International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR)
ISSN: 2582-7618 (Online), Volume-4 Issue-3, April 2024

Controlled Release of Doxorubicin, a Chemotherapy Medication using Papaya Enzyme 'Papain' Immobilized on ZIF-8

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Abstract: ***An enzyme-mediated controlled drug delivery system is envisioned using a metal-organic framework (MOF). The pH-dependent affinity of the enzyme towards the drug Doxorubicin is investigated. Doxorubicin is a chemotherapy medication for cancer treatment. Papain, a papaya enzyme belonging to the cysteine family, was chosen as the drug carrier for biomineralisation. Zeolitic imidazolate frameworks (ZIFs) are synthesised as the matrix for enzyme immobilisation. An encapsulation strategy was used for the immobilisation of both the enzyme and the drug into the carrier support. Encapsulation is envisioned as a one-pot synthesis. Both the systems DOX@ZIF-8 and DOX@Pap@ZIF-8 were found to be pH-sensitive for drug release. The DOX molecules are safely stored in the carrier matrix. Essentially no release of DOX was observed under physiological conditions. A papain-mediated drug delivery system is expected to be more specific and compelling in administering drugs at the right time and in the correct dose anywhere in the body than DOX@ZIF-8.***

Keywords: ***Controlled Drug Release, Metal Organic Framework, Doxorubicin, ZIF-8, Papaya Enzyme, Papain, Enzyme Immobilization and Biomineralization.***

I. INTRODUCTION

Drug delivery refers to the approaches, formulations, technologies, and administration of medication in the body, ensuring the correct dose is administered at the right time to achieve the desired therapeutic effect. Drug release rates have implications for the therapeutic effects of DDS. Delivering drugs at a controlled rate, a sustainable rate, and targeted delivery are beautiful methods that are followed enthusiastically. Controlled drug delivery systems and sustainable drug delivery systems are two well-established strategies for administering medication to patients. In controlled release, the drug releases over time irrespective of concentration, whereas in sustained release, the drug releases slowly over time. As the name suggests, targeted drug delivery is designed to concentrate the medication at, site-specific locations within subcellular organelles [1].

Manuscript received on 11 March 2024 | Revised Manuscript received on 27 March 2024 | Manuscript Accepted on 15 April 2024 | Manuscript published on 30 April 2024.

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It is challenging in a complex cellular network of an organism to administer medication at the correct dose at the right time. Targeted delivery of drugs enables the drug molecule to reach its desired site more efficiently by reducing the dose and minimising side effects [2]. This inherent advantage makes the targeted drug delivery system highly preferred and facilitates the field of pharmaceuticals [3]. Accurate targeting of the drug to the cells or tissue of choice is a fundamental aspect of drug delivery. Drug targeting systems control the fate of a drug entering the body.

For over two decades, the delivery of bioactive agents from polymeric materials, particularly aliphatic polyesters based on lactic and glycolic acids, has garnered considerable attention from researchers across the scientific community. Nowadays, enzyme-mediated drug delivery systems are gaining increasing attention. To recover and recycle the enzyme carrier, various strategies for enzyme immobilisation are available, including covalent binding, crosslinking, adsorption, encapsulation, and entrapment. Biomimetic mineralisation is one of the most widely adopted methods for encapsulating bioactive molecules within protective exteriors. Metal-organic frameworks (MOFs) have great potential [6] for constructing immobilised enzymes with higher loading and catalytic activity, facilitating the fabrication and application of novel biocomposites.

In this work, we aim to utilise enzyme-mediated drug delivery. Zeolitic [11] imidazolate frameworks, ZIFs —a class of metal-organic frameworks topologically isomorphic with zeolites — are used as the support for immobilisation. ZIFs are composed of tetrahedrally-coordinated transition metal ions like Fe, Co, Cu and Zn connected by imidazolate linkers. Papain, a papaya enzyme belonging to the cysteine family, is selected as the enzyme mediator [7]. There are so many reports on papain-mediated controlled drug release. Doxorubicin, DOX, a chemotherapy medication for cancer treatment, is chosen as the drug. Investigations on the pH dependency of DOX release were carried out using encapsulated papain and mere ZIF-8, and a comparison is made on the role of papain in drug release [8] [9].

II. EXPERIMENTAL

A. Materials

$Zn(NO_3)_2 \cdot 6H_2O$ (99.0%), 2-methylimidazole (2-MeIm, 99.0%), and papain from Carica papaya were purchased from Sigma-Aldrich, India. DOX and *p*-nitrophenyl acetate were obtained from Merck, Mumbai. All other chemicals were purchased from local suppliers and purified according to the standard procedures

reported in the literature before application.

B. Instrumentation

Spectral analysis was performed using a Shimadzu FT-IR-8400S spectrophotometer. The thermal stability of the samples was assessed using a Shimadzu Simultaneous DTA-TG. Scanning electron microscopic studies were carried out using JEOL-JSM840A. Kinetic studies were carried out using a Shimadzu UV-2450 spectrophotometer.

C. Synthesis of Papain-MOF Composite Pap@ZIF-8

In a typical reaction, papain (50 mg) was added into a solution of 2-methylimidazole (1.25 M, 40 mL) in deionised water. A separate solution of $Zn(NO_3)_2 \cdot 6H_2O$ (0.31 M, 4 mL) was also prepared. These two solutions were combined and then agitated at 600 rpm for 30 min at room temperature. The precipitate was collected by centrifugal separation and washed at least three times with a mixture of ethanol and H_2O. The resulting papain-loaded ZIF-8, designated as Pap@ZIF-8, was utilised for drug release studies. Pure ZIF-8, the MOF framework without the enzyme, was also prepared for comparative research. The synthesis of pure ZIF-8 followed the same procedure as the preparation of papain-loaded ZIF-8, but in the absence of the enzyme solution [5].

D. Synthesis of DOX@ZIF-8

Stock solutions of the drug were prepared in deionised water by varying the concentration of DOX to 2, 6, and 10 mg/mL. A slightly alkaline solution of zinc nitrate was prepared by dissolving Zn(NO3)2·6H2O (0.2 g, 0.66 mmol) in deionised water (0.8 mL). The pH of the solution was adjusted to 8 by adding a saturated solution of NaOH. Then, the DOX stock solution (4 mL) was added to the zinc nitrate solution and stirred for a minute. After stirring, a solution of 2-methylimidazole (2 g, 4.62 mmol) prepared in deionised water (8 mL) was added dropwise. The reaction mixture was stirred for an additional 15 minutes. The precipitate was collected by centrifugal separation and washed several times with a mixture of ethanol and water. The product was obtained in a powder form and dried at room temperature under vacuum. The DOX loading was adjusted by varying the concentration of DOX in the stock solution. DOX-free ZIF-8 was also synthesized similarly for comparison, using deionised water (4 mL).

E. DOX loading on Pap@ZIF-8

The drug, DOX (10 mg), was dissolved in distilled water-ethanol mixture (50 mL) and added into **Pap@ZIF-8** sample (50 mg). The mixture was then shaken well for five hours in a water bath shaker. After that, the mixture was centrifuged at 100 rpm for 5 minutes to remove any unloaded DOX. The remaining DOX in supernatant solution was measured spectrophotometrically at 479 nm.

F. Kinetics of DOX loading on Pap@ZIF-8

The drug loading experiments were performed in 100 mL conical flasks, and the contents were shaken at a pre-set temperature. The absorbance of the drug solution, Ao, was measured before the addition of the support. The aqueous samples were withdrawn at preset time intervals from the reaction mixture, and their absorbances were measured using a spectrophotometer.

G. Kinetics of controlled drug release from DOX@Pap@ZIF-8 at different pHs

The DOX release profile from Pap@ZIF-8 was measured at a pH value of 7.4. In a typical experiment, the sample DOX@Pap@ZIF-8 (1 mg) was added to a PBS buffer solution (5 mL, 0.01 M) and shaken well to form the reaction mixture for 10 h. At specific time intervals, 1 mL aliquots of the sample were withdrawn, and after suitable dilution, the concentration of the released drug was measured using a UV spectrophotometer at 479 nm.

H. Release of DOX from DOX@ZIF-8 particles at different pHs

A typical release system was prepared by suspending a DOX@ZIF-8 sample (10 mg) in buffer solutions (20 mL, pH 7.4, 6.5, 6.0, 5.0, and 4.0, respectively) at room temperature. The release system was then maintained at room temperature under shaking at 150 rpm. One millilitre of release medium was sampled at each time point, and UV/V is spectrophotometry was used to determine the percentage of DOX that had been released. Afterwards, the sample was returned to the original release system.

I. Stepped release experiment

In a stepped release experiment, the DOX@ZIF-8 sample (10 mg) was tested in a buffer solution of PBS (10% (v/v), 20 mL, pH 7.4) at room temperature for 7 days. The pH of the solution was then adjusted to 6.5 with dilute HCl (0.6 M) and left for an additional 7 days. The pH of the solution was adjusted stepwise over 3 days to 6.0, 5.0, and 4.0 with dilute HCl (0.6 M). The amount of DOX loaded was determined from the UV/Vis absorbance at 479 nm.

The release percentages of DOX were calculated according to the formula.

$$\textbf{Release \% of DOX} = \frac{\textbf{Amount of released DOX}}{\textbf{Total amount of loaded DOX}}$$

J. Regeneration and reusability of Pap@ZIF-8

The regeneration and reusability of the samples were investigated. After the completion of the drug release, the spent Pap@ZIF-8 was filtered, washed several times (5 × 10 mL) with distilled water and treated with PBS buffer (5 × 10 mL, pH 4.5). Then, the procedure was repeated, reloading, drying, and reusing for another cycle of the experiment.

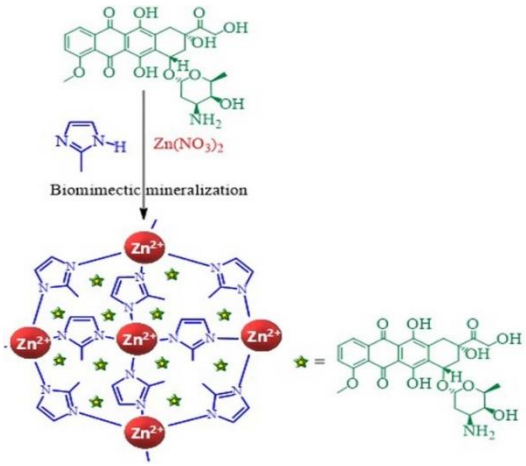
III. RESULTS AND DISCUSSIONS

A. One-Pot Synthesis of DOX Encapsulated in ZIF Crystals-DOX@ZIF-8

The potential applications of MOFs have been utilised in the synthesis of drug delivery systems. MOFs are highly tunable hybrid materials composed of metal coordination points and organic bridging ligands. They are typically synthesized under mild conditions via coordination-directed self-assembly processes. The large porosity, with tunable pore sizes, shapes, and functionalities, of MOFs is responsible for their application in loading and releasing multiple drug molecules. MOFs can be effectively synthesised through a one-

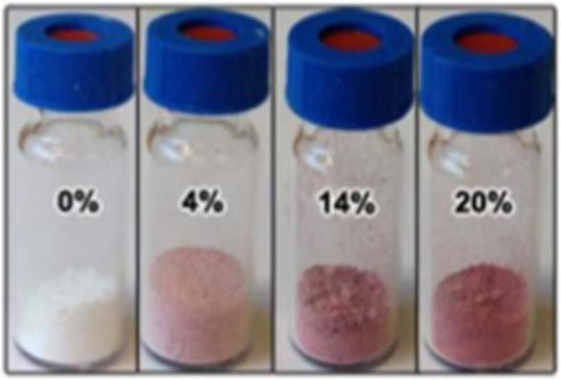
pot synthesis process. Initially, metal ions and target molecules self-assemble to form coordination polymers [4]. Then the organic linker is added to disassemble the metal ions from the target molecules. Subsequently, MOFs are formed by the assembly of the metal ions and linkers. The synthesis of **DOX@ZIF-8** molecules is depicted in **Fig. 1**.

We chose DOX, a typical anti-cancer drug used in the treatment of breast and ovarian cancers, as the target molecule. DOX molecules have functional groups that form weak coordination bonds with Zn^{2+} ions in aqueous media. Initially, Zn(II) ions form a coordination complex with the target DOX molecules; then the organic ligand 2-methylimidazole affords **DOX@ZIF-8** frameworks. The target DOX molecules are essentially encapsulated during the formation of MOFs, resulting in hierarchical MOFs. The high surface area, high surface activity, and exceptional chemical and thermal stability make ZIF-8 frameworks an attractive candidate for drug delivery.



[Fig.1: Synthesis of Papain Encapsulated ZIF-8]

DOX@ZIF-8 particles were synthesized in pure aqueous solutions. In a typical synthesis, a solution of zinc nitrate and stock solution of DOX was mixed well by stirring. Then, a solution of the organic ligand, 2-methylimidazole, was added dropwise while stirring. The reaction mixture was then placed in a water bath shaker to ensure uniform mixing. DOX@ZIF-8 particles formed were collected by centrifugal separation. Washed several times with ethanol-water mixture, filtered and dried under vacuum. The DOX loading was estimated spectroscopically, with loadings of up to 20% achieved (Fig. **2**).



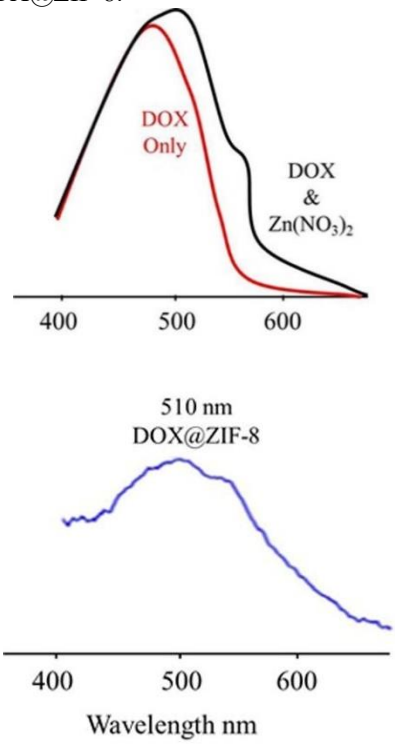
[Fig.2: DOX@ZIF-8 Particles with 0, 4, 14 and 20% DOX Loadings]

B. Characterization of DOX@ZIF-8

The synthesised DOX-encapsulated ZIF was subjected to spectral and morphological analysis. Morphological features were assessed by SEM analysis. The encapsulation of DOX within the ZIF frameworks was confirmed by spectroscopy.

i. Confirmation of synthesis of DOX@ZIF-8

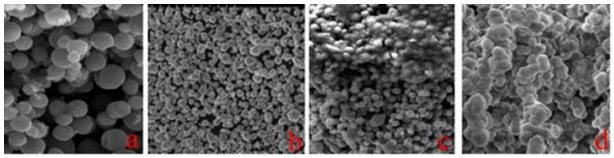
The absorbance characterises pure doxorubicin at 480 nm. During the formation of DOX@ZIF-8, DOX is thought to be coordinated with Zn(II) ions. Hence, a red shift in absorbance is expected. The UV-Vis spectra of a pure DOX solution, a mixed solution of DOX with Zn(NO3)2, and the solid-state UV/Vis spectra of DOX@ZIF-8 were taken. From the spectral analysis, it is clear that; doxorubicin shows a characteristic absorbance at 480 nm and the red shift in absorbance to 487 nm due to its co-ordination with Zn^{2+} ions (**Fig. 3**). The solid-state UV/Vis spectrum of DOX@ZIF-8 showed a characteristic absorbance at 510 nm and indicates that there is no coordination bond between DOX and Zn^{2+} ions in DOX@ZIF-8.



[Fig.3: The UV/Vis Spectra of DOX Solution, Mixed Solution of DOX and Zinc Nitrate and Solid-State UV/Vis Spectra of DOX@ZIF-8]

ii. Scanning electron microscopy

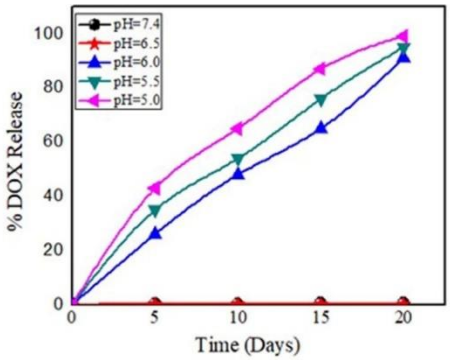
Scanning electron microscopy helps assess the morphological features of samples. SEM analysis of the samples having 0-20% DOX loading was carried out to determine the difference in their morphologies. The SEM photographs showed that the DOX@ZIF-8 materials consisted of isolated crack-free particles of diameter 70-300 nm (**Fig. 4**). As the concentration of DOX molecules increases, the surface becomes more and more rough.



[Fig.4: DOX@ZIF-8 Particles with 0, 4, 14 and 20% DOX Loadings]

C. pH-responsive release of DOX from DOX@ZIF-8 particles

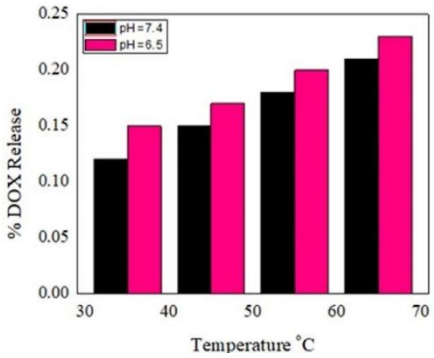
The kinetics of the release of encapsulated DOX molecules from the drug delivery system DOX@ZIF-8 was investigated in detail. The drug release was conducted at different pH levels ranging from 5.0 to 7.4. Phosphate-buffered saline (PBS) of varying concentrations was used to maintain the desired pH level. Our blood pH 7.4 is taken as the limiting value. The pH-responsive cumulative release of DOX was investigated using DOX@ZIF-8 with a 20% DOX loading as the model system. **Figure 5** shows the pH-responsive cumulative release of DOX. At specific time intervals, 1 mL aliquots of the sample were withdrawn, and after suitable dilution, the concentration of the released drug was measured using a UV spectrophotometer at 479 nm. Kinetic studies were continued for 2-3 weeks. At room temperature, at a pH equal or higher than 6.5 in PBS, no release of DOX from DOX@ZIF-8 was observed even after 15 days. At lower pH, in the range of 5.0-6.0, a steady release rate was noticed. An incubation period was observed during the initial stage, characterised by a poor release of loaded DOX. More than 75% of the loaded DOX was found to be released during the initial 9-10 days. The dissolution of ZIF-8 in the PBS buffer is expected to be the reason for the release of drugs from DOX@ZIF-8 at low pH. The dissolution of the peripheral DOX-free shells of ZIF-8, which act as a protective capsule around the DOX@ZIF-8, in the PBS is responsible for the induction period.



[Fig.5: The Typical Release System]

D. Storage stability of DOX@ZIF-8 particles

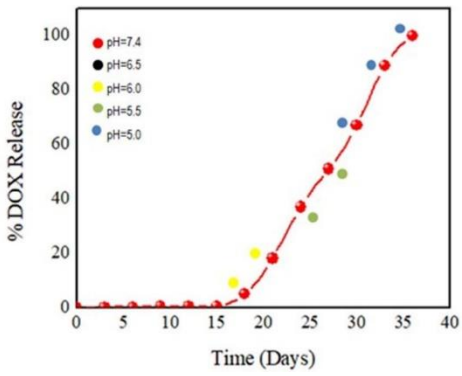
Storage stability and lifetime are the most challenging properties of enzymes [10] and conventional drug delivery systems. We investigated the storage stability of DOX molecules within the framework of ZIF-8. The DOX@ZIF-8 sample was incubated at temperatures ranging from 30 to 70 °C and assessed for the kinetics of DOX release as described above. Two different pH levels, viz. 6.5 and 7.4 are selected for this storage stability assessment. DOX@ZIF-8 system is found to be stable for several days. No DOX is released is noticed even at 60 °C during 7 days in PBS at pH 7.4 and 6.5 (**Fig. 6**). From the graph, it is clear that, even after several days of storage at higher temperatures, less than 0.25% of the drug is released. Hence, one of the most essential advantages of the designed DOX@ZIF-8 system is that the DOX molecules are safely stored with essentially no release under physiological conditions. However, more than 75% of DOX was released under physiological conditions during 9-10 days. The unique release property of DOX@ZIF-8 makes it an interesting potential pH-responsive drug delivery system for cancer therapy.



[Fig.6: Effect of Temperature on % DOX Release]

E. Study on the cumulated release of DOX molecules

Cumulated drug release studies were carried out in the pH range of 5.0 to 7.4. The cumulative release of DOX molecules was found to be very low, or even less than 1%, as the pH was lowered from 7.4 to 6.5. A sharp increase in drug release was observed upon lowering the pH value to 6.0, 5.5, and finally to 5.0 (**Fig. 7**).



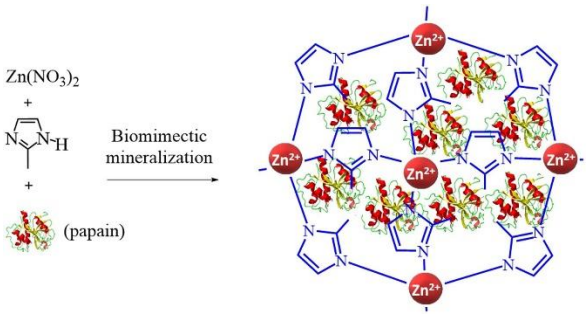
[Fig.7: The Stepped Release System]

The graph provides firm confirmation that DOX@ZIF-8 is a promising pH-responsive drug delivery system. The free DOX molecules completely dissolved within less than an hour at room temperature in PBS, with a pH range of 5.0-7.4. Indeed, the release of the DOX molecules encapsulated in ZIF-8 is responsible for the slow-release rate of DOX molecules. However, in the acidic medium of pH = 5.0, release of zinc ions is expected as a result of the dissolution of the DOX@ZIF-8 particles. About 80% of the zinc ions have been released after 11 days. Thus, the release of drug molecules is

undeniably triggered by pH, through breakage of the coordination bonds between zinc and imidazolate.

F. Synthesis of Pap@ZIF-8

In natural processes, biomineralisation is attributed to the specific ability of amino acids, peptide fragments, and more complex biological entities to concentrate inorganic cations, such as Ca2+ and Zn2+, to seed biominerals. Furthermore, biomimetic mineralisation involves the combination of organic and inorganic substances with distinct thermal properties. The biomimetic mineralisation approach is most widely used to embed papain in zeolite imidazolate framework-8 at room temperature. In this synthetic process, zinc nitrate, 2-methylimidazole, and papain were ground in trace amounts of ethanol at room temperature for 10 minutes to prepare a papain-embedded porous nanomaterial, ZIF-8. The synthetic process is depicted in Fig. 8.

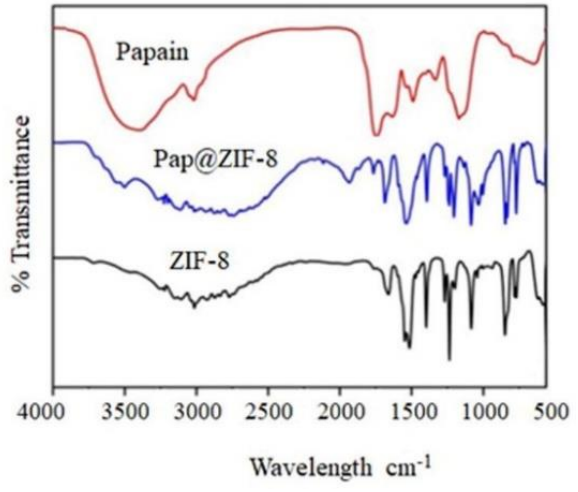


[Fig.8: Synthesis of Papain Encapsulated ZIF-8]

G. Characterization of Pap@ZIF-8

i. FTIR Spectral Analysis

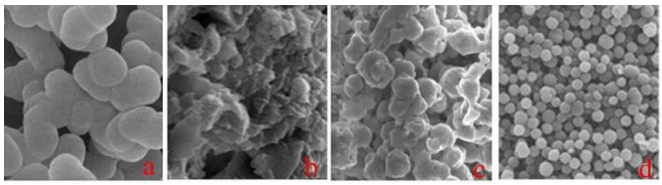
FTIR spectral analysis was carried out to characterise the binding interactions between the enzyme, organic ligand and metal ions. The zeolite imidazolate framework, me-ZIF-8, exhibits characteristics at 1577 and 421 cm-1, even in the absence of enzyme or drug molecules. The characteristic peaks of papain are observed at 3441 and 1658 cm^{-1}. Pap@ZIF-8 exhibits the distinct components of both me-ZIF-8 and papain, indicating the presence of these two components in the composite Pap@ZIF-8 (**Fig. 9**).



[Fig.9: FTIR Spectral Analysis of free and Papain-Loaded ZIF-8]

ii. SEM analysis

SEM analysis of $Zn(NO_3)_2$, ZIF-8, Pap@ZIF-8 before treatment with distilled water and Pap@ZIF-8 after treatment with distilled water were conducted to ensure the difference in morphological features. Fig. 10 visualizes the microscopic architecture and morphology of ZIF-8 with and without papain. An irregular and aggregate morphology was observed for ZIF-8 nanoparticles compared to Zn(NO3)2. In the presence of papain, the morphology appeared irregular. The excessive papain molecules could be observed around Pap@ZIF-8 before washing with distilled water. The surface of Pap@ZIF-8 became rougher after washing with distilled water. This can be explained by the removal of excessive papain adsorbed onto the support.



[Fig.10: The SEM Images of (a) Zn(NO3)2, (b) ZIF-8, (c) Pap@ZIF-8 Before Treatment with Distilled water &(d) Pap@ZIF-8 after Treatment with Distilled Water]

iii. Estimation of enzyme loading capacity

The enzyme loading capacity of the framework is a key parameter that determines the efficiency of the encapsulated system. The enzyme loading capacity of the ZIF-8 matrix was estimated spectroscopically at 280 nm as per the equation:

$$\text{Enzyme loading } \% = \frac{[A - B]}{A} \times 100$$

Where A and B were the absorbance shown by papain at 280 nm for the initial solution and washing solution, respectively.

The papain loading was found to be 35%.

iv. Estimation of catalytic efficiency of the loaded enzyme

Using *p*-NPA as the substrate, the enzyme loading in ZIF-8 was calculated based on its catalytic activity. The hydrolysis of *p*-nitrophenyl acetate was carried out using both free and immobilised papain in a PBS buffer of pH 7.5. The absorbance of released *p*-nitrophenol was monitored spectrophotometrically at 410 nm. In the present work, the enzyme loading was calculated to be 72 mg/g Pap@ZIF-8.

H. DOX Loading on Pap@ZIF-8 Support

Enzyme-mediated drug delivery systems are more compatible with physiological conditions. The DOX loading experiments were carried out in ethanol-water mixture. Initially, the DOX molecules were dissolved in ethanol-water mixture and then added to the entrapped matrix of Pap@ZIF. The reaction mixture was then shaken well for five hours in a water bath shaker. After that, the unloaded DOX from the reaction mixture was removed by centrifugation at 100 rpm for 5 min. The remaining DOX molecules in the supernatant solution were measured spectrophotometrically at 479 nm.

The DOX loading content was calculated using the following formulas:

$$\textbf{DOX loading content}\ (\%) = \frac{\textbf{Weight of DOX in Pap@ZIF}}{\textbf{Weight of Pap@ZIF}} \times 100$$

The DOX loading experiment was carried out using 50 mg weight of Pap@ZIF samples. The amount of DOX attached was weighed as 8.75 mg. Consequently, the DOX loading content was evaluated to be 18% per mg of the Pap@ZIF-8.

I. Kinetics of DOX loading

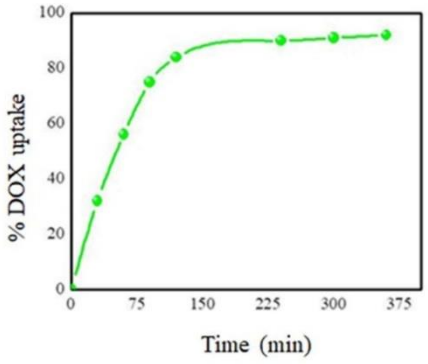
The drug loading experiments were conducted at a preset temperature. The initial absorbance (Ao) of the drug solution was measured before the addition of the carrier support, Pap@ZIF-8. The reaction mixture was shaken gently in a water bath shaker to ensure uniform mixing. The aqueous samples were withdrawn at definite time intervals from the reaction mixture, followed by measurement of the absorbances using a spectrophotometer. The percentage decrease in DOX in solution was evaluated as;

$$\%\ \textbf{Decrease in DOX in solution}\ (\%\ \mathbf{D}) = \frac{\mathbf{A_t^{265}}}{\mathbf{A_0^{265}}} \times 100$$

Hence, the % loading was estimated using the equation:

$$\%\ \textbf{loading of DOX on Pap@ZIF} - 8 = 100 - (\%\ \mathbf{D})$$

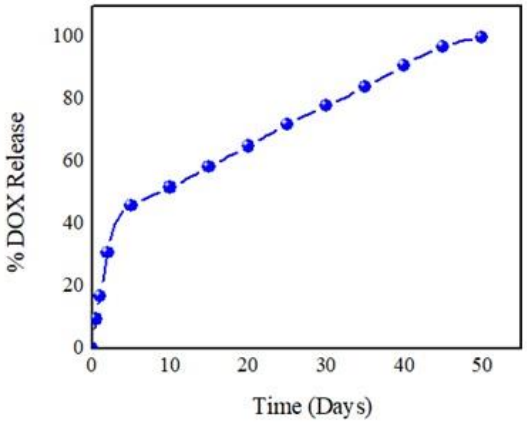
The plot of rate of DOX loading is depicted in **Fig. 11.** Initially, a gradual increase in drug loading was observed. The initial poor loading efficiency may be attributed to problems such as dissolution and diffusion. However, an equilibrium between the DOX molecules and the Pap@ZIF-8 matrix is essential for efficient drug loading. However, 50% of the DOX was loaded onto the Pap@ZIF-8 matrix within one h. The DOX loading reached its maximum within 150 minutes. A maximum of 85% of the DOX was loaded at 150 min. Then saturation in DOX loading was observed. However, 100% DOX loading was not noticed even after 7-8 hours.



[Fig.11: Rate of DOX loading on Pap@ZIF-8]

J. Controlled Release of Loaded DOX From Pap@ZIF-8

Controlled and continuous drug release is essential for the successful administration of pharmaceuticals. The controlled release of loaded DOX molecules [12] from Pap@ZIF-8 was investigated. The loaded DOX was suspended in PBS buffer of pH 5.0 and stirred at room temperature. Aliquots were taken periodically and the release rate of DOX was monitored spectrophotometrically at 479 nm. An initial burst, followed by a slow release of DOX, is observed under these reaction conditions. These results indicate that keeping the drug at the desired therapeutic level in the body can avoid frequent administration. Consequently, the administration of DOX at the right time, at the correct dose, and with specificity and efficiency throughout the body is the primary advantage of the drug delivery system based on Pap@ZIF-8 compared to DOX@ZIF-8 (**Fig. 12**).



[Fig.12: Plot of % release of DOX from Pap@ZIF-8]

K. Regeneration and Reusability of the Drug Delivery System

i. Extraction of DOX@ZIF-8

The regeneration and reusability of the drug delivery carrier system was investigated. The spent DOX@ZIF-8 was dispersed in an ethanol solution, and the mixture was then refluxed at 85°C for 2 hours. The solid was recovered by centrifugation, washed with ethanol and then dried. The above extraction procedure was repeated four times. The system was found to be effective for 10 cycles of repeated DOX delivery, with minimal loss in efficacy.

ii. Extraction of DOX@Pap@ZIF-8

The regeneration and recycling procedures were also repeated with an enzyme-mediated drug delivery system. After the completion of the drug release, the spent Pap@ZIF-8 was filtered, washed several times with distilled water and treated with PBS buffer. Then, the procedure was repeated, reloading, drying, and reusing for another cycle of the experiment. The synthesised drug delivery system was found to be effective even after 15 cycles of analysis.

IV. CONCLUSION

Biomimetic mineralisation is a valuable approach for enzyme immobilisation. In a one-pot synthesis, highlighting the atom-economic principle of green chemistry, zeolitic [13] imidazolate frameworks with encapsulated enzymes and drugs were prepared successfully. Papain, a papaya enzyme belonging to the cysteine family, was selected as the carrier enzyme. Doxorubicin, a chemotherapy medication used in cancer treatment, was chosen as the target molecule for

biomineralisation. The ZIFs were characterized by FTIR and SEM analysis. Both the systems DOX@ZIF-8 and DOX@Pap@ZIF-8 were found to be pH-sensitive in terms of drug release. The release of drugs at low pH is associated with the dissolution of ZIF-8. The induction period is related to the dissolution of the peripheral DOX-free shells of ZIF-8, which act as a protective capsule around the DOX@ZIF-8. A critical advantage of the DOX@ZIF-8 system is that the DOX molecules are safely stored with essentially no release under physiological conditions. An initial burst, followed by a slow release of DOX, is observed at low pH at room temperature. These results indicate that keeping the drug at the desired therapeutic level in the body can avoid frequent administration. The administration of medications at the right time, at the correct dose, and with specificity and efficiency anywhere in the body is the main advantage of the drug delivery system based on DOX@Pap@ZIF-8 compared to DOX@ZIF-8. The synthesised drug delivery system was found to be effective even after 15 cycles of analysis.

ACKNOWLEDGEMENT

We are grateful to the Institute for Intensive Research in Basic Sciences (IIRBS) and the School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India, for providing facilities for spectral analysis and incubation studies.

DECLARATION STATEMENT

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

- **Conflicts of Interest/ Competing Interests:** Based on my understanding, this article has no conflicts of interest.
- **Funding Support:** This article has not been funded by any organizations or agencies. This independence ensures that the research is conducted with objectivity and without any external influence.
- **Ethical Approval and Consent to Participate:** The content of this article does not necessitate ethical approval or consent to participate with supporting documentation.
- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Author's Contributions:** The authorship of this article is contributed equally to all participating individuals.

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