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PHARMAKEFTIKI, 37, I, 2025 | 91-101 RESEARCH ARTICLE

Inhalable Mucoadhesive Nanoparticles of Favipiravir as Drug Delivery Systems to the Lungs in Dry Powder Formulations

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ABSTRACT

The inhalation route provides the best alternative to parenteral or oral drug delivery for systemic drug delivery. The drug reaches the lungs through the inhalation technique. It offers numerous advantages, including a rapid onset of action, high bioavailability, improved patient compliance, self-administration, non-invasive nature, and reduced drug degradation due to bypassing first-pass metabolism. This work aimed to develop favipiravir mucoadhesive nanoparticles and deliver them through a dry powder inhaler, which could be the alternative route for administering favipiravir. We prepared the nanoparticles using the nanoprecipitation method, utilizing poly (lactic-co-glycolic acid) and chitosan as biodegradable and mucoadhesive polymers. We used an X-ray diffractometer to identify the crystalline changes of favipiravir in the nanoparticle, and we tested the physical status with a differential scanning calorimeter analysis. The prepared nanoparticle has a spherical shape, 104.6 nm in size, and a zeta potential of -12.7 mV. The nanoparticle has 72.06% entrapment efficiency, with a significant,

sustained drug release of 85.1% at 48 h. With a dry powder inhaler that gave off a dose of 87.02% and a mass median aerodynamic diameter of 2.90 μm, the mucoadhesive nanoparticle did almost no damage to the epithelial integrity. The prepared nanoparticle showed a significant mucoadhesive strength of 77.1% with lung mucosa. Favipiravir mucoadhesive nanoparticle-loaded dry powder inhaler could be the best route for administering COVID-19 to patients.

1. Introduction

The drug delivery to the systemic via inhalation route is the best alternative for parenteral or oral drug delivery. The drug reaching the lungs through the inhalation technique has a lot of advantages, such as the rapid onset of action[1,2], high bioavailability[3], improved patient compliance[4], self-administration, non-invasive nature5, and less drug degradation due to bypass first-pass metabolism.[6] The pulmonary route has been used for the local delivery of drugs like antibiotics, proteins, peptides, chemotherapy therapeutics, enzymes, deoxyribonucleases, and vaccines.[7] This route has many challenges: poor deposition in the respiratory airway, fast clearance from the airway, and incompetent penetration via epithelial and mucosal barriers, which could be the reason for the frequent administration of the drug. Those limitations have been overcome by inhalation techniques using nanocarriers, which have greater empowerment to encapsulate the molecules. It helps penetration into the epithelial and mucosal barriers and enhances the residence of drugs within the pulmonary tract. There are many nanocarrier materials used for the delivery of drugs to the lung; among them, chitosan, PLGA, polycaprolactone, and polylactic acid are the preferred polymers used for encapsulation and enhancing the sustained release of drugs. Favipiravir is a broad-spectrum antiviral prodrug actively metabolized as nucleoside triphosphate by the host. The adenosine nucleoside analog (ANA) could cease the viral RNA polymerase, avoid proofreading by viral exo-nucleases, and subsequently inhibit viral RNA production. There are many ways to deliver favipiravir into the human body, such as intravenous (IV), intramuscular (IM), and oral administration, which have been well-studied.[11] It was found not to be suitable for oral delivery. Besides, it has poor hepatic stability and bioavailability due to the first-pass effect. The IM route also faces many challenges, including variable release from muscle and slow acting in peripheral blood mononuclear cells[12]. Moreover, it is insoluble in water, and Sulfobutylether-β-cyclodextrin (SBECD) is used as a solubility enhancer in IV injection. Since the kidneys excrete SBECB, it is contradicted in patients with severe renal impairment.[13] In the COVID-19 pandemic, the coronavirus has predominantly affected the respiratory tract, especially in the deep lungs. [14] Thus, the inhalation technique might be the promising route to maximize the direct delivery of the drug into deep lungs, bypassing the first-pass metabolism, and it could boost the local antiviral activity. Moreover, many patients could be treated by low doses with less cost than IV injections. Mucoadhesive nanoparticles are administered through the nasal mucosa. They could protect the drug from enzymatic degradation, increase the epithelium uptake, enhance the drug dissolution rate, intensify the contact of the formulation within the mucosa, and act as a controlled release system.[15] Therefore, this work aimed to develop favipiravir mucoadhesive nanoparticles and deliver them through a dry powder inhaler, which could be the alternative route for administering favipiravir.

2. Materials and Methods

Hetero Healthcare Ltd., India provided a gift sample of Favipiravir, a pure substance. PLGA

(lactide: glycolide (50:50), mol wt 30,000-60,000) and chitosan (mol wt:160 kDa) were purchased from Labkart Scientific Solutions, India. Dimethyl Sulfoxide (DMSO), Dichloromethane (DCM), and Tween 60 were purchased from Finar Chemicals, India. All the reagents and solvents used in this research were analytical grade.

2.1. Formulation of favipiravir mucoadhesive nanoparticle:

Favipiravir-loaded mucoadhesive nanoparticles were formulated by the nanoprecipitation method.[16] chitosan was dissolved separately with 0.25% v/v of acetic acid in a bath sonicator for 5 min. About 100 mg of PLGA and 10 mg of favipiravir were dissolved separately in DMSO. The homogeneous mixture was added dropwise into double the volume of the aqueous solution containing 1% v/v of Tween 80. The mixture was subjected to an ultra-probe Q700 sonicator (Qsonica, Cole-Parmer India Pvt. Ltd., India) at 60 kHz for 2 min. The nanoparticle was precipitated in the non-solvent system (water), and the resulting precipitated suspension was stirred in an uncovered condition for 8 h at room temperature. The formed nanoparticle was washed by centrifuge (Remi, India) at 1000 rpm for 30 min to detach the un-entrapped drugs. Finally, the nanoparticle was freeze-dried at -20˚C using a laboratory lyophilizer (Borg Scientific, India) to obtain a dry powder.

2.2. X-ray diffraction (XRD) and Differential Scanning Calorimetry (DSC) studies

An X-ray diffractometer was utilized to identify the crystalline changes of favipiravir in the nanoparticle, and the physical status was tested by DSC analysis.

2.3. Estimation of encapsulation efficiency

The percentage of favipiravir entrapped within the nanoparticles was indirectly determined by centrifugation; the supernatant solution containing free drug was estimated by the RP-HPLC method.[17]

2.4. Determination of size, polydispersity index, zeta potential, and surface morphology of nanoparticle

The nanoparticle's size and polydispersity index (PDI) as evaluated by the dynamic light scattering technique.[18] The zeta potential was examined based on electrophoretic mobility under an electric field using Zetasizer (Malvern Panalytical Ltd., UK). A scanning electron microscope (SEM) was used to study the morphology of nanoparticles. A small number of nanoparticles was spread on a metal stub and coated with conductive gold by Hitachi 1010 ion sputter. The prepared specimen could be observed under the Hitachi 3000 N SEM (JSM 5610 LV SEM, JEOL, Japan) chamber. The image was clicked at an acceleration voltage of 20 kV with a chamber pressure of 0.6 mmHg.

2.5. In-vitro drug release studies

In-vitro drug release of nanoparticles was determined using a cellulose dialysis tube [19] (Himedia Laboratory, India). The nanoparticle (drug equivalent of 2 mg) was dispersed in 5 mL of water and loaded into a dialysis tube composed of cellulose (molecular weight cutoff 35 kDa). Then, the dialysis tube was immersed in a 300-mL dissolution medium at 37 °C under a magnetic stirring of 75 rpm. An adequate volume of the samples was eluted at a different time interval and replaced by an equal volume of the fresh receptor fluid. The eluted sample was centrifuged, and the supernatant's drug content was estimated using the HPLC technique.

2.6. Lung epithelial integrity test

Pulmonary epithelial cell integrity was calculated in the Calu-3 type cell line.[20] In the description, 1×10^5 Calu-3 cells were placed in a polycarbonate-made insert of 24 trans-well plates. The nutrients were supplied to cells with Eagle's essential medium composition with 10% Fetal Bovine Serum (FBS). The transition of epithelial electrical resistance

was monitored daily with a changing medium using an EVOM^2 epithelial voltage/ohm meter (Lab India Instruments, India), and the device value was maintained at 900–1000 ohm/cm^2, which indicates the monolayer formation. About 1 mg/mL of favipiravir-loaded mucoadhesive nanoparticles were dispersed in the growth medium. The electrical resistance was monitored at different time intervals (0, 0.5, 1, 2, 4, 6, 12, and 24 h), with the plain medium as a negative control.

2.7. DPI dispersion performance test

The US Pharmacopoeia provides aerosol performance criteria as previously reported; the aerosol dispersion performance was assessed using the Next Generation Impactor (NGI).[21] The NGI with a stainless-steel induction port and the gravimetric insert cups were used to determine the aerosol dispersion parameters of the DPI. Prior to each experiment, the airflow rate (Q) was measured and adjusted in a flow meter. The NGI was linked to a critical flow controller through a vacuum pump and set to a constant Q = 60 L/minute flow rate. The effective cutoff diameter for each impactor stage for the NGI Q = 60 L/minute was calibrated by the manufacturer and is as follows: stage one (8.06 m); stage two (4.46 m); stage three (2.82 m); stage four (1.66 m); stage five (0.94 m); stage six (0.55 m); and stage seven (0.34 m). To reduce the re-entrapment of particles, the glass fiber filter was kept in the stainless steel and the gravimetric insert cups for stages one through seven. Hard gelatin was loaded with 10 mg of nanoparticles and firmly placed into the induction port of the high-resistance DPI device. The mass of particles deposited into each stage was calculated gravimetrically for each run by measuring the glass filter's mass difference after particle deposition. A mathematical program was used to calculate the mass mean aerodynamic diameter (MMAD) and geometric standard deviation (GSD), fine particle fraction (FPF), respirable fraction (RF), and emission dose (ED). All experiments were performed in a triplicate manner (n = 3).

2.8. Determination of mucoadhesive binding efficiency of nanoparticles

The nanoparticle-mucin binding efficiency was calculated using the UV spectrophotometric method.[22] Briefly, 0.5% v/v mucin was prepared by dissolving mucin in phosphate buffer (pH 6.5). From this, 1 mL of mucin solution was mixed with 2 mL of nanoparticle suspension (10 mg nanoparticles were suspended in 2 mL of distilled water). The mixture was incubated in an incubator at 37 °C for 2 hours and then centrifuged for 2 hours at 20 °C at 35,000 rpm. The supernatant was extracted, the free mucin was calculated, and the spectra were recorded at 255 nm using a UV-visible spectrophotometer (Jasco, Japan). The favipiravir nanoparticle was compared with blanks (Chitosan nanoparticle and PLGA nanoparticle, respectively). The mucin binding efficiency was determined using the following formula.

Mucin binding efficiency (%)=

$$= \frac{Supernatant\ containing\ free\ mucin\ \ x100}{total\ mucin}$$

2.9. Statistical analysis

A two-tailed t-test calculated the nanoparticle evaluation data for statistical significance, and the value was statistically significant when 'p-value ≤ 0.05.

3. Results and Discussion

The pulmonary route of drug delivery is a safe technique for managing pulmonary conditions such as COPD, asthma, lung infections, and cystic fibrosis.[23] Pulmonary route difficulties have been successfully managed by different inhalation devices, namely, dry powder inhalers (DPIs), nebulizers, metered dose inhalers (MDIs), and soft-mist inhalers (SMIs).[23] Among them, DPI is a well-recognized device for the pulmonary delivery of pharmaceutical drugs. Many studies have tried different methods for preparing mucoadhesive polymeric nanoparticles with their

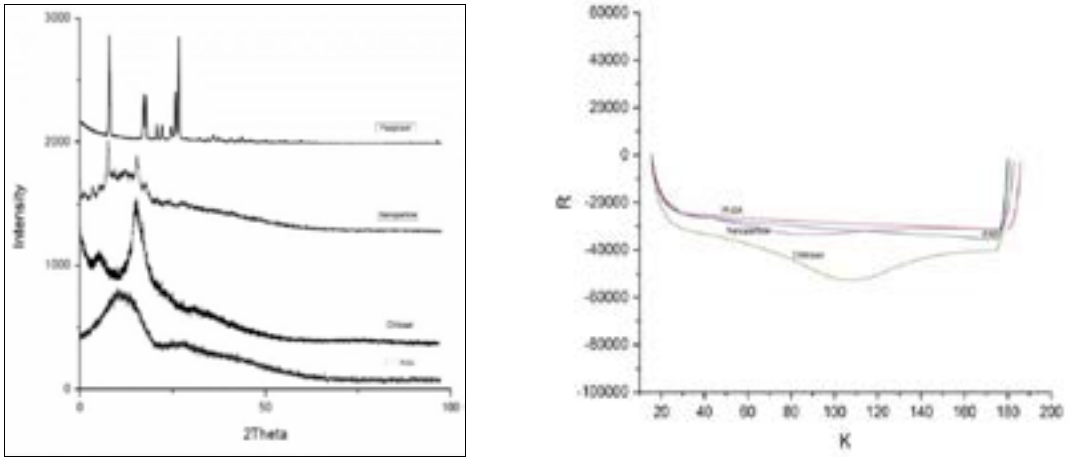
appropriateness for pulmonary delivery.[24] It has been formulated by the nanoprecipitation technique with the help of the Ouzo effect.[25] The effect processed by organic solvent-containing components was precipitated in an aqueous solution to the produced nanoparticles. This process would be enhanced by low frequency in the ultraprobe sonicator. When both phases are mixed properly, the organic phase is back-to-back dispersed as drops within the non-solvent phase to produce nanoparticle precipitate. It is caused by interfacial agitation and thermal variability in the mixture.[26] The X-ray diffraction patterns of the favipiravir nanoparticle are shown in Figure 1(a). The XRD spectra of favipiravir showed 6 sharp peaks at (2θ) 5.37°, 8.45°, 12.61°, 14.45°, 16.05°, and 22.20°, and these sharp peaks confirmed that favipiravir is crystalline. Similarly, chitosan showed two distinguished peaks at 9.63 and 20.53 (2θ), proving its semi-crystalline nature. The favipiravir nanoparticle spectra have less intense peaks, and reference favipiravir is encapsulated as amorphous. The drug loading and co-solvent type did not affect the morphology of favipiravir in nanoparticles. The nanoparticle is smaller, and it causes broad peaks in the diffraction. The peaks have broadened by a few crystalline planes, which, in turn, causes a disappearance of intensity in the diffraction patterns.[27] During the nanoprecipitation, favipiravir was completely embedded in the PLGA and Chitosan network structure. In the observed favipiravir nanoparticle, three sharp peaks disappeared as with pure favipiravir due to the conversion of crystallinity to amorphous nature. The amorphous nature of favipiravir showed excellent absorption and drug release kinetics. The DSC spectra were used to estimate favipiravir glass transition temperature (Tg) in the formulation. The thermograms of favipiravir and nanoparticles are shown in Figure 1(b). Favipiravir showed two exothermic peaks at 135.7 and 173.5 °C and three endothermic peaks at 31.3, 121.7, and 132.6 °C. These sharp peaks confirmed that the favipiravir is crystalline in nature. On the other hand, nanoparticles showed a broad exothermic peak at 138.1 °C, and no other sharp peaks were observed, which concludes that favipiravir was a matrix in polymeric network structure as an amorphous form. Favipiravir nanoparticles provide better dissolution properties in lung fluid that enhance the drug's bioavailability and efficacy when administered through the pulmonary route.[28]

The SEM technique characterized the surface morphology of the nanoparticles. It showed that the formulated nanoparticle has a spherical shape with a smooth surface (Fig.2(a)). When the nanoparticle was administered to the biological fluid, the nano-sized particles were smoothly surrounded by bio-macromolecules, which would modify all the biological characteristics and be eliminated from the biological system by opsonin action. [29] The nanoprecipitation technique formed a uniform shape and size of nanoparticles by the Ouzo effect, which was enhanced by the ultrasonication technique.

Zeta sizer analyzed the nanoparticle size, zeta potential, and PDI of favipiravir nanoparticles. These properties could be the main factor in drug diffusion, permeation, and nanoparticle stability. [30] The prepared nanoparticle has a size of 104.6 nm with a PDI of 0.02 (Fig 2(b)). The zeta potential of the nanoparticle is an important factor for particle-particle aggregation,[31] and the prepared Favipiravir nanoparticles showed -12.7 mV (Fig 2(c)), which is more stable without any aggregation. The ideal zeta potential value of the most stable nanoparticle would be between ±10 and 30 mV. [32] The negative zeta potential value was higher in the nanoparticle preparation due to terminal carboxyl functional groups in the polymers. Lowering the negative zeta potential value is directly proportional to nanoparticle stability. Hence, the prepared mucoadhesive favipiravir nanoparticle was stable.

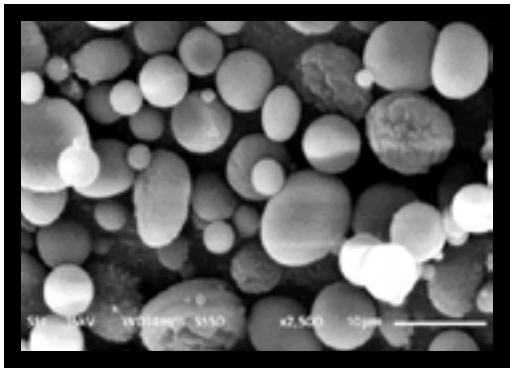
The entrapment of favipiravir in the nanoparticle showed 72.06 ± 0.2% and confirmed that the quantity of the drug would reach the lung to prevent viral replication. The % entrapment efficiency would depend on the concentration of PLGA and chitosan. Since the multi-block copolymer shows more entrapment efficiency than the polymeric chain. More hydrocarbon chains might be the reason for high entrapment efficiency[33].

The drug release profile of favipiravir mucoadhesive

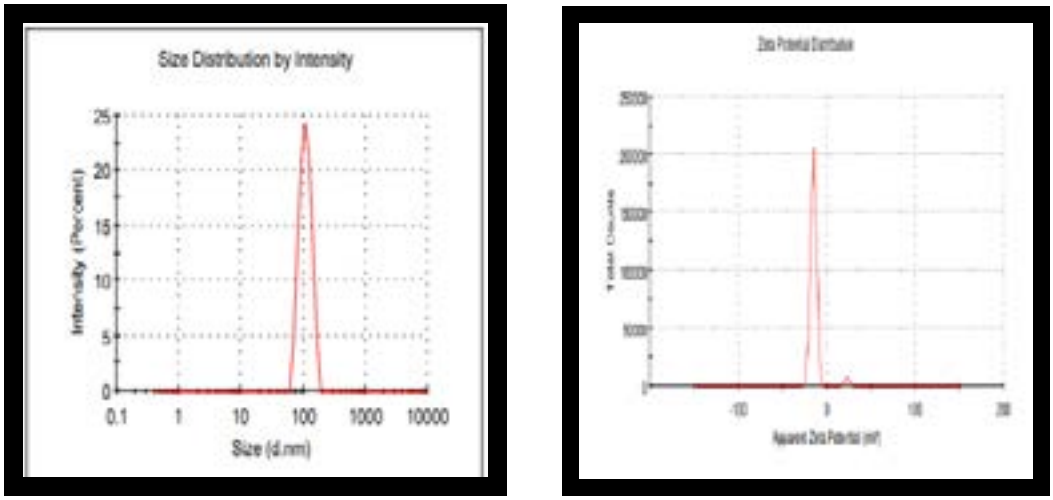


(a) (b)

Figure 1 *(a) XRD spectra of favipiravir mucoadhesive nanoparticles. (b) DSC spectra of favipiravir mucoadhesive nanoparticles*



(a)



(b) (c)

Figure 2 *(a). SEM image of favipiravir mucoadhesive nanoparticles (b). Particle size of favipiravir mucoadhesive nanoparticles. (c). Zeta potential of favipiravir mucoadhesive nanoparticles*

nanoparticles was evaluated by a cellulose dialysis tube, and the released favipiravir was analyzed using by HPLC technique. The precipitated mucoadhesive nanoparticle has a strong matrix network structure of the polymer, and the favipiravir has been entrapped in the matrix, showing significant, sustained release. About 16.2 ± 1.02% drug was released initially at 2 h (Fig. 3). It shows that the low percentage of the drug was released due to the formation of a more solid wall around the drug by polymeric lacto-glycolic-acid linkage.[34] It possesses sustained release for an extended period. At the end of 48 h, a significant amount (85.1 ± 4.18%) of the drug has been released. It confirms that sustained release over the period and that sustained release over the period and stretching satisfied bioavailability. The release follows zero-order kinetic mechanisms ($R^2 = 0.9095$), and Higuchi's equation linearity showed that drug release would be a diffusion mechanism. Peppa's linearity showed non-Fickian anomalous transport from the polymeric matrix into the diffusion medium.

The trans-epithelial electrical resistance of the monolayer of cells in the medium was calculated as 100%, and the resistance was measured after incubating with nanoparticles. Calu-3 cells expressed a 3 ± 0.02% decline at 24 h as compared with the negative control (Figure 4). Pulmonary epithelial integrity is significantly associated with the permeation of virion to the systemic circulation. A high value of trans-epithelial electric resistance showed increased destruction of epithelial membrane integrity.[35] However, favipiravir-loaded mucoadhesive nanoparticles showed negligible differences compared with normal cells. The nanoparticles have been prepared with biodegradable and biocompatible polymers such as PLGA and chitosan, and these polymers do not stimulate proinflammatory cytokines.

The DPI aerosol dispersion of favipiravir mucoadhesive nanoparticles was measured using NGI certified by the USP. Figure 5 shows that detectable deposition occurs in all stages, including the lowest. The DPI aerosol dispersion of nanoparticles exhibited significant deposition on stages demonstrated for middle and deep lung delivery. Favipiravir nanoparticles showed higher deposits in stage 6 (55.14 ± 8.70%) and lesser in stage one (2.18 ± 0.03%). The ED value is very high (87.02 ± 1.38%) and represents aerosolization efficiency; the RF value was 86.18 ± 0.14%. The FPF value is less than 50% (36.14 ± 1.02%), and the marketed formulation of DPI ranges from 10 to 20%. The MMAD value of the nanoparticle was 2.90 ± 0.03, which is ideal for high lung deposition targeting smaller airways (values in the range of 2.5 to 3) (Table 1). The NGI, in conjunction with the DPI device, displayed significant aerosol dispersion performance, indicating that the formed nanoparticles would be ideal for targeted distribution as aerosolized powders by DPI. This confirms the significant potential of DPI aerosols for effectively delivering favipiravir nanoparticles to deep airways in the lungs.[36]

Table 1. DPI Aerosol dispersion performance test

S.No	Aerosol performance parameters	Values (n=3)
1	ED (%)	87.02 ± 1.38
2	FPF (%)	36.14 ± 1.02
3	RF (%)	86.18 ± 0.14
4	MMAD (μm)	2.90 ± 0.03
5	GSD	2.08 ± 0.02

GSD: geometric standard deviation;

ED: emitted dose; MMAD: mass median aerodynamic diameter;

FPF: fine particle fraction; RF: respirable fraction.

All the data was denoted by the mean ± standard deviation (n = 3).

There are several well-established techniques for assessing the mucoadhesion strength of dosage forms, such as pills, gels, and films. Texture analysis is one procedure used to measure the mucoadhesive strength of dosage form in mucous membranes. This approach, which would be precise and robust, may not be appropriate

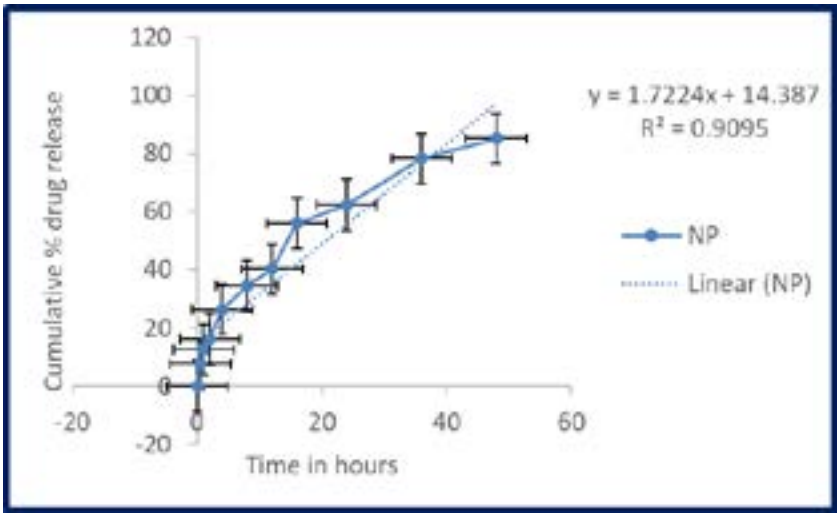


Figure 3. *In-vitro release profile of Favipiravir mucoadhesive nanoparticles.*

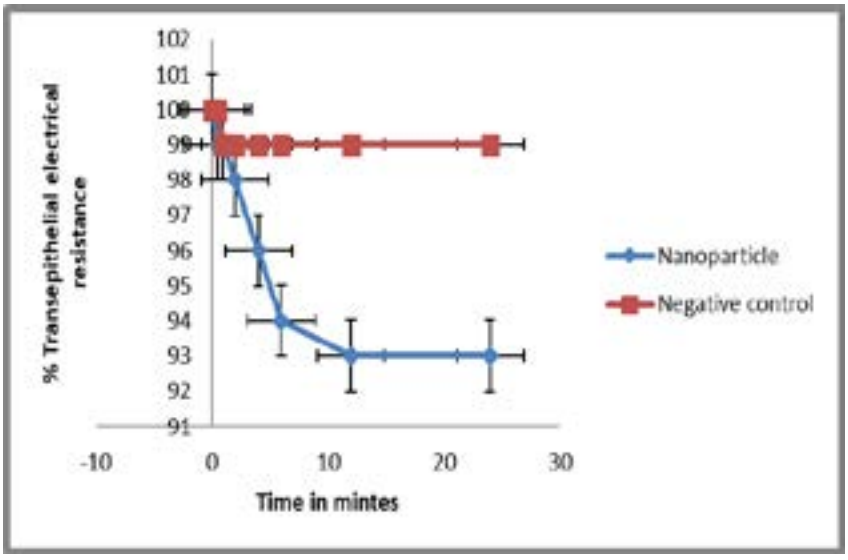


Figure. 4. *Trans-epithelial electrical resistance of favipiravir mucoadhesive nanoparticle*

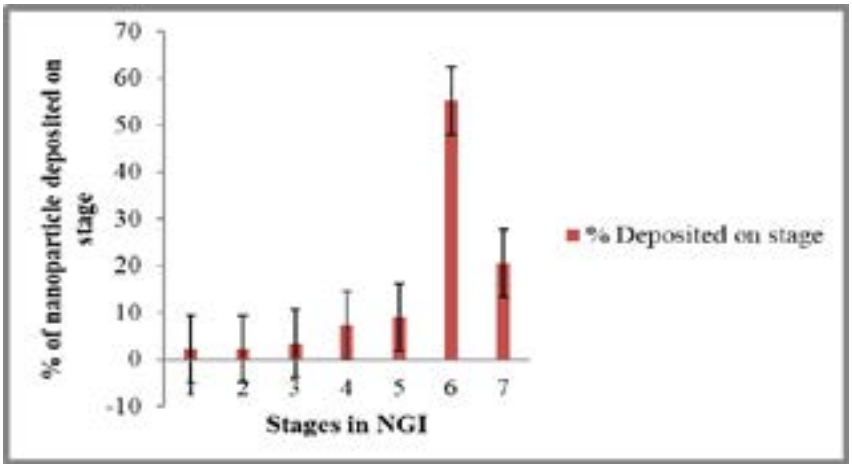


Figure 5. *DPI Aerosol dispersion performance test showed a percentage of favipiravir deposited in every stage of the next-generation impactor.*

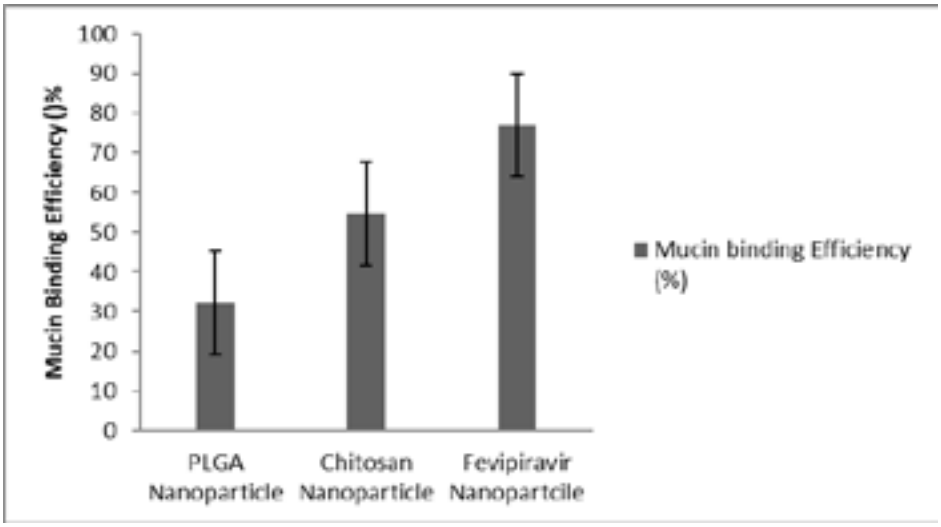


Figure 6. *Mucin binding efficiency of mucoadhesive nanoparticle*

for analyzing nanoparticles, microparticles, or nanocarriers in the nano-size range. [37] As a result, the emphasis has shifted to other methodologies that can measure the mucoadhesive strength by using spectral properties, viscosity, surface characteristics, diffusion coefficient, and so on to evaluate the strength of nanoparticles with mucin. The spectroscopic investigation is the easiest approach for analyzing the interaction of nanoparticles with mucin.

The mucoadhesive nature of favipiravir nanoparticles would be compared with plain chitosan and PLGA nanoparticles, respectively (Fig 6). PLGA nanoparticle showed the lowest binding strength of 32.2 ± 1.2%, and chitosan nanoparticle showed 54.6 ± 5.08% compared to favipiravir nanoparticle, which had the greatest binding effectiveness of 77.1 ± 6.7%. This mucoadhesive nanoparticle has been prepared with the combination of PLGA and chitosan, and these polymers have hydrogen bonds with hydrophilic functional groups (-OH- and -NH_2) in mucin and electrostatic attraction between the positive charge of polymers and the negative charge of sialic acid present in mucin. Even though all the nanoparticles showed mucous binding efficiency, depending on the type of polymer charge and hydrogen bonding, the ideal characteristics of mucoadhesive polymers would be rapid adherence from the mucosal layer without any change in their physical nature, minimize interference of drug release, be biodegradable without producing any toxic by-products, and enhance penetration of the active agents.[38] PLGA, an FDA-approved polymer, has potential as a treatment carrier due to its biocompatibility and biodegradability. However, it cannot specifically interact with cells or proteins, causing drug accumulation issues. To address these limitations, chitosan (CS) was modified to form PLGA nanoparticles, which can form hydrogen and covalent bonds due to its –OH and –NH2 groups. This allows for targeted drug delivery in different human body regions.[39-41]

4. Conclusion

We approached the delivery of favipiravir mucoadhesive nanoparticles by dry powder inhaler. In this attempt, the prepared nanoparticles have the appropriate particle size to reach the lungs and significant mucoadhesive strength on the pulmonary tract with substantial DPI dispersion properties. Therefore, a favipiravir mucoadhesive nanoparticle-loaded dry powder inhaler could be the best and alternative route of administration for the COVID-19 patient population. □

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