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A novel approach for oral delivery of ZINC using Zinc Sulfate solid lipid nanoparticles

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Abstract:

One potential tool to improve the absorption of zinc (Zn) is the use of solid lipid nanoparticles (SLNs). In this study, Zn-containing SLNs were prepared to explore a novel oral formulation and investigate its physicochemical properties, toxicity, and accelerated stability study. SLNs were fabricated using stearic acid as lipid and chitosan as coating for LNPs via double emulsion solvent evaporation followed by lyophilization. In vitro evaluations were performed before and after lyophilization. Zinc loading was optimized at 30% zinc (w/w) versus lipid, with drug loading efficiency (DL) of up to 90%. The physicochemical properties of Zn-SLNs, including particle size (from 83.7 ± 1.8 to 183.8 ± 5.0 nm), dispersion index (PDI), zeta potential (about 8.2 mV), and differential scanning calorimetry (DSC), were investigated. Morphological characteristics were evaluated using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD). Drug release studies showed that 75%, 83%, and 90% of zinc were released from the SLN-Zn-Chitosan formulation within 24 hours, respectively. A cytotoxicity assay (MTT) performed at 24, 48, and 72 hours showed no cytotoxic potential with cell viability at 100% of control cells. The accelerated stability study to evaluate particle size, zeta potential, and drug loading (%EE) at 6 months showed no significant changes, which could indicate the suitability of Zn-SLNs for oral applications.

Keywords: Chitosan; MTT; Nanoparticles; Solid lipid nanoparticles; Zinc sulfate

1. Introduction

Nanomaterials represent a versatile platform for biomedical applications, attributable to their distinctive properties such as high surface area, tunable size, and customizable functionality. These characteristics significantly enhance drug delivery systems by improving solubility, facilitating targeted delivery, and modulating release profiles, thereby augmenting therapeutic efficacy while minimizing adverse effects [1].

Among various nanocarriers, lipid-based nanoparticles, polymeric nanoparticles, and metal-organic frameworks have shown promising results in delivering therapeutic agents effectively. Additionally, nanomaterials are being explored for co-delivery of drugs and diagnostic agents, paving the way for the ragnostic applications [2].

Solid lipid nanoparticles (SLNs), in particular, have gained attention for their biocompatibility, stability, and ability to encapsulate both hydrophilic and lipophilic drugs. The use of SLNs in oral drug delivery is of great interest due to their potential to protect sensitive drugs from gastrointestinal degradation and improve bioavailability [3].

SLNs are noted for their better drug stability and prolonged release compared to liposomes. SLNs enhance compliance through stability and controlled release, while liposomes, despite being biocompatible, face stability challenges. Further studies on absorption and side effects are needed for a comprehensive comparison, but SLNs generally provide better stability and delivery. Zn-SLNs are typically more stable due to their solid lipid matrix, which minimizes leakage and degradation of encapsulated zinc. In contrast, zinc liposomes may be less stable due to their aqueous core, leading to potential leakage over time [4–7].

Using solid lipid nanoparticles (SLNs) for zinc supplementation has several advantages over conventional methods such as traditional zinc supplementation tablets or liquid formulations because SLNs are promising alternatives to traditional zinc supplementation methods by increasing bioavailability, providing controlled release, reducing toxicity, enabling targeted delivery and improving patient compliance by using single dose instead of multiple dose [8].

Zinc (Zn) is a crucial trace element for various metabolic processes and is essential for human health [9]. It is the second least abundant element in the body after iron, playing key roles in gene function, hormone synthesis, and structural integrity of chromatin and membranes [10, 11]. Zn deficiency significantly impacts organs, including the immune, muscular, central nervous, digestive, and reproductive systems. The World Health Organization reports that around 31% of the global population is Zn deficient, with a prevalence of 4% to 73% [12]. Nearly 2 billion people are affected, contributing to major health issues like diarrhea and infections. In developing countries, Zn deficiency ranks among the top ten causes of health burdens [13].

Therefore, Zn sulfate ($ZnSO_4$) is recommended by the WHO as a water-soluble compound in dosage forms (e.g., syrup and dispersible tablets) and is commonly used as an authorized supplement that is the least expensive in the European Union [14]. However, it is worth mentioning that $ZnSO_4$ has strong metallic properties and is bitter and astringent [14], and nausea and abdominal pain are the most common side effects of Zn therapy [15].

In the early 1990s, lipid nanoparticles (LNPs) were first invented by Müller, and then almost 5 years later, in 1995, SLNs were introduced as an alternative to nanoemulsions, liposomes, and polymeric NPs as a novel drug delivery system [16]. The formula of SLNs has been developed for various intravenous injections, oral, topical (dermal), and pulmonary consumption [17]. Yang et al. (1999) and Demirel et al. (2001) demonstrated the drug release characteristics and systemic bioavailability of oral drug delivery in SLNs [18]. The particle sizes of SLN are usually limited to the range of $50-1000$ nm. Considering their advantages, such as NP size, environmentally friendly characteristics, economical production (cost-benefit), and the simplicity of increasing the production batch size, SLNs have attracted the attention of researchers [19].

Although several studies have focused on the synthesis of SLNs containing Zn oxide in topical forms, there is no report on the oral delivery of these particles, especially when using the hydrophilic compound $ZnSO_4$ heptahydrate ($ZnSO_4{\cdot}7H_2O$). According to the mentioned cases, there are currently no oral Zn solid lipid nanoparticles, and in this research, Zn is prepared for oral consumption using SLNs where lipids shield the Zn. It should be noted that in this formulation, lipids protect Zn and prevent its easy interaction with the mucosa of the digestive system. As a result, it will be possible to reduce side effects when taking oral Zn. The physicochemical properties of SLNs containing Zn, including particle size distribution, zeta potential, differential scanning calorimetry (DSC), drug loading (DL) capacity, drug entrapment efficiency (%), and Fourier transform infrared spectroscopy (FT-IR) analysis, underwent investigation. In addition, morphological characteristics were evaluated by a microscope, X-ray diffraction (XRD) spectroscopy, drug release profile, and cytotoxicity assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide: MTT). The main advantage of the SLN technique is the protection of Zn by the lipid shell, which leads to a lack of direct contact of Zn with the mucosa of the gastrointestinal tract and thus a reduction in side effects such as diarrhea [14, 20].

Also, compared to traditional zinc delivery systems such as zinc sulfate formulations, zinc-loaded solid lipid nanoparticles (Zn-SLNs) offer notable advancements in terms of absorption, therapeutic efficacy, and patient compliance. Conventional zinc sulfate often suffers from limited bioavailability due to extensive first-pass metabolism, necessitating higher dosages that can induce gastrointestinal disturbances [20]. Zn-SLNs, on the other hand, exhibit enhanced intestinal absorption owing to their reduced particle size and the ability to shield zinc from enzymatic degradation, which improves systemic availability [21]. Furthermore, the amorphous structure of Zn-SLNs promotes the solubility of Zinc, while their controlled release kinetics maintain stable plasma concentrations, thereby minimizing the need for frequent dosing [22]. In addition, the lipid-based matrix of Zn-SLNs serves to mask the undesirable metallic taste of zinc and reduces gastrointestinal irritation, which are common drawbacks of traditional zinc supplements, thereby improving patient compliance, particularly during extended treatment regimens [20]. These characteristics make Zn-SLNs a more efficient, bioavailable, and patient-friendly alternative to conventional zinc delivery systems.

2. Materials and methods

2.1 Materials

Stearic acid 98%, chitosan (high molecular weight), and polyvinyl alcohol 98% were purchased from Sigma Aldrich (Germany); dichloromethane (99% v/v), zinc sulfate heptahydrate USP grade ($ZnSO_4{\cdot}7H_2O$), hydrochloric acid 37%, dimethyl sulfoxide (DMSO, 99.9% v/v), and methanol (99.85% v/v) were purchased from Merck (Germany).

Purified water was used wherever required. All other utilized chemicals and solvents were of analytical grade.

2.2 Preparation of SLNs

There are normally six main techniques for the formulation of SLNs, including high-pressure homogenization (high or low temperature), microemulsion, high-speed stirring, solvent diffusion technique, ultrasound method, and the like [23]. This research used a double emulsion solvent evaporation method with a hydrophilic molecule to form Zn-loaded SLNs.

SLNs were prepared using stearic acid as the main lipid by the double emulsion solvent evaporation process. To this end, 100 mg of stearic acid was dissolved in a 10-mL mixture of dichloromethane (methanol at a ratio of 1:1 v/v) [18]. This mixture was heated in a water bath at 60 °C for 3 min and then sonicated (Ultrasonic Homogenizer, Topsonics, Iran) at a temperature of 60 °C, a power of 20 kHz, and 100 W for 7 min. The mixture was subsequently homogenized with a Heidolf DIAX 900 homogenizer (Germany) at a speed of 21,000 rpm and a temperature of 60 °C for 4 min.

To prepare the Zn-containing aqueous phase, first, 10% w/v polyvinyl alcohol was prepared at 60 °C, and then $ZnSO_4$ (10, 20, and 30% by weight of total lipid weight) was added to it. Eventually, 0.2% w/v chitosan was added to the aque-

ous phase and then sonicated and homogenized similar to oily phase conditions [18]. Further, the aqueous phase was prepared without increasing $ZnSO_4$. The composition of SLN formulations is provided in Table 1.
To create the initial emulsion, the oily phase was carefully added to the aqueous phase using ultrasonication/homogenization. Subsequently, the initial emulsion was mixed with 20 mL of 1% w/v polyvinyl alcohol solution and homogenized in the same manner [18]. The solvent of the double emulsion solution was evaporated in a rotary evaporator while simultaneously being placed in an ice bath for 7 min. Next, the obtained SLNs were washed twice with water and then placed in a freezer at −80 ℃ for 24 hrs. Finally, they were lyophilized using a desktop freeze dryer (Biolab, Freeze Dryer, BFBT-103-B, USA) to provide the long-term stability of SLNs. Mannitol 1% was used as a cryoprotectant.

2.3 Characterization of (properties) of SLNs

2.3.1 Investigation of particle size and zeta potential

Zn-loaded SLNs before and after lyophilization were characterized by a Malvern Zetasizer Nano ZS instrument (UK) to assess the particle size, polydispersity index (PDI), and zeta potential. Three duplicates of each sample were collected.

2.3.2 FT-IR spectroscopy

FT-IR spectroscopy was performed as a qualitative identification technique using a Shimadzu FT-IR-8300 E spectrophotometer (Japan). The samples were analyzed in a wavenumber ranging from 400 to 4000 cm^{-1}, with KBr disks utilized as blank backgrounds.

2.3.3 Differential scanning calorimetry (DSC)

About 10 mg of NPs were weighed and enclosed in an aluminum container for the DSC experiment. The samples were placed in aluminum containers and heated in a range of $40-300$ °C at a rate of 10 °C/min under nitrogen gas (with a flow of 10 mL/min) using a DSC STA6000 PerkinElmer differential scanning calorimeter (USA).

2.3.4 XRD spectroscopic analysis

XRD analysis was conducted by copper radiation under the conditions of 40 kV and 40 mA at different angles (2θ) from 5 to 80° (Xpert Pro Panalytical Almelo Diffractometer, Netherlands).

2.3.5 Morphology of the Zn-loaded SLNs

About 10 mg of lyophilized Zn-SLNs was dispersed in 1 mL of distilled purified water [24]. Then, 2 µL of the solution was placed on the glass surface after drying and coated with a thin layer of gold. This act avoids electrostatic charging during the process. The prepared suspension was examined by utilizing field emission scanning electron microscopy (FE-SEM; Zeiss SIGMA VP-FESEM, Germany). Zn-SLN dispersion droplets were applied to the copper network for transmission electron microscopy (TEM) imaging (Philips EM208S 100KV, Netherlands), and staining was performed using a phosphotungstic acid solution (2%) [18].

2.3.6 Measurement of drug entrapment and DL efficiencies

Entrapment efficiency (%) and DL (%) of Zn in different Zn-SLN formulations were determined by centrifuging various samples for 30 min at 4 °C. The formulated SLNs were centrifuged using a Hettich® Universal 320/320R centrifuge (Germany) for 4 min at 15,000 rpm or 21,000 rcf at 4 °C using AMICON ULTRA-15 15 ML-100 KDa tunes. The supernatants were evaluated, and the amount of free Zn was estimated by inductively coupled plasma optical emission spectroscopy (PerkinElmer Optima 8000) [25].

2.3.7 Dissolution profile and drug release test

The dialysis membrane approach served as the basis for the drug release analysis [26, 27]. The United States Pharmacopoeia was used to modify the selection of the dissolving medium and other variables (USP 42, NF 37). SLN samples were placed in a 12-14 kDa molecular weight cut-off dialysis tubing (Sigma) [26–28] and immersed in a dissolution medium (500 mL of 0.1 N hydrochloric acid at 37 °C) [28] under a dissolution apparatus condition (paddle) at 75 rpm (Erweka DT 720, Germany). About 5 mL of the sample was collected from the dissolution medium at different times (0.5, 1, 2, 4, 6, 8, 12 and 24 hrs). The Zn content of samples was assayed by inductively coupled plasma optical emission spectroscopy [25].

2.3.8 Cytotoxicity assay (MTT)

For the cytotoxicity assay, the best formulation (prepared of Zn solid lipids named SLN-Zn-Chi 3) was evaluated using the MTT technique [15, 25]. The MTT analysis is a favorite and standard procedure for measuring cell viability [29]. Its basis forms formazan color in reducing the MTT compound tetrazolium salt [30].
About 10,000 cells from the gastric (AGS) cell line were grown in 96-well plates placed in an incubator for 24 and 48 hrs [31]. Before the experiment, the nanoparticle culture medium solutions were sonicated in an ultrasonic bath. SLN-Zn-Chi-3 was divided into 15.625, 31.25, 62.5, 125, 250, and 500 µg/mL concentrations [31]. The AGS cell was dispersed in growth media.
The culture medium solutions were placed in an incubator in the dark for 3 hrs [32]. The medium containing MTT was then removed and replaced with 100 µL of dimethyl sulfoxide to dissolve the formazan crystals while shaking the plate [33, 34]. The absorbance of samples was measured at 570 nm with a reference filter of 630 nm by an enzyme-linked immunosorbent assay plate reader, and its amount was recorded. The viability of the cell was indicated as a percentage of unprocessed control cells ($> 100\%$).

2.3.9 Accelerated stability study

The optimal formulation (SLN-Zn-Chitosan 3) was selected to conduct an accelerated stability study over a period of six months [35]. Factors such as temperature, humidity, and light exposure can significantly influence the physical and chemical stability of Zn-SLN, potentially resulting in aggregation or degradation. Consequently, the SLN-Zn 3 sample, which demonstrated greater suitability following initial

evaluations, was stored in a sealed opaque polyethylene container for six months under accelerated stability conditions of (40 °C ± 2 °C/75% RH ± 5% RH). Assessments of particle size, zeta potential, and drug loading efficiency (EE%) were conducted at 0, 3, and 6 months.

3. Results and discussions

3.1 Effect of initial zinc content on SLN incorporation efficiency

To optimize the initial concentration of Zn in the SLN formulation based on the indication by the National Institute of Health, adult men and women need 11 and 8 mg of Zn per day, respectively, and children suffering from malnutrition need $2-4$ mg/kg of body weight. On the other hand, most of the tablets and syrup involving Zn are administered for diarrhea because 50% of deaths caused by Zn deficiency are related to diarrhea. The WHO has recommended 10 mg for $<$ 3-year-old children suffering from diarrhea. About 20 mg of Zn should be given daily for 10-14 days in older children (3-5 years old).

Therefore, the formulation was prepared to choose different concentrations of Zn (10%, 20%, and 30% by weight of lipid). The results revealed that increasing Zn concentration from 10 to 30% led to an increase in the percentage of Zn in the composition of SLN.

3.2 Particle size, PDI, and zeta potential

The size and PDI of the particles created before and after lyophilization underwent measurement. Particle size was in the range of 83.7 ± 1.8 to 183.8 ± 5.0 nm, with a zeta potential of 8.2 ± 0.43 to 12.5 ± 0.54 mV for the formulated SLN (Table 1).

Particle surface charge is determined by zeta potential analysis, which is considered a reliable indicator of formulation stability, especially for emulsions and colloidal suspensions [18]. The surface charge of stearic acid-based SLNs was found to be negative, which aligns with previous research findings. The incorporation of Zn into SLNs did not significantly affect the particle surface charge, as evidenced by the comparison of the zeta potential between blank SLNs and Zn-loaded SLNs (Table 2). The presence of chitosan on the lipid particle surface resulted in a positive charge. This positive zeta potential of the lipid particles facilitates repulsion between the particles, thereby reducing the likelihood of aggregation. Additionally, positively charged particles are known for their greater affinity to bind to cells and

Table 1. Composition of solid-lipid-nanoparticle (SLN) formulations.

Formulation	Lipid concentration (mg)	Zinc concentration (%, w/w lipid)	Chitosan concentration (%, w/v)
SLN	100	-	0.2
SLN-Zn 1	100	10	0.2
SLN-Zn 2	100	20	0.2
SLN-Zn 3	100	30	0.2
SLN-Chitosan	100	-	0.2
SLN-Zn-Chitosan 1	100	10	0.2
SLN-Zn-Chitosan 2	100	20	0.2
SLN-Zn-Chitosan 3	100	30	0.2

Note. SLN: Solid lipid nanoparticle; Zn: Zinc.

Table 2. Size and PDI of SLNs.

Formulation Code	Before lyophilization		After lyophilization		
	Size (nm)	PDI	Size (nm)	PDI	Zeta potential (mV)
SLN	175.06±5.1	0.395±0.075	183.8±5.0	0.553±0.05	-9.77±0.97
SLN-Zn 1	154.2±1.3	0.545±0.15	173.4±3.8	0.531±0.048	-10.5±1.1
SLN-Zn 2	162.77±4.7	0.625±0.14	175.7±9.9	0.504±0.04	-12.1±0.47
SLN-Zn 3	147.61±2.7	0.438±0.08	164.2±8.2	0.39±0.27	-9.62±0.75
SLN-Chitosan	172.7±3.6	0.465±0.2	182.7±5.8	0.441±0.01	12.5±0.54
SLN-Zn-Chitosan 1	100.0±1.0	0.127±0.05	146.3±4.8	0.550±0.01	9.3±0.67
SLN-Zn-Chitosan 2	88.8±0.9	0.353±0.09	137.6±3.6	0.542±0.07	8.6±0.38
SLN-Zn-Chitosan 3	83.7±1.8	0.430±0.07	132.3±0.8	0.521±0.04	8.2±0.43

Note. PDI: Polydispersity index; SLN: Solid lipid nanoparticle; Zn: Zinc; SD: Standard deviation. The values are expressed as means ± SDs (n = 3).

subsequent cellular uptake [18].

3.3 FT-IR

Figure 1 (SLN-Zn-Chitosan 1 (black), SLN-Zn-Chitosan 2 (red), and SLN-Zn-Chitosan 3 (blue)) illustrates the FTIR spectra of synthesized Zn-SLNs. The essential mode of vibration at 3432 cm^{-1} is likely to be attributed to O-H stretching and deformation due to water adsorption on the metal surface [36]. Furthermore, the vibration at 3432 cm^{-1} is likely to be a result of the combination of NH_2 at chitosan and OH group stretching vibration [37].
The band at 2924 cm^{-1} is attributed to C-H stretching vibration (the characteristic band of stearic acid) [38], and the 2853 cm^{-1} band is assigned to the asymmetric stretching of CH_3, and CH_2 of the chitosan polymer [39]. The 1735, 1350 and 1463 cm^{-1} correspond to the C=O symmetric and asymmetric stretching vibrations, respectively [39]. Additionally, the absorption at 501 to 722 cm^{-1} is related to Zn [39], while the bond at 1105 cm^{-1} and 1240 cm^{-1} is attributed to the C-O or C-O-C stretching vibration [39, 40].

3.4 DSC

The DSC examination was conducted on lyophilized formulations of SLN-Zn-Chitosan 1, SLN-Zn-Chitosan 2, and SLN-Zn-Chitosan 3, which contain stearic acid as the main lipid. The melting point of $ZnSO_4 \cdot 7H_2O$ is 100 °C. In addition, this peak can overlap with stearic acid with melting points at $60 - 70$ °C , indicating that the unmodified stearic acid used in this study has crystalline properties, which is in line with the findings of previous studies [41]. The thermogram of drug-loaded SLNs (figure 2 (2a) SLN-Chitosan, (2b) SLN-Zn-Chitosan 1, (2c) SLN-Zn-Chitosan 2, and (2d) SLN-Zn-Chitosan 3) does not exhibit a peak corresponding to zinc sulfate. This suggests that zinc is not present in its amorphous state within the SLNs [41].

3.5 XRD

X-ray diffractograms of lyophilized SLN-Zn-Chitosan 1, SLN-Zn- Chitosan 2, and SLN-Zn- Chitosan 3 structures are shown in figure 3. X-ray diffraction (XRD) patterns indicated that SLNs have an amorphous form. The diffractograms of stearic acid based on a previous study represented sharp peaks at 7.07°, 20.60°, 21.71°, and 24.05°, which is a reason for the lipid crystalline nature [38]. Based on the results of a previous study by Chandra Dey et al., the broad peaks at 9.81° and 20.48° demonstrated typical fingerprints of semi-crystalline chitosan [38].
The findings of a study on the crude sample of $ZnSO_4 \cdot 7H_2O$ diffraction peaks by Harzali et al. revealed that the main peaks at 5.143°, 20.143°, 35.143°, 50.143°, and, 65.143° were related to Zn [42]. Here, the intensities of Zn peaks in the Zn-SLNs are weak, which can be due to covering Zn with lipids. In conclusion, the XRD pattern of SLNs appears to be less crystalline and indicates the amorphous nature of SLN-Zn-Chitosan formulations [38].

3.6 Morphological studies (FE-SEM and TEM)

FE-SEM and TEM imaging of Zn-SLN revealed more or less spherical NPs dispersed in a bed of a united lipid/polymer structure, highlighting the formation of SLNs (figures 4 (4a) SLN-Zn-Chitosan 1, (4b) SLN-Zn-Chitosan 2, and (4c) SLN-Zn-Chitosan 3) and figure 5). The stable formulation properties of SLNs were further confirmed by the morphology and shape of the particles, as well as the absence of non-agglomerated particles [43].

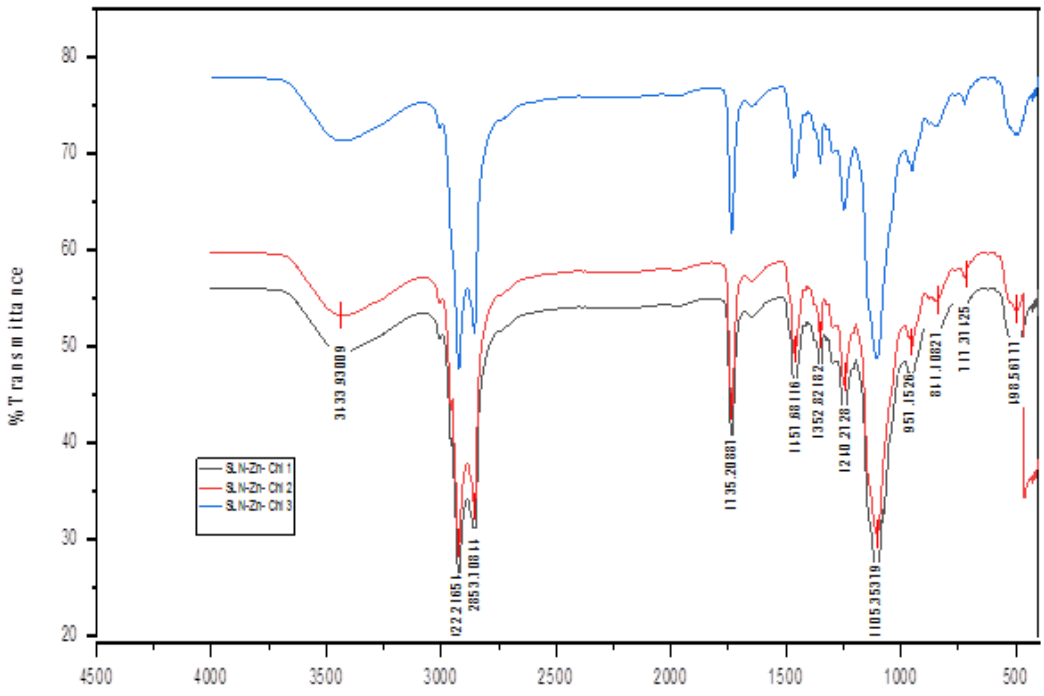


Figure 1. FT-IR spectra of SLN-Zn-Chitosan 1 (black), SLN-Zn-Chitosan 2 (red), and SLN-Zn-Chitosan 3 (blue).

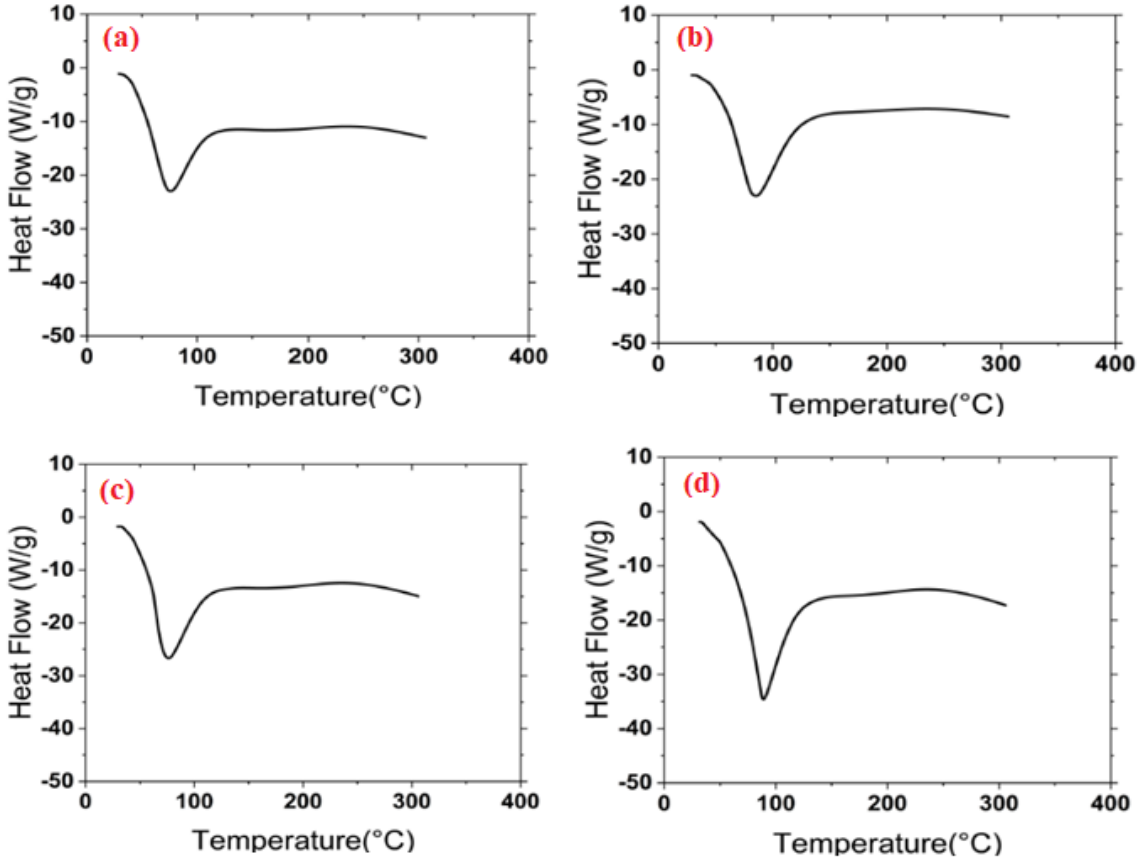


Figure 2. DSC thermograms of SLNs: (a) SLN-Chitosan, (b) SLN-Zn-Chitosan 1, (c) SLN-Zn-Chitosan 2, and (d) SLN-Zn-Chitosan3.

3.7 *In vitro* release profile study (drug release study)

In vitro drug release studies were conducted on various Solid Lipid Nanoparticles (SLNs) as depicted in figure 6, demonstrating a prolonged release profile over a 24-hour period. Specifically, SLN-Zn-Chitosan 1, SLN-Zn-Chitosan 2, and SLN-Zn-Chitosan 3 achieved drug release percentages of 75%, 83%, and 90%, respectively, with SLN-Zn-Chitosan 3 exhibiting the most favorable profile at 90%. This investi-

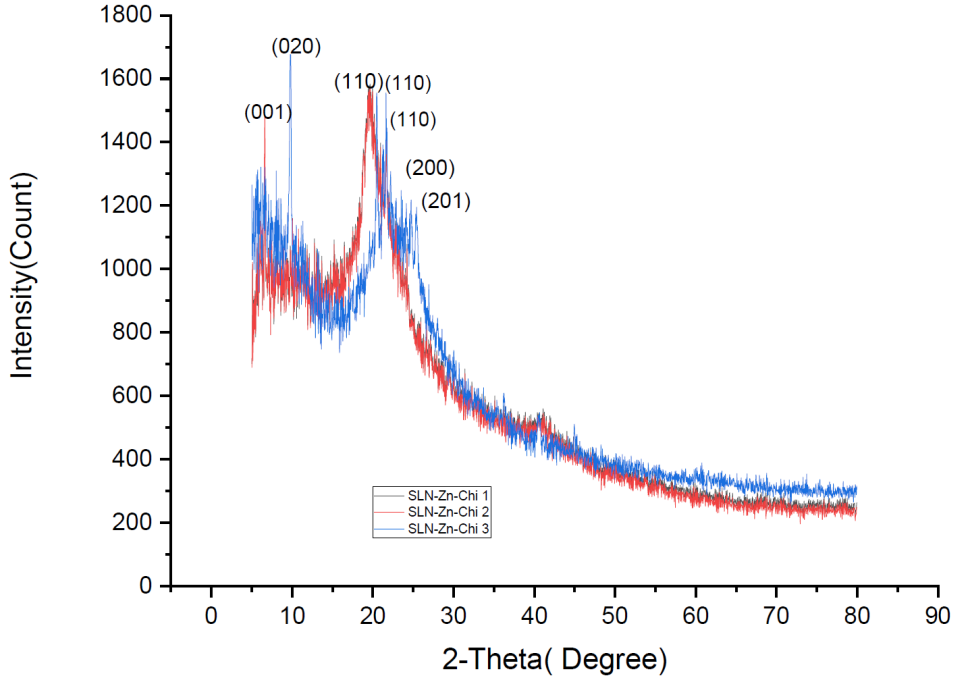


Figure 3. XRD patterns of the SLN-Zn-Chitosan 1(black), SLN-Zn-Chitosan 2 (red) and SLN-Zn-Chitosan 3 (blue).

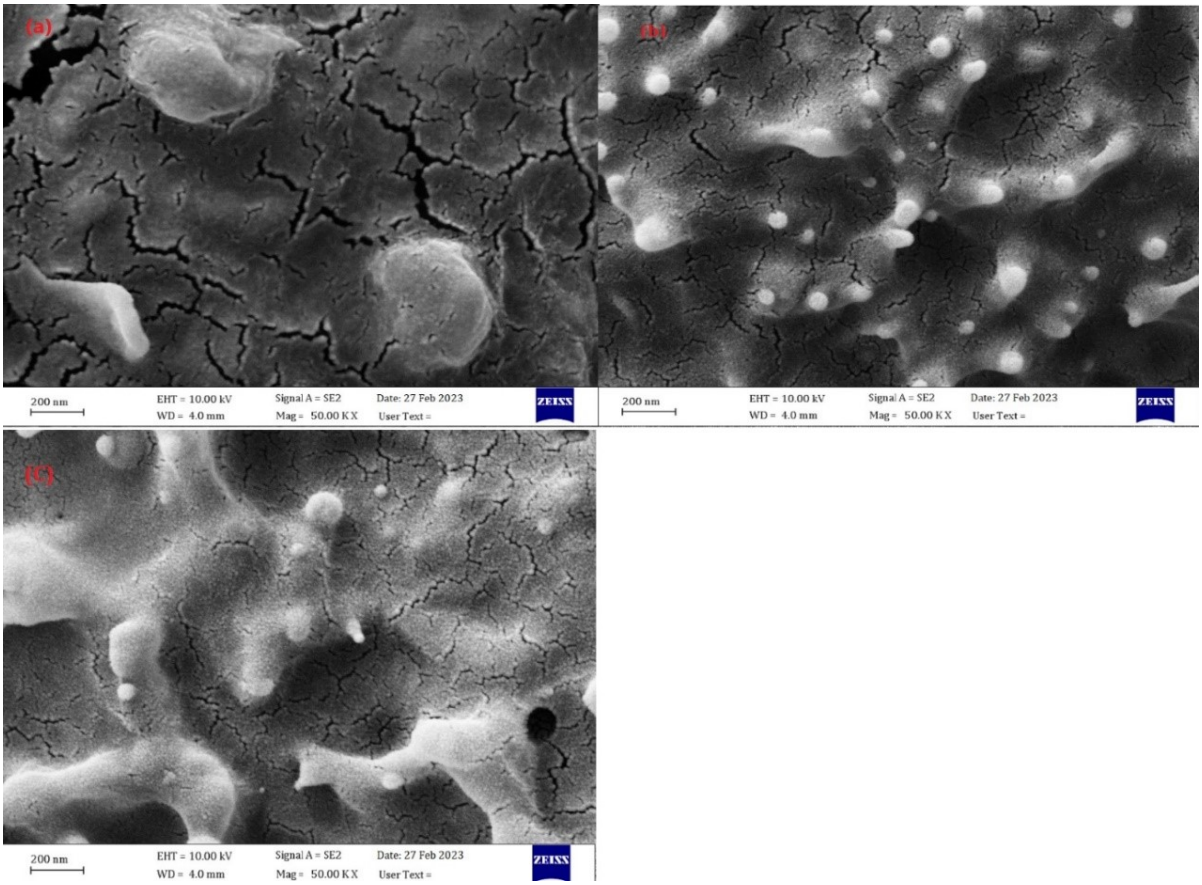


Figure 4. FE-SEM images of (a) SLN-Zn-Chitosan 1, (b) SLN-Zn-Chitosan 2, and (c) SLN-Zn-Chitosan 3.

gation reveals that SLNs are capable of maintaining drug release for extended durations, primarily attributable to the lipids' capacity to modulate drug diffusion within the solid matrix. Such a controlled release mechanism facilitates a gradual and uniform release of the active pharmaceutical ingredient over 24 hours, ultimately resulting in prolonged therapeutic effects and a decrease in dosing frequency. Consequently, patients experience improved stability of medication levels in their system, thereby enhancing adherence to the treatment regimen [4]. The results of in vitro drug release are summarized in Table 3.

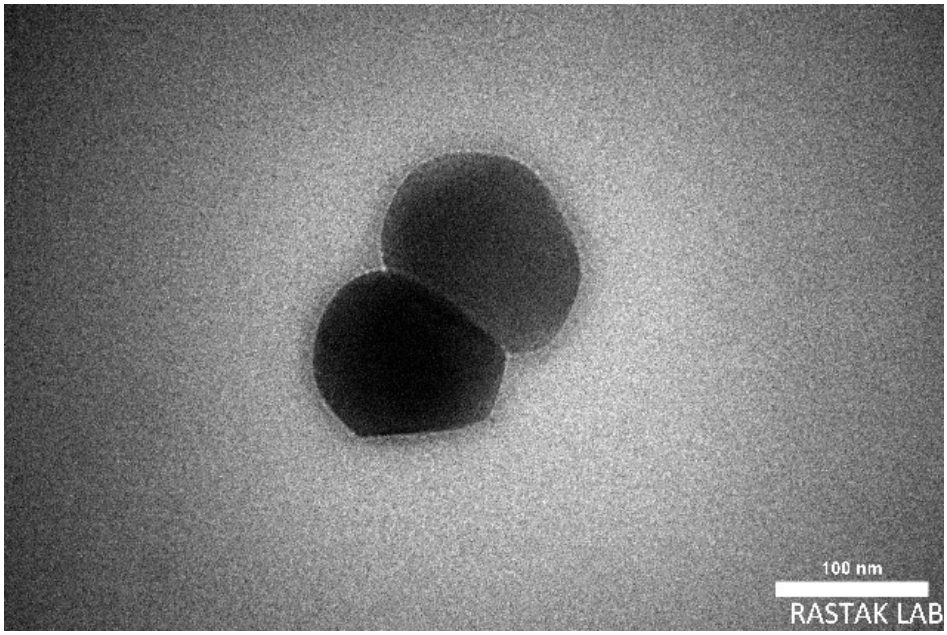


Figure 5. TEM images of SLN-Zn-Chitosan 3.

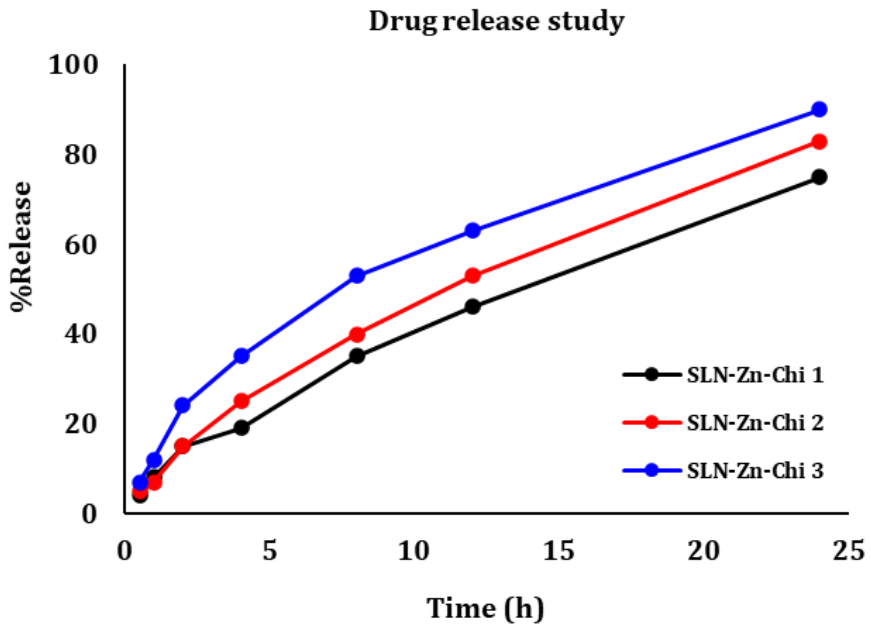


Figure 6. *In vitro* release profile study of Zn-loaded SLNs.

3.8 DL efficiency (EE%)

DL efficiency, or EE (%), is a phrase that is called the percentage of drugs that are excellently trapped in NPs. The EE% was calculated by equation (1).

$$EE\% = \frac{\text{Total Drug conc.} - \text{Supernatant Drug conc.}}{\text{Total Drug conc.}} \times 100 \quad (1)$$

To calculate the amount of medicine enclosed in NPs, the loading capacity of the load is measured based on the weight of the NPs. The Zn was included in varying quantities, specifically 10%, 20%, and 30%, in a weight percentage of the entire lipid matrix. The effectiveness of trapping the medicine inside the NPs ranged from roughly 80% to 92% (Table 4 and figure 7). The greatest trapping efficiency was related to SLN-Zn-Chitosan 3.

3.9 Cytotoxicity assay (MTT)

The best formula (SLN-Zn-Chitosan 3) was selected to perform the MTT assay and cytotoxicity evaluation at two time points (24, 48 and 72 hrs). The results revealed that SLN formulations do not have any potential cytotoxic effects at any time point (Table 5 and figure 8). Cell viability was at least 100% of control cells. Therefore, the results showed the absence of any cytotoxic effects and the potential of prepared SLN formulation for oral Zn delivery. It can be inferred that the Zn-SLN composition has cytocompatibility [44].

3.10 Accelerated stability study

The optimized formulation (SLN-Zn-Chitosan 3) was subjected to a stability study evaluating particle size, zeta potential, and drug loading (%EE) at 0, 3, and 6 months (Table 6). The results showed that there was no significant change over 180 days, indicating good stability. The formulation also had desirable physical properties for potential applications.

Table 3. *In-vitro* release profile study of Zn-loaded SLNs.

Time (h)	% Mean Drug Release (n = 6)		
	SLN-Zn-Chitosan 1	SLN-Zn-Chitosan 2	SLN-Zn-Chitosan 3
0.5	3	5	7
1	8	7	12
2	15	15	24
4	19	25	35
8	35	40	51
12	46	53	62
24	75	83	90

Note. SLN: Solid lipid nanoparticle; Zn: Zinc.

Table 4. EE% of zinc in the structure of prepared SLNs.

Formulation name	EE (%)
SLN	-
SLN-Zn-Chitosan 1	80±2.0
SLN-Zn-Chitosan 2	81±2.5
SLN-Zn-Chitosan 3	92±2.1

Note. EE: Encapsulation efficiency; SLN: Solid lipid nanoparticle; Zn: Zinc; SD: Standard deviation. The results are expressed as means ± SDs (n = 3).

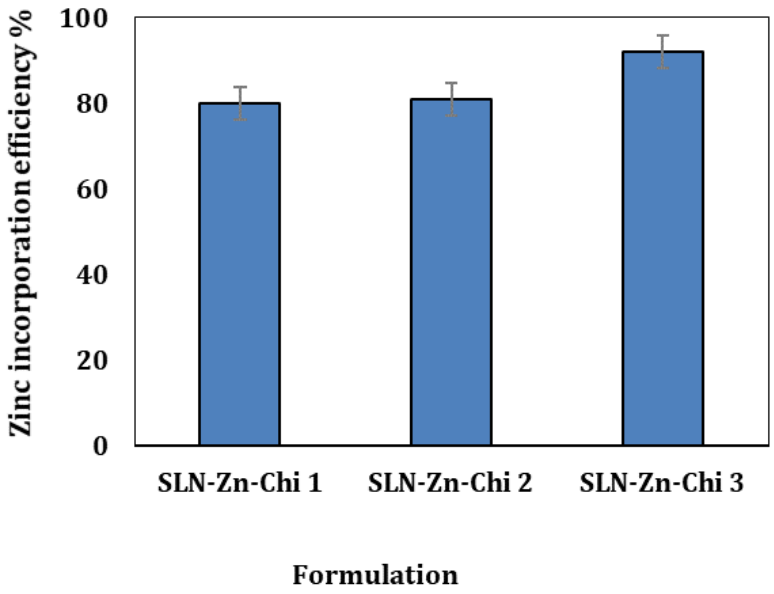


Figure 7. Zinc incorporation efficiency.

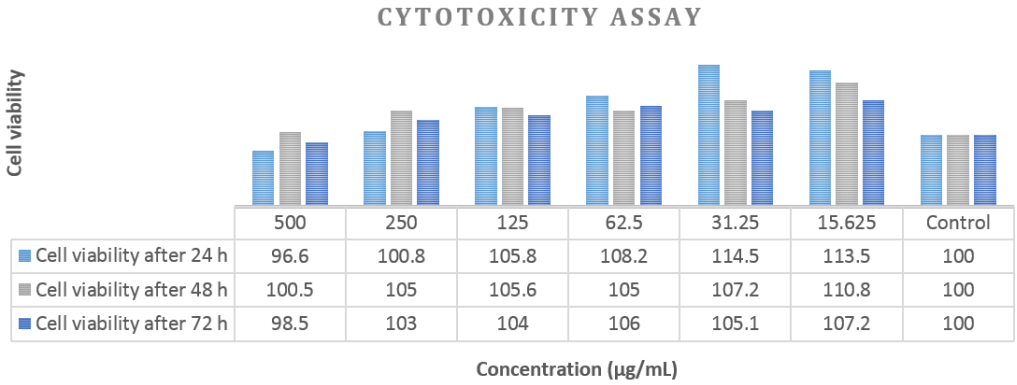
4. Conclusion

In this research, the oral composition of solid Zn lipid NPs was first developed as a substrate for the oral delivery of Zn. Developing an oral Zn delivery system based on lipids was the main subject. Accordingly, $ZnSO_4 \cdot 7H_2O$, chitosan, and stearic acid were used as components for the preparation of SLNs. Zn loading was optimized at 30% Zn (w/w), and chitosan was 0.2% against lipid, showing a 90% prolonged release after 24 hrs. Physicochemical and release studies in laboratory conditions indicated the achievement of the research goal. Considering that Zn deficiency affects growth, the prepared SLNs before or after lyophilization can be utilized as non-toxic food supplements in the form of tablets, capsules, sachets, oral solutions, a solution, or oral syrup before lyophilization.

Table 5. Cytotoxicity assay of the prepared SLN-Zn-Chitosan 3 formulation after 24, 48 and 72 hrs.

Concentration (µg/mL)	500	250	125	62.5	31.25	15.625	Control
Cell viability after 24 h	96.6	100.8	105.8	108.2	114.5	113.5	100
SD (n = 6)	8.3	6.3	6.1	6.3	1.2	6.2	3.9
Cell viability after 48 h	100.5	105	105.6	105	107.2	110.8	100
SD (n = 6)	5.2	4.2	5.1	6.2	6.6	6.1	2.6
Cell viability after 72 h	98.5	103	104	106	105.1	107.2	100
SD (n = 6)	7.1	5.6	6.7	6.5	5.4	4.9	3

Note. SLN: Solid lipid nanoparticle; Zn: Zinc; SD: Standard deviation. The results are expressed as means ± SDs (n = 3). The results are presented as means ± SDs (n = 6).



	500	250	125	62.5	31.25	15.625	Control
Cell viability after 24 h	96.6	100.8	105.8	108.2	114.5	113.5	100
Cell viability after 48 h	100.5	105	105.6	105	107.2	110.8	100
Cell viability after 72 h	98.5	103	104	106	105.1	107.2	100

Figure 8. SLN cytotoxicity assay: Relative cell viability for SLN-Zn-Chitosan 3 formulation after 24,48 and 72 hrs.

Table 6. Accelerated stability study of the prepared SLN-Zn-Chitosan 3 formulation (40°C ± 2 °C/75% RH ± 5% RH).

Formulation name	Initial	After 3 months	After 6 months
Size (nm)	132.3±0.8	134.6±0.33	136.7±0.5
PDI	0.521±0.04	0.579±0.05	0.548±0.05
Zeta potential (mV)	8.2±0.43	8.62±0.5	8.25±0.22
EE (%)	92±2.1	90±1.1	89±1.4

Note. PDI: Polydispersity index; SLN: Solid lipid nanoparticle; Zn: Zinc; SD: Standard deviation, EE: Encapsulation efficiency. The values are expressed as means ± SDs (n = 3).

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Authors contributions

Authors have contributed equally in preparing and writing the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors assert that they do not have any identifiable conflicting financial interests or personal relationships that might be perceived to influence the work presented in this paper.

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