RESEARCH ARTICLE
FORMULATION OF AMORPHOUS TERNARY SOLID DISPERSION OF POORLY SOLUBLE
DRUG-DAPAGLIFLOZIN

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Abstract:
The Formulation of solid dispersions can be easily prepared with adequate physical properties and required particle size and release characterstics which might be an advantage for the solubility enhancement. Screening design helped in identifying the significant characterstics such as particle size and the ratios of drugs and excipients, that affected the response variables. Solid dispersion system of Dapagliflozin used could improve the solubility and dissolution rate of Dapagliflozin. The above study demonstrated the use of PEG 4000, PVP K-30 and HPMC in combination for the formulation of solid dispersions in solubility and dissolution enhancement by kneading method. PXRD and DSC studies indicated the transformation of crystalline Dapagliflozin to amorphous form by solid dispersion technology. The aqueous solubility and dissolution study shows a remarkable improvement in solubility, where F3 was found to have the maximum solubility 2.535±0.09 mg/mL

1. Introduction:[1-2] Diabetes Mellitus
Type 1 diabetes
In type 1 diabetes, there is an absolute deficiency of insulin resulting from autoimmune destruction of β cells. Without insulin treatment, such patients will ultimately die with diabetic ketoacidosis (3). Type 1 diabetes often develops suddenly and can produce symptoms such as polydipsia, polyuria, enuresis, lack of energy, extreme tiredness, polyphagia, sudden weight loss, slow-healing wounds, recurrent infections and blurred vision with severe dehydration and diabetic ketoacidosis in children and adolescents. The symptoms are more severe in children compared to adults (2).

Type 2 diabetes
Type 2 diabetes is accompanied both by insulin resistance (which precedes overt disease) and by impaired insulin secretion, each of which are important in its pathogenesis. Such patients are often obese and usually present in adult life, the incidence rising progressively with age as B-cell function declines. Treatment is initially dietary, although oral hypoglycaemic drugs usually become necessary, and about one-third of patients ultimately require insulin (4).

People living with type 2 DM are more vulnerable to various forms of both short- and long-term complications, which often lead to their premature death. This tendency of increased morbidity and mortality is seen in patients with type 2 DM because of the commonness of this type of DM, its insidious onset and late recognition (5).

Treatment
Diet is the main treatment combined with increased exercise. However, patient's insulin is mainly used for controlling diabetes mellitus. Patients not responding to insulin as well as dietary control are administered oral hypoglycemic agents, they are used to control symptoms from hyperglycemia, as well as to limit microvascular complications (6). The main oral hypoglycemic agents are metformin (a biguanide), sulfonylureas, SGLT2 inhibitor and other drugs that act on the sulfonylurea receptor, and glitazones.

Table No 1 Anti diabetic drugs

Class	Examples	Principal mode of action
Sulphonylureas	Gliclazide, Glibenclamide, Glipizide, Glimepiride	Stimulate insulin secretion (acting 12-24 hours)
SGLT-2 inhibitors	Dapagliflozin, Canagliflozin	Increased glucosuria through the inhibition of SGLT-2 in the kidney
Biguanides	Metformin	Improves insulin action

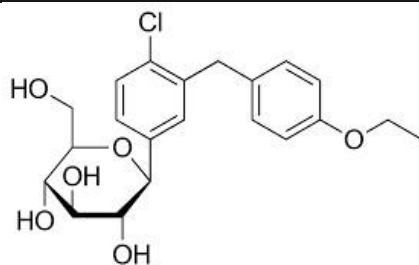| Thiazolidinediones | Pioglitazone, Rosiglitazone | PPAR-gamma agonists, increase insulin action |
| Alpha-glucosidase inhibitor | Acarbose | Slows down rate of carbohydrate ingestion |

Fig 1 Structure of Dapagliflozin

2. Drug Profile[2-3]

IUPAC name : (2S,3R,4R,5S,6R)-2-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-6- (hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol

Category	:	Anti-diabetic drug
Molecular weight :		408.88 g/mol
Molecular Formula :		C21H25ClO6
Description	:	White crystalline powder
Melting Point	:	74^0- 78^0 C
Log P	:	2.11
Storage	:	Store at room temperature away from heat, light and moisture.
Half-life	:	12.2 hours
Solubility	:	Soluble in water, ethanol, DMSO and dimethyl formamide.
Absorption	:	Orally administered dapagliflozin is rapidly absorbed generally achieving peak

plasma concentrations within 2 hour.

Metabolism: Predominantly in the liver and kidneys by uridine diphosphate-glucuronosyltransferase-1A9 to the major metabolite dapagliflozin 3-O-glucuronide (this metabolite is not an SGLT2 inhibitor at clinically relevant exposures).

Elimination: Occurs mainly via renal excretion, with 61 % of a dapagliflozin dose being recovered as this metabolite in urine.

3. Mechanism of Action:[5-8]
4. MATERIALS AND METHOD

Materials	Name	Suppliers
Drug	Dapagliflozin	Dr. Reddy's Laboratories, Hyderabad
Excipients	Hydroxy propyl methyl cellulose (HPMC)	Central Drug House, Mumbai
	Polyethylene Glycol 4000 (PEG)	
	Polyvinyl Pyrrolidone K-30(PVP)	
Chemicals	Sodium Chloride	Central Drug House, Mumbai
	Potassium chloride	
	Disodium hydrogen phosphate	
	Potassium dihydrogen phosphate	
	Methanol	

5. PREFORMULATION STUDIES[10-15]

Preformulation is the physicochemical characterization of the new drug substance alone in order to produce useful information for subsequent formulation of a stable and a biopharmaceutically suitable dosage form. It is the initial step in development of dosage form of drug molecules. The aim of preformulation testing is to provide useful data to the formulator in developing stable and bioavailable dosage forms.

5.1 Melting point determination

Melting point is the temperature at which the pure liquid and solid exist in equilibrium. In practice it is taken as an equilibrium mixture at an external pressure of 1 atmosphere; this is sometimes known as normal melting point. The thiele's tube method of melting point determination was used in the present study.

5.2 FTIR spectrum of Dapagliflozin

Approximately 1–2mg of Dapagliflozin powder, physical mixtures, and solid dispersions formula were placed in a mortar and then crushed until homogeneous then formed pellets with a pressure of 800 mPa under vacuum and analyzed by Fourier-transform infrared (FTIR) spectrophotometer. Absorption spectra were recorded at wave number 500–4000 cm^{-1}.

5.3 Solubility studies of Dapagliflozin

Samples that equal with 50mg Dapagliflozin were weighed and dissolve with 25mL phosphate buffer (pH 7.4) in Erlenmeyer. Samples were put in orbital shaker for 24 hours. After the samples were filtered using filter paper (Whattman 0.45 µm), absorbance of filtrates were measured using UV spectrophotometer at the maximum wavelength 227 nm.

6. UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF DAPAGLIFLOZIN

6.1 Determination of Ʌmax of Dapagliflozin

A stock solution of 1 mg/ml of Dapagliflozin was prepared by dissolving 100 mg of drug in 100 ml of methanol (1000 µg/ml-stock I). 5 ml of stock I was further diluted to 50 ml using methanol (100 µg/ml-stock II). From stock II the aliquots are prepared and was diluted to 10 ml using methanol in a volumetric flask to obtain a concentration of 5-25 µg/ml. the resultant solution was scanned for Ʌmax between 200-400 nm and Dapagliflozin showed maximum absorbance at 227 nm.

6.2 Calibration curve of Dapagliflozin in methanol

A stock solution of 1 mg/ml of Dapagliflozin was prepared by dissolving 100 mg of drug in 100 ml of methanol (1000 µg/ml stock-1). 5 ml of stock I was further diluted to 50 ml using methanol (100 µg/ml-stock II). From stock II, 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml were taken and volume was diluted to 10 ml using methanol. The dilutions were scanned for maximum absorbance in UV-Visible double beam spectrophotometer in the range of 200-400 nm using methanol as blank.

7. COMPATIBILITY STUDIES[18-20]

A proper design and formulation of a dosage form requires considerations of physical, chemical and biological characterstics of both drug and the excipients used in the formulaion. Compatibility between active ingredient and the excipients must be established to produce a stable, efficacious, attractive and safe product. The compatibility study was performed using Fourier Transform Infrared Spectroscopy.

Compatibility between the drug and the excipients was recorded by FTIR, using Shimadzu FT-IR spectrophotometer. The potassium bromide (KBr) pellet method was employed for the study. The samples were thoroughly blended with dry potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the sample cell and the spectrum was recorded between 500-4000 cm-1. The FTIR spectra of pure drug and drug with excipients were recorded and compared with each other.

7. FORMULATION AND DESIGN OF SOLID DISPERSION

7.1 Formulation design

Screening design was inculcated to get different experimental runs using design JMP software version 13, which analyses main effects, certain interactions and also helps to identify the significant factors from those which are not significant. The factors and levels are mentioned in the table- 4. The lower and higher levels of independent factors were selected based on the reported literature and initial screening experiments conducted.

7.2 EVALUATION OF SOLID DISPERSION[19]

Physical evaluation and % yield determination

The percentage yield of solid dispersion of all the formulations were calculated by considering the final weight of the product after drying in comparison to total weight of drug, polymer and other excipients like Hydroxypropyl methyl cellulose (HPMC), Polyethylene glycol 4000 (PEG), Polyvinyl pyrrolidone K-30 (PVP) used for the preparation of solid dispersion. Percentage yield of the product was calculated by using the following formula given below:

W0

% Practical yield =WT× 100

Where, W0- Practical mass of Dapagliflozin Solid Dispersion

WT- Theoretical mass

7.3 Percentage Drug content

An accurate weight of solid dispersion equivalent to 40 mg of Dapagliflozin was dissolved in 20 mL of methanol in 100 mL volumetric flask, the volume was made to the mark with phosphate buffer solution pH 7.4 and the solution was filtered through Whatman filter paper No. 40. The above solution was further diluted with phosphate buffer solution pH 7.4.

7.4 Solubility studies

Samples that equal with 50mg Dapagliflozin were weighted and dissolve with 25mL phosphate buffer (pH 7.4). Samples were put in orbital shaker for 24 hours. After the samples were filtered using filter paper (Whattman 0.45 µm), absorbance of filtrates were measured using UV spectrophotometer at the maximum wavelength 227 nm.

7.5 Dissolution Study[20]

Dissolution test was run using dissolution apparatus type 1 (basket) at 150 rpm for 2 hours. Dissolution medium was 900 mL phosphate buffer (pH 7.4) and temperature of the medium was set at $370^0C \pm 0.5^0$ C. Dissolution test was performed with a test sample that was equivalent amount of Dapagliflozin. Samples were taken about 5 mL at 5, 15, 30, 45, 60, 90 an 120 minutes. In order to maintain a fixed volume, 5 mL of dissolution medium was added at the same temperature. Absorptions of liquid samples were determined using UV spectrophotometer at maximum wavelength.

7.6 Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) is a highly sensitive technique to study the thermotropic properties of many different biological macromolecules and extracts. This method measures the thermodynamic properties of thermally induced transitions and has been applied to a variety of biological macromolecules such as lipids or proteins. Differential Scanning Calorimetry is primarily used to determine the energetics of phase transitions and conformational changes and allows quantification of their temperature dependence. [5] Technical improvements over time have resulted in high sensitivity instruments, which also make DSC a very relevant tool for investigating the thermodynamic properties of various pharmaceutical products, such as, biopolymers, proteins, peptides, and lipid carriers. Both sample and reference are maintained at the same temperature throughout the experiment. Generally, the temperature program for the DSC analysis is designed such that the sample holder temperature increases linearly as a function of time.

The basic principle underlying behind this technique is that when the sample undergoes a physical transformation such as phase transitions more or less heat will need to flow to it than the reference to maintain both at same temperature. Less or more heat flow to the sample depends on whether the process is exothermic or endothermic. As the sample undergoes endothermic reaction more heat is required to raise the same temperature such as the sample undergoes exothermic reaction less heat is required to raise the same temperature. By observing the difference in heat flow between sample and reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions.

7.7 Scanning Electron Microscopy

Surface morphology of the specimen can be determined by using a scanning electron microscopy. SEM is a type of electron microscope the produces images which result from interactions of the electron beam with atoms at various depths within the sample. A cathode, which is the source of electrons, corresponds the lamp of the light system and is usually a tungsten wire filament. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample.

7.8 X-Ray Diffraction

X-ray diffraction (XRD) is a powerful nondestructive technique for characterizing crystalline materials. It provides information on structures, phases, preferred crystal orientations (texture), and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects. X-ray diffraction peaks are produced by constructive interference of a monochromatic beam of X-rays scattered at specific angles from each set of lattice planes in a sample. The peak intensities are determined by the distribution of atoms within the lattice. Consequently, the X-ray diffraction pattern is the fingerprint of periodic atomic arrangements in a given material. This review summarizes the scientific trends associated with the rapid development of the technique of X-ray diffraction over the past five years pertaining to the fields of pharmaceuticals, forensic science, geological applications, microelectronics, and glass manufacturing, as well as in corrosion analysis.

8. RESULT:

8.1 PREFORMULATION STUDIES

Melting point determination

Table 4: Melting point of Dapagliflozin

Sl. No.	Reported	Observed		
		Trial-1	Trial-2	Trial-3
1	$167-170^0C$	168^0C	167^0C	168^0C

The melting point of Dapaglizozin was found to be 168^0 C.

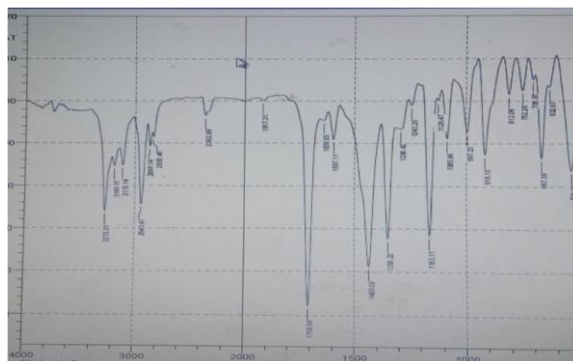**FTIR Spectrum of Dapagliflozin**

Fig 2 FTIR spectrum of Dapagliflozin

Table 3: FTIR spectrum data of Dapagliflozin

Sl.No.	FTIR Spectrum	Functional Group	Stretching peak range (cm-1)	Observedpeak range (cm-1)
1.	Dapagliflozin	C-H	stretching	3200-2900
		aliphatic		
		N-H stretching	3500-3200	3273.31
		N-H bending	1800-2500	1597.11
		SO2NH stretching	1600-1300	1350.22
		C=O stretching	2000-1700	1708.99
		S=O stretching	1400-1100	1163.11

Solubility studies of Dapagliflozin

The results of the solubility study showed an increase in the solubility of Dapagliflozin in physical mixtures and solid dispersions formulas. F3 has the highest (2.535mg/mL) solubility of Dapagliflozin.

Table 4: Solubility studies

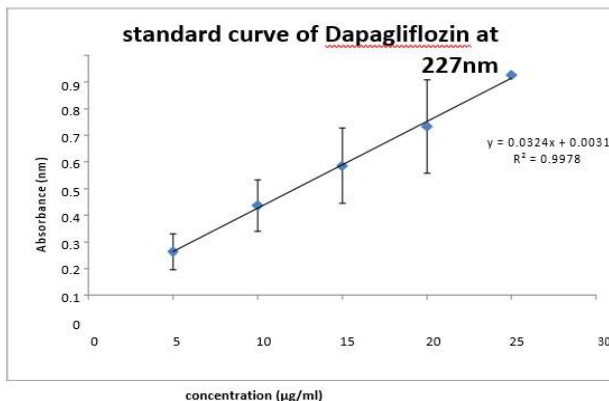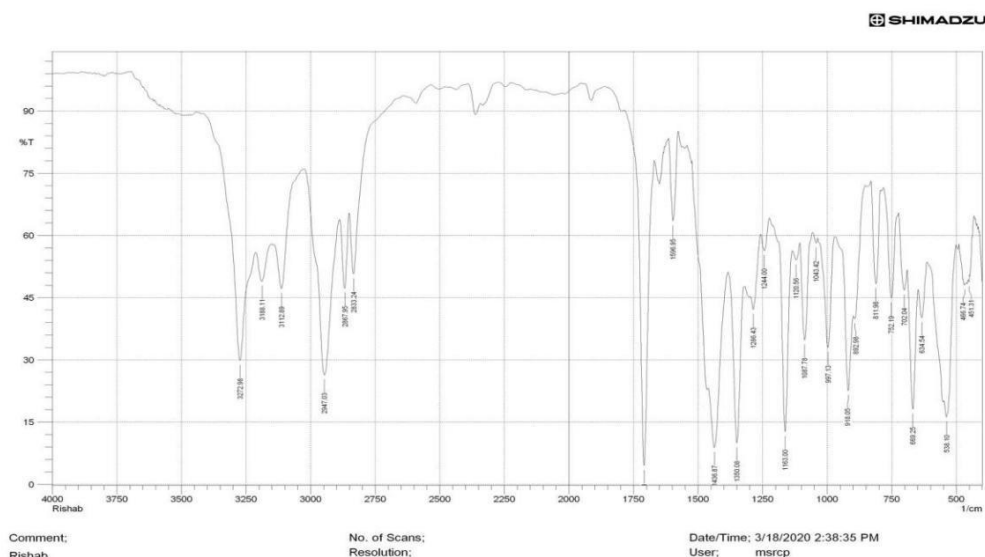| Sample	Solubility (mg/mL)
Dapagliflozin	0.917± 0.04
F1	2.408 ± 0.08
F2	2.250 ± 0.15
F3	2.535 ± 0.09
F4	2.250 ± 0.09
F5	2.110 ± 0.13

Fig 3 Standard graph of Dapagliflozin

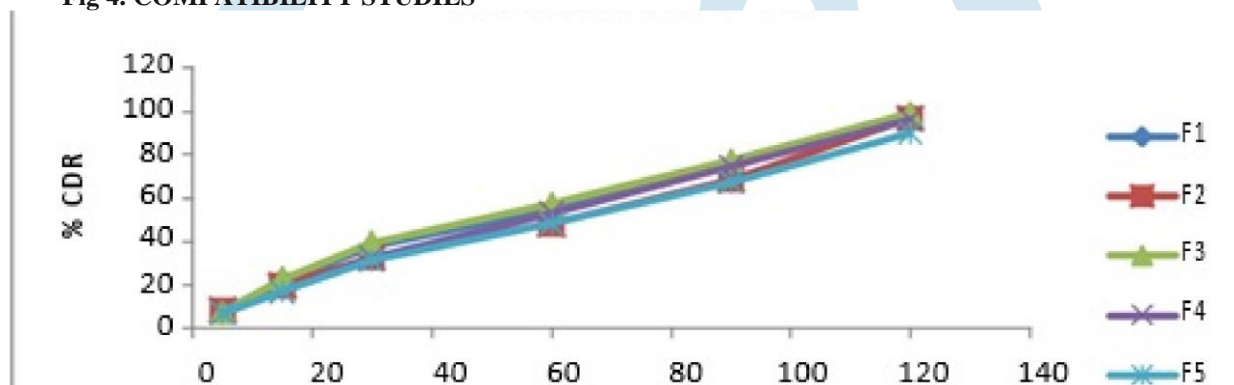**Fig 4. COMPATIBILITY STUDIES**

Fig 5. In vitro drug release profile

CONCLUSION:

The study concluded that kneading technique can be successively adopted for preparation of Dapagliflozin Solid dispersion. Thus, formulation of solid dispersions can be easily prepared with adequate physical properties and required particle size and release characterstics which might be an advantage for the solubility enhancement. Screening design helped in identifying the significant characters such as particle size and the ratios of drugs and excipients, that affected the response variables. Solid dispersion system of Dapagliflozin used could improve the solubility and dissolution rate of Dapagliflozin. The above study demonstrated the use of PEG 4000, PVP K-30 and HPMC in combination for the formulation of solid dispersions in solubility and dissolution enhancement by kneading method. PXRD and DSC studies indicated the transformation of crystalline Dapagliflozin to amorphous form by solid dispersion technology. The aqueous solubility and dissolution study shows a remarkable improvement in solubility, where F3 was found to have the maximum solubility 2.535±0.09 mg/mL as well as in drug dissolution of this new Dapagliflozin solid dispersion in combination with polymer where F3 was found to be having the maximum dissolution rate 98.99%.

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