Novel pediatric suspension of nanoparticulate zafirlukast for the treatment of asthma: Assessment and evaluation in animal model

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Abstract
The aim of the present study was to formulate the novel pediatric suspension containing nanoparticulate Zafirlukast (ZFR) for the treatment of pediatric asthma. The ZFR loaded nanoparticles (NPs) were formulated by ionotropic external gelation method using tripolyphosphate (TPP) and Tween 80. NPs were characterized for drug loading, encapsulation efficiency, surface morphology, saturation solubility, particle size, zeta potential and polydispersity index (PDI). The optimized NP formulation was used for the development of suspension. The suspensions were characterized for pH, viscosity, sedimentation volume, dissolution and drug content. The encapsulation efficiency of NPs was found between 93.24% and 98.57% with drug loading in the range of 26.50–35.90%. All formulations were found of nanosized in nature (150–220 nm) with zeta potential (19.18–21.41 mV). PDI of all NP formulations was found less than 0.3. The NPs were spherical and smooth in nature. The saturation solubility of ZFR was enhanced nearly 16 times as compared to pure ZFR. The optimized suspension showed almost 100% ZFR release within the period of 30 min. The reduction in lung resistance (RL) was found nearly four fold as compared to the control group animals. The study supported the efficacy of suspension containing nanoparticulate ZFR in asthmatic animals.

1 | INTRODUCTION

Asthma is a persistent airway inflammatory condition that is marked by reversible blocking of airflow and bronchospasm. Popular asthma signs include wheezing, coughing, chest tightness, and breathlessness [1]. The underlying disorder that may develop in adult and pediatric patients is asthma. Their first asthmatic exposure can be terrifying, for some children with asthma: intense wheezing, a tight chest, and shortness of breath can easily catch their active, young bodies off balance [2]. Childhood asthma, also referred to as pediatric asthma, is known as one of the most prevalent yet difficult to diagnose, which causes severe diseases in infants. As claimed by the Centre for Disease Control and Prevention, about 6 million children may be affected by the disease [3]. This type of asthma is characterized by the dysfunctioning of muscles coupled with chronic inflammation. This inflammation may be the product of widespread inflammatory cell invasion of the airway, including T cells, mast cells. In some patients, it can manifest as wheezing, fast breathing, cough, diminished stamina, the sensation of exhaustion, and fatigue [4]. Childhood asthma displays some major asthmatic attack trends, such as seasonal, after exposure to typical asthma stimuli, at night or early morning, between or after physical activity. Any of the signs of an illness can vary from those of adults [5]. Diagnosis of the condition is quite complicated since most other disorders (such as rhinitis, sinusitis, and infections of the respiratory tract) often include signs of asthma. Using a lung function examination, also called spirometry, which measures the volume and duration of air exhaled by the infant, the diagnosis may be carried out. Disease management entails monitoring and management through the use of multiple agents. Leukotriene inhibitors, theophylline, inhaled corticosteroids, and cromolyn sodium can be some of the therapeutic agents [6]. A safe, easy to use, and patient-friendly pediatric drug delivery system is essential for the management of pediatric asthma. Oral liquid suspension is the most accepted and compatible dosage form for pediatric patients due to the higher
compatibility [7]. Oral liquid formulas are usually offered to young children so they are easy to swallow, such as solutions and suspensions. They are, however, typically packaged in multidose packages that require storage and specific dose calculations. The precision of dosing is strongly determined by the volume to be measured and the type of dosing instrument to be measured. A minimum volume is yet to be specified for precise dosing with an oral syringe, measuring spoon, or a cup [8].

Nowadays, nanoparticle engineering processes have been developed and reported for pharmaceutical applications to increase the dissolution rate of low-soluble drugs, which in turn may lead to substantial increases in the bioavailability [9]. Nanoparticle engineering enables poorly soluble drugs to be formulated as particles alone or with a combination of pharmaceutical excipients. By decreasing the particle size from a micron to a nanometer scale, there is a significant increase in the surface area [10].

Chitosan is a biocompatible and biodegradable polymer. The degree of deacetylation and the supply of amino groups affect chitosan degradation. Furthermore, the US Food and Drug Administration (FDA) and the European Union (EU) have approved chitosan for use in dietary supplements and wound dressings. The toxicity of chitosan, on the other hand, increases as the charge density and degree of deacetylation rises [11]. So far there is no evidence of demonstrating human toxicity of chitosan-based formulations. Several animal toxicity tests, on the other hand, have shown that the chitosan is effective and safe in vivo and in vitro. Aluani et al. findings were backed up by an in vitro cytotoxicity analysis, which concluded that chitosan NPs are a safe carrier for quercetin in oxidative stress-related injuries [12]. Considering all these potential and safe characteristics of the chitosan polymer, the development of nanoparticles would be beneficial.

Pure zafirlukast (ZFR) is described as a fine, white to pale yellow, amorphous powder. It is practically insoluble in water (0.000962 mg/ml), slightly soluble in methanol, and freely soluble in tetrahydrofuran, dimethyl sulfoxide, and acetone. The poor solubility of drugs can be enhanced by using the formulation of nanoparticles and by increasing the bioavailability [13]. Drugs with poor bioavailability are not able to reach minimum effective concentration, so they fail to exhibit the therapeutic effect. Considering the low aqueous solubility and bioavailability of ZFR, the nanoparticles would be a potential carrier system to overcome the problems associated with this drug [14]. ZFR is an antagonist of the orally treated leukotriene receptor that is used to treat asthma chronically. While ZFR is usually well tolerated, there is always a headache and upset of the stomach. Few rare side effects can occur such as liver failure, which may be life-threatening. ZFR is used to regulate and avoid asthma-induced symptoms (such as wheezing and shortness of breath) [15]. An extensive literature search revealed that there is currently no suspension dosage form of ZFR available for pediatric patients. There have been no records of pediatric suspensions containing the nanoparticulate ZFR. So, there is an urgent need to develop the suspension dosage form which would be beneficial for pediatric patients with improved compliance. The purpose of this study was to develop an oral pediatric suspension of ZFR nanoparticles for the successful treatment of childhood asthma.

2 | MATERIALS AND METHODS

2.1 | Materials

Zafirlukast (ZFR) was purchased from Baoji Guokang Bio-Technology Co., Ltd. (Baoji, Shaanxi, China), chitosan (CS), mannitol, xanthan gum, sodium benzoate, aerosil, and cherry flavour were purchased from Sigma Aldrich, USA. Triplyphosphate (TPP) and acetic acid were purchased from Shanghai Chemical Co. (Shanghai, China).

2.2 | Development of ZFR loaded chitosan nanoparticles (ZFR-CS-NPs) [16]

ZFR-CS-NPs were developed by the ionotropic gelation method. The CS was dissolved in 3% acetic acid under continuous stirring to form a clear and viscous polymeric solution. Tween 80 as a surfactant was added at different concentrations under stirring for 45 min. ZFR was dissolved in methanol at a concentration of 7.5 mg/ml and added dropwise to the polymeric solution using a syringe fitted with a needle. The whole system was stirred until the organic solvent gets evaporated. TPP previously dissolved in double distilled water was added dropwise to drug polymeric solution for crosslinking of the nanoparticles. The whole system was stirred overnight for crosslinking of TPP and CS to get a whitish suspension of nanoparticles. This suspension was centrifuged at −50°C at 25000–30000 rpm to separate nanoparticles. The supernatant solution was used for the determination of the encapsulation efficiency of nanoparticles. The separated wet nanoparticles were freeze-dried by adding trehalose as cryoprotectant and used for further analysis. Formulation batches of ZFR loaded NPs are presented in Table 1.

After performing a preliminary demonstration of experimental trials it was found that concentration of chitosan, TPP, and Poloxamer have a visibly significant influence on drug release and encapsulation efficiency. Therefore, these three parameters are considered to be independent parameters. A 3-factor,
TABLE 2 Composition of ZFR-CS-NPs suspension

| Batch                  | SUS1 | SUS2 | SUS3 | SUS4 |
|------------------------|------|------|------|------|
| ZFR-CS-NPs eq. to 10 (%) | 3    | 3    | 3    | 3    |
| Sucrose (%)            | 76.65| 76.45| 76.25| 76.05|
| Mannitol (%)           | 20   | 20   | 20   | 20   |
| Xanthan gum (%)        | 0.2  | 0.4  | 0.6  | 0.8  |
| Sodium benzoate (%)    | 0.1  | 0.1  | 0.1  | 0.1  |
| Aerosil (%)           | 0.025| 0.025| 0.025| 0.025|
| Flavour (%)            | 0.025| 0.025| 0.025| 0.025|
| Total                  | 100  | 100  | 100  | 100  |
| Distilled water to get 75 ml volume | 40 ml | 40 ml | 40 ml | 40 ml |

the 2-level full factorial design was applied to optimize the nanoparticles and to find out the effect of independent variables on dependent variables percentage encapsulation efficiency and particle size.

2.3 Preparation of suspension containing ZFR-CS-NPs [17]

ZFR-CS-NPs containing suspension were prepared by using a simple blending method of excipients. ZFR-CS-NPs equivalent to 10 mg was weighed accurately and co-sifted with sucrose, mannitol, xanthan gum, and sodium benzoate through sieve 40 and blended in a polybag for 20 min. Aerosil and flavour were co-sifted through sieve 60 and added to previously blended material and again mixed for 10 min in a polybag for proper mixing. All formulation batches are presented in Table 2. The prepared blend was filled (30.0 g) in HDPE bottles and 40 ml distilled water was added to each bottle to reconstitute the dry blend to give a final volume of 75 ml and it was used further for analysis.

2.4 Characterization of nanoparticles (NP)

2.4.1 Yield of the nanoparticles

The yield obtained during nanoparticle preparation was calculated by using the following formula [18].

\[
\% \text{Yield of the NP} = \frac{\text{Weight of NP}}{\text{Wt of ZFR} + \text{Total Wt of excipients}} \times 100
\]

2.4.2 ZFR loading in NP

ZFR-CS-NPs equivalent to 10 mg was dissolved in 3% acetic acid to separate the entrapped ZFR from polymer. Nearly 15 ml methanol was added to this solution and stirred under a magnetic stirrer for 20 min to extract the ZFR. This solution was filtered through 0.45-micron disc filters and the drug content was determined using a UV spectrophotometer at 320 nm. ZFR content from NP was determined using the following formula [18].

\[
\% \text{ZFR loading} = \frac{\text{Total ZFR} - \text{Free ZFR}}{\text{Nanoparticle weight}} \times 100
\]

2.4.3 ZFR entrapment efficiency in NP

The nanoparticulate suspension was centrifuged at 20,000 RPM for 30 min and the supernatant solution was analysed to determine free MTL using a UV spectrophotometer at 320 nm, and the encapsulation efficiency was determined using the following formula [18].

\[
\% \text{ZFR E.E} = \frac{\text{Total ZFR} - \text{Free ZFR}}{\text{Total ZFR amount}} \times 100
\]

2.4.4 Surface morphology, zeta potential, particle size distribution (PDI), and polydispersity index (PDI)

The surface characteristics of the ZFR were evaluated using scanning electron microscopy (SEM). The ZFR-CS-NPs suspension was diluted 1–10 times with purified water and sonicated for 10 min. The samples were analysed by a Malvern particle size analyser. PSD, PDI, and zeta potential of ZFR-CS-NPs were determined [18].

2.4.5 Saturation solubility

Nanoparticles play an important role in the solubility enhancement of poorly soluble drugs. The saturation solubility of ZFR and ZFR-CS-NPs were evaluated in an incubated shaker. The pure ZFR was dissolved in 15 ml of distilled water till no more amount of ZFR and ZFR-CS-NPs gets dissolved (saturation level). Both solutions in triplicate were shaken for 24 h at 37°C. Both solutions were centrifuged at 25,000 RPM for 20 min. The resulting solutions were filtered through 0.45-micron disc filters to get a clear solution. The ZFR content was determined by using UV spectrophotometer at 320 nm [19].

2.5 Evaluation of suspension containing ZFR-CS-NPs [20]

2.5.1 pH of the suspension

The pH of the formulated suspension was determined by using calibrated pH meter. The average of 10 samples was determined and also the final pH of the suspension.
2.5.2 Sedimentation volume (SV)

The sedimentation volume of the suspension was determined by pouring 50 ml suspension in a measuring cylinder, volume was determined at a specific time interval and the final volume was measured. The sedimentation volume was determined using the following formula.

$$SV = \frac{\text{Initial volume (Iv)}}{\text{Final volume (Fv)}}$$

2.5.3 Re-dispersibility

It was determined by measuring the number of strokes to re-disperse the sediment formed at the end of 7 days.

2.5.4 Viscosity

The viscosity of the suspension was determined at room temperature using an Ostwald viscometer. The measurements were done in triplicate and the average value was considered as the final viscosity.

2.5.5 Dissolution test

In vitro dissolution was carried out by pouring 5 ml of suspension through a syringe to determine the amount of ZFR released at a specific time interval using type II dissolution apparatus (Paddle) at 50 RPM in pH 7.4 phosphate buffer. The temperature of the media was maintained at 37 ± 0.5°C throughout the dissolution test. The aliquots of dissolution medium (5 ml) were withdrawn at 5, 10, 15, 20, 30, and 60 min time intervals and replaced with an equal amount of dissolution media to maintain the sink condition. The withdrawn samples were filtered through a disc filter and the amount of ZFR released at each time point was determined by a UV spectrophotometer at 320 nm wavelength.

2.5.6 ZFR content from suspension

The suspension (5 ml) was dissolved in the mixture (20 ml) of ethanol: methanol (20:80) and the resulting solution was filtered through a 0.45-micron disc filter to get a clear solution. These solutions were analysed after appropriate dilutions and at 320 nm by UV spectrophotometer to determine the drug content.

2.5.7 Animal model for asthma and evaluation of efficiency [21]

The asthmatic model was developed using BALB/c female mice (5–10 weeks old). All animal handling and experiments are carried out in compliance with the institute's approval and guidelines. All laboratory animals were kept for 15 days and fed a well-balanced diet as well as plenty of water. After 15 days, ovalbumin (20 µg/dose) was injected to induce eosinophilic asthma. This population of asthmatic animals (n = 30) was divided into three (n = 10) groups. Group I was the monitoring group, group II was the group that received pure ZFR, and group III was the group that received no ZFR (Optimized suspension containing nanoparticles).

2.5.8 Airway hyper responsiveness (AHR) measurement [22]

Pentobarbital sodium injection (intraperitoneal) was used to anesthetize group I–III animals, and a tracheostomy tube was inserted. A micro syringe was used to administer methacholine at doses ranging from 2 to 14 mg/ml intravenously into a jugular vein. Data collection was used to evaluate AHR. The findings were compared to those obtained with a phosphate buffer in saline.

3 RESULTS AND DISCUSSIONS

3.1 Evaluation of ZFR-CS-NPs

The development of appropriate dosage forms for pediatric patients is a major concern for scientists, pharmaceutical companies, and medical professionals. Due to non-compliance and swallowability issues, most widely available dosage forms are refused by pediatric patients. In this case, suspensions are the preferred dosage option for these patients. Such reconstituted suspensions can be easily swallowed with better patient compliance. Furthermore, the inclusion of a sweetener such as mannitol and sucrose brings sweetness to the formulation, increasing patient compliance. Film coated tablets of ZFR (10 mg and 20 mg) are available in the market under the brand name Accolate.

ZFR CS nanoparticles were developed by using an ionotropic external gelation method using TPP as a counter ion. During the development of the NPs CS (polymer), tween 80 (surfactant), and TPP (counter ion), concentrations were varied. Each batch was evaluated for the yield by measuring the actual weight of the nanoparticles and comparing it with the practical weight. All batches showed a higher yield of the nanoparticles in the range of 96.25–98.90%. These observations indicated the minimum loss of the excipients at the time of manufacturing of the nanoparticles. The ZFR loading in the nanoparticles was found in the range of (26.50–35.90%). The higher drug loading signifies the proper and complete crosslinking of TPP (negative charge) with CS (positive charge). Also, similar observations were found in the case of encapsulation efficiency (93.24–98.57%). All batches showed excellent nanosized dimensions ranging from 150 to 220 nm along with stability in terms of zeta potential of 19.18–21.41 mV and helped to act as the separate particle in suspensions (see Figure 1). The formulations with a zeta potential of 30 mV to ±30 mV are regarded as
TABLE 3  Physicochemical parameters of MTL loaded CS NPs

| Batch | Yield (%) | EE (%) | DL (%) | PSD (nm) | Zeta potential (mV) | PDI | Solubility (mg/ml) |
|-------|-----------|--------|--------|----------|--------------------|-----|-------------------|
| F1    | 97.24     | 93.24  | 26.50  | 150      | 19.18              | 0.21| 110               |
| F2    | 96.25     | 95.37  | 30.12  | 165      | 20.45              | 0.20| 125               |
| F3    | 98.50     | 97.20  | 31.24  | 200      | 20.67              | 0.19| 150               |
| F4    | 98.90     | 98.57  | 35.90  | 220      | 21.41              | 0.10| 250               |

3.2 Saturation solubility

ZFR is characterized by very aqueous water solubility leading to lower bioavailability [25]. The saturation solubility of pure ZFR was determined in distilled water and it was observed to be 15 mg/ml. The saturation solubility of ZFR in nanoparticulate form was nearly 16 times enhanced (250 mg/ml) in comparison to pure ZFR. The solubility enhancement effect was coupled with the use of CS nanoparticles due to the potent solubility enhancement property. The solubility of all formulations is presented in Table 3. CS nanoparticles have already been utilized as potent solubility enhancers for poorly water soluble drugs. Yi-Dan Chen et al. also developed prednisolone loaded CS nanoparticles and formulated oral dispersible tablets. They also found the solubility enhancement of prednisolone using nanoparticles and these results are comparable to our results [26].

3.3 Evaluation of suspension containing ZFR-CS-NPs

The F4 nanoparticulate formulation was found to be optimized based on the physicochemical properties observed. This F4 batch was used for the further development of suspension using a simple manual blending method. Table 4 represents all physical parameters of formulated suspensions. All physical parameters of the suspensions were found within range. The pH of the formulation was found neutral (7.2–7.6). The physical stability was also found to be best as sedimentation volume was approaching 1. After 7 days the dispersibility was found between 1 and 3 strokes which indicated a better sign for suspension. Also, the viscosity of the suspension was found dependent on xanthan gum concentration and a direct relation was observed between gum concentration and viscosity (12.35–23.11 poise). The drug content was also found to be excellent and it was ranged between 95.42% and 99.89%.
TABLE 4  Physical evaluation of suspension containing ZFR-CS-NPs

| Batch | pH  | Sedimentation volume (SV) | Redispersibility | Viscosity (Poise) | Drug content (%) |
|-------|-----|---------------------------|------------------|------------------|-----------------|
| SUS1  | 7.2 | 0.8                       | 3                | 12.35            | 95.42           |
| SUS2  | 7.4 | 0.6                       | 3                | 14.45            | 96.57           |
| SUS3  | 7.6 | 0.7                       | 2                | 16.18            | 98.25           |
| SUS4  | 7.5 | 0.9                       | 1                | 23.11            | 99.89           |

3.4  | In vitro ZFR release from suspension

ZFR release from suspension was studied in pH 7.4 phosphate buffer. The rapid and immediate release from SUS1 was observed and compared to other formulations. This formulation showed nearly 100% ZFR release for 30 min (see Figure 3). Other formulations also showed rapid and immediate release as compared to formulations. This immediate drug release pattern is beneficial for the treatment of asthma as drugs would be immediately available in systemic circulation to initiate the therapeutic effect. The nanoparticles in suspensions would help in increasing the solubility of ZFR, thereby enhancing the permeability through GI cells [27].

The ZFR release profiles of the suspensions were subjected to mathematical modeling (drug release kinetics) and regression coefficient $R^2$ (0.9687–0.9912) was found maximum for the Higuchi model. The value of “$n$” in Korsmeyer’s Peppas equation was found between 0.0 and 0.50, showing drug releases by the Fickian diffusion mechanism through polymeric chains of chitosan polymer.

3.5  | Measurement of airway hyper responsiveness (AHR)

Lung resistance was developed by nebulizing the methacholine concentration (2–14 mg/ml) and AHR was measured. The animal group treated with PBS buffer had shown significant enhancement in the RI with increasing the concentration of methacholine (Figure 4). But the animals treated with SUS1 had shown a drastic reduction in RI which was nearly fourfold as compared to control groups. This enhanced activity was due to the presence of ZFR nanoparticles in suspension formulation that played an important role in the enhancement of solubility and permeability of the ZFR that lead to maximum availability of the ZFR at the site of action. This entire scenario helped to achieve greater inhibition of the leukotriene receptors. Leukotrienes are the chemicals that cause swelling and tightening of the lung muscles that lead to the development of asthma. ZFR is a leukotriene receptor antagonist which acts by blocking the action of leukotriene D4 in the lungs and decreases the inflammation and relaxation of smooth muscles of the lungs [28].

The pediatric formulations have gained greater interest in recent years due to the various challenges associated with such dosage forms [29]. So researchers, scientists, and medical practitioners are constantly working on it to develop patient-friendly pediatric dosage forms. Currently, most of the pediatric dosage forms are not accepted due to patient non-compliance and difficulties in swallowing the dosage forms [30]. Among all available pediatric dosage forms, suspensions are widely accepted and recommended due to greater patient compliance. Suspensions due to the presence of sweetening agents and flavours provide greater compliance to the patients [31]. In such a scenario, the suspension with ZFR nanoparticles would be beneficial for the effective management of pediatric asthma.
4 | CONCLUSION

Novel pediatric suspension of ZFR in the form of NPs are successfully prepared using a simple blending method. The efficacy of the suspensions was also found promising in asthmatic animals. The presence of CS and ZFR in NPs enhanced the solubility as well as release pattern from suspensions which made immediate ZFR available at the site of action. Such technology is quite beneficial for childhood asthmatic patients.

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