

# Iron oxide (Fe<sub>3</sub>O<sub>4</sub>) and Ciprofloxacin Loaded Magnetic Nanoparticles for Magnetic Drug Targeting

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**Abstract:** Iron oxide (Fe<sub>3</sub>O<sub>4</sub>) pristine and ciprofloxacin loaded nanoparticles are synthesized successfully using co-precipitation method. X-ray diffraction analysis confirms the pure phase formation of the synthesized Fe<sub>3</sub>O<sub>4</sub> particles. The grain size of the Fe<sub>3</sub>O<sub>4</sub> calculated from the SEM images comes out to be 50 nm. UV-VIS spectrophotometric analysis indicates the accuracy of the results in which an absorption maximum of blank and ciprofloxacin containing iron oxide (Fe<sub>3</sub>O<sub>4</sub>) particle was observed at 260 and 265 nm respectively. The spectrum band shows the maximum absorption band shift of around 4-5 nm which may be explained by the drug conjugation to the Fe<sub>3</sub>O<sub>4</sub> nanoparticles. The spectrum indicates the formation of Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles having black color and obtained material is found with energy band gap (E) of 2.12 eV and 2.19 eV. On the basis of the findings from dynamic light scattering (DLS) results, it can be concluded that overall particle size of magnetic iron oxide nanoparticles is observed as 765 nm. In the present study the polydispersity index (P.I.) of bare Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles is 0.221, which indicate the hydrodynamic property of the synthesized particles.

**Keywords:** Magnetic nanoparticles; UV-VIS spectrophotometer, Magnetic drug targeting.

**1. Introduction:** Magnetic nanoparticles and micron sized spheres are widely used in biomedical applications as their unique properties are granted by their high surface area and behavior in the presence of a magnetic field. Recently, magnetite Fe<sub>3</sub>O<sub>4</sub> as magnetic material is being widely used in human treatment because of its stable quality, high magnetic responses and easy achievement [1-5]. Due to the biocompatibility, the most common material is iron oxide (magnetite, Fe<sub>3</sub>O<sub>4</sub>) these days. Looking to the importance of these magnetic particles present study has been under taken to explore the possibility of such biocompatible material [6-10]. Present study reveals the preparation of magnetic iron oxide Fe<sub>3</sub>O<sub>4</sub> nanoparticles, optimization of the synthesis parameters, and characterization of the synthesized product. In accordance of the objective, a general synthesis of magnetic Fe<sub>3</sub>O<sub>4</sub> nanocrystals via a simple co precipitation method applied herein provides a promising preparative approach to such

magnetic particles. The structure, morphology and magnetic properties of as-prepared were characterized by X Ray Diffraction (XRD), Scanning Electron Microscope-Energy Dispersive X Ray Spectrometry (SEM-EDS) and Vibrating Sample Magnetometer (VSM). The result of XRD characterization indicated Fe<sub>3</sub>O<sub>4</sub> as the product. SEM and TEM image showed that Fe<sub>3</sub>O<sub>4</sub> nanoparticles have the mean diameter 5-20 nm. The EDS spectra showed strong peaks of Fe and O. Magnetic characteristics of Fe<sub>3</sub>O<sub>4</sub> nanoparticles indicated super paramagnetic properties. Magnetite nanoparticles were synthesized through co-precipitation of aqueous solutions of ferric and ferrous chloride ions in the presence of 9.26 kDa dextran. The CaCo-2 cell model was employed as the biological system. X-Ray diffraction was used to characterize the structure of the synthesized nanoparticles, showing a crystallite size of 9.0 nm. FTIR spectroscopy indicated the presence of dextran in the magnetite samples. SQUID

magnetometry was employed to measure the DC magnetization response of the samples, demonstrating superparamagnetic behavior at room temperature, with a saturation magnetization of about 50 A/m, and a magnetic core diameter of about 7.0 nm. TEM measurements confirmed the average size of approximately 9.0 nm. Viability and apoptosis experiments in CaCo-2 cells in contact with 0.15 mg/ml of nanoparticles were determined at different contact times. No cytotoxic effects were observed. In other study, Drug carrying amino silane coated magnetic nanoparticles were synthesized and used as a flexible magnetic targeted delivery of antibiotics. Magnetic nanoparticles were prepared by chemical co-precipitation method and the surfaces were coated by 3-aminopropyletriethoxy silane. The characteristics of the Effects of pH and temperature on the release of the drugs were studied. The drug loading efficiency was found 93.4 and 91.1 % for ofloxacin and ciprofloxacin, respectively.

**2. Materials and Methods:** Ciprofloxacin (CPF) attached Iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles (NPs) were synthesized using surface adsorption method. Ciprofloxacin was the drug to be used and polyethylene glycol (PEG) was used as a surface coating polymer to bind the drug to the surface of the iron oxide nanoparticles. Polyethylene glycol was added to 20 ml of magnetite fluid (140 mg/ml) and stirred for 10 min. Two tablets of 500 mg ciprofloxacin were then dissolved in 10 ml of de-ionized water and added to the 20 ml of solution. The solution was then sonicated for 2 hours at 500 °C. The microspheres were then collected from the emulsified solution by centrifugation at 4500 rpm. The microspheres were then decanted, diluted with distilled water and centrifuged again at 4500 rpm for 3 to 4 times. Particles collected were dried in oven at 80 °C for 24 hours leading to formation of PEG-CPF- $\text{Fe}_3\text{O}_4$  NPs microspheres. The conditions for the preparation of  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles and Ciprofloxacin drug loaded  $\text{Fe}_3\text{O}_4$  nanoparticles were optimized, and developed

$\text{Fe}_3\text{O}_4$  magnetic nanoparticles. Further, X-Ray Diffraction (XRD), elemental analysis, and Fourier Transform Infrared Drug delivery behavior of the amino silane coated MNPs was studied by selecting ofloxacin and ciprofloxacin as model drugs.

### 3. Results and discussion:

**3.1. X-ray diffraction:** The X-ray diffraction patterns of both Iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles and PEG-CPF- $\text{Fe}_3\text{O}_4$  NPs microspheres synthesized at 80 °C are recorded from 20° to 80° with X-ray diffractometer using  $\text{Cu-K}\alpha$  ( $\lambda=1.549 \text{ \AA}$ ) having an accelerating voltage of 30 kV. Data are collected with a counting rate of 4°/min. The powder X-ray diffraction patterns of ciprofloxacin attached iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles is shown in Figure 1 and the relative comparison with the pure iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles is shown in Figure 2. Most of the characteristic lines in the diffraction patterns were generally prominent and sharp. Proper sample preparation helps to attain accurate peak positions for qualitative analysis. If the sample surface is irregular or if it is displaced from the focusing circle, peak locations and intensities may be varied. Identification of a structure from developed powdered  $\text{Fe}_3\text{O}_4$  diffraction pattern is based upon the position of peaks and their relative intensities.

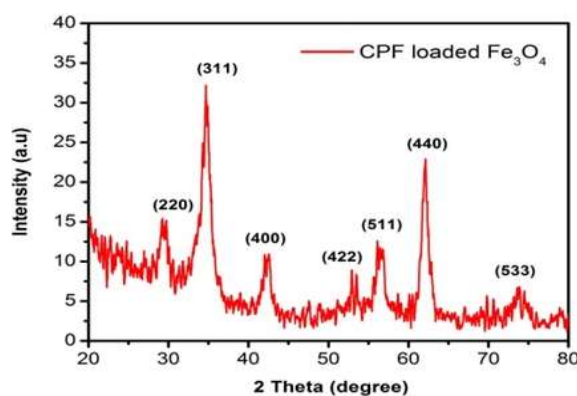


Figure 1. XRD pattern of CPF loaded  $\text{Fe}_3\text{O}_4$  nanoparticles.

A series of characteristics peaks observed in the XRD pattern of PEG-CPF-NPs microspheres corresponds to (220), (311), (400), (422), (511), (440) and (533) Bragg reflection and indicate that the iron oxide magnetic nanoparticles is well crystalline  $\text{Fe}_3\text{O}_4$  (JCPDS card No. 03-0863) [11]. These peaks are characteristic of the cubic magnetite structure as corroborated with the standard data of JCPDS. Using the Scherrer equation the average crystallite sizes of the magnetic  $\text{Fe}_3\text{O}_4$  containing ciprofloxacin are found to be as  $\sim 8$  nm. The results of X-ray diffraction (XRD) of iron oxide and ciprofloxacin attached iron oxide nanoparticles revealed crystallinity of the developed product and accordingly the diffraction pattern and profiles of prepared particle are expressed in the Figure 1 and 2. From the peaks of both samples (Figure 2) it is evident that there are no major differences between the two patterns. However, ciprofloxacin containing iron oxide particles indicate that the intensity of major characteristic peaks specifically at  $34.70^\circ$  and  $56.22^\circ$  decreases and peaks become broad, which indicate the decrement in crystallinity of iron oxide nanoparticles by attaching the CPF particle.

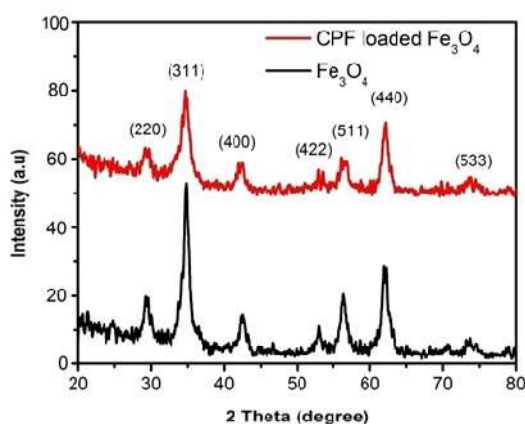


Figure 2. XRD pattern of  $\text{Fe}_3\text{O}_4$  and CPF loaded  $\text{Fe}_3\text{O}_4$ .

**3.2. Scanning Electron Microscopy:** The surface morphology of the developed Ciprofloxacin attached  $\text{Fe}_3\text{O}_4$  nanoparticles was characterized by scanning electron microscopy (SEM) and shown in the Figure 3. As per the SEM images, the iron oxide

magnetic  $\text{Fe}_3\text{O}_4$  nanoparticles- containing Ciprofloxacin (CPF) confirms that the samples taken into consideration during the study period are spherical in shape and are highly dense in nature. The nanoparticles are showing large degree of accumulation. It is also noticed through SEM images that the whitish material of CPF is attached with blackish iron oxide nanoparticles. It is further observed through SEM image that grain size of drug loaded iron oxide NPs decreases  $\sim 40$  nm as compared to 50 nm of pristine  $\text{Fe}_3\text{O}_4$  due to coating of PEG over the iron oxide NPs, which reduce the agglomeration of  $\text{Fe}_3\text{O}_4$  NPs.

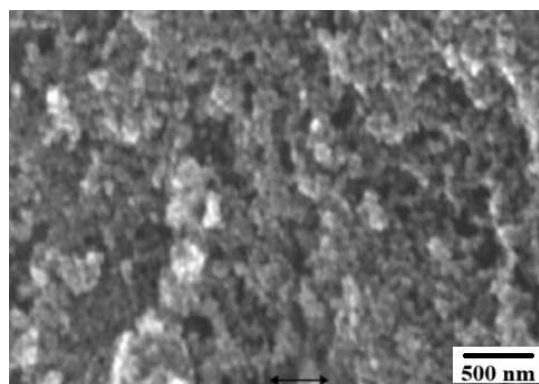
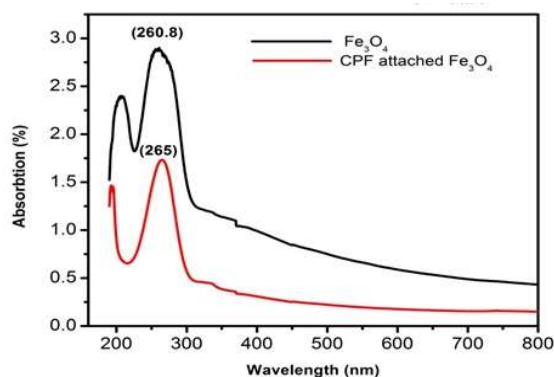


Figure 3. SEM micrograph of Ciprofloxacin attached  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles.

**3.3. UV-Visible Spectroscopy:** The absorption spectrum obtained through UV-Visible spectroscopy of  $\text{Fe}_3\text{O}_4$  and ciprofloxacin loaded iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles is presented in Figure 4. The absorption intensity start to take off or where the absorbance value is minimum at 566.3 nm in visible range between 370 nm to 800 nm wavelengths (Figure 4). However, the presence of absorption maxima of iron oxide ( $\text{Fe}_3\text{O}_4$ ) and ciprofloxacin containing iron oxide ( $\text{Fe}_3\text{O}_4$ ) particle was observed at 260.8 nm and 265 nm respectively. The spectrum band shows the maximum absorption band shift of around 4-5 nm which may be explained by the drug conjugation to the  $\text{Fe}_3\text{O}_4$  nanoparticles [12]. The spectrum indicates the formation of  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles having black color and obtained material is found with energy band gap (E) of 2.12 eV and 2.19 eV according to equation as  $E \text{ (eV)} = 1240/(\text{wavelength in nm})$ .



**Figure 4. UV spectra of  $\text{Fe}_3\text{O}_4$  and ciprofloxacin attached  $\text{Fe}_3\text{O}_4$  particles.**

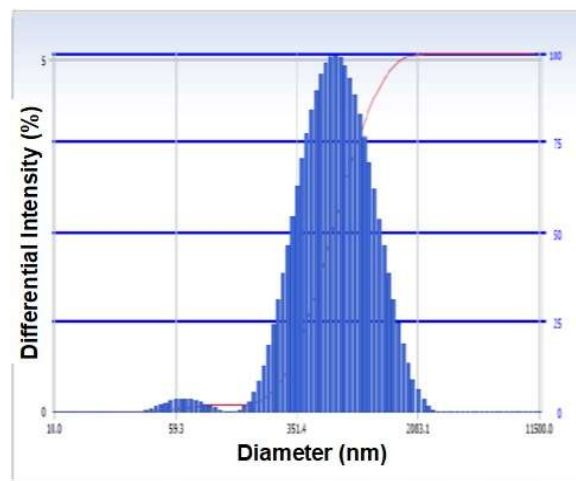
The findings of the present study regarding UV-VIS spectrophotometric analysis are in the support of the observations of the researchers who found the band gap energy of the iron oxide nanoparticles was 2.3 eV of iron oxide nanoparticles developed by co-precipitation method [13].

### 3.4. Particle Size Distribution Using Dynamic Light Scattering (DLS):

DLS measures the light scattered from a laser that passes through a colloidal solution and by analyzing the modulation of the scattered light intensity as a function of time, the hydrodynamic size of particles and particle agglomerates can be determined. Larger particles diffuse slower than smaller particles and the DLS instrument measures the time dependence of the scattered light to generate a correlation function that can be mathematically linked to a particle size. The analytical results as shown in figure 5, revealed that the diameter (d) of the particles containing drug Ciprofloxacin was 619 nm having polydispersity index (P.I.) 0.263 whereas, the intensity distribution (D) was higher (90%) as 1172 nm followed by D (50%) as 591nm and D (10%) as 293 nm. Overlay normalized Intensity distribution of magnetic iron oxide nanoparticles attached with drug Ciprofloxacin and Intensity distribution of drug Ciprofloxacin attached iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles is shown in Figure 6 and Table 1. On the basis of the findings of the present analysis it can be concluded that overall magnetic iron oxide nanoparticles were

observed in 765 nm (Pure) and 619 nm (with drug Ciprofloxacin), therefore, further efforts are needed not only for highly crystalline particles formation but also mono disperse size distribution.

Polydisperse nanoparticles solutions or stable solutions of aggregated nanoparticles have no visible particulates and no particle settling. This class of materials includes dried nanoparticles dispersed with sonication. Polydispersity index is a measure of the width of the particle size distribution. Polydispersity indices less than 0.1 are typically referred to as "monodisperse". Typically, the DLS measures diameters of the particles with a polydispersity index of 0.3 or below. In the present study the polydispersity index (P.I.) of  $\text{Fe}_3\text{O}_4$  nanoparticles and drug Ciprofloxacin attached  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles were as 0.221 and 0.263 respectively Therefore efforts should be made for further analysis to develop monodisperse magnetic nanoparticles of  $\text{Fe}_3\text{O}_4$  used for targeted drug delivery system.



**Figure 5. Intensity distribution of ciprofloxacin attached iron oxide ( $\text{Fe}_3\text{O}_4$ ) NPs.**

### 3.5. Bulk Density and Tapped Density:

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it was handled. The particles can be packed to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the

results, it is essential to specify how the determination was made. Density was measured to predict the flow properties and its compressibility which requires that loose powders be compacted into a durable solid form with the correct mechanical strength, porosity and other characteristics. It is observed that loose bulk density (LBD) and tapped density of ciprofloxacin attached  $\text{Fe}_3\text{O}_4$  nanoparticles was  $0.453 \text{ g/cm}^3$  and  $0.548 \text{ g/cm}^3$ . Compressibility index (CI) was also found from the results of loose bulk density and tapped bulk density, and it was reported as 15.41 for  $\text{Fe}_3\text{O}_4$  and 17.33 for ciprofloxacin attached  $\text{Fe}_3\text{O}_4$  nanoparticles, which indicate good flow property of the magnetic particles [14].

magnetic response of  $\text{Fe}_3\text{O}_4$  magnetic powder. In the present study magnetic response of the magnetic CPF attached  $\text{Fe}_3\text{O}_4$  sample powder in the magnetic field was analyzed and evaluated not only to assess the importance of this selected targeted drug in clinical application but also to give more direct data for further research of targeted drug Ciprofloxacin. Present data revealed that the maximum intensity of the ferromagnet itself was 206.5 mT tested at 0 cm position from ferromagnet. However, the number of the ferromagnets which were changed to investigate the higher magnetic response and it was found that all the ferromagnets had the same magnetic performance, therefore, the distance between ferromagnets and CPF

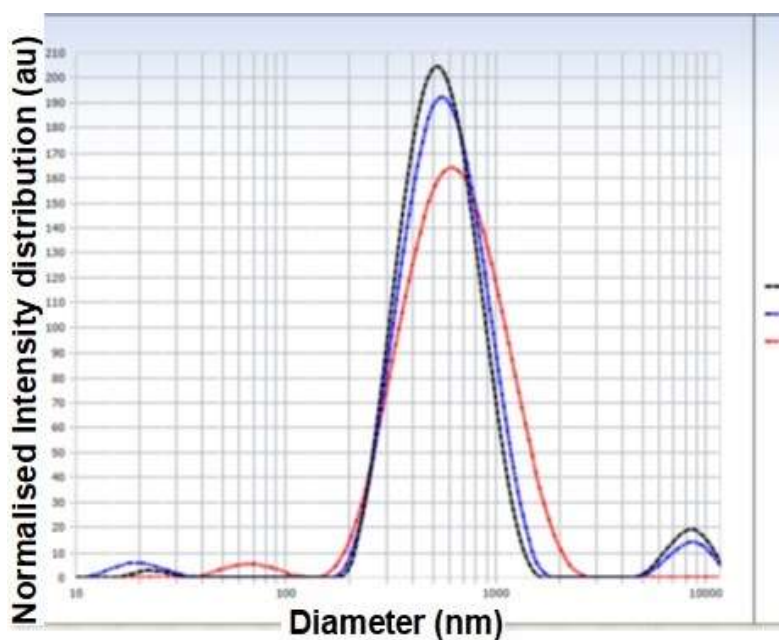


Fig.6: Normalized Intensity distribution of ciprofloxacin attached iron oxide NPs.

### 3.6. Magnetic Field Intensity and Magnetic Response:

The magnetic density of ferromagnet was measured by using Gaussmeter whereas the relative magnetic response of ciprofloxacin attached  $\text{Fe}_3\text{O}_4$  nanoparticles magnetic nanoparticles were measured using an electronic balance. To this context magnetic  $\text{Fe}_3\text{O}_4$  powder (0.05 g) was placed in the balance 2 cm below a magnet of known strength and the reading on the balance in the unit of g was used as an index of the

attached  $\text{Fe}_3\text{O}_4$  magnetic powder was fixed at 2 cm. Present study showed that the intensity of magnetic field and the magnetic response of CPF attached  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles weaken as the distance increases, moreover, the magnetic response depends on the magnetic intensity. The magnetic response increases nearly 60 times when the distance is changed from 3 cm to 1 cm. Thus, in the clinical application, the distance between ferromagnet and drug Ciprofloxacin has been



**Table 1: Intensity distribution table of magnetite iron oxide (Fe<sub>3</sub>O<sub>4</sub>)drug Ciprofloxacin containing nanoparticles**

No.	Data	Repeat No	Ave Diameter (nm)	Polydispersity index	D(10%) (nm)	D(50%) (nm)	D(90%) (nm)
1	S <sub>1</sub>	1	670.4	0.262	305	522	965
2	S <sub>2</sub>	2	669.9	0.257	299	541	1002
3	S <sub>3</sub>	3	619.9	0.263	293	591	1172

highlighted especially in the present study (Table 2).

The data revealed that, a 32.6 mT magnetic field intensity produced by four ferromagnets was sufficient to excite the dipole moments of the 0.05 g CPF attached Fe<sub>3</sub>O<sub>4</sub> magnetic powder which was 2 cm away from the ferromagnets. From the data as obtained during the study period indicated and concluded that the obtained ciprofloxacin attached Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles were accomplished and qualified as the magnetic carriers for further tests in targeted-drug therapy.

From the results obtained during the study

nanoparticles) defined here as a small object that behaves as a whole unit in terms of its transport and properties and that's why their magnetic response may be slightly higher than that of Fe<sub>3</sub>O<sub>4</sub> (pure) sample.

LBD: Loose Bulk Density, TBD: Tapped Density, Magnetic Response\*/g is out of 0.05 g magnetic Iron Oxide powder with the ferromagnets 2 cm away.

**4. Conclusion:** In summary, magnetic Iron oxide (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles were synthesized successfully using co-precipitation of a Fe (II) and Fe (III) precursor mixture in basic aqueous

**Table 2: Magnetic density and Magnetic Response of ciprofloxacin attached Fe<sub>3</sub>O<sub>4</sub> nanoparticles**

Magnetite Iron Oxide Fe <sub>3</sub> O <sub>4</sub> NPs	LBD (g/cm <sup>3</sup> )	TBD (g/cm <sup>3</sup> )	Magnetic field intensity of ferromagnet (mT)	Magnetic Response*/g	Compressibility index
Fe <sub>3</sub> O <sub>4</sub>	0.461	0.545	206.5	0.0487	15.41
Fe <sub>3</sub> O <sub>4</sub> -containing Ciprofloxacin	0.453	0.548	206.5	0.0501	17.33

period support the fact that the nano powder are agglomerates of nanoparticles, or nanoclusters. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Synthesized grown iron oxide nanoparticles in the term of nano powder (Black colour) and their aggregates (ciprofloxacin attached to magnetic iron oxide

solution and then the synthesized Fe<sub>3</sub>O<sub>4</sub> nanoparticles attached with the ciprofloxacin drug stabilized with a polymer poly ethylene glycol (PEG). XRD analysis confirms the pure phase formation of the synthesized product. XRD analysis of the present study reveals that the peaks of both samples do not have major differences between the two patterns. which indicate the decrement in crystallinity of iron

oxide nanoparticles by the attachment of the CPF particle. As per the SEM images, the iron oxide magnetic nanoparticles of  $\text{Fe}_3\text{O}_4$  pure and  $\text{Fe}_3\text{O}_4$  - containing Ciprofloxacin, confirms the shape of the powdered material as spherical. The grain size of the  $\text{Fe}_3\text{O}_4$  pure and  $\text{Fe}_3\text{O}_4$  - containing Ciprofloxacin calculated from the SEM images comes out to be 50 nm and 40 nm respectively. UV-VIS spectrophotometric analysis indicate the accuracy of the results in which an absorption maxima of blank and ciprofloxacin containing iron oxide ( $\text{Fe}_3\text{O}_4$ ) particle was observed at 260.8 and 265 nm respectively. The spectrum indicates the formation of  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles having black color and obtained material is found with energy band gap (E) of 2.12 eV and 2.19 eV. On the basis of the findings from dynamic light scattering (DLS) results, it can be concluded that overall particle size of magnetic iron oxide nanoparticles is observed as 765 nm (Pure) and 620 nm (with drug Ciprofloxacin). In the present study, the polydispersity index (P.I.) of bare and drug Ciprofloxacin containing  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles is 0.221 and 0.263 respectively, which indicate the hydrodynamic property of the synthesised particles. Present findings indicate good flow property of the magnetic particles because of the compressibility index (CI) 15.41% and 17.33% for  $\text{Fe}_3\text{O}_4$  and  $\text{Fe}_3\text{O}_4$  containing Ciprofloxacin magnetic nanoparticles, respectively. The CI was worked out from the loose bulk density and tapped bulk density. Present data reveals that, a 32.6 mT magnetic field produced by four ferromagnets was sufficient to excite the dipole moments of the 0.05 g  $\text{Fe}_3\text{O}_4$  magnetic powder 2 cm away from the ferromagnets.

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