FORMULATION OPTIMIZATION AND EVALUATION OF NOVEL INJECTABLE, THERMORESPONSIVE AND CYTOCOMPATIBLE GEL FOR SUSTAINED DRUG DELIVERY

A THESIS IS SUBMITTED TO

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY, GUWAHATI, ASSAM

IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF

MASTER OF PHARMACY (M.PHARM)
IN
PHARMACEUTICS

SUBMITTED BY:-

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UNDER THE SUPERVISION OF
DR. PULAK DEB (ASST. PROFESSOR, HOD, DEPARTMENT OF PHARMACEUTICS, GIPS)

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE, HATHKHOWAPARA, AZARA, GUWAHATI-781017, KAMRUP, ASSAM, INDIA

Dedicated to my lovely Parents who always sacrifice to shape my future...

DECLARATION

I hereby declare that the topic incorporated in this dissertation entitled "**FORMULATION OPTIMIZATION AND EVALUATION OF NOVEL INJECTABLE, THERMORESPONSIVE AND CYTOCOMPATIBLE GEL FOR SUSTAINED DRUG DELIVERY**" final project report being submitted in partial fulfilment of the requirement for the award of Degree of Masters of Pharmacy (M Pharm) in Pharmaceutics of Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS) affiliated to Assam Science and Technology University, Guwahati, Assam is a bonafied assignment which has been carried out by me under the guidance and supervision of Dr. Pulak Deb, Assistant Professor, Department of Pharmaceutics (GIPS) and Principal Dr. Suvakanta Dash (GIPS). The work embodied in this thesis is original and has not been submitted in part or full for the award of degree, diploma or fellowship of any other university or institution.

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This is to certify that the topic incorporated in this research thesis "**FORMULATION OPTIMIZATION AND EVALUATION OF NOVEL INJECTABLE, THERMORESPONSIVE AND CYTOCOMPATIBLE GEL FOR SUSTAINED DRUG DELIVERY"** being submitted by **BIBHUTI SONOWAL**, Roll no-1505211008, Regd. no- 007505215, in the partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmaceutics is a genuine and legitimate research work carried out at Department of Pharmaceutics Laboratory, **Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS)** affiliated to **Assam Science and Technology University, Guwahati, Assam, India** during the academic year 2016-2017.

I wish him all success in life.

Signature

Date:

Place: Guwahati

Prof. (Dr.) Suvakanta Dash
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CERTIFICATE FROM THE GUIDE

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I wish him all success in life.

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CERTIFICATE FROM THE H.O.D

This is to certify that the topic incorporated in this research thesis "**FORMULATION OPTIMIZATION AND EVALUATION OF NOVEL INJECTABLE, THERMORESPONSIVE AND CYTOCOMPATIBLE GEL FOR SUSTAINED DRUG DELIVERY"** being submitted by **BIBHUTI SONOWAL**, Roll no-1505211008, Regd. no- 007505215, in the partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmaceutics is a genuine and legitimate research work which has been carried out under my direct supervision and guidance at Department of Pharmaceutics Laboratory, **Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS)** affiliated to **Assam Science and Technology University, Guwahati, Assam,** during the academic year 2016-2017.

I wish him all success in life.

Signature

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ACKNOWLEDGEMENT

I take the privilege and pleasure to acknowledge the contributions of many individuals who have been inspirational and supportive throughout my work. I express my cordial and humble thanks to Dr. Pulak Deb sir, HOD & Asst. Professor, Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Sciences, Azara, Guwahati for his valuable guidance, support and everlasting encouragement. I will ever remain indebted to him for having inculcated in me a quest for excellence, a sense of humility and honesty. I am grateful to Dr. Suvakanta Dash sir, Honourable Principal, Girijananda Chowdhury Institute of Pharmaceutical Science for providing the necessary facilities to carry out my work and his constant support and encouragement. I take this opportunity to express my sincere thanks to the library staff, all the teaching and the non teaching staff of this institution specially Mr. Bipul Sarma, Mr. Diganta Barua, Mr. Dipankar Das. A special thanks goes to my friend Iman Bhaduri for his help and support to assemble the parts and completion of the task. Last but not least, I wish to express my gratitude towards "God-almighty" and my Parents, brother and my sister, who gave me the strength and courage to fulfill my dream and has showered upon one their choicest blessings. Finally I consider this is an opportunity to express my gratitude to all the people involved directly or indirectly with the successful completion of my work.

Date:
Place:

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ABSTRACT

The aim of the present work was to formulate and evaluate injectable, thermosensitive *in situ* gelling system of furosemide. Furosemide is a loop diuretic, which exhibits short half life, when given in the form of conventional injectable solutions. To overcome this, an attempt has been made to formulate temperature sensitive *in situ* gelling system of furosemide to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in temperature. The furosemide *in situ* gelling system was formulated by cold method using polyethylene oxide and carbopol 934P which acted as drug carrier and viscosity enhancing agent respectively. All the formulations were evaluated and the results of the study showed that 0.7% to 0.9% of polyethylene oxide produces consistent, maximum and sustained drug release. The formulations were clear liquid appropriate for injection of subcutaneous route. Gelation temperature all the formulations were found in between 32^0c-42^0c and gelation time varying from 2-10 minutes. pH was found to be around 7.4. Viscosity was found out which showed high viscosity upto 13600 (at rpm 50) during gelation at 37^0c and low viscosity at 25^0c at the same rpm. The drug content of the prepared formulation was found to be within the range of 89-99.9%. The optimized formulations F4, F5 & F6 showed cumulative drug release of 97.97%, 99.04% & 97.04 respectively with a sustained effect of 13 hours. The increase in concentration of polymer (polyethylene oxide) increases the concentration of formulations. Thus in this research work the results obtained after the evaluation of the formulations indicate that the optimized formulations i.e. F4, F5 & F6 were found to be ideal having thermosensitive parameter to produce sustained effect via administration of subcutaneous route.

Keywords: Injectable *in-situ* gel, thermo sensitive, sol-gel transition, sustained release.

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LIST OF ABBREVIATIONS

ID:	Intradermal
IM:	Intrmuscular
SC:	Subcutaneous
SDDS:	Sustained release drug delivery system
LET:	Liposomal encapsulation technology
FDA:	Food and Drug Administration
ADF:	Agar Diffusion Technique

PEG-PLA-PEG: Polyethylene Glycol-Polylactic acid- Polyethylene Glycol

PEO-PPO-PEO: Polyethylene oxide-Polypropylene oxide-polyethylene oxide

PEG-PLGA-PEG: Polyethylene oxide-Polylactic Glycolic acid-Polyethylene Glycol

LCST:	Lower Critical Solution Temperature
UCST:	Upper Critical Solution Temperature
DSC:	Differential Scanning Calorimetry
FT-IR:	Fourier transform infrared spectroscopy
HCL:	Hydrochloric acid
PEO:	Polyethylene oxide
NaOH:	Sodium Hydroxide
CP:	Centipoise
UV-VIS:	Ultraviolet - Visible
Conc. **X** DF:	Concentration **X** Dilution factor

LIST OF PUBLICATION

I. Poster Presentation

Presented Poster at **"North East Pharmaceutical Convention"** on 6th-7th May, 2017, organized by **North East Pharmaceutical Society**, hosted by Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati-17.

- **"Formulation optimization and evaluation of novel injectable, thermo responsive and cytocompatible gel for sustained drug delivery".**
  Author's name: **Bibhuti Sonowal*, Dr. Pulak Deb**

II. Review Article Published

II. (A) The review article entitled "**Studies on *In-Situ* Forming Thermo Sensitive Injectable Polymeric gel for Sustained Drug Delivery**" has been published in vol.10, June 2017, in **Research Journal of Pharmacy and Technology (RJPT).**

- Sonowal B., Deb P., Dash S. "**Studies on *In-Situ* Forming Thermo Sensitive Injectable Polymeric gel for Sustained Drug Delivery**", Research Journal of Pharmacy and Technology (RJPT). 2017; vol. 10(6): 1-8.

II. (B) The review article entitled **"Recent advances of smart polymers for sustained release drug delivery**", has been published in vol. 4, June 2017, in **European Journal of Biomedical and Pharmaceutical Sciences (EJBPS).**

- Sonowal B., Ray P., Deb P., Dash S. **"Recent advances of smart polymers for sustain release drug delivery"**, European Journal of Biomedical and Pharmaceutical Sciences (EJBPS). 2017; vol. 4(6): 543-548.

III. Research Article Published

- The research article entitled, **"Formulation optimization and evaluation of novel injectable, thermo responsive and cytocompatible gel for sustained drug delivery",** has been accepted for publication in the upcoming Issue of August, 2017, by International Journal of ChemTech Research**.**

I. Poster Presentation on "**Formulation optimization and evaluation of novel injectable, thermo responsive and cytocompatible gel for sustained drug delivery".**

CATEGORY-II

FORMULATION OPTIMIZATION AND EVALUATION OF NOVEL INJECTABLE, THERMO RESPONSIVE AND CYTCOMPATIBLE GEL FOR SUSTAINED DRUG DELIVERY

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ABSTRACT
The aim of the present work was formulation and evaluation of *in situ* gelling system of furosemide. Furosemide is a loop diuretic, which exhibits short half life, when given in the form of conventional injectable solutions. To overcome this, an attempt has been made to formulate temperature sensitive *in situ* gelling system of furosemide to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in temperature. The furosemide *in situ* gelling system formulated by using polyethylene oxide which is a viscosity enhancing agent and acted as drug carrier. The developed formulation was stable, non-irritant and provided sustained release over 8 hour period and it is a viable alternative to conventional injectable.
Keywords: Injectable *in-situ* gel, furosemide, polyethylene oxide, temperature sensitive, sustain release.

INTRODUCTION
Gel is a combination of two phases containing mostly liquid yet behave like solids due to a three-dimensional cross-linked network within the liquid. The viscous liquid which are designed to be injected are called injectable gel. Thermo-responsive polymers can be used for prolong release of drugs through parenteral route.

OBJECTIVES
To formulate and evaluate novel injectable and cytocompatible gel using thermo responsive gel for sustained drug delivery.

EXPERIMENTAL METHOD
Different concentration of poly ethylene oxide were taken and dissolved in distilled water to prepare injectable gel using cold method as shown in table 1. The drug furosemide was dissolved in low molecular Sodium Hydroxide and added to polymer solution. Dilute Hydrochloric acid was used as a pH adjustifier.

CHARACTERISATION OF INJECTABLE *IN SITU* GELS
Drug-excipients compatibility Study
1. Fourier Transform Infrared (FTIR) Spectroscopy:
Infrared spectra of mixture of polyethylene oxide and furosemide were taken by Fourier Transform Infrared Spectrophotometer. The FTIR graph combination of drug with excipients were recorded using KBR pellets as shown in *table 4.*
2. Appearance and pH of the Formulation: The prepared formulations were observed by visual inspection. The pH of all formulations was found out.
3. Viscosity: Viscosity of each formulations were determined through LDVE-Brookfield viscometer.
4. Syringe Ability Study: Desired amount of optimized formulations were allowed to pass through 20-25 gauge needle to check the syringe ability of the formulations.
5. Estimation of Drug Uniformity: Formulations containing 1 mg of drug was taken 10 ml volumetric flask made up the volume to 10 ml with PBS 7.4. Absorbance values were measured
with suitable dilutions at 277 nm. Concentrations of drug were calculated from the standard calibration curve prepared in Phosphate buffer 7.4
6. Gelation time and gelation temperature: The temperature required for phase transition of sol to gel and its time is studied.
7. *In-vitro* Diffusion Study: *In-vitro* diffusion was determined by Franz diffusion cell. Cellophane membrane was sandwiched securely between donor and acceptor compartment.

Composition of *in-situ gels. Table 1.*

Formulation code	Polyethylene oxide(%w/v)	Furosemide (gm)	Sodium Hydroxide (0.05 Molar) mL	Hydrochloric acid (0.05 Molar) mL	Distilled Water mL
F1	0.5	0.3	10	qs to 10	qs
F2	0.6	0.3	10	qs to 10	qs
F3	0.7	0.3	10	qs to 10	qs
F4	0.8	0.3	10	qs to 10	qs
F5	0.9	0.3	10	qs to 10	qs
F6	1.0	0.3	10	qs to 10	qs
F7	1.1	0.3	10	qs to 10	qs
F8	1.2	0.3	10	qs to 10	qs
F9	1.3	0.3	10	qs to 10	qs

Results and Discussions:

APPEARANCE, CLARITY pH AND DRUG CONTENT: The appearance of all formulations were transparent and clear in color. The pH of all the formulations were found to be within the range of 7.4 ± 0.05. The drug content of optimized formulations (F1 –F6) was found to be within the range 94% to 99%.
SYRINGE ABILITY: The formulations were found easily passable through needle size of 20-25 gauge size.

VISCOSITY: Viscosity of different formulations were found at 37 ^{0}C and were recorded as shown in *table 2.*

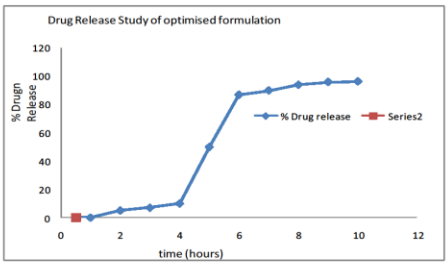
RPM	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	1702	1806	1220	1400	1700	2770	3200	3120	3400
5	1100	1501	1501	1501	1501	1998	1501	2501	2600
10	700	805	715	834	912	912	912	1212	1900
20	400	549	498	298	311	780	657	657	987
50	90	110	110	110	167	400	498	457	357
100	72	80	69	51	42	190	190	210	290

GELATION TIME AND GELATION TEMPERATURE: The gelation temperature and gelation time for all the formulations were found to be within 35^0 C to 42^0 C and 2 to 10 minutes respectively.

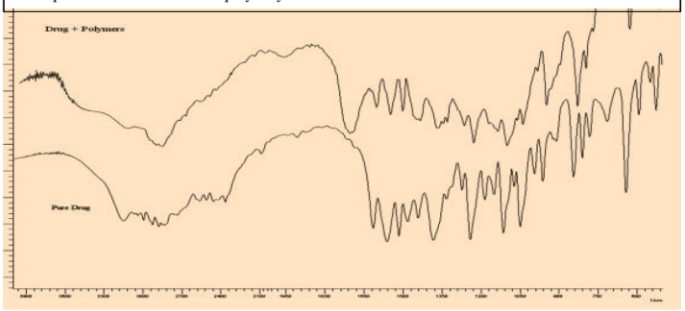
In-vitro Diffusion Study: F4 showed sustained release of drug i.e. maximum release of drug takes place after 4 hours upto 8 hours. The graph for drug release is shown in *table 3.*

table 3.

time (hours)	% Drug release
0.5	0
1	0
2	5
3	7
4	10
5	50
6	87
7	90
8	94
9	96
10	96.4
11	96.6
12	96.9



FTIR Spectra of Furosemide with poly ethylene oxide *table 4.*



CONCLUSIONS: From the study it can be concluded that temperature sensitive injectable gel can be used to achieve sustained drug release. All the formulations had gelation and releases the drug at physiological body temperature. Thus the prepared formulation was proved to be easy to administer, reduce frequency of dose, and increase patient compliance.

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RECENT ADVANCES OF SMART POLYMERS FOR SUSTAIN RELEASE DRUG DELIVERY

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ABSTRACT
Polymers are the core ingredient in the formulation and development of drug product. They have certain characteristics in designing and modification of the formulation in drug delivery system. This delivery system may require polymers in the form of carrier, colloidal or as nanoparticles which show great advantages in drug loading as well as in releasing of active drug moiety. Smart polymers are slightly different from such polymers because smart polymers have the ability to transform its microstructure or physical structure with the change in temperature, pH, biological enzyme, radioactive agents etc. Natural as well as synthetic polymers have been used in making the production and formulation system a very simple and low cost. Through these polymers the designing and formulation system of controlled drug delivery have became quite easy for the researcher. Targeted release and sustain release of drug can be obtained by overcoming the difficulties like poor bioavailability and short half life drug.

KEYWORDS: smart polymer, stimuli responsive, control release, targeted delivery.

INTRODUCTION

Polymers play an important role in the formulations of drug delivery system due to their improved pharmacokinetic properties. Especially in the field of smart drug delivery, polymer has been widely used because it can deliver therapeutic agents directly into the intended site of action, with high efficacy. Smart polymers have shown immense progress in the sustained and controlled delivery of drugs. Some systems like hydrogels swell and release the drug when exposed to intended environment. They work passively in minimizing drug degradation and improving circulation time. Another important issue is the safe excretion of the drug.[1,2] Due to advanced scientific pharmaceutical technology, controlled drug release system has been in a dynamic progress and this delivery model can be categorized into following classes.

(1) Drug delivery through rate-programmed

The recent advances in the smart rate-programmed drug delivery systems have vital roles, such as greater drug-loaded nano/micro-particle encapsulation ability, overcome pre-systemic metabolism, enhanced bioavailability and environmental responsive properties for various applications. An example of new rate pre-programmed drug delivery system is transdermal patch which delivers a particular concentration of drugs to the blood circulation via the skin. The rate-programmed drug delivery system releases drug molecules in a controlled manner by pre-determining the rate.[3,4]

(2) Activation-modulated drug delivery

These types of polymers depend on the surrounding environment and change their physical or microstructure. These polymers are generally divided into physical, chemical and biological stimuli. Stimuli responsive polymers are those which have the ability to transform itself with the change of temperature, pH, radioactive agents, biological enzymes etc. Smart polymers are therefore becoming a bonanza to deliver the active drugs by the means of transdermal route, injectables etc. Smart polymers thus can be used for sustaining and controlling release of drug for the poorly absorbed and half life drugs.[4,5]

(3) Feedback-regulated drug delivery

This self-regulated or feedback-controlled drug delivery is maintained and controlled by feedback information, without any external stimulation, and utilized several approaches to control the release rate. This drug delivery method has been applied to the development of various controlled delivery systems such as bio-erosion regulated, bio-responsive regulated and self regulating drug delivery systems. Among this one of the concepts has been involved in the smart controlled delivery systems. One of the concepts state that the drug release is activated by a triggering agent, such as a biochemical

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REVIEW ARTICLE

Studies on *In-Situ* Forming Thermo Sensitive Injectable Polymeric gel for Sustained Drug Delivery

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ABSTRACT:
The in situ injectable gel is a polymeric formulation which provides several advantages when compared to conventional injectable drug delivery systems. These types of formulations are actually prepared by smart polymers i.e. that change its microstructure according to environmental change. These polymers remain in sol form before administration into the body, but once administered, undergo gelation in situ, to form a gel. Thus the in situ forming polymeric formulations are dependent on biodegradable smart polymers. The formation of gel or gelation occur for many reasons depending on factors like temperature modulation, electrical sensitivity, pH change, presence of ions, ultra violet irradiation and enzyme sensitive from which the drug gets released in a predetermined or controlled manner. Thus this article presents a detailed review of these types of polymeric formulation composed of thermo responsive polymers. Heat sensitive or thermo responsive polymers are those which have a discontinuous change of their physical properties with temperature modulation and thus undergo physical change inside the human body. The production and the formulation system is a very simple and low cost where various synthetic as well as natural thermo sensitive polymers are used for the formulation of in situ gel. The in situ gel dosage form having good stability, biocompatibility and increasing patient compliance makes more reliable and acceptable drug delivery system.

KEYWORDS: In situ gel, gelation, thermo responsive, biodegradable, sustained release.

INTRODUCTION:

Gel:

Gel is defined as a two-component or combination of two phases which mostly contain liquid, but they behave like solids because of three-dimensional cross-linked network within the liquid. [1] Usually, the solvent is the major component of the gel system. Gels are those formulations that are used for several routes of administration. They are useful in different formulations of topical, vaginal, rectal as well as injectable administration. [2] [3]

Novel Injectable Gel:

The development of novel inject able in situ forming (gel) drug delivery systems has been a quite interesting topic as it has received a considerable interest over the last decade. The drugs which are designed to be injected in the form of viscous liquid are called injectables. The injection of gel follows the appropriate route for administration called subcutaneous route. It is a method of infusion to put the viscous liquid into the body, usually with an appropriate syringe and through the skin to a sufficient depth so that the material can be administered easily and freely into the body. [3] The viscous liquid turns to gel in the surrounding tissue of body and start releasing the drug in a sustain manner. Since considerable attention has been paid to the development of injectable gel system over the last few years, so this interest has been spreading due to the various advantages shown by these delivery systems

III. Acceptance Letter of Research Article on **"Formulation optimization and evaluation of novel injectable, thermo responsive and cytocompatible gel for sustained drug delivery"** by International Journal of ChemTech Research.

Paper .no = 163 /july/letter of acceptance /charges/undertaking to be signed.

Inbox x

Journal sphinx <submitpaper@rediffmail.com> Jul 5

to bibhutigips, me

01/06/2017

Respected Bibhuti Sonowal Sir/Mm,

Greetings.

We would like to inform you that,
Your paper titled, " FORMULATION OPTIMIZATION AND EVALUATION OF NOVEL INJECTABLE, THERMO RESPONSIVE
AND CYTOCOMPATIBLE GEL FOR SUSTAINED DRUG DELIVERY "

is selected for publication in: Vol. 10,Number 6, 2017, [Upcoming Regular issues, 2017] Of International Journal of ChemTech Research (to get released on
25 June /2017).

YOUR PAPER NUMBER = 163 June list/ V10N7 CT.

International Journal of ChemTech Research has been approved by UGC, for publishing the Research papers.[UGC website: See list 1, Sr. NO.= 140].

CHEMTECH and PHARMTECH Journals are in Top 10 as per the latest report produced by CII-ICI-CSIR/NISCAIR-Govt of India.

LIST OF SCIENTIFIC CHAPTERS

1 INTRODUCTION

1.1 GEL

A gel is a solid jelly like material which mostly contains liquid, yet they behave like solids due to a three-dimensional cross-linked network within the liquid. Gels are a dispersion of molecules of a liquid within a solid in which the solid is the continuous phase and the liquid is the discontinuous phase. Gels are defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state. Gels are an excellent formulation for several routes of administration. They are useful as liquid formulations in oral, topical, vaginal, rectal as well as injectable administration. [1,4,5]

1.2 INJECTABLES

The drugs which are designed to be injected are called injectables or it can be defined as an infusion method of putting fluid into the body, usually with a syringe or a hollow needle which is pierced through the skin to a sufficient depth for the material to be administered into the body. An injection follows a parenteral route of administration that is, administration via a route other than through the digestive tract. Since the process inherently involves a small puncture wound to the body (with varying degrees depending on injection type and location, medication type, needle gauge which require skills of the individual administering the injection). The injectable gels are those which possess phases of viscous liquid and solid jelly substances incorporated in polymer matrix in different environmental conditions. The injectable gels are of different types which can be classified as follows. [5,7,17]

1.2.1 Types of injectable gel

i. Hydrogels

A hydrogel is a network of polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are highly absorbent (they can contain over 90% water) natural or synthetic polymeric

networks. Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content. Common uses for hydrogels include:

i. They are used as scaffolds in tissue engineering and may contain human cells to repair tissue. They mimic 3D microenvironment of cells.
ii. Hydrogel-coated wells have been used for cell culture.
iii. Environmentally sensitive hydrogels (also known as 'Smart Gels' or 'Intelligent Gel) have the ability to sense changes of pH, temperature or the concentration of metabolite and release their load as result of such a change.
iv. They are used for sustained-release drug delivery systems.
v. Hydrogels that are responsive to specific molecules, such as glucose or antigens, can be used as biosensors, as well as in Drug Delivery System.
vi. Contact lenses (silicone hydrogels, polyacrylamides, polymacon).
vii. They are used in rectal drug delivery and diagnosis.
viii. Dressings for healing of burn or other hard-to-heal wounds. Wound gels are excellent for helping to create or maintain a moist environment.
ix. Materials mimicking animal mucosal tissues to be used for testing mucoadhesive properties of drug delivery systems.

Common ingredients include polyvinyl alcohol, sodium polyacrylate, acrylate polymers and copolymers with abundance of hydrophilic groups. Natural hydrogel materials are being investigated for tissue engineering; these materials include agarose, methylcellulose, hyaluronan, and other naturally derived polymers. Hydrogels show promise for use in agriculture, as they can release agrochemicals including pesticides and phosphate fertilizer slowly, increasing efficacy and reducing runoff and at the same time improve the water retention of dried soils such as sandy loams. [2,3,6]

ii. Organogels

An organogel is a non-crystalline, non-glassy thermo reversible (thermoplastic) solid material composed of a liquid organic phase entrapped in a three-dimensionally cross-linked network. The liquid can be, for example, an organic solvent, mineral oil, or vegetable oil. The solubility and particle dimensions of the structure are important characteristics for the elastic properties and firmness of the organogel. Often, these systems are based on self-assembly of the structural molecules. (An example of formation of an undesired thermo reversible network is the occurrence of wax crystallization in petroleum). [2,3,6]

iii. Xerogels

A xerogel is a solid formed from a gel by drying with unhindered shrinkage. Xerogels usually retain high porosity (15–50%) and enormous surface area (150–900 m^2/g), along with very small pore size (1–10 nm). When solvent removal occurs under supercritical conditions, the network does not shrink and a highly porous, low-density material known as an *aerogel* is produced. Heat treatment of a xerogel at elevated temperature produces viscous sintering (shrinkage of the xerogel due to a small amount of viscous flow) and effectively transforms the porous gel into a dense glass.[2,3,6]

iv. Nanocomposite hydrogels

Nanocomposite hydrogels are also known as hybrid hydrogels, can be defined as highly hydrated polymeric networks, either physically or covalently crosslinked with each other and/or with nanoparticles or nanostructures. Nanocomposite hydrogels can mimic native tissue properties, structure and microenvironment due to their hydrated and interconnected porous structure. A wide range of nanoparticles, such as carbon-based, polymeric, ceramic, and metallic nanomaterials can be incorporated within the hydrogel structure to obtain nanocomposites with tailored functionality. Nanocomposite hydrogels

can be engineered to possess superior physical, chemical, electrical, and biological properties. [2,3,6]

1.2.2 Routes of administration

There are three main routes of administration of injectables used in humans and animals. The routes are as follows.

i. **Intradermal (ID) injection**- Intradermal injection is a technique which is used to deliver the drugs into the dermis, or the skin layer underneath the epidermis. The advantage of such systems is their usability, allowing the tool to be used also by untrained staff. They also evoke less pain for the patient, and the shortness of the injection needle makes injections safer. [6,7]

ii. **Intramuscular (IM) injection**- An intramuscular injection is a technique used to deliver a medication deep into the muscles. In an intramuscular injection, the medication is delivered directly into a muscle. Many drugs are administered intramuscularly which are absorbed into the muscle fairly quickly, while others are more gradual. Injections to the buttocks are known to reach the bloodstream quickly due to the large amount of muscular tissue and corresponding blood supply. [6,7]

iii. **Subcutaneous (SC) injection**- A subcutaneous injection is a technique used to administer as a bolus into the subcutis, the layer of skin directly below the dermis and epidermis, collectively referred to as the cutis. Subcutaneous injections are highly effective in administering vaccines and medications as well as for the sustained drug release of drugs in the body. Long-acting forms of subcutaneous/intramuscular injections are available for various drugs and are called depot injections. It is a technique of injection that deposits a drug in a localized mass, called a depot, from which it is gradually absorbed by surrounding tissue. Such injection allows the active compound to be released in a consistent way over a long period. Depot injections are usually either solid or oil-based. [6,7]

1.3 NOVEL INJECTABLE GEL

The injectable of gel follows the appropriate route for administration called **subcutaneous route**. It is an infusion method of putting viscous liquid into the body, usually with a syringe and a hollow needle which is pierced through the skin to a sufficient depth for the material to be administered into the body. The viscous liquid in the surrounding tissue of body turns to gel and start releasing the drug slowly. The development of injectable gel systems has received considerable attention over the past few years. This interest has been sparked by the advantage shown by these delivery systems such as ease of administration, reduced frequency of administration improved patient compliance and comfort.

In this chemical procedure, the 'sol' (or solution) gradually evolves towards the formation of a gel-like diphasic system containing both a liquid phase and solid phase whose morphologies range from discrete particles to continuous polymer networks. In the case of the colloid, the volume fraction of particles (or particle density) may be so low that a significant amount of fluid may need to be removed initially for the gel-like properties to be recognized. This can be accomplished in any number of ways. The simplest method is to allow time for sedimentation to occur, and then pour off the remaining liquid. Centrifugation can also be used to accelerate the process of phase separation.

Removal of the remaining liquid (solvent) phase requires a drying process, which is typically accompanied by a significant amount of shrinkage and densification. The rate at which the solvent can be removed is ultimately determined by the distribution of porosity in the gel. The ultimate microstructure of the final component will clearly be strongly influenced by changes imposed upon the structural template during this phase of processing.

Afterwards, a thermal treatment or firing process is often necessary in order to favor further polycondensation and enhance mechanical properties and structural stability via final sintering, densification and grain growth. One of the distinct advantages of using this methodology as opposed to the more traditional processing techniques is that densification is often achieved at a much lower temperature.

The precursor sol can be either deposited on a substrate to form a film (e.g., by dip coating or spin coating), cast into a suitable container with the desired shape (e.g., to obtain monolithic ceramics, glasses, fibers, membranes, aerogels) or used to synthesize powders (e.g., microspheres, nanospheres). The sol-gel approach is a cheap and low-temperature technique that allows for the fine control of the product's chemical composition. Even small quantities of dopants, such as organic dyes and rare earth elements, can be introduced in the sol and end up uniformly dispersed in the final product. It can be used in ceramics processing and manufacturing as an investment casting material, or as a means of producing very thin films of metal oxides for various purposes. [8,9,14,16]

1.3.1 Particles and polymers

The sol-gel process is a wet-chemical technique used for the fabrication of both glassy and ceramic materials. In this process, the sol (or solution) evolves gradually towards the formation of a gel-like network containing both a liquid phase and a solid phase. Typical precursors are metal alkoxides and metal chlorides, which undergo hydrolysis and polycondensation reactions to form a colloid. The basic structure or morphology of the solid phase can range anywhere from discrete colloidal particles to continuous chain-like polymer networks.

The term colloid is used primarily to describe a broad range of solid-liquid (and/or liquid-liquid) mixtures, all of which contain distinct solid (and/or liquid) particles which are dispersed to various degrees in a liquid medium. The term is specific to the size of the individual particles, which are larger than atomic dimensions but small enough to exhibit Brownian motion. If the particles are large enough, then their dynamic behavior in any given period of time in suspension would be governed by forces of gravity and sedimentation. But if they are small enough to be colloids, then their irregular motion in suspension can be attributed to the collective bombardment of a myriad of thermally agitated molecules in the liquid suspending medium, as described originally by Albert Einstein in his dissertation. Einstein concluded that this erratic behavior could adequately be described using the theory of Brownian motion, with

sedimentation being a possible long-term result. This critical size range (or particle diameter) typically ranges from tens of angstroms (10^{-10} m) to a few micrometres (10^{-6} m).

i. Under certain chemical conditions (typically in base-catalyzed sols), the particles may grow to sufficient size to become colloids, which are affected both by sedimentation and forces of gravity. Stabilized suspensions of such sub-micrometre spherical particles may eventually result in their self-assembly—yielding highly ordered microstructures reminiscent of the prototype colloidal crystal: precious opal.

ii. Under certain chemical conditions (typically in acid-catalyzed sols), the interparticle forces have sufficient strength to cause considerable aggregation and/or flocculation prior to their growth. The formation of a more open continuous network of low density polymers exhibits certain advantages with regard to physical properties in the formation of high performance glass and glass/ceramic components in 2 and 3 dimensions. [14,16]

In either case (discrete particles or continuous polymer network) the sol evolves then towards the formation of an inorganic network containing a liquid phase (gel). Formation of a metal oxide involves connecting the metal centers with oxo (M-O-M) or hydroxo (M-OH-M) bridges, therefore generating metal-oxo or metal-hydroxo polymers in solution.

In both cases (discrete particles or continuous polymer network), the drying process serves to remove the liquid phase from the gel, yielding a micro-porous amorphous glass or micro-crystalline ceramic. Subsequent thermal treatment (firing) may be performed in order to favor further polycondensation and enhance mechanical properties.

With the viscosity of a sol adjusted into a proper range, both optical quality glass fiber and refractory ceramic fiber can be drawn which are used for fiber optic sensors and thermal insulation, respectively. In addition, uniform ceramic powders of a wide range of chemical composition can be formed by precipitation. [17]

1.4 SUSTAIN RELEASE DRUG DELIVERY SYSTEM

Sustained release drug delivery system (SDDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action. The term therapeutic substance also applies to an agent such as gene therapy that will induce in vivo production of the active therapeutic agent. Drug delivery system is an interface between the patient and the drug. It may be a formulation of the drug to administer it for a therapeutic purpose or a device used to deliver the drug. This distinction between the drug and the device is important, as it is the criterion for regulatory control of the delivery system by the drug or medicine control agency. If a device is introduced into the human body for purposes other than drug administration, such as therapeutic effect by a physical modality or a drug may be incorporated into the device for preventing complications resulting from the device, it is regulated strictly as a device. There is a wide spectrum between drugs and devices, and the allocation to one or the other category is decided on a case by case basis. Sustained release (SR) preparations are not new but several new modifications are being introduced. They are also referred to as "long acting" or "delayed release" when compared to "rapid" or "conventional" release preparations. The term sometimes overlaps with "controlled release," which implies more sophisticated control of release and not just confined to the time dimension. [6,7,14,15]

1.4.1 Advantages of sustained release drug delivery system [11,12,13]

i. The frequency of drug administration is reduced.

ii. Patient compliance can be improved.

iii. Drug administration can be made more convenient as well.

iv. The blood level oscillation characteristic of multiple dosing of conventional dosage

form is reduced.

v. Better control of drug absorption can be attained, since the high blood level peaks that may be observed after administration of a dose of a high availability drug can be reduced.

vi. The characteristic blood level variations due to multiple dosing of conventional dosage forms can be reduced.

vii. The total amount of drug administered can be reduced, thus:

· Maximizing availability with minimum dose;

· Minimize or eliminate local side effects;

· Minimize or eliminate systemic side effects;

· Minimize drug accumulation with chronic dosing.

viii. Safety margins of high potency drugs can be increased and the incidence of both local and systemic adverse side effects can be reduced in sensitive patients.

ix. Improve efficiency in treatment.

· Cure or control condition more promptly

· Improve control of condition

· Improve bioavailability of some drugs.

1.4.2 Disadvantages of sustained release drug delivery system [11,12,13]

i. Decreased systemic availability in comparison to immediate release conventional dosage forms.

ii. Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions.

iii. Reduced potential for dose adjustment of drugs normally administered in varying strengths.

iv. Poor *in vitro – in vivo* correlation.

v. Probability of dose dumping.

vi. Cost of single unit higher than conventional dosage forms.

1.4.3 The following are the rationale of developing Sustained Release[11,12,13]

- **i.** To extend the duration of action of the drug.
- **ii.** To reduce the frequency of dosing.
- **iii.** To minimize the fluctuations in plasma level.
- **iv.** Improved drug utilization.
- **v.** Less adverse effects.

1.5 SUSTAINED RELEASE INJECTABLE GEL

The sustained release injectable gel is viscous liquid which reduces the rate of release of drug into tissues after administration of drug. The advantages of using a long-acting depot injection include increased medication compliance due to reduction in the frequency of dosing, as well as more consistent serum concentrations. A significant disadvantage is that the drug is not immediately reversible, since it is slowly released. [4]

Prolong release of drugs after IM or SC administration may occur due to use of stimuli responsive or "**smart**" polymers. "**smart**" polymers are macromolecules that display a significant physiochemical change in response to small changes in their environment such as temperature, pH, light, magnetic field, ionic factors, etc. The changes are reversible, and therefore, the smart polymers are capable of returning to its initial state as soon as the trigger is removed. Some sustained release injectable formulations are as follows. [13,12,14]

1.5.1 Injectable Suspension

Injectable suspensions are dispersed, heterogeneous system consisting insoluble drug molecules and excipients which need to be resuspensed or redispersed in either aqueous or vegetable oil vehicles before administering to patient. To achieve a pharmaceutically acceptable suspension, it should take into consideration the below mentioned checkpoints.

- **iii.** They should maintain their sterility, pyrogenicity, resuspendibility, syringe ability, stability and isotonicity.

iv. They should be either being formulated as ready to use injection or as reconstitution prior to use.

iii. Suspensions should usually contain solids between 0.5-5.0 percent, having particle size less than 5 micrometer for I.M or S.C. administration.[12,23,24]

1.5.1.1 Merits of Injectable Suspension[19,20]

i. It is a carrier for insoluble drug.

ii. It eliminates first past metabolism.

iii. It is resistance to hydrolysis and oxidation as drug present in solid form.

1.5.1.2 Demerits of Injectable Suspension[19,20]

i. It is difficult to maintain physical stability.

ii. It limits the formulator for selecting excipients.

iii. It is difficult to maintain aseptic condition and manufacturing process.

1.5.2 Injectable Emulsion

An emulsion is a thermodynamically unstable dispersion of two or more immiscible liquids stabilized by a surfactant or emulsifier coating the droplets and prevents coalescence by reducing interfacial tension or creating a physical repulsion between the droplets.

Common types of emulsions are found in parenteral drug delivery systems

i. **Water in oil emulsions (W/O)** used in sustained release of steroids and vaccines by intramuscular injection.

ii. **Oil in water (O/W)** or lipid emulsions can be administered by a variety of parenteral routes (for example subcutaneous, intramuscular and intra-arterial) but are mainly injected intravenously in parenteral nutrition applications.

Mostly, injectable emulsions are oil-in-water emulsion. They are milky white in appearance and have an average globule size of 1.0 micrometer to 5 micrometer. Emulsions are primarily used for parenteral nutrition and infused intravenously. These are terminally sterilized with the sterilization cycle designed to maintain globule size distribution.[20,21]

1.5.2.1 Advantages of injectable emulsions [19,20]

The physicochemical advantages of using emulsions as a drug delivery system is

i. The solubilisation of drugs with low aqueous solubility.
ii. Stabilization of labile drugs against hydrolysis or oxidation.
iii. Therapeutic advantages can be exhibited by a decrease of toxicity of drug.
iv. Other therapeutic benefits can be the elimination of irritation and toxicity associated with formulations containing high organic solvents or alkaline conditions (e.g. phenytoin).
v. Continuous infusion, sustained release and potential drug targeting applications.

1.5.3 Microspheres and Microparticles

1.5.3.1 Microspheres

It can be defined as the reservoir type system in which micron size(tiny particles) core material/internal phase(may be solid, liquid or gas as drug, cell, microorganism, proteins or peptides, enzymes, hormones etc.) is enclosed in a thin layer of wall/ shell material/external material(usually polymer) using a suitable microencapsulation method. [21,22]

1.5.3.2 Microparticles

Microparticles are spherical encapsulated particles with size ranging between 1 to 1000 μm. For injection purpose, micro particles smaller than 125μm are preferred as shown in figure 2. Micro particles depend on biodegradable polymers that have been extensively investigated for controlled release delivery system over last 3 decades. Micro particles can be injected through rarely used needle and alleviate the pain during injection. [22]

1.5.4 Liposomes

Liposomes are small, spherical, bilayer phospholipid vesicles of size range 30 nanometer to micrometers.They are amphipathic in nature, so can transport both hydrophilic and hydrophobic drugs. [26] They are extensively used as carrier for numerous cosmetic and

pharmaceutical industries. Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify sitespecific drug delivery to tumor tissues, liposomes have increased rate both as an investigational system and commercially as a drug- delivery system. Many studies have been conducted on liposomes with the goal of decreasing drug toxicity and/ or targeting specific cells. Liposomal encapsulation technology (LET) is the newest delivery technique used by medical investigators to transmit drugs that act as curative promoters to the assured body organs. [21,22,23]

1.5.4.1 Advantages of Liposome [22]

- **i.** Reduced toxicity of encapsulated drug
- **iii.** Site avoidance of drug
- **iv.** Increased therapeutic index efficacy of drug.
- **v.** Increased stability.

1.5.4.2 Disadvantages of Liposome [22]

- **i.** Low solubility
- **ii.** Short half life
- **iii.** Leakage and fusion
- **iv.** Production cost is high

1.5.5 Niosomes

Niosomes are the highly ordered vesicular structure with bilayer membrane made up of Non-ionic surfactant with or without incorporation of cholesterol. The closed bilayer vesicular structure of niosome formed by the self assembling of non – ionic surfactants in the presence of aqueous media. Niosomes are very small, and microscopic in size. Their size lies in the nanometric scale. Although they structurally similar to liposomes. Niosomes have both advantages and disadvantages.[18]

1.5.5.1 Advantages [18,19]

- **i.** Improve oral bioavailability of poorly absorbed drugs.
- **ii.** Osmotically active and chemically stable.
- **iii.** Act as depot for short acting peptide drugs.
- **iv.** Enhance skin penetration of drug.
- **v.** Improve therapeutic performance

1.5.5.2 Disadvantages [18,19]

- **i.** Physically instable Aggregation
- **ii.** Fusion
- **iii.** Leaking of entrapped drug
- **iv.** Hydrolysis of encapsulated drug

1.5.6 In situ forming implants

In situ forming implants based on a drug-containing polymer semi-solid or solution, which after entering into the body undergo chemical or physical change to form a unit implant for the controlled drug delivery. The development of in situ forming implants was originated by Dunn in the early 1980s. They used injectable depot system loaded with antibiotics for local treatment of periodontal diseases. Thereafter, approval of Eligard® containing drug leuprolide acetate by FDA spurred interest in In-situ forming implant system development. According to different formation mechanism, this system can be classified into different categories. [18,19]

1.5.6.1 Thermoplastic Pastes

Polymers with low melting point can be instilled into body as a melt and form depot upon cooling to the body temperature. The melting point or glass transition temperature of the polymers should fall in range from 25-65 °C and the intrinsic viscosity of the polymers should be in range from 0.05 to 0.8 dl/g (25 °C). [49] Before injecting into body, the polymers are gently heated above their melting point. The drug is admixed with molten polymers without application of solvents. Original thermoplastic pastes are formulated

from monomers such as D, L-lactide, glycolide, dioxanone, ε-caprolactone, trimethyl carbonate. The admixing of drug in this system can be achieved by simple mixing at room temperature without using any organic solvent. [18,19]

1.5.6.2 Insitu cross-linked polymer system

The formation of solid polymers or gels is achieved by in situ cross-link of the introduced macromers. It is initiated by the reaction involving photon absorption or ionic interaction between multivalent anions and cation macromers. The advantage of this system involves rapid polymerization rates at physiological temperature due to photoinitiated reaction. Along with advantages of this system it also have some disadvantages that is, the use of free radical initiator causes the risk of tumor. [17,24]

1.5.6.3 Thermally induced gelling systems

This system is based on polymers that undergo abrupt changes in solubility in response to the variation in environment temperature. Triblock copolymer PEG-PLA-PEG has been also applied in thermally gelling system and are claimed to have good biodegradability and biocompatibility. [54] The aqueous solution of polymer have low viscosity at room temperature but once enters the body, it turns into a gel with very high viscosity. [17,24]

1.5.6.4 pH induced gelling system

The change of polymer solubility in aqueous medium can also be achieved in response to the change in environmental pH. Acidic solutions of chitosan when subjected to alkaline pH form viscous gels. The in situ gel formation has been employed for controlled delivery of several drugs via oral or parenteral routes. [17,24]

1.6 SMART POLYMERS FOR SUSTAINED RELEASE DRUG DELIVERY

Smart polymers, or stimuli-responsive polymers, are in the vanguard of drug administration technology since they have show an active response to small signs and changes in the surrounding environment, which translates into significant changes in their microstructure and in the physiological and chemical proprieties, as desired. Smart polymers are biocompatible, non-thrombogenic, strong, resilient, flexible, easy shaping

and coloring. They keep the drug's stability and are easy to manufacture, good nutrient carriers to the cells, easily changed using cell adhesion ligands and it is possible to inject them *in vitro* as liquid to create a gel with the body temperature. [4,5,18]

1.6.1 Thermoreversible Polymers

Commercially available block copolymers of poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) (PEO-PPOPEO)—Pluronics also known as Poloxamers (BASF, Mount Olive, NJ)—are the best-known examples of thermally gelling polymers. Aqueous solutions of PEO-PPOPEO copolymers demonstrate phase transitions from sol to gel (low temperature sol-gel boundary) and gel to sol (high temperature gel-sol boundary) with monotonically increasing temperature when the polymer concentration is above a critical value. The mechanism of gelation of PEO-PPO copolymers is still being debated among polymer physicists. For example, an intrinsic change in the micellar properties such as aggregation number or micellar symmetry causes aqueous solutions of Poloxamer to form gels. This hypothesis is supported by a decrease in the critical micelle concentration with increasing temperature. Later, based on a 13C-NMR study, dehydration of poly(propylene oxide) blocks was proposed as a cause of gelation of aqueous solutions of Poloxamer. Recently, mechanistic studies on the phase transitions and characterization of the solution and gel states for Poloxamer were reported using various instrumental techniques. Based on these studies, shift in equilibrium from unassociated unimers to micelles was suggested as a gelation mechanism of Poloxamer aqueous solution. As the temperature increases, the micelle volume fraction (fm) increases, resulting in a gel when (fm) is larger than 0.53. Examples of applications of injectable formulations of the PEO-PPO-PEO copolymers in tissue engineering are described in Applications of Selected Injectable Gels in Tissue Engineering. Methyl cellulose and hydroxypropyl cellulose are typical examples. Recently, novel biodegradable triblock copolymers of polyethylene glycol and poly(lactic/glycolic acid or PEGPLGA-PEG) were developed. Aqueous solutions of these copolymers exhibit sol-to-gel transition at body temperature. Subcutaneous injection of the copolymer formulation resulted in the formation of a transparent gel in situ. Small aliquots (0.4 ml) of a 33wt. % aqueous solution of PEG-PLGA-PEG (550- 2810-550) triblock copolymer were injected

subcutaneously in rats using a 22-gauge needle. The gel maintained a three-dimensional shape at the implant site, rather than spreading to form a sheet like structure. This finding indicates that the gel formation rate is fast. Structural integrity of the gel was maintained for more than 1 month. The sol-to-gel transition occurring at about 30°C makes this system an ideal candidate for an injectable matrix for cell delivery or an injectable drug delivery system that may be formulated at room temperature and forms a gel at body temperature. [25]

The signs or stimuli that trigger the structural changes on smart polymers can be classified in three main groups:

i. Physical stimuli (temperature, ultrasounds, light, mechanical stress).
ii. Chemical stimuli (pH and ionic strength).
iii. Biological stimuli.

1.6.1.1 Physical stimuli polymers (Thermo responsive polymers) [12,23,7]

The physical stimuli i.e. temperature sensitive polymers are generally called as thermo responsive polymers. These smart polymers are sensitive to the temperature and change their micro structural features in response to change in temperature. These are most safe polymers in drug administration systems and biomaterials. Thermo-responsive polymers present in their structure a very sensitive balance between the hydrophobic and the hydrophilic groups and a small change in the temperature can create new adjustments.

An important feature of this kind of polymers is the critical solution temperature. If the polymeric solution has a phase below the critical solution temperature, it will become insoluble after heating, i.e., it has one lower critical solution temperature (LCST). Above the critical solution temperature (LCST), the interaction strengths (hydrogen linkages) between the water molecules and the polymer become unfavorable, it dehydrates and a predominance of the hydrophobic interaction occurs, causing the polymer swelling. The LSCT can be defined as the critical temperature in which the polymeric solution shows a phase separation, going from one phase (isotropic state) to two phases (anisotropic state). The hydrophilic or hydrophobic incorporation on a polymeric system of this kind has as consequence the changing of the LCST. [12,23,7]

1.6.1.2 Chemical stimuli (pH responsive polymers) [17,18]

All pH-sensitive polymers consist of pendant acidic or basic group that can either accept or release a proton in response to changes in environmental pH. Polymers with a large number of ionisable groups are known as polyelectrolyte's. Polyelectrolyte's are classified into two types: weak polyacids and weak polybases. Weak polyacids accept protons at low pH and release protons at neutral and high pH. The environmental pH changes, the pendant acidic group undergoes ionization at specific pH called as pKa. This rapid change in net charge of the attached group causes alteration in the molecular structure of the polymeric chain. This transition to expanded state is mediated by the osmotic pressure exerted by mobile counter ions neutralized by network charges. pH-sensitive polymers containing a sulphonamide group are another example of polyacid polymers. These polymers have pKa values in the range of 3–11 and the hydrogen atom of the amide nitrogen is readily ionized to form polyacids. Narrow pH range and good sensitivity is the major advantage of these polymers over carboxylic acid based polymers. [12,23,7]

1.6.1.3 Bioresponsive polymers

Biologically responsive polymer systems are increasingly important in various biomedical applications. The major advantage of bioresponsive polymers is that they can respond to the stimuli that are inherently present in the natural system. Bioresponsive polymeric systems mainly arise from common functional groups that are known to interact with biologically relevant species, and in other instances the synthetic polymer is conjugated to a biological component. Bioresponsive polymers are classified into antigen responsive polymers, glucose-sensitive polymers and enzyme responsive polymers. [23,10,11]

1.7 CYTOCOMPATIBLE GEL

Biocompatibility is determined as the ability of a material to co-exist and perform with a natural substance in a specific biological application. The purpose of performing

biocompatibility testing is to determine the fitness of a device for human use, and to see whether use of the device can have any potentially harmful physiological effects. Typically, material characterization and analysis of a device's components are conducted prior to any biological testing. This involves extracting leachable materials from the device or components at an elevated temperature, and analyzing the leachable extracts for potentially harmful chemicals or cytotoxicity. [19,22]

1.7.1 Cytocompatibility Study

The Cytocompatibility tests involve the exposure of substances extracted from test material to cell culture lines. Cell cultures are extremely sensitive to minute quantities of leachable chemicals and readily display characteristic signs of toxicity in the presence of potentially harmful leachables. The tests are frequently used during product planning stages to qualify the use of a material and as a periodic check for routinely used materials to ensure that no shift in quality has occurred. Cytocompatibility in vitro testing is also required in testing the biocompatibility of materials. Typical testing programs will utilize the ISO test method to meet international regulatory requirements. The screening test method can be performed to characterize materials or to evaluate new materials against established ones. There are three Cytocompatibility evaluation tests commonly used for medical devices. [11,19,22,23]

(a) The Direct Contact Process: It is recommended for low density materials, such as contact lens polymers. In this method, a piece of test material is placed directly onto cells growing on culture medium. The cells are then incubated. During incubation, leachable chemicals in the test material can diffuse into the culture medium and contact the cell layer. Reactivity of the test sample is indicated by malformation, degeneration and lysis of cells around the test material. [11,19,22,23]

(b) The Agar Diffusion assay: It is appropriate for high density materials, such as elastomeric closures. In this method, a thin layer of nutrient-supplemented agar is placed

over the cultured cells. The test material (or an extract of the test material dried on filter paper) is placed on top of the agar layer, and the cells are incubated. A zone of malformed, degenerative or lysed cells under and around the test material indicates cytotoxicity. [11,19,22,23]

(c) The MEM Elution assay: It uses different extracting media and extraction conditions to test devices according to actual use conditions or to exaggerate those conditions. Extracts can be titrated to yield a semi-quantitative measurement of cytotoxicity. After preparation, the extracts are transferred onto a layer of cells and incubated. Following incubation, the cells are examined microscopically for malformation, degeneration and lysis of the cells. [11,19,22,23]

1.8 DRUG PROFILE[24,25,2,1]

1.8.1 Furosemide

It is a potent loop diuretic is an anthranilic acid derivative. It prevents the body from absorbing too much salt and instead of allowing to pass through urine. It is used to treat fluid retention (edema) in people with congestive heart failure, liver disease or kidney disorder. In addition it is highly effective in hypertension. The formulation has been modified with tri-block polymer to extend the release of drug.

i. **Synonyms**- Frusemide
ii. **Proprietary name**- Lasix, Diucontin-K®, Tebemid, Fursimide, Frusemene®
iii. **Dose** – 10-20 mg for adults (intravenous)
iv. **Storage** – Store at room temperature. Protect from light. Exposure to light may cause discoloration.
v. **Class** – Loop Diuretic
vi. **Indication-**

Diuretic- It is used to remove water from the body by increasing the amount of urine the kidneys produce.

Edema- Lasix is indicated in adults and pediatric patients for the treatment of edema associated with congestive heart failure, cirrhosis of the liver.

Hypertension- It may be used in adults for the treatment of hypertension alone or in combination with other antihypertensive agents.

vii. Contraindication-

Drug/drug interaction-

a. Furosemide with **amino glycoside antibiotics** produces ototoxic potential.
b. Furosemide with **ethacrynic acid** produces ototoxicity.
c. Furosemide with **angiotensin converting enzyme** inhibitor may lead to severe hypotension.
d. Furosemide with **cyclosporine** may increase the risk of gouty arthritis.

Drug/Food interaction-

a. Furosemide ↔ Alcohol (Ethanol) (Furosemide and ethanol may have additive effects in lowering blood pressure)

Drug/disease interaction-

a. Furosemide ↔ Anuria (The use of loop diuretics is contraindicated in patients with anuria)
b. Furosemide ↔ Cirrhosis (Loop diuretic therapy should be initiated in the hospital under strict observation in patients with liver cirrhosis and ascites. Sudden alteration of fluid and electrolyte balance may precipitate hepatic encephalopathy and coma in such patients)
c. Furosemide ↔ Renal Dysfunction (Impaired effectiveness and possible delayed excretion of loop diuretics may occur in patients with severe renal dysfunction. These individuals may require high dosages that are associated with an increased risk of electrolyte abnormalities)
d. Furosemide ↔ Electrolyte Losses (The use of loop diuretics, particularly at high dosages or during chronic therapy, is commonly associated with loss of electrolytes, including potassium, sodium, chloride, magnesium, and calcium. Potassium and magnesium depletion may lead to cardiac arrhythmias and cardiac arrest)

viii. Mechanism of Action - Furosemide works by blocking the absorption of sodium, chloride, and water from the filtered fluid in the kidney tubules, causing a profound increase in the output of urine (diuresis).

1.8.2 Physico-Chemical Properties Of Furosemide[24,25,2]

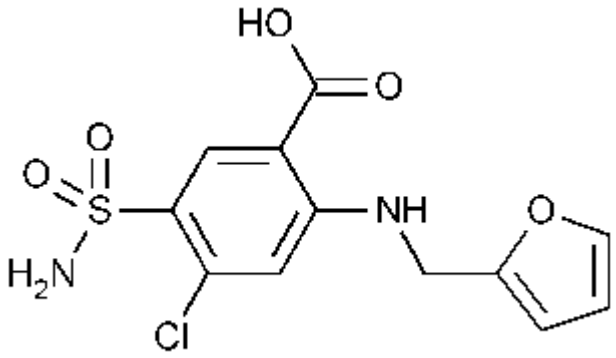
a. Origin of the substance- Synthetic

b. Chemical names 4-Chloro-N-furfuryl-5-sulphamoylanthranilic acid

c. Molecular formula- $C_{12}H_{11}ClN_2O_5S$

d. Molecular weight- 330.7 g/mol

e. Chemical structure-



1.8.2.1 Physical properties

a. Color- White or slightly yellow

b. State/form- Solid-crystals, Solid-powder

c. Description- Odorless

d. Solubility- Practically insoluble in water; very slightly soluble in chloroform; soluble in alcohol; slightly soluble in ether; freely soluble in acetone; dimethyl formamide; methyl alcohol and solutions of alkali hydroxides.

e. Melting Point- 220^0C

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2. LITERATURE REVIEW

2.1 Literature Review of Injectable Gel

2.1.1 Kiran Rajendra B et. al. 2015, formulated Atrigel technology for the administration of poorly bioavailable drugs through parenteral route. Parenteral delivery provides rapid onset even for the drug with narrow therapeutic window, but to maintain the systemic drug level repeated installation are required which cause the patient discomfort. Therefore, a delivery system that combines the simplicity and reliability as well as ease of administration of microparticles is desired. In situ gel forming systems designed to provide drug release in sustained manner.

2.1.2 Kulwant Singh et. al. 2013, studied *in situ* gelling system of cytarabine (an anti-cancer agent), which exhibits less oral absorption, less site targeted ability and show toxic effect (higher amount), when given in the form of conventional injectable solutions. To overcome this, they formulated pH-triggered or ion exchange *in situ* gelling system of cytarabine to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in pH. The cytarabine *in situ* gelling system formulated by using poly acrylic acid (Carbopol 934 NF) in combination with sodium alginate, which acted as viscosity enhancing agent. The developed formulation was stable, non-irritant and provided sustained release over 24-hour period and it is a viable alternative to conventional injectable.

2.1.3 Dharmesh Si sodi et. al. 2014, formulated Atrigel system which consists of biodegradable polymers dissolved in a biocompatible carrier. When the liquid polymer system is placed in the body using standard needles and syringes, it solidifies upon contact with aqueous body fluids to form a solid implant. Controlled release drug system and implants can provide systemic, local, or targeted therapy. The most frequently used solvent is, *N*-methyl-2-pyrrolidone (NMP) because of its solvating ability and its safety/toxicology profile. The advantages are less invasive technique, direct delivery to a target area, protection of drug, sustained drug release.

2.1.4 Gowda D.V. et. al. 2014, formulated injectable in situ gel matrix containing metoprolol succinate by cold method, using the thermosensitive polymer Pluronic F 127 (20%) together with carbopol 934P, HPMC and SCMC. The drug polymer study by FTIR was found compatible. The formulations for clarity, sterility, gelation temperature, gelation time, viscosity, drug content and *in-vitro* drug release were found satisfactory.

2.1.5 Manju Nagpal et. al. 2010, studied Atrigel: A potential parenteral controlled drug delivery system. The Parenteral administration route is the most effective and common form of delivery for active drug substances with poor bio-availability and the drugs with a narrow therapeutic index. It has advantages like quicker onset of action in case of emergency, target the drug quickly to desired site of action, prevention of first pass metabolism etc. The application of advanced drug delivery technology to parenteral administration lead to development of liposomes, nanosuspensions, solid implants etc. to overcome limitations of conventional parenteral delivery. Solid implants are reported

to produce very reproducible release profiles. However, because of their size, they require surgical implantation or the use of large trochars to administer the product. Delivery systems consisting of microparticles can be injected into the body using conventional needles and syringes and have been the most widely accepted biodegradable polymer system for parenteral use. This article compiles the information on the *in-situ* gel forming system i.e. ATRIGEL technology designed to provide drug release in sustained manner.

2.1.6 Nikita Karode et. al. 2015, designed injectable formulations into a sustained release injectable gel to get rid of patient incompliance in the case of frequent dosing.The concept benefits not only rapid onset of action but also helps inducing frequent dosing and maintains a proper systemic level of drug. This article will briefly cover current options for extended release injectable drug delivery system consisting microsphere, implants, suspension, emulsion, liposome, noisome, protein and peptides.

2.1.7 Danhong Long et. al. 2016, prepared and evaluated phospholipid-based injectable gel for the long term delivery of leuprolide acetate. The formulation was a gel system using biocompatible materials (SPME), including soya phosphatidyl choline (SPC), medium chain triglyceride (MCT) and ethanol. The system displayed a sol state with low viscosity in vitro and underwent in situ gelation in vivo after subcutaneous injection. An *in-vitro* release study was performed using a dialysis setup with different release media containing different percentages of ethanol. The stability of LA in the SPME system was investigated under different temperatures and in the presence of various

antioxidants. In vivo studies in male rats were performed to elucidate the pharmacokinetic profiles and pharmacodynamic efficacy. A sustained release of LA for 28 days was observed without obvious initial burst in vivo. The pharmacodynamic study showed that once-a-month injection of LA-loaded SPME (SPME-LA) led to comparable suppression effects on the serum testosterone level as observed in LA solution except for the onset time. These findings demonstrate excellent potential for this novel SPME system as a sustained release delivery system for Leuprolide Acetate.

2.1.8 Rassoul Dinarvand et. al. 2013, studied and developed a controlled delivery system for PEGylated octreotide using a Poloxamer based in situ gel forming polymer. The designed drug delivery system contained low concentration of Poloxamer 407 with polyvinyl alcohol (PVA) as a polymeric additive. Rheological measurements of gel vehicle formulations indicated that the in situ gel forming system with optimum sol–gel transition temperature of 28.7 ^{0}C could be formed using a combination of P407 and PVA at ratio of 15–10% (w/v). Using buffering agents, it was possible to shift the sol–gel transition to lower or higher temperatures. The in vitro release profiles of octreotide and PEGylated octreotide from the selected P407/PVA formulations were measured using a membrane-less device. PEGylated octreotide showed slower release rate from the gel system with different release kinetic compared to octreotide. In animal studies, a sustained release rate was achieved with both PEGylated and non-PEGylated octreotide, but longer delivery was observed for PEGylated octreotide. Tissue histopathological studies confirmed the biocompatibility of the delivery system for PEGylated octreotide, supporting the suitability of P407/PVA mixture as an injectable drug delivery system.

2.2 Literature Review of Smart Polymers

2.2.1 Aguilar et. al. 2007, experimented on Smart Polymers and Their Applications as Biomaterials Living systems respond to external stimuli adapting themselves to changing conditions. Polymer scientists have been trying to mimic this behavior for the last twenty years creating the so called smart polymers. These are defined as polymers that undergo reversible large, physical or chemical changes in response to small external changes in the environmental conditions, such as temperature, pH, light, magnetic or electric field, ionic factors, biological molecules, etc. Smart polymers have very promising applications in the biomedical field as delivery systems of therapeutic agents, tissue engineering scaffolds, cell culture supports, bioseparation devices, sensors or actuators systems. This chapter is focused on pH and temperature sensitive polymers and their most recent and relevant applications as biomaterials in drug delivery and tissue engineering. Dual-stimuli-responsive materials will also be presented because of their high potential in the biomedical field.

2.2.2 Reum Bo Lee et. al. 2001, studied on Polymeric Nanocarriers Responding to Physical or Biological Signals. The physicochemical properties of stimuli-responsive polymers change with physical or biological signals, such as pH, enzyme concentrations, and temperature. These polymers have attracted considerable attention in the field of drug delivery. The drug carrier system, which was revolutionized by the introduction of these polymers, has recently provided a new paradigm of maximizing the therapeutic activity of drugs. This review highlights recent studies regarding stimuli-responsive drug

carriers tailor-made for effective cytosolic drug delivery, with particular emphasis on tumor treatment.

2.2.3 Rushi Ghizal et. al. 2010, studied on Smart Polymers and Their Applications. They found that Smart polymers are materials that respond to small external stimuli. These are also referred as "stimuli responsive" materials or "intelligent" materials. The stimuli include salt, UV irradiation, temperature, pH, magnetic or electric field, ionic factors etc. Smart polymers are very promising applicants in drug delivery, tissue engineering, cell culture, gene carriers, textile engineering, oil recovery, radioactive wastage and protein purification. The study is focused on the entire features of smart polymers and their most recent and relevant applications.

2.2.4 Huaping Tan et. al. 2010, reviewed different smart polymers in Injectable, Biodegradable Hydrogels for Tissue Engineering Applications. They found that Hydrogels have many different applications in the field of regenerative medicine. Biodegradable, injectable hydrogels could be utilized as delivery systems, cell carriers, and scaffolds for tissue engineering. Injectable hydrogels are an appealing scaffold because they are structurally similar to the extracellular matrix of many tissues, can often be processed under relatively mild conditions, and may be delivered in a minimally invasive manner. This review will discuss recent advances in the field of injectable hydrogels, including both synthetic and native polymeric materials, which can be potentially used in cartilage and soft tissue engineering applications.

2.2.5 Parekh Hejal B et. al. 2012, reviewed Novel In situ Polymeric Drug Delivery System. The reviewer found that, In situ forming polymeric formulations are drug delivery systems that are in sol form before administration in the body, but once administered, undergo gelation in situ, to form a gel. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, electrical sensitivity, enzyme sensitive from which the drug gets released in a sustained and controlled manner. Routes of administration are oral, ocular, rectal, vaginal, injectable and intraperitoneal. Various biodegradable polymers that are used for the formulation of in situ gels include gellan gum, alginic acid, xyloglucan, pectin, chitosan, poly (DL lactic acid), poly (DL-lactide-co-glycolide) and poly-caprolactone. Recent developments in the field of polymer science and technology has led to the development of various stimuli sensitive hydrogels like pH, temperature sensitive, which are used for the targeted delivery of proteins to colon, and chemotherapeutic agents to tumors. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. From a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing.

2.2.6 Honey Priya James et. al. 2014, reviewed Smart polymers for controlled delivery. They studied that Smart polymers have enormous potential in various applications. In particular, smart polymeric drug delivery systems have been explored as “intelligent” delivery systems able to release, at the appropriate time and site of action, entrapped drugs in response to specific physiological triggers. These polymers exhibit a non-linear

response to a small stimulus leading to a macroscopic alteration in their structure/properties. The responses vary widely from swelling/contraction to disintegration. Synthesis of new polymers and crosslinkers with greater biocompatibility and better biodegradability would increase and enhance current applications. The most fascinating features of the smart polymers arise from their versatility and tunable sensitivity. The most significant weakness of all these external stimuli-sensitive polymers is slow response time. The versatility of polymer sources and their combinatorial synthesis make it possible to tune polymer sensitivity to a given stimulus within a narrow range. Development of smart polymer systems may lead to more accurate and programmable drug delivery. In this review, we discuss various mechanisms by which polymer systems are assembled in situ to form implanted devices for sustained release of therapeutic macromolecules, and we highlight various applications in the field of advanced drug delivery.

2.2.7 Hugo Almeida et. al. 2012, studied Temperature and pH stimuli-responsive polymers and their applications in controlled and self regulated drug delivery. They found that stimuli-responsive polymers is steadily gaining increasing momentum especially in the fields of controlled and self-regulated drug delivery. Stimuli responsive or "smart" polymers are macromolecules that display a significant physiochemical change in response to small changes in their environment such as temperature, pH, light, magnetic field, ionic factors, etc. The changes are reversible, and therefore, the smart polymers are capable of returning to its initial state as soon as the trigger is removed. They have become one important class of polymers and their applications have been increasing

significantly in the last three decades. Smart polymers have very promising applications in the biomedical field as delivery systems of therapeutic agents, tissue engineering scaffolds, cell culture supports, bioseparation devices, etc. The versatility and untapped potential of smart polymeric materials makes them one of the most promising interfaces of chemistry and biology.

2.3 Literature Review of Furosemide

2.3.1 Espinosa Bosch M. et. al. 2013, studied the Analytical Determination of Furosemide. It is an anthranilic acid derivative which is a potent diuretic widely used in the treatment of congestive heart failure and edema. FUR works by blocking the absorption of salt and fluid in the kidney tubules, causing a profound increase in urine output (diuresis). Due to the considerable use of FUR, analytical determination is frequent. In this manuscript, a review of the methods developed for determination of FUR from the year 2008 is presented.

2.3.2 Satendra Kumar et. al. 2010, studied Preformulation of Furosemide. The development of this injectable and rapidly acting highly efficacious diuretic was a breakthrough. Its maximal natriuretic effect is much greater than that of other classes. The diuretic response goes on increasing with increasing dose: up to 10 L of urine may be produced in a day. It is active even in patients with relatively severe renal failure. The onset of action is prompt (i.v. 2-5 min., i.m. 10-20 min., oral 20-40 min.) and duration short (3-6 hours).

2.3.3 Dhumansure Nagendrakumar et. al. 2015, formulated Sustained Release Matrix Tablets of Furosemide. They developed and evaluated once-daily sustained release matrix tablets of furosemide using hydroxypropylmethyl cellulose K4M, xanthan gum and sodium alginate as release retarding polymers. The formulations were prepared by using different drug: polymer ratios (1:1, 1:2, 1:3, 1:4 and 1:5) and the prepared matrix tablets were evaluated for pre-compression parameters like angle of repose, bulk density, tap density and post-compression parameters and *in vitro* drug release studies etc. and concluded that the formulation SF_2 containing drug: sodium alginate ratio of 1:2 offered the required *in vitro* drug release rate according to the official limits of drug release as per USP and selected as a promising formulation among the fifteen formulations which shows 85% drug release in 12 hrs.

2.3.4 Ashish Deshmukh et. al. 2010, formulated and evaluated Self Micro emulsifying Drug Delivery System (SMEDDS) Of Furosemide. They developed the Self Micro emulsifying Drug Delivery System (SMEDDS) of Furosemide which is a Class IV molecule according to BCS (Biopharmaceutical Classification System), having low solubility and low permeability. Prepared optimized SMEDDS of Furosemide composed of CAPTEX 500 as oil and Cremophore EL as surfactant in 20:80 ratio. Optimized SMEDDS of Furosemide showed increase in dissolution rate of Furosemide in Simulated Gastric Fluid (SGF) and 5.8 pH phosphate buffer, irrespective of pH compared the Marketed Tablet LASIX Ò (Furosemide 40 mg).

2.4 Literature Review of Formulation and Evaluation of gel

2.4.1 Giovanni Filippo Palmieri et. al. 1997, formulated Thermosensitive Self-Assembling Block Copolymers as Drug Delivery Systems**.** self-assembling block copolymers (poloxamers, PEG/PLA and PEG/PLGA diblock and triblock copolymers, PEG/polycaprolactone, polyether modified poly(Acrylic Acid) with large solubility difference between hydrophilic and hydrophobic moieties have the property of forming temperature dependent micellar aggregates and, after a further temperature increase, of gellifying due to micelle aggregation or packing. This property enables drugs to be mixed in the sol state at room temperature then the solution can be injected into a target tissue, forming a gel depot *in-situ* at body temperature with the goal of providing drug release control. The presence of micellar structures that give rise to thermoreversible gels, characterized by low toxicity and mucomimetic properties, makes this delivery system capable of solubilizing water-insoluble or poorly soluble drugs and of protecting labile molecules such as proteins and peptide drugs.

2.4.2 Dalia S Shaker et. al. 2012, prepared in-situ Injectable Thermosensitive Gel Based On Poloxamer As A New Carrier For Tamoxifen Citrate and evaluated poloxamer based *in-situ* injectable thermosensitive gel of Tamoxifen citrate (TMC) compared to orally administered TMC regarding retention in different tissues. The inclusion complexes of TMC with ß-cyclodextrin (ß-CD), hydroxypropyl ß-cyclodextrin (HP-ßCD) and sulfobutyl-7-ether ß-cyclodextrin (SBE-ß-CD) were prepared by solvent evaporation method and evaluated for drug excipient compatibility tests as well as *in-vitro* release studies. Poloxamer analogs were mixed in different ratios and evaluated for gelation temperature and rheological properties. Finally, the optimized thermosensitive hydrogel formula was evaluated for *in-vitro* drug release as well as *in-vivo* drug retention in rat tissues, plasma and liver. Results: TMC/SBE-ß-CD complex showed the highest drug release rate. The optimum concentrations of poloxamer analogs for the in situ gel-forming delivery system were 20% (w/v) Poloxamer 407 (F127) and 15% (w/v) Poloxamer 188 (F68) that exhibited sol–gel transition at 36.5oC ± 0.5C. TMC/SBE-ß-CD complex incorporated in the optimized thermosensitive gel base exhibited elevated drug level in cancer tissues and low level in plasma and liver compared to oral TMC suspension. Conclusion: The optimized formula of TMC/SBE-ß-CD hydrogel could

contribute in elevating drug level at targeted tissues and improving drug anticancer activity.

2.4.3 Krishna Burugapalli et. al. 2002, experimented on *in-v*itro Cytocompatibility Study of a Medical device. This research was part of a European Commission funded research project for the development of a new generation of titanium based orthopedic biomaterials. In particular, hip implants alloy were fabricated by the powder metallurgy route. An advanced surface treatment technology of Laser Engineered Net Shaping (LENS) was used to apply a ZrO/Zr coating on the implant surfaces where relative movement takes place between the implant and the bone, in order to enhance the wear resistance. The present work was conducted to evaluate the cytotoxicity of the uncoated and ZrO2/Zr coated Ta alloy in both indirect and direct contact of human embryonic kidney 293T (HEK293T) cells. The cellular response was quantified by cell viability assessments using Alamar Blue assay and cell attachment. Cytotoxicity experiments showed that the responses of the uncoated and ZrO/Zr coated alloy on surviving cells were nontoxic. HEK293T cells displayed appropriate survival rates, good cell adhesion and cell spreading. These results confirmed that the Ta alloy, in both coated and uncoated conditions possesses good cytocompatibility, which allowed adhesion and proliferation of HEK293T cells to occur in both indirect and direct contact cell culture methods by Alamar Blue assay.

2.4.4 Daniel Rey de et. al. 2015, Characterized and studied on *in-vitro* Cytocompatibility of an Acid-Etched Titanium Surface. The aims of this study were to characterize the microstructure of a commercially pure titanium (cpTi) surface etched with HCl/H2SO4 and to investigate its *in-vitro* cytocompatibility. cpTi showed a grooved surface and ae-cpTi revealed a surface characterized by the presence of micropits. Surface parameters indicated that the ae-cpTi surface is more isotropic and present a greater area compared to T-cpTi. The oxide film thickness was similar between both surfaces; however, ae-cpTi presented more Ti and o and less C. osteoblastic cell proliferation, alkaline phosphatase activity, and bone-like nodule formation were greater on T-cpTi than on ae-cpTi. These results show that acid etching treatment produced a surface with different

topographical and chemical features compared to the turned one, and such surface modification affected negatively the *in vitro* cytocompatibility of cpTi as demonstrated by decreasing culture growth and expression of osteoblastic phenotype.

2.4.5 Priyanka Patel et. al. 1998, studied the Drug-Excipient compatibility and first step for dosage form development. Study revealed that drug-excipient compatibility represent an important phase in the preformulation stage of the development of all dosage forms. The potential physical and chemical interactions between drugs and excipients can affect the chemical, physical, therapeutical properties and stability of the dosage form. The present review contains a basic mode of drug degradation, mechanism of drug- excipient interaction like physical, chemical and biopharmaceutical. Different Thermal and Non-thermal method of analysis, Tools and software for incompatibility is also discussed. Once the type of interaction is determined we can take further steps to improve the stability of drug and dosage form. From review, we conclude that consequent use of thermal and non-thermal method provide data for drug- excipients interaction which can further help in selection of excipient for the development of stable dosage form.

2.4.6 Young-Wook Won et. al. 2010, studied the Prolongation and enhancement of the anti-apoptotic effects of PTD-Hsp27 fusion proteins using an injectable thermo-reversible gel in a rat myocardial infarction model. They observed that Ischemic heart disease has emerged as a leading cause of death worldwide. Conventional surgery-based therapy for this disease, especially myocardial infarction, requires additional pharmaceutical agents using heart's endogenous protective mechanism to suppress the progress and recurrence of the disease.. Injectable thermo-reversible gel system was developed and found to be effective in stabilizing and retarding the release of the PTD-Hsp27 fusion proteins both in vitro and in vivo. PTD-Hsp27-loaded thermo-reversible gels were locally administered to the heart muscle in a ligation/reperfused rat myocardial infarction model and the long-term therapeutic efficacy was observed by measuring the inhibition of apoptosis and the area of fibrosis.

2.4.7 Min Ley Pua et. al. 2013, studied Redox-active injectable gel using thermo-responsive nanoscale polyion complex flower micelle for non invasive treatment of local inflammation. Therefore, to enhance the local retention time of ROS scavengers, we developed a redoxactive injectable gel (RIG) system by using poly[4-(2,2,6,6-tetramethylpiperidine-N-oxyl)aminomethylstyrene]-bpoly(ethyleneglycol)-b-poly[4 (2,2,6,6-tetramethylpiperidine-N-oxyl)amino methylstyrene] triblock copolymer, which possesses ROS scavenging nitroxide radicals as side chains of the PMNT segment. Cationic PMNT segment in PMNT-PEG-PMNT forms polyion complexes with anionic poly(acrylic acid) (PAAC) to form a flower-like micelle (ca. 79 nm), which exhibits in situ thermo-irreversible gelation under physiological conditions. We confirmed the prolonged site-specific retention time of RIG by performing in vivo non invasive electron spin resonance imaging and quantitative evaluation. These findings indicate the potential of RIG as an innovative approach for treatment of local inflammation.

2.5 Literature Review of Optimization Technique

2.5.1 Abhishek Dutt Tripath et. al. 2015, worked on Statistical Optimization of Parameters Affecting Polyhydroxybutyrate Recovery by Dispersion Method from Alcaligenes Cells and Its Characterization In the present study, efforts have been made to optimize the three process variables i.e., incubation time, temperature of separation and hypochlorite concentration for enhanced polyhydroxybutyrate (PHB) recovery from Alcaligenes cells which may serve as precursor for bio-plastic production. Screening of bio-plastic (PHB) producing microorganism was done by TEM (Transmission electron microscopy). Initially, three recovery methods were applied to achieve efficient PHB recovery which included; Alkali treatment, ATPS (Aquas two phase separation (PEG 6000)/ KH2PO) and chloroform-hypochlorite dispersion (1:1, v/v). Maximum PHB recovery of 95 ± 0.5% with 97.0 ± 0.29% purity was obtained by dispersion method. In order to enhance the PHB recovery further, dispersion process was optimized using design expert (DX 8.0.6) software. PHB recovered under optimized physical condition comprising: incubation temperature 37°C, incubation time 28 h and hypochlorite concentration of 30.0, gave maximum PHB recovery of 98.2 ± 0.05% against the predicted yield of 99.0

± 0.05% with 99.0% purity. Characterization of recovered polymer as PHB was done by FTIR (Fourier transform infrared microscopy) and NMR (Nuclear magnetic resonance spectroscopy).

2.5.2 Veronica Maria de Araujo Calad et. al. 2010, studied the importance of Design of Experiments (DOE) in process modeling in food science and technology in the last ten years, the use and applications of mathematical modeling have increased in chemistry and food science and technology. However, it is still common to find researchers using the 'one at a time' approach to test and select variables to develop and optimize products and processes. In this regard, the objectives of this review are to provide some statistical information related to mathematical modeling of processes using design of experiments followed by multiple regression analysis, the so-called response surface methodology (RSM), and to discuss some recent published researches based on RSM optimization of products and processes, with special attention to microbiology, sensory analysis, food development and nutrition.

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3. AIM AND OBJECTIVES

3.1 AIM

- To formulate and optimize a novel injectable, thermo responsive & cytocompatible gel for sustained drug delivery.
- To evaluate the injectable gel for *in-vitro* drug release.

3.2 OBJECTIVES

- To study the Physico-chemical properties of drug.
- To study the Preformulation parameters.
- To formulate injectable gel using smart polymers.
- To optimize the formulation by standard statistical optimization method.
- To study the post formulation parameters.
- To evaluate drug release (*in-vitro) o*f the formulation.
- To study the biopharmaceutical properties of the formulation.

3.3 Rational of the study

The Parenteral route of administration is the most efficient for delivery of active drug substances with poor bio-availability and the drugs with a narrow therapeutic index. But parenteral route offers rapid onset of action with rapid declines of systemic drug level. It requires frequent injection, which ultimately leads to patient discomfort. So to overcome these problems by reducing total number of injection throughout the effective treatment, a sustained release novel injectable and biocompatible gel has been formulated by using smart polymer. These biodegradable *in situ* injectable drug delivery system offer attractive opportunities and provides sustained release of drug with less pain and less invasive technique thereby reducing frequency of administration and improving patient compliance.

4. MATERIAL AND METHODS

4.1 MATERIAL USED

Furosemide was purchased from Yarrow Chem, Mumbai, India. Polyethylene oxide was bought was from Yarrow Chem., Mumbai, India. All other chemicals and solvents used in this study were of analytical grade.

Table 1. : Materials used and the list of manufacturer/ Supplier for the preparation of formulations

MATERIALS	MANUFACTURERS/SUPPLIERS
Furosemide	Yarrow Chem, Mumbai
Polyethylene Oxide	Yarrow Chem, Mumbai
Carbopol 934	Balaji Drugs, Mumbai
Triethanolamine	Marc Company
Sodium Hydroxide	Balaji Drugs, Mumbai
Hydrochloric acid	Balaji Drugs, Mumbai

4.2 INSTRUMENTS USED

Table 2. Instruments used for the preparation and evaluation of formulation.

INSTRUMENTS	COMPANY NAME & MODEL
Digital Weighing Balance	Denver Instruments
UV- visible spectrophotometer	Shimadju, Model: UV 1800240V
Fourier Transform Infrared Spectrometer	Bruker, Model: 10059736
Differential Scanning Calorimeter	Parkine almer 2000
Digital Brookfield Viscometer	DVE viscometer
Water bath	JSGW Thermostat water bath
Ph meter	Systronic Digital Ph meter 335
Digital blender	IKA RW 20 digital

4.3 METHODOLOGY

4.3.1 Determination of absorption maxima (λ max) in phosphate buffer 7.4

100 mg of drug was accurately weight and dissolved in 100 ml of phosphate buffer 7.4 in volumetric flask. 10 ml of the solution was transferred to 100 ml volumetric flask and diluted upto the mark (standard working solution). The spectra of the solution were run in 200-400 nm in UV- visible spectrometer. [1,2,3]

4.3.2 Preparation of standard curve of furosemide in phosphate buffer 7.4

10 mg of furosemide was weighed accurately and transferred to a 100 ml volumetric flask which is then dissolved in 100 ml phosphate buffer pH 7.4 to prepared stock solution (100µg/ml). Then 10 ml of above solution was taken and diluted it with 100 ml phosphate buffer pH 7.4 in 100ml volumetric flask to prepare the solution (10µg/ml). Volumes of 2, 4, 6, 8 & 10 ml were taken in 10 ml volumetric flask from the prepared solution and diluted upto the mark with pH 7.4 phosphate buffers. Absorbance of the resulting solution was measured at 277.0 nm against a blank solution prepared without drug using Shimadzu UV Spectrophotometer. [1,2,3]

4.3.3 Drug-Excipients Compatibility Study

4.3.3.1 Differential Scanning Calorimetry (DSC)

DSC can be used to determine the nature and specification of crystallinity of drug and excipients through measurement of glass transition temperature and melting point temperature and their associated enthalpies. This technique has been used to study the physical and chemical interaction between drug and excipients. Firstly, required amount of furosemide was taken to obtain DSC curve and a physical mixture of furosemide and poly ethylene oxide was also performed using DSC4000**,** Perkin Elmer. Samples were taken and sealed in aluminium pans and analyzed in an atmosphere of air at flow rate of 25 mL/min. A temperature range of 30°C to 400°C was used where rate of heating was 10°C/min. [3,4,5]

4.4.3.2 Fourier transform infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectra of furosemide was performed individually and a physical mixture of both drug and polyethylene oxide were recorded using potassium bromide mixing method on FTIR instrument. Required amount of furosemide and polymer individually was kept on the sample holder and scanned from 400 $^{-cm}$ to 4000 $^{-cm}$ using FT-IR, Alpha, Bruker, Germany, to evaluate the physical state of the drug. [5,7,8]

4.3.4 Preparation of thermo sensitive *in situ* gel formulations

For the preparation of *in situ* gel formulations, cold method was followed. Polyethylene oxide was first added to distilled water with continuous stirring for several hours and kept in refrigerator and stirred periodically until clear homogenous solutions were obtained (approximately 24 hrs). By the time swelling took place and homogeneous distribution of particles were seen. Carbopol 934 was added to the polyethylene oxide solution in certain concentrations in some particular formulations uniformly. Required quantity of furosemide was dissolved in slightly basic solution of sodium hydroxide separately and then it was added to polymer solutions under constant stirring until a uniform solution was obtained. Finally dilute hydrochloride acid was added to adjust the pH. Thus different concentrations of polymer and composition of other ingredients used for formulation of *in situ* gel are shown in Table 3. . [9,10,11]

Table 3. : Composition of prepared in-situ gel

Formulation code	Furosemide (gm)	Polyethylene oxide (gm)	Carbopol 934 (gm)	NaOH solution (pH 10.5) (ml)	Dilute HCl (ml)	Distilled Water (ml)
F1	0.3	0.4	0.01	qs	qs	qs
F2	0.3	0.5	0.03	qs	qs	qs
F3	0.3	0.6	0.04	qs	qs	qs
F4	0.3	0.7	-	qs	qs	qs
F5	0.3	0.8	-	qs	qs	qs
F6	0.3	0.9	-	qs	qs	qs
F7	0.3	1.0	0.001	qs	qs	qs
F8	0.3	1.1	0.002	qs	qs	qs
F9	0.3	1.2	0.001	qs	qs	qs

4.4 CHARACTERISATION OF THERMO SENSITIVE IN-SITU GEL

4.4.1 Appearance

All prepared formulations were evaluated by visual inspection. They were critically observed for clarity and transparency. [12]

4.4.2 Gelation Temperature

The estimation of gelation temperature was done by heating the solution in a thin walled tube that was placed in a low temperature digital water bath with gentle shaking. A thermometer was placed in the sample solution and heated at the rate of 1°C/min with continuous stirring. The temperature or the point where gel formation was observed with no flow of liquid was considered as sol-gel transition temperature. [8,13]

4.4.3 Gelation Time

The gelation time was determined by test tube inverting method. Solution was taken in a thin walled tube and kept at the respective gelation temperature on a water bath. The test tube was taken out every 1 min and inverted to observe the state of the sample. The gelation time was determined by flow of the sample. [7,14]

4.4.4 Gel Melting Temperature

The sample was taken in a test tube. On heating causes gel and further heating causes liquefaction of gel and form viscous liquid and it starts flowing, this temperature was noted and regarded as gel melting temperature. [8,15,16]

4.4.5 pH Determination

This parameter is one of the important factors in the formulation of *in situ* gel because the solubility and stability of the formulation are directly related to it. All the formulations were prepared and then evaluated for pH by using digital pH meter. [7,8,14]

4.4.6 Syringe Ability

All prepared formulations were transferred into an identical 5 ml syringe placed with 20 gauge needle to a constant volume (1 ml). The solutions which were easily passed from syringe was termed as pass and difficult to pass were termed as fail. [8,15,17]

4.4.7 Viscosity Determination

The viscosity of all the prepared formulations was measured using Digital Brookfield viscometer (LVDV-E). The measurements were carried out using spindle no.64. The readings are taken in centipoises (CP) against shear rate or rotation per minute. [16,17]

4.4.8 Percent Drug Content Determination

Accurately weighed amount of gel equivalent to 2mg of drug was taken in a 100ml volumetric flask. Phosphate buffer (pH 7.4) was added to it and kept on magnetic stirrer to dissolve the drug. The volume was made to 100ml with phosphate buffer (pH 7.4) and filtered using 0.45µm filter paper. 10ml aliquot of the above solution was taken and diluted to 100ml with phosphate buffer (pH 7.4). The absorbance of sample solution was determined at 277 nm against phosphate buffer (pH 7.4) by using UV-Visible Spectrophotometer-1800 (Shimadzu, Japan). [7,8,17]

4.4.9 In vitro Drug Release Studies

The in vitro release of furosemide from the formulations was studied through cellophane membrane using Franz diffusion cell. The dissolution medium was phosphate buffer (pH 7.4).Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 2 cm diameter). A selected volume of the formulation was accurately pipette into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 100 ml of dissolution medium maintained at 37± 0.5°C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred continuously using magnetic stirrer. Aliquots, a sample was withdrawn at regular intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and were analyzed by UV-VIS spectrophotometer. [7,15,16,17]

4.5 REFERENCES

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5. RESULTS AND DISCUSSIONS

5.1 Estimation of drug in Analytical solution

5.1.1 Determination of absorption maxima (λ max)

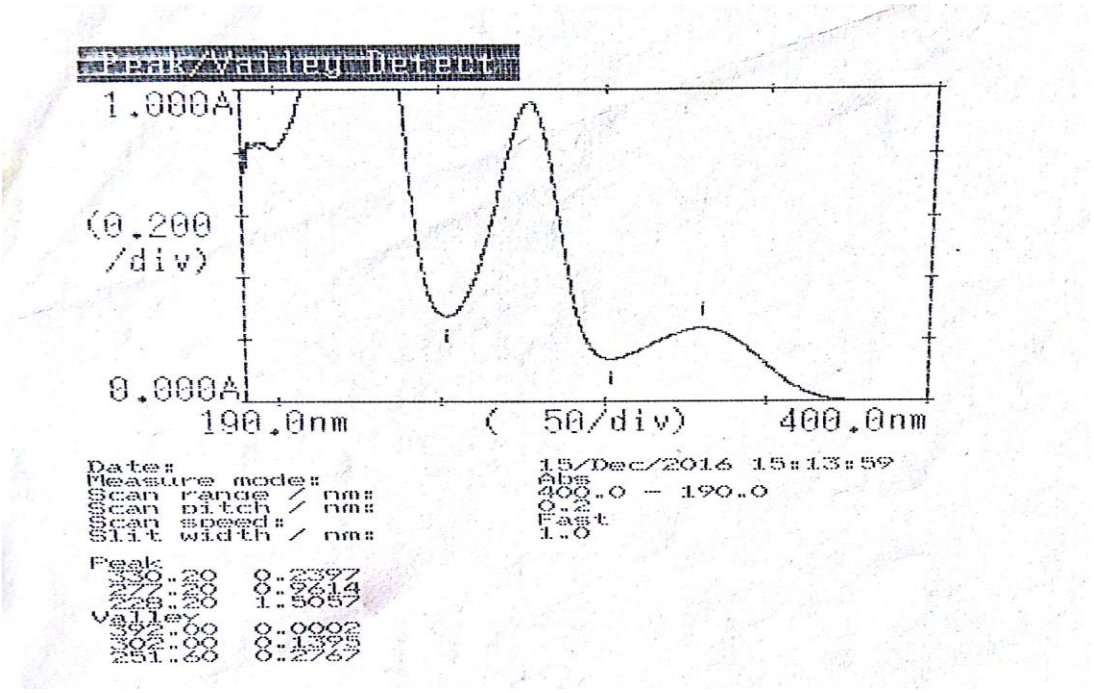


Figure 1. Maximum Absorption Spectra (λ max) in phosphate buffer pH 7.4

5.1.2 Preparation of standard curve of furosemide at phosphate buffer pH 7.4

Table 4. It shows the values for concentration and absorbance

Sl no.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.1199
3	4	0.2011
4	6	0.3011
5	8	0.3874
6	10	0.4982

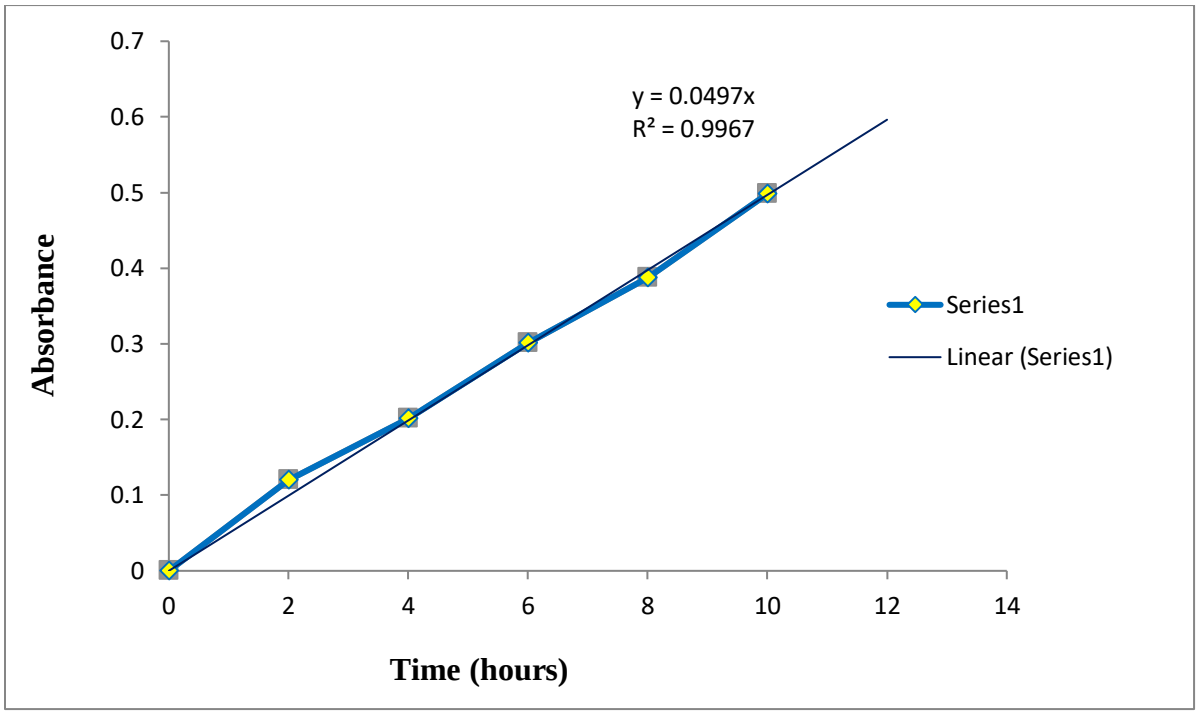
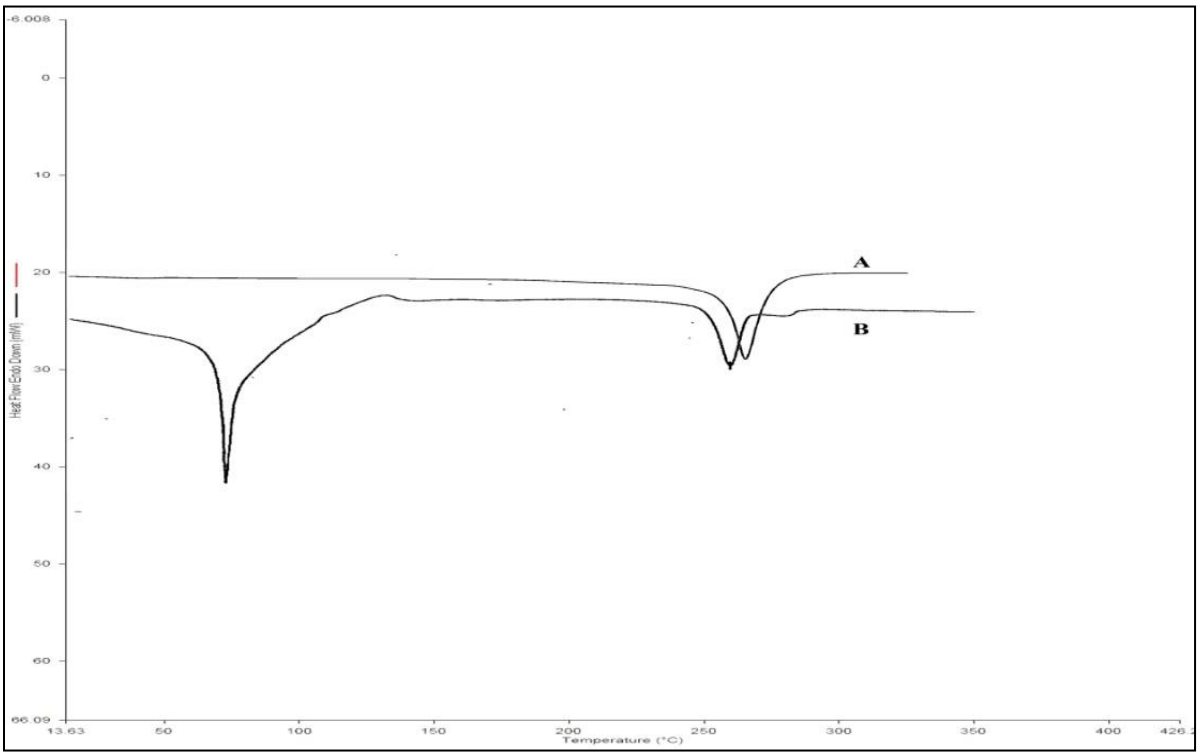


Figure 2. Standard Curve of Furosemide

5.2 Drug Excipients compatibility Study

5.2.1 Differential Scanning Calorimetry (DSC)

The DSC results provided both qualitative and quantitative information about the physicochemical state of the drug present in formulation. The thermograph of pure furosemide showed a melting endothermic peak at 128^0C while in the thermograph of mixture peaks it was observed at 128^0C. The DSC thermo grams of the mixture showed distinct endothermic peaks for furosemide and the polymer. This corresponds to the peaks of individual drug and polymer without exhibiting any modification which indicates that the drug did not interact with excipients used in the injectable gel. This confirmed that the presence of other excipients did not affect the drug stability. The thermograph is shown in Figure 3 indicating A & B for thermogram curve for drug and drug-polymer mixture respectively.



***A & B shows the thermogram of drug and drug-polymer respectively**

Figure 3. Thermogram (DSC) of furosemide and furosemide-polymer mixture

5.2.2 Fourier transform infrared (FT-IR) Spectroscopy

The FT-IR spectra of physical mixture of Furosemide and polyethylene oxide and optimized formulation of injectable are shown in Figure 4. From this, drug-excipients compatibility is clearly observed. The spectrum in the figure indicates A & B for drug and drug-polymer mixture respectively.

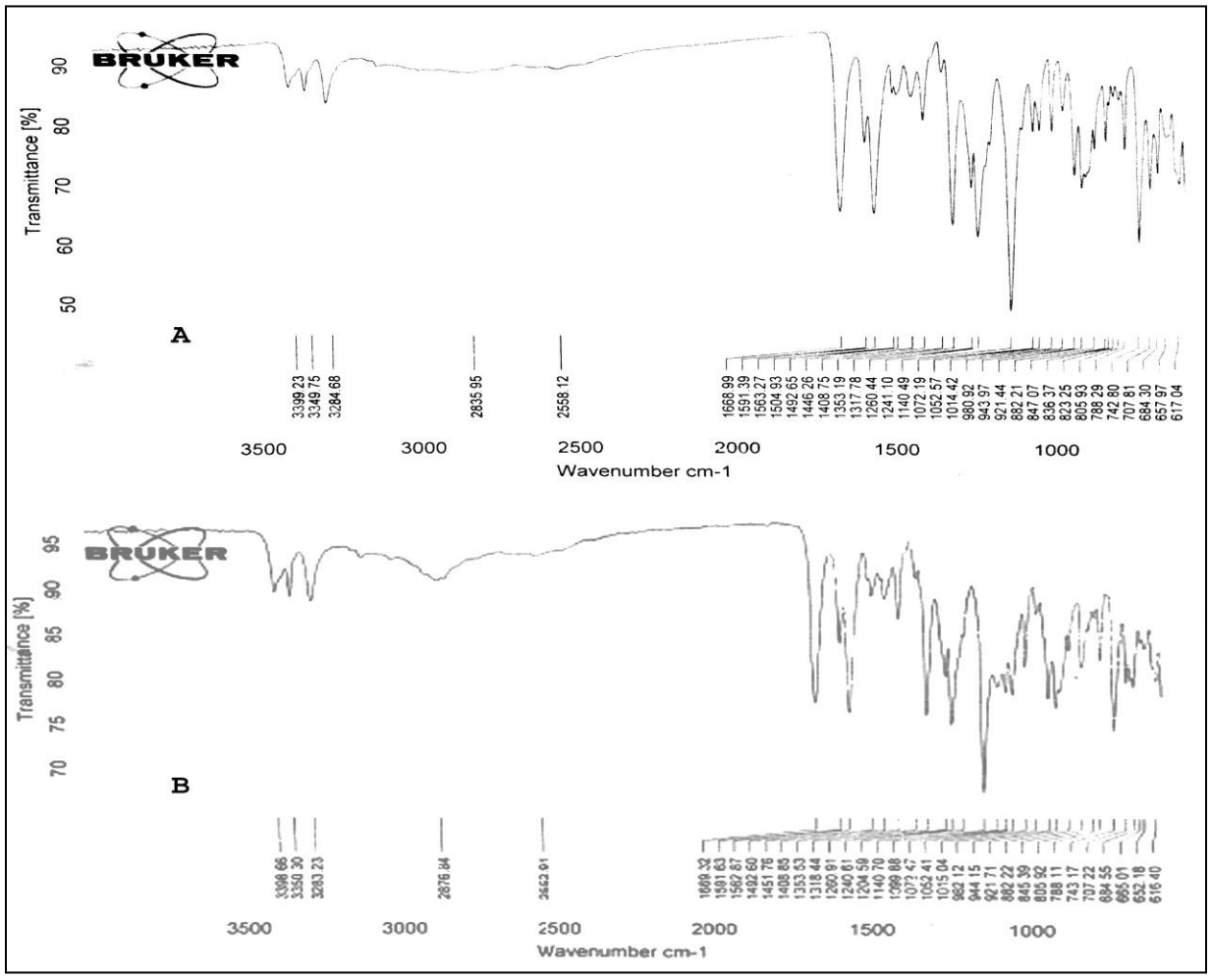


Figure 4. FTIR study of furosemide with polymer mixture

5.3 EVALUATION OF *IN SITU* GELS

5.3.1 Appearance

All the formulations were checked for clarity. The clarity of all the formulation was evaluated by visual inspection under white background and was found clear.

Table 5. Visual appearance of all formulation

.

Formulation code	Appearance
F1	clear
F2	clear
F3	clear
F4	clear
F5	clear
F6	clear
F7	clear
F8	clear
F9	clear

5.3.2 Gelation Temperature

The prepared formulations showed a wide range of gelation temperature. Gelation temperature of all the formulations is shown in Table 6.

Table 6. It shows the gelation temperature, gelation time and gel melting temperature

Formulation code	Gelation temperature (^{0}C)	Gelation time (min)	Gel melting temperature (^{0}C)
F1	39.3 ± 0.5	15-20	69.4 ± 0.5
F2	38.6 ± 0.5	15-20	67.6 ± 0.5
F3	35.4 ± 0.5	15-20	68.5 ± 0.5
F4	36.5 ± 0.5	5 - 10	70.5 ± 0.5
F5	35.6 ± 0.5	5 - 10	72.7 ± 0.5
F6	34.7 ± 0.5	5 - 10	74.2 ± 0.5
F7	31.4 ± 0.5	4-8	78.1 ± 0.5
F8	32.3 ± 0.5	4-8	77.7 ± 0.5
F9	33.3 ± 0.5	4-8	78.8 ± 0.5

From the table 6. it was found that that as the concentration of polymer increases the gelation temperature of the formulation decreases. The gelation temperature as recorded has been found between 31^0C and 39^0C.

5.3.4 Gelation Time

The time for gelation of all the formulations were observed and noted. The thermosensitive viscous liquid when in contact with temperature higher than physiological body temperature converts to gel. The time for gelation of all the formulations were found to be In different ranges as shown in Table 6.

5.3.5 Gel Melting Temperature

The temperature for melting of gel has been recorded. From the study it is found that formulations with lower polymeric concentration melt at lower temperature. The values found are indicated in Table 6.

5.3.6 pH

Table 7. Values for pH of all formulations

Formulation code	pH	
	Initial	Final
F1	3.6 ± 0.6	6.7 ± 0.4
F2	3.8 ± 0.4	6.9 ± 0.5
F3	3.5 ± 0.7	7.4 ± 0.6
F4	4.0 ± 0.4	7.4 ± 0.4
F5	3.7 ± 0.6	7.3 ± 0.4
F6	3.7 ± 0.6	7.4 ± 0.6
F7	3.6 ± 0.4	8.1 ± 0.7
F8	3.8 ± 0.7	8.0 ± 0.4
F9	3.8 ± 0.5	8.1 ± 0.5

The formulations were evaluated for pH as per described in material and methods. pH of formulation code such as F3, F4, F5, F6 were close to physiological pH. All other pH values were noted as shown in Table 7.

5.3.7 Syringe Ability

Syringe ability of all the prepared formulations were checked as per material and methods. Syringe ability of all formulations is shown Table 8. . The formulation code F1 to F6 was found to pass through the needle gauze size of 20. The formulations (F7, 78, 79) were found to be too thick and viscous that fails to pass through needle of gauze 26.

Table 8. It shows the Syringe ability of the formulations

Formulation code	Syringe ability (pass/fail)
F1	Pass
F2	Pass
F3	Pass
F4	Pass
F5	Pass
F6	Pass
F7	Fail
F8	Fail
F9	Fail

5.3.8 Viscosity

Viscosities of all formulations were measured using Brookfield digital Viscometer. The gel under study was placed in the spindle S64 at different rpm at 37^0C. As the RPM increases, viscosity decreases from 13600 to 380 in F4, 14880 to 430 in F5 and 16240 to 480 in F6. The graph of viscosities of the optimized formulations (i.e. F4, F5, F6) are cited in Figure 5.

Table 9. Viscosity of the formulations at 37^0C

RPM	Viscosity (cps) at 37^0C								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	7976	8980	9032	13600	14880	16240	19300	20330	21470
5	3210	4372	5064	9892	10320	11220	16930	18920	19000
10	1212	1948	2190	5220	6562	7420	10560	12120	12300
20	190	290	340	1830	1920	2040	4450	5000	5620
50	148	169	180	380	430	480	1440	1620	1700

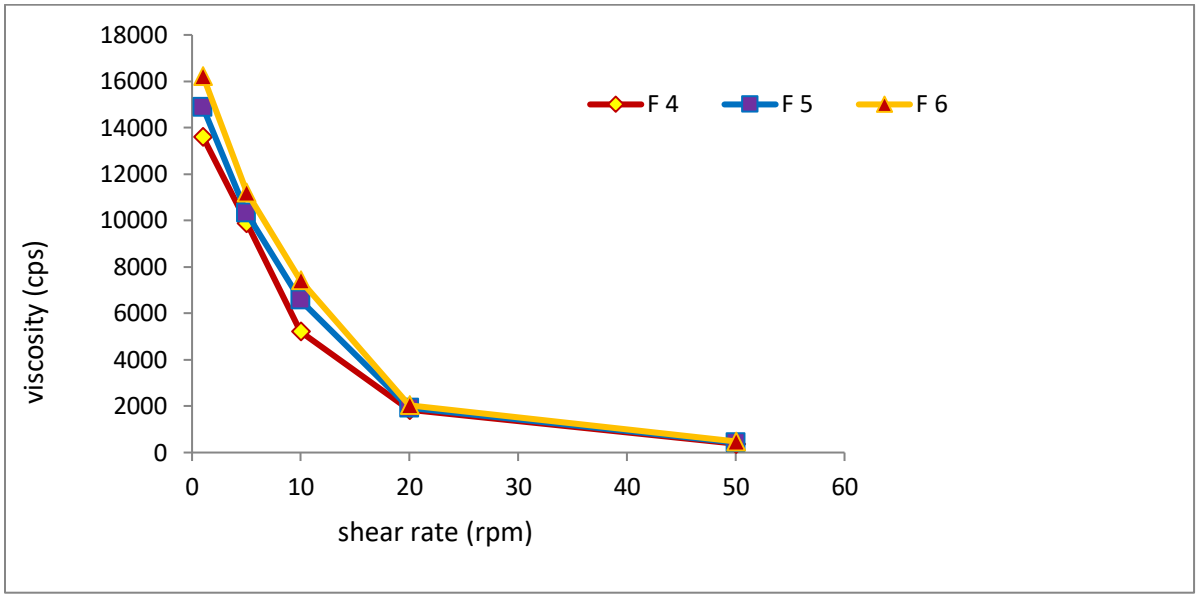


Figure 5. Graphical Representation showing viscosity of optimized formulations at 37^0C

Table 10. It shows the viscosity of the formulation at 25^0C

RPM	Viscosity (cps) at 25^0C								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	4820	4940	5500	6000	5890	6500	8980	8590	9890
5	2090	2140	2540	3530	3694	3800	6000	6422	7240
10	1220	1600	1590	2000	2242	2640	3400	3624	5020
20	280	380	460	950	1050	1220	2012	2680	3040
50	65	68	70	95	105	120	240	280	390

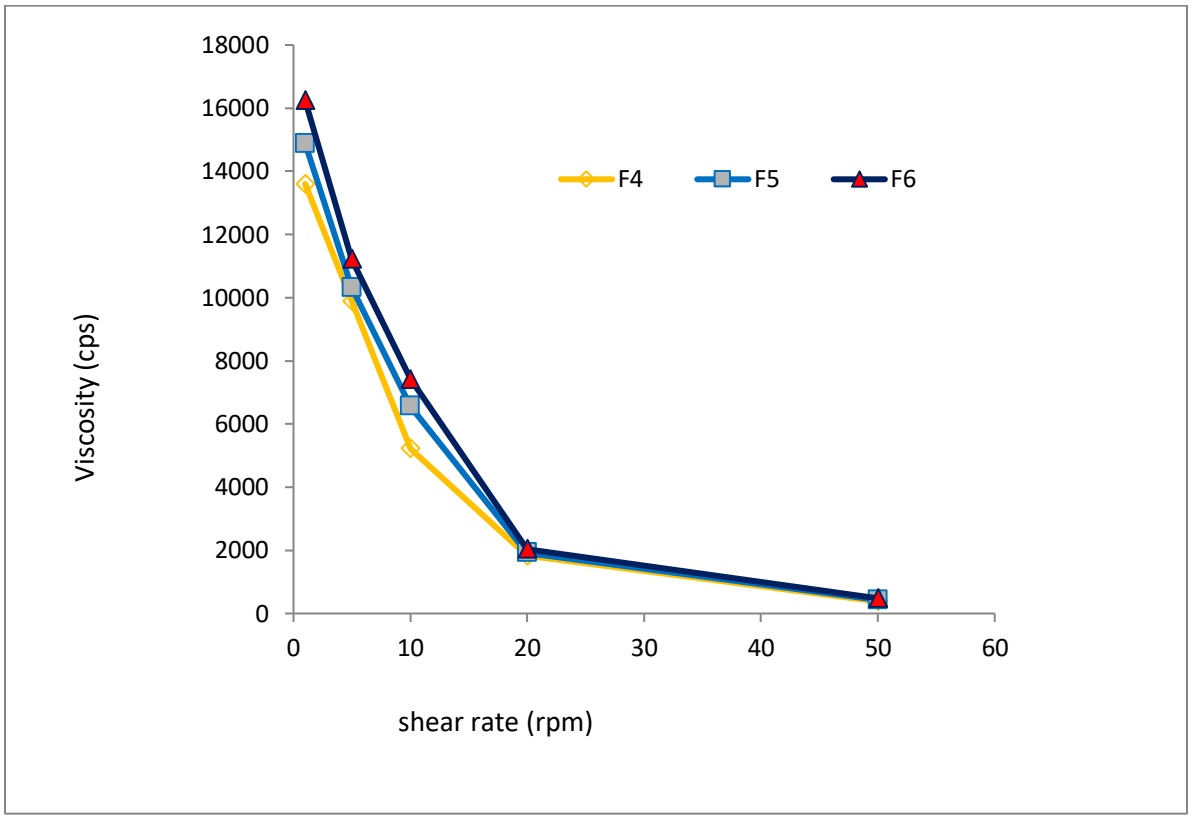


Figure 6. Graphical Representation showing viscosity of optimized formulations at 25^0C

5.3.9 Percent Drug Content Determination

Drug content of all batches was found to be in the range of 89.91 to 99.79. This indicates the uniformity of drug content. The rejected formulations were kept aside and all other formulations were considered for in vitro drug release study. All the drug content percentage are stated in Table 11.

Table 11. It shows the Percentage drug content of the formulations

Formulation code	Percentage (%) drug content
F1	97.06
F2	96.77
F3	96.73
F4	98.91
F5	99.79
F6	95.16
F7	RF
F8	RF
F9	RF

Note: RF stands for rejected formulation

5.3.10 *In vitro* Drug Release Studies

The *in vitro* release of furosemide from the formulations was studied and evaluated as per the process indicated in materials and methods. The formulations having optimum viscosity with highest percentage of drug release are shown Table 3. Graphical representation of cumulative drug release is shown in Figure 5.3.10

Table 12. Percentage cumulative release study of formulation 4 (F4)

Time (hours)	Absorbance	Concentration (µg/ml)	Conc. X DF	Release in 100 ml	Cummulative Release	% Cummu Release
1	0.0225	0.4591	4.591	459.10	459.10	2.295
2	0.0586	1.1959	11.95	1195.9	1200.49	6.00
3	0.1078	2.200	22.00	2200.0	2211.95	11.05
4	0.1764	3.600	36.00	3600	3622.00	18.11
5	0.2548	5.200	52.00	5200	5236.00	26.18
6	0.3724	7.600	76.00	7600	7652.00	38.26
7	0.5390	11.00	110.0	1100	11076.0	55.38
8	0.6762	13.80	138.0	13800	13910.0	69.55
9	0.7644	15.60	156.0	15600	15738.0	78.69
10	0.8330	17.00	170.0	17000	17156.0	85.78
11	0.9114	18.60	186.0	18600	18770.0	93.80
12	0.9310	19.00	190.0	19000	19186.0	95.93
13	0.9506	19.40	194.0	19400	19590.0	97.95

Table 13. Percentage cumulative release study of formulation 5 (F5)

Time (hours)	Absorbance	Concentration (µg/ml)	conc. X DF	Release in 100 ml	Cummulative Release	% Cummu Release
1	0.0245	0.5	5	500	500	2.5
2	0.0784	1.6	16	1600	1605	8.05
3	0.1372	2.8	28	2800	2816	14.08
4	0.2156	4.4	44	4400	4428	22.14
5	0.2744	5.6	56	5600	5600	28
6	0.3920	8.0	80	8000	8056	40.28
7	0.5850	11.94	119.4	11940	12020	60.1
8	0.6693	13.66	136.6	13660	13779	68.89
9	0.7765	15.848	158.48	1584.8	15984	79.92
10	0.8592	17.536	175.36	17536	17694	88.47
11	0.9025	18.420	184.20	18420	18595	92.97
12	0.9485	19.538	193.58	19358	19542	97.71
13	0.9607	19.608	196.08	19608	19808	99.04

Table 14. Percentage cumulative release study of formulation 6 (F6)

Time (hours)	Absorbance	Concentration (µg/ml)	conc. X DF	Release in 100 ml	Cummulative Release	% Cummu Release
1	0.0107	0.2183	2.183	218.3	218.3	1.09
2	0.0392	0.80	8.00	800	802.18	4.01
3	0.0882	1.80	18.00	1800	1808.0	9.04
4	0.1470	3.0	30.0	3000	3018.0	15.09
5	0.1960	4.0	40.0	4000	4030.0	20.15
6	0.3724	7.6	76.0	7600	7640.0	38.2
7	0.4580	9.2	92.0	9200	9276.0	46.38
8	0.5782	11.8	118.0	11800	11892	59.46
9	0.6860	14.0	140.0	14000	14118	70.59
10	0.8232	16.80	168.0	16800	16940	84.7
11	0.8918	18.20	182.0	18200	18368	91.84
12	0.9152	18.67	186.0	18670	18852	94.26
13	0.9437	19.25	192.5	19250	19436	97.18

Table 15. Percentage cumulative release of optimised formulation

Time (hours)	%age of cumulative drug release		
	F4	F5	F6
1	2.295	2.5	1.09
2	6	8.05	4.01
3	11.05	14.08	9.04
4	18.11	22.14	15.09
5	26.18	28	20.15
6	38.26	40.28	38.2
7	55.38	60.1	46.38
8	69.55	68.89	59.46
9	78.69	79.92	70.59
10	85.78	88.47	84.7
11	93.85	92.97	91.84
12	95.93	97.71	94.26
13	97.95	99.04	97.18

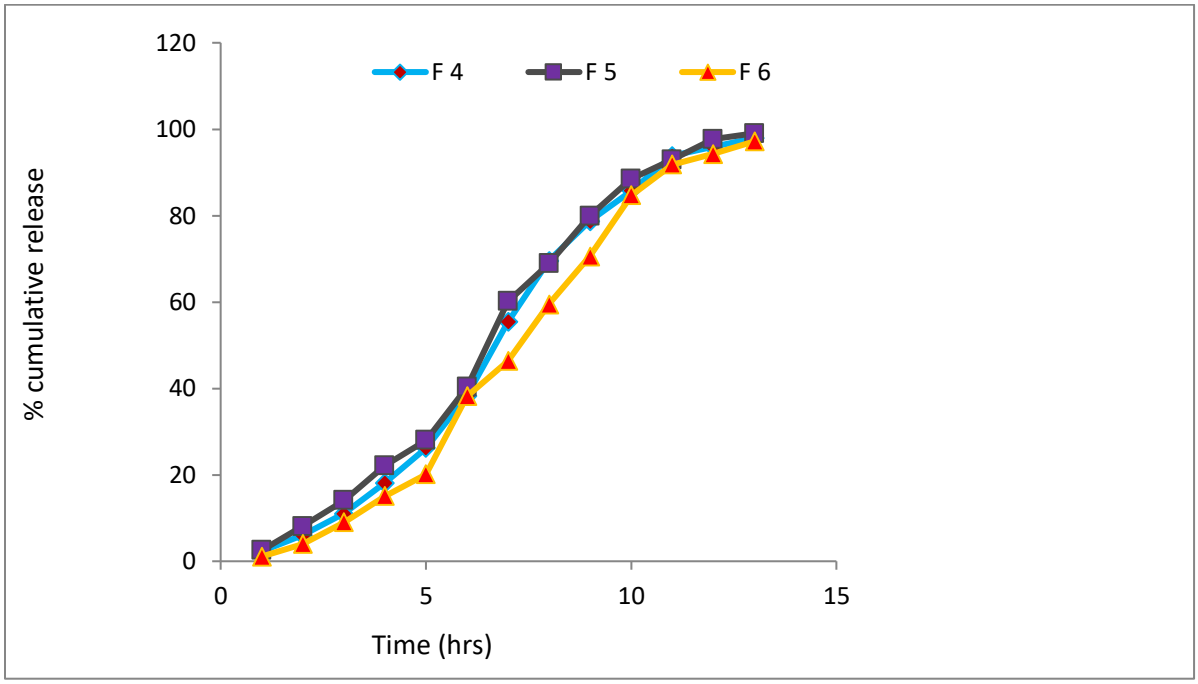


Figure 7. Graphical representation of cumulative release of drug from optimized formulations

From the graphical presentation of cumulative drug release versus time; formulation code of F4, F5 & F6 showed sustained release of drug till 13 hours with release percentage of 99%.

5.3.11 Mathematical Models for the Diffusion Data.

All the release data of *in vitro* drug release study was applied in various kinetic models and the release mechanism was found to be diffusion which is reported in Table 16. All the formulations showed a greater linearity (r^2= 0.96-0.98) indicating the korsemeyer-peppas model of diffusion.

Table 16. Mathematical Models for the diffusion data

Formulation code	Zero-order (r^2)	First-order (r^2)	Higuchi (r^2)	Korsemeyer-Peppas (r^2)	(n)
F4	0.9417	0.8015	0.7038	0.9628	1.248
F5	0.9546	0.8170	0.7283	0.9671	1.248
F6	0.9279	0.7830	0.6701	0.9712	1.248

6. CONCLUSION

The novel injectable thermo sensitive *in situ* gelling drug delivery was successfully formulated by using polyethylene oxide and carbopol 934P. Thermo sensitive gel was formulated by cold method using heat sensitive polymer called polyethylene oxide with the excipients of carbopol 934P. Polymer with percentage of 0.4% to 1.2% was formulated upon which 0.7%, 0.8% and 0.9% showed proper thermo sensitivity in physiological body temperature. 0.4% to 0.6% of the formulated polymer showed higher gelation temperature to solidify in-vitro and required more time to form gel, whereas 1%, 1.1% and 1.2% of the polymer showed a heavy solidified gel upon application of physiological temperature and the time for gelation and gelation temperature was very less compared to formulation code of F4, F5 and F6. The gel melting temperature of all the formulations ranges from 67^0c to 78^0c. The formulated injectable *in situ* gelling systems were further characterized for appearance, clarity, pH, rheological character, *in-vitro* drug release in PBS 7.4 fluid. The formulated gel was observed for visual appearance under white background. Clarity of all the formulations was clearly visible and no foreign particles were seen under inspection. Digital pH meter was used to find out the pH of the formulations which were almost close to physiological body pH and it ranges from 6.7 to 8.1. The prepared gel was passed through syringe of gauze size 26 to check the syringe ability of viscous liquid. Formulation code of F1 to F6 was found acceptable but F7, F8 and F9 were rejected as those were too viscous to pass through the selected syringe. Viscosity of the prepared formulation (F1, F2 & F3) was observed with digital viscometer at room temperature (25^0c) which showed below the ideal range whereas the formulation (F4, F5 & F6) showed optimum viscosity. The rejected formulation F7, F8 & F9 showed very high viscosity above the ideal range. Percentage drug content of F1 to F6 was found in between 95% to 99%. *In-vitro* Cumulative drug release of the optimized formulations was found to be 97.95%, 99.04% and 97.18% for F4, 75 and F6 respectively. Thus from the study it can be concluded that temperature sensitive injectable gel can be used to achieve sustained drug release over 13 hours period of time. The formulation was slightly viscous liquid at room temperature and

underwent rapid gelation upon raising the temperature. So, this formulation system is an alternate way to conventional parenteral formulation of present drug to improve the bioavailability through its longer residence time and ability to sustain drug release. By reducing the frequency of administration this formulation may improve patient compliance.

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REVIEW ARTICLE

Studies on *In-Situ* Forming Thermo Sensitive Injectable Polymeric gel for Sustained Drug Delivery

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ABSTRACT:
The in situ injectable gel is a polymeric formulation which provides several advantages when compared to conventional injectable drug delivery systems. These types of formulations are actually prepared by smart polymers i.e. that change its microstructure according to environmental change. These polymers remain in sol form before administration into the body, but once administered, undergo gelation in situ, to form a gel. Thus the in situ forming polymeric formulations are dependent on biodegradable smart polymers. The formation of gel or gelation occur for many reasons depending on factors like temperature modulation, electrical sensitivity, pH change, presence of ions, ultra violet irradiation and enzyme sensitive from which the drug gets released in a predetermined or controlled manner. Thus this article presents a detailed review of these types of polymeric formulation composed of thermo responsive polymers. Heat sensitive or thermo responsive polymers are those which have a discontinuous change of their physical properties with temperature modulation and thus undergo physical change inside the human body. The production and the formulation system is a very simple and low cost where various synthetic as well as natural thermo sensitive polymers are used for the formulation of in situ gel. The in situ gel dosage form having good stability, biocompatibility and increasing patient compliance makes more reliable and acceptable drug delivery system.

KEYWORDS: In situ gel, gelation, thermo responsive, biodegradable, sustained release.

INTRODUCTION:
Gel:
Gel is defined as a two-component or combination of two phases which mostly contain liquid, but they behave like solids because of three-dimensional cross-linked network within the liquid. [1] Usually, the solvent is the major component of the gel system. Gels are those formulations that are used for several routes of administration. They are useful in different formulations of topical, vaginal, rectal as well as injectable administration. [2] [3]

Novel Injectable Gel:
The development of novel inject able in situ forming (gel) drug delivery systems has been a quite interesting topic as it has received a considerable interest over the last decade. The drugs which are designed to be injected in the form of viscous liquid are called injectables. The injection of gel follows the appropriate route for administration called **s**ubcutaneous route. It is a method of infusion to put the viscous liquid into the body, usually with an appropriate syringe and through the skin to a sufficient depth so that the material can be administered easily and freely into the body. [3] The viscous liquid turns to gel in the surrounding tissue of body and start releasing the drug in a sustain manner. Since considerable attention has been paid to the development of injectable gel system over the last few years, so this interest has been spreading due to the various advantages shown by these delivery systems

such as ease of administration, reduced frequency of administration improved patient compliance and comfort. [4]

There are three main routes of administration of injectables used in humans and animals. The routes are as follows.

1. Intradermal (ID) Injection:

It is a process in which the drugs are delivered into the dermis**,** or the skin layer underneath the epidermis layer. The advantage is that, its usability allows the tool to be used by untrained staff. They also have less pain for the patient during use, and the shortness of the injection needle makes injections safer.

2. Intramuscular (IM) Injection:

An intramuscular injection is a process which delivers medication deep into the muscles. The medication is delivered directly into a muscle where the drugs are administered intramuscularly and are absorbed into the muscle quickly compared to other conventional system which is more gradual. Some injections are given to the buttocks which reach the bloodstream quickly due to the large amount of muscular tissue and corresponding blood supply.

3. Subcutaneous (SC) Injection:

A subcutaneous injection is a process used to administer as a bolus into the sub cutis that is the layer of skin directly below the dermis and epidermis. This technique is highly effective in administering vaccines and medications as well as for the sustain release of drugs in the body. Long-acting forms of subcutaneous or intramuscular injections are available for various drugs and are called depot injections. It is also a technique of injection where drugs deposit in a localized mass, called a depot and is slowly absorbed by surrounding tissue.[5] [6]

Sustained Release Drug Delivery System:

The term sustained release or prolong release can be defined as the system that is designed in such a way that it releases the dosage form slowly and continuously to a long period of time after administration of single dose and produce therapeutic effect. The main fundamental of prolong release is to sustain the drug action at a predetermined rate by maintaining a relatively constant, effective therapeutic drug level in the body by decreasing the undesirable or adverse side effects. [3] [4]

Advantages of Sustained Release Drug Delivery System:

1. Duration of action of the drug is extended.
2. The dosing frequency of conventional dosage form is reduced quite easily.
3. The total amount of drug administration can be reduced by maximizing availability with minimum dose.
4. Minimizes the drug accumulation with chronic dosing.
5. Improve efficacy in treatment by controlling and reducing fluctuations in drug level.
6. Improve bioavailability of some drugs.
7. The drug absorption can be attained much better and effective.
8. Administration of dosage form can be made more convenient. [7] [8]

Disadvantages of Sustained Release Drug Delivery System:

1. In comparison to immediate release conventional dosage forms the systemic availability of injectable sustained release system is decreased.
2. Toxicity, poisoning or hypersensitivity reactions are a major drawback for retrieval of drugs after administration.
3. Reduced potential for dose adjustment of drugs normally administered in varying strengths.
4. The flexibility in dosage regimen for adjustment is limited.
5. The *in vitro – in vivo* correlation is poor.
6. The half life of the active compound must be short.
7. A large amount of drug is must to maintain a sustained effective dose for the short half life drugs.
8. The absorption in not effective in lower small intestine. [7] [8]

Sustained Release Injectable Gel:

Prolong or sustained release of drugs through injection after IM or SC administration may occur due to use of stimuli responsive or “smart” polymers. “Smart” polymers are actually macromolecules having certain critical physical as well as chemical properties to change in response to small changes in their environment such as temperature, pH, light, magnetic field, ionic factors, etc. The changes are always reversible, and therefore, the smart polymers are capable of returning to its initial state sooner after the trigger is immediately removed. [9]

Smart Polymers for Sustained Release Drug Delivery:

Smart polymers also called as intelligent polymers are in the vanguard of drug administration technology because those polymers show active response by changing its physical and chemical structure to small signs and changes in the surrounding environment, which transforms into significant changes in the physiological and chemical properties. These polymers are biocompatible, non-thrombogenic, strong, resilient, flexible, easy shaping and coloring. They keep the drug stable and to manufacture easily. The good nutrient

carriers to the cells using cell adhesion ligands is possible to inject *in vitro* as liquid and changes itself to gel within the body at physiological body temperature.
The smart polymers that change the structural can be classified in three main groups as:

1. Chemical stimuli (pH and ionic strength).
2. Biological stimuli (enzymes and biomolecules).
3. Physical stimuli (ultrasound, light, mechanical stress, temperature). [13] [11] [15]

Chemical Stimuli (pH responsive polymers):
A good example of chemical stimuli is pH sensitive polymer which consists of pendant basic or acidic group that have the ability to accept or release a proton with the changes in environmental pH. Polymers with a large number of ionisable groups are known as polyelectrolyte's. They are classified into two types: weak polyacids and weak polybases. At low pH weak polyacids accept protons and at neutral and high pH it releases protons. With the change of environmental pH the pendant acidic group undergoes ionization at specific pH called as pKa. Thus the molecular structure of the polymeric chain gets altered with the rapid change of the attached group. This transition to expanded state is mediated by the osmotic pressure exerted by mobile counter ions neutralized by network charges. PH-responsive polymers containing a sulphonamide group are another good example of polyacid polymers. These polymers have pKa values in the range of 3–11 and the hydrogen atom of the amide nitrogen is readily ionized to form polyacids. Narrow pH range and good sensitivity is the major advantage of these polymers over carboxylic acid based polymers. [11] [13] [15]

Biological Polymers:
Biologically responsive polymer systems are increasingly important in various biomedical applications. The major advantage of bioresponsive polymers is that they can respond to the stimuli that are inherently present in the natural system. Bioresponsive polymeric systems mainly arise from common functional groups that are known to interact with biologically relevant species, and in other instances the synthetic polymer is conjugated to a biological component. Bioresponsive polymers are classified into antigen responsive polymers, glucose-sensitive polymers and enzyme responsive polymers. [11] [15]

Physical Stimuli Polymers (i.e. Thermo Responsive Polymer):
The physical stimuli i.e. temperature sensitive polymers are generally called as thermo responsive polymers. These smart polymers are sensitive to the temperature and change their micro structural features in response to change in temperature. These are most safe polymers in drug administration systems and biomaterials. Thermo-responsive polymers present in their structure a very sensitive balance between the hydrophobic and the hydrophilic groups and a small change in the temperature can create new adjustments. [16] These are the polymeric structures sensitive to both temperature and pH, they are obtained by the simple combination of ionization and hydrophobic (inverse thermo sensitive) functional groups. This approach is mainly achieved by the copolymerization of monomers bearing these functional groups, combining temperature sensitive polymers with polyelectrolyte's or by the development of new monomers that respond simultaneously to both stimuli. [12] [13] [14]

Mechanism of Thermo Sensitive Polymers:
Critical solution temperature is one of the important parameter that is considered in this type of polymer. The polymeric solution is categorized into lower critical and upper critical solution temperature. The polymeric solution changes at certain temperature and it is called as critical temperature. If such solution doesn't change and remain same below that temperature, the so-called lower critical solution temperature become insoluble after heating. Such behavior is typical for the polymers that form hydrogen bonds to water and has wide range of biological applications such as cell patterning, smart drug release, DNA sequencing and others. In this approach control of the polymer temperature response in water is done by varying chemical composition of the monomer. The polymeric solution where the phase changes above certain temperature is called upper critical solution temperature (UCST). [14] [16] The reversible solubility of temperature sensitive smart polymers is caused by changes in hydrophobic/hydrophilic balance of uncharged polymer induced by increasing temperature or ionic strength. The uncharged polymers are soluble in water due to the hydrogen bonding with water molecules. The efficiency of hydrogen bonding reduces with increase in temperature. The phase separation of polymer takes place when the efficiency of hydrogen bonding becomes insufficient for solubility of macromolecule. The polymers with a lower critical solution temperature (LCST) are the most used on drug delivery systems. The therapeutic agents as drugs, cells or proteins can be mixed with the polymer when this is on its liquid state (temperature below the transition temperature) being able to be injected in the human body on the subcutaneous layer or in the damaged area and forming a gel deposit on the area where it was injected after increasing the temperature. This kind of pharmaceutical system delivers the drug on a controlled way without being too invasive. Smart polymers that are sensitive to the temperature can be used to increase chemotherapeutic agents in solid tumors. The accumulation of temperature sensitive polymeric

systems in solid tumors is due to the increased impermeability effect to the tumor vascular net retention and to the use of an external impulse (heat source) on the tumor area. This temperature increase promotes the changing of the microstructure of the polymeric system, turning it into gel and releasing the drug, thus increasing the drug in the intra-tumoral area and the therapeutic efficiency, and reducing the side effects. [13] [16] [17]

Examples of Thermo Sensitive Polymers:

Some temperature sensitive polymers are poly (N-isopropylacrylamide) (PNIPAAM); poly (oxyethylene-oxypropylene-oxyethylene) triblock copolymers (PEO-PPO-PEO); poly (ethylene glycol) - poly (lactic acid) - poly (ethylene glycol); poly (N-alkyl substituted acrylamides) and poly (N-isopropyl acrylamide) and poly (N-vinylalkylamides) like poly (N-vinyliso-butyramide). [13]

Poly (N-isopropyl acrylamide)

PNIPAM is the mostly used and prominent candidate of thermo responsive polymer. It is a biocompatible polymer and changes structure itself under different physical temperature. The lower critical solution temperature (LCST) of PNIPAM is thus a candidate for controlled and sustained release application and is independent of the molecular weight and the concentration. PNIPA possesses an inverse solubility and phase separation takes place upon heating. It changes the hydrophilicity and hydrophobicity at temperature below the lower critical solution point. [13]

PNIPA have been used in gel actuators and by converting the stimuli to mechanical motion. Upon heating the solution above LCST, phase transformation takes place from hydrophilic to hydrophobic sate. It has been used in the tissue engineering as temperature sensitive scaffolds for the manipulation of cell sheets. Poly (NIPAAm-co-acrylic acid) & poly (NIPAAm-co-AA) gels have been applied as extracellular matrix for pancreatic islets in biohydrid pancreas. [13] [19]

Triblock copolymer (PEO-PPO-PEO) or Poloxamers and its derivatives:

Triblock copolymer has been extensively used in the delivery of active drugs. The hydrophilic polymer part i.e. PEO separates from the hydrophobic part (PPO) after the increment of lower critical solution temperature. At certain ratios, the two different polymers behave individually in situ and thus gelation occurs with the corresponding physiological temperature. It is used for tissue engineering as well as smart delivery of drugs. [19] [20]

The use of water soluble vehicles with high viscosity (known as hydrophilic gels) are one of the best ways to obtain a controlled delivery system. It represents an area which is important in scientific research. The copolymer blocks based on PEO-PPO sequences constitutes one family of triple blocks of commercialized copolymers with the following names. [21]

Pluronics, Poloxamers, Tetronics are non ionic polymers having different composition ratios of poly (oxyethylene-oxypropylene-oxyethylene) (PEOn-PPOn-PEOn), with many pharmaceutical uses. These polymers are composed by white granules soluble in water with no odor or taste. They present a sol-gel transition phase below or near the physiologic body temperature and a gel-sol transition around 50º C in relatively highly concentrations. The triple block copolymers PEO-PPO-PEO get into gel at body temperature in concentrations above 15% (m/m); however this kind of formulas, when injected by intraperitoneal via, presents high toxicity and increases the plasma concentration of cholesterol and triglycerides.[22] [24]

The Poloxamers normally used are: 188 (F-68), 237 (F-87), 338 (F-108) and 407 (F-127). “F” refers to the polymer in the form of flakes. Pluronics® and Tetronics® are polymers approved by FDA to be used as food additives, pharmaceutical ingredients, and drug carriers in parenteral systems, tissue engineering and agricultural products. PluronicF-127 (Polaxamer 407, PF-127) can also be used as carrier in several routes of administration, including oral, cutaneous, intranasal, vaginal, rectal, ocular and parenteral. In the last years, PF-127 has had a special role on dermal and transdermal drug delivery systems. Pluronic® F127 (PF-127) or poloxamer 407 (P407) (copolymer polyoxyethylene$_{106}$-polyoxypropylene$_{70}$-polyoxyethylene$_{106}$) contains about 70% of ethylene oxide which contributes to its hydrophilicity. PF-127 is a copolymer with 12,000 Daltons, a PEO/PPO with ratio of 2:1 which is non toxic and low viscosity below 4ºC and forming a semi solid gel at body temperature. PF-127 is more soluble in cold water than in hot water due to the hydrogen linkages at low temperatures. [23]

Applications of thermo sensitive polymers:

Smart drug delivery:

The “smart” polymeric carrier is used to deliver drugs. These carriers allow delivery of the drug at the right time and concentration by only releasing the drug in response to an external stimulus. For example the polymer chains of a carrier may expand as a result of the temperature increasing, thus enabling the drug to diffuse out and be released from the carrier. [25] Stimuli occurring externally of internally include temperature, electric current, pH etc. When an enzyme is immobilized in smart hydrogel, the product of enzymatic reaction could themselves trigger the gel’s phase transition. The swelling or shrinking of smart polymer beads in response to small

change in pH or temperature is used to control the drug release, because diffusion of the drug out of beads depends upon the gel state. These smart polymers become viscous and cling to the surface. And as a result it provides an effective way to administer drugs, either topically or mucosal or injectable, over long timescales by dissolving them in solution, which contain hydrophobic regions. [25] [26]

Tissue Engineering:

Thermo responsive polymers in this field are commonly used in two situations such as substrates that enable the cell growth and proliferation and as injectable gels, for *in situ* of the scaffold. The first situation states that, the thermo responsive ability of the polymers is used to regulate the cells' attachment and detachment from a surface. In fact, in one study, the polymer surface was even reusable for repeated cell culture. The second application involves the encapsulation of cells in 3D structures in the body. The *in situ* formation of cell or scaffold contrast compared to the *in vitro* formation of the construct allows the delivery of encapsulated cells, nutrients and growth factors to defects of any shape using minimally invasive techniques. Specifically, the thermo responsive polymer is mixed at room temperature with the cells and then injected into the body. Upon injection due to the temperature increase (to 37 C) that is above the polymer's LCST, the polymer forms a physical gel. The cells are encapsulated within the 3D structure of the gel. [25]

For example: Poly-N-Isopropylacrylamide based hydrogels are non-adherent below the LCST and adhere above the LCST; at high temperature bioactive molecule can be entrapped and subsequently released upon lowering the temperature. Temperature-sensitive hydrogels have gained considerable attention in the pharmaceutical field due to ability of the hydrogels to swell or shrink as a result of changing the temperature of the surrounding fluid. Numerous researchers studied various applications of these hydrogels such as on-off drug release regulations, biosensors and intelligent cell culture dishes. [11] [25]

Stimuli-responsive surfaces:

The change in the surface properties of the thermo responsive polymers from hydrophobic above the critical temperature to hydrophilic below it has been used in tissue culture applications. Mammalian cells are cultivated on a hydrophobic solid culture dishes and are usually detached from it by protease treatment, which also causes damage to cells. This is rather an inefficient way in that only some detached cells are able to adhere onto new dishes because the rest are damaged. At temperature of 37°C, a substrate surface coated with grafted poly (N- Isopropylacrylamide) is hydrophobic because this temperature is above the critical temperature of the polymer and cells grow well. However when the temperature is decreased by 20°C, the surface becomes hydrophilic, and the cells can be easily detached without any damage. The cells can be used for further culturing. The cells are detached maintaining the cell-cell junction. This enables the collection of the cultured cells as a single sheet which is highly effective when transplant to patients due to tight communication between cells and cells. [11] [15]

Gene therapy:

It aims at the treatment of many genetic diseases as it is a technique for correcting defective genes slowing down tumor growth and stopping neurodegenerative diseases. Specifically, the delivery of the appropriate, therapeutic gene (DNA) into the cells and finding of the gene that will replace, repair or regulate the defective gene that causes the disease. Nonviral gene carrier of two types which are to be cationic in nature in order to form complexes with anionic DNA and the complex has to have net positive charge to interact with the anionic cell membrane and undergo endocytosis. During attachment of coil forming endosome, binding of carrier and DNA is to be high and he complex should be easy to dissociate to move the DNA into nucleus to initiate transcription. In particular, in studies where PEI with grafted PNIPAM, chitosan grafted with PNIPAM. Further by the use of temperature sensitive polymer more selective gene expression is possible in terms of site, timings and duration. [11] [12] [25]

Applications of polymers based on their structure

Hydrogels:

Hydrogels mainly constitute 99% w/w water to polymer which is actually a polymer networks dispersed *in water* to form semi solid states called gels. These gels can be either covalently linked polymer networks or physical gels. With reference to thermo responsive polymers, covalently linked networks exhibit a change in their degree of swelling in response to temperature. When cross linked into hydrogels, the coil-to-globule transition causes a rapid decrease in the volume of the gel resulting in a fast release of entrapped drug and solvent followed by a more linear, diffusion controlled release. The swelling kinetics of co-networks of NIPAM with BuMA, P (NIPAAm-*co*-BuMA), commenting on the need for zero order drug release profiles and found that after a burst release of drug from the outer part of the hydrogel a sustained release can be obtained. Other thermo responsive monomers have been utilized for the preparation of hydrogels including PDMAAm. PDMAAm-*co*-Poly (methoxyethyl acrylate) and showed that at body temperature this hydrogel releases drug following a Fickian diffusion process with a linear relationship in respect to the square root of time. Co-

networks of PNIPAM, poly (HEMA) and a lactic acid monomer were synthesized by Ma et al. and found to exhibit LCSTs of 10–20°C with PNIPAM contents of 80% or more. The gels had high tensile strength and degraded over several months with no cytotoxic byproducts when used in tissue engineering. [15] [21] [25]

Micelles:

Block copolymers are a combination of hydrophilic and hydrophobic monomers and allows the formation of ordered structures in solution called micelles. Micelles are useful for encapsulating hydrophobic drugs and delivering them into an aqueous environment. [12] [21] Several studies have focused on using PNIPAM as the thermo responsive block in the formation of thermo responsive micelles. Micelles of P(NIPAM-*co*-DMAAm)-*b*-PLA, where PLA was poly(lactic acid), and showed that these micelles were able to internalize into cells above their LCST, specifically due to the increased interaction between the hydrated NIPAM outer sphere and the cells. Degradable copolymers of (HEMAmlactate) to form micelles above a critical micelle temperature dependant on the polymer LCST. Thermo responsive star block copolymer based on L-Lactide and NIPAM. These star polymers were found to self assemble into large micelle structure in water which showed a fast on/off drug switching with temperature. [22] [25] [27]

Films:

Copolymer films of PNIPAM and poly (*N*-butylacrylamide) to give a sustained release of drugs from the film over a considerable time period. They showed the released amounts of drug loaded at room temperature to be inversely proportional to the hydrophobic monomer content once heated to 37 C. The possibility of using a copolymer of PNIPAM with PAAm as a stimuli responsive membrane for the control of permeation of molecules for numerous applications like drug delivery. Cell growth and selected cell detachment was shown to be achievable with this approach. The production of plasma polymerized PNIPAM films onto micro heater arrays produced using photolithography. This method allows for localized heating and specific area detachment of cells with many possible applications. An interesting 3D cell culture method was envisaged. [12] [25]

Particles:

Nanoparticles of thermo responsive polymers possessed sensitivity to a reducing environment, such as the intracellular cytoplasm, by reduction of the disulfide bonds in the polymer chain resulting in breakdown of the nanoparticles. Coated insoluble nanoparticles with PNIPAM rendering them stable in aqueous solutions with temperature dependant solution properties and suggested uses in drug delivery and biological sensing. PVC and PVC-*graft*-PEG microgels were formed by heating the polymer above its LCST and using salicylic acid as a crosslinker. The salicylic acid formed hydrogen bonds between the polymer chains forming a physical hydrogel. By adding a solution of polymer and drug to a solution containing the crosslinker at temperatures greater the LCST, hydrogel particles were formed which showed sustained release. Interestingly, the PEG graft copolymers showed a slower drug release due to an increase in hydrogen bonding and hence increase packing from the PEG chains. [12] [25]

Interpenetrating networks:

Interpenetrating networks specifically, an interpenetrating network of PAA and PAAm forms hydrogels that swell above their upper critical solution temperature, UCST, due to hydrogen bonding between the two different networks being disrupted at higher temperatures allowing the networks to swell. Recent work on the same IPN with grafted β-cyclodextrin showed a faster thermo response and lower UCST (35 C) and a lowered effect of salt on the swelling. Incorporation of a model drug, ibuprofen, showed a positive drug release with a controlled rate above and below the UCST. [12] [25]

Evaluation and Characterization of in situ gel system

Viscosity and Rheology:

Viscosity and rheological properties of in situ forming drug delivery systems may be assesses using Brookfield rheometer or other viscometers such as Ostwald's viscometer. The viscosity of these formulations should be such that no difficulties are envisaged during their administration by the patient, especially during parenteral and ocular administration. For in situ gel forming systems incorporating thermo reversible polymers, the sol-gel transition temperature may be defined as that temperature at which the phase transition of sol meniscus is first noted when kept in a sample tube at a specific temperature and then heated at a specified rate. Gel formation is indicated by a lack of movement of meniscus on tilting the tube. Gelling time is the time for first detection of gelation from sol. [28] [29]

Gel Strength:

This parameter can be evaluated using a rheometer. Depending on the mechanism of the gelling of gelling agent used, a specified amount of gel is prepared in a beaker, from the sol form. This gel containing beaker is raised at a certain rate, so pushing a probe slowly through the gel. The changes in the load on the probe can be measured as a function of depth of immersion of the probe below the gel surface. [28] [30]

In vitro drug release studies:
For the in situ gel formulations to be administered by oral, ocular or rectal routes, the drug release studies are carried out by using the plastic dialysis cell. The cell is made up of two half cells, donor compartment and a receptor compartment. Both half cells are separated with the help of cellulose membrane. The sol form of the formulation is placed in the donor compartment. The assembled cell is then shaken horizontally in an incubator. The total volume of the receptor solution can be removed at intervals and replaced with the fresh media. This receptor solution is analyzed for the drug release using analytical technique. For injectable in situ gels, the formulation is placed into vials containing receptor media and placed on a shaker water bath at required temperature and oscillations rate. Samples are withdrawn periodically and analyzed. [29] [31]

Drug Content Uniformity:
Percentage of drug content is evaluated with uniformity. The uniformity of drug distribution displays the ability to release drug *in-vitro* and *in-vivo*. It is generally investigated by UV spectrophotometer after certain dilution. The results found are co-related to *in vitro* as well as *in-vivo* drug release and shows the performance of drug content of the formulation. [28]

Fourier transforms infra-red spectroscopy and thermal analysis:
During the process of gelation, the nature of interacting forces can be evaluated using this technique by employing potassium bromide pellet method. Thermo-gravimetric analysis can be conducted for in situ forming polymeric systems to quantitative the percentage of water in hydrogels. Differential scanning calorimetry is used to observe if there are any changes in thermogram as compared with the pure ingredients used thus indicating the interactions. [30] [31]

Texture analysis:
The consistency, cohesiveness or the firmness of hydrogels are analyzed using texture analyzer. The analyzer generally indicates the syringe ability of sol and determines whether the formulation can be administered in vivo to humans or animals. Higher values of adhesiveness of gels are needed to maintain an intimate contact with surfaces like tissues. [33] [32]

Gelation temperature and Gelation time:
The gelation temperature is one of the important parameter to be noted for injectable gel. It is defined as the exact temperature when the viscous liquid changes its phase to sol form after its contact with the environmental temperature. Generally in-situ formation of gel takes place with the physiological body temperature. The time required for gelation of viscous liquid or *in-vitro* and *in-vivo* phase transformation with the change in temperature is called gelation time. The experiment can be performed by visual measurement. [28]

CONCLUSIONS:

The "smart-polymers" or stimuli-responsive polymers have a very wide range of applications such as modified release of drugs, tissue engineering, bio-separation devices, active membrane etc. However intelligent polymers like thermo sensitive polymers offer great advantages and benefits in different way and in biomedical uses which includes from tissue engineering to the delivery of active drug molecules. Thermo sensitive polymer which aims to deliver the drugs in a controlled or sustained manner at the physiological body temperature has a great impact in the pharmaceutical field. The uses of such polymers are suitable candidate for formulation of pharmaceutical dosage form including modified release (i.e. sustained or prolong release) and distribution of the active drug to a specific target and thus reducing the side effects or adverse systemic reactions. This type of pharmaceutical formulation provides other advantages like reduction in therapeutic doses, and a consequent patient compliance. In addition such systems became quite easy for production in pharmaceutical industry. The versatility of thermo responsive polymers makes the pharmaceutical excipients a class of potential materials in the field of pharmacy and chemistry.

LIST OF ABBREVIATIONS:

ID : Intradermal
IM : Intramuscular
SC : Subcutaneous
UCST : Upper critical solution temperature
LCST : Lower critical solution temperature
PNIPAAM: Poly (N-isopropylacrylamide)
PEO-PPO-PEO: polyethylene oxide- polypropylene oxide - polyethylene oxide
NIPAAm-co-AA: Poly (NIPAAm-co-acrylic acid)
PEI : Polyethyleneimine
PDEAM : Poly (N, N-diethylacrylamide)
BuMA : Butyl methacrylate
HEMA : H+droxyethyl methacrylate
PEG : Poly (ethylene glycol)
PVC : Poly vinyl glycol
PLA : Poly lactic acid
PDEAM : Poly (N,N-diethylacrylamide)
PDMAAm: Poly (dimethyl acrylamide)
PVC : Poly vinyl chloride
DNA : Deoxyribonucleic acid

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RECENT ADVANCES OF SMART POLYMERS FOR SUSTAIN RELEASE DRUG DELIVERY

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ABSTRACT
Polymers are the core ingredient in the formulation and development of drug product. They have certain characteristics in designing and modification of the formulation in drug delivery system. This delivery system may require polymers in the form of carrier, colloidal or as nanoparticles which show great advantages in drug loading as well as in releasing of active drug moiety. Smart polymers are slightly different from such polymers because smart polymers have the ability to transform its microstructure or physical structure with the change in temperature, pH, biological enzyme, radioactive agents etc. Natural as well as synthetic polymers have been used in making the production and formulation system a very simple and low cost. Through these polymers the designing and formulation system of controlled drug delivery have became quite easy for the researcher. Targeted release and sustain release of drug can be obtained by overcoming the difficulties like poor bioavailability and short half life drug.

KEYWORDS: smart polymer, stimuli responsive, control release, targeted delivery.

INTRODUCTION
Polymers play an important role in the formulations of drug delivery system due to their improved pharmacokinetic properties. Especially in the field of smart drug delivery, polymer has been widely used because it can deliver therapeutic agents directly into the intended site of action, with high efficacy. Smart polymers have shown immense progress in the sustained and controlled delivery of drugs. Some systems like hydrogels swell and release the drug when exposed to intended environment. They work passively in minimizing drug degradation and improving circulation time. Another important issue is the safe excretion of the drug.[1,2] Due to advanced scientific pharmaceutical technology, controlled drug release system has been in a dynamic progress and this delivery model can be categorized into following classes.

(1) Drug delivery through rate-programmed
The recent advances in the smart rate-programmed drug delivery systems have vital roles, such as greater drug-loaded nano/micro-particle encapsulation ability, overcome pre-systemic metabolism, enhanced bioavailability and environmental responsive properties for various applications. An example of new rate pre-programmed drug delivery system is transdermal patch which delivers a particular concentration of drugs to the blood circulation via the skin. The rate-programmed drug delivery system releases drug molecules in a controlled manner by pre-determining the rate.[3,4]

(2) Activation-modulated drug delivery
These types of polymers depend on the surrounding environment and change their physical or microstructure. These polymers are generally divided into physical, chemical and biological stimuli. Stimuli responsive polymers are those which have the ability to transform itself with the change of temperature, pH, radioactive agents, biological enzymes etc. Smart polymers are therefore becoming a bonanza to deliver the active drugs by the means of transdermal route, injectables etc. Smart polymers thus can be used for sustaining and controlling release of drug for the poorly absorbed and half life drugs.[4,5]

(3) Feedback-regulated drug delivery
This self-regulated or feedback-controlled drug delivery is maintained and controlled by feedback information, without any external stimulation, and utilized several approaches to control the release rate. This drug delivery method has been applied to the development of various controlled delivery systems such as bio-erosion regulated, bio-responsive regulated and self regulating drug delivery systems. Among this one of the concepts has been involved in the smart controlled delivery systems. One of the concepts state that the drug release is activated by a triggering agent, such as a biochemical

substance, in the body via some feedback mechanisms and the release rate has been determined by triggering agent concentration. The concentration or the agent needs to be at a certain level and if that exceeds, the release is activated. This kind of drug delivery system utilizes several approaches for the rate-control release such as temperature-responsive polymers, pH-responsive polymers, enzyme-substrate reactions etc. Multi-functional polymer can be incorporated into hydrogel and can be used for feedback-regulated drug delivery system which can protect the drug from dangerous environments such as enzymes and low pH in the stomach. [4,5]

(4) Site-targeting drug delivery system

Site-targeted drug delivery systems are complex of multiple steps of diffusion and partitioning. A variety of both natural and synthetic water-soluble polymers have been used for biomedical applications. These polymers have been used routinely in biopharmaceutics because of the effectiveness in controlled drug release. The traditional formulations are not significantly efficient at targeting molecules, thus the new and smart drug delivery systems are being studied to overcome the problem. The goal of the smart drug delivery systems is to allow a localized drug delivery, at the same time; it does not affect the healthy tissues and no unwanted effects. The drugs composed of micro or nano-sized particulate system, which is able to spread through the systemic circulation, and transport through various body organs and body areas such as arteries, veins, and capillaries and even cross membrane barriers. The nanoparticles transport and targeting tissue are the complex process, so the transportation and communication have been viewed by the molecular communication paradigm.[4,5]

Few examples of site-targeted drug delivery system are: the kidney site-targeted drug delivery systems, it acted as a smart delivery to enhance drug efficacy and safety in the therapeutics of kidney diseases. By this smart drug delivery treatment provides that reduces inflammation and reduce the formation of excess fibrous to proximal tubular cells, it can protect systemic infection and renal tubular inflammations. So targeting the renal proximal tubular cells is the novel and efficient routes to cure kidney disease. Kidney-targeted drug delivery system can overcome from the various obstacles such as kidney transplantation, urethral obstruction, diabetes, and other some important kidney disease. [6,7,8]

SUSTAINED RELEASE DRUG DELIVERY SYSTEM

The term sustained release can be defined as the system that is used to deliver the drug particles at pre-determined time and frequency to a longer period of time after administration of a single dose producing therapeutic effect. The main aim of the concept sustain release is to prolong the drug action at a predetermined rate by maintaining a relatively constant, effective therapeutic drug level in the body by reducing the undesirable or adverse side effects.[9]

Advantages of Sustained Release Drug Delivery System

i. Duration of action of the drug is extended as compared to conventional dosage form.
ii. The frequency of dose is reduced by sustaining the dosage form.
iii. The total amount of drug or the drug load per dose can be reduced by maximizing availability with minimum dose.
iv. Minimizes the drug accumulation with chronic dosing.
v. Improve efficacy in treatment by controlling and reducing fluctuations in drug level.
vi. Improve bioavailability of some drugs.
vii. The drug absorption can be attained much better and effective.
viii. Administration of dosage form can be made more convenient.[10,11]

Disadvantages of Sustained Release Drug Delivery System

i. The flexibility in dosage regimen for adjustment is limited.
ii. The *in vitro – in vivo* correlation is poor.
iii. A large amount of active drug is required to maintain a sustained dose for the drugs of short half life.
iv. Toxicity, poisoning or hypersensitivity reactions are a major drawback for retrieval of drugs after administration.
v. The absorption in not effective in lower small intestine. [10,11,12]

SMART POLYMERS AND THEIR RECENT APPLICATIONS

Smart polymers have been widely used because of its potent characters like self transformation of one phase to another by coming in contact with surrounding environmental change. The change may include temperature, pressure, pH etc. These polymers are biocompatible, flexible, easy shaping, coloring etc. Because of such variant parameters the formulated dosage form remains stable and safe. [13]

Smart polymers can be classified into three main heading as.

i. Biological responsive polymers (enzymes and biomolecules).
ii. Chemical responsive polymers (pH and ionic strength).
iii. Physical responsive polymers (ultrasound, light, mechanical stress, temperature).

Biological responsive polymers

The polymers that are biologically responsive have an important space in the field of pharmaceutical manufacturing. They are significant in various

biomedical applications. Some of the major advantages are cited below.

Bioresponsive polymers possess the ability to respond to the stimuli that are inherently present in the natural system. They mainly arise from common functional groups that are known to interact with biologically relevant species, and in other instances the synthetic polymer is conjugated to a biological component. Bioresponsive polymers are classified into antigen responsive polymers, glucose-sensitive polymers and enzyme responsive polymers.[14, 15, 16]

Chemical responsive polymers (pH and ionic strength)

Chemical responsive polymers include pH sensitive and ionic strength etc. The pH responsive polymer consists of pendant basic or acidic group that have the ability to accept or release a proton with the changes in environmental pH. And those types of polymers with a large number of ionisable groups are known as polyelectrolytes. They are classified into two types: weak polyacids and weak polybases. The weak polyacids accept protons at low pH and releases protons at neutral as well as high pH it. Thus the molecular structure of the polymeric chain gets altered with the rapid change of the attached group. This transition to expanded state is mediated by the osmotic pressure exerted by mobile counter ions neutralized by network charges. pH-responsive polymers containing a sulphonamide group are another good example of polyacid polymers. These polymers have pKa values in the range of 3–11 and the hydrogen atom of the amide nitrogen is readily ionized to form polyacids. Narrow pH range and good sensitivity is the major advantage of these polymers over carboxylic acid based polymers.[14,15,16]

Physical responsive polymers (temperature responsive polymer)

Temperature sensitive polymers are those that change their structure with the change in surrounding temperature whether *in-vitro* or *in-vivo*. These are the polymeric structures sensitive to both temperature and pH, they are obtained by the simple combination of ionization and hydrophobic (inverse thermo sensitive) functional groups. This approach is mainly achieved by the copolymerization of monomers bearing these functional groups, combining temperature sensitive polymers with polyelectrolyte's or by the development of new monomers that respond simultaneously to both stimuli.[14,15,16]

APPLICATIONS

Drug delivery systems

Stimuli responsive polymers are used to deliver drug substances in the gastrointestinal tract (GIT) between stomach and colon. This condition is suitable for pH-sensitive polymers for colon specific drug delivery. The most common approach utilizes enteric polymers that resist degradation in acidic environment and release drug in alkaline media due to the formation of salt. There are several examples of these kind of polymers already commercialized, i.e. Eudragit L, Eudragit S from Rohm Pharma GmBH (based on methacrylic acic and methyl methacrylate). Researchers have designed more sophisticated pH-sensitive polymers in order to take advantage of the pH changes that occur in nature.[16,17]

Gene carriers

Polyelectrolytes have high potential as biomaterials in delivering oppositely charged molecules. One of the most promising applications of pH-sensitive polymers is as Nonviral gene carriers. Naked DNA is very difficult to incorporate into the cells because it is negatively charged and it has a very large size at physiological conditions. Liposomes and polycations are the two major classes of chemical (non-viral) gene delivery methods to condense DNA in charge balanced nanoparticles that can be carried into cell. Im *et al*[38] prepared a self-destroying, biodegradable, polycationic polyester, poly(*trans*-4-hydroxy-L-proline ester) (PHP ester), with hydroxyproline, a major constituent of collagen, gelatine, and other proteins, as a repeating unit. PHP ester formed soluble polymer/DNA complexes with average diameters of less than 200 nm. These complexes could transfect the mammalian cells, being comparable to the transfection obtained with PLL, the most common polymer for gene delivery.[16,17]

Polymers with dual stimuli-responsiveness

There are certain polymers which are sensitive to both temperature and pH by the simple combination of ionisable and hydrophobic (inverse thermosensitive) functional groups. It has mainly been achieved by the copolymerization of monomers bearing these functional groups combining thermosensitive polymers with polyelectrolytes or by the development of new monomers that respond simultaneously to both stimuli. Well defined and tailored polymers were obtained covering a wide range of properties. They developed different materials by fermentation, which showed clear environmental advantages. The ELPs presented a modulated pH- and temperature sensitivity covering the most interesting range of biomedical applications. ELPs have also been modified with photoresponsive molecules as azobenzenes getting photosensitive macromolecules.[16,17]

VARIOUS POLYMERS USED IN DRUG DELIVERY

PLGA (Polylactic-co-glycolic acid)

Biodegradable polymers have played an important role in the delivery of drugs in a controlled and targeted manner. Polylactic-co-glycolic acid (PLGA) is one of the extensively researched synthetic biodegradable polymers due to its favorable properties. A wide range of PLGA-based drug delivery systems have been reported for the treatment or diagnosis of various diseases and disorders. PLGA can be processed into almost any shape and size, and can encapsulate molecules of virtually any

size. It is soluble in wide range of common solvents including chlorinated solvents, tetrahydofuran, acetone or ethyl acetate. The change in PLGA properties during polymer biodegradation influences the release and degradation rates of incorporated drug molecules. PLGA physical properties themselves have been shown to depend upon multiple factors, including the initial molecular weight, the ratio of lactide to glycolide, the size of the device, exposure to water (surface shape) and storage temperature.[18]

Poloxamers

Poloxamer is a block copolymer which is well known for its thermo reversible property has several other properties used in several formulations. It has the property to optimize the drug release from its formulation with a sol-gel transition. The block copolymer poloxamer in aqueous media exhibits micellar structures which can convert into gel like structures based on their length, concentration and temperature. The increase of temperature above the critical micelle concentration (CMC) and critical micelle temperature (CMT) leads to the reduction of solubility of propylene oxide chain (PPO) blocks in aqueous solution and simultaneous increase of concentration and temperature will lead to the core shell type of micelle formation. The dehydrated micelle core helps in the incorporation of hydrophobic drug in aqueous poloxamer solution. After the incorporation of drug, the gelation process can be achieved by increasing the temperature and concentration above the critical gel temperature (CGT) and critical gel concentration (CGC) respectively. Here the physical entanglement and packing of micellar structures are the reason for gelation.[19,20]

Other Advantages

Ease of manufacture and characterization

Smart polymers used in sustain drug delivery system are easy to manufacture and characterize producing very low cost. They are strong, resilient, flexible, biocompatible, biodegradable, non-thrombogenic, easy shaping, and colouring. They have the capacity to maintain the drug stability of the drug delivery systems. They are good nutrient carriers as well.[21]

Management of toxicity

Management of toxicity is another important advantage that can be obtained by the use of smart polymers. The unnecessary exposure of the drug throughout the body with increasing risk of its toxicity and adverse drug reactions can be easily escaped. The best way to circumvent is to make magic bullets, which can go and act on predetermined targets. With advances in polymer sciences and nanotechnology, designing intelligent sustained release drug delivery system, with objectives of targeted actions and thereby reduced toxicity has now become a reality. Other advantages of using sustained release drug delivery system is that only a small dose of the drug will serve the purpose effectively thereby reducing dosage frequency, improving patient compliance, and minimizing the expenditure in case of expensive active pharmaceutical ingredients. For example, Amphotericin B, when administered as a bare drug needs to be given in high concentration and patients are subjected to toxicity. This is easily resolved by incorporating it in SDDS. These SDDS will release the drug completely to the affected area thereby relieving the patient of the disease symptoms with invariably less dosage frequency and reduced cost.[21]

Biodegradable smart polymers as drug delivery systems

Smart polymers have a very good advantage of being biodegradable. Once it enters into the system after releasing the drug to which it is bound at the target site it degrades and gets eliminated from the body without causing any harmful or toxic effects. This biodegradable quality of smart polymer renders its usage safe and desirable as a drug delivery system. Its biodegradable nature also prevents intervention to remove it from the body once the drug's targeted release is achieved. This helps in ensuring patient compliance as well as is a cost effective innovative approach of drug delivery system.[21]

CONCLUSION

Smart polymers have become quite advantageous in the control delivery of drugs. They are promising polymers which provide beneficial effect to drugs having short half life, narrow therapeutic window and drugs that are therapeutically active at low plasma concentration. The self- transformation to another phase at different environmental condition is the ultimate mechanism possessed by the smart polymers. So designing formulation accordingly and releasing active drugs at a predetermined rate is the best utilization of smart polymers at present day. Control and sustained delivery of therapeutic drugs thus can be achieved by these types of polymers which also have advantages like biocompatibility, response time to stimuli, burst release etc.

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