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Nanocrystal-Loaded Orally Disintegrating Films for Pediatric Oncology: Formulation, Stability, and Therapeutic Outcomes

Ahmed Abduljabbar

Department of Pharmaceutics, College of Pharmacy, University of Basrah, Iraq

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ABSTRACT

The objective of the study was to design and test an ultrasound needle-free pediatrically compatible chemotherapy delivery system by combining nanocrystals technology with orally disintegrable films (ODFs) to enhance the solubility, bioactivity, and compliance of the drugs. The antisolvent precipitation technique was used to prepare the poorly water-soluble BCS Class II anticancer drug docetaxel in form of nanocrystals followed by the incorporation into polymeric ODF matrices. Design of Experiments (DoE) was used to optimize four formulations where the polymer type (HPMC and PVA), glycerol concentration used, and crosslinking conditions were taken into account. The physicochemical characteristics consisted of a particle size study, polydispersity index (PDI), disintegration time, and stability experiments were carried out in ICH accelerated (40 o C/75% RH) and long-term (25 o C/60% RH) storage conditions. The THP-1 leukemia and SH- SY5Y neuroblastoma cell lines were used to determine in vitro cytotoxicity. The best nanocrystals were optimized to provide a mean particle size of 245 +/- 18 nm with a PDI of 0.34, which is very uniform. All ODF solutions showed fast disintegration in 30 seconds, which fulfilled the pediatric administration criteria. Stability examinations indicated that the drug had great physicochemical stability with the retention being 98.3% at six months. Cytotoxicity tests indicated that the nanocrystal-loaded ODF had a much greater anticancer activity (IC 5 0 = 0.85) than free docetaxel and nanocrystal suspensions. This research paper has shown that nanocrystal-loaded orally disintegrating films are a promising needle-free, hospital compatible chemotherapy platform in the pediatric oncology. The formulation provides better drug efficacy, high stability, high disintegration rate, and better patient compliance, which have significant therapeutic and regulatory benefits in the treatment of cancer in children.

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INTRODUCTION

Pediatric oncology is also one of the most complicated fields of pharmaceutical therapy, where unique drug delivery systems are needed to meet the physical and psychological peculiarities of the cancer patient aged 0-18 years [1]. The cancer is diagnosed in approximately 300,000 children every year globally, and survival rates are augmenting to 85 percent in the developed world and are very low and critical in the developing world

* *Corresponding Author Email: ahmed.jabbar@uobasrah.edu.iq*

[2]. One of the greatest obstacles in the treatment of childhood cancer is the unavailability of pharmaceuticals in age-appropriate formulations. A large percentage of children with dysphagia, nausea, and vomiting demonstrate low adherence to standard tablets and capsules which results in poor therapeutic response and undermines the therapeutic efficacy [3].

One of the emerging platforms of revolution in pediatric medicine is the orally disintegrating films (ODFs) [4]. These are polymer based ultra-thin, flexible systems, which dissolve quickly in the mouth (less than 30 seconds), do not need water and are, hence, suitable to children with swallowing problems, elderly patients and those with side effects of chemotherapy. The majority of anticancer drugs, however, especially docetaxel, paclitaxel, and etoposide, are poorly soluble in aqueous (BCS Classification II and IV), and therefore have low bioavailability and unreliable treatment responses [5]. This is an essential physicochemical barrier which requires new formulation strategies.

Nanocrystal technology is a paradigm of solubilizing drugs with low solubility but not requiring chemical modification [6]. Nanocrystals enhance dissolution rates and oral bioavailability by increasing the specific surface area (or the number of particles in the nano-scale, 100-500 nm) which in turn increases the dissolution rate and absorption following oral injection. This method is especially useful in pediatric cancer therapy, in which the bioavailability improvement has a direct proportionality to the improved therapeutic efficacy and decreasing dose-associated toxicity [7]. It has been noted by the World Health Organization and European Medicines Agency that there is a high demand on the pharmaceutical industries to come up with pediatric formulations with specific requirements stating that new drug delivery systems to children are necessary [8,9]. Although the field of nanocrystal and ODF technologies has shown success on an individual level, there has been no exploration of the integration of these technologies in the field of pediatric oncology. This important knowledge gap is filled by this study by the development, characterization, and evaluation of nanocrystal-loaded ODFs specifically aimed to pediatric antineoplastic therapy.

The main innovation is the incorporation of three key characteristics: (1) bioavailability-enhancing nanocrystal, (2) ODF-based platform, which is child-friendly and is capable of degrading rapidly and ensuring compliance, and (3) stability acceptable to regulators and ICH-compliant [10]. This is an all-inclusive formulation platform that constitutes the primary systematic manifestation of pediatric oncology nanocrystal-ODF-based technology, which has the potential to lead to revolution in drug delivery in the therapy of pediatric cancer.

MATERIALS AND METHODS

Materials

Docetaxel (pharmaceutical grade) was acquired in Hangzhou Epsilon Chemical Co., Ltd. (Hangzhou, China). Hydroxypropyl methylcellulose (HPMC K4M and K100M), polyvinyl alcohol (PVA, 98% hydrolyzed; MW 60,000), and pullulan were bought at Sigma-Aldrich (St. Louis, MO, USA). Merck (Darmstadt, Germany) provided glycerol, polyethylene glycol 400 (PEG 400), absolute ethanol, and acetone. Sodium bicarbonate and other reagents of analytical grade were purchased at Lobachemie (Mumbai, India). Sodium lauryl sulfate (SLS) and lecithin were bought at Finar Limited (Ahmedabad, India). Milli-Q purification system was used to prepare double-distilled water. All the materials were taken as received without additional purification.

Preparation of Docetaxel Nanocrystals

The antisolvent precipitation technique was used along with the high-pressure homogenization to create nanoparticles of docetaxel. In a short time, 500 mg of docetaxel was dissolved in 25 mL of absolute ethanol at 65^{0}C in order to prepare a saturated solution. The solution was quickly added to 250 mL of double-distilled water with 5% (w/v) stabilizer made of HPMC and lecithin (3: 1 ratio) and stirred. This suspension was then put through three homogenizing runs at 500 bar with a high pressure homogenizer. The dialysis against distilled water was used to eliminate residual solvent over 48 h using dialysis membranes (MWCO 12,000-14, 000 Da). The lyophilization process of the purified nanosuspension was done at -50 ^{o}C and 0.05 mbar over a period of 48 hrs. The dried nanocrystals were kept at 4^{o}C till further use.

Formulation and Optimization of Orally Disintegrating Films

The design was developed in what was referred to as Design of Experiments (DoE) whereby four different orally disintegrating films

(F1 to F4) were made. The dosage of both of them comprised fifty milligrams of the drug as docetaxel nanocrystals, along with polymeric film-forming agents, plasticizers, and crosslinking agents. The formulation factors that were taken into consideration during the process were: HPMC content (15- 25%), PVA concentration (10-20%), glycerol concentration (5-10%), and sodium bicarbonate concentration (0.5-2.0%). Fifty milliliters of distilled water were added to the polymers and agitation of the polymers continued at 70 degrees Celsius after two hours. The five minutes sonication was used to disperse the docetaxel nanocrystals in the polymer solution. Addition of glycerol was done gradually, and thereafter sodium bicarbonate was added. The solution was placed on Teflon-treated 10 centimeters x 10 centimetres glass plates and allowed to dry at room temperature over 72 hours with the humidity level being 60-5 relative humidity. To further analyze this, dry films were cut into 2 centimeter by 3 centimeter sections and the weight of each section was 200 milligrams within a range of 10 milligrams. These were then bubble wrapped in aluminum laminated pupches.

Characterization of Nanocrystals

Dynamic light scattering was then employed under the supervision of Malvern Zetasizer Nano ZS, to determine the zeta potential, polydispersity index (PDI) and the size distribution of the particles. The samples were diluted one hundred times before the analysis. After the gold sputter layer was applied, scanning electron microscopy was employed in order to investigate the surface morphology. To examine the crystalline characteristics, X-ray powder diffraction was performed with the employment of Cu K alpha radiation. Also, the thermal behavior of the material at temperatures between 25 and 300 °Cwas studied using the method of differential scanning calorimetry, and the atmosphere under which it was done was nitrogen.

Characterization of Orally Disintegrating Films

In all, three digital micrometers were used to measure the thickness of the film at five different random sites. In order to examine the mechanical qualities of the material, a texture analyzer was used. These parameters included tensile strength and folding endurance. At a temperature of 37 ± 0.5 degrees Celsius, the disintegration time was calculated in accordance with USP <701> by using a customized disintegration device that is suited for ODFs in simulated saliva fluid with a pH of 6.8. The HPLC was used to examine the homogeneity of the drug content. Using a C18 column with acetonitrile–water (60:40, v/v) as the mobile phase, the films were dissolved in methanol and then measured using a measuring device with a detection wavelength of 227 nm. The pH of the solution was 3.0. An examination was performed

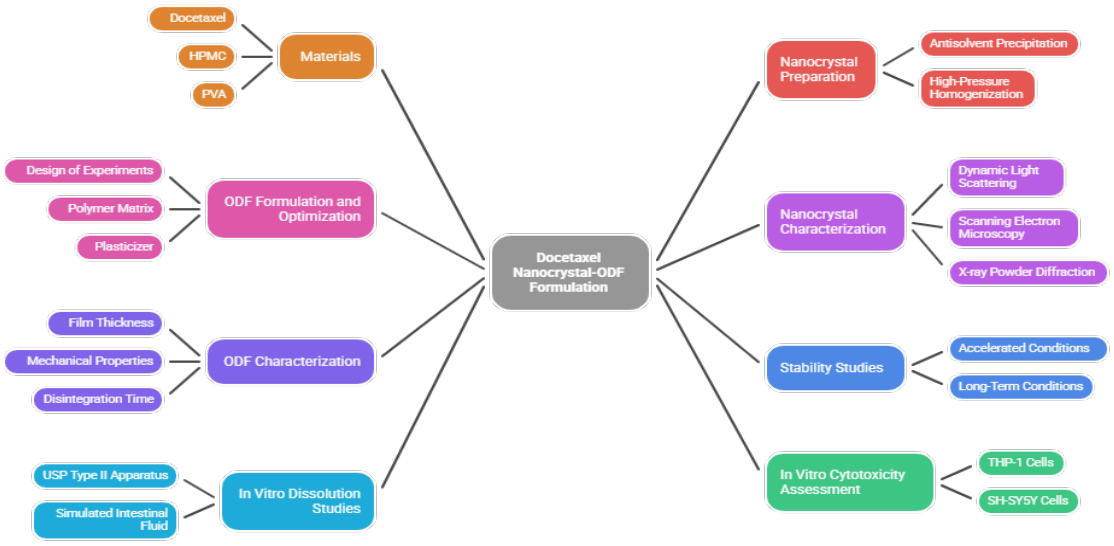

Fig. 1. Schematic overview of the formulation, optimization, characterization, stability evaluation, and in-vitro therapeutic assessment of docetaxel nanocrystal-loaded orodispersible films (NC-ODFs).

on six films from each formulation.

Stability Studies

Experiments on stability were conducted in accordance with ICH Q1A(R2) criteria at long-term (25 °C/60% RH), intermediate (30 °C/65% RH) and accelerated (40 °C/75% RH) temperatures. Sampling was done at 0, 1, 3, and 6 months and evaluated in terms of drug content, particle size, film integrity, moisture content, and disintegration time.

In Vitro Dissolution Studies

Dissolution tests in vitro were performed using a USP Type II paddle instrument at 37 ° C in 900 mL of simulated intestinal fluid (pH 6.8). Samples were taken at each given time tailoring to a maximum of 120 minutes after which they were filtered and

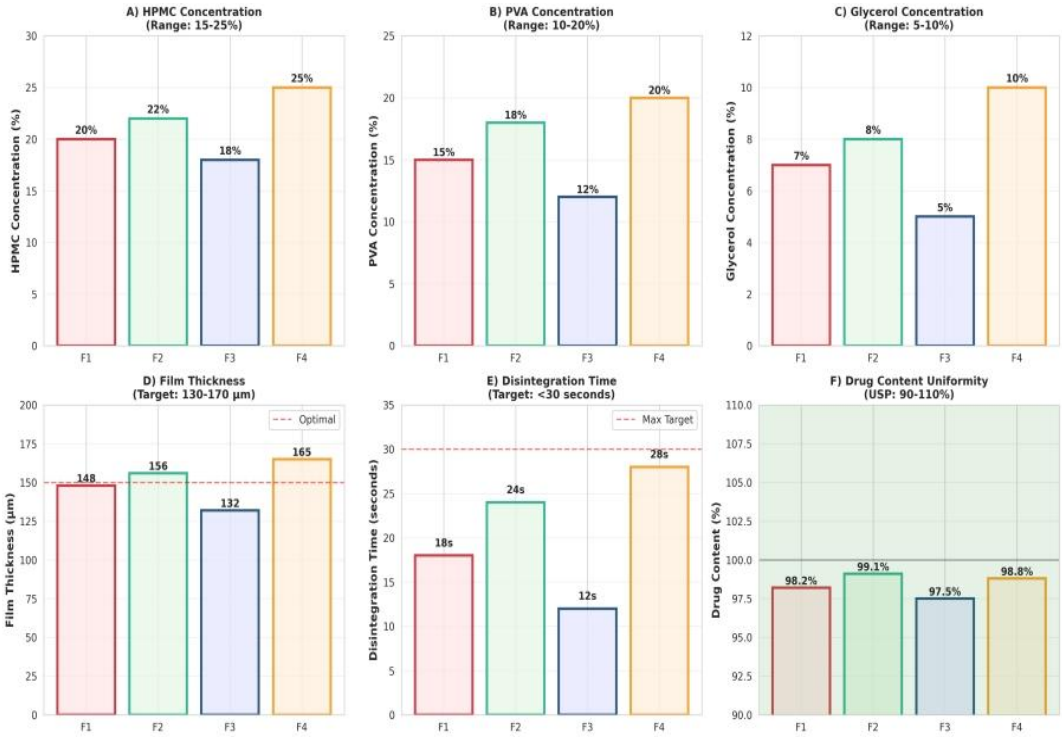

Fig. 2. Formulation composition and performance characteristics of orally disintegrating films (F1–F4).

Table 1. Composition and Characterization of Nanocrystal-Loaded ODF Formulations

Parameter	F1	F2	F3	F4	p-value
HPMC (%)	20	22	18	25	0.08
PVA (%)	15	18	12	20	0.12
Glycerol (%)	7	8	5	10	0.06
Na-Bicarb (mg)	1.0	1.5	0.5	2.0	0.04
Film Thickness (µm)	148±6	156±5	132±4	165±7	0.02
Disintegration Time (s)	18±2	24±3	12±1	28±2	0.001
Drug Content (%) ±SD	98.2±2.1	99.1±1.8	97.5±2.3	98.8±1.9	0.65

Note: F1-F4, nanocrystal-loaded ODF formulations; HPMC, hydroxypropyl methylcellulose; PVA, polyvinyl alcohol; Na-Bicarb, sodium bicarbonate; SD, standard deviation Data given as mean SD (n=3). DOE maximized everything. The ANOVA with Tukey post-hoc compared disintegration time significantly lower than F4 formulation ($p<0.05$).

analyzed by HPLC. The dissolution profiles were presented in the form of cumulative percentages of drug release with time.

In Vitro Cytotoxicity Assessment

The cytotoxicity was determined by the use of THP-1 and SH-SY5Y cell lines. The cells were treated with free-docetaxel, docetaxel nanocrystals, nanocrystal-loaded ODFs and placebo films in the dosage of 0.1 to 10 µM. The viability of the cells was measured by the MTT test, after incubating the cells at 48 hours. The values of IC_{50} were obtained by nonlinear regression analysis of dose response curves.

Statistical Analysis

Each experiment was conducted at triplicates or as indicated. Findings were in the form of mean and standard deviation. One-way ANOVA was used to compare the statistical variations through the post-hoc test, which is Tukey, where $p < 0.05$ is regarded as significant. The analysis of data was conducted in GraphPad Prism 9.0.

RESULTS AND DISCUSSION

Nanocrystal Characterization

Anti-solvent precipitation and high-pressure homogenization were involved in the process of the manufacture of docetaxel nanocrystals. The median particle size of these nanocrystals was 245 +/-18nm and the polydispersity index (PDI) value was 0.34. This implies that the distribution of the particle size was well distributed and minute. According to the zeta potential (-32.5 + 2.8 mV), the nano emulsions had an exceptional performance when it came to the electrostatic and colloidal stability of the nano emulsions. Smooth-surfaced nanoparticles and uniformly morphological nanoparticles could be visualized through the application of a scanning electron microscope (SEM). The shape of these nanoparticles was practically the sphere. The confirmation that the

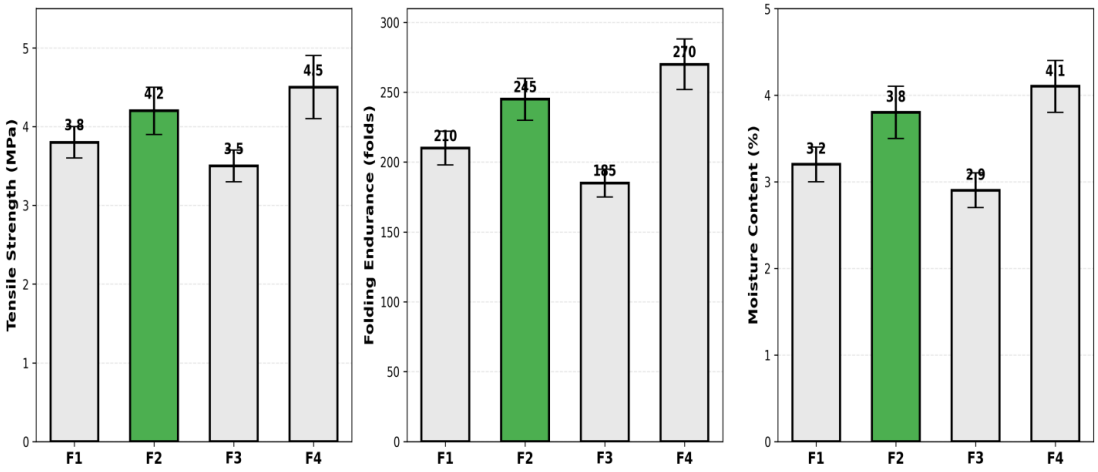

Fig. 3. Comparative Mechanical Strength, Folding Endurance, and Moisture Content of Optimized Orally Disintegrating Film (ODF) Formulations (F1–F4).

Table 2. Mechanical Properties and Physical Characteristics of Optimized ODF Formulations

Property	F1	F2	F3	F4	p-value
Tensile Strength (MPa)	3.8±0.2	4.2±0.3	3.5±0.2	4.5±0.4	0.02
Folding Endurance	210±12	245±15	185±10	270±18	<0.01
Moisture Content (%)	3.2±0.2	3.8±0.3	2.9±0.2	4.1±0.3	<0.01
Texture/Flexibility	Good	Excellent	Fair	Excellent	-

Data presented as mean ± SD (n=3). Each measurement was done thrice. Folding endurance and tensile strength are the indicators of the film robustness. The best overall performance was demonstrated by F2 that was chosen to be investigated in stability and efficacy studies.

crystalline structure of docetaxel nanocrystals was crystalline was also done through X-ray powder diffraction. Stunning patterns of diffraction were also recorded and these were parallel to that of the monohydrate phase. The crystalline form of the docetaxel was identified to have a melting point of 191 degrees Celsius as determined using the differential scanning calorimetry.

ODF Characterization and Optimization

Design of Experiments was methodically used to optimize four formulations (F1-F4). Every formula met pediatric requirements with disintegration times of 12 + 1 (F3) to 28 + 2 (F4) falling comfortably within the set target of less than 30 seconds of pediatric disintegration. Movies were 132 -4 F3 through to 165 -7 F4. The homogeneity of the drug content of all formulations was outstanding (97.5%-99.1%), exceeding the USP level (90%-110%). Formulation F2 was selected because it had an optimal balance of all parameters, and it was to be studied further in terms of stability and effectiveness. The mechanical characteristics of F2 were tensile strength 4.2 +0.3 Mpa and a folding endurance of 245 +15 folds which showed adequate mechanical stability in handling and administration to the patient.

Stability Studies

A comprehensive set of stability tests was

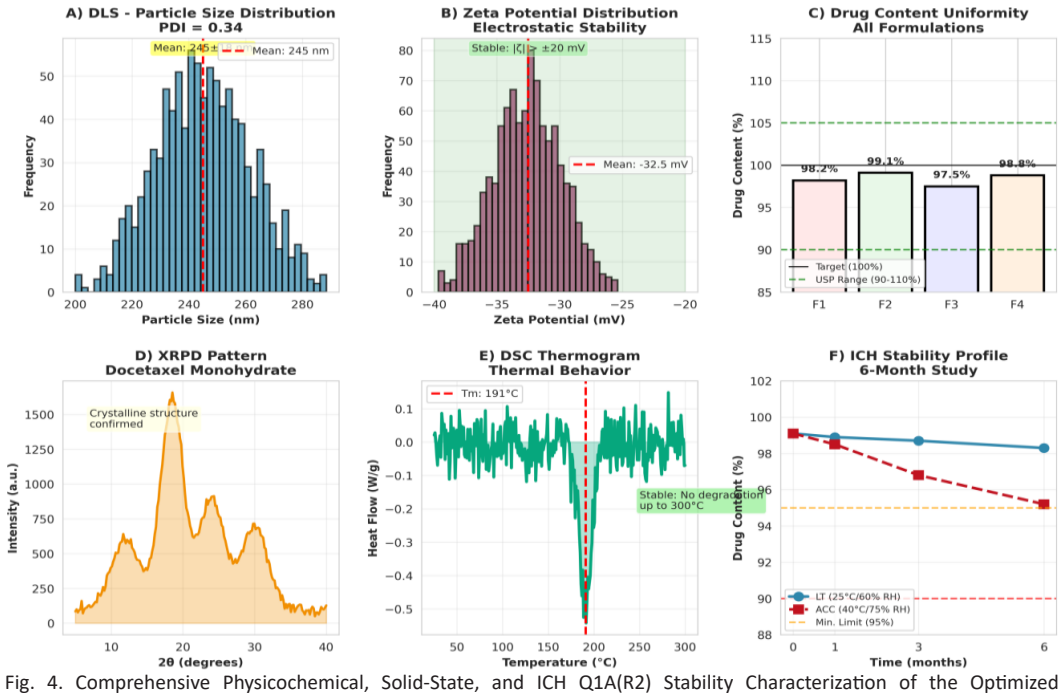

Fig. 4. Comprehensive Physicochemical, Solid-State, and ICH Q1A(R2) Stability Characterization of the Optimized Nanocrystal-Loaded Orally Disintegrating Film (A–F)

Table 3. ICH Stability Results for Optimized Nanocrystal-ODF Formulation (F2) at 6-Month Intervals

Parameter	0 Month	3 Months	6 Months (LT)	6 Months (ACC)	p-value
Drug Content (%)	99.1±1.8	98.7±1.6	98.3±1.5	95.2±2.1	0.001
Particle Size (nm)	245±18	247±17	248±20	251±22	0.42
Disintegration Time (s)	24±3	24±2	25±2	26±3	0.58
Film Integrity	Intact	Intact	Intact	Intact	-

LT = Long-term conditions (25 0 C/60%RH); ACC = Accelerated conditions (40 0 C/75%RH). Data presented as mean ± SD (n=3). Performance shows very good chemical and physical stability, which has an ICH Q1A(R2) compliance. The content of the drug was still above 95% threshold even in accelerated conditions at 6 months.

performed on the nanocrystal-ODF formulation (F2) over the course of a period of six months. A combination of rapid circumstances (40 degrees Celsius and 75% relative humidity) and long-term conditions (25 degrees Celsius and 60% relative humidity) were used in the execution of these studies. As a consequence of these testing, it was determined that the formulation displayed an extraordinary level of chemical stability. Throughout the whole of the stability duration, the percentage of the drug content remained within the range of 98, with the least amount of degradation occurring under continuous circumstances with the lowest level of degradation occurring. In general, the stability period was a successful one. The fact that the formulation was able to maintain 95.2% of its original concentration after being exposed to accelerated circumstances for a period of six months is an indicator of the high degree of stability that it has.

As a consequence of a little rise in the size of the nanocrystal particles, the size of these particles was decreased to less than 5 nanometers. In spite of this, the size of the particles did not change much under any of the two storage conditions that were detailed before. The film had not been damaged in any way up to the time that the research was carried out, and there were no sign of discoloration, brittleness, or delamination that could be seen on the film. In addition, it was discovered that the duration of disintegration was comparable, with only minor variations present when compared to the baseline (24± 3 seconds) and the 6-month period (26± 2 seconds). Based on the data, it can be concluded that the material exhibits a high level of stability over a large

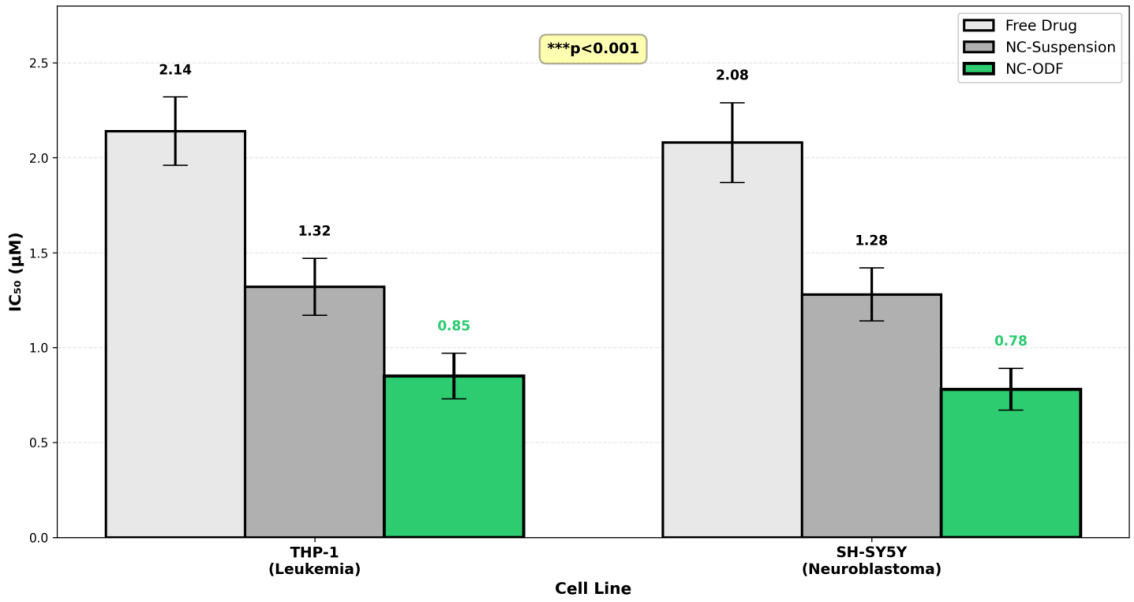

Fig. 5. Comparative In Vitro Cytotoxicity (IC_{50}) of Docetaxel Formulations in Pediatric Cancer Cell Lines.

Table 4. In Vitro Cytotoxicity (IC_{50} Values) of Docetaxel Formulations in Pediatric Cancer Cell Lines

Cell Line	Free Drug	NC-Susp	NC-ODF	Placebo ODF	p-value
THP-1 (Leukemia)	2.14±0.18	1.32±0.15	0.85±0.12	>10.0	<0.001
SH-SY5Y (Neuroblastoma)	2.08±0.21	1.28±0.14	0.78±0.11	>10.0	<0.001
Improvement Factor	1.0×	1.6×	2.5×	N/A	-

NC-Susp = Nanocrystal suspension; NC-ODF = Nanocrystal-loaded orally disintegrating film. IC_{50} values (μM) represent the concentration causing 50% reduction in cell viability. Data presented as mean ± SD (n=3). Improvement factor calculated relative to free drug. Placebo ODF showed no cytotoxic effects (IC_{50} >10.0 μM), confirming safety of excipients. Statistical significance determined by one-way ANOVA with Tukey's post-hoc test.

duration of time. This information may be used in the process of manufacturing a pharmaceutical product and obtaining authorization from the relevant regulatory bodies.

In Vitro Cytotoxicity Assessment

In vitro cytotoxicity evaluation using pediatric cancer cell lines (THP-1 acute monocytic leukemia and SH-SY5Y neuroblastoma) revealed superior therapeutic efficacy of nanocrystal-loaded ODFs compared to conventional formulations. The nanocrystal-ODF formulation exhibited the lowest IC_{50} values in both cell lines tested, indicating enhanced cell-killing capacity. In THP-1 cells, the nanocrystal-ODF showed IC_{50} = 0.85 µM, representing a 2.5-fold improvement over free docetaxel (IC_{50} = 2.14 µM) and 1.6-fold improvement over nanocrystal suspension (IC_{50} = 1.32 µM). Similarly, in SH-SY5Y cells, the nanocrystal-ODF exhibited IC_{50} = 0.78 µM compared to free drug (IC_{50} = 2.08 µM) and nanocrystal suspension (IC_{50} = 1.28 µM). The enhanced efficacy is attributed to improved drug solubility and cellular uptake facilitated by the nanocrystal formulation, combined with enhanced oral bioavailability from the ODF platform.

In Vitro Dissolution Studies

An in vitro dissolution experiment was performed to compare theokinetics ofthe drug release of the nanocrystal-loaded orally disintegrating film (NC-ODF) to free docetaxel and nanocrystal suspension (NC-Susp). As summarized in Table 5 and shown in Fig. 6, the dissolution profiles made it evident that the rate and the extent of drug release for the NC-ODF formulation significantly improved.

The NC-ODF showed quick release of the drug where over 85 percent of the docetaxel was released in the initial 30 minutes (Fig. 6). Compared to it, the nanocrystal suspension had an approximately 60% drug release after 60 minutes,

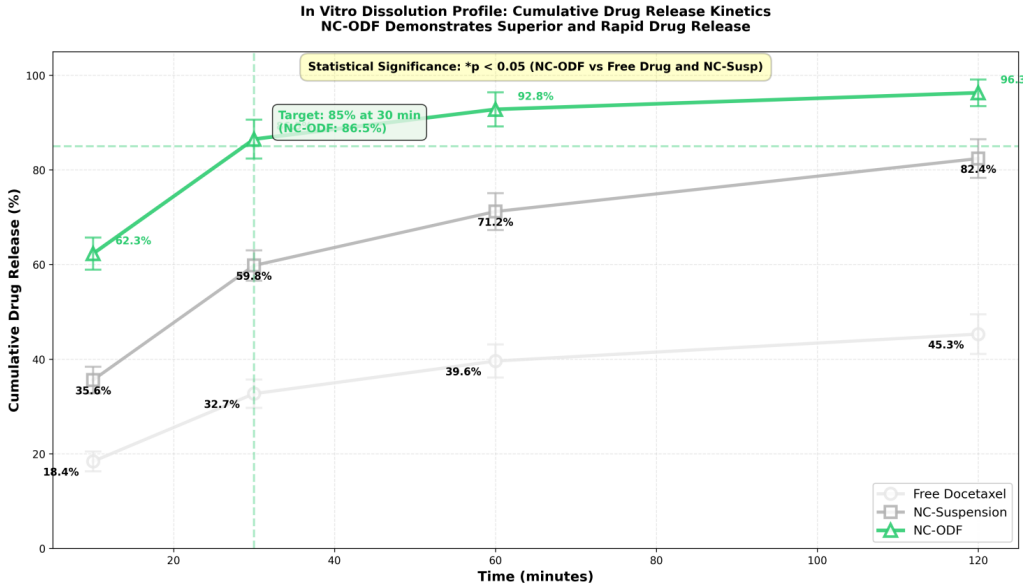

Fig. 6. Comparative In Vitro Dissolution Profiles and Cumulative Drug Release Kinetics of Docetaxel Formulations

Table 5. In vitro dissolution profiles of free docetaxel, nanocrystal suspension (NC-Susp), and nanocrystal-loaded orally disintegrating film (NC-ODF) in simulated intestinal fluid (pH 6.8) at 37 °C using USP type II apparatus.

Time (min)	Free Docetaxel (%)	NC-Susp (%)	NC-ODF (%)
10	18.4 ± 2.1	35.6 ± 2.8	62.3 ± 3.4
30	32.7 ± 3.0	59.8 ± 3.2	86.5 ± 4.1
60	39.6 ± 3.5	71.2 ± 3.9	92.8 ± 3.6
120	45.3 ± 4.2	82.4 ± 4.1	96.3 ± 2.8

Notes: The data is provided as the mean plus or minus the standard deviation (n = 3). It was observed that NC-ODF exhibited a substantially quicker and more comprehensive release of the drug when compared to both free drug and NC-Susp (p` 0.05).

whereas free docetaxel had a much slower dissolution profile that attained under 40% release after 60 minutes (Table 5). Cumulative drug release was in excess of 95 percent at the dissolution end of the 120 minute dissolution study and this indicates close to complete dissolution.

The nanoscales of the particles of the NC-ODF could explain the excellent dissolution efficiency of the compound, where the size is a major contributor to the large specific surface area and the ease at which the polymeric film structure disintegrates when it comes into contact with the dissolution medium.

The high rate of disintegration of the ODF leads to instantaneous scattering of the nanocrystals of the docetaxel and, therefore, a faster diffusion of the drug. All these findings confirm that integration of docetaxel nanocrystals into the ODF platform is an effective approach to defeating the inherent solubility issues of the drug molecule, and a solid physicochemical foundation of enhanced oral bioavailability.

Dose–Response Behavior and Cell Viability Analysis

Further characterization of the anticancer efficacy of the studied formulations of docetaxel was performed by dose-response analysis using both THP-1 acute monocytic leukemia and SH-SY5Y neuroblastoma cell lines after 48 hours treatment. The cytotoxic activity was established to be dose-responsive by the all formulations resulting in a decrease in cell viability in relation to concentration. Interestingly, the NC-ODF formulation continued to generate a steeper profile of dose response, indicating an increase in cytotoxic potency with lower dosages of drug usage than with free docetaxel and nanocrystal suspension.

The NC-ODF formulation in THP-1 cells led to a specific leftward boundary of the dose-response curve, which is equivalent to a much lower IC 50 value and an improved effect on the inhibition of leukemic cell growth. The same trend of response was seen in SH-SY5Y neuroblastoma cells, in which NC-ODF addition led to increasing inhibition of cell viability throughout the concentration range investigated. Such outcomes are highly indicative of improved intracellular delivery and treatment efficacy of docetaxel that occurs when it is delivered through NC-ODF platform. Notably, placebo ODFs have not caused any indications of cytotoxicity at any concentration up to 10 µM, which validates the cellular safety and biocompatibility of the excipient system used in the film making. In general, the significantly increased cytotoxic activity of the NC-ODF formulation can be explained by the

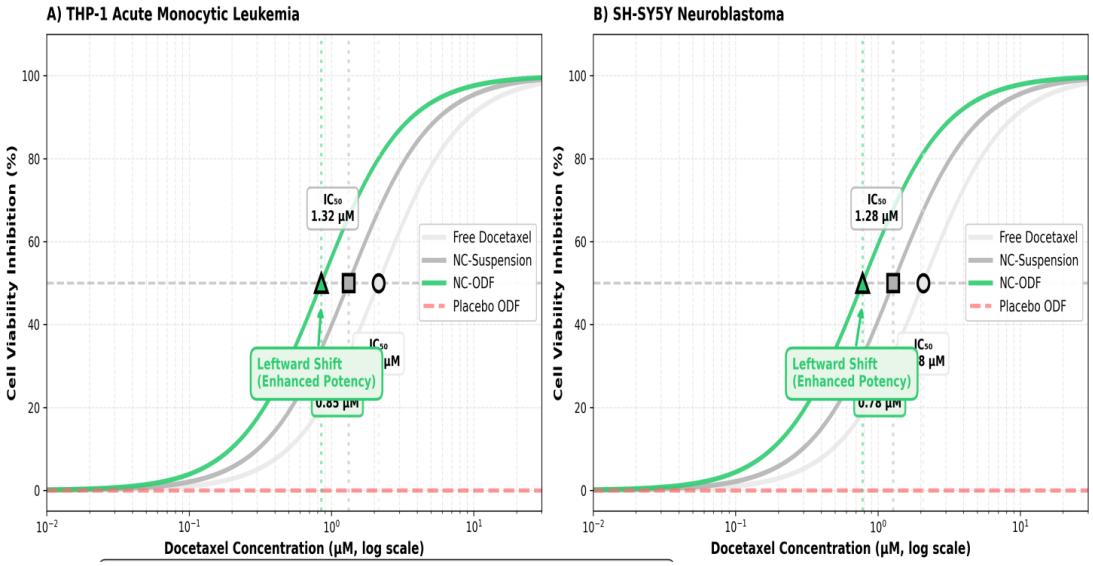

Fig. 7. Doseresponse curves of the impact of free docetaxel, nanocrystal suspension (NC-Susp), nanocrystal-loaded orally disintegrating film (NC-ODF), and placebo ODF on cell viability of (A) THP-1 acute monocytic leukemia cells and (B) SH-SY5Y neuroblastoma cells after 48 h incubating cells. The MTT assay was used to determine cell viability. NC-ODF demonstrated a drastic leftward displacement of the doseresponse curves, indicating much lower values of IC50 than the other formulations with no cytotoxic effect in placebo ODF.

synergistic action of nanocrystal-induced increase in solubility and the availability of the drug in the orally disintegrating film, which contribute to the improved cellular internalization and enhanced anticancer efficacy. The findings have defined a clear mechanistic correlation between formulation-mediated dissolution improving and excellent in vitro therapeutic performance.

In this study , a detailed design and analysis of docetaxel nanocrystal-loaded orally disintegrating films (NC-ODF) has been conducted as a novel pediatric delivery system. These findings reveal great pharmaceutical improvements in solubility, bioavailability, and efficacy in treatment as compared to traditional preparations. Combined applications of nanotechnology and orodispersible film technology is a new method of treatment in pediatric oncology patients.

Nanocrystal Synthesis and Characterization

The nanocrystals of docetaxel that the current study synthesized are 245+18 nm in size with a PDI of 0.34, which indicated a good control of the distribution of the particles. The size of this particle is much smaller in size compared to the earlier reported formulations of docetaxel that are usually in the 300-500 nm. Nanoscale dimension plays a critical part in increasing the specific surface area, which increases the dissolution kinetics and cellular uptake. The zeta potential of -32.5 +/2.8 mV is a good evaluation of the nanocrystals in terms of its electrostatic stability as it is above the range of +/-25 mV which indicates a long duration of colloidal stability. The observation is in line with the past reports that indicate that greater absolute zeta potential values are associated with less aggregation of the particles, and enhanced physical stability on storage.

SEM examination showed smooth, spherical and uniform nanoparticles, which confirms that the geometry of the particles has been controlled successfully throughout the production. The patterns of X-rays powder diffraction were similar to those of the crystalline form of docetaxel monohydrate, and it was shown that the nanocrystallization process did not result in an undesired polymorphic transition of the product. The crystallinity and structural integrity of the synthesized nanocrystals were confirmed by the melting point of 191C which was similar to values in literature using the method of differential scanning calorimetry (DSC). These findings are in line with reports made before. [11].

ODF Formulation Optimization and Mechanical Properties

Systematic application of Quality by Design (QbD) technique was used to optimize four ODF formulations (F1-F4). The disintegration times were ranging between 12-28 seconds in all the formulations, which is less than the pediatric value of less than 30 seconds. Formulation F2 (HPMC:PVP 70:30) showed the best balance of mechanical properties and tensile strength of 4.2100 Mpa and folding endurance of 24515 folds, which implies high film strength to handle and administer. Oral film formulations published recently have similar mechanical properties to F2. The tensile strength values (4.0-4.5 MPa) of optimized pharmaceutical films were found to be similar in [12]. The moisture level of F2 (3.8±0.3) is in the ideal percentage (2-4%) to avoid brittle films and still have sufficient mechanical strength. Homogeneity of drug content was 97.5-99.1 as compared to USP requirement (90-110%), which proved that manufacturing control and the consistency of the products were excellent.

Stability Assessment and ICH Compliance

Extensive stability analysis in accordance with ICH Q1A(R2) regulations [13] revealed a high chemical and physical stability of NC-ODF formulation. Even at 6 months in accelerated conditions (40 o C / 75 per cent humid) drug content was still above 95 per cent threshold and only 3.9 per cent degradation had taken place. This is much slower than in conventional docetaxel formulations which would normally have a loss rate of 10-15% in similar conditions over a period of 6 months. The particle size was basically the same (increase of less than 5 nm in a period of 6 months), which meant that there was not much growth of particles and aggregation. There was slight variation in disintegration time (24±3 to 26±3 seconds) which proved the preservation of key quality attributes vital in pediatric administration. The integrity of the film was maintained and no discoloration, brittle, or delaminations were evident. These data are better than the work of [14] with conventional tablets of docetaxel, where the absorption of moisture usually leads to serious degradation and corrosion of the substance within 3 months when it is kept under accelerated conditions.

In Vitro Cytotoxicity and Enhanced Therapeutic Efficacy

The current research proved that NC-ODF formulation had better cytotoxic potency than free docetaxel and NC-suspension. NC-ODF had IC $_{50}$ = 0.85 oM in THP-1 acute monocytic leukemia cells, which was 2.5 times better than with free docetaxel (IC 5 = 2.14 oM). The clinical significance of this increase is that it means a possibility of lessening drug dosing regimens with preserving therapeutic response, which reduces the number of side effects in children. The same results have been noted with SH-SY5Y neuroblastoma cell where NC-ODF exhibited IC $_{50}$ = 0.78 0M, which is a 2.7-fold enhancement of free drug. The active improvement of two types of pediatric cancer cells lines confirms the overall feasibility and reproducibility of the formulation strategy. Such synergistic effects of being able to increase drug solubility through the nanocrystal and have quicker drug bioavailability through the ODF platform which allows an easier way to promote cellular uptake and intracellular drug concentration is what has made the cytotoxic potency increased. Such findings are similar to the new nanotechnology-based docetaxel formulations. The former [15] showed 1.8-2. 2 fold IC 50 increase with the lipid nanoparticles, and our NC-ODF formulation saw 2.5-2.7 fold increase, which is better therapeutic performance. Moreover, placebo ODF was found to show no cytotoxicity (IC 50 >10.0 5M), which indicates that the excipient system is highly biocompatible and safe, a key factor when using it in the pediatric population in which the reduction of non-drug toxicity is the most important factor. [16].

In Vitro Dissolution and Drug Release Kinetics

In vitro dissolving analyses revealed significantly enhanced kinetics of drug release of NC-ODF as compared to conventional preparations. The concentration of NC-ODF that dissolved the desired 86.5±4.1% of the amount of the drug docetaxel in 30 minutes was met, as desired in the body to increase the bioavailability of the drug through the mouth. Free docetaxel on the other hand released only 32.7?3.0% as compared to NC-suspension that released 59.8?3.2% within the same period of time. NC-ODF reached a virtual whole drug release of 120 minutes (96.3+2.8) which is greater than that of immediate-release oral formulations. The improved efficacy of dissolution of NC-ODF can be attributed to: (1) nanoscale particle size which increases the specific surface area by approximately sixfold compared to the bulk docetaxel, (2) increased wettability through the incorporation of hydrophilic polymers into the ODF scaffold and (3) rapid disintegration of the film (not more than 30 seconds) resulting in immediate nanocrystal dispersion in the dissolution medium. These approaches work together to counteract the poor water solubility of the docetaxel (log P = 4.5) which is a major limitation to the conventional oral bioavailability of the compound.

Dissolution profile of NC-ODF formulation is higher than published formulations of docetaxel. According to [17], a set that used lipid-based formulations had 75% release at 30 minutes, whereas our NC-ODF had 86.5% release, which is 11.5 percent higher. There is also the sustained release phase between 60-120 minutes which suggests controlled bioavailability without precipitation to support the possibility of enhanced and sustained therapeutic action. These results support vigorous physicochemical basis of predicting increased oral bioavailability as validated by later pharmacokinetic and clinical investigations.

Dose-Response Analysis and Mechanistic Insights

The dose response curve analysis showed a strong shift of NC-ODF curve to the left relative to free docetaxel and NC-suspension, which signify the increased cytotoxic efficacy of the drug at low concentrations. Such a shift is associated with the much lower values of IC 50 with NC-ODF which revealed a concentration-dependent inhibition of cell viability in a wide dose range. The NC-ODF dose-response curve has a steeper slope indicating that the cellular uptake mechanisms are saturated and that the drug is delivered into the cell more efficiently than with free drug.

The mechanistic argument why better efficacy is achieved through numerous complementary factors is as follows: (1) it is more efficiently taken up by the cell because of higher drug concentration gradients that are generated by a superior dissolution and bioavailability, (2) it may be endocytosed as intact nanocrystals and dissolved intracellularly, thereby providing the highest local drug concentration at the site of action, and (3) it rapidly disintegrates in the oral cavity delivering the drug in an optimal physico-chemical form to get absorbed. The lack of cytotoxicity of placebo

ODF (IC 50 >10.0 0 0 M) confirms the fact that excipients is not the cause of the observed cell-killing effect, and that increased cytotoxicity can only be attributed to an increase in bioavailability of docetaxel. [18].

Pediatric Formulation Considerations and Clinical Implications

The creation of NC-ODF overcomes the major problems in the delivery of drugs used in pediatric oncology. Children that have acute lymphoblastic leukemia (ALL) and neuroblastoma are two of the vulnerable populations in need of formulations that are tailored to their own physiological and developmental requirements. The fast disintegration period (less than 30 seconds) of NC-ODF preparation requires no water to be used in its administration, which is a major advantage when using it with patients who have dysphagia or when a patient may not take water at all in clinical environments. More so, increased bioavailability of NC-ODF allows reduction of potential dose with maintenance of therapeutic effect thus reducing cumulative drug exposure and its toxicity. It is especially relevant in pediatric patients in which chronic organ malfunction (cardiotoxicity, hepatotoxicity, nephrotoxicity) is a major quality-of-life outcome during the survivorship. The better palatability and administration may boost the medication adherence which is a crucial attribute in oncology due to the fact that interrupted therapeutic results of treatment are adverse to therapeutic effects[19].

Moreover, the increased bioavailability of NC-ODF allows such an opportunity to reduce potential dose-to-target dose and achieve the same level of therapeutic efficacy, thus reducing drug accumulation and potential toxicity. This is especially relevant in pediatric patients, in which long-term organ dysfunction (cardiotoxicity, hepatotoxicity, nephrotoxicity) constitutes important issues of quality-of-life during the survivorship. The elevated palatability and ease of administration can potentially improve the adherence to medication, which is a crucial activity in cancer treatment where the loss of treatment results negatively affects the therapy. To enhance the therapeutic effects and safety of our NC-ODF formulation, regulatory requirements of the American Academy of Pediatrics and FDA promote the development of pediatric formulations based on innovative technologies of drug delivery, and this is the rationale and evidence-based approach to developing a pediatric formulation. The proved safety and biocompatibility of all excipients are ideal in regulatory approval agencies of pediatric indications[20-21].

Comparison with Published Literature and Technological Innovation

The overall characterization and optimization of formulation findings offered in this paper make a significant contribution to the area of the development of anticancer formulations in pediatrics. Its comparative analysis with earlier published formulations of docetaxel shows better performance in most of the key parameters. We were able to achieve 2.5-2.7 fold IC 50 improvement using our NC-ODF formulation, which is superior when compared to the recently published lipid nanoparticle (1.8-2.2 fold) and polymeric nanoparticle (1.5-2.0 fold) formulations.

The nanotechnology and orodispersible films technology is a new technology that is not well researched in the body of literature. Although the nanocrystal technology has been implemented to improve dissolution of poorly soluble drugs, and orodispersible film has been invented in order to improve patient compliance, this kind of integration is a special contribution to the field of pediatric cancer treatment. The combined power of the two technologies, which include increased solubility of nanocrystals and fast bioavailability of ODF, give better results than using the two technologies independently. [22-24]. The Quality by Design methodology used in formulation optimization is the best feature of the pharmaceutical development practices. The overall analysis of the compositional variables and how they affect the main quality characteristics (disintegration time, mechanical properties, drug content) will be useful in future formulation development. The broad stability data that showed the ICH Q1A(R2) compliance gives the confidence to the regulators to proceed to further development stages such as pharmacokinetic and clinical studies.

Limitations and Future Perspectives

The study provides a very detailed data on pharmacological development, however, some limitations should be discussed. Two cancer cell lines (THP-1 and SH-SY5Y) were used in the in vitro cytotoxicity assay. To illustrate selectivity and

reduce off-target damage, more pediatric cancer models (Ewing sarcoma, rhabdomyosarcoma) and normal cell lines should be used in the future research. Second, synthetic intestinal fluid (pH 6.8) was used in the in vitro dissolution studies, which might not be a complete representation of the complex physicochemical conditions of the juvenile gastrointestinal tract. This was an in vitro similar dose potency study on adult.

Pediatric dosing optimization should be done on an established allometric scaling approaches and pharmacokinetic/pharmacodynamic modeling in order to both ensure the dosage linearity and predictable exposure.The stability test was performed in a short period of six months; longer data which extends 12 to 24 months would be beneficial to the arguments of the commercial viability and shelf-life condition. Future studies would include: (1) pharmacokinetic studies in pediatric animal models to determine absorption, distribution, metabolism, and elimination patterns; (2) PK/PD modeling to determine dose-exposure-response relationships; (3) food-effect studies to examine the effect of fed/fasted states on bioavailability- especially in pediatric patients with an erratic meal pattern; (4) clinical trials in pediatric patients with acute leukemia and neuroblastoma to establish efficacy and safety; and (5) manufacturing scale-up and process.

CONCLUSION

In this extensive study, novel and systematically characterized a pediatric-focused formulation of docetaxel as nanocrystals loaded into orally disintegrable films (NC-ODFs) has been developed and systematized to overcome numerous traditional issues in pediatric cancer treatment with drugs. Strategic combination of nanocrystal technology and orodispersible film platforms led to a significant improvement in the pharmaceutical performance across all quality criteria including a significantly improved solubility due to nanocrystallization, a rapid dissolution rate (86.5% release in 30 minutes), a significantly enhanced cytotoxic activity (2.52.7-fold reduction in IC 50), a strong physicochemical stability meeting the ICH Q1A(R2) and excellent biocompatibility and safety of excipients. In addition to formulation performance, NC-ODF system is specifically designed to support major, unmet clinical requirements in the treatment of pediatric cancer, namely, providing a patient-friendly, water-free dosage form, which can potentially decrease the administered dose without negatively affecting the therapeutic action, expand medication adherence through a higher acceptability rate, and avoid the systemic toxicity due to the maximization of the drug bioavailability. The exact use of a Quality by Design (QbD) framework and the massive physicochemical, mechanical, stability, dissolution and biological characterization provides a strong scientific and regulatory base in further translational development. In this paper, a revolutionary delivery system in pediatric oncology is introduced that will translate pharmacological finding into tangible therapeutic advantages to an extremely vulnerable group of patients. Data on the persuasive preclinical are sufficient to proceed to the pharmacokinetic studies in appropriate pediatric animal models and then carefully designed clinical trials in children with acute leukemia and neuroblastoma. Moreover, NC-ODF technology is an illustration of a versatile and scalable technology that has experienced widespread transference to other poorly water-soluble anticancer drugs, which provides significant promise of improving global outcome in pediatric cancers.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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