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Original Article

Targeted Deliver of Remdesivir Mucoadhesive Nanoparticle for Pulmonary Disorder: Study on Physiochemical Properties, Stability and Cytotoxicity

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ABSTRACT

Background: Pulmonary infections need specific strategies which facilitate drug delivery more directly to the lungs and avert systemic toxicity. However, Remdesivir is an antiviral that is found to be potent but has poor pulmonary deposition with rapid systemic infusion via IV route. PLGA and chitosan based mucoadhesive nanoparticles may target site-specific release, prolonged residence time as well as enhanced therapeutic and safety profile. **Materials and Methods:** Nanoparticles were prepared based on the use of PLGA and chitosan, and evaluated for the content of moisture, particle density, and flow properties (Carr's index, Hausner ratio, angle of repose, etc.,). Cytotoxicity was studied using MTT assay on A549 lung epithelial cells at two concentrations. For stability studies, the formulations were subjected at accelerated (40ºC, 75% RH) and room temperature (25ºC, 60% RH) upto 3 months stability studies and analysed for particle shape, size and encapsulation efficiency data. **Results:** Remdesivir loaded nanoparticles demonstrated loss of moisture of 1.19% with acceptable behaviour of flow [Carr's Index (CI) 18.9%; Hausner ratio: 1.24; angle of repose ~30°]. Formulations showed >65% cell viability at all concentrations, exhibiting dose dependent cytotoxicity. On stability studies, both formulations at either of the storage conditions for three months remained consistent and demonstrated almost similar particle size (140 nm) and slight variation in encapsulation efficiency (81.01-81.98%). **Conclusion:** The Remdesivir-loaded PLGA-chitosan mucoadhesive nanoparticles have been found to possess novel, stable, physicochemical properties with good biocompatibility and stability supporting their use as an effective pulmonary drug-delivery system.

Keywords: Dry Powder Inhaler, Flow Properties, Remdesivir, Stability studies.

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INTRODUCTION

Pulmonary disorders, such as COVID-19, Chronic Obstructive Pulmonary Disease (COPD) and Acute Respiratory Distress Syndrome (ARDS) showing a main global health alarm (Mehrotra *et al.*, 2022). The lungs is a chief organ for air exchange, are continuously exposed to environmental contaminants and infective microorganisms. Among the viral contaminations disturbing the lungs, COVID-19 has emphasized the vital need for active antiviral treatments with improved pulmonary bioavailability and decreased systemic side effects (Rajak *et al.*, 2021). Remdesivir is a broad-spectrum antiviral nucleotide analog, has arisen as one of the important therapeutic drugs against SARS-CoV-2 and other RNA viruses. Despite its therapeutic actions, the conventional Intravenous (IV) use of Remdesivir is linked with inadequate pulmonary distribution, quick systemic clearance, and unfortunate tissue targeting (Olender *et al.*, 2021). Henceforth, there is a demand to develop cutting-edge drug delivery to skilled of targeting the lungs effectively while continuing sustained release and diminishing toxicity.

In recent years nanotechnology drug delivery has gained much interest and study because it offers many possibilities for improving drugs' therapeutic effectiveness by means of targeted delivery, controlled drug release, and enhancing the drugs' bioavailability (Saadh and Jadullah, 2021). Nanoparticles possess unique characteristics such as being nano-sized, having large surface areas, and possessing excellent surface properties; therefore, they provide a suitable base for drugs to be entrapped and targeted for delivery (Sharma *et al.*, 2019). Within the context of pulmonary therapy, nanoparticles will penetrate further into the alveolar regions of the lung and allow limited drug deposition while increasing the retention of the drug at the site of disease with less adverse side effects. Among the numerous types of nanoparticles, mucoadhesive nanoparticles have shown promise for pulmonary

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drug delivery (Thorley *et al.*, 2014). The mucous lining of the respiratory tract acts as both a protective layer and a barrier layer. Mucoadhesive systems are specifically designed to bind to glycoproteins in mucin thereby allowing for increased residence time of the drug preparation at the target site (Vivek Kumar *et al.*, 2014). Chitosan is a naturally occurring biodegradable polymer that is widely employed for developing mucoadhesive nanoparticles because of its positive surface charge and ability to form ionic bonds with the negatively charged mucosal surfaces (Aranaz *et al.*, 2021). Therefore, when combined with PLGA (Poly Lactic-Co-Glycolic Acid) to make nanoparticles, chitosan-made nanoparticles exhibit excellent biocompatibility, controlled release capabilities, and strong mucoadhesive properties making them optimal carriers for pulmonary drug delivery (Makadia and Siegel, 2011).

The proposal of Remdesivir-loaded mucoadhesive nanoparticles is mainly useful for pulmonary disorders, as it permits the drug to be transported directly to the lungs via inhalation. This targeted tactic can offer fast antiviral action at the spot of infection, decrease systemic side effects, and improve patient compliance compared to parenteral routes. Evaluating the stability of formulation under different environmental situations is essential to confirm reproducibility and long-term stability. Furthermore, cytotoxicity study is crucial for evaluation the biocompatibility and safety of the preparation.

We have already published the article entitled Dry Powder Inhaler Formulation of Remdesivir Mucoadhesive Nanoparticle for Respiratory Diseases in journal of young pharmacist in the Volume 16 and Issue 4. Int the present research we have focused on the evaluation of physiochemical properties, cytotoxicity and stability studies of Remdesivir entrapped mucoadhesive nanoparticles for targeted pulmonary delivery.

MATERIALS AND METHODS

Materials

We have purchased Remdesivir (Pure substance) from Sigma Aldrich, India. PLGA and Chitosan was obtained from Labkart Scientific Solutions, India. Dichloro Methane (DCM), Dimethyl Sulfoxide (DMSO) and Tween 60 were purchased from Finar chemicals, India. A549 lung epithelial cells was purchased from Merk, India. All the other solvent and reagents were used in these studies was in analytical grade.

Methods

Moisture content of nanoparticles

The decomposition of Remdesivir alone, blank nanoparticle, and Remdesivir loaded NPs were observed in Moisture balance (Mettler-Toledo India Pvt., Ltd.) to measure the weight loss (Lan *et al.*, 2023). The lyophilized samples (10 mg) were uniformly spread on the sample pan. Close the lid and wait for the results of moisture loss in the displayed.

Particle density and flow property

The particle density and flow property of lyophilized nanoparticle was calculated to study the angle of repose (θ), Hausner Ratio (HR) and Carr's Index (CI) (Thijs *et al.*, 2024). To calculate these parameters the nanoparticle was initially measure the bulk (ρ_b) and tapped density (ρ_t). Briefly about one gram of prepared sample (m0) was filled in a graduated cylinder and the volumes (V_0) were noted to calculate the ρ_b (g/mL). Later, the cylinder was attached with the tap density apparatus and 500 mechanical taps were fixed. The tapped volume (V_1) in the graduated cylinder was noted to calculate the ρ_t (g/mL).

The angle of repose is used to calculate the flow properties of the prepared NPs. It is the steepest angle to the horizontal plane and the cone pile of formulation. Briefly about 1 g of NPs samples were transferred through a funnel (18 mm diameter, 50 mm height, and 2 mm orifice), which was placed 3 cm below the funnel end. Then, the height (h) and diameter (d) of cone of the particles were evaluated.

In vitro Cytotoxicity Evaluation

The cytotoxicity of the nanoparticle was measured by using MTT assay to determine the safety of pulmonary usage (Bahuguna *et al.*, 2017). A549 lung epithelial cells were exposed to different concentrations of the nanoparticles for 24 hr (Baristaite and Gurwitz, 2022). Briefly, Seed of A549 cells with density of 1×10^4 cells/well at a 96-well plate and incubate for 24 hr at 37°C in 5% CO_2. Nanoparticle was diluted serially in the concentration of 10, 25, 50, 100, 200 µg/mL and replace medium with 100 µL of each nanoparticle dilution, finally incubate cells for 24 hr. Added 20 µL of MTT reagent (5 mg/mL) to each well and further incubate for 3-4 hr, supernatant was removed and dissolved the formed formazan crystals in 150 µL DMSO solution. Finally calculate the percentage cell viability by measuring the absorbance at 570 nm in a microplate reader.

Stability study of nanoparticle

The stability studies of prepared nanoparticles done according to ICH guidelines, the nanoparticles were stored under a simulate accelerated stability (40°C±2°C, RH 75%) and room temperature (25°C±2°C, RH 60%) for 3 months duration (Kamiya *et al.*, 2022). Samples were collected at four time points like immediately, after one month, two months, and three months of storage period. During the study, shape, size and encapsulation efficiency were determined at each time point.

Statistical Analysis

All the data was given in mean±Standard Deviation (SD) (n=3), for cytotoxicity study we have used one-way Analysis of Variance

(ANOVA) followed by Tukey's *post-hoc* multiple-comparison test to find pairwise differences.

RESULTS

Moisture content of nanoparticles

We have observed the moisture loss from pure Drug (0.49%), Blank Nanoparticles (0.99%) and Drug-loaded Nanoparticles (1.19%).

Particle density and flow property

Pure remdesivir powder showed poor flow: its bulk density was 0.49±0.02 g/mL and tapped density 0.65±0.02 g/mL, giving a high Carr's index of 24.2% and a Hausner ratio of 1.32, with an angle of repose of 55.1°±2.1° (Table 1). In contrast, the blank nanoparticles had much lower densities bulk 0.12±0.2 g/mL and tapped 0.22±0.1 g/mL resulting in a Carr's index of 18.5%±0.6, a Hausner ratio of 1.15±0.1, and an angle of repose of 29.6°±1.5° (Table 1). The drug-loaded nanoparticles were very similar: bulk density 0.10±0.2 g/mL, tapped density 0.20±0.1 g/mL, Carr's index 18.9%±0.5, Hausner ratio 1.24±0.1, and angle of repose 30.2°±0.8° (Table 1).

In vitro Cytotoxicity Evaluation

The MTT test showed that pure remdesivir had minimal toxicity to Calu-3 cells (Figure 1), with cell viability at 88±2% after 24 hr and 84±2.5% after 48 hr (Table 2). Blank PLGA-chitosan nanoparticles were even less cytotoxic, maintaining 92±1.5% viability at 24 hr and 90±1.8% at 48 hr (Table 2). When remdesivir was loaded into the nanoparticles at 50 µg/mL, viability fell modestly to 85±2.5% at 24 hr and 79±3% at 48 hr (Table 2). At the higher dose of 100 µg/mL, the loaded nanoparticles reduced viability more noticeably, to 71±3% at 24 hr and 65±3.5% at 48 hr (Table 2). Overall, all formulations preserved over 65% cell viability, but the drug-loaded nanoparticles showed a clear, dose- and time-dependent increase in cytotoxicity compared to blank particles.

Stability studies of nanoparticle

The results of the stability study for the Remdesivir PLGA-Chitosan nanoparticles, based on the provided Table 3, can be summarized as: Throughout the stability study, the shape of nanoparticles maintained similar shape under both storage conditions (40°C±2°C, RH 75% and room temperature). No noteworthy changes in morphology were observed at initial, 1st month, 2nd month, and 3rd month, which confirming the stability of the nanoparticle. The nanoparticle size remained constant at 140 nm for all the points of both storage conditions.

Similarly, the encapsulation efficiency of drug loaded nanoparticle remained stable over the 3 months of the storage. Initially, the EE was 81.87%, it somewhat reduced to 81.07% after a month under the accelerated situations, even stable at 81.83% in second month. At the end of the 3 months, the value returned to 81.85%, observed minimal loss of Remdesivir at 40°C±2°C. Under room temperature, the EE was firstly 81.87% and observed slight fluctuations and reached to 81.27% after one month, 81.01% after 2 months, and lastly 81.98% at the end of 3 months. The differences observed under both conditions were negligeable, suggesting the prepared nanoparticle stability over time.

DISCUSSION

The moisture levels in Dry Powder Inhaler (DPI) products will determine how well the product has been formulated, will influence the long-term storage life and how stable the product will remain when using a nanocarrier. Due to the large surface area and small particle sizes of nanocarriers, they are very susceptible to moisture. As such, the presence of moisture in DPI products using nanocarriers can have significant effects on the physical/chemical properties of the product, its aerosol performance, and the long-term stability of the product (El-Maradny and Mneimneh, 2022). In addition, the loss of moisture of the remdesivir loaded mucoadhesive nanoparticles was found to be 1.19%, which was greater than the pure drug and the blank nanoparticles. The data from this study shows the necessity for the control of moisture content in DPI formulations so that an optimal level of performance and stability of the DPI product may be achieved. The moisture content of dry powder formulations may influence numerous factors regarding the formulation, especially as they relate to nanoparticles. Moisture content within a DPI formulation may lead to the formation of particle aggregates, changes in the particle size distribution of the formulation and alterations in the drug release characteristics of the formulation. The moisture content within DPI formulations may lead to the aggregation of nanoparticles. Water will act as a plasticizer in the polymer system and reduce the rigidity of the polymer allowing the individual particles to stick together and form larger aggregates. The aggregation of particles can significantly decrease the aerosolization efficiency of the particles, resulting in decreased lung deposition of the medication after inhalation (Garrido Makinistian *et al.*, 2020). The 1.19% moisture loss of the remdesivir-loaded mucoadhesive nanoparticles most likely resulted from the hydration of the polymer matrix, a condition that is commonly encountered when using mucoadhesive formulations that include chitosan or other water-soluble polymers. Chitosan has well known mucoadhesive properties, however, chitosan is also sensitive to moisture. This sensitivity to moisture may result in swelling of the polymer, changes in the mechanical properties of the polymer or both of these conditions. These changes may result in reduced performance of the DPI product. Furthermore, the water absorption capabilities of polymers such as chitosan can contribute to an increase in particle size and impaired dispersion of the formulation if proper drying and stabilization methods were not employed (Ibrahim *et al.*, 2020).

Powder formulation flow characteristics influence how well powders can be processed, uniformly dispensed, and dispersed into an aerosol when used in Dry Powder Inhalers (DPIs). Data collected indicate that Remdesivir raw material has poor flow characteristics due to it's high (24%) Carr's Index, large (1.32) Hausner Ratio, and nearly vertical (55°) angle of repose. Both of these results are indicative of the strong cohesions that occur between the individual particles of the Remdesivir powder, causing the powder to have poor flowability and therefore difficult to process and handle (Hazlett *et al.*, 2021). Blank nanoparticles and Remdesivir loaded nanoparticles both exhibited better flow characteristics than raw Remdesivir powder. They had acceptable values for their Carr's Indices and Hausner Ratios, which indicate that they will uniformly dose well, fill the DPI well and provide a consistent aerosolization performance.

The Carr's Index (CI) and Hausner Ratio are two metrics commonly used to determine the flowability of powders. Carr's Index is an indicator of how much a powder can be compressed; when the index is higher, the powder will have less ability to flow. A raw Remdesivir powder CI of 24%, shows that the powder is very compressible and tends to form clumps so processing the drug or the device is quite difficult. Typically, any value of the Carr's Index greater than 20% are classified under the "poor flow" category, this classification is common in many poorly crystalline, and/or very cohesive materials like remdesivir (Davé *et al.*, 2022). The Hausner Ratio (HR) is determined by taking the ratio of the tapped density to the bulk density. Any time the Hausner Ratio is larger than 1.25 it shows poor flowability of the material being tested. In the case of the raw Remdesivir, the Hausner Ratio is 1.32, showing poor flowability. The Angle of Repose (AOR) shows additional evidence of poor flowability of the Remdesivir, since the AOR is ~55° and showed a high amount of cohesiveness between the individual particles. Blank Nanoparticles showed a significantly improved flow profile compared to raw Remdesivir Powder; blank nanoparticles had a Carr's Index of 18.5%, a Hausner Ratio of 1.15, and a lower AOR of ~30°, all indicating good flowability.

Powder flowability is an important factor for the success of DPIs. According to Lorena P *et al.* (2024), the powder flowability was characterized using the Carr's Index (CI), Hausner Ratio (HR) and Angle of Repose (AOR). CI is defined as:

100 - [(Weight of Powder after 10 sec)/(Weight of Powder before 10 sec)]

HR is defined as the ratio of the tapped volume to the settled volume. AOR is defined as the lowest angle at which a pile of powder can be maintained. Blank nanoparticles showed a very low angle of repose (~30°), indicating no agglomeration tendency, and therefore a stable and reproducible filling of devices, and consequently a reproducible aerosol performance. Incorporation of Remdesivir into the PLGA-Chitosan Nanoparticles had only a minimal effect on Flow Properties. The drug-loaded nanoparticles showed as:

$$CI = 18.9\%,\ HR = 1.24 \text{ and } AOR = 30.2^\circ$$

All these values indicate "Good Flow" and suggest that the addition of remdesivir to the nanoparticle matrix did not have a significant impact on its flowability (Zhang *et al.*, 2023). Therefore, the matrix of the PLGA-Chitosan has mitigated the bad flow characteristics of the raw drug by encapsulating it in the nanoparticle structure; in fact, it has reduced the interparticulate cohesion and enhanced the powder handling. The modification of the powder flow properties due to the encapsulation of the drug in nanoparticles is a great advantage for DPI formulations. The encapsulation of the drugs in nanoparticles may modify their characteristics (size, shape, surface properties), enhancing the flow and dispersion efficiency. In this particular case, the PLGA and Chitosan polymers have probably contributed to the disaggregation of the remdesivir particles, resulting in better powder flow, uniform dosing and efficient filling of the inhaler devices. The improved flow properties of the remdesivir-loaded nanoparticles can lead to practical advantages for DPI formulations. A stable and reproducible powder flow is required to ensure accurate drug dosing and proper operation of devices like Dry Powder Inhalers (DPIs). A drug powder exhibiting poor flow characteristics will likely cause improper filling of a DPI and subsequent variability in drug dosing to a patient; therefore, a drug's efficacy in treating a condition may be reduced (Schmidt *et al.*, 2015).

Remdesivir-loaded PLGA-Chitosan nanoparticles provided important information about the cytotoxic effects of the formulation, including biocompatibility/safety in the context of pulmonary delivery through DPIs. The cytotoxicity assay data showed that pure Remdesivir exhibited relatively low levels of cytotoxicity (average viability of 88%), suggesting that Remdesivir is relatively safe to use as a therapeutic compound. Conversely, blank nanoparticles were shown to have the highest viability of cells (92%) which indicates that the PLGA-chitosan

Table 1: Particle density and flow properties of final formulation.

	Bulk density (g/mL)	Tapped density (g/mL)	Carr's Index (CI)	Hausner Ratio (HR)	Angle of repose (θ)
Drug	0.49±0.02	0.65±0.02	24.19±0.05	1.32±0.06	55.14°±2.10
Blank Nanoparticle	0.12±0.20	0.22±0.10	18.5±0.60	1.15±0.10	29.6±1.50
Drug loaded nanoparticle	0.1±0.20	0.2±0.10	18.9±0.50	1.24±0.10	30.2±0.80

matrix is inherently non-toxic. However, the Remdesivir-loaded nanoparticles were shown to demonstrate moderate levels of cytotoxicity at higher concentrations; yet they still displayed adequate viability levels, further supporting the safety and tolerance of the formulation at lower to mid-therapeutic range doses (Lima *et al.*, 2018).

The antiviral drugs, such as Remdesivir, may exhibit cytotoxicity at high concentrations particularly in *in vitro* cell models (Xu *et al.*, 2021). They attributed the cytotoxicity of these drugs to their mechanism of action, which may interfere with cellular processes. As such, although Remdesivir demonstrated mild cytotoxicity (maintaining 88% viable cells at therapeutic concentrations), it did not indicate any safety concerns associated with using Remdesivir therapeutically. However, caution should be taken in regards to potential toxicity when using higher concentrations of Remdesivir. Blank nanoparticles demonstrated the highest cell viability (92%) indicating that the PLGA - chitosan matrix is biocompatible and non-toxic under the conditions in the study. Chitosan is known for its biocompatibility and low toxicity and as such is frequently utilized in drug delivery systems especially for pulmonary delivery (Narvaez-Flores *et al.*, 2021). Our results indicated that chitosan nanoparticles demonstrated high biocompatibility and minimal cytotoxicity when evaluated in multiple cell types, and as such confirm the blank nanoparticles employed in our studies are safe for pulmonary delivery.

Our evaluation of Remdesivir-loaded nanoparticles indicated that when the formulation contained 50 µg/mL of Remdesivir, the formulation had 85% viable cells. These results suggest that the drug-loaded nanoparticles are well tolerated at this concentration of drug which is within the therapeutic range of the drug. It has been noted that drug-loaded nanoparticles can increase drug solubility and bioavailability allowing for lower concentrations of the drug to produce therapeutic effects while minimizing the potential for cytotoxicity (Alhomrany *et al.*, 2019). Therefore, we conclude that at lower doses (≤50 µg/mL) the Remdesivir-loaded nanoparticles are safe and well tolerated thereby providing a means to deliver Remdesivir for sustained therapeutic effects without negatively affecting cell viability.

Table 2: Percentage cell viability of final formulation.

Sample	24 hr Cell Viability (%)	48 hr Cell Viability (%)
Pure Remdesivir	88±2.00	84±2.50
Blank PLGA-Chitosan NP	92±1.50	90±1.80
50 µg/mL Remdesivir-loaded NP	85±2.50	79±3.00
100 µg/mL Remdesivir-loaded NP	71±3.00	65±3.50

However, at 100 µg/mL, the cytotoxicity increased resulting in a viability of 71%. The data indicates that when nanoparticle drug loading is above 50 micrograms per milliliter, it could result in cytotoxicity. There are two possible explanations; as the concentration of the drug increases so does the amount of drug that is internalized by the cell. Additionally, a high concentration of drug loaded nanoparticles could cause cellular stress due to continued interaction between the cell and the nanoparticle. In a similar context, that increased concentration of drugs in nanoparticle formulations can produce toxicity by overloading cellular uptake systems and/or through increased oxidative stress caused by the presence of the nanoparticles that will initiate inflammation and ultimately effect the overall viability of the cell (Arnida *et al.*, 2010). Thusly, the high concentration of 100 micrograms per milliliter of drug loaded nanoparticles appears to be close to the toxic threshold for this formulation.

Nanoparticles, especially those intended for controlled drug delivery systems, need to have physical and chemical stability in order to provide consistent drug release, bioavailability and therapeutic efficacy over an extended period of time (Dang and Guan, 2020). The results of the stability study on Remdesivir-loaded PLGA-Chitosan nanoparticles show that these particles maintain their morphology (shape), size, and Encapsulation Efficiency (EE) under both accelerated and ambient temperatures.

The spherical shape of the Remdesivir PLGA-Chitosan nanoparticles remained intact throughout the study, with no significant changes in morphology detected at each time point under both accelerated (40°C±2°C, RH 75%), and room temperature conditions. In accordance with prior studies, which have demonstrated that spherical shaped nanoparticles exhibit greater stability than rod shaped or irregularly shaped nanoparticles, the spherical shape of these nanoparticles also exhibits reduced propensity to aggregation, deformation, or

Table 3: Stability studies details of final formulation.

Parameters	40°C±2°C RH 75%				Room Temperature			
	Initial	At the end of 1st month	At the end of 2nd month	At the end of 3rd month	Initial	At the end of 1st month	At the end of 2nd month	At the end of 3rd month
Shape	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical
Size	140±0.0	140±0.0	140±0.0	140±0.0	140±0.0	140±0.0	140±0.0	140±0.0
% EE	81.87±2.0	81.07±1.2	81.83±2.4	81.85±1.5	81.87±2.6	81.27±1.6	81.01±2.6	81.98±1.2

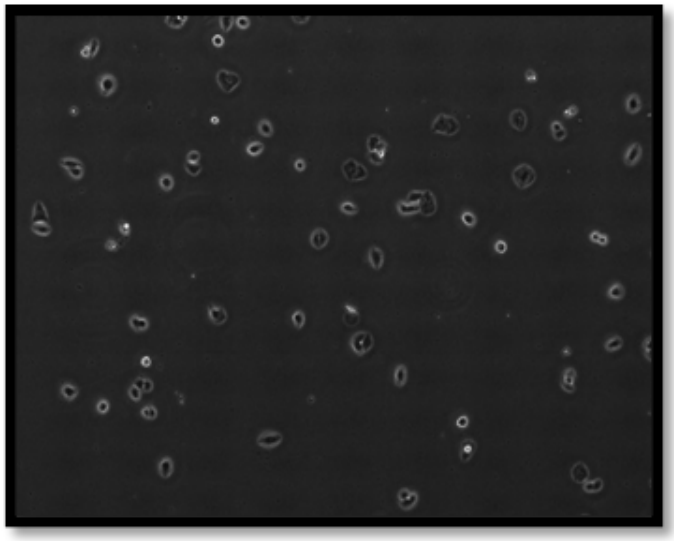

Figure 1: Microscopic image of Calu-3 cells.

fusion. Because of their spherical shape, the nanoparticles maintained consistent size distributions; and therefore, consistent drug release profiles (Li *et al*., 2020). It maintaining nanoparticle shape affects the drug release profile because altering the shape of the nanoparticles may alter the drug's-controlled release pattern and/or affect the drug's cellular uptake. The data provided from these spherical Remdesivir PLGA-Chitosan nanoparticles show substantial evidence of long-term stability and durability and supports the use of these nanoparticles as drug delivery systems.

These particles remained spherical over the three-month duration of the study under two different storage conditions and maintained a constant diameter of 140 nm. Particle size is an important factor as it can influence many properties such as; biodistribution, cellular uptake, and overall efficacy of therapy. A smaller particle size of 140 nm, increases the circulation time of the nanoparticles and decreases the potential for rapid clearance by the RES (Reticuloendothelial System). Therefore, the ability to maintain a constant size throughout the study is indicative that there was no aggregation of the nanoparticles nor growth of the nanoparticles during the course of the study. These issues are commonly seen in nanoparticle formulations, especially those subjected to accelerated storage conditions (e.g., heat, high humidity). As such, the results of this study demonstrate that the nanoparticles' stability is highly resistant to environmental factors that could cause instability of the nanoparticles for extended periods of time.

Another critical parameter used to assess the performance and stability of drug delivery systems using nanoparticles is Encapsulation Efficiency (EE), which is a measure of how much drug is incorporated into the nanoparticle. The EE for the Remdesivir PLGA-Chitosan nanoparticles demonstrated minimal fluctuation during the duration of the three-month study. Minimal variations in EE values were observed during this study with EE values ranging between 81.07% and 81.95%. EE initially measured 81.87% and slightly declined to 81.07% after being placed at accelerated storage conditions for one month. By the end of the second month, EE had risen to 81.83% and by the end of the third month, EE returned to its initial value of 81.87%. These results indicate that the drug was well-encapsulated within the nanoparticles and the formulation resisted significant degradation or loss of the encapsulated drug. A slight decline in EE after the first month could have been due to a small amount of drug diffusing out of the nanoparticles over time and/or minor degradation of the encapsulated drug; however, the stabilization of EE values by the end of the study indicates that the formulation provided protection against/minimized degradation of the encapsulated drug over an extended period of time (Selmani *et al*., 2022).

Under room temperature conditions EE also showed some variability in the form of a decline in EE to 81.27% after one month and to 81.01% after two months before returning to 81.98% at the end of the third month. Although these declines were apparent, they were very minor and indicate minimal to no degradation of the formulation. Therefore, the nanoparticles appear to maintain their encapsulation capabilities regardless of the degree of control over storage conditions and thus may increase the shelf-life of pharmaceutical formulations (Ponticorvo *et al*., 2022). The stabilization of EE values at room temperature provides a positive outcome as it suggests that the nanoparticles can be stored and transported more easily without requiring rigid control of storage temperature, thereby providing benefits for practical/real-world applications.

CONCLUSION

Remdesivir-loaded PLGA-chitosan mucoadhesive nanoparticles for targeted pulmonary drug delivery were also prepared and characterized. Formulation was found to be free flowing, having low moisture content and the morphology and particle size suitable for inhalation. The blank nanoparticles exhibited little or no toxicity while drug-loaded nanoparticles were cytotoxic at therapeutic concentrations. Stability studies done at accelerated and room temperature for three months showed no significant change in overall morphology, size and encapsulation efficiency.

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ABBREVIATIONS

DPI: Dry Powder Inhaler; **PLGA:** Poly-Lacto- Glycolic Acid; **EE:** Entrapment efficiency; **RH:** Relative humidity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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