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Synthesis, Characterization and Drug Delivery Profile of Magnetic PLGA-PEG-PLGA/Maghemite Nanocomposite

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Summary: The antibiotic cotrimoxazole was associated to the multi-block copolymer containing poly(D,L-lactic-glycolic acid) (PLGA) and poly(ethylene glycol) (PEG) segments, PLGA-PEG-PLGA, aiming to reach a controlled drug release system. Block copolymer was synthesized via polycondensation of lactic acid and glycolic acid with PEG in situ. In turn, maghemite was synthesized through the co-precipitation method. The drug cotrimoxazole was inserted in the composite through melting mixing method. Several techniques were used to characterize the materials. The materials were characterized by Nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and magnetic force, this last according to the methodology developed by our group. In addition, dissolution profile was studied. These dissolution tests were performed with and without magnetic field, aiming to study the influence of the magnetic field on the dissolution profile. The dissolution was monitored and quantified using the ultraviolet-visible spectrophotometry (UV-Vis), following the USP method for cotrimoxazole tablets. Results demonstrated that nanocomposites presented a good magnetic force, able to keep the magnetic composite trapped in a specific place or tissue. Furthermore, in the presence of a magnetic field, the magnetic nanoparticles were able to perform a magnetic constriction of the material, making the drug release faster than in the absence of the magnetic field. This phenomenon may be useful to perform a fine tuning of the system, allowing the easier adjust of the speed and amount of released drug, useful to improve medical treatments and even the welfare of the patients.

Keywords: block copolymer; cotrimoxazole; drug delivery; maghemite; magnetic composites

Introduction

The spatial and kinetic control of the drug delivery offers many advantages when compared to the conventional release.[1] These controlled drug delivery systems usually use biocompatible magnetic nanoparticles, as maghemite,[2–4] to promote the spatial control and synthetic biodegradable polymers as drug carriers to promote the kinetic control.[5] Among the polymers, PLGA is the most used for this purpose because of its well known safety use in humans.[6]

PLGA is a nontoxic and biocompatible polyester, approved by the FDA.[7] However its properties can be improved by combination with other polymers, such as PEG.[8] Blends of PEG and PLGA improve the rate of degradation and the hydrophilicity, decreasing the acidity of PLGA degradation products. Hydrophilic PEG segments in the copolymers of PLGA can also improve the diffusivity of the water.[8]

The drug cotrimoxazole is a combination of two antibiotics, trimethoprim and

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sulfamethoxazole in a fixed ratio 5:1. This combination is widely used in the treatment of infections.[9] A recent study shown that the use of cotrimoxazole in prophylaxis for pneumonia in HIV infected patients reduces morbidity and mortality, being recommended by the World Health Organization (WHO).[10] All these treatments would benefit by the possibility of controlling the release profile of the drug mentioned.

To the best of our knowledge, this work illustrates for the first time the production of magnetic composites based on PLGA-PEG-PLGA containing cotrimoxazole prepared by melt mixing. Several techniques were used to characterize the materials, such as ^{1}H-NMR, FTIR, XRD, and magnetic force. In addition, the drug delivery was studied with and without the presence of the magnetic field, proving the influence of the magnetic constriction on the dissolution profile of the magnetic nanocomposites.

Experimental Part

Materials

Lactic acid (85–90%), glycolic acid (57%), sulfuric acid, hydrochloric acid, ammonium hydroxide, ferric chloride and sodium sulfite were purchased from VETEC. PEG (Mn 6000) was purchased from SIGMA-ALDRICH. The trimethoprim was manufactured by the laboratory Dinalab and sulfamethoxazole by the laboratory Andhra and provided by Farmaguinhos (Fiocruz-Brazil). The drug Bactrim F ® Roche was purchased at a conventional pharmacy. All materials were used as received. Cotrimoxazole (CTZ) was prepared by mixing the drugs trimethoprim and sulfamethoxazole in a ratio of 5:1 in weight.

Synthesis of the Block Copolymer

The PLGA-PEG-PLGA block copolymer (BC) was synthesized from lactic acid (50 ml) and glycolic acid (62 ml) in equimolar ratio (1:1), in the presence of PEG (5 g). Sulfuric acid (0.25 mL) was used as catalyst, in a closed system under nitrogen atmosphere and slight vacuum. The reaction medium was kept at 150 °C under magnetic stirring for 10 h.

Synthesis of Maghemite

Maghemite was prepared as described by our group elsewhere.[4,11–17] Aqueous solutions of hydrochloric acid (2 M), ferric chloride (2 M), and sodium sulfite (1 M) were prepared. Into a beaker, under continuous agitation, 30 ml of the ferric chloride solution and 30 ml of deionized water were added. Soon afterwards, 20 ml of the sodium sulfite solution was added to the beaker, still under continuous agitation. The reaction product was precipitated by slowly adding 51 ml of concentrated ammonium hydroxide into the beaker under continuous stirring. The medium was poured after 30 min. The obtained particles were washed several times using distilled water and finally dried at 60 °C in an oven at 250 °C for 1 h.

Preparation of Block Copolymer/Drug and Magnetic Composite/Drug Systems

Two samples of the block copolymer were molten at 120 °C. This temperature was chosen because it is considerably lower than the degradation temperature of the drugs (Sulfamethoxazole 205 °C; Trimethoprim 310 °C).[18] The cotrimoxazole was incorporated in the first copolymer sample at the same amount of commercial Bactrim F® (960 mg). Obtained material was named BC-CTZ. The second copolymer / drug sample was prepared following the last steps plus the addition of 5% w/w of maghemite, to form the sample named Mag-CTZ.

Dissolution Test of Cotrimoxazole

The dissolution tests were performed following the United States Pharmacopeia (USP) method for Sulfametoxazole/Trimethoprim tablets (900 ml of HCl 0.1N, apparatus I, 75 rpm).[20] First the test was conducted with commercial Bactrim F® tablets, and then with a known mass of BC-CTZ and Mag-CTZ, equal to 1g. The sampling times used were: 0,25 h; 0,5 h; 1 h; 2 h; 2,25 h; 2,5 h; 3 h; 4 h; 4,25 h; 4,5 h; 5 h and 6 h. The drug delivery profile of Mag-CTZ

was also studied in the presence of magnetic field, produced by a Nd magnet, which was placed under the basket (apparatus I - USP) of the dissolution equipment providing a magnetic field equal to 4000 Gauss on the sample. All these tests were performed in triplicate.

Materials Characterization

Block copolymer was studied using Hydrogen-1 Nuclear magnetic resonance (^{1}H-NMR) on a Varian® equipment model Oxford 300. Samples were dissolved in deuterated chloroform (80 mg in 0.7 ml).

The copolymer, the samples BC-CTZ, Mag-CTZ and the magnetic nanoparticles were characterized by Fourier Transform Infrared (FTIR) using KBr pellets. The analysis were performed on a Varian equipment model 3100 FTIR Excalibur Series using a resolution of 4 cm^{-1} and 20 scans from 4000 to 400 nm.

The copolymer, the samples BC-CTZ, Mag-CTZ and the magnetic nanoparticles were characterized by X-Ray Diffraction (XRD). Tests were performed using a Rigaku Miniflex X-ray diffractometer in a 2θ range from 2° to 80° by the method FT (fixed time). Used steps were equal to 0.05° and time of 1s, using a tube voltage and current equal to 30 kV and 15 mA, respectivelly. The radiation used was CuKα = 1.5418 Å.

Magnetic force tests were performed using a homemade experimental setup, described elsewhere.[19] This setup is constituted by an analytical balance Shimadzu AY-220, a voltage source ICEL PS-4100, a digital multimeter ICEL MD-6450, a gaussmeter GlobalMag TLMP-Hall-02; a homemade sample holder and a home-made electromagnet. System calibration was performed in the absence of magnetic material. First, using the ampermeter and the gaussmeter, a current versus magnetic field calibration was performed. Soon afterward a current versus mass calibration was also performed. Obtained results were used to predict part of the presented error. Magnetic force tests were performed following the mass variation of the sample in the presence of different magnetic field, produced by the electromagnet. Then, the apparent variation of mass of the sample in the presence of magnetic field was calculated subtracting the mass of the sample in the presence of magnetic field from the mass of sample. The magnetic force (opposite to gravitational one) was calculated according to Equation (1).

$$F_m = \Delta m \cdot g \quad (1)$$

where F_m is the magnetic force, Δm is the apparent variation of mass in the presence of the magnetic field and g is the acceleration of gravity. As reference, the magnetic force of a cobalt (II) chloride hexahydride standard sample was calculated according to this method and obtained result is equal to (0.18 ± 0.02) mN at (838 ± 1) Gauss.

Ultraviolet-visible spectrophotometry (UV-Vis) measurements were performed at 206 nm using a monochromator Biospectro spectrophotometer model SP-220. The spectra of the solutions with known concentrations of cotrimoxazole were collected and used to set up the analytical curve (absorbance versus concentration curve).

Results and Discussion

Figure 1 shows the ^{1}H-NMR spectrum of the copolymer. In this spectrum, the peak at 3.64 ppm is assigned to methylene present in PEG. In turn, the peak at 1.5 ppm is associated with the repetitive methyls groups of the lactic acid. Finally, the peaks at 5.2 ppm and 4.8 ppm are related to the $CH\text{-}CH_3$ from lactic acid and CH-H from glycolic acid, respectively, confirming the synthesis of the block copolymer.[21]

FTIR was also used to characterize the materials and obtained spectra are shown in Figure 2. The FTIR spectrum of the maghemite (see Figure 2(a)) shows characteristic peaks at 3400 cm^{-1} corresponding to the stretching of OH bond on FeOH. In turn, the peaks at 630 and 570 cm^{-1} are assigned to the Fe—O stretching for phases α-Fe_2O_3 and ε-Fe_2O_3, respectively.[22] The FTIR spectrum shown in Figure 2(b)

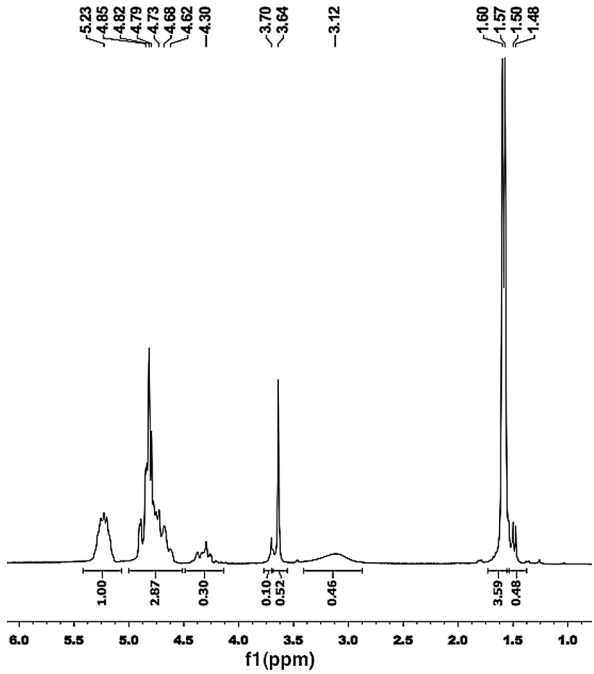

Figure 1.
^{1}H-NMR spectrum of the copolymer.

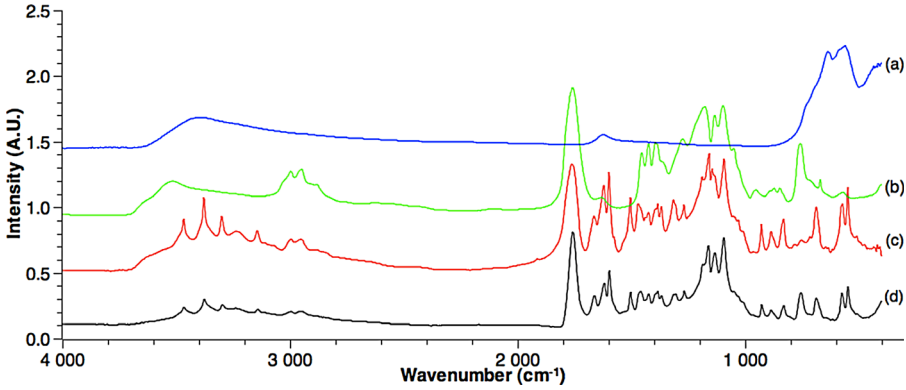

Figure 2.
FTIR spectra of maghemite (a), copolymer (b) Mag-CTZ (c) and BC-CTZ (d).

corresponds to PLGA-PEG-PLGA copolymer. The peak at 1752 cm^{-1} is attributed to the stretching vibration of the C=O, the peak at 1182 cm^{-1} can be assigned to the ether group and the peaks at 1130 cm^{-1} and 1452 cm^{-1} are attributed to CO bond and CH bond of the methyl group, respectively. In turn, the spectrum shown in Figure 2(c) corresponds to the sample Mag-CTZ. This spectrum presents peaks described for copolymer and maghemite. In addition, this spectrum and the next one, which corresponds to the block copolymer containing the drug (see Figure 2(d)), also shown the characteristic peaks of the cotrimoxazole, in 1150 and 1070 cm^{-1}

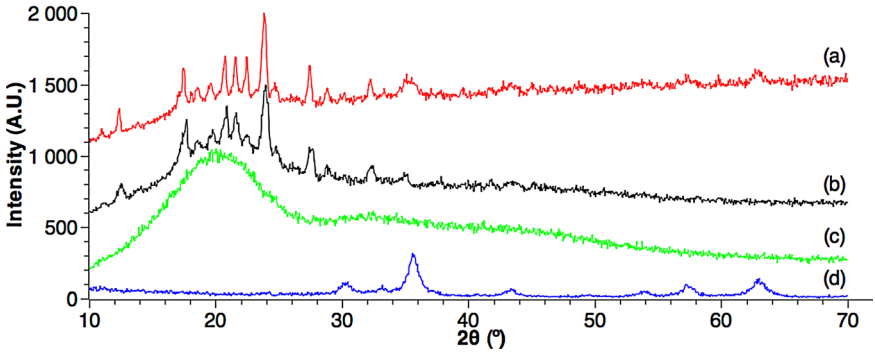

Figure 3.
X-ray diffraction patterns of Mag-CTZ (a), BC-CTZ (b), copolymer (c) and maghemite (d).

related to the stretching vibration of the ether C—O bonds, in 3400 cm^{-1} related to NH_2 in free aromatic amines. I addition, this spectrum also shown other characteristic bands of the cotrimoxazole:the stretch of C=N bond at 1615–1700 cm^{-1}, the stretch near 1340 cm^{-1} regarding the C—N stretching vibration of aromatic and the stretch correspondent to SO_2 bond in 1350 cm^{-1}.[22]

Samples were also studied using XRD and the diffractograms obtained are shown in Figure 3. Pure maghemite presented characteristic peaks in 30.2°, 35.6°, 43.3°, 53.3°, 57.3° and 62.9°, which correspond to crystal planes (220), (311), (400), (422), (511), and (440) in the spinel structure of an orthorhombic cell, respectively.[2,19,23] Moreover, these nanoparticles have a crystallite size (Lc) equal to 12 ± 1 nm, determined by the Scherrer equation[24] using the peak corresponding to the (311) crystal plane.[19,25,26]

The diffractogram of PLGA-PEG-PLGA copolymer, shown in Figure 3(b) presents only amorphous halos, mainly due to the presence of the small amount of the PEG, equal to 3.5% of the total mass. The diffraction of the sample BC-CTZ, shown in Figure 3 (c) presents the amorphous halo from the copolymer and the crystalline peaks from the drug cotrimoxazole, located at 2θ equal to 12.23°, 16.50°, 18.29°, 20.74°, 24.76°, 26.58°, 28.33° and 32.49°.[18] The XRD pattern of the sample Mag-CTZ, shown in Figure 3 (d) also presents the amorphous halo from the copolymer and the corresponding peaks of the drug. In addition, this XRD pattern also shown the peaks of the maghemite, described earlier.

The results of the magnetic force test are show in the Figure 4. Pure maghemite presented a magnetic force very high (see Figure 4 (a)), which is important to several applications, such as the targeting of the drug delivery.[27] On the other hand, when maghemite is incorporated into the copolymer forming the nanocomposite the magnetic force increases (see Figure 4 (b)) suggesting that the nanoparticles are dispersed in the copolymer.

The dissolution of the Bactrim F® commercial tablets, the polymer and the composite (with and without the presence of the magnetic field) containing the drug

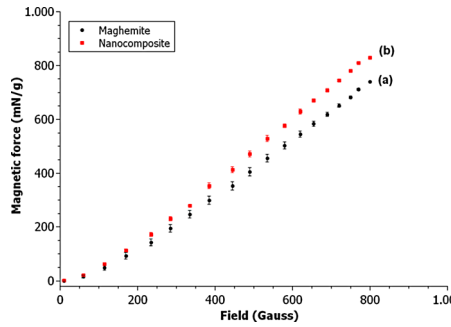

Figure 4.
Magnetic force test of the pure maghemite (a) and Mag-CTZ (b).

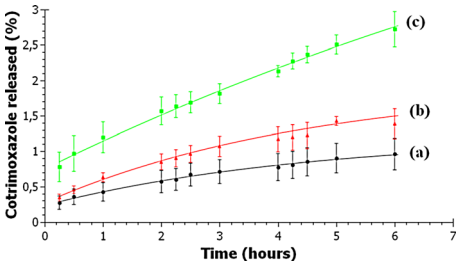

Figure 5.
Cotrimoxazole released along experimental time: system BC-CTZ without magnetic field (a); system Mag-CTZ studied in the absence (b) and presence (c) of the magnetic field.

were monitored by UV-Vis analysis and results are shown in Fig. 5. In the first case, using the Bactrim F®, the drug released achieved 100% in one hour. The results obtained from the other cases are shown in Figure 5.

Figure 5 shows that, for all of the studied cases, the dissolution profile remained sustained and none of the samples exceeded 3% of cotrimoxazole released along experimental time. Therefore, all samples can be considered as useful as drug delivery systems. Figure 5 also allows to infer that the presence of the nanoparticles in the composite produced a faster release, in comparison with copolymer. This may be occurring due to the capability of nanosized metal oxides posses to act as polymer degradation agents,[28] decreasing the molar mass, responsible by the faster release profile. The magnetic field also seems to produce interference into the dissolution profile of the drug. In addition, under a magnetic field, as shown in a previous work of the group,[1] the magnetic nanoparticles are able to produce a magnetic constriction of the material, making the drug release faster than in the absence of the magnetic field. This magnetic constriction phenomenon is shown in Figure 6. For that it was used 2 beakers, each containing 40 ml of

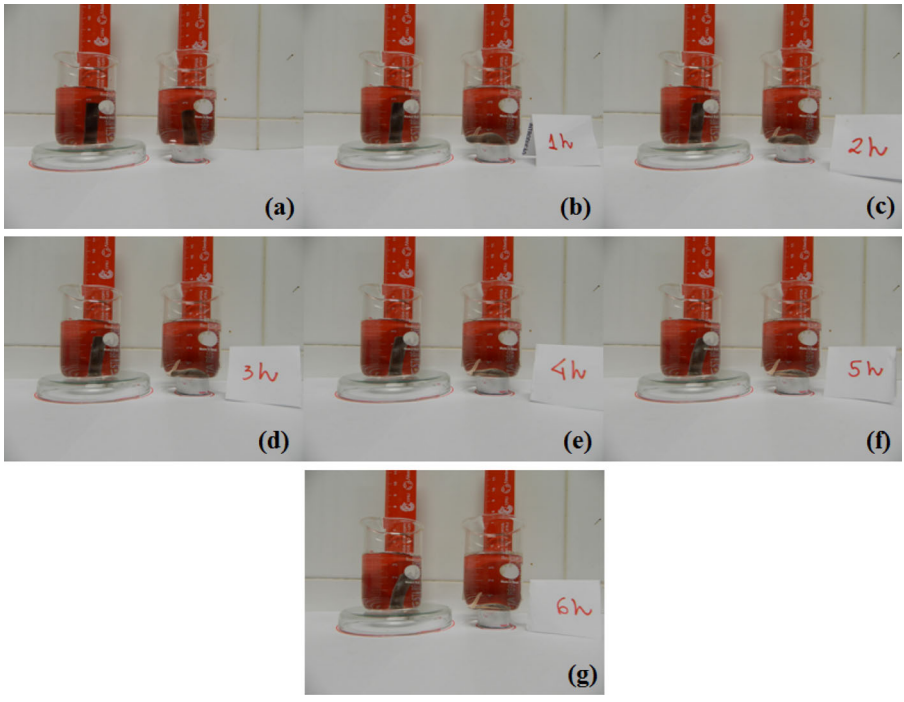

Figure 6.
Magnetic constriction experiment: 0 hour (a), 1 hour (b), 2 hours (c), 3 hours (d), 4 hours (e), 5 hours (f) and 6 hours (g) of experiment without (left) and with (right) the presence of the magnetic field produced by a Nd magnet.

hydrochloric acid (0.1N) and a sample of the composite, one was submitted to a magnetic field, and the other was used as control. As can be seen, after 1 hour of the experiment, the one submitted to the magnetic field is completed misshaped, while the control did not suffer any change. Therefore, the presence of the magnetic filler associated with the use of the magnetic field could be useful to perform a fine tuning of the system, allowing the easier adjust of the speed and amount of released drug, which may improve medical treatments.

Conclusion

The PLGA-PEG-PLGA copolymer, maghemite, and the nanocomposites containing the drug were successfully synthesized and characterized. The release profile of cotrimoxazole inserted in the copolymer and the nanocomposites were monitored by ultraviolet analysis and shown to be well sustained. In the interval 6 hours, the nanocomposite seems to release the drug a little faster than the copolymer and this behavior can be attributed to the fact that nanosized metal oxides acted as polymer degradation agents, decreasing the molar mass. In addition, in the presence of a magnetic field, the magnetic nanoparticles are able to perform a magnetic constriction of the material, making the drug release faster than in the absence of the magnetic field, which is an interesting result, able to provide differentiated health treatments, useful to improve the welfare of the patients.

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