Multifunctional Magnetite Nanoparticles for Drug Delivery: Preparation, Characterisation, Antibacterial Properties and Drug Release Kinetics

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Abstract

Multifunctional nanoparticles (NPs) with magnetic (M) and antibacterial properties were prepared for drug delivery purposes by a method involving co-precipitation synthesis. Partial and complete substitutions of ferrous ions (Fe^{2+}) by copper ions (Cu^{2+}) were carried out for the preparation of the magnetite NPs, which are designated as $\text{Cu}_{0.5}\text{M}$ and CuM , respectively, in this work. In addition, chitosan and ciprofloxacin were hybridized with the NPs from the previous step to achieve multifunctional properties. XRD, TEM, SEM/EDAX, VSM and FTIR were subsequently employed to characterize various properties of the prepared NPs, namely, crystallinity, nanostructure (size), particle morphology, elemental mapping, magnetic strength and chemical composition. Antibacterial properties of the NPs were tested against *Bacillus cereus* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria) and *Candida albicans* (yeast). Efficiency of the ciprofloxacin release was also studied for the drug-loaded NPs. It is demonstrated that the obtained NPs possess mixed phases with crystalline structures that are affected by the degree of Cu ion substitution (5-10nm (M), 2.5-3.5nm ($\text{Cu}_{0.5}\text{M}$) and 11-16nm (CuM)). Saturation magnetization values of the NPs were recorded as 38.7, 3.5 and 1.3 emu/g, respectively. It was also found that the introduction of Cu ions in the NP samples improved the significance of their antibacterial activity, especially against *Escherichia coli*. Chitosan and ciprofloxacin were found to have stronger effects against *Bacillus cereus* and *Escherichia coli* and lesser effects against *Candida albicans*. However, the samples containing chitosan, ciprofloxacin and the higher Cu ion concentration exhibited strong influence against *Candida albicans*. During a study period of 30-days, the amounts of released drug from the tested NPs were 85, 26 and 20% of the originally loaded amount, respectively. Owing to the findings in this paper, the developed NPs are considered to have good potential for drug delivery applications and to study them further such as in pre-clinical studies.

Keywords: Nanoparticles; magnetite; copper-substitution; drug delivery vehicles; antibacterial activity; drug release kinetics.

1. Introduction

Nanoparticles (NPs) have been successfully implemented for disease diagnosis, *in vivo* molecular imaging and improved efficacy for therapeutic delivery (Suk et al., 2016, Mahdavinia et al., 2019, Piñeiro et al., 2020). The applications of NPs are particularly advantageous in achieving these goals due to their small particle size, large surface area, ability for *in vivo* drug delivery and, tunable electronic, optical and magnetic properties (Yang et al., 2012). Consequently, a plethora of NPs with different characteristics have been prepared using different materials which have prompted a growing interest for their use in biomedical engineering and imaging (e.g., Yu et al., 2012, Mahdavinia et al., 2019). Due to the bio-compatible and superparamagnetic nature, magnetic nanoparticles (MNPs) are emerging as excellent candidates for biomedical applications

such as magnetic targeting, real-time monitoring of diseases using magnetic resonance imaging (MRI) (Yang et al., 2012) and, hyperthermia treatment of cancer (Yu et al., 2012, Arora et al., 2017, Spirou et al., 2018, Hosu et al., 2019). Modified MNPs have also been observed to inhibit microbial development (Rodrigues et al., 2019). According to previous researchers, a possible antibacterial activity mechanism of metallic NPs action is the electromagnetic attraction between the NPs and the microorganisms, due to positively charged NPs and negatively charged microbes (Spulber et al., 2018). Several antimicrobial agents based on transition metals (d-block) have been investigated for antimicrobial agents (e.g., Cr, V, Co, Ti, Cu, Ni, Fe, Zn, Ag, W, Cd, Hg, Au) (Slavin et al., 2017). In particular, ferrite (Fe_3O_4) NP-based structures are of importance in targeted drug delivery (Turner et al., 2017). The unique properties of the NPs, viz., large saturation magnetization, superparamagnetic nature, chemical inertness, increased surface-to-volume ratio, morphology control through synthesis and biocompatibility of metal–ferrite nanocomposites/NPs have led towards a great motivation for developing new Fe_3O_4 NPs for drug delivery (Piñeiro et al., 2020).

MNPs are prone to aggregation due to the presence of magnetic dipole-dipole interactions or Van der Waals forces which lead to their detection and clearance from the human body by the mononuclear phagocytic system (MPS2) before they can reach their target sites, thus, limiting their biomedical applications (Urquijo et al., 2011, Yu et al., 2012, Aisida et al., 2020). Suk et al. (2016) have utilized magnetite NPs coated with various polymers to prevent their oxidation and aggregation with a view to introduce a steric or electrostatic repulsion between them. MNPs with a biopolymer coating allow for a sequestration of drug molecules which improve the biocompatibility of the NPs while allowing the attachment of the targeting moieties. A common natural polymer utilized as a biomaterial and in drug delivery vehicles is chitosan. Chitosan consideration in biomedicine field is based on the existence of its amine groups. Also, it can be delivered into humans with relatively higher concentrations and its biosafety is maintained as proven through rat models (Chiu et al., 2020). It can be easily crosslinked and modified into gels which can carry biologically-active molecules. Accordingly, chitosan was utilized combined with MNPS for medication selective delivery (Pham et al., 2016). This innovation permits onsite conveyance as the movement of covered MNPs loaded with a drug molecule is adjusted by external magnetic field.

Additionally, several forms of chitosan have been utilized in different *in vitro* and *in vivo* studies proving their broad antimicrobial activities against various microorganisms that include yeasts, fungi, bacteria and algae. For example, several applications for chitosan were explored for their positive influence in bone and skin regenerations (El-Sayed et al., 2020; Liu et al., 2018). It is also worthy to highlight that the inclusion of antibiotics in implantable or injectable systems must be ensured to avoid bacterial infections that might occur during the drug-loaded NP delivery process.

Keeping these in mind, we have selected ciprofloxacin as the drug to load on the NPs. This is because ciprofloxacin is efficient in controlling different types of infections and inflammations (Korzeniowska et al., 2020). However, the unique features of chitosan as mentioned above, its magnetic nanoparticulate form especially when doped with transition metals (Spulber, et al., 2018, Turner, 2017) and antibacterial properties make a hybridization between a multifunctional material with different properties attractive. In this work, incorporating ciprofloxacin in the chitosan/MNPs matrix was the point of interest for antibacterial and drug release study where the key parameter affecting these was the copper(Cu) ion concentration. Cu was chosen as a model for transition metal owing to its noticeable bacterial activity. In addition, Cu has good chelation properties with chitosan.

Different methods have been used for preparing NPs, e.g., combustion-based synthesis (Abd El-Wahab et al., 2014), hydrothermal (Justin et al., 2010), sol–gel (Srivastava et al., 2009), solid-state reaction (Wang et al., 2002), electrochemistry (Dierstein et al., 2001), microemulsion (Han et al., 2004), hard template (Wang and Xia, 2006), spray pyrolysis (Wang et al., 2004), reverse micellar formation (Ahmad et al., 2006) and precipitation process (Karthik et al., 2011). Most of these methods have drawbacks for NPs preparation, such as elevated costs, high pressures and temperatures requirements, low yields, generation of large amount of toxic and expensive solvents hampering their implementation on an industrial scale (Cruz et al., 2018). Considering these, co-precipitation synthesis was selected in this work to prepare NPs as they are facile, inexpensive and quick, and it's efficient in up scaling NP production methods. It concedes nanomaterials with low amounts of impurities through an eco-accommodating course, without requiring unsafe solvents or preparation under high temperature or pressure (Cruz et al., 2018). In addition, it is expected that the existence of another metal other than the iron in the magnetite structure will induce several alterations for the final properties of the developed MNPs such as physico-chemical features, particle diameter, morphology and magnetization performance as well. Antibacterial activity, drug loading efficiency and release kinetics are also expected to be influenced by the same factors along with chitosan/ciprofloxacin hybridizations. Therefore, this research is motivated to explain in detail the expected alterations by these factors on the developed MNPs.

At the beginning of the paper, we describe the fabrication and depiction of magnetite NPs (M), partial substitution of ferrous ions by Cu ion ($\text{Cu}_{0.5}\text{M}$), and, a complete substitution of ferrous ions by Cu ions(CuM). Also, the preparation and characterization of the previous samples coated with chitosan ChM (sample M with chitosan), $\text{ChCu}_{0.5}\text{M}$ (sample $\text{Cu}_{0.5}\text{M}$ with chitosan), ChCuM (sample CuM with chitosan) were investigated. Introducing the antibiotic ciprofloxacin to the NPs samples containing chitosan, CipChM (ChM with ciprofloxacin), $\text{CipChCu}_{0.5}\text{M}$ ($\text{ChCu}_{0.5}\text{M}$ with ciprofloxacin), CipChCuM (ChCuM with ciprofloxacin) was also conducted. Finally, we examine the antibacterial

activities of all the prepared samples and, study the drug release kinetics and the release efficiency from the drug loaded NPs.

2. Materials and methods

2.1. Chemicals, reagents and materials

$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Loba Chemie), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (S D Fine-Chem Limited, SDFCL), $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (Laboratory Rasayan Co.), NaOH pellets (SDFCL), chitosan (MW=100,000-300,000Daltons; Acros Organics, Germany), ciprofloxacin 98.0% (HPLC grade chemical; Fluka, Neu-Ulm, Germany; MW = 331.34 g/mol). All microorganisms used in this work were obtained from the non-profit organization *American Type Culture Collection* (ATCC, Rockville MD, USA) and Northern Utilization Research and Development Division, United State Department of Agriculture, Peoria, Illinois, USA (NRRL). The bacteria used in this work were Gram-positive bacterial strains (*Bacillus cereus* ATCC-6629), Gram-negative bacterial strains (*Escherichia coli* ATCC-51659) and yeast (*Candida albicans* ATCC-10231). The bacterial strains were revived for bioassay by sub-culturing in fresh nutrient broth medium for 24 hours before the tests. The yeasts were cultured on potato dextrose agar (PDA) for 7 days at 28°C before the antibacterial tests were carried out.

2.2. Preparation of magnetite and Cu substituted solutions

Fe_3O_4 NPs (M) were prepared utilizing the precursor salts ferrous sulphate and ferric nitrate at 1:2 molar ratio as follows. Iron (II) sulphate and iron (III) nitrate solutions were prepared by dissolving 14 gm and 40.4 gm of the salts, respectively, in 1L of distilled water supplemented with nitrogen gas purging. Nitrogen gas was selected for purging due to its inertness to the solutions and its easy availability. After complete dissolution (transparent solutions) of the salts, the two solutions were mixed. When the mixture temperature reaches $\approx 70^\circ\text{C}$, 5% NaOH solution was titrated onto the beaker containing the mixture until complete precipitation of the black precipitate of Fe_3O_4 is achieved (the complete precipitation is indicated by testing the filtrate solution against drops of NaOH). The prepared sample was then washed with distilled water many times. The partial Cu substituted sample, $\text{Cu}_{0.5}\text{M}$, was prepared by dissolving 6.04 gm of Cu nitrate in 500 ml distilled water under a nitrogen gas atmosphere and 7 gm of ferrous sulphate in 500 ml distilled water under a nitrogen gas atmosphere. After that the mixture was added to the same concentration and volume of iron (III) nitrate solution as done for the M preparation. Then, it was prepared under the same conditions as the previous sample. In the absence of ferrous ions, they are substituted by Cu ions by dissolving 12.08 gm of Cu nitrate in 1L distilled water (via the above nitrogen purging) and 40.4 gm of ferric nitrate in 1L distilled water. The samples were synthesized as $\text{Cu}_{0.5}\text{M}$ and CuM samples for partial substitution (50 and 100% of ferrous ions).

2.3. Drug loading and release

Ciprofloxacin was loaded on the fabricated NPs through physical hybridization (Mabrouk et al., 2019). For this objective, a normalized (1%^{w/v}) solution of chitosan was utilized in which 10%^{w/w} ciprofloxacin was dispersed. In the mixture, an appropriate amount of M or Cu_{0.5}M or CuM powder was blended. The whole system was then dehydrated at 40°C for overnight (20h). The same procedure was repeated with the above condition, except for the drug inclusion to evaluate the chitosan influence on the final features of the obtained MNPs. Ciprofloxacin release from the drug containing NPs were determined by immersing 200 mg of the dried drug-loaded samples (containing ~20 mg of ciprofloxacin) in 50 mL of phosphate buffer solution (PBS) (pH=7.4) at 37°C. At specific time intervals, 5 mL of the solution was withdrawn, and it was replaced with an equivalent volume of PBS. UV visible spectrophotometer (Jasco V-730) was used to determine the amount of released drug by monitoring the absorbance at the wavelength of λ_{max} =272 nm. The ciprofloxacin loading and encapsulation efficiency were calculated according to the following equations.

$$\% \text{ Drug loading} = \frac{\text{Weight of drug in formulation}}{\text{Weight of total formulation}} \times 100 \quad (1)$$

$$\% \text{ Encapsulation Efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100 \quad (2)$$

2.4 Physical characterization

To identify the influence of Cu presence on the physical characteristics and to prove its inclusion into the developed MNPs, X-ray diffraction analysis (XRD, model BRUKER Axs, D8ADVANCE, Germany), was applied for each sample. The 2 θ diffractogram curves were found in the range 2 – 90° and 2°/min scan speed. As a reference for the identification of the designed phases, the report of the Joint Committee on Powder Diffraction Standard (JCPDS, 1970) was used.

2.5 Chemical characterization

The chemical interactions that take place upon the inclusion of Cu and after chitosan/ciprofloxacin hybridization were confirmed using Fourier transformer infrared (FTIR) spectrophotometer (model FT/IR-6100 type A, USA). Thin discs were prepared by mixing KBr with very small quantity of the sample by molding and compression. These discs were then scanned in range of 4000-400 cm⁻¹.

2.6 Morphology, size and elemental analysis

Scanning electron microscopy coupled with energy-dispersive X-ray microanalysis, (SEM/EDXA model FEJ quanta 250 Fei-Holland) at 15 kVm, transmission electron microscope (TEM) were utilized to determine the influence of the inclusion of Cu and after chitosan/ciprofloxacin hybridization

2.7. Magnetic properties

Vibrating sample magnetometer (VSM) at room temperature was utilized to determine the influence of Cu ion concentration on the final magnetic feature of the MNPs. Specimens were put inside appropriately positioned detecting coils and were allowed to experience sinusoidal movement, i.e., precisely vibrated. The subsequent magnetic motion changes instigate a voltage in the detecting coils that is corresponding to the magnetic moment of the example.

2.8 Antibacterial experiments

Agar plug diffusion technique (Perez et al., 1999) was utilized to evaluate the *in-vitro* antibacterial activity of the prepared NPs. 0.1ml sample from an 18 hr broth culture of the above-mentioned bacteria was adjusted to a turbidity equivalent of 0.5 McFarland standards (Zamora and Perez-Gracia, 2012). This was then administered into a sterile petri-dish named with the bacterial species. Molten sterile Muller-Hinton was used to aseptically fill the plates and delicately shake the microorganisms to disperse them homogeneously in the medium in the plate. The agar plates were allowed to solidify, after which the same weights of 15 powder samples were put as disks on the agar surfaces, then the plates were allowed to diffuse for 2 hr at 4°C. The experiment was conducted in triplicates. All plates were incubated at 37°C for 24 hours for bacterial strains and at 28-30°C for 48 hr for fungal strain. Diameters of the cleared bacterial zones in the petri dishes were measured in millimeters (e.g., see Anitha and Muthukumaran, 2020).

2.9 Drug release kinetics

The kinetics of ciprofloxacin release mechanism were evaluated by using a mathematical model. Korsmeyer–Peppas model was utilized to regress the ciprofloxacin release kinetics from the tested MNPs and fit the obtained data. The general form of the model was selected as it was originally developed for drug release from NPs coated with polymer or its main component is polymeric particles of spherical shape in which the drug delivery is governed by polymer disintegration or diffusion of the drug (Korsmeyer et al., 1986). The Korsmeyer–Peppas model is given as follow:

$$M_t/M_\infty = Kt^n \quad (3)$$

where, M_t/M_∞ is the fraction of drug released at time t , K is the rate constant and, n is a parameter defining the drug release mechanism. If the release profiles follow quasi-Fickian diffusion, the value of $n < 0.5$, for Fickian diffusion $n = 0.5$, and for non-Fickian or anomalous drug transport, $n = 0.5–1.0$. Therefore, a regression of the release data can provide an indication on the type of the release kinetics.

3. Results and discussion

3.1 Preparation of magnetite and Cu substituted samples

The optimized co-precipitation method was utilized successfully to produce Cu-free and Cu-substituted MNPs. These nanoparticles were hybridized with chitosan/ciprofloxacin to develop multifunctional MNPs with impressive antibacterial activities and medication delivery vehicles as it will be discussed in the following sections.

3.2. Crystallinity and phase formation of the prepared NPs

To study and verify the crystalline patterns of the synthesized Fe_3O_4 , X-ray powder diffraction analysis was carried out and the results are given in Figure 1. The effect of the substitution of ferrous by Cu ions, partially and completely, in magnetite sample (M) is represented by $\text{Cu}_{0.5}\text{M}$ and CuM , respectively, as shown in Figure 1a.

Figure 1 XRD patterns of a) magnetite M, partial substitution of ferrous by copper $\text{Cu}_{0.5}\text{M}$, complete substitution of ferrous by copper CuM , b) Ch CuM and Cip-Ch CuM represent sample M with chitosan and chitosan-ciprofloxacin, respectively. c) Ch $\text{Cu}_{0.5}\text{M}$ and Cip-Ch $\text{Cu}_{0.5}\text{M}$ represent sample $\text{Cu}_{0.5}\text{M}$ with chitosan and chitosan- ciprofloxacin, respectively d) Ch CuM and Cip Ch CuM represent sample CuM with chitosan and chitosan-ciprofloxacin, respectively.

It can be seen that the sample M gives its patterns at $2\theta = 30.1^\circ, 35.4^\circ, 43.1^\circ, 53.4^\circ, 57^\circ$ and 62.6° , which are corresponding to standards peaks of Fe_3O_4 according to PDF 89-0691. Introducing Cu, partially, into the structure produced compound with low crystallinity compared to the pure magnetite. The two phases Fe_3O_4 and CuFe_2O_4 were formed according to PDF 89-3854 and 340425, respectively. However, the complete substitution of ferrous by Cu produced a semi crystalline mixture of CuFe_2O_4 and CuO according to PDF 89-0691 and 89-5899, respectively. CuO is the major phase in this sample. The presentation of Cu diminished the intensity of peaks for all Cu/MNPs samples may be because of its lower crystallinity or molecule size distinction (Pouran et al., 2016). From the past research (Abdellatif et al. 2017), it is evident that the metallic Cu ordinarily demonstrates a characteristic peak at $43.4, 50.5$ and $74.2^\circ 2\theta$. Moreover, the coating with chitosan or with ciprofloxacin did not change the patterns of the starting materials significantly (Figure 1b, c and d). Table1 shows the crystallite size and the formed phases of the three samples M, $\text{Cu}_{0.5}\text{M}$ and CuM .

Table1. The crystallite size and the formed phases of the three samples M, $\text{Cu}_{0.5}\text{M}$ and CuM

Sample	M	$\text{Cu}_{0.5}\text{M}$		CuM	
Phases	Fe_3O_4	Fe_3O_4	CuFe_2O_4	CuO	CuFe_2O_4
Average crystallite size(nm)	10.4	22.5	25.3	14	27.8

3.3. Characterization of Chemical Composition

From Figure 2, it can be seen that for the iron oxide (magnetite) possess only a major band at 560 cm^{-1} . The intensity of this band decreases with increasing the substitution of ferrous by Cu ions with increase in the same time the bands at 1145 and 1632 cm^{-1} , which related to the formation of Cu ferrite. These results were in line with the earlier report which detected FTIR bands for magnetite substituted with Cu (Masudi et al., 2020). The bands at $1482, 1388$ and 1294 cm^{-1} are related to the coated layer of chitosan loaded ciprofloxacin on Cu-ferrite (Sanchayanukun and Muncharoen, 2020).

Figure 2 FTIR of a) magnetite (M), partial substitution of ferrous by copper ($\text{Cu}_{0.5}\text{M}$), complete substitution of ferrous by copper (CuM), b) ChCuM and Cip-ChCuM represent sample M with chitosan and chitosan-ciprofloxacin, respectively, c) $\text{ChCu}_{0.5}\text{M}$ and $\text{Cip-Ch Cu}_{0.5}\text{M}$ represent sample $\text{Cu}_{0.5}\text{M}$ with chitosan and chitosan-ciprofloxacin, respectively, d) ChCuM and CipChCuM represent sample CuM with chitosan and chitosan-ciprofloxacin, respectively.

3.4. Characterization of NP Morphology

The morphological properties of the samples M, $\text{Cu}_{0.5}\text{M}$ and CuM and their modification by adding chitosan and ciprofloxacin are shown in Figure 3(a–i). The magnetite NPs are observed to contain agglomerated spherical particles which are similar in size as shown in Figure 3a. According to earlier research, magnetite particles of spherical shape are expected to occur owing to the dissolution-recrystallization phenomenon (Shahid and Choi, 2020, Jiang et al., 2020). The mechanism by which magnetite particles take the spherical structure is owed to an electrons exchange process that takes place between the ferric and ferrous ions during their interactions (Shahid and Choi, 2020). On the other hand, increasing the amount of Cu in samples, $\text{Cu}_{0.5}\text{M}$ and CuM, strongly affects the morphology and particle size as shown in Figure 3(d and g) as the

particles lose its spherical shape and become harder (Emídio et al., 2020). Figure 3(b, e and h) shows that adding chitosan to M, Cu_{0.5}M and CuM samples helps in dissociation of the agglomerated particles which is greatly obvious in the sample Ch M (Figure 3b). Addition of ciprofloxacin to the previous samples, ChM, ChCu_{0.5}M and ChCuM, increases again the connection between the particles in the shape of big granules as shown in Figure 3(c, f and i). Earlier researchers introduced similar effect for the chitosan coating on magnetite NPs (Bezdzorozhev et al., 2017, Morales et al., 2019, Sanchayanukun and Muncharoen 2020).

Figure 3 SEM images of a) magnetite, b) magnetite coated with chitosan and c) magnetite coated with chitosan loaded with ciprofloxacin drug; d) Cu_{0.5}M, e) Cu_{0.5}M coated with chitosan and f) Cu_{0.5}M coated with chitosan loaded with ciprofloxacin drug; g) CuM, h) CuM coated with chitosan and i) CuM coated with chitosan loaded with ciprofloxacin drug.

3.5 Characterization of NP Structures (size)

TEM images confirmed the nanostructures(size) of all prepared samples. Fine NPs were found with narrow particle size distribution. These results are in a good agreement with those obtained from XRD. The transition sample (Cu_{0.5}M) between M and CuM has low crystallinity and smaller particle diameters. It was revealed early that magnetite NPs were synthesized through chemical precipitation technique, which produced NPs with diameters below 5nm (Wu et al., 2008). These MNPs possess well-defined superparamagnetic performance and are most favorable for cells interlunations, which is useful for medication guided delivery.

Figure 4 TEM images of a) M, b) Cu_{0.5}M c) CuM

3.6. Characterization of Elemental Mapping in NPs

To identify the elemental distribution in the samples, EDAX mapping for M, Cu_{0.5}M and CuM samples was performed. The elemental analyses of the magnetite particles and sample M confirmed the basic constituents of the magnetite, i.e., oxygen and iron (Figure 5a). An increase in the intensity of Cu peaks from the sample Cu_{0.5}M to the sample CuM was also observed due to an increasing amount of Cu in the sample (Figure 5 (b,c)). The degree of colors brightness is mostly the same in all the examined specimens, which indicates that the components of these MNPs are homogeneously distributed in the specimens. These findings provide further confidence that a successful synthesis of the MNPs and the Cu substitutions using the facile chemical precipitation method have been achieved. It must be stated that the utilization of transition metals such as Cu²⁺, Ca²⁺ and Mg²⁺ for biomedical application carries another concern into the picture, which is the harmfulness and the need to stay away from metal draining from the nanoconjugates (Colombo et al., 2012). Thus, an excellent elemental distribution and polymer coating procedure for the NPs ought to be considered as performed in the current study. This will ensure the adjusting of the leached inorganic part from developed MNPs to the encompassing media to expand their sustainability in biological conditions.

Figure 5 SEM images, elemental mapping and chart of a) M, b) $\text{Cu}_{0.5}\text{M}$ and c) CuM

3.7. Characterization of NP magnetization

Diminishing particles aggregation venture is an advantage that is possessed by MNPs characterized as superparamagnetic, in addition to maintaining magnetized upon removing the magnetic field. There are two reasons behind the great consideration of superparamagnetic NPs in biomedical applications, especially, in bioimaging and theranostics fields. The first reason is the very fine particle diameters that result in high surface-to-volume ratio. By this property the MNPs guarantee maximum drug/molecules loading and higher cell internalization. The second reason is the superparamagnetic feature that makes them externally controlled and directed to the specific diseased site (Rebolledo et al., 2008, Wang et al., 2010).

Figure 6 Magnetization hysteresis loops recorded for samples M, Cu_{0.5}M and CuM at room temperature

Figure 6 shows the measurements of the magnetic field-dependence of the magnetization of the particles of M, Cu_{0.5}M and CuM samples at room temperature. The saturation magnetization is determined around 38.7, 3.5 and 1.3 emu/g for samples M, Cu_{0.5}M and CuM, respectively, which is smaller than previously reported value for bulk magnetite (92 emu/g). The magnetization value is directly related with the particle size and any increase in particle size may lead to higher magnetization value (Sharifi, et al., 2012, Shahid and Choi, 2020). The very low magnetization of sample CuM may be explained in terms of phase formation as the predominant phase is CuO. These features demonstrate that the synthesized magnetite in current research is a stable magnetic material at room temperature. However, sample M exhibits superparamagnetic behavior, owing to their relative moderate nanoscale (approximately 20 nm), i.e., Fe₃O₄NPs can be in the superparamagnetic regime if the particle size is less than 20 nm (Sharifi, et al., 2012). This finding is in consistent with our TEM results. Magnetization decreases with the increase in Cu concentration for samples Cu_{0.5}M and CuM.

3.8. Antibacterial properties

The bacterial activity of the prepared samples against *Bacillus cereus*, *Escherichia coli* and *Candida albicans* are showed in Figure 7. The results show that the highest bacterial activity was for CipChM sample against *Escherichia coli*. No bacterial activity was observed for ChM sample against the tested bacterial strains; however, pure magnetite was weakly sensitive towards *Escherichia coli*. The partial substitution of ferrous by Cu increases the inhibition zones from 0 to 12mm, from 12 to 33mm and from 0 to 25mm against *Bacillus cereus*, *Escherichia coli* and *Candida albicans*, respectively. When ferrous was completely substituted by Cu, the inhibition zones reached to 26, 34 and 23mm against the *Bacillus cereus*, *Escherichia coli* and *Candida albicans* populations, respectively. The addition of chitosan to pure magnetite did not show any antibacterial influence on the strains. Other chitosan coated samples, Ch Cu_{0.5}M and ChCuM increased the inhibition rings against *Bacillus cereus* while decreased against *Escherichia coli*. Additionally, the antibacterial activity of the sample ChCu0.5M decreased against *Candida albicans* while increased with ChCuM sample. Generally, supplement the sample with ciprofloxacin drug had the most intense bacterial activity towards all the tested strains (Muthukumaran, 2020). Our samples were classified according to the strength of their bacterial activity (Table 2), and the antibiotics standards are summarized in Table 3. The inhibition zones of some samples were shown in Figure 8. Cu doped materials revealed similar results in early reported research (Anitha and Muthukumaran, 2020).

Table 2 Antibacterial activity strength of the tested samples against *Bacillus cereus*, *Escherichia coli* and *Candida albicans*

Sample name	Strength of antibacterial activity (diameter of inhibition zone, mm)		
	<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
M	Resistant	Weakly sensitive (12)	Resistant
ChM	Resistant	Resistant	Resistant
CipChM	Strongly sensitive(43)	Strongly sensitive(48)	Moderately sensitive(27)
Cu _{0.5} M	Weakly sensitive(12)	Strongly sensitive(33)	Moderately sensitive(25)
ChCu _{0.5} M	Strongly sensitive(30)	Strongly sensitive(32)	Weakly sensitive(14)
CipChCu _{0.5} M	Strongly sensitive(41)	Strongly sensitive(42)	Moderately sensitive(23)
CuM	Moderately sensitive(26)	Strongly sensitive(34)	Moderately sensitive(23)
ChCuM	Strongly sensitive(34)	Strongly sensitive(31)	Strongly sensitive(32)
CipCuM	Strongly sensitive(40)	Strongly sensitive(39)	Strongly sensitive(29)

Table 3. The antibacterial activity strength of the standards against *Bacillus cereus*, *Escherichia coli* and *Candida albicans*

Standards	Strength of antibacterial activity (diameter of inhibition zone, mm)		
	<i>Candida albicans</i>	<i>Escherichia coli</i>	<i>Bacillus cereus</i>
TE 30	Moderately sensitive(19)	Moderately sensitive(18)	Resistant
NV 30	Moderately sensitive(20)	Weakly sensitive(16)	Resistant
CIT 5	Resistant	Resistant	Weakly sensitive(13)
CH 50	Resistant	Resistant	Strongly sensitive(32)

Figure 7. Antibacterial activity evaluations of different samples against *Bacillus cereu* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria) and *Candida albicans*(yeast).

Figure 8 Inhibition zones of samples a) M, ChM and CipChM against *Escherichia coli*; b) $\text{Cu}_{0.5}\text{M}$, $\text{ChCu}_{0.5}\text{M}$ and $\text{CipChCu}_{0.5}\text{M}$ against *Escherichia coli*; c) CuM , ChCuM and CipChCuM against *Escherichia coli*; d) M, ChM and CipChM against *Bacillus cereu*; e) $\text{Cu}_{0.5}\text{M}$, $\text{ChCu}_{0.5}\text{M}$ and $\text{CipChCu}_{0.5}\text{M}$ against *Bacillus cereu*

3.9. Drug release kinetics

The ciprofloxacin release kinetics were studied to determine the performance of the prepared samples, namely, CipChM, $\text{CipChCu}_{0.5}\text{M}$ and CipChCuM , on the drug release behavior. The drug loading process was not followed up with any washing or purifications steps. Therefore, the ciprofloxacin loading efficiency in practice and theoretical calculated ones are similar in this work. As shown in Figure 9, a burst release of ciprofloxacin within the first 8 h was observed in CipChM sample. During this time, $\text{CipChCu}_{0.5}\text{M}$ and CipChCuM samples released a lower amount of ciprofloxacin. Introduction of Cu ion into the samples resulted in more efficient complexation process. During this time, $\text{CipChCu}_{0.5}\text{M}$ and CipChCuM samples gave convergent lower amounts of released ciprofloxacin. The initial fast release of ciprofloxacin in CipChM sample may be attributed to the loss of cross linker. In addition, not all of the ciprofloxacin dispersed in chitosan solution is trapped inside the magnetite NPs, which result in quick release for weakly adsorbed ciprofloxacin on the magnetite.

The above interpretation is consistent with the antibacterial result for the sample ChM which did not have any bacterial activity as the chitosan could not be found on the surface. As mentioned above, the release process was proceeded at a neutral solution of phosphate buffer solution adjusted pH 7.4. At this pH, ciprofloxacin has the zwitterionic form due to the presence of carboxylic (CO_2H) and secondary amine (NH_2^-) group (Paluch et al., 2013). In turn, the anionic groups of CO_2H of ciprofloxacin react with the positively charged amine groups of chitosan to form complexes, which may be a reason for adsorption of ciprofloxacin on chitosan. On the other hand, amine groups of chitosan chelate with Cu ions. It was demonstrated that Cu considered as one of the most interacting metal with chitosan (Rhazi et al., 2002, Brunel et al., 2013). These reasons may explain the slow ciprofloxacin release for the samples CipChCu_{0.5}M and CipChCuM. After 8 h, a sustained release of ciprofloxacin was observed from CipChM into the medium. However, a sustained and prolonged release of ciprofloxacin was achieved from CipChCu_{0.5}M and CipChCuM. Starting from 25h, the released ciprofloxacin from CipChCuM was noticed to be slightly higher than of CipChCu_{0.5}M. During a 30-day period, the total amounts of released drug from CipChM, CipChCu_{0.5}M and CipChCuM were 85, 26, and 20%, respectively.

Figure 9 Ciprofloxacin release from CipChM, CipChCu_{0.5}M and CipChCuM

3.10 Mechanism of ciprofloxacin release

The obtained parameters after applying the above-mentioned rate expression for the ciprofloxacin release data from the fabricated MNPs are presented in Table 4. It was found that the ciprofloxacin release from the Cu-free and partial substituted followed the Ritger-Peppas models for non-swelling expanding framework. Based on the Korsmeyer-Peppas model fitting, for spherical particles a value $n > 0.5$ is characteristic for Fickian diffusion. This implies that the medication discharge followed both controlled dissemination and disintegration mechanisms. Furthermore, the release from Cu-full substituted sample followed the non-Fickian diffusion model as affirmed by the R^2 esteems and $n < 0.5$. Similar explanation was reported for mesoporous silica nanospheres coated with PEG polymers (Moodley and Singh, 2019). Thus, the medication moved from the magnetite surface into the encompassing PBS. These outcomes affirmed our thoughts that the discharge conduct and its mechanism were constrained by chitosan degradation, which is accompanied with ciprofloxacin release. However, a highly appropriate model could be a multi-faceted model that incorporates the effects of ciprofloxacin diffusion co-efficient, electrostatic forces between ciprofloxacin and chitosan matrix, and the heterogenous structure of developed MNPs medication guided delivery system.

Table 4 Release kinetics parameters of different ciprofloxacin-loaded MNPs

Formula code	R ² -value [†]		Korsmeyer-Peppas model		n	K*	RE _{0-720h} [‡]	(%)
	Zero-order	Fickian diffusion	Korsmeyer-Peppas model	t ₅₀ ^{**} (hours)				
CipCh M	0.766	0.929	0.931	228.061	669.839	0.287	14.056	901.225
CipCh Cu _{0.5} M	0.774	0.944	0.970	1308.511	2490.867	0.515	1.204	239.988
CipCh CuM	0.741	0.924	0.916	1803.334	3418.88	0.452	1.387	203.5115

n is the diffusion exponent,

*K is the release rate constant,

**t₅₀ is time required for 50% of the drug to be released

*** t₉₀ is time required for 90% drug release

‡RE_{0-720h} is the release efficiency of drug from 0 to 720 hours

†R²-value is the value for regression co-efficient

Conclusion

We have developed MNPs multi-functional systems through using facile chemical precipitation technique, free and substituted with Cu. Partial and full substitution of Cu in magnetite structures was confirmed by FTIR and EDAX analyses. The morphology, particle diameters and magnetization of the fabricated MNPs were influenced by the presence and the concentration of Cu ions. These MNPs were tested for their magnetization, antibacterial activity and medication delivery rate as potential systems for selective treatments. It worthy to highlight, that the developed MNPs herein were proved to be good selective medication vehicles themselves, which could be adjusted by controlling the Cu substitution percentage and the polymer masking process. Moreover, the ciprofloxacin (antibiotic model drug) kinetics release from the developed MNPs was constrained by chitosan degradation, which is accompanied with ciprofloxacin release. These combined features of the developed MNPs promise their applications in drug delivery field after evaluating their *in vivo* metabolic forms and quantification of the dispersion of the MNPs in animal model.

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