Nanomed. J., 4(1): 25-36, Winter 2017

ORIGINAL RESEARCH PAPER

Poly (methacrylic acid-co-acrylic acid)-grafted polyvinylpyrrolidone coated Magnetic nanoparticles as a pH-responsive magnetic Nano-carrier for controlled delivery of antibiotics

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ABSTRACT

Objective(s): Pharmaceutical industries are leading to improved medications that can target diseases more effectively and precisely. Researchers have intended to reformulate drugs so that they may be more safely used in human body. The more targeted a drug is, the lower its chance of triggering drug resistance, a cautionary concern surrounding the use of broad-spectrum antibiotics. The aim of this paper is to introduce efficient drug delivery vehicles which can perform both targeted and controlled antibiotic release by synthesis of magnetic pH-responsive polymer.

Materials and Methods: Iron oxide nanoparticles were synthesized by chemical co-precipitation technique and primary coated with 3-trimethoxysilylpropylamine (APTS). APTS coated MNPs was used in the reaction medium for synthesis of a pH-responsive poly (MAA-co-AAc)-grafted PVP. The prepared vehicle was characterized by TEM, XRD, FT-IR, TGA, DSC and elemental analysis. Drug loading, release, kinetics and mechanism of the system were evaluated.

Results: The results for drug release showed that the release of antibiotics in pH 5.5 and 7.2 could be effectively sustained, while about 92 % of the drugs were released at pH 1.2. Considerations demonstrate the tendency of drug release by Fickian mechanism and diffusion controlled release.

Conclusion: The results indicate that the prepared magnetic nano-carrier could be suitable for site-specific antibiotic delivery through oral administration.

Keywords: *Ciprofloxacin, Drug delivery, Fe_3O_4 nanoparticles, Ofloxacin, pH-responsive polymer*

How to cite this article
Amoli-Diva M, Pourghazi K. Poly (methacrylic acid-co-acrylic acid)-grafted polyvinylpyrrolidone coated Magnetic nanoparticles as a pH-responsive magnetic Nano-carrier for controlled delivery of antibiotics. Nanomed J. 2017; 4(1): 25-36. DOI:10.22038/nmj.2017.8049

INTRODUCTION

Oral administration is one of the most preferred drug delivery ways because of convenience and no cross-infection [1]. Many different kind of polymeric systems are proposed as drug carriers for oral delivery with the aim of site-specific delivery such that the drug is released at controlled rate and at the desired time [2-5]. Stimuli-responsive polymers are a group of materials that swell considerably in external stimuli and demonstrate extraordinary capacity [5, 6]. Being insoluble, these networks can retain a large amount of water, which contribute to their good blood compatibility, beside maintain a certain degree of structural elasticity and integrity [7, 8]. These materials exhibit phase transitions in response to change in pH, ionic strength, temperature, light, magnetic or electric field and etc. [9]. The most important stimuli used for drug delivery are pH and temperature [10, 11]. The temperature has to be altered externally within narrow limits in most cases except maybe hyperthermia therapy, but the pH changes extensively within the body [12, 13]. The

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Note. This manuscript was submitted on October 28, 2016; approved on December 13, 2016

obvious pH changes in the gastro-intestine track from acidic in the stomach (pH 1-3) to basic in the intestine (pH 5-8) make the pH a suitable stimulus and considered for oral delivery of any kind of drug [14-16].

Nowadays, development of nano-structured materials in the field of biology and medicine caused interesting applications. Iron oxide nanoparticles (Fe_3O_4 and Fe_2O_3), in particular, have received special attention for their strong magnetic properties and low toxicity [17, 18].

Magnetic drug targeting has been used to improve localized drug delivery by application of an external magnetic field.

For a given period of time, the magnetic field retained until receiving very high concentrations of the therapeutic agents near the target tissue without any toxic effects to surrounding tissues or the whole body [19]. By combination, magnetic nanoparticles (MNPs) covered with a layer of biocompatible polymeric shell have been reported to be effective drug carriers [20, 21]. The polymer coatings have found to reduce aggregation problem of uncoated nanoparticles and lower toxicity [22]. Furthermore, the therapeutic drug concentration is maintained for sustained periods of time in polymeric network and prevents drug degradation. In addition, control of release kinetics of the drug from MNPs can be easily modulated by altering formulation parameters such as polymer molecular weight and composition, drug: polymer ratio, etc [23].

It is reported that there is a relationship between the release rate of the drug and the degree of interaction between the polymer and the drug [24, 25].

Drug-polymer interaction may be physical through hydrogen bonding or chemical through the formation of insoluble complexes between them. Otherwise, changing in the interactions occurs when the carrier comes in contact with the release medium and affects the rate and extent of drug release. However, it is remains difficult to predict precise drug release profile.

The three main mechanisms of releasing from the polymeric magnetic MNPs are diffusion, degradation, and swelling followed by diffusion [26].

Diffusion occurs due to concentration gradient between release medium and carrier, thus migrate out of the drug. Degradation occurs when drug molecules, which trapped by the polymer, release due to hydrolyzes of polymer chains. Finally, in swelling controlled release, when the polymer placed in the release fluid, swells to increase porosity, enabling the drug to diffuse from network.

pH-responsive polymers can swell when the pH approaches the dissociation constant (pK_a) of the ionic monomer incorporated within the network [27]. Many magnetic polymeric core-shell nano-carriers were used for drug delivery [28-31].

PVP based polymers have been used as drug delivery systems. The choice of PVP was due to its long-standing and safe record in pharmaceutical applications. Additionally, it has been reported that PVP enhance drug circulatory time in plasma [32, 33].

Presence of functional groups like -COOH make the polymer more suitable to bind with the drugs, methacrylic acid and acrylic acid are suitable monomers to form cationic polymer grafted nonionic PVP. This co-polymer will contain acidic carboxyl groups that partially dissociate in water, producing a flexible coil structure.

The designed matrix dosage form can absorb water molecules, which is accompanied by polymer chain relaxation and causes the drug is entrapped in the polymeric network and released over a long period of time.

In this study, iron oxide nanoparticles were synthesized by chemical co-precipitation technique and primary coated with 3-trimethoxysilylpropylamine (APTS).

The secondary coating was performed using pH-responsive poly (MAA-co-AAc)-grafted PVP. The structure and morphological characterization was performed out by TEM and XRD. The chemically covalent interactions were investigated by FT-IR and elemental analysis and TGA, and DSC studied thermal stability. This carrier was loaded with two antibiotics, ciprofloxacin and ofloxacin, as model drugs (Fig. 1).

The loading kinetics and in-vitro drug release profile of the drugs were studied in three different pH (1.2, 5.5, and 7.2) buffered solutions at 37ºC. Different parameters affecting on the release of the drugs such as the amount of MNPs, initiator, cross-linker, and MAA content were evaluated.

The swelling behavior and drug release kinetics and mechanism were also studied.

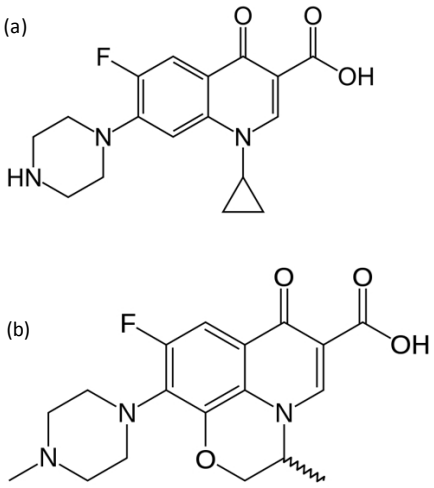


Fig. 1. Chemical structure of (a) ciprofloxacin and (b) ofloxacin

Experimental

Materials

The chemicals were listed below were purchased from Merck Company and used without further purification. Ferrous chloride hexahydrate ($FeCl_3.6H_2O$), ferric chloride tetrahydrate ($FeCl_2.4H_2O$), aqueous ammonia (25%), 3-trimethoxysilyl propylamine (APTS), methanol, acetic acid, sodium acetate trihydrate ($CH_3COONa.3H_2O$), sodium hydroxide, potassium hydrogen phosphate, Glycerol (about 87%), hydrochloric acid, potassium chloride, acrylic acid (AAc), methacrylic acid (MAA), and ethylene glycol dimethacrylate (EGDMA, 98%). Ciprofloxacin and ofloxacin were received as a gift from Faculty of Pharmacy, Tehran University. Deionized water was used throughout the experiment. 2, 2´-Azobis isobutyronitrile (AIBN, 98%) was purchased from ACROSS and polyvinylpyrrolidone (PVP, Mw40 000) was purchased from Rahavard Tamin chemical Co. (Iran, Saveh).

Instrumentation

A CM120 and anEM2085 Phillips transmission electron microscopes (Netherlands) with an accelerating voltage of 100 kV were used for characterization of particle size and morphology of APTS-Fe_3O_4 nanoparticles and polymer coated MNPs respectively. Phase characterization was performed using Phillips PW-1800 X-Ray diffraction. Characterization of Fe_3O_4 nanoparticles, APTS coated Fe_3O_4 nanoparticles, polymer coated MNPs, and drugs loaded magnetic nano-carrier were carried out by Perkin Elmer FT-IR spectrometer, Spectrum one Bv5.3.0.

Elemental analysis was performed onto the APTS-coated Fe_3O_4 nanoparticles, polymer coated MNPs, and drugs loaded magnetic nano-carrier\using Perkin Elmer 2400 SERESE II. The thermal behavior of poly (MAA-co-AAc)-grafted PVP and poly (MAA-co-AAc)-grafted PVP coated MNPs were characterized by differential scanning calorimetry and thermo gravimetric analysis, STA-1500, Rheometric Scientific. UV-Vis spectra of released drugs and other solutions were carried out by UV-240 Shimadzu UV-Vis spectrophotometer. A Metrohm 827 pH meter was used for measuring and adjusting the pH of the solutions. A water bath Memmert WB10 was used to precise control of the temperature (37 ± 0.1 °C).

Coating of Fe_3O_4 nanoparticles with APTS

Core-shell strategy was applied to synthesis the nanoparticles. First, the APTS coating was performed as follow.

The Fe_3O_4 nanoparticles were prepared via chemical co-precipitation method with some modification [34].Briefly, 11.6 g ferrous chloride hexahydrate ($FeCl_3.6H_2O$) and 4.3 g ferric chloride tetrahydrate ($FeCl_2.4H_2O$) were dissolved in 250 mL deionized water under nitrogen atmosphere with vigorous stirring as the temperature rises to 85 °C. Then 40 mL of 25% aqueous ammonia was added to the solution.

The color of the solution turned immediately from orange to black. Then the solution left to reach ambient temperature and washed with deionized water and 0.02 M sodium chloride to neutralize. Then, the magnetic suspension was placed in a 250 mL round bottom flask and allows participates to settle. The supernatant was removed, and an aqueous solution of 80 mL 10 % (v/v) APTS, followed by 60 mL glycerol was added.

The mixture was then stirred and heated at 90 °C for 2 h under nitrogen atmosphere. After cooling, the suspension was washed sequentially with 200 mL deionized water(three times), 100 mL methanol (two times) and 200 mL deionized water (three times). The MNPs dried to powders at 50 °C in oven for further coating with the polymer.

Coating of APTS- MNPs with poly (MAA-co-AAc)-grafted PVP

The free radical polymerization was carried out in 50 mL conical flask. 1 mL of 0.37% PVPin methanol and 1mL 0.49 mM of AAc were dissolved in 12 mL methanol. Variable amount of MAA (0.7-3 mL, 0.39 mM) was added simultaneously. Variable amount of EGDMA (0.7-3 mL, 0.39 mM) as cross-linker, variable amount of AIBN (0.3-1.2 mmol) as initiator and variable amount of MNPs (0-100 mg) were added to the reaction mixture after addition of monomers. Then the mixture was bubbled with N_2 gas to remove dissolved oxygen and continuously stirred under the nitrogen atmosphere for 15 min. The cross-linking reaction was completed within six hours at 60 ºC in water bath. After cooling, the polymeric particles were separated from the polymerization medium and washed three times with 200 mL methanol and five times with 250 mL deionized water. The UV-Vis spectra of the supernatant were compared with the reagents. Elimination of all reagent peaks in the supernatant confirms the removal of unreacted reagents. The collected nanoparticles were dried in oven at 50 ºC to obtain fine powder and stored in desiccators before use. Polymer with 20% (v/v) of MAA and EGDMA and 0.6 mmol of AIBN was used as plain and that with 20% (v/v) of MAA, 15% (v/v) of EGDMA, 0.6 mmol of AIBN and 10 mg of MNPs was used as the nano-carrier for further study of swelling and loading behaviors.

Characterization

The size and morphology of APTS-Fe_3O_4 nanoparticles and polymer coated MNPs was characterized using TEM images. The images were obtained by placing one drop of each sample on a carbon plate. XRD technique was used to confirm the synthesis of APTS coated Fe_3O_4 nanoparticles using Cu-Ká radiation wavelength 1.540598 Aº, matching peak position and relative intensities to the Joint Committee on Powder Diffraction Standards (JCPDS card, file No. 79-0418). To confirm the structure of APTS-Fe_3O_4 nanoparticles, polymer coated MNPs, plain polymer, ciprofloxacin-loaded and ofloxacin-loaded magnetic nanoparticles, FT-IR spectra were recorded in transmission mode in the 400-4000 cm^{-1} region for sample dispersed in KBr pellets. Stepwise increase in organic compartments (carbon, hydrogen, oxygen and nitrogen), which observed by elemental analysis, was used to confirm the coatings of MNPs and loading of the drugs. The volume-phase transition behavior of the plain polymer and polymer coated MNPs were investigated by TGA and DSC using STA techniques. It is capable of providing direct weight measurements by time-programmed thermogravimetric analysis (TGA) as well as heat consumption release by differential scanning calorimetry (DSC) to detect thermal events that do not involve mass change. The method combines the benefits of thermal analysis and differential scanning calorimetry techniques into a single experiment. Scans were run from ambient temperature to 800 ºC at a heating rate of 10 ºC min^{-1}. Plain polymer and polymer coated MNPs were dried at 50 ºC for 24 h and homogenized into fine powder before used.

Swelling behavior

To determine the pH-dependent swelling properties of the plain polymer, weighed dry samples were immersed in buffered solutions with pH of 1.2-9.0. When the swelling was equilibrated, the swollen polymer separated from the solution, their surface water was removed with tissue paper and weighed. This procedure was repeated for each pH value. The following equation was used to determine the swelling ratio:

$$swelling\ ratio\ = (W_t - W_0)/W_0$$

Where, W_t is the weight of the swollen polymer and W_0 is the weight of the polymer before swelling experiments. Each swelling process was carried out for approximately 148 h. Then, the samples were removed from the solution, weighted and dried for the FT-IR studies (not mentioned here).

Drug content and encapsulation efficiency

A 0.5 g of dried polymer and polymer coated MNPs were weighed and various amount of ciprofloxacin and ofloxacin solutions were added to make different sorbent to drug weight ratio. The solutions were stirred well to mix homogeneously and left to equilibrate for 24 h at room temperature. Unbounded ciprofloxacin and ofloxacin were determined by UV-Vis spectrophotometry at 279 and 298 nm. The supernatant from unloaded polymer and polymer coated MNPs were used as blank. As necessary, appropriate dilutions were performed to ensure that the absorbance of the drugs was in the range of Beer's

law. The % drug content and encapsulation efficiency were calculated using the following equations:

$$Drug\ content\ (\%) = (W_{Drug\ loaded}/W_{carrier}) \times 100$$

$$Encapsulation\ efficiency(\%) = (W_{Drug\ loaded}/W_{Total\ drug}) \times 100$$

Where, $W_{Drug\ loaded}$ is the weight of ciprofloxacin or ofloxacin encapsulated in the carrier. $W_{caeeier}$ is the weight of each carrier and $_{Total\ drug}$ is the total weight of free ciprofloxacin or ofloxacin. The drug-loaded carriers were washed five times with deionized water to remove unloaded drugs for further release studies.

In-vitro drug release

The in-vitro release experiments were performed in three buffered solutions at 37 ºC. The pH values were simulated to gastric fluid (pH 1.2), duodenum and intestine fluids (pH 5.5 and 7.2). Ciprofloxacin and ofloxacin loaded carriers from loading procedure were immersed into 25 mL of each buffered solution.

At certain time intervals, 6 mL of the supernatant was removed and the same volume of buffer solution was replaced. The amount of ciprofloxacin and ofloxacin released from the nano-carries were determined by spectrophotometry. The effect of different parameters on the drug release was also investigated. The data mentioned hereis the average of three replicate experiments.

Kinetic modeling of drug release

Consideration of data obtained in release experiments was easier when mathematical formulas, which can express the release results as a function of carrier characteristics, are used. Among various kinetics models, the release profile of ciprofloxacin and ofloxacin were fitted to zero-order, first-order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, and Beaker-Lonsdale models. The zero-order model describes the systems were drug release is independent of its concentration and can be represented by the equation:

$$Q_t = Q_0 + K_0 t$$

Where Q_t is the amount of drug released at time t, Q_0 is the initial amount of the drug in solution (Q_0=0 in most times) and K_0 is the rate constant. The first-order model describes the release of drug from systems where release rate is concentration dependent and can be expressed by the equation:

$$\text{Log } C = \log C_0 - K_1 t/2.303$$

Where C_0 is the initial concentration of drug and K_1 is the first-order rate constant. This relationship can be used to describe release kinetics from systems containing water-soluble drugs in porous matrix. Higuchi model is the first example of a mathematical model aimed to describe the release of drugs from insoluble matrix. The simplified Higuchi model can be expressed as:

$$Q = K_H T^{\frac{1}{2}}$$

Where K_H is the Higuchi release constant reflecting the design variables of the system.

Korsmeyer-Peppas derived a simple equation, which described drug release from polymeric system which can represent as:

$$M_t/M_\infty = K_{KC}\ t^n$$

Where $M_t/M_¥$ is a fraction of drug released at time t, K_{KC} is the release rate constant and n is the release exponent. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model. The n value is used to characterize different release mechanism for different shaped matrix as shown in Table 1.

The Baker-Lonsdale model was developed from the Higuchi model and describes the drug release from spherical matrixes according to the equation:

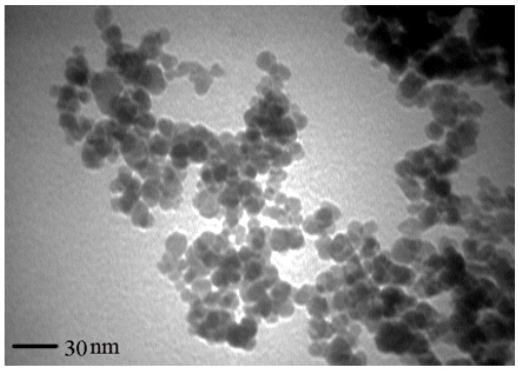
$$\frac{3}{2}[1-(1-\frac{M_t}{M_\infty})^{\frac{3}{2}}]\frac{M_t}{M_\infty} = Kt$$

Where, K is the release constant corresponding to the slope.

RESULTS AND DISCUSSION

Characterization

The TEM images of APTS-Fe_3O_4 nanoparticles and polymer coated MNPs are shown in Fig. 2. APTS-Fe_3O_4 nanoparticles image (Fig. 2a) shows good size distribution with average diameter of 8.6 ±1.6 nm and The TEM image of polymer coated MNPs (Fig. 2b) show that the polymer coated MNPs have spherical shape yet with average size of 19 ±2.1 nm. X-ray diffraction pattern of APTS coated Fe_3O_4 nanoparticles were shown in Fig. 3 and the indexing results are summarized in table 2. The pattern indicates that the



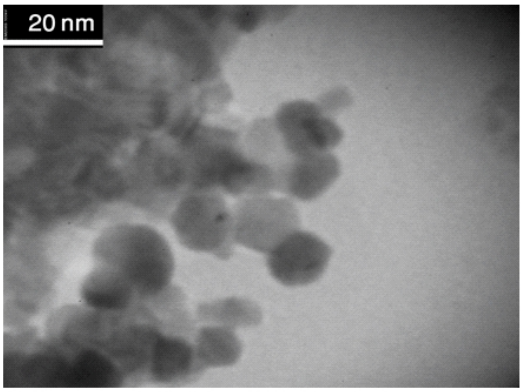


Fig. 2. TEM images of (a) APTS- Fe_3O_4 NPs and (b) polymer coated MNPs

nanoparticles have a cubic structure and modification did not change the crystal structure of the MNPs.

FT-IR is an appropriate technique to study the chemical interaction or adsorption of the reagents. The FT-IR spectra of APTS coated Fe_3O_4 nanoparticles, polymer coated MNPs, plain polymer, and ciprofloxacin and oflaxacin loaded magnetic nano-carrier are shown in Fig. 4. The absorption band of Fe-O-Si, which indicates the bonding of APTS on the

Table 1. Release exponent value and related mechanism

Drug release mechanism	Release exponent(n) for cylindrical shape	Release exponent(n) for spherical shape	Release exponent(n) for film shape
Fikian diffusion	0.45	0.43	0.5
Anomalous (non Fikian)	0.45 < n < 0.89	0.43 < n < 0.85	0.5 < n < 1
Case-II transport	0.89	0.85	1
Super case-II transport	n < 0.89	n < 0.85	n < 1

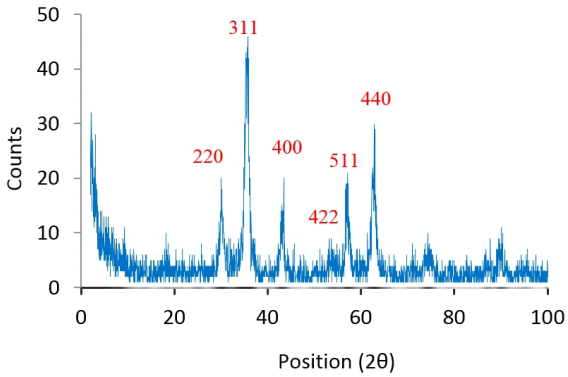


Fig. 3. The XRD pattern of prepared APTS coated MNPs

magnetic nanoparticles, cannot be seen in the FT-IR spectrum because it appears at around 585 $cm^{-1}$and therefore overlaps with Fe-O vibration band of the nanoparticles. However, two bands at 1125, 1039 and 979 cm^{-1} assigned to Si-O-H and Si-O-Si vibration bond. Similarity in the spectra of the plain polymer and polymer coated MNPs (Fig. 4b and c)beside the presence of characteristic peak of Fe_3O_4 nanoparticles absorption peak along with its absence in plain polymer confirm the coating of the polymer onto the APTS coated nanoparticles. Loading of each drug was confirmed with addition of absorption peaks in 1625 cm^{-1} (Fig. 4d) and 1622 cm^{-1} (Fig. 4e) which is assigned to C=C ring stretching of ciprofloxacin and ofloxacin. Fig 5 shows the TGA-DSC plots of the plain polymer and polymer coated MNPs. From Fig. 5a, a weight loss with endothermic peak at about 60 °C may attribute to the physically adsorbed water. The three exothermic peaks at 345, 388 and 445 may attributed to the degradation of PVP and poly carboxylic acid network. As can be seen in Fig. 5b, polymer coated MNPs show good thermal stability until 300°C. Then, the degradation

Table 2. Comparison of APTS-MNPs peak position (2q) values and diffracting plane index with naked Fe_3O_4 nanoparticles

Sample/ phase	Peak position (2θ) (experimental)	Peak position (2θ) (standard)	Diffraction plane (h k l)
Fe_3O_4 (cubic)	30.1	30.1	2 2 0
	35.53	35.5	3 1 1
	43.08	43.1	4 0 0
	53.48	53.7	4 2 2
	57.16	57.3	5 1 1
	62.73	62.7	4 4 0

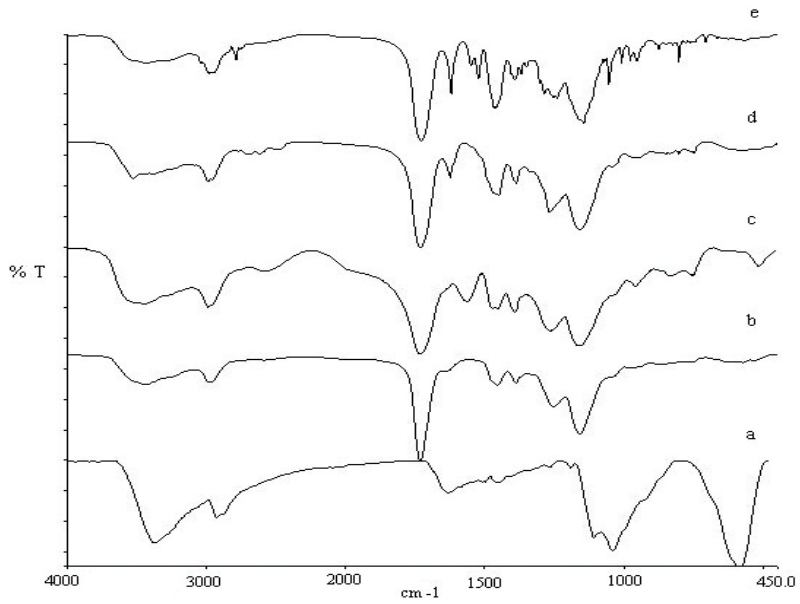


Fig. 4. FT-IR spectra of (a) APTS-Fe_3O_4 NPs,(b) polymer coated MNPs, (c) plain polymer, (d)ciprofloxacin loaded nano-carrier and (e) ofloxacin loaded nano-carrier

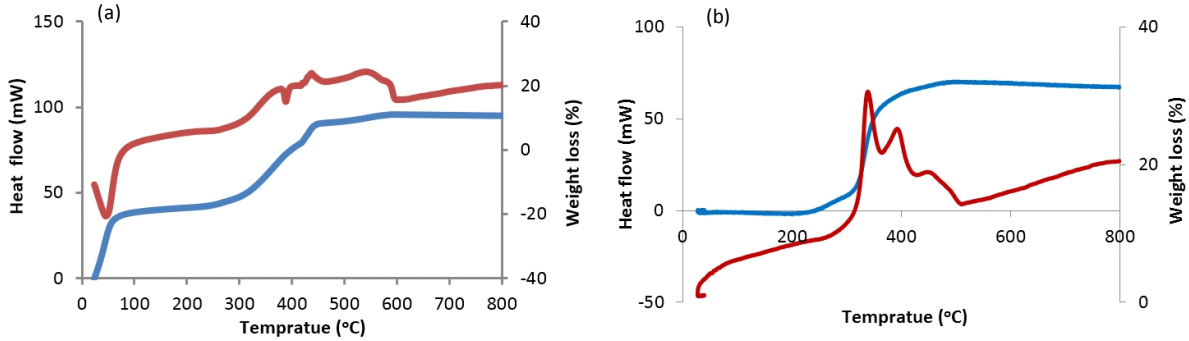


Fig. 5. TGA-DSC plots of (a) plain polymer and (b) polymer coated MNPs

will occur. Furthermore, elemental analysis was performed onto APTS-Fe_3O_4NPs, polymer coated MNPs and ciprofloxacin-loaded and ofloxacin-loaded magnetic nano-carriers.

The results show an increase in the amount of organic compounds (hydrogen, carbon and nitrogen), which confirm the functionalization of MNPs with APTS, coating with the polymer, and loading of the drugs.

Swelling studies

The pH-sensitive swelling studies of poly (MAA-co-AAc)-grafted PVP in different buffer solutions were shown in Fig. 6.

As can be seen from the Fig, increase in the pH of buffer solution was caused the increase in swelling ratio of the polymer up to pH 8.5 to form a gel media. Both PMAA and PAAc monomers are weak acids with pK_a 7.0 and 6.6 respectively[35]. In the pHs lower than the pK_a, high concentration of the H^+ ions cause most carboxylic groups were in the form of protonated non-ionic (-COOH) groups. Hydrogen bonding constructed by the carboxylic acid groups together and with –C=O groups of PVP led to the stronger interaction between polymer chains and decrease the swelling (gel collapsing).

As the pH increased above the pK_a, ionization of the carboxylic groups occurs and the electrostatic repulsion between the same charged groups are favor of the swelling. Other authors reported similar observation previously [34, 36].

Table 3. Loading characteristics of plain polymer and polymer coated MNPs for ciprofloxacin and ofloxacin

Sorbent	Sample	Weight ratio (Sorbent: Drug)	Drug content (%)		Encapsulation efficiency (%)	
			CIPRO	OFLOX	CIPRO	OFLOX
Plain polymer	Sample I	2.5:1	36.4-38.8	38.4-39.2	91-97	96-98
	Sample II	10:1	8.8-9.0	9.1-9.4	88-90	91-94
	Sample III	25:1	3.3-3.5	3.4-3.5	83-89	87-89
Polymer coated MNPs	Sample I	2.5:1	34.8-36.4	35.6-37.7	87-91	89-94
	Sample II	10:1	7.9-8.7	8.1-9.1	79-87	81-91
	Sample III	25:1	2.7-3.1	2.9-3.2	68-77	73-80

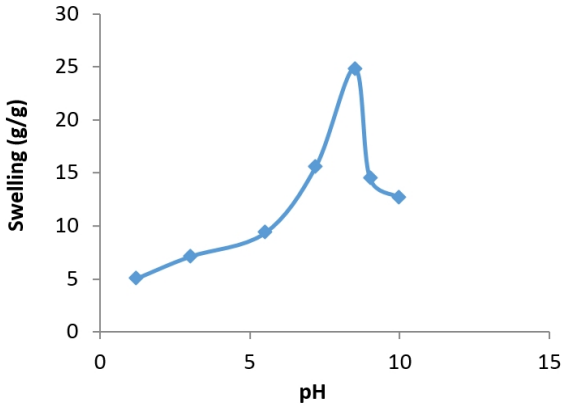


Fig. 6. The swelling behavior of poly (MAA-co-AAc)-grafted PVP in different pH

Drug content and encapsulation efficiency

The drug content and encapsulation efficiency of plain polymer and polymer coated MNPs (carriers) were determined by varying the weight ratio of the carrier to drugs. Table 3 summarizes the loading characteristics of different weight ratio for plain polymer and polymer coated MNPs. The long entrapment time (24 h) would allow a good chance for interaction between the polymer and the drug. For plain polymer, when the weight ratio of the carrier to drug was 2.5:1, the drug content was about 37.6-38.8 % with encapsulation efficiency of 94-97 %. With increasing the carrier to drug ratio (i.e. the amount of the drug per carrier mass unit decreased), a decrease in encapsulation efficiency occurs. The same behavior was observed for polymer coated MNPs with slightly decreased in drug content to about 35.6-36.6 % (encapsulation efficiency of 89-91 %). This may occurs due to lower polymer content in polymer coated MNPs carrier with the same weight as plain polymer which decreases the overall interactions of the drug with the carrier.

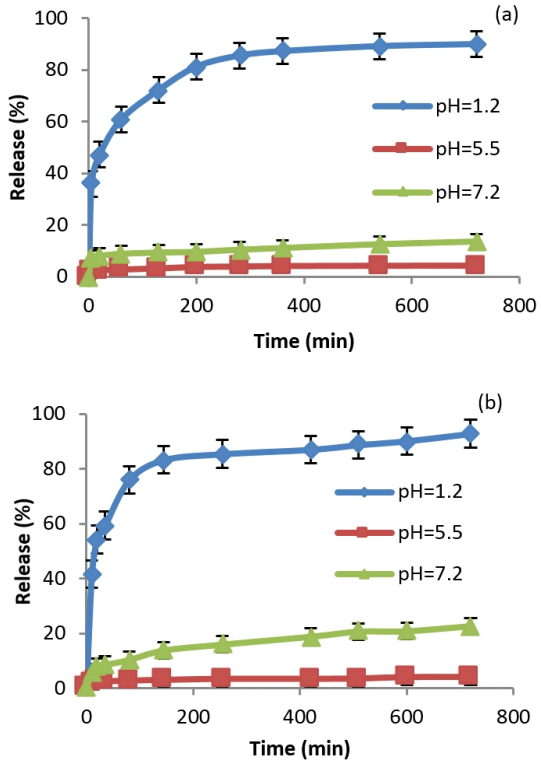


Fig. 7. The release profile of (a) ciprofloxacin and (b) ofloxacin in different pH

Drug release studies

Effect of pH

The drug release profiles of ciprofloxacin and ofloxacin for polymer coated MNPs were shown in Fig. 7. As could be seen, the drug release at pH 1.2 is much faster than at pH 5.5 and 7.2. As mentioned before, when the pH is increased from 1.2 to 7.2 (above the pK_a of The PMAA and PAAc), an increases in dissociation of polymer carboxylic groups occurs, which facilitate the interaction of anionic carboxyl groups of the polymer with amine group of the drugs

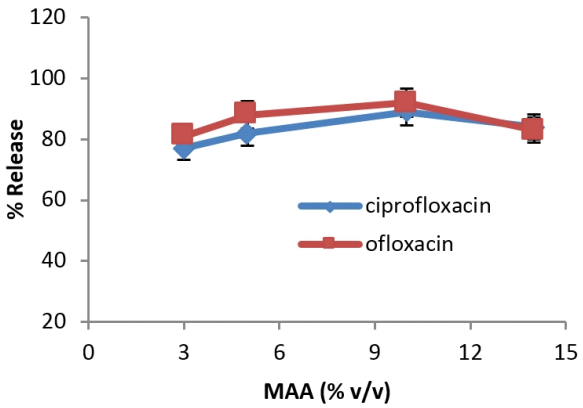


Fig. 8. Effect of amount of MAA on the release of ciprofloxacin and ofloxacin

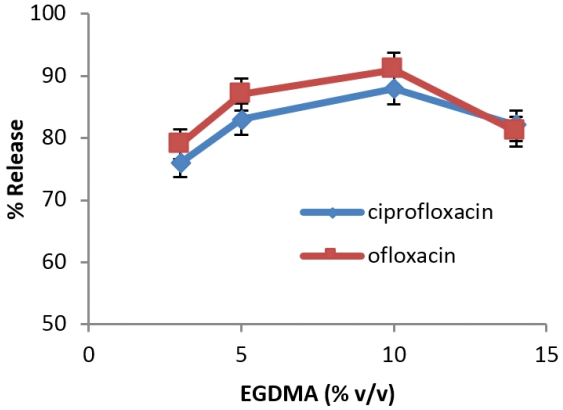


Fig. 9. Effect of amount of EGDMA on the release of ciprofloxacin and ofloxacin

and probably interaction of carboxyl group of the drugs with the amine groups of APTS. Furthermore, when the interactions between the polymer and the drugs occur, an insoluble complex is formed, which further retards the release. In addition, at pH values above the pK_a's, the carboxylic acid groups of the polymer ionized and repulsion of the chains cause expansion of the polymer and form a gel that slows drug release rate. The sudden dip in release rate of ciprofloxacin and ofloxacin in pH 5.5 and 7.2 may be explained in part by these phenomena. The effect of decrease in drug release with increasing pH, i.e. increase in the interaction of anionic polymers with cationic drug which decrease the amount of drug release, were observed by many authors [37-39].On the other hand, the swelling of the polymer increases with increasing the pH value, thus, a little increase in atorvastatin release % was observed in pH 7.2 with respect to pH 5.5 may explained by increasing diffusional pass due to expanding the polymer.

Effect of the amount of MNPs

The effect of amount of MNPs on the ciprofloxacin and ofloxacin release was studied by varying the amount of MNPs incorporated in the carrier in the range of 10-100 mg.

Experimental results indicate that the amount of the drugs released from the carrier decreased with increasing the amount of MNPs. This observation can be explained by decreasing in polymer content of the carrier with increasing in the MNPs amount, which decreases total interactions of the drugs due to lower pore density. Thus decreased the amount of drug released.

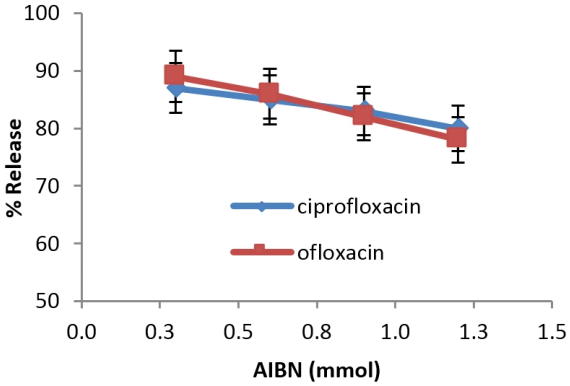


Fig. 10. Effect of amount of AIBN on the release of ciprofloxacin and ofloxacin

Effect of methacrylic acid content

Variation in the amount of MAA can alter the amount of carboxylic acid groups in the polymeric shell and altering in chemical structure can affects on the release of the drugs. Thus the amount of MAA varies in the range of 3-14 % (v/v), in presence of 10 mg Fe_3O_4 nanoparticles. As could be seen from Fig.8, increase in MAA content up to 10 % (v/v) raises the drug released from the carrier because of increase in chain porosity, therefore hydrodynamic free volumes increase which increases the drug release. A drop in the release after this point could be explained, as the polymeric chains on the MNPs were largely crowded and the release of drug molecules not allowed.

Effect of the amount of cross-linker

The effect of cross-linker was studied by varying the amount of hydrophobic EGDMA cross-linker in the range of 3-14 % (v/v) in presence of 10 mg Fe_3O_4

nanoparticles. The release results are shown in Fig. 9. The amount of cross-linker affects on the porosity and free volume accessible to the drugs. From Fig. 9, the release of the two drugs increases with increasing EGDMA amount up to 10%, which may be explained by network loosening that, occurs in lower cross-linker density. Decrease in drug release after this point may be explained by hydrophobic nature of EGDMA, which may affect on the interaction of the drugs with the polymer. In addition, decrease in free volume accessible occurs due to more density cross-linked polymer with increasing in the amount of EGDMA.

Effect of the amount of initiator

The amount of AIBN as initiator was varied in the range of 0.3-1.2 mmol in presence of 10 mg Fe_3O_4 nanoparticles to study the effect of amount of initiator on the release of ciprofloxacin and ofloxacin. Varying in the amount of initiator can affect on molecular weight of prepared polymer. The experimental results are shown in Fig. 10. Our observation reveals a decreasing pattern with a gentle slope, which may be due to small pore size polymer constructed with higher amount of initiator. These events can also affects on the loading efficiency and drug content of the carrier. Obtained results from loading experiments (not shown here) confirmed this observation.

Kinetic modeling of drug release

Different semi-empirical kinetic equations (zero-order, first-order, Higuchi, Korsmeyer-Peppas Hixson-Crowell and Baker-Lonsdale) were applied to interpret the release rate from the matrix. The model best fitted to the release data was evaluated by correlation coefficient (R^2). For ciprofloxacin release, the release data of plain polymer and polymer coated MNPs at pH 1.2 and 5.5 followed Korsmeyer model (R^2 between 0.95-0.99). But for pH 7.2, the data primary followed Higuchian release model and secondary followed first-order model. The release exponent values from Korsmeyer-Peppas equation demonstrating the tendency of drug release by Fickian or mechanism and showing effect of diffusion phenomena for controlled drug release for all experiments. In the case of ofloxacin, all release data followed Korsmeyer model (R^2 between 0.96 to 0.98) except for release of ofloxacin from plain polymer at pH 7.2, which primarily followed Higuchian release pattern (R^2 =0.99) and secondarily followed Korsmeyer model (R^2 =0.98). However, for all other experiments, Higuchian release were observed to be the best in second step, the release exponent values (n) from Korsmeyer-Peppas equation which are in the range of 0.19-0.38 demonstrating Fickian mechanism and diffusion controlled release. Furthermore, comprising between cumulative drug release in different pH and swelling behavior of the polymer confirm that among different physical and chemical phenomena which affect the drug release kinetics, diffusion phenomena predominate in the studied drug delivery system. In low pH which swelling is in the minimum value, the maximum release was occurred. In addition, plot of Korsmeyer-Peppas kinetic rate constant versus pH reveals that at pH 1.2, the average of K value were high and when the pH was increased to 5.5, the rate constant decreased to a minimum value. After which the rate constant a little increased. Therefore, there is a direct relationship between total percent drug released and K. increase in rate constant from pH 5.5 to 7.2 maybe as a result of increase in diffusional pass, therefore increase in release of drugs.

CONCLUSION

In this study, a new pH-responsive polymer, poly (methacrylic acid-co-acrylic acid)-grafted polyvinyl-pirrolidone was successfully coated on the surface of APTS coated Fe_3O_4 nanoparticles. Free radical polymerization was used to form core-shell structure. The size and morphology of synthesized APTS-MNPs and polymer coated APTS-MNPs was shown the spherical nanoparticles with mean diameter of 8.6 and 19 nm respectively. X-Ray diffraction pattern confirms the appropriate synthesis of the APTS-MNPs and FT-IR analysis show the graft of APTS-MNPs with polymer and loading of two drugs. TGA and DSC were also applied to study the thermal behavior of the plain polymer and polymer coated MNPs. Two antibiotics, ciprofloxacin and ofloxacin were selected as model drugs to investigate loading and release study of the carrier. Three different buffered Solutions with pH 1.2, 5.5, and 7.2 were selected as a release medium and all the release experiments were done at 37 ºC to simulate the body conditions. The results show that the amount of drug release was about 92 % in pH 1.2. In pH 5.5 virtually no drug released from the carrier, while in pH 7.2, a little increase in drug

release was observed due to increasing in swelling of the polymer. The effect of different parameters on the release of the drugs and kinetic and mechanism of the release were also investigated. The results indicate that the prepared magnetic nano-carrier could be suitable for site-specific antibiotic delivery through oral administration.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Mohammad Mashhadizadeh for his scientific supports and information.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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