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Research paper

PEGylated green halloysite/spinel ferrite nanocomposites for pH sensitive delivery of dexamethasone: A potential pulmonary drug delivery treatment option for COVID-19

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ABSTRACT

Dexamethasone (Dex) is used in drug regimen for treatment of Coronavirus disease (COVID-19). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) fusion and entry into the cell occurs at pH 5.5. In our present study, we have identified a green, cheap clay based halloysite (Hal) nanoformulation with release capability of Dex at such interactive pH condition. 30%ZnFe$_2$O$_4$/Hal and 30%NiFe$_2$O$_4$/Hal were prepared by one-pot synthesis technique. Dex (5% wt/wt) was functionalized over both nanocomposites. Finally, polyethylene glycol (PEG) was coated over ZnFe$_2$O$_4$/Hal/Dex and NiFe$_2$O$_4$/Hal/Dex nanocomposite using lyophilization technique ($0.08\mu\text{g/mg}$ of nanocarrier). The release ability of Dex was studied under pulmonary infection and normal pH conditions (pH = 5.6 and 7.4). The characterization study using X-ray diffraction (XRD) and UV–visible diffuse reflectance (DRS) spectra confirmed the presence of spinel ferrites over Hal. Nitrogen adsorption isotherm showed the surface area of ZnFe$_2$O$_4$/Hal (75 m$^2$/g), pore volume (0.27 cm$^3$/g) with average pore size (14.5 nm). Scanning electron microscope/Energy dispersive spectroscopy (SEM-EDS) and Transmission electron microscopy analysis revealed a textural change in halloysite tubular type indicating drug adsorption and PEG adhesion. DRS spectra indicated an intergrowth of zinc ferrite nanoparticles on the halloysite nanotubes. Interestingly, ZnFe$_2$O$_4$/Hal/Dex/PEG exhibited a high Dex release ability (17.5%, 168 h) at pH = 5.6 relevant to SARS-CoV-2 fusion entry into the cell pH condition of 5.5. Comparatively, the nanocomposite showed a less Dex release (＜5%) release for 168 h at neutral pH = 7.4. The drug release kinetics were studied and the obtained data were fitted for the release constant and release exponent, using the Korsmeyer-Peppas model. To test the compatibility of our nanocomposites, we performed the cell viability assay (MTT) using HEK293 cells. Our results showed that at 0.3 mg/ml, Dex-loaded nanocomposite had a statistically significant improvement in cell viability compared to Dex alone. These results suggest that our nanocomposite has prevented the toxic effect of Dex and has huge potential to act as pulmonary drug delivery system for targeted lung infection therapeutics.

1. Introduction

Coronavirus belonging to large family of viruses cause illnesses of a wide range of severity. COVID-19 is caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). COVID-19 pandemic related mortality is continuously increasing irrespective of the

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preventive measures taken worldwide. Up to now, 250 million cases have been registered with total deaths more than five million (https://www.worldometers.info/coronavirus, Cutler and Summers, 2020). COVID-19 infection is reported to occur at lower respiratory mucosa region (Subbarao and Mahany, 2020). Spike proteins of COVID-19 interact preferentially with ACE2 receptor expressed in cells of human (lung and other vital organs) (Andersen et al., 2020; Zhang et al., 2021a; Izza et al., 2021).

The treatment strategies of SARS-CoV-2 infection includes drugs that specifically target host cell interactions or proteins generated by virus (Subbarao and Mahany, 2020). The proteins evolution of virus effectively targets the receptor termed as angiotensin-converting enzyme (ACE2), a prime target identified for nanotherapeutic intervention (Bonam et al., 2021). Up to now, there is no effective antiviral drug available to combat COVID-19. One strategy involves blocking the virus entry by blocking specific CoV receptors using protease inhibitors (Camostat mesylate, Umifenovir, lopinavir/ritonavir) (Heinrich et al., 2020), immunotherapies involving TNF inhibitor (Thalidomide, Adalimumab), IL-6 receptor antagonist (Sarilumab, Tocilizumab), IL-6 antagonists (Silmituzumab) (Hassomikarimi et al., 2021). A drug combination including a corticosteroid drugs, immunosuppressant, antibiotics, antioxidant along with traditional medications was reported to be effective to some extent in controlling the lung infection and cytokines (Zhang et al., 2021b). Several drugs like Remdesivir, Ribavirin, Favipiravir, Interferons, Lopinavir/ritonavir, Chloroquine, Umifenovir, Ivermectin, Dexamethasone, Azataviran, Imatitinib, Ruxolitinib, Fluvoxamine, Baricitinib, Tocilizumab, Eculizumab, Molnupiravir, hydroxychloroquine/azithromycin and 2-deoxy-o-glucose has been tested to treat COVID-19 infection (Jean et al., 2020; Kumar and Trivedi, 2021). Dexamethasone is the most studied glucocorticoid compound has been used along the treatment regime of COVID-19. The drug is effective in reducing the mortality (6 mg for 5–10 days), while recent study suggested such corticosteroid treatment with more controlled trial study and need of optimizing the dose regime (Noreen et al., 2021; Pinzon et al., 2021). However, the treatment with dexamethasone also impact several side effects like hormonal disturbance, oedema, weight gain, sleep disorder and dose related adverse side effects (Cole, 2020). Dexamethasone also cause diabetes related complications, immune suppressor and cause hypertension. Poor gastrointestinal stability, low bioavailability, poor transport behavior and side effect on other organs like kidney limit its therapeutic effectiveness (Alessi et al., 2020; Huang et al., 2017).

Pulmonary drug delivery research has been rising steadily in interdisciplinary core area for treatment of lung diseases. Such therapeutic route increases the targeted drug delivery, reduces the effective dose, easily adsorbed on available large lung surface area (Rangaraj et al., 2019; Strzempek et al., 2019). The most striking features of pulmonary nanotherapeutics is the ability of nanoparticle to accommodate several components into nano structure to generate multifunctional modality. Pulmonary drug delivery system has been developed to treat of lung infection asthma, chronic pulmonary diseases. One of the prerequisite of such delivery system is that it should able to increase the bioavailability, strong adhesion ability, rapid diffusion through mucus, sustained drug release capacity and delivering drugs specifically to bronchi and alveoli (Bonam et al., 2021). For instance, several nanocarriers based on metal organic framework Fe-MIL-100 (Strzempek et al., 2019), liposomes (Yildiz-Peköz and Ehrhart, 2020), micelles (Pham et al., 2021), and inorganic nanoparticles (Xu et al., 2020) has been reported for treating pulmonary infections. Magnetic nanoparticles, superparamagnetic iron oxide nanoparticles (SPIONs) based drug delivery system has shown to improve the targeted delivery to desired position with the help of external magnetic field (Saadat et al., 2020; Stocke et al., 2015). Magnetic micrords fabricated with poly (ethylene oxide) and poly (l-lactide) polymers are shown to increment the drug accumulation and improve targeted release of drugs at the lower airways (Nikolau et al., 2021). ACE-2 surface rich membrane based nanomaterials synthesized by extrusion technique was reported to be effective in blocking virus entry into host renal tubular cells and reduce the SARS-CoV-2 infection (Wang et al., 2021). Inspite of recent advancement in vaccines and treatment related to antiviral therapy, a continuous rise in the mortality related to COVID-19 clearly shows the need for developing an effective targeted pulmonary drug delivery system.

A nanotechnology tool with diagnostic tool sensing SARS-CoV-2 specific cell entry through interactive pH is yet to be reported. In addition, an effective pulmonary delivery capable of carrying and releasing antiviral agent is required. Dexamethasone is used in drug regimen for treatment of COVID-19. High dose increases mortality rate, poor gastrointestinal stability, low bioavailability, poor transport behavior limit its therapeutic effectiveness. For example, a low dose administration of dexamethasone is proposed to overcome acute respiratory distress syndrome (Mahaase, 2021). Halloysite (AlSiO$_2$(OH)$_4$·2H$_2$O), a natural clay mineral similar to kaolin is deposited abundantly worldwide. The nanoclay is an ecofriendly green tubular material of with cheaper cost of production (Duan et al., 2020). Basically built with kaolinite structure has layered structured (d$_{001}$ value of 10 Ångstrom) occupied inbetween by water molecules (Barman et al., 2020). The external adsorption site (specific surface area) of Hal can be modified by treatment with HCl for effective drug incorporation and influence the release rate of antioxidant such as curcumín (de Abreu Pereira et al., 2021). Halloysite with high tubular surface area, biocompatibility, functionalamal and siloxane moieties has been used as a controlled drug release carriers for cancer (Mo et al., 2021), tissue engineering (Gokoua et al., 2021), diabetes mellitus (Massaro et al., 2018), dental (Karczewski et al., 2021), herbicide (Tan et al., 2015), etc. Gold nanoclusters supported on silane modified Hal has been described as an effective nanocomposite inducing non-toxic effect at desired concentration range (25–50 µg/ml) suitable for tumor imaging and cancer therapy (Gorba- chevskii et al., 2021). The present study put forward an important strategy by designing a nanosystem that can indentify such virus-ACE2 interactions pH 5.5 with a potential to attack the virus by delivering a known antiviral drugs in slow and controlled manner. Zinc ferrite or nickel ferrite/Hal with magnetic resonance imaging property was investigated for potential pulmonary drug delivery including COVID-19. The textural and chemical environment of both ferrite system and Dexamethasone was analyzed using different characterization techniques. The technique is simple, reproducible, and scalable. The study shows that the developed pulmonary drug delivery system is more effective in releasing drug into pulmonary infectious pH condition of 5.6 matching with the pH of virus interacting with ACE-2 receptor.

2. Material and methods

Zinc acetate dihydrate (99.999%), Iron(III) nitrate nonahydrate (BioReagent), Nickel (II) nitrate hexahydrate (99.999%), Halloysite nanoclay (Kaolin clay) and Dexamethasone (>98%) were purchased from Sigma Aldrich and used as-such. Uniformly dispersed spherical silica was obtained from Superior silica, USA. The surface area of ZnFe$_2$O$_4$/Silica was of 56 m$^2$/g.

All the reagents used in in vitro study were of high quality reagents manufactured under good manufacturing practices. Gibco cell culture products were obtained from Thermo Fisher Scientific: DMEM (Dulbecco’s Modified Eagle Medium), heat-inactivated fetal bovine serum (HI-FBS), 100× Penicillin Streptomycin, and 100× MEM NEAA (MEM non-essential amino acids) were obtained from Thermo Fisher. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was purchased from Sigma-Aldrich (catalogue M2129).

2.1. One pot synthesis of spinel ferrite/Hal nanocomposites

30%ZnFe$_2$O$_4$/Hal and 30%NiFe$_2$O$_4$/Hal were synthesized using one pot synthesis method. The procedure for preparing 30%ZnFe$_2$O$_4$/Hal involves addition of 1.01 g of iron nitrate nonahydrate and 0.54 g of Zinc...
acetate dihydrate in 60 ml of deionized water with continuous stirring for 10 min. Afterwards, 1.4 g of Hal was added and stirred further for 30 min. Then, the precipitated mixture was dissolved in 5 ml of alkaline solution (2.0 M NaOH) and continued stirring for 10 min. The mixture was then aged at 90 °C for 2 h with constant stirring. After that, the particles were filtered, washed, dried at 80 °C overnight and calcined at 500 °C for 5 h (5 °C/min). 30%NiFe$_2$O$_4$/Hal, 1.03 g of iron nitrate nonahydrate and 0.74 g of nickel nitrate solutions were taken and dissolved in 60 ml water with consistent stirring for 10 min. After dissolution, 1.4 g of Hal was added and stirred for 30 min. The mixture was then added to 5 ml of alkaline solution (2 M NaOH) and stirred for 10 min. Then temperature increased 90 °C and aged for 2 h with stirring. After that, the particles were filtered, washed, dried at 80 °C overnight and calcined at 500 °C for 5 h (5 °C/min).

2.2. Spinel ferrite/Hal/Dex (5% wt/wt)

For drug functionalization, 30 mg of Dex was initially dissolved in 10 ml of methanol: PBS (7.2) mixture (1:2) and stir for 20 min, then prepared nanocomposite (600 mg) was added and stirred overnight under ice cool environment and then filtered, washed with 5 ml of methanol/PBS mixture solution. The samples were then air dried for 24 h followed by vacuum drying at 50 °C for 24 h. The amount of Dex loaded was calculated using UV-visible spectroscopy at 243 nm. For instance, the initial Dex and Hal were weighted accurately, dissolved in methanol/PBS mixture (10 ml) and stirred (50 rpm) overnight. The solution mixture was then filtered using Buchner funnel with sintered disc G4 type and washed with additional 5 ml of methanol/PBS solution. The sample was then completely dried under vacuum filtration. Further the sample was air dried at ambient condition for 24 h and again dried under vacuum at 50 °C. The filtrant was analyzed and estimated the remaining Dex on the precipitate was determined to be 0.12 mmol/g of nanocarrier.

2.3. Spinel ferrite/Hal/Dex/PEG (0.08 μl/mg of nanocarrier)

For PEG coating, 40 mg of PEG (Molecular weight = 400) was added in 10 ml of deionized water, stirred for 20 min under argon atmosphere, then 20 mg of ZnFe$_2$O$_4$/Hal/Dex or NiFe$_2$O$_4$/Hal/Dex nanofluids were added and stirred under ice cool environment for 24 h. Then the solution mixture was freeze dried using lyophilization technique.

2.4. Characterization techniques

The phase of support carriers such as ZnFe$_2$O$_4$/Hal and NiFe$_2$O$_4$/Hal were identified using benchtop XRD (Miniflex 600, Rigaku, Japan). The textural features including BET surface area, pore size and pore volume was measured using nitrogen adsorption technique (ASAP-2020 plus, Micromeritics, USA). The ferrite nanoparticle chemical coordination was analyzed using DRX spectroscopy analysis (JASCO, V-750, Japan). The functional groups of spinel ferrite, DEX and PEG coating in nanofluidation was determined using FT-IR spectroscopy (L160000A, Perkin Elmer, USA). The spinel ferrite/halloysite morphology and various elements were analyzed using SEM (JSM-6610LV, JEOL) equipped with energy dispersive spectroscopy (EDS) and transmission electron microscopy (TEM) (FEI, Morgagni 268 at 80 kV, Hillsboro, Oregon, USA).

2.5. Drug release study

The drug release trend of Dex over ZnFe$_2$O$_4$/Hal and NiFe$_2$O$_4$/Hal nanofluids were investigated using dialysis membrane technique. The nanofluidation loaded with Dex (30 mg) with 3 ml of PBS solution was packed inside the dialysis tubing (MWCO = 14,000 Da) and placed in 47 ml of PBS (pH 5.6 or pH 7.4). The temperature was maintained at 37 °C. 10 ml of solution was withdrawn at specific period of time and replaced with equal volume of fresh solution. The release content was identified at specific wavelength of 243 nm. Prior to analysis, calibration curve for dexamethasone was established. At first, an initial stock solution was prepared with concentration of 1000 μg/ml for Dex. Various concentration of aliquots 5, 10, 15, 20, 25 and 30 μg/ml was prepared with makeup volume to 10 ml using release medium PBS solution (pH = 5.6 or 7.4) and calibration curve established against blank at maximum absorption wavelength of 243.5 nm. Linear regression was found to be $y = 0.0373x + 0.0104$, where $y$ corresponds to absorbance and $x$ to the concentration of released drug (μg/ml). The correlation coefficient is 0.992 for maximum absorption. The release study was repeated in triplicates.

2.6. Cell cultures

To assess the compatibility and effectiveness of our nanocomposites, we used the human embryonic kidney cells HEK293 cell lines. Cells were cultured in a DMEM medium with 10% HI-FBS, 1% Penicillin Streptomycin, and 1% MEM NEAA. Cells cultures were maintained in a cell incubator at 37 °C with 5% CO$_2$ in humidified conditions. For the MTT cell viability assay, cells were seeded in a 96-well plate with a density of 20,000 cells/well. After 24 h from seeding, cells were treated with the conditions and treatment duration discussed in the following section.

2.7. Cell treatment

We first performed a dose response curve. HEK293 cells were treated with the following conditions: Zn/Hal, Hal, Dex, ZnFe$_2$O$_4$/Hal/Dex, ZnFe$_2$O$_4$/Hal/Dex/PEG, and the no treatment control group. Treatment concentrations of Zn/Hal, Hal, ZnFe$_2$O$_4$/Hal/Dex, ZnFe$_2$O$_4$/Hal/Dex/PEG, and PEG were: 0.0375, 0.075, 0.15, and 0.3 mg/ml. Treatment concentrations of Dex was determined based on the drug loading experiment in which a simple calculation was used to reflect the actual quantity of Dex that was adsorbed on the nanocomposites. According to the loading experiment, 1 mg of ZnFe$_2$O$_4$ nanoparticles contains 0.0483 mg of Dex. Therefore, if ZnFe$_2$O$_4$/Hal/Dex concentration was 0.3 mg/ml, then there is 0.01449 mg/ml of adsorbed Dex. Thus, the treatment concentrations of Dex were: 0.00181, 0.00362, 0.00725, and 0.01449 mg/ml. We first performed a dose curve treatment for 48 h. After selecting the optimal dose we performed the treatment at different timepoints: 24, 48, and 72 h.

2.8. Cell viability (MTT)

We used the MTT cell viability assay to assess the compatibility and effectiveness of our nanocomposites. The assay assesses the viability of cells via evaluation of mitochondrial function as MTT is converted to formazan (Mosmann, 1983). After washing cells with PBS, MTT working solution was added at a final concentration of 0.5 mg/ml. Cells were incubated at 37 °C for 3 h after which 0.04 N HCl isopropyl alcohol was added to solubilize the formazan crystals. Change in color was measured using SYNERGY-neo2 BioTek ELISA reader at 570 nm. An initial reading (before addition of MTT) was recorded and subtracted from the final reading to avoid background interference from NPs. Each condition was performed in three technical repeats with three biological repeats ($n$ = 3). A no treatment negative control and an MTT background control (no cells) were also included. Analysis was performed by comparing treatment conditions with the no treatment negative control. Percentage of cell viability was measured as follows:

\[
\text{Cell Viability} \% = \left( \frac{\text{absorbance of condition}}{\text{absorbance of negative control}} \right) \times 100.
\]

2.9. Microscopic examination

Cells were plated on a chamber slide and treated with the treatment
conditions for 24, 48, and 72 h. Nikon Eclipse TS100 (inverted microscope) was used to capture brightfield images.

3. Statistics

The cell viability assay was performed in three independent experiments (n = 3). Statistical analysis was performed using Prism 9.1 software (GraphPad, La Jolla, CA). The analysis was performed using one-way ANOVA with Dunnett’s post hoc test. Error bars ± S.E.M. Statistical significance and p values are listed in figures as tables.

4. Results & discussion

The phase analysis of Dex, Hal, ZnFe$_2$O$_4$/Hal, ZnFe$_2$O$_4$/Hal/Dex, NiFe$_2$O$_4$/Hal/Dex, NiFe$_2$O$_4$/Hal/Dex/PEG and ZnFe$_2$O$_4$/Hal/Dex/PEG were investigated using XRD analysis (Fig. 1). Dex exhibited several major intense crystalline peaks at 2θ value ranging between 6.7°-40° with an intense peak at 14.2°. ZnFe$_2$O$_4$/Hal spectra showed both the presence of clay and Zinc spinel ferrite coexisting with each other. Several diffraction peaks appears corresponding to (220), (311), (222), (400), (422) and (440) planes at 2θ range (25–60°), confirming the zinc and nickel based spinel ferrite structures. The cubic spinel structure of zinc ferrite with Fd3m structure matches well with JCPDS data no. 04–015-7060. The spinel particle size was determined by using Scherer equation using the intense (311) peak. ZnFe$_2$O$_4$ nanoparticle was of 8.6 nm, while NiFe$_2$O$_4$ particle size was of 6.1 nm, respectively. After Dex loading over ZnFe$_2$O$_4$/Hal and NiFe$_2$O$_4$/Hal, the drug showed a reduced reflections of Dex, which signals transformation of crystalline drugs to nanosize form. However, the presence of weak peaks of Dex indicates still a few crystalline nature of drug is preserved in conjugation with the nanotubes of halloysite. Such peaks of dexamethasone was observed with NiFe$_2$O$_4$/Hal/Dex/PEG and ZnFe$_2$O$_4$/Hal/Dex/PEG even after wrapping with PEG.

The textural properies of Hal, ZnFe$_2$O$_4$/Hal, ZnFe$_2$O$_4$/Hal/Dex and ZnFe$_2$O$_4$/Hal/Dex/PEG determined using nitrogen adsorption isotherm was shown in Fig. 2(a-d). Hal and modified nanocomposites showed a type IV isotherm with H3 hysteresis pattern at higher relative pressure between 0.7 and 1.0, indicating both micro and mesopore characterisitcs (Zhang et al., 2021c). As presented in Table 1, the surface area of halloysite was observed to be 76 m$^2$/g (Fig. 2a), which only slightly changes to 75 m$^2$/g after nanocomposite formation with zinc ferrite. However, the pore volume decreases from 0.33 cm$^3$/g to 0.27 cm$^3$/g and average pore size distribution from 17.7 nm to 14.5 nm, respectively (Fig. 2b). This suggest the occupation of zinc ferrite at external surface of nanotube also contribute to the surface area and not clogging interior lumen portion of clay during nanocomposite formation. A recent work by Masindi et al. (2021) proved that adsorption metal ions rather increases the surface area after adsorption over halloysite and correlated to the radial diameter of nanoparticles. However, after Dex loading on

![Fig. 1. XRD pattern of (a) Dex, (b) Hal, (c) ZnFe$_2$O$_4$/Hal, (d) ZnFe$_2$O$_4$/Hal/Dex, (e) NiFe$_2$O$_4$/Hal/Dex, (f) NiFe$_2$O$_4$/Hal/Dex/PEG and ZnFe$_2$O$_4$/Hal/Dex/PEG.](image1)

![Fig. 2. Nitrogen adsorption isotherm pattern of (a) Hal, (b) ZnFe$_2$O$_4$/Hal, (c) ZnFe$_2$O$_4$/Hal/Dex and (d) ZnFe$_2$O$_4$/Hal/Dex/PEG.](image2)

| Table 1: Physical properties of catalysts and supports. |
|----------------------------------------------------------|
| **Nanocomposite:** | **BET surface area [m$^2$/g-catalyst]a** | **Pore volume [cm$^3$/g-catalyst]b** | **Average pore diameter [nm]c** |
|---------------------|-------------------------------|------------------|-------------------------------|
| 30%ZnFe$_2$O$_4$/Hal| 76                            | 0.33             | 17.7                          |
| 30%NiFe$_2$O$_4$/Hal| 75                            | 0.27             | 14.5                          |
| ZnFe$_2$O$_4$/Hal   | 40                            | 0.14             | 13.8                          |
| ZnFe$_2$O$_4$/Hal/Dex| 18                          | 0.09             | 20.5                          |
| NiFe$_2$O$_4$/Hal   | 54                            | 0.22             | 16.6                          |
| NiFe$_2$O$_4$/Hal/Dex/PEG| 23                      | 0.12             | 17.7                          |

a BET surface area.

b Pore volume and average pore diameter measured using BJH isotherm.

c Pore volume and average pore diameter measured using BJH isotherm.
ZnFe$_2$O$_4$/Hal, the surface area decreases to 40 m$^2$/g along with pore volume (0.14 cm$^3$/g) and pore diameter (13.8 nm). Such a significant textural changes could be ascribed to the loading of drugs inside the lumen portion of Hal (Vinokurov et al., 2018). Further decrease in surface area to 18 m$^2$/g and pore volume to 0.09 cm$^3$/g is observed with PEG coating. The decreasing surface texture, indicates PEG interaction with external hydroxyl groups of Hal. Similarly, in case of NiFe$_2$O$_4$/Hal, the surface area after NiFe$_2$O$_4$ composite formation was of 54 m$^2$/g, which then decreases to 23 m$^2$/g after Dex and PEG wrapping. Pore volume reduction occurs from 0.22 cm$^3$/g to 0.12 cm$^3$/g, while pore diameter remains at about 18 nm, which reflects the loading of drugs inside lumen of Hal. Gorbunova et al. (2020) has reported that coating with PEG tends to interact with hydroxyl group of silanol groups forming a hydrogen bonding, results in polycondensation.

Fig. 3 shows the diffuse reflectance spectra of Hal, Dex, ZnFe$_2$O$_4$/Hal/Dex, ZnFe$_2$O$_4$/Hal/Dex/PEG, NiFe$_2$O$_4$/Hal/Dex, and NiFe$_2$O$_4$/Hal/Dex/PEG. For comparison, the Hal and Dex was also presented. Both nanotube halloysite and Dex showed a UV light absorption peak in the range between 200 and 400 nm. The peak at 263 nm of Hal is attributed primarily to the layered coordination of SiO$_4$ and Al$_2$O$_3$ species. Dex showed a strong absorption at about 200–300 nm and addition peak appears at about 350 nm. For Dex and PEG wrapped over ZnFe$_2$O$_4$/Hal and NiFe$_2$O$_4$/Hal, an enhancement in peak absorption occurs expanding to visible region up to 800 nm. In case of ZnFe$_2$O$_4$/Hal/Dex/PEG, the zinc ferrite nanoparticles exhibited a strong UV absorption at 354 nm corresponding to Zn$^{2+}$ species and Fe$_2$O$_4$ species (Talebi et al., 2016). As such Hal had no visible light absorption, while spinel ferrite loading on Hal, the spectrum spread across the visible light attributing to the nanosized spinel ferrites. This clearly indicates the nanosized ferrite intergrowth on the halloysite nanotubes. There are reports that indicates presence of zinc in metallopeptidase angiotensin system play a vital role in cardiovascular homeostasis, acute inflammation and autoimmune disorders (Shih et al., 2014; Nehme et al., 2019). Also, zinc supplementation enhance the drug efficiency of chloroquine/hydroxychloroquine combination to treat COVID-19 (Derwand et al., 2020). The extended absorption of zinc ferrite based halloysite loaded with Dex exhibited only a slight differences with PEG wrapping and indicates a potential pulmonary nanocomposite formulations.

Fig. 4 shows the FTIR spectra of Dex, ZnFe$_2$O$_4$/Hal/Dex, NiFe$_2$O$_4$/Hal/Dex, NiFe$_2$O$_4$/Dex/PEG, and ZnFe$_2$O$_4$/Hal/Dex/PEG, respectively. ZnFe$_2$O$_4$/Hal showed a broad absorption peak at about 3390 cm$^{-1}$ corresponding to hydroxyl group. The FTIR characterization tool is used to confirm the presence of spinel ferrite and an effective interaction of Dex and PEG with Hal. The presence of two bands at about 426 cm$^{-1}$ and 554 cm$^{-1}$ corresponds to the characteristic peaks of octahedrally coordinated Zn$^{2+}$ species and tetrahedral Fe$^{3+}$ species (Fe–O stretching) of ZnFe$_2$O$_4$ while hydroxyl groups was observed at about 2800–3000 cm$^{-1}$ (Puspitasari et al., 2021). Dex showed a characteristics peaks between 400 and 4000 cm$^{-1}$. The presence of weak peaks at 1708 cm$^{-1}$ (C=O), 1664 cm$^{-1}$ (-COO), 1625 cm$^{-1}$ (-C=O), clearly shows the successful interaction of Dex into ZnFe$_2$O$_4$/Hal nanof ormulation (Lee et al., 2017). Similar overlapping of such peaks at 1712 (-C=O), 1667 (-COO), 1625 (-C=O) over ZnFe$_2$O$_4$/Hal/Dex/PEG and NiFe$_2$O$_4$/Hal/Dex/PEG, clearly shows the attachment of Dex after PEG coating through lyophilization. Compared to Dex (standard), a significant reduction in the stretching peaks of Dex in ZnFe$_2$O$_4$/Hal/Dex and NiFe$_2$O$_4$/Hal/Dex indicates the incorporation of drugs inside the lumen portion of Hal. Conversely, the presence of PEG was identified with characteristic peaks of (CH$_2$) asymmetric stretching at about 2913 cm$^{-1}$. Furthermore, a significant decrease in hydroxyl stretching peaks at about 3400 cm$^{-1}$ represents an effective PEGylation. The morphological characteristics of ZnFe$_2$O$_4$/Hal and ZnFe$_2$O$_4$/Hal/Dex/PEG was analyzed using SEM. A tubular nano clay corresponding to ZnFe$_2$O$_4$/Hal was clearly seen in agglomerated form (Fig. 5a). In case of freeze dried ZnFe$_2$O$_4$/Hal/Dex/PEG sample, addition of Dex and PEG clearly changed the textures of tubular clay indicating the drug embemendment and PEG adhesion (Fig. 5b). Particularly, the cluster size of ZnFe$_2$O$_4$/Hal (22.71 ± 28.82 μm) increases to several micrometers after PEG coating (26.86 ± 36.89 μm). In case of EDS analysis, the chemical composition of nanof ormulation was confirmed with the elements presence of Dex (C, O), PEG (C, O), zinc ferrite (Zn, Fe), oxygen, silicon and aluminium peaks corresponding to that of Hal (Fig. 5c). The spinel ferrite, Dex and PEG distributions on Hal were mapped using SEM-EDS analysis (Fig. 5d-f). EDS spectra showed the presence of C, O, Al, Si, Zn and Fe elements. The EDX mapping pattern shows the well distributed spinel ferrite species at 30% composite with Hal. The densification of C and O elements revealed with Dex and PEG. Some agglomerations are observed which can be attributed to wrapping of PEG over Hal. The detailed morphology and analysis of the two composites (ZnFe$_2$O$_4$/Hal and ZnFe$_2$O$_4$/Hal/Dex/PEG) were performed further by using transmission electron microscopy (TEM). The results of the TEM analysis are displayed by Fig. 5g-l). TEM images revealed the obvious tubular structure and morphology of the nanoclay (Hal) decorated with spherically shaped ZnFe$_2$O$_4$ nanoparticles (Fig. 5g and h). The tubular nature of the clay is judged by a brigh contrast of each clay particle as compared to their outer dark sides. The size of the
nanoparticles is measured by extracting the intensity profile of the indicated particle as marked with a yellow arrow (Fig. 5 i). The average size of the ferrite particles was found nearly 10 nm. A group of ZnFe$_2$O$_4$ nanoparticles attached with nanocalys are marked by white arrows. TEM images of the ZnFe$_2$O$_4$/Hal/Dex/PEG specimen showed a compact but less visible morphology for individual nanoparticles and clay as compared to ZnFe$_2$O$_4$/Hal nanoclay, suggesting a possible coating of Dex/PEG over ZnFe$_2$O$_4$/Hal nanoclay (Fig. 5 j and k). Furthermore, the tubular morphology (bright contrast) of the clay/nanoparticles is less visible due to coating of PEG. SAED pattern confirmed the co-existence of spinel ferrite nanoparticles with clay, the possible diffracting reflections of Dex/PEG over ZnFe$_2$O$_4$/Hal nanoclay are indexed as: (220), (311), (422) and (440) (see Fig. 5 l). In summary, TEM analysis confirming the successful preparation of ZnFe$_2$O$_4$ decorated tubular clay and coating of Dex/PEG over this composite.

Fig. 6 shows the Dex release over ZnFe$_2$O$_4$/Hal and NiFe$_2$O$_4$/Hal in as-such and PEGylation form under lung infection pH condition of 5.6 and neutral pH 7.4. In case of ZnFe$_2$O$_4$/Hal, the release of Dex was found to depend on the pH conditions. An pH sensitive (5.6 vs 7.4) release of Dex was observed. At neutral pH 7.4, less Dex was found to be released (<5%). It is worth noting that at pH 5.6, controlled release of Dex occurs and also the release is in sustained manner (<20%). This shows an excellent pH stimuli responsive drug delivery pattern. In order to confirm this, we have studied the release pattern over ZnFe$_2$O$_4$/Silica, substituting Hal with spherical silica support. Evidently, with silica support, which contains a silanol (Si-OH) and siloxane groups (Si-O-Si) shows a pH insensitive Dex release. The Dex release of about 2.3% occurs at both pH conditions. Jackson et al. (2004) have reported that in presence of telodendrimer nanocarrier, the release of Dex in sustained manner reduced the lung inflammation compared to pure drug.
reason for such a sustained release over Hal could be attributed to the charge distributions at internal and external surface of nanotube. Aluminium rich lumen part is positively charged, while external surface consisting Si-O-Si group is negatively charged. Dex containing hydroxyl groups is evidently interacting with active site of Hal. FTIR confirms the presence of Dex inside the lumen part with a significant reduction in stretching peak of Dex (Fig. 4). Further, the appearance of carbonyl functional moieties confirms the effective coating of PEG around Dex loaded ZnFe$_2$O$_4$/Hal. Therefore, such a charge distributions exhibits an electrostatic and van der Waals interactions and influence the drug adsorption and release behavior (Lisuzzo et al., 2021). It is further reported that a drug Ibuprofen loaded inside the lumen part of Hal with strong affinity assist a prolonged release, while fast release occurs with weak hydrogen bonding of drugs residing on the surface of Hal (Tan et al., 2014). In our study, the observed release pattern of Dex clearly shows the presence of such strong electrostatic interaction could occur between Hal and Dex and presence of drug inside the lumen part is well reflected with a prolonged release of Dex. UV–visible analysis confirms that the sample ZnFe$_2$O$_4$/Hal/Dex contains 0.12 mmol of Dex per gram of nanocarrier. In our synthesis step, PEG coating was carried out in the final step in water medium. The water in the sample was removed by vacuum freeze drying using lyophilization technique. This technique found to be an effective method to preserve the Dex loaded at the initial stage without any disintegration and assist a sustained Dex release.

It is known that Coronavirus entry into the cell is a pH dependent process (ACE-2 receptor mediated process). SARS-CoV-2 fusion, entry into the cell occurs at pH 5.5 (Pindiprolu et al., 2020). In case of pulmonary infection, a nanocarrier is needed that is sensitive and effective to release drug at pH 5.0–6.0. The delivery of the drugs to a particular site such as the pulmonary system, as it forms the main site for SARS-CoV-2 invasion, is important as poor absorption and low bioavailability effects are present. Similarly, the release of Dex over Nickel ferrite/Hal was also found to depend on the pH conditions. Hence our drug carrier which is sensitive to pH 5.6, could effectively delivery drug at this point of pH dependent process.

The strategy of pulmonary delivery of corticosteroids for lung infection can improve the therapeutic index and reduce the side effects. An effective drug delivery system is generally characterized by the stimuli responsive release of drugs in required therapeutic doses at infection site. However, the presence of several barriers in lungs especially in conducting airways (mucociliary escalation) and in respiratory region (alveolar macrophage clearance) along with immune cells system decreases the therapeutic efficacy of drug delivery system (Liu et al., 2020). The polymeric-drug conjugates are reported to improve the drug absorption and distribution mechanism (pharmokinetics) and sustained release ability of drug in lungs (Luo et al., 2016). Alnaief et al. (2020) has used an alginate/chitosan nanocomposite synthesized using emulsion gelation technique as pulmonary drug delivery device. The nanocomposite formation of biodegradable copolymer PLGA and polysaccharide chitosan was reported to enhance the bronchial mucus and lung tissue adhesion and effectively deliver an anti-parathyroid agent (Yamamoto et al., 2005). In case of polyethylene glycol (PEG) coating on nanocarrier (PEGylation), the mucus penetrating ability increases rather than adhesion by avoiding interaction with mucin fibers (He et al., 2019). Furthermore, the presence of PEG is reported to increase the residence time of nanoparticles and reduce the cytokine based inflammations (Patil et al., 2018). Our findings here shows that the Zn/Hal/PEG nanocomposite preferencially shows the ability to release Dex at pH 5.6 and therefore has a good potential to target the virus during interaction with ACE-2 receptor, where similar pH level prevails therefore facilitating Dex release to neutralize before the replication process.

The drug release profiles were examined using the Korsmeyer-Peppas model, expressed using the equation:

$$R^% = kt^n$$

Where R% is the drug percentage release at time (t), k and n are the kinetic rate constant and the release exponent, respectively. The
obtained fitting parameters together with their 95% confidence intervals are shown in Table 2.

For the ZnFe\(_2\)O\(_4\)/Hal at the different pH of 5.6 and 7.4, the kinetic rate constant (k) reduced with decrease in the pH value. This indicates that, the rate of drug release using this material is enhanced with increase in pH. The release exponent (n) signified fickian (n ≈ 0.45) and non-fickian (0.45 < n < 0.89) diffusion mechanisms for pH = 7.4 and 5.6, respectively. Increase in pH reduces the rate of drug release using NiFe\(_2\)O\(_4\)/Hal. The pH has no effect on the diffusion mechanism as both release exponents n < 0.45, confirming fickian mechanism. For ZnFe\(_2\)O\(_4\)/Hal/PEG, lower pH increases the rate of drug release as observed from the higher rate constant. The release exponent (n) at both pH signifies fickian diffusion mechanism. Interestingly, for NiFe\(_2\)O\(_4\)/Hal/PEG, the kinetic rate constant increases with increase in pH, signifying improved rate of drug release at higher pH. Fickian diffusion mechanism was followed at all the two pH. Conclusively, adding PEG to the composite materials increases the rate of drug release especially for ZnFe\(_2\)O\(_4\)/Hal material as observed from the kinetic rate constants of the before and after adding PEG.

To assess the compatibility and effectiveness of our nanocomposites, we performed the MTT cell viability assay. Cells were treated with the following conditions: Zn/Hal, Hal, Dex, ZnFe2O4/Hal/Dex, ZnFe2O4/Hal/PEG, and PEG. Treatment concentrations were as follows: 0.0375, 0.075, 0.15, and 0.3 mg/ml. In addition, cells were treated with the Dex at concentrations to reflect that loaded in our nanocomposite as follows: 0.00181, 0.00362, 0.00725, and 0.01449 mg/ml. To choose the optimal dose, we explored specific doses at different time points. We first performed a concentration curve with treatments given for 48 hours (Fig. 7). Then we selected specific concentration to be treated at different time points. Cells were treated with dose 2 (0.075 mg/ml - Fig. 8), or dose 4 (0.3 mg/ml - Fig. 9) for 24, 48, and 72 h. Upon analysis of our results, we decided that dose 4 (0.3 mg/ml) was the optimal dose as it shows a distinct separation between the cell viability curves. This was confirmed with statistical analysis of dose 4 treatment for 24, 48, and 72 h (Fig. 9.B,C,D) and the morphological assessment of treated cells (Fig. 10). Our analysis shows that when comparing our nanocomposites with Dex, there is a statistically significant improvement in cell viability of HEK293 cells at 24 and 48 h. These results suggest that our nanocomposites are able to prevent the toxic effects of Dex while in blood circulation until it reaches the target tissue. Thus, our nanocomposites are a good candidate as a delivery vehicle for Dex.

In the initial stage, an antiviral drug combination included hydroxychloroquine, zinc along with azithromycin. Subsequently, remdesivir was reported to be effective in improving the recovery rate of patients with doses of 200 mg on first day, followed by 100 mg/day for 9 days (Beigel et al., 2020). Dex, a synthesized glucocorticoids compound has been used along the treatment regime of COVID-19. In a clinical trial study (Open-lable trial), Dex was administrated for patients without any respiratory support. A total of 482 patients treated with Dex and 1110 patients treated in usual group died in a period of 28 days. A combinational antiretroviral potease inhibitors with the dose regimen of Lopinavir (400 mg)/ritonavir (100 mg), for the period of 14 days was effective to treat COVID-19. In some cases, interferon alfa-2B (100,000–200,000 IU/kg) also used in combination through nebulization (Venkatasubbaiah et al., 2020).

A study finds that in a group of 138 patients administrated with either tofacitibn or Dex, a total of 44 patients expired. In a group, which treated only with tofacitibn reported a 31.8% death, while 68.2% patients succumb in group of Dex. The death proportions were found to be high with Dex. However, the addition of Dex (10 mg/day, 1–2 days) along with tofacitibn (10 mg, 7–10 days) shown to improve the patients recovery rate (Hayek et al., 2021). In another study, a group of patients in total of 197 were treated with a drug combo of baricitinib-Dex and Dex alone. In that, a combo group contains 123 patients, while remaining 74 patients received Dex alone. The mortality rate was found to be significantly reduced in a group treated with baricitinib-Dex (20.3%) than Dex alone (40.5%) (Perez-Alba et al., 2021).

Fatima et al. (2020) has observed that corticorsaoid including dexam and methyl are effecting in moderate to severe ill patients. In total of 100 patients, the 35 patients with some on ventilator support (20%) recovered with Dex (8 mg/day, 5 days), while 6 patients died. In case of 65 patients with some on ventilator (12.3%) receiving methylprednisolone (1 mg/kg/day, 2 doses/day, 5 days) recovered, while 10 patients has expired.

Importantly, Dex-remdesivir combo along with critical treatment protocols (blood plasma and ventilation), was reported to be effective in treating severely infected pregnant patients. The patient in ventilation received a varied doses of Dex (20 mg/5 days, 10 mg for another 5 days) followed by remdesivir (200 mg × 1, 100 mg/day for 9 days) (Jacobson et al., 2021). Dex has demonstrated to be effective in reducing the mortality, while recent study caution such corticosteroid treatment with more controlled trial study and need of optimizing the dose regimen (Noreen et al., 2021).

These results suggest that the Hal based nanocomposite has an innate property of a pH sensitive release ability of Dex. A sustained release pattern could be effective at pulmonary infection site and PEG coating can further prevent the toxic effects of Dex to normal cells while in blood circulation until it reaches the target tissue (Scheme 1). Thus, our nanocomposites are a good candidate as a delivery vehicle for Dex.

5. Conclusions

Pulmonary drug delivery systems are being developed to treat of lung infections.

COVID-19 is caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). One of treatment strategy is developing a pulmonary drug delivery system with capability of delivering antiviral drugs specifically targeting host cell interactions or proteins generated by virus. The nanocarrier particles with sizes between 60 and 300 nm is reported to diffuse freely in tracheal mucus of mouse. Biocompatible polymer polyethylene glycol coating of nanoparticle of 100 and 200 nm shown to penetrate respiratory mucus. In present study, a green and eco-friendly clay based halloysite with particle size 50–70 nm have been successfully used as pulmonary nanocarrier for delivery of Dex. ZnFe\(_2\)O\(_4\)/Hal and NiFe\(_2\)O\(_4\)/Hal nanocomposites are successfully synthesized using one-pot method. The technique is simple, reproducible, and scalable. X-ray diffraction, diffuse reflectance and elemental mapping analysis showed the cubic spinel structure with particle size ranging between 6 and 8.6 nm. FTIR confirmed the vibrations of spinel ferrite, Dex conjugation and PEGylation over halloysite. The study shows that the developed ZnFe\(_2\)O\(_4\)/Hal/DEX/PEG drug delivery system is more effective in releasing drug Dex at pulmonary infectious pH condition. It is known that Coronavirus entry into the cell is a pH dependent process (ACE-2 receptor mediated process). SARS-CoV-2 fusion, entry into the cell occurs at pH 5.5. Hence our drug carrier

| Materials     | k/h  | n    | z     |
|---------------|------|------|-------|
| ZnFe\(_2\)O\(_4\)/Hal (pH 7.4) | 1.6188 ± 0.1724 | 0.1671 ± 0.0331 |
| ZnFe\(_2\)O\(_4\)/Hal (pH 5.6) | 1.3668 ± 0.2055 | 0.5415 ± 0.0459 |
| NiFe\(_2\)O\(_4\)/Hal (pH 7.4) | 1.5794 ± 0.1371 | 0.1593 ± 0.0258 |
| NiFe\(_2\)O\(_4\)/Hal (pH 5.6) | 3.6050 ± 0.3010 | 0.0866 ± 0.0249 |
| ZnFe\(_2\)O\(_4\)/Hal/PEG (pH 7.4) | 2.1683 ± 0.2957 | 0.1914 ± 0.0415 |
| ZnFe\(_2\)O\(_4\)/Hal/PEG (pH 5.6) | 2.3090 ± 0.2697 | 0.2332 ± 0.0343 |
| NiFe\(_2\)O\(_4\)/Hal/PEG (pH 7.4) | 2.4441 ± 0.2608 | 0.2241 ± 0.0333 |
| NiFe\(_2\)O\(_4\)/Hal/PEG (pH 5.6) | 1.9441 ± 0.1994 | 0.3249 ± 0.0321 |
which is sensitive to pH 5.6, could effectively deliver drug at this point of pH dependent process. The drug release kinetics at the studied pH values revealed that, the rate of drug release is dependent on the spinel ferrite/Hal nanocomposite and diffusion mechanism is mostly independent of the pH. Our MTT results showed that Dex-loaded nanocomposite at 0.3 mg/ml had a statistically significant improvement in cell viabilities and prevented the toxic effect of Dex. Therefore, the system can be used as pulmonary drug delivery of nanomedicine like dexamethasone, and other potential antiviral drugs.

Fig. 7. A) Cell viability of HEK293 cells treated with the stated conditions using different concentrations for 48 h. B) Statistical significance and p values for each condition compared with the no treatment control.

| Treatment Group          | Concentration |
|--------------------------|---------------|
|                          | 0.0375 mg/ml  |
|                          | 0.075 mg/ml   |
|                          | 0.15 mg/ml    |
|                          | 0.3 mg/ml     |
| Significant P value      |               |
| ZnFe₂O₄/Hal             | No            |
|                          | 0.1340        |
|                          | 0.1373        |
|                          | Yes           |
|                          | 0.0457        |
|                          | Yes           |
|                          | 0.0004        |
| Dex                      | Yes           |
|                          | 0.0103        |
|                          | Yes           |
|                          | <0.0001       |
|                          | Yes           |
|                          | <0.0001       |
| ZnFe₂O₄/Hal/Dex          | No            |
|                          | 0.0750        |
|                          | Yes           |
|                          | 0.0138        |
|                          | Yes           |
|                          | 0.0011        |
|                          | Yes           |
| ZnFe₂O₄/Hal/Dex/PEG      | Yes           |
|                          | 0.0157        |
|                          | No            |
|                          | 0.5166        |
|                          | Yes           |
|                          | 0.0011        |
|                          | Yes           |
|                          | <0.0001       |

Fig. 8. Cell viability of HEK293 cells treated with the stated conditions using (A) dose 2 (0.075 mg/ml), and (B) dose 3 (0.15 mg/ml) for 24, 48, and 72 h.
Fig. 9. A) Cell viability of HEK293 cells treated with the stated conditions using dose 4 (0.3 mg/ml) for 24, 48, and 72 h. Statistical analysis for each group compared with either the control (no treatment) or Dex treated for: B) 24 h, C) 48 h, or D) 72 h.

![Fig. 9](https://example.com/image9)

| Treatment group | Control | Dexamethasone |
|----------------|---------|----------------|
| Significant?   | p value | Significant?   | p value |
| PEG            | No      | 0.5931         | Yes     | <0.0001 |
| ZnFe₂O₄/Hal    | Yes     | <0.0001        | Yes     | 0.0030  |
| Hal            | Yes     | <0.0001        | No      | 0.1628  |
| Dex            | Yes     | <0.0001        | –       | –       |
| ZnFe₂O₄ + Hal + Dex | Yes  | <0.0001        | Yes     | 0.0041  |
| ZnFe₂O₄ + Hal + Dex + PEG | Yes | 0.0012 | Yes | 0.0002 |

Fig. 10. Brightfield images using an inverted microscope of HEK 293 cells treated with the stated conditions using dose 4 (0.3 mg/ml) for 24, 48, and 72 h.

![Fig. 10](https://example.com/image10)

**Author statement**

B. Rabindran Jermy developed the idea, formulated, characterized spinel ferrite/Hal/PEG nanocomposite, characterizations, acquire financial support. Vijaya Ravinayagam developed the conceptualization of drug formulation, delivery, *in vitro*, interpretation. Dana Almohazy, *in vitro* study, dose optimization, experimental discussion and writing part. Ms. Alamoudi, involved in *in vitro* experimental part. H. Dafalla performed the SEM-EDX, elemental mapping analysis. Dr. Sultan Akhtar performed TEM experiments, analysis and wrote discussion part relevant to microscopic technique. Gazali Tanimu performed kinetics study for drug release. All authors reviewed and approved the final manuscript.
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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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B.R. Jermy et al.

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Scheme 1. PEGylated ZnFe₂O₄/Hal nanocomposite for delivering dexamethasone for pH sensitive Dex release.

SARS-CoV-2
Fusion entry cell pH 5.5

Pulmonary
Drug delivery
In-vitro study

ZnFe₂O₄/Hal/Dex/PEG

ZnFe₂O₄/Hal

Dexamethasone (Dex)

Low bioavailability
Toxin at high dose

Pulmonary
Drug delivery

In-vitro study

HEK 293 cells

ZnFe₂O₄/Hal/Dex (0.3mg/ml)

Cell viability
Toxic effect of Dex

OH

OH

Zn(CH₃COO)₂·2H₂O

Fe(NO₃)₃·9H₂O

Halloysite (Hal)

ZnFe₂O₄/Hal

O

O

NH₂

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