avoid the effect of presence of unreacted monomer and byproducts of the polymer synthesis in the absorbance change, the amount of naltrexone was calculated from the absorbance of the solution after subtracting the absorbance of the supernatant produced of NIPs in the same conditions.

To study the kinetic of template extraction, naltrexone was removed using acetic acid/methanol by batch method.

2.6. Selectivity studies

To estimate the selectivity of the naltrexone imprinted particles, the loading capacity of MIPs toward methadone was also studied. Methadone was chosen due to their molecular structures similar to naltrexone to some extent and its ability to form hydrogen bond with MIPs. The procedure followed for binding experiment of methadone was similar to that of naltrexone.

2.7. Drug reloading to the MIPs by soaking procedure

In order to investigate the reloading capacity of MIPs, naltrexone was reloaded to the soxhelted MIPs by soaking method. As brief, after removal of the naltrexone from the MIPs by soxhelt method, they were dried at room temperature. 0.0350 g of MIPs were immersed in 25 ml of phosphate buffer with a known concentration of naltrexone (0.00239 M) and soaked for 1 day at room temperature while stirring. To study kinetic of naltrexone reloading on MIPs, sampling was carried out with 1 h interval. Samples were centrifuged for 15 min (15,000 rpm) and the naltrexone concentration in the supernatant was monitored. Any particles present in the samples withdrawn were returned to the medium after centrifugation and analysis of the drug concentration. NIPs were also subjected to the reloading study with equal initial concentration and condition. The amount of naltrexone reloaded to the polymer was calculated by subtracting the amount of free drug from its initial value.

2.8. In vitro release study

Release studies were carried out for both drugs entrapped during MIPs synthesis and for drugs reloaded to the soxhelted MIPs in water media (phosphate buffer). The nanoparticles were dispersed in flasks containing phosphate buffer (pH 7.2) at 37 °C for 24 h with shaking. These conditions were maintained throughout the experiment. Samples were withdrawn from solution medium at appropriate time intervals to determine the amounts of drug released.

2.9. Mechanism and mathematical modeling of drug release from MIPs particles

There is a number of reports deal with the mathematical modeling of drug release from polymeric systems. The following equation is one of the generalized empirical equations which is widely used to describe drug release (Ritger and Peppas, 1987).

$$\frac{M_t}{M_\infty} = kt^n \quad (2)$$

where M_t/M_∞ is the fractional release of drug at time t (M_t) and the drug released at equilibrium time (M_∞), k is the constant characteristic of the drug–polymer system, and n is the diffusion exponent characteristic of the release mechanism. The slope and the intercept of the plot drawn between $\ln M_t/M_\infty$ and $\ln t$ give the value of n and k, respectively. Normal Fickian diffusion is characterized by $n = 0.5$ and Case II diffusion by $n = 1.0$. A value of n between 0.5 and 1.0 indicates a mixture of Fickian and Case II diffusion, which is usually called non-Fickian, or anomalous, diffusion.

The binding characteristics of MIPs were also studied using different adsorption isotherm models including Hguchi model, Ritger–Peppas model, Peppas–Sahlin model, Ritger–Peppas model ($m = 0.5$), and Zero-order.

3. Results and discussion

3.1. Computational selection of the functional monomer

One of the most important advantages of MIP as drug delivery systems is the possibility to enhance loading capacity and selectivity by selection of a monomer with high affinity toward template. The computational design is a promising approach for selection of adequate monomers for MIP. In this work, five functional monomers Acrylamide (AAM), methylacrylamide (MAAM), MAA, allylamine, and AA were theoretically selected as possible functional monomers. Monomers selection was based on their functionalities able to form non-covalent interactions especially H–bonding with naltrexone.

Geometries of monomers, naltrexone and their corresponding complexes were fully optimized using the density functional theory (DFT) method at the B3LYP level of theory in conjugate with the 6-31+G(d) basis set (Becke, 1993; Lee et al., 1988). All of the calculations were performed with the Gaussian 03 program (Frisch et al., 2003). First, semiemprical and Hartree–Fock (HF) methods were employed to optimize the complexes as an initial guesses. The obtained results were improved using the B3LYP calculation method and used to calculate the relative energy of collisional complexes. Using such level of the method gives the opportunity to generate a wave function in a suitable form to execute the topological analysis atom in the molecule by means of the AIM2000 series program (Bader, 1990; Biegler-Kônìng et al., 2000). Finally, the counter poise (CP) procedure was used to correct the interaction energy for basis set superposition error (BSSE). The optimized molecular structure of naltrexone, the five functional monomers, and the most stable complexes of naltrexone–AA, naltrexone–MAAM, naltrexone–MAA, naltrexone–AAM, and naltrexone–Allylamide is presented in Fig. 1 and Fig. 1S in supplementary data.

Table 1
Bond length, bond angles and topological analysis of AA, naltrexone, and their complexes.

Species	$\rho(r)$ (eÅ^{-3})	$\nabla\rho(r)$(eÅ^{-3})	Bond lengths(Å)	Ellipticity
Acrylic acid and naltroxone complex				
H56–O52	0.2959	−1.3728	1.0021 (0.0212)	0.01193
H56–O18	0.04014	0.1343	1.7540	0.01215
C17–O18	0.3589	−0.2805	1.2465 (0.0086)	0.07287
C17–C14	0.2372	−0.4257	1.5315 (−0.0083)	0.05550
C14–O15	0.2077	−0.2305	1.4933 (−0.0094)	0.02998
O53–O15	0.006104	0.02358	3.1665	0.3733
C8–O15	0.2394	−0.3846	1.4233 (0.0139)	0.03566
C8–C9	0.2952	−0.6835	1.3957 (0.0067)	0.2104
C9–O3	0.2733	−0.5153	1.3656 (−0.0215)	0.01549
O3–H30	0.3036	−1.4179	0.9966 (0.0178)	0.01708
H30–O53	0.04426	0.16815	1.6794	0.01342
O53–C50	0.36203	−0.4720	1.2497 (0.0116)	0.00775
C50–C49	0.2580	−0.5300	1.4756 (0.0056)	0.06689
C50–O52	0.2863	−0.5416	1.3475 (−0.0367)	0.05053
6-RCP	0.004988	0.02492		−1.7484
8-RCP	0.005274	0.02332		−1.6471
Naltrexone				
H30–O3	0.3267	−1.5554	0.9788	0.01908
C9–O3	0.2647	−0.3579	1.3871 [1.36]	0.001896
C9–C8	0.3081	−0.7545	1.3891 [1.38]	0.2210
C8–O15	0.2482	−0.4236	1.4094 [1.36]	0.02904
C14–O15	0.2133	−0.2627	1.5027 [1.44]	0.03683
C14–C17	0.2378	−0.4249	1.5398 [1.53]	0.05395
C17–O18	0.3807	−0.1561	1.2379 [1.21]	0.03485
Acrylic acid				
H56–O52	0.3127	−1.45332	0.9809 [0.952]	0.017843
C50–O52	0.2954	−0.4331	1.3842 [1.355]	0.076494
C50–C49	0.2549	−0.5135	1.4700 [1.476]	0.06662
C50–O53	0.3829	−0.4768	1.2381 [1.220]	0.02584

The extent of bond lengths in pretenses refers to deviation bond length of complex from corresponding bond in monomer or naltrexone and the value in bracket refers to experimental value.

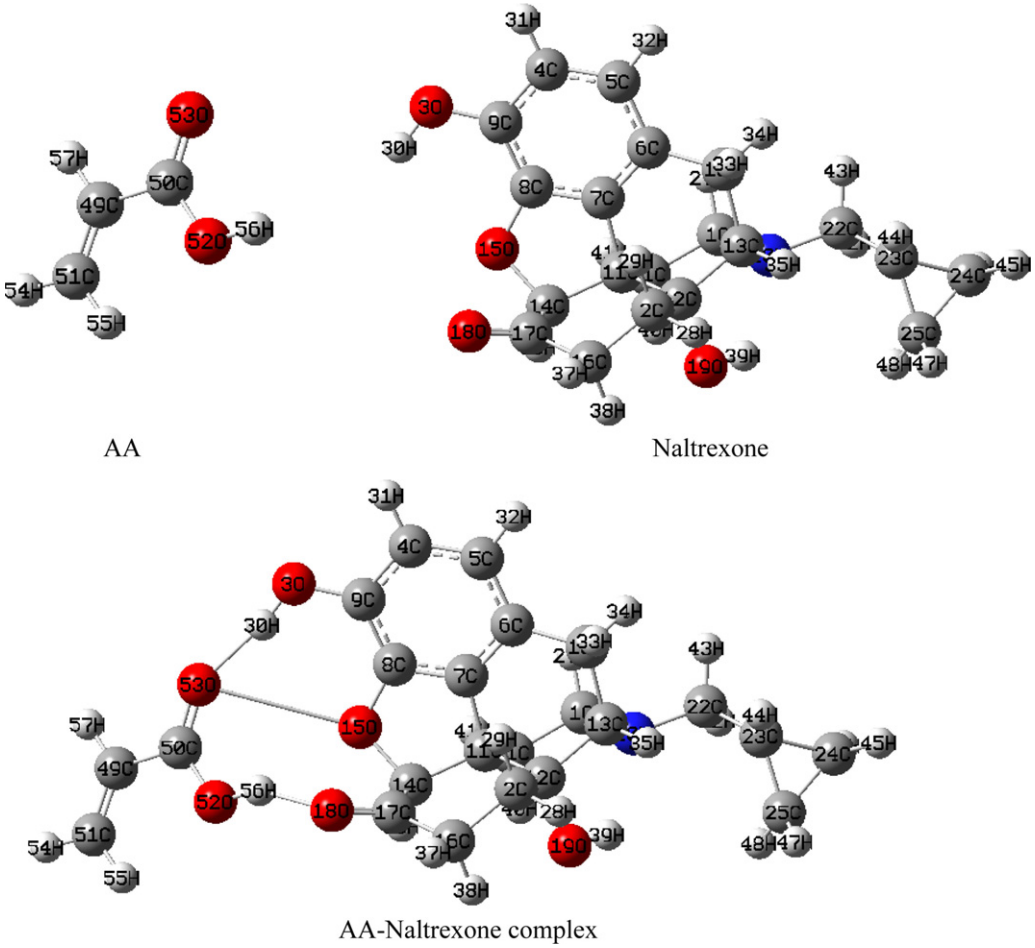

Fig. 1. Optimized molecular structure of acrylic acid, naltrexone and their complex: bond lengths presented in Table 1.

3.1.1. Molecular structure calculations

The most relevant calculated bond lengths of the monomers, naltrexone and their complexes are listed in Table 1. The bond lengths of some species have been compared with the experimental values. The comparison shows that the bond lengths of all molecules calculated at B3LYP level are relatively in good agreement with the experimental data.

The calculation showed variation in the AA–naltrexone complex bond lengths compared to the free AA and naltrexone which is indicative of the complex formation. For instance, variation in H56–O52 and C50–O53 bond lengths of acrylic acid and its complex with naltrexone were calculated to be 0.0212 and 0.1094 Å, respectively (Table 1). This difference can be explained by the new O18–H56, O53–O15, and H30–O3 bonds which form two 6 and 8 member ring structure. Since the lengths of newly formed bonds were longer than the covalent bonds, they seem to be van der Waals bonds. Similarly, calculations demonstrated the complex formation between other monomers with naltrexone.

3.1.2. Relative stability of the complexes

Table 2 presents the total energies and relative energies of complexes with respect to the monomers and template at the B3LYP level of computation. To perform calculation, only one stable van der Waals complex formed through the interaction of two or three atoms of each monomer with appropriate atoms of naltrexone was considered. The results revealed that the obtained collisional complexes of naltrexone with AA, MAA, allylamine, AAM, and MAAM exhibited 20.694, 21.562, 20.507, 20.106, and 17.184 kcal/mol less energies than that of the original reagents (Table 2).

3.1.3. Atom in molecules theory (AIM)

Theory of AIM developed by Bader is based on the CP of the molecular electronic charge density, $\rho(r)$. It has been proven that AIM provides valuable information about many different chemical systems by analysis of the molecular electron density distribution. The application of the atoms and molecules theory to understand the nature of the bonds in deeper detail is an interesting approach. Atoms in molecules theory is based on the CP of the molecular electronic charge density, $\rho(r)$. These are points where the electronic

Table 2
The total energy and relative energy of species obtained at B3LYP level.

	Total energy (Hartree)	Relative energy (kcal/mol)
Naltroxene–AA complex	−1398.31748	−20.694
Naltroxene–MAA complex	−1304.44298	−21.562
Naltroxene–allylamine complex	−1437.62819	−20.507
Naltrexone–AAM complex	−1378.494385	−20.106
Naltrexone–MAAM complex	−1417.781117	−17.184
Naltrexone	−1131.24041	
AA	−267.07707	
MAA	−306.38778	
Allylamine	−173.20257	
AAM	−247.2219297	
MAAM	−286.513319	

density gradient ($\nabla^2\rho(r)$) vanishes and which are characterized by the three eigenvalues ($\lambda_i, i = 1, 2, 3$) of the Hessian matrix of $\rho(r)$. The first two eigenvalues correspond to the perpendicular curvatures and the latter provides curvatures along the internuclear axis. The CP's are labeled (r, s) according to their rank, r, (number of non-zero eigenvalues) and signature s (the algebraic sum of the signs of the eigenvalues).

Four types of CP are of interest in molecules: (3, −3), (3, −1), (3, +1) and (3, +3). A (3, −3) point corresponds to a maximum in $\rho(r)$ characterized by $\nabla^2\rho(r) < 0$ and occurs generally at the nuclear positions. A (3, +3) point indicates electronic charge depletion and it is characterized by $\nabla^2\rho(r) > 0$. It is known as bond critical point (BCP). The (3, +1) points, or ring critical points (RCP), are saddle points. Finally, a (3, −1) point or bond critical point, BCP, is generally found between two neighboring nuclei indicating the existence of a bond between them. BCPs and RCPs were analyzed in this work. The trajectories of $\rho(r)$ that start at BCP and terminate at such nuclei define a 'bond path'. Then, a 'molecular graph' is the network of bond paths and it is interesting to see that the molecular graph for a molecule at equilibrium geometry is identified with the corresponding network of chemical bonds. The charge density along a bond path attains its minimum value at the BCP and the associated curvature or eigenvalue of the Hessian of ρ at r, λ_3, is thus positive. On the other hand, the charge density in an interatomic surface attains its maximum value at the BCP and two directed long axes perpendicular to the bond path are thus negative.

The Laplacian $\nabla^2\rho(r)$ is associated with the charge density and it is the sum of the curvatures in the electron density along any orthogonal coordinate axes at the point (r). The sign of $\nabla^2\rho(r)$ indicates whether the charge density is locally depleted ($^2\rho(r) > 0$) or locally concentrated ($\nabla^2\rho(r) < 0$). Thus, when the negative curvatures dominate at the BCP, the electronic charge is locally concentrated within the region inter atoms leading to an interaction named as covalent or polarized bonds and being characterized by large ρ values, $\nabla^2\rho(r) < 0$ and $|\lambda_1|/\lambda_3 > 1$. On the other hand, if the positive curvature is dominant, the electronic density is locally concentrated in each of the atomic basins. The interaction is now referred to as a closed-shell and it is characteristic of highly ionic bonds, hydrogen bonds and van der Waals interactions. It is characterized by relatively low ρ values, $\nabla^2\rho(r) > 0$ and $|\lambda_1|/\lambda_3 < 1$.

Other interesting parameter is the ellipticity (ε) defined as $(\lambda_1/\lambda_2) - 1$. It is indicative of the similarity between the perpendicular curvatures (λ_1 and λ_2) at the BCP. In terms of the orbital model of electronic structure, the ε provides a quantitative measure of the π-bond character and of the delocalization electronic charge.

The topological analysis of the electronic charge density was performed for all bonds in the complexes, monomers and naltrexone. Calculations exhibited that three cites corresponding with H30–O3, O53–O15 and H56–O18 were responsible for AA–naltrexone and MAA–naltrexone complexes formation. Table 1 and Fig. 1S in supplementary data represent the topological characteristic in the BCPs and RCPs of all species involved in the study. The data shows that the newly formed bonds strength for monomers containing acid functional group was stronger than the monomers with amine functional moiety. This is presumably coming from the atoms electronegativity and complexation ability of acid and amine groups. Among two acidic monomers, it was shown that the newly bonds in MAA–naltrexone complex are stronger than that of AA–naltrexone complex.

According to the theory of AIM, the positive value of $\nabla^2\rho(r)$ for the newly formed bonds describe the intermolecular interaction character. All complexes showed RCPs. In the acid form monomers, two RCPs were calculated which indicate that two ring structures are formed.

The ε is a measure of the ratio of the rate of density decrease in the two directions perpendicular to the bond path at the BCP, the general shape of the bond and the degree of π-character. A value of zero indicates a symmetrical distribution of density about the bond path, such as found in standard single and triple bonds, while large values indicate a preference for density build up in a particular orientation. The values of the ε for AA–naltrexone are listed in Table 1.

For the O53–O15 and H52–H30 bonds, high values of ε ascribe to the symmetrical van der Waals bonds of the coordination cites (Table 1 and Fig. 1S in supplementary data) and also, high value of ε for C8–C9 bonds in various species show high π bonding character which are in consistent with the experimental predictions. Whereas low values of ε belong to the other bonds especially hetroatome containing newly bonds with the asymmetrical distribution of electron charge density or low π bonding character.

In essence, computational results indicate that the acid form monomers interact with the naltrexone stronger than the amine form monomers. So it can be concluded that the AA and MAA monomers are more favorable monomers for MIP preparation than the others. On the other words, the MIP synthesized with AA and MAA are expected to possess the highest loading capacity and selectivity to naltrexone compared to the MIP prepared with other monomers. To identify the most appropriate monomer among AA and MAA to prepare MIP, experimental approach was considered.

3.2. MIPs synthesis and characterization

On the basis of theoretical calculations, two monomers (AA, MAA) strongly interacting with the template were identified and used for the synthesis of nanoparticles of MIPs. As a matter of fact, two sets of molecular imprinted and non imprinted polymers (AA–MIP, AA–NIP and MAA–MIP, MAA–NIP) were prepared under similar condition. MIPs were characterized by FTIR. For instance, Fig. 2 illustrates the FTIR spectrum of MIP–AA, naltrexone, NIP–AA and physical mixture of NIP–AA and drug. FTIR spectrum of MIPs–AA shows a broad absorption band at 3445.1 cm^{-1} which is due to –OH stretching and indicating the acid functional group in this polymer. The infrared absorption bands at 1734.4 cm^{-1} due to C=O stretching of the acid and at 1161.4 cm^{-1} due to the C–O stretching of acid and the CH_2 asymmetric stretching vibration at 2982.2 cm^{-1} were also observed in the spectra. One surprising observation was that there was no significant difference between MIP and NIP spectrum and the absorption bands corresponding to the naltrexone were not observed in the MIP spectra. A possible explanation for this phenomenon could be the low amount of the drug compared to the polymer. In order to confirm this assumption, physical mixtures of drug and NIP were also prepared by mixing the powders together. The drug/NIP ratio in the physical mixtures was the same as in the MIP. As it can be seen from Fig. 2, the absorption bands corresponding to naltrexone in the physical mixture spectrum were also not detectable.

The hydrodynamic diameter of nanoparticles was 358 nm (Fig. 3). Polydispersity index and ζ potential of MIP particles were determined to be 0.86 and 6.28 mV, respectively.

3.3. Evaluation of the MIPs loading capacity (Q)

The evaluation of the MIPs loading capacity was carried out by measuring the template extracted from MIPs. The results showed that Q_{MIP-AA} was 75 mg naltrexone/g MIP, which was more than that of MIP–MAA ($Q_{MIP-MAA}$ = 34 mg naltrexone/g MIP). The kinetic of naltrexone extraction was also monitored during extraction process by batch method (Fig. 4). As it is clear the extraction of template using acetic acid/methanol was almost complete in 6 h, as there is no change in concentration of naltrexone. As it can be seen

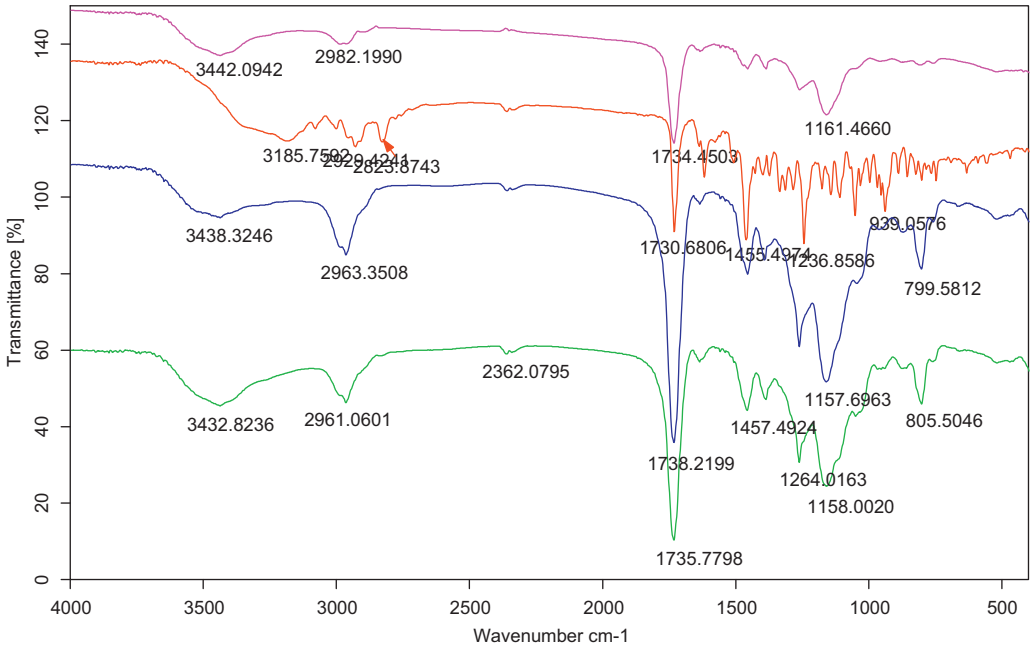

Fig. 2. FTIR spectra of MIP–AA (purple), naltrexone (red), NIP–AA (blue), and physical mixture of NIP–AA and drug (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

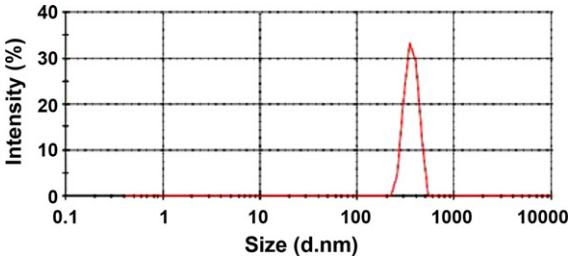

Fig. 3. Hydrodynamic diameter of MIP–AA particles.

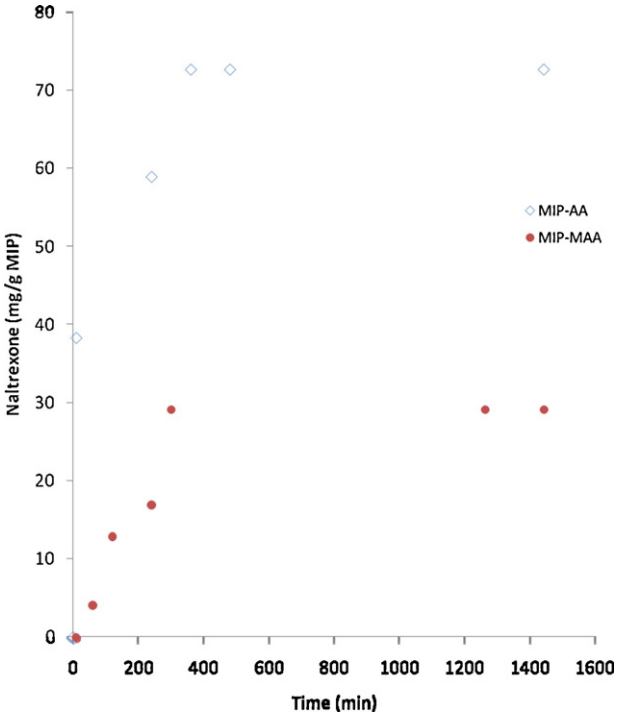

Fig. 4. Naltrexone extraction kinetics from MIPs particles.

the efficiency of MIPs–AA is quite higher than that of MIPs–MAA. Apparently, this can be associated with the optimum association of AA with the template. It is obvious that although the intensive interaction of monomer with template would lead to the high incorporation of drugs into the MIP, but on the other hand it would make it difficult for template to be extracted from MIP, so it can be concluded that the best monomer is the one capable of moderate interaction with template. The result was well consistent with the computational results, which confirmed the validity of the model and method proposed to benefit screening molecularly imprinted systems rapidly in an experiment-free way instead of trial and error approach. In addition, these values are acceptable for practical application of MIP as drug delivery device.

3.4. Evaluation of the MIPs reloading performance

The time course of reloading using the soxhelted MIPs synthesized with the two monomers, AA and MAA, is shown in Fig. 5. The reloading capacity for MIP–AA and MIP–MAA were obtained to be 250 and 120 mg naltrexone/g MIPs. A possible explanation for this behavior could be the intensive interaction of MIPs with naltrexone which subsequently leads to significant surface adsorption of drug on nanoparticles. The curve of MIPs indicated that the adsorption of naltrexone increased quickly with time and then reached equilibrium. In the first step, the adsorption rate was fast, and the contact time to nearly reach equilibrium was 10, and 5 min for AA–MIP and MAA–MIP, respectively. The observation can be explained in terms of small size of particles which facilitates loading process.

3.5. Selectivity studies

Probably, the most important feature of MIPs is their selectivity. In order to evaluate the selectivity of the synthesized MIPs, methadone, which is an analogue of naltrexone, to a certain extent, and possesses capability to form hydrogen bonds with MIPs was considered in this study (Fig. 6). As it can be seen, the binding observed for naltrexone in MIP–AA and MIP–MAA were 2.5 and 1.3 times as high as that of methadone, respectively. This can be attributed, in part, to the unique shape of the template cavity

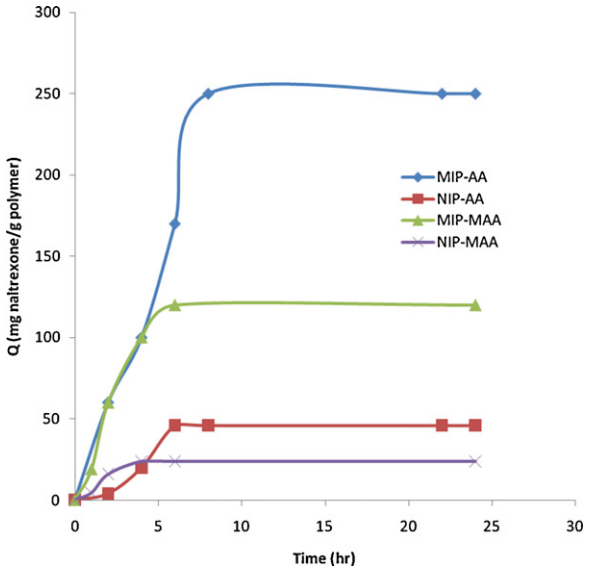

Fig. 5. Reloading kinetics of naltrexone into MIPs and NIPs particles.

created on the MIP particles which play an important role in the template selective binding to the MIPs. On the other words, the difference in adsorption of naltrexone to MIP–AA and MIP–MAA devices indicated that in case of MIPs–AA specific binding occurred more than MIP–MAA. Fig. 5 reports that naltrexone binds to naltrexone imprinted MIP–AA to a greater extent than that of MIP–MAA but this difference was not observed for their corresponding NIPs. This trend may be explained by the fact that the NIPs adsorption arises from nonspecific adsorption of particles. Overall, these findings can be considered to be further evidence that AA appear to be the most useful monomer to form stable complex with naltrexone. This is in agreement with computational studies demonstrating that the observed effects are a consequence of the optimal association of drug with monomer.

3.6. Release dynamics of the drug from MIPs

Two methods were followed in order to achieve the loaded MIPs: drug incorporation during the process of generation of the nanoparticles and hence trapping of the drug in the polymeric matrix (MIPs), or adsorption of naltrexone into soxhelted MIP particles by

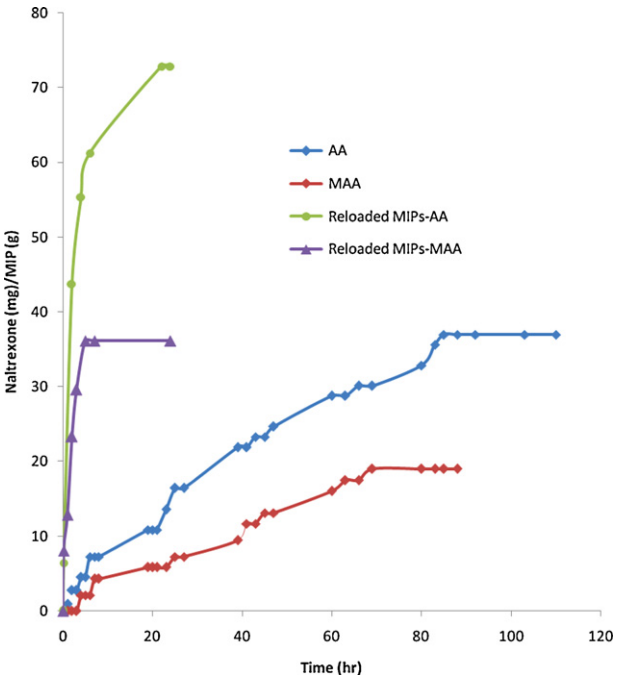

Fig. 7. Release profile of naltrexone from MIPs and reloaded MIPs.

incubation in the drug solution (reloaded MIPs). The release behavior of both particles was evaluated.

The profile of the release of naltrexone per gram of the MIPs and reloaded MIPs is presented in Fig. 7. The overall amount of drug released from MIP–AA, and MIP–MAA was 37, and 19 mg/g_{MIP} in about 85 h, respectively which corresponds to 49% and 56% of total encapsulated drug of MIP–AA and MIP–MAA, respectively (Fig. 8). The total amounts of naltrexone released from reloaded AA–MIP, and MAA–MIP were 72.8, and 36.1 mg/g_{MIP} in 22 h, respectively. It can be seen that the amount of drug released from the reloaded MIPs–AA was higher and faster than that of the MIP–MAA. The increased release of naltrexone from reloaded MIPs may arise due to higher loading of drug in this polymer, whereas in case of reloaded nanoparticles the release was fast. It is of great importance to note that the drug release in the MIPs was in a controlled manner. This observations can be explained by the fact that the most loading of reloaded MIPs comes from surface adsorption which shows burst

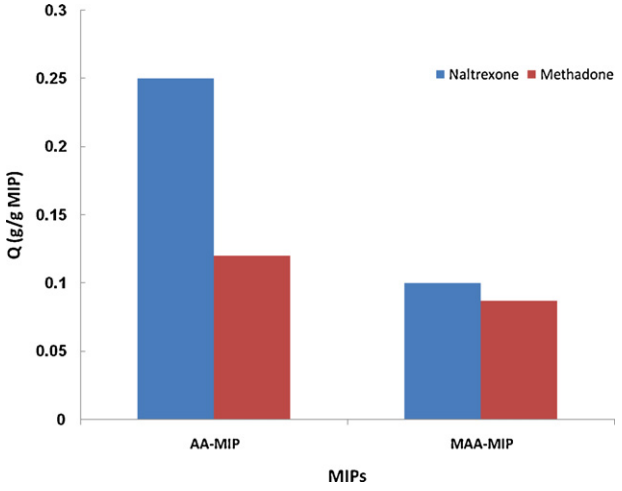

Fig. 6. Comparison loading capacity of MIPs toward naltrexone and its analogue, methadone.

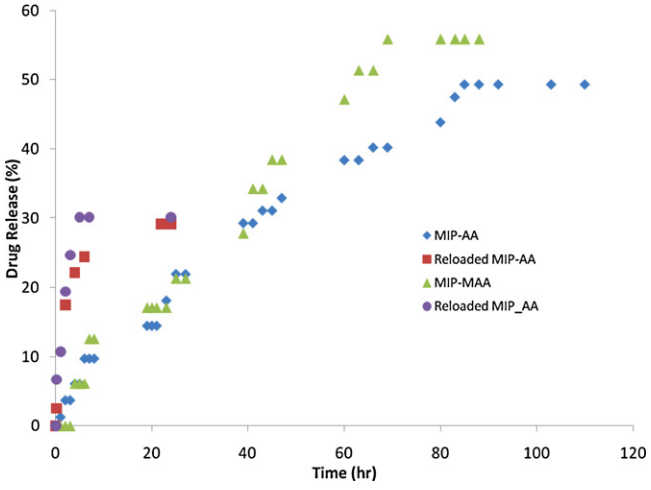

Fig. 8. comparison of the percent of naltrexone release from MIPs and reloaded MIPs.

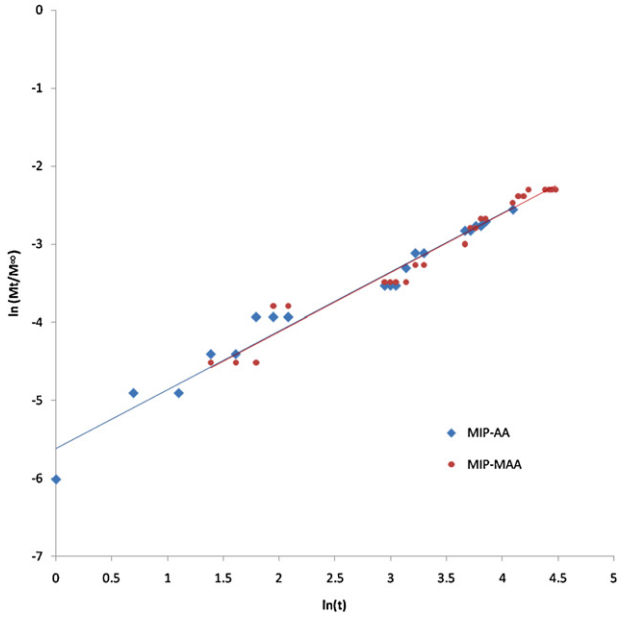

Fig. 9. Plot for the evaluation of the diffusion exponent n and the MIPs characteristic constant k.

release, but in case of MIPs, naltrexone incorporated in the MIP cavity were released which is controlled by different phenomenon.

In order to investigate of release mechanism of drugs from MIPs, the values of the diffusion exponent n and the MIPs characteristic constant k for polymers in phosphate buffer were evaluated from the slope and intercept of the plot $\ln M_t/M_\infty$ vs. $\ln t$ (Fig. 9). It is clear that a value of $n = 0.7$ were obtained for both release case, which indicates that both MIPs were quite similar in their drug release behavior which may result from non-Fickian type diffusion mechanism. The MIPs characteristic constant k for both MIPs was calculated to be 5.6.

3.7. Modeling release kinetics

The binding characteristics of MIPs were also studied using different adsorption isotherm models: including Hguchi model, Ritger–Peppas model, Peppas–Sahlin model, Ritger–Peppas model ($m = 0.5$), and Zero-order. These mathematical models are valid only for the first 60% of the drug release. To distinguish the models that described the data properly from those that did not fit the data correctly, the sum of the squared residuals (SSR) was obtained. The model that best explains the experimental data is the one that shows the minimal value for the SSR. However, since a larger number of model parameters could lead to a higher probability of obtaining a smaller SSR value, it was necessary to use a discriminatory criterion that was independent of the number of parameters that each model had. For this reason, the Akaike Information Criterion (AIC) was applied. The AIC can be defined as

$$\mathrm{AIC} = N(\ln SSR) + 2p$$

where N is number of experimental data points, SSR is the sum of the squared residuals and p is the number of parameters. The model that provides the best fit for the experimental data is selected based on the smallest value for the AIC. Additionally, the fit of the predicted curve to the experimental data and the validity of the calculated parameters were also examined. The findings showed that MIPs prepared gives rise to a binding isotherm that is accurately modeled by the Ritger–Peppas model (Table 3).

Table 3
Different model parameters fitted to the release profile of MIPs.

	Higuchi model		Ritger–Peppas model			Peppas–Sahlin model				Ritger–Peppas model ($m = 0.5$)			Zero-order	
	kH	AIC	$k1$	N	AIC	$k1$	$k2$	M	AIC	$k1$	$k2$	AIC	kd	AIC
MIP–AA[a]	0.407	0.34	0.395	0.517	2.08	0.312	0.0989	0.378	7.79	0.389	0.007	2.05	0.177	0.26
MIP–AA[b]	0.272	−1.6	0.594	0.176	−8.08	0.542	−0.0717	0.476	−5.87	0.522	−0.066	−7.85	0.177	3.31
MIP–MAA[a]	0.466	−7.1	0.403	0.643	−3.75	0.306	0.306	0.487	−1.7	0.317	0.088	−3.75	0.266	−2.80
MIP–MAA[b]	0.447	−6.42	0.412	0.571	−3.7	0.290	0.1	0.407	−1.73	0.388	0.032	−2.54	0.233	−1.7

[a] Corresponding to the release of naltrexone from MIPs.
[b] Corresponding to the release of naltrexone from reloaded MIPs.

4. Conclusion

The aim of the present study was to prepare nanoparticles of molecular imprinted polymers (MIPs) with high loading capacity for naltrexone as drug carrier. A computational protocol was employed to select the best monomer for MIP preparation based on density functional theory (DFT) method at the B3LYP level of theory in conjugate with the 6-31+G(d) basis set. Screening the virtual library of some vinilic monomers showed that acrylic acid (AA) and methacrylic acid (MAA) were the most appropriate monomer. The validity of computational results was verified by synthesis of two sets of MIPs using AA, and MAA monomers. The experimental results were in agreement with the computational design and showed that the MIP synthesized using AA (MIP–AA) exhibited the higher loading capacity to naltrexone (75 mg of drug/g of MIP) compared to MIP–MAA (34 mg of drug/g of MIP). In vitro release dynamics of the drug was also investigated and modeled. The results suggested a non-Fickian type diffusion mechanism for drug release. The results of the computational modeling as well as the experimental results on the formation of the MIPs provide a possible way to enhance the loading capacity of polymeric drug carriers.

Acknowledgments

We are most grateful for the continuing financial support of this research project by Zanjan University of Medical Sciences and Zanjan University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.12.054.

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