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Preparation and Comparative Evaluation of Fe+2/Fe+3 and Mg+2/Fe+3 LDHs as Promising Nanocarriers for Class II and Class IV Drugs

Shahad Majeed Nidhal Maraie Ashour H. Dawood
College of Pharmacy – Al-Mustansiriya University, Baghdad, Iraq

Abstract

Application of NPs is a promising nanocarriers strategy for development of new drug delivery system. To accomplish such strategy, two types of NPs of layered double hydroxides (Fe+2/Fe+3) and (Mg+2/Fe+3) were synthesized, each one was separately loaded with two types of drugs, montelukast sodium (class II) and cefdinir (class IV) by application of two methods of drug loading (ion-exchange and intercalation methods). The final dried powder were characterized using Fourier transform infrared spectra (FT-IR), Scanning electron microscopy (SEM), Transmission Electron Microscopy (TEM), Thermal analysis (TGA), Zeta Potential analyzer, Powder X-ray diffraction pattern (PXRD) and Energy-dispersive X-ray spectroscopy (EDX), and evaluated by measuring solubility, percentage yield and drug loading capacity, in vitro dissolution, in vitro diffusion and biological activity. The results indicated that Fe+2/Fe+3 LDHs NPs is more suitable nanocarrier for class IV as well as class II drugs since it gave smaller particle size, improved permeability, higher loading capacity and improved biological activity therefore, it could be used to give sustained release pattern that can prolong the action of the drug in the body.

Keywords: Fe+2/Fe+3-LDHs, Mg+2/Fe+3-LDHs, drug loading, cefdinir, montelukast

1. Introduction

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization is on nanometer scale. At present 95% of all new potential therapeutics have poor pharmacokinetics and biopharmaceutical properties (Gadad 2011). Therefore there is a need to develop suitable drug delivery system that distribute the therapeutically active drug molecule only to the site of action, without affecting healthy organs and tissues. Nanocarriers are ultrafine particles where drug or biologically active material dissolved, entrapped or encapsulated (Singh & Abha 2013). Layered double hydroxides (LDHs), also known as anionic clays, are host-guest materials that have recently gained much attention. They consist of stacks of positively charged mixed metal hydroxide layers that require the presence of interlayer anions to maintain overall charge neutrality (Feitknecht 1942). One subclass of LDHs is that where the charge balancing anion is organic (Lagaly & Beneke1991). There are several methods for organic drug incorporation within LDHs including intercalation method and ion-exchange method (Bingxin 2004).

In this study two types of LDHs (Fe+2/Fe+3 and Mg+2/Fe+3) and two different methods for drug incorporation (intercalation and ion-exchange) were applied for montelukast (class II) a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma as well as to relieve symptoms of seasonal allergies (Soni 2012), and cefdinir (class IV) a third generation cephalosporin with a broad spectrum of activity against enteric gram-negative rods (Drug Topics 2009). Characterization including SEM, TEM, PXRD, EDX, Zeta potential, TG analysis and FT-IR were applied and comparative evaluation between the prepared compounds including; percentage yield, loading capacity, dissolution (in-vitro release), diffusion, solubility and biological activity in order to emerge a promising nanocarriers for poorly soluble drug as well as low permeability drug to achieve suitable drug release, site-specific action at therapeutically optimal rate and dose regimen (Kipp 2004).

2. Experimental Section

2.1 Materials:

All chemical used; Cefdinir (B.D.H, UK), Montelukast Sodium (Siga, USA), FeCl3 (B.D.H, UK), HCl (B.D.H, UK), FeSO4.7H2O (Scharlu, Germany), Mg(NO3)2.6H2O (B.D.H, UK), NaOH (B.D.H, UK).

Synthesis of Layered Double Hydroxides Nanoparticles:

The preparation of Fe+2/Fe+3 LDHs and Mg+2/Fe+3 LDHs NPs was done by mixing 1:2 molar ratio of Fe+2/Fe+3 (25 ml of 0.02 M of FeSO4.7H2O solution and 25 ml of 0.04 M of FeCl3 solution) for Fe+2/Fe+3 LDHs and mixing 25 ml of 0.06 M of Mg(NO3)2.6H2O solution and 25 ml of 0.02 M of FeCl3 (3:1 ratio) for

Mg+2/Fe+3 LDHs. In both cases 2.1 ml 0.5 N HCl was added and a drop wise titration is done with 2 M NaOH with vigorous stirring at 80 ◦C until the pH elevated from 2 to 9 with change in the color of the solution from clear yellow to dark brown suspension which is then left at room temperature for 24 hours. The suspension is filtered and continuously washed with deionized water until the filtrate became neutral. The obtained solid residue was dried at 50◦C in oven for 4 hours (Marcin 2012).

2.2 Preparation of LDHs Loaded Drug

Two methods for each drug were applied for loading on LDHs including; intercalation method and ion-exchange method, where each drug separately (Cefdinir and Montelukast) loaded on Fe+2/Fe+3 LDHs and Mg+2/Fe+3 LDHs.

For Cefdinir; Intercalation method was used to prepare Fe+2/Fe+3 and Mg+2/Fe+3 LDHs-loaded cefdinir, where 25 ml 0.125 M of FeSO4.7H2O solution mixed with 25 ml 0.5 M FeCl3 solution (1:3 ratio) for Fe+2/Fe+3 LDHs-loaded cefdinir and 25 ml of 0.5 M of Mg(NO3)2.6H2O solution mixed with 25 ml of 0.16 M of FeCl3 solution (4:1 ratio) for Mg+2/Fe+3 LDHs-loaded cefdinir.. In both cases 2.1 ml 0.5 N HCl was added and 20 ml of saturated solution of cefdinir (0.25 M) in 2 M NaOH were prepared and put in a burette and then added as drop wise titration to the first mixture with vigorous stirring at 80◦C until the pH became 7 and a dark black precipitate appeared. The suspension left for 24 hours at room temperature then filtered and washed with deionized water. The obtained solid residues were dried at 50 ◦C in oven for 4 hours (. Hui Zhang 2006).

For Montelukast; Ion-exchange method was used to prepare Fe+2/Fe+3 and Mg+2/Fe+3 LDHs-loaded montelukast, by mixing 25 ml 0.125 M of FeSO4.7H2O solution and 25 ml of 0.5 M FeCl3 solution (1:3 ratio) for Fe+2/Fe+3 LDHs-loaded montelukast and 25 ml of 0.5M Mg(NO3)2.6H2O solution with 25 ml of 0.16 M of FeCl3 (4:1ratio) solution. In both cases 2.1 ml 0.5 N HCl was added then titrated drop by drop with 2 M NaOH with vigorous stirring at 80◦C until the pH became 9, the mixture color changed from clear yellow into dark brown. After 1 hour of stirring at room temperature, a saturated aqueous solution of 0.25 M of montelukast was added gradually drop by drop to the mixture, the color changed to become pale yellow with the same pH, left over night at room temperature, then filtered and washed with deionized water several times, until the filtrate became neutral. The obtained solid residues were dried at 50◦C in oven for 4 hours (Soni 2012).

2.3 Characterization of the prepared nanoparticles and their corresponding drug loaded

Fourier transform infrared spectra (FT-IR) (8400S Shimadzu Japan) were recorded in the range 4000 – 500 cm-1 using the KBr pellet technique. The morphology and sizes of the synthetized LDHs NPs were observed with an S 4300 (UK) scanning electron microscopy (SEM) and 2010f (USA) Transmission Electron Microscopy (TEM). Thermal analysis (TGA) (Shimadzu Japan) at a heating rate 10◦C/min. Compounds stability analyzed by using Zeta Potential analyzer (USA) with applied voltage range (80-150 V). Powder X-ray diffraction pattern (PXRD) (Shimadzu Japan) were recorded using 220V/50Hz diffractometer equipped with Cu-Ka radiation (λ=1.5418 A◦) at a voltage of (40 kV) and a current of (30 mA), the instrument was operated in the continuous scan and sample were analyzed in the range (5-50◦) at scanning speed of (5◦/min) and (2 Θ) axis. Elemental analysis for carbon, hydrogen and metal ions was performed using Energy-dispersive X-ray spectroscopy (EDX) (UK).

2.4 Determination of Percentage Yield and Drug Loading Capacity

The percentage yield of the reaction after incorporation of the drug with the prepared NPs was calculated as percentage of the actual weight of nanocarriers obtained to the total weight of the starting materials used in the reaction.

Determination of loading capacity (entrapment efficiency) for each type of the prepared NPs loaded drug is done by dissolving 25 mg of Fe+2/Fe+3 LDHs and Mg+2/Fe+3 LDHs loaded drug separately in 50 ml phosphate buffer solution (pH 7.4) respectively then the prepared mixture samples were sonicated for about 1 hour until a clear solution was obtained and the mixtures left at room temperature overnight, then filtered and the content of each drug determined spectrophotometrically at λ max 287 nm for cefdinir and λ max 293 nm for montelukast using double beam UV- Visible spectrophotometer. The percentage loading capacity was calculated as percentage of actual amount of drug to the theoretical drug content (Katherine 2004).

2.5 In Vitro Drug Release Study

The in vitro release of cefdinir and montelukast from the prepared Fe+2/Fe+3 and Mg+2/Fe+3 LDHs-loaded drug were studied using USP type II (paddle type) dissolution test apparatus. Fifty milligrams of each type was introduced separately into a dissolution vessel containing 250 ml of pH 7.4 buffer and the dissolution test apparatus was run at 50 rpm for maximum up to 5 hours at a temperature 37± 0.5 ◦C. Five milliliters samples were withdrawn from dissolution medium with disposable syringe at predetermined time intervals of 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300 minutes and replenished with 5 ml pre-warmed fresh medium at each time then the samples were filtered. Cefdinir and montelukast contents in the aliquots were determined spectrophotometrically using double beam UV-Visible spectrophotometer at the specified wave length for each drug. The experiments were conducted in triplicate at each time interval and the average was recorded (Hui Zhang 2006).

2.6 In Vitro Diffusion Study

The diffusion study was modified by using dialysis bag technique. The dialysis bag technique is done by dispersing 50mg of the LDHs-loaded cefdinir and 50 mg of the LDHs-loaded montelukast each one separately in 10mL of phosphate buffer pH 7.4, placing in a dialysis bag (MWCO: 12–14 kDa, surface area of 22.5 cm2), which was then submerged in a paddle dissolution apparatus containing 250 mL of the same buffer maintained at 37 ◦C and stirred at 100 rpm. At designated time intervals, 5mL aliquots were collected, filtered and replaced with fresh media. The drug concentrations were determined using a UV-Visible spectrophotometer at the specified wave length for cefdinir 287 nm and montelukast 293 nm, the experiments were conducted in triplicate at each time interval and the average was recorded (Galli 2006).

2.7 Antibacterial Test for Cefdinir

This was done by preparing 1µg/ml of cefdinir [according to the reported in vitro minimum inhibitory concentration (MIC)], as well as 5µg/ml and 25µg/ml from Fe+2/Fe+3 LDHs-loaded Cefdinir and Mg+2/Fe+3 LDHs-loaded Cefdinir in phosphate buffer respectively which is containing 1 µg/ml cefdinir according to the percent drug content of cefdinir in Fe+2/Fe+3 LDHs-loaded Cefdinir and MG+2/Fe+3 LDHs-loaded Cefdinir. In addition to 5µg/ml of Fe+2/Fe+3 LDHs and Mg+2/Fe+3 LDHs NPs (without cefdinir). Each sample was tested on two types of Gram –ve bacteria (E. colli , Pseudomonas aeruginosa) and two types of Gram +ve bacteria (Basillus subtilis, Staphylococcus aureus) (ATCC) by culturing on nutrient agar and the zone of inhibition is measured after 24 hours incubation period at 37 ◦C (Carolina 2007).

3. Results and Discussion

3.1 Synthesis of the Layered Double Hydroxides Nanoparticles:

The synthesis of the Fe+2/Fe+3 and Mg+2/Fe+3 LDHs NPs compounds revealed the solubility of trivalent iron oxide is smaller than that of divalent iron oxides, Fe+3 hydrolyzes and forms hydroxide species. The hydrolysis can be induced by heating the solution and the complete hydrolysis results in the formation of a trivalent iron oxide-hydroxide.

3.2 Preparation of Layered Double Hydroxides Loaded Drug:

The final solid resultant powder of Fe+2/Fe+3 LDHs-loaded cefdinir was 7.5 g, and 2.5 g for Mg+2/Fe+3 LDHs-loaded cefdinir, this difference in the weight of the final solid powder is due to the difference in the molar ratio of the divalent, trivalent and the drug used in the preparation (Gursky 2006), where the molar ration for Fe+2/Fe+3 LDHs-loaded cefdinir was (1:4:2), while the molar ratio for Mg+2/Fe+3 LDHs-loaded cefdinir was (3:1:1). The ion-exchange method cannot be applied for cefdinir due to its low aqueous solubility.

The drug loading of montelukast with LDHs NPs was done by ion-exchange method since, montelukast sodium is freely soluble in water so it was added to the freshly prepared mixture of the double layered hydroxides NPs after one hour from the preparation. The final solid resultant powder weight was 6.5 g for Fe+2/Fe+3 LDHs-loaded montelukast and 2 g for Mg+2/Fe+3 LDHs-loaded montelukast. This difference in the weight of the final solid powder is due to the difference in the molar ratio of the divalent, trivalent and the drug used in the preparation, where the molar ratio for Fe+2/Fe+3 LDHs-loaded montelukast was (1:4:2), while the molar ratio for Mg+2/Fe+3 LDHs-loaded montelukast was (3:1:1).

Cefdinir and montelukast anions are believed to be intercalated physically not only into the cationic LDHs interlayer space, but also may adsorbed onto the surface of the LDHs, and the charge balance between the divalent/trivalent LDH interlayer spaces ultimately determined the amount of intercalated montelukast (Carolina 2007).

The percentage yield of the prepared Fe+2/Fe+3 LDHs-loaded cefdinir and Mg+2/Fe+3 LDHs-loaded cefdinir was 84.65% and 26.44% respectively, the percentage yield of the prepared Fe+2/Fe+3 LDHs-loaded montelukast and Mg+2/Fe+3 LDHs-loaded montelukast was 60.86% and 20.40% respectively.

The determination of the percentage drug loading (entrapment efficiency) of cefdinir and montelukast were studied using phosphate buffer solution in pH 7.4. The result showed that %(entrapment efficiency) of cefdinir in Fe+2/Fe+3 LDHs-loaded cefdinir and in Mg+2/Fe+3 LDHs-loaded cefdinir equal to 17.52% w/w and 3.09% w/w respectively. While the entrapment efficiency of montelukast in Fe+2/Fe+3 LDHs-loaded montelukast and in Mg+2/Fe+3 LDHs-loaded montelukast equal 9.114% w/w and 4.006% w/w respectively.

It is found that in both cases (cefdinir and montelukast) the percentage drug loading in Fe+2/Fe+3 LDHs NPs is larger than that in Mg+2/Fe+3 LDHs NPs, this could be attributed to the similarity of the divalent and trivalent cationic irons size and shapes that provide a larger interlayer space for the anion to be intercalated allowing multisite for intercalation so providing more loading capacity (Dengke 2010).

Also the loading capacity of cefdinir on Fe+2/Fe+3 LDHs NPs is significantly higher than that of montelukast on the same LDHs NPs, this could be attributed to the difference in molecular weight of both drugs, where the molecular weight of cefdinir (395.42 g/mole) is less than that of montelukast (586.148 g/mole), also cefdinir have more structural functional groups that can give more intercalation sites with LDHs NPs (Drug Topics 2009).

3.3 Characterization of the prepared nanoparticles and their corresponding drug loaded:

The prepared LDHs NPs (Fe+2/Fe+3 and Mg+2/Fe+3 LDHs) were characterized using the transmission electron microscope which demonstrated that the average particle size of the Fe+2/Fe+3 and Mg+2/Fe+3 LDHs NPs were 45 nm and 78 nm respectively. The TEM images in (Figure 1) showed that the particle shape of each one were spherical (Zolfaghar 2008).

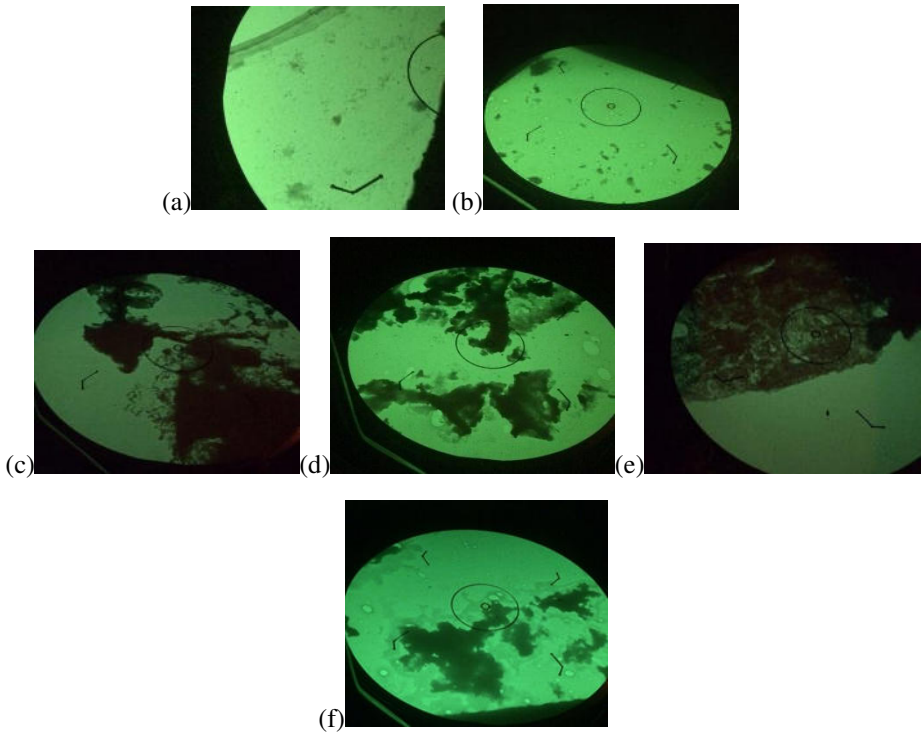


Figure 1: TEM images of the prepared (a) Fe+2/Fe+3 LDHs (b) Mg+2/Fe+3 LDHs (c) Fe+2/Fe+3 LDHs-loaded Cefdinir (d) Mg+2/Fe+3 LDHs-loaded Cefdinir (e) Fe+2/Fe+3 LDHs-loaded Montelukast (f) Mg+2/Fe+3 LDHs-loaded Montelukast

The TEM images for LDHs after loading (Figure 1) demonstrates that the black spots of cefdinir and montelukast dispersed in the spherical matrix of the LDHs (both Fe+2/Fe+3 and Mg+2/Fe+3 LDHs NPs) indicating the intercalation of drug within the LDHs NPs with increase in the particle size, where the average particle size of Fe+2/Fe+3 LDHs changed from 45 to 62 nm and 65 nm after loading of cefdinir and montelukast respectively, and that of Mg+2/Fe+3 LDHs changed from 78 to 88 nm and 90 nm after loading of cefdinir and montelukast respectively, which is in a good consistency of higher loading capacity of Fe+2/Fe+3 LDHs NPs for both drugs (Zolfaghar 2008).

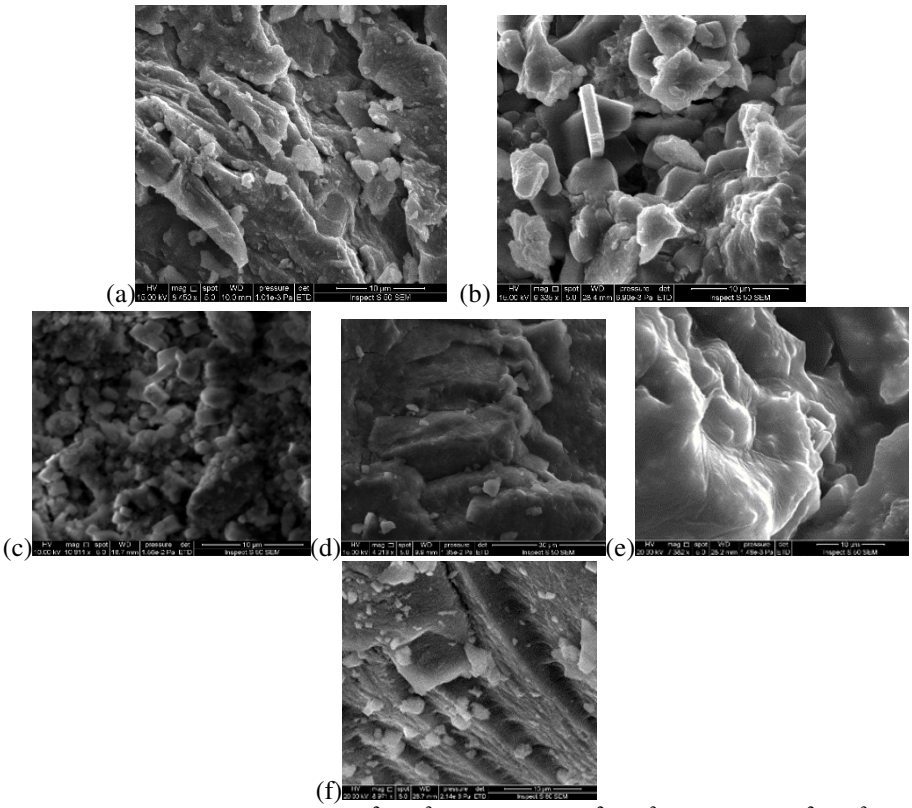


Figure 2 SEM images of the prepared (a) Fe^{+2}/Fe^{+3} LDHs (b) Mg^{+2}/Fe^{+3} LDHs (c) Fe^{+2}/Fe^{+3} LDHs-loaded Cefdinir (d) Mg^{+2}/Fe^{+3} LDHs-loaded Cefdinir (e) Fe^{+2}/Fe^{+3} LDHs-loaded Montelukast (f) Mg^{+2}/Fe^{+3} LDHs-loaded Montelukast

The SEM images of the prepared LDHs NPs displayed in (Figure 2) (A) for Fe+2/Fe+3 LDHs and (B) for Mg+2/Fe+3 LDHs, provide information about the particle morphology, where layered structure is visualized. The Fe+2/Fe+3 LDHs showed smaller particles with better homogenous distribution than Mg+2/Fe+3 LDHs which showed digger irregular shaped particles with sharp edges and observable breaks or holes (Xu 2005).

The SEM imaged after loading of LDHs NPs with drug Figure 4 showsed the changes in the morphology of the particles for Fe+2/Fe+3 and Mg+2/Fe+3 LDHs-loaded cefdinir and montelukast respectively, where an obvious shape transformation from small ball shape into larger cubic and octahedral shape that indicated intercalation of drug with in the LDHs NPs (drug topics 2009). It is observed that in Mg+2/Fe+3 LDHs-loaded drug showed layered agglomerates with larger holes between the particles and this may affect release pattern of the drug.

Table 1 EDX analysis composition of the prepared LDHs

LDHs Type	C%	O_2 %	Na	Fe	Cl	Mg	N	S	%Drug Content*
Fe^{+2}/Fe^{+3} LDHs	_	21.33	1.84	64.76	3.92	_	_	_	_
Mg^{+2}/Fe^{+3} LDHs	_	25.42	10.23	**44.75**	**5.84**	**13.72**	_	_	_
Fe^{+2}/Fe^{+3} LDHs-loaded cefdinir	**14.87**	_	**20.77**	**13.43**	**29.11**	_	**8.46**	**9.79**	**17.52% Cefdinir**
Mg^{+2}/Fe^{+3} LDHs-loaded cefdinir	**1.38**	_	_	**0.54**	_	**5.92**	**1.14**	_	**3.09% Cefdinir**
Fe^{+2}/Fe^{+3} LDHs-loaded montelukast	**14.87**	_	**20.77**	**13.43**	**29.11**	_	**8.46**	**9.79**	**9.11% Montelukast**
Mg^{+2}/Fe^{+3} LDHs-loaded montelukast	**1.38**	_	_	**0.54**	_	**5.92**	**1.14**	_	**4.00% montelukast**

***** based on UV-Visible measurements.

The energy dispersive X-ray spectrometry (EDX) analysis of the prepared LDHs before and after loading (Figure 3) and (Table 1) shows the presence of the essential divalent and trivalent cations of the LDHs and functional groups of the drugs (Zolfaghar 2008).

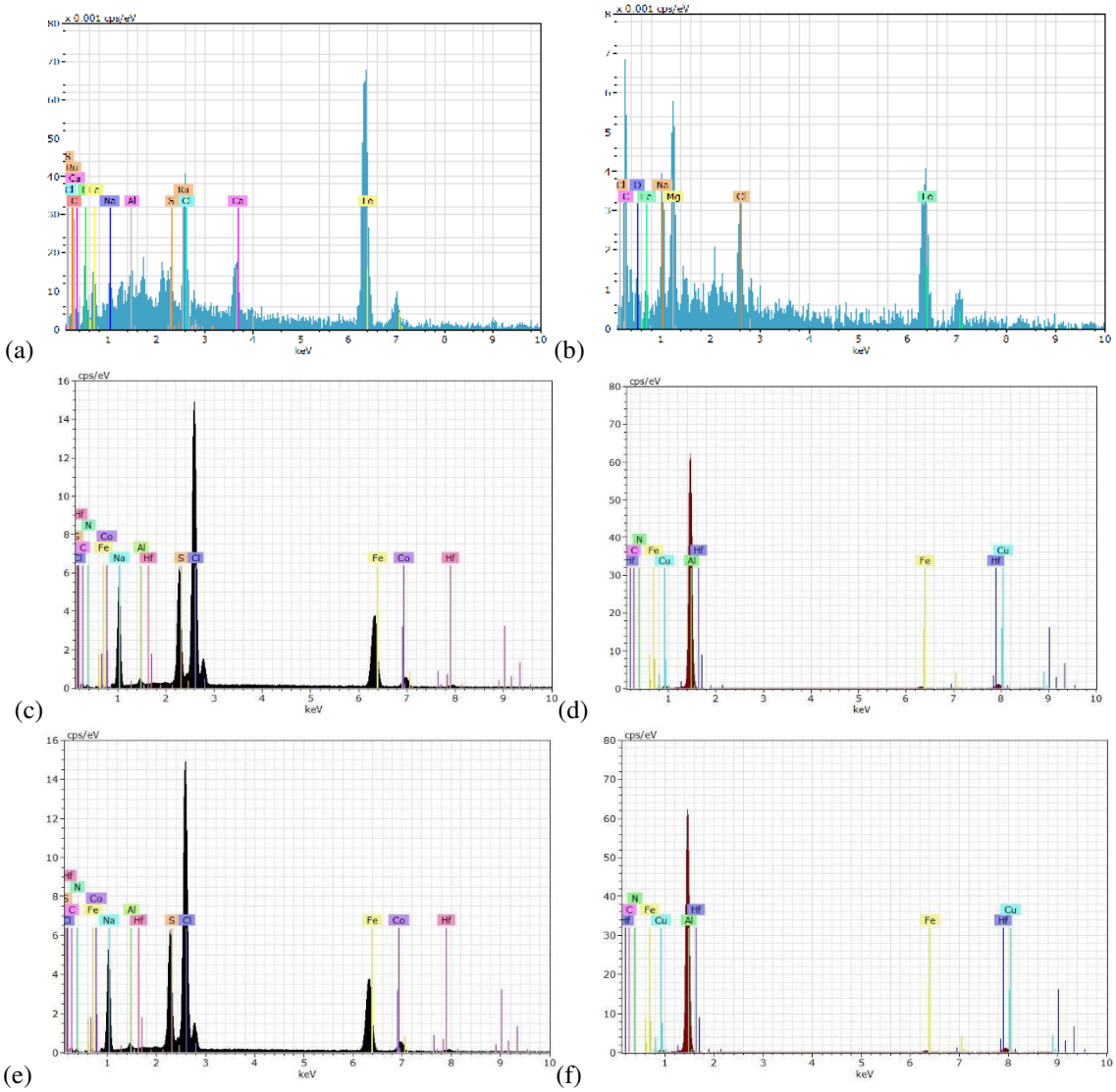


Figure 3 EDX analysis of the prepared (a) Fe^{+2}/Fe^{+3} LDHs (b) Mg^{+2}/Fe^{+3} LDHs (c) Fe^{+2}/Fe^{+3} LDHs-loaded Cefdinir (d) Mg^{+2}/Fe^{+3} LDHs-loaded Cefdinir (e) Fe^{+2}/Fe^{+3} LDHs-loaded Montelukast (f) Mg^{+2}/Fe^{+3} LDHs-loaded Montelukast

The FT-IR spectrum for pure cefdinir and the prepared LDHs-loaded cefdinir displaced in (Figure 4), in which the FT-IR spectrum for the prepared LDHs exhibit the disappearing of the hydroxyl groups bands and show the broad band at (3400 cm-1) attributed to the primary and secondary amines as well as the bands at (1789, 1645 and 1525 cm-1) for the carboxyl and C=N stretching, the disordering in the IR spectrum indicating the loading of cefdinir with the nanocompound takes place (Saurabh 2013, Juliana 2004).

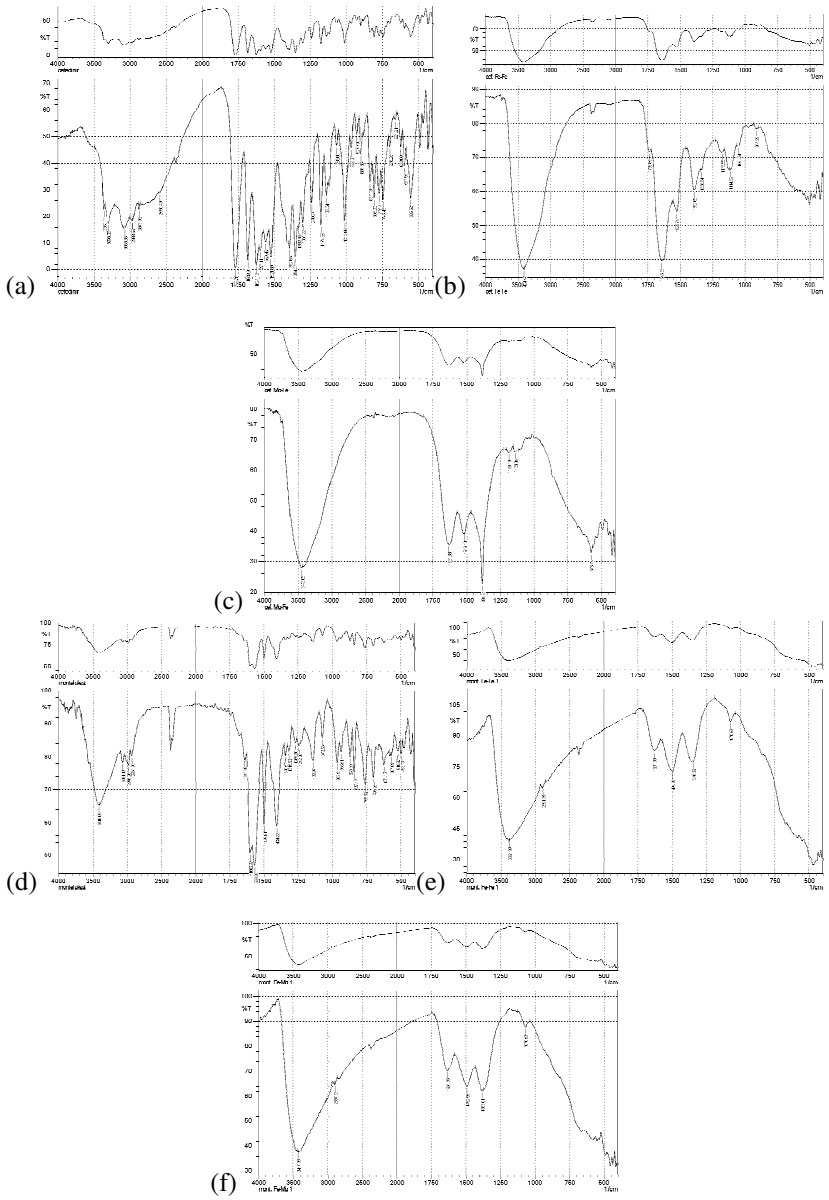


Figure 4 FT-IR spectra for (a) pure Cefdinir (b) Fe^{+2}/Fe^{+3} LDHs-loaded Cefdinr (c) Mg^{+2}/Fe^{+3} LDHs-loaded Cefdinir (d) Montelukast (e) Fe^{+2}/Fe^{+3} LDHs-loaded Montelukast(f) Mg^{+2}/Fe^{+3} LDHs-loaded Montelukast

The FT-IR spectrum for pure montelukast and the prepared LDHs-loaded montelukast displaced in (Figure 4), in which the FT-IR spectrum for the prepared LDHs shows the disappearing of the hydroxyl group for carboxylic moiety and the broad band showed in new region at (3381 cm-1) due to the hydroxyl group moiety and the other groups was shifted to (1631 and 1494 cm-1) indicating the loading and chelating of montelukast with the double Layered NPs was occured [Juliana 2004, Priyanka 2012).

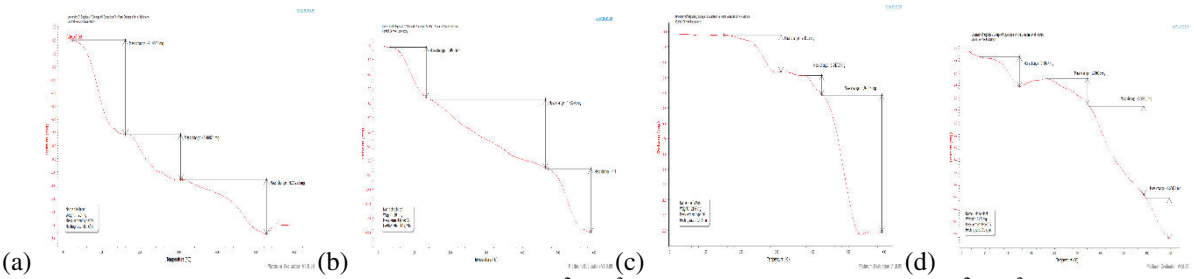
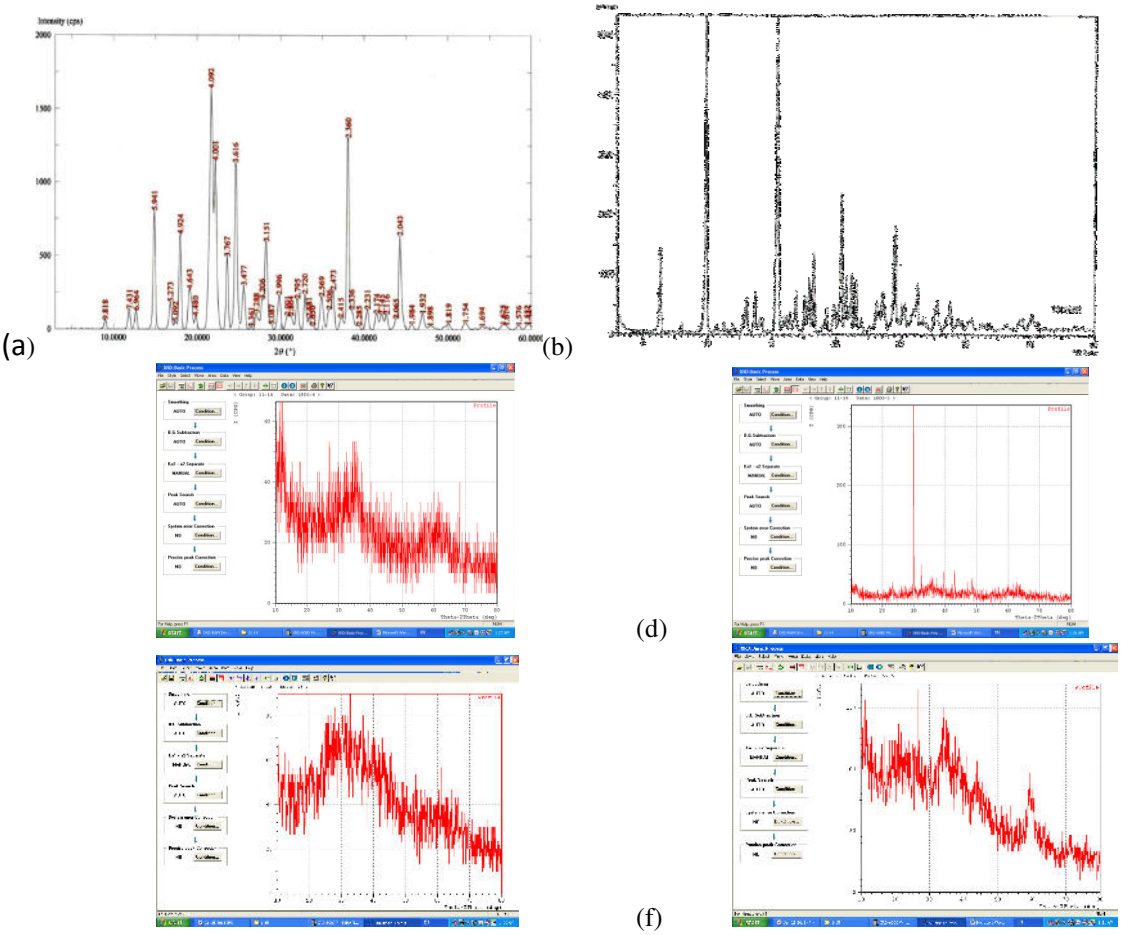


Figure 5 Thermal analysis of the prepared (a) Fe^{+2}/Fe^{+3} LDHs-loaded Cefdinir (b) Mg^{+2}/Fe^{+3} LDHs-loaded Cefdinir (c) Fe^{+2}/Fe^{+3} LDHs-loaded Montelukast (d) Mg^{+2}/Fe^{+3} LDHs-loaded Montelukast

Thermal analysis revealed the changes during heat processing of NPs powder, (Figure 5) showed the changes that taken place from zero to 600 ◦C in atmosphere for the prepared LDHs NPs that showed three to four steps weight loss until complete drug decomposition and LDHs layer dihydroxylation. It provided that the thermal stability of intercalated cefdinir and montelukast molecules in both LDHs NPs were enhanced, which agreed with reported data for naproxen-LDHs NPs [125]. In addition to that the stability of Mg+2/Fe+3 LDHs NPs is slightly higher than that in Fe+2/Fe+3 LDHs by comparing the % of mass loss and temperature of second step which involves decomposition of the drug. The increase in the stability of LDHs-loaded drug structure results from the increased interlayer attraction through bridging of anions which possess also hydrogen bonding and provides more sites available to interact with the LDHs layers (Zhang 2005).



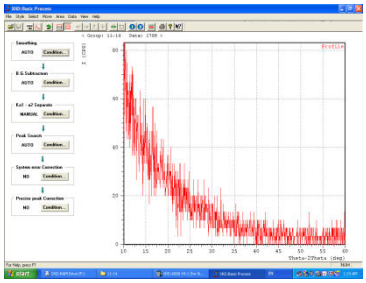
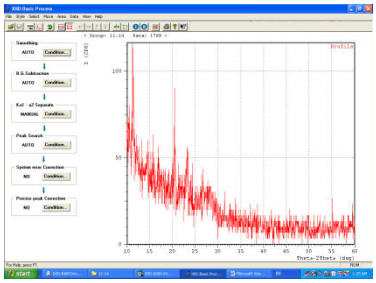
(g)  (h) 

Figure 6 The X-ray patterns of (a) pure cefdinir (b) pure montelukast (c) Fe^{+2}/Fe^{+3} LDHs (d) Mg^{+2}/Fe^{+3} LDHs (e) Fe^{+2}/Fe^{+3} LDHs-loaded Cefdinir (f) Mg^{+2}/Fe^{+3} LDHs-loaded Cefdinir (g) Fe^{+2}/Fe^{+3} LDHs-loaded Montelukast (h) Mg^{+2}/Fe^{+3} LDHs-loaded Montelukast

The reported X-ray diffraction patterns of the pure cefdinir and montelukast (Figure 6), displayed the presence of numerous narrow and symmetrical characteristic diffraction peaks with high intensity that indicated the crystalline structure of the drugs [131,132]. While X-ray diffraction pattern of the prepared LDHs NPs before and after drug loading showed no sharp peak and less intensity of the diffraction peaks, indicating the amorphous structure of the prepared NPs (Lewin 2001).

Table 2 Zeta Potential Values for the prepared LDHs NPs before and after loading

LDHs NPs Type	Zeta Potential (mV) ± SD	Mobility (µ/s)/(V/cm) ±SD	Frequency (Hz) ±SD	Frequency Shift (Hz) ±SD
Fe^{+2}/Fe^{+3} LDHs	-43.54 ±2.93	-2.87±0.19	226.36±1.69	- 23.87±1.84
Mg^{+2}/Fe^{+3} LDHs	-24.67±1.40	-1.63±0.09	236.84±0.72	- 13.02±0.80
Fe^{+2}/Fe^{+3} LDHs-loaded Cefdinir	-26.38±0.81	-1.74±0.05	235.50±0.43	- 15.50±0.85
Fe^{+2}/Fe^{+3} LDHs-loaded Montelukast	-26.27±4.22	-1.73±0.28	235.74±2.31	- 13.71±1.30
Mg^{+2}/Fe^{+3} LDHs-loaded Cefdinir	-23.27±1.59	-1.58±0.11	237.17±0.89	- 11.99±2.01
Mg^{+2}/Fe^{+3} LDHs-loaded Montelukast	-28.61±1.22	-1.89±0.08	234.71±0.72	- 16.70±0.46

The zeta potential measurement for the prepared Fe+2/Fe+3 and Mg+2/Fe+3 LDHs NPs before and after loading with drugs assigned in (Table 2), and the criteria of stability of NPs are measured when the values of zeta potential ranged from higher than +20 mV to lower than -20 mV. The values of the zeta potential of the prepared LDHs NPs before and after loading provided satisfactory evidence about their little tendency towards aggregation when its zeta potential in the negative scale and below -20 mV. This behavior unambiguously suggested the presence of strong electric charges on the particle surfaces to hinder agglomeration. These values were found to fall in the negative side for the LDHs before and after loading. This result suggested that the LDHs NPs particles and their LDHs-loaded drug were stable with no tendency to aggregates and this in accordance with the results reported for colloidal nanoparticles behavior (Mohammed 2014).

3.4 In Vitro Drug Release Study:

The release of the cefdinir and from the Fe+2/Fe+3 LDHs-loaded cefdinir (Figure 7) was slow without burst effect and persistent to give 98% release within 5 hours, T80 % release was within 240 min and T50 % release was within 120 min. This slow and sustained release process may be explained on the basis of the slow ion exchange process between the intercalated anions and phosphate anions in the buffer. While the release of cefdinir from the Mg+2/Fe+3 LDHs-loaded cefdinir was faster where T50 % release within 20 min, T50 % with 60 min and T100 % release within 150 min. This could be attributed to the presence of layered agglomerates with large holes between the particles in the Mg+2/Fe+3 LDHs-loaded cefdinir as shown from its SEM analysis that permit faster penetration of dissolution medium compare to Fe+2/Fe+3 LDHs-Loaded cefdinir which showed more compact structure (Dengke 2010).

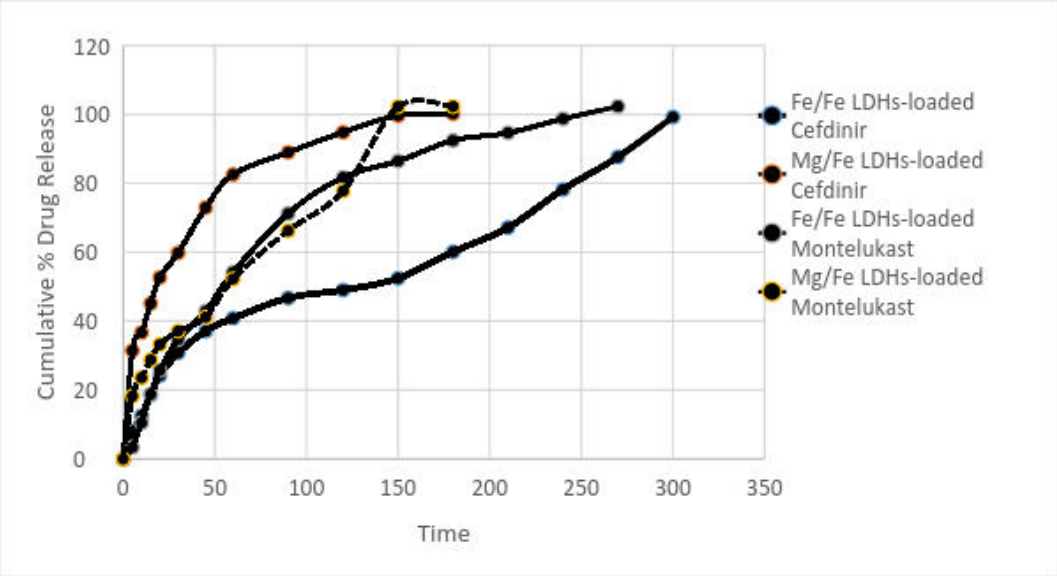


Figure 7: In Vitro drug release of Cefdinir and amaontelukast from the prepared LDHs-loaded drug

Same results were obtained with the release of montelukast from Fe+2/Fe+3 and Mg+2/Fe+3 LDHs-loaded drug where slower release pattern was obtained from Fe+2/Fe+3 than Mg+2/Fe+3 LDHs and the release pattern presented in (Figure 7).

It was found that Higuchi order model was best fitted for most LDHs-loaded drug NPs, indicated by highest regression value (R2=0.9764). This result indicated that most prepared LDHs NPs exhibit diffusion mechanism in drug release accompanied by acceptable regression value for zero order. Kinetic model which best fit zero order and Higuchi's diffusion equation were most suitable for controlled release formulation (Sharma 2004).

3.5 In Vitro Drug Diffusion

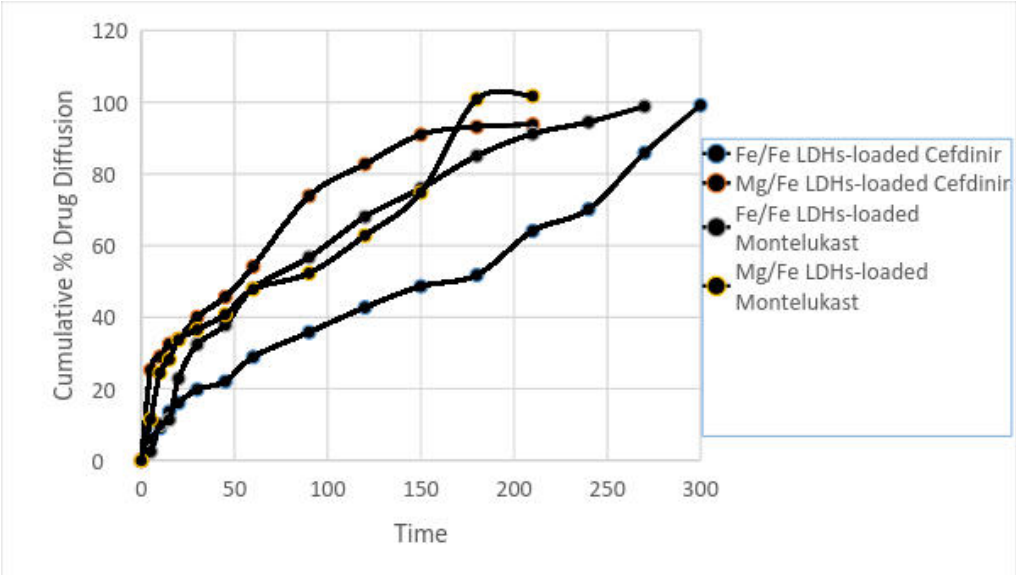


Figure 8 In Vitro drug diffusion of Cefdinir and Montelukast from the prepared LDHs-loaded Drug

The in vitro diffusion curves for both cefdinir and montelukast from the prepared Fe+2/Fe+3 and Mg+2/Fe+3 LDHs-loaded drug (Figure 8) was identical with the in vitro release study indicating that the released drug is readily pass freely through the dialysis membrane and this is due to the small particle size of the drugs loaded on

the LDHs NPs that improves the permeability of the drug through semipermeable membrane and this is particularly important for class IV drugs which is characterized by low permeability, that may improve its bioavailability and drug action.

3.6 Antibacterial Test for Cefdinir

Table 3 Antibacterial activity of pure cefdinir and the prepared LDHs NPs represented by zone of inhibition (mm)

Sample Name	***E. Coli***	***Pseudomonas***	***Bacillus***	***St. Aureus***
Control(buffer 7.4 pH)	_	_	_	_
Fe^{+2}/Fe^{+3} LDHs	_	_	_	_
Mg^{+2}/Fe^{+3} LDHs	_	_	_	_
Fe^{+2}/Fe^{+3} LDHs-loaded cefdinir	25 mm	35 mm	41 mm	44 mm
Mg^{+2}/Fe^{+3} LDHs-loaded cefdinir	20 mm	43 mm	37 mm	42 mm
Pure Cefdinir	23 mm	35 mm	35 mm	40 mm

The antibacterial activity (Table 3) of pure cefdinir and the prepared LDHs NPs on two types of Gram –ve bacteria (Escherichia colli, Pseudomonas aeruginosa) and two types of Gram +ve bacteria (Basillus subtilis, Staphylococcus aureus) showed an obvious increase in the antibacterial activity for the Fe+2/Fe+3-cefdinir LDHs NPs compared to the pure cefdinir on the both Gram –ve and Gram +ve bacteria. The Mg+2/Fe+3-cefdinir LDHs NPs displayed increasing in antibacterial activity for all used bacteria except E. colli. The increasing in the antibacterial activity of the LDHs NPs loaded with cefdinir is attributed to the fact that the prepared nanocarriers are neutral hybrid can enter cells by moving across negatively charged cell membrane without repulsive electrostatic interactions that would be experienced by guest anion alone in addition to its small size (nanosize) (Nalawade 2009). The test also done for Fe+2/Fe+3 and Mg+2/Fe+3 LDHs NPs (without drug) and no antibacterial activity appeared. Therefore, incorporation of cefdinir in LDHs NPs leads to increase its permeability through bacterial cells and to improve its antibacterial activity and this study is in a good agreement with diffusion study previously mentioned.

4. Conclusions

Intercalation of class II drug (cefdinir) and class IV drug (montelukast) with LDHs NPs via intercalation method and ion-exchange method provides a promising nanocarriers for poorly soluble and low permeable drug with the ability to control drug release, site-specificity. The SEM and TEM analysis reveals more compact and non-porous structure of Fe+2/Fe+3-LDHs NPs With smaller particle size than Mg+2/Fe+3-LDHs NPs. The PXRD demonstrates the amorphous structure of the prepared LDHs before and after drug loading. Thermal analysis demonstrates the obviously improved thermal stability of intercalated organic compounds after intercalating into LDH interlayer due to the host–guest interaction. The zeta potential analysis provided satisfactory evidence about the prepared LDHs little tendency towards aggregation.

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