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## **Controlled Release of Lawsone-intercalated Zn-Al-layered Double Hydroxide**

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Lawsone (2-hydroxy-1,4-naphthaquinone) is the biologically active compound which have profound effect on human health. This compound has becoming more important in medicinal chemistry especially in the area of drug development. In this study, a drug-inorganic nanostructured material involving pharmaceutically active compound of lawsone was intercalated between the layers of Zn-Al layered double hydroxide by co-precipitation and ion exchange methods. Powder X-ray Diffraction (XRD) and Fourier Transform Infrared spectra (FTIR) analysis indicate a successful intercalation of lawsone between the layers of layered double hydroxide. TG-DTA analyses shows that the thermal stability of intercalated organic species is largely enhanced due to host-guest interaction as compared to pure form before intercalation. The deintercalation or release of lawsone was found to be rapid initially followed by a more sustained release thereafter and this behavior was found to be dependent on the pH of the release medium. The results suggest that the layered double hydroxide can be used as potential carrier for lawsone with controlled delivery capability.

**Key words:** Lawsone layered double hydroxide, drug-inorganic composite, characterizations, controlled release

## INTRODUCTION

Recently, enormous demands on the inorganic-organic hybrid materials are increasing due to its various new applications. This is because the hybrid materials can show better properties as compared to their original material. The hybrid material can be used in various applications. For instant, layered double hydroxide hybrid material was reported can be used as catalyst for liquid-phase aldol condensation (Roelofs *et al.*, 2001) and as controlled release formulation of an herbicide (Hussein *et al.*, 2005).

Layered double hydroxides are layered materials having positively charged layers and interlayer charge compensating anions. The chemical composition of this material can be represented by the general formula of  $[M^{II}_{(1-x)}M^{III}(OH)_2]^{x+}A^{z-}_{xz} \cdot yH_2O$  or  $[M^I M_2^{III}(OH)_2]^+ A^{z-}_{1/z} \cdot yH_2O$  where  $M^I$ ,  $M^{II}$  and  $M^{III}$  are mono, di- and trivalent cations, respectively, occupying octahedral positions in hydroxide layers and  $A^{z-}$  is an interlayer charge-compensating anion. These materials have good anion exchange properties which can be used as fundamental in preparing a controlled release formulation and biocompatible materials (Constantino *et al.*, 2008; Tyner *et al.*, 2004; Li *et al.*, 2004). The formulation prepared from layered double hydroxide gives some advantages as compared to its counterpart such as prolonged duration of released, minimize adverse reactions and higher stability of the active agents in the formulation (Constantino *et al.*, 2008).

The preparation of inorganic-organic hybrid materials of layered double hydroxides can be carried out by two different methods namely direct or indirect method. In the direct method, the organic compound can be intercalated into the layers of layered double hydroxides by co-precipitation reaction (Yasin *et al.*, 2011). By used of this method, layered double hydroxides and the organic compound are included in the mother liquor, followed by aging process to form a well ordered nanolayered structure. By used of indirect method, the insertion of organic compound can be done by first preparing the layered double hydroxide followed by modification and finally insertion of the organic compound into the interlayer (Choy *et al.*, 2001).

Lawsone (2-hydroxy-1,4-napthaquinone) is an active compound which can be isolated from the leave of *henna*. In Asia, human have used henna for centuries for hair dyeing and skin painting. Recently, lawsone is becoming more important due to its profound effect on human health. In the present study, lawsone was selected as active organic compound species and intercalated into the layers of Zn and Al layered double hydroxide by both

direct and in-direct method. In our previous paper, we have reported the characterizations of this compound using Powder X-ray diffraction and Fourier transform infrared (Yasin *et al.*, 2011). In addition to our previously reported paper, we carried out further investigations on the characterization using thermo gravimetric analysis as well as to study and discuss the controlled release property of the resulting intercalated compound.

## MATERIALS AND METHODS

**Synthesis of Zn-Al-NO<sub>3</sub>-layered double hydroxide (ZAN-LDH):** All chemicals used in this synthesis were of analytical grade and used without any further purification. Co-precipitation method was adopted to synthesize Zn-Al-NO<sub>3</sub> (ZAN-LDH) in this study. In the preparation of Zn-Al-NO<sub>3</sub>, an aqueous solution of Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O (0.1 M) was added to Al(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O (0.025 M) to give Zn<sup>2+</sup>/Al<sup>3+</sup> ratio, R = 4. Aqueous solution of NaOH and Na<sub>2</sub>CO<sub>3</sub> (2.0 M) were then added to the mixture dropwise, with vigorous stirring at room temperature and the pH was adjusted to 10.0±0.2. The precipitate formed was aged at 70°C in an oil bath shaker for 24 h, cooled, centrifuged, washed several times with deionized water and dried in an oven for 48 h. The resulting ZAN-LDH was ground into powder and keep in sample bottles for further used and characterizations.

**Synthesis of ZAN-Law (cop) using co-precipitation method:** An aqueous solution (100 mL) of sodium hydroxide (NaOH) (1.52 g, 0.003 mol) and lawsone (1.03 g, 0.003 mol) was added drop wise to a solution (250 mL) containing zinc nitrate (Zn(NO<sub>3</sub>)<sub>2</sub>) (1.53 g, 0.006 mol) and aluminium nitrate (Al(NO<sub>3</sub>)<sub>3</sub>) (0.75 g, 0.002 mol) under nitrogen atmosphere with vigorous stirring until the final pH of 10. The resulting slurry was aged at 28°C for 48 h in an oil batch shaker. The resultant slurry will then filtered, wash with de-ionized water until the final pH of 7 and finally dried at room temperature for 12 h.

**Synthesis of ZAN-Law (ie) using ion exchange method:** Synthesis of ZAN-Law using ion exchange method was done under nitrogen atmosphere in which 0.5 g of ZAN-LDH was added to 50 mL of a 0.06 M aqueous lawsone solution. The pH of the mixture solution was held constant at 10 by simultaneous addition of 2 mol L<sup>-1</sup> sodium hydroxide solutions. The exchange process was stirred vigorously for 24 h at room temperature. The precipitate formed was washed several times with de-ionized water and finally dried at room temperature for 12 h.

**Controlled release study:** Phosphate buffer solution at pH 7.0, 4.0 and pH 10 were used as aqueous release medium in this study. The release of lawsone from the interlayer of layered double hydroxide was performed by adding 0.025 g of ZAN-Law into 200 mL of aqueous release medium at room temperature. The paddle rotation speed was set at 125 rpm. Two mL of sample was withdrawn at pre-determined intervals and pass through 0.45  $\mu\text{m}$  membrane filter before the concentration of lawsone released into an aqueous release medium was measured using UV-Vis spectrometer which performed at 265 nm wavelength.

**Characterizations:** The PXRD spectra were recorded on a X'pert PRO PAN powder diffractometer using filtered Cu-K $\alpha$  radiation ( $\lambda = 1.54\text{\AA}$ ) at 40 kV and 200 mA and samples scan were done at  $5^\circ\text{-}90^\circ 2\theta \text{ min}^{-1}$  at  $0.003^\circ$  steps. All solid samples were mounted on an alumina sample holder. Basal spacing (d-spacing) were determined via powder technique. Fourier Transform Infrared (FTIR) spectra were recorded by a Perkin Elmer 1725X spectrophotometer in the range of  $4000\text{-}400 \text{ cm}^{-1}$ . Finely ground 1% samples in KBr powder were compressed to obtain a pellet and the pellet was then used to obtain the IR spectrum. Thermo Gravimetric Analysis (TGA) were carried out on a Du Pont Instrument 951 Thermo gravimetric analyzer using a heating rate of  $10^\circ\text{C min}^{-1}$  under nitrogen atmosphere with a flow rate at  $50 \text{ mL min}^{-1}$ . A temperature range between  $50\text{-}800^\circ\text{C}$  was used in this study.

## RESULTS AND DISCUSSION

**Characterizations:** Powder X-ray diffraction analysis of original ZAN-LDH and resulting intercalated material by used of ion-exchange (in-direct method) and co-precipitation (direct method) intercalated with lawsone are given in Fig. 1. As shown in the Fig. 1, the PXRD pattern of original ZAN-LDH layered double hydroxide and its resulting intercalated material synthesized by used of both methods show high crystallinity recorded by the narrow width of the reflection peaks especially at 003 and 006 reflections. The  $d_{003}$  value for original ZAN-LDH was recorded at  $8.9 \text{ \AA}$  which demonstrated the general feature of layered double hydroxide (Hussein *et al.*, 2002) while the  $d_{003}$  values recorded for ZAN-Law (cop) and ZAN-Law (IE) were  $8.7 \text{ \AA}$  and  $7.6 \text{ \AA}$ , respectively. The resulted  $d_{003}$  values that obtained from both techniques show that the intercalation process by used of co-precipitation is better as compared to ion-exchange method. The interlayer spacing occupied by the lawsone between the layers of layered double hydroxides was found to be  $3.9 \text{ \AA}$ . The

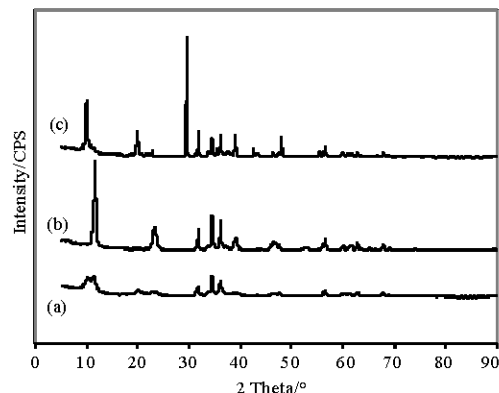


Fig. 1(a-c) : PXRD patterns for ZAN, a: ZAN-Law (ie) b: and c: ZAN-Law (cop)

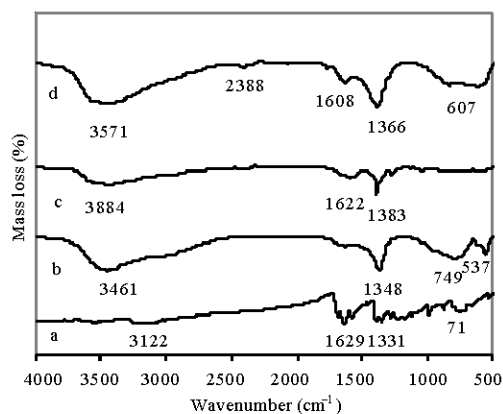


Fig. 2(a-d) : FTIR spectra for a: Lawsone, b: ZAN-Law (ie), c: ZAN-Law (cop) and d: ZAN

obtained value is based on the standard thickness of the brucite like sheets that recorded at  $4.8 \text{ \AA}$  and therefore the proposed orientation of the lawsone between the layers of layered double hydroxide is flat and monolayer since the thickness of the benzene ring is recorded at  $3.7 \text{ \AA}$  (Hussein *et al.*, 2009).

Figure 2 shows the Fourier transform infrared (FTIR) spectra for lawsone, ZAN-LDH, ZAN-Law (IE) and ZAN-Law (cop). As shown in Fig. 2a for a lawsone spectrum, a band attributed to the phenolic OH vibrations can be observed around  $3200 \text{ cm}^{-1}$ . A strong stretching vibration of benzene ring skeleton can be observed around  $1600 \text{ cm}^{-1}$  and vibration due to C = O and C = C can be observed around  $1510 \text{ cm}^{-1}$ . Other bands can be attributed to various functional groups present in lawsone molecule.

Figure 2b shows the FTIR spectrum for ZAN-LDH that prepared using co-precipitation technique. A broad band centered at around  $3400\text{ cm}^{-1}$  is due to the presence of OH stretching of the hydroxyl group of LDH and physically adsorbed water molecule. The band around  $1640\text{ cm}^{-1}$  is due to H-O-H bend vibrations. As shown in the figure, the presence of nitrate group can be observed as the sharp peak at  $1385\text{ cm}^{-1}$ . A band around  $600\text{ cm}^{-1}$  can be attributed to the Al-OH and Zn-Al-OH bending vibration (Hussein *et al.*, 2002).

Figure 2c and 2d show the FTIR spectrum for ZAN-Law (cop) and ZAN-Law (ie), respectively. As explained earlier, XRD diffractogram (Fig. 1) shows that lawsone is intercalated in the layer of LDH and therefore a combination of both lawsone and ZAN-LDH spectra is expected. The FTIR spectrum of ZAN-Law synthesized using both techniques show that it resembles a mixture of each spectrum of lawsone and ZAN-LDH. This indicates that both functional groups of lawsone and ZAN-LDH are present in ZAN-Law and clearly indicates the intercalation of lawsone inside the layer of LDH. Also shown in the figure is a band at  $1385\text{ cm}^{-1}$  for ZAN-Law (cop) became less broad due to intercalation of lawsone which removed some of nitrates from the layer of LDH. Generally, the band around  $600\text{ cm}^{-1}$  that is attributed to Al-OH and Zn-Al-OH bending vibration became smaller than that of ZAN-LDH which also confirmed the intercalation of lawsone through an interaction between the interlayer lawsone and hydroxyl groups of LDH layers.

TG/DSC thermogravimetric analyses profile for lawsone, ZAN-Law (cop) and ZAN-Law (ie) are reported in Fig. 3. For lawsone, thermal studies show that three main thermal events are observed. The first slow event in the temperature range between  $90\text{-}120^\circ\text{C}$  is attributed to the melting process of lawsone which corresponds to a sharp endothermic peak at ca.  $198.6^\circ\text{C}$ . The followed mass loss in the temperature range between  $150\text{-}250^\circ\text{C}$  is due to the decomposition and subtle combustion of lawsone which correspond to weak endothermic peak at ca.  $213.6^\circ\text{C}$ . The last event recorded at temperature between  $350\text{-}450^\circ\text{C}$  is due to the strong combustion of lawsone corresponding to exothermic peak at ca.  $417^\circ\text{C}$ .

For lawsone that intercalated in the layer of layered double hydroxide using co-precipitation method (ZAN-Law (cop)), four thermal events are observed. The first event recorded at temperature range between  $50\text{-}150^\circ\text{C}$  which can be attributed to the loss of surface adsorbed water molecules and interlayer water that corresponds to DSC curve which shows two endothermic peaks at ca.  $182.4$  and  $264.7^\circ\text{C}$ , respectively. The followed loss of mass at temperature between  $150\text{-}250^\circ\text{C}$  can be attributed to the loss of residual intercalated water

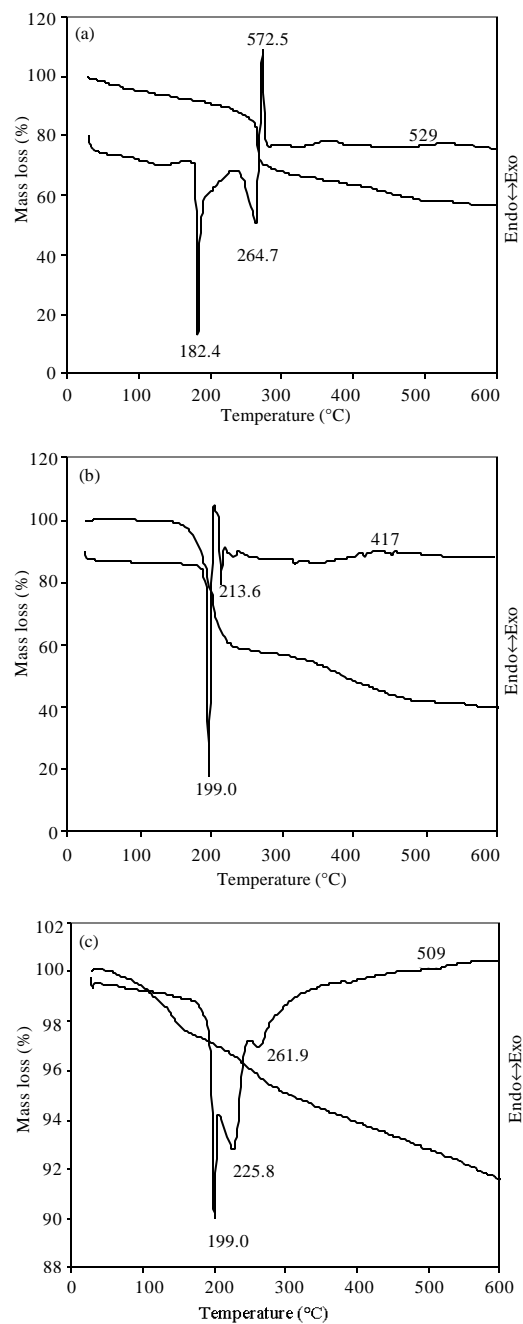


Fig. 3(a-c): TG-DSC curves for (a) Lawsone, (b) ZAN-Law synthesized by co-precipitation method and (c) ZAN-Law synthesized by ion-exchange method

and trace dehydroxylation of the LDH layer. The loss of mass that recorded at temperature range between  $250\text{-}300^\circ\text{C}$  is due to the dehydroxylation of layered double hydroxides accompanying with the formation of layer

double oxide and the partial decomposition of intercalated lawsone (Ni *et al.*, 2008). The recorded loss of mass is corresponds to a sharp exothermic peak recorded at ca. 272.5°C Fig. 3b and 261.9°C Fig. 3c, respectively. The followed loss of mass at temperature between 400-550°C can be attributed to the major decomposition of intercalated lawsone that are corresponds to ca. 529°C (Fig. 3b and 509°C Fig. 3c. TG/DSC thermogravimetric analyses indicate that, the temperature region of Law-LDH is higher as compared to lawsone. It shows that thermal stability of lawsone in ZAN-Law (cop) and ZAN-Law (ie) is clearly enhanced due to the host-guest interaction involving hydrogen bonding (Xia *et al.*, 2008).

**Controlled release study:** Lawsone can be de-intercalated from the interlayer of LDH through ion-exchange with the surrounding anions, such as phosphate. A series of phosphate buffer solutions with different pH values were used to observe the pH effect on the release rate of lawsone from the interlayer of layered double hydroxide. Figure 4 shows the release profile of lawsone from the interlayer of layered double hydroxide into the release medium at different initial pH values.

As shown in the Fig. 4, the released rate of lawsone into aqueous release medium increased with increased in contact time between the intercalated compound and aqueous release medium. The release rate was found to be rapid for the first one hour for all pH and followed by a more sustained released thereafter. Equilibrium was achieved after one hour. This is true for all release medium with initial pH of 4, 7 and 10. The release rate was found to

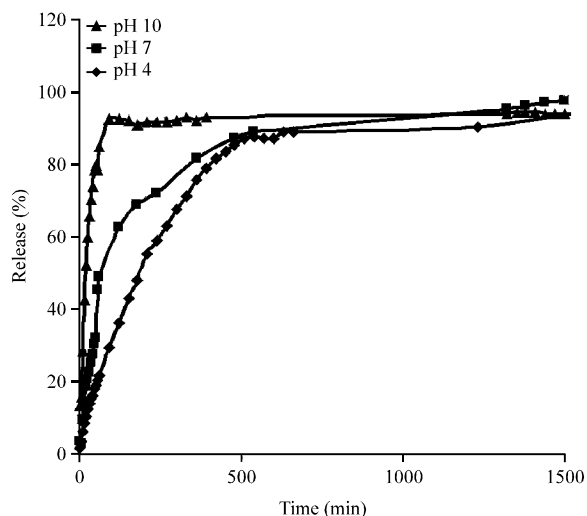


Fig. 4: Release profile of lawsone from ZAN-Law (cop) at different pH values

be very rapid for the first one hour and recorded a slower rate thereafter and the release process still continued after 3 h. A maximum percentage of released are achieved at 90 minutes for pH 10, 480 minutes for pH 7 and 540 min for pH 4.

The percentage of lawsone released from ZAN-Law into the aqueous release medium solutions at initial pH 10 was found to be the lowest, as shown in Fig. 4. The highest percentage of lawsone that released was achieved in neutral and acidic aqueous release medium solution. At equilibrium it was estimated that 90 and 82% of lawsone could be released from ZAN-Law into the aqueous release medium solution with initial pH of 7 and 4 respectively. The results was in agreement with the reported previously study (Constantino *et al.*, 2008) which explained that the intercalation drug was unionized in the acidic environment and ionized in neutral and alkaline environment. The explanation reflected the results that obtained from the released study which shown that the lowest percentage of released was recorded at pH of 4. This might be due to the remaining lawsone that is still in the interlayer of layered double hydroxide which unable to ionized and performed ion exchange while in neutral and alkaline aqueous release medium the lawsone will be ionized and able to perform ion exchange which contributed to the high percentage of released.

## CONCLUSION

The drug-inorganic composite, ZAN-Law with Zn/Al = 4 has been successfully synthesized by using co-precipitation and ion exchange method. PXRD analysis of ZAN-Law prepared by both methods show that the basal spacing ( $d_{002}$ ) shifted to a lower  $2\theta$  angles indicating the intercalation of lawsone in the layer of LDH. FTIR study shows that the drug-inorganic composite (ZAN-Law) resemble the spectra of ZAN-LDH and lawsone indicating the presence of both functional groups. The percentage released of lawsone was found to be dependent on the pH of the release medium. The highest percentage of released was achieved in alkaline and neutral release medium. The present study may suggest that the LDH can be used as an alternative medium for a drug delivery system especially in controlling the release rate of lawsone.

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