Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 45 No. 1 (2024

Development and Evaluation of Metformin HCL Injectable Controlled-Release Hydrogel for The Management of Diabetes

Dixit Thakur[1], Ankita Pokhriyal[2], Pranshu Tangri[3], Arvind Negi[4]

[1]*Research Scholar, Department of Pharmaceutics, GRD (PG) Institute of Management &Technology, Uttarakhand University, Dehradun, India.*

[2]*Associate Professor, Department of Pharmaceutics, GRD (PG) Institute of Management &Technology, Uttarakhand University, Dehradun, India.*

[3]*Professor, Department of Pharmaceutics, GRD (PG) Institute of Management &Technology, Uttarakhand University, Dehradun, India.*

[4]*Professor, Department of Pharmacognosy, GRD (PG) Institute of Management &Technology, Uttarakhand University, Dehradun, India.*

Abstract: **-** Chronic metabolic condition called diabetes mellitus causes high blood glucose levels as a result of poor glucose control. Metformin is one of the main oral antidiabetic drugs used in current treatment strategies for diabetes control. These techniques, meanwhile, frequently run into problems with patient compliance, dose optimisation, and systemic adverse effects. Controlled-release drug delivery systems have attracted a lot of interest because they provide prolonged and localized drug release, boosting therapeutic efficacy while reducing side effects, to overcome these constraints. Injectable hydrogels offer potential as vehicles for controlled drug delivery because of their biocompatibility, customizable characteristics, and capacity to hold and release therapeutic molecules. It was noted that the formulations (F6 to F11) contained between 96.3% and 98.90% of the medication. A study of the formulation's medication content showed a 99.97% purity rate. Investigations were also done on the hydrogel's biocompatibility. The produced hydrogel's IR spectra have shown that a cross-linking network exists between the injected components.

Keywords: *CDDS (Controlled Drug Delivery System), injectable hydroge and Metformin*

1. Introduction

Controlled Drug Delivery Systems (CDDS) have improved their effectiveness over the past 50 years due to their improved accessibility, low bioavailability, and minimal therapeutic indexes. Injectable hydrogel technology has been developed to overcome these limitations, as they are flexible and biocompatible with living tissue. Shear-thinning injectable hydrogels have shown potential for entrapping and distributing therapeutic drugs like Metformin. Diabetes mellitus, a metabolic disease, is a major global health concern. Controlled-release drug delivery systems have gained interest due to their ability to maintain constant therapeutic doses, minimize side effects, and optimize drug release kinetics. Injectable hydrogels, which include multidimensional crosslinked networks, can be used for regulated medication administration.

This research paper discusses creating and testing a metformin HCl injectable controlled-release hydrogel for diabetic treatment, highlighting the potential of hydrogel-based compositions to address healthcare concerns and contribute to the growing body of knowledge about controlled-release medication delivery methods.

2. Objective

The present work and the research are planned with the accordance of the following mentioned objectives below:

The current study is planned to develop an ideal parental drug delivery system suitable for diabetic patients in the treatment of type 2 diabetes.

i. **Selection of model drug and Excipients for the formulation of fast and responsive onset action of metformin:** with current formulations, using metformin orally and through the parenteral route is now routine practice. Metformin still requires some improved breakthroughs despite the numerous improvements in oral formulations, such as the introduction of prolonged and sustained-release tablets. The drawback of orally usable metformin is that its repeated dosages are required to be administered after a certain amount of time. It is necessary to make preparations using a contemporary strategy in order to lessen this attribute or complication. Model excipients and methods like injectable prolonged-release hydrogels are necessary to do this.

ii. **To reduce the period of the administration of metformin through the oral route:** The most popular method of administering any medication is through the oral route. However, when taken orally, the medication or preparation must be taken often or repeatedly, as appropriate. In addition, it has been noted that when the drug is administered via the oral route in higher doses compared to the parental route, there is less pharmacological activity. It has been observed and demonstrated that the parental route helps the medicine act more quickly than the oral route. According to the study, the medicine must be administered in bigger doses more frequently via the oral route to resolve this problem this research was carried.

iii. **Reduce the dose frequency of metformin among the patients:** Since oral medications must overcome numerous obstacles, such as first-pass metabolism and other factors that cause some of the API to deteriorate, oral medications must be taken more frequently and in higher doses than those administered via parenteral routes. In contrast, the medication is directly deposited into the systemic circulation when administered by the parental route. Because of this, the drug's pharmacological action can be achieved in relatively modest dosages.

iv. **Prevention of drug loss:** To prevent metformin from being lost after oral delivery is one of the earlier goals of this investigation. Drug compounds classified as BCS Class III are thought to be more sensitive to the effects of excipients. These medications may absorb at certain sites since they are not thought to be extremely permeable.

v. **Improvement in the wound healing process:** It's important to be aware of the warning signs since wounds typically recover and deteriorate more slowly in diabetics. Despite the reality that wounds, grazes, scuffs and blisters can occur everywhere on the body, the lower extremities are among the more commonly injured parts of the body. A tiny foot cut that worsens fast might become an ulcer. Foot ulcers that go untreated can develop and become serious. fifteen per cent of the population have diabetes and suffer foot ulcers. An elevated blood sugar level impairs the function of your immune system, hinders the flow of oxygen and nutrients from reviving cells, and triggers aggravation in the cells in your body. These effects prevent wounds from healing.

vi. **To introduce metformin in the form of injectable hydrogels:** While metformin is an extremely popular and effective drug for treating diabetes with type II, there hasn't been much study or debate on injectable metformin hydrogels. The enhanced formulation and modernization of the ancient techniques in the form of injectable hydrogels suggest that this topic needs to be explored in the modern world of fresh discoveries and improvements. Therefore, the primary goal of this study is to introduce a novel and improved method of metformin delivery through the use of a new parental preparation in the form of injectable hydrogels.

vii. **Optimization of the formula and composition of metformin:** Metformin has been used extensively for a very long time to treat type II diabetes. Traditionally, oral intake of metformin has been employed. However, it has a low absorption rate and it has to face numerous physiological obstacles in order to reach the systemic circulation, from which the appropriate use of metformin has not yet been determined and applied. It is now necessary to optimize the formulation of this treatment in a different and better approach given the world of fresh discoveries and improvements.

viii. **To increase the bioavailability of metformin for a prolonged period of time:** There are many physiological barriers that come to the role when the drugs are taken orally and make an impact on the bioavailability of the drug in the blood and on the other hand when a drug is supplied via the parental route, allowing it to integrate

directly into the body's systemic circulation bypassing these barriers then we may manufacture the majority of medications prolonged-release, sustained-release medications by making a few necessary changes to the excipients, such as polymers of the preparation, prior to formulation. As a result, we can change the timing and rate of the medication's release into the blood. All of these adjustments can make metformin injectable preparation accessible for a very long time.

ix. To introduce the new treatment of diabetes by the tissue engineering

x. To facilitate factors, like:

i. Flexibility
ii. Versatility
iii. stimuli-responsive nature

For pediatric, geriatric, bedridden, nauseated, or noncompliant patients, recent technological advancements have produced a viable dosage alternative to the oral route hydrogel represents one of the most current developments in the world of dosage forms., which makes the use of drugs more reliable and easily usable with numerous benefits.

Orally dissolving tablets are becoming increasingly popular due to their ease of administration and lack of water requirement. Tablets that can be chewed are palatable and can be taken by mouth. The films that dissolve when swallowed can be injected into the mouth and deliver more effective therapeutic effects because they dissolve in a matter of seconds. Despite these factors, these modes of administration have lots of drawbacks which are generally irreparable. Most Cardiovascular agents, neuroleptics, analgesics, anti-allergist, and medications are all potential drug candidates for this system.

When administered, such a dosage form quickly breaks down; dispenses the medication, and makes its rapidly bioavailable with prolonged effect.

Few indications that specifies the objectives and implies on the fact that why this study is necessary.

i. Bowel side effects from oral route: Metformin HCL is a type of medication that, when taken orally, can cause gastrointestinal side effects such as bowel side effects within a few weeks.

ii. Low bioavailability from oral route: When metformin is taken orally, its bioavailability drops to about 40%:60%, and the remainder of the drug is unable to demonstrate its intended pharmacokinetic effect.

iii. Half-life: Because the half-life of metformin is also very short—between two and four hours—it has been needed to convert into a hydrogel or any other form which is suitable to extend its half-life.

iv. Absorption: Metformin is absorbed through the upper small intestine (duodenum and jejunum) at a rate of less than 50% oral bioavailability. It then travels to the liver, is essentially circulated unbound, and is finally eliminated by the kidneys so in order to make improvement in the metformin's response it is priorly needed to be changed to some another form

v. To reduce the dose and frequency of the dose: Diabetes patients are required to take their medications at regular intervals, and in some cases, the frequency of the dose must be taken orally, which can be a time-consuming and aggravating process at higher concentrations. To avoid this, it is necessary to investigate the hydrogel of metformin in order to prolong the action and reduce the frequency of repeated doses

vi. To introduce a new and better route of administration of Metformin: The oral administration of metformin has a number of irreversible drawbacks; in order to address these issues, a new method with a number of benefits is more necessary.

vii. Enhanced Therapeutic Efficacy: By delivering regulated and long-term release at the site of action, injectable hydrogels have the potential to enhance the therapeutic effects of metformin. Better disease treatment may result from this, particularly in circumstances when extended and localized medication administration is necessary.

viii. Overcoming the Limitations of Oral Administration: Metformin may cause gastrointestinal adverse effects, a low bioavailability, and inconsistent drug absorption when used orally. These restrictions can be circumvented by creating an injectable hydrogel preparation, which offers a different route for consumption that avoids the GI tract and assures greater medication delivery and bioavailability.

viii. **Consumers Compliance**: Injectable hydrogels can improve consumer compliance and convenience by reducing the duration of administering medication. Metformin can be constantly administered via long-lasting injectable formulations, allowing for a lower dosage as well as minimizing the risk of missing doses.

ix. **Achievement of Localized Action**: Because injectable hydrogels may be created to target specific tissues or areas of interest, they allow for localized and targeted medicine administration. This can be useful for treating cancers, healing wounds, and rebuilding tissue, all requiring precise pharmaceutical delivery.

x. **Therapies with the combination**: Injectable hydrogels provide the opportunity to mix metformin with other therapeutic drugs or nanoparticles inside the hydrogel matrix, leading to combination treatments and synergistic effects. This enables the creation of combination medicines, which harness the complementary properties of metformin alongside additional medications for better therapeutic results.

xi. **Personalised medicine**: by modifying the product's properties, including release kinetics as well as medication concentrations, injectable hydrogels may be personalized to match specific patient demands. This makes it possible to use personalized medicine techniques, where the hydrogel composition may be altered to meet the needs of each patient, so improving the treatment outcome.

xii. **Improvement in outcomes**: by exploring metformin injectable hydrogels, researchers may address the aforementioned issues, advance the area of drug administration, widen the usage of metformin beyond its current limitations, and improve patient outcomes in a number of diseases and conditions.

xiii. **Environmental stimuli respondent:** in the realm of biomaterials and medication delivery, injectable hydrogels that have environmental stimuli-responsive characteristics have sparked considerable attention. In reaction to environmental factors including pH, sunlight, temperature, and enzymes, these hydrogels may cycle through changes in their physical or chemical properties.. Because of their adaptability, encapsulated pharmaceuticals or therapeutic agents may be released precisely, making them ideal for focused and on-demand medication administration. Here are some instances of injectable hydrogels' responsiveness to environmental stimuli:

a. Injectable Hydrogels responsive to Ph
b. Injectable Hydrogels responsive to Temperature
c. Injectable Hydrogels responsive to Light
d. Injectable Hydrogels responsive to Enzymes
e. Injectable Hydrogels responsive to Redox reaction

Future perspectives of Injectable hydrogels: Bioactive and Biomimetic Hydrogels: Future advancements in injectable hydrogels will focus on developing bioactive and biomimetic hydrogel systems. These hydrogels can incorporate bioactive signals to increase tissue regeneration while simulating the extracellular matrix, or extracellular matrix (ECM)., wound healing, and organ restoration. Integration of growth factors, peptides, or stem cells within the hydrogel structure can facilitate tissue-specific regeneration and improve therapeutic outcomes.

i. **Intelligent Drug Delivery Systems:** Injectable hydrogels will continue to evolve as intelligent drug delivery systems. Integration of advanced technologies, such as nanomaterials, microencapsulation, or responsive nanocomposites, can further enhance the functionalities of hydrogels. These systems will enable precise spatial and temporal control over drug release, triggered by specific stimuli or external signals, enabling on-demand and targeted therapies.

ii. **Bio responsive Hydrogels:** The development of bio responsive hydrogels that can actively interact with the biological environment holds immense potential. These hydrogels can sense and respond to specific biological signals, such as biomarkers or disease indicators, enabling real-time monitoring and adaptive drug delivery. Such systems have the potential to revolutionize the treatment of diseases that necessitate dynamic adjustments in drug release based on changing physiological conditions.

iii. **Translational Applications:** Injectable hydrogels' transition from research in laboratories to therapeutic use is a significant future aspect. Further investigation and clinical trials will focus on demonstrating safety, efficacy, and long-term stability of injectable hydrogels in human subjects. Regulatory approval and commercialization

of therapies based on injectable hydrogels will pave the way for their widespread utilization in healthcare settings.

iv. Overall, the future prospects of injectable hydrogels encompass personalized medicine, combination therapies, bioactive and biomimetic designs, intelligent drug delivery systems, bio responsive hydrogels, and translational applications. Ongoing progress in material science, formulation techniques, and understanding of biological interactions will drive the advancements and application of injectable hydrogels in various domains of medicine and healthcare.

The prospective of fast wound healing: The potential of utilizing injectable hydrogels containing metformin for promoting rapid wound healing is highly promising. Here are the main points regarding this perspective:

i. **Improved Healing Abilities:** Incorporating metformin, a commonly used antidiabetic medication, into an injectable hydrogel offers the opportunity to enhance wound healing capabilities. The hydrogel serves as a vehicle for the targeted and controlled release of metformin, facilitating its direct delivery to the wound site. This localized administration can significantly augment the therapeutic effects of metformin on the wound-healing process.

ii. **Anti-inflammatory and Antimicrobial Properties:** Metformin has been found to possess anti-inflammatory and antimicrobial properties, both of which play vital roles in wound healing. By reducing inflammation and preventing infections, metformin creates a conducive environment for effective wound healing. When entrapped within an injectable hydrogel, metformin can be released gradually, ensuring a sustained presence of the drug at the wound site.

iii. **Modulation of Cellular Behaviour:** Metformin has demonstrated the ability to modulate the behaviour of various types of fibroblasts, endothelial cells, immune cells, and others involved in the healing of wounds. It promotes cell proliferation, migration, and angiogenesis, facilitating tissue regeneration and blood vessel formation. By encapsulating metformin within an injectable hydrogel, controlled and localized release can be achieved, enabling continuous supply and targeted action of the drug.

iv. **Remodelling of the Extracellular Matrix (ECM):** Metformin has been shown to influence the remodelling of the ECM, a critical component in wound closure and tissue repair. It enhances the synthesis and deposition of collagen, supports the maturation of newly formed blood vessels, and improves the overall quality of healing tissue. Injectable hydrogels act as scaffolds, facilitating ECM remodelling and providing an appropriate microenvironment for metformin's effects on tissue regeneration.

v. **Combination Therapies:** Injectable hydrogels offer the potential for combining metformin with other therapeutic agents or growth factors to further enhance wound healing. By incorporating additional bioactive substances within the hydrogel matrix, synergistic effects can be achieved, leading to accelerated healing, minimized scarring, and improved tissue regeneration.

In summary, the utilization of injectable hydrogels containing metformin for expediting wound healing holds great promise. It enables direct delivery of metformin to the wound site, sustained release of the drug, anti-inflammatory and antimicrobial actions, modulation of cellular behaviour, and promotion of ECM remodelling. Further research and clinical investigations are necessary to validate the efficacy and safety of this approach. However, if successful, it could significantly enhance wound healing outcomes and advance patient care.

To introduce the self-healing property in the medication: The self-healing property of injectable hydrogels brings several benefits, such as independent restoration, prolonged lifespan, enhanced mechanical stability, improved biocompatibility, simplified fabrication and handling, and versatile applications. These advantages make self-healing injectable hydrogels highly desirable for a wide range of practical applications in medicine, biomaterials, and other fields where their unique properties can enhance performance and durability.

making them highly advantageous for various applications. Here are the main advantages:

i. **Independent Restoration:** Injectable hydrogels with self-healing capability can autonomously restore their structure and integrity when damaged. This means that if the hydrogel experiences physical breakage or mechanical stress, it can spontaneously reassemble and regain its original form without external assistance. This self-repairing ability eliminates the need for manual repairs or replacements, enhancing convenience and reliability.

ii. **Prolonged Lifespan:** The self-healing feature extends the lifespan and durability of injectable hydrogels. Even when minor damage like cracks or fractures occurs, the hydrogel can self-repair, preventing further deterioration. This prolongs the functional lifespan of the hydrogel, reducing the frequency of replacements and increasing cost-effectiveness.

iii. **Enhanced Mechanical Stability:** Self-healing injectable hydrogels exhibit improved mechanical stability, making them more resistant to deformation and mechanical stress. The self-repair process reinforces the structural integrity, minimizing the risk of structural failure or collapse under external forces. This heightened mechanical stability expands the potential applications of injectable hydrogels, particularly in scenarios involving load-bearing or high mechanical demands.

iv. **Improved Biocompatibility:** Self-healing injectable hydrogels offer enhanced biocompatibility. The self-repair mechanism enables the hydrogel to seal gaps or defects within its structure, preventing the infiltration of harmful substances or microbial colonization. This feature reduces the risk of infection, inflammation, or adverse reactions when the hydrogel interacts with biological systems, enhancing safety and biocompatibility.

v. **Simplified Fabrication and Handling:** The self-healing nature of injectable hydrogels simplifies their fabrication and handling processes. During manufacturing, the hydrogel can be easily moulded or shaped, and any defects or imperfections can be repaired through self-healing. This simplifies production, making it more efficient and cost-effective. Moreover, the self-healing property facilitates easier handling and manipulation of the hydrogel in practical applications, as it can spontaneously recover from minor damages or deformations.

vi. **Versatile Applications:** The self-healing property broadens the potential applications of injectable hydrogels. They may be utilised in many different applications, including tissue engineering., drug delivery, biosensing, and soft robotics. The ability to self-repair enables injectable hydrogels to adapt to dynamic and challenging environments, making them suitable for applications that require resilience, flexibility, and long-term stability.

Both solid and liquid-like properties: Injectable hydrogels possess a distinctive quality that gives them characteristics resembling both solids and liquids. This duality makes them highly advantageous for a range of applications. Here is an alternative explanation of the solid and liquid-like properties of injectable hydrogels:

i. **Solid-Like Property:** Injectable hydrogels exhibit solid-like behaviour, enabling them to maintain their shape and integrity under specific conditions. They have a three-dimensional network structure that imparts mechanical strength and stability. This solid property allows the hydrogel to serve as a scaffold or support matrix for cells, tissues, or drug delivery systems. It ensures that the hydrogel retains its structure even when subjected to external forces.

ii. **Liquid-Like Property:** Injectable hydrogels also demonstrate liquid-like behaviour, which means they can flow and fill irregularly shaped spaces. Unlike traditional solid materials, injectable hydrogels exist in a gel or gel-like state, giving them fluidic characteristics. This property facilitates easy injection or delivery through minimally invasive techniques, enabling the hydrogel to conform to the shape and contours of the target area. Once injected, the hydrogel can adapt to the surrounding environment, establishing close contact and optimal coverage. The combination of solid and liquid-like properties in injectable hydrogels offers numerous advantages. They can be conveniently administered in minimally invasive procedures, ensuring precise placement and targeted delivery. The solid-like characteristic provides mechanical support and stability, while the liquid-like attribute allows for easy handling and adaptation to complex anatomical structures. Injectable hydrogels are adaptable for a variety of applications, comprising tissue engineering, medication delivery, the healing of wounds, and regenerative medicine, thanks to this special combination.

3. Methods

Preparation of chitosan-dialdehyde cellulose injectable hydrogel

Preparation of dialdehyde cellulose/DAC [10,11,12,13,14]

Procedure:

i. First, 6.10g of sodium metaperiodate and 10.00g of xanthan gum were measured.

ii. Now, 500 mL of deionized water was added together with each of them. Potassium chloride was used to further catalyse the mixture; 1.4g

iii. Periodate mixture was securely covered in aluminium foil to stave against oxidation.

iv. The mixtures subsequently mechanically agitated gently for the specified reaction durations at 20°C in the dark at 1300 rpm.
v. With the help of distilled water, the extra periodate was broken down.
vi. The products (DAC) were collected, cleaned by centrifugation, and dried by air.
vii. IR spectra proved the dialdehyde cellulose that had been synthesised.
viii. For later usage, dried DAC was stored in water at 5°C.

Preparation of injectable Hydrogel:

Procedure

Batch Optimisation:

The eleven separate batches were created using a range of chitosan, dialdehyde cellulose, as well as metformin concentrations. (Table 1.1)

By taking into account the cross-linking reaction between dialdehyde cellulose and chitosan, the final batch of hydrogel was optimised. (formulation F6, Table 1.1)

IR spectra verified the cross-linking of the DAC and chitosan. The regulated drug release pattern was made clear by the cross-linking.

Table 1.1 Optimized formulation formula of metformin

Sr. No.	**Drug and Excipient**	**F 1**	**F 2**	**F 3**	**F 4**	**F 5**
1.	Chitosan	1.5g	1g	2g	4g	0.5g
2.	Di aldehyde Cellulose	1.5g	1.5g	1.5g	3.5g	3.5g
3.	Sodium Metaperiodate	0.17g	0.18g	0.20g	0.30g	0.18g
4.	Metformin	50mg	50mg	50mg	50mg	50mg
5.	Water	50ml	50ml	50ml	50ml	50ml

Cont.

Sr. No.	**Drug and Excipient**	**F 6**	**F 7**	**F 8**	**F 9**	**F 10**	**F 11**
1.	Chitosan	0.5g	0.2g	2g	4g	0.5g	4.5g
2.	Di aldehyde Cellulose	2.5g	1.5g	1.5g	5.5g	4.5g	4g
3.	Sodium Metaperiodate	0.18g	0.18g	0.20g	0.50g	0.18g	0.50
4.	Metformin	50mg	50mg	50mg	50mg	50mg	50mg
5.	Water	50ml	50ml	50ml	50ml	50ml	50ml

1. Characterization or evaluation parameters of injectable hydrogels

pH and visual texture analysis [15,16]

By using clarity testing equipment and visual inspection, the clarity of a prepared solution is determined. Results are on Table 1.9

Syringe ability study:

The syringe ability test is passed by formulations that readily pass through the needle of 21,23,25,27-gauge. Results are displayed in Table 1.10

Rheological Study:

Rheological studies were performed upon the solution or upon the formulation by using Brookfield viscometer at 20,40,60,70 RPM respectively. Results for the rheological study are displayed in Table 1.11

In-vitro gelling and content of uniformity study[45]

Drug content uniformity estimation1 mg drug formulation was placed in a 10 ml volumetric flask, mixed with buffer 7.4, adjusted up to 10 ml with buffer 7.4, and then filtered. At (max) 281 nm, absorbance values were recorded with appropriate dilutions. Results for this study are displayed in Table 1.12

In-vitro dissolution study[20,21]

Utilising USP apparatus II at 37°C and 100 rpm in phosphate buffer saline with a pH of 7.4, the in vitro released pattern was examined. To ensure sink condition and the amount of drug could be assessed by spectroscopy at 281 nm throughout the research, 5 ml of aliquots were obtained from the dissolving media at various times (0.5, 1, 2, 4, 6, 8, 10, & 24 hour) and replenished with fresh buffer. The results for the study are in Table 1.13

In-vitro diffusion study[38,39]

In vitro diffusion was measured using the Franz diffusion cell. The cellophane membrane is tightly sandwiched between the donor and receptor compartments in Franz diffusion cells, towards the donor region, the epidermal site. The receptor compartment was filled with buffer solution during the course of the study, which was thermostatically maintained at 37°C (1°C). The donor cell is wrapped with paraffin film and coated with foil made of aluminium to limit exposure to light. before the experiment begins.[17] 5ml of aliquots are removed and replenished with a comparable amount of fresh buffer at defined intervals of time (0.5, 1, 2, 4, 6, 8, 10, and 24 hour) to guarantee sink condition as well as drug content may be evaluated spectrophotometrically. The results for the study are in Table 1.14

Texture analysis of Metformin Injectable Hydrogel:[18,19]

Using a texture analyser, the hardness, consistency, and cohesiveness of the formulation are evaluated.. The graphs from the texture analyser are displayed in several Figures 1.19-1.24.

Spectral analysis of Formulation (F6):

In order to characterize any potential interactions between the medication and the excipients, the FTIR analysis of final formulation F6 was carried out. Figure 1.26 shows the formulation (F6)'s FTIR spectrum.

In-vitro Gelling Temperature Studies from Solution to Gel phase Transition:

The in-situ gel-forming temperature for the formulation was determined to be 37°C after screening at various temperatures and circumstances The results for the study are in Table1.16

Arrhenius model accelerated stability investigation:[30,31,32,33]

As per the International Conference on Harmonisation (ICH), formulations are placed in vials of ambient hue. with sealed with foil made up of aluminium to undergo quick stability accelerated study evaluation at 402 °C and 75 percent RH. The guidelines as well as control samples were held at 2-80 degrees Celsius, and the experimental results collected at various temperatures may be examined using the Arrhenius equation. The outcomes for the study are in Table 1.17

Swelling Behaviour Study:[36,37]

By measuring Hydrogel's swelling index in phosphate buffer (pH--7.4) at 37°C, the capacity of the medium to absorb water was evaluated. The findings demonstrate that there had been no discernible improvement in the swelling ability after 60 minutes.

Differential Scanning Calorimetry Study:[40,41,42]

This study was performed and the results are entitled in the table 1.18 and figure 1.29

Materials:

Metformin, Sodium Hydroxide, Sodium Phosphate, Methanol, Phosphoric acid, Sodium Metaperiodate, Xanthan Gum, and Chitosan were obtained from the GRD IMT Dehradun and all of the chemicals were of high analytical grade.

4. Results

Preformulation Studies:

Table 1.2 Solubility Study of Metformin in Different Solvents

Sr. No.	Solvents	Solubility mg/ml	Part of solvent required per part of solute	Temperature Degree Celsius/Room temperature
1.	Distilled water	Freely soluble	From 1 to 10	Room Temperature
2.	Methanol	Freely soluble	From 1 to 10	Room Temperature
3.	Ethanol	Slightly soluble	From 100 to 1000	Room Temperature
4.	Chloroform	Practically Insoluble	10,000 and over	Room Temperature
5.	Acetone	Practically Insoluble	10,000 and over	Room Temperature
6.	Ether	Practically Insoluble	10,000 and over	Room Temperature
7.	Tap water	Freely soluble	From 1 to 10	Room Temperature

Table 1.3 Melting Point trials

Sr. No.	Melting point (°C)	Average (Mean_+ S.D) n=3)
1.	210	
2.	215	
3.	221	
4.	222	**222.5**

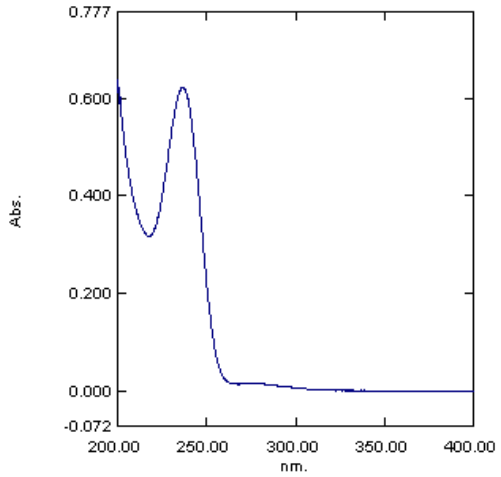

Figure 1.5 UV Spectra of Metformin

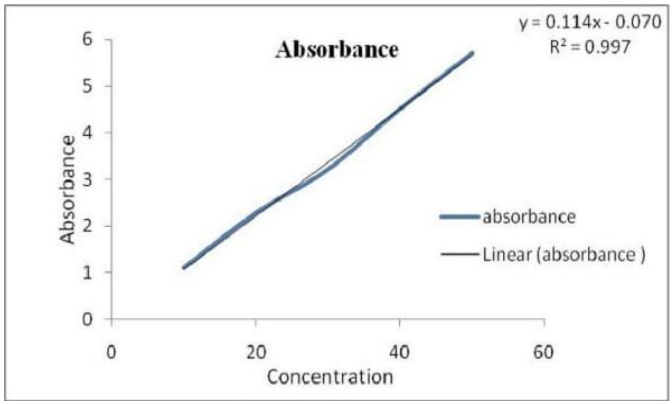

Figure 1.6 Calibration curve of metformin at 234nm

Table 1.4 Study of Metformin with Respect to Linearity

Sr. No.	Concentration (ppm)	Absorbance
1.	10.00	2.103
2.	20.00	1.279
3.	30.00	3.124
4.	40.00	3.540
5.	50.00	4.621

Mean	2.3697
Standard Deviation	2.8166
Relative Standard Deviation	0.5490
Percent RSD	54.80
Slope	0.07876

Table 1.4 (a) Recovery Studies Validation of Metformin

e	Concentration (ppm)	Absorbance	Mean	SD	RSD	Percent RSD
1.	90%	3.820 3.790 3.784	3.794	0.01323	0.00357	0.35
2.	120%	4.250 4.280 4.270	4.268	0.01777	0.00405	0.40
3.	100%	3.914 4.001 3.927	3.946	0.04650	0.01171	0.17

Table 1.5 Intraday Accuracy or precision for Metformin

Sr. No.	Concentration (ppm)	Absorption-l	Absorption-ll	Absorption-lll
1.	20	2.233	2.231	2.198
2.	20	2.223	2.261	2.250
3.	20	2.273	2.261	2.250
4.	20	2.260	2.151	2.256
5.	20	2.257	2.291	2.260
6.	20	2.255	2.195	2.226
Average		2.2450	2.2255	2.2420
SD		0.0144	0.0333	0.0248
RSD		0.0064	0.0150	0.0111
Percent RSD		0.66	1.51	0.10
Average Percent Relative Standard Deviation 1.09				

Table 1.6 Interday Accuracy or Precision of Metformin

Sr. No.	Concentration (ppm)	Observation Day-l	Observation Day -ll	Observation Day -lll
i.	Twenty	2.233	2.269	2.282
ii.	Twenty	2.223	2.261	2.270
iii.	Twenty	2.273	2.264	2.347
iv.	Twenty	2.260	2.251	2.281
v.	Twenty	2.250	2.295	2.262
vi.	Twenty	2.255	2.286	2.270
Avg.		2.2450	2.225	2.273
SD		0.0148	0.0087	0.0067
RSD		0.0065	0.0037	0.028
Percent RSD		0.66	0.38	0.28
Average Percent RSD 1.09				

Where, (n=6)

Table 1.7 Gelling, Density, and Viscosity behaviour of chitosan solution

Formulation	Chitosan Concentration (%w/v)	Gelling capacity (n=3)	Density (g/cc) ± SD*	Viscosity (cP) ± SD* $\mu_1 = \mu_s t_1 p_1 \div t_s p_2$
F 1	0.51	++	1.045±0.1	4.268±0.086
F 2	0.71	+++	1.033±0.11	5.392±0.52
F 3	1.0	+++	1.057±0.11	9.240±0.055
F 4	1.22	+++	1.099±0.11	11.251±0.45
F 5	1.52	+++	1.110±0.06	14.225±0.24
F 6	1.73	+++	1.217±0.05	22.619±0.34
F 7	2.0	++	1.132±0.11	28.358±0.046

Where-, **+:** Dissolves but Gels slowly

++: Gelation takes place with immediate effect and it persists for a few hours

+++: Persistence for an extended period as well as gelation occurs immediately

***n = 3**

Drug-excipient compatibility studies

Figure 4.24 to 4.29displays the distinct infrared (IR) spectrum of drug and polymer mixtures as well as the spectra of the individual drugs and polymers. Given that all of the functional frequencies were present, this demonstrates that there is no relationship between metformin & polymers if one compares to the infrared range of the medicine in its purest form.

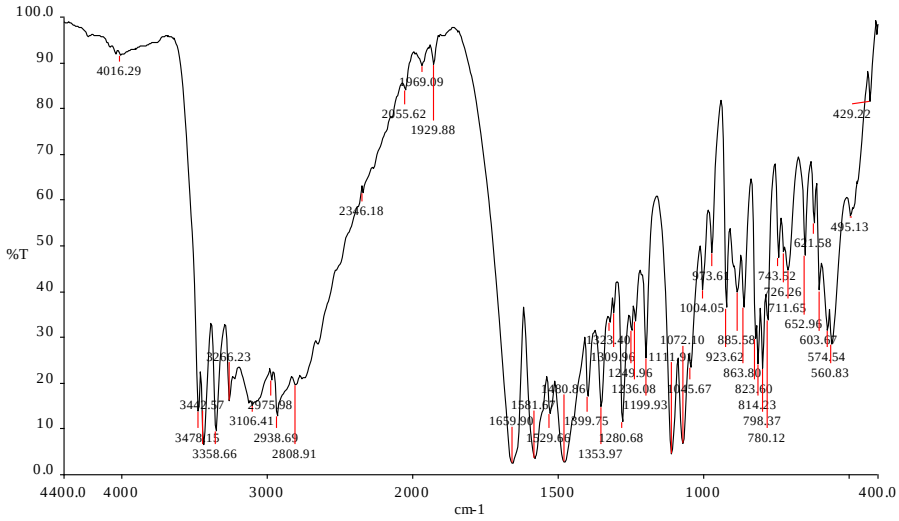

Figure 1.7 Drug Metformin's FTIR Spectrum.

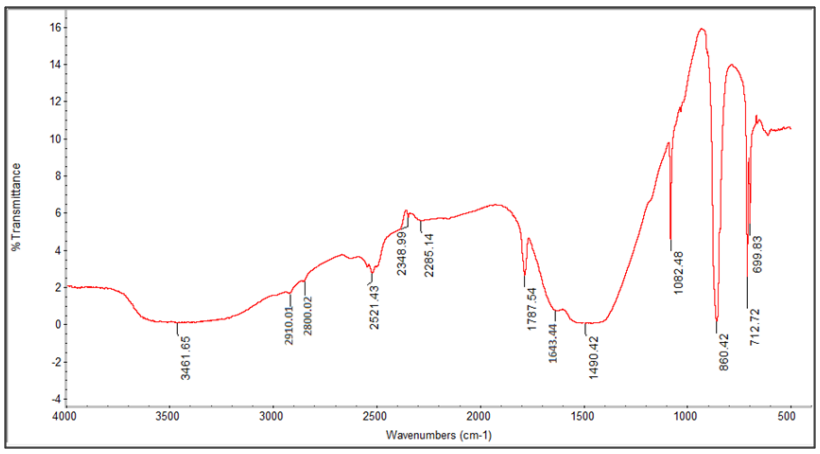

Figure. 1.8 Chitosan's FTIR Spectrum.

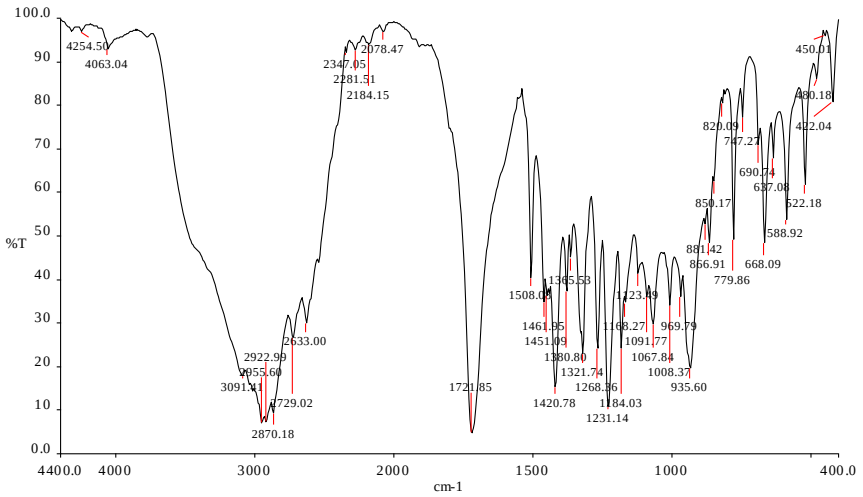

Figure. 1.9 Dialdehyde Cellulose FTIR Spectrum.

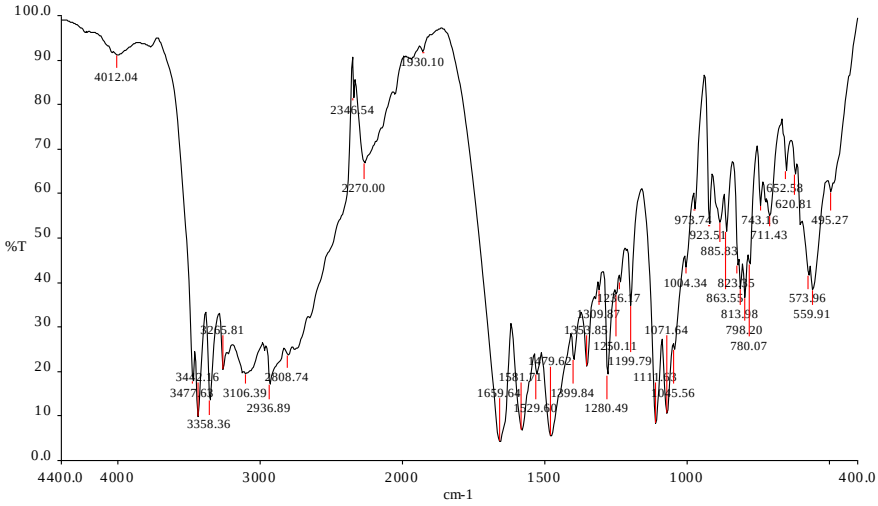

Figure 1.10 Sodium Metaperiodate FTIR Spectrum.

Table 1.8 Interpretation of FTIR spectra and determination of drug purity

Polymer with Drug	Peak of Drug (sample)	Pure drug	Peak of Polymer	Melting point	Findings
Drug + chitosan (1:1)	561, 552.86, 782, 794, 811, 873.81,1034, 1189.94, 1365.94, 1453, 1571.83, 1639.71, 3938.89, 3256.13	572, 547, 643, 413, 729, 792, 845, 464, 973, 1004, 1071, 1220, 1324, 1283, 1559, 2582, 1558, 2939, 3156, 3565	512, 833, 1142, 1139, 1314, 1939, 2116, 3579	226^0 C	No interaction found
Drug + Dialdehyde cellulose (1:1)	560, 652.86, 782, 794, 811, 873.81,1034, 1189.94, 1365.94, 1453, 1571.83, 1639.71, 3938.89, 3256.13	562, 577, 653, 713, 779, 798, 815, 864, 973, 1004, 1071, 1200, 1354, 1483, 1529, 1582, 1658, 2939, 3106, 3265	522, 588, 668, 779, 925, 1232, 1422, 1722, 2871	226^0 C	No interaction found

Evaluation of final formulation

The finished formulations underwent tests for texture analysis, stability research, in-vitro gelling, drug content, pH measurement, syringe ability, rheological investigations using Brookfield viscometer, and in-vitro dissolution and diffusion release.

i. **pH and Visual Texture analysis of the injectable hydrogel:**

Table 1.9 pH and Visual Texture analysis of the injectable hydrogel

Formulation code	pH of sol (n=3)	Nature of Gel
F1	3.18 ± 0.02	Transparent
F2	4.86 ± 0.03	Transparent
F3	5.11 ± 0.05	Transparent
F4	5.18 ± 0.04	Transparent
F5	6.19 ± 0.05	Transparent
F6	6.93 ± 0.01	Transparent
F7	6.81 ± 0.02	Transparent
F8	6.63 ± 0.03	Transparent
F9	6.42 ± 0.04	Transparent
F10	6.28 ± 0.03	Transparent
F11	6.19 ± 0.01	Transparent

ii. **Syringe ability study of metformin injectable hydrogel:**

Table 1.10 Syringe ability study of metformin injectable hydrogel

Formulation No	Gauge of needle	Result
F6	21, 23, 25, 27	Easily Syringe able
F7	21, 23, 25, 27	Easily Syringe able
F8	21, 23, 25, 27	Easily Syringe able
F9	21, 23, 25, 27	Easily Syringe able
F10	21, 23, 25, 27	Easily Syringe able
F11	21, 23, 25, 27	Easily Syringe able

iii. **Rheological Studies of sol using Brookfield viscometer at 20, 40, 60 and 70 rpm**

Table 1.11 Rheological Studies of sol using Brookfield viscometer at 20, 40, 60 and 70 rpm

Formulation	Viscosity (cP) of Metformin Injectable Hydrogel formulation at angular velocity (rpm)			
	20	40	60	70

F1	2023.00	1316.00	866.00	531.66
F2	1936.00	1413.66	757.33	565.00
F3	1846.16	1113.00	613.00	147.33
F4	1755.31	1113.00	513.66	216.33
F5	1685.33	925.00	424.00	277.33
F6	2026.00	1463.00	876.66	513.00
F7	1933.66	1267.66	735.66	346.00
F8	1657.61	1046.00	580.66	374.66
F9	1583.01	847.00	335.00	194.00
F10	1434.31	664.00	255.00	117.66
F11	1114.33	443.33	255.33	127.00

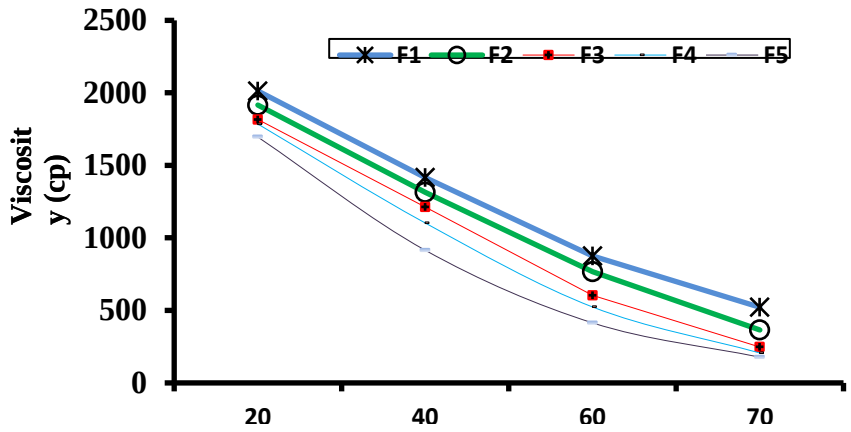

Figure 1.11 Using a Brookfield viscometer, the determined rheological profile of sol

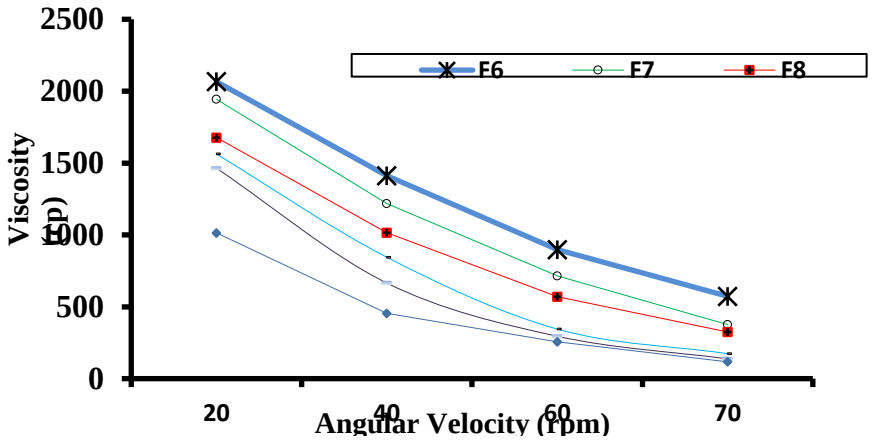

Figure 1.12 Using a Brookfield viscometer, the determined rheological profile of sol

Figure 1.13 Using a Brookfield viscometer, the hydrogel sample appearance

iv.In vitro gelling study and drug content uniformity:

Table 1.12 In vitro gelling study and drug content uniformity

Formulation code	**Gelling capacity (n=3)**	**Drug content percentage ± S.D (n=3)**
F6	+++	98.8± 0.002
F7	+++	98.9± 0.001
F8	+++	96.3 ± 0.001
F9	+++	98.6 ± 0.002
F10	+++	97.6 ± 0.003
F11	+++	98.5 ± 0.003

(+: Slowly gels and dissolves, ++: Immediate gelation that lasts for a few hours, +++: Immediate gelation that lasts for a long time.)

v.In-vitro Dissolution study:

Table 1.13 Dissolution Percent Cumulative Release of Metformin

Time (hours)	**Percent Cumulative drug release ± SD***					
	F6	F7	F8	F9	F10	F11
0.5	4.6±2.16	3.72±0.17	6.9±1.52	6.92±0.14	7.42±0.20	6.75±0.12
1	9.8±0.24	14.92±0.37	11.48±0.31	14.3±0.22	13.21±0.25	17.23±0.22
2	22.6±0.32	17.54±0.38	22.97±0.51	25.7±0.36	23.59±0.26	23.49±0.32
4	28.2±0.11	28.32±0.43	23.96±0.62	35.1±0.45	30.78±0.35	34.17±0.42
6	31.4±0.22	35.4±0.56	35.45±0.85	36.2±0.26	37.2±0.43	37.49±0.26
8	37±0.13	4250.14	68±0.8	44.9±0.37	44.3±0.38	44.3±0.44

10	42±0.23	46.6±0.42	43.5±0.2	49.2±0.42	51.7±0.66	50.0±0.27
24	58±0.11	62±0.34	60.1±0.13	62.2±0.38	65.2±0.12	63.6±0.42

*n=3

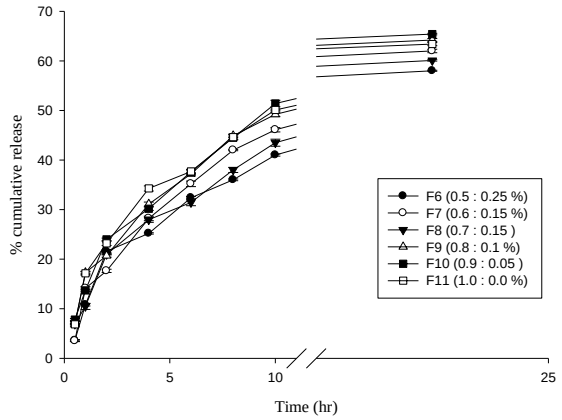

Figure 1.14 Metformin cumulative release from improved formulations

In-vitro Diffusion Study:

Table 1.14 Mathematical Models for the Diffusion Data of Final formulation

Sr.No.	Code o Formulation	Zero Order rf^2	First Order r^2	Higuchi r^2	Korsemeyer and Peppas n
1	F6	.840	.934	.985	.712
2	7	.859	.928	.971	.717
3	8	.910	.974	.982	.647
4	9	.839	.962	.963	.622
5	10	.855	.951	.991	.574
6	11	.817	.910	.946	.633

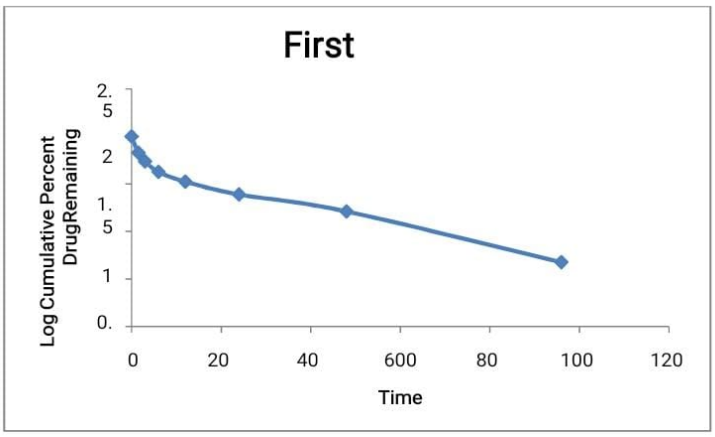

Figure 1.15 Plots of the log cumulative percent medication remaining against the First order of time

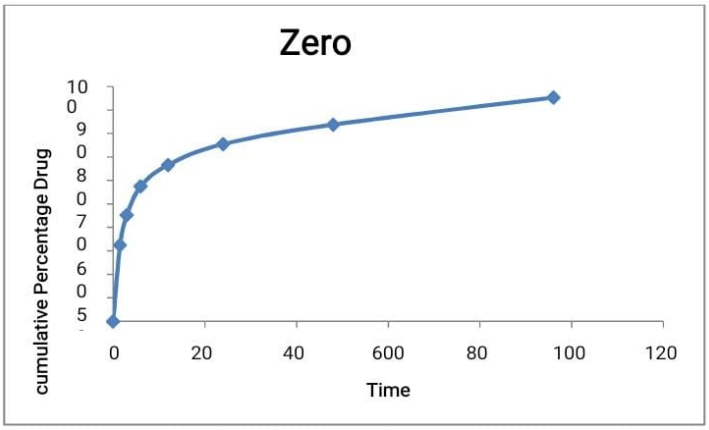

Figure 1.16 Plots of the log cumulative percent medication released against the Zero order of time

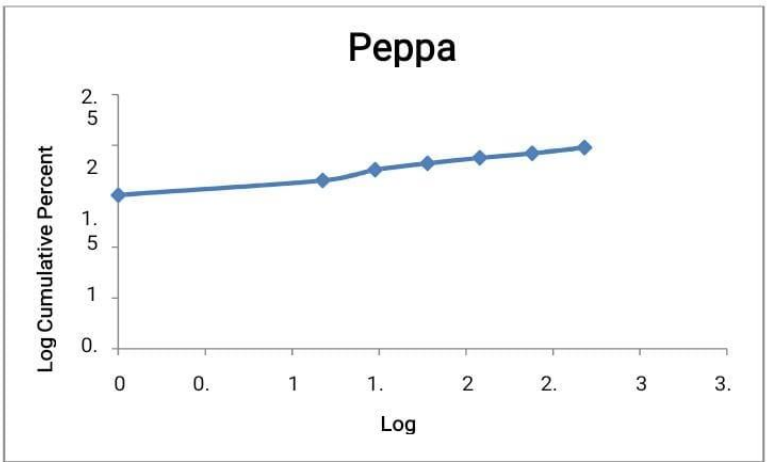

Figure 1.17 Plots of the log cumulative percent medication released against the Log Time (Peppas Plot)

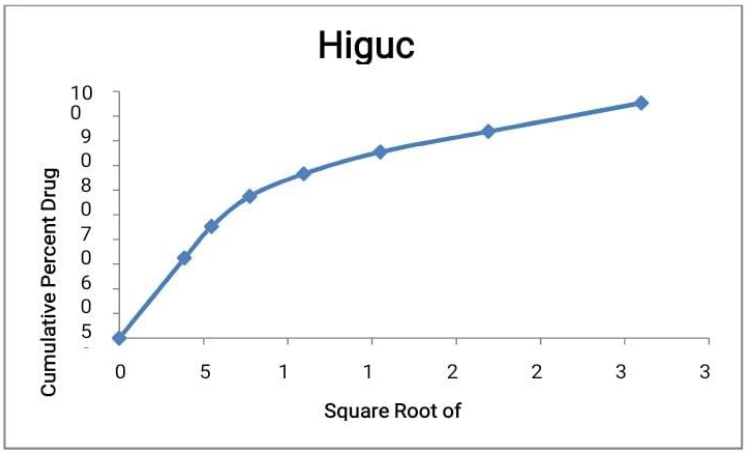

Figure 1.18 Plots of the log cumulative percent medication released against the Square root Time (Higuchi's Plot)

VI.Texture analysis of Metformin Injectable hydrogel:

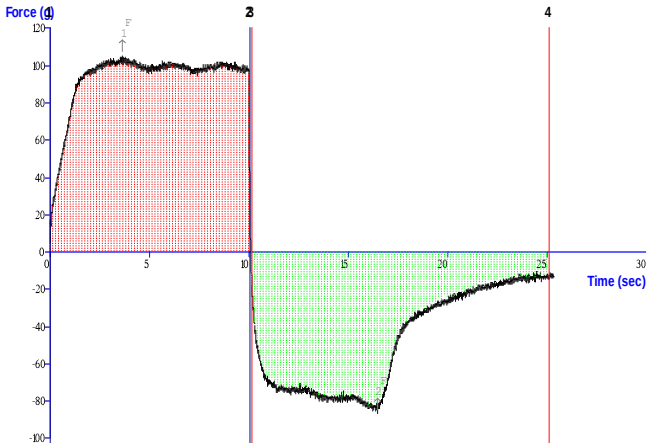

Figure 1.19 Formulation F6 Metformin Injectable Hydrogel consistency graph

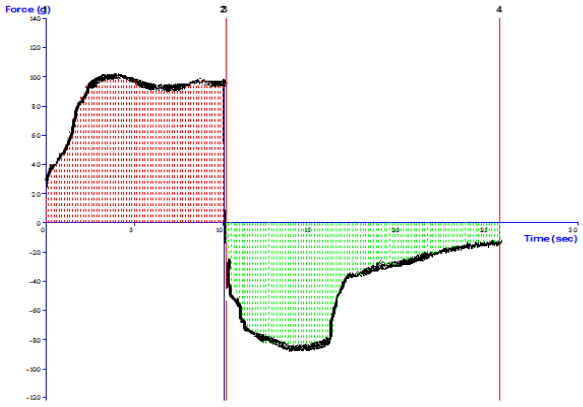

Figure 1.20 Formulation F7 Metformin Injectable Hydrogel consistency graph

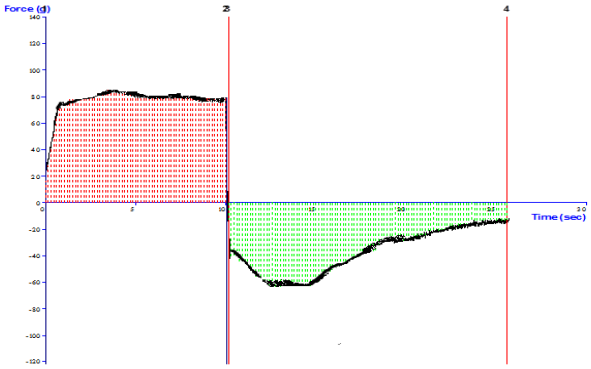

Figure 1.21 Formulation F8 Metformin Injectable Hydrogel consistency graph

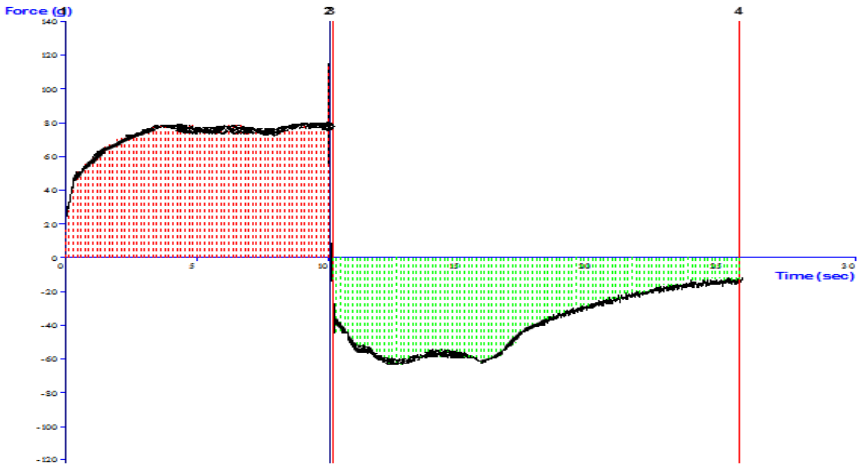

Figure 1.22 Formulation F9 Metformin Injectable Hydrogel consistency graph

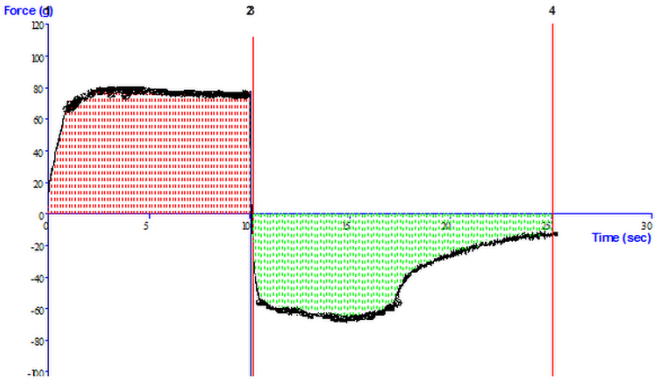

Figure 1.23 Formulation F10 Metformin Injectable Hydrogel consistency graph

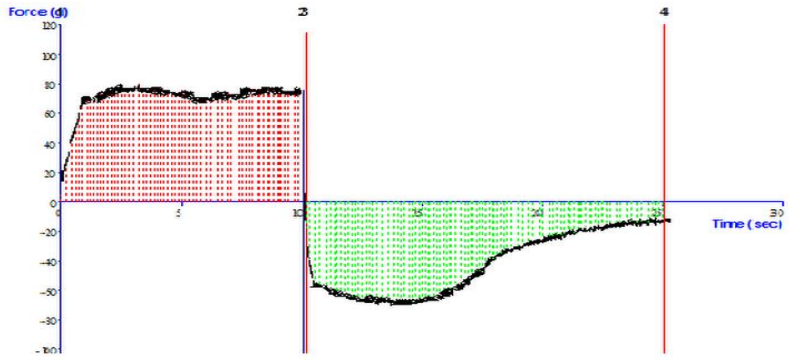

Figure 1.24 Formulation F11 Metformin Injectable Hydrogel consistency graph

Figure 1.15 Visual Appearance texture analysis of Metformin Injectable Hydrogel

Table 1.15 Texture analysis of Metformin Injectable Hydrogel

S. No.	Code of Formulation	Firmness Mean max. Positive force	Consistency Mean Positive area	Cohesiveness Mean max. Negative force
1	F6	129.81	1089.30	- 94.81
2	F7	94.31	921.14	- 83.47
3	F8	81.25	723.12	- 62.12
4	F9	78.62	713.23	- 64.25
5	F10	81.41	728.46	- 65.42
6	F11	76.32	739.41	- 60.42

Analysis on the Spectral basis of formulation (F6):

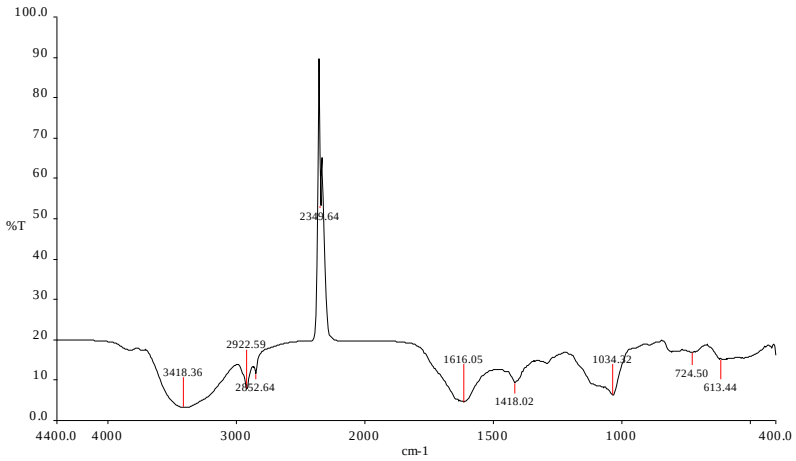

Figure 1.26 Analysis on the Spectral basis of final formulation (f6)

In-vitro Gelling Temperature Studies from Solution to Gel phase Transition:

Table 1.16 In-vitro Gelling Temperature Studies from A solution for the gel phase transition

Sr. No	Temperature Range (Degree Celsius)	Condition
1.	5-8	Solution phase
2.	10-11	Solution phase
3.	12-13.09	Solution phase
4.	14-19	Solution started to thicken
5.	20	Thick-Solution phase
6.	20-28	Semi Gel-Solution phase
7.	28-35	Semi Gel-Solution phase
8.	36	Gel Easily Flowing
9.	37	Complete Gel Transition

Accelerated Stability Studies:

The preservation conditions for Formulations F6 through F11 varied. The experimental samples were maintained at 40°C and ambient temperature, whereas the control samples remained between 2 and 80°C. At 0, 7, 15, & 21 days, as well as at the end of a month, spectrophotometric analysis was used to measure the gel's drug concentration. At day zero, the formulation's drug level was assumed to be 100%. Transparency, simplicity, along with any physical separation are all evaluated along with the solution.

Table 1.17 Results of research on accelerated stability

Code of formulation	Storage condition	Clarity and transparency	percentage of gel content at various times				
			0 day	7 days	15days	21days	30days
F6	4 degree Celsius RT 40^{0}C	Clear and transparent	97.9	97.75 95.99 93.44	98.72 99.74 94.92	98.54 99.65 93.79	96.52 95.44 94.65
F7	4 degree Celsius RT 40^{0}C	Clear and transparent	97.9	97.81 96.93 94.25	99.71 98.82 95.97	97.89 96.79 94.64	96.81 95.70 91.59
F8	4 degree Celsius RT 40^{0}C	Clear and transparent	94.3	95.31 97.57 96.34	96.94 94.50 95.72	95.73 93.15 93.49	96.66 91.93 97.56
F9	4 degree Celsius RT 40^{0}C	Clear and transparent	99.6	97.51 94.97 93.35	97.96 97.93 96.90	95.94 95.97 93.69	95.89 94.81 96.75
F10	4 degree Celsius RT 40^{0}C	Clear and transparent	97.6	98.51 97.96 95.82	95.96 96.94 94.98	94.95 95.87 91.59	94.91 94.76 91.48
F11	4 degree Celsius RT 40^{0}C	Clear and transparent	98.5	99.35 96.97 97.52	98.95 94.92 93.97	97.95 96.86 94.77	95.85 92.78 91.62

Swelling Behaviour Study:

By measuring Hydrogel's swelling index in phosphate buffer (pH--7.4) at 37°C, the capacity of the medium to absorb water was evaluated. The findings demonstrate that there had been no discernible improvement in the swelling ability after 60 minutes.

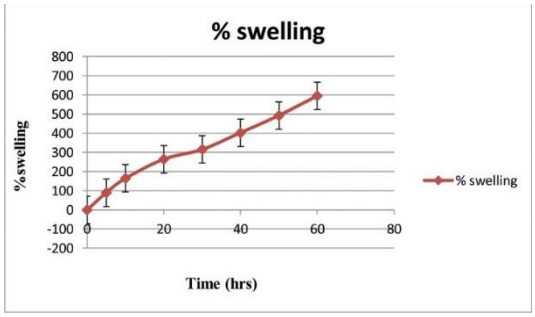

Figure 1.28 Swelling Behaviour Study final formulation.

Differential Scanning Calorimetry Study:

Report: Differential scanning calorimetry (DSC) was used to determine the melting points of the hydrogel formulation or TPP, which were 102.57°C and 117.53°C, respectively, indicating the amorphous nature of the compounds. Because of its better-organized organisation, the formed hydrogel exhibits a strong peak at 103.57C. The chitosan, hydrogel preparation DSC spectra were summarised as follows.

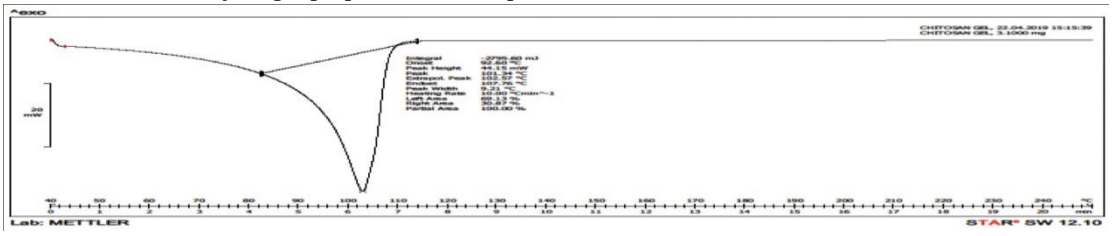

Figure 1.29 Differential Scanning Calorimetry Study

Table 1.18 Differential Scanning Calorimetry Study

Sr. No	Selected Parameter	Results
1.	Onset	93.61 degree Celcius
2.	Height of Peak	43.15 MW
3.	Peak	102.25 degree Celsius
4.	Peak (extrapol)	101.23 degree Celsius
5.	End set	108.86 degree Celsius
6.	Width of peak	9.20
7.	Range of heating	100 gree Celsius min^{-1}

5. Discussion

Metformin was combined with chitosan and dialdehyde cellulose to create an injectable hydrogel. The hydrogel had a 99.96% quality rate and was opaque with a pale, creamy hue. It was suitable for injection and showed significant shear-thinning behaviour. The hydrogel's high drug level ensured efficient drug loading and potency. It had a 99.65% trapping efficiency. The hydrogel's melting points indicated amorphous nature, with a strong peak at 103.57°C. Metformin's sustained drug release was 87.25% over 84 hours. This hydrogel formulation offers controlled medication release and antidiabetic action.

Refrences

[1] Tha1. mbi T, Li Y, Lee DS. Injectable hydrogels for sustained release of therapeutic agents. J Control Rel 2017;267:57-66.

[2] Li J, Mooney DJ. Designing hydrogels for controlled drug delivery. Nat Rev Matter 2016;1:160-71.

[3] Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. Polymer 2008;49:1993-2007

[4] Chirani N, Gritsch L, Motta FL, Fare S. History and applications of hydrogels. J Biomed Sci 2015;4:75-85.

[5] Ganji F, Vasheghani-Farahani E. Hydrogels in controlled drug delivery systems. Iran Polym J 2009;18:63-88.

[6] Hanafy NA, Leporatti S, El-Kemary MA. Mucoadhesive hydrogel nanoparticles as smart biomedical drug delivery system. Appl Sci 2019;9:825.

[7] Chai Q, Jiao Y, Yu X. Hydrogels for biomedical applications: Their characteristics and the mechanisms behind them. Gels 2016;3:6.

[8] Park KM, Park KD. Injectable hydrogels: Properties and applications. Encycl Polym Sci Technol 2016;15:1-6.

[9] Pertici V, Pin-Barre C, Rivera C, Pellegrino C, Laurin J, Gigmes D, et al. Degradable and injectable hydrogel for drug delivery in soft tissues. Biomacromolecules 2019;20:149-63.

[10] Prabaharan M. Chitosan derivatives as promising materials for controlled drug delivery. J Biomater Appl 2008;23:5-36.

[11] Seliktar, Designing cell-compatible hydrogels for biomedical applications. *Science* 336, 1124–1128 (2012). 10.1126/science.12148

[12] Radomsky M, Liu L, and Iwamoto Z, (2000), "Sustained Release Injectable Products," Interpharm Press., 181.

[13] Radomsky M.L, Brouwer G, Floy B.J, Loury D.J, Chu F, Tipton A.J, and Sanders L.M, (1993), "The controlled release of Ganirelix from the Atrigel injectable implant system," Proc Intern Symp Control Rel Bioac Material., 20.

[14] Ralph And Amlalo, (2005), "In Vitro Release Of Ketoprofen From Proprietary And Extemporaneously Manufactured Gels," Rhodes University Grahamstown.,1.

[15] Ranade V.V, and Hollinger M.A, (1996), "Drug Delivery Systems," Boca Raton: CRC Press.

[16] Rathbone M.J, P.J, Burns C.R, Ogle S, Burggraaf and C.R, Bunt, (2000), "Controlled Release Drug Delivery Systems for Estrous Control of Domesticated Livestock, in Controlled Release Veterinary Drug Delivery," Biological and Pharmaceutical Considerations, Rathbone M.J, and Gurny R, Editors. 1st ed., Elsevier Science: Amsterdam. 201.

[17] Ravivarapu H.B, Moyer K.L, and Dunn R.L, (2000), "Sustained activity and release of leuprolide acetate from an in situ forming polymeric implant," AAPS Pharm Sci Tech., 1, 145.

[18] Rozier A, Mazuel C, Grove J, Plazonnet B, and Gelrite, (1989), "Novel, ion activated, in situ gelling polymer for ophthalmic vehicles : Effect on bioavailability of timolol," Int. J. Pharm., 57, 163.

[19] Sajid Ali M.D, Swati Singh, Awdhesh Kumar, Sant Singh, Tahir M.D, and Gurudutta Pattnaik, (2010), "preparation and invitro evaluation of sustained release matrix tablets of phenytoin sodium using natural polymers" Int. J. Pharmacy and Pharmaceutical Science., 2, 174.

[20] Saleem M.A, and Ahmed S.I, (1999), "Tephrosia purpurea ameliorates benzoyl peroxides-induced cutaneous toxicity in mice: diminution of oxidative stress," Pharm Pharmacol Commun., 5, 455.

[21] Samuel B, Adams J.R, Mohammed F, Shamji, Dana L, Nettles, Priscilla Hwang, and Lori A, and Setton, (2009), "Sustained Release of Antibiotics from Injectable and Thermally Responsive Polypeptide Depots" J Biomed Mater Res B Appl Biomater., 90, 67

[22] Sawhney A.S, Pathak CP, and Hubell J.A, (1993), "Bioerodible hydrogels based on photopolymeerizpoly(ethyleneglycol)-copoly(alpha-hydroxy acid) diacrylate macromers, Macromolecules," 26, 581.

[23] Sechoy O, Tissie G, Sebastian C, Maurin F, Driot J.Y, and Trinquand C.A, (1984), "new long acting ophthalmic formulation of carteolol containing Alginic acid," Int J Pharm.,207, 109.

[24] Sharifi S, Mirzadeh H, Imani M, Rong Z, Jamshidi A, Shokrgozar M, Atai M, and Roohpour N, (2009), "Injectable in situ forming drug delivery system based on poly(epsilon-caprolactone fumarate) for tamoxifen citrate delivery: Gelation characteristics, in vitro drug release and anti-cancer evaluation," Acta Biomater.,5,1966.

[25] Shively M.L, Bennett A.T, Coonts B.A, Renner W.D, and Southard J.L, (1995), "Physico–chemical characterization of polymeric injectable implant delivery system," J Control Release., 33, 237.

[26] Shoaib M.H, Taneez J, Merchant H.A, and Yousuf R.I, (2006), "Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC.," Pak. J. Pharm. Sci.,19, 119.

[27] Singh U.V, Udupa N, Kamath R, and Umadevi P, (1990), "Enhanced Biodegradable in situ forming implants and methods of antitumor efficacy of methoteraxate poly (lactic-co-glycolic) producing the same, US Pat. 4938763," acid injectable gel implants in mice bearing sarcoma-180, 136.

[28] Smart J.D, Kellaway I.W, and Worthington H.E, (1984), "An in vivo investigation of mucosa adhesive materials for use in controlled drug delivery," J Pharm Pharmacol., 36, 259.

[29] Smirnov I.V, Zavyalov G.G, And Eremin S.V, (2000), "A Comparative Study Of The Kinetics Of cytarabine hydrolytic deamination in aqueous solutions," Pharmaceutical Chemistry Journal., 34, 451.

[30] Soskolne W.A, (1997), "Subgingival delivery of therapeutic agents in the treatment of periodontal diseases," Crit. Rev. Oral Biol. Med., 8, 164.

[31] Srividya B, Cardoza R.M, and Amin P.D, (2001), "Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system," J. Control Release.,73, pp. 205.

[32] Stile R.A, Burghardt W.R, and Healy K.E, (1999), "Synthesis and characterization of injectable poly (N isopropylacrylamide)-based hydrogels that support tissue formation in vitro," Macromolecules., 32, 7370.

[33] Suisha F, Kawasaki N, Shirakawa M, Yamatoya K, and Attwood D, (1998), "Thermally reversible xyloglucan gels as vehicles for rectal drug delivery," J. Control Release., 56, 75.

[34] Sun Q, Eduardo A, Silva,Aixia Wang, James C, Fritton, David J, Mooney, Mitchell B, Schaffler Paul M, Grossman, and Sanjay Rajagopalan, (2000), "Sustained Release of Multiple Growth Factors from Injectable Polymeric System as a Novel Therapeutic Approach Towards Angiogenesis," Pharmaceutical Research., 27, 264.

[35] Swati Rawat, Warade, and Lahoti, (2010), "In Situ Gel Formulation of Ornidazole for the Treatment of Periodontal Disease," Current Pharma Research.,1, 60.

[36] Taylor, and Bagley, (1975), "Rheology of Dispersions of Swollen Gel Particles," J. Polym. Sci., Polymer Physics Edition, 13, 1133.

[37] Taylor, and Bagley, (1977), "Tailoring Closely Packed Gel-Particles Systems for Use as Thickening Agents," J. Appl. Polym. Sci., 21, 113.

[38] Tripathi K.D, (2008), "Essential of medical pharmacology ," Jaypee publisher.,6, 1. Chap. 1

[39] Tripathi K.D, (2008), "Essential of medical pharmacology ," Jaypee publisher.,6, 819. Chap. 62.

[40] Veyries M.L, Couarraze G, Geiger S, Agnely F, Massias L, Kunzli B, and Faurisson F, (1999), "Rouveix B. Controlled release of vancomycin from Poloxamer 407 gels," Int. J. Pharm., 192, 183.

[41] Vyas J, Ghedia T, Gajjar V, and Christian V, (2011), "A review on Novel in-situ Polymeric drug delivery system," International journal of Pharm Tech Research., 3(5), 53.

[42] Wang W.S, Tzeng C.H, and Chiou T.J, (1997),uJpn. J. Clin. Oncol.27, 154.

[43] Wenge LiuJ, Andrew MacKayc, Matthew R. Dreherd, Mingnan Chena, Jonathan R, McDaniela, Andrew J, Simnicka, Daniel J, Callahana, Michael R, Zalutskyb, and Ashutosh Chilkotia, (2010), "Injectable intratumoral depot of thermally responsive polypeptide radionuclide conjugates delays tumor progression in a mouse model,"Journal of Control Release., 144, 2.

[44] West J.L, and Hubell J.A, (1995), "Localized intravascular protein delivery from photopolymerized hydrogels," Proc Int Symp Control Rel Bioact Mater., 22, 17.

[45] Westhaus E, and Messermith P.B, (2001), "Triggered release of calcium from lipid vesicles: a bioinspired strategy for rapid gelation of polysaccharide and protein hydrogels," Biomaterials22, 453.