Ibuprofen Intercalation and Release from Different Layered Double Hydroxides

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Structured Abstract

Background: The chemical composition of Layered Double Hydroxides (LDHs) affects their structure and properties. The method of ibuprofen (IBU) intercalation into LDHs may modify its release, reduce adverse effects, and decrease the required dosing frequency.

Methodology: This study investigates the effects of four different LDHs; MgAl-LDH, MgFe-LDH, NiAl-LDH and NiFe-LDH on *in vitro* release of IBU intercalated by co-precipitation and anionic-exchange.

Results: MgAl-LDH was the most crystalline and substitution of either cation decreased LDH order. FT-IR spectra and pXRD confirmed the intercalation of IBU within the lamellar structure of MgAl-LDH and MgFe-LDH. Intercalation of IBU by anion-exchange resulted in slower, partial, drug release compared co-precipitation.

Conclusions: The chemical composition of LDHs affects their crystallinity, IBU intercalation and subsequent release.

Keywords

- Layered double hydroxides
- LDH
- Ibuprofen
- Anionic Exchange
- Co-precipitation
- Drug Release

1. Introduction

Layered double hydroxides (LDHs) are inorganic lamellar solids often referred to as hydrotalcite-like minerals [1]. They are sometimes referred to as anionic clays due to their physical and chemical similarities with clay mineral [1] but LDHs have anions between octahedral layers [2], whereas clay minerals have cations between octahedral-tetrahedral layers [1].

The layers of LDHs are assembled from octahedral sheets of divalent and trivalent metal hydroxides bound together through edge-sharing. The charge imbalance across the sheet, attributed to the di- and trivalent metal cations, results in a net positive charge [2]. LDH sheets can be stacked on top of each other with anions and water molecules between the sheets to counterbalance the positive charge. These interlayer anions are commonly carbonate, halides, nitrates or sulphates [2]. Water molecules and other anionic species can also reside in the interlayer space from the synthesis of the LDH sheets, or through incorporation methods such as anionic-exchange [1]. As expected, the interlayer distance depends on the size, charge and arrangement of the anionic species within the interlayer space [1].

The chemical composition of LDHs is generally described as $[M^{II}_{1-x}M^{III}_{x}(OH)_2][X^{q-}_{x/q}\bullet nH_2O]$, where M^{II} is the divalent cation such as Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+} and M^{III} is the trivalent cation such as Al^{3+}, Mn^{3+}, Fe^{3+}, Co^{3+}, Ni^{3+} [1]. These metal cations must be of similar ionic radius to Mg^{2+}ions to be able to fit in the brucite-like ($Mg(OH)_2$) layers [1]. Additionally, it has been suggested that the charge density ($M^{III}/(M^{II}+M^{III})$) which relates to the anionic exchange capacity must be between 0.2 and 0.33, with the M^{II}/M^{III} ratio being between 2 and 4.37 to get a pure LDHs structure [2]. The chemical variation of LDHs is diverse due to the possible ratios and combinations of divalent and trivalent cations, in addition to the choice of anions that can be incorporated between the layers [3]. Moreover, preparations of LDHs containing quaternary and monovalent cations have also been reported [4,5].

LDHs can act as drug carriers due to their positively charged layers and interlayer anions that can be exchanged for negatively charged drug compounds for storage and subsequent release in a controlled manner [6,7]. Current developments in drug-delivery systems strive to optimise drug-release by means of maintaining a therapeutic concentration of the drug at the targeted site for an extended period of time, thus prolonging the therapeutic effects, reducing the dosing frequency and minimising dose-related adverse effects [3]. Recent years have seen a growing interest in the

pharmaceutical applications of LDHs as controlled drug delivery systems [4,8]. LDH chemical composition affects the layer structure and properties, which affects the intercalation and release of drug molecules [9]. Therefore, it is possible to optimise the chemical composition of LDHs to design controlled release drug nanocarriers that are also biocompatible *in vivo* [10,11]. Furthermore, the application of LDH materials in the biomedical field expands beyond their nanoparticle drug carrying properties and includes, amongst other applications, the use of LDHs in biomaterials for tissue engineering [12] and applications as biosensors [13], as well as formulation into hybrid polymer containing nanocomposite hydrogels [14], films [15] and beads [16].

The most common intercalation methods described are co-precipitation (co) and anionic-exchange (ex) [4] but can also be achieved through reconstruction, hydrothermal precipitation and transformation methods [4,17,18]. These intercalation methods produce different LDH-drug composites in terms of their structure, bonding, purity and amount of intercalated drug [19], which consequently influence drug release rate and characteristics [20].

LDHs offer many advantages as drug carriers due to a high adsorptive capacity, low toxicity [1], ability to improve drug stability [21], and being easy and inexpensive to prepare. In recent years, LDHs have been used to successfully intercalate many drugs and biomolecules including anti-inflammatory drugs [3,4], antihypertensive drugs [22], antimicrobials [23], anticancer drugs [24], and DNA fragments [25].

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of molecules indicated for the treatment of pain and inflammation [26]. However, these drugs are limited by their low water solubility [27] which can restrict their dissolution and absorption in the body. A study on naproxen and flurbiprofen showed a substantial increase in water solubility and improved drug bioavailability when intercalated within MgAl-LDH [19]. Similarly, Capsoni *et al.* found that co-precipitating carprofen with Zn_2Al-LDH significantly increased the drugs solubility potentially improving subsequent absorption [28]. The solubility of NSAIDs are also increased when LDHs were included as additives, but to a lesser extent than when intercalated within the layers [29]. Furthermore, the LDH layers can also act as a barrier and provide gastrointestinal protection against the adverse effects of NSAIDs [30].

Several studies also demonstrated that LDHs can modify and prolong the release of the intercalated NSAIDs [3,4]. For example, Ambrogi *et al.* intercalated ibuprofen (IBU) into MgAl-LDH

and observed slower *in vitro* release compared to the commercial formulation [31]. Li *et al.* revealed that the dissolution of fenbufen was slower when intercalated by co-precipitation in MgAl-LDH and MgLi-LDH [32]. However, the MgAl-LDH was concluded to be the more effective delivery system as its release rate was significantly slower. The chemical composition of LDHs affect drug intercalation. For example, del Arco *et al.* showed that fenbufen intercalated successfully in MgAl-LDH via co-precipitation, anionic exchange, and reconstruction but it was only intercalated into MgAlFe-LDH by co-precipitation and anionic-exchange [3]. Subsequently, MgAl-LDH released its intercalated drug fully while MgAlFe-LDH released 93% of its intercalated drug at a slower rate [29].

Another study revealed that the release rate of naproxen decreased when the charge density of the LDHs delivery system increased [33]. Williams and O'Hare suggested that the release of NSAIDs from LDHs is affected by the pH of the release medium, demonstrating a slower release at pH 7 than at pH 4 due to acidity causing hydrolysis of the LDH sheets [9]. Additionally, the impact of interlayer space size on drug loading and subsequent release has been demonstrated by Djaballah *et al.* [5] who demonstrated suitability of the very short interlayer space in ZnTi-LDH to deliver low-dose therapy of intercalated IBU.

These studies show that the intercalation of NSAIDs into LDHs for a modified release system depends on multiple factors, including the chemical composition and charge density of the LDHs, the intercalation method and the pH of the release medium. These variables collectively influence the LDH structure and orientation of the drug molecules within the interlayer space, which will consequently affect the rate and amount of drug released. Williams and O'Hare suggest it is possible to optimise these factors to obtain an optimal modified release formulation of the drug, although these have not yet been fully investigated [9]. Current interests include further improving the drug delivery potential of LDHs and research into this had included surface coating the drug loaded LDH nanoparticles with mesoporous silica [34].

The aim of this work was to investigate and characterise the effects of four different metal compositions of LDH sheets (MgAl-LDH, MgFe-LDH, NiAl-LDH and NiFe-LDH) on the intercalation and *in vitro* drug-release of IBU using two different intercalation methods: co-precipitation and anionic-exchange.

2. Materials and Methods

2.1 LDH-IBU composite preparation

2.1.1. Co-precipitation of LDH-IBU composites

Co-precipitation (co) was used to prepare the following four metal compositions of LDH-IBU composites: MgAl-LDH-IBU(co), MgFe-LDH-IBU(co), NiAl-LDH-IBU(co), and NiFe-LDH-IBU(co).

This involved preparing the relevant metal salts solution (molar ratio metal ion^{2+}/metal ion^{3+} =1:2) [31]. Firstly, 0.025 mol of divalent metal chloride salt ($MgCl_2$ or $NiCl_2$) and 0.0125 mol of trivalent metal chloride salt ($AlCl_3$ or $FeCl_3$) were dissolved in 50 mL of deionised water. Secondly, a caustic solution of the drug was prepared by dissolving 0.0125 mol of IBU into a solution containing 5 M NaOH (3 mL) and deionised water (50 mL).

The metal salts solution was added dropwise from a burette into a stirring caustic solution of IBU. Additions of 5 M NaOH solution were made as necessary to maintain the mixture at pH 9. The exact volume of 5 M NaOH (8 – 14mL) solution added was recorded to establish the final volume of the resultant mixture. Once all of the metal salts solution was added, the viscous resultant mixture was left to stir for one hour following which it was centrifuged at 25000 rpm for 20 minutes and the pellets were dried. After drying, the solid products were grounded into fine particles using a mortar and pestle, and the weights of the solids were recorded and the yields calculated according to the following equation:

$$Yield\ \ (\%) = \frac{mass\ \ of\ \ LDH\ \ obtained\ \ (g)}{total\ \ mass\ \ of\ \ metal\ \ salts\ \ and\ \ IBU\ \ used\ \ (g)} x100$$

The supernatant was kept to assay the amount of IBU not intercalated, as detailed in section 2.3 below.

2.1.2 Anionic-exchange of LDHs with IBU

The anionic exchange (ex) method was used to prepare the following four metal compositions of LDH-IBU composites: MgAl-LDH-IBU(ex), MgFe-LDH-IBU(ex), NiAl-LDH-IBU(ex) and NiFe-LDH-IBU(ex).

This involved a two-step process; the first step involved the co-precipitation of MgAl-LDH, MgFe-LDH, NiAl-LDH and NiFe-LDH exactly as detailed above, but excluding the IBU in the caustic solution. The second step involved equilibrating 1 g of the LDHs with 2 g of IBU dissolved in a

148 solution containing 5 M NaOH (10 mL) and deionised water (74 mL). This mixture was covered with
149 foil paper, heated to 60 °C [31] and stirred vigorously on a hotplate stirrer for 3 hours. After 3
150 hours, the mixture was left to cool before centrifuging at 25000 rpm for 20 minutes. After
151 centrifugation, the pellets were dried the solid products were grounded into fine particles using a
152 mortar and pestle, and the weights of the solids were recorded. The supernatant (3 mL) was kept
153 to assay the amount of IBU not intercalated, as detailed in section 2.3.
154
155 **2.1.3 Preparation of the physical mixes**
156 Different metal salts and IBU were mixed together in a mortar and pestle to prepare physical
157 mixes equivalent to each of the LDHs synthesised. The amounts used reflected the 2:1 ratio of the
158 divalent and trivalent metal salts. Additionally, the amount of IBU used was equivalent to the
159 amount of IBU determined from the co-precipitated LDH-IBU composites (section 2.3).
160
161 **2.2 Characterisation of LDH-IBU composite**
162 The composites were analysed on a Perkin Elmer Spectrum 1000 Fourier-Transform Infrared
163 (FT-IR) Spectrophotometer with a Pike Miracle ATR attachment in the range of 4000 to 600 cm^{-1}.
164 The power X-ray diffractograms (pXRD) of the composites were collected on a Rigaku Mini-Flex X-
165 ray diffractometer using Cu Kα radiation of wavelength 1.54 Å in the scan range 2θ: 3° to 30°.
166 The LDHs and LDH-IBU composites were analysed using FT-IR spectroscopy and pXRD. The
167 physical mixes were analysed using FT-IR spectroscopy only.
168
169 **2.3 Determination of amount of IBU intercalated**
170 All UV analysis was completed on the Thermo Spectronic Genesys 10 UV-Visible
171 Spectrophotometer at wavelength 265 nm.
172
173 **2.3.1 Back-exchange method (carbonate-ion exchange)**
174 The amount of IBU intercalated into each LDH-IBU composite was determined by carbonate-
175 ion back-exchange. This involved exchanging higher affinity carbonate ions with intercalated IBU,
176 thus releasing the drug out of the LDHs for quantifying with UV spectroscopy.
177 0.005 mol sodium carbonate decahydrate (1.4307 g) was dissolved in phosphate buffered

saline (50 mL, pH 7.4). This mixture was heated to 80 °C before adding 500 mg of LDH-IBU. Then, the mixture was sealed with foil, stirred and maintained at 80 °C for 4 hours using a magnetic hotplate stirrer. When cooled to room temperature, 3 mL of the mixture was pipetted out, centrifuged and its supernatant was analysed in a quartz cuvette under UV spectroscopy at 265 nm to determine the mass of IBU released from 500 mg of LDH-IBU. This was then used to calculate the amount of IBU loaded into the various composites using the following equation:

$$IBU\ \ loading\ \ (mg/g\ \ LDH) = \frac{mass\ \ of\ \ IBU\ \ determined\ \ by\ \ back-exchange\ \ (mg)}{mass\ \ of\ \ LDH\ \ used\ \ in\ \ back-eachange(g)}$$

2.3.2 IBU intercalation efficiency

The amount of IBU not intercalated into the various composites was determined by measuring the amount of IBU remaining in the supernatant of the intercalation mixture after centrifugation. The supernatant was analysed in a quartz cuvette under UV spectroscopy at 265 nm to determine the mass of IBU not intercalated. This was deducted from the initial mass of IBU added and used to calculate the percentage intercalation efficiency using the following equation:

$$IBU\ \ intrcalation\ \ efficiency\ \ (\%) = \frac{mass\ \ of\ \ IBU\ \ used\ \ (mg) - mass\ \ IBU\ \ not\ \ intercalated\ \ (mg)}{mass\ \ of\ \ IBU\ \ used\ \ (mg)} x100$$

2.4 *In vitro* drug release

A sample of each physical mix and co-precipitated and anion exchanged LDH-IBU composite (230 mg) was suspended in separate round-bottom flasks containing phosphate buffer saline (PBS, 200 mL, pH 7.4) under constant stirring, in an incubator at a constant physiological temperature (37 ± 5 °C). The mass of the sample suspended was equivalent to approximately 100 mg of IBU, as estimated from the preliminary back-exchange. This mass/volume ratio was chosen to correspond to the sink conditions, based on the solubility of IBU at pH 7.4 [31].

Once, the samples were suspended, aliquots (1 mL) of dissolution medium were taken at 5 minutes interval up to one hour. The aliquots were then centrifuged and their supernatant were analysed under UV spectroscopy at 265 nm. One millilitre of PBS was replaced after each aliquot sample was removed to maintain sink conditions. The dissolution tests were repeated and the average absorbance values were used to determine the concentration of IBU released.

The amount of IBU released was calculated as a percentage over the total amount of IBU in

230 mg of the physical mix, or the total amount of intercalated IBU in 230 mg of LDH-IBU composite.

3. Results and Discussion

3.1 Intercalation of IBU in LDHs by co-precipitation and anionic-exchange

During the co-precipitation of LDHs a pH of 9 was maintained. This alkaline environment was required to create a supersaturated conditions for the hydroxide ions to displace the metal salts and form a precipitate [35]. Precipitation occurred when the NaOH solution was added into mixtures of metals salts with and without IBU. However, a larger addition of NaOH solution was generally required to maintain pH 9 during the co-precipitation of LDHs with IBU due to the acidic nature of IBU [20].

Table 1. Yield of LDHs prepared without ibuprofen (IBU) and via co-precipitation with **IBU**.

LDH composite	Yield (%) with no IBU	Yield (%) co-precipitated with IBU
MgAl-LDH	85	79
MgFe-LDH	71	65
NiAl-LDH	70	62
NiFe-LDH	77	60

Co-precipitation of LDHs with IBU produced a lower yield than without IBU (table 1). This indicates that LDHs form more easily with chloride anions than IBU anions, which suggests that the presence of IBU disrupts the formation of LDH layers due to its relatively larger size and hydrophobic nature. Bulky anions are capable of moving the layers out of alignment as a consequence of the turbostratic effect [2], thus less layers can be assembled during co-precipitation. Additionally, MgAl-LDH and co-precipitated MgAl-LDH-IBU had a higher yield compared to the other co-precipitated composites. This indicates that these metal ions are more efficient in forming LDHs, which was expected as MgAl-LDHs have a similar composition to the natural mineral hydrotalcite [4].

The co-precipitation process resulted in considerably higher IBU intercalation efficiencies than anionic-exchange (table 2). This is likely due to the varying intercalation mechanism, as explained

231 below with the difference in interlayer spacing. Similar findings have been reported by other
232 groups, Djebbi *et al.* describe a lower adoption of berberine chloride into MgAl-LDH prepared by
233 ion-exchange compared to equivalent co-precipitation methods [36].

234

Table 2. Ibuprofen (IBU) intercalated

	co-precipitated (co)				anion-exchanged (ex)			
	MgAl-LDH-IBU(co)	**MgFe-LDH-IBU(co)**	**NiAl-LDH-IBU(co)**	**NiFe-LDH-IBU(co)**	**MgAl-LDH-IBU(ex)**	**MgFe-LDH-IBU(ex)**	**NiAl-LDH-IBU(ex)**	**NiFe-LDH-IBU(ex)**
IBU intercalation efficiency (%)	90.31	87.98	76.36	58.14	29.50	27.50	25.50	23.00
IBU loading (mg/g LDH composite)	420	490	396	314	368	342	296	252

235 In both methods, MgAl-LDH and MgFe-LDH intercalated more IBU than NiAl-LDH and NiFe-
236 LDH, which may be due to the increased order and crystallinity of MgAl-LDHs and MgFe-LDH
237 compared to Ni containing LDH (see section 3.4). Ambrogi *et al.* (2001) reported to have achieved
238 an IBU content of 50% by anionic exchange. MgAl-LDH was also reported to have intercalated
239 fenbufen with a drug content of 51% when co-precipitated at pH 8, and 61% when precipitated at
240 pH 13 [32]. This indicates that more drug molecules are intercalated at higher basicity, as the layers
241 are more regular [32].

242 The IBU loading in the various composites (table 2) was deduced from the amount of IBU
243 back-exchanged. In both types of composite materials (co-precipitated and ion exchanged) there
244 was residual drug remaining on the LDHs that was not released during the back-exchange.
245 However it is notable that IBU was relinquished from the anion-exchange prepared composites
246 more readily, which suggests a stronger adsorption of IBU in the co-precipitated LDH composites.

247

248 **3.3 Characterisation of LDHs and physical mixes with FT-IR spectroscopy**

249 The FT-IR spectrum of IBU (figure 1e) consists of the characteristic C=O stretching vibrations
250 at^{-1} due to the free carboxylic acid group, the C-H alkyl stretching at 2954-2868 cm^{-1} due to the
251 aliphatic C-H groups [37], and the skeletal stretching vibrations between 1507-1418 cm^{-1} due to
252 the C-C bonds in the aromatic ring [38]. These characteristic absorptions were also observed in the

physical mixes (figure 1d), suggesting that simply mixing IBU with the metal salts or LDHs does not result in intercalation. The FT-IR spectra of LDHs (figure 1a) display weak peaks at 1393-1357 cm^{-1} indicating the presence of carbonate [3] and implies that carbonate anions were adsorbed onto the LDHs from the atmosphere and dispersion media due to their strong affinity [2].

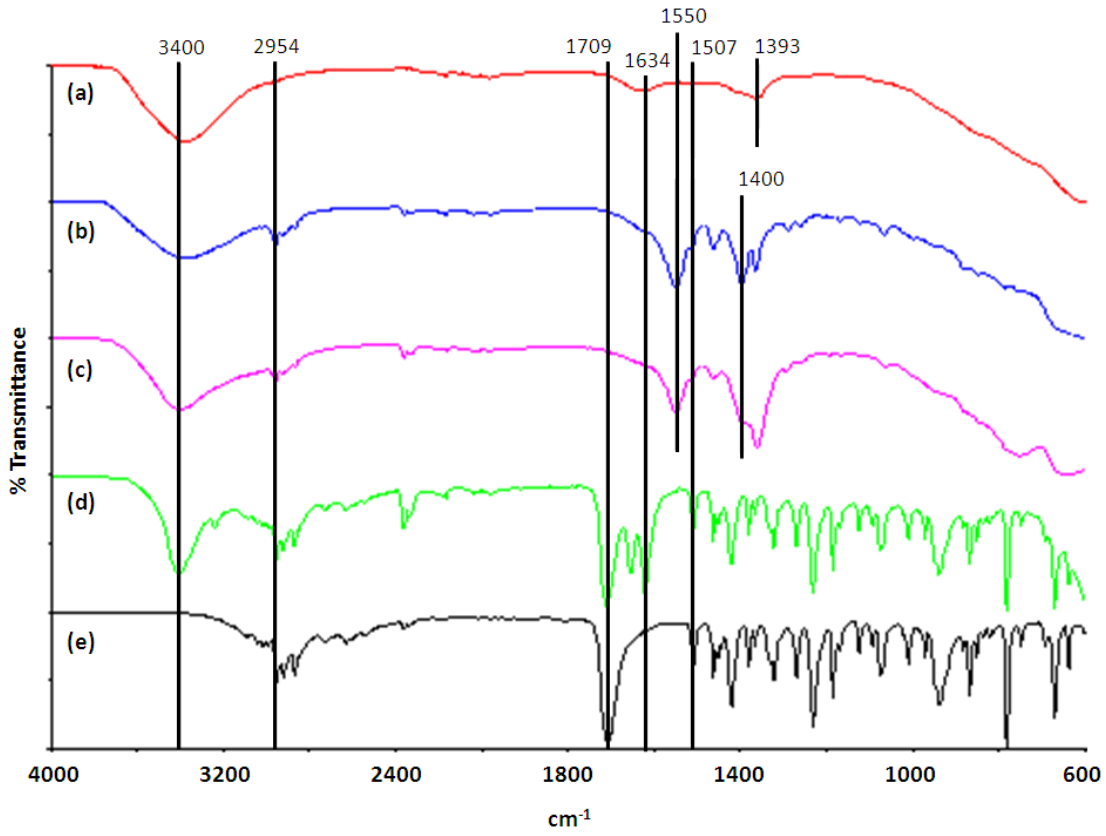

Figure 1. FT-IR spectra of (a) the MgAl-LDH synthesised, (b) the co-precipitated MgAl-LDH-IBU, (c) the anion-exchanged MgAl-LDH-IBU, (d) a physical mix of $MgCl_2$, $AlCl_3$ and IBU and (e) IBU

The aliphatic C-H stretching was present on the spectra of the physical mix sample and LDH-IBU composites (figures 1b, c and d), but not of the LDHs without IBU (figure 1a). This establishes the presence of IBU in the physical mixes and LDH-IBU composites. The LDHs and LDH-IBU contain OH groups as shown by the broad FT-IR peak between 3400 and 3335 cm^{-1} relating to the hydroxyl groups within the LDH layers, and to the interlayer and adsorbed water [39]. The bending modes of OH bonds are only seen on the spectra of LDHs at 1634-1629 cm^{-1}, as there are no IBU molecules to obscure it. The broadening of the OH stretching peak indicates that OH groups are hydrogen bonded [38].

The FT-IR absorption due to the free acid group of IBU is no longer visible on the spectra of LDH-IBU composites, confirming immobilisation of IBU onto the surface of LDHs. FT-IR absorption modes due to the asymmetric and symmetric stretching of the carboxylate anion group (COO^-) are seen at 1556-1528 cm^{-1} and 1408-1360 cm^{-1}, respectively (table 3). This implies that the negatively-charged carboxyl group of IBU interacts with the positively charged layers of the LDHs. Similar changes in FT-IR spectra were reported with the intercalation of fenbufen [32] and indomethacin [37,40] into MgAl-LDH. Conversely, it was observed by del Arco *et al.* that this change did not occur with the intercalation of meclofenamic acid into MgAl-LDH because its sodium salt was used [3].

The FT-IR spectra of the LDH-IBU composites were similar (figure 2), which suggests that IBU interacts with these LDHs in the same manner regardless of intercalation method or LDH composition. The carbonyl stretching from IBU disappears in both the LDH-IBU composites and the COO^- peaks occur at similar wavelengths. In addition, the FT-IR absorbance modes for the LDHs remained in their original positions, indicating the structure of the LDHs remained unchanged during and after the intercalation of IBU.

Table 3. Absorption peaks of interest on the FT-IR spectra of ibuprofen (IBU), co-precipitated (co) LDH-IBU and anion-exchanged (ex) LDH-IBU composites.

	FT-IR absorption peaks (cm^{-1})		
	v(C=O)	$v_{as}(COO^-)$	$v_s(COO^-)$
IBU	1709, s, sh	-	-
MgAl-LDH-IB(co)	-	1548, s, sh	1396,s, sh
MgFe-LDH-IBU(co)	-	1552, s, sh	1408, s, sh
NiAl-LDH-IBU(co)	-	1546, s, sh	1397, s, sh
NiFe-LDH-IBU(co)	-	1548, s, sh	1395, s, sh
MgAl-LDH-IBU(ex)	-	1548, m, sh	1362, s, sh
MgFe-LDH-IBU(ex)	-	1556, m, sh	1361, s, sh
NiAl-LDH-IBU(ex)	-	1542, m, sh	1363, s, sh
NiFe-LDH-IBU(ex)	-	1538, m, sh	1360, s, sh

s = strong, m = medium, sh = sharp, v_s = symmetrical stretching, v_{as}= asymmetrical stretching

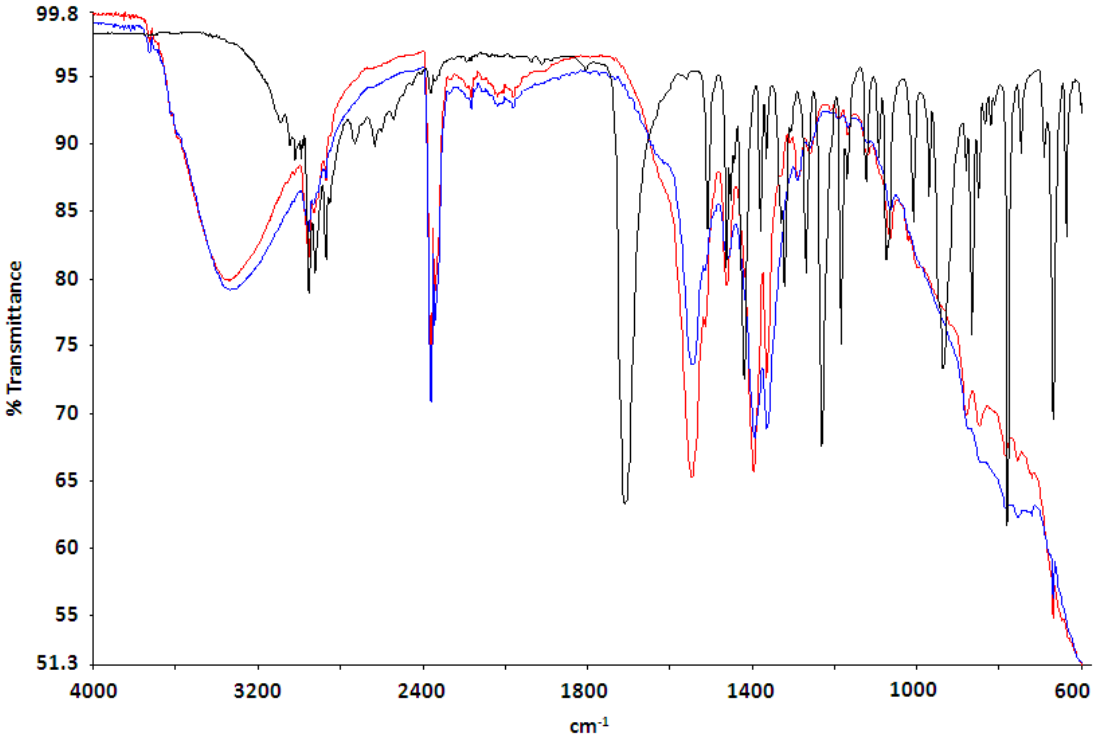

Figure 2. FT-IR spectra of co-precipitated (co) NiAl-LDH-IBU(co) (red), anion-exchanged (ex) NiAl-LDH-IBU(ex) (blue) and ibuprofen (IBU) (black).

3.4 Characterisation of LDHs with pXRD

MgAl-LDH was the most crystalline structure, followed by MgFe-LDH, NiAl-LDH and NiFe-LDH in descending order of crystallinity, with the latter two materials showing no observed diffraction at around 11 deg. 2θ (figure 3). This implies that the two Mg-containing materials are laminar crystalline LDH structures and whereas the two Ni-containing materials have formed amorphous metal oxides. This is due to the charge to size ratio of the metal ions which affects the layer charge density [41], and therefore influences the stacking of the layers. These data suggest the combination of magnesium and aluminium cations produce superior layer charge density than the other metal combinations. This finding is also supported by the existence of the only natural LDH, hydrotalcite, which consists of magnesium and aluminium ions [41]. The high crystallinity of MgAl-LDH would also explain its high yield compared to the other LDHs (table 1).

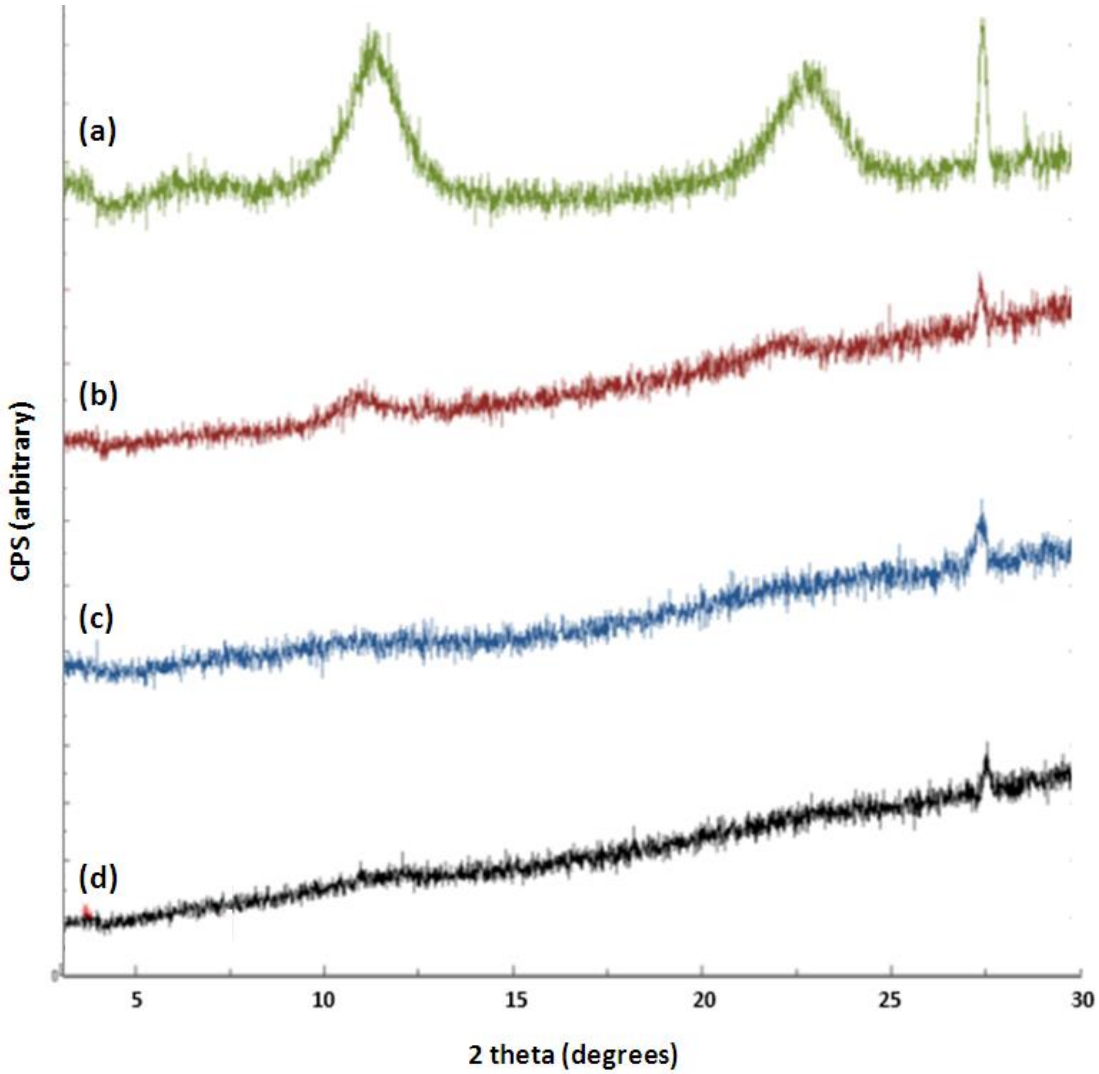

Figure 3. Diffractograms of synthesised LDHs (a, green) MgAl-LDH, (b, red) MgFe-LDH, (c, blue) NiAl-LDH, and (d, black) NiFe-LDH

The method of synthesis affects the crystallinity of the LDHs, and consequently the orientation of IBU within the interlayer space. MgAl-LDH has a d_{003} value of 0.778 nm (table 4) which represents the size of one cationic layer and the interlayer space and is consistent with other LDHs reported in the literature [31,42]. Upon intercalation of IBU by co-precipitation and anionic exchange, the d_{003} value increased by 1.627 nm and 1.564 nm, respectively, demonstrating placement of IBU within the interlamellar space. This increase in interlayer space is similar to that seen with other organic anions of a similar size to IBU, such as fenbufen [32] and indomethacin [40] .

Table 4. Characteristics of peak d_{003} on the diffractograms of the LDHs, co-precipitated LDH-IBU composites and anionic-exchanged LDH-IBU composites.

LDHs composite	d_{003} (nm) for LDH	d_{003} (nm) for LDHs co-precipitated with IBU	d_{003} (nm) for LDHs anion-exchanged with IBU
MgAl-LDH	0.778	2.405	2.342
MgFe-LDH	0.796	2.425	0.796
NiAl-LDH	No diffraction	2.425	No diffraction
NiFe-LDH	No diffraction	No diffraction	No diffraction

Additionally, the increase in d-values for MgAl-LDHs containing IBU suggest that IBU formed a tilted bilayer between the layers [41], with its carboxylate groups interacting with the cationic surface and its primary axes perpendicular to the layers [32]. This arrangement has also been reported with the co-precipitation of ketoprofen and MgAl-LDH, which produced a similar expansion of the interlayer spacing by 1.72 nm [38].

The orientation of IBU occupies less interlayer space when intercalated via anionic-exchange than co-precipitation, as evident by the difference in expansion of interlayer space (table 4). This is likely because the co-precipitation allows the formation of hydroxides layers around IBU molecules, which would therefore encapsulate more anions, and widen the initial interlayer space. However, anionic-exchange intercalates IBU into LDHs that already contains small chloride anions in the interlayer space, which can inhibit absorption, hence reduced yield, and expansion of the interlayer space.

After intercalation of IBU via anion exchange into MgAl-LDH, the d_{003} reflection became more intense and sharper (diffractograms not shown). This suggests that the MgAl-LDH layered structure became more ordered after intercalation. The d_{003} reflection of co-precipitated MgAl-LDH-IBU composite is less intense than that of the MgAL-LDH and anionic exchanged MgAl-LDH-IBU equivalents, suggesting that co-precipitation produces LDH-IBU composites that are less ordered. This implies that IBU disrupts the stacking of the cationic layers during co-precipitation, which does not occur with anionic-exchange process as the LDH layers are already formed and associated before the intercalation of IBU. Huang *el al.* report an improved crystal structure when IBU-LDH materials are prepared by the hydrothermal precipitation method compared to the traditional co-precipitation method also applied in this study [43] implying that harsher conditions are required

to overcome the issue of IBU hindering the formation of ordered layers.

MgFe-LDH exhibit basal reflections that are very broad, asymmetrical and have a low intensity (figure 3b) and represents a poorly crystalline structure with minimal layers [38]. The interlayer spacing is found to be 0.796 nm, which is the same value for MgFe-LDH reported by Gasser [44]. Magnesium and iron cations are not as efficient at forming structured LDHs as magnesium and aluminium cations under the same synthesis conditions. This is shown by the weaker reflections compared to MgAl-LDH, which is likely due to iron cations being larger than aluminium cations [45], which could create distortions within the cationic LDH layers [41].

Similarly, on intercalation of IBU into MgFe-LDH by co-precipitation, the interlayer space increased by 1.629 nm. This confirms the successful intercalation of IBU by co-precipitation, as the interlayer space expanded by the same distance as with the intercalation of IBU in MgAl-LDH. It also suggests that MgFe-LDH-IBU has the same bilayer arrangement of IBU as MgAl-LDH-IBU. Again, the reflections of MgFe-LDH-IBU(co) are less intense than MgAl-LDH-IBU(co) due to the distortion caused by the larger aluminium cations.

On the contrary, the intercalation of IBU into MgFe-LDH by anionic-exchange was unsuccessful, as the d_{003} value remained the same. This could be due to the irregular structure of the MgFe-LDH making it challenging for IBU to intercalate. Although, IBU did not intercalate into MgFe-LDH, its FT-IR spectra indicate that IBU still formed bonds with the LDH, meaning IBU was adsorbed onto the outer surfaces of the LDH particles.

The diffractograms of NiAl-LDH and NiFe-LDH do not exhibit any diffractions around 11 deg. 2θ, thus an ordered layered structure was not formed. Nickel cations have a smaller ionic radius than magnesium cations [45], which increases its charge density and makes them more strongly bound to chloride anions; requiring more vigorous method to successfully synthesise nickel containing LDHs [46,47]. NiAl-LDH and NiFe-LDH have previously successfully been prepared using the co-precipitation method however the intercalation anion was carbonate [48,49] suggesting that chloride anions are not conducive to LDH formation for nickel containing materials.

As a lamellar structure was not formed, the intercalation of IBU by anionic-exchange was unsuccessful for both NiAl-LDH and NiFe-LDH, as evident from the absence of reflections in these diffractograms. While the intercalation of IBU into NiFe-LDH by co-precipitation was also unsuccessful, the pXRD analysis suggests that NiAl-LDH was able to intercalate IBU by co-

precipitation as it had an interlayer space of 2.425 nm. This value is similar to the other IBU co-precipitated LDHs in this study (MgAl-LDH-IBU and MgFe-LDH-IBU), which suggest that a bilayer of IBU had formed. In turn, this suggests IBU anions help the formation of the layered structure, which could not be formed with chloride anions alone.

3.5 *In vitro* drug release

The IBU release profiles in phosphate buffer saline (pH 7.4) from co-precipitated and ion-exchanged LDH-IBU was performed on the five LDHs showing d_{003} reflections on their diffractograms (figure 4). The IBU release profile differed for each LDH-IBU composite tested showing that differences in LDH chemical composition and IBU intercalation method also affected the final drug release behaviour. The physical mixes of the parent LDHs (MgAl-LDH, MgFe-LDH and NiAl-LDH) and IBU did not show release profiles (data not shown) as all the IBU present in the mix had dissolved once suspended in the phosphate buffer saline.

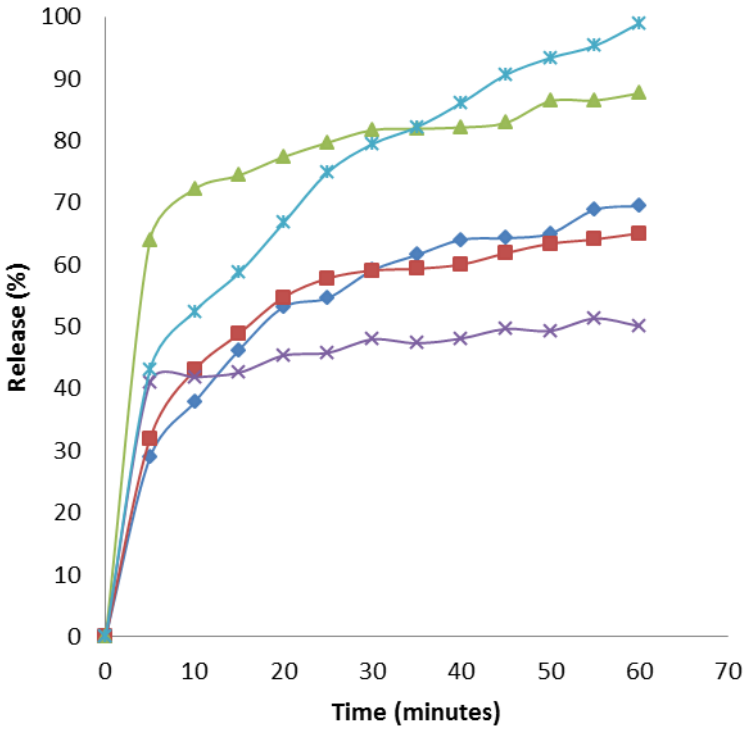

Figure 4. Drug release profiles of ibuprofen (IBU) from LDH-IBU composites of co-precipitated (co) MgAl-LDH-IBU(co) (dark blue diamonds), MgFe-LDH(co) (green triangles), and NiAl-LDH-IBU(co) (light blue asterix), and anionic-exchanged (ex) MgAl-LDH-IBU(ex) (red squares) and MgFe-LDH-IBU(ex) (purple crosses).

All the LDH-IBU composites tested showed an initial burst release within the first 5 minutes that corresponds to the release of IBU from the edges and external surfaces of the LDH particles [31,50]. The initial release is greatest with MgFe-LDH-IBU(co), which may indicate that the majority of its IBU was associated with the outer surfaces of this LDH. This is supported by the pXRD analysis that revealed poorer crystallinity compared to the other composites. MgFe-LDH-IBU(ex) showed limited release, which suggests most of the IBU available for release was relinquished in the initial burst phase. pXRD of this LDH did not suggest any IBU was intercalated but had adhered onto the outer surfaces of the LDH.

A slower release rate of IBU followed the initial burst and corresponds to the phosphate ions in the solution exchanging with the adsorbed IBU. LDHs are semi-rigid lamellar solids and demonstrate a reduction in interlayer spacing when larger anions are exchanged with the smaller anions [31]. As the intercalated IBU is exchanged for phosphate ions at the outer edges the interlayer space reduces, inhibiting exchange with IBU deeper within the LDH structure. This can explain the slow and partial release of IBU in anionic-exchanged LDHs as they have more crystalline layers. Co-precipitated and anionic-exchanged MgAl-LDH-IBU have similar crystallinity, which may explain their similar release profiles.

It is also likely that differences in the chemical composition and charge density of LDH layers will affect the strength of interaction with IBU and therefore affect how easily the IBU can be liberated thereafter [9,33]. Extrapolating this theory would suggest that IBU was held most strongly within anionic-exchanged composites, especially MgFe-LDH-IBU(ex), and less strongly within co-precipitated composites.

It is also proposed that $H_2PO_4^-$ reacts with exposed hydroxyl groups of LDHs to produce a hydroxyphosphate [51]. This is known as a solid state grafting reaction which obstructs IBU release from deep within the layers due to the strong bonds between phosphate ions and cationic LDH layers [50,51].

The release of IBU from MgAl-LDH-IBU was also studied by Ambrogi *et al*. [31], who established modified release of IBU. The release rate of IBU from MgAl-LDH-IBU was found to be 60% over 20 minutes, which is similar to the data presented here showing 54% drug released over the first 20 minutes. This demonstrates MgAl-LDH can be used as IBU drug carriers for modified release.

4. Conclusion

IBU intercalates into LDHs by interaction between its negatively charged carboxylic acid group and the cationic surface of the LDHs. Anionic-exchange of IBU onto a formed LDH generally produces more crystalline and ordered materials compared to co-precipitating the LDH with IBU. Intercalated IBU is initially released rapidly from the LDHs outer surfaces, then more slowly by ion-exchange with phosphate ions in the dissolution medium. Formation of LDH-IBU composites via ion exchange generally results in slower, partial, drug release compared to its co-precipitated counterparts, which may be explained by intensity of LDU and IBU interactions.

The chemical composition of LDHs affects the crystallinity of the overall particle structure, which affects the intercalation of IBU and its subsequent release profile. Mg^{2+} and Al^{3+} ions form the most crystalline LDHs. The substitution of Mg^{2+} cations with higher charge-density Ni^{2+} cations makes it difficult to synthesise LDH layers. Substitution of Al^{3+} cation with Fe^{3+} cation distorts the layers due to its larger atomic radius. Therefore, Mg^{2+} and Al^{3+} ions were found to have the best charge densities to form the cationic layers of LDHs.

This study demonstrates that MgAl-LDH has the optimal metal composition of LDHs to act as a host for modifying release of IBU out of the four LDHs synthesised. Further research into the use of MgAl-LDH as a drug carrier could yield interesting and promising materials for optimising patient care.

5. Future perspectives

The arena of drug delivery is vast and ever expanding with novel approaches, materials and technologies emerging from the research. This is justified by the extensive requirements for modern drug delivery vehicles to improve patient outcomes, support adherence to medicines, and reduce adverse effects. There are a large number of promising materials being investigated and applied to the field, each with their set of desirable physicochemical and biological properties. Current knowledge of the LDH materials provides an understanding of their chemical diversity and the adaptability of their physical properties. It is this diversity which is the foundation of their exploitation in biomedical applications.

449 The particle size dependent cellular uptake demonstrated by LDH materials make them
450 particularly interesting for drug delivery [52]. Further exploration of the biocompatibility,
451 pharmacokinetics and toxicity of LDH-drug hybrids [53] is required before their true potential is
452 acknowledged and advances made. The benefits of combining drug molecules with LDHs range
453 from improved drug solubility and bioavailability to overcoming drug resistance. Thus, utilising
454 such inorganic materials as novel delivery vehicles provides a platform for not only reducing the
455 use of animal and petroleum based materials in such applications but provides scope for bettering
456 the therapeutic effect of the drug molecules themselves.

457 In addition to delivery of drugs, the application of these low-cost materials extends to other
458 fields of biomedicine including LDH-polymer scaffolds for improved cell regeneration [12], LDH-
459 immobilised enzyme biosensors [54] as well as gene delivery vectors [55] further widening the
460 importance of research into these inorganic layered materials.

461

462 ## 6. Executive Summary

463 Intercalation of ibuprofen in LDHs

464 • Adsorption of IBU into LDHs was achieved via co-precipitation and anion exchange.
465 • Co-precipitation of LDHs with IBU produced a lower yield than without IBU, implying larger
466 anions may inhibit successful formation of LDH layers.
467 • The co-precipitation of LDHs with IBU resulted in considerably higher drug intercalation
468 efficiencies and a stronger adsorption of IBU compared to the anion-exchanged counterparts.

469 Characterisation of LDHs

470 • FT-IR spectra and pXRD confirmed the intercalation of IBU within the lamellar structure of
471 MgAl-LDH and MgFe-LDH. An ordered layered structure for NiAl-LDH and NiFe-LDH was not
472 formed.

473 Drug release

474 • LDH chemical composition and IBU intercalation method also affected the final drug release
475 behaviour.
476 • An initial burst release was observed for all LDH-IBU composites within the first 5 minutes that
477 corresponds to the release of IBU from the edges and external surfaces of the LDH particles

- A slower release rate of IBU followed the initial burst and corresponds to the phosphate ions in the solution exchanging with the adsorbed IBU.

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8. Disclosures:

None

9. Ethical conduct of research statement

Not applicable

10. References

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** of considerable interest

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