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Exploring The Potential of Solid Lipid Nanoparticles to Improve the Oral Bioavailability of Niclosamide: A Pharmaceutical and Stability Evaluation

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ABSTRACT

Background: Niclosamide, a BCS Class II drug with poor water solubility, suffers from limited oral bioavailability, necessitating innovative delivery strategies to enhance its therapeutic efficacy. **Objective:** This study aimed to develop and optimize solid lipid nanoparticles (SLNs) of Niclosamide to improve its solubility, stability, and bioavailability. **Methods:** Niclosamide-loaded SLNs were prepared using the solvent emulsification-diffusion method. Optimization was performed by varying lipid-to-drug ratios, surfactant concentrations, and stirring times. The SLNs were characterized for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency (EE), drug loading capacity (DLC), and morphology. Stability studies were conducted at refrigerated and room temperatures for three months. In vitro drug release was assessed using the dialysis bag method, and in vivo pharmacokinetics were evaluated in rabbits using high-performance liquid chromatography (HPLC). **Results:** Optimized SLNs (NSED-2) showed a particle size of 208.6±2.2 nm, PDI of 0.376±0.04, and zeta potential of -34.11±1.2 mV. EE and DLC were 85.4±0.04% and 3.18±0.04%, respectively. In vivo, NSED-2 demonstrated a 2.04-fold increase in peak plasma concentration (Cmax: 4.07±0.124 µg/mL) and a 10.59-fold increase in area under the curve (AUC0→24: 21.19 µg·h/mL) compared to the marketed product. **Conclusion:** Niclosamide-loaded SLNs significantly enhanced drug solubility, stability, and oral bioavailability, offering a promising platform for improving the delivery of poorly water-soluble drugs.

INTODUCTION

The majority of active pharmaceutical ingredients (APIs) developed by the pharmaceutical industry exhibit poor water solubility, which severely limits their oral bioavailability and consequently compromises therapeutic efficacy and safety (1). Niclosamide (NCL), a well-established oral anthelmintic drug, exemplifies this challenge, as it belongs to the Biopharmaceutics Classification System (BCS) Class II, characterized by low solubility and high permeability (2). Its aqueous solubility is reported at 0.23 µg/mL, significantly hindering its absorption in the gastrointestinal tract (3). Various approaches have been explored to enhance the solubility and bioavailability of BCS Class II drugs, including chemical modification, solid dispersion, and the development of nano-carrier systems. Among these, the reduction of particle size to the nanoscale has emerged as a promising strategy to improve dissolution rates, enhance permeability, and ultimately increase bioavailability (4).

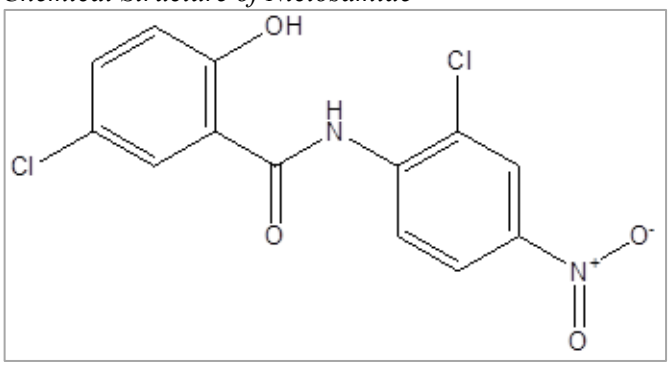
Solid lipid nanoparticles (SLNs) have garnered significant attention as a versatile drug delivery platform, particularly for poorly soluble drugs like Niclosamide. These nanoparticles offer several advantages, including improved drug stability, controlled release, and the potential for enhanced gastrointestinal absorption due to their nano-dimensions

and bioadhesive properties (5,6). Niclosamide's traditional use as an anthelmintic agent targeting tapeworm infections has been well-documented; however, recent studies have highlighted its potential anticancer activity, although the precise mechanisms remain elusive (7,8). Despite its therapeutic promise, the clinical utility of Niclosamide is limited by its poor aqueous solubility and consequent suboptimal bioavailability, necessitating innovative approaches to optimize its pharmacokinetic profile (9,10).

The solvent emulsification diffusion (SED) technique, a method that avoids high-temperature processing, has proven effective in the fabrication of SLNs. This technique involves the diffusion of an organic solvent containing dissolved lipids into an aqueous phase, leading to the formation of nanoparticles. Its efficiency in encapsulating hydrophobic drugs, such as Niclosamide, while maintaining drug stability and enhancing bioavailability, makes it a method of choice for this study (11,12). The current research aimed to develop an optimized SED-based process for the preparation of Niclosamide-loaded SLNs. The formulation was characterized in terms of particle size, zeta potential, entrapment efficiency, drug loading, and stability, with a focus on enhancing in vitro dissolution and in vivo bioavailability. Additionally, the study addressed potential compatibility issues among formulation components, employing Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) to confirm the absence of interactions and to assess the physical state of the drug within the SLNs (13,14).

Figure 1

Chemical Structure of Niclosamide



This investigation represents a significant step in leveraging SLNs to overcome the inherent limitations of Niclosamide's solubility and bioavailability. By optimizing formulation parameters and evaluating the pharmacokinetics of the SLNs in vivo, this study contributes valuable insights into the potential of nanotechnology to improve the therapeutic performance of poorly water-soluble drugs. The findings provide a robust foundation for further exploration of SLNs as a platform for oral drug delivery, with implications for both Niclosamide and other challenging APIs (15,16).

MATERIAL AND METHODS

The materials and methods of the study were meticulously designed to ensure reliability and reproducibility. Niclosamide was generously provided by Shaigan Pharmaceuticals Pvt. Ltd, Pakistan, while other chemicals, including stearic acid and Tween-80, were obtained from Acros Organics Thermo Fisher Scientific, New Jersey, USA. Ethanol was sourced from Sigma-Aldrich Continental, USA, and Polyvinylpyrrolidone-29000 (PVP) from Crescent Chemical Company, New York, USA. All reagents used were of analytical grade. (Institutional Review Board (IRB) Number DREC/20160503-14)

The preparation of unloaded solid lipid nanoparticles (SLNs) employed the solvent emulsification-diffusion (SED) technique. Twelve formulations (BFSe-1 to BFSe-12) were developed by varying the concentrations of stearic acid, Tween-80, and PVP, along with stirring times. Stearic acid was dissolved in ethanol, and the aqueous phase contained Tween-80 and PVP dissolved in deionized water. The organic and aqueous phases were mixed at 1200 rpm using a magnetic stirrer, followed by dilution with deionized water, leading to nanoparticle formation. The organic solvent was removed using a rotary evaporator under reduced pressure (60 mbar). Zeta size and polydispersity index (PDI) were determined using a Zetasizer Nano ZS-90 (Malvern Instruments, UK). Table 1 provides details of the unloaded SLNs formulations.

Table 1

Unloaded SLNs Formulations

Formulation	Stearic Acid (g)	Tween-80 (g)	PVP-29000 (g)	Stirring Time (min)
BFSe-1	2.0	1.0	0.0	5
BFSe-2	1.0	1.0	0.0	5
BFSe-3	2.0	3.0	0.0	5
BFSe-4	1.0	2.0	0.0	5
BFSe-5	1.0	1.9	0.1	5
BFSe-6	1.0	1.8	0.2	5
BFSe-7	1.0	1.7	0.3	5
BFSe-8	1.0	1.6	0.4	5
BFSe-9	1.0	1.5	0.5	5
BFSe-10	1.0	1.6	0.4	10
BFSe-11	1.0	1.6	0.4	15
BFSe-12	1.0	1.6	0.4	20

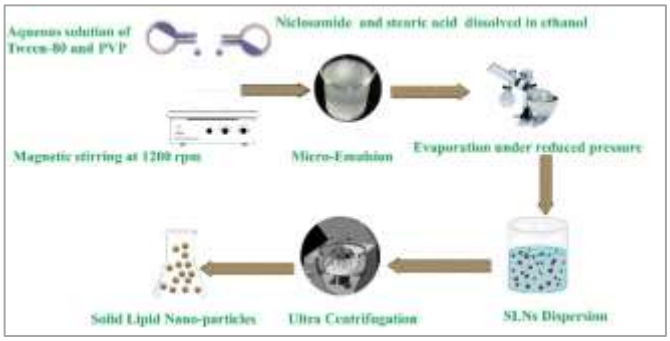
The formulation of Niclosamide-loaded SLNs (NLC-SLNs) was based on the optimized BFSe-11 conditions. Niclosamide and stearic acid were dissolved in ethanol, while the aqueous phase consisted of Tween-80 and PVP in water. The organic phase was added to the aqueous phase under stirring at 1200 rpm, followed by solvent removal using a rotary evaporator. Five formulations (NSED-1 to NSED-5) were prepared with varying drug-to-lipid ratios, as shown in Table 2.

Table 2
Niclosamide-Loaded SLNs Formulations

Formulation	Niclosamide (mg)	Stearic Acid (g)	Tween-80 (g)	Stirring Time (min)	PVP-29000 (g)
NSED-1	200	1.0	1.6	15	0.4
NSED-2	100	1.0	1.6	15	0.4
NSED-3	66.6	1.0	1.6	15	0.4
NSED-4	50.0	1.0	1.6	15	0.4
NSED-5	40.0	1.0	1.6	15	0.4

The prepared SLNs were lyophilized using a freeze dryer (Heto PowerDry LL1500, Thermo Electron Corporation, USA) with a 5% fructose solution as a cryoprotectant. After pre-freezing at -20°C, the samples were freeze-dried at -75°C for 48 hours to ensure stability and longevity.

Figure 2
Schematic diagram of Solvent Emulsification Diffusion Method



The entrapment efficiency (EE) and drug loading capacity (DLC) of the SLNs were calculated using the following equations after centrifuging the samples to separate the un-entrapped drug:

$$EE\ (\%) = \frac{\text{Total Drug} - \text{Unentrapped Drug}}{\text{Total Drug}} \times 100$$

$$DLC\ (\%) = \frac{\text{Entrapped Drug}}{\text{Total Weight of Nanoparticles}} \times 100$$

Characterization of SLNs included zeta size, PDI, and zeta potential measurements using a Zetasizer Nano. Surface morphology was analyzed via scanning electron microscopy (SEM) at 30,000× magnification. Differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) were performed to evaluate the thermal properties and physical state of the nanoparticles, respectively. FTIR spectroscopy assessed the compatibility between Niclosamide and excipients.

Stability studies were conducted by storing the optimized formulation (NSED-2) at refrigerated (5±3°C) and room temperature (25±2°C) for three months. Zeta size and PDI were monitored over time, and data were analyzed using a two-tailed t-test ($P<0.05$).

For in vitro release studies, the dialysis bag method was employed. NLC-SLNs dispersion (1 mL) was placed in dialysis bags (12–14 kDa) and immersed in phosphate buffer (pH 7.4) at 50 rpm. Samples were collected at specific intervals and analyzed spectrophotometrically. Release kinetics were determined using mathematical models.

In vivo pharmacokinetics were assessed in rabbits, following ethical approval (DREC/20160503-14). The study involved two groups of six rabbits each, administered either NSED-2 or marketed Niclosamide (Mesan®) at a dose of 100 mg/kg. Plasma samples were collected at various time points and analyzed using HPLC. Pharmacokinetic parameters, including area under the curve (AUC), peak concentration (Cmax), and time to peak concentration (Tmax), were calculated using standard equations.

Statistical analysis of all data was performed using one-way ANOVA and t-tests, with significance set at $P<0.05$. This comprehensive methodological framework ensured reliable and reproducible results, providing valuable insights into the efficacy of SLNs in enhancing Niclosamide bioavailability.

RESULTS

The results of this study are presented in a structured and detailed manner, supported by statistical analysis to validate the findings.

The optimization of unloaded solid lipid nanoparticles (SLNs) was conducted by varying formulation parameters. The formulation BFSe-11 demonstrated the most optimal properties, with a zeta size of 212.2±2.5 nm and a polydispersity index (PDI) of 0.3953±0.003. When Niclosamide was loaded into SLNs, the optimized NSED-2 formulation exhibited a slightly smaller zeta size of 208.6±2.2 nm, a PDI of 0.376±0.04, and a zeta potential of -34.11±1.2 mV, indicating good stability and uniformity. The results are detailed in Table 3 and depicted in Figures 1 and 2.

Table 3
Zeta Size, PDI, and Zeta Potential of SLNs

Formulation	Zeta Size (nm)	PDI	Zeta Potential (mV)
BFSe-11	212.2±2.5	0.3953±0.003	Not Applicable
NSED-2	208.6±2.2	0.376±0.04	-34.11±1.2

The entrapment efficiency (EE) and drug loading capacity (DLC) of the formulations were analyzed, with the highest EE (85.4±0.04%) and DLC (3.18±0.04%) observed in the NSED-2 formulation (drug-to-lipid ratio of 10:1). In comparison, NSED-1, with a 5:1 ratio, exhibited significantly lower EE (38.2%) and DLC (2.03%). These findings are summarized in Table 4 and illustrated in Figure 3.

Table 4
Entrapment Efficiency and Drug Loading Capacity of SLNs

Formulation	Drug-to-Lipid Ratio	Entrapment Efficiency (%)	Drug Loading Capacity (%)
NSED-1	5:1	38.2±0.02	2.03±0.01
NSED-2	10:1	85.4±0.04	3.18±0.04

Morphological studies using SEM revealed that the SLNs of the NSED-2 formulation were spherical, with smooth surfaces and an average particle size below 210 nm. Minor aggregation was observed, as shown in Figure 4

Differential scanning calorimetry (DSC) analysis demonstrated a reduction in the melting point of Niclosamide from 229°C in its pure form to 180°C in the NSED-2 formulation, indicating a transition from a crystalline to an amorphous state. These findings are illustrated in Figure 5.

Powder X-ray diffraction (PXRD) analysis supported the DSC results, showing sharp peaks for pure Niclosamide that were significantly reduced in intensity in the NSED-2 formulation, confirming its amorphous nature (Figure 6).

Fourier-transform infrared (FTIR) analysis revealed no significant interactions between Niclosamide and excipients, as evidenced by the absence of new peaks or shifts in characteristic frequencies in the NSED-2 spectrum compared to the pure drug (Figure 7).

The stability study indicated no significant changes in the zeta size and PDI of NSED-2 stored at refrigerated temperatures over three months. However, samples stored at room temperature exhibited a gradual increase in size and PDI due to potential degradation of the lipid matrix. Statistical analysis showed a significant difference between storage conditions ($P<0.05$). The stability data are detailed in Table 5.

Table 5
Stability Study of NSED-2 Formulation

Day	Size (nm) (5±3°C)	Size (nm) (25±2°C)	PDI (5±3°C)	PDI (25±2°C)
1	208.6	208.6	0.376	0.376
30	209.1	219.3	0.378	0.400
90	211.5	255.5	0.375	0.557
Mean	209.61	228.25	0.37	0.45
SD	1.09	17.77	0.001	0.07

In vitro drug release studies revealed a cumulative release of 100% for NSED-2, following zero-order kinetics with a release exponent (n) of 0.906, indicating a Super Case-II transport mechanism. Release profiles and kinetic parameters are presented in Table 6 and Figure 8.

Table 6
Drug Release Kinetics of SLNs

Formulation	Zero Order (R^2)	First Order (R^2)	Higuchi Model (R^2)	Release Exponent (n)
NSED-2	0.9941	0.9954	0.9941	0.906

In vivo pharmacokinetic analysis in rabbits demonstrated that the NSED-2 formulation significantly improved Niclosamide bioavailability compared to the marketed product. The Cmax of NSED-2 was 4.07±0.124 µg/mL, approximately double that of the marketed drug (1.99±0.124 µg/mL). Similarly, the AUC0→24 of NSED-2 (21.19 µg·h/mL) was over tenfold higher than that of the marketed formulation. These results are tabulated in Table 5 and depicted in Figure 9.

Figure 3
Size and PDI of Unloaded SLNs

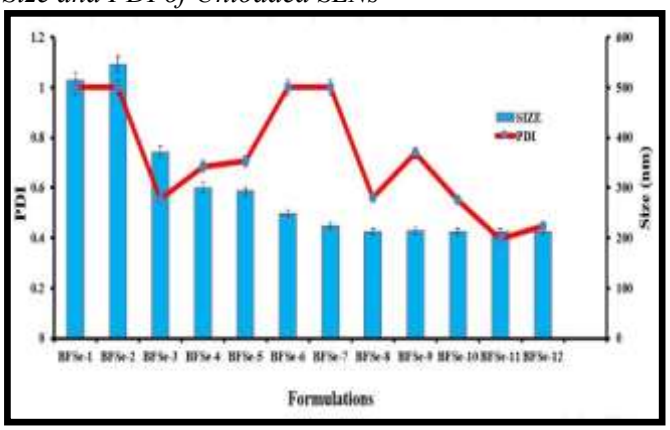


Figure 4
Zeta Size of NSED-2

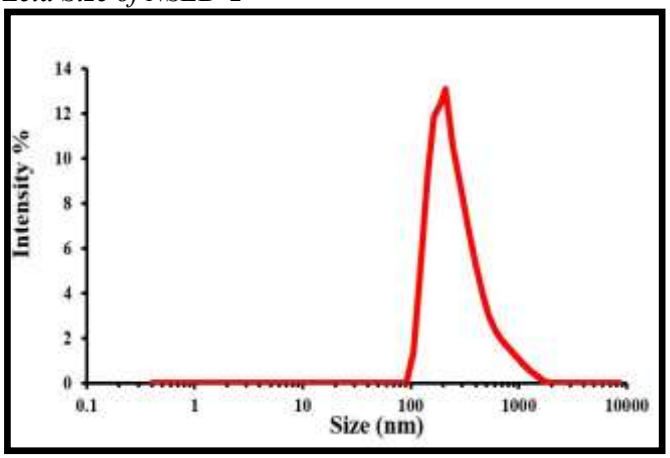
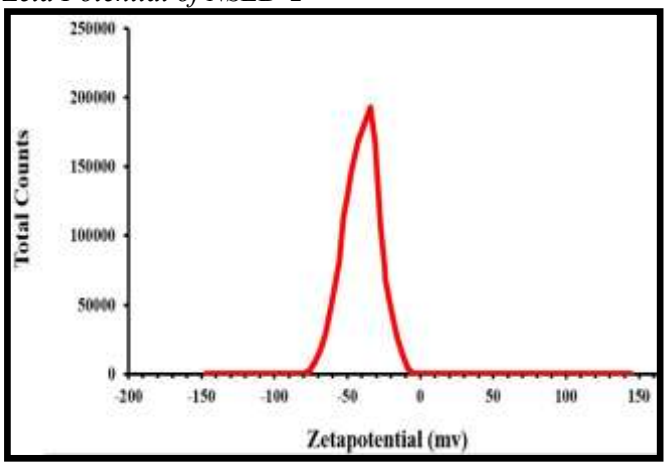


Figure 5
Zeta Potential of NSED-2



Entrapment efficiency(EE) and drug loading capacity(DLC)

Figure 6

EE and DLC of NLC loaded SLNs

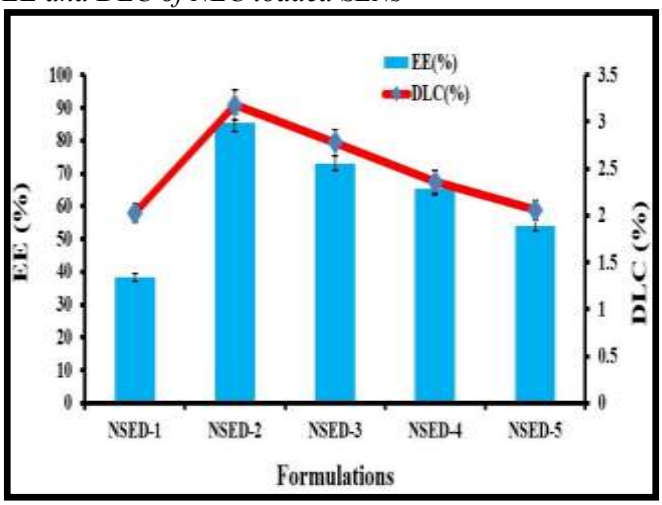


Figure 7

SEM image of NSED-2 formulation

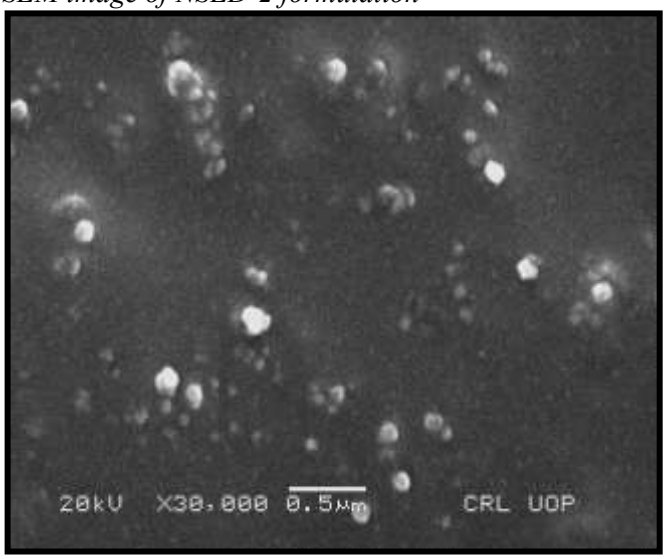


Figure 8

DSC thermogram of NLC and NSED-2

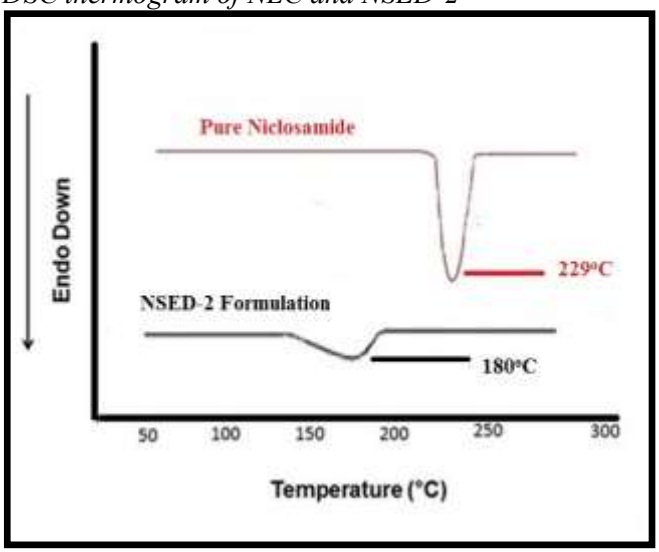


Figure 9

P-XRD spectra of Niclosamide and of NSED-2

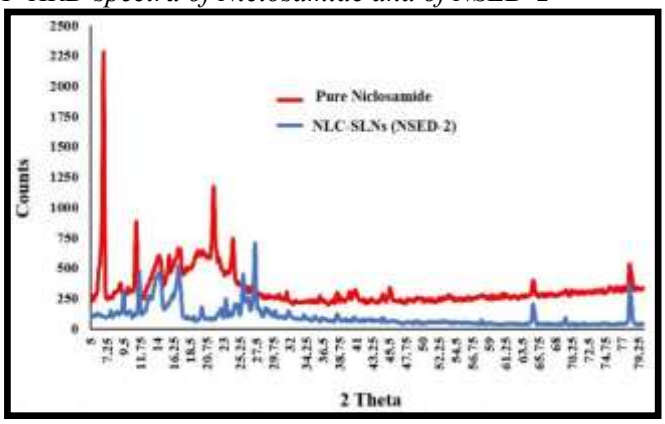


Figure 10

FT-IR spectra of Niclosamide(A) and NSED-2(B)

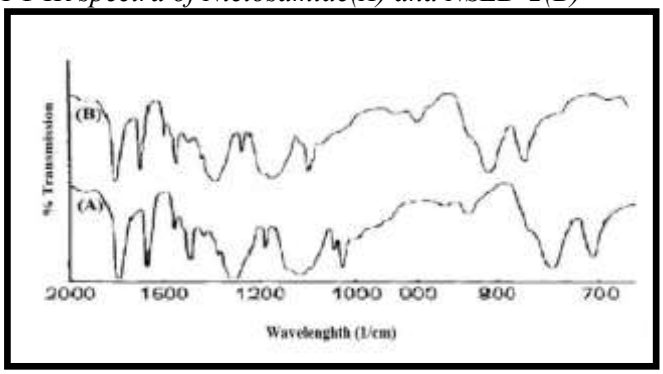


Figure 11

Zeta Size analysis at different temperatures as function of time.

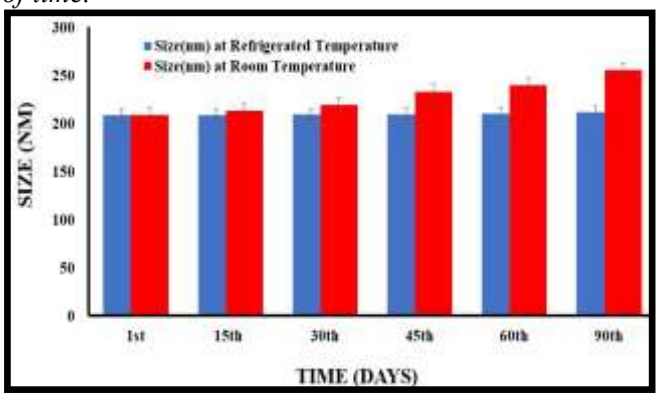


Figure 12

PDI analysis at different temperatures as function of time

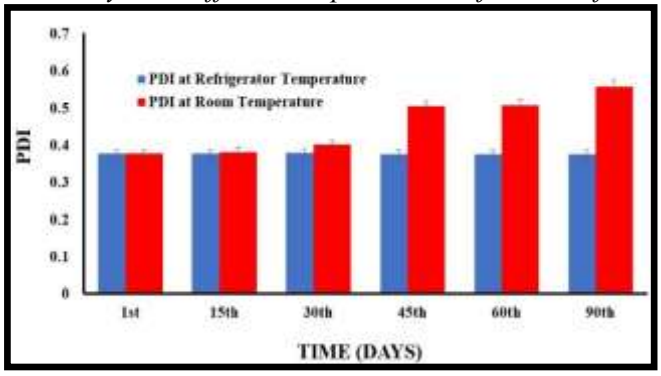


Figure 13
Cumulative drug release from NLC loaded SLNs formulations

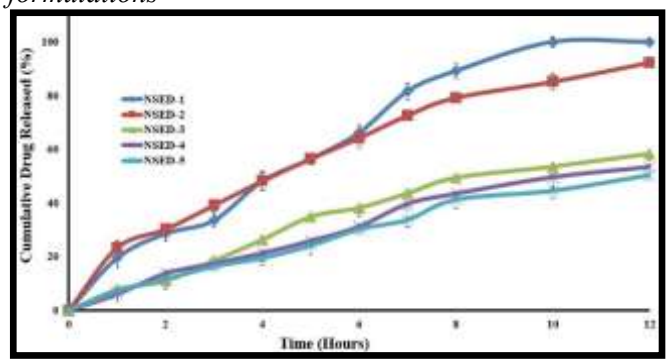


Figure 14
Concentration

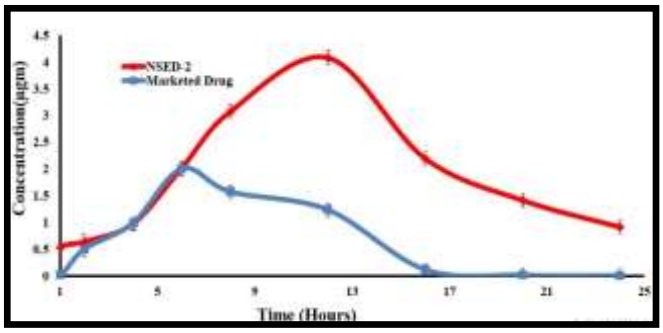


Table 7
Pharmacokinetic Parameters of NSED-2 and Marketed Drug

Parameter	NSED-2	Marketed Drug
Cmax (µg/mL)	4.07±0.124	1.99±0.124
Tmax (h)	12±0.2	6±0.3
AUC0→24 (µg·h/mL)	21.19	2.005
Relative Bioavailability (Fr)	10.59	-

The enhanced pharmacokinetic performance of NSED-2 highlights the potential of SLNs as an effective drug delivery system for poorly soluble drugs like Niclosamide.

DISCUSSION

The study successfully optimized the formulation of Niclosamide-loaded solid lipid nanoparticles (SLNs) to enhance its oral bioavailability, leveraging the solvent emulsification-diffusion (SED) method. The optimized unloaded SLNs (BFSe-11) demonstrated a zeta size of 212.2±2.5 nm and a polydispersity index (PDI) of 0.3953±0.003, providing a stable foundation for drug loading. Following incorporation of Niclosamide, the NSED-2 formulation achieved a reduced zeta size of 208.6±2.2 nm, a PDI of 0.376±0.04, and a zeta potential of -34.11±1.2 mV, indicative of favorable colloidal stability. These values fell within acceptable ranges reported in similar studies, where nanoparticle systems with a zeta potential beyond ±30 mV exhibited enhanced stability due to electrostatic repulsion (38, 39).

The high entrapment efficiency (85.4±0.04%) and drug loading capacity (3.18±0.04%) of NSED-2 underscored the compatibility of Niclosamide with the lipid matrix, which was further supported by FTIR analysis. The absence of significant changes in characteristic peaks indicated no chemical interaction between Niclosamide and the excipients, consistent with previous findings that confirmed the inert nature of stearic acid and Tween-80 in similar formulations (40). Additionally, the amorphous nature of the nanoparticles was evidenced by differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). The reduction in the melting point of Niclosamide from 229°C to 180°C in the NSED-2 formulation corroborated reports that crystalline drugs often transition to an amorphous state during nanoparticle formation, enhancing solubility and bioavailability (41, 42).

The stability study highlighted that the NSED-2 formulation maintained its size and PDI at refrigerated temperatures over three months, demonstrating its robustness. However, samples stored at room temperature showed a gradual increase in size and PDI, likely due to the degradation of the lipid matrix or Ostwald ripening, a phenomenon commonly observed in amorphous nanoparticles (39, 42). These findings suggest that storage conditions play a critical role in preserving the physicochemical properties of SLNs, aligning with recommendations in prior stability studies of lipid-based nanoparticles (43).

In vitro drug release studies revealed a zero-order release profile for the NSED-2 formulation, with cumulative release reaching 100%. The release followed a Super Case-II transport mechanism, characterized by relaxation and erosion of the lipid matrix, consistent with established kinetic models for SLN systems (36). The controlled and sustained release behavior observed in this study was comparable to other lipid nanoparticle systems designed for poorly soluble drugs, reinforcing the potential of SLNs to improve therapeutic efficacy (35).

The in vivo pharmacokinetic analysis demonstrated a significant improvement in Niclosamide absorption with the NSED-2 formulation compared to the marketed product. The NSED-2 formulation exhibited a 2.04-fold increase in peak plasma concentration (Cmax) and a 10.59-fold increase in the area under the curve (AUC0→24), reflecting enhanced bioavailability. These findings were attributed to the nanoscale size of the SLNs, which likely facilitated bioadhesion to the gastrointestinal tract and improved permeation across intestinal barriers. Similar studies have reported that nanoparticles can prolong residence time in the gastrointestinal tract and enhance drug absorption through increased surface contact and interaction with

the mucosal membrane (44, 45). Furthermore, the presence of surfactants like Tween-80 may have contributed to the enhanced permeability by modulating membrane fluidity, as previously suggested in the literature (46).

Despite the promising results, certain limitations were acknowledged. The study primarily focused on oral bioavailability, leaving other potential routes of administration unexplored. While the SLNs demonstrated stability at refrigerated conditions, their performance under varying environmental stresses, such as humidity and temperature fluctuations, warrants further investigation. Additionally, the in vivo studies were limited to a small animal model, and extrapolation to human pharmacokinetics requires caution. Future studies could explore scaling up the formulation for clinical applications, assessing its efficacy across diverse patient populations, and investigating its potential for other routes of administration, such as parenteral delivery.

The strengths of this study included the use of a robust and reproducible SED technique, comprehensive physicochemical and pharmacokinetic evaluations, and a clear demonstration of the advantages of SLNs in improving the bioavailability of poorly soluble drugs. The findings contribute to the growing body of evidence supporting the utility of nanotechnology in pharmaceutical sciences. By addressing solubility and stability challenges, SLNs offer a promising platform for enhancing the therapeutic potential of drugs like Niclosamide, paving the way for further advancements in nano-medicine.

CONCLUSION

The study demonstrated that Niclosamide-loaded solid lipid nanoparticles (SLNs) significantly enhanced the drug's solubility, stability, and oral bioavailability, offering a robust solution to the limitations of poorly water-soluble drugs. The optimized formulation (NSED-2) exhibited controlled release, excellent stability under refrigerated conditions, and a notable improvement in pharmacokinetics compared to the marketed product. These findings highlight the potential of SLNs as an innovative drug delivery platform with promising implications for improving therapeutic outcomes in human healthcare, particularly for managing diseases requiring enhanced oral drug absorption and sustained release. Further clinical exploration could solidify its role in advancing patient care.

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