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Enhancing the Dissolution Rate of Dolutegravir Sodium Using Nanosuspension Technology and a 3^2 Factorial Design

Bing Xie[1], Yaping Liu[1], Xiaotong Li[1], Pei Yang[2*], Wei He[3]

[1]School of Pharmacy, China Pharmaceutical University, Nanjing 2111198, China.
[2]School of Science, China Pharmaceutical University, Nanjing 2111198, China.
[3]Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai 200443, China.

***E-mail** ✉ pharmyp@163.com

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ABSTRACT

Dolutegravir sodium is an effective antiretroviral medication used for the treatment of HIV. The drug is classified as BCS class II, indicating its low solubility at the typical gastric pH. The purpose of this research was to identify and optimize the key operational and formulation factors affecting the drug's properties, using a 3^2-factorial design. Nanosuspensions were prepared using PVP K30, polyvinyl alcohol, polxammer 407, and polxammer 188. The study focused on polymer concentration as the formulation variable and homogenization speed as the manufacturing variable. Particle size and saturation solubility were the main parameters evaluated. The analysis of variance showed that both the polymer concentration and homogenization speed significantly affected the nanosuspension's particle size and saturation solubility. The results, including contour plots and polynomial equations, provide useful insights into the optimization of the independent variables for the formulation of nanosuspensions with the desired properties.

Keywords: Solubility enhancement, Nanosupension, Dolutegravir sodium, Antiviral drug, Factorial design

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Introduction

The effectiveness of a drug largely depends on its ability to dissolve and release from its formulation, directly impacting its bioavailability. A significant challenge in the pharmaceutical industry is improving the solubility of many newly discovered active pharmaceutical ingredients, as a large number are poorly soluble or insoluble in water [1]. The solubility and particle size of a drug are critical determinants of its dissolution rate. Research on poorly soluble drugs has shown that reducing the particle size to sub-micron levels can significantly improve dissolution and, in turn, enhance bioavailability [2, 3].

Over the last decade, nanoparticle technology has been increasingly explored for medical applications [4]. Nanosuspensions, which consist of finely dispersed solid drug particles in a liquid phase, have become a key focus. Techniques such as high-speed homogenization, pearl milling, and precipitation are commonly used to create nanosuspensions, whether in aqueous or non-aqueous environments [5]. In addition, the process of nanosuspension formulation can alter the crystalline structure of the drug, often increasing the proportion of amorphous material, or sometimes producing entirely amorphous particles [6, 7].

Dolutegravir sodium (DTGS) is a novel integrase strand transfer inhibitor used to combat HIV. By inhibiting the integration of HIV DNA into host DNA, DTGS prevents viral replication. It is rapidly absorbed following oral administration, and its long half-life of approximately 11-12 hours in HIV-positive adults allows it to be taken once daily without the need for a boosting dose. As a BCS Class II drug, DTGS has low aqueous solubility but high permeability and minimal protein binding [8, 9].

Given this, the objective of the current study was to assess whether formulating DTGS as a nanosuspension could improve its dissolution rate, leading to faster absorption and a quicker therapeutic response, which is beneficial for the treatment of HIV.

Materials and Methods

Materials

DTGS was provided as a gift sample by Emcure Pharmaceutical Pvt. Ltd., Ahmedabad, India. PVP K30, polyvinyl alcohol, polxammer 407, and polxammer 188 were generously provided by BASF, Mumbai. All other materials used were of analytical grade.

Preparation of nanosuspension

The nanosuspension was formulated using the solvent-antisolvent technique. Poloxamer 188 was dissolved in water, while the drug was dissolved in methanol in separate containers under magnetic stirring. The drug solution was then slowly added to the polymeric solution using a syringe. The mixture was subjected to high-speed homogenization at 10,000 rpm for thirty minutes to reduce particle size. The nanosuspension, containing 6 g of mannitol, was rapidly frozen using liquid nitrogen and subsequently lyophilized for 24 hours at room temperature [10].

Experimental design

To evaluate the preparation and processing conditions of the nanosuspensions, preliminary tests were performed to determine the effects of various factors, including polymer concentration, solvent selection, homogenization time, and homogenization speed **(Table 1)**. A factorial design was employed to assess the influence of these formulation and processing variables on the nanosuspension's characteristics. The polymer concentration and homogenization speed were identified as the most significant variables influencing particle size and stability. These factors were tested at three levels: +1, 0, and −1. The type and quantity of polymer, solvent volume, and drug concentration were kept constant throughout all trials. The experiments were conducted using Design-Expert 13 software, which generated a total of nine experimental conditions. To minimize bias, the experiments were carried out in a randomized order, with each batch having a size of 25 g [11, 12].

Table 1. Factor level and responses for 3^2 factorial design investigation

	Batch	Level of poloxamer 188 (mg)	Level of shearing speed (RPM)	Particle size (nm)	Saturation solubility (µg/ml)
1	NS1	0.5% (-1)	5000 rpm (-1)	384.0 ± 5.2	175.83 ± 1.15
2	NS2	0.5% (-1)	7500 rpm (0)	325.0 ± 4.0	183.24 ± 1.09
3	NS3	0.5% (-1)	10000 rpm (1)	356.0 ± 4.7	195.36 ± 1.32
4	NS4	1 % (0)	5000 rpm (-1)	467.0 ± 3.2	145.75± 0.53
5	NS5	1 % (0)	7500 rpm (0)	324.0 ± 11.0	140.78 ± 1.21
6	NS6	1 % (0)	10000 rpm (1)	375.8 ± 3.2	136.12 ± 1.43
7	NS7	1.5% (1)	5000 rpm (-1)	348.9 ± 7.6	194.90 ± 1.28
8	NS8	1.5% (1)	7500 rpm (0)	230.6 ± 8.2	231.92 ± 1.34
9	NS9	1.5% (1)	10000 rpm (1)	189.0 ± 4.0	288.24 ± 1.32

Characterization of nanosuspension

Measurement of particle size and zeta potential

The particle size and zeta potential of the nanosuspension were analyzed using the Malvern Zetasizer ZS200. Each sample underwent a minimum of three measurements, with the average value being used for data interpretation. The response surfaces were derived from these average values to better understand the formulation characteristics [13].

Saturation solubility test

The saturation solubility of the nanosuspension was determined by placing it in a container and stirring continuously for 48 hours using a mechanical shaker. Following this, the suspension was transferred to a four ml centrifuge tube, where it was subjected to centrifugation at 5,000 rpm for twenty minutes. After centrifugation, the supernatant was filtered, diluted with dissolving fluid, and analyzed using UV-visible spectrophotometry. All tests were performed in triplicate to ensure consistency [14, 15].

FTIR (Fourier transform infrared) spectroscopy

FTIR spectroscopy was performed on the nanosuspension using a Bruker FTIR Alpha II Spectrophotometer. The spectra were recorded over a wavelength range of 4000–400 cm^{-1} to study the chemical interactions and functional groups present in the formulation [16].

Differential scanning calorimetry (DSC) analysis

The thermal behavior of the drug, stabilizers, physical mixtures, and lyophilized nanosuspension was studied using DSC. A Shimadzu DSC-60 calorimeter was employed, with samples heated in aluminum pans under nitrogen at a rate of 5 °C/min from 50 °C to 400 °C. An unfilled aluminum pan was used as a reference point for comparison [17].

Powder X-ray diffraction (PXRD) analysis

PXRD was utilized to determine the crystalline form of both the drug and the lyophilized nanosuspension. Diffraction patterns were recorded using a Miniflex II X-ray diffractometer (Rigaku Co., Tokyo, Japan) to analyze the crystallinity and structural changes in the nanosuspension [18].

In-vitro drug release study

In-vitro dissolution tests were performed with the USP 24 paddle apparatus. To minimize foaming during the process, the dissolution medium was carefully added to the jar. The test was conducted at a temperature of 37 ºC with consistent paddle rotation speed. A dose-equivalent amount of nanosuspension was added to the dissolution vessel. At pre-defined time intervals, 5 ml of the sample was withdrawn, filtered, and analyzed spectrophotometrically. After each withdrawal, the vessel was replenished with 5 ml of fresh medium. The tests were repeated three times to ensure accuracy, and the data were averaged [6].

Scanning electron microscopy (SEM) analysis

The surface morphology of the lyophilized nanosuspension was observed using scanning electron microscopy (SEM) to assess the physical characteristics of the formulation [19].

Accelerated stability testing

The optimized formulation underwent a stability study over six months at accelerated conditions of 40 ± 2 °C and 75 ± 5% relative humidity, following ICH guidelines. The formulation was stored in amber-colored USP type I vials, sealed with aluminum caps and rubber plugs. After the stability period, dissolution testing was performed, and the in vitro drug release profile was evaluated to determine the stability of the formulation [20].

Results and Discussion

Statistical analysis

The regression coefficients were incorporated into the formula to construct a robust model. Regression analysis was performed using Design–Expert software to analyze the results effectively.

The fitted equation for mean particle size (nm)

$$Y1 = 230-44.54X1-21.00X2\ 37.50X12+23.19X22+71.94X1X2 \quad (1)$$

The fitted equation for saturation solubility (µg/ml)

$$Y2 = 231+29.86X1+6.87X2+33.25X12-5.31X22-40.81X1X2 \quad (2)$$

The polynomial equations (1) and (2) describe the influence of independent variables (X1 and X2) on the response variables. It was evident that each independent variable had a significant effect on the outcomes. The negative sign in the coefficients signifies that an increase in the factor results in a decrease in the response, and conversely, a decrease in the factor corresponds to an increase in the response. The absolute value of the coefficient indicates the magnitude of the impact, with larger values representing greater influence.

Contour plots **(Figure 1)** display the variations in mean particle size (nm) and saturation solubility, derived from a set of predetermined values, as shown in **Figure 2**. These plots revealed a complex, nonlinear relationship between the independent variables and the responses, further supporting the multidimensional nature of the effects [21].

FTIR

The FTIR spectra of the DTGS nanosuspension showed only minor alterations compared to the pure drug, with broadened and slightly shifted peaks. These changes are consistent with findings from other similar studies [22].

DSC

The DSC analysis of the bulk drug revealed a sharp melting peak at 340 °C, beginning at 327 °C. However, the lyophilized DTGS nanosuspension exhibited a broader peak at 317 °C, signifying its transition into an amorphous state **(Figure 1)** [23].

Particle size and zeta potential

The particle size of the optimized nanosuspension was measured to be 144 nm, with a zeta potential of -26.9 mV, as shown in **Figure 2** [24].

PXRD

In the PXRD analysis, the raw DTGS displayed distinct crystalline peaks at specific 2Θ angles (13.74, 15.19, 18.9, 17.4, 18.94, 19.1, and 21.68), indicating its crystalline form. However, the lyophilized DTGS nanosuspension did not show these sharp peaks, aligning with prior studies, suggesting a shift towards amorphousness **(Figure 2)** [25].

In-vitro drug release study

The in-vitro dissolution study demonstrated a rapid release of 97.55% of the drug from the DTGS nanosuspension within 45 minutes, compared to only 32.98% release from the unprocessed drug. This clearly illustrates the substantial improvement in solubility and dissolution rate through nanosuspension formulation **(Figure 2)** [26].

SEM

Scanning electron microscopy (SEM) revealed that DTGS particles initially had a flat, elongated, and irregular shape, with sizes ranging from 4 to 30 micrometers. After undergoing lyophilization into a nanosuspension, the particles significantly reduced in size to around 200 nm, possibly due to hydrophobic interactions with mannitol, which served as a cryoprotectant [27].

Stability study

Following six months of storage under accelerated conditions (40 ± 2 °C, 75% RH), the optimized formulation showed a drug content of 98.94 ± 0.23% and a cumulative drug release of 97.63 ± 0.23%, which were nearly identical to the initial values. These findings confirm the stability of the formulation after six months [28, 29].

A summary of the optimized formulation composition **(Table 2)**, its physicochemical properties **(Table 3)**, in-vitro release data including CDR and release kinetics **(Table 4)**, and the stability study outcomes **(Table 5)** can be found in **Table 2**.

Table 2. Optimized batch formulation

1	**Amount of DTGS**	**20 mg**
2	Amount of polaxamer 188	150 mg
3	Solvent-antisolvent Ratio	1:20

4	Shearing speed	10,000 rpm
5	Shearing time	30 mins
6	Amount of mannitol 1:1 (cryoprotectant)	170 mg

Table 3. Physicochemical parameters of optimized batch

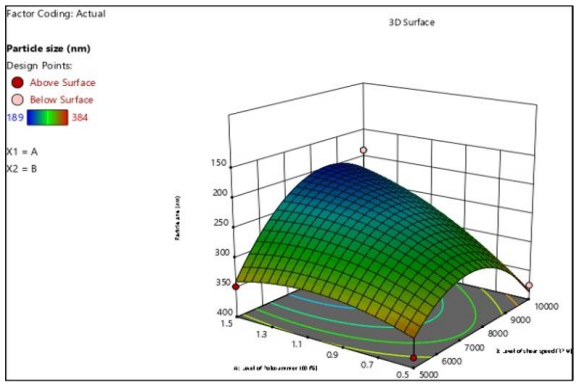
Sr. No.	Parameters	Results
1	Particle size	144.5 nm
2	Zeta potential	-26.9 mV
3	PDI	0.393
4	Drug content	98.87%
5	Saturation solubility	288.67 (µg/ml)
6	In vitro drug release	97.54 % uptil 45 mins

Table 4. *In vitro* release % CDR and release kinetic

Formulations	% Cumulative drug release in 45 min	Release kinetics R^2
NS optimized	97.55 ± 0.49	R^2 = 0.997 (Zero order)
Pure drug	32.98 ± 0.24	R^2 = 0.996 (Zero order)

Table 5. Stability study of nanosuspension

Formulation	% Drug content (40 ± 2 °C/75% RH)	% CDR (40 ± 2 °C/75% RH)
Optimized formulation	98.94 ± 0.23	97.63 ± 0.23



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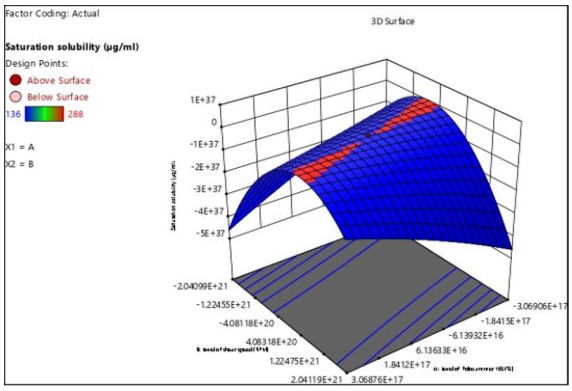

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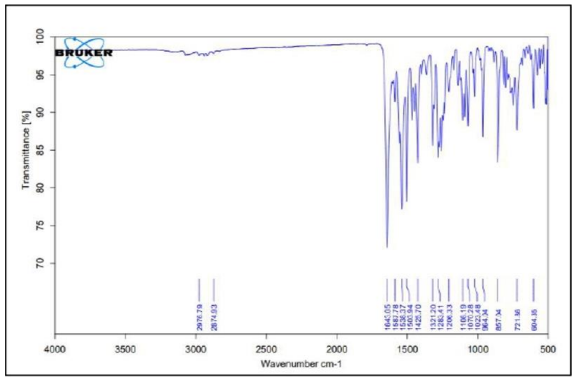

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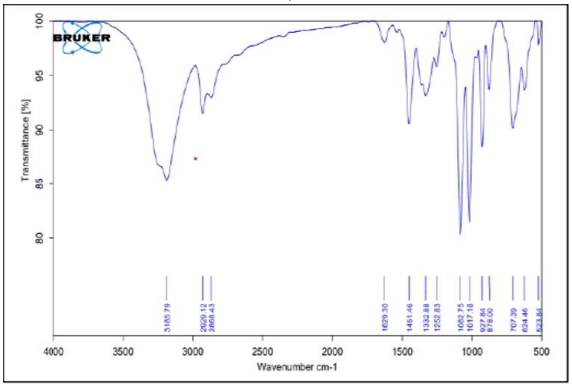

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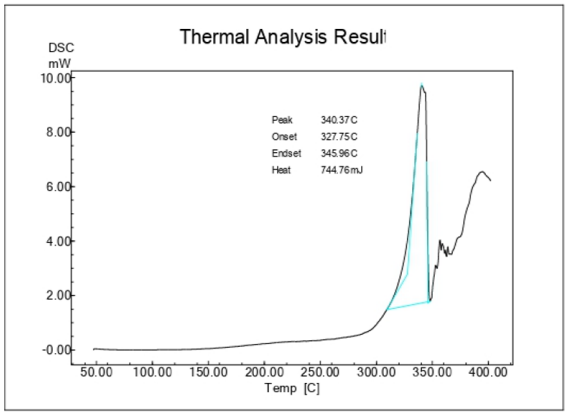

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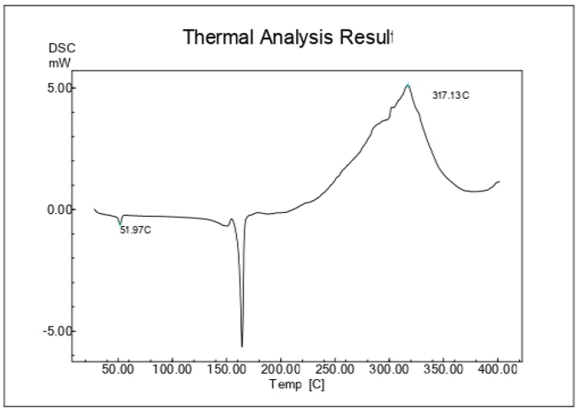

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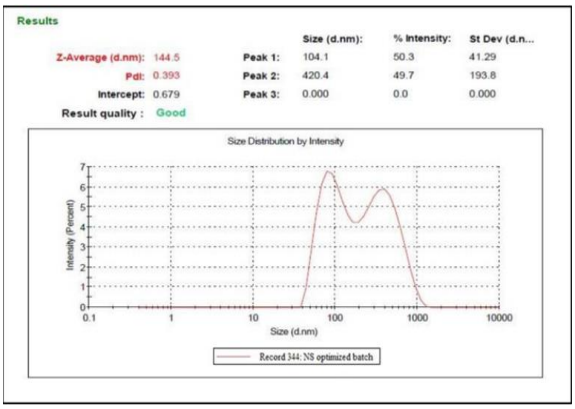
Figure 1. a) Counter plot of particle size, b) Counter plot of saturation solubility, c) FTIR of DTGS, d) FTIR of DTGS nanosuspension, e) DSC thermograph of DTGS, and f) DSC of DTGS nanosuspension.



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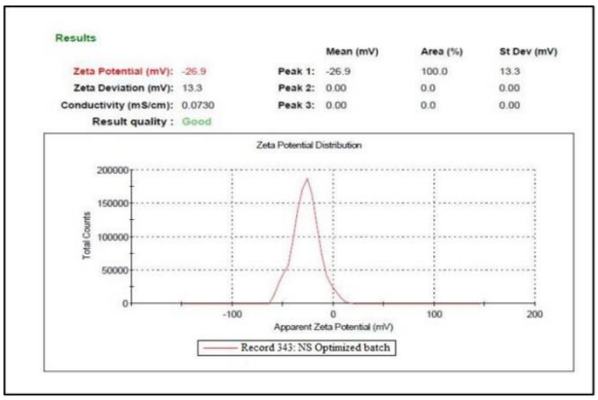

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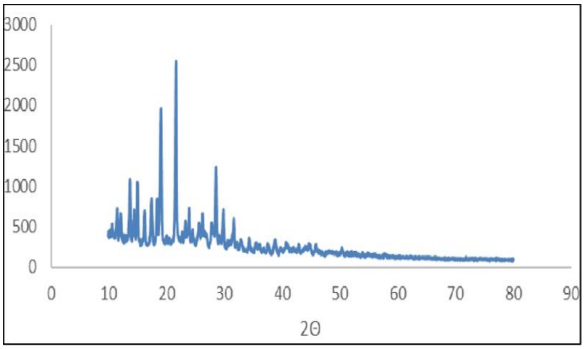

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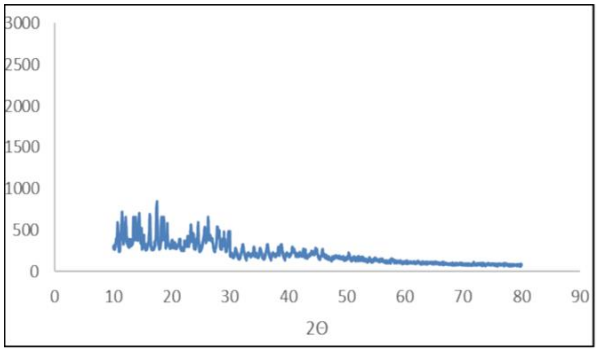

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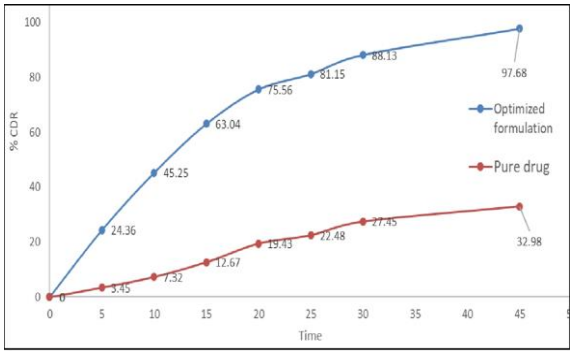

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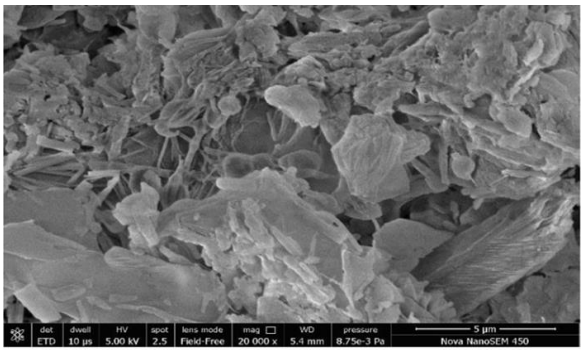

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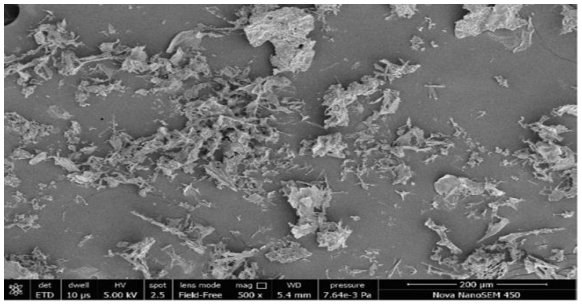

g)

Figure 2. a) Particle size, b) Zeta potential graph of nanosuspension, c) PXRD of DTGS, d) PXRD of lyophilized nanosuspension of DTGS, e) in vitro drug release in phosphate buffer 6.8, f) SEM of DTGS, and g) SEM of lyophilized nanosuspension of DTGS

Conclusion

The development of DTGS nanosuspensions was successfully achieved through high-speed homogenization. The key variables, such as homogenization speed and surfactant concentration, were found to have a major influence on the particle size distribution. With optimized conditions, nanosuspensions were created with particle sizes under 200 nm and a low polydispersity index. A significant improvement in the dissolution rate of DTGS was observed, demonstrating the potential of this advanced delivery method as an effective alternative to traditional DTGS formulations.

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Conflict of Interest: None

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Ethics Statement: None

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