Preparation and Characterization of Spherical Agglomerates of diabetic drug by direct compression method and a comparative evaluation with innovative tablets

A Consultancy Project

Inception Source Pvt Ltd – Hyderabad

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May-2019

St.Pauls College of Pharmacy

(Approved by AICTE, PCI and Affiliated to Osmania University)

2018-2019

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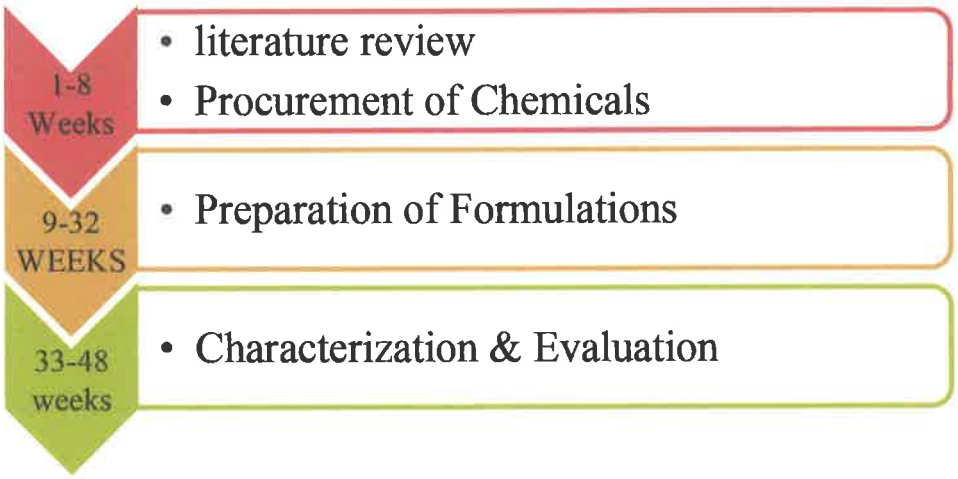

Cost Analysis

S.No	Parameter	Amount in Rupees
1	Man power	79,450
2	Consumables	95,550
3	Contingencies	25,000
4	Overhead Charges	15,000
Total		215000

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Chapter I

Introduction-Need for the Study

1.1 Direct tableting: API are used in Direct tableting techniques by tablet manufacturers over the past few decades that provides compressed form of tablets which are cost reliable.

The sustainability of APIs in the current market along with the addition of excipients and tableting equipment have made solid dosage forms especially tablet manufacturers to increase their demand and supply as it is the convenient dosage form used by maximum no. of patients. Many advantages are offered by these dosage forms like ease of manufacturing, accurate dosing, convenience in administration and stability criteria when compared to oral liquid formulations, tamperproofness to that of capsules, patient acceptability towards tablets than parenteral etc. that had an impact on making it more popular than others.

"Direct Compression" is the term that is referred as the process through which tablets are directly compressed from the powder blends of active ingredients and excipients that are suitable for its preparation. Pre-treatment is not required for powder blends when wet or dry granulation method is carried out. Out of 100 per cent of APIs less than 20% of drugs can be directly compressed into tablets while the rest of the ingredients lack flow or lubricating properties or cohesion which is required for production of tablets by DC. By using directly compressible adjuvants satisfactory results can be obtained by tablet processing.

Direct tabletting of active pharmaceutical ingredients (APIs) is applicable when powders have a better flow property and compression criteria, which is a problem for major of the active ingredients that have poor compressibility and flow characteristics. On addition of excess amount of diluents or by performing wet or dry granulation satisfactory results can be obtained.

1.2 DIABETES MELLITUS

Diabetes mellitus is a Greek word which is commonly called as diabetes. It is a chronic metabolic disorder where in sugar levels in blood are high (i.e. above from the normal range) because enough insulin is not produced in the body. Diabetes occurs in pancreas as it produces the insulin hormone (it is the organ that speeds up the transfer of sugar from blood and deliveries into muscle, liver and fat tissues).[3]If body doesn't have enough insulin then sugar accumulates in blood stream which results in diabetes mellitus. Diabetes is the most hazardous disease.

There are 3 major types of DM

- Type 1 diabetes –ID- DM/ juvenile onset DM (5-100%)

- Type 2 diabetes - NID DM/ adult onset DM (90-95%)
- Pre-diabetic stage (IGT)
- Gestational diabetes
- Other types (1-2%):
    - ✓ Genetic syndromes (affecting insulin secretion or action)
    - ✓ Endocrinopathies (Cushing's syndrome, pheochromocytoma, Acromegaly, glaucoma, thyrotoxicosis)
    - ✓ Disease of pancreas -cancer, chronic pancreatitis
    - ✓ Drug or chemical induced - beta blockers, corticosteroids, thiazide diuretics)
    - ✓ Viral Infections

DIABETES- TYPE 1:

Diabetes type 1 also called as IDD. It usually occurs in childhood and hence called juvenile onset diabetes This Type 1 diabetes occurs due to absolute deficiency of insulin. Other reasons include genetic predisposition. It is the condition of auto immunity where the body attacks own pancreas with antibodies. Ketoacidosis is the first manifestation of the disease which is observed in some children and adolescents. Some of the patients have insulinopenia permanently and are also exposed to ketoacidosis but do not have evidence of auto immunity. Number of medical risks are associated with type 1 diabetes mellitus many of them includes diabetic retinopathy (damage to the tiny blood vessels in eyes), diabetic neuropathy (nerves) and nephropathy (kidney damage) and also includes heart risks.

TYPE 2 DIABETES:

This occurs due to inadequate compensatory of insulin secretory response. It is the most prevalent type of diabetes mellitus. It is called maturity onset of diabetes mellitus because it starts with adulthood. It is also known non-insulin dependent diabetes. It causes major health disturbances especially in blood vessels that are smaller in size in the body, kidney, nerves and eyes and also heart diseases. In this type the pancreas generally produces some insulin, but it is not sufficient for the body needs or body cells and are resistant to body, so it is called as insulin resistance (lack of sensitivity to insulin). Obesity is the main risk factor for cause of diabetes mellitus.

GESTATIONAL DIABETES (GD):

DM that occurs during pregnancy is called as GD. GD is usually diagnosed in second or third trimesters of pregnancy, since high blood sugar levels in mother are being circulated through placenta to foetus. GD is necessary to be regulated inorder to save the foetal development and growth. During

method of adding bridging liquid, agitation speed, temperature, to obtain highest yield of spherical particles/crystals.

1.3.1. Advantages of Spherical Agglomeration

1. Flow properties are improved and compression characteristics of the drug which can be directly compressed into tablet and micromeritic property of the drug crystals can be drastically improved which include changes in the size, shape, crystal habit and porosity of drug.

2. The bioavailability of hydrophobic drugs is enhanced with the help of SA, crystalline form is converted into other polymorphic forms that have higher solubility and hence resulted in better bioavailability and this technique can be employed for masking the taste of bitter drugs (ATH &Enoxacin)

1.3.2. Applications

1. By this technique dissolution, solubility, bioavailability of poorly soluble drugs can be enhanced,Flowability and compressibility of drug can be improved by this technique and taste masking of bitter drugs.

2. Used in preparation of other novel drug delivery system like micro particle, micro sponges, micro balloons, micro pellets and also in controlled drug delivery systems.

pregnancy the action of insulin is influenced by hormonal changes, which simultaneously increases glucose levels in the body

DIAGNOSIS:

- Fasting plasma glucose test:

  Normal fasting blood sugar level 70 – 100 mg per dl

  FPGL is 126 mg/dl or high is known as diabetes

- Oral glucose tolerance test:

  A liquid which is containing 75 grams of glucose is taken by a person and the blood sugar level is above 200 mg/dl is diagnosed as diabetes.

- Hemoglobin A1C (glycohaemoglobin) test:

  It is used to measure the average glucose levels in blood for a duration of 60-90 days. The Hemoglobin A1C level is above 6.5% then the person is diagnosed as diabetes.

INSULIN:

Insulin is a hormone that is secreted from pancreas situated behind and below the stomach and helps in body metabolism and controlling the blood sugar levels, pancreas secreted insulin into the blood stream. Insulin circulates allowing sugar enter into the blood stream. Insulin decreases the amount of sugar in blood circulation. Insulin regulates fat and glucose use and storage in body.

It controls blood glucose level by taking glucose which is stored in liver muscle and fat cells as glycogen, liver can store up to 5% of its mass as glycogen.

1.3 SPHERICAL AGGLOMERATION

Spherical agglomeration is a unique procedure that increases particle size in which fine crystal formed by different crystallization method are aggregated with the help of bridging liquid to form the spherical crystals and they possess improved the micromeritic properties of powder. The spherical agglomerates can be then directly compressed into tablets. This method was first prepared by Kawashima et al. in 1986. SC is the more effective method as it involves less labour cost, processing time is less, low energy consumption is required, less equipment are required and also less space is required and it saves time and reduces economic risk. The particle design technology is used in industrial sectors to improve the 1^{o} and 2^{o} characteristics of particles. (1^{o} properties like crystal form, crystal habit, particle shape, size, crystal density and porosity. 2^{o} properties like flow ability, compressibility, packability, compatibility reduction in air entrapment, wettability and flow). Spherical agglomeration technique is also preferred to improve the solubility, bioavailability and dissolution of poorly soluble and poorly compressible drugs. This process is simple and requires consideration of drug solubility and agglomerating conditions. Parameters which are to be optimized for SA method are amount and

Chapter II

2.1 Review of literature

JyothiThati *et al.*, has performed Spherical agglomerates of benzoic acid have now been successfully prepared by semi-batch, agitated vessel, drowning-out crystallization in water–ethanol–toluene mixtures. With increasing feeding rate the particle size decreases and the fracture force increases the morphology remains unchanged. Using toluene since the bridging liquid contributes to improved product properties rather than chloroform. To reveal the mechanisms of the synthesis of the agglomerates, experiments have been already performed. The outcomes show that the properties of the particles change, gradually but substantially over the length of the process. Particle size and number increases alongside increasing feed. The spherical shape develops gradually but does not appear immediately, and is shown to be quite definitely the consequence of the agitation of the slurry.

Mudit Dixit *et al.*, conducted their study on Ketoprofen, which is an anti-inflammatory drug that exhibits poor water solubility and flow properties. By the neutralization method Spherical agglomerates were prepared. Crystallization medium useful for spherical agglomerates of ketoprofen contained 1 M Sodium hydroxide; 0.25 M hydrochloric acid; chloroform (bridging liquid) in the ratio of 20:55:25, respectively. By differential scanning calorimetry, Infrared spectroscopy, X-ray diffractometry and scanning electron microscopy the spherical agglomerates were characterized. Micromeritic and dissolution behaviour studies were carried out. Process variables such as quantity of bridging liquid, stirring time and duration of stirring were optimized. The spherical agglomerates of dissolution profile was compared with pure sample and recrystallized sample. By direct compression and evaluated for tablet properties Tablets were prepared using spherical agglomerates. Decreased crystalline and improved micromeritic properties were exhibited by spherical agglomerates. The dissolution of the spherical agglomerates was improved compared with pure sample. The dissolution profiles of ketoprofen tablets prepared using spherical agglomerates exhibit greater dissolution behaviour than tablets prepared by powder raw material.

SarfarazMd *et al.*, researched on aceclofenac-disintegrant agglomerates with improved solubility, flow and compression characteristics by a novel crystallo-co-agglomeration (CCA) technique. By using a three solvent system comprising of acetone: DCM: water, Aceclofenac agglomerates were prepared. Acetone-water containing PEG 6000, HPC and disintegrants like sodium starch glycolate (SSG), crospovidone (CP) and croscarmellose sodium (CCS) in different concentrations were used since the crystallization medium. The agglomerates were characterized by FTIR, DSC, PXRD, SEM studies and were evaluated for flow, packing and tableting properties and drug release. DSC and XRPD studies indicated that aceclofenac particles, crystallized in the presence of HPC, PEG 6000 and

disintegrant didn't undergo structural modifications. The dissolution rate of aceclofenac from the agglomerates could possibly be controlled by the quantity of included disintegrant, being enhanced since the latter was increased. Among most of the formulations studied, F-9 prepared by incorporation of CP (18.43%) had shown short disintegration time (18.03 sec) and maximum drug release.

A.R. Tapas*et al.,* prepared Felodipine tablets by quasi emulsion solvent diffusion technique to enhance the dissolution rate of felodipine using spherical agglomeration technique with acetone, water and dichloromethane nearly as good solvent, poor solvent and bridging liquid, respectively. In agglomeration process Inutec SP1 was used as an emulsion stabilizer and as hydrophilic polymer. After crystallization process The FTIR and DSC results showed no change in the drug. Sharp peaks in the diffractograms of spherical agglomerates with minor reduction in height of the peaks were showed by PXRD studies. Enhanced solubility in comparison to untreated powder possibly because of the partial conversion to amorphous form was showed by Spherical agglomerates.

AlladiSaritha *et al.,* prepared meloxicam tablets by using spherical agglomeration technique. The aim of their study is to boost the dissolution rate and to transform the meloxicam crystals in to spherical agglomerates. Conventional crystals have lesser dissolution rates when compared to spherical agglomerates of meloxicam. By differential scanning calorimetry, Infrared spectroscopy, X-ray diffractometry and scanning electron microscopy characterizes the Spherical agglomerates. The DSC results indicated that decline in melting enthalpy related to disorder in the crystalline content. The changes in crystallinity showed by XRD studies. And the IR spectroscopy revealed that there is no pure drug without impurities. The optimized spherical agglomerates provide rapid anti-inflammatory activity that was revealed by the pharmacodynamic activity.

Sachin kumarpatil *et al.,* using methanol, chloroform and water nearly as good solvent, bridging liquid and poor solvent, agglomerates were prepared. Direct compressible tablets of the agglomerates showed appropriate hardness, friability, and weight variation and disintegration time with improved drug release than conventional marketed tablets. Pharmacokinetic study indicated rapid absorption with higher bioavailability of the drug from the prepared tablets of agglomerates than marketed tablet (Glyburide; Sandoz). Hence, the tablets prepared with the agglomerates of Glibenclamide may reduce the sum total dose of drug and could improve the patient compliance by reducing the dose-related side effects.

2.2 AIM &OBJECTIVE OF THE RESEARCH WORK

AIM

The aim of the current investigation is to prepare, characterize and evaluate spherical agglomerates of Anti diabetic drugs with increased bioavailability characteristics prepared by direct compression method and also a comparative evaluation with marketed tablets for its evaluation.

OBJECTIVE

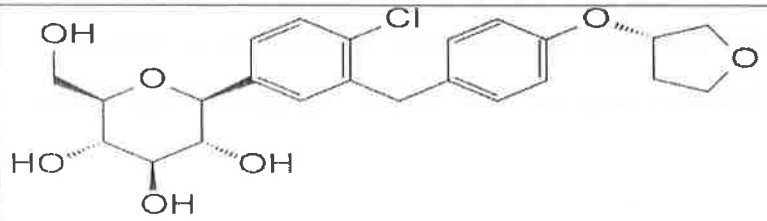
The objective of the current research work embodies

- ✓ Preparation and formulation of selected drug candidates (Glipizide&Empagliflozin) using spherical agglomeration technique.
- ✓ To characterize the prepared formulations for DSC studies, XRD studies, FT-IR studies and SEM
- ✓ To evaluate the potentiality of different polymers used in the formulations for its dissolution criteria, bioavailability and other parameters
- ✓ To optimize the formulation for its desired release profile both in *in-vitro* and *in-vivo*

III. Drug & Excipient profile

3.1Drug Profile

Table: Empagliflozin

Structure	
Trade Name	Jardiance
Chemical Name	D Glucitol 1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetra hydro 3furanyl]oxy]methyl]phenyl]
CAS number	864070-44-0
Molecular Formula	$C_{23}H_{27}CLO_7$
Molecular Weight	450.912gm/mol
IUPAC name	(2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol
Therapeutic Category	It belongs to SGLT2 inhibitors. It comes under the category of Class –III drugs in BCS classification
Description	*It is* non-hygroscopic powder and white to yellowish in colour.
Solubility	It is soluble in organic solvents, slightly *soluble* in water, sparingly *soluble* in methanol, slightly *soluble* in ethanol and acetonitrile; *soluble* in 50% acetonitrile/water; and insoluble in toluene.
Mechanism	Empagliflozin is a SGLT-2 inhibitor. For reabsorption of glucose from kidneys (Glomerular filtrate) SGLT2 co-transporters are responsible. The glucuretic effect resulting from SGLT2 inhibition decreases renal absorption and lowers the renal threshold for glucose which results in increased glucose excretion
Dose and dosage forms	10mg, 25mg in morning with or without food Tablet, Film coated

plasma concentration, Volume of distribution, Plasma protien binding, Metabolism, Elimination	1.5 hours 73.8L 86.2% protein bound Metabolized by 3 metabolites are 2-O-, 3-O- and 6-O-Glucronide. 41.2% eliminated in faeces & 54.4% eliminated in urine.
Biological Half life	5.6-12.4 h
Storage	It is stored at room temperature and away from heat and moisture.

3.2 Excipient profile

3.2.1Caesalpiniaspinosa

Caesalpiniaspinosa is a small leguminous tree or thorny shrub which is native to Peru. It is commonly called Tara, the scientific name for Tara spinosa was Caesalpiniaspinosa. It is specially cultivated for increased source of tannins, which is based on a galloylatedquinic acid structure.

TABLE: CAESALPINIA SPINOSA

Name	*Caesalpiniaspinosa*
Common names	Tara, Spiny hold back, Taya, algarroba tanino
Kingdom	Plantae
Order	Fabales
Family	Fabaceae
Genus	Tara
Species	*Tara spinosa, Caesalpiniaspinosa.*
Description	*T. spinosa* grows at a height of 2–5 m tall and its bark has scattered prickles which is dark grey and contain twigs that are hairy
Viscosity	5,500 cps , up to 145 °C (can withstand high temp conditions)
Uses	Used in controlled release medications, food additives

Other polymers like HPMCK100M, Ethyl cellulose and Sodium alginate were also used in different formulations.

IV.Methodology

Plan of Work

To achieve the above mentioned aim and objective of the research work, the plan has been divided into various distinct phases

Phase I- Review of Literature

Phase II- Collection of plant for extracting natural polymer and Identification and sourcing of raw materials

Phase III- Design, Development and Characterization

Phase IV- Performing evaluation for the prepared batches both *in vitro* and *in vivo*.

Table:1List ofequipment

S.No.	Nameoftheequipment	Model
1.	ElectronicweighingBalance	CWS602
2.	Dissolution testapparatus	Electrolab TDT08L
3.	Friabilator	Electrolab
4.	Compressionmachine	CadmachYAW-300
5.	Stabilitychamber	VS511
6.	Tablethardnesstester	MonsantohardnesstesterSHT-17
7.	Ultra violetspectrophotometer	Labindia3000+
8.	Differential scanningcalorimetry	DSC50
9.	X-raydiffraction	BrukerD5005
10.	Fouriertransformer infra-red	FTIR-8033

	spectrophotometer	
11.	Verniercalipers	Cd-6"Cs
12.	pHmeter	pHCal10
13.	Highperformanceliquidchromatography	--
14.	Tapped densitymeter	ETD-1020

Table4.2:Listof chemicals

S.No.	nameofthechemical	Grade/variety	Manufacturername
1.	Ethylcellulose	Rand D grade	BASF
2.	Sodiumalginate	Rand D grade	BASF
3.	Hydroxy propyl methylcellulose	Rand D grade	BASF
4.	Magnesiumstearate	Technicalgrade	Sigma-Aldrich
5.	Water	HPLC	Merck
6.	Acetone	HPLC	BRUCE
7.	Chloroform	Analyticalgrade	Sigma-Aldrich
8.	Methanol	HPLC	Sigma-Aldrich
9.	Dichloromethane	Analyticalgrade	Merck

4.1 Extractionandcharacterizationofnaturalpolymer

4.1.1. Extractionofnaturalpolymer

Fig1: ***Caesalpiniaspinosa*****plant**

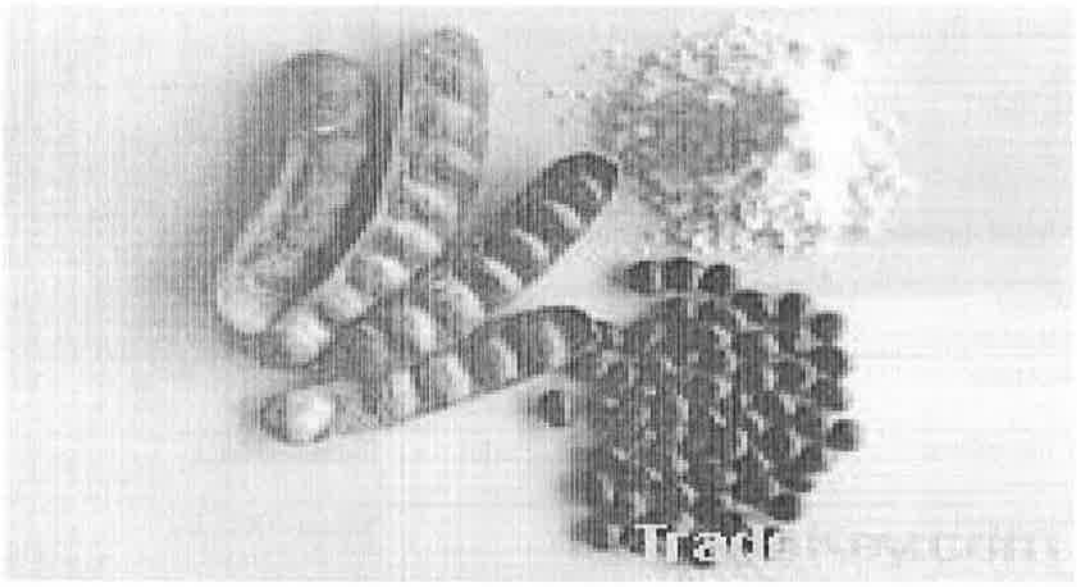


Fig2: ***Caesalpiniaspinosa*****seeds**

4.1.1.a. Collectionandauthenticationofplants

The seeds of C*aesalpeniaspinosa*were collected from in and around areas ofNelloredistrict.TheplantswereauthenticatedbyProf.K.MadhavaChetty,department of botany, SV University, Tirupathi, Chittoor district, Andhra Pradesh,Indiaand seedsspecimensampleswere keptinthe laboratoryforfurtheruse.

4.1.1. b.Extractionofnaturalmucoadhesivematerials[38]

500mgofseedswerecollected andwashed thoroughlywithwaterand dried

⇩

Driedseedsweresoakedindistilledwater(2500ml)for 24hrs

⇩

Boiled for one hour with continuous stirring at 2000rpm and then kept aside in order to releasethenaturalgumintowater

⇩

Seedsweresqueezedusingmuslinclothandmarcwasseparatedunder vacuumat60^{0}C

⇩

Acetone was added to precipitate the natural mucoadhesive materials-separatedbyfiltrationanddriedat60^{0}C inhotairoven

⇩

Thematerialwaspassed throughsieveno.100 andstored at roomtemperature

Fig3:Flowchartpresentationofextractionof naturalpolymer-CSNMM

4.1.1.c. Percentageyield ofnaturalmaterial

Itwascalculatedfromtheratiobetweentheinitialweightandthefinalweightofthe extractedMMM.ThepercentageyieldofCS-NMMis15%w/w.

4.1.2. GeneralcharacterizationofCS-NMM.

4.1.2.a. Identificationtestsforhydrocolloid

- A small amount of extracted material was mixed with ruthenium red anda cover slip was placed on its top. After a few seconds, lead acetatesolution was added. A blotting paper was used to remove the excessamountofstrain.Hydrocolloidwasstainedpinkcolour.
- A small amount of extracted material was mixed with corallin soda andwasplacedonaglassslide.25%sodiumcarbonatesolutionwasaddedto it and a cover slip was placed on it. Hydrocolloid was stained pinkcolour.
- A small amount of extracted material was mixed with distilled water andboiledforfewminutes.Aftercoolingagelatinousmasswasobserved.[79]

4.1.2.b. PhytochemicalconstituentsinCS-NMM-identification

The extracted material was subjected to qualitative chemical analysis by using astandardprocedure.[80,81]Theresults areshown intable5.1.

4.1.2.c. DeterminationofpHofextractedmucoadhesivematerial

OnegramoftheextractedmaterialofCS-NMMwasdissolvedin100mldistilledwaterandtheirpHwasmeasuredusingadigitalpHmeter.[80,81]

4.1.2.d. DeterminationofswellingindexofCS-NMM

Procedure:CS-NMM(200mg),wasplacedonapetri-dishand10mlofdistilled water was added and the mixture was shaked vigorously at 10min for 1hr. Itwas allowed to stand for 3 hours at room temperature. In between the process, at aninterval of every 1 hour the remaining water in Petri-dish was discarded and theincrease in weight of the CS-NMM was noted. The weight increase was observedbecause of the sticky hydrogel property of the material extracted.[82]The procedure wasrepeated for three times and mean value was noted. The swelling index was calculatedfortheCS-NMMinwater,atpH1.2and7.4.Thereport ismentionedintable5.2.

4.1.2. e.Meltingpoint determinationofCS-NMM

Afinely powderedmaterial wasfilledintoonecapillary tubewhich wassealed onsingleside.Itisintroducedintoameltingpointapparatus.[82]

4.1.2.f. Determinationofviscosityof1%w/vCS-NMM.

1% w/v aqueous solution of the extract was prepared. The viscosity of thesolution was measured at different temperatures using ostwald's viscometer. [82] Theresultswere depictedintable 5.3.

Viscosity(η_1)= $\eta_2 \times (d_1t_1/d_2t_2)$

Where, η_1 =Viscosity of natural mucoadhesive material solution, η_2= Viscosity ofwater, d_2= Density of water, d_1= Density of sample solution, t_2 = Time of flow ofwater(sec),t_1=Timeofflowofsamplesolution.

4.1.2. g.Fouriertransforminfraredspectroscopy(FTIR)

IR was recorded by KBr samples of CS-NMM the frequency range 4000-400cm^{-1} using a Shimadzu,model 8033(USA).[83] FTIR spectrum was shown asfig. 5.1andinterpretationofdatawas explainedinthe table5.4.

4.1.2.h. Differentialscanningcalorimetry(DSC)

DSCwasperformedbytaking10mgofthe samplebyusingDSC-50Shimadzuautomaticthermalanalyzer.[84]DSCspectrumismentionedinfig.5.2

4.1.2.i. SolubilitystudiesofCS-NMM

The solubility study was conducted by using different solvents. The results ofsolubilitystudiesis shownintable5.5

4.1.2.j. Nuclearmagneticresonance

^{13}CNMR

^{13}C-NMR spectrum was recorded using Bruker II 600 spectrometer in 2%deuterated acetic acid in D_2O solution for CS-NMM. The measured NMR spectrumwas interpreted and reported to confirm the presence of polysaccharides.[85]The^{13}C -NMRspectrumofCS-NMMisshowninfig.5.3.

^{1}HNMR

^{1}H-NMRspectrumwasrecordedusingBrukerAC250tecmagDSPect(modified)fourier transform NMRspectrometer at400.13 MHzin 1Mmethanolsolution at 32°C.NMR spectrum was interpreted to calculate the no. of protons usingchemicalshiftvalue(PPM)i.e.thechemicalshiftingrange.[86,87] The^{1}H-NMRspectrumofCS-NMMis showninfig.5.4.

4.1.3. Antimicrobialactivity

The extract of *Caesalpiniaspinosa*was tested for its antimicrobial effect against somecommon microorganisms and for growth promoting properties.The sterility test wasperformed as per Indian Pharmacopoeia. Sterility test was done in order to detect thepresenceofagainst Staphylococcusaureusinthepreparedextract.Asimpleprocedure like agar disc diffusion technique is employed to perform sterility test. Aspecified quantity of sample was incubated aseptically at 250°C and 45%RH usingautoclave at 121°C for 15 min; then allowed to cool to 45°C before pouring into theagar plate. The pH of the agarmedium wasmaintained at 7.4. The stock solution ofthe extract was prepared on each occasion by careful weighing and dissolving insuitable volume of dimethyl sulphoxide (DMSO) to get a concentration of 100 mg/ml.A tablet of tetracycline was dissolvedin appropriate volume of water to get5 mg/mlofstocksolutionandthegrowthofmicroorganismsinthemediumwascheckedfor

14 days and sterilizedThe plates were left at room temperature for solidification.Each plate, a single well of 6 mm diameter was made using a sterile borer. Theextracts were freshly reconstituted with suitable solvents (dimethyl sulphoxide) andtested at various concentrations. The samples were placed in 6-mm diameter well.Antibacterial assay plates were incubated at 37±2°C for 24 h, standard disc (6 mmdiameter) with cephalosporin (5µg/ml) was used as a positive control for antibacterialactivity plates were kept in laminar flow for 30 minutes for pre diffusion of extract tooccur and then incubated at 37°C for 24 hours. Resulting zone of inhibition wasmeasured at the end of incubation period to clarify the presence of microbial growth.Positive controls were equally set up by using solvents and test organisms withoutextracts. The tube with least concentration of extract without growth after incubationwastakenandrecordedastheminimuminhibitoryconcentration

4.1.4. Stabilitytestingstudies

The physical and chemical stability of a producthavebeen tested under definedstorage conditions and the shelf-life was established. Quality of crude drug materialdepends upon the content variation and stability during storage. Environmental factorssuch as temperature, light, air (specifically oxygen, carbon dioxide and water vapors)and humidity may affect stability. Similarly, factors such as particle size, pH, theproperties of water and other solvents employed results from can influence stability.Hence an accelerated stability studies were performed as per ICH guidelines for aperiod of 6 months. These studies were performed on the extract taken. The results areillustratedintable:5.7.

Acceleratedconditions:

i)at25 °C ± 2 °C/60% RH ± 5% RHii)40 °C±2

°C/65%RH±5%RH

iii)40°C±2 °C/75%RH ±5%RH

iv)8°C±2 °C/60%RH ±5%RH foraperiodof6months

4.2 Preliminarystudiesforthepreparationofsphericalagglomerates

In spherical crystallization method as reported by Kwashima et al., [17]was chosen forpreparationofsphericalagglomerates bydirectcompressionmethod.

Theflowdiagramofthemethodisoutlinedbelow:

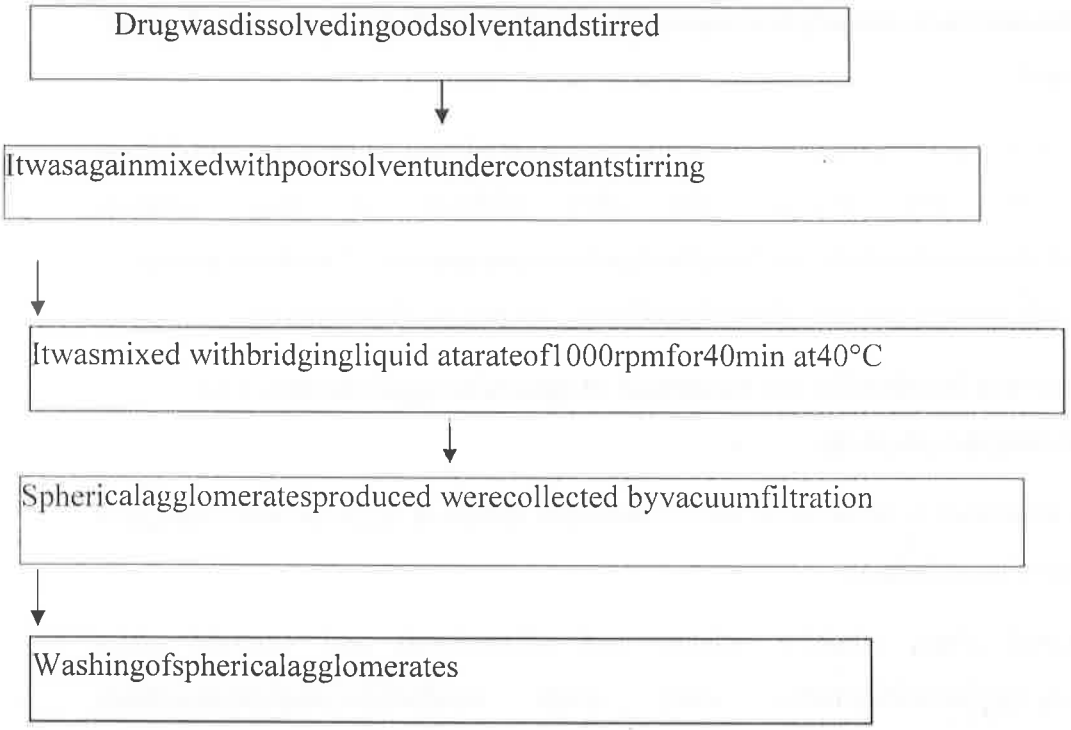


Fig4: Flowdiagramforthepreparation sphericalagglomerates

In this method, generally a good solvent is required to dissolve the drug. Bridgingliquid helps in collecting the crystals that are suspended within the system that formsbridges among the liquid interface among the crystals because of capillary negativepressure and interfacial tension that is exerted between the interface of solid andliquid. A poor solvent is required for precipitation of drug. Hence, preliminary trialsweretaken asfollowsforselection of agood solvent,bridgingliquidandpoorsolvent.

4.2.1 Selectionofbridgingliquid,poorsolvent and goodsolvent

The solubility of the drugs was determined in different solvents by adding 100 mg ofdrug to 1 ml of solvent with stirring. The list of solvents used for studying solubility isshownintableno.5.7alongwiththe solubilityobservations.

4.2.2.Selectionofbridgingliquids:

Thebridgingliquidmustbemiscible withthegoodsolventselected–dichloromethane. Hence, miscibility studies of dichloromethane was studied with n-hexane,chloroformandglycerineinproportionsof1:1,1:2,1:3,1:4and1:5respectively.

4.2.3 Preliminaryselectionofprocessparametersaffectingtheformationofspherical agglomerates

Various factors affecting this process are stirring rate, type of non-solvent, rate ofaddition of drug solution and stirring time after addition of drug solution. Hence,differenttrialsweretakenforidentifyingthesignificantparameters.[21]Theobservations with respect to each process are also shown in table no. 5.9 and 5.10respectively.

4.2.4 Mechanism involved in the formation of spherical agglomerates and factorsaffectingthesphericity

The following mechanism is involved in the formation of spherical agglomeratesthroughthe sphericalcrystallizationtechnique.

- Internal phase (dichloromethane and chloroform) and external phase (water)aremiscible,butfor avery small timebeforecompletemiscibility takesplace,internalphasemayexistas globules.
- Diffusion of for dichloromethane and chloroform may take place from theglobules into water. In addition, diffusion of water can take place from theexternal phase into globules. Both diffusion processes may be responsible forthe formationofdrugparticlesduetoanti-solventeffect.
- The spherical shape of particles may be due to formation of particles withinglobuleswhicharegenerallysphericalinnature.
- The degree of sphericity may be influenced by the life span of the globule.Longer the life span of globule,more sphericalmay be the agglomerates.Timedurationforwhichtheinternalphaseexistasglobules,maybeinf luencedbyitsviscosity.Astheviscosityincreases,lifespanoftheglobules increases. Chloroform in the internal phase helps in increasing theviscosityoftheinternalphase.[24]

4.3 Preparationandcharacterizationofsphericalagglomerates

4.3.1 Preparationofsphericalagglomerates

Agglomerates of drug were preparedby spherical crystallization method.10mg drug was tend to get dissolved in dichloromethane. Chloroform is added to thesolutioncontainingdrugandthoroughlymixed.Themixtureofdrug,dichloromethaneandchlo roformwasaddedatarateof1ml/mintowaterstirredata

rate of 1000 rpm using magnetic stirrer. Stirring was continued for a period of 40minutes after complete addition of mixture. The particles of drug obtainedin thewater were separated by vacuum filtration and dried at 40°C. Particles were washedwith water (25 ml each 3 times) to make them free from solvents. The agglomeratesobtainedwerefreeflowingandofsphericalinshape.[24]

4.3.2 Characterizationofsphericalagglomerates

The prepared spherical agglomerates of drugs were characterized[33] as per the detailsmentionedbelow.

i) DSCstudy

A DSC study was performed inorder to detect polymorphic transition and thermalproperties of the drug while crystallization occurs. These changes can be measured byusing thermal analyser. About 3-5mg of drug was mounted on aluminium sealed pans,andtemperaturewasfixedfrom25°C-250°C/min,undernitrogenfixedenvironment. The DSC apparatus must be calibrated using Indium which is a puremetal,beforeeverystudy[33,88].

ii) FT-IRspectroscopy

The FT-IR spectra were measured using a Shimadzu, model 8033(USA) which ismaintained at ambient temperature. The drug samples that should be detected weredispersedinKBrpowderandpelletsweremadebyapplying5tonpressure[33,89].

iii) X-Raydiffractionstudies

X-Ray powder diffraction studies were analyzed at room temperature using Brukerdiffractometer,whereCuactsasanodematerialandgraphitemonochromator,whichis operated ata voltage of 40mA,45kV.Itis used todetermine the conversions ofstate i.e. from crystalline to amorphous forms and interactions between drug andexcipientsusedarenotedifany.[33]

iv) Scanningelectronmicroscopy(SEM)

SEM(Shimadzu-LV-5600,USA,withmagnificationof 250X)photographswereconsideredin ordertoconfirmsphericalnature
andalsotomeasurethesurfacetopographyofthepreparedcrystals[90].

4.3.3.Preformulationstudies

These studies are essential for determining chemical and physical characteristics ofdrug substances along with its combination of other excipients.It is the preliminarystep to develop any of the desired dosage forms. With the help of thispreformulationparameters,wecanassessthenatureand characterofthedrugand itsrelease.

Someofthepreformulationstudiesarelistedbelow:

Organoleptic evaluation, particle size distribution, bulk and tapped density, diameter,carr'sindex,hausnersratio,angleofrepose,drug–excipientcompatibilitystudies[91].

4.3.4 Organolepticcharacteristics:

This can be evaluated by color, odor, taste, and elegance of the tablet, its size andshape, surface texture, unique identification marks etc were usually done by physicalexamination.The resultsweregivenintable 4.3.

Table:4.Organolepticpropertiesofpreparedtabletsofandempagliflozin

S no.	Properties	Observation
1.	Description	Roundinshape
2.	Colour	White
3.	Taste	Tasteless
4.	Odour	Odourless
5.	Elegance andsurfacetextur e	Smooth

4.3.5 Flowproperties

i. (Angleofrepose):

It is defined as maximum possible angle obtained between surface of pile of powderand the horizontal plane.[92,93] Through fixed funnel method angle of repose can bedetermined. Funnel is fixed at a specific height.(2.5cm) on to a burette stand. Thesample of powder is allowed to pass through the funnel allowing it to form a pile. Nomoregranulesareaddedwhenthepilereachestheedgeofthefunnel.Thisregionis

encircledtomeasureradius.Thesameprocedureisrepeatedforthreemoretimesandtheaverageval ueisnoted.It canbe calculated byusing thefollowing equation

Angle of repose (ɵ) =Tan^{-1}

(h/r)Where,h=heightofpiler=radiusof

thebaseofthepileɵ=angleofrepose

Table:4Angleofreposeandcorrespondingflowproperties

Angleofrepose	Flowproperty
<25	Excellent
25-30	Good
30-40	Passable
>40	VeryPoor

Determinationofbulkdensity:

Sufficient amount of powder (W) is weighed and is poured into a measuring cylinderwhich is graduated and volume (V_0) can be measured along with bulk density byfollowingformula[92],

Bulk density(BD)=Weightofthepowder/volume ofpowder

Tappeddensitydetermination:

Sufficient amount of powder (W) is weighed and is poured into a measuring cylinderwhich was fixed to the 'tapped densitometer' and tapped for number of times (500,750 and 1250) until the variation in the volume after consecutive tappings was □ 2%which is graduated and volume (V_0). The final reading was represented by (V_f). Thetapped density, carr's index, hausner's ratio were calculated using the volume ofblend.[92]

Tapped density= W/V_fg/ml

Carr'sindexorcompressibilityindex:

Carr's index is also known as compressibility. It is indirectly related to the relativeflow rate, cohesiveness and particle size. It is simple, fast and popular method ofpredictingpowderflowcharacteristics.[92,93]

Carr'sindex(%) =[(Tappeddensity-bulkdensity)/tappeddensity]X100

Table5:Carr'sindexandcorrespondingflowproperties

Carr'sindex(%)	Flowcharacter
<15	Excellent
16-18	Good
18-21	Fairtopassable
23-25	Poor
33-38	Verypoor
>40	Veryverypoor

Hausner'sratio: Theflowpropertiesof powder areindicated anditis ratiooftappeddensitytobulkdensity[94,95].

Hausner'sratio = Tapped density/bulkdensity

Table6: Limitsofhausner'sratio

Hausner'sratio	Typeofflow
<1.25	Goodflow
1.26-1.34	Passableflow
1.35-1.45	Poor
1.46-1.59	Verypoor

4.4 Finaltabletcompression(directcompression):

In this process, the optimized sustained release granules were introduced initially in tothe die cavity and later a slight pre-compression was done so as to distribute the layersuniformly.

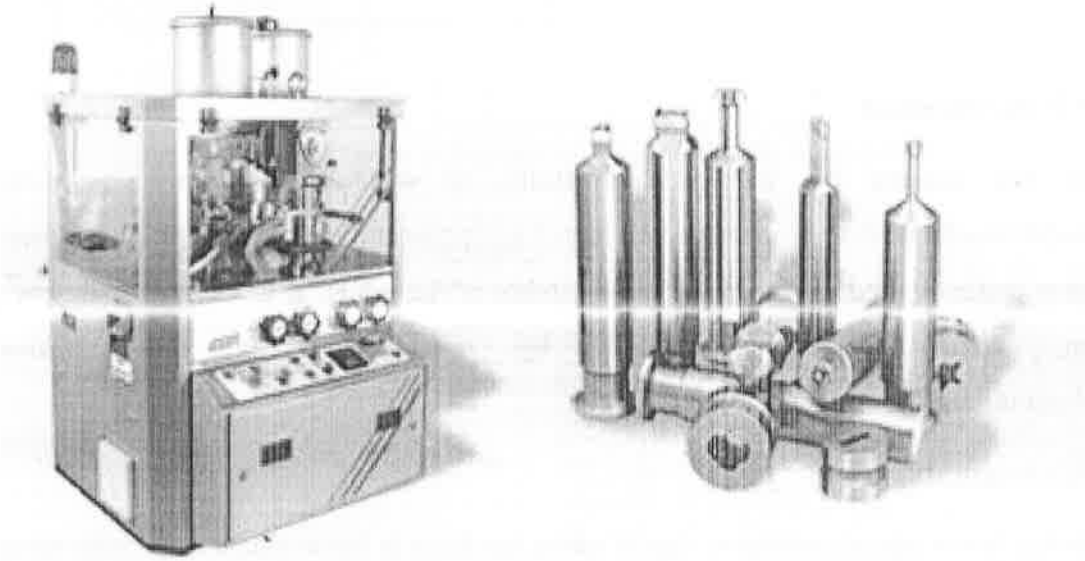


Fig5:Tabletpunchingmachine

4.5 Postcompressionstudies

Evaluationoftablets:

Theevaluationincludesthediameter,weight,shape,thickness,size,hardness,friability,floatinglo gtimeand *invitro*-dissolutioncharacters.[96,97,98,99]

4.5.1 Weight variation: The tablet weight was determined to verify that a tabletwhich is being weighed is according to the prescribed criteria.20 tablets were weighedand average weight was calculated to its individual weight as per the USP weightvariationtestprocedures.TheweighedtabletsshouldmeettheUSPlimitsnotexceedin g more than 2 tablets of its individual weights and no single tablet shoulddiffer by more than 2 times the percentage limit. USP limits of % deviation of tabletare shownbelow.

Table7:Limitsfortabletweightvariationtest (USP29-NF34)

Averageweightoftablet(mg)	%Differencepermitted
130orless	±10%
From130–324	±7.5%
> 324	±5%

4.5.2 Hardnesstest

Hardness test ensures the stability and ability to withhold the stress or shear strengthwhilemechanicalshocksoccurredwhilepackaging,handlingandtransportation.Monsanto hardness tester is used to determine the hardness of the tablet. It is denotedby kg/cm^2. Themean values were determined by considering 3 tablets that werepickedrandomlyfromthebatch.

4.5.3 Friability

The friability test is almost similar to that of tablet hardness in the evaluation ofwithholding capability of the tablet prepared. Roche friabilator is the instrument usedfor determine friability.[100]The tablets were weighed and kept in the apparatus andwere subjected to rotation atan rpm of 100. The tablets were weighed after therevolutionsandcomparedtoitsinitialweight.Friabilityisexpressedin%.

Limits: weight loss of not more than 1% of the original

weight.Thepercentagefriabilitywascalculated usingtheformula:

% friability = $(W_1-W_2) / W_1$ X

100W_1=Initialweight

W_2=Finalweightoftablet

iv*In-vitro* dissolution studies: The dissolution criteria used for studying the drugreleasefromthetablets:

TABLE 8:*In-vitro*dissolutionparametersforempagliflozin

Apparatus	USP 26apparatustypeII(Paddle)
Agitationspeed(rpm)	75rpm(for empagliflozin)
Medium	0.1NHCland7.5 pHphosphatebuffer
Volume	900ml
Temperature	37.0± 0.5^{0}C
Time	0.5,1,2, 4,6, 8,10and12hours
Wavelengths	276nmand272nm

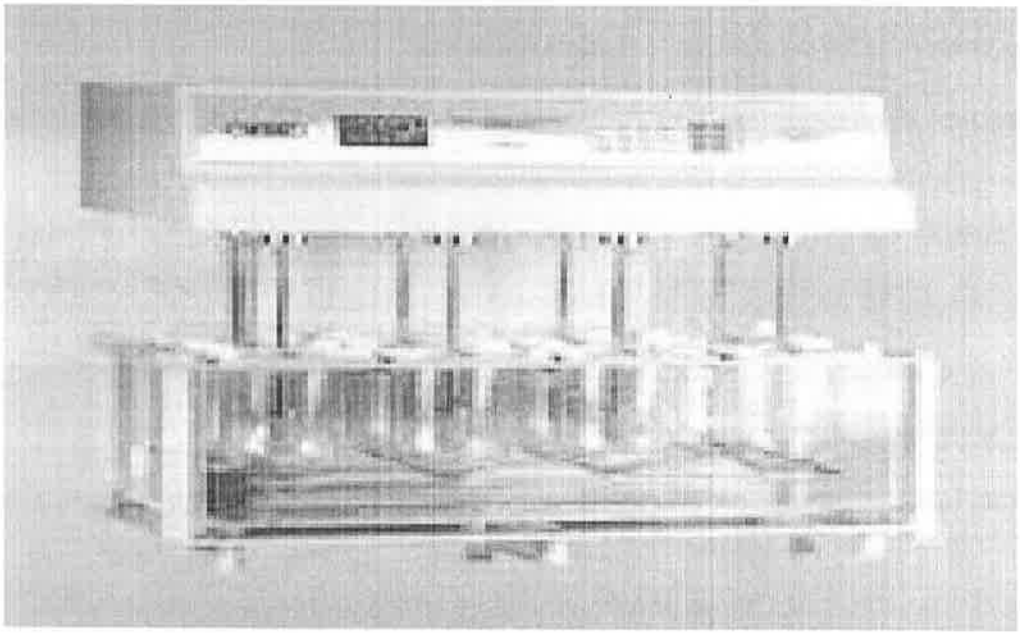


Fig6:USP26apparatustypeII(paddle)-dissolutionapparatus.

4.6 Kinetic-models

Kineticmodelsdepicts the drugreleaserate kineticsof preparedformulations withrespecttodissolutionprofile,namely,zeroorder,firstorder,andhiguchirespectively.

$Q_t=Q_0+K_0t$ ..... (1)

where,Qt-amountofdrug releasedattimet;

Q_0-amountofdruginthesolutionat t =0,(usually,Q0=0)andK_0-

zeroorderreleaseconstant.

$\log Q_t = \log Q_\alpha + (K_1/2.303)t$ .....(2)

Q_α-totalamountofdruginthematrixandK_1-

thefirstorderkineticconstant.

$Q_t = KH.t^{1/2}$ .....(3)

where,KH-Higuchirateconstant.

Further, to characterize the mechanism of drug release from matrices, dissolution datawere analyzedusingthe equationproposedbyKorsmeyerandPeppas.

$Q_{(t-l)} / Q_\alpha = KK(t-l)n$ ..... (4)

where, Q_t-theamountofdrugreleasedintimet,

l-lag time(*l*=2 hours)

Q_α-totalamountofdrugthatmustbereleasedatinfinitetime

KK - constant comprising the structural and geometric characteristics of the tablet,andn istherelease exponentindicating thetypeofdrugreleasemechanism.

To determine the exponent n, points in the release curves where $Q_{(t-l)}/Q_\alpha > 0.6$, wereonlyutilized.Ifnreachesto0.5,the releasemechanismisfickian.

If n reaches to 1, the release mechanism is zero order and on the other hand if $0.5 < n < 1$,non-fickian(anomalous)transportcanbeobtained.

Anomalous (non-fickian) transportusually refers todrug releaseby addingbothdiffusion and erosion of the polymeric matrix. The criteria suggested in the selectionof''bestmodel''wastheonewiththehighestcoefficientofdetermination(r^2)[101].

Table:9Interpretationofdiffusionalreleasemechanisms

Releaseexponent(n)	**Drugtransportmechanism**
$0.45 \leq n$	Fickiandiffusion
$0.45 < n < 0.89$	Non-fickiantransport
0.89	CaseIItransport
$n > 0.89$	Supercase IItransport

4.7 OptimizationOfFormulationParameters

Preparation of spherical agglomerates are affected by many process variables andneeds to be optimized for optimal response. The process variables like stirring speed,stirring time and rate of addition of drug solution identified on the basis of abovepreliminarytrialswerefixed andkeptconstantthroughoutthe study.

Rateofadditionofdrugsolution-

1ml/minStirringspeed-1000rpm

Stirringtimeafteradditionofentiredrugsolution– 40min

Inthepresentstudy,theindependentvariableslikevolumeof dichloromethane,volume of water and % of chloroform in dichloromethane were chosen based on theresultsobtainedfromthepreliminarystudies conducted.

4.7.1 Selection ofbestbatch

During the optimization of a multivariable process, such as spherical agglomeration,the responses were taken into consideration in order to produce a product of desiredcharacteristics.

4.8 Stabilitystudies

The selected formulation was subjected to stability studies as per ICH guidelines. ThetabletswerepackedinHDPEbottlesandwerestored at following conditions.

Table10:Conditionsofstabilitystudies

Study	Storagecondition	Minimumtimeperioddatacovered
Acceleratedconditions	40°C±2°C/75%±5%RH	4months

4.9 *In-vivo*evaluationofthepreparedtablets:

Thepharmacokineticparameterswereestimatedforthe*in-vivo*studyfortheoptimizedformulationandmarketedtabletforcomparisionofthe parameters.

Pharmacokineticparameterswereusedfordeterminationofparameterssuchasmaximumconcentrationofserum(C_{max}),time toreachthemaximumconc.of

serum(T_{max}),areaobtainedundertheplasma-concentrationtimecurve(AUC),volume of distribution (V_d), half-life ($t_{1/2}$), mean residence time (MRT) and clearance(Cl_T).

4.9.1. Groupsforthe*in-vivo* study:

*In-vivo*studywasperformed,makingfourgroupsofhealthyalbinorabbits.Eachgroupconsistsoffourr abbits (n=4).

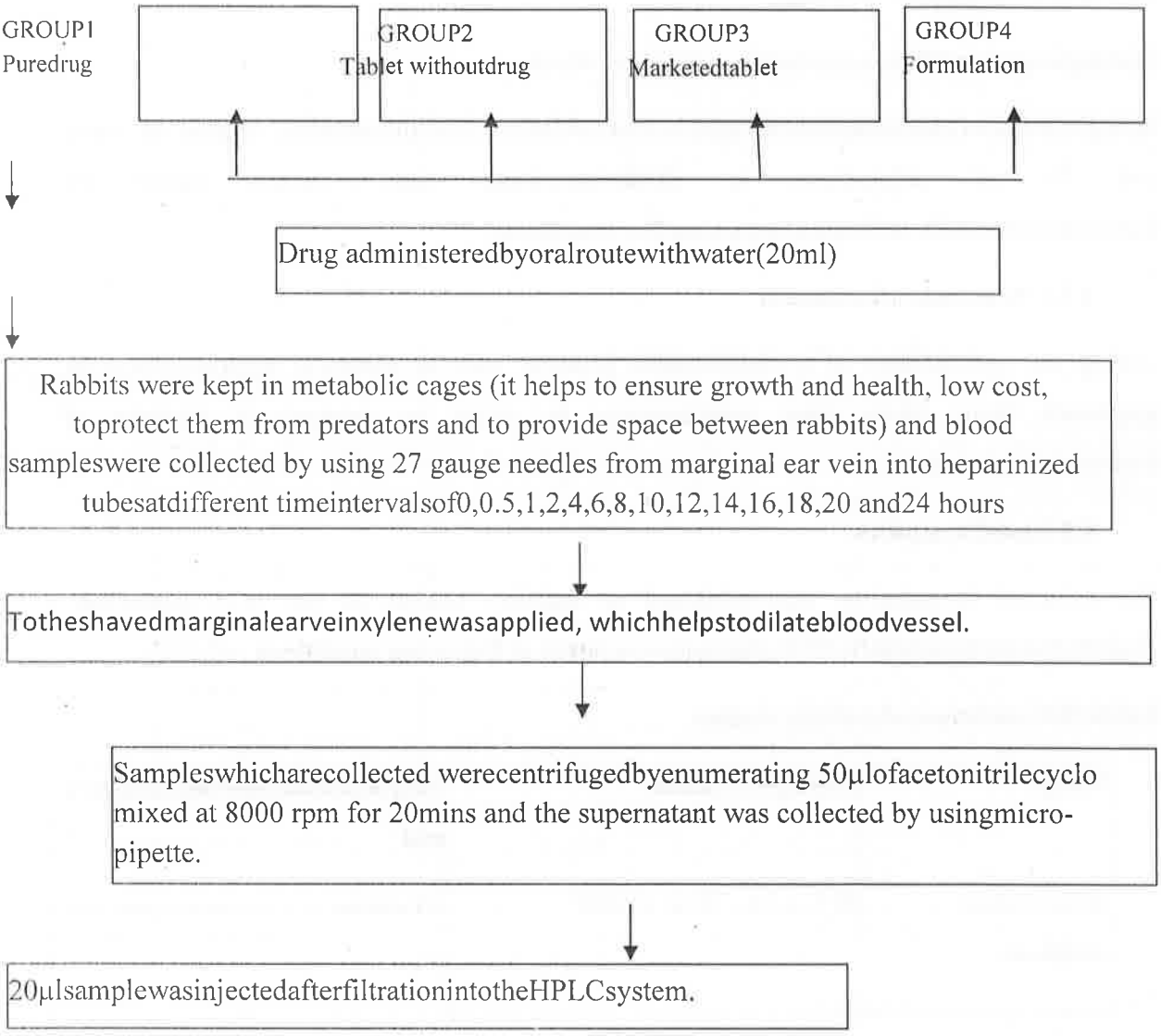


Fig7: Flowchartrepresentationofpreparationofsamplesolutions

4.9.2. Experimental methodologyHPLCanalyticalmethodd evelopment

Glipizide andempagliflozincontentpresentin the plasma was estimatedbyusingHPLCmethodanda calibrationcurvewasplotted.[107]

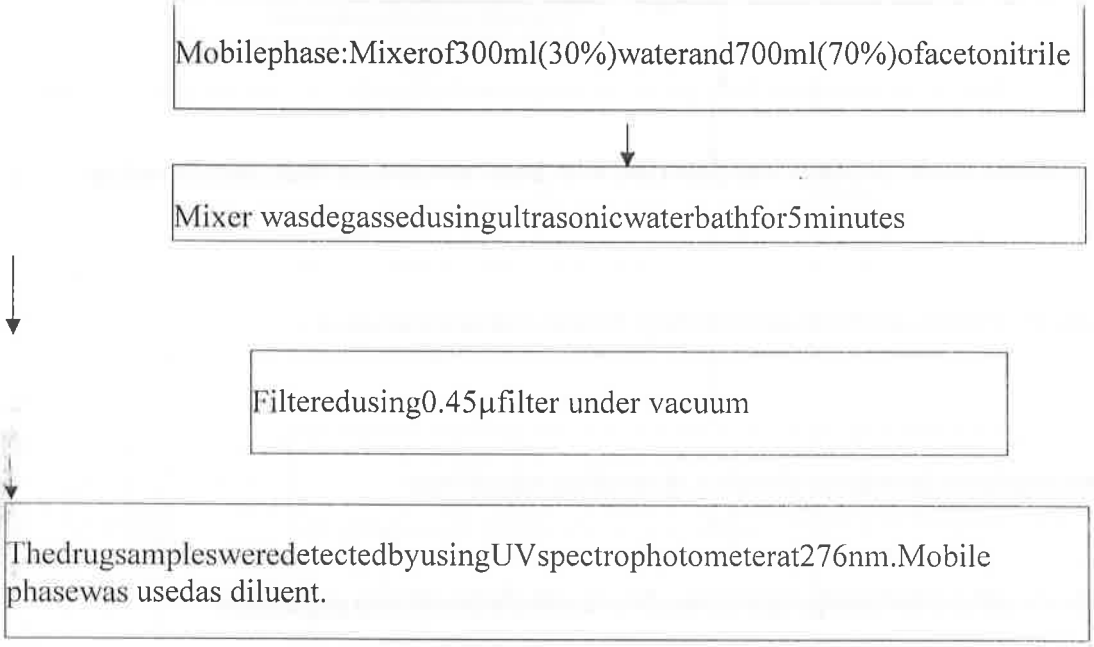


Fig8:FlowchartrepresentationofHPLCanalyticalmethoddevelopmentforglipizide

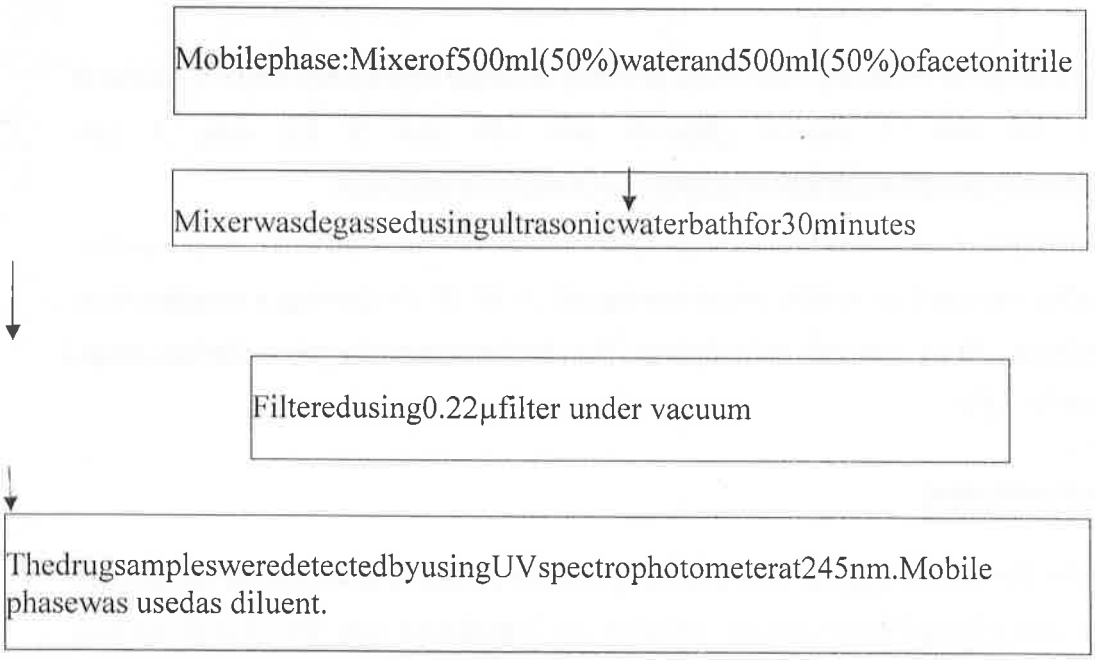


Fig9:FlowchartrepresentationofHPLCanalyticalmethoddevelopmentforempagliflozin

Preparationofstandardsolutionofempagliflozin

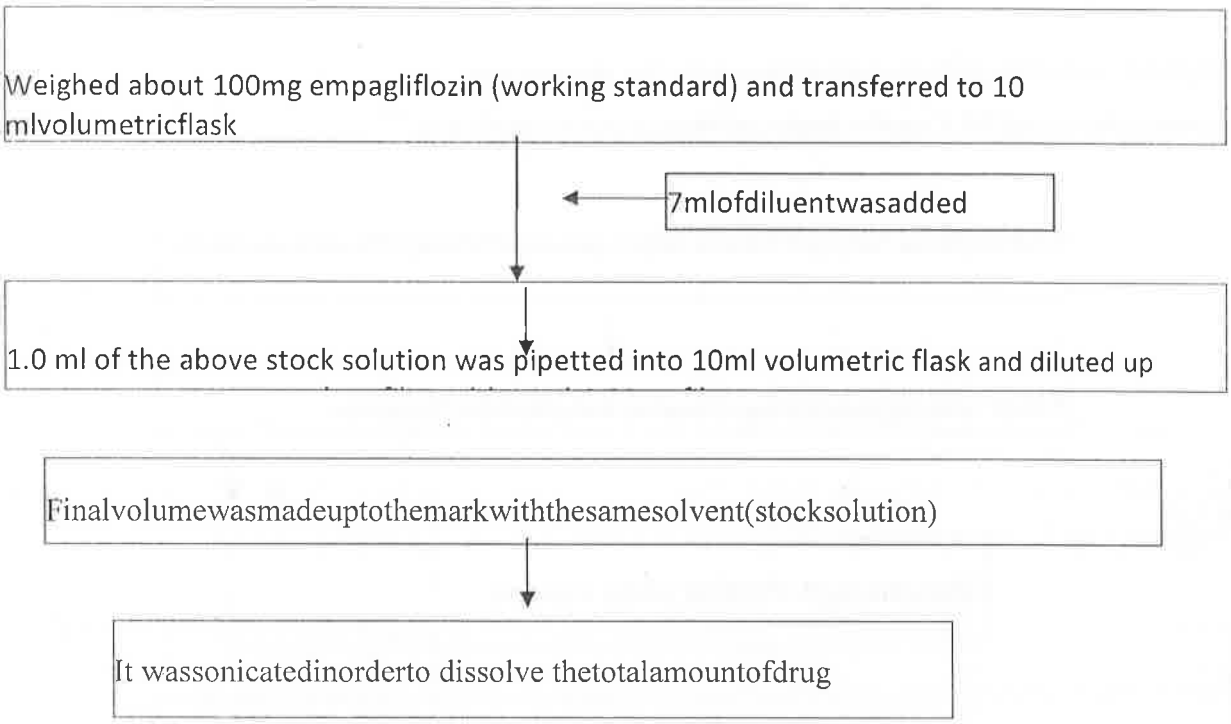


Fig.11:Flowchartrepresentationofpreparationofstandardsolutionsforempagliflozin

4.9.3. Analyticalinstrumentandmethod

HPLC (2695 seperation module), with PDA detector utilizing INERTSIL ODS C_{18}column (250 mm x 4.6 mm, 5 µm-for glipizide and 150 mm X 4.6 mm, 5 µm-forempagliflozin)reversephasechromatographyisappliedforestimationof drugs.Columntempalongwithinstrumenttempiscontrolledatnormaltemperature.Acetonitrile: phosphate buffer was used as mobile phase having pH 3 (50:50 v/v),having a constant flow rate of 1.5 ml/min, 20 µl -volume of injection. The detectionwavelengthwas245nm,temp-maintainedat20^{0}C ±2^{0}C.

HPLCmethodvalidation

10µl of sample free blank plasma and glipizide sample solution and 20µl of samplefree blank plasma and empagliflozin sample solution are introduced into the system toknow aboutthe specificity.Byinjecting 20µl solution thechromatograms weredeveloped.Thepeakareawasestimatedforeachdrugsolution.Thestandardgraph

was plotted and correlation coefficient was determined. To estimate the inter andintraday precision repeatedly, the same procedure is repeated for 6 times. Robustness,LODandLOQwere validatedsimultaneously.

4.9.4. Assesmentofpharmacokineticparameters

To assess the pharmacokinetic parameters of empagliflozin test and control, noncompartmentalmethodwaspreferredwiththehelpofThermoscientificKINETICA5.2so ftware (plasma concentrationvs.timedata).

4.9.5. Statisticalanalysis

Statistical data was analyzed using graph pad prism 6 software data. Paired t-testwas usedforfindingthe similarity of PK parameters of testand control samplesand a value of $p<0.05$ was found to be significant and ANOVA was considered toestimate anydifferences PKcriteriainagroup.

V.Results& Discussion

5.1Generalcharacterization ofCS-NMM.

Identificationtestsforhydrocolloid

Inference: Theresultsprovedthat theextractedmaterialwashydrophilic innature.

Phytochemical constituents in CS-NMM- identification

Table11.Phytochemicalconstituentsidentified

Tests	Results
	CS-NMM
Alkaloids	–
Carbohydrates	+
Flavonoids	+
Tannins	+

–Absence +Presence

Inference:CS-NMMexhibitedthepresenceofcarbohydrates,flavonoidsandtannins.

5.1.2.a. DeterminationofpHofextractedmucoadhesivematerial

ThepH wasdetermined as6.5 ±0.5.

Inference: The pH of 1%w/v solution of CS-NMM was found to be 6.5. The materialextracted was slightly acidic in nature. Hence, the formulations that are prepared byusingthispolymercanbecompatibleandwillnotcauseanyirritationwhenadministeredintotheGIT

Table 12.DeterminationofswellingindexofCS-NMM

Table5.2:SwellingindexofNMM

Parameter	Result
	NMM
Swellingindexafter3hr	
Distilledwater	17.37± 0.51
pH–1.2	15.83± 0.30
pH–7.4	13.19± 0.47

$$\text{Swellingindex}=[(w_2-w_1)/w_1]$$

Where,w_1=weightofNMMbeforeswelling,w_2=weightofNMMafterswelling

Inference: The swelling index study revealed that the material absorbs more or lesstentimes ofwaterbyits weight.

5.1.2. e.Meltingpoint determinationofCS-NMM

Themeltingpointwasrecordedas339.9°C.

Inference: The high range of melting point i.e. 339.9° C .represents that the material isheatstable withinvarious ranges of temperature,hence canbe usedin differentpharmaceuticalformulations.

5.1.2.f. Determination ofviscosityof1%w/vCS-NMM.

Table13:Viscosityof1%w/vsolution ofCS-NMM

Temperature(°C)	Viscosity inpoise
	CS-NMM
37°C	0.0149
45°C	0.0126
60°C	0.0083

Inference: It was revealed that as temperature increased, the viscosity of CS-NMMhasbeendecreased.

5.1.2. g.Fouriertransforminfraredspectroscopy(FTIR)

FTIRspectrumwasshownasfig.5.1andinterpretationofdatawasexplainedinthetable5.4.

Fouriertransforminfraredspectroscopy(FTIR)

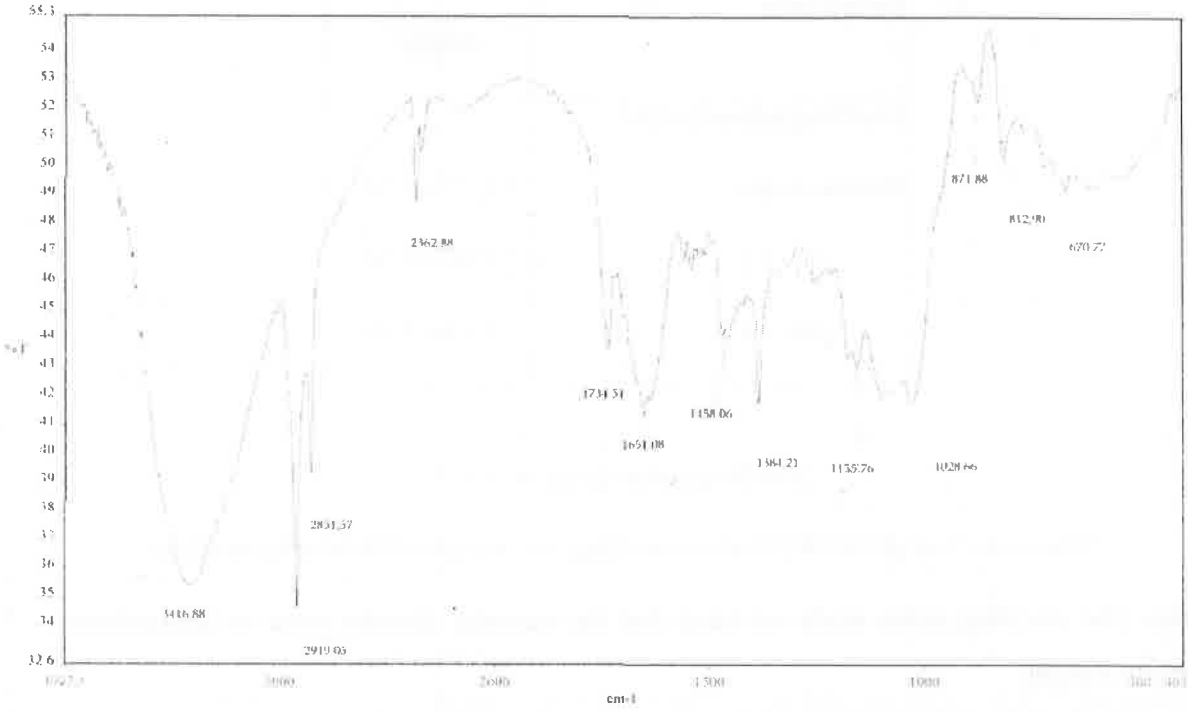


Fig.12. FTIR spectrum of CS-NMMTable5.4:FT-IR dataofCS-NMM

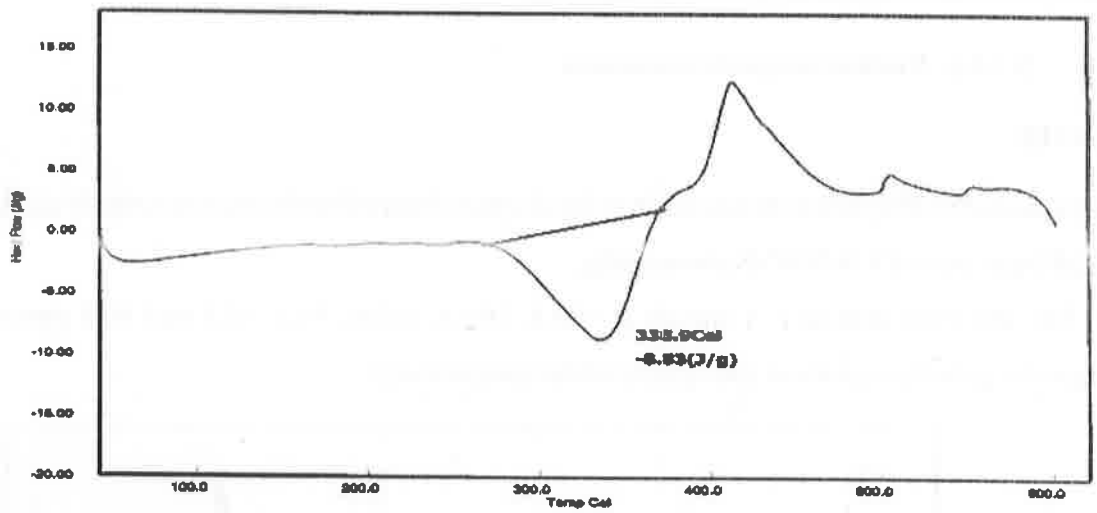
Wavenumber(cm^{-1})	Characteristicband
3416.88	N–H**(S)**
2919.05	(methylor methylene) C– H**(stretch)**
2851.57	C–H **(S)** (Aldehyde)/COOH
1734.51	C= O **(S)**
1458.06	C– H**(bending)**(CH_2)
1384.21	C–H **(B)**(CH_3)

1155.76	C– O(**S**)ether
1028.66	C–O **(B)**(ether/alcohol/esters/anhydrides)
871.88	C–H**(OOP)**foraromaticring
812.90	
670.77	C–Br/C–I**(Outofplane)**

Inference: A single band is seen at 3416.88cm^{-1}which is assigned to the stretching ofH-bonds to the amide group of the adjacent intra-sheet chain. The strong bands areseenintherangeof1458.06exhibitsthecharacteristicresemblancewiththepolysaccharides.

5.1.2.h. Differentialscanningcalorimetry(DSC)

Fig.13:DSC spectrumofCS-NMM



Theoptimum temperaturerequiredtomelttheCS-NMM wasfoundtobe338.9°C.DSC spectrumismentionedinFig.5.2

Inference: As temperature increased the heat flow also increased, indicating that theweightloss has occurred. It was depicted by sharp drop in the curve at 338.9° C forthe sample.

5.1.2.i. SolubilitystudiesofCS-NMM

Thematerial(CS-NMM)wasfoundtobefreelysolubleinhotwater.Theresultofsolubilitystudiesis shownintable5.5

Table14:SolubilityofCS-NMM

Solventsused	Solubility of naturalmucoadhesivemate rials
ColdWater	-
Hotwater	+
n-Hexane	-
Methanol	-
Ethylacetate	-
Ethanol	-

Soluble–(+);Insoluble–(−)

Inference: The solubility has been checked by using different polar and non-polarsolvents. It was found that the substance was soluble in hot water. It was practicallyinsolubleinnon-polarsolventssuchasethanol,benzene,hexane,etc.

5.1.2.j. Nuclearmagneticresonance

^{13}CNMR

ThemeasuredNMRspectrumwasinterpretedandreportedtoconfirmthepresenceofpolysaccharides.The^{13}C-NMRspectrumof CS-NMMisshowninfig.

5.3. The spectrum depicts C-1 signals at 105.4, 103.4, 100.0, 99.3, 93.6 and 90.5 ppmthat are assignedto galactose,glucose andxyloseresiduesrespectively.

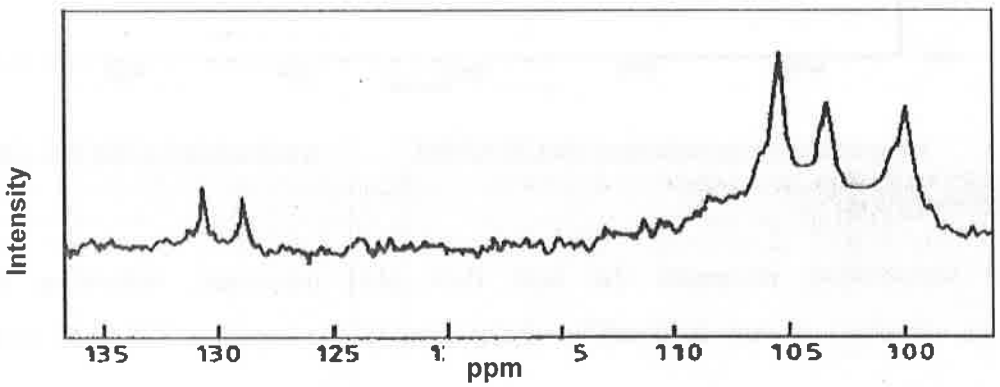


Fig.14:^{13}C-N.M.RspectrumofCS-NMM

^{1}HNMR

^{1}The^{1}H-NMRspectrumofCS-NMMisshownin Fig.5.4.

Inference: The protons of ^{1}H–NMR spectrum is depicted in table 5.6. From this datawe can have an evidence of polysaccharides in the extracted material which is furtherconfirmedbyrepeatedOHandCOOHgroups.

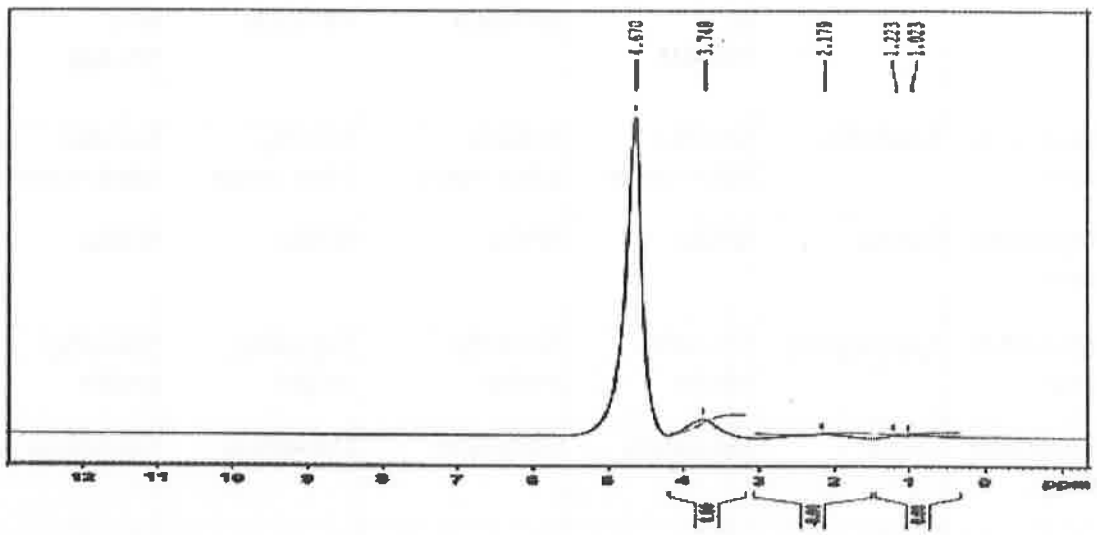


Fig. 15 :^{1}H - NMR spectrum of CS-NMMTable 5.6:Dataof^{1}H-NMRspectrumforCS-NMM

Chemicalshift (PPM)	Natureofprotons	Approximatenumberofprotons
1.023	R-CH_3	1
1.223	R-CH_2-R	1
2.179	R-(C=O)-CH_3/ R_2-N-CH_3	1
3.748	R-CH_2-X (X-Cl, Br, I) /R-O-CH_2-R/ R-(C=O)-O-CH_2-R/ HO-CH_2-R	2
4.670	R_2-C=CH_2	2

Inference: The chemical composition of the CS-NMM was identified by the ^{13}Cand^{1}HNMR.Thespectrumisshowninthefig.5.3to5.4.Thereportsuggeststhe

presenceofOHandCOOHgroups.TheIRandNMRspectrumshowedthepresenceofpolysaccharidesi nCS-NMM.

5.1.3. Stabilitytestingstudies

Table:15. PhysicalstabilitystudiesofextractedCS-NMM

Model	Parameter	25°C± 2 °C/60%RH ± 5%RH	40 °C ± 2 °C/65%RH ±5%RH	40°C±2 °C/75%RH ±5%RH	8°C± 2 °C/60%RH ± 5%RH
Powderedextract	Solubility	Soluble inhot water	Soluble inhot water	Soluble inhot water	Soluble inhot water
Powderedextract	Colour	White	White	White	White
Powderedextract	Appearance	Smoothpowder	Smoothpowder	Smoothpowder	Smoothpowder
Powderedextract	Odour	Odourless	Odourless	Odourless	Odourless
Powderedextract	Sterilitystudies	Absence ofmicrobial growth	Absence ofmicrobial growth	Absence ofmicrobial growth	Absence ofmicrobial growth

5.2 Premilinarystudiesforthepreparationofsphericalagglomerates

5.2.1 Selectionofbridgingliquid,poorsolvent andgoodsolventTable 16:Solubilitystudies ofdrugs

	Glipizide	Empagliflozin
Composition	Observations	Observations
100mgdrug +1mldichloromethane	Soluble	Soluble
100 mg drug +1 mlacetone	Soluble	Slightlysoluble
100 mg drug + 1 mlmethanol	Insoluble	Sparinglysoluble
100 mgdrug+ 1mlalcohol	Insoluble	Sparinglysoluble
100 mg drug + 1 mlwater	Insoluble	Almostinsoluble
100mgofdrug+1mloftoluene	Insoluble	Insoluble

From the above studies, dichloromethane was selected as a good solvent (internalphase)while waterisselectedasa poorsolvent(externalphase).

5.2.2.Selectionofbridgingliquids:

Dichloromethane was found to be miscible with chloroform in proportions from 1:1 to1:5, while it was immiscible with glycerine and n-hexane in different proportions. So,chloroformwas selectedasabridgingliquid.

5.2.3Preliminaryselectionofprocessparametersaffectingtheformationofsphericalagglomerates

Table17:Preliminarytrialsfordrugsphericalagglomerates

S.No.	Procedure	Observations
1.	100mg of the selected drug was dissolvedin1 ml of dichloromethane. The drug solutionwas added dropwise at a rate of 1 ml/min to50ml of water stirred on a magnetic stirrer at600rpm.	Irregular shaped crystals of drug wereobtainedimmediately.
2.	100 mgofdrugisdissolvedin1mlof dichloromethane.Thedrugsolutionwasadded dropwise at a rate of 1 ml/min to 50 mlofwatercontaining50%v/vofchloroformstirred ona magneticstirrerat600rpm.	Spherical agglomerates of drugparticleswere obtained.
3.	100 mg of drug is dissolved in 1 ml ofdichloromethane.To thissolution10mlof chloroform was added. The drug solution wasadded dropwise at a rate of 1 ml/min to 50mlof water stirred on a magnetic stirrer at 200rpm,400rpm,600rpmand1000rpmrespectively.	The results of characterization ofproductobtained bydifferentbatcheswere shownintableno.5.8.
4.	100mgofdrugisdissolvedin1mlofdichloromethane.To this solution 1ml,3mland5mlofchloroformwas added.Thedrug solution was added dropwise to 50 ml ofwaterataarateof1ml/min.stirredona magnetic stirrer at 1000 rpm to find out theoptimumstirringtime(10,20,30and40minutes)afteradditionofentire drugsolution	Theresultsofproductcharacteristics obtainedbyvariousbatcheswereshownintableno.5.7.

Here,chloroform increasedtheviscosity of theinternal phaseandstabilizedthesphericalagglomerates ofdrugformed.

Table5.10:Effectofstirringspeed oncharacteristicsofsphericalagglomerates

Stirringspeed	Characteristicsoftheproduct		
	Sphericity	Matrix	No.ofparticles
400	+	+	+
600	++	++	++
800	++	+	+++
1000	+++	--	++++

From the above results, it was decided to keep the stirring speed at 1000 rpm forfurther studies since spherical agglomerates with good sphericity without any matrixofparticles wereobtainedatthis speed.

Table18:Effectof%v/vchloroformininternalphaseonproductcharacteristics

ml ofchloroform in internalphase	Stirring time afteradditionofdrug solution(minutes)	Sphericity	Particleagglomeration	Matrixofparticles	No.ofparticles
20	10	+	+	+	+
20	20	+	++	++	+
20	30	++	++	-	++
20	40	++	++	++	++
30	10	+	++	+	++
30	20	++	++	++	++
30	30	+++	++	+	++++
30	**40**	**+++**	**+++**	**--**	**++++**
40	10	+	+	+	++
40	20	+	++	+	++
40	30	++	++	++	++
40	40	++	+++	++	+++

From the above results, the stirring time was fixed at 40 minutes after the addition ofentire drug solution. Stirring time of 40 minutes gave a product with more sphericalagglomerateswithgoodsphericitywithoutanymatrixofparticles.

5.3 Organolepticcharacteristics:

Table19:Organolepticpropertiesofpreparedtabletsofempagliflozin

S.No.	Properties	Observation
1.	Description	Roundinshape
2.	Colour	White
3.	Taste	Tasteless
4.	Odour	Odourless
5.	Elegance andsurfacetexture	Smooth

5.7. Preparationandcharacterizationofempagliflozinsphericalagglomerates

A total of 24 formulations were made using different polymers namely c*aesalpiniaspinosa*, HPMC K100M, ethyl cellulose, sodium alginate out of which F1-F12 wereformulated using spherical agglomeration technique and F13-F24 were formulatedwithAPIofempagliflozin

Table20:Formulationchart

	Sphericalagglomeratesofempagliflozin												EmpagliflozinAPI											
Ingredients(mg)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**	**F16**	**F17**	**F18**	**F19**	**F20**	**F21**	**F22**	**F23**	**F24**
Drug	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
MCC	128.5	118.5	108.5	128.5	118.5	108.5	128.5	118.5	108.5	128.5	118.5	108.5	128.5	118.5	108.5	128.5	118.5	108.5	128.5	118.5	108.5	128.5	118.5	108.5
Caesalpiniaspinosa	10	20	30	–	–	–	–	–	–	–	–	–	10	20	30	–	–	–	–	–	–	–	–	–
HPMCK100M	–	–	–	10	20	30	–	–	–	–	–	–	–	–	–	10	20	30	–	–	–	–	–	–
Sodiumalginate	–	–	–	–	–	–	10	20	30				–	–	–	–	–	–	10	20	30			
Ethylcellulose	–	–	–	–	–	–	–	–	–	10	20	30	–	–	–	–	–	–	–	–	–	10	20	30
Mgstearate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total(mg)	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150

The salient features of the above chart reveals that the polymers are used in the formulation because they prolong the drug release which furthersuits the rate limiting characteristics of the drug, MCC is used to improve the bulkness of the tablet. The tablets were directly compressed to itstotalweightof150mg.Thepreparedtabletswerefurtherevaluatedforitpre-compressionandpostcompressionparameters.

5.7. Micromeriticproperties

All the prepared formulations of empagliflozin spherical agglomerates and API weresubjected to preformulation studies and thevalues obtained were within thelimits.The values weregivenintable: 5.62.

Table 21:Preformulationstudiesforempagliflozin

Formulationcode	Bulkdensity (g/cm^3)	Tappeddensity (g/cm^3)	Carr'sindex (%)	Hausnersratio	Angleofrepose (Θ)	Diameter(mm)
F1	0.33±0.01	0.38±0.05	13.33±0.11	1.15±0.02	33.12±0.11	7.01±0.02
F2	0.35±0.03	0.38±0.03	6.98±0.09	1.08±0.06	27.32±0.12	7.08±0.01
F3	0.31±0.01	0.35±0.04	10.42±0.16	1.12±0.08	31.11±0.11	7.03±0.03
F4	0.33±0.05	0.37±0.05	10.87±0.07	1.12±0.07	31.52±0.16	6.92±0.01
F5	0.36±0.02	0.38±0.08	7.14±0.07	1.08±0.03	27.69±0.15	7.11±0.02
F6	0.32±0.06	0.38±0.02	14.89±0.12	1.18±0.05	35.28±0.09	6.89±0.03
F7	0.35±0.02	0.38±0.05	9.30±0.03	1.10±0.01	29.31±0.17	6.92±0.02
F8	0.33±0.03	0.37±0.06	8.89±0.06	1.10±0.08	29.17±0.16	7.14±0.04
F9	0.37±0.01	0.43±0.01	14.63±0.17	1.17±0.06	34.63±0.19	7.06±0.02
F10	0.32±0.09	0.36±0.08	10.64±0.16	1.12±0.09	31.88±0.14	6.95±0.02
F11	0.38±0.05	0.43±0.02	10.26±0.11	1.11±0.03	30.51±0.13	7.02±0.03
F12	0.33±0.04	0.38±0.04	11.11±0.12	1.13±0.07	32.27±0.06	7.04±0.01
F13	0.41±0.03	0.47±0.02	13.51±0.16	1.16±0.06	33.71±0.03	7.19±0.02
F14	0.43±0.06	0.48±0.01	11.43±0.09	1.13±0.04	32.64±0.13	6.85±0.01
F15	0.31±0.03	0.36±0.06	12.50±0.07	1.14±0.01	33.7±0.07	7.13±0.03
F16	0.33±0.05	0.37±0.04	10.87±0.02	1.12±0.07	31.29±0.02	6.91±0.02
F17	0.27±0.01	0.30±0.05	10.71±0.06	1.12±0.02	31.75±0.04	7.03±0.01
F18	0.33±0.04	0.38±0.04	13.33±0.03	1.15±0.05	33.95±0.03	7.09±0.01
F19	0.36±0.01	0.43±0.07	16.67±0.05	1.20±0.03	37.27±0.13	7.15±0.03
F20	0.39±0.06	0.45±0.09	13.16±0.07	1.15±0.06	33.62±0.02	7.13±0.01
F21	0.48±0.02	0.58±0.03	16.13±0.05	1.19±0.03	36.38±0.03	7.03±0.02
F22	0.43±0.03	0.50±0.06	14.29±0.03	1.17±0.07	34.96±0.11	6.92±0.04
F23	0.41±0.01	0.45±0.05	10.81±0.01	1.12±0.04	31.69±0.15	6.89±0.01
F24	0.35±0.02	0.39±0.06	11.63±0.06	1.13±0.03	32.56±0.14	7.05±0.03

Inference: In pre-compression parameters the bulk density of empagliflozin it waswithin the range of 0.27 ± 0.01-0.48 ± 0.02.Carr's index was found to be within therange of 6.98-16.67 ± 0.09, hausner's ratio was in the range 1.08- 1.18 ± 0.06. Angleofreposewasfoundtobewithintherangeof27.32-37.27±0.12(Theflowproperty

was found to be in passable limits) and diameter was found to be within the range7.01-7.15± 0.01.

Postcompressionparameters

All the prepared formulations of empagliflozin spherical agglomerates and API weresubjected to post compression studies and the values obtained were within the limits.The values weregivenintable:5.63.

Table22:Postcompressionstudiesforempagliflozin

Formulationcode	Weight Variation(%)	Thickness(mm)	Hardness(Kg/cm^2)	Friability(%)	Drugcontent(%)
F1	Pass	2.52±0.06	8.23±0.11	0.32±0.01	96.01±0.14
F2	Pass	2.57±0.01	8.10±0.02	0.15±0.05	96.82±0.18
F3	Pass	2.49±0.04	8.31±0.05	0.41±0.03	99.85±0.13
F4	Pass	2.52±0.08	8.17±0.02	0.27±0.04	97.03±0.21
F5	Pass	2.55±0.06	7.96±0.07	0.35±0.02	97.05±0.16
F6	Pass	2.57±0.04	8.21±0.03	0.16±0.04	97.11±0.18
F7	Pass	2.52±0.02	8.13±0.06	0.24±0.02	97.36±0.13
F8	Pass	2.54±0.07	8.31±0.03	0.12±0.05	97.28±0.13
F9	Pass	2.46±0.02	7.89±0.07	0.05±0.03	98.31±0.19
F10	Pass	2.57±0.01	8.06±0.03	0.26±0.06	96.29±0.13
F11	Pass	2.51±0.07	7.94±0.02	0.36±0.04	97.69±0.16
F12	Pass	2.48±0.03	8.16±0.01	0.41±0.03	97.85±0.16
F13	Pass	2.46±0.01	8.32±0.06	0.28±0.06	97.31±0.18
F14	Pass	2.55±0.06	8.16±0.11	0.22±0.04	98.03±0.14
F15	Pass	2.53±0.04	8.17±0.14	0.16±0.07	99.56±0.13
F16	Pass	2.46±0.01	8.25±0.08	0.19±0.04	96.93±0.17
F17	Pass	2.42±0.03	8.17±0.03	0.07±0.05	97.52±0.14
F18	Pass	2.51±0.02	8.35±0.02	0.31±0.03	97.67±0.17
F19	Pass	2.47±0.07	7.8±0.01	0.39±0.05	98.34±0.14
F20	Pass	2.56±0.04	8.09±0.03	0.16±0.04	98.52±0.18
F21	Pass	2.39±0.02	8.28±0.04	0.26±0.04	99.34±0.14
F22	Pass	2.55±0.01	8.35±0.01	0.24±0.02	96.04±0.19
F23	Pass	2.41±0.04	7.86±0.04	0.17±0.01	96.08±0.21
F24	Pass	2.52±0.01	8.19±0.07	0.12±0.03	96.38±0.27

Inference: Inpostcompressionparameterstheweightvariationforalltheformulationswerefoundtobe withinthelimits.Thethicknesswasintherangeof 2.39mm -2.57mm±0.04.Hardnesswasfoundtobeintherangeof7.8-8.35kg/cm^2

±0.01,friabilitywasfoundtobeintherangeof 0.05–0.41±0.03%anddrugcontentwasfoundtobeinthe rangeof96.01-99.85±0.21%.

5.8. Dissolutionstudiesandkineticstudiesforempagliflozinformulations

5.8.1. Dissolutionstudiesforempagliflozinformulations:

ThepreparedformulationsweresubjectedtodissolutionstudiesforF1-F12formulations

Table23:*In vitro*drugreleaseprofileforF1-F12

time hrs	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0 hrs	0	0	0	0	0	0	0	0	0	0	0	0
0.5hrs	8	9	12	6	8	9	5	7	10	5	9	7
1 hrs	18	15	21	17	18	15	16	18	13	16	18	15
2 hrs	25	22	32	28	26	24	27	22	25	22	26	27
4 hrs	38	41	46	39	40	37	33	41	35	40	36	33
6 hrs	55	48	61	57	45	52	56	47	50	54	44	56
8 hrs	59	67	72	60	65	68	58	62	69	64	63	60
10 hrs	60	80	87	64	75	83	62	65	81	78	83	75
12 hrs	72	86	97	68	81	90	70	77	85	81	88	90

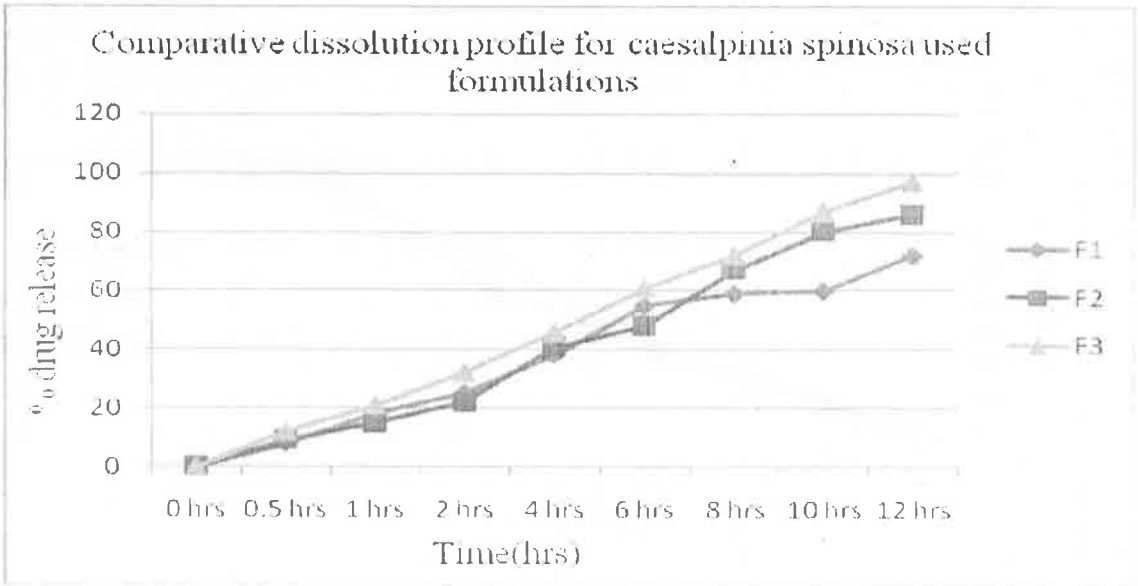


Fig17: Comparativedissolutionprofilesforformulations F1-F3

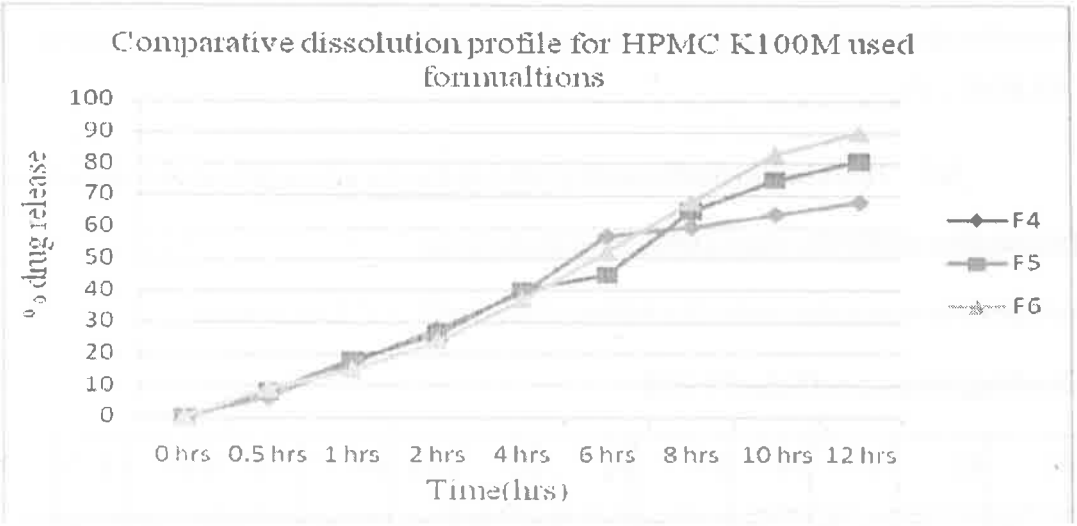


Fig18:ComparativedissolutionprofilesforformulationsF4-F6

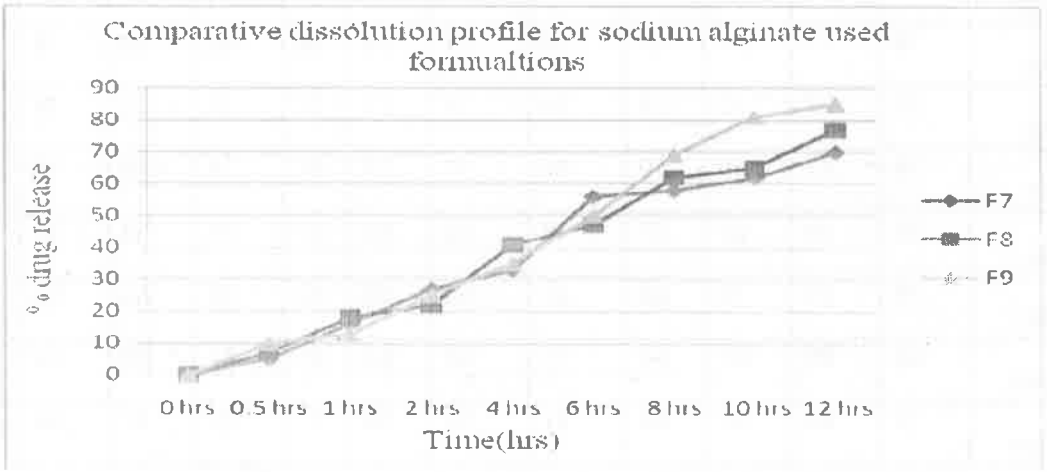


Fig19:ComparativedissolutionprofilesforformulationsF7-F9

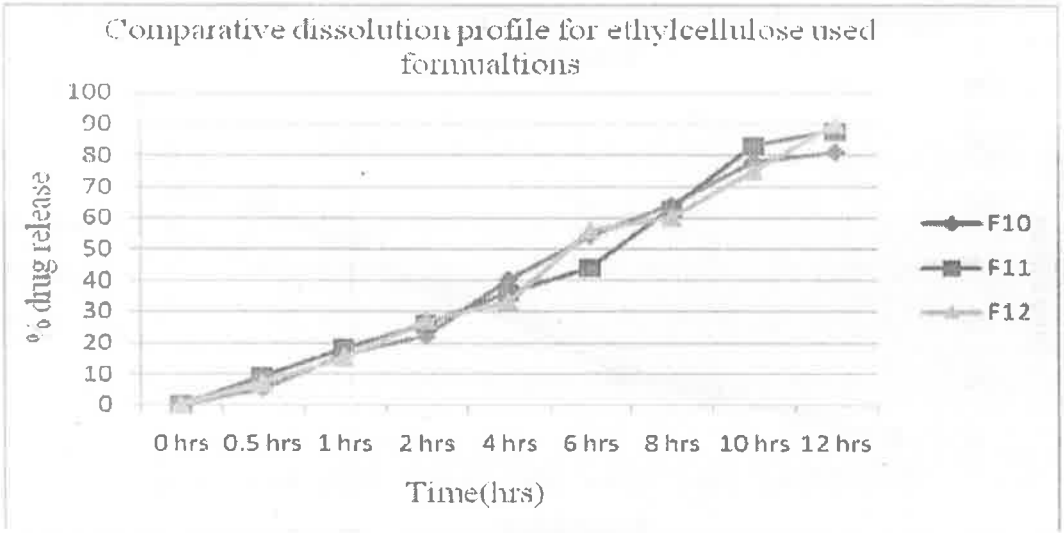


Fig20: ComparativedissolutionprofilesforformulationsF10-F12

Table24:*Invitro*drugreleaseprofileforformulationsF13-F24

time hrs	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24
0 hrs	0	0	0	0	0	0	0	0	0	0	0	0
0.5hrs	10	11	11	5	8	9	7	9	8	4	5	9
1 hrs	19	20	19	18	15	12	11	15	17	15	13	11
2 hrs	28	29	30	25	22	27	20	26	25	28	20	25
4 hrs	45	42	44	39	35	40	30	35	41	34	36	38
6 hrs	60	59	60	55	50	45	49	55	50	45	56	52
8 hrs	65	71	70	69	60	63	53	62	69	50	65	60
10 hrs	67	86	85	72	84	80	58	67	72	53	73	72
12 hrs	70	88	89	79	84	83	66	75	76	67	78	75

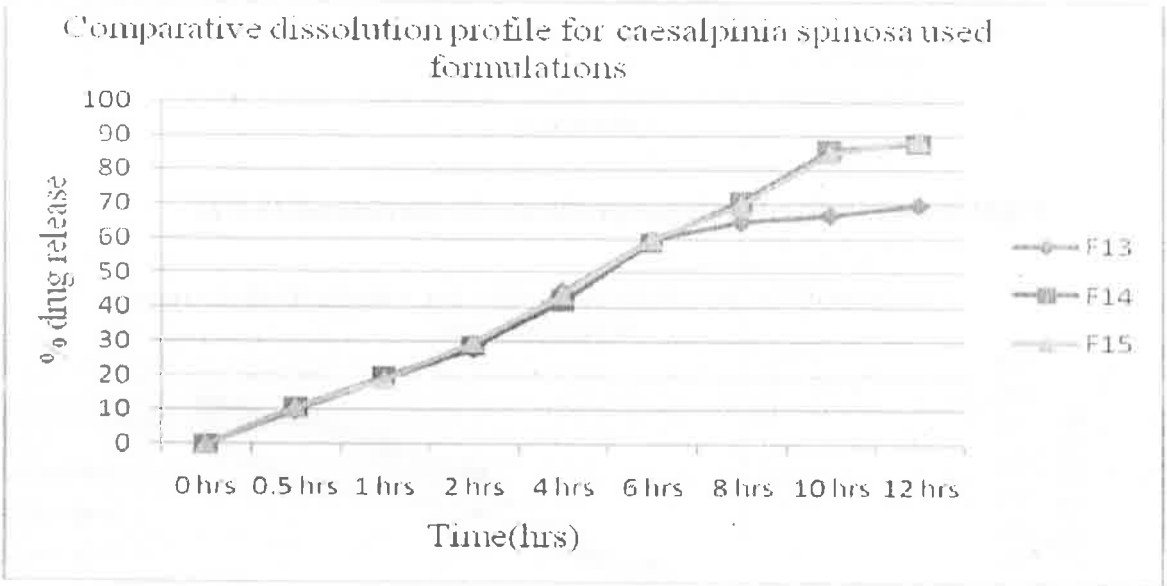


Fig21:Comparativedissolutionprofiles forformulationsF13-F15

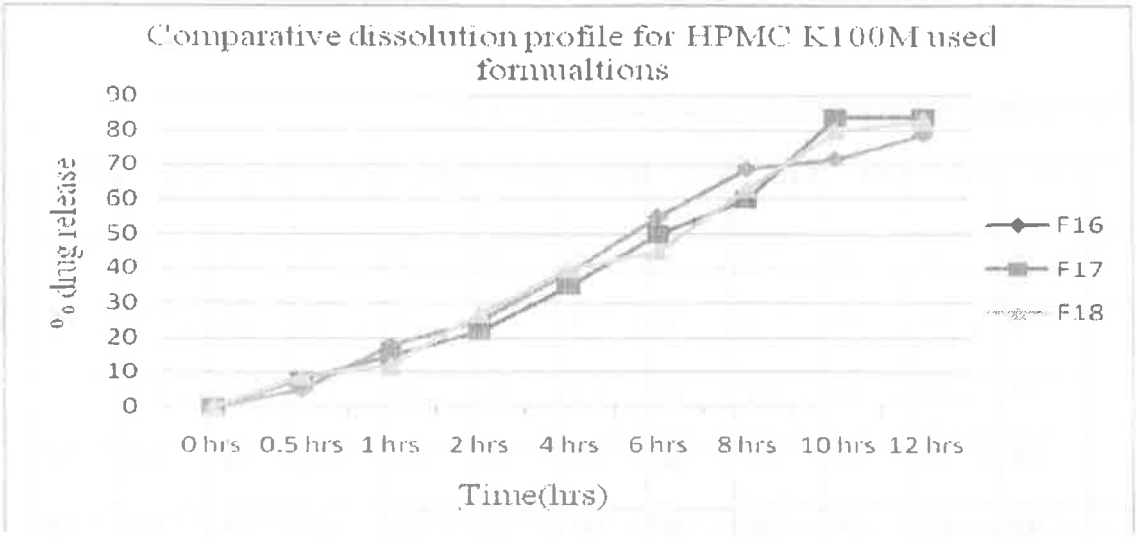


Fig22:Comparativedissolutionprofilesforformulations F16-F18

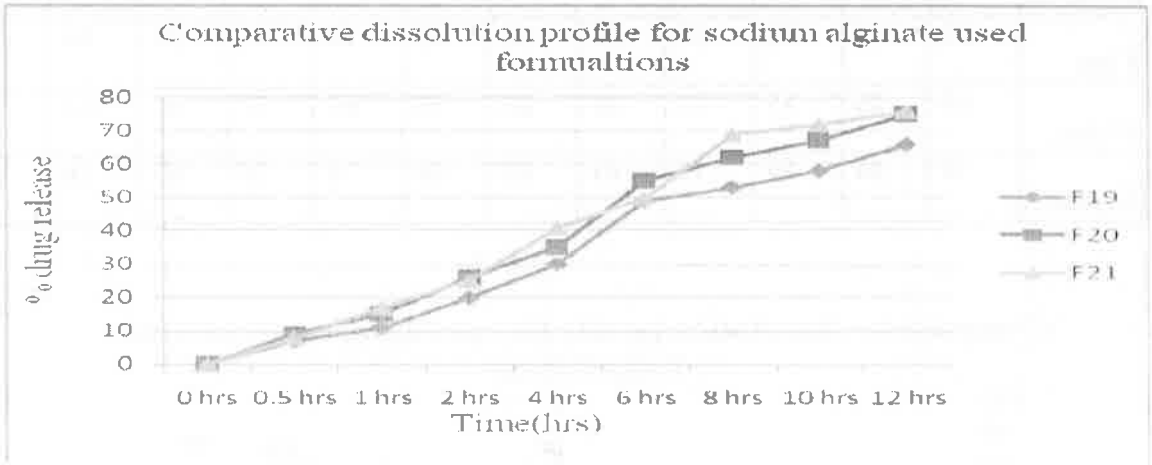


Fig23:Comparativedissolutionprofilesforformulations F19-F21

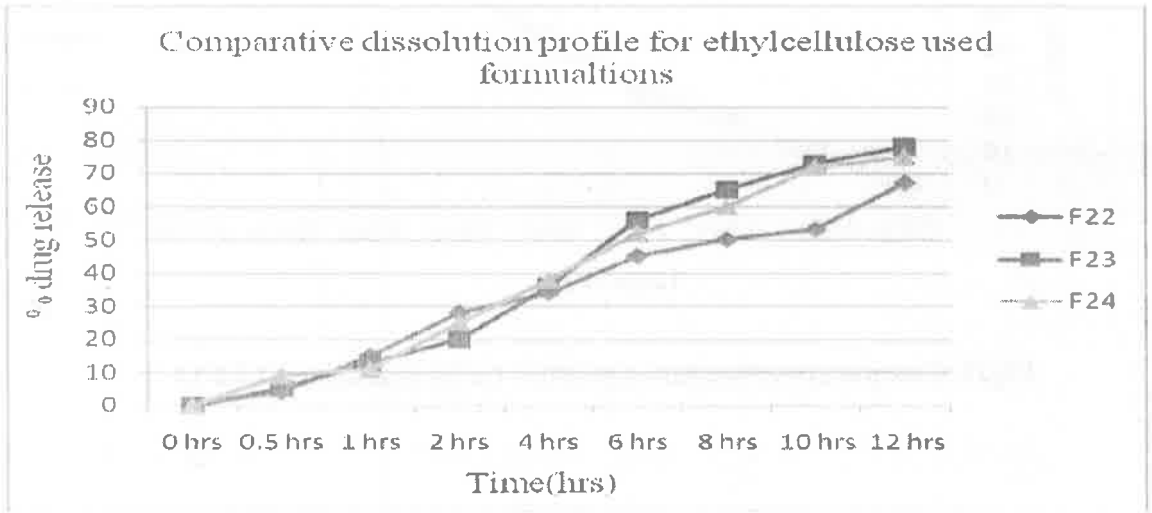


Fig24: Comparativedissolutionprofilesforformulations F22-F24

5.12.1. Firstorder

Thepreparedempagliflozinformulationsweresubjectedtodissolutionstudiesandthefollowingrepresentsfirstorderkineticsfor F1-F24formulations

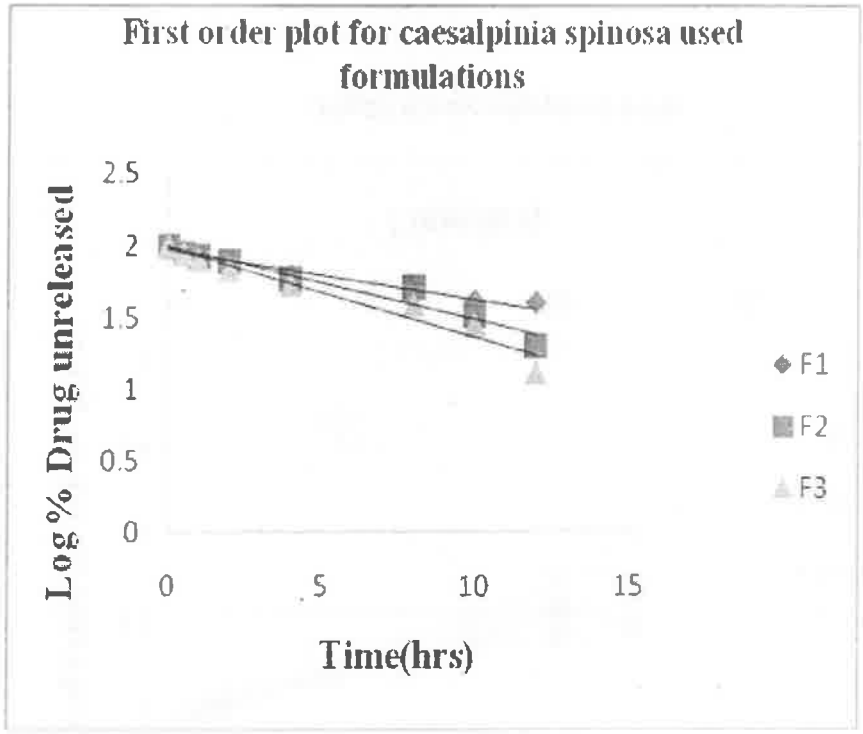


Fig24 First orderplotF1-F3

Time(hrs)	F1	F2	F3
0 hrs	2	2	**2**
0.5hrs	1.963788	1.959041	**1.944483**
1 hrs	1.913814	1.929419	**1.897627**
2 hrs	1.875061	1.892095	**1.832509**
4 hrs	1.792392	1.770852	**1.732394**
6 hrs	1.653213	1.716003	**1.591065**
8 hrs	1.612784	1.518514	**1.447158**
10 hrs	1.60206	1.30103	**1.113943**
12 hrs	1.447158	1.146128	**0.477121**
R^2	0.960	0.939	**0.944**

Table24:FirstorderdataF1-F3

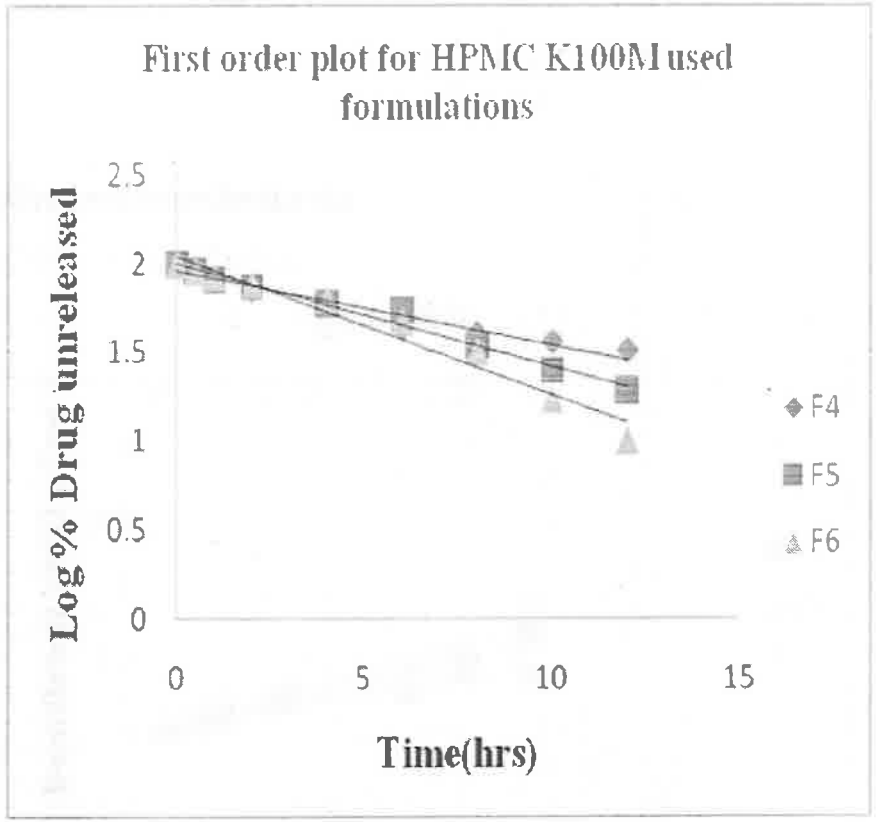


Fig25 FirstorderplotF4-F6

Time(hrs)	F4	F5	F6
0	2	2	2
0.5	1.973128	1.963788	1.959041
1	1.919078	1.913814	1.929419
2	1.857332	1.869232	1.880814
4	1.78533	1.778151	1.799341
6	1.633468	1.740363	1.681241
8	1.60206	1.544068	1.50515
10	1.556303	1.39794	1.230449
12	1.50515	1.278754	1
R^2	0.956	0.980	0.958

Table25:FirstorderdataF4-F6

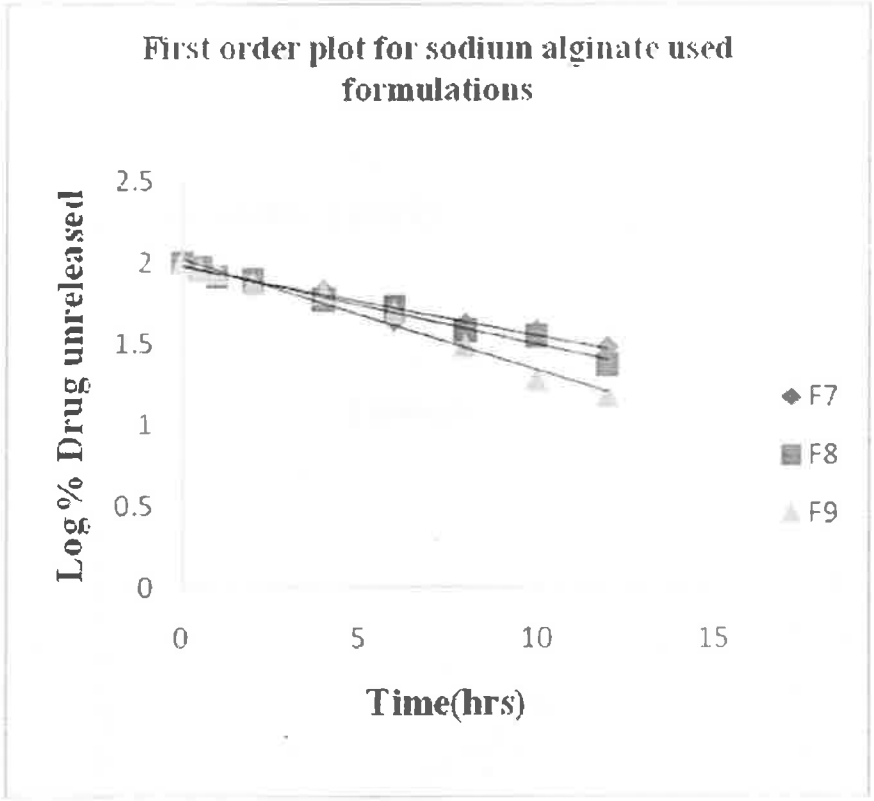


Fig26 FirstorderplotF7-F9

Time(hrs)	F7	F8	F9
0	2	2	2
0.5	1.977724	1.968483	1.954243
1	1.924279	1.913814	1.939519
2	1.863323	1.892095	1.875061
4	1.826075	1.770852	1.812913
6	1.643453	1.724276	1.69897
8	1.623249	1.579784	1.491362
10	1.579784	1.544068	1.278754
12	1.477121	1.361728	1.176091
R^2	0.967	0.984	0.975

Table 26:FirstorderdataF7-F9

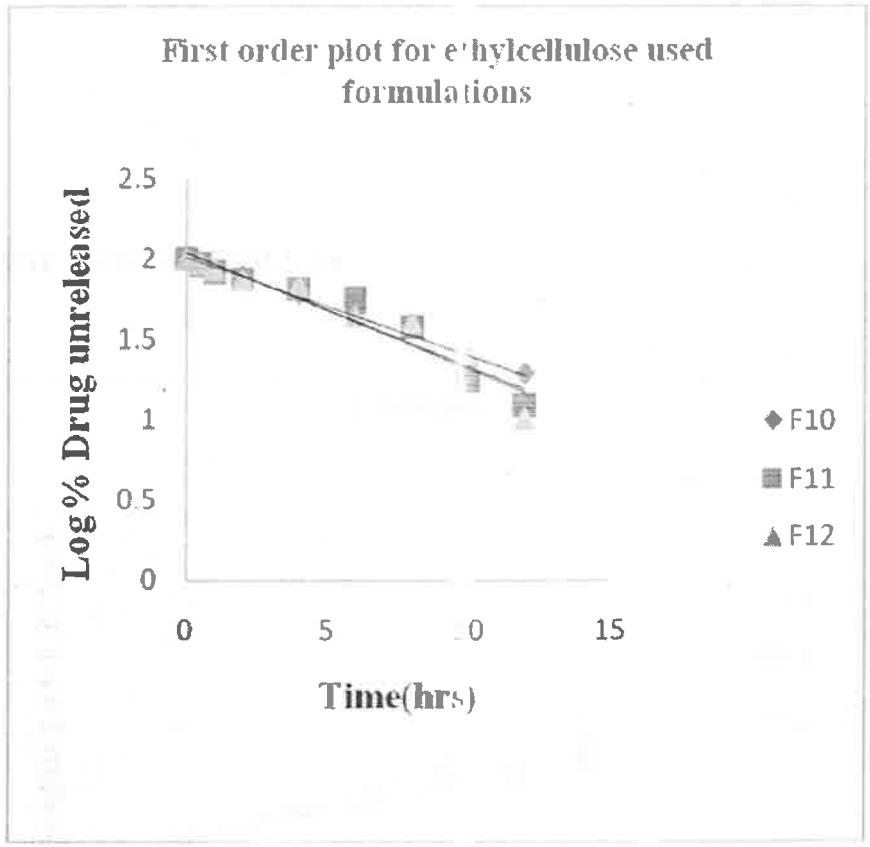


Fig27 First orderplot F10-F12

Time(hrs)	F10	F11	F12
0	2	2	2
0.5	1.977724	1.959041	1.968483
1	1.924279	1.913814	1.929419
2	1.892095	1.869232	1.863323
4	1.778151	1.80618	1.826075
6	1.662758	1.748188	1.643453
8	1.556303	1.568202	1.60206
10	1.342423	1.230449	1.39794
12	1.278754	1.079181	1
R^2	0.990	0.933	0.919

Table27:First orderdataF10-F12

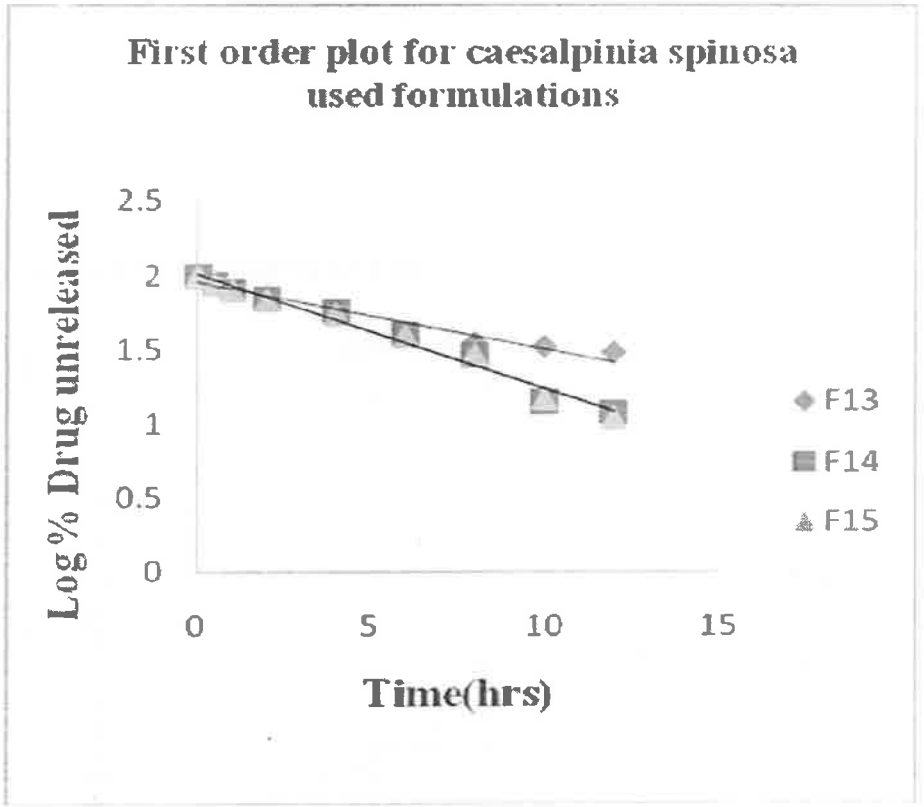


Fig28 FirstorderplotF13-F15

Time(hrs)	F13	F14	F15
0	2	2	2
0.5	1.954243	1.94939	1.94939
1	1.908485	1.90309	1.908485
2	1.857332	1.851258	1.845098
4	1.740363	1.763428	1.748188
6	1.60206	1.612784	1.60206
8	1.544068	1.462398	1.477121
10	1.518514	1.146128	1.176091
12	1.477121	1.079181	1.041393
R^2	0.946	0.975	0.979

Table28:FirstorderdataF13-F15

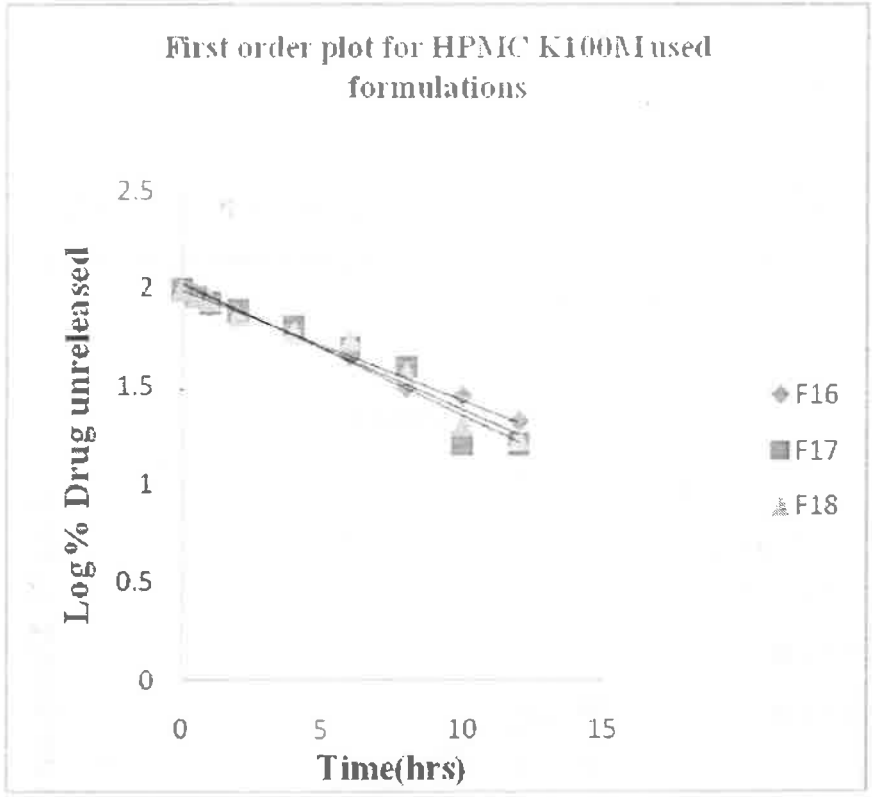


Fig29 FirstorderplotF16-F18

Time(hrs)	F16	F17	F18
0	2	2	2
0.5	1.977724	1.963788	1.959041
1	1.913814	1.929419	1.944483
2	1.875061	1.892095	1.863323
4	1.78533	1.812913	1.778151
6	1.653213	1.69897	1.740363
8	1.491362	1.60206	1.568202
10	1.447158	1.20412	1.30103
12	1.322219	1.20412	1.230449
R^2	0.992	0.936	0.963

Table29:FirstorderdataF16-F18

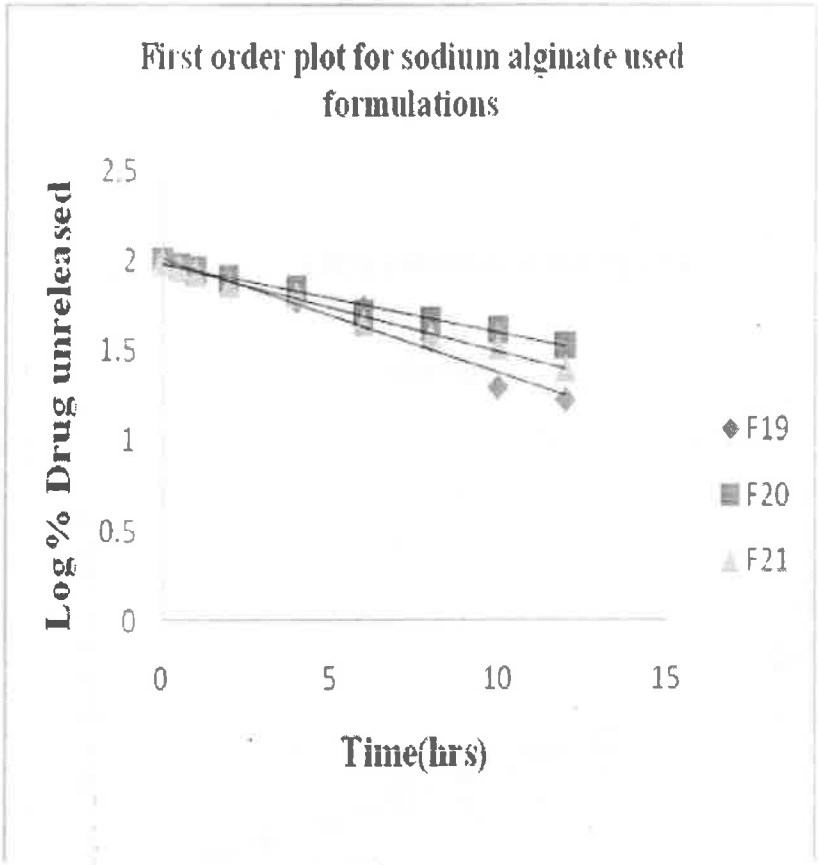


Fig30 FirstorderplotF19-F21

Time(hrs)	F19	F20	F21
0	2	2	2
0.5	1.959041	1.968483	1.959041
1	1.944483	1.94939	1.929419
2	1.863323	1.90309	1.869232
4	1.778151	1.845098	1.812913
6	1.740363	1.70757	1.653213
8	1.568202	1.672098	1.579784
10	1.30103	1.623249	1.518514
12	1.230449	1.531479	1.39794
R^2	0.963	0.980	0.991

Table30:FirstorderdataF19-F21

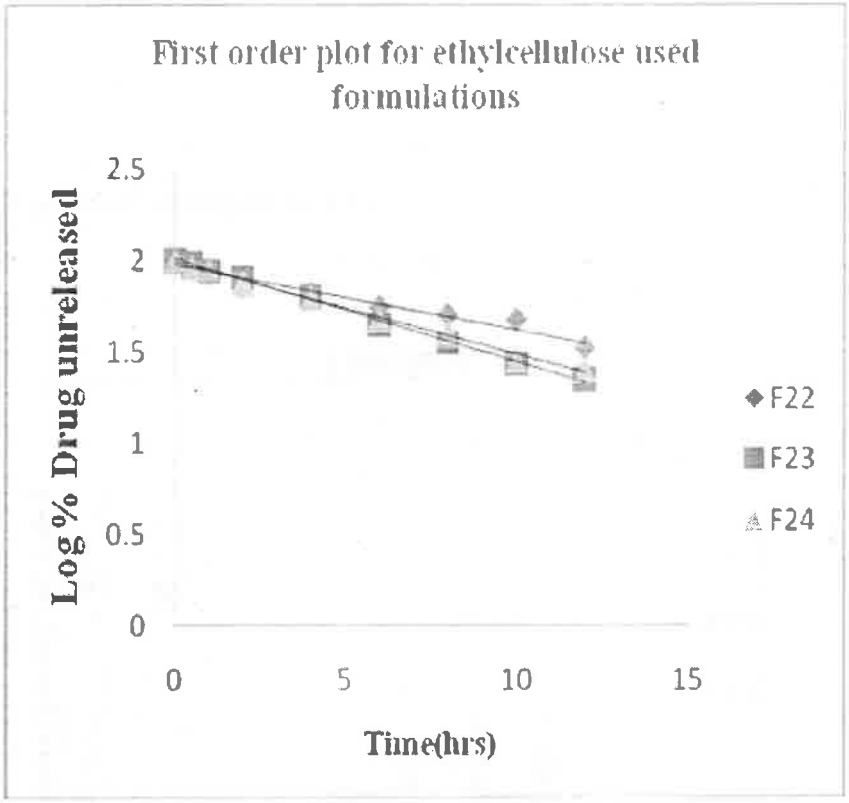


Fig31 FirstorderplotF22-F24

Time(hrs)	F22	F23	F24
0	2	2	2
0.5	1.982271	1.977724	1.959041
1	1.929419	1.939519	1.94939
2	1.857332	1.90309	1.875061
4	1.819544	1.80618	1.792392
6	1.740363	1.643453	1.681241
8	1.69897	1.544068	1.60206
10	1.672098	1.431364	1.447158
12	1.518514	1.342423	1.39794
R^2	0.961	0.996	0.994

Table31 :FirstorderdataF22-F24

5.12.2. Higuchidata

ThepreparedempagliflozinformulationsweresubjectedtodissolutionstudiesandthefollowingrepresentsHigu hikineticsforF1-F24formulations

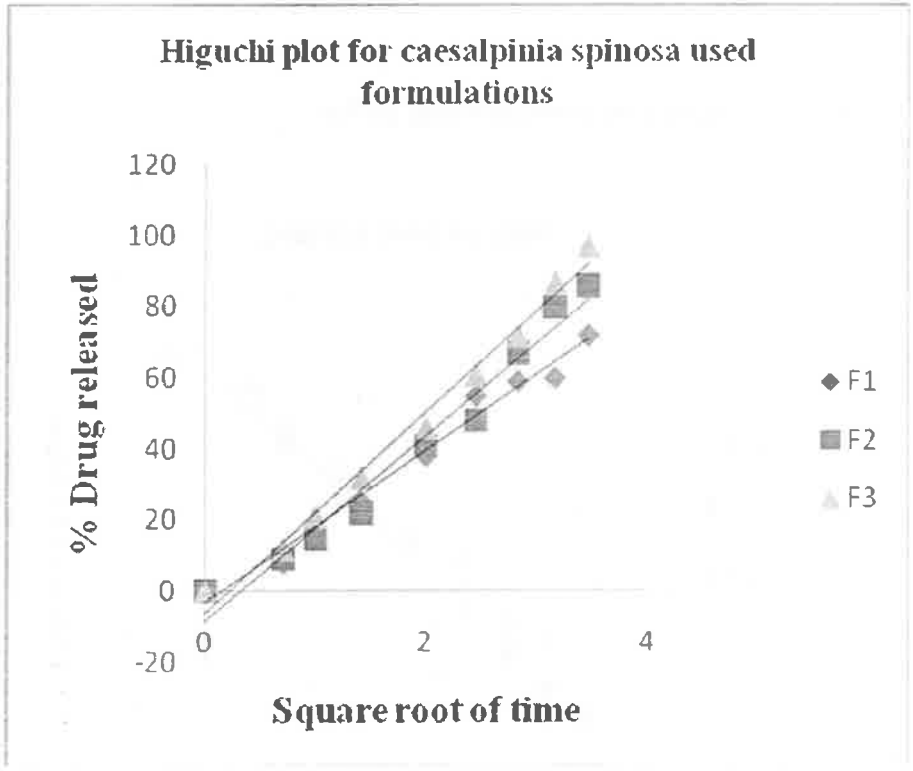


Fig32Higuchi'splot forF1-F3

Time(hrs)	SQRt	F1	F2	**F3**
0	0	0	0	**0**
0.5	0.707107	8	9	**12**
1	1	18	15	**21**
2	1.414214	25	22	**32**
4	2	38	41	**46**
6	2.44949	55	48	**61**
8	2.828427	59	67	**72**
10	3.162278	60	80	**87**
12	3.464102	72	86	**97**
R^2		0.983	0.970	**0.987**

Table:32 Higuchi'sdataF1-F3

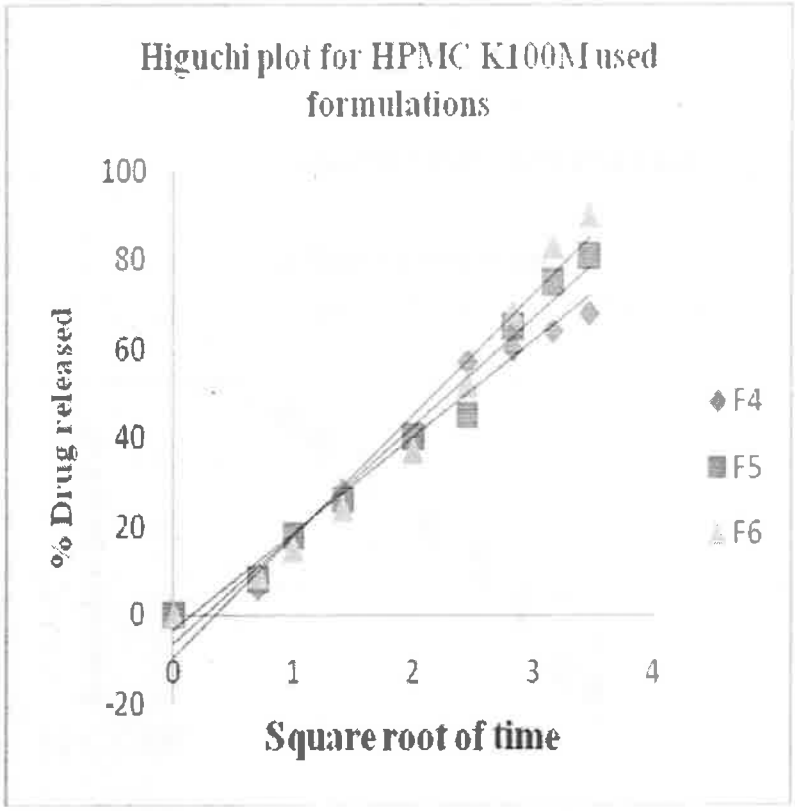


Fig33 Higuchi'splot forF4-F6

Time(hrs)	SQRt	F4	F5	F6
0	0	0	0	0
0.5	0.707107	6	8	9
1	1	17	18	15
2	1.414214	28	26	24
4	2	39	40	37
6	2.44949	57	45	52
8	2.828427	60	65	68
10	3.162278	64	75	83
12	3.464102	68	81	90
R^2		0.977	0.976	0.967

Table:33Higuchi'sdataF4-F6

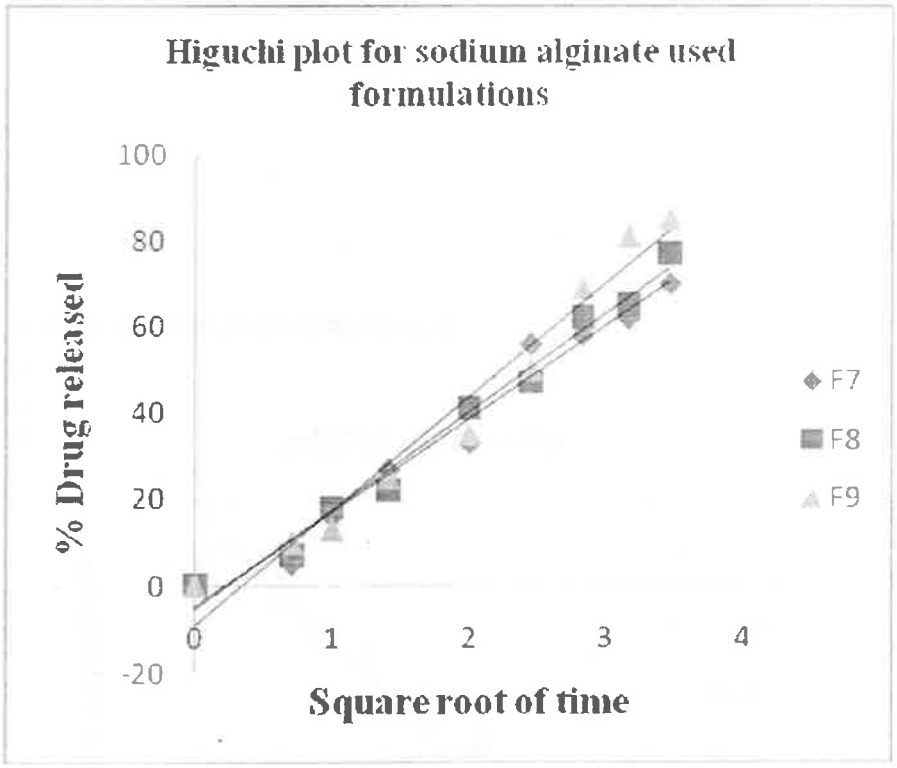


Fig34 Higuchi'splot forF7-F9

Time(hrs)	SQRt	F7	F8	F9
0	0	0	0	0
0.5	0.707107	5	7	10
1	1	16	18	13
2	1.414214	27	22	25
4	2	33	41	35
6	2.44949	56	47	50
8	2.828427	58	62	69
10	3.162278	62	65	81
12	3.464102	70	77	85
R^2		0.972	0.982	0.965

Table: 34Higuchi'sdataF7-F9

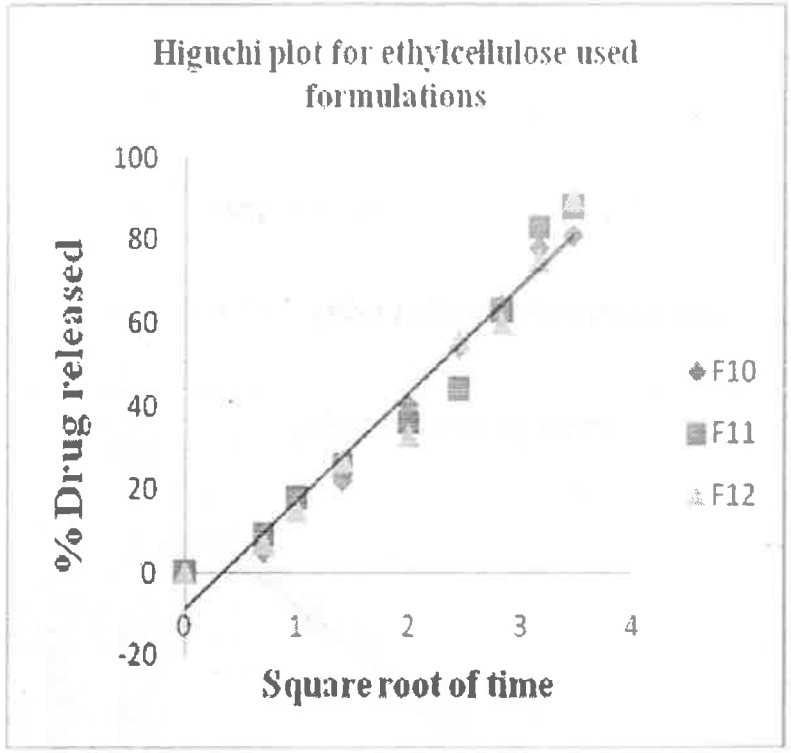


Fig35 Higuchi'splot forF10-F12

Time(hrs)	SQRt	F10	F11	F12
0	0	0	0	0
0.5	0.707107	5	9	7
1	1	16	18	15
2	1.414214	22	26	27
4	2	40	36	33
6	2.44949	54	44	56
8	2.828427	64	63	60
10	3.162278	78	83	75
12	3.464102	81	88	90
R^2		0.978	0.950	0.962

Table35Higuchi'sdataF10-F12

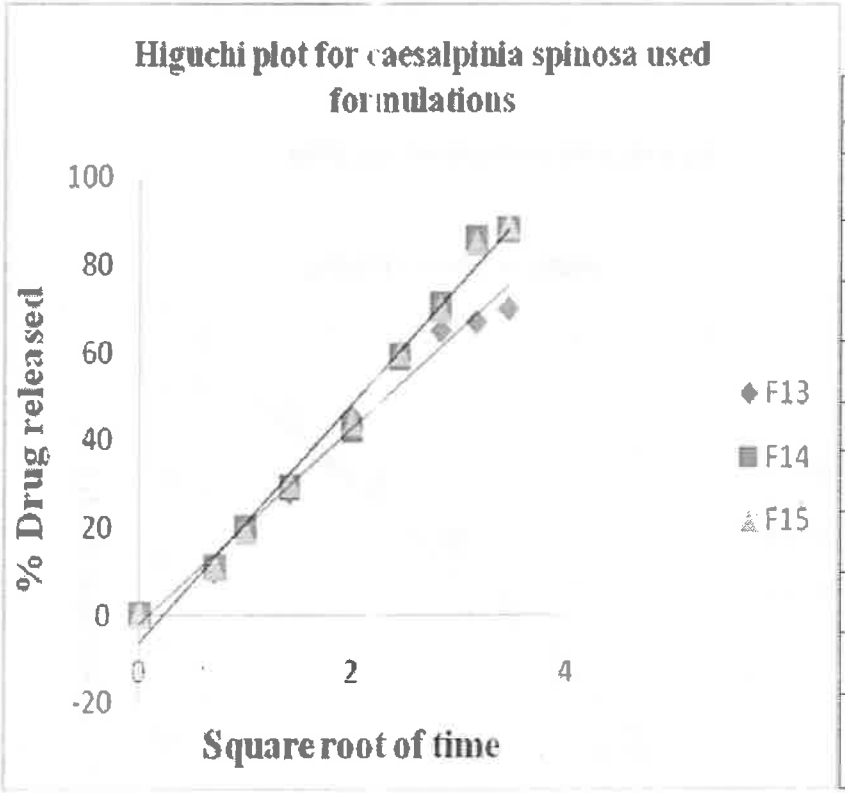


Fig36Higuchi'splot forF13-F15

Time(hrs)	SQRt	F13	F14	F15
0	0	0	0	0
0.5	0.707107	10	11	11
1	1	19	20	19
2	1.414214	28	29	30
4	2	45	42	44
6	2.44949	60	59	60
8	2.828427	65	71	70
10	3.162278	67	86	85
12	3.464102	70	88	89
R^2		0.977	0.984	0.988

Table:36Higuchi'sdataF13-F15

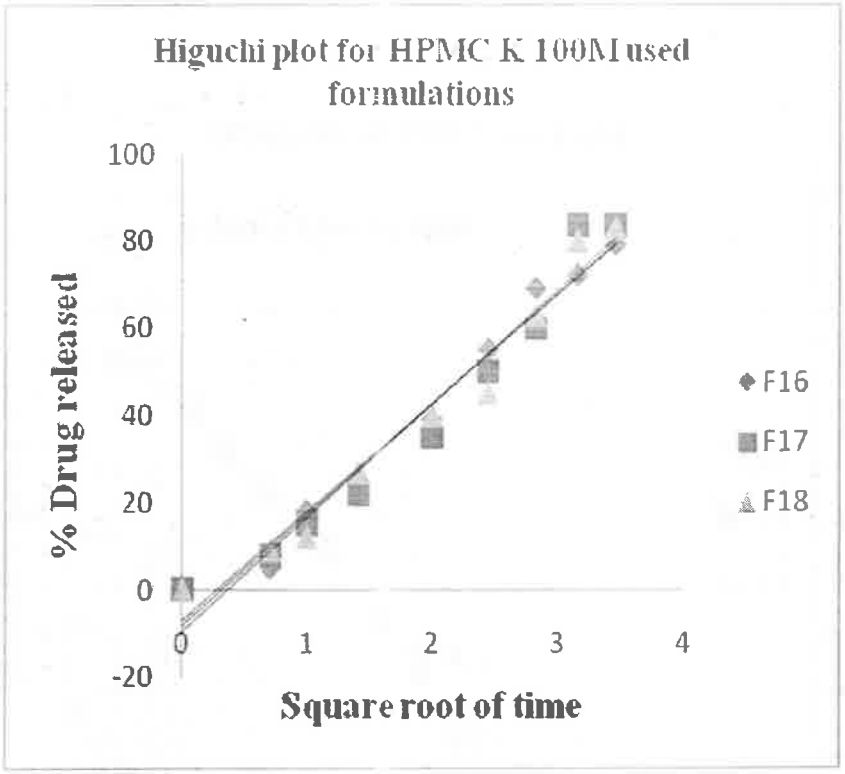


Fig37Higuchi'splot forF16-F18

Time(hrs)	SQRt	F16	F17	F18
0	0	0	0	0
0.5	0.707107	5	8	9
1	1	18	15	12
2	1.414214	25	22	27
4	2	39	35	40
6	2.44949	55	50	45
8	2.828427	69	60	63
10	3.162278	72	84	80
12	3.464102	79	84	83
R^2		0.980	0.956	0.965

Table:37Higuchi'sdataF16-F18

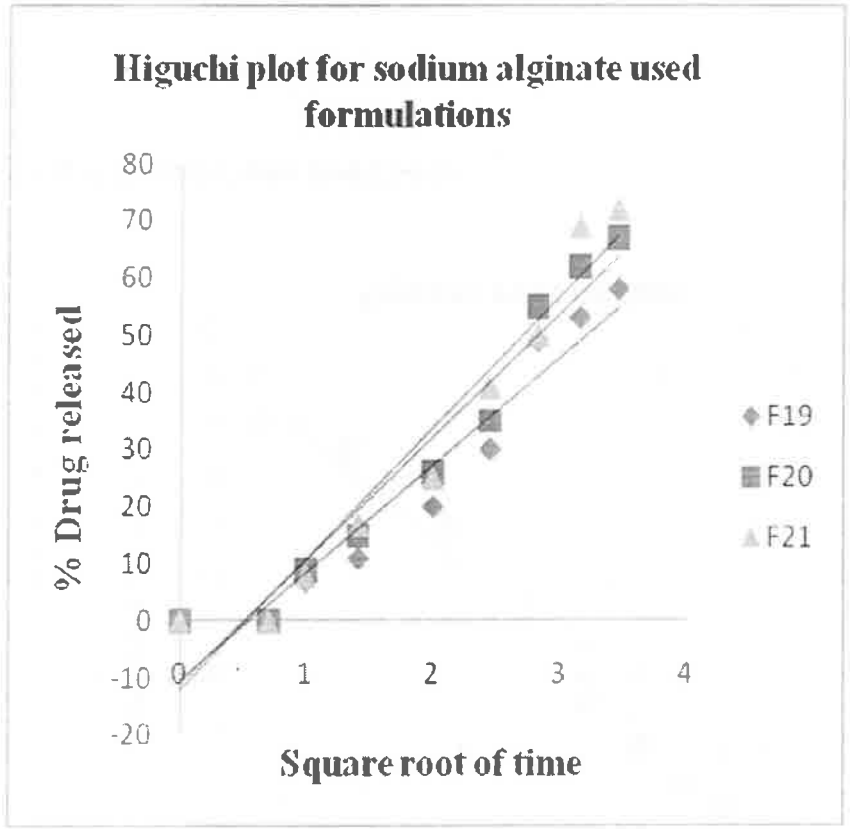


Fig38 Higuchi'splot forF19-F21

Time(hrs)	SQRt	F19	F20	F21
0	0	0	0	0
0.5	0.707107	7	9	8
1	1	11	15	17
2	1.414214	20	26	25
4	2	30	35	41
6	2.44949	49	55	50
8	2.828427	53	62	69
10	3.162278	58	67	72
12	3.464102	66	75	76
R^2		0.931	0.944	0.940

Table:38Higuchi'sdataF19-F21

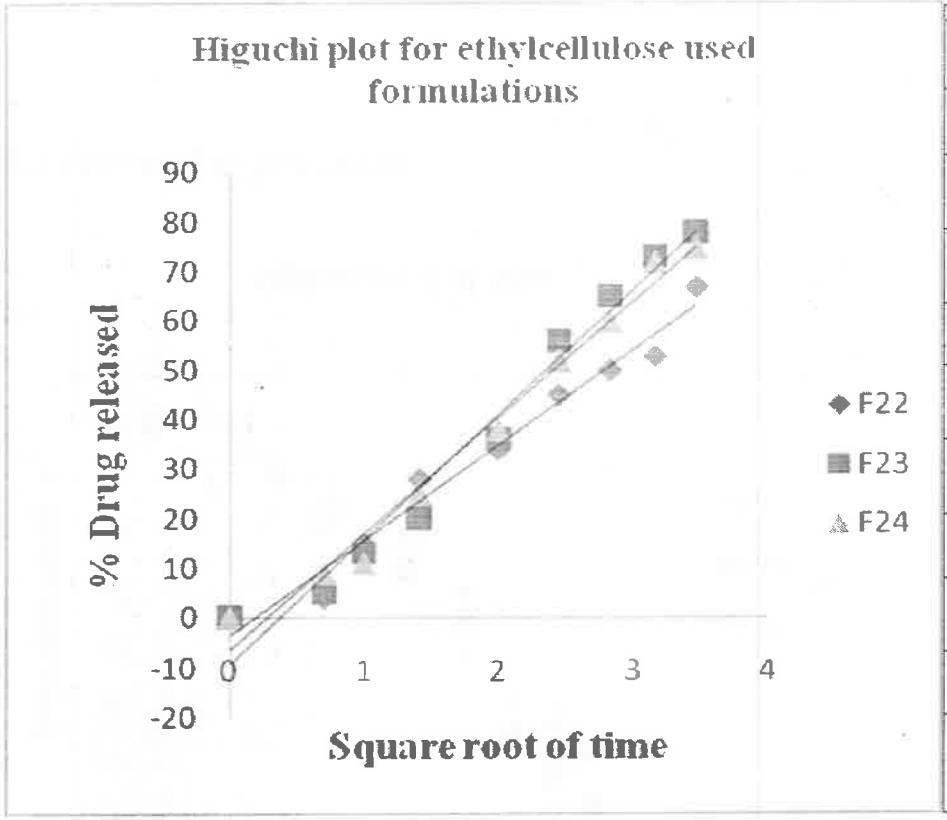


Fig39 Higuchi'splot forF22-F24

Time(hrs)	SQRt	F22	F23	F24
0	0	0	0	0
0.5	0.707107	4	5	9
1	1	15	13	11
2	1.414214	28	20	25
4	2	34	36	38
6	2.44949	45	56	52
8	2.828427	50	65	60
10	3.162278	53	73	72
12	3.464102	67	78	75
R^2		0.975	0.972	0.983

Table:39Higuchi'sdataF22-F24

5.12.3. Peppasdata

Thepreparedempagliflozinformulationsweresubjectedtodissolutionstudiesandthefollowingrepresentspeppaskineticsfor F1-F24formulations

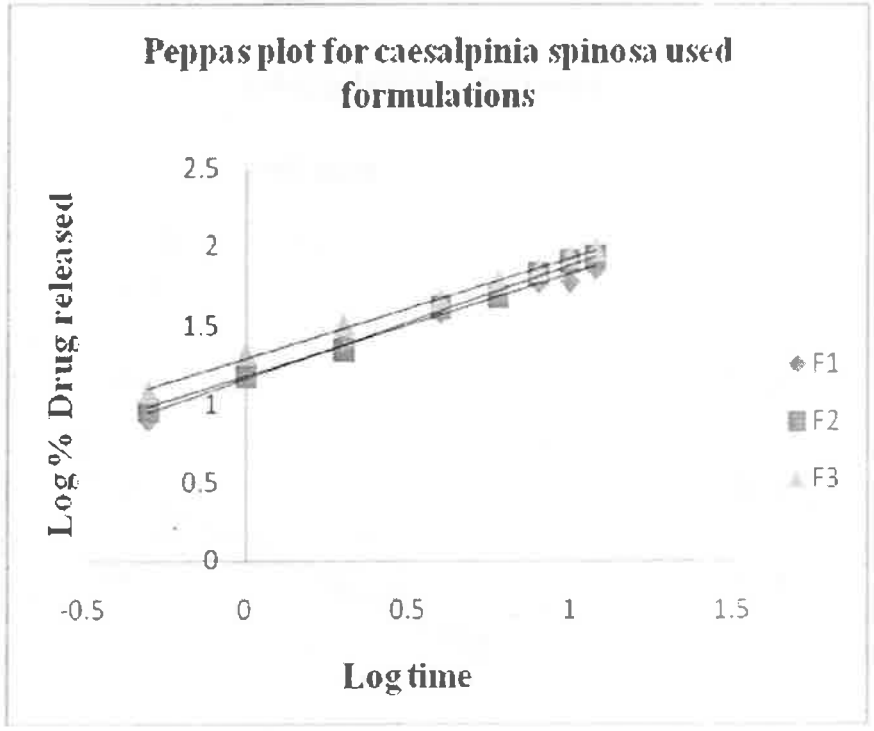


Fig40 Peppasplot forF1-F3

Time(hrs)	F1	F2	**F3**
-0.30103	0.90309	0.954243	**1.079181**
0	1.255273	1.176091	**1.322219**
0.30103	1.39794	1.342423	**1.50515**
0.60206	1.579784	1.612784	**1.662758**
0.778151	1.740363	1.681241	**1.78533**
0.90309	1.770852	1.826075	**1.857332**
1	1.778151	1.90309	**1.939519**
1.079181	1.857332	1.934498	**1.986772**
R^2	0.974	0.995	**0.996**

Table40:Peppasdata forF1-F3

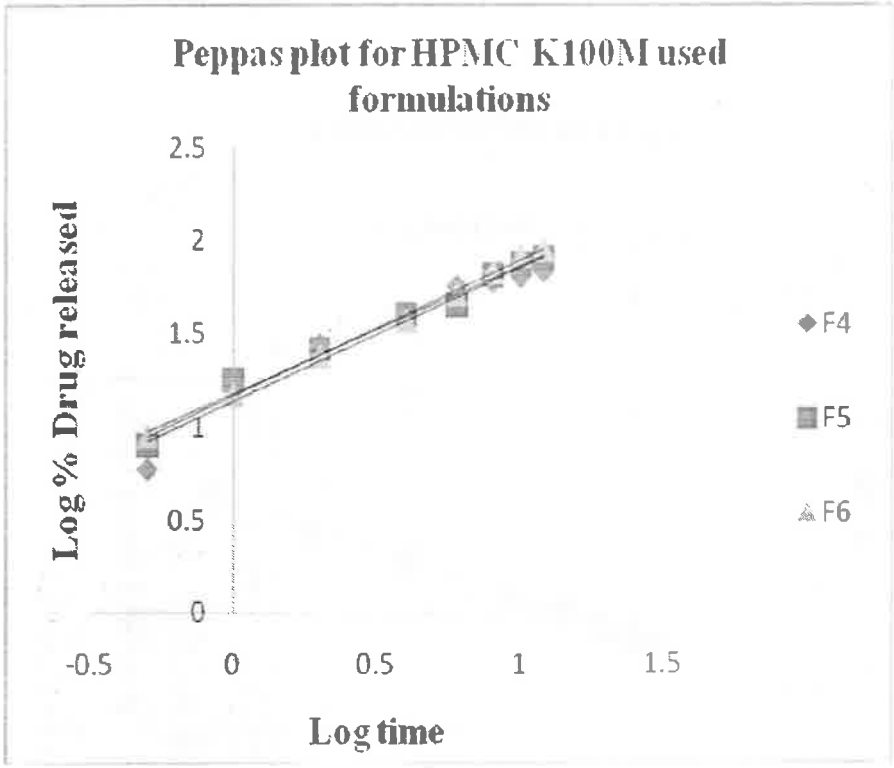


Fig41Peppasplot forF4-F6

Time(hrs)	F4	F5	F6
-0.30103	0.778151	0.90309	0.954243
0	1.230449	1.255273	1.176091
0.30103	1.447158	1.414973	1.380211
0.60206	1.591065	1.60206	1.568202
0.778151	1.755875	1.653213	1.716003
0.90309	1.778151	1.812913	1.832509
1	1.80618	1.875061	1.919078
1.079181	1.832509	1.908485	1.954243
R^2	0.946	0.981	0.997

Table41:PeppasdataforF4-F6

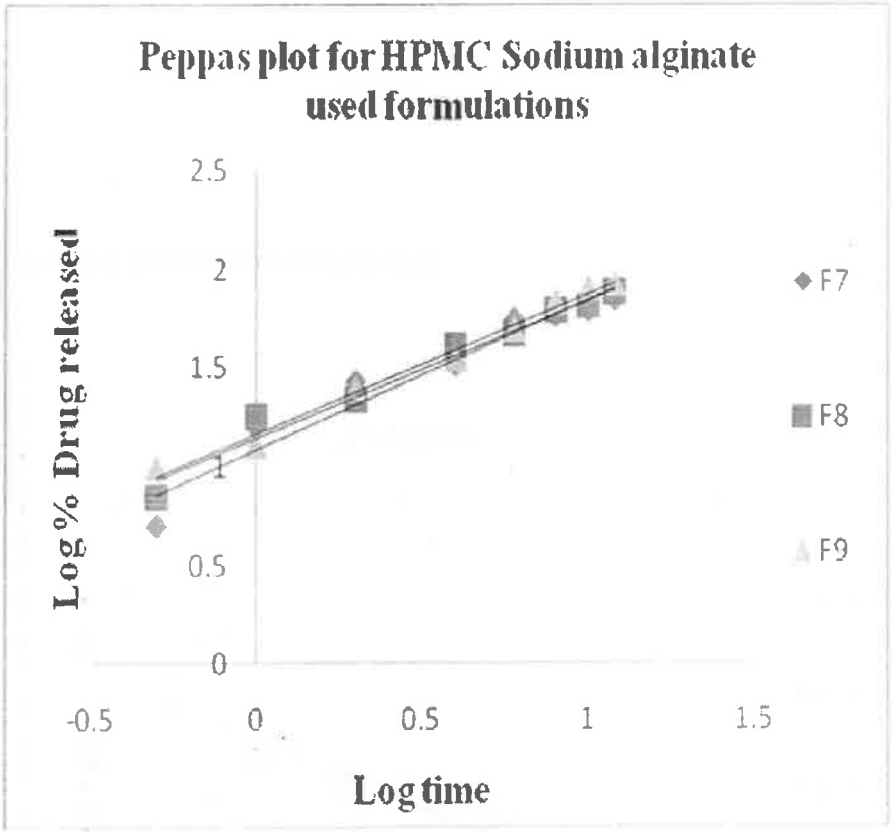


Fig5.91Peppasplot forF7-F9

Time(hrs)	F7	F8	F9
-0.30103	0.69897	0.845098	1
0	1.20412	1.255273	1.113943
0.30103	1.431364	1.342423	1.39794
0.60206	1.518514	1.612784	1.544068
0.778151	1.748188	1.672098	1.69897
0.90309	1.763428	1.792392	1.838849
1	1.792392	1.812913	1.908485
1.079181	1.845098	1.886491	1.929419
R^2	0.939	0.97	0.988

Table42:PeppasdataforF7-F9

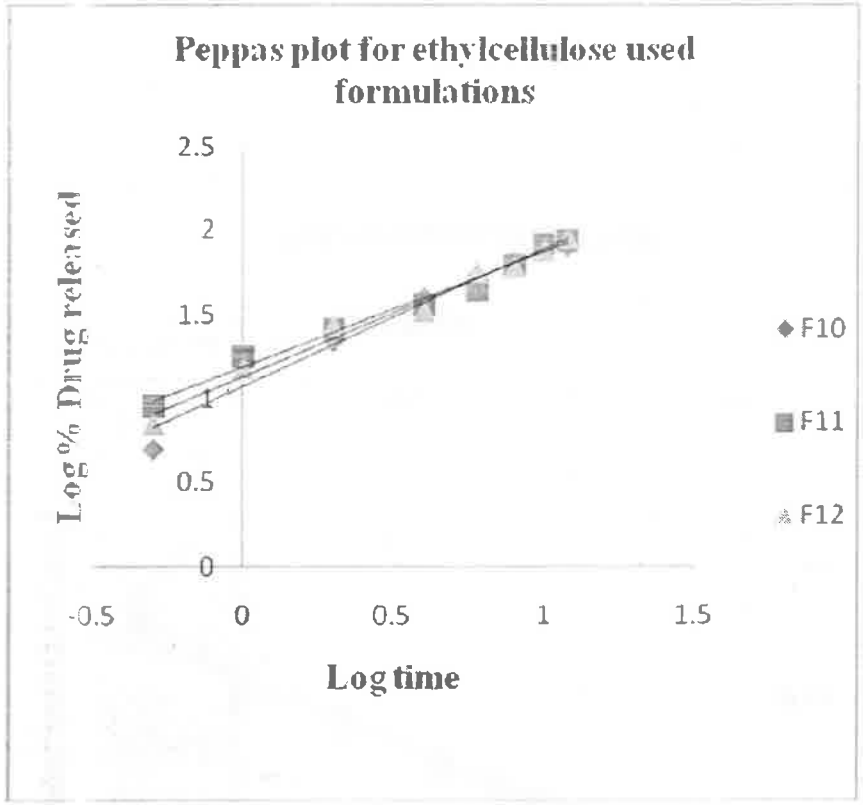


Fig5.92 Peppasplot forF10-F12

Time(hrs)	F10	F11	F12
-0.30103	0.69897	0.954243	0.845098
0	1.20412	1.255273	1.176091
0.30103	1.342423	1.414973	1.431364
0.60206	1.60206	1.556303	1.518514
0.778151	1.732394	1.643453	1.748188
0.90309	1.80618	1.799341	1.778151
1	1.892095	1.919078	1.875061
1.079181	1.908485	1.944483	1.954243
R^2	0.969	0.980	0.982

Table43:PeppasdataforF10-F12

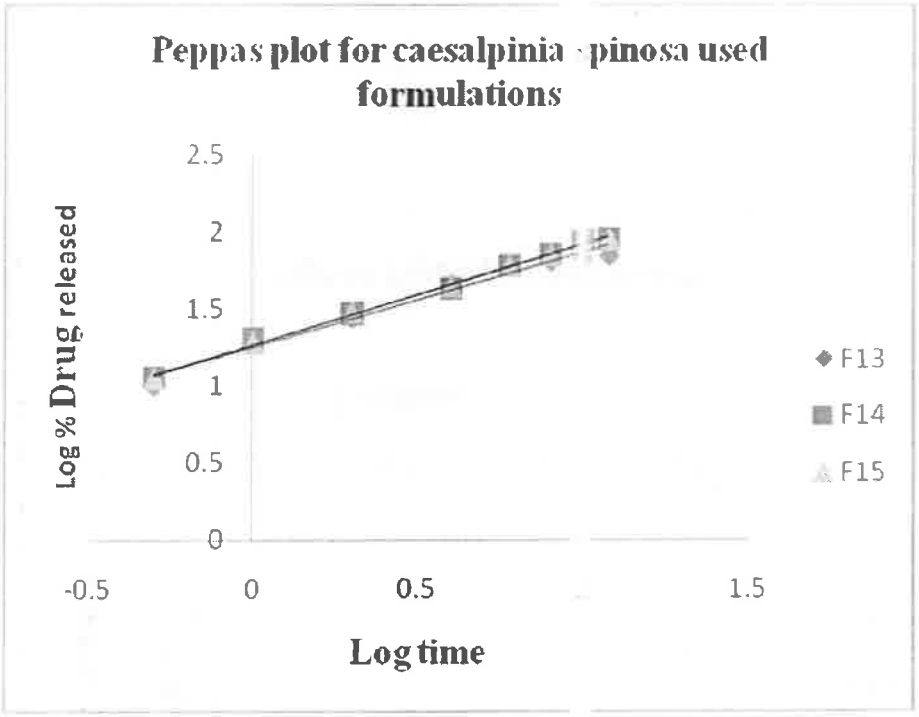


Fig5.93 Peppasplot forF13-F15

Time(hrs)	F13	F14	F15
-0.30103	1	1.041393	1.041393
0	1.278754	1.30103	1.278754
0.30103	1.447158	1.462398	1.477121
0.60206	1.653213	1.623249	1.643453
0.778151	1.778151	1.770852	1.778151
0.90309	1.812913	1.851258	1.845098
1	1.826075	1.934498	1.929419
1.079181	1.845098	1.944483	1.94939
R^2	0.978	0.994	0.997

Table44:Peppasdata-F13-F15

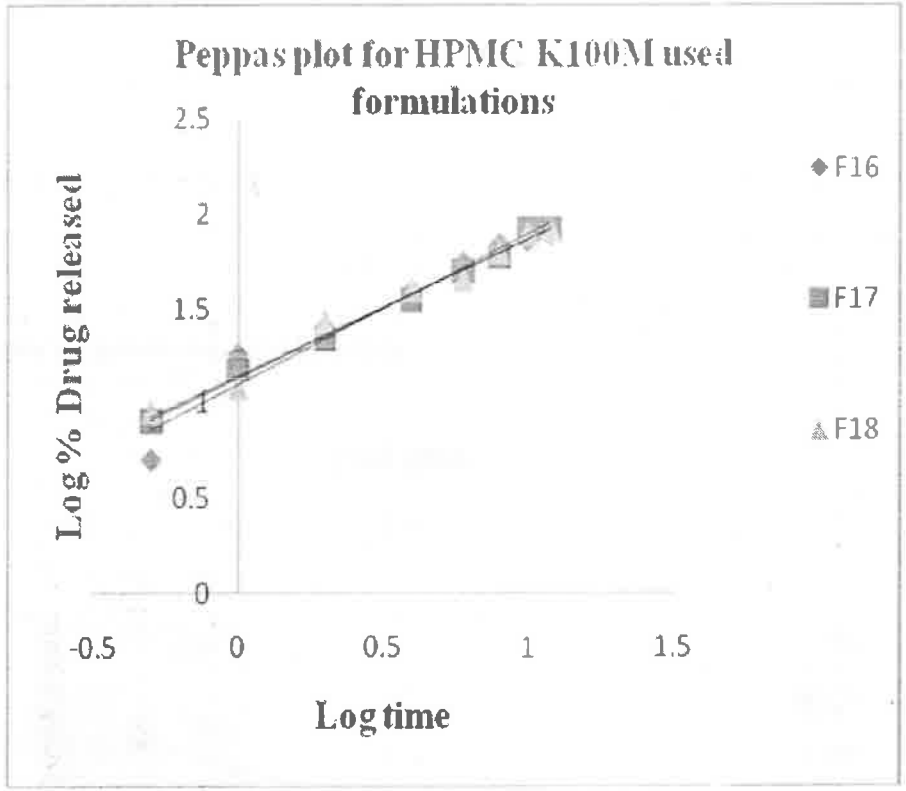


Fig5.94 Peppasplot forF16-F18

Time(hrs)	F16	F17	F18
-0.30103	0.69897	0.90309	0.954243
0	1.255273	1.176091	1.079181
0.30103	1.39794	1.342423	1.431364
0.60206	1.591065	1.544068	1.60206
0.778151	1.740363	1.69897	1.653213
0.90309	1.838849	1.778151	1.799341
1	1.857332	1.924279	1.90309
1.079181	1.897627	1.924279	1.919078
R^2	0.948	0.993	0.984

Table45:Peppasdata-F16-F18

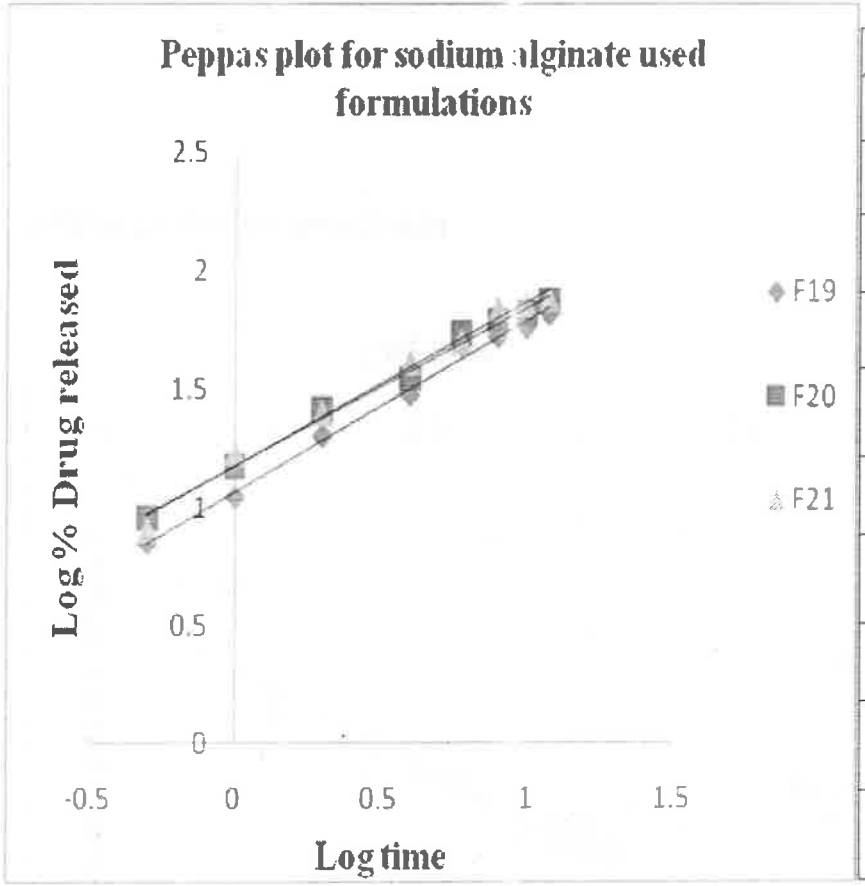


Fig5.95 Peppasplot forF19-F21

Time(hrs)	F19	F20	F21
-0.30103	0.845098	0.954243	0.90309
0	1.041393	1.176091	1.230449
0.30103	1.30103	1.414973	1.39794
0.60206	1.477121	1.544068	1.612784
0.778151	1.690196	1.740363	1.69897
0.90309	1.724276	1.792392	1.838849
1	1.763428	1.826075	1.857332
1.079181	1.819544	1.875061	1.880814
R^2	0.992	0.992	0.986

Table46:Peppasdata F19-F21

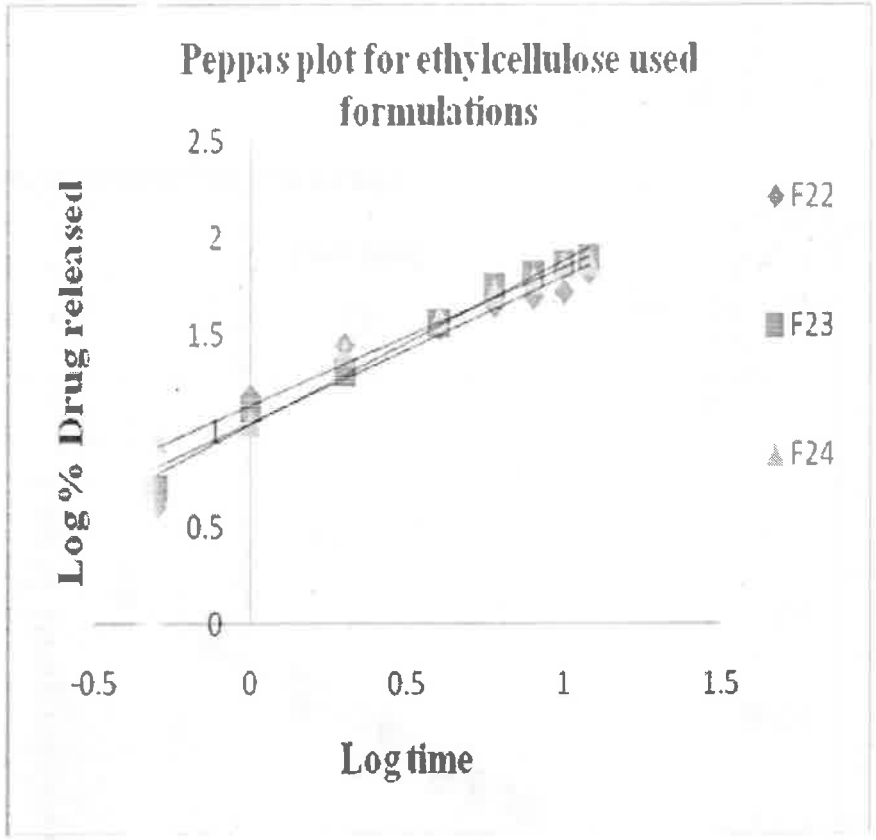


Fig5.96 Peppasplot forF22-F24

Time(hrs)	F22	F23	F24
-0.30103	0.60206	0.69897	0.954243
0	1.176091	1.113943	1.041393
0.30103	1.447158	1.30103	1.39794
0.60206	1.531479	1.556303	1.579784
0.778151	1.653213	1.748188	1.716003
0.90309	1.69897	1.812913	1.778151
1	1.724276	1.863323	1.857332
1.079181	1.826075	1.892095	1.875061
R^2	0.907	0.984	0.984

Table47:Peppasdata-F22-F24

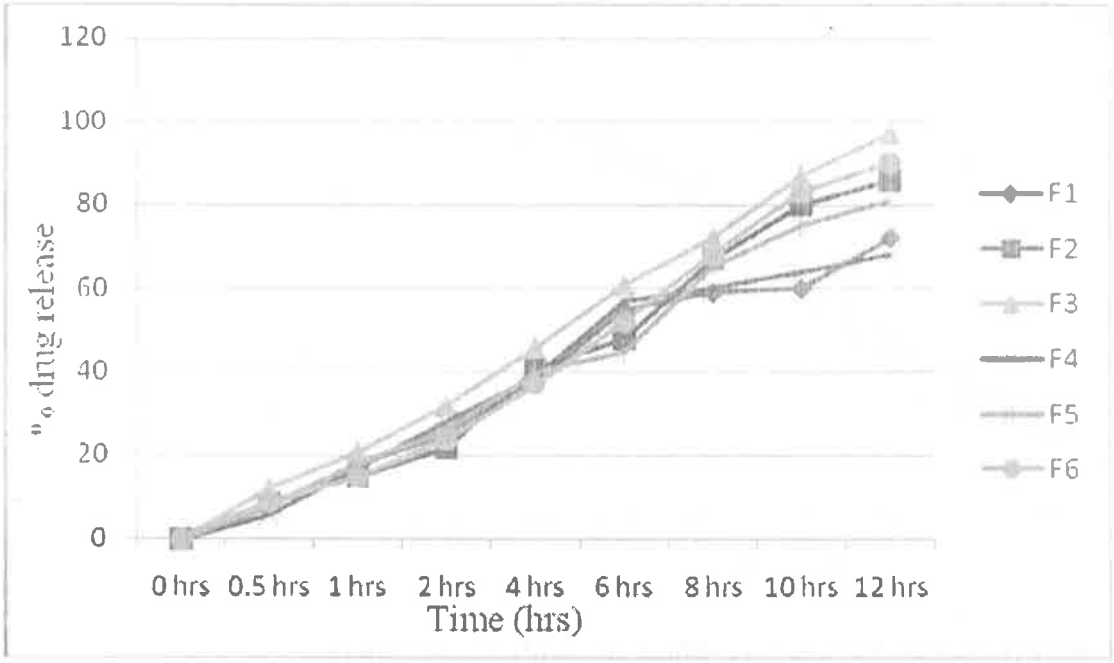


Fig49:DissolutionprofileforempagliflozinformulationsF1-F6

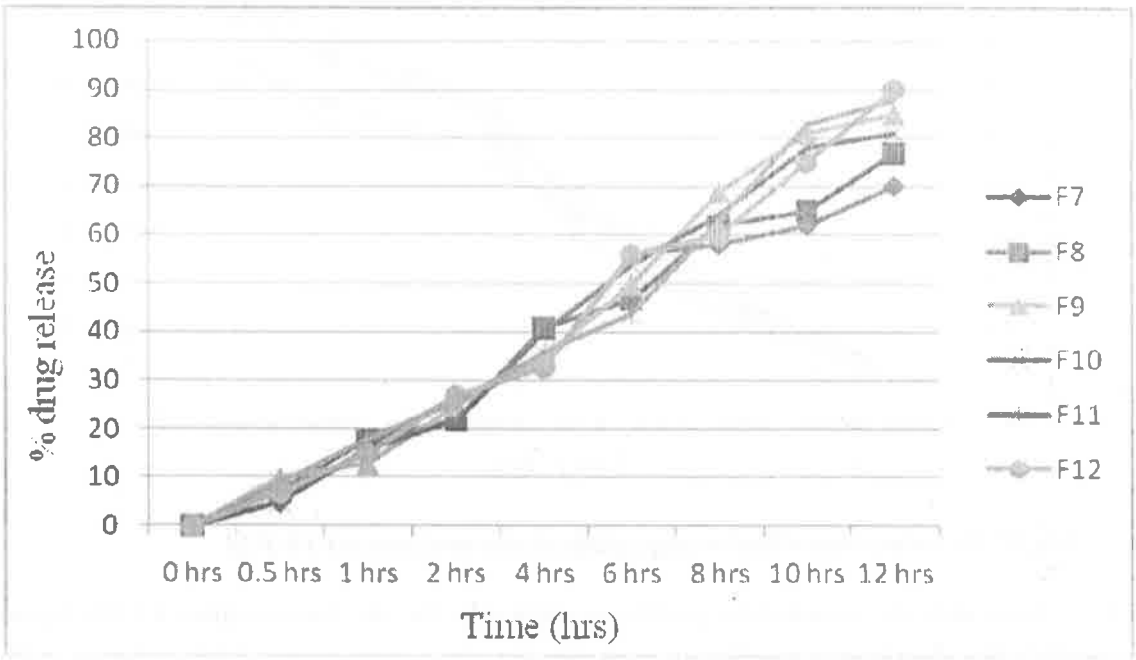


Fig50:DissolutionprofileforempagliflozinformulationsF7-F12

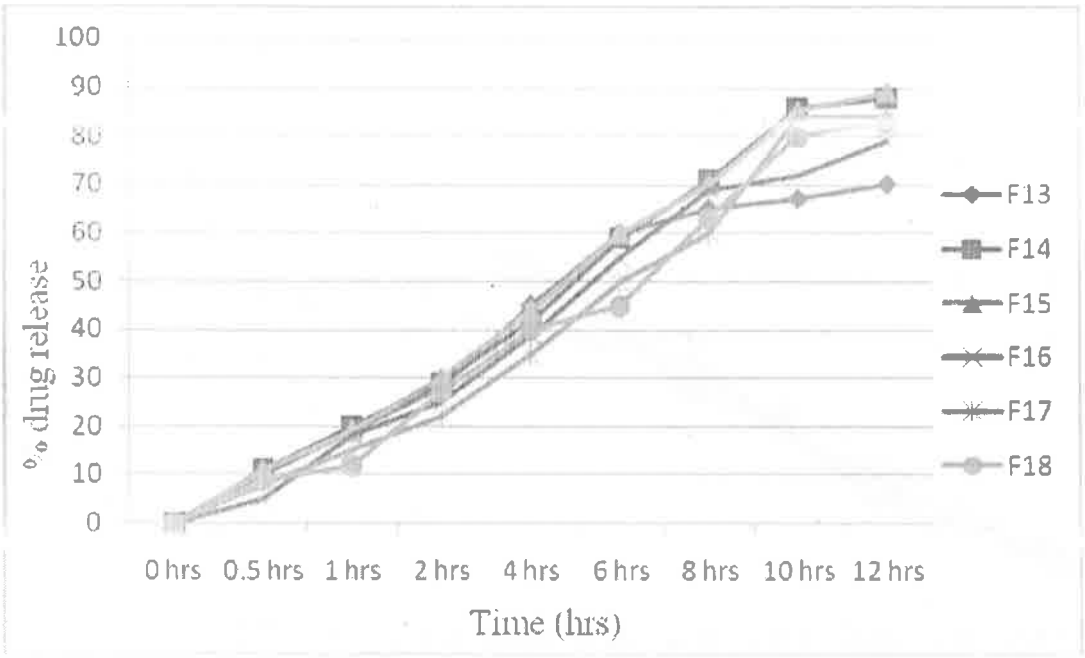


Fig51:DissolutionprofileforempagliflozinformulationsF13-F18

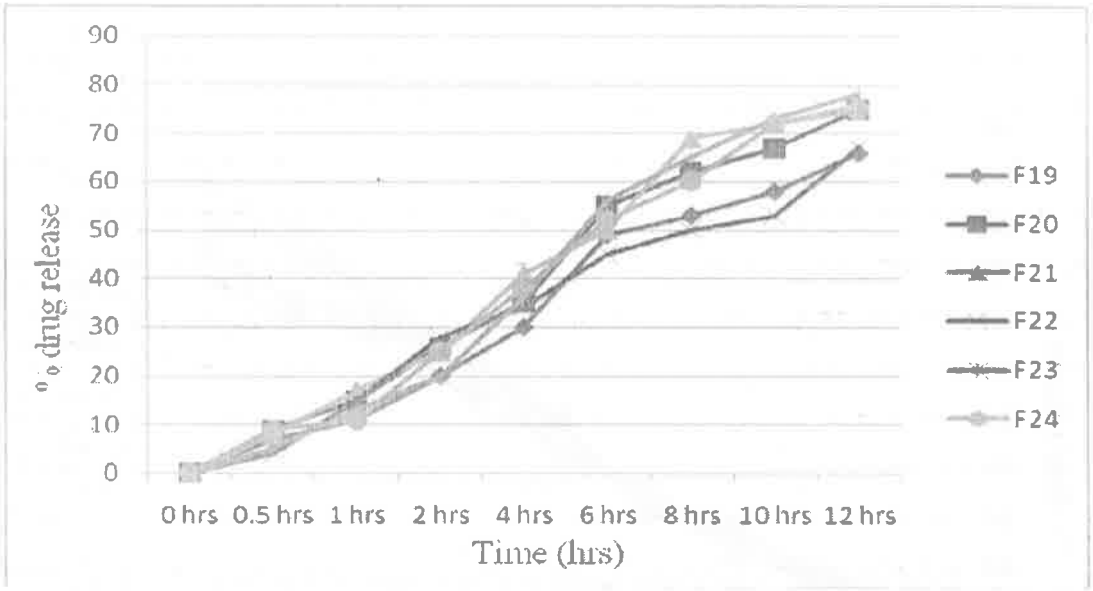


Fig52:DissolutionprofileforempagliflozinformulationsF19-F24

Figure 5.97 illustrates the dissolution profile of glipizide for the formulations F1-F6,figure 5.98 illustrates the dissolution profile of glipizide for the formulations F7-F12,figure 5.99 illustrates the dissolution profile of glipizide for the formulations F13-F18and figure 5.100 illustrates the dissolution profile of glipizide for the formulationsF19-F24.

5.12.4. Kineticstudies:

Drug release profiles were fitted into various kinetic equations and the values aregiven in table: 5.90. The 'n' value for the optimized formulation F3 was found to be0.637. To know the mechanism of drug release from these formulations, the data weretreated according to zero order (cumulative amount of drug released vs time), first-order (log cumulative percentage of drug remaining vs time), Higuchi's (cumulativepercentage of drug released vs square root of time), and Korsmeyer (log cumulativepercentage ofdrugreleasedvslogtime)equations.

Table48:Drugreleasekineticprofileofempagliflozintablets

Formulationcode	Zeroorder	Firstorder	Higuchi	Peppas	nvalue
F1	0.966	0.984	0.991	0.987	0.650
F2	0.993	0.986	0.985	0.997	0.718
F3	**0.993**	**0.943**	**0.993**	**0.998**	**0.637**
F4	0.949	0.979	0.988	0.972	0.715
F5	0.998	0.990	0.988	0.990	0.686
F6	0.996	0.979	0.983	0.998	0.727
F7	0.959	0.983	0.986	0.969	0.757
F8	0.983	0.992	0.991	0.986	0.700
F9	0.992	0.987	0.982	0.994	0.710
F10	0.985	0.995	0.989	0.984	0.824
F11	0.991	0.966	0.975	0.990	0.678
F12	0.990	0.959	0.981	0.991	0.753
F13	0.944	0.973	0.988	0.988	0.613
F14	0.988	0.987	0.992	0.997	0.649
F15	0.988	0.989	0.994	0.998	0.654
F16	0.976	0.996	0.990	0.974	0.796
F17	0.990	0.968	0.977	0.996	0.735
F18	0.988	0.981	0.982	0.992	0.721
F19	0.978	0.981	0.988	0.996	0.723
F20	0.979	0.992	0.992	0.996	0.668
F21	0.976	0.995	0.991	0.993	0.695
F22	0.962	0.980	0.987	0.952	0.762
F23	0.980	0.998	0.986	0.992	0.848
F24	0.983	0.997	0.991	0.992	0.716

Inference: The value of 'n' was found to be 0.637, which indicates that the drugrelease was followed by anomalous (non-fickian) diffusion. The significance of thestudy was to determine the order of kinetics and was concluded that it followed zeroorderkinetics withreferencetotable:4.9.

5.9. Acomparativestudyofempagliflozinoptimizedwithmarketedtablet

The optimized formulation was compared to that of marketed formulation (Jardiance)andthe%drugrelease was notedfor12hours.

Table49:Comparisonofdissolutiondataofformulation*F3*withthecorrespondingmarketedtablets(Jardiance-10mg)

Time(hrs)	%Drugreleaseofmarketed tabletcontaining empagliflozin	%Drug releaseforthebestformulation, *F3* *(Presentstudy)*
0	0	0
0.5	10±0.14	12±0.02
1	18±0.23	21±0.31
2	30±0.19	32±0.37
4	45±0.34	46±0.41
6	58±0.15	61±0.36
8	71±0.18	72±0.23
10	84±0.21	87±0.09
12	97±0.19	97±0.17

Eachvaluerepresentsthemean±standarddeviation(n=3)

The % drug release for marketed formulation was found to be 97±0.19 and for theoptimizedformulation,F3the%drugreleasewasfoundtobe97±0.17.Thedissolution study was comparable with the marketed tablet and the satisfactory resultswere obtained. From the *in vitro* studies the works have been extended to the nextphase.

5.10.1. FT-IRstudies

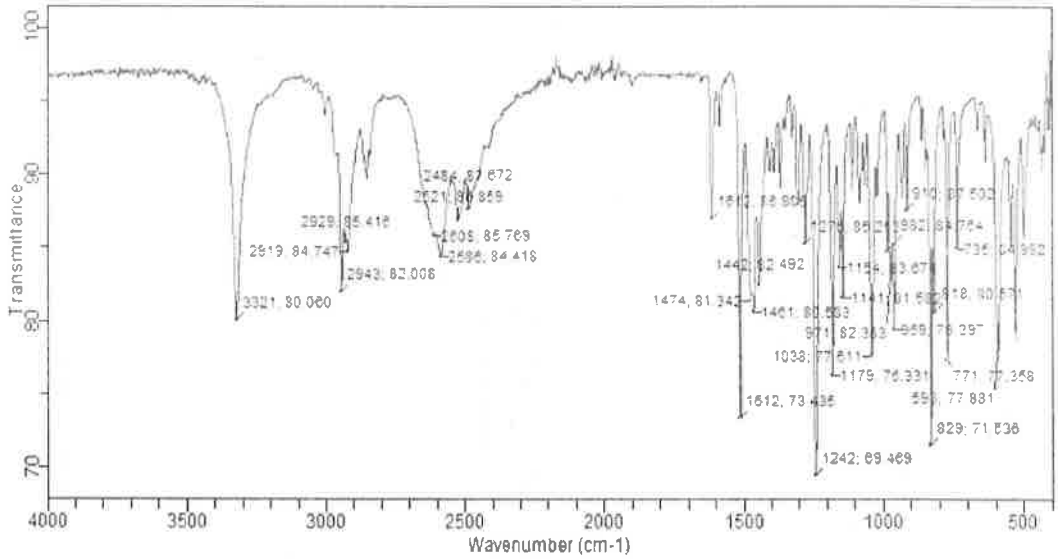


Fig53: FTIRspectraforbestfromulation-F3

Inference: Thephysicalmixtureshowedidenticalspectrumwithrespecttothespectrum of the pure drug,indicating there is nochemical interactionbetween thedrug molecule and polymer used. Results indicated that drug is compatible with thepolymersusedintheinvestigation.

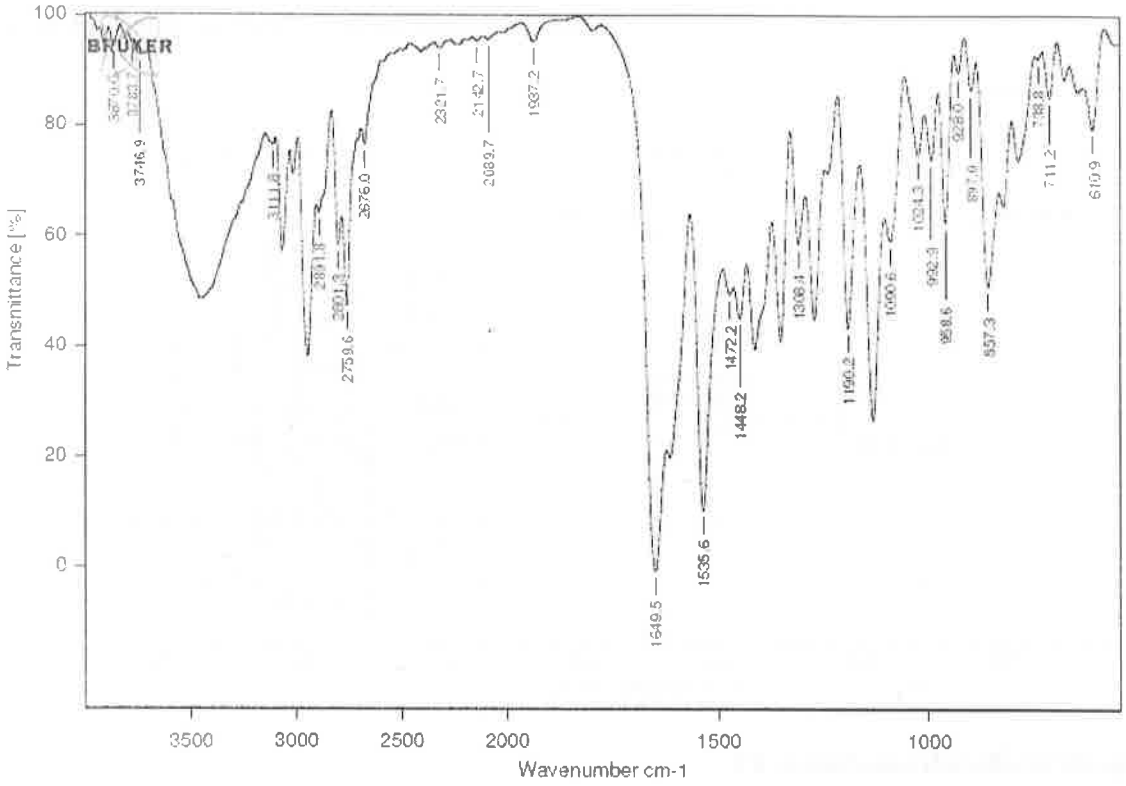


Fig54:FTIRspectraforpureempagliflozinTable 5.92:Pure empagliflozin

Wavenumberincm^{-1}	Characteristicbond
610.9	C-X(Bromide)
857.3	C-H(Aromaticoutofplanebend)
1536.6	C=C(Aromatic)
1649.5	C=C(Alkene)
2795.6	C-H(Alkanesstretch)

Inference:

The IR spectra of pure empagliflozin showed all the principal IR absorption peaks atwave numbers 610 cm^{-1}, 857 cm^{-1}, 1536.6 cm^{-1}, 1649.5 cm $^{-1}$, 2795.6 cm^{-1}respectivelyconfirmingthepurityofthedrug.

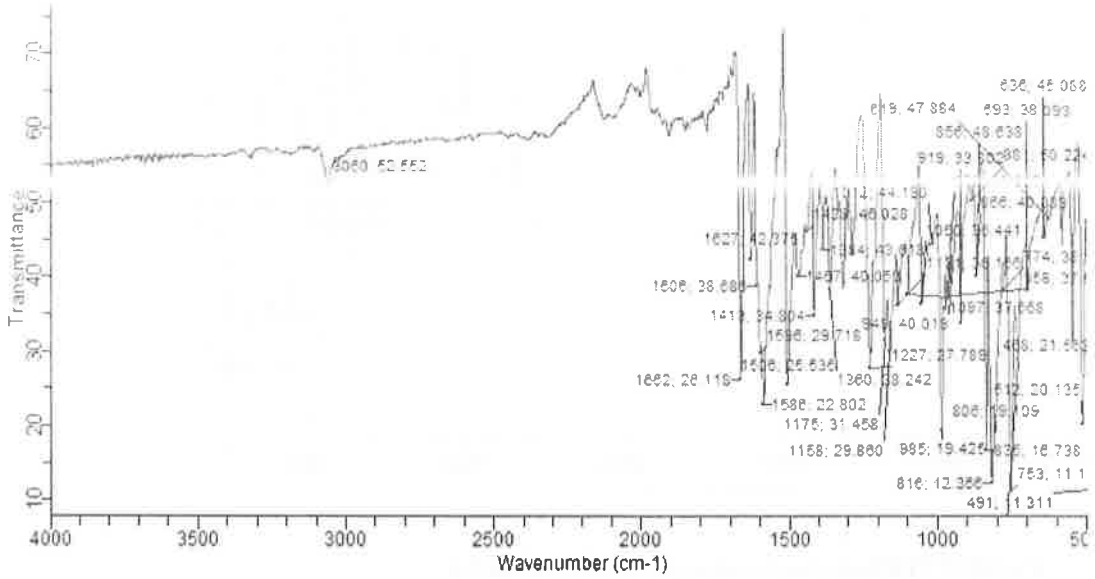


Fig55:FTIRspectrafordrug+ethylcellulose

INFERENCE

TABLE5.93:FTIRspectraanalysisfordrug+ethylcellulose

Wavenumberincm^{-1}	Characteristicbond
753:11	C-X(Chloride)
985:19	Aromatic-outofplane bend
1586:22	Aromatic
1662:26	Amide

Inference: The FTIR spectrum of pure drug showed characteristic amide peaks atwave numbers C-X at 610 cm^{-1}, C-H aromatic plane bend at 857 cm^{-1}, C=C at 1536.6cm $^{-1}$, 1649.5 cm $^{-1}$,. There were no new bands observed in the spectrum, whichconfirmsthatnonewchemicalbondswereformedbetweenthedrugandthepolymer.

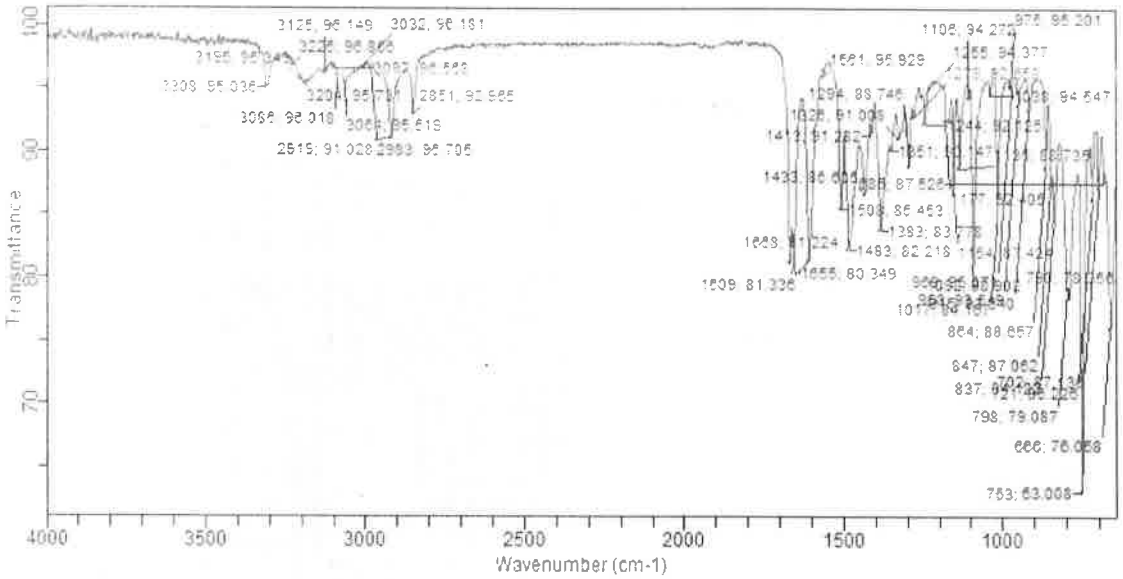


Fig56:FTIRSpectrafordrug+HPMCK100M

INFERENCE

Table5.94:FTIR spectraanalysisfordrug +HPMCK100M

Wavenumberincm^{-1}	Characteristicbond
753:63	C-Cl(Stretch)
864:88	C-C(Stretch)
1154:87	C-F(Stretch)
1383:83	C-H(Bendinplane)
1483:82	O-H(Stretch)
1655:80	C=N(Stretch)

Inference: FT-IR studies were carried out to know the compatibility. FT-IR resultsrevealed that there was no significant difference in the peaks of drug and HPMCK100M in the formulation compared to pure drug as shown in figure 5.104. It wasfound that there was no interference to the drug with excipients and polymer used inthe formulations.

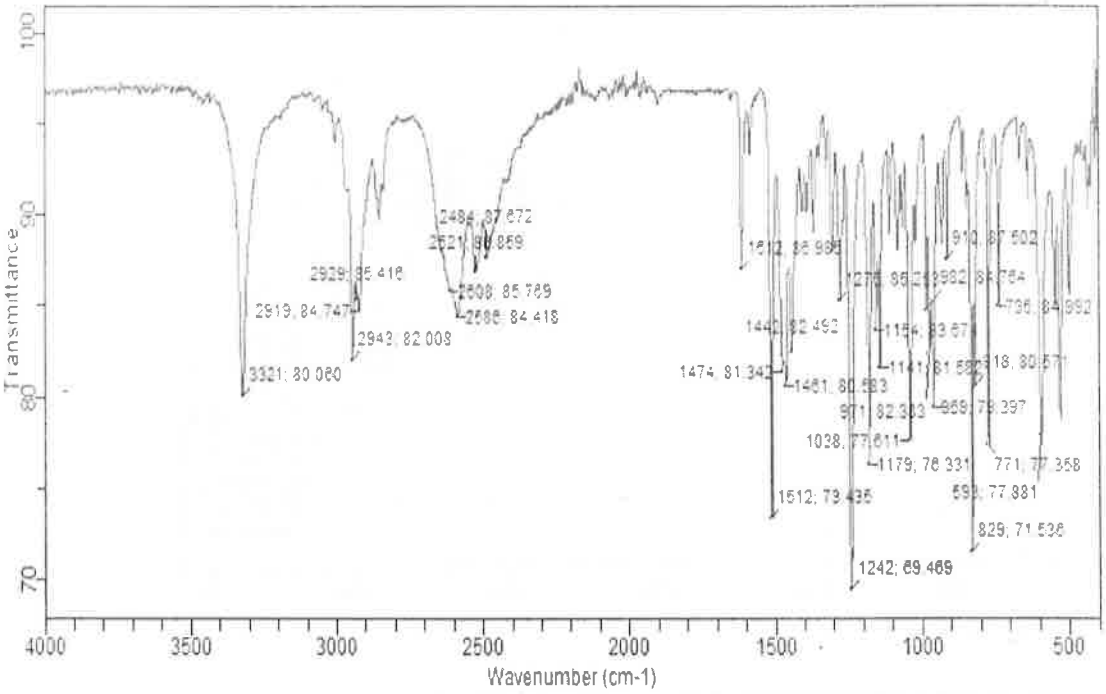


Fig57:FTIRspectrafordrug+*Caesalpiniaspinosa*

INFERENCE

The IR spectral analysis of empagliflozin alone showed that the principal peaks wereobserved at wave numbers at 610 cm^{-1}, 857 cm^{-1}, 1536.6 cm^{-1}, 1649.5 cm^{-1}, 2795.6cm^{-1}respectively confirming the purity of the drug. In the IR spectra of the physicalmixture of empagliflozin and caesalpinia spinosa the major peaks were at 829.71,1179.76, 1512.73 and 1612.8 cm^{-1}. However, some additional peaks were observedwith physical mixtures, which could be due to the presence of polymer and doesnoteffecttheefficacyofthedrug.

Table5.95:FTIRspectraanalysisfordrug+*Caesalpiniaspinosa*

Wavenumberincm^{-1}	Characteristicbond
598:77	C-Br (Stretch)
829:71	C-C(Stretch)
1179:76	C-C(Stretch)
1512:73	N-H(Bending)
1474:81	C-H(Bendoutofplane)
1612:86	C=N(Stretch)

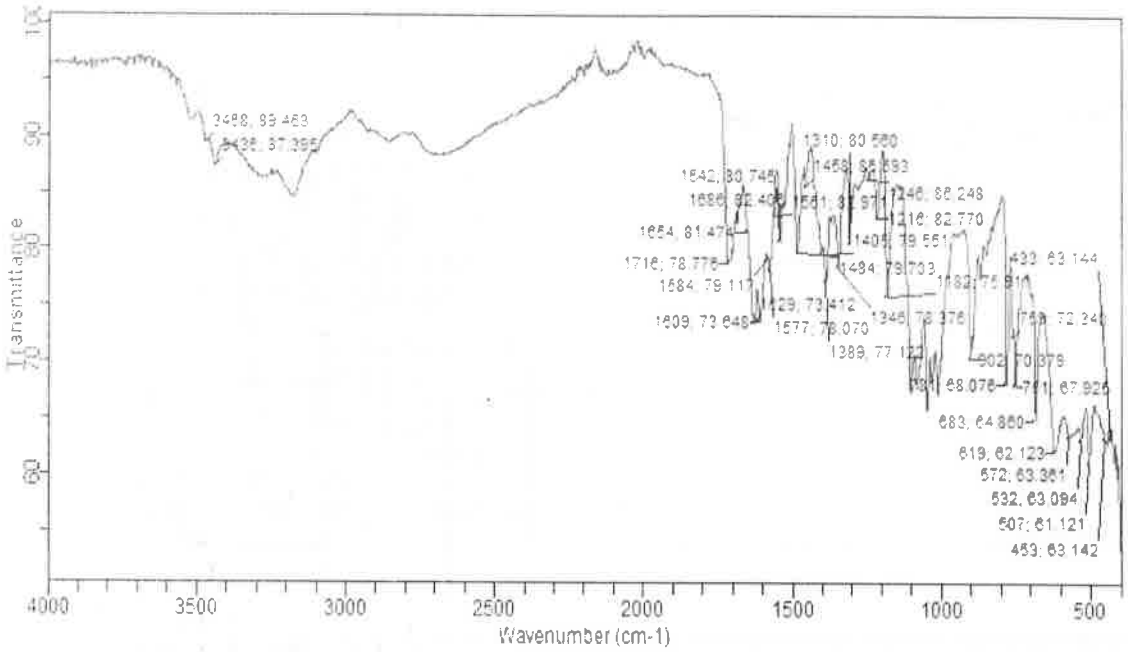


Fig5.8:FTIRSpectrafordrug+sodiumalginate

INFERENCE

Table5.96:Drug+sodiumalginate

Wavenumberincm^{-1}	Characteristicbond
453:63	C-I(Stretch)
619:62	C-Cl(Stretch)
683:64	C-H(Rocking)
1389:77	C-H(Bendinoutofplane)
1577:78	N-H(Bending)
1609:73	C-C(Stretch)
1716:78	C=O(Stretch)

Inference: The FT-IR spectrum showed many intense, absorption peaks that are dueto the different functional groups present in the molecules. In the IR spectra wavenumber of 453.63 cm^{-1} disclosed the presence C-I stretching, the wave number of1716.78 cm^{-1} showed the presence of C=O stretching, the wave number 1577.78 cm^{-1}showed the presence of N-H bending, the wave number 1609.73 cm^{-1} indicated thepresence of C-C stretch indicating thatalmostsame peaks weremaintained withrespect tothepuredrug,thusindicatingthat thepolymeriscompatiblewiththedrug.

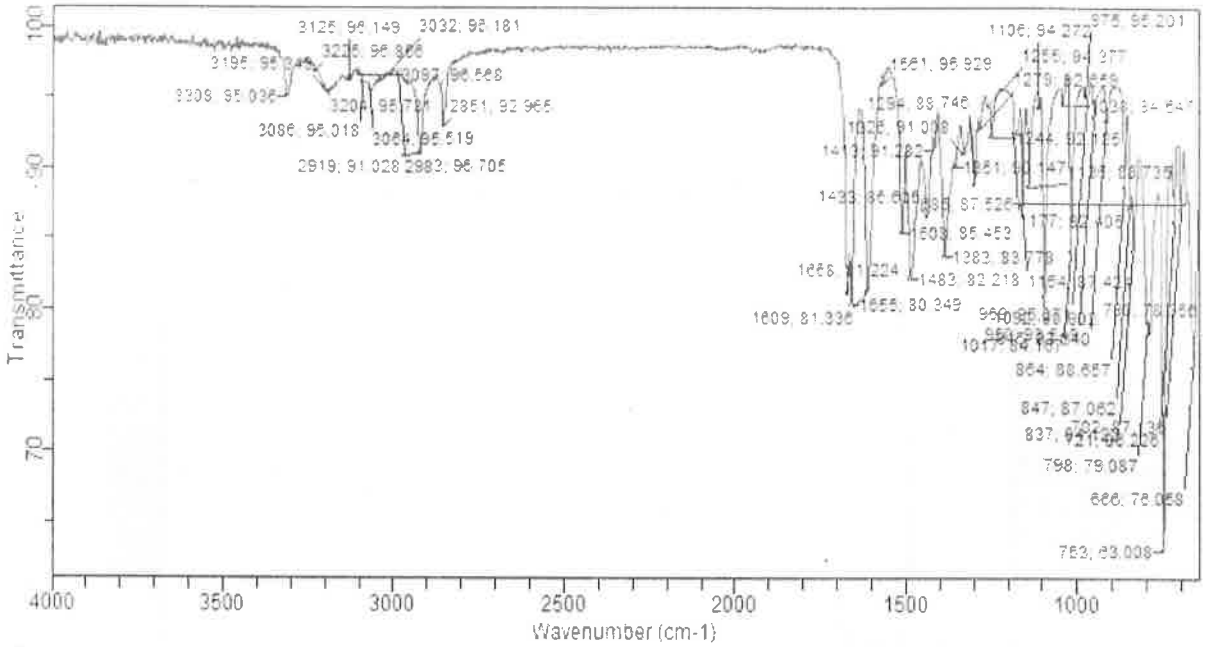


Fig59: FTIRspectrafordrug+magnesium stearate

INFERENCE

Table5.97:Drug+magnesiumstearate

Wavenumberincm^{-1}	Characteristicbond
753.63	C-X(Chloride)
798:79	C-H(Bendoutofplane)
1017:84	C-F(Stretch)
1383:83	C-F(Stretch)
1668:81	C-C(Stretch)
1609:81	C-C(Stretch)

Inference: Physical mixture of drug and magnesium stearate was characterized byFTIRspectralanalysisforphysicalaswellaschemicalalterationofthedrugcharacteristics.Fromth ewavenumbers,itwasconcludedthattherewasnointerference of the functional groups as the principal peaks of the drug were unalteredindrugpolymerphysical mixtures,indicatingtheywerecompatiblechemically.

5.10.2. XRDstudies

TheXRDstudieswereperformedforbestformulationandforpureempagliflozinwhichwereillustrate dinfig:5.108 and5.109respectively.

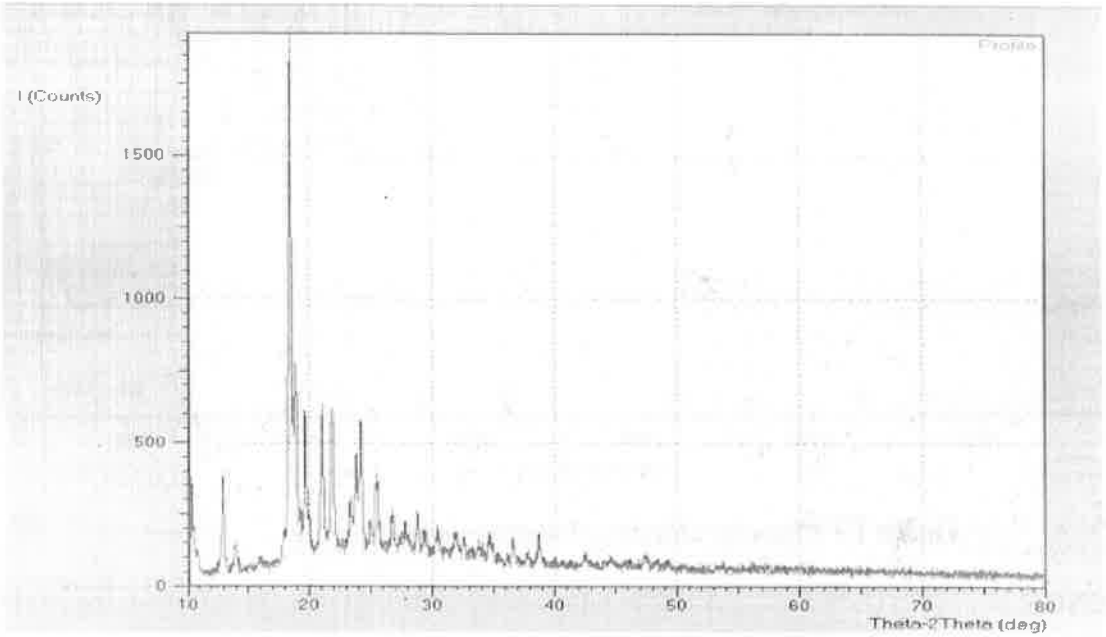


Fig60: X-raydiffractionspectrabest formulationF3

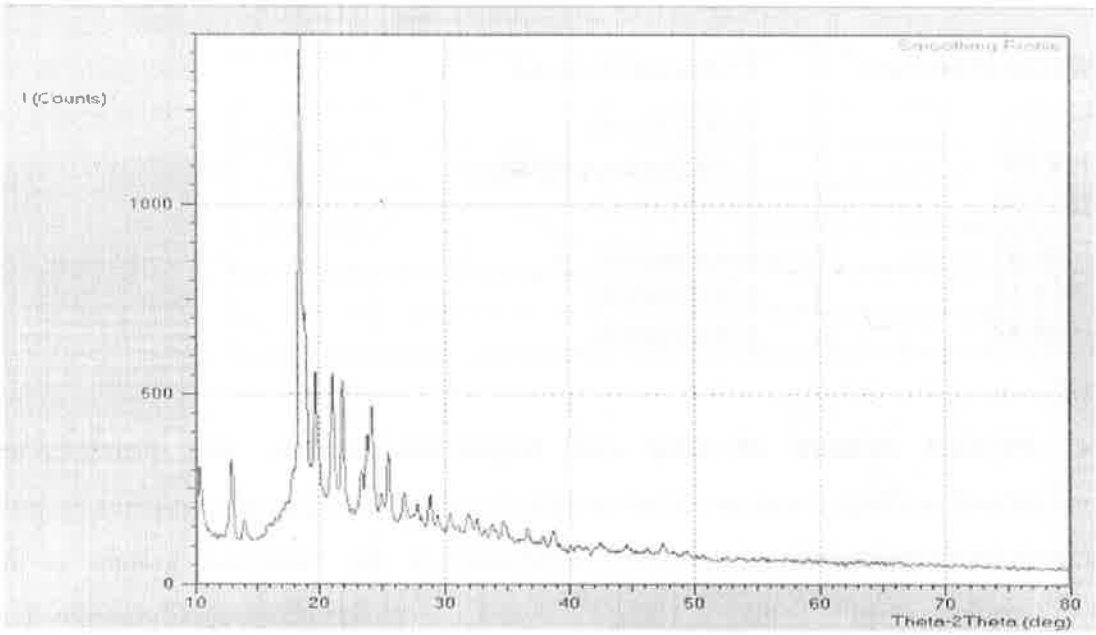


Fig61:X- raydiffractionspectraforpureempagliflozin

Inference:theX-

raydiffractionspectraofpuredrugexhibitspeaksat2θanglethatshowedatypicalcrystallinepattern.

5.10.3. SEMresults

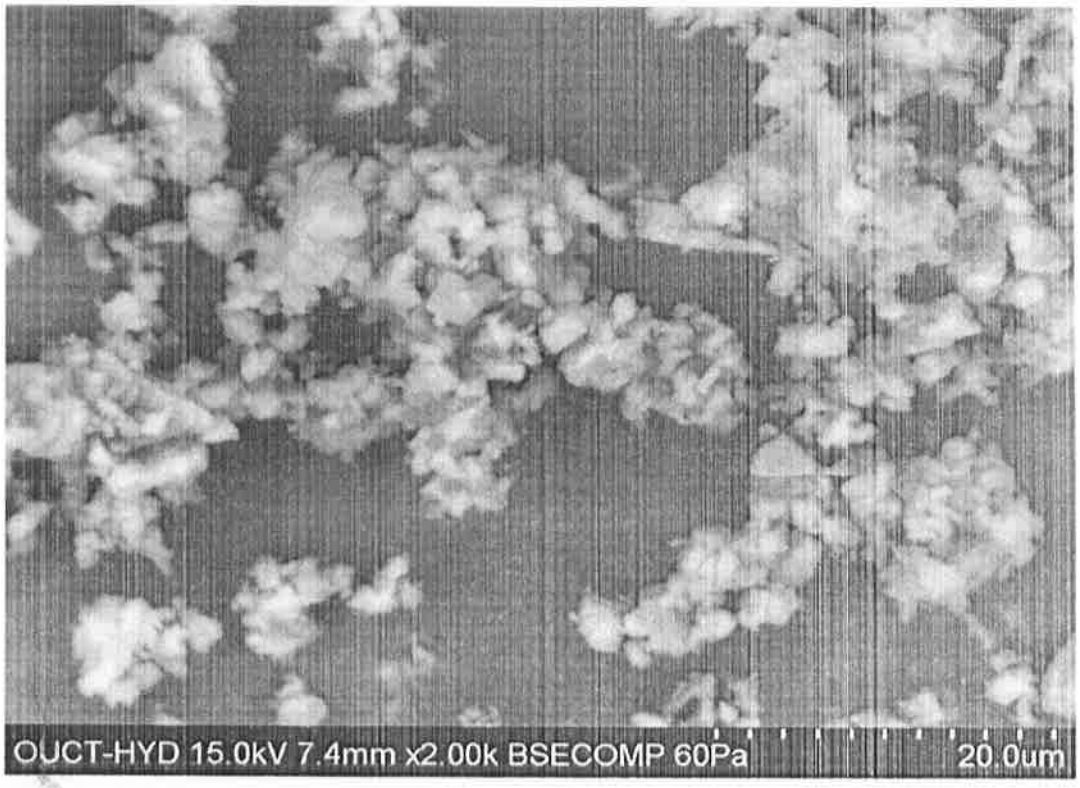
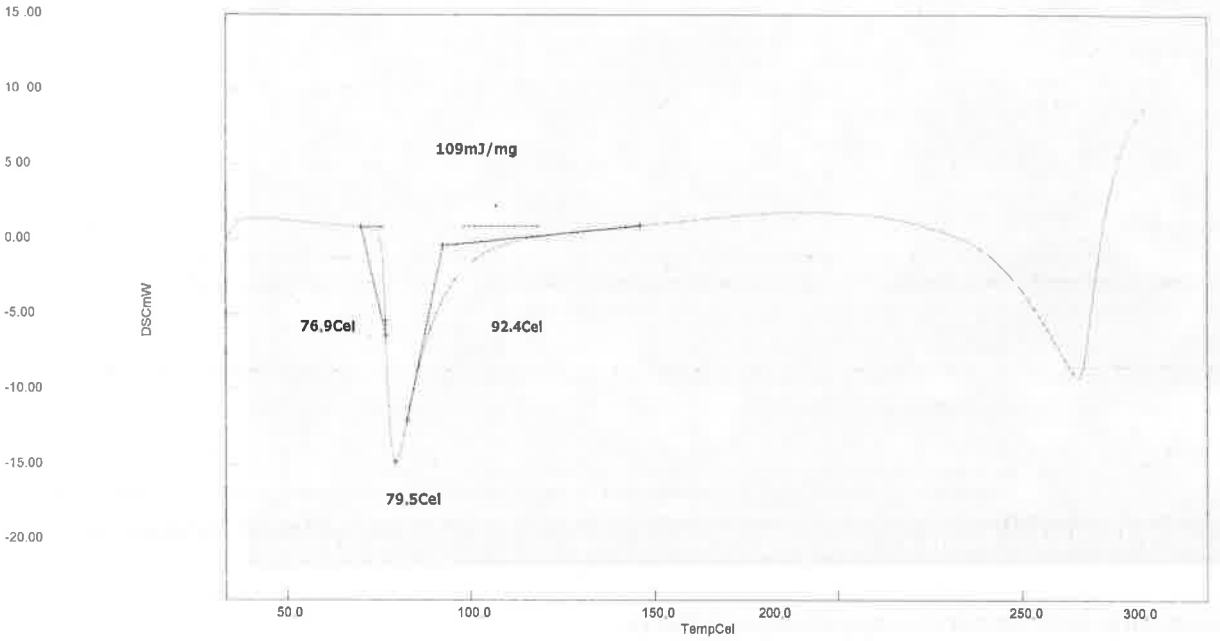


Fig62:Scanningelectronmicroscopyofempagliflozin

Crystals of puresampleare of smallestsize(4-10μm)andhaveirregularshapes.Recrystallizationproductcrystalshaveintermediatesize(9-15μm).Theagglomerates were formed by coalescence of the microcrystalline precipitates, so theagglomerates had a rugged surface. Agglomerates obtained were spherical in its shapewithsize198μm-670μm.[20,21]

5.10.4. DSCspectralstudies

The DSC studies were performed for pure drug and optimized formulation whichwereillustratedinFig:5.111and5.112respectively.



F63 DSCofpureempagliflozin

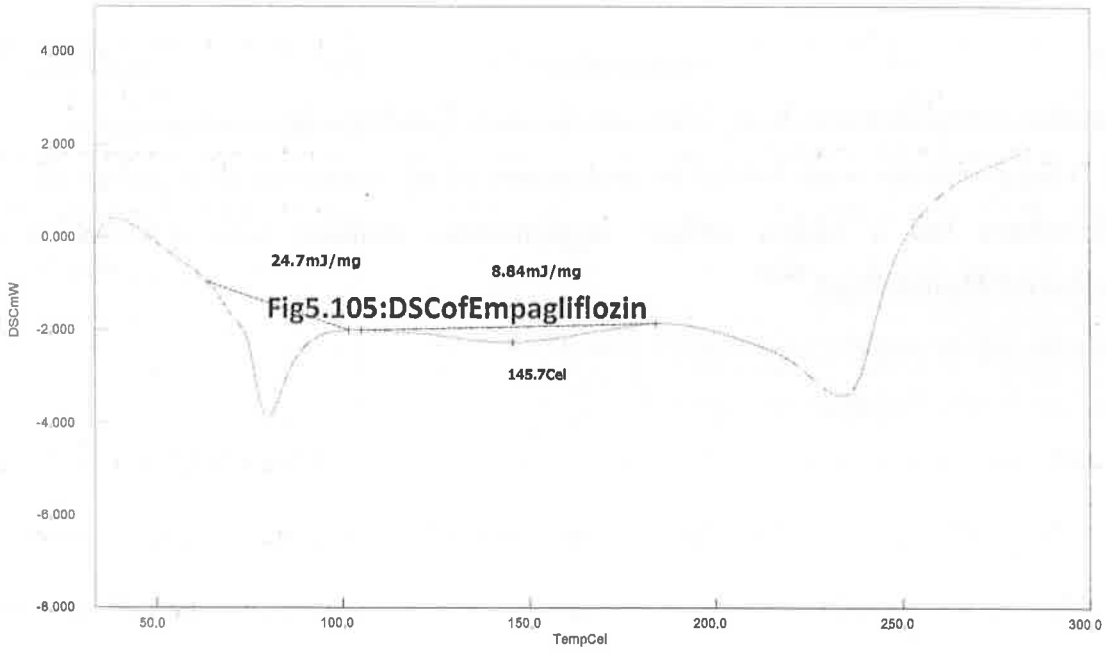


Fig5.105:DSCofEmpagliflozin

Fig64DSCofbestformulation-F3

Table50CompatibilityprofileofempagliflozinanditsexcipientswithrespecttoDSC

Sample	Appearanceof newpeaks	Fadeof existingpeaks	Shiftingof peaks
Empagliflozin+*caesalpinia spinose*	No	No	No
Empagliflozin+HPMC	No	No	No
Empagliflozin+sodiumalginate	No	No	No
Empagliflozin+ethylcellulose	No	No	No
Empagliflozin+ magnesium stearate	No	No	No

INFERENCE:

Itwasobservedthatthereisnointeractionbetweendrugandexcipents,hencetheycanbeusedintheprepa rationofempagliflozintablets.

5.11. *In vivo*data:

This chapter discusses (explains) the description regarding PK parameters ofempagliflozin optimized spherical agglomerates tab which are formulated as controlreleasedmedicationfordiabeticconditions.Thecurrentaimistoperformpharmacokineticpar ametersinrabbitsandtodeterminethetimecourseofempagliflozinconcentrationinbloodsamplesin mathematicalexpressions,simultaneously to compare these with the innovative preparation of empagliflozintablets. The prepared formulation is administered in order to check the bioavailabilitylevelsinnewlydeveloped dosage formswhencompared withthe marketedones.

The CDDS is designed in order that it could have the prolonged drug releasefor extended period of time. Empagliflozin formulations were prepared and amongstthe formulations and the batch using *caesalpinia spinosa* was choosed as optimizedone(F3)fromthe*in-vitro*dissolutionstudiesanditwascomparedwith*invivo*

evaluation in rabbits. As this formulation showed least amount of empagliflozin drugrelease up to 7-12 hours in a controlled releasemanner and itis choosed for thisstudy. Tocompensate these results, *in vivo* PK parameters were planned. So thecurrent study was aimed to perform *in vivo* studies and compare them with *in vitro*results in order to prove the sustained drug delivery of empagliflozin of selectedformulation of spherical agglomerates prepared by using natural polymer *ceasalpiniaspinosa*.

5.11.1. Estimationofdruginrabbitplasma:

Inalbinorabbitsweighingabout2kg,*in-vivo*studywasperformed.1688/PO/c/13/CPCSEA.Proposalno.526,dated06.03.2017.(ForIAECApprovalcopypleasereferappendixno:iii)

Groupsforthe*in-vivo* study:

In vivo study was carried out making 4 groups of albino rabbits. Each groupconsistsoffourrabbits (n=4).

I :Control-with drug

II :Control -without drug

:placeboIII:Positivecontrol-marketedtablet.

IV: Formulation -compression of spherical agglomerates tablet (Thetabletwaspowderedandcalculatedaccordingtoanimaldose[106]andadministeredthroughoralroutewith20mlofwater)

Preparationofsamplesolutions:

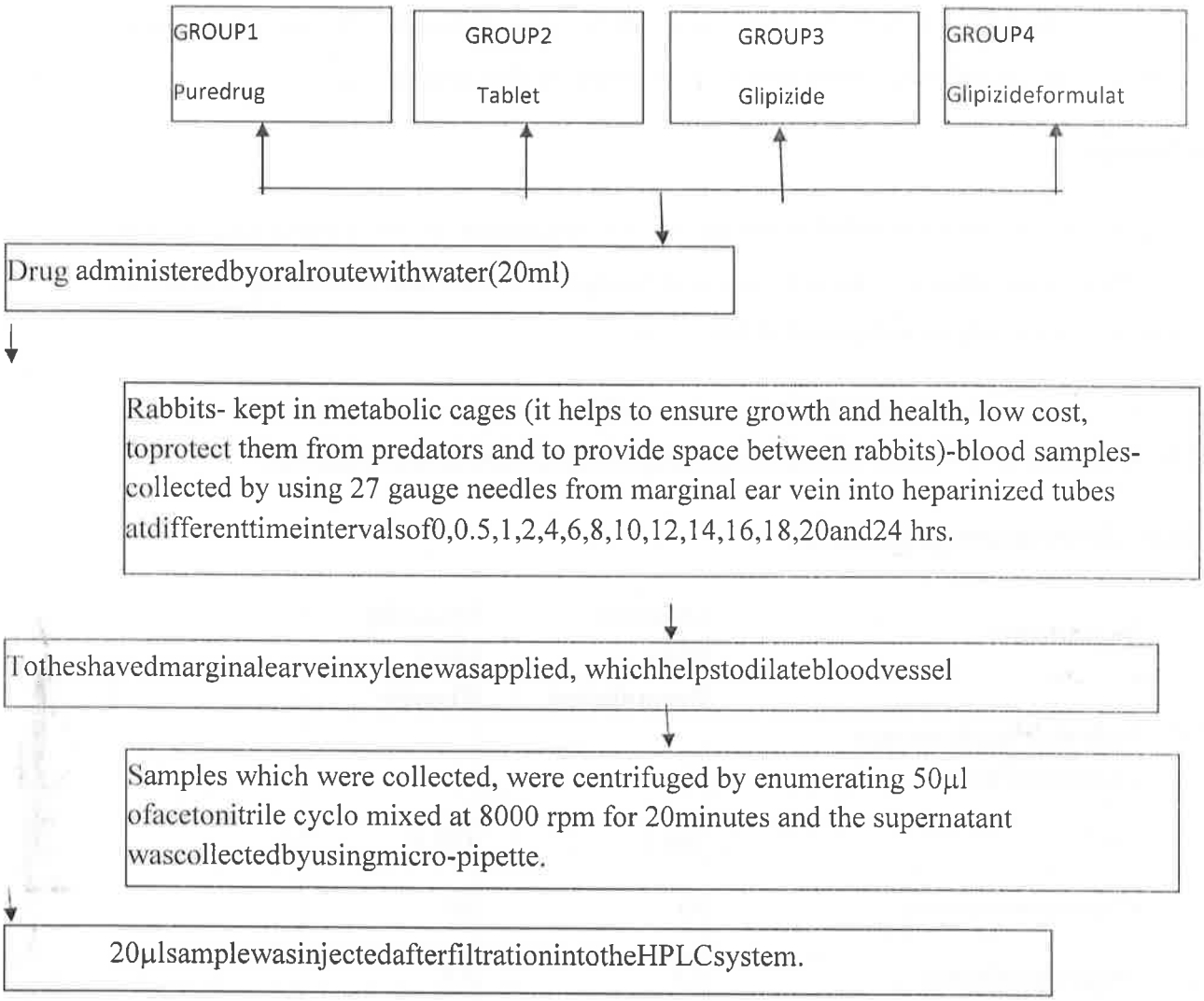


Fig5.113:Flowchartrepresentationofpreparationofsamplesolutionforempagliflozin

5.11.2. Pharmacokinetic evaluation in rabbitsInstitutionalanimalethicalcommitteeapproval

The considered protocol of theempagliflozinsustained release tablets inhealthyrabbits that is approved by IAEC of Sanzyme labs pvt ltd. Telangana, India bearingthe Regno1688/PO/c/13/CPCSEA.Proposalno.526,dated06.03.2017.

Subjects:

2.0 to 2.5 kg, healthy, 16 New Zealand white rabbits were preferredin thecurrentpre-clinical study andall the animalswere under observation before 10 daysofthestudy.

Studydesign

The current study involves parallel design for the assessment of PK criteria.New Zealand white rabbits were randomly divided into four batches, for each batchconsisting 4rabbits.*In-vivo*studies-compositionwasdepictedintable 5.100.

Half of marketed empagliflozin10 mg tablets were taken by one group andtheothergroupreceivedformulated10mgempagliflozin(optimizedformulation).

Table51:*Invivo*tablets-composition

Ingredients	**Quantity (mg)**	**Quantity (mg)**
	Formulation	**Placebo**
Spherical agglomerates ofempagliflozin	10	----
MCC	108.5	108.5
Caesalpiniaspinosa	30	30
Magesiumstearate	1.5	1.5
Totalweight(mg)	150	140

Feed wasnotprovided toall the groups of rabbits prior tohalf day and after oneday of drug administration whereas water can be provided as and when required.Specimens were kept in metabolic cages and blood samples are collected using 27gauge needle from the marginal ear vein into heparinized tubes at time intervals of0,0.5, 1, 2,4, 6, 8,10, 12, 14,16, 18, 20and24hours.

Marginal ear vein was shaved using xylene, as it dilates the blood vessel. Sampleswerecentrifugedbyadding 50µlofacetonitrilecyclomixedfor30minutesat8000

rpm and the supernatant liquid was collected by micropipette. After the filtrationprocess20µlofsamplewasinjectedintotheHPLC.

5.11.3. Resultsanddiscussion:

Analyticalmethoddevelopment:HPLC

The HPLC method development was done and validated and the run timewas made to eight (8) min. Empagliflozin shows linearitybetween 100µg/ml to1000 µg/ml concentration and calibration curve shows coefficient of correlation of0.999empagliflozinretentiontime was observedat3.928mins.

LINEARITY

Table52:Linearityofempagliflozin

S.No	Linearitylevel	Concentration(µg/ml)	Area
1	I	100	18072
2	II	400	38742
3	III	600	58502
4	IV	800	77747
5	V	1000	98657
Correlationcoefficient			**0.999**

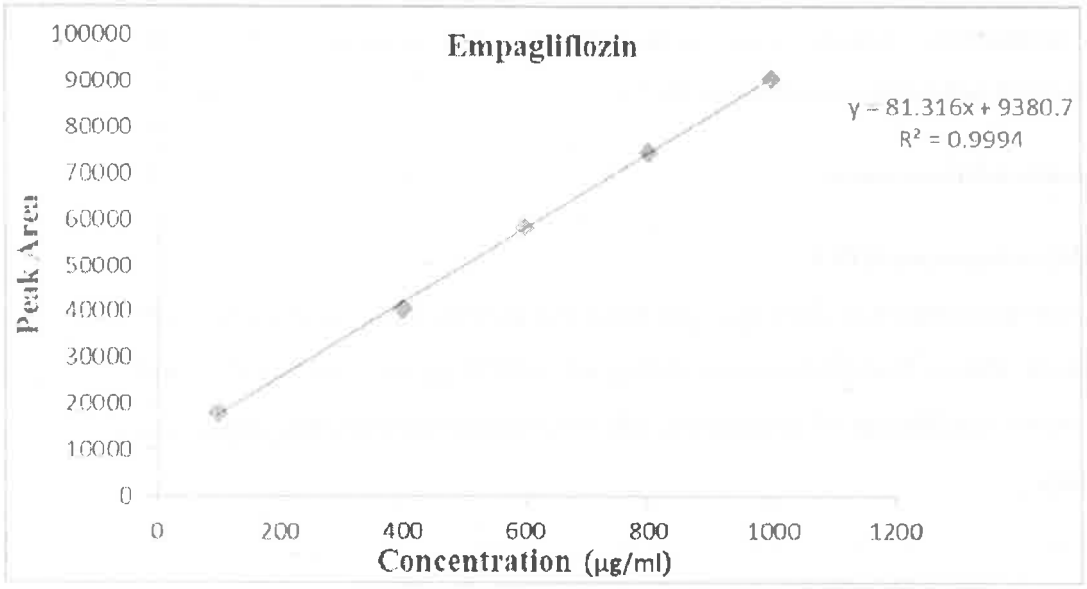


Fig64:Graphicalpresentationoflinearityof empagliflozin

ValidationofHPLCmethod

The presentwork proved that the empagliflozin peak in blank plasma sampleisnotpresentbutitis seen inchromatogram of drug solution. The range of %recovery was found to be 99.0- 100.1% for each level. % RSD for the assays was0.614%forempagliflozin(lessthan thestandardvalue - 2%).Linearregressioncoefficientofempagliflozinis0.999.

LOD values infer the signal to noise ratio (S/N) to 2.98 that was within thelimits i.e., 3. The LOQ values for S/N ratio was 9.98 (□10-within the limits). TheresultsofLODandLOQwere150µg/mland450µg/mlrespectively.Organiccomposition of mobile phase andflow rate alteration didnot have any influenceinthisprocessindicatingthemethod foritsrobustnessat±10%deviation.

HPLC–Processvalidation

Table53: HPLCmethodvalidationvalues

Precisiondeterminationbyinjecting600µg/mlconcentration	
Injection	**Peakarea**
1	58502
2	57426
3	58134
4	57956
5	58023
6	58213
S.D	356.796
RSD	0.614

Intradayandinterdayprecisionvalues		
Concentration (µg/ml)	**Peakarea**	
	Intraday(n=3)	**Interday(n=3)**
0	0	0
100	18072.36± 4.325	18072.28± 4.136
400	38472.15± 6.002	38462.25± 6.008
600	58502.54± 5.119	57426.24± 2.196
800	77747.12± 5.106	76548.6± 6.186
1000	98657.51± 2.162	98564.8± 2.121

Robustness			
S.No	**Parameterchange**	**System suitabilitydetermination**	
		USPplatecount	**USP tailing**
Changeinflowrate(ml/min)			
1	1.35	5547	1.0
2	1.5	5678	0.8
3	1.65	5547	0.9
Changeintheorganiccompositioninthemobilephase			
1	10% less	5298	1.2
2	Actual	5101	1.3
3	10%more	5399	1.4

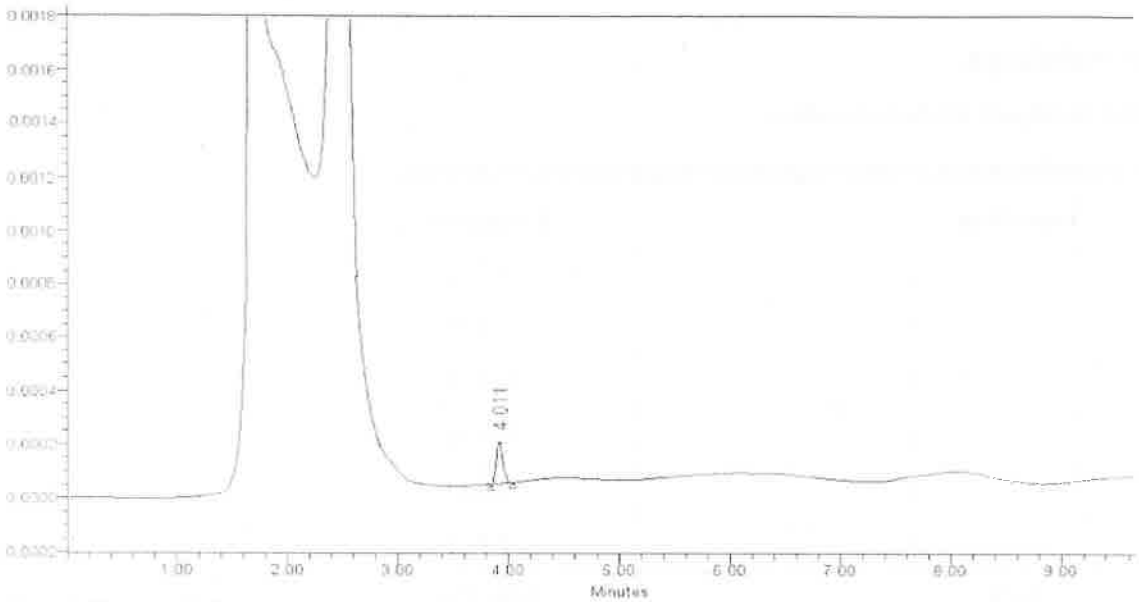


Fig65:Empagliflozinstandardcurveat100µg/mlconc-chromatogram

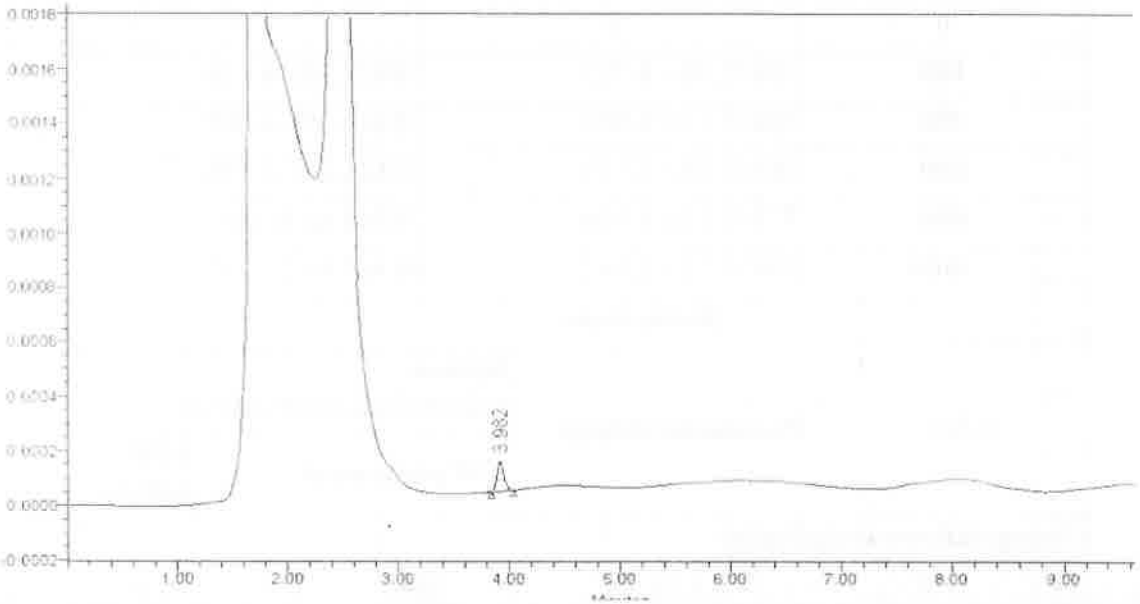


Fig66:Empagliflozinstandardcurveat 400µg/mlconc- chromatogram

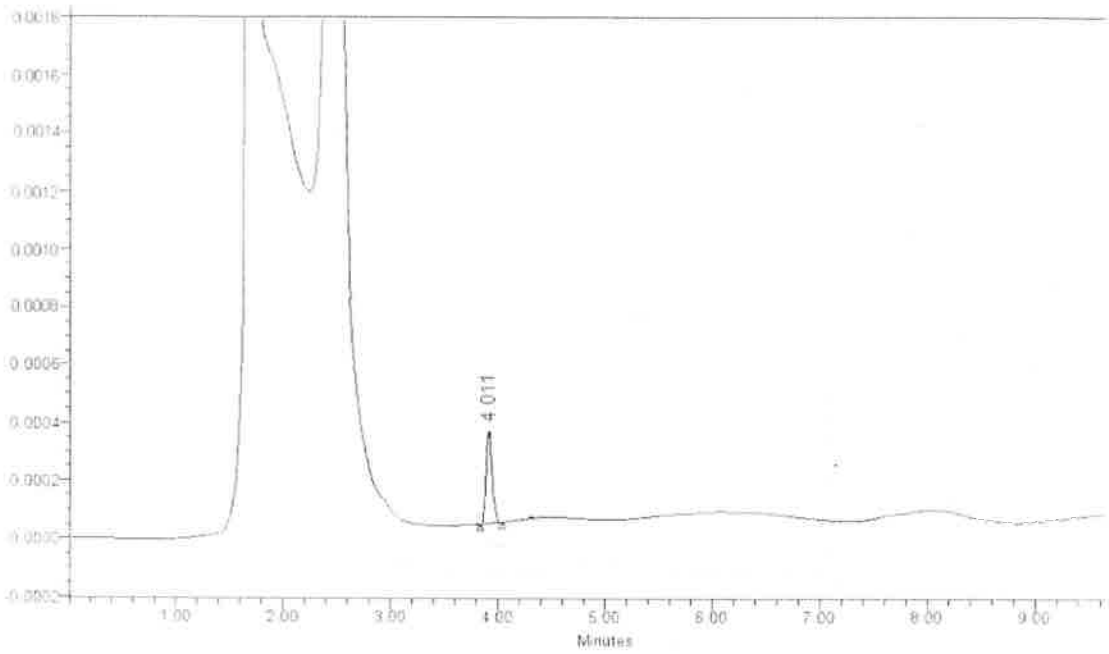


Fig67:Empagliflozinstandardcurveat600µg/mlconc-chromatogram

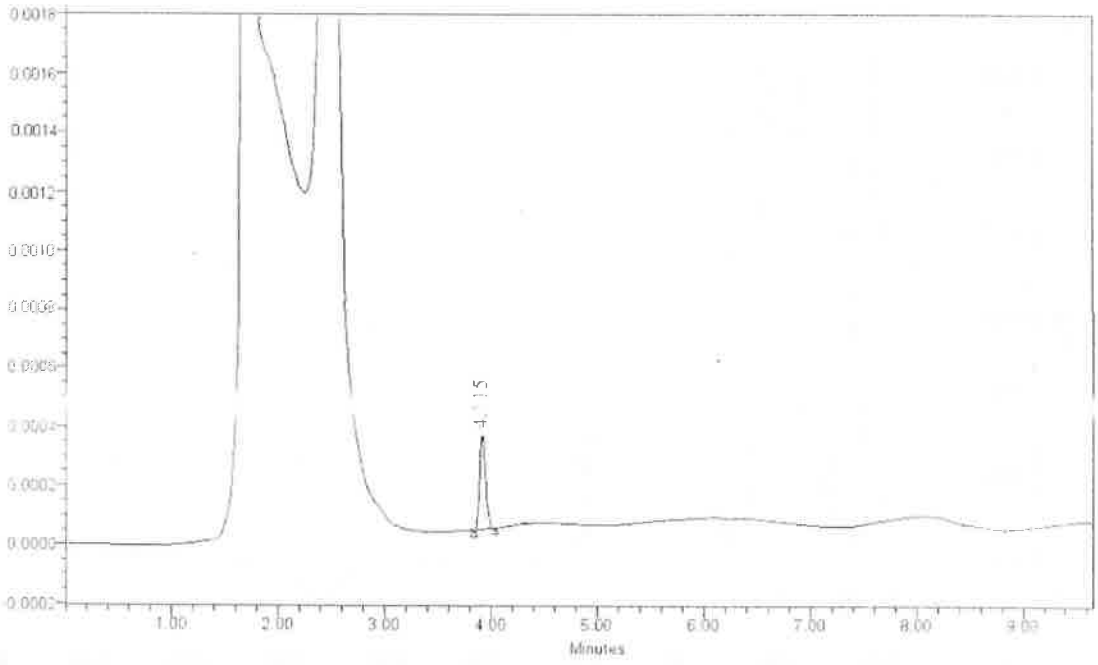


Fig68:Empagliflozinstandardcurveat800µg/mlconc-chromatogram

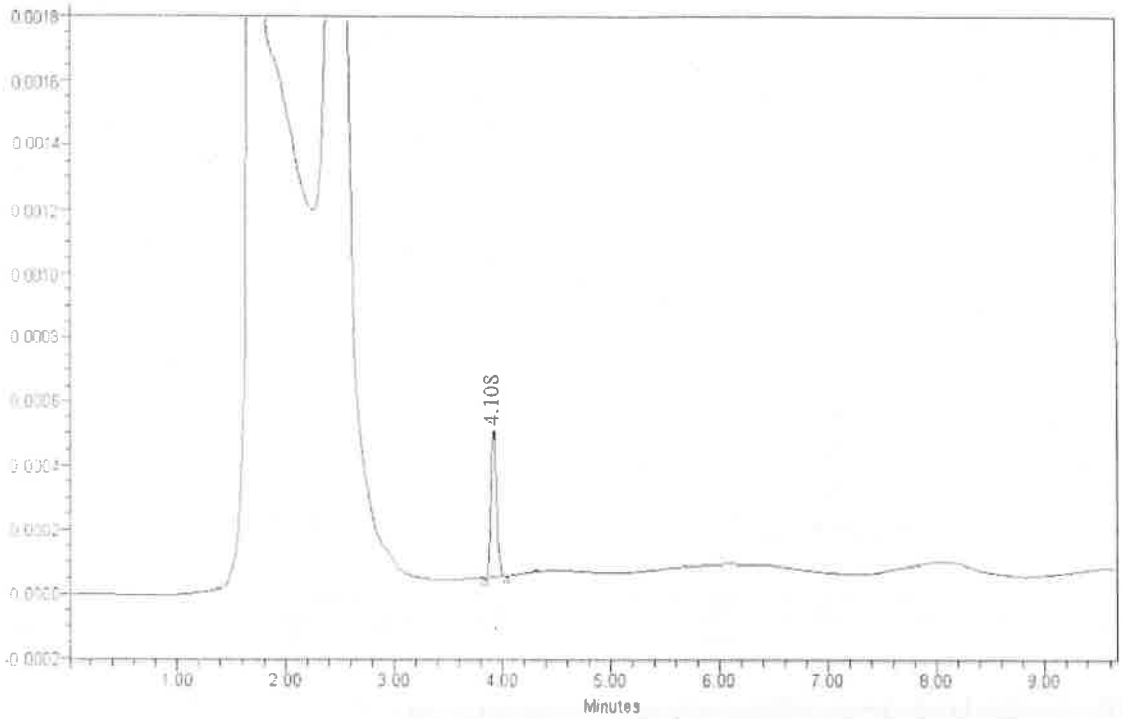


Fig69:Empagliflozinstandardcurveat1000µg/mlconc- chromatogram

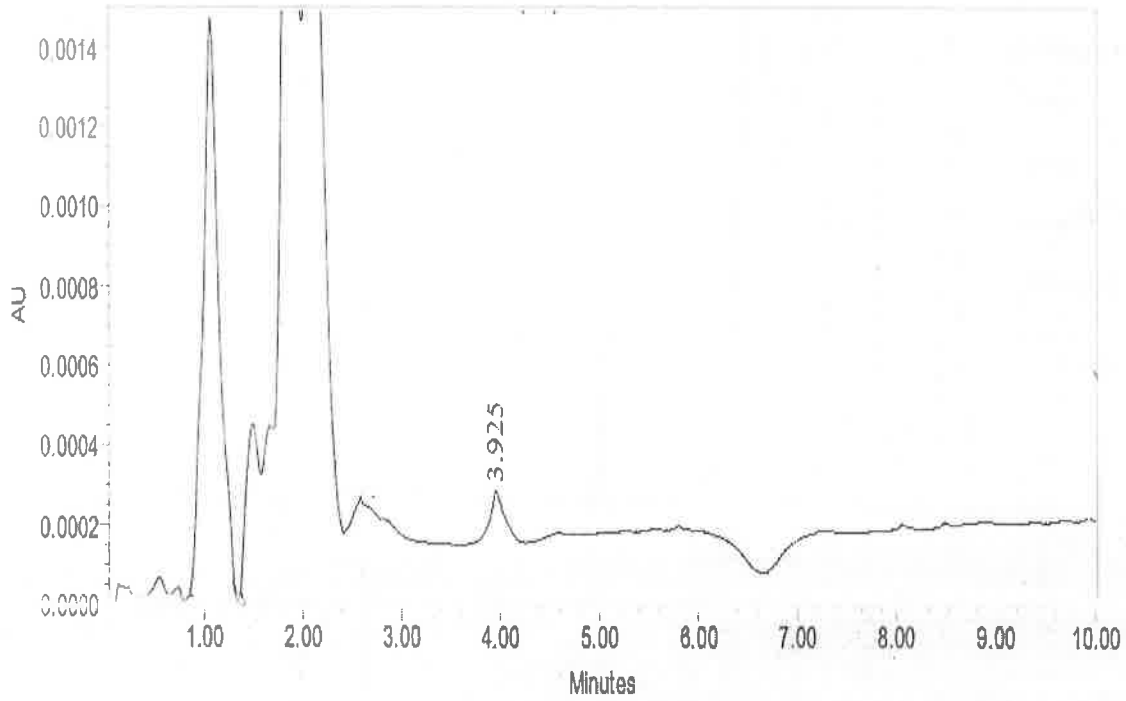


Fig70:Empagliflozintestanimal-sampleHPLCchromatogram(Formulation)at1hour

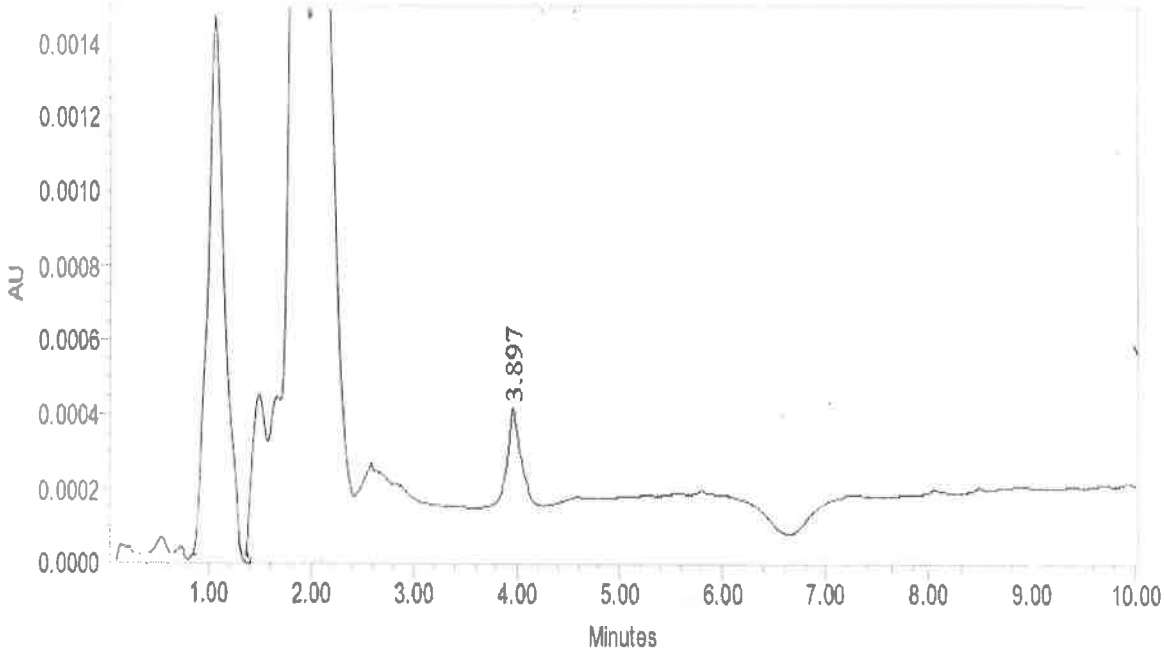


Fig 71: Empagliflozin test animal -sample HPLC chromatogram(Formulation)at2nd hour

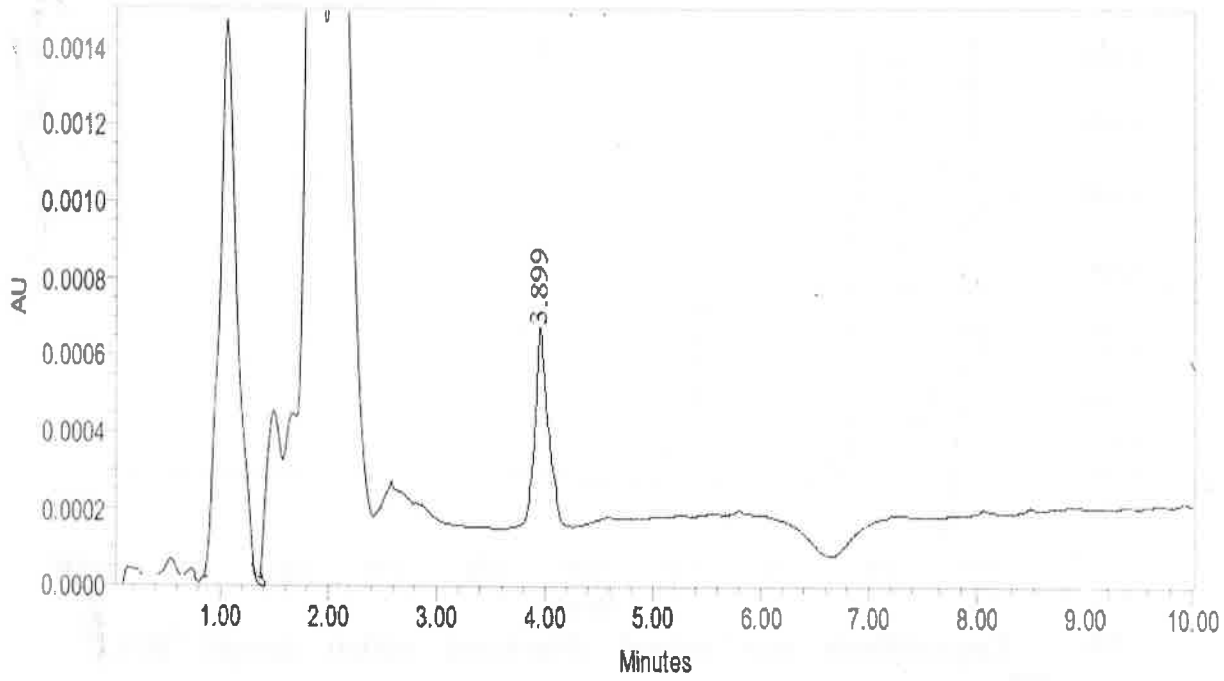


Fig72: Empagliflozin test animal - sample HPLC chromatograms(Formulation)at4th hour.

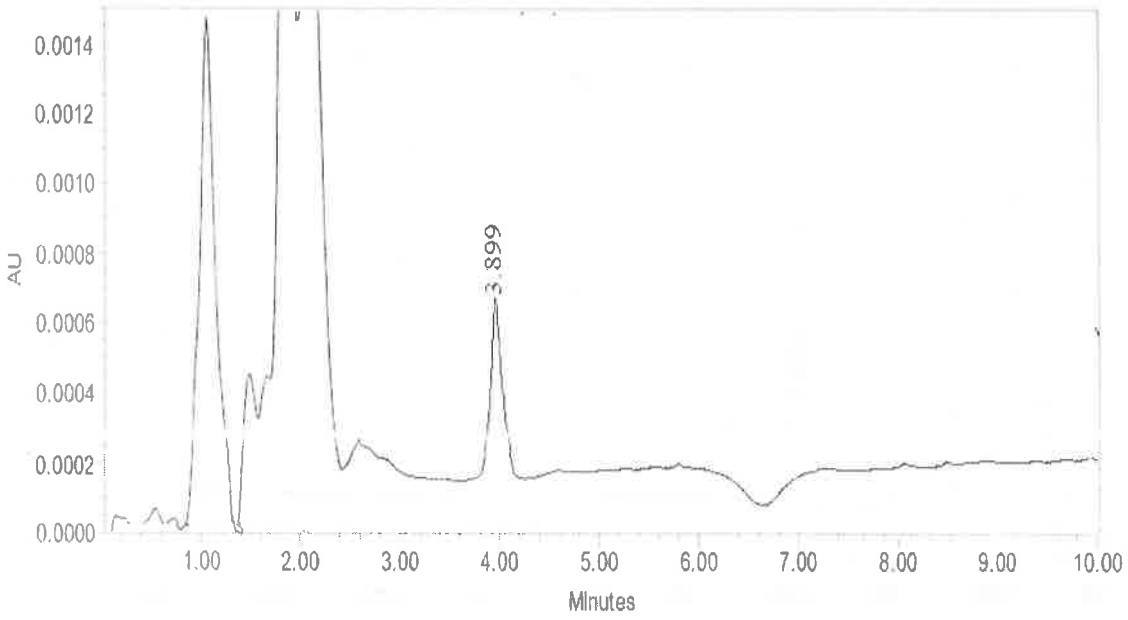


Fig 73: Empagliflozin test animal - sample HPLC chromatograms(Formulation)at8th hour.

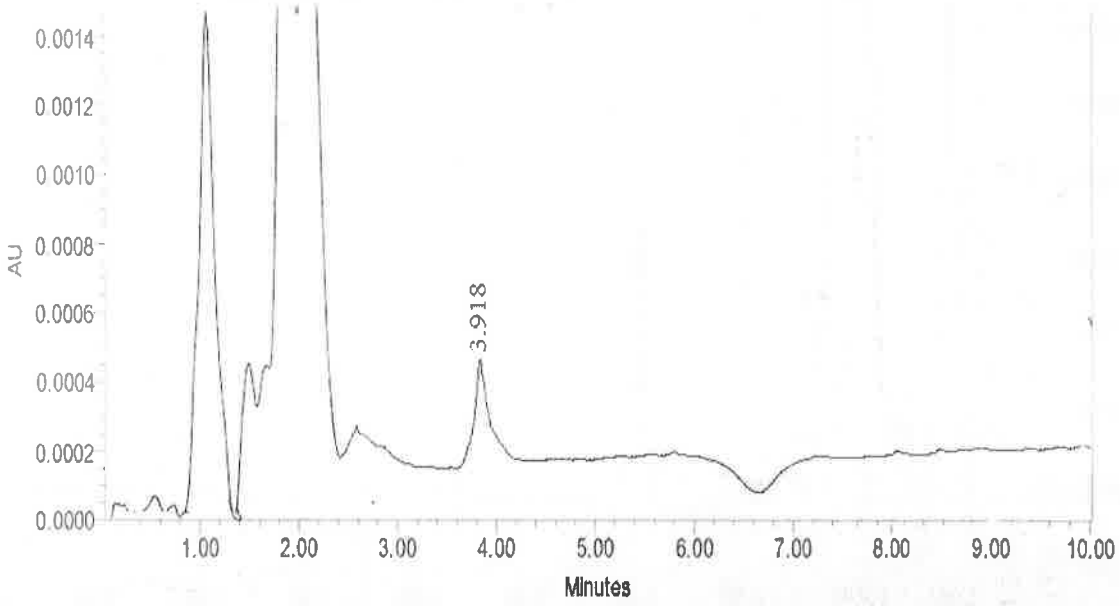


Fig 74: Empagliflozin test animal (Marketed tablet) sample HPLC chromatogramsat2nd hour.

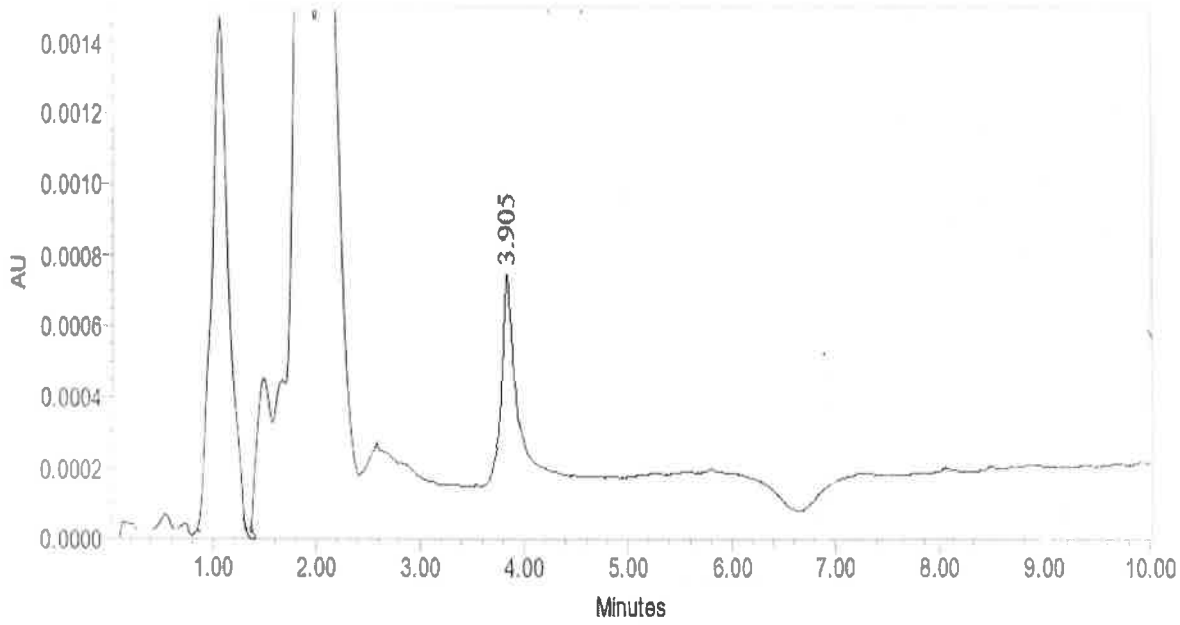


Fig75:Empagliflozintestanimal4(Marketedtablet)sampleHPLCchromatogramsat4hours.

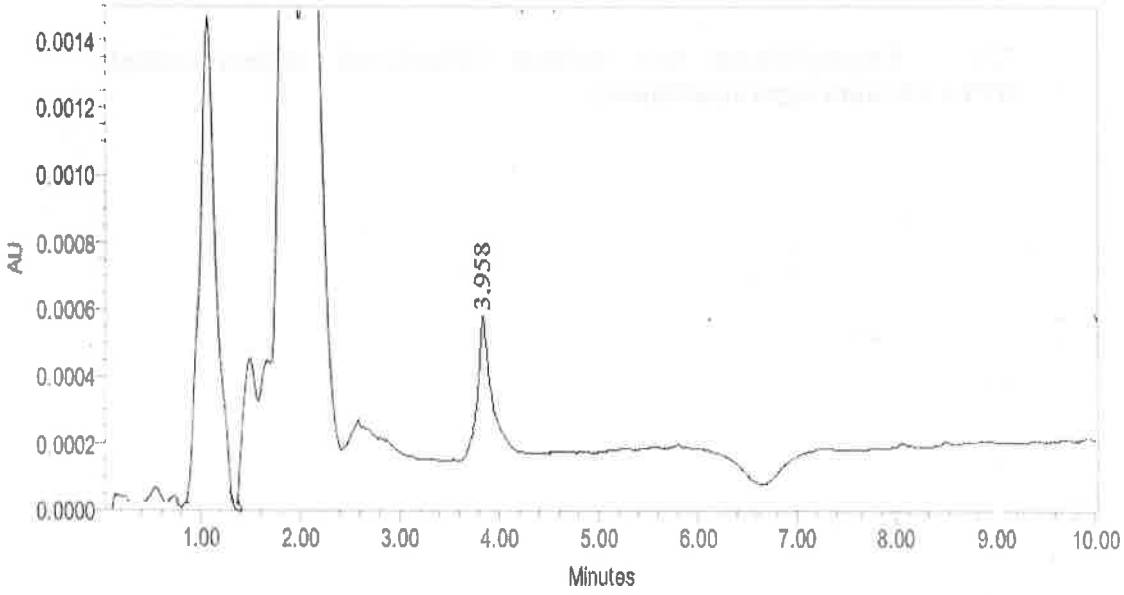


Fig 76: Empagliflozin test animal (Marketed tablet) sample HPLCchromatogramsat6hours.

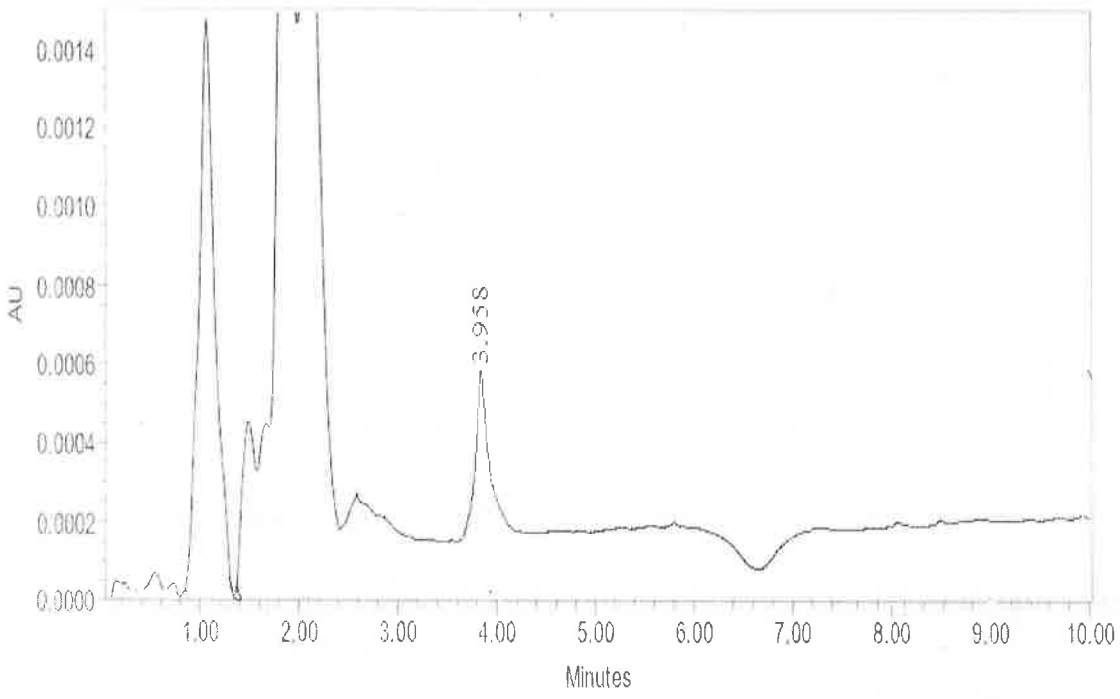


Fig 77: **Empagliflozin test animal (Marketed tablet) sample HPLCchromatogramsat8hours.**

Table54:Plasmaconcentrationsofempagliflozin(Optimizedformulation)atdifferenttimeintervals

Rabbits	Plasmaconcentration(µg/ml)	Time(hours)												
		0	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**24**
1		0	165	210	341	458	390	278	195	142	120	98	0	0	*
2		0	158	204	360	490	378	269	182	150	124	89	0	0	*
3		0	159	205	352	480	390	260	189	160	118	86	0	0	*
4		0	163	213	360	510	374	263	190	156	120	84	0	0	*
N	Statisticalparameters	0	4	4	4	4	4	4	4	4	4	4	4	4	4
Mean		**0**	161.25	208	353.25	484.5	383	267.5	189	152	120.5	89.25	0	0	*
SD		0	3.304	4.243	8.995	21.626	8.246	7.937	5.354	7.832	2.517	6.185	0	0	*
Min		0	158	204	341	458	374	260	182	142	118	84			
Median		0	161	207.5	356	485	384	266	189.5	153	120	87.5			
Max		0	165	213	360	510	390	278	195	160	124	98			
%CV		0	2.049	2.04	2.55	4.46	2.15	2.97	2.83	5.15	2.09	6.93	0	0	*

Table5.103:Pharmacokineticparametersofoptimized formulation											
Subjects	T_{max}(hours)	C_{max}(µg/ml)	$t_{1/2}$(hr)	MRT (hr)	Clml/min	Vd(ml)	AUC_{0-t} (µg-hrml^{-1})	AUC extrapolate(µg-hrml^{-1})	$AUC_{0-\infty}$ (µg-hrml^{-1})	TotalAUMC (µg-hrml^{-1})	K_{el}(Hr^{-1})
1	4	458	8.70	0.08	2.424	39.446	4125.30	7.802	135.26	6352.24	0.079
2	4	490	8.38	0.065	2.434	29.452	4108.10	7.352	123.52	6474.45	0.082
3	4	480	8.31	0.084	2.436	29.246	4104.66	7.163	129.47	6247.32	0.085
4	4	510	8.07	0.078	2.427	28.279	4119.71	7.215	132.21	6412.58	0.083
Statisticalparameters											
N	4	4	4	4	4	4	4	4	4	4	4
Mean	4	484.5	8.365	0.076	2.430	31.606	4114.443	7.383	130.115	6371.648	0.082
SD	0.000	21.626	0.260	0.008	0.006	5.252	9.688	0.290	4.992	96.743	0.003
Min	4	458	8.07	0.065	2.42	28.28	4104.66	7.16	123.52	6247.32	0.08
Median	4	485	8.345	0.079	2.43	29.35	4113.91	7.28	130.84	6382.41	0.08
Max	4	510	8.7	0.084	2.436	39.446	4125.3	7.802	135.26	6474.45	0.085
%CV	0.00	4.46	3.11	10.71	0.23	16.62	0.24	3.93	3.84	1.52	3.04

Table55:Plasmaconcentrationofmarketedformulation

Subjects		Time(Hours)													
		0	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**24**
1	Plasmaconcentration(µg/ml)	0	220	352	498	365	220	198	120	85	64	35	NA	NA	NA
2		0	217	360	458	372	240	186	118	88	67	34	NA	NA	NA
3		0	206	380	456	362	246	188	126	87	64	39	NA	NA	NA
4		0	214	345	487	362	248	184	124	86	65	35	NA	NA	NA
N	Statisticalparameters	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mean		**0**	214.250	359.250	474.750	365.250	238.500	189.000	122.000	86.500	65.000	35.750	*	*	*
SD		0	6.021	15.130	20.998	4.717	12.793	6.218	3.651	1.291	1.414	2.217	*	*	*
Min		0	206.00	345.00	456.00	362.00	220.00	184.00	118.00	85.00	64.00	34.00	*	*	*
Median		0	215.50	356.00	472.50	363.50	243.00	187.00	122.00	86.50	64.50	35.00	*	*	*
Max		0	220	380	498	372	248	198	126	88	67	39	*	*	*
%CV		0	2.81	4.21	4.42	1.29	5.36	3.29	2.99	1.49	2.18	6.20	*	*	*

(CV=Coefficientofvariation,SD=Standarddeviation, NA=Notapplicable)

Table56:PKcriteriaofjardiance

Pharmacokineticparameters											
Subjects	T_{max}(Hr)	C_{max}(µg/ ml)	$t_{1/2}$(h r)	MRT (hr)	Cl ml/min	Vd(ml)	AUC_{0-t} (µg-hrml^{-1})	AUC extrapolate(µg-hrml^{-1})	$AUC_{0-\infty}$ (µg-hrml^{-1})	TotalA UMC (µg-hrml^{-1})	K_{el}(hr $^{-1}$)
1	2.0	498	6.71	0.085	3.03	29.448	3291.85	6.602	325.12	5987.25	0.103
2	2.0	458	5.33	0.086	3.53	27.174	2832.66	4.416	458.45	5879.57	0.129
3	2.0	456	5.55	0.081	3.50	28.081	2853.06	4.866	460.32	5632.21	0.124
4	2.0	487	5.31	0.078	3.50	26.835	2856.06	4.567	450.47	5786.28	0.130
Statisticalparameters											
N	4	4	4	4	4	4	4	4	4	4	4
Mean	2.000	474.750	5.725	0.083	3.390	27.885	2958.408	5.113	423.590	5821.328	0.122
SD	0.000	20.998	0.666	0.004	0.240	1.168	222.538	1.010	65.785	150.462	0.013
Min	2.00	456.00	5.31	0.08	3.03	26.84	2832.66	4.42	325.12	5632.21	0.10
Median	2.00	472.50	5.44	0.08	3.50	27.63	2854.56	4.72	454.46	5832.93	0.13
Max	2	498	6.71	0.086	3.53	29.448	3291.85	6.602	460.32	5987.25	0.13
%CV	0.00	4.42	11.63	4.48	7.09	4.19	7.52	19.76	15.53	2.58	10.38

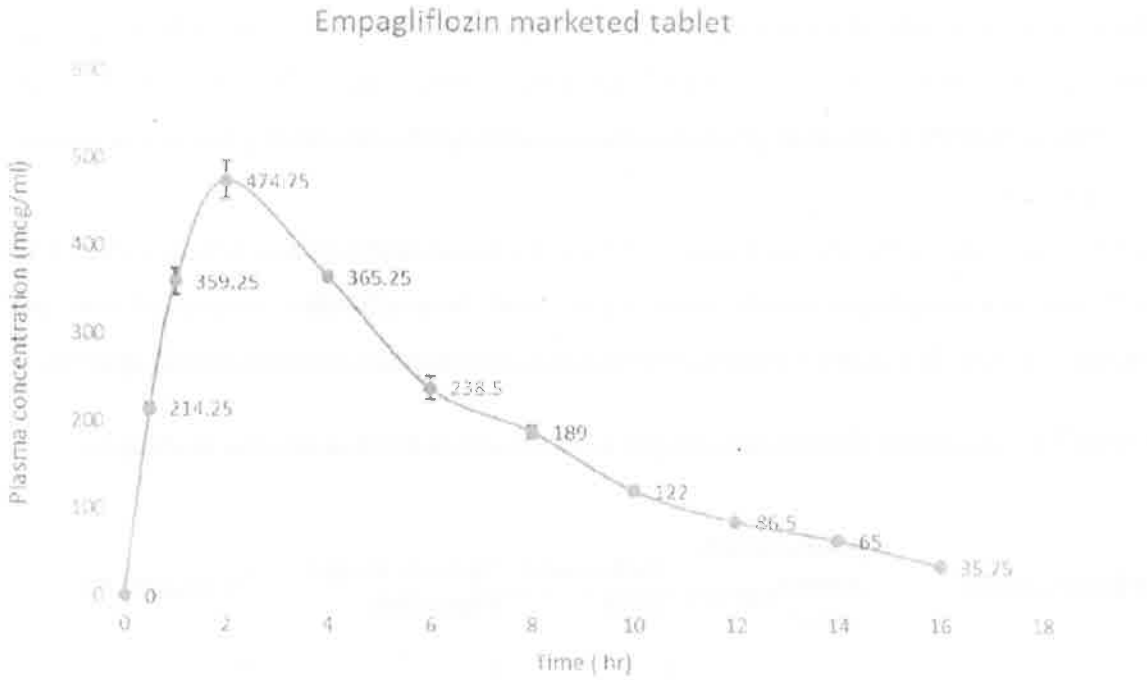


Fig78:Plasmaconcentrationvs.timeofmarketedempagliflozininrabbitplasma

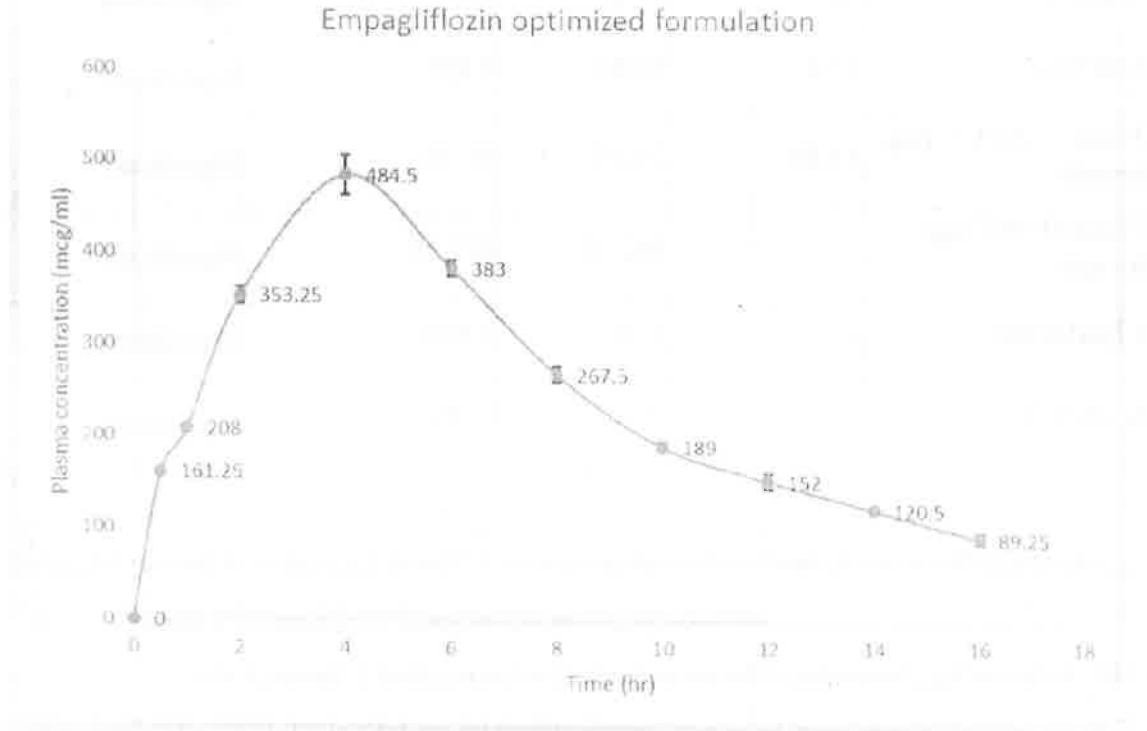


Fig79:Plasmaconcentrationvs.timeoftestempagliflozinsphericalagglomeratesinrabbits

5.15.7.Pharmacokineticparametersevaluation

These criteria are important for assuring the values of bioavailability, like max C_{max}.T_{max}, area under curve, V_d, $t_{1/2}$, mean residence time and Cl_T. Fig 5.115- fig 5.119showstheHPLCchromatogramsforreferenceempagliflozininrabbitplasmaconcentrati on.Fig5.120-fig5.127showstheHPLCchromatogramsoftestformulationinrabbitplasmalevels.Table5.102 and5.104illustratesplasmaconcentration values and bioavailability criteria of test and reference tablet. The graphwasplottedwithmeanandstandarddeviationofallthefourrabbits.

Table57:Comparativebioavailabilityparametersofstandardandtestformulations

PKparameter	Reportedliterature survey [18,36,47]	Referencetablet	Sphericalagglomerates	't'testat0.05
C_{max}(µg/ml)	3.33	4.754	4.845	Notsignificant
T_{max}(hours)	3.8	2.0	4.0	Significant
$t_{1/2}$(hrs)	5.54	5.725	8.36	Significant
MRT(h)	17.1	0.083	0.076	Significant
Total AUC (µg-hr/ml)	53.89	29.58	41.14	Significant
TotalAUMC(µg-hr/ml)	-	582.13	637.16	Significant
Cl(ml/min)	-	3.39	2.430	Significant
K_{el}(hrs^{-1})	-	0.122	0.082	Significant

C_{max}ofempagliflozinmarketandtestformulationswere4.754±0.1µg/mland4.845±0.01µg/ml withacceptablynodeviation($P<0.05$)andaPvalueof 0.085.ValuesofT_{max}forempagliflozinmarketandtestwere2.0±0.35hours,4.0± 0.73hourscorrespondinglywitheloquentconflict($P<0.05$)andaPvalue0.0005.standardandtest$t_{½}$ valueswere5.725±0.531hrs,8.36±1.59hrs,specificallywith

acceptable deviation (P<0.05) and a P value is 0.0002. MRT values of standard andalso test were found to be 0.083± 0.034 hrs and 0.076± 0.028 hrs with acceptabledeviationwithaPvalueis0.002.$AUC_{0-\infty}$valueswere29.584±20.04µg-hr/ml, 637.164 ± 49.44 µg-hr/ml respectively for standard and test with acceptable deviation(P<0.05)andPvalueis<0.0001.

As inter and intra subject deviation is present, a difference in individual $T_{max}$and C_{max} variables is observed. The same case is seen in both innovative and preparedsamples too. The consequences of PK parameters showed that the innovative productand testwere absolutely differentproving that theformulated productshowed thedrughassustainedrelease.

5.15.8Stabilitystudiesfortheoptimizedformulationofempagliflozinforaperiodof120days

Stability testing was performed for a period of 4 months at accelerated conditions of40°C±2°C/75% ±5%RHfor the optimizedformulation andthedissolutionparameters were evaluated for 12hr drug release. The following table: 5.107 depictsthedatafordissolutionstudiesandfig:5.130depictsitsreleaseingraphicalrepresentation

Table:58Stabilitystudiesofempagliflozinoptimizedformulation

Time (hrs)	30day DR	60day DR	90day DR	120day DR
0	0	0	0	0
0.5	11±0.17	11±0.23	11±0.14	10±0.01
1	20±0.31	19±0.03	18±0.18	18±0.05
2	34±0.25	33±0.13	32±0.08	31±0.02
4	45±0.18	44±0.15	43±0.34	42±0.18
6	62±0.02	59±0.06	58±0.12	59±0.24
8	74±0.23	72±0.18	72±0.03	73±0.21
10	86±0.34	84±0.24	82±0.19	82±0.12
12	97±0.14	97±0.72	97±0.01	97±0.11

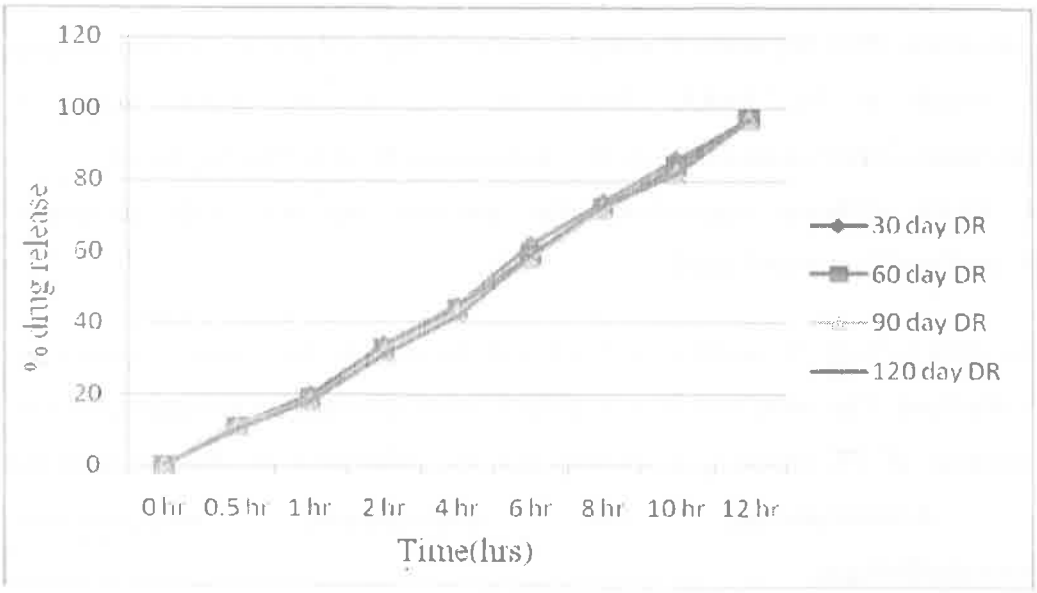


Fig:80 Stabilitystudiesindicatingthedrugreleaseofempagliflozin

Inference: StabilitystudiesconductedasperICHguidelinesforoptimizedformulation at accelerated conditions (40□C ± 2□C / 75% ± 5% RH) for 120 days.There was no significant change in the physical property and percent of drug releasewaswithinthelimits duringthe stabilityperiod.

Chapter VI. Outcome of the Project

Controlled release medication has gained a very pivotal role in the pharmaceuticaldosage forms. A new and surprisingly simple, safe and inexpensive formulation forcontrolled release tabletis preferred which shows a release pattern for the activesubstance in a programmed rate of approximately zero order. It not only prolongs theduration of drug release but also increases the retention of drugs in the body thus bymaintainingasteadystateconcentrationlevelsandthusarelativelyuniformtherapeutic blood level of the active material is thereby maintained for that period oftime. Diabetes is one of the diseases in the world which is being considered as majormetabolic disorders where high blood glucose levels are present in the body for aprolonged period of time. Many formulations were introduced into the market forcombating its risks in life. Proper care should be taken in selecting and administeringthe drug based on dose and frequency of administration. Spherical agglomerationprocess is used as a novel technique in order to increase the bioavailability of poorlysoluble drugs. To form the spherical crystals or agglomeratesparameters like amountof bridging liquid used, addition of poor solvent and good solvent are processed. Tooptimize theformulations,speed of agitation,stirring rate, andtemperature weremaintained. The use of natural polymers like *caesalpiniaspinosa*which is commonlyknown as taragum, are preferred for its controlled release nature to prolong the drugeffectin the plasma. In addition to the controlled release, unique techniques likespherical agglomeration is advantageous to have a sustained action by increasing theglobule size and by increasing the bioavailability of poorly soluble drugs. Polymerslike HPMC, ethyl cellulose and sodium alginate were used under different viscositygrades.

Based on the investigations the following conclusions were drawn

Spherical agglomeration technique has been proved its efficiency in increasingthesolubility anddissolution rateof poorly watersolubledrugs whichisaverybigconfrontinthepharmaceuticalmarket.

- The present study was aimed to develop and evaluate anti-diabetic sustainedrelease oraltabletsofempagliflozin.

Onthebasisofliteraturesurveyandcompatibilitytests,excipientslikemicrocrystallinecellulose,magnesiumstearate wereused.

- C_{max} ofempagliflozin market and test formulations were 4.75 ± 0.1 µg/ml and4.845±0.01µg/mlwithacceptablynodeviation(P<0.05)andaPvalueof 0.085.ValuesofT_{max}forempagliflozinmarketandtestwere2.0± 0.35hours, 4.0 ± 0.73 hours correspondinglywith eloquent conflict (P<0.05) and a Pvalue 0.0005. Standard and test $t_{½}$ values were 5.725 ± 0.531 hrs, 8.36 ± 1.59hrs, specifically with acceptable deviation (P<0.05) and a P value is 0.0002.MRTvaluesofstandardandalsotestwerefoundtobe0.083±0.034hrsand 0.076±0.028hrswithacceptabledeviationwithaPvalueis0.002.$AUC_{0-\infty}$valueswere295.84±20.04µg-hr/ml,637.16±49.44µg-hr/mlrespectivelyforstandardandtestwithacceptabledeviation(P<0.05)andPvaluei s <0.0001.

- As inter and intra subject deviation is present, a difference in individual $T_{max}$and C_{max} variables is observed. The same case is seen in both innovative andpreparedsamplestoo.Theconsequencesofpharmacokineticparametersshowed that the innovative product and test were absolutely different provingthat the formulated product showed the drug has extended release. The graphwasplottedwithmeanandstandarddeviationofallthefourrabbits.

- Itconcludesthatdirectcompressionofsphericalcrystallizationofanti-diabeticdrugs withselectivepolymersisan efficientmethodtoimprovecompressibilityandalsodissolutionprofileofpreparedtablets.

7.2 RECOMMENDATIONSANDFUTURESCOPEOFTHESTUDY

Theresultsofcurrentresearchindicatedthatthepreparationofempagliflozin tablets by spherical agglomerates method improved the solubility anddissolution rate. All the prepared spherical agglomerates of both the drugs exhibitedimproved flow and are spherical in shape. The spherical agglomeration techniquealong with natural polymer had its success in prolonged release rate of the drugs. Allthe formulations thatwere prepared by spherical agglomeration method compliedwith the Indian Pharmacopoeial standards in its kinetics and stability studies criteria.Out of all the polymers used *Caesalpiniaspinosa*has better sustainability and showedbetter dissolution rate thus

increased bioavailability is observed in rabbit models. Therate of release of drug had its prolonged action i.e., sustained release when combinedwith natural polymer i.e., *Caesalpiniaspinosa.* Hence the objective of the currentresearchwas achievedbysphericalagglomeratetechnique.

In this thesis, sustained release studies were performed. Other types of controlledrelease medications can also be done by substituting some other type of natural orsynthetic polymers with other drugs with respective to disease and its classification.Other formulationslikepatches,filmsetccanbestudiedforcommercialization.

REFERENCES

[1]. http://www.pharmatips.in/Articles/Pharmaceutics/Tablet/Introduction-Of-Direct-Compression-Tablet.aspx

[2]. ParulSaini, Anoop Kumar and SharadVisht. Spherical Agglomeration: A Novel Technique of Particulate Modification & Developing Niche Drug Delivery System. IJBR Vol.6 No.2 July-December 2013, pp.86-101 @ International Science Press, (India) 86.

[3]. https://en.wikipedia.org/wiki/Diabetes_mellitus

[4]. https://my.clevelandclinic.org/health/diseases/7104-diabetes-mellitus-an-overview

[5]. Diagnosis and Classification of Diabetes Mellitus American Diabetes Association Diabetes Care 2004 Jan; 27(suppl 1): 5-10.

[6]. https://www.webmd.com/diabetes/guide/types-of-diabetes-mellitus#1.

[7]. http://googleweblight.com/i?u=https://www.mayoclinic.org/disease-conditions/type-1-diabetes/symptoms-causes/syc-20353011&hl=en-IN.

[8]. https://www.webmd.com/diabetes/guide/types-of-diabetes-mellitus#2.

[9]. https://my.clevelandclinic.org/health/diseases/7104-diabetes-mellitus-an-overview.

[10]. https://www.webmd.com/diabetes/guide/risk-factors-for-diabetes#1.

[11]. U.Satyanarayana, U Chakrapani. Biochemistry. 4th edition. 2013. Copublished by Reed Elsevier India Pvt Ltd and books and Allied Pvt Ltd. Pg: 669-670.

[12]. S. K. Putta and P. Srikumar. Spherical crystallization and its process optimization. Journal of Chemical and Pharmaceutical Research, 2016, 8(7):611-623

[13]. ArindamChatterjee, Madan Mohan Gupta, and BirendraSrivastava International Journal of Pharmaceutical Investigation Spherical crystallization: A technique use to reform solubility and flow property of active pharmaceutical ingredients 2017 Jan-Mar; 7(1): 4–9. doi: 10.4103/jphi.JPHI_36_16

[14]. . Patil SV, Sahoo. Spherical Crystallization: a Method to Improve Tabletability Research Journal of Pharmacy and Technology 2(2): April.-June. 2009. pg.234-237.

[15]. NitanBharti et al., Spherical Crystallization: A Novel Drug Delivery Approach, Asian Journal of Biomedical and Pharmaceutical Sciences; 3(18) 2013, pg no 10-16

[16]. Parida R et al., overview of spherical crystallization in pharmaceutical, International Journal of Pharma and Bio Sciences, Vol.1/Issue-3/Jul-Sep.2010.

[17]. JyothiThati, et al., the mechanisms of formation of spherical agglomerates European journal of pharmaceutical sciences 2011/3/18 42(4)Pages 365-379

[18]. Mudit Dixit et al., Preparation and characterization of spherical agglomerates of ketoprofen by neutralization method. International Journal of Pharma and Bio Sciences, Vol-1, Issue-4, Oct-Dec.2010, Pg: 395-406).

[19]. Pritishkurumkar et al., preparation of spherical crystal agglomerates via crystallo-co-agglomeration technique, Digest Journal of Nanomaterials and Biostructures Vol. 7, No. 3, July - September 2012, p. 1223 – 1236.

[20]. SarfarazMd et al., Particle Design of Aceclofenac-Disintegrant Agglomerates for Direct Compression by Crystallo-Co-Agglomeration Technique. Asian Journal of Pharmacy and Technology 1(2): April-June 2011; Page 40-48.

[21]. A.R. Tapas et al., enhanced dissolution rate of felodipine using spherical agglomeration with Inutec SP1 by quasi emulsion solvent diffusion method'Research in Pharmaceutical Sciences 2009 Jul-Dec; 4(2): 77–84.

[22]. Alladisaritha et al., Enhancement of Dissolution and Anti- inflammatory Activity of Meloxicam by Spherical Agglomeration Technique, Journal of Pharmaceutical Sciences and Research Vol.4(1), 2012, pg.no 1657-1661.

[23]. SachinkumarPatil et al., Directly Compressible Glibenclamide Tablet Prepared from Spherical Agglomerates: A Comparative Evaluation with Marketed Tablet. Journal of Pharmaceutical Science and Technology, Volume 3 (Issue 1) 2013; pg:31-36.

[24]. Sunil Kumar et al., a comparative evaluation of direct compression and wet granulation methods for formulation of stavudine tablets, Journal of Global Trends in Pharmaceutical Sciences / 5(3)-(2014) 2000-2003.

[25]. Fadke J et al., Formulation Development of Spherical Crystal Agglomerates of Itraconazole for Preparation of Directly Compressible Tablets with Enhanced Bioavailability, American Association of pharmaceutical scientistsPharmSciTech. 2015 Dec;16 (6):1434-44.

[26]. GadhaveMV et al., Preparation and Characterization of Spherical Crystals of Embelin to Improve the Solubility and Micromeritic Properties, International Journal of Pharmaceutical and Clinical Research 2014; 6(4): 363-369.

[27]. PavitraSolanki et al., Designing & development of spherical agglomerates of ibuprofen paracetamol blend for improved tableting and dissolution, International Journal of Therapeutic Applications, Volume 8, 2012, 8-13

[28]. P. K. Kulkarni et al., spherical agglomerates of mefenamic acid by solvent change method. International journal of pharmaceutical sciences Vol-2, Issue-2, 2011 pg:111-125.

[29]. Ian J Neeland et al., Empagliflozin reduces body weight and indices of adipose distribution in patients with type 2 diabetes mellitus Diabetes & Vascular Disease Research 2016, Vol. 13(2) 119–126.

[30]. IlkkaTikkanen et al ., Empagliflozin reduces blood pressure in patients with type 2 diabetes and hypertension. Diabetes Care 2015 Mar;38(3):420-428.

[31]. Hans-Ulrich Häring *et al.,*Empagliflozin as add-on to metformin plus sulfonylurea in patients with type 2 diabetes: a 24-week, randomized, double-blind, placebo-controlled trial. Diabetes Care. 2013 Nov;36(11):3396-404.

[32]. Matthew J. Budoff et al., Effects of canagliflozin on cardiovascular risk factors in patients with type 2 diabetes mellitus. International Journal of Clinical Practice 2017 May; 71(5): e12948.

[33]. Bode B et al., Long-term efficacy and safety of canagliflozin over 104 weeks in patients aged 55-80 years with type 2 diabetes. Diabetes, Obesity and Metabolism. 2015 Mar;17(3):294-303.

[34]. ParthasarathiKeshavarao et al., Spherical Agglomeration of Naproxan by Solvent Change Method, S. Journal of Pharmaceutical Sciences,. 4(1): 01-08.

[35]. IzabelaPolowczyk et al., Spherical agglomeration of acetylsalicylic acid, E S Web of Conferences e sconf /2016 3 8.

[36]. Parmarshital S et al., spherical agglomeration a novel approach for solubility and dissolution enhancement of simvastatin, Asian Journal of Pharmaceutical and Clinical Research, Vol 9, Issue 6, 2016, 65-72.

[37]. M. Dixit et al., preparation and characterization of spherical agglomerates of piroxicam by neutralization method, American journal of drug discovery and development, 2011,1 (3), 188-199.

[38]. V.B. Yadav et al., effect of different stabilizers and polymers on spherical agglomerates of gresiofulvine by emulsion solvent diffusion (ESD) system, international journal of pharm tech research, vol(2), 2009, 149-158.

[39]. Glipizide https://en.m.wikepedia.org/wiki/glipizide

[40]. Glipizide https://www.drug bank.ca/drugs/DB01067

[41]. Glipizidehttps://reference.medscape.com/drug/glucotrol-glipizide-342704#10

[42]. MedlineplusEmpagliflozinhttps://medlineplus.gov/druginfo/meds/a614043.html

[43]. Empagliflozin. https://www.diabetes.co.uk/diabetes-medication/jardiance.html

[44]. Empagliflozin Rx list. https://www.rxlist.com/jardiancedrug.htm#description

[45]. Empagliflozinhttps://reference.medscape.com/drug/jardiance-empagliflozin-999907

[46]. Caesalpiniahttp://tropical.theferns.info/viewtropical.php?id=Caesalpinia+spinosa

[47]. A handbook by sustainable polymers: processing and applications.edited by Vijay Kumar Thakur, ManjuKumari.

[48]. A book by M. Paz Arriaz, frontiner in horticulture, medical and aromatic plants: the basics of industrial applications, volume 1, .

[49]. Ethylcellulosehttps://www.intechopen.com/books/cellulose-medical-pharmaceutical-and-electronic-applications/application-of-cellulose-and-cellulose-derivatives-in-pharmaceutical-industries.

[50]. Ethylcellulosehttps://googleweblight.com/i?u=https://pubchem.ncbi,nih,gov/compound/ 24832091&hl=en-IN.

[51]. Ethylcellulosewww.fao.org

[52]. Hydroxy propyl methyl cellulose.https://www.drugs.com/inactive/hydroxypropyl-methylcellulose-162.html

[53]. Hydroxy propyl methyl cellulose https://www.sigmaaldrich.com/catalog/product/sigma/56340?lang=en&region=IN

[54]. Hydroxy propyl methyl cellulose https://labdoor.com/article/what-is-hydroxypropyl-methylcellulose

[55]. Nikhil K Sachan et al., Sodium alginate: the wonder polymer for controlled drug delivery, Journal of Pharmacy Research 2009, 2(8),1191-1199.

[56]. Sodium alginate https://www.drugs.com/international/sodium-alginate.html

[57]. Jan Karlsen et al., Sodium alginate in Drug Delivery Systems, Drug Development and Industrial Pharmacy, 28(6), 621–630 (2002).

[58]. Magnesium stearate https://en.wikipedia.org/wiki/Magnesium_stearate

[59]. Magnesium stearatehttp://shodhganga.inflibnet.ac.in/bitstream/10603/28951/11/11_chapter%205.pdf

[60]. Magnesium stearate shodhganga.inflibnet.ac.in/bitstream/10603/61806/10/11_chapter%204.pdf

[61]. Microcrystalline cellulose https://www.drugs.com/inactive/microcrystalline-cellulose-48.html

[62]. Microcrystalline cellulose http://www.fao.org/docrep/w6355e/w6355e0l.htm

[63]. Clare E. M. McEnroe, A book on Microcrystalline cellulose. The Functionality of Microcrystalline Cellulose and Carrageenan in a Low Fat Processed Cheese Spread.1996, pg: 360.

[64]. Satani R. R et al., design and development of compressed coated as chronomodulated system for hypertension, International Bulletin of Drug Research., 4(6): 45-59, 2014

[65]. Gita Chaurasia et al., a review on pharmaceutical preformulation studies in formulation and development of new drug molecules,International Journal of Pharmaceutical Sciences and Research (2016), vol 7, issue 6 pg no, 2313-2320.

[66]. Rinalmistry et al., determination of angle of response of pharmaceutical materials based on image processing using labview, International Journal ofAdvanced Research in Electrical, Electronics and Instrumentation Engineering , vol 6, issue 3, march 2017, pg no 1125-1131.

[67]. Manoj Kumar et al., development and characterization of aceclofenac enteric coated tablets, International Journal of Research and Development in Pharmacy and Life Sciences, , 4(6), October- November 2015, 1861-1866

[68]. Particle.dk/methods-analytical-laboratory/bulk-and tapped-density/

[69]. Amrutha JV et al., pre and post compression studies of tablets. Indian Journal of Inorganic Chemistry 2016; 11(4), pg no 100- 109.

[70]. Hitesh Chaturvedi et al., post compression evaluation parameters for tablets-an overview. European Journal of Pharmaceutical and Medical Research, 2017, 4(11), 526-530.

[71]. Haritha B et al., A Review on Evaluation of Tablets, journal of formulation science and Bioavailability 2017, Volume 1, Issue 1, pg 1-5.

[72]. Zia-ur-Rahman et al., Post-Market In-Vitro Comparative Evaluation of Quality Control Parameters of Paracetamol Compressed Tablets Manufactured in Local

Industrial Zones of Kpk Pakistan, The Pharma Innovation, Vol. 2 No. 3 2013, pg 11-15.

[73]. PranatiSrivastava et al., formulation and evaluation of paracetamol tablets to assess binding property of orange peel pectin, International Journal of Pharmaceutical Sciences Review and Research, Volume 3, Issue 1, July – August 2010, pg 30-34.

[74]. Suvakanta Dash et al., kinetic modeling on drug release from controlled drug delivery system. ActaPoloniaePharmaceutica- Drug Research, vol 67 issue 3 2010 pg no 217-223

[75]. PrakashGoudanavar et al., Development and in vitro characterization of esomeprazole floating gastro retentive microspheres, Journal of Applied Pharmaceutical Science Vol. 3 (03), pp. 071-077, March, 2013.

[76]. M. Mohan varma et al., design and evaluation of gastroretentive floating matrix tablets of tramadol hydrochloride, International Journal of Chemistry and Pharmaceutical Sciences, vol. 1 (4) 2012.

[77]. Zaida Urban-Morlan et al., Preparation of Ethyl Cellulose Nanoparticles by Solvent-Displacement Using the Conventional Method and a Recirculation System, The Journal of the Mexican Chemical Society 2015, 59(3), pg173-180.