Novel Glibenclamide–Phospholipid Complex for Diabetic Treatment: Formulation, Physicochemical Characterization, and *in-vivo* Evaluation

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ABSTRACT

**Introduction:** Formulation of phospholipid complex is an ideal approach to improve the solubility of poorly soluble drugs. **Objectives:** This study has been aimed to prepare a novel glibenclamide-phospholipid complex by using the solvent evaporation technique. **Materials and Methods:** Because glibenclamide is a weakly soluble medication, complexing it with phospholipids is an excellent way to enhance its solubility. The phospholipid complex of Glibenclamide was produced using the solvent-evaporation technique to enhance its oral efficacy. The formulation was characterized and evaluated by various parameters including FTIR, DSC, PXRD, SEM, TEM, *in vitro* drug release, and *in vivo* pharmacokinetic studies in Wistar rats plasma. According to studies, the Glibenclamide phosphates combination is significantly more water-soluble than the physical combination and pure Glibenclamide. The oral bioavailability of the glibenclamide-phospholipids complex was measured by using HPLC in Wistar rats’ plasma. **Results:** There was no substantial interaction between the medication and the phospholipid in the formulation, according to the FTIR and PXRD findings. The morphology of the formulation was verified by SEM and TEM investigations, indicating that the crystalline form had been converted to an amorphous form. The glibenclamide-phospholipids complex had a greater peak plasma concentration (5.1 vs. 3.8 g/mL), its AUC was higher (14.65 vs. 11.81 μgh/L), and its T1/2 was longer (2.4 vs. 3.1 hr), showing that it enhanced drug dissolution rate. **Conclusion:** The findings showed that increasing the oral bioavailability of water-insoluble medicines by phospholipid-complexation is a potential approach. The results showed that phospholipid-complexation may be used to enhance the oral bioavailability of water-insoluble drugs. **Keywords:** Glibenclamide, Glibenclamide–phospholipid complex, Pharmacokinetic, Oral bioavailability.

INTRODUCTION

Diabetes mellitus (DM) is a serious illness indicated by high levels of glucose (hyperglycemia) that need medical treatment.¹ Diabetes mellitus (DM) causes a variety of serious consequences, including nephropathy, neuropathy, cardiovascular risk, high blood pressure, an abnormal lipid profile, and retinopathy, making it the seventh greatest cause of death in the United States.² Diabetic people with Type 2 diabetes accounted for approximately 90% of all cases. Type 2 diabetes is a metabolic disease associated with decreased pancreatic insulin production as well as insulin resistance or diminished insulin action, predominantly in the liver and muscle cells.³ Therefore, one of the most major problems in the twenty-first century is the prevention and treatment of Type 2 diabetes to minimize complications, mortality, and healthcare expenditures.⁴ The method of delivery, solubility are important requirements for therapeutic effectiveness. It also presents significant difficulty for the pharmaceutical industry designing innovative medicines, because 40% of the active ingredients reported are.
either insoluble or poorly soluble in aqueous systems.\(^5\) According to the International Diabetes Federation (IDF), diabetes prevalence was estimated to be 7.7% in 2019 and anticipated to increase to 9.5 percent in 2045 with a population count of 77 million.\(^6,7\) Glibenclamide is a sulfonylurea oral hypoglycemic drug of the second generation which is used to treat type 2 diabetes.\(^8\) Sulfonylurea act by binding and blocking ATP-sensitive potassium channels in pancreatic beta cells, due to an increase in intracellular \(K^+\) ions, membrane depolarization, opening of voltage-dependent \(Ca^{2+}\) channels, intracellular \(Ca^{2+}\) influx, and increased insulin secretion depicted in Figure 1.\(^9\) By boosting insulin release from pancreatic beta cells and increasing the sensitivity of peripheral tissue to insulin, it induces hypoglycemia. The World Health Organization’s Model List of Essential Medicines included glibenclamide in 2015. Glibenclamide has a lipophilic Physicochemical profile and is classified as category II of the Biopharmaceutical Classification System (low solubility, high permeability, pKa-3.79). The glibenclamide dissolution is considered the rate-limiting stage, as absorption after oral administration approaches 45 percent of the initial dose.\(^10\) Glibenclamide has a short half-life of 4hr that specifies a high dosage frequency.\(^11\) Several techniques have been developed to solve these problems like incorporation in solid dispersions, amorphization,\(^12\) nanoparticles, nanoemulsions, formation of inclusion complexes,\(^13\) transdermal patch,\(^14\) floating tablets,\(^10\) and self nanoemulsifying drug delivery system.\(^11\) The solubilizing characteristics of phospholipids are not the only benefits of their potential as a carrier system; however, being major components of the cellular membrane, also have great biocompatibility.\(^15\) Phospholipids are also known for having amphiphilic structures. Phospholipids have self-assembly, emulsifying, and wetting properties due to their amphiphilicity.\(^16\) However, phospholipids’ solubilizing properties are not the sole advantages of their potential as a carrier system; phospholipids in the cell membrane play a key role in maintaining membrane flexibility and have the potential to improve the water solubility of poorly soluble medicines, while at the same time enhancing membrane permeability for pharmaceuticals with low membrane permeation. To promote drug absorption across biochemical barriers, phospholipids (such as phosphatidylcholine) are used in drug delivery systems due to their amphiphilic nature. As a result, glibenclamide oral bioavailability may be enhanced by employing a glibenclamide–phospholipid complex. Researchers have shown in the past that phospholipids can change the polarity of certain compounds with low bioavailability and improve their therapeutic efficacy.

We have developed innovative glibenclamide–phospholipid complex formulations based on the aforesaid advantages of phospholipid complex to improve the solubility of the poorly soluble drug. Glibenclamide was complexed with phosphatidylcholine (Lipoid 75s). The formulation was characterized by using, FTIR, DSC, PXRD, SEM, and TEM, in vitro dissolution, in vivo pharmacokinetic parameters. Glibenclamide solubility and the physical combination of the two were compared. The pharmacokinetics and oral bioavailability of glibenclamide and the glibenclamide–phospholipid complex were studied in Wistar rats. The complexation of glibenclamide with phospholipids in the efficient management of diabetic drugs, according to our information, is first reported glibenclamide–phospholipid complex.

**MATERIALS AND METHODS**

**Materials**

The sample of Glibenclamide was procured as a gift from ISFAL analytical lab Moga Punjab, India. Phosphatidylcholine (Lipoid S-75) was purchased from HPLC Pvt. Ltd., Mumbai. It included approximately 60 percent (w/w) hydrogenated phosphatidylcholine. DCM and methanol of HPLC grade were procured from SDFCL Pvt. Ltd. (Mumbai, India). All the chemicals used were pure and were of analytical grade.

**Preparation of glibenclamide and phospholipid physical composition**

A physical combination in an identical ratio (1:1) was formed by carefully grinding glibenclamide and phospholipid in a mortar for 10 to 15 min. The material was preserved in desiccators after being filtered through a 20-mesh sieve.

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*Figure 1: Mechanism of action of glibenclamide.*
Glibenclamide–phospholipid complex synthesis

A 1:1 ratio of glibenclamide to phospholipids exists in the glibenclamide–phospholipid complex. A precise amount of glibenclamide and phospholipids were mixed and dissolved in 100 mL of DCM and refluxed at 60°C for 24 hr with continual stirring on a water bath. The finished product was dried using a rotary evaporator (Labtech, Private Limited, India). The dried residues were collected and kept overnight in the desiccators and passed through a 100-mesh sieve to produce a uniform particle size. The glibenclamide–phospholipids complex was finally stored at room temperature in a light-resistant colored glass container. Figure 2 depicts the methodology adopted for the preparations of glibenclamide–phospholipid complex.

Physicochemical characterization of the glibenclamide–phospholipids complex

Fourier transforms infrared spectroscopy (FTIR)

Glibenclamide was mixed in a ratio of 1:100 with dried spectroscopic grade KBr in a pestle mortar and pressed into a KBr pellet. The KBr pellet was analyzed using an FTIR spectrophotometer (Shimadzu, Japan) by spectral scanning in the wavenumber region of 4000–400 cm⁻¹. FTIR spectra for phospholipids, a physical combination of glibenclamide and phospholipid, and a glibenclamide–phospholipid complex were procured in the same way. The spectra were analyzed for characteristic peaks to identify any possible interaction between glibenclamide and phospholipid.

Differential scanning calorimetric (DSC)

Using the DSC instrument (DSC6000) PerkinElmer, USA, the thermograms of glibenclamide, phospholipids, physical combination, and glibenclamide–phospholipid complex were conducted. The temperature axis and cell constant of the DSC were calibrated in the medium. A heating rate of 20°C/min was employed with nitrogen purging (100 ml/min) throughout a temperature range of 50–250°C. With an empty aluminum pan serving as a control, samples (4–6 mg) were weighed into an aluminum pan and graded as pinhole-sealed.

Powder X-ray diffraction (PXRD)

PXRD patterns of glibenclamide, phospholipids, physical mixture, and glibenclamide–phospholipid complex were evaluated using Cu K-alpha-1 XRD instrument (Model saxspace, Anton Paar) at room temperature. Data was collected in continuous scan mode across an angular range of 2 to 50 degrees.

Scanning electron microscopy (SEM)

The surface morphology of glibenclamide, phospholipid, physical mixture, and the glibenclamide-phospholipid complex was studied using an SEM instrument (ZESIS EVO40, Germany). Before the examination, samples were taped with double-sided adhesive tape to aluminum material and sputter-coated with a thin gold 3-5nm coating for 75s and at 40 W. After scanning the samples with an electron beam with an acceleration potential of 1.2 kV, the images were obtained in secondary electron mode.

Transmission electron microscopy (TEM)

TEM investigations were used to evaluate the glibenclamide-phospholipid complex internal morphology. A drop of glibenclamide–phospholipids complex having various pH 1.2, 6.8, and water was put on a carbon film-covered copper grid for the experiment. Philips Technai-20, Japan instrument was used for this purpose. The copper grid was put onto a sample holder and placed in the transmission electron microscopes vacuum chamber, where it was examined under a low vacuum and TEM pictures were taken.

Solubility studies

To test the solubility of glibenclamide and glibenclamide–phospholipid complex, an excess quantity of these was added to 10 ml of distilled water and n-octane in sealed glass vials at 37°C. The liquids were agitated for one hour followed by extraction of 1 ml of the solution and centrifuged at 1200 rpm for 5 min to remove excess glibenclamide. Each experiment was repeated three times in total. The concentration of glibenclamide in a 20ml aliquot of the final solution was determined using UV–spectrophotometer at a wavelength of 274 nm.
**Dissolution studies**

The dissolution of glibenclamide and glibenclamide – phospholipid complex in HCl (pH 1.2) and phosphate buffer (pH 6.8) was studied using USP type-II automated dissolution test equipment (Lab, India). The samples containing 20 mg of glibenclamide, were agitated at 50 rpm at 37±0.5°C in a dissolving vessel containing 900 mL buffer. To maintain the sink’s condition, aliquots of the material were removed and refilled regularly with new dissolving media. Using the UV method at 274 nm, the concentration of glibenclamide in the samples was measured.

**Determination of glibenclamide content in phospholipid complex**

HPLC was used to determine the glibenclamide content in the glibenclamide–phospholipid complex (Shimadzu Europa Gmbh, Germany). 10 mg of glibenclamide–phospholipid complex was dissolved in 10 ml of methanol and then tested. The stationary phase was a Phenomenex C_{18} column (250 mm 4.6 mm, 5 m) that was operated at ambient temperature. The mobile phase was made up of 80:20 ACN: ammonium acetate buffer (2 mM). The flow rate remained constant at 1 ml/min. The samples were evaluated using HPLC at a wavelength of 274 nm.

**Oral in vivo pharmacokinetic studies**

**Chromatography**

RP-HPLC was used to determine the plasma concentrations of glibenclamide (Shimadzu Europa Gmbh, Germany). Phenomenex C_{18} column (250 mm 4.6 mm, 5m) kept at room temperature was used as the stationary phase. The mobile phase consisted of a 30:70 mixture of ammonium acetate and acetonitrile. The flow rate was kept constant at 1.0 mL/min, and samples were analyzed using an HPLC system.

**Glibenclamide extraction and sample preparation**

200 mL plasma was placed into a micro-centrifuge vial and 500 mL methanol was added to extract glibenclamide. The solution was vortexed quickly, then centrifuged at 10000 rpm for 10 min, the supernatant was separated, and 20-μL samples were tested by HPLC.

**Animals and dosing**

Male Wistar rats (150–250 g) were obtained from an experimental animal facility, department of pharmaceutical sciences, Guru Jambheshwar University Science and Technology, Hisar, Haryana, IAEC/2020/29-38: Dated 18-11-2020. The study protocol was approved by the institutional animal ethics committee (IAEC). They were split into four groups (n = 6/group/time point), The first group (Normal), the second group (Diabetic Control), the third group for glibenclamide at a dose of 5 mg/kg, and the other fourth group for the glibenclamide-phospholipid complex at a dose corresponding to 20 mg/kg of glibenclamide. Blood samples from the retro-orbital plexus of four groups of rats were collected into micro-centrifuge vials containing heparin at several periods at 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hr time points under light ether anesthesia. Following that, plasma was isolated and stored at 20°C before analysis following centrifugation at 10,000 rpm for 10 min. Glibenclamide content was calculated using the HPLC method. Maximum concentration (C_{max}) and time to attain maximum concentration (T_{max}) are derived directly from the plasma concentration-time curve.

**RESULTS AND DISCUSSION**

**Glibenclamide content in phospholipid complex**

A 1:1 molar ratio was used to make the glibenclamide–phospholipids complex. Glibenclamide–phospholipid complex was made using drug and phospholipids ratios of 1:0.5, 1:1, 1:2, 1:3, and 1:4. When the phospholipids percentage was more than 1:1, a viscous material resulted. However, when it was less than one, the drug concentration in the complex was less than 3% (w/w). This occurrence might be due to a shortage of phospholipids or the drug’s surface-active characteristics. Model membranes have been demonstrated to be destroyed and solubilized by cationic drug molecules, resulting in a reduction in complex formation. As a consequence, an equimolar ratio of drug and phospholipid was chosen for the glibenclamide–phospholipids complex formulation. The medication content and % yields were 82.3% and 73.45%, respectively.

**Fourier transforms infrared spectroscopy (FTIR)**

All the significant peaks corresponding to glibenclamide were present in the FTIR spectra of physical combination, and formulations of the glibenclamide-phospholipids complex (Figure 3). The distinctive absorption peaks of glibenclamide in the physical mixture were found at 1738 cm^{-1} and 1599 cm^{-1} demonstrating the additive effect. The strength of four distinctive absorption peaks of glibenclamide at 1623 cm^{-1}, 1526 cm^{-1}, 1165 cm^{-1}, and 1455 cm^{-1} were all the same in the spectra of the glibenclamide-phospholipids complex. It represents the compatibility of the drug with the lipid without the appearance of any
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significant interaction. These observations ensure the stability of the drug in the formulations. These findings imply that during the complex formation, some partial interaction between glibenclamide and phospholipids occurred.

**Differential scanning calorimetry (DSC)**

Differential scanning calorimetry is a common technique for detecting drug-excipient incompatibility since it gives information about feasible interactions. The DSC curves of Glibenclamide (A), Phospholipid (B), and Physical combination (C), Glibenclamide-phospholipids complex (D) are shown in (Figure 4). Because of its crystalline structure, glibenclamide has a pronounced endotherm sharp peak at 178.72°C. The physical mixture of glibenclamide and phospholipid has the first endothermic peak appearing at 146.36°C. The Glibenclamide-phospholipids complex has one sharp endothermic peak at 141.65°C and this peak was observed at lower temperatures than phospholipid and glibenclamide respectively. It suggested that there is fractional amorphization of the drug during formulation or it may be resulted due to the existence of hydrophobic interaction between the drug and phospholipid. It happens because upon raising the temperature, glibenclamide and phospholipids partially combine to produce a complex with a lesser melting point than the individual candidates. The initial peaks of glibenclamide and phospholipids have been eliminated from the DSC curve of the glibenclamide–phospholipid complex. By comparing the four DSC curves, it is clear that glibenclamide and phospholipids interact in some way, such as the creation of hydrophobic bonds and van der Waals forces.18

**Powder X-ray diffraction (PXRD)**

The powder X-ray diffraction patterns of glibenclamide, physical mixture, phospholipid, and glibenclamide–phospholipid complex are shown in (Figure 5). Because of its crystalline structure, glibenclamide has distinct peaks at 2θ angles of 7.34, 9.89, 10.93, 11.89, 14.73, 15.58, 16.84, 17.44, 18.43, 19.16 20.98, 21.71, and 24.32. Physical mixture and glibenclamide both showed these peaks. Their intensities, on the other hand, were lowered in the glibenclamide–phospholipid complex, indicating a decrease in glibenclamide crystallinity.21,22

**Scanning electron microscopy (SEM)**

SEM was used to investigate the shape and crystal pattern of glibenclamide and the glibenclamide–phospholipid complex (Figure 6). Rod-like crystals were visible of free glibenclamide (Figure 6A), but in the case of the phospholipid complex, (Figure 6D), no such crystal pattern was observed. Because the drug and phospholipid were completely miscible, the particles had an irregular shape and a smooth surface. In the physical mixture, both the drug and the phospholipid are easily recognizable (Figure 6C). As a result, surface morphology research might be used to derive the complex development.

**Transmission electron microscopy (TEM)**

The glibenclamide-phospholipid complex was revealed as small, black structures in water (Figure 7A), whereas the glibenclamide complex in pH 1.2 (Figure 7B) was
a hexagonal structure with irregular particles with no discernable shape like structure. At pH 6.8 (Figure 7C) the glibenclamide-phospholipid complex in phosphate buffer exhibited was hexagonal, with a dark inner core surrounding by a lighter envelope, most likely made up of phospholipids. At pH 7.4 glibenclamide-phospholipid complex (Figure 7D) showed loose aggregates.

**Solubility studies**

The solubility of glibenclamide, physical mixture, and glibenclamide-phospholipid complex determined in water and n-octanol at 25°C has been shown in (Table 1). In comparison to glibenclamide, the results show that the glibenclamide-phospholipid complex has better water solubility. This increase in solubility might be related to the development of complexes.

**Dissolution studies**

Glibenclamide and glibenclamide-phospholipid complex dissolution patterns in pH 1.2, pH 6.8, and pH 7.4 are shown in (Figure 8). In contrast to glibenclamide and glibenclamide-phospholipid complex showed improved dissolution in both mediums. Glibenclamide-phospholipid complex dissolution was 99 percent after two hours in different pH compared to 81 percent for glibenclamide. The pH of the dissolving media has a significant impact on glibenclamide dissolution, which coincides with the solubility difference. In acidic pH, glibenclamide is a basic molecule with enhanced solubility. In both acidic and basic environments, the compound improved solubility and dissolution.

**Pharmacokinetic Parameters**

The concentration of glibenclamide in rat plasma was measured up to 24 hr after oral administration to compare the pharmacokinetic profiles of the pure and complexed drugs. Liquid-liquid extraction was used for sample preparation on the plasma. (Figure 9) shows the mean plasma concentration-time profiles of glibenclamide and glibenclamide-phospholipid complex. Glibenclamide-phospholipid complex (20 mg/kg) and

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<th>Samples</th>
<th>Apparent solubility (μg/mL)</th>
<th>Water</th>
<th>n-Octanol</th>
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<tr>
<td>Glibenclamide</td>
<td>119.14</td>
<td>57.92</td>
<td></td>
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<tr>
<td>Physical mixture</td>
<td>141.71</td>
<td>72.67</td>
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<tr>
<td>Glibenclamide-phospholipid complex</td>
<td>249.64</td>
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Glibenclamide (5 mg/kg) were orally given to rats (n = 6). Various determined pharmacokinetic parameters have been summarized in (Table 2). The results revealed that glibenclamide-phospholipid complex had a C\text{max} of 5.1 μg/mL with a T\text{max} of 2.01 hr after oral administration, whereas glibenclamide had a C\text{max} of 3.8 μg/mL with a T\text{max} of 4.01 hr. The AUC of the glibenclamide-phospholipid complex and free glibenclamide, on average, were 14.65 and 11.81 μgh/mL, respectively. The increased bioavailability of glibenclamide-phospholipid complex can be ascribed to two factors; one is the maintenance of cell membrane fluidity phospholipids as they are present in cell membranes and the other is improved glibenclamide-phospholipid complex solubility leading to improved rate and/or extent of drug absorption across the intestinal mucosa. As a result, phospholipids have played a significant role in enhancing drug absorption through the intestinal mucosa.

CONCLUSION

Our research might lead to new trends for improving the solubility of poorly soluble drugs. The current study demonstrated how phospholipids may be used to build a complex that improved glibenclamide absorption. A simple and repeatable technique was used to successfully produce glibenclamide–phospholipid complex. FTIR, DSC, PXRD, SEM, and TEM, were used to confirm the complex formation of the glibenclamide-phospholipid complex, and the investigations revealed that the drug and phospholipid joined and created some type of connection through hydrogen bonds or van der Waals forces. Glibenclamide–phospholipid combination has a greater solubility in water than pure glibenclamide, according to solubility experiments. When compared to the pure drug solution, the glibenclamide-phospholipid complex displayed increased AUC and MRT, suggesting that glibenclamide bioavailability in rats may be improved.

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Declared none

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ABBREVIATIONS


REFERENCES


PICTORIAL ABSTRACT

SUMMARY

The Glibenclamide-Phospholipid Complex was prepared by using the solvent evaporation technique. The formulation was characterized and evaluated by various parameters including FTIR, DSC, PXRD, SEM, TEM, in vitro drug release and in vivo pharmacokinetic studies in Wistar rats plasma. This work is significant in order to improve the solubility of poorly soluble drugs specially drugs belonging to BCS Class-II. The findings showed that increasing the oral bioavailability of water-insoluble medicines by phospholipid-complexation is a potential approach. The results showed that phospholipid-complexation may be used to enhance the oral bioavailability of water-insoluble drugs.