Layered Double Hydroxide Nano-carrier for Controlled Delivery of Drug Molecules

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of

Bachelor of Technology

by

Ramu Ranjan Meher
(Roll No. 108CR038)

Department of Ceramic Engineering
National Institute of Technology,
Rourkela, Odisha.
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Supervisor:
Dr. Sudip Dasgupta

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Rourkela, Odisha
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National Institute of Technology Rourkela

CERTIFICATE

This is to certify that the thesis entitled, *"Layered Double Hydroxide Nano-carrier for Controlled Delivery of Drug Molecules"* submitted by Mr. **Ramu Ranjan Meher (108CR038)** in partial fulfilments for the requirements for the award of **Bachelor of Technology** degree in **Ceramic Engineering** at National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Date:

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Date: Ramu Ranjan Meher

108CR038

ABSTRACT

Ibuprofen intercalated Mg-Al Layered Double Hydroxide nanohybrids have been prepared for controlled delivery of anti-inflammatory drug molecule ibuprofen (IBU). NSAID such as ibuprofen in anionic form has been intercalated *in situ* into the interlayer space of LDH nano-particle during coprecipitation of hydroxides. LDH-drug nanohybrids have been characterized using DLS, XRD, FTIR, and EDX. The XRD patterns shows a decrease in diffraction angle and anincrease in the interlayer spacing of basal planes of LDH-IBU which is due to the presence of ibuprofen in the interlayer. FTIR analysis indicates that ibuprofen molecules are intercalated into the hydroxide interlayer space and are stabilized by electrostatic forces, intermolecular bonds and Vanderwall's interaction. Stretching vibration of hydroxyl group of ibuprofen at 2980 cm^{-1} in LDH-IBU confirms the presence of ibuprofen in the interlamellar space of LDH-IBU. Drug release behaviour of LDH-drug nanohybrids have been evaluated *in vitro* using UV-Visible spectroscopy in simulated body fluid (SBF) of physiological pH. The drug release behaviour of LDH-IBU nanohybrid shows an initial burst release followed by a slower, steady and sustained release for later time period and importantly more than 95 % of drug was released in 72 hours in SBF.

LIST OF ABBREVIATIONS

Serial Number	Abbreviation	Full form
1	LDH	Layered Double Hydroxide.
2	LDH 9	Layered Double Hydroxide synthesised at pH 9.
3	LDH 10	Layered Double Hydroxide synthesised at pH 10.
4	LDH 11	Layered Double Hydroxide synthesised at pH 11.
5	LDH-IBU	Ibuprofen intercalated LDH synthesised at pH 10.
6	PSD	Particle Size Distribution.
7	XRD	X- Ray Diffraction.
8	FTIR	Fourier Transform Infrared Spectroscopy.
9	EDX	Energy Dispersive X-ray Spectroscopy
10	SBF	Simulated Body Fluid

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Layered Double Hydroxides (LDHs) are generally minerals and synthetically prepared materials that have surface layers formed of positively charged brucite type layer made up of mixed metal hydroxides of divalent and trivalent metals with exchangeable intercalated negatively charged species in between the two surface layers which compensate for the positive charge of the brucite layer.

Chemical composition of LDH is generally expressed as follows:

$$M(II)_{1-x}\, M(III)_x\, (OH)_2\, (A^{n-})_{x/n} \times y\, H_2O$$

Where M(II) is divalent metal cation, M(III) is trivalent metal cation, 'A' is interlayer anionic species, 'n' is charge on interlayer anion, 'x' and 'y' are fraction constants.

LDH have many physical and chemical properties that are surprisingly similar to those of clay mineral. These properties are their layered structure, wide chemical compositions (due to variable isomorphous substitution of metallic cations), variable layer charge density, ion-exchange properties, reactive interlayer space and rheological and colloidal properties. But because of their anion-exchange properties, LDH are known as 'anionic clays'.

Anticancer drug molecules such as Methotrexate, 5-Flurouracil and anti-inflammatory drug molecule such as ibuprofen are negatively charged; hence they can be intercalated into the LDH molecule for the delivery into targeted location in human body. As many of the biomolecules are negatively charged, they can also be intercalated into LDH for gene of DNA delivery.

The brucite type layer is of structure with hydroxyl (OH^-) groups in hexagonal close packingand each divalent metal cation is octahedrally coordinated to six OH^- groups and

these octahedra share edges to form the layers. Because all octahedrally coordinated sites between oxygen layers are occupied by cations, this structure is described as trioctahedral; each OH^- group is surrounded by three occupied octahedral positions.

LDH-drug nanohybrids have a positive zeta potential, therefore the nanohybrid particles can approach and adhere to the negatively charged cell membrane via electrostatic interaction. These nanohybrids are internalized into the cell by phagocytosis (LDH agglomerates, particles larger than 500nm) and endocytosis (individual crystallite of smaller size, < 300nm). Endocytosis leads to quicker uptake of LDH nanoparticles. The cellular uptake can be enhanced by decreasing the particle size, adjusting the zeta potential and conjugating the ligands to enhance the receptor mediated endocytosis process.

Using LDH as drug delivery agent is advantageous because of its easy preparation, particle size control, versatile composition, very good biocompatibility, pharmaceutical antacid behaviour, very low cytotoxicity, surface charge density can be controlled, provide protection to drug molecule, easy attachment of targeting moiety.

Similar to intercalation process, de-intercalation can also occur by ion exchange method with the surrounding ions such as Cl^- and/or phosphates. More possible release pathway is the acidic dissolution of hydroxide layer due to the low pH in the intracellular environment. This is the only pathway for release of big anionic species.

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

LDH shows tremendous promise in its use as a controlled drug delivery system because of its ability to intercalate drug molecule in its interlayer space and its non-toxicity in living tissues. Many researchers have investigated its potential as a carrier of functional biomolecule in recent past.

Jin-Ho Choy *et al* [1] synthesised nanohybrids of LDH, methotrexate (MTX) and LDH, folinic acid by ion exchange reaction. They showed that the intercalated molecules are stabilized in the tilted longitudinal monolayer mode by electrostatic forces.The further carried out cellular uptake test of MTX–LDH nanohybridsin SaOS-2 cell line (Osteosarcoma) *in vitro* by MTT assay and inferred that the proliferation of SaOS-2 cell is suppressedmore stronglyby MTX–LDH hybrid than with MTX alone. Hence they concluded thatLDH increases the drug release rate along with acting as abiocompatible delivery medium.

Zhongliang Wang *et al* [2] synthesised LDH containing 5-Flurouracil (5-FU) by reconstruction method. They stated that the stabilisation of 5-FU in the LDH interlayer is due to electrostatic forces and intermolecular forces. They further studies the release of the drug and found that and rapid release in followed by sustained release of the drug molecule over time.

Manjusha Chakraborty *et al* [3] synthesised nanovector for delivery of anticancerous drug containing methotrexate (MTX) in ZnAl-layered double hydroxide(LDH) by anionexchange method. They produced nanoparticles of range 100–300nm by ion-exchange method. They revealed using small angle XRD that the interplannar spacing has been increased from 8.9 Aº to 21.3 Aº after MTX intercalation. Using thermogravimetric analysis they showed that the thermal stability of the MTX increase when it is intercalated in the LDH. They studied the release profile of the drug inphosphatebuffersaline(PBS) and inferred

that the drug sustained for 48 hours and release occurred by a diffusion model given by Rigter-Peppas.

Jae-Min Oh *et al* [4] intercalated methotrexate (MTX) into MgAl layered double hydroxide by co-precipitation method.To check the toxicity of LDHs they used the normal one (human fibroblast) and the osteosarcoma cell culture lines (Saos-2 and MG-63) in laboratory and inferred that no harm effect is seen on the cells used for a concentration of LDH up to 500 ug/mL. They also partially confirmed that LDH is non-toxic to human cells and it also enhances cellular permeation. Anticancerousactivity of MTX-LDH nanoparticleswere determined using MTT and BrdU bioassay with the bone cancer cell culturelines (Saos-2 and MG-63) and proved that the anticancerous efficacy of MTX-LDH is much higher than that of pristine LDH.

Weishen Yang *et al* [5] studied the thermal behaviour of Mg–Al–CO_3 layered double hydroxide. They suggested that the double layer structure remain intact on heating to 70-190ºC, only the loosely bound interlayer water molecules are released at this temperature. The disappearance of OH^- group in a Al–(OH)–Mg structure begins at 190ºC, and is the conformation is completely lost at 280ºC.The transformation of the LDH structure begins in same temperatures range. They inferred thatdisappearance of OH^- group in a Mg–(OH)–Mg structure begins at 280ºC, and is the conformation is completely lost at the temperature 405ºC.Degradation of LDH structure starts after reaching this temperature value. Carbonate evolution started from lower temperature but high rate of carbonate loss is seen after reaching the temperature of 410ºC and all the carbonates are lost after reaching the temperature 580ºC after which the material become amorphous metal stable mixed oxides.

L. Mohanambe *et al* [6] synthesized nanovectors of nonsteroidal anti-inflammatory drug molecules (NSAID) Ibuprofen, Diclofenac, and Indomethacin in Mg-Al layered double

hydroxide and used molecular dynamics (MD) simulationsto determine the interlayer structure, orientation and geometry of the intercalated species. They confirmed bilayer arrangement of drug molecule in the interlamellar space. The geometry of the Diclofenac and Indomethacin changed on intercalation while there is no change in geometry of ibuprofen. They said the change is geometry is due to electrostatic forces between the electronegative chlorine in drug and the positively charged metal hydroxide layer of the anionic clay. The change in geometry occurs without distorting the layer structure.

Manjusha Chakraborty *et al* [8] synthesised LDH-MTX nanovector by *ex situ* and *in situ* processes. Analysing the XRD pattern they said that MTX molecules are present in interlamellar space and are in stabilizedintiltedlongitudinalconformation. From FTIR spectra they found two hydroxyl peaks of MTX and inferred that MTX was successfully intercalated in the LDH-MTX nanovector. From HRTEM image they confirmed the increase in the interlamellar spacing. Further they suggested that synthesis route is responsible for the size and morphology of the nanovectors.

CHAPTER 3

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

3.1.Preparation of Layered Double Hydroxide Nanoparticles

3.1.1.Batch Preparation:For 0.1 M Mg_2Al Layered double hydroxide.

0.066 M - Magnesium Nitrate

$Mg(NO_3)_2.6H_2O$ → Molecular Mass 256.41

2.563 g required in 150 ml solution.

0.033 M – Aluminium Nitrate

$Al(NO_3)_3.9H_2O$ → Molecular Mass 375.13

1.875 g required in 150 ml solution.

Preparation of 0.2 M and 0.02 M NaOH solution for use in pH control.

3.1.2.Synthesis Method (Co-precipitation Technique):

(i) A solution of mixed metal salts ($Mg(NO_3)_2.6H_2O$ and $Al(NO_3)_3.9H_2O$ in 2:1 ratio) in water was vigorously stirred.

(ii) Along with stirring 0.2 M NaOH solution and 0.02 M NaOH solution was added to induce co-precipitation.

Samples were precipitated at three different pH that is at pH 9.2, pH 10.4 and pH 11.2.

(iii) Then the precipitates were aged at room temperature for 12 hours.

(iv) Then the precipitates were filtered and then washed with water thoroughly.

Washing Procedure:

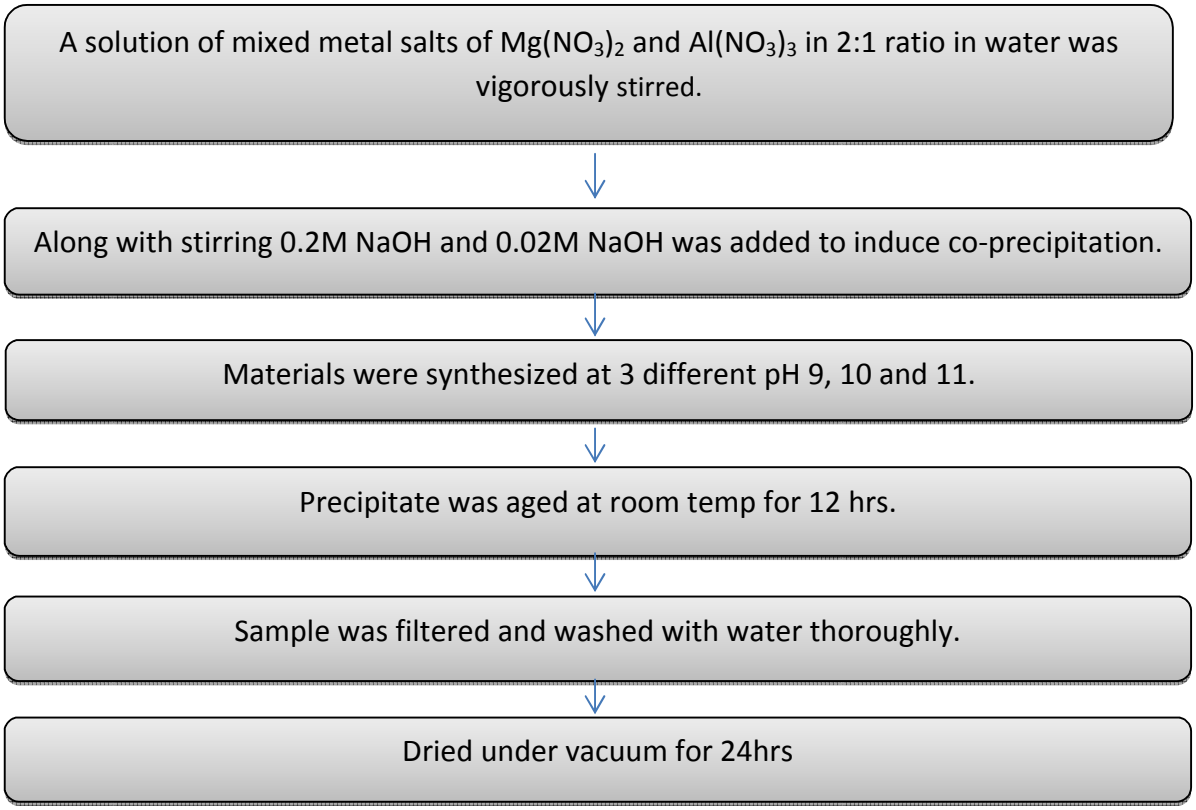
Centrifugation to settle the LDH nanoparticles (8000 rpm for 5 mins), washing the settled precipitate with water to remove molecules and ions present in the surface. Proper washing was done by stirring with addition of water and again centrifuging.

(v) Finally the centrifuges were dried under vacuum for 24 hours.

(vi) Dried sample was crushed and ground using mortar and pastle.

Flowchart: 1 shows the steps of synthesis of LDH by coprecipitation method.

Basic flow chart of the Synthesis Technique of LDH



Flowchart 1: Basic flowchart for synthesis of ibuprofen intercalated LDH

3.2.Preparation of drug ibuprofen intercalated Layered Double Hydroxide Nanoparticles

3.2.1. Batch Preparation: : For ibuprofen intercalated Mg.Al LDH.

0.066 M - Magnesium Nitrate

$Mg(NO_3)_2.6H_2O$ → Molecular Mass 256.41

2.563 g required in 150 ml solution.

0.033 M – Aluminium Nitrate

$Al(NO_3)_3.9H_2O$ → Molecular Mass 375.13

1.875 g required in 150 ml solution.

3.2.2. Synthesis Method (Co-precipitation Technique):

(i) Decarbonated water was prepared by continuously purging nitrogen over deionised water for 3hrs and 15 mins.

(ii) Mixed metal salts ($Mg(NO_3)_2.6H_2O$ and $Al(NO_3)_3.9H_2O$ in 2:1 ratio) in water were added and was vigorously stirred.

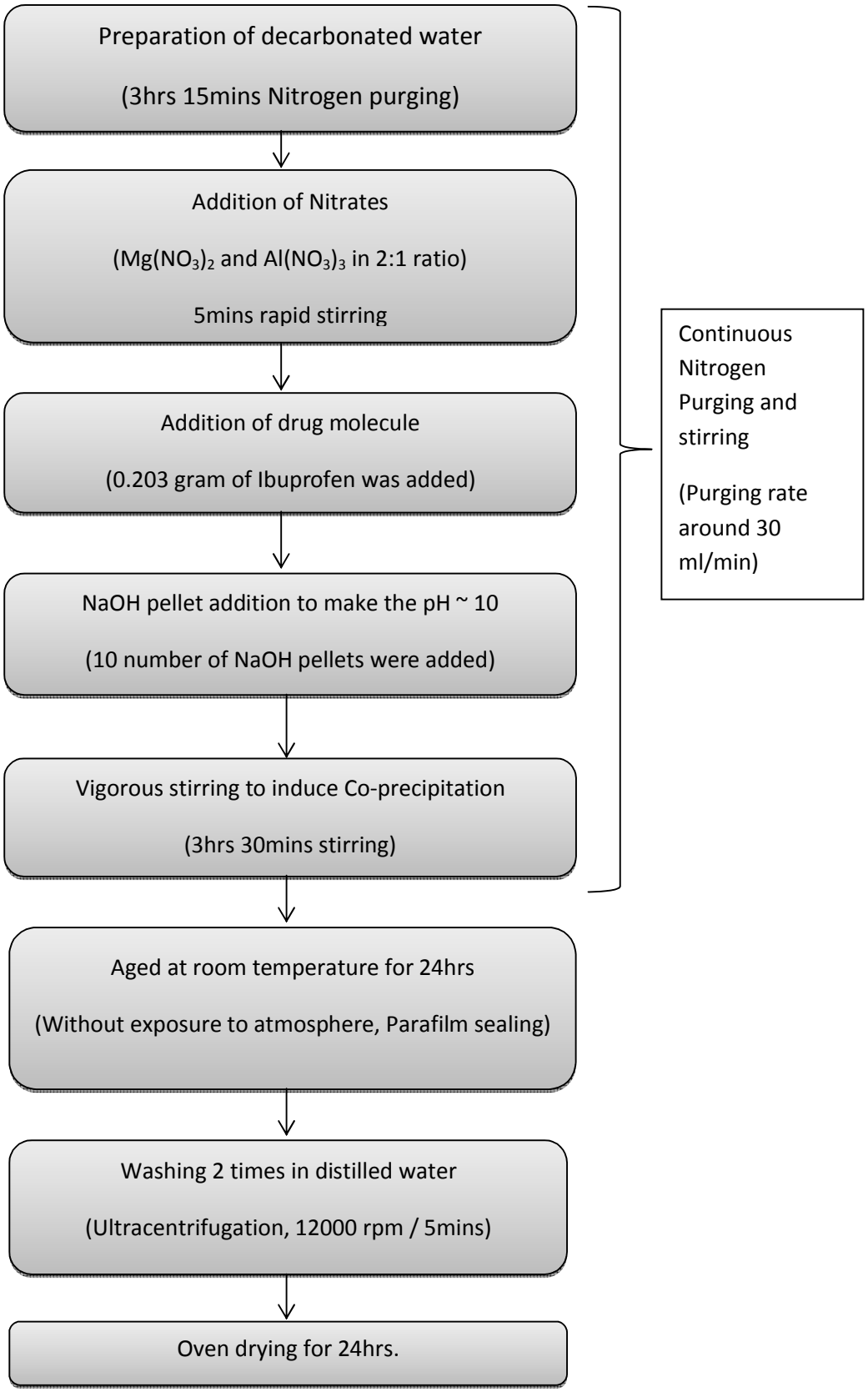
(iii) 0.203 gm. of drug molecule was added with continuous nitrogen purging and vigorous stirring.

(iv) NaOH pellets were added to make pH 10 and induce coprecipitation.

(v) After vigorous stirring for 3 hrs. and 30 mins the precipitate was aged at room temperature for 24 hrs.

(vi) Then the precipitate was washed in deionised water twice by Ultracentrifugation, 12000 rpm / 5mins. Then dried in vacuum for 24 hrs. Flowchart: 2 shows the basic steps of synthesis of LDH-IBU

Basic flow chart of the Synthesis Technique of LDH-IBU



Flowchart 2: Basic flowchart for synthesis of Ibuprofen intercalated LDH nanoparticles.

3.3. Characterisations

3.3.1 Particle Size Analysis

- Particle size analysis of the LDH samples synthesised at different pH was determined using Dynamic Light Scattering (DLS) using the Zetasizer machine.

3.3.2. Energy Dispersive X-ray Analysis

- EDX or Energy Dispersive X-ray analysis of the samples of LDH 9, LDH 10 and LDH 11 were done in Scanning Electron Microscope equipment.

3.3.3. XRD Analysis

- XRD analysis of all the synthesised samples were done and compared to get the knowledge about any change in structure.

3.3.4. FTIR Analysis

- FTIR analysis was done for LDH, drug ibuprofen, and LDH-IBU and the relative plot were determined to get the comparative change in bond structure and vibration due to intercalation of drug molecule in LDH.

3.3.5. Release Rate Study

- Using UV-Visible spectroscopy the linear plot between drug concentration and absorbance was obtained for 260 nm UV radiations. From the linear plot of absorbance vs. concentration the relation between the two parameters were determined.

- Then the drug ibuprofen release study was done in SBF from the LDH-IBU nanohybrid. The absorbance of ibuprofen with respect to time in SBF was determined using UV-Vis spectroscopy and from the absorbance data the concentration of the drug was calculated from the liner plot of absorbance vs. concentration determined earlier.
- SBF preparation was done using A. Cuneyt Tas [7] method.

CHAPTER 4

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1. Particle Size Distribution Analysis:

4.1.1. LDH 9

Intensity distribution

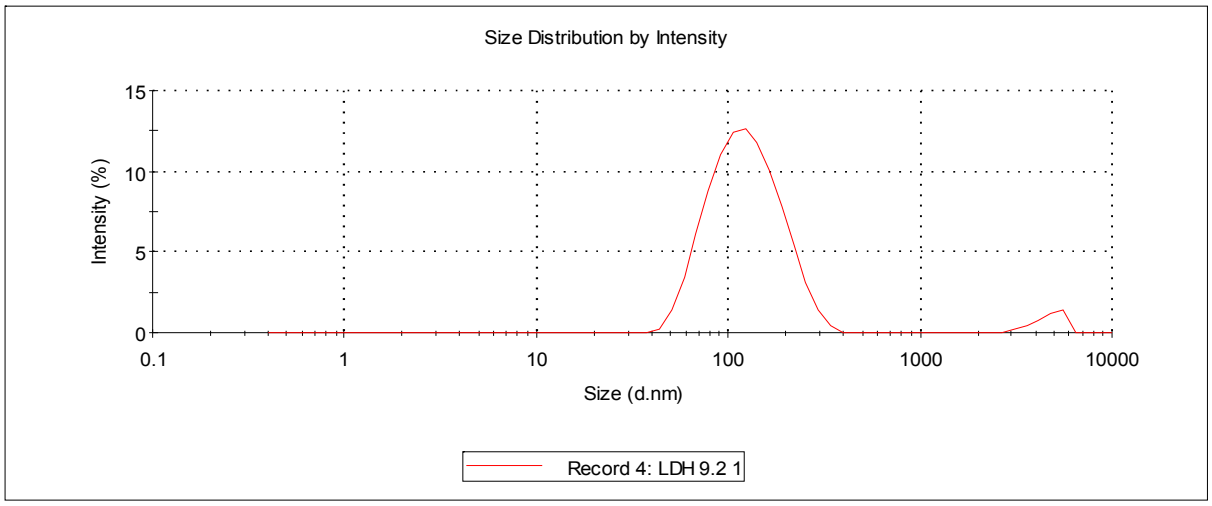


Fig 1: Intensity vs. size plot for LDH synthesised at pH 9.

Volume distribution

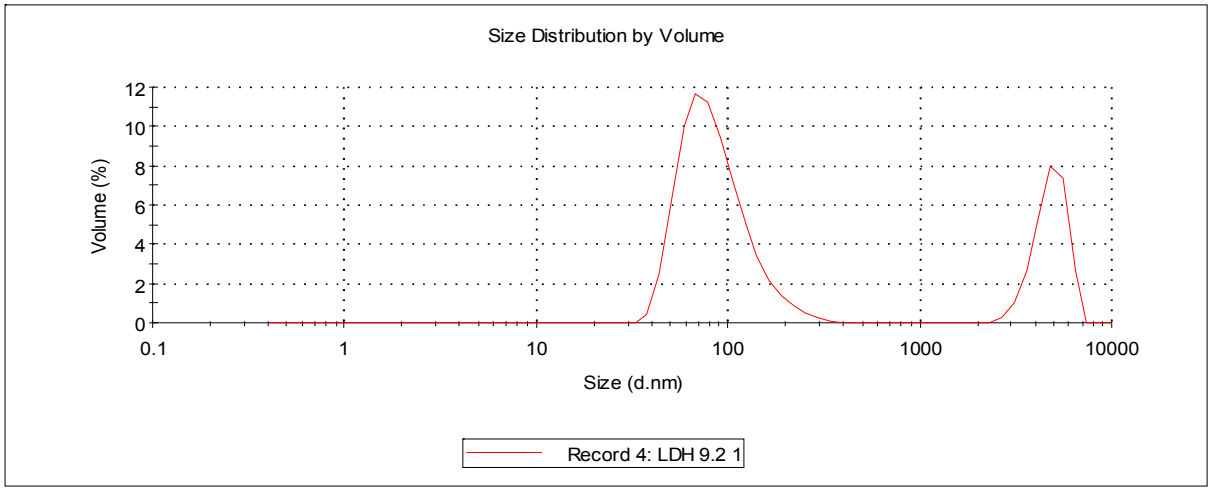


Fig 2: Volume percent vs. size plot for LDH synthesised at pH 9.

Average particle size obtained in this case is 118.8 nm.

Figure 1 represents the intensity vs particle size distribution plot whereas figure 2 shows the volume vs particle size distribution plot. In both the cases the larger particles around 5-6 μm is originated from the bigger dust particle in the powder suspension which is as obviously more intense in volume distribution plot in figure 2. The particle size obtained in this case ignoring the impurities will be less than 100nm.

4.1.2.LDH 10

Intensity distribution

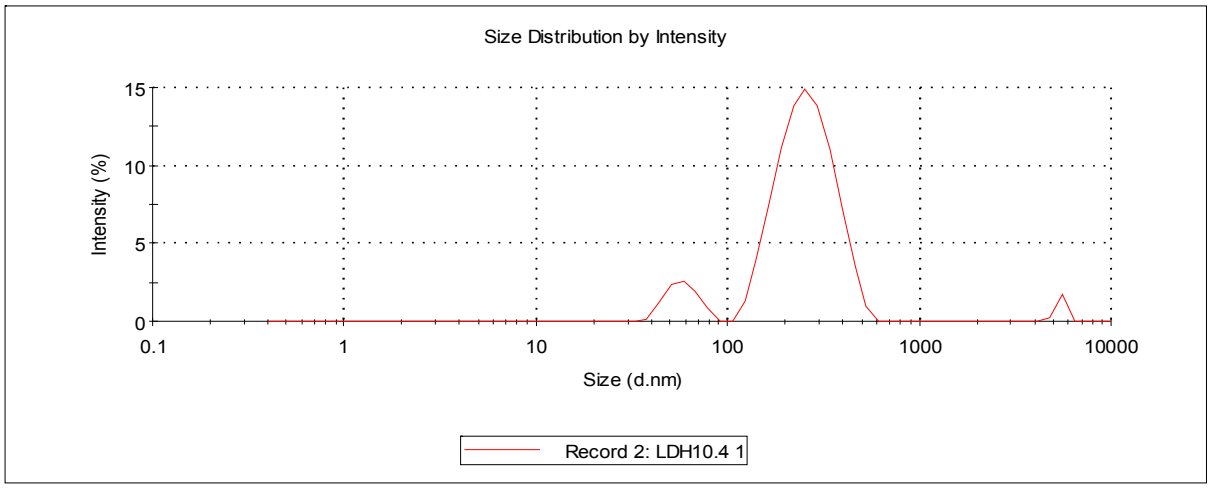


Fig 3: Intensity vs. size plot for LDH synthesised at pH 10.Volume distribution

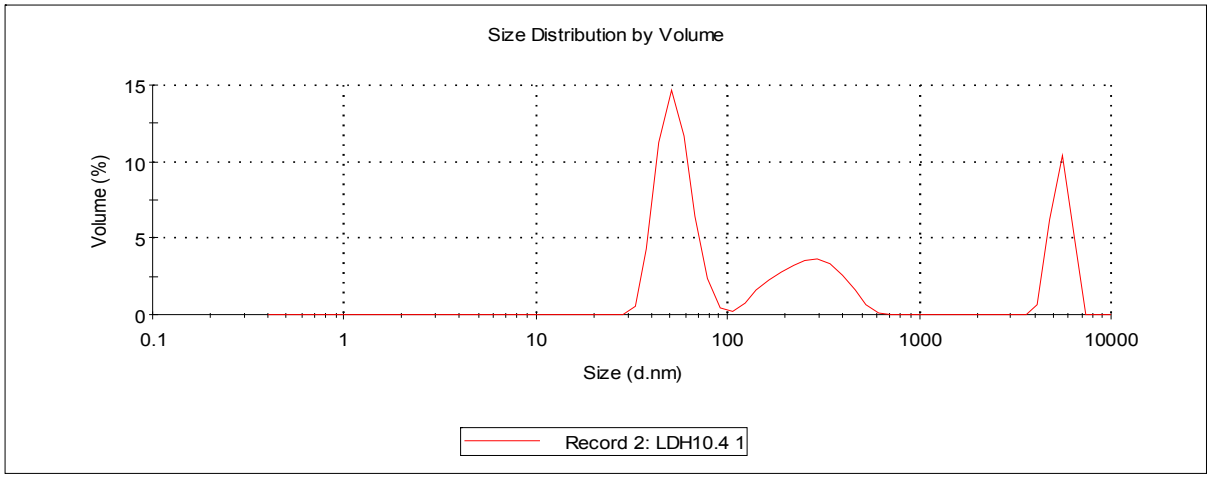


Fig 4: volume percent vs. size plot for LDH synthesised at pH 10.

Figure 3 and figure 4 show the intensity vs particle size and volume vs particle size distribution plot of LDH synthesized at pH 10. LDH 10 powders showed an average particle size of 240 nm. In this case also the particle sizes around 5-6 μm was originated from fewer dust particles in the powder suspension coming from air. Very small particles below 100nm were also present though they were found in smaller concentration.

4.1.3. LDH-11

Intensity distribution

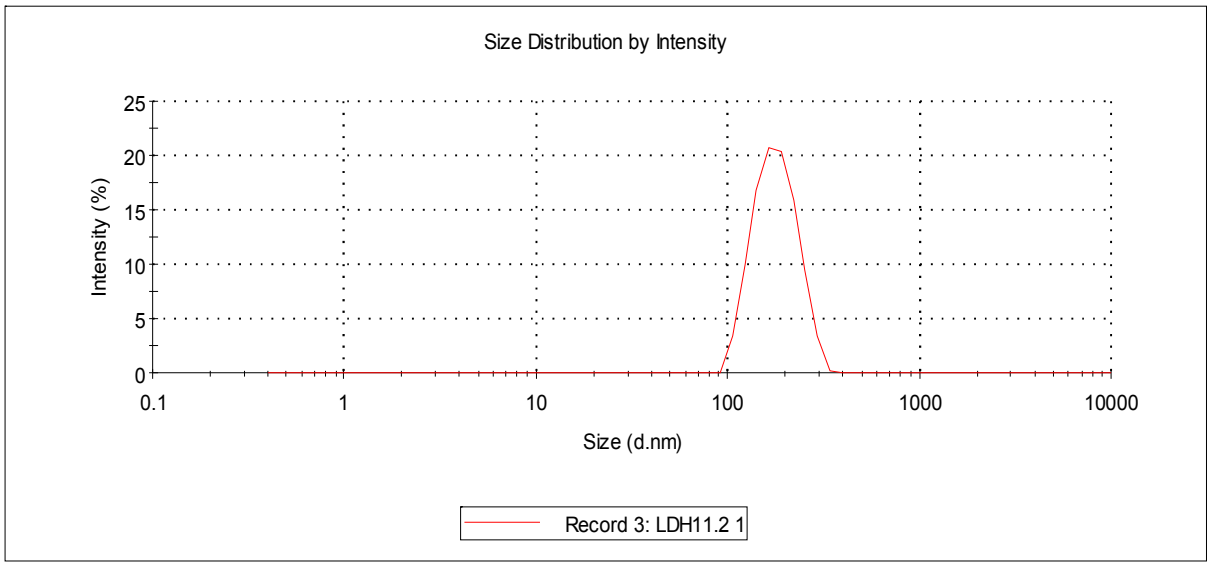


Fig 5: Intensity vs. size plot for LDH synthesised at pH 11.

Volume distribution

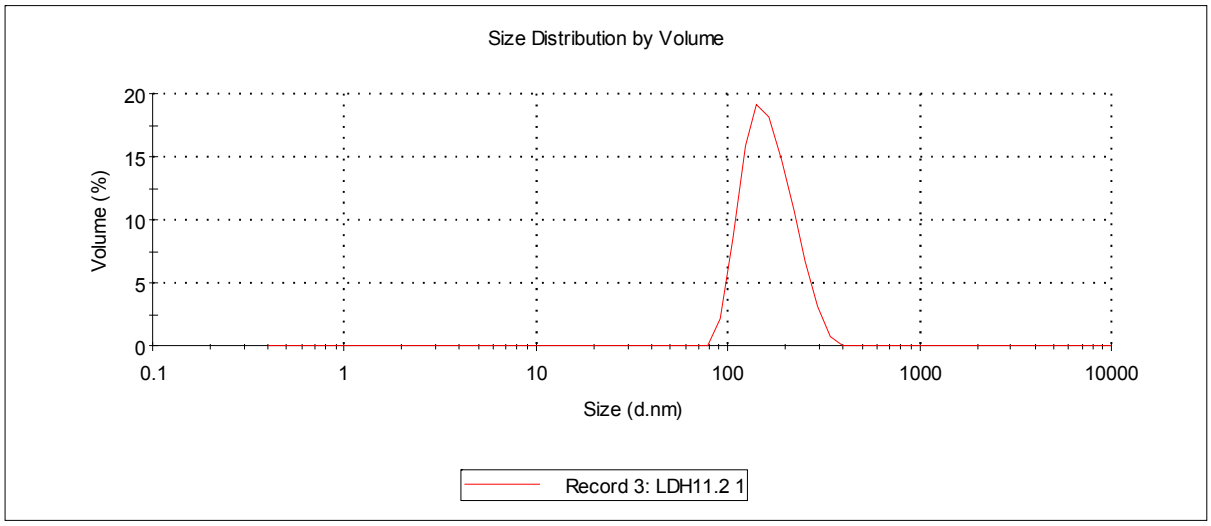


Fig 6: Volume percent vs. size plot for LDH synthesised at pH 11.

Figure 5 and figure 6 show the intensity vs particle size and volume vs particle size distribution plot of LDH synthesized at pH 11 which are unimodal in nature. LDH 11 showed an average particle size of 170.7 nm.

Sample name	Average particle size (nm)	PDI
LDH 9	118.8	0.241
LDH 10	240.1	0.437
LDH 11	170.7	0.063

Table 1: Average particle size and Poly Dispersive Index of LDH synthesised at different pH.

From Table 1, we conclude that the average particle size of 118.8 nm obtained in case of LDH 9 is the smallest, and that of the LDH 10 is the highest and that is 240.1 nm. Obviously LDH 11 showed the lowest value of polydispersive index of 0.063 nm as from table 1.

Particle size is an important aspect of LDH as a drug delivery agent. The particle size determines the following properties:

(i) The rate of absorption of LDH and the transport rate.

(ii) The rate of internalisation into cell. (By phagocytosis or endocytosis)

(iii) The rate of dissolution of LDH in the cytoplasm to release the drug molecule.

All the above aspects suggest that larger particles are not suitable as they decrease the absorption and transport rate. Larger particles enable phagocytosis which is very slower and less efficient process in comparison to endocytosis carried out by smaller particles. Ion exchange of intercalated drug molecules is not possible in case of larger particles, hence smaller particles below 150 nm are desired.

4.2. EDX Analysis

4.2.1. LDH 9

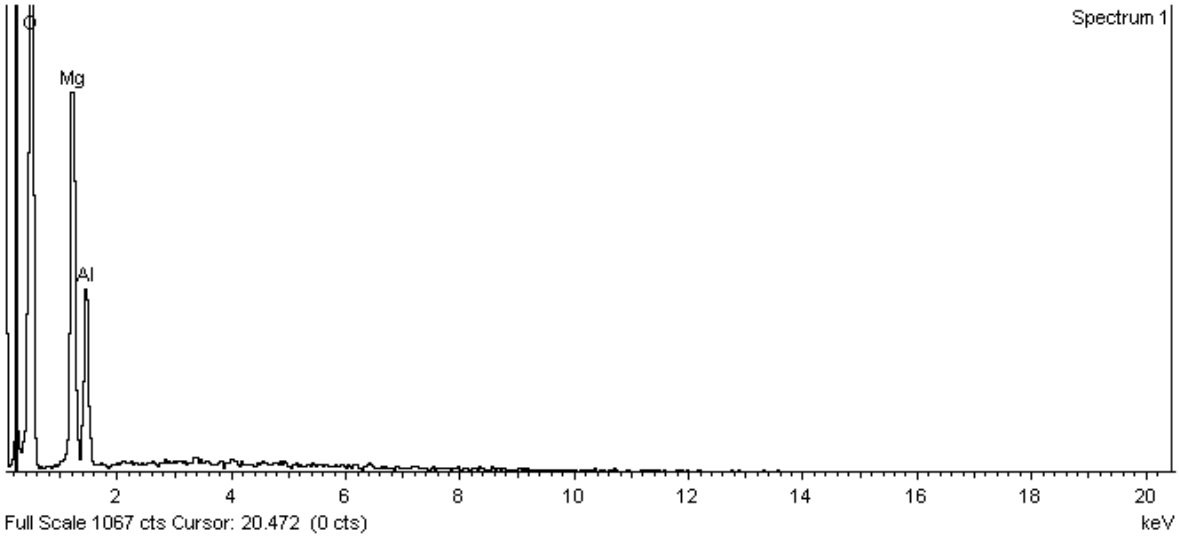


Fig 7: EDX plot of LDH synthesised at pH 9 showing various elements present in the sample and their concentration.

ELEMENT	APP CONC.	INTENSITY CORRN	WEIGHT %	ATOMIC %
O	135.00	1.6023	64.81	74.33
Mg	23.24	0.7681	23.27	17.56
Al	9.43	0.6087	11.92	8.10
TOTALS			100.00	

Table 2: Weight percent and Atomic percent of elements detected by EDX for LDH synthesised at pH 9.

From fig 7 and table 2, the elements detected by energy dispersive x-ray analysis were oxygen, magnesium and aluminium. It suggests that magnesium-aluminium mixed hydroxides were present in the material and that this mixed hydroxides were in ratio close to

2:1 of Mg:Al which was the ratio of the nitrate precursors that we used for the synthesis of LDH.

4.2.2. LDH 10

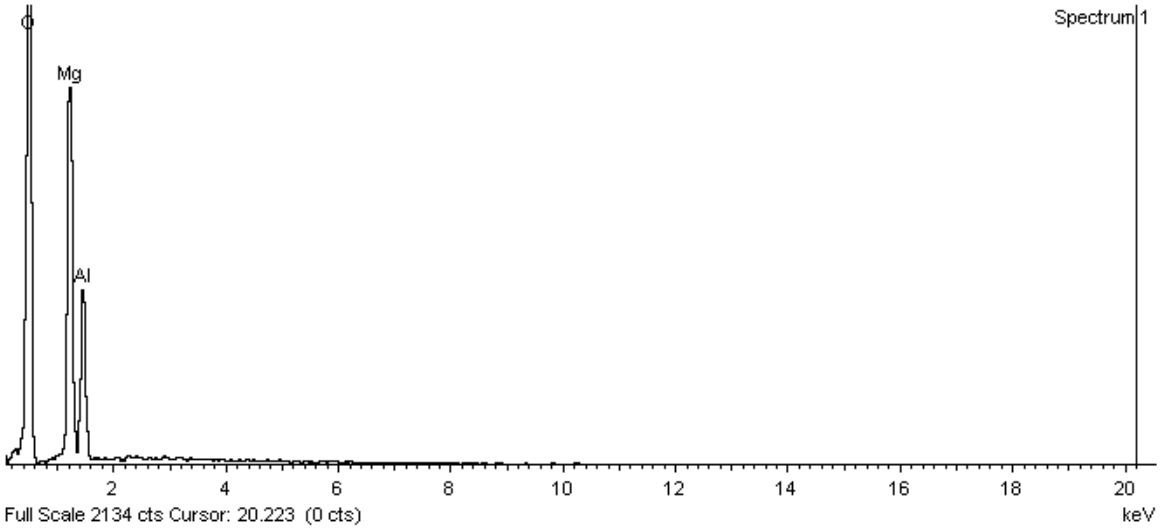


Fig 8: EDX plot of LDH synthesised at pH 10 showing various elements present in the sample and their concentration.

ELEMENT	APP CONC.	INTENSITY CORRN	WEIGHT %	ATOMIC %
O	156.12	1.5866	63.98	73.63
Mg	28.55	0.7737	23.99	18.17
Al	11.20	0.6055	12.03	8.21
TOTALS			100.00	

Table 3: Weight percent and Atomic percent of elements detected by EDX for LDH synthesised at pH 10.

Figure 8 and table 3 show the energy dispersive x-ray analysis of LDH 10. The elements detected by energy dispersive x-ray analysis are oxygen, magnesium and aluminium. The

atomic percent of magnesium was almost double of that of the aluminium which was the atomic ratio of Mg and Al in the precursor used. The oxygen atomic percent was around 74 % that for the formation of the layered double hydroxide.

4.2.3. LDH 11

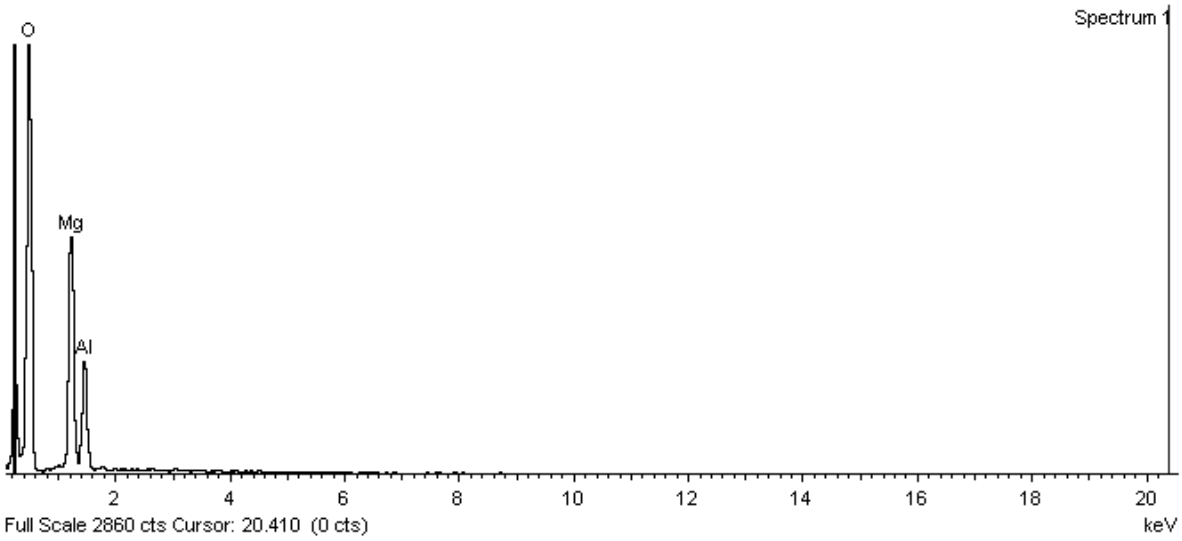


Fig 9: EDX plot of LDH synthesised at pH 11 showing various elements present in the sample and their concentration.

ELEMENT	APP CONC.	INTENSITY CORRN	WEIGHT %	ATOMIC %
O	163.33	1.6605	67.47	76.53
Mg	23.59	0.7506	21.55	16.09
Al	9.82	0.6143	10.97	7.38
TOTALS			100.00	

Table 4: Weight percent and Atomic percent of elements detected by EDX for LDH synthesised at pH 11.

Figure 9 and Table 4 show the energy dispersive x-ray analysis of LDH 11. It suggests that the atomic concentration of oxygen is 76 % and that of magnesium-aluminium are 16 % and around 8% respectively that is in the atomic ratio of 2:1.

From the above EDX plots and tables we confirmed that magnesium aluminium layered double hydroxides were formed with the Mg and Al ratio 2:1.

Hence we got the LDH as

$Mg(II)_{0.66}\ Al(III)_{0.33}\ (OH)_2\ (A^{n-})_{0.33/n} \times y\ H_2O$

Where 'A' is interlayer anion that may be nitrate or other exchangeable anions, 'y' is a fraction constant and n is an integer.

4.3. XRD Analysis

4.3.1. LDH at different pH

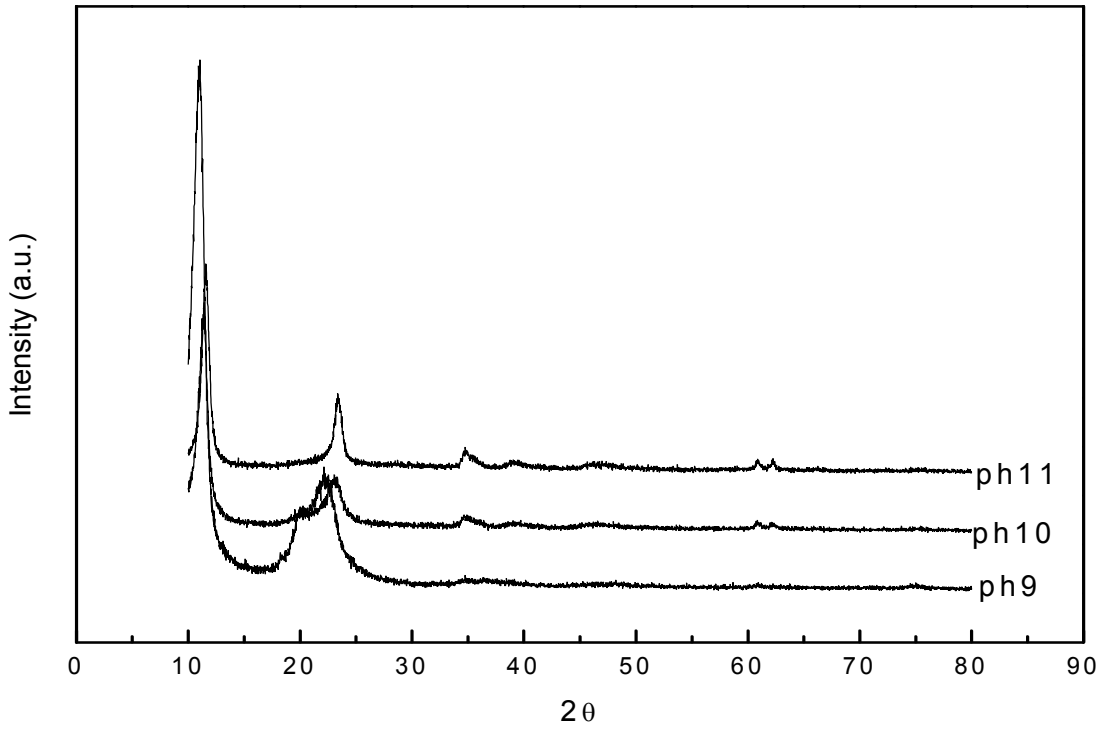


Fig 10: Comparative intensity vs. 2θ plot showing XRD patterns of LDH at different pH.

Figure 10 shows the XRD pattern of as synthesized LDH nanopowder synthesized at different pH of 9, 10 and 11.

(All peaks obtained are of LDH, first peak is (0 0 3), second one (0 0 6), third one (0 0 9)

LDH 9 (From Fig. 10)

Peak 1 $d_{0\,0\,3}$ at 2θ = 10.93º

Peak 2 $d_{0\,0\,6}$ at 2θ = 22.79º

$d_{0\,0\,3} = (\lambda / 2 \sin \theta)$ for n=1 and λ=1.54 Å

so d_{003} = 8.085 Å

LDH 10 (From Fig. 10)

Peak 1 d_{003} at 2θ = 11.49º

Peak 2 d_{006} at 2θ = 23.03º

$d_{003} = (\lambda / 2 \sin \theta)$ for n=1and λ=1.54 Å

so d_{003} = 7.403 Å

LDH 11 (From Fig. 10)

Peak 1 d_{003} at 2θ = 11.57º

Peak 2 d_{006} at 2θ = 23.35º

$d_{003} = (\lambda / 2 \sin \theta)$ for n=1 and λ=1.54 Å

so d_{003} = 7.639 Å

The interplannar spacing d_{003} is around 8 Å for the LDH synthesised at three different pH 9, 10, 11.

4.3.2. LDH-IBU

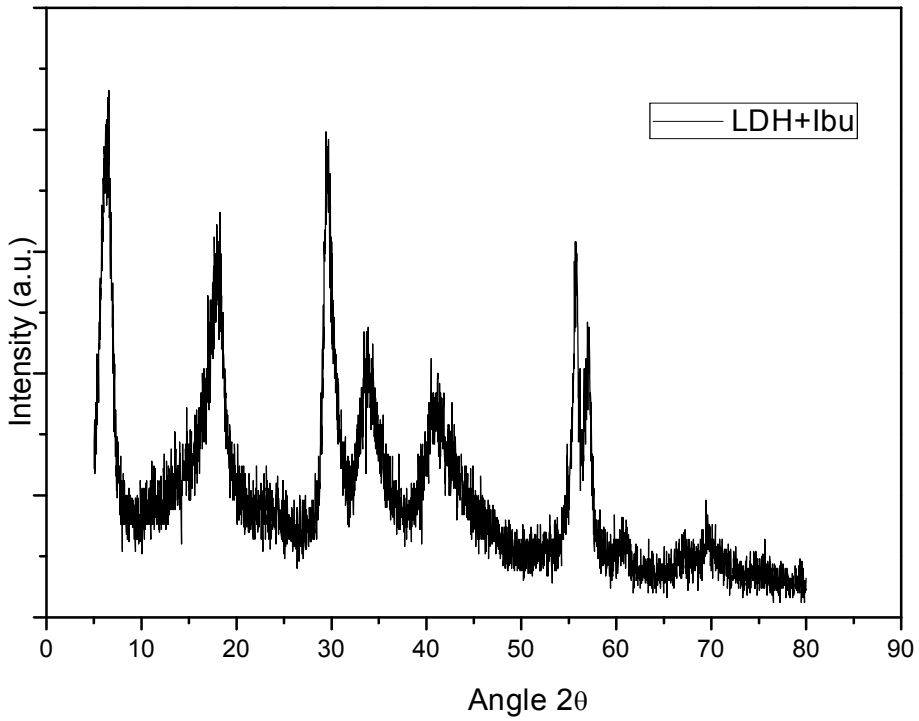


Fig 11: Intensity vs. 2θ plot showing XRD patterns of ibuprofen intercalated LDH synthesised at pH 10. (All peaks are of LDH, first peak is (0 0 3), second one (0 0 6), third one (0 0 9).

Figure 11 shows the XRD pattern of ibuprofen intercalated LDH sample.

LDH-IBU (From Fig. 11)

Peak 1 $d_{0\,0\,3}$ at 2θ = 6.57º

Peak 2 $d_{0\,0\,6}$ at 2θ = 18.25º

$d_{0\,0\,3} = (\lambda / 2 \sin \theta)$ for n=1 and λ=1.54 Å

so $d_{0\,0\,3}$ = 13.437 Å

4.3.3. Relative plot of LDH-IBU and LDH at pH 10

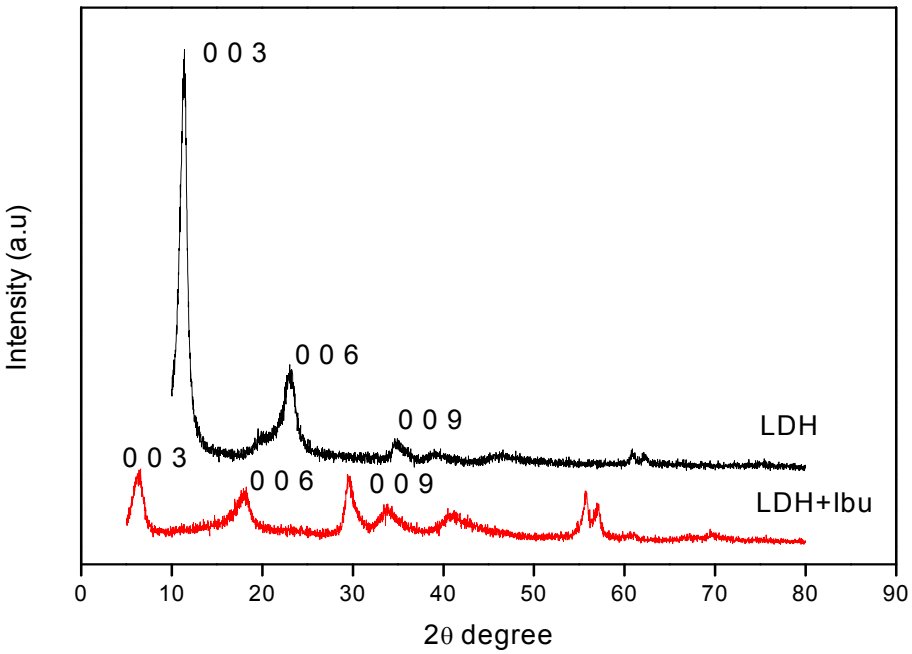


Fig 12: Comparative intensity vs. 2θ plot showing XRD patterns of LDH and LDH-IBU (ibuprofen intercalated LDH) synthesised at pH 10.

Figure 12 shows the comparative XRD pattern of pristine LDH and ibuprofen intercalated LDH sample. A left shift for the peaks of basal plane of ibuprofen intercalated LDH sample is quite evident here.

Table 5: Table showing relative value of first peak 2θ, interplannar spacing and peak intensity for LDH and LDH-IBU.

	LDH	LDH-IBU
2θ for first peak	11.49º	6.57º
$d_{0\,0\,3}$	7.403 Å	13.437 Å
Peak intensity for first peak	High	Low

From Fig 12 and Table 5, there is increase in the interplannar spacing from 7.403 Å in case of LDH to 13.437 Å in case of LDH-IBU due to intercalation of ibuprofen the interlayer spacing which was of greater size than the normally intercalated ions such as carbonates and nitrates. Intercalation of drug molecules in the interlayer space caused expansion in the lattice structure.

There was decrease in intensity of the peaks after the intercalation of drug ibuprofen, because intercalation of drug decreased the crystalinity of the material sample.

The interlayer distance can increase up to 20 Å to accommodate the larger drug/gene molecule with in it, which is a unique property of LDH and one of the important property as a drug carrier.

4.3.4. Schematic Representation Showing Increase in Interplannar Spacing

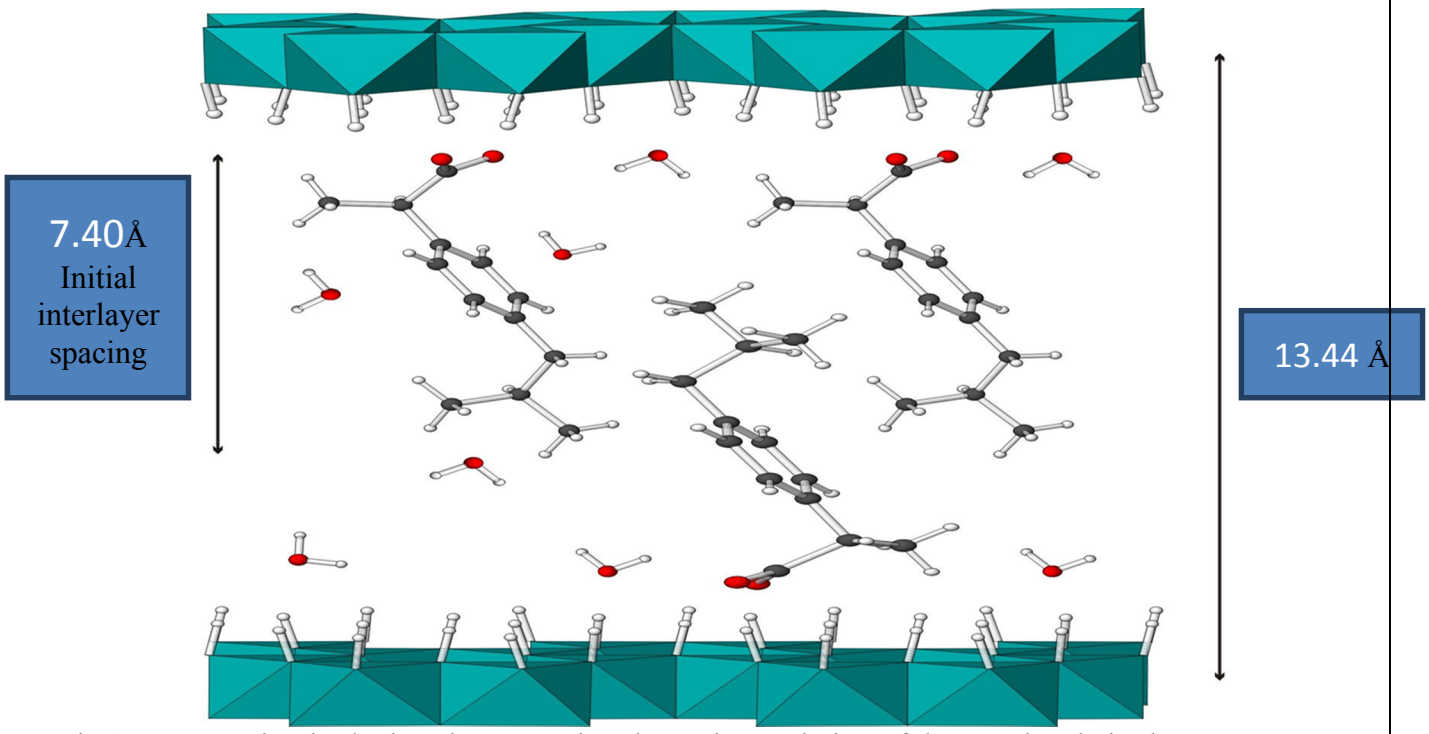


Fig 13: Expansion in the interlayer spacing due to intercalation of drug molecule in the interlayer space.

(Picture courtesy: http://users.ox.ac.uk/~dohgroup/)

The schematic representation (Fig 13) shows that before intercalation the interlayer spacing was 7.40Å and the intercalation of drug molecule increased the interlayer spacing to 13.44 Å due to the large size of the drug ibuprofen. The structure of ibuprofen contains large structure benzene ring which is responsible for expansion of the lattice.

Ibuprofen ($(CH_3)_2CHCH_2C_6H_4CH(CH_3)COOH$) is large in comparison to the CO_3^{2-} and NO_3^- structure. Hence, to accommodate the large structure of the drug molecule the interlayer spacing is increased and drug is intercalated in the interlayer space. Various bonding between the layer structure and the drug molecule are establishedsuch as hydrogen bonding, Vanderwall attraction, electrostatic forces etc. to accommodate the large structure the lattice has to expand.

4. 4. FTIR ANALYSIS

4.4.1. FTIR OF LDH

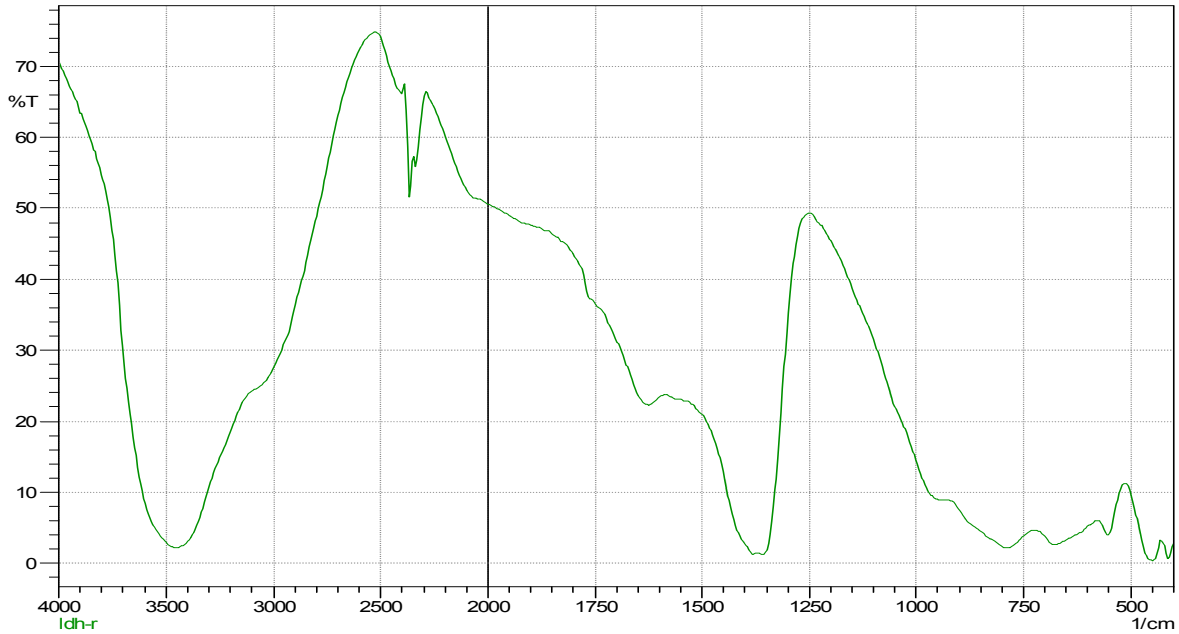


Fig 14: FTIR plot {Transmittance (% T) vs. wave number (1/cm) plot} of LDH.

From Fig 14, occurrence of different peaks can be explained as follows.

- 450 cm^{-1} is due to Lattice vibration of M-OH bond.
- 650 cm^{-1} is due to Lattice vibration of M-O bond.
- 1357 cm^{-1} signifies presence of carbonate (CO_3^{2-}) ions.
- 1380 cm^{-1} signifies presence of nitrate (NO_3^-) ions.
- 1620 cm^{-1} signifies bending vibration of water molecules.
- 3400 cm^{-1} signifies the stretching of hydroxyl group (both from layer and water molecules)

4.4.2. FTIR OF IBUPROFEN

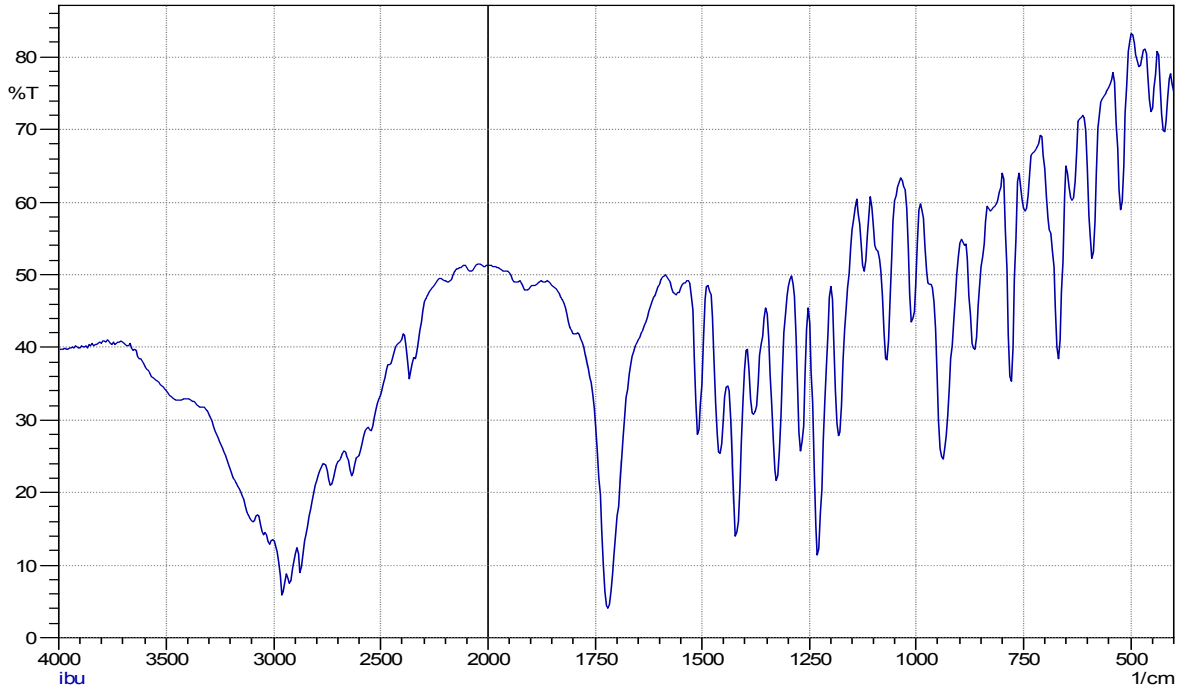


Fig 15: FTIR plot {Transmittance (% T) vs. wave number (1/cm) plot} of drug ibuprofen.

From Fig 15, occurrence of different peaks can be explained as follows.

- 1373 cm^{-1} peak is due to vibration of COO^- group.
- 1510 cm^{-1} peak can also be explained due to vibration of COO^- group.
- 1720 cm^{-1} can be explained as carbonyl stretching of isopropionic acid group.
- 2980 cm^{-1} can be depicted as peak due to hydroxyl stretching.

4.4.3. FTIR OF LDH-IBU

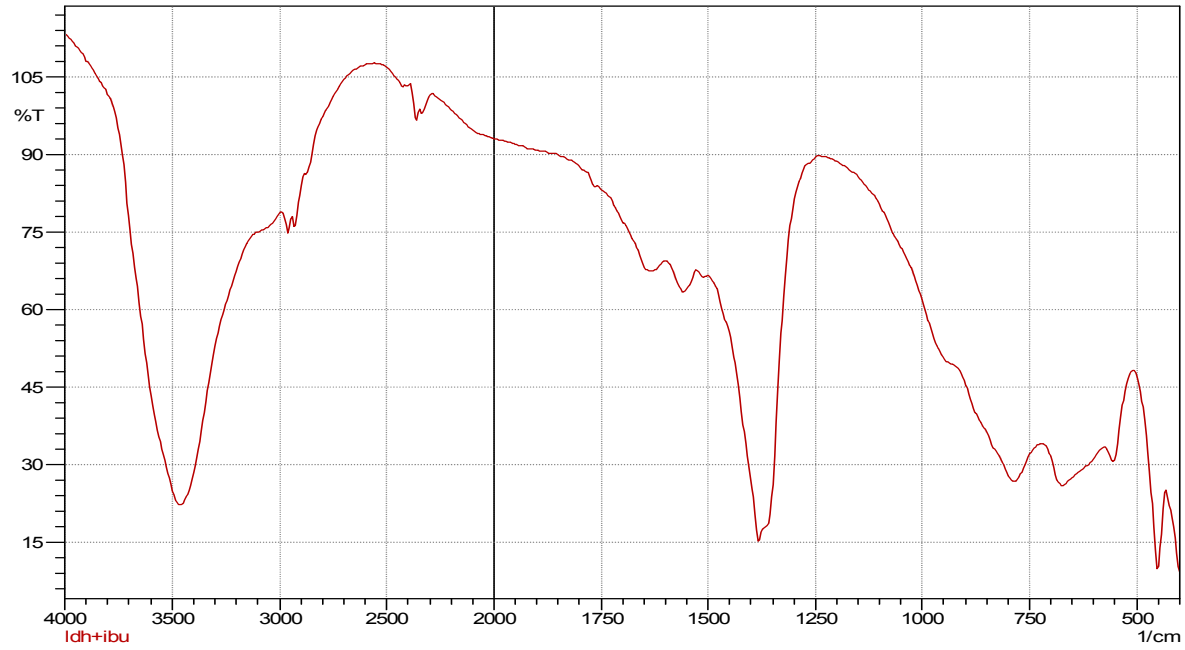


Fig 16: FTIR plot {Transmittance (% T) vs. wave number (1/cm) plot} of ibuprofen intercalated LDH (LDH-IBU).

From Fig 16, occurrence of different peaks can be explained as follows.

- 1380 cm^{-1} sharp peak is due to presence of nitrate (NO_3^-).
- 1550-1600 cm^{-1} peaks can be explained as overlap of peaks (C=C, C=O).
- 1602 cm^{-1} peak is due to stretching vibration of water molecules.
- 1620 cm^{-1} peak signifies increase in bending vibration of water molecules.
- 2980 cm^{-1} signifies the hydroxyl stretching became prominent.

4.4.4. Comparison of FTIR of LDH, IBUPROFEN and LDH-IBU

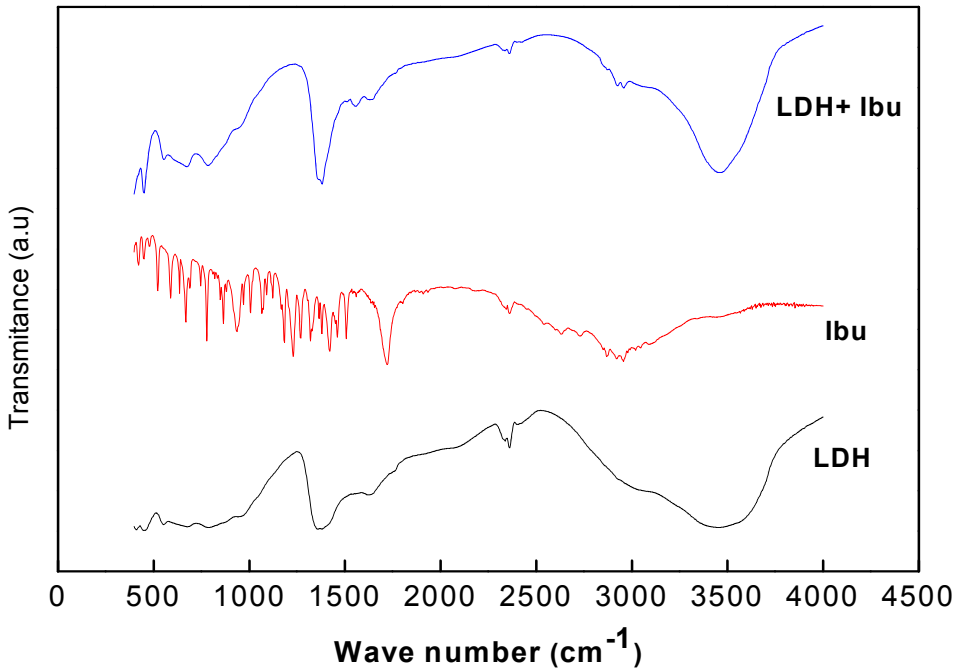


Fig 17: Comparative FTIR plot (Transmittance vs. wave number plot) of LDH, IBUPROFEN, LDH-IBU.

The above plot of Transmittance vs. Wave number is the relative plot of FTIR of LDH 10, drug ibuprofen and LDH-IBU (ibuprofen intercalated LDH).

From the comparative study of FTIR peaks of LDH, IBU and LDH+IBU as in figure 17, we can draw following conclusions:

- 1380 cm^{-1} peak signifies presence of nitrate (NO_3^-).The peak became sharper in case of LDH-IBU than that of LDH which also have 1357 cm^{-1} carbonate peak along with nitrate peaks.
- 1606 cm^{-1} peak is due to stretching vibration of C=C. (This peak is not present in LDH).

- 1620 cm^{-1} signifies increase in the bonding vibration of water molecules. (Less intense peak in LDH).
- 1550-1600 cm^{-1} signifies overlap of peaks of C=C and C=O.
- 450 cm^{-1} signifies that the peak for lattice vibration of M-OH became more intense.
- 660 cm^{-1} signifies that the peak for lattice vibration of M-O became more intense.
- 2980 cm^{-1} peak is due to hydroxyl stretching of molecules of ibuprofen which appeared in LDH-IBU.
- 1357 cm^{-1} and 1510 cm^{-1} peaks are due to occurrence of COO^- group vibration.
- 1720 cm^{-1} signifies carbonyl stretching of ibuprofen that appeared in LDH-IBU.

4.5. Release Rate Study by UV-Visible Spectroscopy

The absorbance of different drug concentration (from 50 µg/mL to 2000 µg/mL) in aqueous solution at pH 10 is determined in the Ultraviolet radiation ranging from 200 nm to 400 nm.

In all the concentration of drug molecules, highest absorbance is achieved in case of 260 nm UV radiation. So, the absorbance vs. concentration plot is determined at 260 nm.

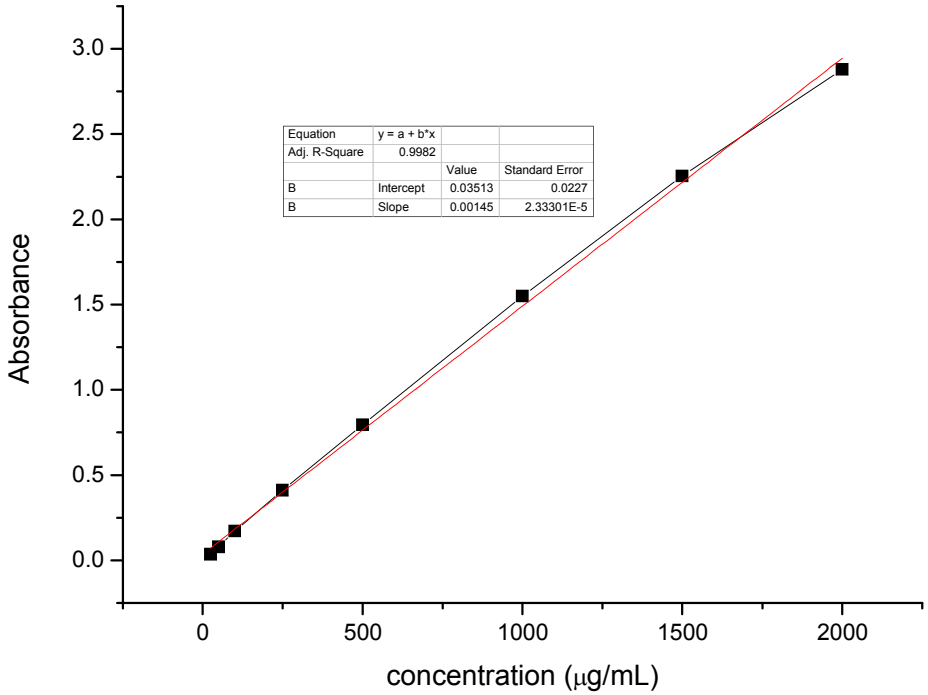


Fig 18: Linear variation of Absorbance with concentration for drug ibuprofen in aqueous solution at pH 10 for UV radiation 260 nm.(Linear fit curve)

From the plot on Fig 18, we get the following equation,

$$y = a + b.\ x$$

Where, 'y' is Absorbance, 'x' is Concentration. 'a' is intercept value is 0.03513 from Fig 18, and 'b' is the slope and value is 0.00145 from Fig 18.

The equation becomes **y = 0.00145.x + 0.0351** and this equation is used to find out unknown concentration of drug molecule.

RELEASE STUDY

The release of the drug molecules in SBF (Simulated Body Fluid) in the given time period is calculated by determining the absorbance by using UV visible spectroscopy and further the concentration the drug is calculated from absorbance using the equation obtained from the linear plot in Fig 18.

TIME (in hour)	ABSORBANCE	CONC. (µg/mL)	CUMMULATIVE % RELEASE
0.5	0.0893	37.3818	20.01
1	0.1297	65.3310	34.95
2	0.1461	76.4966	40.92
4	0.1788	99.0617	52.98
6	0.2040	116.4345	62.29
12	0.2369	139.1379	74.44
24	0.2670	159.9035	85.55
48	0.2850	172.3035	92.19
72	0.2927	172.6276	95.04

Table 6: Variation of Absorbance, Concentration of ibuprofen and Cumulative release of ibuprofen in SBF with respect to elapsed time in SBF.

From the data obtained in the Table 6, we can determine the release rate by plotting the cumulative release vs. time plot, which is shown in Fig 19.

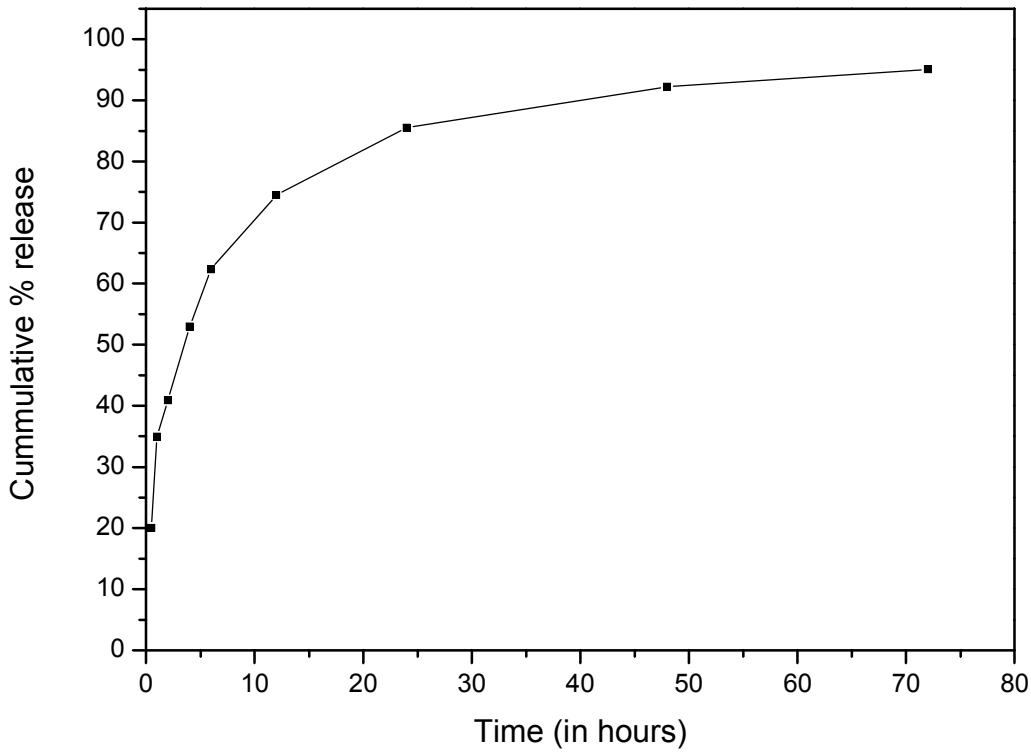


Fig: Release rate of drug Ibuprofen in SBF (Simulated Body Fluid)

Fig 19: Cumulative percent release of drug molecule in SBF (pH 7.4) vs. time.

From Table 6 and Fig 19, we come to the conclusion that, in 1 hour time around 35 % of the drug ibuprofen was released. In 4 hours more than 50 % drug was released. Cumulative drug release after 12 hour was more than 75 %. In 24 hour time more than 85 % drug was released. In 72 hours more than 95% drug release was achieved.

CHAPTER 5

CONCLUSION

CONCLUSIONS

Non-steroid anti-inflammatory drug ibuprofen intercalated Mg-Al layered double hydroxide was synthesized using coprecipitation method. The nanopowders synthesised were in the size range between 100 to 200 nm. XRD analysis showed that there is an increase in d_{003} spacing from 7.40 Å for pristine LDH to 13.44Å for ibuprofen intercalated LDH due to the intercalation of larger ibuprofen molecule in the interlayer space of LDH. FTIR analysis indicated hydroxyl and carbonyl stretching of ibuprofen in LDH-IBU sample confirming the presence of ibuprofen in LDH. The drug release study in *in vitro* simulated body fluid using UV-Vis spectroscopy showed that 50 % drug molecules were released in 4 hours and more than 95 % release was achieved after a time period of 72 hours.

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