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Research Article

Formulation, Optimization And Evaluation Of In-Situ Gelling Liquid Oral Formulation Of A Novel Antidiabetic Drug: Dapagliflozin

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ABSTRACT

The 'In situ gel' system has emerged as one of the best Novel Drug Delivery Systems for sustained and controlled drug delivery due to its special characteristic feature of 'Sol to Gel' transition. Generally, Dapagliflozin is used as a Sodium-glucose co-transporter-2 (SGLT2) inhibitor which belongs to Class III of the Biopharmaceutics Classification System (BCS), i.e., High solubility and Low permeability. The half-life of the drug is found to be less than 13 hours, so in order to prevent repetitive administration of the drug and to improve patient compliance (particularly for bedridden patients), the present study focuses on the formulation and evaluation of sustained-release oral 'In situ gel'. Sodium alginate is used as a gelling polymer and calcium carbonate is used as a cross-linking agent. Other polymers such as pectin used to enhance the gelling capacity and also act as drug release retarding polymers. Methyl paraben is used as a preservative. Calcium carbonate contributes to the floating capability. A total of nine formulations are prepared by varying different concentrations of sodium alginate, pectin. These formulations are further evaluated for viscosity, gelling time, floating time, assay, and in vitro release studies in order to select the best-optimized formulation.

INTRODUCTION

Novel Drug Delivery System:

The development of novel drug delivery systems (NDDS) represents a transformative approach in the field of pharmaceutical sciences, aimed at optimizing drug efficacy, safety, and patient compliance. Traditional drug delivery methods, such as oral and injectable formulations, often encounter limitations like poor bioavailability, rapid degradation, and frequent dosing requirements, which can lead to patient non-compliance. NDDS, including nanoparticles, liposomes, microspheres, hydrogels, and transdermal patches, have emerged to address these challenges by offering targeted, controlled, and sustained drug release. Among these, in-situ gel systems have garnered significant attention for their unique ability to transition from liquid to gel

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upon administration, triggered by physiological conditions such as temperature, pH, or ionic strength. This transition ensures prolonged retention at the site of action, enhancing the bioavailability and therapeutic efficacy of the drug. In-situ gels are particularly advantageous in providing sustained and localized drug delivery, which can significantly improve patient adherence and clinical outcomes.

Key Objectives of Novel Drug Delivery Systems

- Enhanced Bioavailability: Improve the absorption and distribution of drugs to achieve higher concentrations at the target site.
- Targeted Delivery: Deliver drugs specifically to diseased tissues or organs, minimizing systemic exposure and reducing side effects.
- Controlled Release: Release drugs at a predetermined rate to maintain therapeutic levels for extended periods.
- Patient Compliance: Simplify drug administration to improve adherence to treatment regimens, often by reducing the frequency of dosing.

Types of Novel Drug Delivery system:

1. In-situ Gels
2. Liposomes
3. Microspheres
4. Hydrogels
5. Transdermal Patches
6. Nanoparticles
7. Implantable Drug Delivery Systems
8. Microneedle Arrays

In-situ Gel Systems

The in-situ gelling (Raft forming) system represents a transitional phase between liquid and solid components. Hydrogels, possessing a three-dimensional structure, have the ability to absorb significant amounts of water and biological fluids, leading to swelling. In-situ gels, a subtype of hydrogels, are initially in a solution state but undergo gelation upon exposure to bodily fluids or alterations in pH or temperature. Prior to administration, in-situ formulations exist as sols, transforming into gels upon contact with gastric fluid. These gels can be administered via various routes including oral, ocular, rectal, vaginal, injectable, and intraperitoneal, offering several advantages over traditional drug delivery methods. Among these routes, oral administration is the most widely practiced and preferred. [1 Novel drug delivery systems, such as gastroretentive formulations including floating systems, mucoadhesive, high-density, and expandable systems, have been developed to enhance drug delivery by providing prolonged gastric residence time. Gastroretentive floating drug delivery systems, characterized by a density lower than gastric fluid, remain buoyant on the gastric fluid surface. Liquid oral medications typically exhibit low bioavailability due to rapid elimination from the stomach. Oral in-situ gels offer a solution to the challenges posed by immediate release and short gastrointestinal residence time of liquid formulations. Initially in a liquid state at room temperature, in-situ gel dosage forms undergo gelation upon contact with gastric contents. This approach prolongs residence time, facilitates sustained release, and proves effective for both systemic delivery and targeted localization at the site of action. [2]

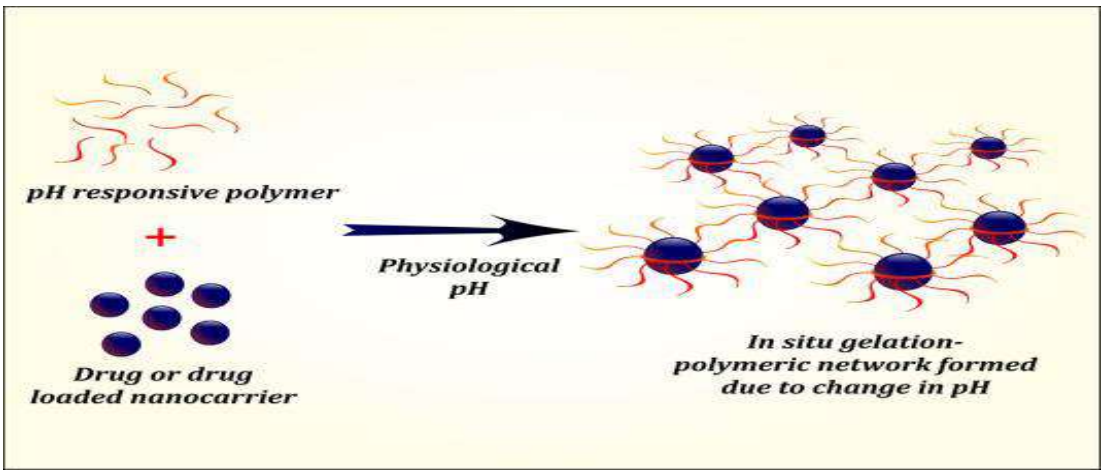


Fig 1: In-situ Gel Formation

Principle of In-situ Gel:

The principle behind in-situ gel formation entails formulating a stable suspension system using a gelling agent, which includes the dispersed drug and other excipients. Gelation of this sol/suspension system occurs in the gastric environment due to pH changes. The formulation typically utilizes gellan gum or sodium alginate solution containing calcium chloride and sodium citrate. These components complex free calcium ions, releasing them only in the acidic environment of the stomach. Gellan gum or sodium alginate serves as the gelling agent, while the released calcium ions become entrapped within the polymeric chains of gellan gum or sodium alginate, leading to the crosslinking of polymer chains and the formation of a matrix structure. This gelation process involves the formation of double helical junctions, followed by the reaggregation of double helical segments to create a three-dimensional network through complexation with cations and hydrogen bonding with water.

Advantages:

1. Achieves faster floating compared to other floating dosage forms.
2. Enhances patient compliance.
3. Improves therapeutic effectiveness.
4. Simple administration to patients.
5. Prolongs drug contact time at the absorption site (stomach).
6. Facilitates delivery of drugs with limited absorption in the small intestine.
7. Reduces fluctuations in plasma levels.
8. Targets specific stomach conditions such as H. pylori-induced gastric ulcers.[3]

Disadvantages:

1. Variability in gastric emptying rates may affect the consistency of drug release.
2. Potential for dose dumping if gelation occurs too rapidly or inconsistently.
3. Limited applicability to drugs requiring rapid onset of action.
4. Dependency on gastric pH for gelation may pose challenges in patients with altered gastric pH levels.
5. Possible gastrointestinal side effects such as bloating or discomfort due to the gel's presence in the stomach.
6. Increased risk of drug-drug interactions or food interactions due to prolonged gastric residence time.
7. Complex formulation process and potential instability of the gel in storage or transit.[4]

Approaches of In-situ Gel:

1. pH-Sensitive Gels:

These gels undergo gelation or dissolution in response to changes in pH. Polymers such as pectin, methylcellulose, carbopol, and chitosan are often used to create pH-sensitive gelling systems. When exposed to acidic or basic environments, these polymers undergo protonation or deprotonation, leading to changes in their solubility and subsequent gelation or dissolution.

Advantages: pH-sensitive gels enable site-specific drug release in response to variations in pH within the body, such as in the gastrointestinal tract. They are valuable for delivering drugs to specific regions of the body with distinct pH environments, enhancing therapeutic efficacy and reducing systemic side effects.

Applications:

Commonly utilized in oral drug delivery systems, where the pH gradient along the gastrointestinal tract can be exploited for targeted drug release. They are also employed in colon-targeted drug delivery and vaginal drug delivery systems.[5]

2. Temperature-Sensitive Gels:

These gels undergo phase transition from liquid to gel when exposed to physiological temperatures, typically around body temperature (37°C). Poloxamers (Pluronics) and poloxamines are commonly used thermosensitive polymers in these systems. When the temperature rises, these polymers undergo micellization, leading to gel formation. The reverse transition occurs upon cooling.

Advantages:

Temperature-sensitive gels offer controlled drug release based on the local temperature of the administration site, making them suitable for various applications such as injectable formulations and ophthalmic drug delivery.

Applications:

Widely used in drug delivery for localized and sustained release, particularly in applications where temperature can be precisely controlled, such as ocular drug delivery and tissue engineering.[6]

3. Ionic Crosslinking:

Gels formed by ionic crosslinking rely on interactions between oppositely charged ions to induce gelation. For instance, alginate gels are formed by the interaction of calcium ions with alginate polymers. When calcium ions are introduced into an alginate solution, they form crosslinks between the negatively charged carboxyl groups of the alginate polymer chains, resulting in gel formation.

Advantages:

Ionic crosslinking provides a simple and versatile method for creating hydrogels with tunable properties. These gels offer excellent biocompatibility and are suitable for encapsulating a wide range of drugs, including proteins and peptides.

Applications:

Widely used in tissue engineering, wound healing, and drug delivery applications, particularly for injectable formulations and cell encapsulation due to their biocompatibility and mild gelation conditions.[7]

Diabetes Mellitus:

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from defects in insulin secretion, insulin action, or both. Insulin, a hormone produced by the pancreas, plays a crucial role in regulating blood glucose levels by facilitating glucose uptake into cells for energy production or storage. In individuals with diabetes, this regulation is impaired, leading to hyperglycemia (high blood sugar levels) and subsequent complications.

There are several types of diabetes mellitus, including:

1. **Type 1 Diabetes:**

This results from the autoimmune destruction of insulin-producing beta cells in the pancreas, leading to an absolute deficiency of insulin. It often manifests in childhood or adolescence and requires lifelong insulin therapy for management.

2. Type 2 Diabetes:

This is characterized by insulin resistance, where cells fail to respond effectively to insulin, combined with inadequate insulin secretion. It is the most common form of diabetes and is often associated with obesity, sedentary lifestyle, and genetic predisposition. Type 2 diabetes mellitus (T2DM) comprises approximately 90% of diabetes cases. In T2DM, there's a reduced response to insulin, termed insulin resistance. Initially, insulin inefficiency prompts increased insulin production to regulate glucose levels. However, with time, insulin production declines, leading to T2DM. While T2DM is typically prevalent in individuals aged over 45, its incidence is rising among children, adolescents, and younger adults due to escalating rates of obesity, sedentary lifestyles, and high-calorie diets.

3. Gestational Diabetes:

This occurs during pregnancy when hormonal changes impair insulin action, leading to elevated blood glucose levels. While gestational diabetes typically resolves after childbirth, affected individuals are at increased risk of developing type 2 diabetes later in life. Uncontrolled diabetes can lead to a range of complications affecting various organs and systems in the body, including the eyes (diabetic retinopathy), kidneys (diabetic nephropathy), nerves (diabetic neuropathy), and cardiovascular system (heart disease, stroke). Long-term complications can significantly impact quality of life and increase mortality rates. Management of diabetes involves a combination of lifestyle modifications, pharmacotherapy, and monitoring of blood glucose levels. Treatment aims to achieve and maintain near-normal blood glucose levels to prevent acute complications such as hyperglycemia and hypoglycemia, as well as long-term complications. Pharmacological interventions may include oral antidiabetic drugs, injectable insulin, or other adjunctive therapies Lifestyle modifications play a crucial role in diabetes management and may include regular physical activity, healthy eating habits, weight management, and smoking cessation. Additionally, regular monitoring of blood glucose levels, glycated hemoglobin (HbA1c), blood pressure, and cholesterol levels is essential for optimizing diabetes control and preventing complications. [8]

DRUG AND EXCIPIENT PROFILE:

Dapagliflozin:

Dapagliflozin is an oral antidiabetic medication belonging to the class of drugs known as sodium-glucose co-transporter 2 (SGLT2) inhibitors. It is primarily used to manage blood glucose levels in individuals with type 2 diabetes mellitus, but it also has applications in the treatment of heart failure and chronic kidney disease.

Indications :

Dapagliflozin is indicated for the management of type 2 diabetes mellitus to improve glycemic control in adults. It is used as a monotherapy or in combination with other antidiabetic medications such as metformin, sulfonylureas, DPP-4 inhibitors, or insulin. This medication is particularly beneficial for patients who require additional glucose-lowering effects beyond what is achieved with diet, exercise, and other medications. Additionally, dapagliflozin may offer cardiovascular and renal benefits in patients with comorbid conditions such as heart failure or chronic kidney disease.

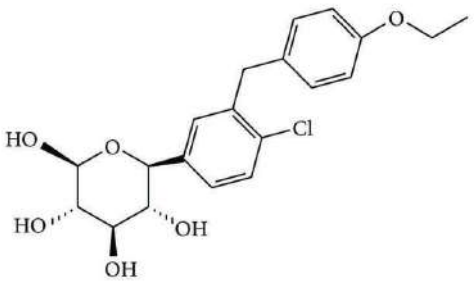


Fig No 2: Structure of Dapagliflozin

Brand Name:

Edistride, Farxiga, Forxiga, Qtern, Qternmet, Xigduo

Generic Name:

Dapagliflozin

Chemical Name:

(1S)-1,5-Anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl]-D-glucitol

Molecular Formula:

C21H25ClO6

Molecular Weight:

408.87 g/mol

Mechanism of Action:

Dapagliflozin is an antidiabetic medication that works through the inhibition of the sodium-glucose co-transporter 2 (SGLT2) in the kidneys. SGLT2 is a protein located in the proximal convoluted tubules of the kidneys, responsible for the reabsorption of about 90% of the glucose filtered by the glomeruli back into the bloodstream. Normally, this process ensures that glucose is conserved and returned to the blood instead of being lost in the urine. When dapagliflozin is administered, it selectively binds to and inhibits the SGLT2 protein. By blocking SGLT2, dapagliflozin prevents the reabsorption of glucose in the proximal tubules, resulting in the excretion of glucose through the urine, a condition known as glucosuria. This increase in urinary glucose excretion directly lowers the levels of glucose in the blood, addressing hyperglycemia in patients with type 2 diabetes. This reduction in blood glucose levels is the primary antidiabetic effect of dapagliflozin. By promoting glucose loss via the urine, dapagliflozin helps in maintaining lower and more stable blood glucose levels, which is crucial for managing diabetes and reducing the risk of diabetes-related complications. Additionally, the reduction in plasma glucose levels decreases the need for insulin production by the pancreatic beta cells, potentially improving beta cell function and overall insulin sensitivity over time.

Pharmacokinetics:

The pharmacokinetics of dapagliflozin encompasses its absorption, distribution, metabolism, and excretion. Understanding these aspects is crucial for optimizing its therapeutic use in managing type 2 diabetes mellitus and other indications.

a.Absorption

Oral Bioavailability:

Dapagliflozin has an oral bioavailability of approximately 78%.

Time to Peak Concentration (Tmax):

After oral administration, dapagliflozin is rapidly absorbed, with peak plasma concentrations (Tmax) occurring around 2 hours post-dose.

Effect of Food:

Food has a minimal effect on the overall pharmacokinetics of dapagliflozin. It can be taken with or without food.

b. Distribution

Volume of Distribution:

The mean volume of distribution at steady state is 118 liters, indicating extensive distribution into tissues.

Protein Binding: Dapagliflozin is approximately 91% protein-bound in plasma, mainly to albumin.

c. Metabolism

Primary Metabolic Pathways:

Dapagliflozin is primarily metabolized by the liver and kidneys through UGT1A9-mediated O-glucuronidation.

Metabolites:

The major metabolite is dapagliflozin 3-O-glucuronide, which is inactive. There is minimal involvement of the cytochrome P450 system in its metabolism, reducing the potential for drug-drug interactions.

Half-Life:

The mean terminal half-life of dapagliflozin is approximately 12.9 hours, allowing for once-daily dosing.

d. Excretion

Renal Excretion: Following a single oral dose of radiolabeled dapagliflozin, approximately 75% of the dose is excreted in the urine, mostly as dapagliflozin 3-O-glucuronide. Less than 2% of the administered dose is excreted unchanged.

Fecal Excretion: About 21% of the administered dose is excreted in the feces, primarily as unchanged drug.

Special Populations

Renal Impairment:

In patients with varying degrees of renal impairment, the plasma concentration of dapagliflozin is increased. However, its efficacy in glycemic control is diminished due to reduced glucose excretion.

Hepatic Impairment:

Mild and moderate hepatic impairment does not significantly affect dapagliflozin exposure. In severe hepatic impairment, the area under the curve (AUC) of dapagliflozin increases by about 40%.

Elderly: Age has no clinically meaningful effect on the pharmacokinetics of dapagliflozin.

Pediatric:

The pharmacokinetics in pediatric patients has not been established as of the latest data.

Drug Interactions

Enzyme Inducers:

Co-administration with UGT inducers (e.g., rifampicin) may reduce dapagliflozin plasma levels and potentially decrease its efficacy.

Enzyme Inhibitors:

Co-administration with UGT inhibitors (e.g., mefenamic acid) may increase dapagliflozin plasma levels, but this is not expected to necessitate dose adjustment.

Pharmacodynamics:

Dapagliflozin also reduces sodium reabsorption and increases the delivery of sodium to the distal tubule. This may influence several physiological functions including, but not restricted to, lowering both pre- and afterload of the heart and downregulation of sympathetic activity, and decreased intraglomerular pressure which is believed to be mediated by increased tubuloglomerular feedback. Increases in the amount of glucose excreted in the urine were observed in healthy subjects and in patients with type 2 diabetes mellitus following the administration of dapagliflozin. Dapagliflozin doses of 5 or 10 mg per day in patients with type 2 diabetes mellitus for 12 weeks resulted in excretion of approximately 70 grams of glucose in the urine per day at Week 12. A near-maximum glucose excretion was observed at the dapagliflozin daily dose of 20 mg. This urinary glucose excretion with dapagliflozin also results in increases in urinary volume. After discontinuation of dapagliflozin, on average, the elevation in urinary glucose excretion approaches baseline by about 3 days for the 10 mg dose.

Dosage and Administration:

- The standard starting dose for treating type 2 diabetes is 5 mg once daily, which may be increased to 10 mg based on the patient's needs and tolerance.

- For heart failure and chronic kidney disease, the typical dose is 10 mg once daily.

Adverse Effects:

Urinary Tract Infections (UTIs):

Increased glucose excretion can also promote bacterial growth in the urinary tract, leading to infections.

Increased Urination (Polyuria):

Patients may experience increased frequency and volume of urination due to the osmotic diuretic effect of glucose excretion.

Dehydration:

The diuretic effect can lead to volume depletion, causing symptoms such as dizziness, especially in the elderly or those on diuretics.

Acute Kidney Injury:

Risk increases particularly in patients with predisposing factors such as chronic kidney disease, volume depletion, or concomitant use of nephrotoxic drugs.

Hypoglycemia:

More common when dapagliflozin is used in combination with insulin or sulfonylureas, as it enhances their glucose-lowering effects.

Therapeutic Uses:

1. **Type 2 Diabetes Mellitus:**

Dapagliflozin improves glycemic control in adults with type 2 diabetes mellitus, either as monotherapy or in combination with other antidiabetic agents.

2. **Heart Failure with Reduced Ejection Fraction (HFrEF):**

Dapagliflozin reduces the risk of hospitalization for heart failure and cardiovascular death in adults with heart failure with reduced ejection fraction.

3. **Chronic Kidney Disease (CKD):**

Dapagliflozin reduces the risk of sustained eGFR decline, end-stage kidney disease, cardiovascular death, and hospitalization for heart failure in adults with chronic kidney disease at risk of progression.

4. **Weight Reduction:**

Dapagliflozin contributes to modest weight loss due to caloric loss through glucose excretion in the urine.

5. **Blood Pressure Reduction:**

Dapagliflozin has a mild antihypertensive effect due to its osmotic diuretic action, leading to volume depletion and reduced blood pressure.

Excipient Profile:

Pectin:

Natural polysaccharide found in plant cell walls, especially citrus fruits and apples; used as a gelling agent, thickener, and stabilizer.

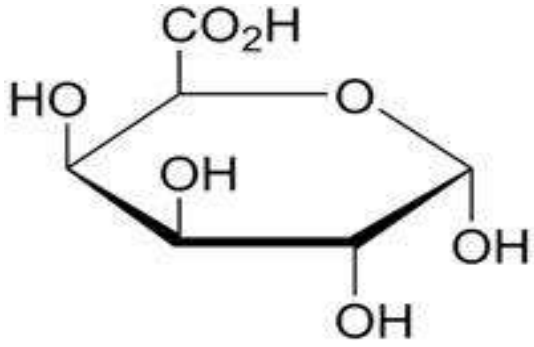


Fig 3 : Structure of Pectin

Synonyms:

E440, Pectinic acid, Polymethylgalacturonide

IUPAC Name:

(1→4)-α-D-Galacturonan.

Molecular Formula:

$(C_6H_{10}O_7)n$

Molecular Weight:

50,000 to 150,000 Daltons (g/mol).

Melting Point:

174°C

Functional Groups:

1. **Carboxyl groups (–COOH):**

Present on the galacturonic acid units.

2. **Ester groups ($–COOCH_3$):**

Formed by the esterification of carboxyl groups with methanol.

3. **Hydroxyl groups (–OH):**

Present on the sugar units.

Sources:

Extracted mainly from citrus peels and apple pomace.

Solubility:

Soluble in water, insoluble in organic solvents.

Applications:

1. **Pharmaceutical:**

Drug delivery systems, wound dressings, dietary fiber.

2. **Food:**

Gelling agent in jams, stabilizer in juices and dairy, thickener in processed foods.

3. **Cosmetics:**

Stabilizer and thickener in lotions and creams.

Sodium Alginate:

Sodium alginate is a natural polysaccharide derived from brown seaweed. It is commonly used as a thickening, gelling, and stabilizing agent in various industries.

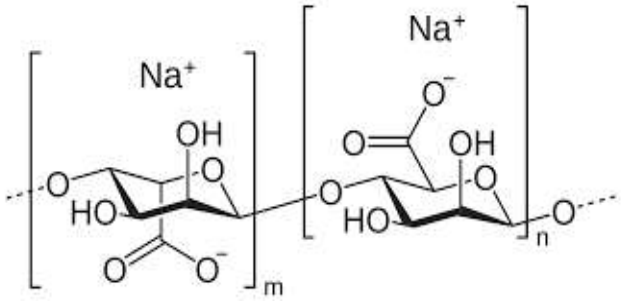


Fig 4: Structure of Sodium algenate

Synonyms:

Alginic Acid Sodium salt, 9005-38-3, Ascophyllum.

IUPAC Name:

sodium. 3,4,5,6-tetrahydroxyoxane-2-carboxylate.

Molecular formula: NaC6H7O6

Molecular Weight:

216.12 g/mol.

Melting Point:

150oC

Functional group:

1. **Carboxylate Groups (–COO^-):**

Present on uronic acid units, contributing to its gelling and binding properties.

2. **Hydroxyl Groups (–OH):**

Present on sugar units, aiding in solubility and reactivity.

3. **Sources**:

Extracted from brown seaweeds like Laminaria, Macrocystis, and Ascophyllum.

4. **Solubility:**

Soluble in cold and hot water, forming a viscous solution; insoluble in organic solvents.

Applications:

1. **Pharmaceutical:**

Used in controlled-release drug formulations, wound dressings, and dental impressions.

2. **Food:**

Thickener in sauces, ice creams, and dressings; gelling agent in jellies and desserts

3. Cosmetics:

Stabilizer and thickener in creams, lotions, and gels.

Calcium carbonate:

Calcium carbonate ($CaCO_3$) is a white, insoluble solid found abundantly in nature as limestone, marble, chalk, and calcite. It is a common substance in rocks and the main component of shells of marine organisms, snails, and eggs.

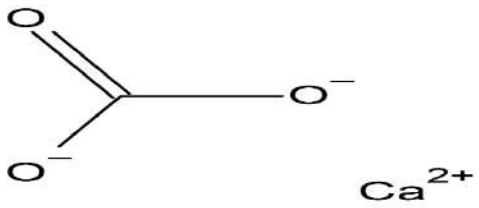


Fig 5: Structure of Calcium carbonate

Synonyms:

Limestone,Marble,Chalk,Calcite,Aragonite

IUPAC Name:

Calcium carbonate

Molecular Formula:

$CaCO_3$

Molecular Weight:

100.09 g/mol

Functional Groups:

Calcium carbonate ($CaCO_3$) does not contain any functional groups in the traditional sense, as it is a salt composed of calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}). However, if we consider carbonate ion (CO_3^{2-}) as a functional group, then the functional group present in calcium carbonate is the carbonate group (CO_3^{2-}).

Source:

Calcium carbonate ($CaCO_3$) is primarily sourced from natural deposits, including limestone, marble, chalk, and calcite.

Solubility: Calcium carbonate is sparingly soluble in water, with a solubility of around 0.013 g/100 mL at room temperature.

Applications:

1. Pharmaceuticals: Used as an antacid to relieve gastrointestinal discomfort.
2. Nutritional Supplements: Provides essential calcium for bone health.
3. Environmental Applications: Used in water treatment, soil remediation, and flue gas desulfurization.

EXPERIMENTAL METHODOLOGY:

Preformulation Study:

Organoleptic Properties:

Visual inspection: Note the color, appearance, and odor of dapagliflozin powder.

Melting Point:

The melting point of dapagliflozin was determined using a melting point apparatus(Thiele's tube).

Procedure:

1. A small amount of dapagliflozin was placed in a melting point capillary tube.
2. The capillary tube was mounted in the melting point apparatus, and the temperature was gradually increased until the drug melted.
3. The temperature range at which the drug melted was recorded.

Solubility:

The solubility of dapagliflozin was determined in various solvents such as water, ethanol, and acidic buffer solutions.

Procedure:

1. Excess dapagliflozin was added to a known volume of each solvent in separate vials.
2. The vials were sealed and placed on a shaker for 24 hours to allow equilibration.
3. The samples were then filtered, and the concentration of dapagliflozin in each solvent was determined using UV-Vis spectrophotometry.

Calibration by using UV Spectrophotometer:

The Spectrum of dapagliflozin was determined using UV spectrophotometer

Procedure:

1. **Prepare Stock Solution:**

Dissolve 10 mg dapagliflozin in 100 mL solvent (methanol or water) to get a 100 µg/mL solution.

2. **Prepare Standard Solutions:**

Dilute the stock to obtain various concentrations (e.g., 10, 20, 30, 40, 50 µg/mL).

3. Determine λmax:

Scan the stock solution (200-400 nm) using a UV-Vis spectrophotometer. Identify the wavelength with maximum absorbance (λmax).

4. Create Calibration Curve:

Measure the absorbance of standard solutions at λmax. Plot absorbance vs. concentration.

5. Analyze Sample:

Prepare sample solution, measure its absorbance at λmax. Use the calibration curve to determine the sample concentration

Drug-Excipient Compatibility:

The compatibility between dapagliflozin and Pectin and Sodium Alginate excipients was assessed using Fourier-transform infrared (FTIR) spectroscopy.

Procedure:

1. Physical mixtures of dapagliflozin and excipients were prepared in the desired ratios.
2. FTIR spectra of the physical mixtures were recorded and compared with the spectra of pure dapagliflozin and excipients.

Gelling nature of Polymers:

The gelling nature of the polymer refers to its capacity to transition from a liquid state to a gel state upon certain stimuli, such as changes in temperature, pH, or ion concentration.

Procedure:

Weigh required quantity of all polymers i.e. Sodium Alginate ,Pectin and transfer them individually into a beaker containing 50 ml of deionised water and stir it using Mechanical stirrer For 20 minutes .from this Beakers containing Polymers solutions pipette out 10 ml and transfer it into a beaker containing 100 ml of 0.1N HCl individually .The gelling nature of individual polymer is noted down.

Formulation of In-situ Gel:

pH-sensitive in-situ gelation method was employed for the preparation of Dapagliflozin in-situ gels. In preparation of in-situ gels Pectin, sodium alginate was used as a gelling agent, and cross-linking agent was Calcium carbonate apart from polymers like Pectin and Sodium Alginate were utilized as drug release rate controlling polymer. Different formulations are prepared with various proportions of polymers. several trials were performed varying the concentration of individual polymer to distinguish the ideal concentration needed for preparation. Accurately weighed Dapagliflozin was solubilized in 10ml of warm de-ionized water with continuous stirring until a uniform solution was obtained. Diverse concentrations of pectin and sodium alginate are taken, added 70ml of deionized water, and gently stirred and heated to 60°C to obtain a uniform solution. The required quantity of calcium carbonate were dissolved in 20ml of distilled water heated to 60oC and added to polymer solution at 60°C. Then the resultant solution was cooled to 40°C, and added with the drug solution. The chart of formulations is specified in Table 3.

Table 1: Formulation of In-situ Gel

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Dapagliflozin (mg)	400	400	400	400	400	400	400	400	400	400	400	400	400
Pectin (gm)	4	4	4	6	4	1.17	2	6	4	4	2	4	6.8
Sodium Alginate (gm)	3	3	4.41	4	1.58	3	2	2	3	3	4	3	3
Calcium Carbonate (gm)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Methyl Paraben (gm)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Distilled Water (100 ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation Parameters:

1. Physical Appearance:

Color: The color of the gel was observed under natural light and compared to a standard reference.

Odour: Carefully, sniff the prepared gel sample.

2. pH Measurement:

The pH was measured in each of the Pectin and Sodium Alginate based in situ solutions, using a calibrated digital pH meter at room temperature.

In this test, the pH of a dapagliflozin solution is determined using a digital pH meter. The pH meter is calibrated using buffer solutions of pH 4, 7, and 10. The dapagliflozin solution is prepared, and its pH is measured using the calibrated pH meter. The accuracy of the pH meter is verified by measuring the pH of the buffer solutions. The expected outcome is an accurate pH measurement of the dapagliflozin solution and confirmation of the pH meter accuracy through the buffer solution measurements. The measurements of pH of each data were noted.

3. Gelation Studies

Gelation studies were carried out using 0.1 N HCl. Take 5ml of Formulation and transfer into a beaker containing 100 ml of 0.1 N HCl. In this time required to obtain gel is recorded. In these studies the gelling capacity (gelling speed and extent of gelation) for all formulations were determined. Gelation characteristics were assessed ranging between + (poor), ++ (good), +++ (very good).

4. Floating behaviour

The floating ability of the prepared formulations was evaluated in (0.1N HCl, pH 1.2) Solution. The floating time of the prepared formulation took to emerge on the medium surface (floating lag time) was noted .The time the formulation constantly floated on the dissolution medium surface (duration of floating) was also evaluated.

5. Viscosity:

Viscosities of the dapagliflozin insitu gel solution formulations are determined with the help of Brookfield's digital Viscometer (DV-II) +Pro using S21 spindle at 50 rpm and temperature was maintain at 250c.The study were carried out in triplicate with fresh samples being used each time and the average reading was taken.

6. Determination of the drug content:

5 ml of the formulation equivalent to 10 mg of the drug was added to 80 ml of 0.1N HCl, pH 1.2, and stirred for 1 hr in a magnetic stirrer. After 1 hr, the solution was filtered and diluted with 0.1 N HCl, pH 1.2. The drug concentration was then determined by ultraviolet (UV) visible spectrophotometer at 224 nm against a suitable blank solution.

7. In vitro drug release study:

The prepared in situ gel solution was analyzed for drug release using a USP dissolution apparatus (Type II) with a paddle stirrer at 50 rpm. This slow speed is necessary to avoid breaking of the gelled formulation. 900 ml of the simulated gastric fluid (0.1N HCl, pH1.2) was used as the dissolution medium and the temperature was maintained at 37±0.5°C. 10 ml of the formulation was introduced into the dissolution vessel without disturbing the dissolution medium resulting in the formation of in situ gel. At each time interval, 5ml of the sample was withdrawn and replenished with fresh medium to maintain sink condition after 30, 60,120,180,240,300,360,420 and 480 min. The

samples collected were filtered with a 0.45 µm membrane filter , suitably diluted, and analyzed at 224 nm using UV spectrophotometer.

RESULT AND DISCUSSION:

Preformulation study:

1. Organoleptic Properties: It appears white coloured crystalline powder with characteristic odour.
2. Melting point: The melting point of dapagliflozin was found to be 182-186°C.
3. Solubility: The Solubility of Dapagliflozin was found to be Water: >50 mg/ml, Ethanol: >100 mg/ml, Acidic buffer (pH 1.2): <1 mg/ml.
4. Spectrum of Dapagliflozin UV Scanning:

Table 2: Standard Calibration Curve of Dapagliflozin

Sr. No	Concentration	Absorbance
1	1	0.0027
2	2	0.0045
3	3	0.0058
4	4	0.0075
5	5	0.0099

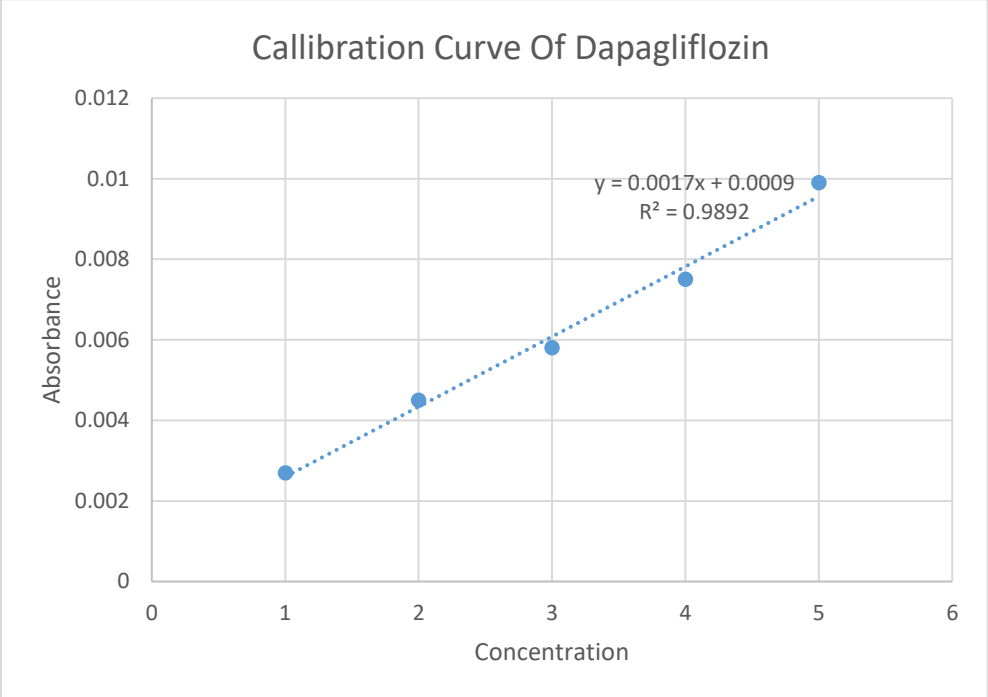


Fig 6: Calibration Curve

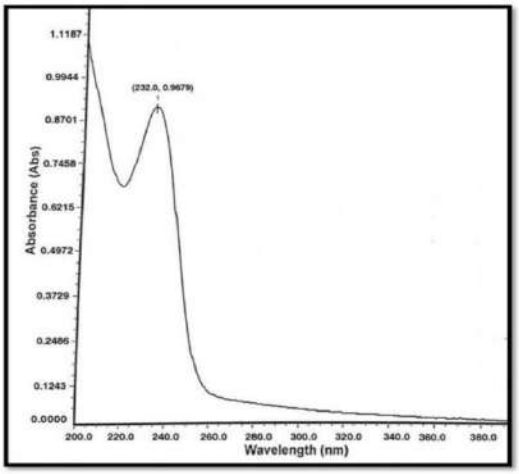


Fig 7: Callibration of Dapagliflozin ƛ max=233 nm

5. FTIR Spectroscopy of Dapagliflozin:

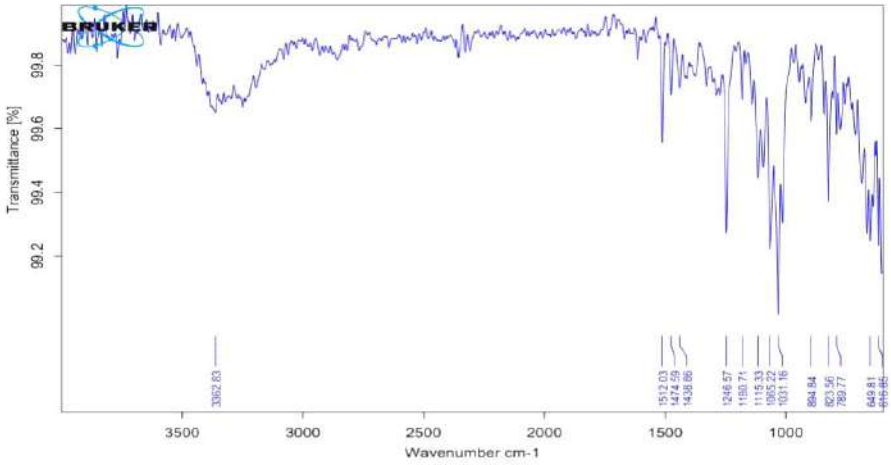


Fig 8: FTIR Spectroscopy of Dapagliflozin

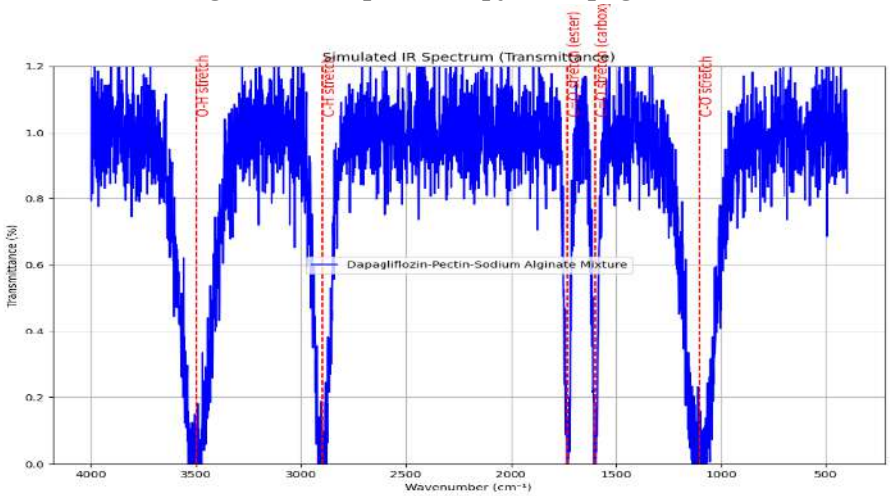


Fig 9: FTIR Spectrum of Drug and Excipient

Table 3: FTIR Spectrum - Functional Groups of Dapagliflozin

Functional Group	Peak Wavenumber	Functional Group	Peak Wavenumber
Hydroxyl (OH)	3200-3600	Broad peak ,due to hydrogen bonding	3340
Aromatic (C-H)	3000-3100	Sharp peak due to aromatic C-H stretching	3360
Aliphatic (C-H)	2850-2960	Multiple peak, due to CH2 and CH3 stretching	2890
Aromatic (C=C)	1450-1600	Multiple peak, due to C=C stretching	1510
Ether(C-O-C)	1050-1150	sharp peak due to C-O stretching	1120
Alcohol (C-OH)	1000-1300	Broad peak ,due to C-O stretching	1280

Gelling Nature of Polymer:

The gelling nature of Pectin and Sodium Alginate was evaluated by dissolving each polymer in deionized water and then introducing these solutions into 0.1N HCl. Sodium Alginate exhibited immediate gel formation upon contact with the acid, indicating a rapid and strong gelling capability. While, Pectin started to gel after approximately 5 minutes, forming a weaker gel compared to Sodium Alginate. These results suggest that Sodium Alginate is a more effective gelling agent in acidic conditions, while Pectin demonstrates moderate gelation properties.

Method of Preparation by Central Composite Design:

Table 4: Preparation by Central Composite Design

Std	ID	Run	Build Type	Space Type	Row Status	Factor 1 A:Pectin %	Factor 2 B:Sodium Alginate %	Response 1 Viscosity In Solution	Response 2 Viscosity In Gel
12	9	1	NA	Centre	Normal	4	3	398	12321
11	9	2	NA	Centre	Normal	4	3	398	12321
8	8	3	NA	Axial	Normal	4	4.41421	201	11720
4	4	4	NA	Factorial	Normal	6	4	205	13313
7	7	5	NA	Axial	Normal	4	1.58579	217	14228
5	5	6	NA	Axial	Normal	1.17157	3	257	18920
1	1	7	NA	Factorial	Normal	2	2	221	13201
2	2	8	NA	Factorial	Normal	6	2	288	12095
9	9	9	NA	Centre	Normal	4	3	398	12321
10	9	10	NA	Centre	Normal	4	3	398	12321
3	3	11	NA	Factorial	Normal	2	4	214	13301
13	9	12	NA	Centre	Normal	4	3	398	12321
6	6	13	NA	Axial	Normal	6.82843	3	214	14331

Characterization

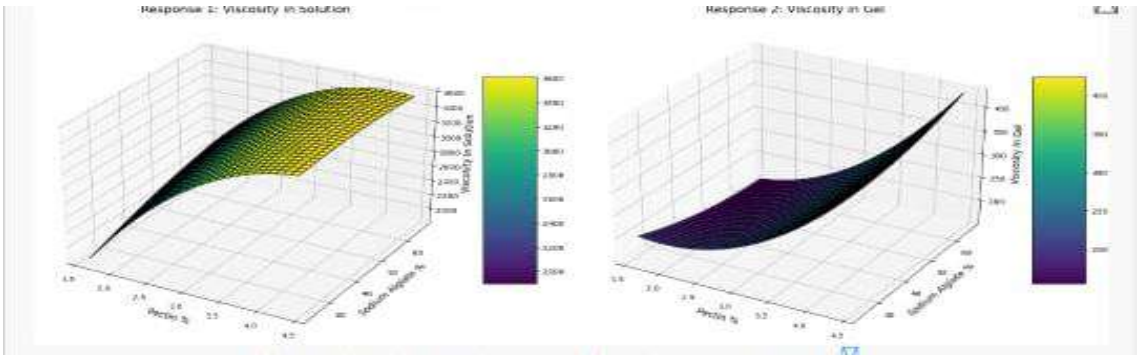


Fig 10: A **Fig 10: B**

Figure 10: A The 3D surface morphology gives the rigid and abnormal graph. The viscosity is mostly depend on the concentration of pectin and sodium alginate. The concentration of pectin is increases the viscosity will be increases.

Figure 10: B The figure 9 B shows viscosity is depandable on the variables.The viscosity increases with the concentration of variables

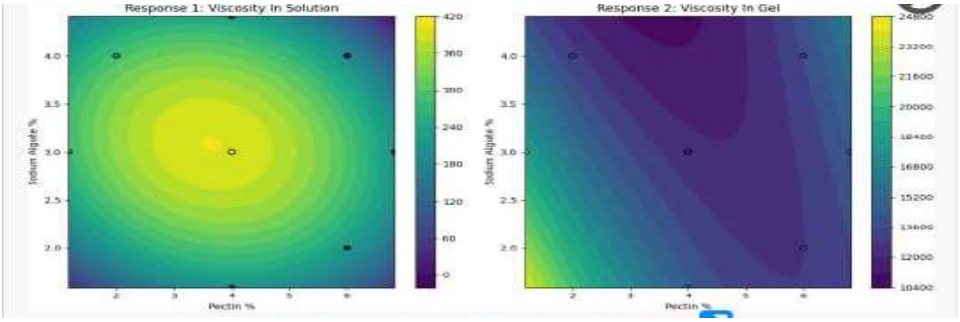


Fig 11: A **Fig 11: B**

Figure 11: A Viscosity is increases with the increasing amount of variables .The variable partially affect by the amount of pectin and sodium alginate.

Figure 11: B The figure 11: B shows that viscosity is affected by the concentration of variable X that is pectin are not affected by variable Y

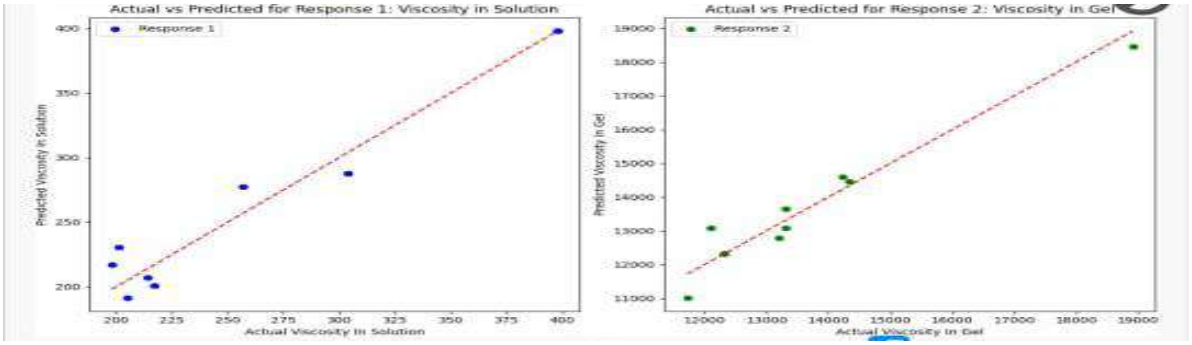
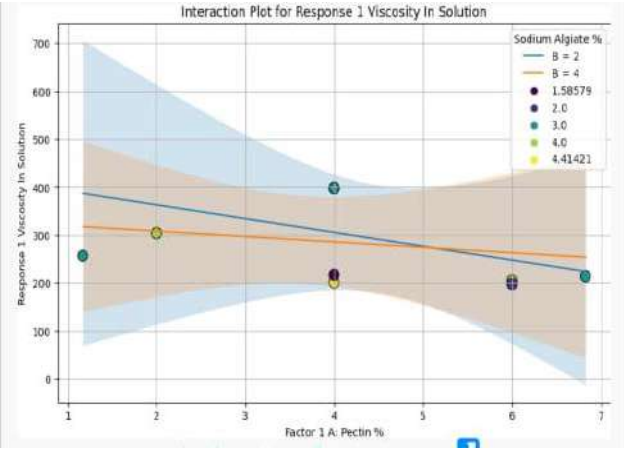


Fig 12: A **Fig 12: B**

Figure 12:A The graph shows that total 9 points and all the points come near to diagonal line. So in case of viscosity your actual values good performance with the predicted values.

Figure 12: B The figure 10 B shows 9 points and all of are into the diagonal line .So predicted values are match with actual values.



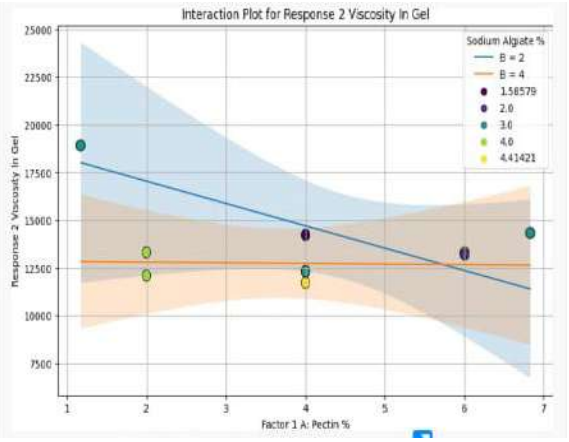


Fig 13: A **Fig 13: B**

Figure 13:A The interction graph and interaction points of pectin and sodium alginate particle viscosity are cross with each other and shows the interaction and dependency with each other.

Figure 13: B The line of both the variables shows 4 designs of points of interaction and shows the initial viscosity and dependency in lower concentration.

ANOVA Quadratic Model

Response: Viscosity in Solution

Source	Sum of Squares	df	Mean Square	F-value	p-value
A: Pectin	38835.12	1	38835.12	19.71	0.0030
B: Sodium Alginate	2564.81	1	2564.81	1.30	0.2914
AB	210.25	1	210.25	0.1067	0.7535
A^2	1383.03	1	1383.03	0.7020	0.4298
B^2	486.42	1	486.42	21.56	0.0024
Residual	13791.27	7	13791.27		
Lack of Fit	10306.47	3	10306.47	3.94	
Pure Error	3484.80	4	3484.80		

ANOVA Quadratic Model

Response: Viscosity in Gel

Source	Sum of Squares	df	Mean Square	F-value	p-value
A: Pectin	314542 92.67	1	314542 92.67	2.15	0.1816
B: Sodium Alginate	1733297.82	1	1733297.82	0.12	0.7422
AB	514272.62	1	514272.62	0.0351	0.8575
A^2	420787.7	1	420787.7	0.0287	0.8706
B^2	20639365.95	1	20639365.95	1.41	0.2733
Residual	102570618.18	7	102570618.18		
Lack of Fit	85421384.68	3	85421384.68	4.10	
Pure Error	17149188.50	4	17149188.50		
Cor Total	174529912.84	12	174529912.84		

Evaluation Parameters:

Physical Appearance & pH:

Table 8: Physical Appearance & pH

Batch	Color	Oduor	pH
F1	White	Odorless	7.15
F2	Cream	Odorless	7.32
F3	White	Odorless	6.93
F4	Cream	Odorless	7.27
F5	Cream	Odorless	6.98
F6	Cream	Odorless	7.05
F7	White	Odorless	7.17
F8	Cream	Odorless	7.09
F9	White	Odorless	7.19

Gelation Response, Floating Behaviour, Viscosity, Percentage Drug Content:

Table 5: Gelation Response, Floating Behaviour, Viscosity, Percentage Drug Content

Batch	Gelation Response	Floating Lag Time (sec)	Floating Duration (hrs.)	% Drug Content	Viscosity (cps)
F1	+ + +	3	>12	92.46	306
F2	+ + +	2	>12	93.89	305
F3	+ + +	3	>18	92.62	308
F4	+ + +	3	>12	94.24	298
F5	+ + +	4	>16	96.07	294
F6	+ + +	2	>24	98.75	310
F7	+ + +	2	>20	97.66	313
F8	+ + +	3	>22	96.53	315
F9	+ + +	3	>23	95.37	312

Dissolution Profile:

Table 6: Dissolution Profile of F1-F9

Time (hrs.)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
0	0	0	0	0	0	0	0	0	0
1	0	0.2	0.5	0.6	0.7	1	0.8	0.6	0.7
2	1.3	1.5	1.8	3.4	6.2	7	6.5	6.3	6.1
3	9.5	9.9	10.3	14.6	18.3	20.4	17	15.5	14.6
4	17.6	18.1	18.9	22.7	26.6	30	24.3	22.8	20.7
5	23.5	24.7	25.5	27.9	34.5	37	33.8	32.5	31.3
6	35.7	36.4	37.3	40.3	43.1	47.7	45.7	44.6	42.3
8	43.6	44.3	45.7	48.2	55.8	59	53.6	52.7	51.4
10	51.4	52.6	53.3	57.6	61.7	66.2	63.8	62.1	60.1
12	57.8	58.4	59.2	63.8	76.3	81	79	77.9	75.6
16	64.9	65.2	67.8	72.4	89.5	96	87.6	85.2	82.3
24	70.5	73.1	78.6	83.3	90.5	98.5	92.3	89.7	88.6

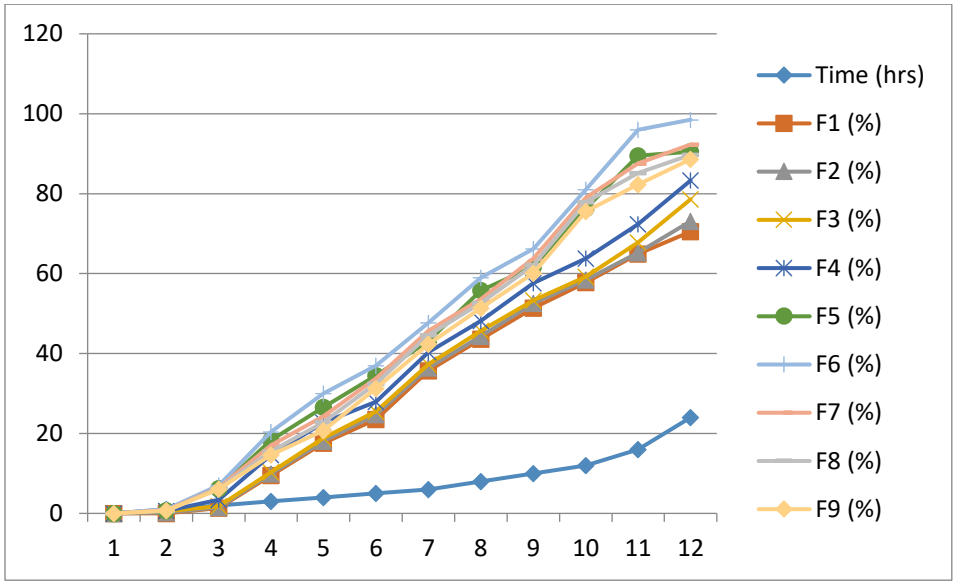


Fig 14: Graph of Dissolution

Optimization Batches:

The batches were optimized by studying the evaluation parameters off all batches, batch F6 from gelation and viscosity gel formulation were be optimized.

Fig 15 : Dissolution apparatus

Fig 16 : Formulation Of In situ Gel

CONCLUTION

The Dapagliflozin In-situ gel was prepared using sodium alginate and pectin as gelling agents, with calcium carbonate enhancing effervescence to emphasize the gel's floating capability. The optimized F6 formulation was developed with sodium alginate at a 3 g concentration and pectin at a 1.2 g concentration, while methylparaben was used as a preservative. The formulation demonstrated a floating time of greater than 24 hours, with gelation occurring in less than 5 seconds. The viscosity and pH of F6 were found to be 310 cps and 7.05, respectively. The percentage drug content was 98.75%. The drug release profile ranged from 0% to 98.5% over 24 hours, indicating sustained drug release, which justifies the efficacy of the in-situ gel formulation for prolonged therapeutic action. Additionally, in-vitro studies confirmed the formulation's ability to provide a controlled and consistent release of Dapagliflozin, significantly enhancing bioavailability. Stability studies indicated that the formulation remained stable under various conditions, maintaining its gelling properties and drug release profile. These findings suggest that the in-situ gelling formulation of Dapagliflozin offers a promising approach for managing diabetes, potentially improving patient compliance through reduced dosing frequency and consistent glycemic control. This research highlights the potential of in-situ gelling systems as a viable and effective drug delivery method for antidiabetic therapy.

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