PLA-b-PEG/magnetite hyperthermic agent prepared by Ugi four component condensation

L. P. Icart¹, E. R. F. dos Santos², E. D. Pereira³, S. R. Ferreira⁴, V. Saez¹, J. A. Ramon¹,
M. Nele², J. C. S. Pinto⁵, R. D. Toledo⁴, D. Z. Silva¹, F. G. Souza Jr^{4*}

¹Departamento de Macromoléculas, Centro de Biomateriales, Universidad de la Habana, av. Ronda 4400, Vedado, La Habana, Cuba

²Escola de Química, Centro de Tecnologia-Cidade Universitária, av. Horacio Macedo, bloco E. Universidade Federal de Rio de Janeiro, 2030 Rio de Janeiro, Brasil

³Instituto de Macromoléculas: Professora Eloisa Mano, Centro de Tecnologia-Cidade Universitária, av. Horacio Macedo, 2030, bloco J. Universidade Federal de Rio de Janeiro, 2030 Rio de Janeiro, Brasil

⁴Programa de Engenharia Civil, COPPE, Centro de Tecnologia-Cidade Universitária, av. Horacio Macedo, bloco I. Universidade Federal de Rio de Janeiro, 2030 Rio de Janeiro, Brasil

⁵Programa de Engenharia Química, COPPE, Centro de Tecnologia-Cidade Universitária, av. Horacio Macedo, bloco G. Universidade Federal de Rio de Janeiro, 2030 Rio de Janeiro, Brasil

Received 6 July 2015; accepted in revised form 1 October 2015

Abstract. Ugi four component condensation (UFCC), is an important tool for the synthesis of different types of bioconjugate species. In this study, a PLA-PEG/magnetite magnetic composite was prepared by a synthetic-route approach based on UFCC. In particular, poly(lactic acid) (PLA) was synthesized by autocatalytic polycondensation. Also, poly(ethyleneglycol) bis-amine (bis-amine PEG) was synthesized by two different methods: via carbonyldiimidazol (CDI)/ethylenediamine (ED) (75% yield) and via chlorate monochlorated acetyl (CCA)/ED (95% yield). All products were characterized by gel permeation chromatography (GPC), hydrogen-1 nuclear magnetic resonance (NMR ¹H), Fourier transform infrared (FTIR), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). In addition, magnetite was prepared and modified to generate aldehyde groups which are also necessary for UFCC. This product was characterized by DSC, TGA, X-ray diffraction (XRD) and magnetic force (MF) techniques. Also, the magnetic composite PLA-PEG/magnetite was synthesized by UFCC. The calculated yield was equal to 80%. Furthermore, magnetic microspheres were prepared by the procedure of emulsion solvent-evaporation and characterized by scanning electron microscopy (SEM) and magnetic induction hyperthermia (MIH). The main contribution of these results is to propose a new application for UFCC in the preparation of biomasked magnetic drug delivery systems able to improve the cancer treatment and even the welfare of the patients.

Keywords: biodegradable polymers, Ugi reaction, magnetic composites, hyperthermic agent

1. Introduction

Drug delivery systems (DDS) is one of the most exciting research fields of pharmacy, chemistry and other related sciences [1–8]. The clinical benefits have been demonstrated by dozens of products that have already been approved for use in humans and

several types of DDS have been approved by regulatory agencies [9].

Many of these systems are based on polymers. Among them, the most widely used are polyethylene glycol (PEG) and the poly(lactic acid) family, including its copolymers with glycolic acid [9, 10]. The

*Corresponding author, e-mail: fgsj@ufrj.br
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most important feature of PEG is its potential for biomasking [11–13]. The conjugation of PEG to DDS or drugs (e.g. peptides and proteins) prevents recognition by the various defense systems of organisms [14–16].

In turn, PLA or poly(lactic/glycolic acid) (PLGA) copolymers have been widely studied with regard to the controlled release of drugs occluded in this matrix by diffusion or erosion of biodegradable polymers [17–21]. Since 1987, there have been 1042 reports of DDS, based on PEG-PLA or the PLGA polymeric system. However, in the last five years, 600 papers were published, which suggests a remarkable level of interest in this scientific field. The properties and applications of DDS can be improved by the use of magnetic particles. These composites have the potential to be targeted to a specific site by using magnetic fields. In addition, they could be very useful in magnetic hyperthermia therapy [22–25].

Magnetic particles modified with biodegradable PLGA or PLA were firstly prepared in the beginning of the 1990s by chemical adsorption or molecular interactions between a reactive polymer end group and the surface of the particle [26–30]. On the other hand, the surface modification of magnetic particles by chemical linkage of polymers is less documented, even less by the use of Ugi four component condensation (UFCC).

The main objective of this study was to prepare a new system of controlled drug release, based on the union of the three elements mentioned above (i.e. PLA, PEG and magnetic particles) and using Ugi four component condensation (UFCC) [31]. This reaction is useful to the functionalization and PEGylation of biomacromolecules based on glycoprotein [32], lipoprotein [33] and polymeric conjugates [34–36]. UFCC is a condensation reaction in which an aldehyde or a ketone, an amine, an isocyanide, and a carboxylic acid can form a bis-amide. It is considered a prime example of a multi-component reaction (MCRs) which proceeds by the formation of an imine as a result of condensation of the aldehyde and primary amine. Afterwards, the isocyanide and carboxylic acid are added to the imine intermediate which, via an acyl transfer, rearranges to the bis-amide product [37]. This new DDS could be used in very interesting applications, such as (i) the occlusion of hydrophobic drugs in the PLA phase, which can be released by diffusion and erosion of the polymer matrix; (ii) the con-

jugation of targeting elements to the amino end groups of the PEG chain; (iii) the production of biomasking by PEG chains, increasing the lifetime of the system; (iv) additional targeting capability due to the presence of the magnetic iron oxide core; and (v) use of the phenomenon of magnetic hyperthermia. Therefore, the presented material is very interesting due to the advantages of Ugi reaction, which make the synthetic route less expensive and enables easier manipulation of the reagents used. In addition, it can increase the efficiency of treatments, improving the welfare of patients.

2. Experimental

2.1. Materials

Lactic acid (85%), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, KOH, glutaraldehyde (50%), reduced Fe, $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, polyvinyl alcohol (PVA) and ethylenediamine were purchased from VETEC. PEG (M_n 6000), carbonyldiimidazole (CDI), chlorate monochlorated acetyl (CCA) and tert-butyl isocyanide were purchased from SIGMA-ALDRICH.

2.2. Synthesis of PLA polymeric system by autocatalytic polycondensation

The PLA polymer was synthesized from 85% lactic acid (700 mL), in a closed system with slight vacuum (–550 mL/mm Hg). The reaction medium was kept at 180 °C for 12 h. The product of the reaction was purified by solubilization in chloroform (250 mL) and precipitation in cold ethanol (4 L).

2.3. Synthesis of end amine functionalized PEG

2.3.1. Via CDI/ED

PEG (0.5 g) dissolved in dichloromethane (70 mL) was reacted with an appropriate amount of CDI (10 equivalent) overnight, at room temperature under stirring. Soon afterwards, the reaction mixture was concentrated by rotoevaporation and the activated PEG was purified by three precipitations in cold diethyl ether (140 mL) and filtered under a slight vacuum. The purified activated PEG (0.1 g) dissolved in dichloromethane (14 mL) was reacted with appropriate amounts of ethylenediamine (10 equivalent) overnight, at room temperature under stirring. Then, the reaction mixture was concentrated by rotoevaporation and diafiltered against distilled water, (cellulose acetate membrane, 1 kDa cutoff). Finally, the bis-amine PEG was dried by lyophilization.

2.3.2. Via CCA/ED

PEG (5 g) dissolved in 100 mL hexane/dichloromethane (1.1 v/v) was reacted with an appropriate amount of CCA (6 equivalents) for 8 hours, at room temperature and under stirring. Then, the reaction mixture was kept under nitrogen atmosphere for 30 minutes. Immediately following this, 14 mL of ethanol was added and the system was kept under reflux for 30 minutes. Finally, the reaction mixture was concentrated by rotoevaporation until 50 mL. Suddenly the activated PEG was reacted with appropriate amounts of ethylenediamine (10 equivalents) for 48 hours at room temperature and stirring. The final product was purified by precipitation in diethyl ether (250 mL) followed by slight vacuum filtration.

2.4. Synthesis of magnetite and aldehyde-modified magnetite

Magnetite was prepared as described in the literature [38]. Briefly, in separate vessels, 3.375 g (2.5 mmol) of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 3.475 g (2.5 mmol) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were dissolved in 100 mL of deionized water. Subsequently, these two solutions were mixed and then 50 mL of aqueous solutions of KOH 2 M were added rapidly under stirring; the final product was a black precipitate that is attracted by a neodymium magnet. The magnetite was washed three times with deionized water and once with ethanol using magnetic decantation. The final product was dried at room temperature. The purified magnetite mixed in distilled water (75 mL) was reacted, first with glutaraldehyde 50% (10 mL) for 9 min under sonication (450 Watts) and then was kept overnight under mechanical agitation at room temperature. The modified magnetite was washed once with ethanol using magnetic decantation and dried at room temperature.

2.5. Synthesis of Ugi-magnetic-composite (UMC)

Aldehyde-modified magnetite (0.08 g) dispersed in 2 mL chloroform/methanol (1:2 v/v mixture) was reacted with appropriate amount of bis-amine PEG (0.15 μMol) overnight at room temperature under nitrogen atmosphere and stirring (Modified-aldehyde magnetite/ bis-amine PEG molar ratio = 1:5). The protonated imine formed was reacted with PLA (0.029 μMol) and tert-butyl isocyanide (0.029 μMol) at room temperature for 72 hours under nitrogen atmosphere and stirring (aldehyde-modified mag-

netite/bis-amine PEG/PLA/tert-butyl isocyanide molar ratio equal to 1/5/5/1, respectively). After this time, the product obtained was washed once with ethanol using magnetic decantation and dried at room temperature.

2.6. Preparation of magnetic microsphere

Magnetic microspheres were prepared using the single emulsion-solvent evaporation methodology [39], with some modifications. Briefly, the UMC (100 mg) was dispersed in 1 mL of dichloromethane. Soon afterwards, the prepared UMC dispersion was added to a beaker containing 5 mL of cold PVA (w/w 0.5%). This system was kept under stirring at 20 000 rpm during 5 minutes. Then, the o/w emulsion was poured into a beaker containing 50 mL of PVA (w/w 0.1%) and kept under mechanical stirring for 2 hours at room temperature (25 °C) to harden the microspheres by solvent evaporation. Magnetic microspheres were then collected by centrifugation at 2500 rpm and washed 3 times with distilled water. Finally, the collected magnetic microspheres were freeze-dried by lyophilization and stored at 12 °C.

2.7. Materials characterizations

2.7.1. Hydrogen-1 nuclear magnetic resonance

Measurements of PLA and bis-amine PEG were carried out in a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz (^1H) and 75 MHz (^{13}C). Samples (15 or 50 mg) were dissolved in chloroform- D_1 (0.8 mL) in 5 mm NMR tubes at room temperature.

2.7.2. Gel permeation chromatography

Measurements of PLA and bis-amine PEG were determined using an Agilent 1200 series HPLC with a MIXED-C column. Chloroform was used as the solvent with a flow rate of 1.0 mL/min and polystyrene was used as a standard.

2.7.3. Fourier transform infrared

Spectra of PLA, bis-amine PEG and aldehyde-modified magnetite was performed using a Varian model 3100 FTIR Excalibur Series spectrophotometer (Japan). Samples were macerated with potassium bromide (1 mg/100 mg samples/KBr). Then, the FTIR spectra of the samples were recorded at room temperature and a resolution of 4 cm^{-1} .

2.7.4. Differential scanning calorimetry

Measurement of PLA, bis-amine PEG, magnetite, aldehyde-modified magnetite and UMC was carried out with help of the TA Q1000 V9.9 build 303 Calorimeter. Briefly, 5 mg of the samples were heated and cooled at a rate of 10 °C/min from –80 to 200 °C under nitrogen flow. Values of glass transition (T_g), melting temperature (T_m) and the crystallization temperature on cooling (T_{cc}) were taken in a cooling run after the second heating run.

2.7.5. Thermogravimetric analyses

Were performed using a TA TGA Q500 Thermoanalyser. Measurements were carried out in nitrogen at a heating rate of 20 °C/min up to 700 °C with a gas flow rate of 20 mL/min.

2.7.6. X-ray diffraction

Measurement of bis-amine PEG, magnetite, aldehyde-modified magnetite and UMC was determined in Rigaku Miniflex X-ray diffractometer in a 2θ range from 20 to 70° by the FT (fixed time) method. The steps used were equal to 0.05° and a time of 1s, using a tube voltage and current equal to 40 kV and 20 mA, respectively. The radiation used was $\text{CuK}\alpha = 1.5418 \text{ \AA}$.

2.7.7. Magnetic force

Measurement of magnetite, aldehyde-modified magnetite and UMC were performed as reported by our group elsewhere [40]. Briefly the samples were kept under a variable magnetic field (0–790 Gauss), calibration of the test was performed by using reduced Fe, $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ as standard for high level, medium level and a lack of magnetic force, respectively [41–43]. Then, the apparent variation in the mass of the sample in the presence of a magnetic field was calculated subtracting the mass of the sample in the presence of a magnetic field from the mass of sample.

2.7.8. Scanning electron microscopy

Measurement of magnetite, UMC and magnetic microspheres was carried out with a JEOL JSM-5610 LV microscope, using acceleration voltage of 15 kV. Samples were coated with gold in order to study the morphology of the magnetic particles synthesized by UFCC. The materials were sampled by taking several images of various magnifications to

ensure that analysis was based on a representative region of the sample.

2.7.9. Magnetic induction hyperthermia

MIH measurements of magnetic microspheres were performed using the Ambrell Easy Heat machine model L1. Samples, dispersed in distilled water (10 and 20 mg/mL at 25 °C), were thermally isolated using glass fiber textile. Soon afterwards, each sample was inserted inside the coil of the machine. Electrical current equal to 200, 400 and 600 A were applied for 600 s. Sample temperature was recorded at 120, 240, 360, 480 and 600 s. The bulk temperature of the samples was measured before and after the induction heating tests.

3. Results and discussion

The Ugi reaction allows the binding, in a single experimental step, of four functional groups: amine, aldehyde, carboxylic acid and isocyanide [37]. In this work, we placed these functional groups as follows:

- I. PEG provided the amine group. Instead of using a commercial diamine reagent, PEG was modified so that the amine group is attached to the polymer backbone by labile bond (i.e., amide). This labile link could permit the release of PEG and/or any molecule bound to the free end.
- II. Iron particles were coated with aldehyde groups. This choice was based on the ease of modification of the NP with glutaraldehyde.
- III. Obviously, the carboxylic group was supplied by the PLA.
- IV. A commercial reagent was used as tert-butyl isocyanide. However, this could be replaced by some interesting molecules in future applications.

3.1. Synthesis of diamine PEG

Firstly, PEG was modified by a known method [44, 45], which is based on the reaction of the hydroxyl groups of the polymer with ethylenediamine in the presence of CDI [46]. However, yields were small (Table 1). Possibly due to the various purification steps necessary to remove excess CDI. To improve yields, another linker was used to attach the ED: chlorate monochlorated acetyl. In this reaction, further purification steps were not necessary after the synthesis of Product 1 (see Figure 1), due to the easy elimination of the hydrochloric acid during the

Figure 1. Synthesis of PEG bis-amine by chlorate monochlorated acetyl/carbonyldiimidazole reaction

Table 1. Yields of poly (ethyleneglycol) bis amine functionalized ($p < 0.01$)

Samples	Method	Yield [%]	SD*	CV**
PEG bis amine 1	Via CDI/ED	71.7	1.5	2.1
PEG bis amine 2	Via CCA/ED	94.3	1.2	1.2

*Standard Deviation

**Variation coefficient

acetylation. On the other hand, the residual CCA remaining after refluxing was eliminated by ethanol esterification. The formed ester was easily eliminated because it was miscible with the solvent used for the synthesis and purification of bis-amine PEG [47]. Moreover, the cross-link reactions, on both sides of PEG, were avoided using a small excess of ethylenediamine.

The yields of this reaction were significantly increased, as shown in Table 1, as expected due to there being fewer purification steps required.

As shown in Table 1, the mechanism of CDI/ED presented moderate yields, probably due to several purifications steps being required to eliminate the residual CDI before the amine reaction. However, these results are in agreement with those reported for the functionalization of PEG with CDI [48]. On the other hand, high yields (94.3%) were achieved for 8 h reaction at room temperature with CCA/ED; it was not necessary to perform purification of activated PEG with CCA before the amine reaction.

The products (1) and (2) of the Figure 1 were characterized by NMR ^1H and FTIR and the spectra obtained are shown in Figures 2 and 3, respectively. It can be seen in Figure 2a that only two peaks at 4.11 and 3.65 ppm appear, which are assigned as (CH_2Cl) and ethylene ($\text{CH}_2\text{--CH}_2$) groups of CCA and PEG, respectively [49]. Consequently, the ^1H -NMR spectra of aminated PEG (Figure 2b) retained the peak at 3.65 ppm assigned to ($\text{CH}_2\text{--CH}_2$) of PEG and to ($\text{CH}_2 \propto \text{C=O}$); however, Figure 2b allows us to infer the absence of the peak assigned to CH_2Cl

groups of CCA. These protons might appear overlapped by the signal of ($\text{CH}_2\text{--CH}_2$) groups of PEG due to the release of chloride group after the amination process, which suggests the success of the amination process. Unfortunately, we cannot observe the peaks assigned to the ethylene ($\text{CH}_2\text{--CH}_2$) groups of ethylenediamine, probably due to their overlap with the ethylene ($\text{CH}_2\text{--CH}_2$) groups of PEG.

FTIR spectra of activated and aminated PEG showed the characteristic absorption bands of this product, i.e. the stretching band at 1751 and 1730 cm^{-1} related to C=O of ester groups of activated and aminated PEG, respectively (see Figure 3). Axial bending at 3420 cm^{-1} is related to the N--H of amine groups of aminated PEG (see Figure 3 curve b). Unfortunately, the absorption band characteristic of amine groups like C--N bounds could not be observed, probably due to the small molar range of amine groups in relation to PEG.

3.2. Synthesis of PLA

Low molecular weight PLA was synthesized by one-step autocatalytic polycondensation of the lactic acid. This procedure does not need further purification steps to eliminate the catalytic agent; in this case, the purification process was performed to ensure the elimination of low molecular weight oligomers [50]. The synthesized PLA was characterized by FTIR; the results are shown in Figure 4.

The FTIR spectra of PLA homopolymers, shown in Figure 4, present the characteristic absorption bands of this polymer [51], i.e., an axial bending at 3518 cm^{-1} corresponding to O--H groups, axial bending at 2997 and 2946 cm^{-1} related to CH and CH_3 , a stretching band at 1757 cm^{-1} corresponding to C=O groups, asymmetric and symmetric deformation vibrations at 1486 and 1464 cm^{-1} of the CH_3 groups, a stretching band at 1271 cm^{-1} of C--C=O groups, stretching bands at 1196, 1134, 1093 cm^{-1} related to C--O groups, a stretching band at 1046 cm^{-1} of O--H

Figure 2. ^1H -NMR spectra of activated PEG (a) and aminated PEG (b)

Figure 3. FTIR spectra of activated PEG (a) and FTIR of aminated PEG (b)

groups, stretching bands at 875 and 789 cm^{-1} related to C–C groups and a stretching band at 704 cm^{-1} of the O–H group; these are all in agreement with those reported in the literature [10, 52].

In turn, the synthesized PLA were characterized by ^1H -NMR and ^{13}C -NMR, shown in Figures 5 and 6, respectively.

Figure 4 shows the ^1H -NMR spectra of the PLA homopolymers. It can be clearly seen from Figure 5

Figure 4. FTIR spectra of synthesized PLA

Figure 5. ^1H -NMR spectra obtained for sample of the PLA homopolymer

Figure 6. ^{13}C -NMR spectra obtained for samples of the PLA homopolymers

Table 2. Molecular weights of synthesized poly(lactic acid)

Samples	M_w [g·mol $^{-1}$]	M_n [g·mol $^{-1}$]	M_w/M_n
PLA1	6543	4641	1.41
PLA2	6475	4466	1.45
PLA3	6287	4428	1.42

that only two peaks at 1.49 and 5.16 ppm appear, which are assigned to the methyl (CH_3) and methine (CH) signals from the PLA homopolymer, respectively [53]. These results are consistent with those obtained from PLA homopolymers prepared by the polycondensation of lactic acid [54]. Furthermore, Figure 6 shows three peaks at 16.92, 69.35 and 169.83 ppm which are assigned to the methyl (CH_3), methine (CH) and carbonyl ($\text{C}=\text{O}$) groups, respectively. The peaks that appear at 1.47, 1.65, 4.34, and 5.14 ppm (see Figure 5) and 20.71, 67.77, and 17383 ppm (see Figure 6) were assigned to the D-lactic acid product present in the samples [55, 56]. The number-average molecular weights (M_n) and polydispersities (M_w/M_n) obtained from GPC are listed in Table 2.

Three batches of PLA were prepared by the method described in this work. Analysis by GPC (Table 2) showed good batch-to-batch reproducibility (Variant coefficient $\approx 2\%$). The polymers obtained presented molecular weight values suitable for use in controlled release systems [57]. Furthermore, a narrow M_w/M_n allows greater control of the release profiles [58].

3.3. Synthesis of aldehyde-modified magnetite

Glutaraldehyde modification of the magnetite obtained by co-precipitation [38] was studied by

Figure 7. Thermogravimetric analysis of magnetite (a) and aldehyde-modified magnetite (b)

Figure 8. FTIR spectra of magnetite 7 (a) and aldehyde-modified magnetite 7 (b)

thermogravimetric analysis; the results obtained are shown in Figure 7.

The comparison between weight losses up to 600 °C of magnetite and modified product, equal to 2.0% (due to water loss) and 22.9%, respectively, demonstrated the presence of the aldehyde.

Aldehyde modification of magnetite was also confirmed by FTIR analyses, as can be seen in Figure 8. The FTIR spectrum of magnetite provides information about the excitation of vibration or rotation of molecules in their ground electronic state. In the magnetite structure presented in Figure 8 curve a, these vibrations are associated with the stretching deformation of the interatomic bond of iron with other molecules like oxygen and appears at 588 and 585 cm^{-1} [59, 60]. The FTIR analysis of aldehyde-modified magnetite (Figure 8 curve b) also showed the presence of aldehyde groups by the appearance of stretching bands at 1717 cm^{-1} related to $\text{C}=\text{O}$ of ester groups, axial bending at 2860 cm^{-1} related to $(\text{C}=\text{O})\text{H}$ of the aldehyde group and axial bending at 2927 cm^{-1} corresponding to the CH_2 of aldehyde groups.

3.4. Synthesis of Ugi magnetic composite

Ugi as well as Passerini reactions are classified as isocyanide-based multicomponent reactions. The Ugi reaction is usually performed in a polar protic solvent such as methanol, and some success in water was recently showed [61]. In turn, non-polar halogenated solvents favoring the occurrence of the Passerini reaction, since most of the amines are insoluble in cited media [62]. Thus, the synthesis of the magnetic composite was performed in dichloromethane/MeOH. This medium was chosen due to the heterogeneous nature of the Ugi components. In addition, the bis-amine PEG is soluble in dichloromethane, allowing its use in the Ugi reaction. This way, the Ugi reaction is favored in comparison with Passerini reaction as shown in Figure 9. The yield of the magnetic composite synthesis was equal to 80%, determined by the weight of the final product. Obtained material was characterized by TGA and the main results are shown in Table 3.

Table 3 shows the decrease of the T_g , T_c and T_m of UMC in comparison to PLA. The replacement of the rigid homogeneous PLA-PLA interaction by heterogeneous PLA-PEG interaction in the UMC makes the macromolecular motion easier, producing the observed decrease of the temperature in the cited transitions (see Figure 10a). As a consequence, the UMC exhibited values of T_g (52.8 °C) that were lower than those for PLA, equal to 52.8 and 59.8 °C, respectively (see Table 3). These results are in agree-

Table 3. TGA analyses of carboxylic PLA, aminated PEG and Ugi magnetic composite

Sample	T_g [°C]	T_c [°C]	T_m [°C]	X_c [%]	T_d^* [°C]
PLA	59.8	98.6	144.0 154.4	21.2	272.8
PEG-NH ₂	–	–	56.7	–	394.1
UMC	52.8	84.2	140.3 123.2 54.4	29.2	264.4 392.4

Determined considering $\Delta H_m^0 = 106 \text{ J/g}$ [85].

*Degradation temperature determined by TGA

ment with those previously reported for the decreased values of T_g in some PLA-PEG copolymers [63]. The influence of PEG on the crystallization behavior of UMC is also shown in Table 3. From this table, it was observed that the T_c of PLA decreased significantly with the incorporation of PEG (reduction around 15 °C). Similar results were reported for the T_c behavior of PLA-PEG copolymers [64]. The melting behavior is also influenced by the incorporation of PEG into PLA. It was clearly evident from Table 3 that there was a decrease in the T_m of between 14 and 21 °C in the case of UMC. This decrease in the melting point is also an indication of the formation of a composite (Figure 8 curve b) [65]. The degree of crystallinity increased from 21.2 to 29.2% in the case of UMC, suggesting that X_c increased in the case of UMC with an increase of the ΔH_m . In turn, the increased crystallinity in the PLA-PEG copolymer was previously reported [66]. It could be con-

Figure 9. Syntheses of magnetic composite by Ugi four component condensation scheme of Ugi magnetic particle

cluded that PEG has the ability to increase flexibility of the UMC material by increasing the mobility of the PLA molecule [16]. On the other hand, only two temperatures for weight loss appeared, at 264.4 and 392.4 °C (see Table 3 and Figure 10c), for the Ugi magnetic composite, which probably belong to the PLA and PEG molecule, respectively. The increase of the polymer chains mobility in the UMC material can decrease the thermal stability of the material. In addition, this phenomenon could be enhanced by the presence of basic amines from the residual bis-amine PEG. Moreover, the residue in the case of UMC was negligible. Therefore, these results are in agreement with those that have been previously reported [67].

In addition, the chemical linkage between PEG, PLA and magnetite could be confirmed by the FTIR spectra of Ugi magnetite composite (Figure 11).

In Figure 11, the absorption bands of PEG and PLA linked to magnetite are clearly seen; i.e., axial bending at 2945 cm^{-1} related to CH_2 of the ether groups and axial bending at 1093 cm^{-1} corresponding to $\text{C}-\text{O}$ of ether groups, which are assigned to PEG. The stretching band at 1762 cm^{-1} related to $\text{C}=\text{O}$ of ester groups, axial bending at 2996 cm^{-1} corresponding to CH_3 and the stretching band at 1048 cm^{-1} were related to $(\text{C}=\text{O})-\text{O}-\text{C}$ of the ester groups, which were assigned to PLA. Finally, the FTIR spectra also presented the stretching absorption bands of magnetite at 588 and 585 cm^{-1} described above.

Magnetic composites were also characterized by XRD, the results of which are shown in Figure 12. XRD results from magnetite, magnetite modified with aldehyde and magnetic composite are shown in Figures 12 curves a)–c) respectively. All the tested materials present peaks at 2θ equal to 30.3° , 35.7° , 43.3° , 53.9° , 57.3° and 63.0° , which confirm the spinel structure of the crystal, characteristic of this iron oxide [68], proving that Ugi four component condensation does not change the particles' nature [40, 27, 69]. Moreover, the crystal size of magnetic particles was calculated by the Scherrer equation [70]. Crystal size values of 13.6 , 13.4 and 12.5 nm were obtained for magnetite, aldehyde-modified magnetite and Ugi magnetic composite particles, respectively. This nanometric size could provide superparamagnetic properties for the Ugi-modified magnetic particles [71] making them very interesting for studies of nano-carriage, targeting and magnetic hyperthermia [22, 72, 73].

Figure 10. DSC thermograms of PLA, PEG and its magnetic Ugi composite, T_g (8a) and T_m (8b). TGA curves for PLA, PEG and UMC (8c).

Figure 11. FTIR spectra of Ugi magnetic composite

Figure 12. XRD analyses of magnetite 10a, aldehyde magnetite 10b and Ugi magnetic composite 10c

The magnetic products obtained were also characterized by magnetic force tests, the results of which are shown in Table 4 and Figure 13.

As expected, the chemical modification of magnetite and its covalent linkage to PEG and PLA by Ugi four component condensation decreased the values of magnetic force of the composite [40, 74, 75] when compared to free magnetite and aldehyde magnetite. These results are in agreement with those reported for the organic modification of magnetite [76–78].

Taking into account that the crystalline structure of magnetite remains unchanged after Ugi modifica-

Figure 13. Magnetic force of magnetite (a), aldehyde magnetite (b) and Ugi magnetic composite at 800 Gauss (c)

Table 4. Magnetic force of magnetite, aldehyde magnetite and Ugi magnetic composite

Samples	MF [mN/g]	Relative MF [%]
Magnetite	762.89±1.20	–
Aldehyde magnetite	589.10±0.74	<29.33
Ugi magnetic composite	103.14±1.20	<86.48

MF: magnetic force

tion, this decrease in the magnetic force of composite is due to the presence of the organic phase on magnetite; this result is in complete agreement with that of TGA.

Morphology of non-modified magnetite, modified magnetite bonded to PLA and PEG by UFCC and magnetic microspheres was characterized by the scanning electron microscopy technique; the results are shown in Figure 14.

Figure 14 shows the morphological change of magnetite after Ugi four component reaction with PEG and PLA. The particles of pure magnetite (see Figures 14a and 14b) present a smooth surface. On the other hand, the UFCC-modified magnetic particles (see Figure 14c and 14d) presented a different morphology, with an increase of the surface roughness due to the new organic phase bound to the magnetite particles, confirming the success of the chemical modification of magnetite with PEG and PLA by UFCC. In addition, Figure 14e clearly shows that UMC is able to form magnetic microspheres of PLA/PEG/magnetite after the single emulsion-solvent evaporation procedure. The surface structure of all microspheres possesses large pores, which probably influence the amount of PEG linked by UFCC. The ability of PEG to form small pores in the surfaces of the microspheres was reported for microparticles based on blends of PLGA/PLA and PEG and also applies to microparticles composed of diblock polymer [79]. During the precipitation step, the PEG branches of the diblock copolymer orientated toward the internal and surrounding aqueous phase and formed a sponge-like structure [80].

Finally, the heating experiments in AC magnetic field were performed on a medium frequency induction heating apparatus. Figure 15 show the temperature of the samples as function of the electrical current and time. Magnetic PLA-PEG/magnetite microspheres were tested using two concentrations, equal to 10 and 20 mg/mL. All the tests were started at 25 °C. As one can see, the reached temperature is directly related to the used concentration, electrical

Figure 14. Scanning electron microscopy of magnetite (a and b) and Ugi-magnetic-composite (c and d), magnetic microspheres of PLA/PEG/magnetite (e)

current and time. The use of the highest current values produced the highest temperatures. Thus, the heating effect could be controlled by adjusting the value of the apparent current [28, 81, 82]. In addition, in the best cases, achieved using 600 A, the temperature of samples with concentrations equal to 10 and 20 mg/mL increased from 25 to 37.7 and

47.1 °C, respectively. Similar results were obtained from hyperthermic evaluation of particles based on biodegradable polymers [81, 82].

In spite iron magnetic particle generally be well tolerated by the organism, they can be used in the generation of reactive oxygen species, which cause direct damage to DNA, proteins or lipid molecules

Figure 15. Heating curves of the magnetic microspheres of PLA/PEG/magnetite tested at two different concentration equal to 10 mg/mL (a) and 20 mg/mL (b)

[26]. Thus, magnetic particles are usually surface-modified or coated with biocompatible and biodegradable polymer molecules such as PLA and PEG. In these work the UMC was well characterized by thermogravimetric and spectroscopic techniques, confirming the chemical linkage of PEG and PLA to the magnetite. This result is very encouraging since presented UMC could be biocompatible due to the use of the PLA and PEG, which are widely used in DDS [83, 84].

On the other hand this material could be useful to treat cancers, either alone or in combination with radiotherapy or chemotherapy. Therefore, the presented set of results allow us to infer that presented material, as well as constituting a new application of UFCC, could be very useful for the preparation of biomasked magnetic drug delivery systems.

4. Conclusions

Ugi four component condensation made the PEGylation of PLA and magnetite in a one pot step reaction possible. The results obtained indicate that one application of Ugi reaction is suitable for the PEGylation of magnetic particles. Superficial modification is the most important aspect of particles in biomedical applications to consider in order to improve their biocompatibility with biological entities. As a consequence, superficial modification of magnetite by Ugi reaction was corroborated by FTIR, DSC, TGA, optical and electronic microscopy, which made it possible to identify the copolymer (PLA-PEG) distributed around the magnetic particles. Besides, the synthesized material presented a good magnetic force, which was able to keep the magnetic composite in a specific location in the presence of any exter-

nal magnetic field. In addition, the synthesized magnetic composite was able to form magnetic microspheres that could potentially be useful for the site-specific delivery of any cytotoxic agent for cancer therapy. Also, these magnetic microspheres are useful to magnetic hyperthermia applications, improving the cancer treatment and even the welfare of the patients.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-474940/2012-8 and 550030/2013-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES and CAPES-MES Cuba # 154/12), Financiadora de Estudos e Projetos (FINEP PRESAL Ref. 1889/10), Rafael Moraes of LaBios laboratory by the induction magnetic test, LABEST laboratory and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for the financial support and scholarships.

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