



Hydroxy double salts loaded with bioactive ions: Synthesis, intercalation mechanisms, and functional performance



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ARTICLE INFO

Article history:

Received 10 December 2015

Received in revised form

29 February 2016

Accepted 10 March 2016

Available online 10 March 2016

Keywords:

Hydroxy double salt

Intercalation

Drug delivery systems

In situ diffraction

Molecular dynamics

ABSTRACT

The intercalation of the anions of diclofenac (Dic), naproxen (Nap), and valproic acid (Val) into three hydroxy double salts (HDSs) has been explored in this work. Experiments were performed with $[\text{Co}_{1.2}\text{Zn}_{3.8}(\text{OH})_8](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ (CoZn- NO_3), $[\text{Ni}_2\text{Zn}_3(\text{OH})_8](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ (NiZn- NO_3) and $[\text{Zn}_5(\text{OH})_8](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ (Zn- NO_3). It proved possible to intercalate diclofenac and naproxen into all three HDSs. In contrast, Val could be intercalated into CoZn- NO_3 but when it was reacted with Zn- NO_3 the HDS structure was destroyed, and the product comprised ZnO. Successful intercalation was verified by X-ray diffraction, IR spectroscopy, and elemental microanalysis. Molecular dynamics simulations showed the Dic and Nap ions to arrange themselves in an “X” shape in the interlayer space, forming a bilayer. Val was found to adopt a position with its aliphatic groups parallel to the HDS layer, again in a bilayer. In situ time resolved X-ray diffraction experiments revealed that intercalation of Dic and Nap into CoZn- NO_3 and Zn- NO_3 is mechanistically complex, with a number of intermediate phases observed. In contrast, the intercalation of all three guests into NiZn- NO_3 and of Val into CoZn- NO_3 are simple one step reactions proceeding directly from the starting material to the product. The HDS-drug composites were found to have sustained release profiles.

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1. Introduction

Layered metal hydroxides (LMHs) are a broad family of materials which have much potential in the delivery of biologically active species. Two commonly occurring types of LMH are hydroxy double salts (HDSs) and layered double hydroxides (LDHs). These have the same key structural features of positively charged layers and charge-balancing anions located in the interlayer region, but they differ in their layer compositions. LDHs contain a mixture of trivalent and mono or divalent metal cations [1–4], while HDSs contain only divalent metal cations [5–7]. The generic formula of an HDS is $[(\text{M}_a^{2+}\text{Me}_b^{2+})(\text{OH})_y] \text{X}_{(2a+2b-y)/n}^n \cdot z\text{H}_2\text{O}$. M and Me are divalent metal ions such as Zn^{2+} , Cu^{2+} , Ni^{2+} , or Co^{2+} [8,9], and X is an anion such as Cl^- , NO_3^- , or CO_3^{2-} [9,10]. The latter can be replaced, through an ion exchange route, with a variety of inorganic and organic anions [11–14].

LDHs have attracted the interest of many researchers for their potential as drug delivery systems. They have been widely

explored *in vitro* and *in vivo* for drug delivery and LDHs loaded with active pharmaceutical ingredients (APIs) have shown improved properties over the free drugs in terms of solubility, absorption, bioavailability and cellular uptake [15–22]. However, much less attention has been paid to HDSs in general and specifically to their potential as delivery systems. The few studies which have been reported suggest that HDSs may have improved properties than LDHs in this regard. HDSs have been intercalated with mefenamate, indole-3-acetate, 4-biphenylacetate, 4-(2,4-dichlorophenoxy) butyrate and 2-(3-chlorophenoxy) propionate and in all cases the guests were released from the HDS hosts over a prolonged period of time, with slower release than observed with LDHs [7,23,24].

In this work, a range of carboxylic acid drugs was explored. These can easily form anions suitable for intercalation. The anti-inflammatory, analgesic, and antipyretic drugs diclofenac (Dic) and naproxen (Nap), together with the anticonvulsant and mood stabilizing active ingredient valproic acid (Val), were selected for investigation. The structures of these drugs are given in Fig. 1.

These guests were incorporated into a range of Zn-containing HDSs, and the resultant intercalates fully characterised. An in situ X-ray diffraction study was performed to investigate the intercalation mechanisms and kinetics. Molecular dynamics

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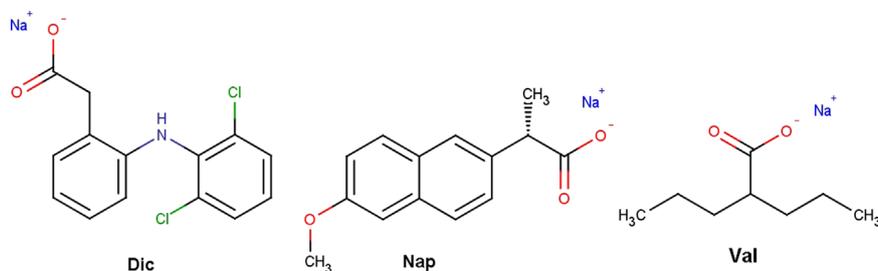


Fig. 1. The chemical structures of Dic, Nap and Val.

simulations were undertaken to aid interpretation of the diffraction data. Finally, the release of the drugs was studied in simulated physiological conditions.

2. Experimental section

2.1. Synthesis

2.1.1. Materials

Zinc oxide, zinc chloride, zinc nitrate hexahydrate, cobalt (II) nitrate hexahydrate, and nickel (II) nitrate hexahydrate were supplied by Fisher Scientific. Diclofenac sodium (Dic) was sourced from Cambridge Bioscience, and naproxen sodium (Nap) and valproate sodium (Val) were purchased from Sigma-Aldrich.

2.1.2. HDS synthesis

The HDSs were synthesised using methods reported previously [10]. The reaction mixtures detailed in Table 1 were prepared in water and stirred for 7 days at room temperature. The solid products were isolated by vacuum filtration, washed with copious amounts of deionised water and a small amount of acetone, then dried in an oven for 4 h at 40 °C.

2.1.3. Intercalation reactions

Intercalation was achieved by combining 0.4 mmol of an HDS with a 4-fold excess of the guest anions. 10 ml of deionised water was added to the solid materials, and the mixture stirred at 60 °C for 3 days. The solid products were filtered under vacuum, washed with deionised water, and dried.

2.1.4. Guest recovery

The ability to recover the guest ions intact after intercalation was investigated in selected cases by reacting ca. 50 mg of the intercalate with approximately 100 mg of Na₂CO₃ in D₂O overnight at 80 °C. The resultant suspension was filtered and the filtrate analysed by ¹H NMR.

Table 1
Summary of HDS synthesis conditions.

HDS	Starting materials	Volume (ml)
[Zn ₅ (OH) ₈ (NO ₃) ₂ ·yH ₂ O] [Zn-NO ₃]	ZnO (4.2 g) + Zn(NO ₃) ₂ ·6H ₂ O (24.1 g)	60
[Zn ₅ (OH) ₈ Cl ₂ ·yH ₂ O] [Zn-Cl]	ZnO (3.0 g) + ZnCl ₂ (7.26 g)	18
[Ni ₂ Zn ₃ (OH) ₈ (NO ₃) ₂ ·yH ₂ O] [NiZn-NO ₃]	ZnO (3.0 g) + Ni(NO ₃) ₂ ·6H ₂ O (8.73 g)	18
[Co _{1.2} Zn _{3.8} (OH) ₈ (NO ₃) ₂ ·yH ₂ O] [CoZn-NO ₃]	ZnO (3.0 g) + Co(NO ₃) ₂ ·6H ₂ O (8.73 g)	18

2.2. Characterisation

2.2.1. X-ray diffraction

X-ray diffraction (XRD) was performed using a Philips PW1830 instrument operating at 40 kV and 25 mA with Cu K α radiation ($\lambda=1.5418$ Å). Samples were finely ground and mounted on aluminium plates for measurement. Patterns were recorded over the 2 θ range from 2° to 20°. Diffracted intensity from the sample holder did not interfere with sample characterisation.

2.2.2. IR spectroscopy

IR spectra were recorded on a Perkin-Elmer Spectrum 100 instrument. Data were collected from 4000 to 650 cm⁻¹ at a resolution of 2 cm⁻¹.

2.2.3. NMR spectroscopy

¹H NMR spectra were obtained on a Bruker Avance-400 instrument at ambient temperature (¹H frequency: 400 MHz). Samples were dissolved in D₂O prior to measurement.

2.2.4. Elemental analysis

C, H, and N contents were determined using the quantitative combustion technique on a Carlo Erba CE1108 elemental analyser.

2.2.5. In situ X-ray diffraction

The first series of in situ XRD measurements was performed on Beamline F3 of the DORIS synchrotron at the Deutsches Elektronen-Synchrotron (DESY), Hamburg, Germany. The energy-dispersive X-ray diffraction (EDXRD) technique was employed here. Further in situ XRD experiments were undertaken at the Diamond Light Source, using Beamline I12 and the Oxford-Diamond In situ Cell (ODISC). These used an angle-dispersive modality, with an X-ray beam monochromated at ca. 0.23 Å and a Pixium area detector. Full details of the equipment used is reported elsewhere [25,26].

Both at DESY and Diamond, 0.4 mmol of the desired HDS was mixed with 1.6 mmol of the guest ion in 10 ml of deionised water and heated to the desired temperature. Diffraction patterns were recorded every 60 s (DESY) or 4 s (Diamond) until no further changes in these were observed. Data analysis was performed by integrating reflections of interest using the F3Tool software (DESY), or by employing Fit2D [27] to convert the as-collected images into one-dimensional patterns, subtracting the background, and applying in-house tools to integrate the reflections of interest (Diamond). Integrated data were subsequently probed using the Avrami-Erofe'ev model [28–31]; more details are given in the results section.

2.2.6. Modelling

Models of the Zn-NO₃ derived HDS systems were built in the space group C2/m, using the structure previously reported by Stahlin and Oswald [32]. In this symmetry, $\beta=93.28^\circ$, $a=19.48$ Å, $b=6.238$ Å and $c=5.517$ Å. All molecular dynamics (MD) simulations were carried out using the Materials Studio v5.5 software

package (Accelrys Software Inc) [33]. Temperature and pressure control were implemented using the Andersen [34] and the Berendsen [35] methods respectively. Coulombic interactions were computed by the Ewald summation technique [36] and van der Waals interactions using a “spline-cut off” method. The simulations were performed with the aid of the LDHFF force field. [37] The time-step was set to be 1 fs, which is deemed to be suitable for the characterisation of thermal motion [38], and the simulation time at 5 ns. For MD work, both water bound to tetrahedral Zn and free interlayer water were included in the simulation, with models of the final products isolated *ex situ* constructed using the formulae $[\text{Zn}_5(\text{OH})_8(\text{Val})_2 \cdot 3\text{H}_2\text{O}]_4$, $[\text{Zn}_5(\text{OH})_8(\text{Nap})_2 \cdot 3\text{H}_2\text{O}]_2$ and $[\text{Zn}_5(\text{OH})_8(\text{Dic})_2 \cdot 4\text{H}_2\text{O}]_2$. The interactions between the layers and free interlayer water are known to be small, and so the differences between the amounts of water observed experimentally and those approximated in this approach should not lead to the introduction of any significant errors. For MD work on the intermediate phases observed *in situ*, the same model as above was used for Dic. In the Nap case free water molecules caused the simulation to fail, and thus only water bound to tetrahedral Zn was included (formula: $[\text{Zn}_5(\text{OH})_8(\text{Nap})_2 \cdot 2\text{H}_2\text{O}]_2$).

3. Results and discussion

3.1. Intercalation into CoZn-NO₃

It proved facile to intercalate the APIs into the CoZn-NO₃ HDS through anion exchange. XRD patterns of the drug intercalates of CoZn-NO₃ are given in Fig. 2. The reaction products show no characteristic basal reflections of the starting material, and a shift of the *h*00 basal reflections to lower angles; this corresponds to an increase in interlayer distance consistent with the incorporation of a larger anion (see Table 2). The interlayer spacings seen are in good agreement with previous results for LDHs: Khan et al. recorded *d*-spacings of 22.3 Å for Dic, 21.5 Å for Nap and 18.7 Å for Val [39]. All the diffraction patterns illustrate peak broadening, indicative of stacking defects.

Elemental analyses of the intercalation compounds (Table 2) indicated complete replacement of nitrate with the API guests had

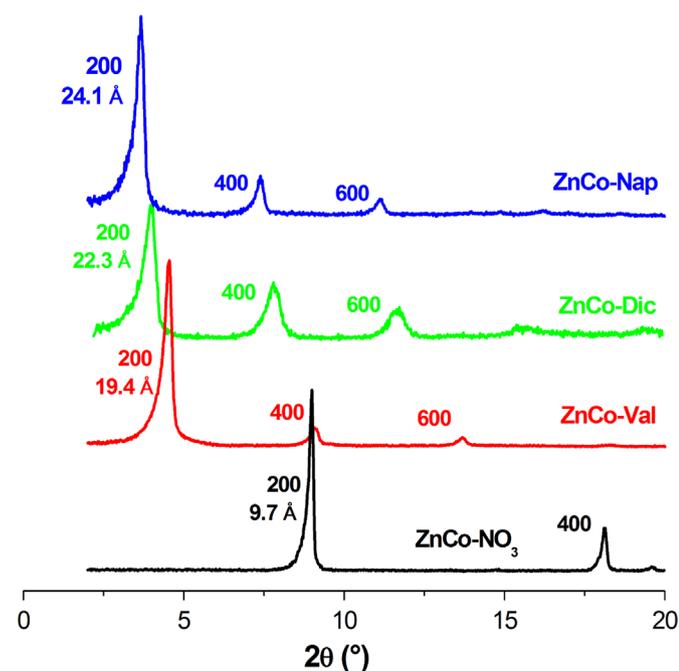


Fig. 2. XRD patterns of CoZn-NO₃ and its intercalates.

been achieved in the majority of cases. In some cases, some carbonate incorporation appears to have occurred, as is common for these systems.

The intercalation compounds were also characterised by IR spectroscopy (see Fig. 3). The spectrum of CoZn-NO₃ shows typical features of an HDS. A broad peak centred at around 3250 cm⁻¹ corresponds to H-bonded OH groups of the HDS layers. The δ-vibration of the interlayer water molecules is located at 1636 cm⁻¹, the NO₃⁻ vibrations at ca. 1373 cm⁻¹, and Co/Zn–O vibrations from the HDS layers are visible below 1000 cm⁻¹. The spectrum of Dic (Fig. 3) displays distinctive symmetric and asymmetric carboxylate bands at 1397 and 1572 cm⁻¹ respectively. An additional peak at 746 cm⁻¹ corresponds to the C–Cl group. The IR spectrum from the CoZn-Dic intercalation compound is a combination of those from the HDS and API, containing a broad OH absorption band at around 3400 cm⁻¹, distinctive carboxylate vibrations at 1580 and 1397 cm⁻¹, and a C–Cl vibration at 746 cm⁻¹. There is no visible peak at 1373 cm⁻¹ for the intercalate, implying that the nitrate ions have been displaced from the structure. The shift in the position of the asymmetric carboxylate band from 1572 cm⁻¹ in Dic to 1580 cm⁻¹ in CoZn-Dic may be attributed to electrostatic interactions between the COO⁻ groups of the anions and the hydroxide layers.

The IR spectra for the other CoZn-NO₃ intercalates also show the disappearance of the nitrate absorption and the presence of characteristic bands from the guest ions (see Supplementary Information, Fig. S1).

3.2. Intercalation into other HDSs

Intercalation into two further HDSs, Zn-NO₃ and NiZn-NO₃, was also explored. Attempts to react Val with Zn-NO₃ were unsuccessful, yielding ZnO as the solid reaction product (see Fig. S2). For the other materials, the XRD and elemental analysis data (Table 2 and Fig. S2) show the intercalation compounds to be largely analogous to those prepared from CoZn-NO₃. In the diffraction patterns of Zn-Dic, NiZn-Dic and the Zn-Nap material it appears that there are two intercalate phases with different *d*-spacings (see Fig. S2 and Table 2). These may be ascribed to two different orientations of the guest ions. IR spectroscopy (Fig. S3) additionally demonstrated successful intercalation of the Dic and Nap anions into all the HDSs.

3.3. Guest recovery

The CoZn-Dic, CoZn-Nap and CoZn-Val intercalates were reacted with Na₂CO₃ in D₂O, and NMR spectra recorded of the filtrates from these reactions. The spectra after deintercalation are observed to be identical to those of solutions of the pure APIs, confirming that the structural integrity of these drug molecules is retained. Exemplar data are given in Fig. S4.

3.4. Guest orientation

Molecular dynamics (MD) simulations were performed to explore the orientations of the guests in the interlayer space. These were performed using the reported structure for Zn-NO₃ [32], since no crystal structures are known for the mixed-metal systems. For Val, simulations indicate a bilayer of ions is formed between the layers, as shown in Fig. 4(a). The model gives an interlayer spacing of 19.1 ± 0.1 Å. This result is in good agreement with the experimental value (19.4 Å), with the small difference between experimental and simulation being well within the range of deviations reported in previous LDH modelling studies (very little modelling work has been done using HDSs) [40–44]. The Val ions adopt a perpendicular position in the interlayer regions, with

Table 2
Interlayer spacings and chemical formulae of the various HDS-drug composites prepared.

ID	d_{200} (Å)	Formula	Elemental analysis (%) Obsd (calcd)
CoZn-NO ₃	9.7	[Co _{1.2} Zn _{3.8} (OH) ₈](NO ₃) ₂ · 2H ₂ O	–
CoZn-Val	19.4	[Co _{1.2} Zn _{3.8} (OH) ₈](C ₈ H ₁₅ O ₂) ₂ · 2.5H ₂ O	C 24.46 (24.43) H 5.55 (5.51) N 0.0 (0.0)
CoZn-Dic	22.3	[Co _{1.2} Zn _{3.8} (OH) ₈](C ₁₄ H ₁₀ Cl ₂ NO ₂) _{1.6} (CO ₃) _{0.2} · 2H ₂ O	C 28.25 (27.83) H 2.77 (2.89) N 1.44 (2.30)
CoZn-Nap	24.1	[Co _{1.2} Zn _{3.8} (OH) ₈](C ₁₄ H ₁₃ O ₃) _{1.8} (CO ₃) _{0.1} · 3.4H ₂ O	C 32.21 (32.49) H 4.12 (4.12) N 0.0 (0.0)
NiZn-NO ₃	9.7	Ni ₂ Zn ₃ (OH) ₈ (NO ₃) ₂ · 2H ₂ O	–
NiZn-Dic	21.2/15.6 ^a	[Ni ₂ Zn ₃ (OH) ₈](C ₁₄ H ₁₀ Cl ₂ NO ₂) _{1.65} (NO ₃) _{0.35} · 2H ₂ O	C 27.91 (27.90) H 2.42 (2.89) N 3.31 (2.82)
NiZn-Nap	24.2	[Ni ₂ Zn ₃ (OH) ₈](C ₁₄ H ₁₃ O ₃) _{1.72} (CO ₃) _{0.14} · 2.7H ₂ O	C 32.21 (32.29) H 4.03 (4.00) N 0.0 (0.0)
Zn-NO ₃	9.7	[Zn ₅ (OH) ₈](NO ₃) ₂ · 2H ₂ O	–
Zn-Dic	22.3/19.5 ^a	[Zn ₅ (OH) ₈](C ₁₄ H ₁₀ Cl ₂ NO ₂) ₂ (C ₁₄ H ₁₁ Cl ₂ NO ₂) _{0.4} · 2H ₂ O	C 33.68 (33.42) H 1.67 (3.01) N 3.05 (2.78)
Zn-Nap	23.6/20.3 ^a	[Zn ₅ (OH) ₈](C ₁₄ H ₁₃ O ₃) ₂ · 2H ₂ O	C 34.87 (35.12) H 3.90 (4.00) N 0.0 (0.0)

^a In some cases, two intercalate phases with different interlayer spacings were observed. The latter d-spacing is that of the minority phase.

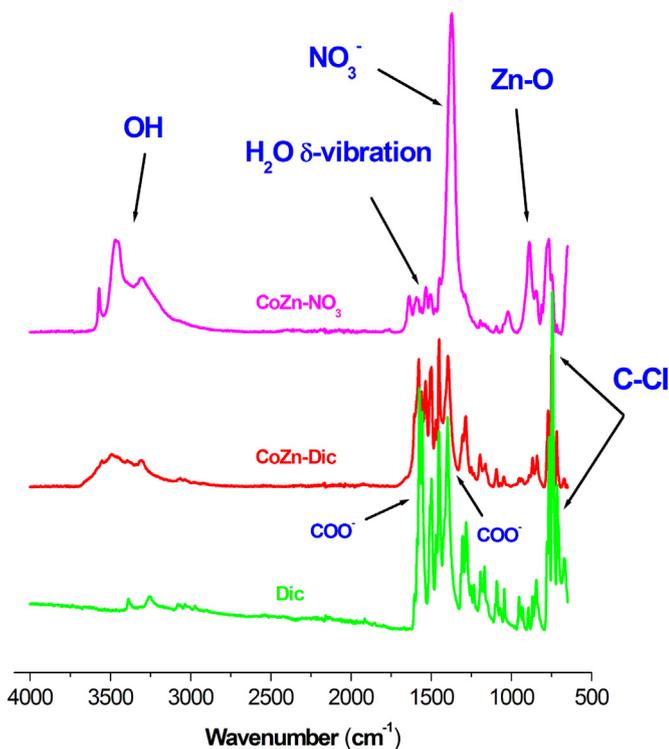


Fig. 3. IR spectra of CoZn-NO₃, CoZn-Dic and Dic.

their carboxylate groups facing the HDS layer and the aliphatic chains towards the centre. The simulation shows that H-bonding occurs between the COO[−] group of Val, water and the HDS layer.

Simulations with Nap ions also suggest a bilayer arrangement of guests between the HDS layers, with a d-spacing of 24.0 ± 0.1 Å (Fig. 4(b)), in very good agreement with the experimental value of 23.6–24.2 Å seen for the major intercalate phase (for Zn-Nap, a second API-loaded phase with a lower d-spacing was observed). The Nap ions form an “X” shape in three dimensions. This keeps the hydrophobic regions of the molecules close together in the

centre of the interlayer, and the hydrophilic regions close to the tetrahedral Zn²⁺ and facing the positively charged layers. Dic ions are similarly predicted to form an “X” shape (Fig. 4(c)), leading to a simulated interlayer spacing of 22.0 ± 0.3 Å (cf. 21.2–22.3 Å observed experimentally). H-bonds are also calculated to be present in the Nap and Dic MD simulations.

3.5. In situ diffraction

3.5.1. Intercalation of Val

Data for CoZn-NO₃ are given in Fig. 5(a). The reaction at 80 °C was seen to be a one stage process, proceeding directly from the starting material to the product. The intercalate exhibits virtually the same d-spacing in situ (19.8 Å) as seen in laboratory syntheses (19.4 Å). No crystalline intermediate phases were visible in the diffraction data.

The intensities of the 200 reflections of the CoZn-NO₃ and CoZn-Val materials were integrated, and converted into extent of reaction, α . This is essentially a normalisation process: $\alpha = I_{hkl}(t)/I_{hkl}(\max)$, where $I_{hkl}(t)$ is the intensity of a reflection hkl at time t, and $I_{hkl}(\max)$ is the maximum intensity of that reflection. A plot of α vs. time is illustrated in Fig. 5(b). The curves of the starting material and product cross at $\alpha \approx 0.45$, which indicates a one stage transformation. This crossing point shows that the diffracted intensity lost from the starting material is matched by intensity gained by the product, and therefore the intercalation reaction has not passed through an intermediate phases (if there were an intermediate stage, the starting material and product curves should cross close to $\alpha \approx 0$).

The Avrami-Erofe'ev model, which has been very widely used with intercalation processes [45–47], was applied to the data for Val intercalation. This states:

$$\alpha = 1 - e^{-(kt)^n}$$

where α is the extent of reaction, t is the time, k the rate constant, and n an exponent which can be used to infer details of the reaction mechanism [10]. A Sharp-Hancock plot of $\ln(-\ln(1-\alpha))$ vs. $\ln t$ can be conveniently used to calculate n and k (Fig. 5(c)). The plot in Fig. 5(c) is linear, indicating that the reaction proceeds via a

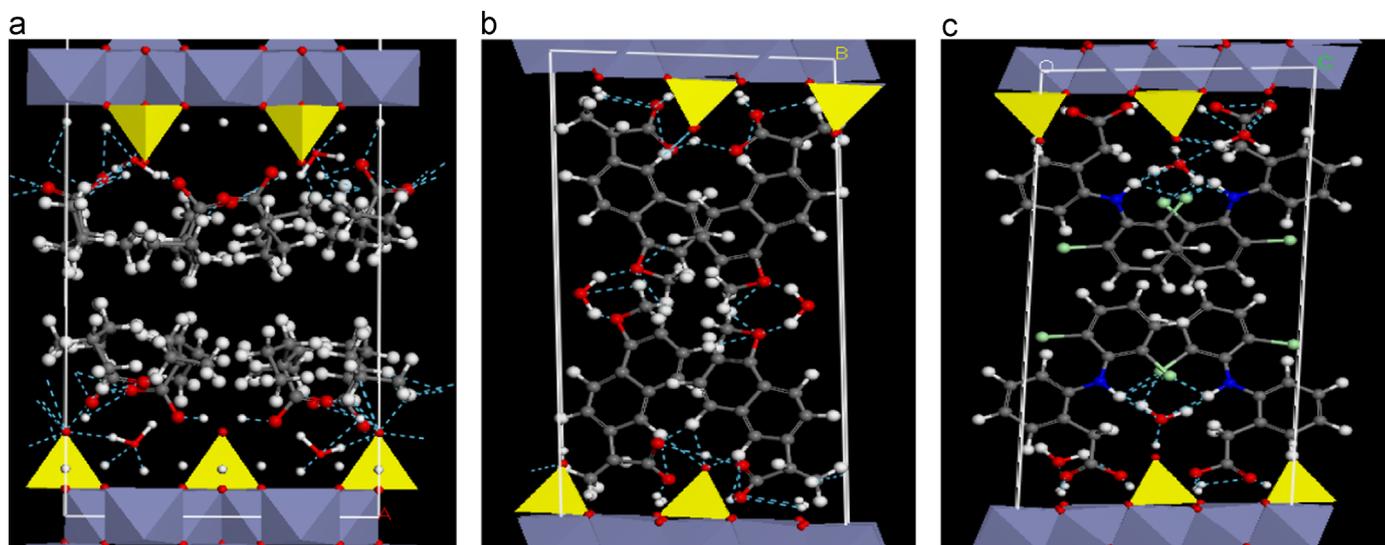


Fig. 4. MD results of HDSs intercalated with (a) Val; (b) Nap; and, (c) Dic. H-Bonds are shown in blue and the tetrahedral Zn^{2+} sites are highlighted in yellow. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

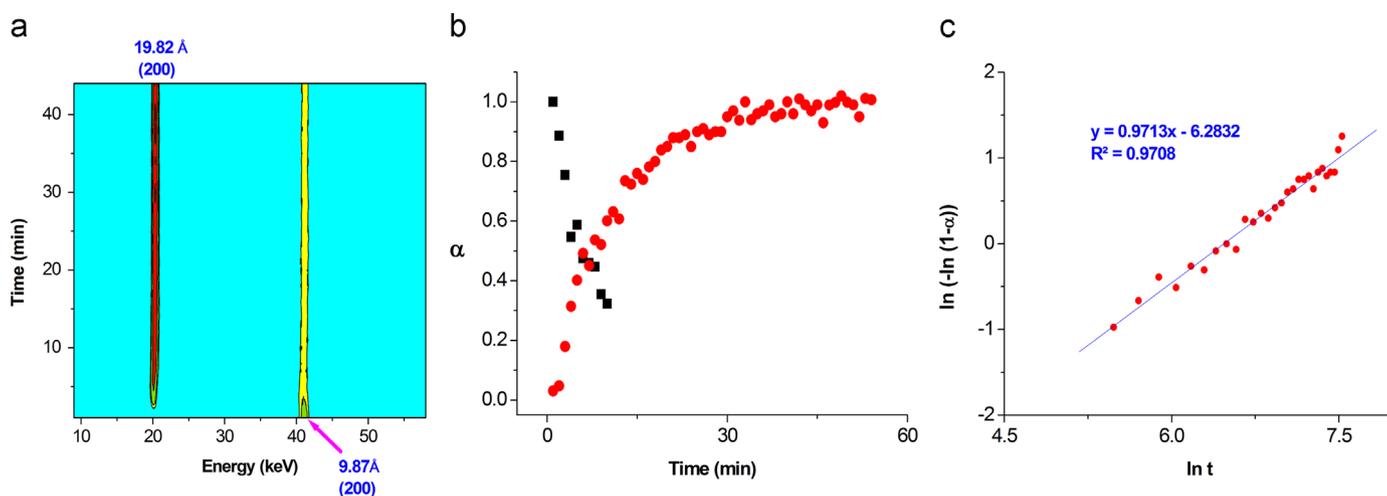


Fig. 5. In situ XRD data for the intercalation of Val into CoZn-NO_3 at 80°C . (a) Contour plot of the raw data; (b) extent of reaction vs. time data showing the changes in intensity of the host (\blacksquare) and product (\bullet) 200 reflections; and, (c) a Sharp-Hancock plot for the product 200 reflection.

Table 3
The results of Avrami-Erofe'ev analyses of the intercalation processes.

ID	T / $^\circ\text{C}$	n	k (10^{-3} s^{-1})	Notes
CoZn-Val	80	1.04	2.96	
CoZn-Dic	80	Avrami-Erofe'ev kinetics not applicable		
NiZn-Dic	75	1.01	1.03	$E_a \approx 28.7 \text{ kJ mol}^{-1}$
	80	1.00	1.18	
Zn-Dic	80	Avrami-Erofe'ev kinetics not applicable		
CoZn-Nap	80	Avrami-Erofe'ev kinetics not applicable		
NiZn-Nap	60	0.98	1.22	$E_a \approx 33.4 \text{ kJ mol}^{-1}$
	70	0.98	1.63	
	75	1.19	2.09	
	80	Avrami-Erofe'ev kinetics not applicable		
Zn-Nap	80	Avrami-Erofe'ev kinetics not applicable		

consistent mechanism over almost the entire course of the reaction, and that the Avrami-Erofe'ev model is suitable to describe this system. The reaction exponent n is observed to be ca. 1 (Table 3). This suggests that the reaction is a nucleation growth process, with instantaneous nucleation followed by two dimensional diffusion control. The layer edges are the nucleation sites, and “instantaneous” nucleation suggests that all these are

saturated with guest ions at the start of the reaction. Two-dimensional diffusion control indicates that, once a pair of layers has been prised apart, the movement of the new guest ions into the interlayer space is facile: thus, diffusion control operates thereafter [10].

3.5.2. Intercalation of Dic

This reaction was studied at 80°C for all three HDSs. Plots of the raw data obtained on DESY are depicted in Fig. 6.

Dic intercalation into NiZn-NO_3 appears to be a straightforward process: the reaction proceeds directly from the starting material to the product, which at 24.3 \AA has a d-spacing somewhat higher than that observed in laboratory synthesis (presumably due to increased hydration levels). This is confirmed by the α vs. time plots of host and product crossing at ca. 0.5 (Fig. S5(a)). An Avrami-Erofe'ev analysis yields $n \approx 1$ (Fig. S5(b)), indicating a mechanism of 2D diffusion control following instantaneous nucleation as discussed above. Performing the reaction at two different temperatures allowed the activation energy to be estimated at ca. 28.7 kJ mol^{-1} .

The CoZn-NO_3 and Zn-NO_3 data are more complex. The d-spacing of the final product in the in situ experiments is 16.3--

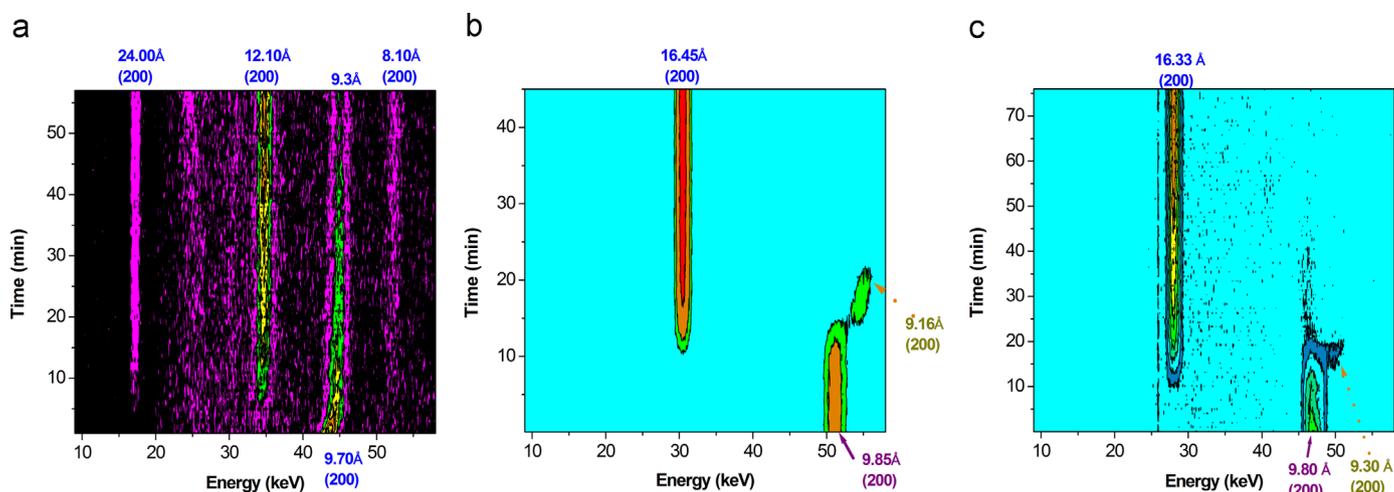


Fig. 6. Plots of the raw data for intercalation of Dic into (a) NiZn-NO₃; (b) CoZn-NO₃; and, (c) Zn-NO₃ at 80 °C.

16.5 Å, much lower than the 22.3 Å observed ex situ. Further, in both Fig. 6(b) and (c), a reflection at around 9.16–9.30 Å is observed to grow in at the same time as the product first appears. This was initially thought to be an artefact, and to verify this hypothesis further experiments were performed with Zn-NO₃ on Beamline I12 of the Diamond Light Source, which has much better resolution. The resultant data are given in Fig. S6: they verify those obtained on DESY, clearly showing the reflection at ca. 9.2 Å to be genuine and not an experimental artefact. MD simulations were carried out to model this phase and predicted a system with Dic ions intercalated in a horizontal arrangement. This gave a d-spacing of 9.28 Å, close to the experimental value. Thus, it seems that at the start of the intercalation process some of the Dic ions intercalate vertically between the layers, while others intercalate horizontally and subsequently rearrange themselves.

To understand why the final product seen in situ has a 200 reflection with a lower d-spacing than observed ex situ, the reaction was run at 80 °C and the solid recovered by rapid filtration after 80 min. The XRD pattern of the quenched product (Fig. S7) shows a virtually identical interlayer spacing to that observed in situ (15.6 Å). The quenched product was returned to the Dic solution and left to stir at 80 °C for approximately 10 h further. The XRD pattern of the material recovered shows a very intense reflection at 20.5 Å, while the phase at 15.6 Å has almost vanished. Thus, we can conclude that the 16.3 Å phase seen in situ is a long-lived intermediate *en route* to the final product (d_{200} = 19.5–22.5 Å). CHN analysis of the material recovered after 80 min shows that only 65% of the initial NO₃⁻ ions had been replaced; further reaction leads to replacement of the remaining ions to yield a fully intercalated product with a higher d-spacing (Table 2). MD simulations (Fig. S8) indicated that the 15.6 Å phase this contains the guest ions arranged in a perpendicular monolayer. The Dic carboxylate groups are close to the tetrahedral Zn²⁺ ions. This arrangement is thought to become unstable when the number of Dic ions in the interlayer region increases and the amount of NO₃⁻ ions declines. As a result, at a critical Dic content the ions rearrange themselves to give a phase with an interlayer spacing of 19.5–22.5 Å.

During the intercalation of Dic into Zn-NO₃ and CoZn-NO₃, we observed an initial drop in the intensity of the starting material 200 reflection, followed by a subsequent increase. This was observed in four independent experiments, both at Diamond (Fig. S6) and DESY, and thus is believed to be a genuine feature of the reaction rather than an artefact arising from, for instance, a failure in stirring. We thus postulated that an additional intermediate phase might be forming, with a d-spacing very similar to that of the starting material.

To confirm this suggestion, experiments were performed with the HDS [Zn₅(OH)₈]Cl₂ · yH₂O (Zn-Cl) which has a different initial interlayer spacing (7.8 Å) but otherwise behaves similarly to Zn-NO₃. Quenching reactions were conducted under the same conditions as used for in situ studies, and the results are shown in Fig. S9. The XRD patterns show clear reflections at 9.3 and 9.9 Å, suggesting that the in situ phenomenon seen during the intercalation of Dic into Zn-NO₃ is caused by an intermediate that has a d-spacing of 9.8–9.9 Å.

Plots of α vs. time for Dic intercalation into CoZn-NO₃ and Zn-NO₃ are depicted in Fig. S10. Although it appears at first sight that the curves from the starting material and the 16 Å intercalate cross at around α = 0.5, the situation is in fact more complex because of the presence of an intermediate with identical d-spacing to the starting material. Attempts were made to fit the Avrami-Erofe'ev model to the data, but these were inconclusive: the resultant Sharp-Hancock plots were decidedly non-linear, indicating a complex reaction which does not proceed via a consistent mechanism throughout (Table 3). This is perhaps not surprising given the number of different phases identified on the reaction coordinate.

3.5.3. Intercalation of Nap

In situ diffraction data for the intercalation of Nap are given in Fig. 7.

As for Dic, the process of Nap intercalation into NiZn-NO₃ is found to be a simple one-step process going directly from the starting material to the product. The host and product α vs. time curves cross at α ≈ 0.5 (see Fig. S11). The product 200 reflection arises at around 24.6 Å (400: 12.4 Å, 600: 8.3 Å). The interlayer spacing observed in situ is thus very similar to that seen ex situ (24.2 Å). At 80 °C, the reaction appears not to follow Avrami kinetics, with highly non-linear Sharp-Hancock plots being seen. At lower temperatures, the reaction is again observed to proceed directly to the product, but linear Sharp-Hancock plots are produced and values for n and k may be extracted (Table 3). n is found to be ca. 1, indicative of 2D diffusion control following instantaneous nucleation as observed previously. The Arrhenius equation was used to estimate the kinetic energy; this was found to be approximately 33.4 kJ mol⁻¹, consistent with a nucleation-controlled reaction.

The intercalation of Nap into CoZn-NO₃ at 80 °C (Fig. 7(b)) shows that the final product observed in situ has essentially the same interlayer spacing as seen ex situ (24.3 Å vs. 24.1 Å). Clear crystalline intermediate phases are visible in the diffraction data,

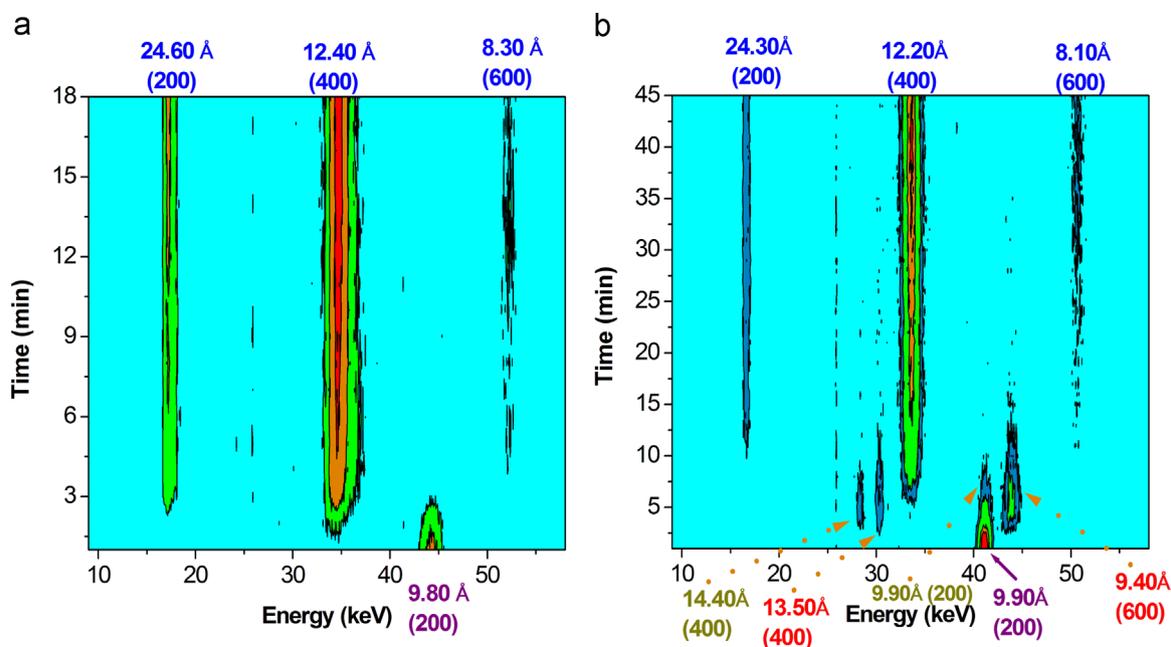


Fig. 7. Plots of the raw data for intercalation of Nap into (a) NiZn-NO₃; and, (b) CoZn-NO₃ at 80 °C.

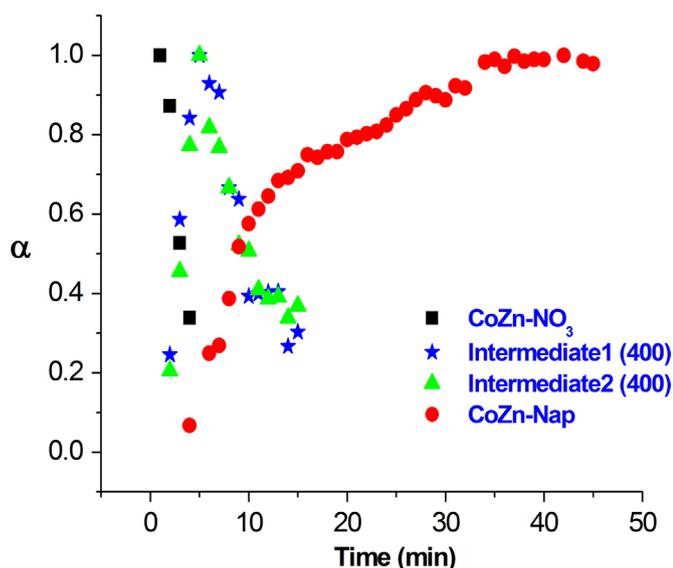


Fig. 8. α vs. time plot for the intercalation of Nap into CoZn-NO₃ at 80 °C. Intermediate 1 refers to the 400 reflection at 14.4 Å and intermediate 2 to that at 13.5 Å.

with three distinctive reflections that appear and disappear synchronously. These are believed to be the 400 (14.4/13.5 Å) and 600 (9.4 Å) reflections of two higher d-spacing intermediate phases (the 200 positions of these at 27 – 29 Å placed them beyond the detection window). Repeating these experiments on Beamline I12 at Diamond (Fig. S12) identified the same features, with some additional reflections corresponding to these intermediate phases resolvable in the patterns. α vs. t curves for this system are given in Fig. 8. The reflections from CoZn-NO₃ and CoZn-Nap cross at $\alpha \approx 0$, consistent with the observation of an intermediate. The starting material vs intermediate curves cross at $\alpha \approx 0.5$, as do the intermediate vs product curves. This indicates a two stage transformation. The results of MD simulations are presented in Fig. S13. The higher d-spacing phases are found to arise from the Nap ions being arranged in a perpendicular bilayer, with a d-spacing of 28.0 Å. This arrangement is presumably unstable, and thus as the reaction continues the Nap ions rearrange themselves to give a

phase with an interlayer spacing of 24.3 Å.

The Sharp-Hancock plot for CoZn-Nap formation was decidedly non-linear, indicating that simple Avrami kinetics are not sophisticated enough to describe this complex system (Table 3).

The intercalation of Nap into Zn-NO₃ produces two product phases of different d-spacings in the laboratory syntheses. This same phenomenon is seen in the time-resolved data (Fig. 9). A phase first grows in at 20.8 Å before a second product phase appears at 24.0 Å. These d-spacings are very similar to those seen *ex situ* (Table 2). Plotting the extent of reaction vs. time (Fig. S14) suggests that the 20.8 Å phase exists in equilibrium with the 24.0 Å phase; although the integrated intensity of the former declines very slightly during the course of intercalation, it then appears to level off. There is a very transient and poorly crystalline intermediate phase which forms at 9.2 Å. This phase is believed on the basis of MD simulations to have the Nap ions intercalated horizontally, analogous to the Dic system discussed above. The data obtained on Diamond (Fig. 9(b)) also show another, very poorly crystalline, intermediate phase present with a 200 reflection at 21.5 Å.

The Sharp-Hancock plot for the 24.0 Å product was non-linear, indicating the non-applicability of Avrami kinetics here. The 20.8 Å phase produced a linear plot but the exponent value was not consistent with any of the possible mechanisms of reaction, and thus again it is thought Avrami kinetics cannot be applied. The simulated structure of the 24.0 Å phase is discussed above; the other phases at 20.8 and 21.5 Å are believed to be similar, but with reduced hydration levels.

3.6. Drug release

A drug release study was carried out on selected systems in phosphate buffered saline (PBS; pH 7.4) to explore the potential of the HDS systems for drug delivery. Dic release (see Fig. 10(a)) was faster from Zn-NO₃ than from the HDSs containing a mixture of divalent metals (CoZn-NO₃ and NiZn-NO₃). The drug was released fully within 6 h from Zn-Dic, while a maximum of around 75% release was observed from NiZn-Dic after this time, and only 85% release was reached after 24 h (Fig. 10(a)). Release from all the HDSs is initially rapid, followed by a slower and more sustained release phase. This two-stage release behaviour has previously

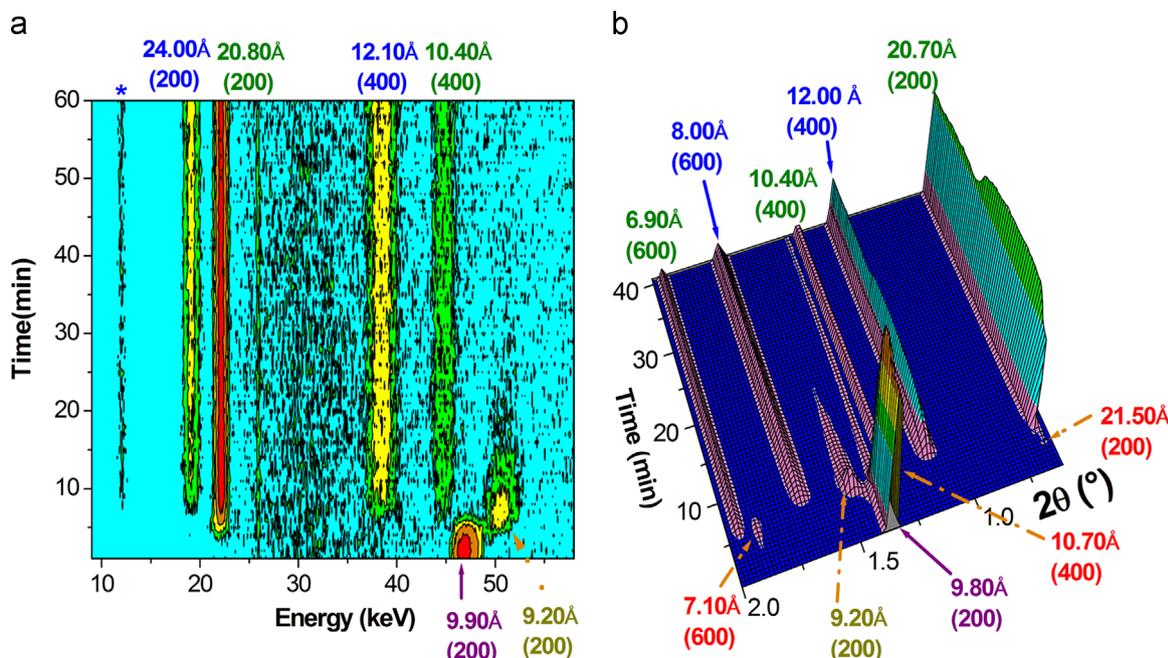


Fig. 9. Time resolved XRD data obtained for the intercalation of Nap into Zn-NO₃ on (a) DESY and (b) Diamond at 80 °C, showing the possible presence of a transient and low crystallinity intermediate phases (labelled in red/orange). The peak marked * is an escape peak from the Ge detector. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

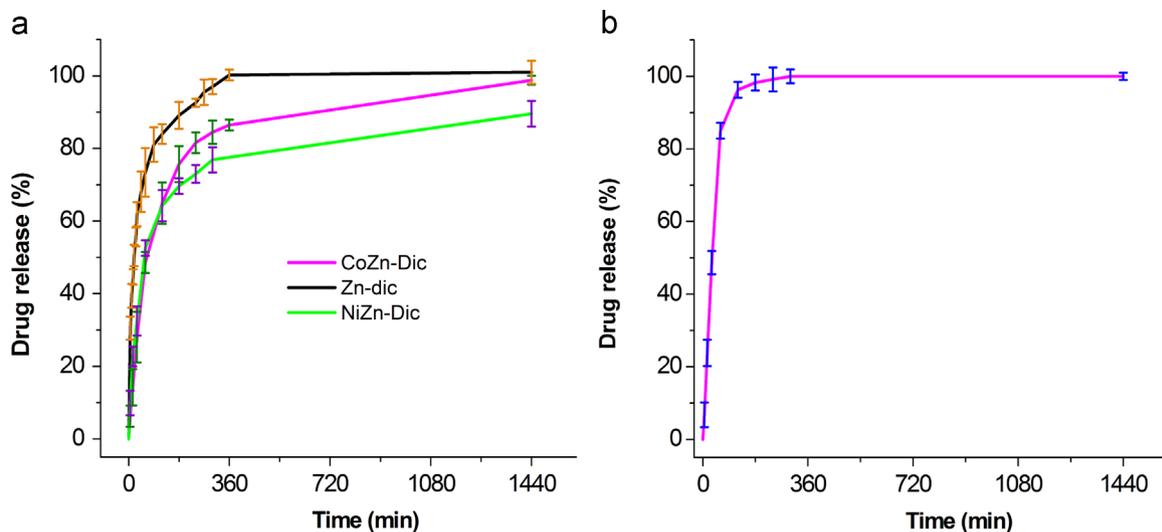


Fig. 10. *In vitro* release of (a) Dic from Zn-Dic, NiZn-Dic and CoZn-Dic; (b) Nap from CoZn-Nap.

been reported from LDH and HDS systems with various drugs, including NSAIDs [48–52]. Similar Dic release profiles have been seen for both HDSs and LDHs [50,53,54]. The metal composition of the layers clearly has an influence on the release rates obtained.

The Nap release profile from CoZn-Nap is given in Fig. 10(b); the full drug loading was released within 5 h. Nap is thus released more rapidly than Dic from the CoZn system, which could be explained by the higher solubility of the former [55,56]. The release profiles seen here are more sustained than those previously observed. LDHs in general have been shown to release their drug cargo more quickly than HDSs and here Nap release proceeds more slowly than has been reported from both LDHs and HDSs, where complete release in less than 100 min was seen [20,57–59].

Trial release experiments were also carried out in conditions that mimic passage through the human gastrointestinal tract (2 h in a pH 1 medium, followed by transfer to a pH 6.8 PBS; see Fig.

S15). Zn-Dic released only 2% of its drug loading in the acidic media, but upon raising the pH to 6.8 this process was accelerated and most of the drug loading freed within 9 h. Release from CoZn-Dic was slower, and was complete after around 21 h. Again, very little release was seen at pH 1. Release from both Zn-Dic and CoZn-Dic was slower at pH 6.8 than 7.4; such a positive correlation between the rate of release from layered systems and pH has been reported previously [60,61]. On the basis of the data reported herein, HDSs seem to have promise for sustained release. Further, the release profiles observed suggest that loading the drug-loaded HDSs into capsules would give formulations meeting the pharmacopeia requirements for intestinal drug targeting [62–64].

4. Conclusions

The anions of diclofenac (Dic), naproxen (Nap), and valproic acid (Val) were intercalated into several Zn-based hydroxy double

salts (HDSs). While Nap and Dic could be intercalated into all the HDS explored, the attempted intercalation of Val into $[\text{Zn}_5(\text{OH})_8](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ ($\text{Zn}-\text{NO}_3$) led to the destruction of the HDS layers to yield ZnO. In contrast, the presence of Co^{2+} in the HDS appears to stabilise the layers and resulted in the successful intercalation of Val into $[\text{Co}_{1.2}\text{Zn}_{3.8}(\text{OH})_8](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ ($\text{CoZn}-\text{NO}_3$). Molecular dynamics simulations suggested that the Dic and Nap ions were arranged in an “X” shape, forming a bilayer arrangement in the interlayer space. Val was determined to adopt a position with its aliphatic groups parallel to the HDS layer, and again form a bilayer.

In situ time resolved X-ray diffraction experiments showed that the intercalation of Dic and Nap into $\text{CoZn}-\text{NO}_3$ and $\text{Zn}-\text{NO}_3$ proceeds by distinct intermediates, while their reactions with $[\text{Ni}_2\text{Zn}_3(\text{OH})_8](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ ($\text{NiZn}-\text{NO}_3$) are simple one step processes proceeding directly from the starting material to the product. The one-step processes obeyed simple Avrami-type kinetics and were found to be nucleation-governed processes, with instantaneous nucleation followed by 2D diffusion control. In contrast, where intermediates were observed Avrami kinetics could not be applied. The d-spacings observed for the intermediates were not consistent with staging processes, and molecular dynamics simulations suggested the intermediate phases to be a result of reorientation of the drug anions between the layers and/or varying hydration amounts. The HDS-drug composites were found to have sustained release profiles, and comply with pharmacopeia requirements in this regard.

The presence of Ni and Co in some of the systems reported in this work comprises a hurdle to their translation to the clinic. Although it is not believed that the materials reported here would be toxic (the amounts of HDS/drug composites required would free quantities of Ni and Co well below LD50 values, even if they dissolved fully), in our future work we will develop a series of HDSs using the most biocompatible metals possible.

Acknowledgments

The authors would like to thank: the Deutsches Elektronen-Synchrotron for the provision of beamtime on DORIS, and Dr Joern Donges and Dr Andre Rothkirch for their assistance during in situ experiments on F3; Diamond Light Source for the provision of beamtime on I12 (EE7782), and Dr Michael Drakopoulos and Dr Christina Reinhard for their help and advice; and, Stephen Boyer of London Metropolitan University for elemental microanalysis measurements. Part of this work was done while AYAK was a visiting student at the Beijing University of Chemical Technology funded by the British Council China / China Scholarship Council Sino-UK Higher Education Research Partnership for PhD Studies, and we thank them for the award of this grant. Finally, A.Y.A.K. thanks his parents for financial and moral support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jssc.2016.03.019>.

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