FORMULATION AND EVALUATION OF TOPICAL GEL CONTAINING SOLID LIPID NANOPARTICLES OF TOLBUTAMIDE

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ABSTRACT

Tolbutamide, a first-generation sulfonylurea oral hypoglycemic agent, is utilized in the management of type 2 diabetes. It promotes insulin secretion by binding to the high-affinity subunit of the ATP-sensitive potassium channel in pancreatic beta cells. Classified as a BCS Class II drug, Tolbutamide has poor solubility and bioavailability. To address these limitations, a topical gel containing solid lipid nanoparticles (SLNs) was developed. Topical gels, applied to the skin, offer greater stability compared to other dosage forms. Initially, SLNs were synthesized using the solvent diffusion method and characterized for particle size, zeta potential, polydispersity index (PDI), entrapment efficiency, in-vitro release, and interaction studies using FTIR, DSC, and XRD techniques. Subsequently, the SLNs were incorporated into a gel formulation and further evaluated. The resulting SLNs exhibited a particle size of 88166.1±3.2 nm, a zeta potential of -18.1 mV, an entrapment efficiency of 91.68%, and an 85.68% drug release within 6 hours. Interaction studies confirmed no interactions between the drug and excipients. The SLNs were then converted into a gel, which was characterized for pH, viscosity, spreadability, and other parameters. The developed gel demonstrated excellent homogeneity, appearing white, translucent, and with good spreadability. This SLN-based topical gel presents an improved formulation for type 2 diabetes, offering a sustained release of the medication over an extended period.

Keywords: Tolbutamide, diabetes 2, solid lipid nanoparticles, topical gel, formulation.

Introduction

Diabetes is a chronic health condition that affects how your body turns food into energy. There are three main types of diabetes: Type 1, Type 2, and gestational diabetes. Each type has different causes and treatments, but all can lead to serious health complications if not managed properly (1).

The causes of diabetes vary depending on the type of diabetes. Here's a detailed look at the primary causes for Type 1, Type 2, and gestational diabetes: Type 1 diabetes is primarily caused by an autoimmune reaction where the body's immune system attacks and destroys the insulin-producing beta cells in the pancreas (2, 3). Type 2 diabetes is primarily caused by insulin resistance, where the body's cells do not respond effectively to insulin, and eventually, the pancreas cannot produce enough insulin to keep blood glucose levels normal. Gestational diabetes occurs during pregnancy and is caused by hormonal changes that make the body less responsive to insulin (4).

The epidemiology of diabetes involves the study of the distribution and determinants of the disease within populations. Here's a comprehensive overview of the current epidemiological landscape of diabetes globally and in specific regions. According to the International Diabetes Federation (IDF), as of 2021, approximately 537 million adults (20-79 years old) were living with diabetes worldwide (5). This number is projected to rise to 643 million by 2030 and 783 million by 2045. Diabetes is a significant cause of morbidity and mortality, contributing to an estimated 6.7 million deaths in 2021. In Asia, particularly China and India, has the largest number of people with diabetes, driven by rapid economic development and lifestyle changes. Predominantly Type 2, with increasing rates of childhood obesity and diabetes. As of recent estimates, India has over 77 million adults living with diabetes, making it one of the countries with the highest number of diabetes cases globally. This figure is projected to reach 134 million by 2045 (6, 7). The prevalence is higher in urban areas compared to rural areas, attributed to lifestyle differences. Urbanization and changes in dietary patterns are significant contributors (8).

Tolbutamide is a first-generation sulfonylurea oral hypoglycemic drug utilized in treating type 2 diabetes (refer to "Antidiabetic Agents"). It enhances insulin release by attaching to the high-affinity subunit (SUR1) of the potassium-sensitive ATP channel (KATP channel) in beta cells. Numerous drug interactions have been documented for tolbutamide and other sulfonylureas. These interactions can either increase or decrease the hypoglycemic effect, though the clinical significance of many of these interactions remains unclear. Some interactions result from the extensive plasma protein binding of these agents, leading to displacement from plasma proteins (9). However, individual variations can occur due to factors such as gastrointestinal transit time, food intake, and liver metabolism. It's essential to note that bioavailability can also be affected by drug interactions with other medications or substances and by the presence of underlying medical conditions affecting gastrointestinal absorption or liver function (10).

To reduce the above drawback preparing solid lipid nanoparticles loaded topical gel and characterized for the treatment of diabetes. Solid lipid nanoparticles (SLNs) loaded into topical gel formulations offer several potential benefits, particularly in the field of dermatology and transdermal drug delivery.

SLNs loaded into topical gels represent a promising drug delivery strategy for the treatment of various dermatological conditions, offering improved drug stability, solubility, targeted delivery, and patient acceptance compared to traditional formulations. However, further research is needed to optimize formulation parameters and evaluate the clinical efficacy and safety of these novel delivery systems.

Material and methods

Materials used:

S. No.	Materials	Source
1	Tolbutamide	Sigma-Aldrich (St Louis, MO, USA)
2	Stearic acid	SD Fine-Chem Ltd., Mumbai, India
3	Ethanol	SD Fine-Chem Ltd., Mumbai, India
4	Methyl and propylparaben	SD Fine-Chem Ltd., Mumbai, India
5	Carbopol 934	Thermo Fisher Scientific and Himedia (Mumbai, India)
6	Poloxomer 188	SD Fine-Chem Ltd., Mumbai, India
7	Triethanolamine	SD Fine-Chem Ltd., Mumbai, India
8	KBr	SD Fine-Chem Ltd., Mumbai, India

Table 1: Material used for preparation of Solid Lipid Nanoparticle

List of equipment's used for preparation of SLNs

S. No.	Equipment's	Source
1	Mechanical stirrer	Hielscher
2	Digital Balance	Excell
3	pH Meter	Systronics
4	Ultra Sonicator	Hielscher
5	UV-visible spectrophotometer	Systronic-2202
6	FTIR	Model 8400S Shimadzu
5	Centrifuge Machine	Simplex Engineers
9	Particle Size Analyser	Delsa Nano C

Table 2: List of equipment's used for preparation of SLNs

Methods

UV spectroscopy study

Determination of λmax of Tolbutamide

The maximum absorption wavelength (λmax) of Tolbutamide was determined using a UV spectrophotometer. To achieve this, 5 mg of the pure drug was dissolved in 50 ml of ethanol. From this solution, 5 ml was pipetted into a 10 ml standard flask and diluted to volume with ethanol. The λmax was then identified using the UV spectrophotometer.

Preparation of Standard Curve of Tolbutamide

To prepare the standard curve, 100 mg of Tolbutamide was accurately weighed and placed in a volumetric flask, then diluted to 100 ml with ethanol to create a 1000 µg/ml solution. From this, 10 ml was transferred into a 100 ml volumetric flask and diluted to volume with ethanol to produce a 100 µg/ml stock solution. Aliquots of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.4 ml, 1.6 ml, 1.8 ml, and 2.0 ml of this stock solution were transferred into 10 ml volumetric flasks and filled to volume with ethanol to achieve concentrations ranging from 2 to 20 µg/ml. The absorbance of these solutions was measured at 248 nm using a UV double-beam spectrophotometer, with ethanol as the blank. A standard curve was constructed by plotting the concentration (µg/ml) on the X-axis against the absorbance on the Y-axis (11).

Preparation of Tolbutamide-Loaded Solid Lipid Nanoparticles (SLNs) Gel

SLNs Formulation

Tolbutamide-loaded SLNs were prepared using a modified solvent diffusion method. In this process, 5 mg of Tolbutamide and a specified amount of stearic acid were heated to 60±3°C in a water bath and dissolved in 10 ml of ethanol. Table 1 shows various formulation batches, where the drug quantity was kept constant, while the amounts of stearic acid and poloxamer-

188 were varied. The organic solution was rapidly dispersed into 50 ml of an aqueous poloxamer-188 solution at 4–8°C under magnetic stirring at 2000 rpm using a syringe. The heterogeneous mixture was then subjected to high-pressure homogenization using an APV 2000 homogenizer at 1200 bars. The SLNs formed instantly and were collected by centrifugation at 2000 rpm for 30 minutes at 4°C (12).

Gel Preparation

To prepare the gel, 1% Carbopol-934 was dissolved in water and mixed with the SLNs formulation under continuous stirring with a mechanical stirrer for 30 minutes. The resulting formulation was manually stirred with the gel phase in a 1:1 ratio until a clear Tolbutamide gel was obtained. Triethanolamine was gradually added to adjust the pH. Lastly, methylparaben sodium (0.02% w/v) and propylparaben sodium (0.1% w/v) were added, and the mixture was continuously stirred until homogeneous (13).

Table 1: Formulation design of tolbutamide loaded SLNs with corresponding parameters like particle size and entrapment efficiency

Batches	**Ingredients**		**Resulting Parameters**	
	Stearic acid (mg)	**Poloxomer-188 (%)**	**Particle size (nm)**	**Entrapment Efficiency (%)**
F1	400	2.0	295.3±7.6	89.59
F2	350	1.5	195.3±2.5	85.6
F3	300	1.0	269.7±4.6	90.29
F4	200	2.0	214.6±3.8	89.52
F5	250	1.5	206.8±2.3	85.86
F6	**200**	**1.0**	**166.1±3.2**	**91.68**

Evaluation of SLNs

Particle Size, PDI and zeta potential Analysis

The particle size of solid lipid nanoparticles (SLNs) is typically measured using a particle size analyzer such as the Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). Before analysis, the sample was diluted with distilled water. The software calculated the average particle size using light scattering methods, and transmission electron microscopy was also employed to assess the particle size (14).

The polydispersity index (PDI) of the sample, which indicates the broadness of the size distribution, was determined using photon correlation spectroscopy.

The zeta potential, which reflects the surface charge of the SLNs, was measured using a zeta sizer instrument equipped with Malvern software. The sample was analyzed at 25°C with the detection angle set to 90 degrees.

Entrapment Efficiency

The entrapment efficiency of the solid lipid nanoparticles (SLNs) was determined using the centrifugation method. The SLNs dispersion was centrifuged at 5000 rpm for 30 minutes in a refrigerated centrifuge to collect the supernatant. The collected supernatant was then filtered to measure the concentration of the free drug. After appropriate dilution with fresh phosphate

buffer saline (pH 7.4), the free drug concentration was measured using UV spectroscopy (15). The entrapment efficiency was calculated using the following formula:
Entrapment efficiency = {Wt. of drug incorporated/Wt. of drug initially taken} × 100.

In Vitro Drug Release study

The drug release behavior of the solid lipid nanoparticles (SLNs) formulation was studied using the dialysis bag membrane method. A 2 ml portion of the SLNs formulation was placed into a dialysis membrane, which was then securely closed by tying knots. The membrane was enclosed in a hollow cylindrical mesh and attached to the paddles of a dissolution apparatus. The paddles were immersed in 900 ml of phosphate buffer saline (PBS, pH 7.4) maintained at 37°C, with a stirring speed of 50 rpm for 6 hours. At predetermined time intervals (0, 5, 15, 30, 60, 120, 240, and 480 minutes), 3 ml samples were withdrawn from the dissolution medium and replaced with an equal volume of fresh PBS to maintain sink conditions. The collected samples were analyzed using UV spectroscopy to determine the amount of drug released at each time point (16).

FTIR (Fourier Transform Infrared Spectroscopy) study

To verify the compatibility of the formulation components, Fourier-transform infrared (FTIR) spectroscopy experiments were conducted. The FTIR spectra of the samples were recorded using the KBr pellet method. The samples were placed in a standard FTIR measurement device. This analysis was performed to investigate the interaction between the drug and the polymer, as well as the integrity and compatibility within the formulation. The infrared spectra were recorded in the range of 4000 cm^{-1} to 600 cm^{-1}. The spectra of the formulation were compared to the spectra of the physical mixtures of all ingredients to detect any spectral changes that might indicate interactions or incompatibilities (17).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a DSC Q100 instrument (Universal V3.8A, TA Instruments, USA). For the analysis, 10 mg samples of the powdered formulation and drug-free SLNs were separately placed in 40 µl aluminum pans. The thermal scan was conducted at a heating rate of 10 °C per minute over a temperature range of 90–150 °C (18).

X- Ray Diffraction

X-ray diffraction (XRD) analysis was conducted to compare the lyophilized formulation and blank SLNs (tolbutamide-free SLNs). The analysis was performed using an X-ray diffractometer (PANalytical B.V., Almelo, Netherlands). The XRD measurements were carried out under CuKα\alphaα radiation, with an operating voltage of 40 kV and a current of 40 mA. The scan parameters included a step size of 0.013 and a scan step time of 8.67 seconds, covering an angle range from 2° to 90° (19).

Characterization of gel

The pH of the gel was measured using a digital pH meter. The physical appearance, including clarity, color, and the presence of any particles, was assessed through visual inspection of the

produced gels. The viscosity of the gels was determined using a Brookfield viscometer equipped with a "T" bar spindle. Measurements were taken at a speed of 5 rpm and at a temperature of 25 ± 2°C. The viscosity values were expressed in centipoise (cP) (20).

Spreadability Characteristics

A wooden block with a pulley attached served as the base for the measurement setup. The spreadability coefficient of the gels was determined by assessing their "Slip" and "Drag" qualities.

A glass slide was securely fixed to one side of the wooden block as the stationary base. Approximately 2 grams of excess gel from the experiment was evenly applied onto this stationary glass slide. Another glass slide of the same dimensions was placed on top of the gel-coated slide. This second slide had a hook attached to it (21).

A 500 mg weight was placed on top of the upper glass slide for five minutes to ensure uniform coating of the gel between the two slides and to remove any trapped air.

A pan, attached to the pulley system, contained a weighted material. The measurement involved applying a specific force to the pulley system and timing how long it took for the upper slide to separate from the gel and move into position. A shorter time interval indicated better spreadability or easier gel spreading. It is computed using the formula below.

$$S = M * \frac{L}{T}$$

Where L is the length of the glass slides, M is the weight fastened to the upper slide, and T is the time needed to discrete the slides.

Result and discussion

UV spectroscopy study

The standard curve of tolbutamide was plotted using ethanol, and the absorbance was measured at 248 nm. The results indicated that tolbutamide followed Beer's law within the concentration range of 2-20 µg/ml. The correlation coefficient (R^2) was found to be 0.9983, demonstrating a high degree of linearity. The calibration curve of tolbutamide is shown in Figure 1.

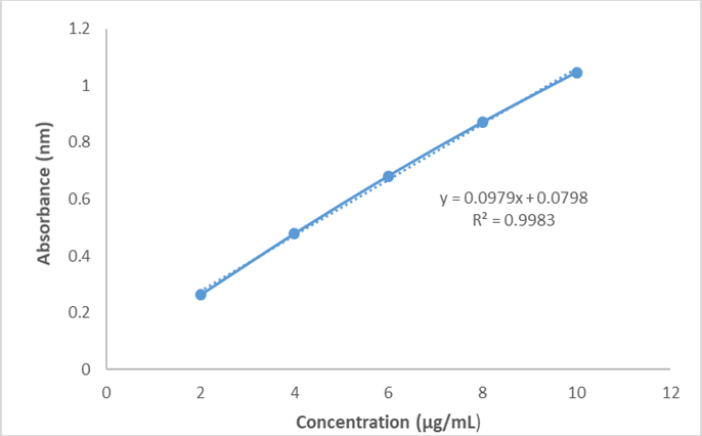

Figure 1: Calibration curve of tolbutamide

Preparation of Tolbutamide loaded solid lipid nanoparticles (SLNs) gel

The solid lipid nanoparticles (SLNs) were prepared using the solvent diffusion method. The batch exhibiting the smallest particle size and highest entrapment efficiency was selected for further analysis. This selected batch demonstrated a particle size of 166.1±3.2 nm and an entrapment efficiency of 91.68%.

Evaluation of SLNs

Particle Size, PDI and zeta potential Analysis:

The impact of polymer (PEG) and surfactant (Tween 80) concentrations on the particle size distribution of the drug-loaded SLNs was investigated. The selected SLN formulation exhibited a particle size of 166.1±3.2 nm. Notable variations in particle size were observed with changes in lipid concentrations.

The SLNs demonstrated a zeta potential value of –18.1 mV, indicating good stability and minimal particle aggregation. This negative zeta potential value suggests that the particles possess sufficient electrostatic repulsion to prevent aggregation, thereby ensuring good quality of the SLN formulation.

Entrapment Efficiency

To achieve optimal encapsulation efficiency, the concentrations of the polymer and surfactant materials were varied. The entrapment efficiency of the SLN dispersions ranged between 85.86% and 91.68%. Among the different formulations, Formulation "F6" demonstrated the highest entrapment efficiency, making it the most effective formulation.

In Vitro Drug Release study

The in vitro drug release profile of the optimized formulation was found to be 85.21% over 6 hours. This extended release could be attributed to the increased concentration of Carbopol 934 (1%), which enhanced the viscosity of the formulation. The drug release profile of the gel is shown in Figure 2.

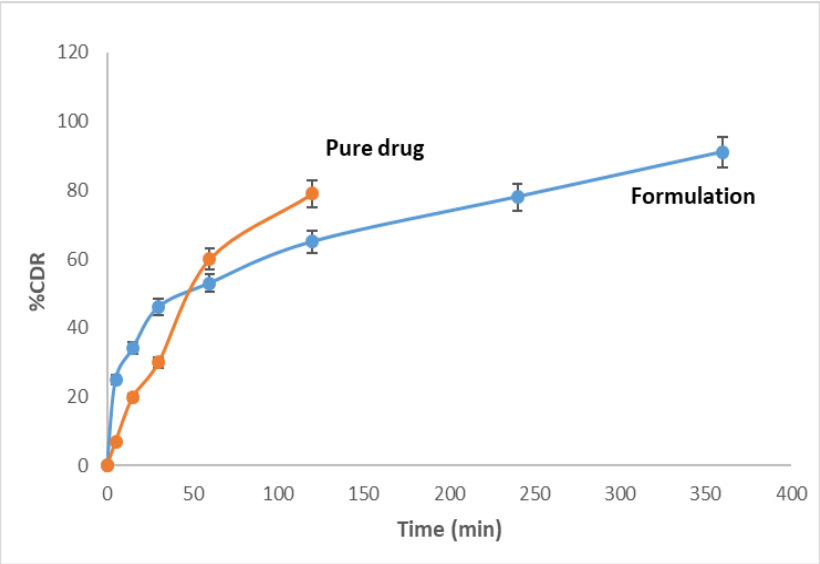

Figure 2: In-vitro drug release study formulation F-2, and pure drug. data expressed as mean±S.D, n=3

FTIR (Fourier Transform Infrared Spectroscopy) study

To verify the compatibility of the drug with the formulation components, infrared (IR) spectroscopy experiments were performed on both the formulation and the physical mixture. The IR spectra revealed no significant shifts or loss of functional peaks between the drug, lipid, and drug-loaded SLNs (as shown in Figures 3 and 4). This indicates that there were no interactions between the drug and the excipients.

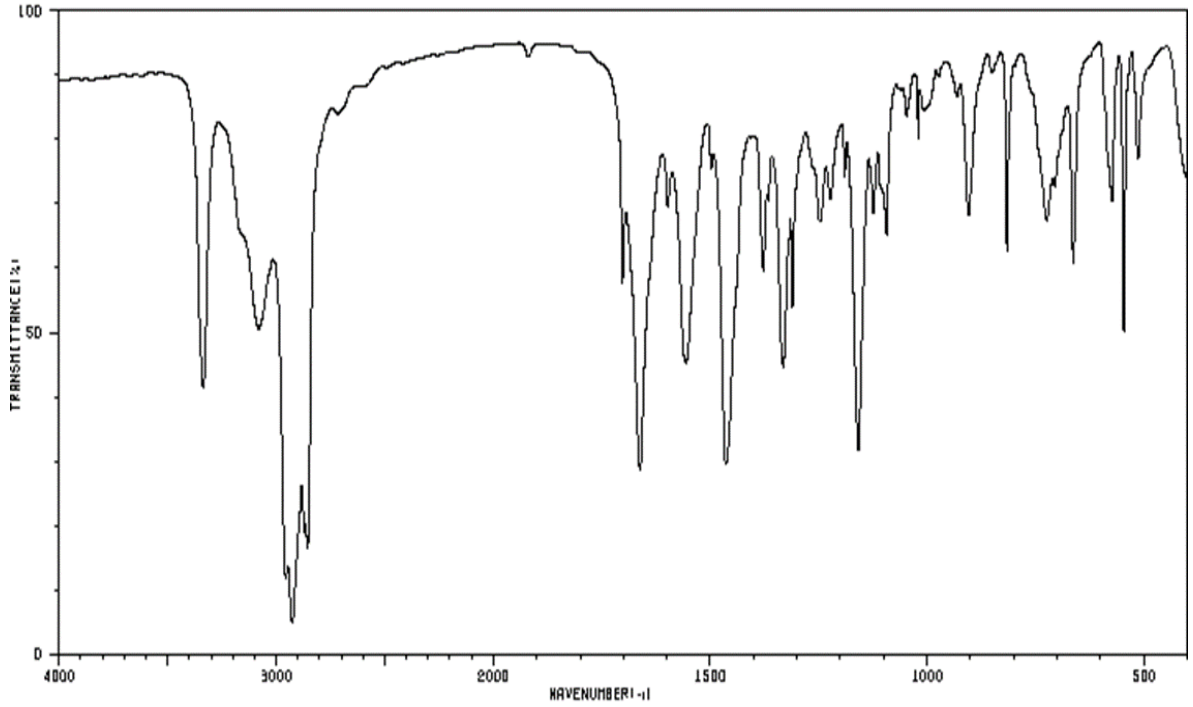

Figure 3: FTIR (Fourier-transform infrared spectroscopy) images of formulation.

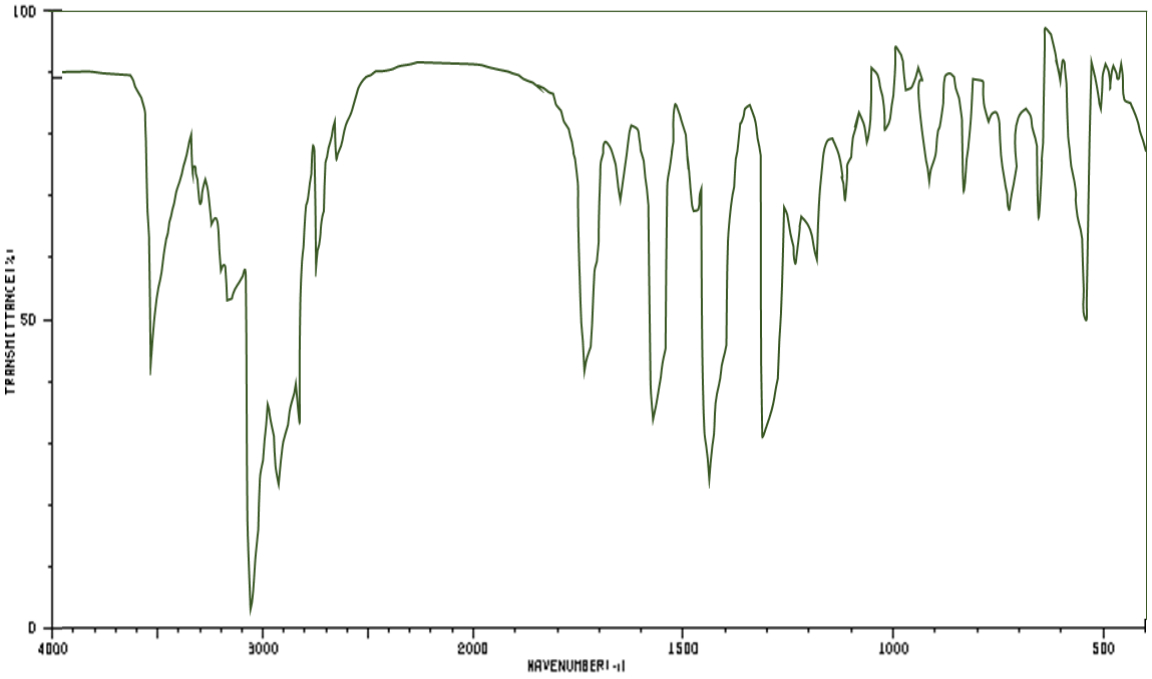

Figure 4: FTIR (Fourier-transform infrared spectroscopy) images of physical mixture.

Differential scanning calorimetry

Thermal analysis using differential scanning calorimetry (DSC) indicates that the obtained complex is well-integrated with water of hydration. The presence of a sharp melting peak within a narrow temperature range of 124–130°C suggests that the melting process occurs distinctly. Additionally, the absence of water of hydration leads to a loss of crystallinity and coordinated water, resulting in simultaneous melting. This observation implies that there is no complex formation between the drug and excipients (as shown in Figure 5).

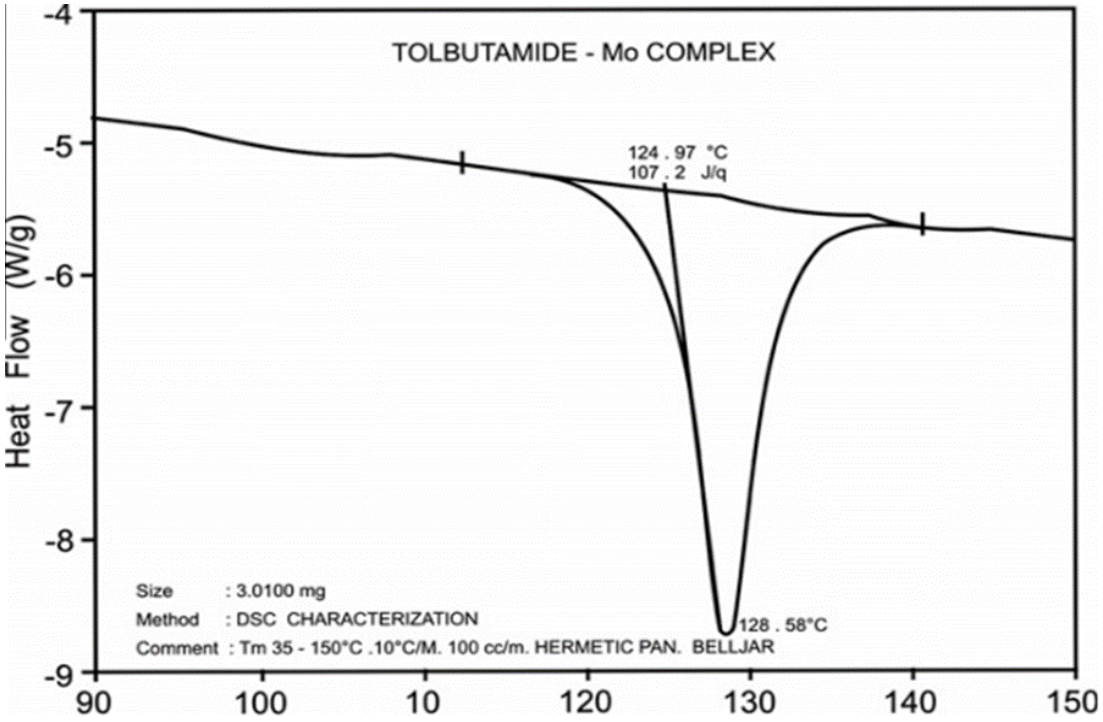

Figure 5: Differential scanning calorimetry of tolbutamide formulation.

X- Ray Diffraction

The XRD spectra of pure tolbutamide, as shown in Figure 6, exhibit distinct peaks at various 2θ intensities, indicating its large crystalline structure. These findings align with the DSC analysis results, which suggest that the drug transitions from a crystalline to an amorphous state over time. A crystalline solid can be transformed into an amorphous solid when bombarded by high-kinetic-energy ions. Amorphous phases can also form through interdiffusion or atomic-scale mixing of crystalline layers under specific compositional and temperature conditions.
The XRD spectra of the SLNs formulation, shown in Figure 7, are nearly identical to those of the pure drug, indicating that there is no significant interaction between the drug and the excipients. This similarity further supports the conclusion that the drug maintains its structural integrity within the SLN formulation.

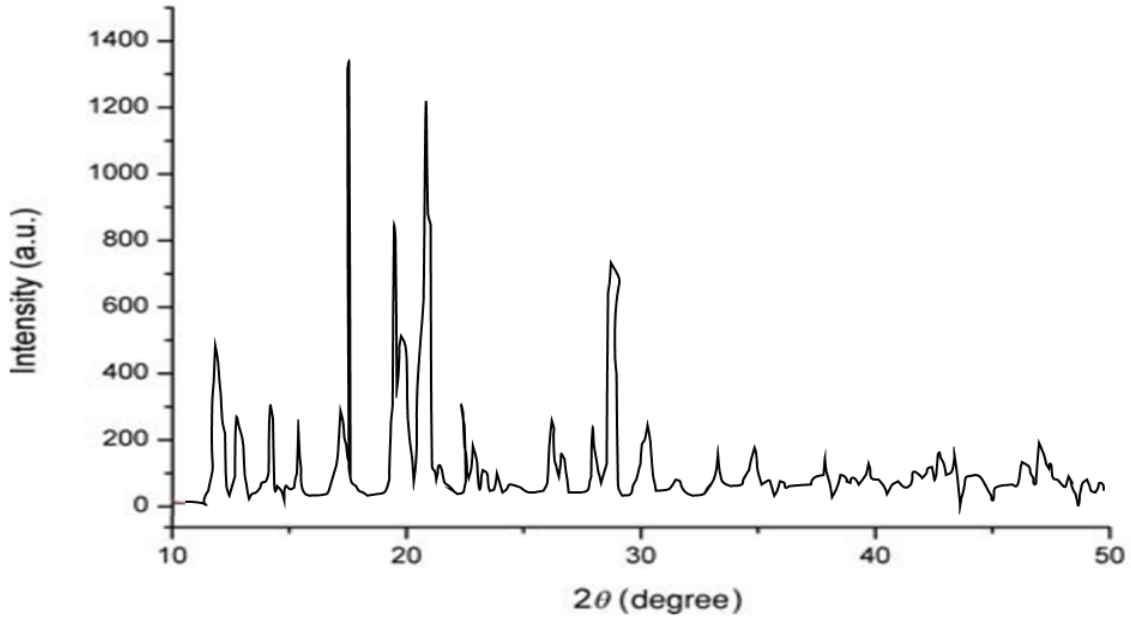

Figure 6: XRD spectra of pure drug tolbutamide.

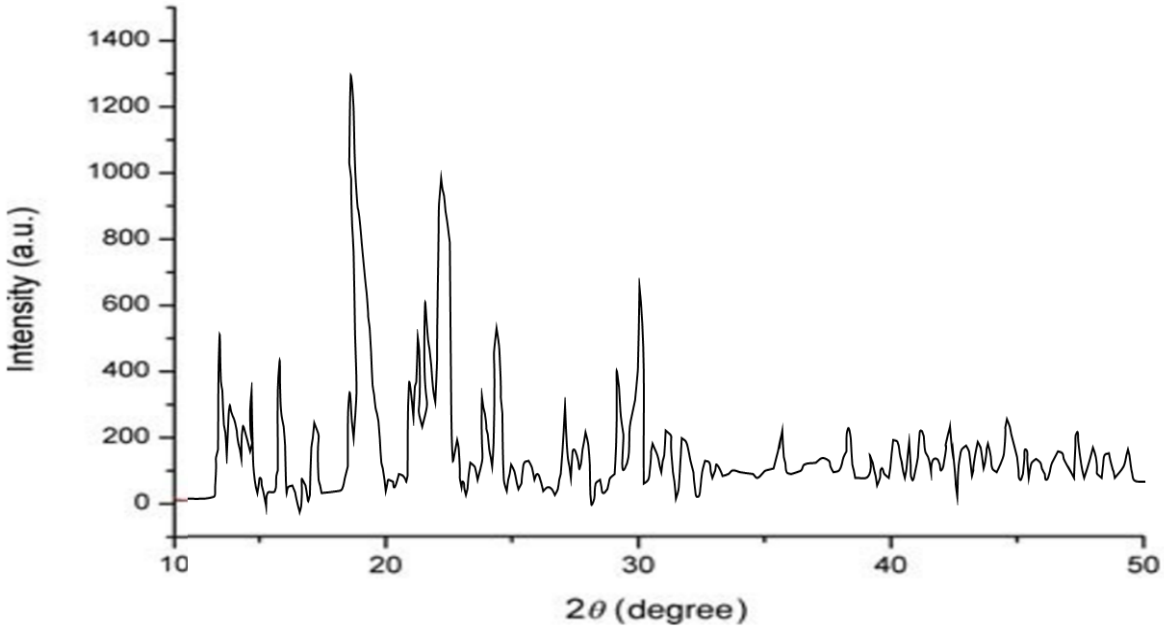

Figure 7: XRD spectra of formulation tolbutamide loaded SLNs.

Characterization of gel

The synthesized gel exhibited excellent homogeneity and appeared as white, translucent gels. The pH of the gel ranged from 6.4 to 7.1, which was deemed satisfactory for topical application. The selected formulation, F2, demonstrated a spreadability of 70.42 g/cm/sec, likely due to the elevated concentration of Carbopol 934.

Conclusion

Solid lipid nanoparticles (SLNs) were produced and evaluated using a solvent diffusion method, yielding several significant outcomes for their effective creation. Among the six formulations, F6 demonstrated superior performance. Both F6 and another formulation were

optimized for producing a continuous release dosage form and were considered the best formulations in this project. Formulation F6 had a particle size of 166.1±3.2 nm and an entrapment efficiency of 91.68%. The in vitro drug release profile of the optimized formulation showed 85.21% release over 6 hours. The tolbutamide nanoparticles produced in this study exhibited appropriate drug loading levels and demonstrated enhanced stability, with a significant reduction in particle size compared to previous findings. The SLNs-loaded gel exhibited excellent homogeneity and appeared as white, translucent gels. The selected formulation, F2, had a spreadability of 70.42 g/cm/sec, likely due to the elevated concentration of Carbopol 934. Finally, the prepared SLNs gel is a promising formulation for the treatment of type 2 diabetes. The gel form is easy to apply and effectively penetrates the skin layer, while the presence of SLNs allows for sustained release of the medicament over a longer period. Future work will focus on in-vivo studies to further confirm these findings and improve accuracy.

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