

An Evaluation of the Impact of Zinc and Copper Metal-Organic Frameworks (MOFs) on the Drug Release Profiles of Piroxicam

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ABSTRACT

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) poorly soluble in an aqueous medium with a slow and gradual dissolution in biological fluids resulting in sub-optimal efficacy in patients. In recent times, Metal-Organic Frameworks (MOFs), a new class of highly tenable hybrid porous materials with unique properties have been explored for usage as drug carriers and other biomedical applications. This study is aimed at studying the solubility, *in vitro* release profile and kinetics of piroxicam loaded Zinc and Copper Metal-Organic Frameworks. Piroxicam was loaded on Zinc and Copper MOFs and tested for its solubility, dissolution and drug release profile. The mechanisms of the release pattern of the drug from Zinc and Copper MOFs were analysed using kinetic models. At the end of the study, Piroxicam-loaded Zn MOFs had solubility of 161.34 ± 0.63 $\mu\text{g/mL}$, while that of Cu MOFs and pure piroxicam had solubility of 154.31 ± 1.27 $\mu\text{g/mL}$ and 134.86 ± 0.48 $\mu\text{g/mL}$ respectively. Within a period of 24 hours, Piroxicam had the highest release from Zn MOFs; with a drug release in the range of 43.70 to 99.10%. Piroxicam was released from the loaded Cu MOFs in the range of 41.20 to 78.3% while the release of pure Piroxicam was in the range of 37.50 to 49.0%. Mechanism of piroxicam release was by Fickian diffusion. Piroxicam was successfully loaded on Zinc and Copper MOFs. Zn MOFs demonstrated a better solubility and dissolution profile as compared to Cu MOFs. These study shows the drug delivery potentials of the Zinc and Copper MOFs in improving the solubility and drug release of piroxicam.

KEYWORDS: piroxicam, solubility, zinc, copper, MOFs, drug release.

INTRODUCTION

The solubility of a drug plays a key role in determining its absorption. Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic properties¹. Piroxicam has long been widely used in the management of inflammation, pains and arthritic conditions, other musculoskeletal and non-rheumatic conditions².

According to the Biopharmaceutics Classification System (BCS) proposed by

Amidon and co-workers³, piroxicam, a BCS Class II drug has a low solubility of about 0.143 mg/mL in aqueous medium⁴. It is characterized by a slow and gradual absorption via the oral route leading to delayed onset of therapeutic effect and thus sub-optimal efficacy in patients. These properties have created a need to modify the solubility and release profile of piroxicam². In recent times, various materials such as liposomes, nano emulsions, nanoparticles, mesoporous silica, silicon, functional hydrogels, or micelles have been used as drug delivery

vehicles⁵. Although these delivery vehicles have posed advantages of providing biocompatibility and the ability to uptake many drugs, limitations of achieving a controlled drug release and a low drug loading capacity exists. It is therefore pertinent to explore advanced techniques in delivering drug particles into the circulation. It is common knowledge that a successful drug delivery requires effectiveness and safety of therapy⁶. Metal-organic frameworks (MOFs) are a new class of highly tenable hybrid materials crafted from metal connecting points and organic bridging ligands⁷. MOFs exhibit many desirable characteristics as drug carriers, including exceptionally high surface areas and large pore sizes for drug encapsulation, intrinsic biodegradability because of relatively labile metal-ligand bonds, and versatile functionality for post synthetic grafting of drug molecules⁸. Over the years, MOFs have been investigated for applications in loading and release of several drug molecules⁷. MOFs can be regarded as optimal drug delivery systems due to the possibility of adjusting the framework's functional groups and tuning of the pore size⁸. Organic materials in the hybrid structure of MOFs provide biocompatibility and capacity of a large amount of drug uptake, while inorganic groups may be optimized for controlled release profiles⁸.

In our earlier study⁹, we synthesised the zinc terephthalate (MOF) and its functionalization by incorporation of amino and cyano groups using ethylenediamine and malononitrile respectively. They were further loaded successfully with piroxicam.

In developing MOFs for drug delivery, the goal is to design carriers that show little toxicity in the body and the biocompatibility of both the metal and bridging ligand must be considered. Generally, more investigations on the

toxicity exhibited reported that the toxicity and *in vivo* study of MOFs should be conducted before clinical translation¹⁰.

In this research, the drug delivery potential of a novel drug delivery system using Zinc and Copper MOFs to modify the solubility and release profile of piroxicam would be explored. The research aims at studying the solubility profile, *in vitro* release profile and kinetics of piroxicam loaded Zinc and Copper Metal-Organic Frameworks.

MATERIALS AND METHODS

Materials

All reagents were purchased from Sigma-Aldrich and were used without further purification.

Methods

Synthesis of Zinc Terephthalate and Copper Terephthalate MOF

Preparation of the MOFs (ZnMOF and CuMOF) was performed as described by Okoro and co-workers¹¹. Zinc acetate dihydrate (0.439 g, 2 mmol) and terephthalic acid (0.166 g, 1 mmol) were dissolved separately in 10 mL of dimethylformamide. Triethylamine (0.3 mL) was added into the Terephthalic acid solution followed by adding zinc salt solution under stirring for 2 h 30 min under ambient temperature. The resulting precipitate was filtered, washed with dimethylformamide and kept overnight to dry. Melting point > 400 °C; the CHN analysis showed ZnOC₁₆H₈O₈ (409.4 g/mol) (found C—46.99%, H—1.78%; calculated C—46.89%, H—1.95%) and the IR (KBr): $\nu_{\text{max}}/\text{cm}^{-1}$ showed: 3605, 3435, 1654, 1600, 663, 545.

Copper terephthalate MOF was prepared by a procedure similar to the synthesis of Zinc terephthalate MOF described above except that Copper acetate monohydrate (0.400 g, 2 mmol) was used instead of Zinc acetate dihydrate.

Melting point > 350 °C; the CHN analysis showed CuOC₁₆H₈O₈ (410.5 g/mol) (found

C—46.90%, H—1.90%; calculated C—46.89%, H—1.95%) and the IR (KBr): $\nu_{\max}/\text{cm}^{-1}$ showed: 3609, 3438, 1645, 1606, 657, 540, 446.

Loading of piroxicam

The piroxicam loading was performed by introducing 100 mg of the dehydrated zinc terephthalate MOF and copper terephthalate MOF (dried at 150 °C in an oven for 5 h) in a 10 mL solution of ethanol containing

300 mg of piroxicam under stirring for 3 days. After drug immobilization, the MOF with the adsorbed drug was separated by centrifugation at 4000 rpm for 20 min and dried under vacuum at 70 °C. Piroxicam was also loaded on the functionalized MOF materials by introducing 100 mg of the zinc terephthalate–EN and zinc terephthalate–MN in 10 mL solution of ethanol containing 300 mg of the drug, respectively, under stirring for 3 days. Subsequently, the suspensions were centrifuged at 4000 rpm for 20 min, and the resultant solids were characterized by

Powder X-ray diffraction (PXRD), SEM and FTIR analysis⁹.

Solubility studies

The solubility test was performed according to the equilibrium solubility method described by Higuchi and Connors¹², 5 mg of pure piroxicam was weighed into 100 mL screw capped conical flasks and mixed with 50 mL of phosphate buffer at pH 7.4. The conical flasks were placed in a thermostatically controlled water bath shaker (LabTech, England) at 37 °C and at a speed of 100 rpm. After 24 hours, the solutions were filtered through 0.45 μm membrane filter and samples were analysed for piroxicam concentration at 334 nm using a UV-double beam spectrophotometer (General Scientific, USA). The piroxicam concentration was extrapolated from already constructed standard piroxicam calibration plot (125 to 300 $\mu\text{g/mL}$ concentration range) in Figure 1. The tests were performed in triplicates and the procedure was repeated using piroxicam loaded Zinc and Copper MOFs (containing 5 mg equivalent piroxicam).

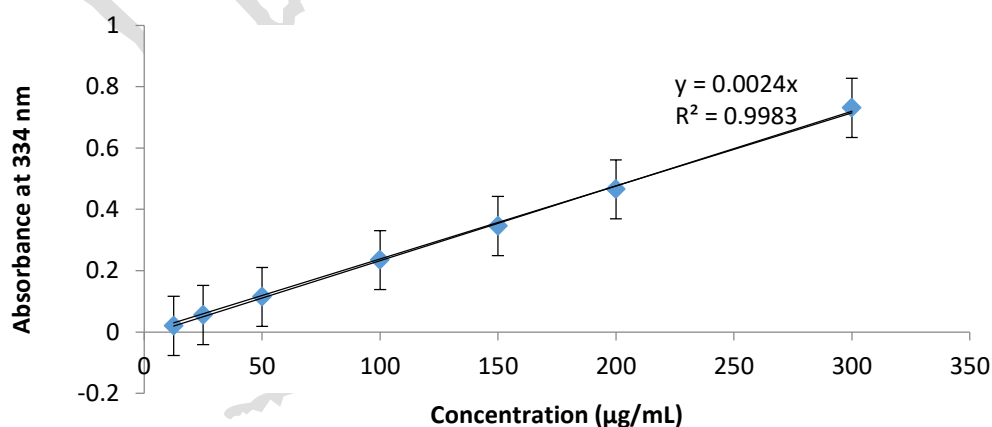


Figure 1: Calibration Curve of Piroxicam in pH 7.4 phosphate buffer

Dissolution and drug release studies

A calibration curve was obtained using pure piroxicam as reference standard. The powder was dissolved in buffer solution (pH 7.4) within a range of concentration of

12.50 and 300.00 $\mu\text{g/mL}$. The correlation was linear ($r^2 = 0.9986$) with a slope of 0.0024. The concentrations of piroxicam were derived from this calibration curve. The dissolution profile of piroxicam was

studied using USP dissolution method; USP apparatus II dissolution test instrument-paddle method (DBK, India), 20 mg of pure piroxicam was re-dispersed in 20 mL of distilled water and placed in a dialysis tubing (Carolina™, USA) of diameter 1" and exclusion size 12000 to 14000 Da; with both ends tied and suspended in 900 mL of phosphate buffer pH 7.4 and maintained at a temperature of 37 ± 0.5 °C. Paddle rotation speed was maintained at 50 rpm. At time intervals, 0.2, 0.3, 0.5, 1, 2, 4, 6 and 24 h, 5 mL aliquots were withdrawn and replaced with 5 mL phosphate buffer to maintain sink condition. The withdrawn samples were filtered through 0.45 µm membrane filter and piroxicam concentration was quantified at 334 nm using UV-double beam spectrophotometer against the dissolution medium as the blank. The same procedure was repeated using piroxicam loaded Zinc and Copper MOFs (containing 20 mg equivalent piroxicam). The procedure was carried out in triplicates. Percentage drug release was calculated using the equation obtained from the calibration curve of piroxicam standard prepared under the same experimental conditions.

Drug release kinetics

The mechanisms of the release pattern of piroxicam from Zinc and Copper MOFs were analysed by kinetic (mathematical) models. Drug release data of *in vitro* dissolution study was fitted into various kinetic models including zero-order, first-order, Higuchi and Korsemeyer-Peppas (first 60% drug release)¹³. Zero order (Eq. 1) describes the system where the release rate of drug is independent of its concentration¹⁴. The cumulative % drug release against time plot was made.

$$C = C_0 - K_0 t \dots\dots\dots (1)$$

Where, C is the amount of drug released or dissolved (if release occurs rapidly after the drug dissolved), C_0 is the initial amount of drug in solution (it is usually

zero), K_0 is zero order rate constant and t is time.

First order (Eq. 2) describes the release from the system where the release rate of drug is dependent on the concentration of the drug¹⁵. The log cumulative % drug release vs. time plot was made.

$$\log C = \log C_0 - Kt/2.303 \dots\dots\dots (2)$$

Where, C_0 is initial concentration of drug, K is first order constant and t is time.

Higuchi (Eq. 3)¹⁶ described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion as presented in equation (3). The cumulative % drug release vs. square root of time plot was made.

$$Q = K_H \times t^{1/2} \dots\dots\dots (3)$$

Where Q is the cumulative amount of drug released and K_H is the Higuchi dissolution constant.

Korsemeyer-Peppas and co-workers (Eq. 4)¹⁷ derived a simple relationship which describes the release of drug from a polymeric system: equation (4). First 60% of drug release data is fitted in Korsemeyer-Peppas model to illustrate the mechanism of drug release:

$$C_t/C_\infty = kt^n \dots\dots\dots (4)$$

Where, C_t/C_∞ is the fraction of drug released at time t, k is the rate constant; and n is the release exponent, whose value is used to determine different mechanisms of release. The log cumulative % drug release vs. log time plot was made.

Correlation coefficient (r) values were calculated for the linear curves obtained by regression analysis of the above plots and the degree of fitness into the mathematical models was used to evaluate the mechanism of drug release.

Calculation of similarity fit factor (f_2)

The similarity factor was calculated and used to compare the dissolution profiles the test and reference which were the drug-MOFs conjugates and reference piroxicam respectively. The f_2 is defined by the formula (Eq. 5) proposed by Moore and Flanner¹⁸,

$$f_2 = 50 \log \left\{ \left(1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right)^{-0.5} \times 100 \right\} \dots (5)$$

Where, T_t and R_t are the percentage drug dissolved of the test products and reference respectively at each sample time, t and n represents the total number of dissolution sample times.

While the values of f_2 are between 0 and 100, a value greater than 50 indicates similarity between the test and reference formulate and a value of 100 indicates they are identical. Generally, the dissimilarity between release profiles increases as the f_2 value becomes smaller¹⁹.

Statistical analysis

Numeric data were presented as mean $\pm$ standard deviation. Unpaired t -test was used to test statistically significant difference between the solubility and drug

release of the loaded MOF's and control. All statistical analyses were performed with GraphPad Prism V5.0 software (GraphPad, CA, USA). Statistical significance level was set at $p < 0.05$

RESULTS

Solubility studies

Solubility test on pure piroxicam (control), piroxicam loaded Zn and Cu MOFs carried out using phosphate buffer showed that piroxicam loaded Zn MOFs had the highest solubility; $161.34 \pm 0.63 \mu\text{g/mL}$, followed by piroxicam loaded Cu MOFs; $154.31 \pm 1.27 \mu\text{g/mL}$, and pure piroxicam (control) had the lowest solubility; $134.86 \pm 0.48 \mu\text{g/mL}$ as shown in Table 1.

Table 1: Solubility results after 24 hours for pure piroxicam control, piroxicam loaded Zn MOFs and piroxicam loaded Cu MOFs in pH 7.4 phosphate buffer

Type of sample	Solubility ($\mu\text{g/mL}$) after 24 hours (n = 3)
Piroxicam control	134.86 ± 0.48
Piroxicam loaded Zn MOFs	161.39 ± 0.64
Piroxicam loaded Cu MOFs	154.31 ± 1.27

Dissolution and drug release studies

The release of piroxicam at physiological conditions (pH 7.4) from Zn MOFs was in the range of 43.7 ± 1.08 to $99.1 \pm 1.11\%$ while the release of piroxicam from Cu MOFs was in the range of 41.2 ± 0.00 to

$78.3 \pm 1.09\%$, which were both higher than the release from piroxicam control which was in the range of 37.5 ± 0.00 to $49.0 \pm 2.20\%$ at the end of 24 h as shown in Figure 2.

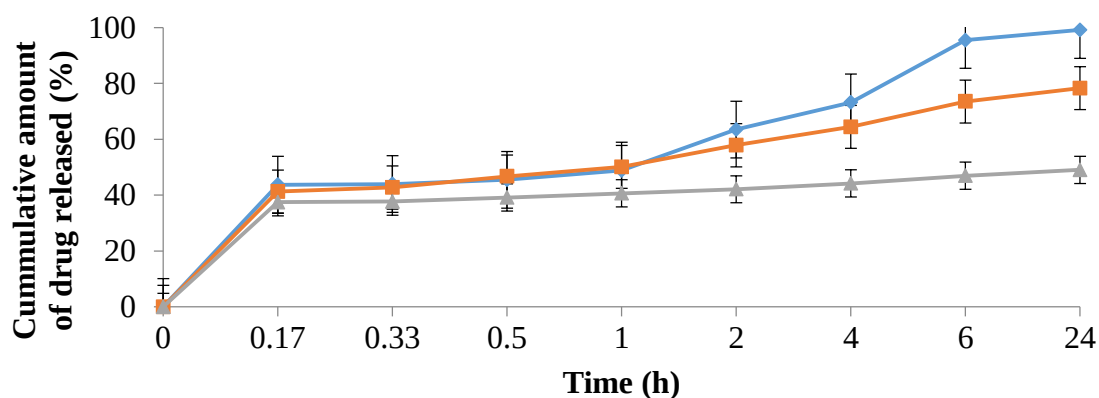


Figure 2: Release profile of pure piroxicam and piroxicam from Zn and Cu MOFs. Each data point represents mean $\pm$ SD (n = 3).

Legend:

Abbreviations

Pxm-control 
 Pxm-Zn-MOF 
 Pxm-Cu-MOF 

Meaning

Pure piroxicam as control
 Piroxicam loaded-Zn Metal Organic Framework
 Piroxicam loaded-Cu Metal Organic Framework

Drug release kinetics. Table 2 shows the coefficient of regression (r) values of different kinetic models.

Table 2: Regression coefficient (r) values of different kinetic models for the release of piroxicam from Zn and Cu MOFs

Formulation	Zero order R^2	First order R^2	Higuchi R^2	Korsemeyer-Peppas R^2	n
Piroxicam control	0.4835	0.6436	0.8729	0.9738	0.05
Piroxicam loaded Zn MOFs	0.6303	0.5794	0.8260	0.8639	0.06
Piroxicam loaded Cu MOFs	0.6266	0.5692	0.8405	0.9623	0.10

Similarity factor

The similarity factor result is as shown in Table 3.

Table 3: Similarity factor parameters of piroxicam loaded MOFs and pure piroxicam

Formulation	Similarity factor f_2
Piroxicam and piroxicam loaded Zn MOF	27.49
Piroxicam and piroxicam loaded Cu MOF	38.01

DISCUSSION

Piroxicam is poorly soluble in water and hence remains floating on the surface of the dissolution medium due to poor contact with the medium resulting in poor dissolution profile. From Figure 2, a rapid release of piroxicam at 10 minutes was observed in both pure and MOFs formulation. However, pure piroxicam maintained slightly the same release pattern beyond 10 minutes since the pure piroxicam was not subjected to release modification. This was similarly reported by Uppuluri and Neela² where a rapid release of piroxicam was observed at initial stages. In a controlled release drug

delivery system, it is often preferred to achieve an initial burst of drug release to obtain the threshold dose that elicits therapeutic action². After the initial rapid release, piroxicam loaded Zn and piroxicam loaded Cu MOFs showed a gradual increase in release and the maximum drug was released in 24 hours. This is consistent with the research carried out by Uppuluri and Neela². The *in vitro* release behaviour of piroxicam from Cu and Zn MOFs showed about 1.5 and 2-fold increase in dissolution respectively. As shown in the solubility test result, piroxicam loaded Zn MOFs had a better

dissolution profile than piroxicam loaded Cu MOFs.

In vitro dissolution profiles of drugs are compared using kinetic or mathematical models. Drugs are released from delivery systems by desorption, matrix diffusion, polymer wall diffusion, matrix erosion, or a combined erosion-diffusion process¹⁹. As shown in Table 2, pure piroxicam (control), piroxicam loaded Zn MOFs and piroxicam loaded Cu MOFs were found to best fit Korsemeyer-Peppas (first 60 % release) with R^2 values of 0.9738, 0.8639 and 0.9623 respectively and n values of 0.05, 0.06 and 0.1 respectively; but did not fit into zero order, first order and Higuchi model. In Korsemeyer-Peppas model, the value of n characterizes the release mechanism of drug. All the formulations had n values < 0.43 , suggesting Fickian diffusion as the mechanism of release of piroxicam from the MOFs within $< 60\%$ dissolution rate.

The drug release profiles of pure piroxicam (control) with piroxicam loaded Zn MOFs (test) and piroxicam loaded Cu MOFs (test) was compared using the f_2 similarity factor. The f_2 value obtained for pure piroxicam (control) and piroxicam loaded Zn MOF was 27.49 while the f_2 value obtained for pure piroxicam (control) and piroxicam loaded Cu MOF was 38.01 as shown in Table 3. The values are lower than 50, which is the f_2 limit value. This suggests a dissimilarity in the release profiles of Zn and Cu MOFs compared to the control. This implies that the *in vitro* release profile of piroxicam loaded Zn MOFs and piroxicam loaded Cu MOFs were not identical with the control (pure piroxicam) as both piroxicam loaded Zn MOFs and piroxicam loaded Cu MOFs had a better dissolution profile than pure piroxicam (control).

These results demonstrate the potentials of Zn and Cu MOFs as drug delivery systems to improve the solubility and dissolution rate of poorly soluble piroxicam. The solubility of piroxicam loaded Zn MOFs

and piroxicam loaded Cu MOFs was higher than the solubility of pure piroxicam (control). The release of piroxicam from Zn and Cu MOFs respectively was also higher than that of pure piroxicam control which showed better dissolution profiles. Fickian diffusion was deduced as the principal mechanism of drug release of piroxicam from both Zn and Cu MOFs. The solubility and dissolution profile of piroxicam was improved using Zn and Cu MOFs as drug delivery devices; with Zn MOFs demonstrating a better release profile than Cu MOFs. The drug loaded metallic MOFs demonstrate great potential in overcoming the limitations and drawbacks of drug release from poorly soluble drugs to achieve good therapeutic efficacy.

CONCLUSION

This study investigated the solubility and drug release profile of piroxicam from Zn and Cu MOFs. The solubility of piroxicam loaded Zn MOFs and piroxicam loaded Cu MOFs was higher than the solubility of pure piroxicam (control). The release of piroxicam from Zn and Cu MOFs respectively was also higher than that of pure piroxicam control which showed better dissolution profile. Fickian diffusion was deduced as the principal mechanism of drug release of piroxicam from both Zn and Cu MOFs. The solubility and dissolution profile of piroxicam was improved using Zn and Cu MOFs as drug delivery devices; with Zn MOFs demonstrating a better release profile than Cu MOFs. The drug loaded metallic MOFs demonstrate great potential in overcoming the limitations and drawbacks of drug release from poorly soluble drugs to achieve good therapeutic efficacy.

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