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Research Article

Green Synthesis and Characterization of Magnetic Antibiotic Delivery System Using Linden Extract

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Abstract

Green chemistry enables iron oxide nanoparticle production with easy, cost-effective and environmentally friendly way. Amoxicillin is used in the treatment of various bacterial infections, however antibiotic resistance is a major problem for treatment. Iron oxide nanoparticles are promising alternatives for overcoming these problems and magnetically targeted antibiotic delivery. In this work, it was aimed to synthesis of iron oxide nanoparticles with green chemistry method utilizing the linden plant, and loading of amoxicillin to these magnetic nanoparticles to be targeted antibiotic delivery system. Iron oxide nanoparticles were prepared using aqueous linden extract and $FeCl_3$ solution. These nanostructures were characterized with FTIR, SEM, and zetasizer. Amoxicillin solutions at varying concentrations were incubated with magnetic nanoparticles for drug adsorption and in vitro drug release from nanoparticles at pH 7.4 was studied. It was found that nanoparticles had spherical morphology (70-88 nm), iron oxide structure and were capped with polyphenols. Optimum amoxicillin amount in magnetic nanoparticles was determined as 100.17 µg drug/ mg nanoparticle. Drug release was faster in the first hours and proceeded in controlled manner. It can be suggested that amoxicillin loaded iron oxide nanoparticles could have potential of magnetically targeted therapy of bacterial infectious disease with low cost and ecofriendly production.

Keywords: magnetic nanoparticle, amoxicillin, green chemistry, linden, targeted antibiotic delivery

Ihlamur Ekstraktı Kullanılarak Yeşil Sentez ile Manyetik Antibiyotik Gönderim Sisteminin Hazırlanması ve Karakterizasyonu

Öz

Yeşil kimya, kolay, ucuz ve çevre dostu yöntemlerle demir oksit nanopartiküllerin üretimini mümkün kılar. Amoksisilin çeşitli bakteri enfeksiyonlarının tedavisinde kullanılmakla birlikte antibiyotik direnci tedavi için büyük bir problemdir. Demir oksit nanopartikülleri gerek bu problemlerin üstesinden gelinmesi için gerekse antibiyotiklerin manyetik hedeflendirilmesi için önde gelen alternatiflerden biridir. Bu çalışmada ıhlamur ekstraktı kullanılarak yeşil kimya temelli demir oksit nanopartiküllerinin sentezlenmesi ve hedefli antibiyotik taşınım sisteminin oluşturulması için bu nanopartiküllere amoksisilin yüklenmesi amaçlanmıştır. Demir oksit nanopartikülleri sulu ıhlamur ekstraktı ve $FeCl_3$ çözeltisi kullanılarak hazırlandı. Bu yapılar FTIR, SEM ve zetasizer ile karakterize edildi. İlaç adsropsiyonu için değişen konsantrasyonlarda amoksisilin çözeltisi manyetik nanopartiküller ile inkübe edildi ve pH 7,4 ortamında ilaç salım çalışması yapıldı. Nanopartiküllerin küresel morfolojiye (70-88 nm) ve demir oksit yapısına sahip olduğu ayrıca polifenoller ile sarılı halde olduğu belirlendi. Nanopartiküllere yüklenen optimum amoksisilin miktarı 100.17 µg ilaç/ mg nanopartikül olarak tespit edildi. İlaç salımının ilk saatlerde daha hızlı ardından kontrollü bir şekilde devam ettiği gözlendi. Düşük maliyet ve çevre dostu üretimle elde edilen amoksisilin yüklü demir oksit nanopartiküllerin bakteriyel enfeksiyon hastalıklarının manyetik hedefli tedavisinde potansiyel taşıdığı düşünülmektedir.

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Anahtar Kelimeler: manyetik nanopartikül, amoksisilin, yeşil kimya, ıhlamur, hedefli antibiyotik gönderimi

1. Introduction

Nanoparticles are particles that have a dimension in the range of 1- 100 nm and possess significant properties owing their small sizes, large surface area to volume ratio, and higher reactivity. Iron oxide nanoparticles are the one of most interesting materials due to their physicochemical features, high magnetism, low toxicity etc. Iron oxide nanoparticles have found wide medical applications including drug delivery, magnetic targeting, hyperthermia, gene therapy etc. Physical and chemical methods are being extensively used for iron oxide nanoparticle production. However these methods have many problems such as the imperfection of the surface structure, use of toxic solvents, generation of hazardous by-products which might harm human health and the environment. Green chemistry methods are differ from those physico–chemical approaches. Green synthesis is a less expensive and free of chemical contamination bottom up approach, where various biological organisms can act as clean, eco-friendly and sustainable materials to produce the stable and well functionalized nanoparticles. These organisms may include plant, bacteria, fungi, yeast, viruses, etc. Among the biological systems, plant mediated synthesis by differenet plant parts (eg. leaf, flower, stem, seed) attracted a remarkable interest due to easy, fast and easy-to scale up process. The plant extracts, mostly polyphenols can play role as natural source for both reducing and stabilizing agent in a one pot synthesis reaction for production of iron nanoparticles when reacted with iron ions (Ebrahiminezhad et al., 2018; Hussain et al., 2016; Saif et al., 2016; Oladotun et al., 2020).

Recently, antimicrobial resistance against antibiotics has become a serious health issue in worldwide and researchers have looked for innovative strategies in antimicrobial therapies. Magnetic nanoparticles are the most promising strategies related to antibiotic therapy and have been used in medical and pharmaceutical areas as drug delivery and hyperthermia agents for bacteria killing. Magnetic nanoparticles could be addressed by means of the influence of an external magnetic field to selected targets. Drug delivery by this way could reduce the drug concentration administered, hence reducing systemic side effects. Moreover, this method increases the drug concentration in the affected tissue, obtaining a better therapeutic effect. (Rodrigues et al., 2019; Xu et al., 2019). Various antibiotics have been used as agents for killing bacteria. Amoxicillin (trihydrate), a semi synthetic antibiotic, has a broad spectum of bactericidal effect against many Gram-positive and Gram-negative, aerobic and anaerobic microorganisms (Palanikumar et al., 2013). There have been many studies (Grumezescu et al., 2014; Mashhadizadeh and Amoli-Diva, 2012; Rouhani and Singh, 2020; Zhang et al., 2020) on designing magnetic delivery of antibiotics including amoxicillin, up to date.

Here, it was aimed to the synthesis of iron oxide nanoparticles with a facile, novel, fabricable green chemistry method utilizing the linden extract and Fe^{3+} precursor, and amoxicillin loading to these magnetic nanoparticles to be targeted antibiotic delivery system for bacterial infectious diseases. Linden plant (*Tilia*) has been known to possess an antifungal, antibacterial and antioxidant activity (Corciova et al., 2018).

There are some works reporting the preparation of inorganic nanoparticles such as silver (Corciova et al., 2018) and copper (Hassanien et al., 2018) nanoparticles from *Tilia* species, however we have found only one research (Rajendrachar et al., 2020) representing magnetite nanoparticle preparation from linden with a different synthesis method from ours. Furthermore, drug loading to green synthesized magnetic nanoparticles using plant extract is a novel approach for drug delivery since lots of works have stated removal or degradation of dye/other chemicals or the antibacterial or cytotoxic etc. activity of the self of iron oxide nanoparticles (Noreen et al., 2020; Qasim et al., 2020). Amoxicillin loaded iron oxide nanoparticles could be a novel magnetically targeted antibiotic delivery system with the potential antibacterial activity of both drug and polyphenol capped iron oxide nanoparticles and magnetic targeting capability.

2. Material and Methods

2.1 Material

Iron (III) chloride ($FeCl_3.6H_2O$) purchased from Isolab. Sodium carbonate (Na_2CO_3) and potassium dihydrogen phosphate (KH_2PO_4) were obtained from Sigma-Aldrich. Folin-Ciocalteu reagent (FCR) was purchased from Merck. Amoxicillin trihydrate was kindly donated by Deva Holding A.Ş. All other chemicals were of analytical grade.

2.2 Biosynthesis of magnetic nanoparticles based on green chemistry

Magnetic nanoparticles were obtained with the modified green synthesis methods described by Mahdavi et al. (2013) and Sun et al. (2014). The washed and dried linden (*Tilia)* was mixed with d-water at 60 g/L concentration (w/v) and the mixture was heated to boiling at 80°C for 1 h. The aqueous mixture was cooled and filtered through filter paper. Then, filtrate was centrifuged at 9000 rpm for 20 min. Supernatant (*Tilia* extract) collected and freshly used for nanoparticle synthesis. $FeCl_3$ solution (0.1 M) was prepared seperately. The aqueous extract was mixed with $FeCl_3$ solution in ratios of 1:1, 1:2, 1:3, 2:1, 3:1, 3:2 (extract: $FeCl_3$ solution, v/v). The mixtures were allowed to incubate in an orbital shaker at 480 rpm for 1 h then, centrifuged at 13000 rpm for 40 min. The iron oxide nanoparticles were obtained in the pellet phase and washed with d-water until the pH reached neutral in order to remove the bioresidues. Iron oxide nanoparticles (NP) were dried in drying oven for characterization analyses.

2.3 Characterization of magnetic nanoparticles

Synthesized nanoparticles were firstly characterized with Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu IR Tracer-100 spectrophotometer) to investigate surface functional bonds. Secondly, Scanning Electron Microscopy (SEM) (Thermo Scientific Apreo S) was used for morphological analyses of nanoparticles in Ege University Central Research Test and Analysis Laboratory Application and Research Center. Then, dilute aqueous nanoparticle suspension was prepared and hydrodynamic particle size and zeta potential of nanoparticles were measured through zetasizer (Malvern Nano ZS 90) in Izmir Katip Celebi University Central Research Labs.

2.4 Preparation of amoxicillin loaded magnetic nanoparticles and characterization

In the first step of amoxicillin (Amox) loading, iron oxide nanoparticles were synthesized at optimum conditions. The optimum ratio for extract:$FeCl_3$ solution mixture was selected as 3:1 (v/v) (the reason explained in Results and Discussion section). Various concentrations of amoxicillin (0.1; 0.3; 0.5; 1.0; 2.0; 3.0 mg/mL) were added as 1 mL on magnetic nanoparticles. Nanoparticles and drug solution were mixed in a ultrasonic bath for 10 min then allowed to incubate in an orbital shaker at 250 rpm for 16 h at 25°C (Ak and Hamarat Şanlıer, 2020). Resulting mixtures were centrifuged at 13000 rpm for 40 min, then supernatants were collected and remaining nanoparticles were washed with d-water for two times to purify. At the end of process, amoxicillin loaded magnetic nanoparticles (Amox-NP) were obtained. Amoxicillin were analyzed in all supernatant specimens. The method described by Singh and Maheshwari (2010) was used for the determination of Amox (Singh and Maheshwari, 2010). In sum, 1.25 mL of 10% (w/v) Na_2CO_3 solution was added to 500 µL of the solution and tubes were left to react in a water bath at 98° C for 40 min. After cooling, 1.75 mL of FCR was added into the each tube and sonicated for 10 min. The final volume was completed to 5 mL with d-water. Absorbance of the solutions was measured at 720 nm using spectrophotometer (Perkin Elmer Lambda 35). Drug loading efficiency (1) and drug loading content (2) were calculated as follows:

$$\text{Drug loading efficiency (\%)} = \frac{\text{Initial Amox amount (µg)} - \text{Unbound Amox amount (µg)}}{\text{Initial Amox amount (µg)}} x100 \quad (1)$$

$$\text{Drug loading content (µg Amox/mg NP)} = \frac{\text{Initial Amox amount (µg)} - \text{Unbound Amox amount (µg)}}{\text{Weight of nanoparticles (mg)}} \quad (2)$$

Drug loading was also considered via FTIR technique for surface interactions.

2.5 In vitro drug release

In vitro drug release from Amox-NP was studied at physiological conditions according to Ak et al. (2018). The release of free Amox was also tested as control group. Amox-NP (containing 1.7 mg Amox) was dispersed in 1 mL of 10 mM pH 7.4 phosphate buffer. Free Amox solution was prepared in buffer at 1.7 mg/mL concentration, too. Both Amox-NP and free Amox solution were taken into dialysis membrane tubings (Sigma Aldrich, MWCO 14.000 Da) and dialyzed against 10 mL of phosphate buffer in a water bath at 37°C with constant shaking. At regular intervals of time, dialyzed sample was taken and changed with a fresh-prewarmed buffer. The released amount of Amox was determined in dialysate samples spectrophotometrically, and the cumulative drug release versus time was expressed using the following equation (3):

$$\text{Drug release (\%)} = \frac{\text{Amount of released Amox (µg)}}{\text{Initial amount of Amox (µg)}} \text{x100} \quad (3)$$

3. Results and Discussion

3.1 Biosynthesis and characterization of magnetic nanoparticles

As known, magnetic nanoparticles can be synthesized from plant extracts which act as low-cost reducing and stabilizing agents at room temperature (Herlekar et al., 2014). In this study linden extract was selected for iron oxide nanoparticle preparation owing to its potential antimicrobial activity. Iron oxide nanoparticle formation was a consequence of

the interaction of Fe^{3+} ions and the reductant groups present in the linden extract. Six different ratios of linden extract:$FeCl_3$ solution (1:1, 1:2; 1:3, 2:1, 3:1, 3:2, v/v) were mixed for preparation. After mixing of linden extract with ferric chloride solution, the immediate formation of iron oxide nanoparticles was proven with the help of darkening of the solution. The smaller amount of particle formations were observed when those ratios of 1:1, 1:2; 1:3, 2:1, 3:2 were mixed. Apart from those ratios, much more amount of particles were formed by using 3:1 ratio. The reason of this could be explained with the polyphenol content of used linden plant which reduces Fe^{3+} ions. In addition, Martínez-Cabanas et al. (2016) claimed that iron oxide nanoparticle mixtures with higher extract content resulted in higher magnetic behaviour and stability properties. Therefore, further studies continued using the ratio of 3:1.

In order to characterize the structures, firstly FTIR was used to check the possible molecules that are responsible for reducing and capping in the linden extract. The FTIR spectrum of linden extract is shown in Figure 1A. The broad band around 3300 cm^{-1} can be related to the stretching vibrations of the O-H groups in water, alcohol, and phenols which indicates the strong hydrogen bonding. The strong peak around 1635 cm^{-1} can be assigned to the C=C ring stretching in flavonoids and polyphenols (Hassanien et al., 2018; Senthilkumar and Sivakumar, 2014). The bands around 1450 cm^{-1} can be associated with the C-H bending vibrations. In addition, the peak observed around 1340 cm^{-1} can be ascribed to aromatic C−OH bending mode (Karade et al. 2017). All the peaks showed the presence of polyphenolic structure of linden extract. FTIR spectrum of NP is given in Figure 1B. The occurrence of the Fe-O bond around 611 cm^{-1} confirmed the formation of iron oxide in the structure of NP. This means reduction of Fe^{3+} by polyphenols. The similar streching vibration attributed to Fe-O bond was shown in a work described by Karade et al. (2017). The appearance of magnetic properties could be associated with the formation of crystalline structured iron oxides (Martínez-Cabanas et al., 2016). Besides, several peaks at 1340, 1450, 1630 cm^{-1} were also present in the structure of NP meaning presumably capping activity of iron oxide surface by polyphenols.

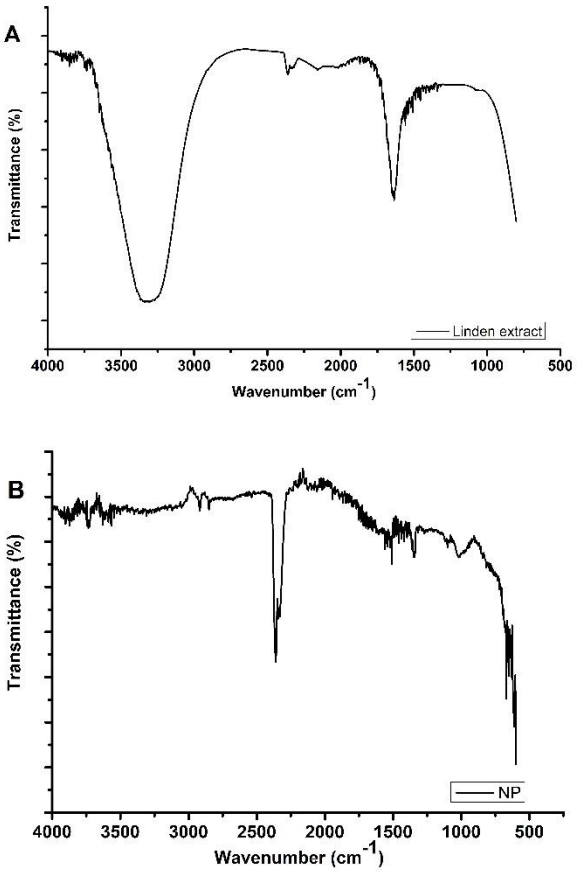
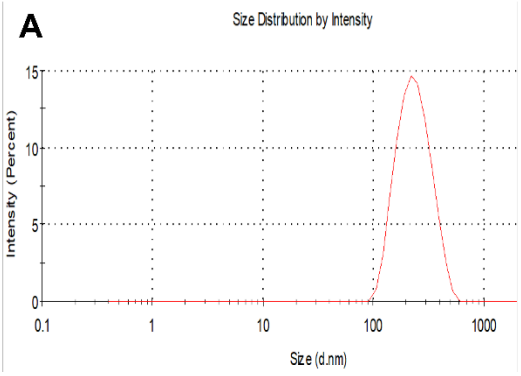
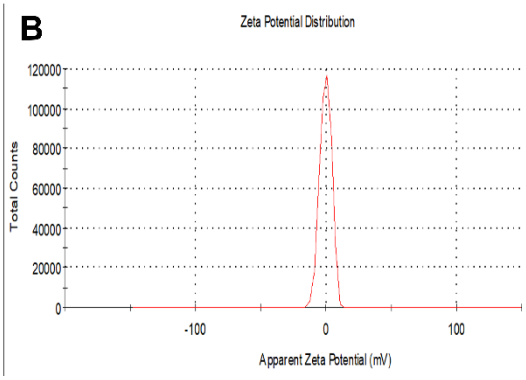


Figure 1. FTIR spectra of A) Linden extract and B) NP prepared with the initial ratio of 3:1 (extract:$FeCI_3$, v/v).

According to the hydrodynamic size distribution analysis (Figure 2A), NP were 255.3 nm± 9.21 in size with the polydispersity index of 0.408 ± 0.061. The zeta potential of

NP was measured and given in Figure 2B. The zeta potential of NP was found to be -0.297 mV± 0.074. Some aggregation was thought to be present due to the magnetic attraction of molecules with eachother. The morphology of NP was also examined with SEM investigations (Figure 2C). At a range of 70-88 nm particle diameter and almost spherical shape of particles were observed. The results revealed the successful biosynthesis of nanosized iron oxide particles. In similar, in a report stated by Wang et al. (2014) iron nanoparticles were synthesized from eucalyptus leaf extract and the nanoparticles appeared spheroidal with a diameter ranging from 20-80 nm. In addition the fewer aggregates in the nanoparticles was implied though the crucial role of polyphenols as the controlling the aggregation and capping.





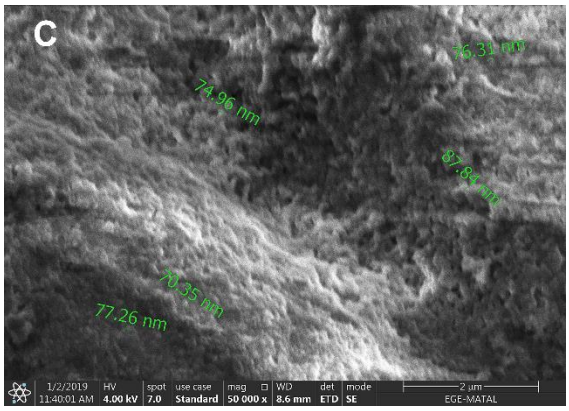
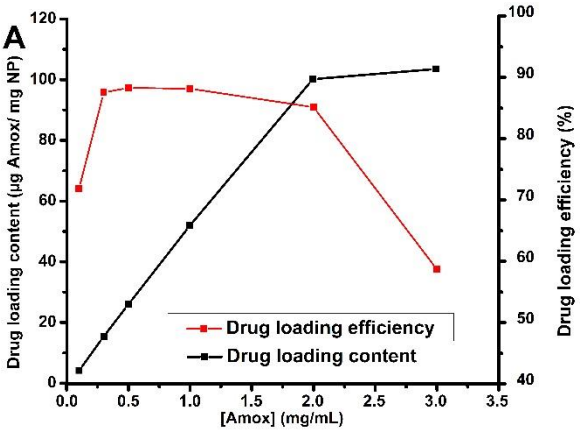


Figure 2. A) The hydrodynamic size distribution, B) zeta potential diagram and C) SEM image of green synthesized NP.

3.2 Preparation of amoxicillin loaded magnetic nanoparticles and characterization

Amoxicillin, β-lactam antibiotic, was loaded to iron oxide nanoparticles so as to generate magnetically targeted and green chemistry based antibiotic delivery system for bacterial infectious. The attachment of drug to NP was achieved by adsorption of antibiotic to NP via combination of weak interactions (hydrogen bond, ionic and Van der Waals interactions etc.) between surface functional groups (amine, carbonyl, hydroxyl) and ring structure of drug with phytochemicals around NP. The effect of drug concentration on loading capacity was studied. Figure 3A shows the drug encapsulation efficiency and loaded drug content for Amox. Drug loading efficiency was found to be maximum at 0.5 mg/mL Amox concentration (88.30 %), in addition efficiency at 2 mg/mL concentration was nearly same (85.14 %). After that dose efficiency decreased dramatically. On the other hand, drug loading content at 2 mg/mL concentration (100.17 µg/mg) was very close to the next concentration, 3 mg/mL of drug (103.50 µg/mg). Thus, the Amox concentration of 2 mg/mL was thought to be optimum amount to be added during drug

loading and further studies continued with that initial dose. Drug loading was also verified with FTIR analyses. FTIR of amoxicillin trihydrate and Amox-NP are given in Figure 3B and Figure 3C, respectively. In the FTIR spectrum of Amox the broad absorption bands in the range 3560–3028 cm^{-1} assign the presence of the surface hydroxyl groups and N-H streching and the signals at 2968 cm^{-1} and 2358 cm^{-1} correspond to benzene ring C-H streching (Palanikumar et al., 2013). The bands at 1614 cm^{-1}, 1683 cm^{-1} and 1770 cm^{-1} show C=O streching, -$CONH_2$ streching and –COOH streching, respectively. The peaks at 1573 cm^{-1} belongs to C-H bending and 1311 cm^{-1} indicates C-N streching. Moreover, the signal at 846-600 cm^{-1} corresponds to aromatic CH_2 bending (Thombre and Gide, 2016). The FTIR spectrum of Amox-NP represented mixed bands typical of empty nanoparticles and Amox. The signals around 3300 cm^{-1} ascribing hydroxyl groups and N-H streching, and at 2360 cm^{-1} indicating benzene ring C-H streching obviously increased when compared to the peaks of empty nanoparticles. Besides, the intensity of peaks at 1683 and 1770 were also higher. FTIR analysis confimed the succeeded loading of Amox to iron oxide nanoparticles.



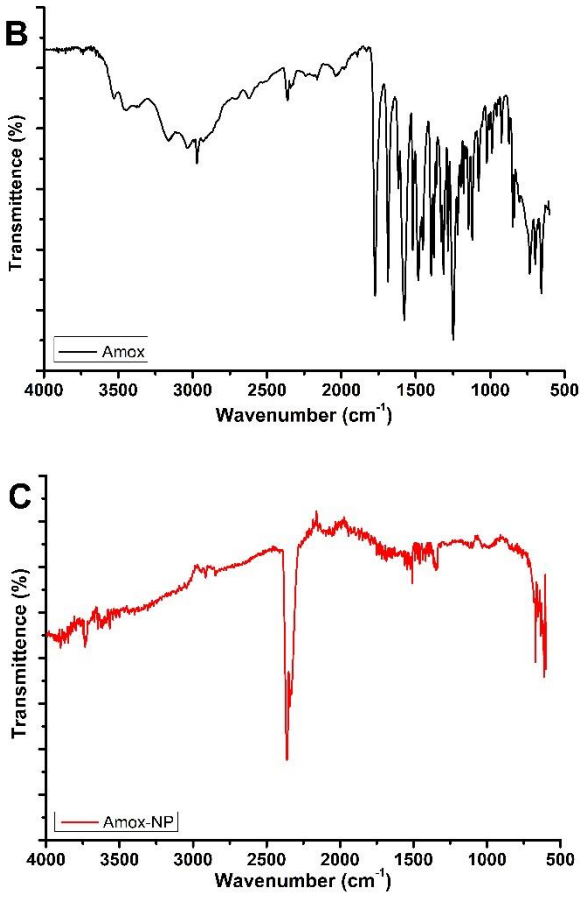


Figure 3. A) The effect of initial [Amox] on loading efficiency and amount of amoxicillin in NP. FTIR spectra of B) amoxicillin trihydrate and C) amoxicillin loaded NP.

3.3 In vitro drug release

Release profile of Amox from free form and Amox-NP were studied under physiological conditions (pH 7.4, 37°C). Figure 4A displays cumulative release of free Amox and Figure 4B shows cumulative release of Amox-NP. Amox release in the first 1.5 hours found as 21.17% from magnetic nanoparticles and whereas the release was 48.68% from free form. After the rapid release of the weakly bonded Amox around the core of the magnetic nanoparticle, release continued slower. At the end of 5 h, while the release was 80.47% from free Amox, the release from nanoparticles was 58.54%. It was probably due to the controlled release of drug included in nanoparticle

system. Like our study Arif and coworkers prepared cysteine conjugated chitosan/poly (malic acid) multifunctional nanoparticles as targeted amoxicillin delivery to eradicate *Helicobacter pylori* and found that approximately 20% of drug release in the first hour and 65% of drug release in 5 h with at pH 7.0 (Arif et al., 2018).

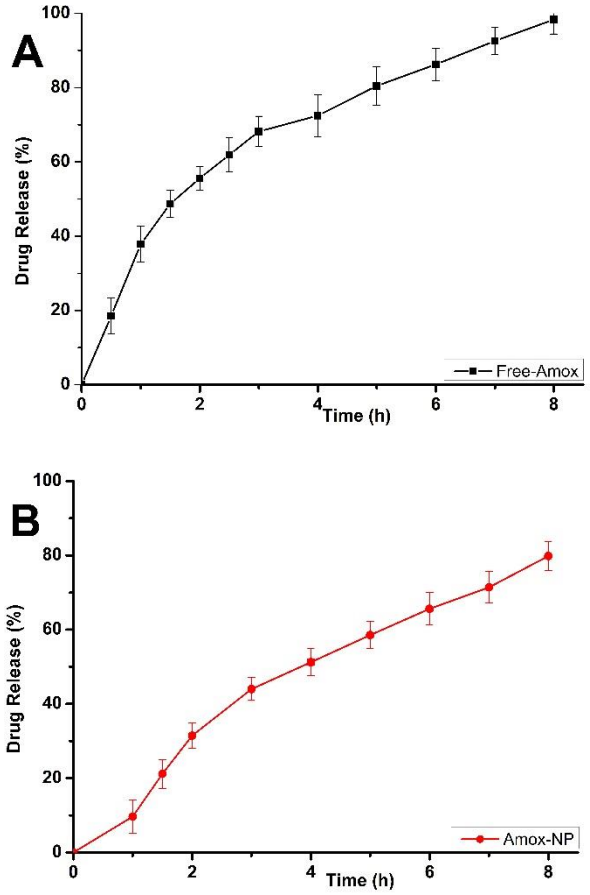


Figure 4. Cumulative amoxicilin release (%) from A) free form and B) Amox-NP in pH 7.4 phosphate buffer at 37°C.

4. Conclusion

In this study, iron oxide nanoparticles were synthesized from linden extract for being drug nanocarrier with an eco-friendly and cost-effective way. The linden was preferred owing to its potential antimicrobial activity. Formation of these magnetic nanoparticles occurred thanks to polyphenol content of the plant. Amoxicillin was attached to nanoparticles with an easy method. Iron oxide nanoparticles exhibited favorable properties such as nanometer sized. Amoxicillin was loaded to nanoparticles with high efficiency and drug release from Amox-NP occurred with a biphasic profile including an initially rapid phase followed by slower release. All the data suggested that amoxicillin loaded iron oxide nanoparticles synthesized based on green chemistry could be a novel drug carrying system for magnetically targeted antibiotic delivery against bacterial infectious disease.

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