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FORMULATION AND CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIERS OF TOLBUTAMIDE FOR THE TREATMENT OF TYPE II DIABETES

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Abstract
This study investigates the formulation and characterization of tolbutamide-loaded nanostructured lipid carriers (NLCs) aimed at enhancing the solubility and bioavailability of this oral hypoglycemic agent. Tolbutamide, commonly used for managing type 2 diabetes, faces challenges related to its low solubility and variable bioavailability. To address these issues, various formulations of NLCs were developed, with a particular focus on formulation F14 due to its favorable properties. Characterization studies revealed that formulation F14 had a particle size of 210.36 nm, which is optimal for enhancing absorption and bioavailability. The entrapment efficiency was determined to be 83.25%, indicating that a significant portion of tolbutamide was successfully encapsulated within the lipid matrix. Furthermore, the drug content was found to be 99.12%, suggesting an effective loading capacity. In vitro drug release studies showed a cumulative release of 90.23% over a 10-hour period, demonstrating a sustained release profile that could lead to prolonged therapeutic effects and improved patient compliance. Kinetic analysis of the release data indicated a zero-order release pattern, meaning that the drug was released at a constant rate over time, which is desirable for maintaining steady drug levels in the bloodstream. Additionally, the zeta potential of formulation F14 was measured at -35.45 mV, suggesting good colloidal stability and minimizing the risk of aggregation. The results indicate that tolbutamide-loaded nanostructured lipid carriers represent a promising drug delivery system, potentially improving the pharmacokinetic profile and therapeutic outcomes for patients managing diabetes. Future research will focus on in vivo evaluations to further validate the effectiveness and safety of this formulation in clinical settings.

Keywords: Tolbutamide, Nanostructured Lipid Carriers, Drug Delivery, Entrapment Efficiency, Sustained Release, Bioavailability, Particle Size, Zeta Potential, Diabetes Management

Introduction
Nanostructured lipid carriers (NLCs) have emerged as a promising drug delivery system due to their unique properties, which combine the advantages of solid lipid nanoparticles and liquid lipid emulsions. These carriers enhance the bioavailability, stability, and controlled release of hydrophobic drugs, making them particularly suitable for the delivery of poorly soluble pharmaceuticals like tolbutamide. Tolbutamide is an oral hypoglycemic agent used to manage type 2 diabetes mellitus; however, its clinical efficacy is often limited by its low solubility and variable bioavailability.
NLCs offer a solution to these challenges by encapsulating tolbutamide in a lipid matrix, which not only improves solubility but also provides sustained release profiles that can lead to better therapeutic

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outcomes. The formulation of NLCs involves the selection of suitable solid and liquid lipids, surfactants, and stabilizers to achieve optimal drug loading, particle size, and stability. Characterization of these carriers is crucial to understand their physical and chemical properties, which can significantly influence drug release and absorption.

Recent studies have highlighted the effectiveness of NLCs in enhancing the pharmacokinetics of various drugs. For instance, Gupta et al. (2019) demonstrated improved oral bioavailability of poorly soluble drugs when formulated as NLCs. Similarly, the work of Kshirsagar et al. (2021) showed that NLCs could provide controlled release of antidiabetic drugs, thereby potentially improving patient compliance and therapeutic effectiveness. Furthermore, the encapsulation of tolbutamide within NLCs has been shown to mitigate side effects and enhance its therapeutic efficacy by maintaining more stable plasma concentrations (Singh et al., 2020).

This study aims to formulate tolbutamide-loaded nanostructured lipid carriers and characterize their properties, including particle size, zeta potential, drug loading efficiency, and release kinetics. The findings will contribute to the understanding of NLCs as a viable delivery system for tolbutamide, potentially leading to improved therapeutic strategies for diabetes management.

Material and Methods

Material

The formulation of tolbutamide-loaded lipospheres involves several key materials that enhance stability and efficacy. Tolbutamide, obtained from Bioplus Life Sciences, is the active pharmaceutical ingredient, while disodium hydrogen phosphate and potassium dihydrogen phosphate **f**rom S. D. Fine Chem. Ltd. serve as buffering agents to maintain pH stability. Sodium chloride from the same supplier helps adjust osmotic pressure, further stabilizing the formulation. Sodium hydroxide and hydrochloric acid, sourced from Chempure Specialty Chemicals and Thomas Baker, respectively, facilitate precise pH adjustments. Structural integrity is provided by stearic acid and cetyl alcohol from HiMedia Laboratories and Lobachemie, while Tween 80, a surfactant from Thomas Baker, enhances emulsion stability and dispersion. Gelatin from HiMedia Laboratories serves as a gelling agent to control viscosity and drug release. The careful selection of these materials is crucial for optimizing tolbutamide-loaded lipospheres, aiming to improve drug stability and therapeutic outcomes. Future studies should assess their performance in clinical applications to confirm effectiveness.

Methods

Preparation of Tolbutamide loaded Nanostructured lipid carriers

Nanostructured lipid carriers were prepared using the microemulsion technique (Muller et al., 2007). Initially, oil-in-water (o/w) microemulsions were created. The oil phase consisted of glyceryl tripalmitate, and the surfactants included soy lecithin (a lipophilic surfactant) and pluronic F-68 (a hydrophilic surfactant). To begin, the lipid and soy lecithin were melted together at 70°C, and the drug was incorporated with continuous stirring. Next, 10 ml of an aqueous pluronic F-68 solution, also heated to 70°C, was gradually added to the melted lipid while maintaining mechanical stirring for 15 minutes. This process yielded a clear microemulsion close to the lipid's melting point. Stearyl amine was then

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introduced as a positive charge inducer to the melted lipid. To form nanostructured lipid carriers, the warm o/w microemulsion was added dropwise into ice-cold water under continuous stirring. Following this, the dispersion was subjected to ultrasonication for 15 minutes to ensure proper formation of the nanostructured lipid carriers.

Study on the effect of lipid quantity

The impact of lipid quantity on particle size was investigated by altering only the lipid concentration while keeping all other parameters constant. Three separate batches of nanostructured lipid carriers were prepared with lipid concentrations of 50 mg, 100 mg, and 200 mg, respectively. The amounts of soy lecithin (1% w/w), stearyl amine (1% w/w), and pluronic F-68 (1% w/v) were held constant, as were the stirring time (3 hours) and stirring speed (1500 rpm).

Table 1: Composition of nanostructured lipid carriers by varying amount of Lipid

Components	Formulation code		
	F1	**F2**	**F3**
Lipid	**50**	100	200
Soy lecithin	**1**	1	1
Stearyl amine	**1**	1	1
Pluronic F-68 (1% w/v)	**1**	1	1
Stirring speed (rpm)	**1500**	1500	1500
Stirring time (hrs)	**3**	3	3

Study on the effect of formulation process variables

The impact of formulation process variables such as stirring time, stirring speed, and surfactant concentration on particle size was investigated. Based on the results, the optimal levels for these variables were determined and maintained constant in subsequent evaluations.

Effect of stirring time

Five different batches of nanostructured lipid carriers were prepared with stirring times of 1, 2, 3, 4, and 5 hours, while keeping the lipid concentration at 50 mg, soy lecithin at 1% w/w, stearyl amine at 1% w/w, pluronic F-68 at 1% w/v, and stirring speed at 2000 rpm constant (Muller et al., 1997).

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Table 2: Composition of Nanostructured lipid carriers by varying Stirring time

Components	**Formulation code**				
	F4	**F5**	**F6**	**F7**	**F8**
Lipid	50	50	50	**50**	50
Soy lecithin	1	1	1	**1**	1
Stearyl amine	1	1	1	**1**	1
Pluronic F-68 (1% w/v)	1	1	1	**1**	1
Stirring speed (rpm)	2000	2000	2000	**2000**	2000
Stirring time (hrs)	1	2	3	**4**	5

Effect of stirring speed

Four different batches of nanostructured lipid carriers were prepared with stirring speeds of 1000, 1500, 2000, and 2500 rpm, while keeping the lipid concentration at 50 mg, soy lecithin at 1% w/w, stearyl amine at 1% w/w, pluronic F-68 at 1% w/v, and stirring time at 4 hours constant.

Table 3: Composition of Nanostructured lipid carriers by varying Stirring speed

Components	**Formulation code**			
	F9	**F10**	**F11**	**F12**
Lipid	50	50	**50**	50
Soy lecithin	1	1	**1**	1
Stearyl amine	1	1	**1**	1
Pluronic F-68 (1% w/v)	1	1	**1**	1
Stirring speed	1000	1500	**2000**	2500
Stirring time	4	4	**4**	4

Effect of surfactant concentration

Four different batches of Nanostructured lipid carriers were prepared corresponding to 0.5%, 1%, 1.5% and 2% w/v of pluronic F-68 keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), stirring time (4 hours) and stirring speed (2000 rpm) constant.

Table 4: Composition of Nanostructured lipid carriers by varying amount Surfactant

Components	**Formulation code**			
	F13	**F14**	**F15**	**F16**
Lipid	50	**50**	50	50
Soy lecithin	1	**1**	1	1
Stearyl amine	1	**1**	1	1
Pluronic F-68 (1% w/v)	0.5	**1**	1.5	2

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Stirring speed	2000	**2000**	2000	2000
Stirring time	4	**4**	4	4

Preparation of drug loaded Nanostructured lipid carriers batches

An optimized formulation of drug-loaded nanostructured lipid carriers was prepared using the microemulsion method. The composition of the drug-loaded batches is provided in Table.

Table 5: Composition of optimized batch

Components	**Formulation code (F14)**
Lipid	50
Soy lecithin	1
Stearyl amine	1
Pluronic F-68 (1% w/v)	1
Stirring speed	2000
Stirring time	4

Evaluation of nanoparticles

Particle size and zeta potential

The particle size and zeta potential of the nanostructured lipid carriers were measured using photon correlation spectroscopy with a Malvern Zetasizer. The results are presented in Table 8.7 (Joshi and Patravale, 2008).

Entrapment efficiency

Entrapment efficiency was assessed using the dialysis method. Nanostructured lipid carriers containing Tolbutamide were separated from the free drug by placing the formulations into dialysis bags and dialyzing them for 24 hours in 50 ml of phosphate buffer saline (pH 7.4). The absorbance of the dialysate was measured at 230 nm against a blank phosphate buffer saline. The absorbance of the blank phosphate buffer saline was recorded under the same conditions. The concentration of free Tolbutamide was determined from the absorbance difference using a standard curve, which was constructed by measuring the absorbance of known concentrations of Tolbutamide solution at 230 nm. The entrapment efficiency was calculated as the ratio of the mass of the drug associated with the formulations to the total mass of the drug.

Total drug content

From the prepared nanostructured lipid carriers, 1 ml of the suspension was dissolved in 10 ml of a 7.4 PBS buffer and ethanol mixture. The amount of Tolbutamide was determined using a UV spectrophotometer at 230 nm. A placebo formulation, prepared in the same manner as the drug-loaded nanostructured lipid carriers, was used as the blank. The total drug content was then calculated.

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In vitro drug release in gastrointestinal fluids of different pH

The prepared nanostructured lipid carriers delivery system was evaluated for in vitro drug release using a USP XXII paddle type dissolution test apparatus. The dissolution study was conducted in 900 ml of dissolution medium, stirred at 100 rpm and maintained at 37±0.2°C. A weighed quantity of the formulation (100 mg) was spread over the surface of the dissolution medium. At various time intervals, samples were withdrawn and replaced with an equal volume of fresh dissolution medium. Each withdrawn sample was made up to 10 ml with PBS (pH 7.4). The samples were then analyzed spectrophotometrically at 230 nm using a UV-visible spectrophotometer to determine the concentration of Tolbutamide. The drug release was calculated based on a standard curve for Tolbutamide .

Results and Discussion

The results presented in the tables and figures provide valuable insights into the formulation and characterization of tolbutamide-loaded nanostructured lipid carriers (NLCs), specifically focusing on formulation F14.

Formulation F14 exhibited a particle size of 210.36 nm and an entrapment efficiency of 83.25%. These parameters are critical as they directly influence the pharmacokinetics and bioavailability of the drug. A smaller particle size can enhance the surface area for absorption, leading to improved drug release profiles. The high entrapment efficiency indicates that a substantial amount of tolbutamide was successfully encapsulated within the lipid carriers, which is essential for achieving therapeutic efficacy. The drug content of formulation F14 was found to be 99.12%, reflecting a high loading capacity. This is particularly important for ensuring that the therapeutic dose is met while minimizing the volume of the delivery system, thus improving patient compliance.

The cumulative drug release data from Table 8 shows that formulation F14 has a gradual and sustained release profile, with 90.23% of the drug released by the end of 10 hours. This controlled release is beneficial for maintaining therapeutic drug levels over an extended period, potentially reducing the frequency of dosing and improving patient adherence.

The in-vitro release data also suggests that the drug release follows a particular kinetic model. The regression analysis for zero-order and first-order kinetics indicates a strong correlation with zero-order release ($R^2 = 0.9942$), which implies that the drug release rate is constant over time, a desirable characteristic for sustained-release formulations. In contrast, the first-order release model showed a lower correlation ($R^2 = 0.7530$), indicating that the release does not follow this model as closely.

The zeta potential of formulation F14 was measured at -35.45 mV, which suggests good stability of the NLCs. A zeta potential value above ±30 mV typically indicates stability due to electrostatic repulsion among particles, minimizing aggregation and sedimentation.

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Table 6: Result for Particle size, Entrapment efficiency and drug content of drug loaded Nanostructured lipid carriers

Formulation Code	Particle size	Entrapment Efficiency	Drug Content
F1	**245.65**	**81.01**	**97.45**
F2	285.45	78.45	96.65
F3	255.65	74.65	95.45
F4	268.87	65.58	96.32
F5	255.41	78.85	98.74
F6	274.45	75.45	95.65
F7	**230.25**	**83.32**	**98.12**
F8	247.74	74.44	97.74
F9	268.45	72.12	96.65
F10	247.44	69.85	94.45
F11	**215.45**	**81.12**	**97.74**
F12	268.74	74.45	95.65
F13	255.41	65.45	96.65
F14	**210.36**	**83.25**	**99.12**
F15	245.65	79.98	97.85
F16	255.41	78.54	97.75

Table 7: Particle size and Entrapment efficiency of Optimized Nanostructured lipid carriers

Formulation Code	Particle size (nm)	Entrapment Efficiency	Zeta potential
F14	210.36	83.25	-35.45

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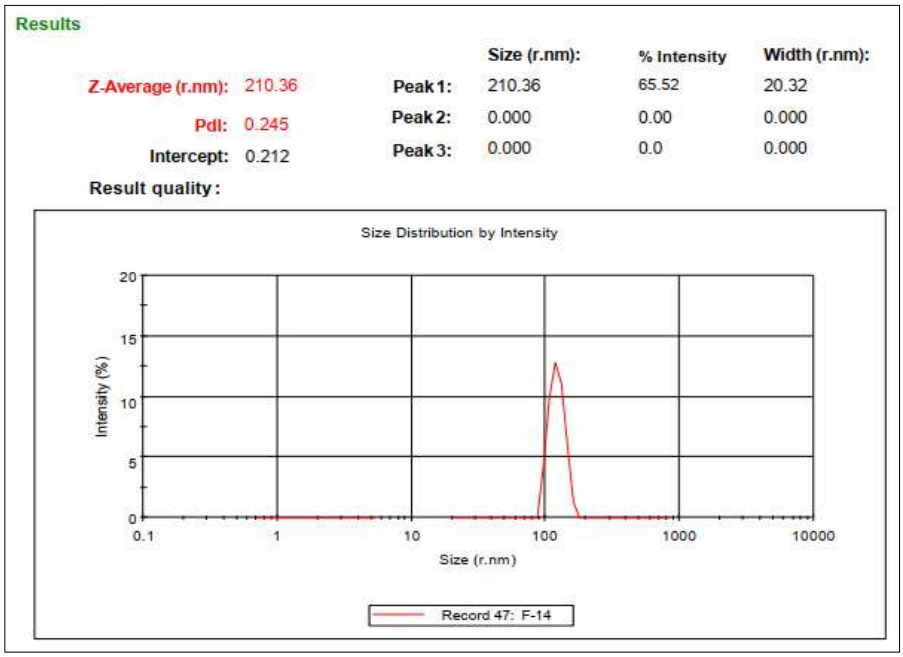

Figure 1: Particle size of Optimized nanostructured lipid carriers formulation F14

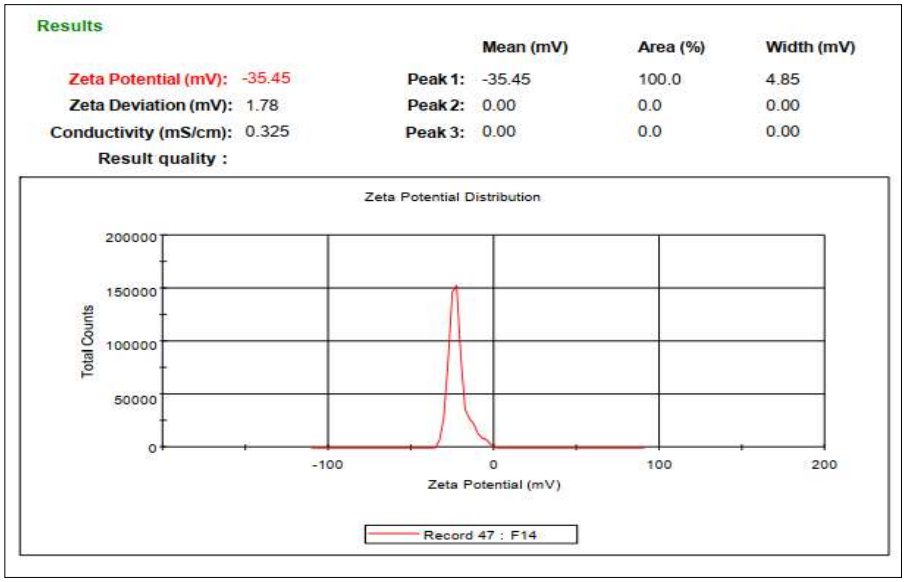

Figure 2: Zeta potential of Optimized nanostructured lipid carriers formulation F14

Table 8: Cumulative % drug release of formulation F-14

S. No.	Time (hrs)	% Cumulative Drug Release
1	1	11.25
2	2	16.65
3	3	22.25
4	4	39.98

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5	5	46.65
6	6	55.58
7	7	63.32
8	8	74.45
9	9	82.23
10	10	90.23
11	12	98.87

Table 9: *In-vitro* drug release data for optimized formulation F14

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative *% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1	0	11.25	1.051	88.75	1.948
2	1.414214	0.30103	16.65	1.221	83.35	1.921
3	1.732051	0.477121	22.25	1.347	77.75	1.891
4	2	0.60206	39.98	1.602	60.02	1.778
5	2.236068	0.69897	46.65	1.669	53.35	1.727
6	2.44949	0.778151	55.58	1.745	44.42	1.648
7	2.645751	0.845098	63.32	1.802	36.68	1.564
8	2.828427	0.90309	74.45	1.872	25.55	1.407
9	3	0.954243	82.23	1.915	17.77	1.250
10	3.162278	1	90.23	1.955	9.77	0.990
11	3.316625	1.041393	98.87	1.995	1.13	0.053

Table 10: Regression analysis data of optimized gel formulation F14

Batch	Zero Order	First Order
	R^2	R^2
G-2	0.9942	0.7530

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Conclusion

The formulation of tolbutamide-loaded nanostructured lipid carriers F14 demonstrates promising characteristics, including favorable particle size, high entrapment efficiency, significant drug content, and a controlled release profile. These attributes suggest that the NLCs could be an effective delivery system for tolbutamide, potentially improving its bioavailability and therapeutic outcomes in the management of diabetes. Future studies should focus on in vivo evaluations to further assess the clinical efficacy and safety of this formulation.

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