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Formulation, Characterization and Evaluation of Temperature Sensitive Controlled Release Hydrogels of Methotrexate.

Swatantra Kumar Singh Kushwaha*, Prashant Kumar, and Awani Kumar Rai.

Pranveer Singh Institute of Technology, Kanpur, India,

ABSTRACT

The use of a novel injectable biocompatible and biodegradable Methotrexate formulation for controlled release is described. The drug delivery vehicle is an autogelling pH sensitive formulation, which is based on the natural biopolymer chitosan. The primary objective of this research work was to Prepare and evaluate the temperature induced and pH induced gelling injectible hydrogels of Methotrexate for cancer drug delivery. For the preparation of Temperature induced gelling Hydrogels Chitosan and β-Glycero Phosphate were used. Preformulation studies of Methotrexate and polymers were carried. Preformulation studies included solubility, X-Ray Diffractometry and FTIR etc. Preformulation studies showed that there is no interaction b/w drug and excipients to formulate the in-situ forming hydrogels. The drug release was found that cumulative percent drug release were 73.39%, 77.74 %, 85.3%, 94.48%, and 98.10% for formulation $F1_T$, $F2_T$, $F3_T$, $F4_T$ and $F5_T$ respectively after 8 hours. The *in vitro* release data shows that the formulation $F5_T$ has better sustained effect than other formulations. The pyrogen testing result was found that the average rise in temperature for individual rabbits was 0.5, 0.35 and 0.4 respectively for $F2_T$ formulation while 0.3, 0.45 and 0.5 respectively for $F4_T$ formulations (less than 0.6). Hence formulations passed the pyrogen test. All these findings shows that chitosan/ β-Glycero Phosphate gel to be a safe, effective, homogeneous, injectable and stable formulation for delivery of Methotrexate and this approach represents an attractive technology platform for the delivery of other clinically important Anticancer drugs.

Keywords: Anticancer, hydrogels, kinetics, temperature, sensitive, release

Corresponding author

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INTRODUCTION

Cancer is a disease characterized by an aggressive growth of cells which divide without normal limitations, invade and destroy adjacent tissues, and spread to distant anatomic sites through a process called metastasis, a major cause of death of cancer [1]. Cancer is currently the cause of 12% of all deaths worldwide. In 2008 approximately 12.7 million cancers occurred and 7.6 million people died of cancer worldwide [2]. This makes it the leading cause of death in the developed world and the second leading cause of death in the developing world. Cancer prevalence in India is estimated to be around 2.5 million, with over 8, 00,000 new cases and 5, 50,000 deaths occurring each year due to this disease in the country. The common sites for cancer in India are oral cavity, lungs, esophagus and stomach in males and cervix, breast and oral cavity among females.

Cancer is caused by a combination of environmental and genetic factors. Cancer is influenced by environment, lifestyle and diet on one hand, heredity and spontaneous mutations on the other. In normal cell growth there is a finely controlled balance between growth–promoting and growth-restrataining signals such that proliferation occurs only when required [3].

Hydrogels are three-dimensional, cross-linked networks of water-soluble polymers. Hydrogels can be made from virtually any water-soluble polymer, encompassing a wide range of chemical compositions and bulk physical properties. Furthermore, hydrogels can be formulated in a variety of physical forms, including slabs, microparticles, nanoparticles, coatings, and films. As a result, hydrogels are commonly used in clinical practice and experimental medicine for a wide range of applications, including tissue engineering and regenerative medicine, diagnostics, cellular immobilization, separation of biomolecules or cells, and barrier materials to regulate biological adhesions [4].

Hydrogels show minimal tendency to adsorb proteins from body fluids because of their low interfacial tension. Further, the ability of molecules of different sizes to diffuse into (drug loading) and out of (drug release) hydrogels allows the possible use of dry or swollen polymeric networks as drug delivery systems for oral, nasal, buccal, rectal, vaginal, ocular and parenteral routes of administration. Several terms have been coined for hydrogels, such as ‘intelligent gels’ or ‘smart hydrogels’ [5]. Hydrogels are ‘smart’ or ‘intelligent’ in the sense that they can perceive the prevailing stimuli and respond by exhibiting changes in their physical or chemical behavior, resulting in the release of entrapped drug in a controlled manner [6-9].

Chitosan [10-12] is soluble, mucoadhesive and active as an absorption enhancer in its protonated form because the pKa of the amine groups of Chitosan is 6.2, chitosan at neutral pH hardly carries a charge, has a low solubility and is therefore essentially inactive. Because of the presence of functional groups (amine and hydroxyl) various chemical chitosan derivatives have been synthesized and studied for different applications. The chitosan have shown in situ gelling properties due to the formation of inter and intra molecular disulfide bonds at physiological pH and temperature [13].

MATERIALS AND METHODOLOGY

MATERIALS

Methotrexate was obtained from East and West Pharma Pvt. Ltd. Roorkee. Chitosan (Deacetylation degree DDA = 80%), β- Glycero phosphate were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India and Glyceryl monooleate (GMO) was obtained from Estelle Chemicals Pvt. Ltd., Ahmednagar, India. Demineralized and double distilled water was used. All chemicals and reagents used were of analytical grade.

METHODS

Preparation of In-situ forming Temperature sensitive Hydrogels

Temperatue sensitive hydrogels were prepared by the method invented by [14] *Chenite et al. (2004)*, known as BST (Biosyntech) Gel platform technology. It is based on the neutralization of acidic chitosan solution with a polyol counterionic dibase salt such as β glycerophosphate. Chitosan/glycerophosphate (C/GP) is a

thermosensitive solution which is liquid at room temperature and solidifies into a hydrogel at body temperature. For the preparation of acidic solution of chitosan 0.1M acetic acid was used.

For the preparation of Temperature Sensitive Hydrogels, Chitosan solution of desired concentration was prepared according to **table 1** by stirring accurately weighed quantity of Chitosan along with suitable quantity of 0.1M Acetic acid for 3 hours. Drug solution was prepared by dispersing weighed amount of drug in solution of β -Glycero Phosphate prepared in Phosphate buffer pH7.4. In 5 ml of chilled Chitosan solution, 5 ml of drug and β -Glycerophosphate solution was added drop wise with continuous stirring to obtain clear and homogenous liquid in a final volume of 10 ml. The final solutions were mixed an additional 10 min at 4^0C and were filtered by membrane filtration using cellulose membrane.

Table 1: Formulation design for Temperature sensitive Hydrogels

Contents	Quantity (% w/w)				
	$F1_T$	**$F2_T$**	**$F3_T$**	**$F4_T$**	**$F5_T$**
Drug	0.5	0.5	0.5	0.5	0.5
Chitosan	1.5	1.5	1.5	1.5	1.75
β-Glycero Phosphate	10	12	15	18	15
0.1 M Acetic Acid	QS	QS	QS	QS	QS
Phosphate buffer pH 7.4	QS 10 gm	QS 10 gm	QS 10 gm	QS 10 gm	QS 10 gm

Physicochemical characterization

X-ray Diffractometry

The crystal X-ray scattering measurements for the obtained sample of Methotrexate were performed to determine the solid structure of Drug. XRD Patterns were obtained with a Seifert Germany ISO debyeflex 2002 apparatus (Japan) using Cu-K α radiation (λ = 1.541 nm), a voltage of 40 kV and a 100 mA current. Samples were scanned from 0–60^0 2θ for qualitative studies and the scanning rate was 4^0/ min.

FTIR Spectroscopy

The FT-IR spectrum of the obtained sample of the drug was compared with the standard FT-IR spectra of the pure drug.

Determination of λmax

λmax for the given sample of drug was determined by using UV-Vis spectrophotometer(UV-1700 Pharma Spec).

Standard Calibration curve of Methotrexate

Accurately weighed 20 mg Methotrexate Hydrochloride was dissolved in 100 ml of Phosphate buffer pH 7.4 to get the solution of 200µg/ml. This solution was further diluted with buffer to get the final stock solution of 20µg/ml. From this stock solution aliquots of 1, 2, 3, 4, 5, 6 & 7 ml were withdrawn and further diluted to 10 ml with buffer to obtain a concentrations range of 2 to 14µg/ml. The absorbance of the solutions was measured at 258 nm by using UV-Vis spectrophotometer (UV-1700 Pharma Spec). A graph of Concentration vs. Absorbance was plotted.

***In vitro* Gelation Studies**

All temperature sensitive formulations were evaluated for gelling capacity. The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of pH 7.4 Phosphate buffer freshly prepared and equilibrated at 37^0 C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. The lowest scores (+) were assigned to those products in

which the phase transition occurred only after 60-90 sec. and the formed gels collapsed within 1-2 hrs. The highest scores (+++) were assigned to those products for which the phase transition commenced within 60-90 sec. and the gels so formed were stable for about 7-8 hrs. The moderate scores (++) were assigned to the products, which could form the gel in 60-90 sec. but failed to maintain gel structure for more than 3 hrs.

***In vitro* Viscosity Studies**

Viscosity is the main prerequisite of an *in situ* gelling system. Viscosities of Liquid formulations as well as gelled formulations both are important in determining the effectiveness of In-situ forming Hydrogels. Where an in-situ gelling formulation should have an optimum viscosity that will allow easy injection into the body as a liquid with minimum pain, which would undergo a rapid sol-to-gel transition; at the same time Viscosity of instilled formulation is an important factor in determining residence time of drug in the body. All temperature sensitive formulations were evaluated for viscosity of liquid formulations and viscosity of gelled formulations. Viscosity was measured using a Brookfield viscometer (DV-II + Pro) in the small volume adapter. The viscosity of prepared Temperature sensitive Hydrogel formulations measured at 10 rpm was used for purposes of comparative evaluation.

***In vitro* Release Studies**

The *in vitro* release of Methotrexate from all the temperature sensitive formulations was studied through cellophane membrane using a USP-I dissolution testing apparatus. The dissolution medium used was pH 7.4 Phosphate buffer freshly prepared. A 2-ml volume of the gelled formulation was accurately kept in Cellophane membrane, previously soaked overnight in the dissolution medium to form a cellophane pouch. Cellophane membrane pouch having drug was put in the cylindrical basket. The cylindrical basket was attached to the metallic driveshaft and suspended in 900 ml of dissolution medium maintained at 37± 1°C .The dissolution medium was stirred at 50 rpm. Aliquots, each of 5-ml volume, were withdrawn at regular intervals and replaced by an equal volume of the dissolution medium. The aliquots were analyzed by UV-Vis spectrophotometer (UV-1700 Pharma Spec) at 258 nm.

Drug Release Kinetics studies

The Drug release data obtained from all Temperature induced gelling formulations was fitted into various mathematical models given below in order to determine the Drug release kinetics of prepared formulations:

- Cumulative percent drug released Vs. Time (Zero order rate kinetics).
- Log Cumulative percent drug retained Vs. Time (First order rate kinetics).
- Cumulative percent released Vs. Root T [Higuchi's classical diffusion equation (Higuchi matrix)].
- (Percentage Retained) $^{1/3}$ Vs. Time (Hixson-Crowell erosion equation).

To find out the mechanism of drug release, 60 % drug of release data was first fitted in the *Korsmeyer-Peppas model*. Where Log of cumulative percent drug released was plotted against Log Time. The model was used to study the drug release mechanism by analyzing 'n' as the diffusion exponent. According to this model if 'n' is b/w 0.45 to 0.5 the Fickian mechanism, 0.5 to 0.8 the Non-Fickian and if 0.8 to 1.0 Case-II transport i.e. a zero-order mechanism is governing the drug release mechanism from the gels.

Sterility Testing

All Parenteral preparations should be sterile therefore the test for sterility is very important evaluation parameter. Two formulations from Temperature induced gelling formulations were randomly selected and subjected for sterility testing. Liquid Nutrient broth media containing Peptone (10gm), Meat extract (10gm), Sodium chloride (0.50gm) and Distilled water 1000 ml was prepared. pH of media was adjusted to 7.2 ± 0.2 and it was autoclaved at 125^{0}C for 30 minutes. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to Liquid Nutrient broth media (200 ml). The liquid was mixed with the media. The inoculated media were incubated for 15 days at 37°C temperature and 75±5% relative humidity.

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Pyrogen Testing

An Injectible formulation must be Pyrogen free. After sterilization the end products or metabolic products of bacteria might be left in formulation, which may act as Pyrogen. Hence Pyrogen testing is also necessary in ophthalmic formulations. Two formulations from Temperature induced gelling formulations were randomly selected and subjected to Qualitative Fever Response Test in rabbits. Pyrogen testing was performed according to IP. Three rabbits weighing 2.50 kg were selected for the purpose. Formulation in a dose of 10ml/kg of body weight was injected in the ear vein of rabbit and injection was completed within 10 seconds. Rectal temperature after giving the formulation was recorded at 1, 2 and 3 hr. and Rise in temperature was determined.

Stability Studies

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. Two from Temperature induced gelling formulations were subjected to stability studies at ambient humidity conditions for a period of one month. The samples were withdrawn after 7, 15 and 30 days and were evaluated for Drug content.

RESULTS AND DISCUSSION

Standard calibration curve of Methotrexate

Fig 1 shows a representative standard calibration curve with slope and regression coefficient of 0.069 and 0.998 respectively. The curve was found to be linear in the range of 2-14µg/ml at λmax 258 nm. The calculation of the drug content, in-vitro release, and stability studies are based on this calibration curve.

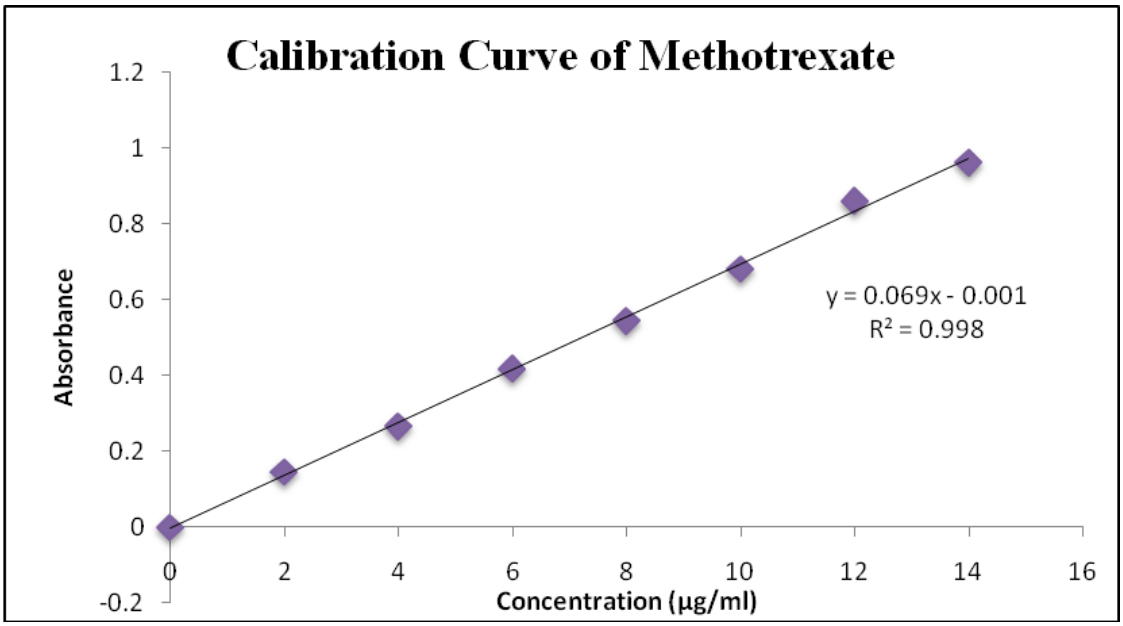

Fig 1: Standard Calibration curve of Methotrexate in Phosphate Buffer pH 7.4

IR Spectroscopy

The IR spectrum of the pure sample of Methotrexate recorded by FTIR spectrometer is shown in Fig. 2, which was compared with standard IR spectra of Methotrexate. Preformulation studies were carried out to study the compatibility of pure drug Methotrexate with the polymers Chitosan and β-Glycero Phosphate prior to the preparation of hydrogels. The individual IR spectra of the pure drug and Physical mixtures of

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Methotrexate with polymers are shown in the Fig. 2 and 3, which indicate no interaction between Methotrexate and polymers when compared with infrared spectrum of pure drug, as all functional group frequencies were present.

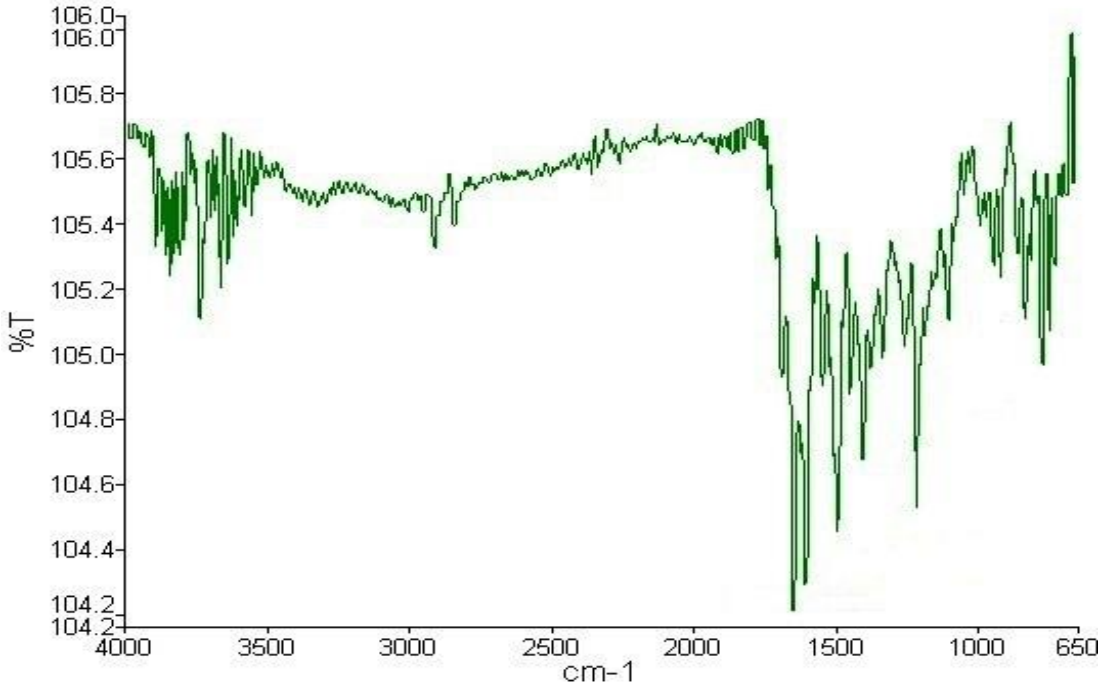

Fig 2: IR Spectra of Pure Methotrexate

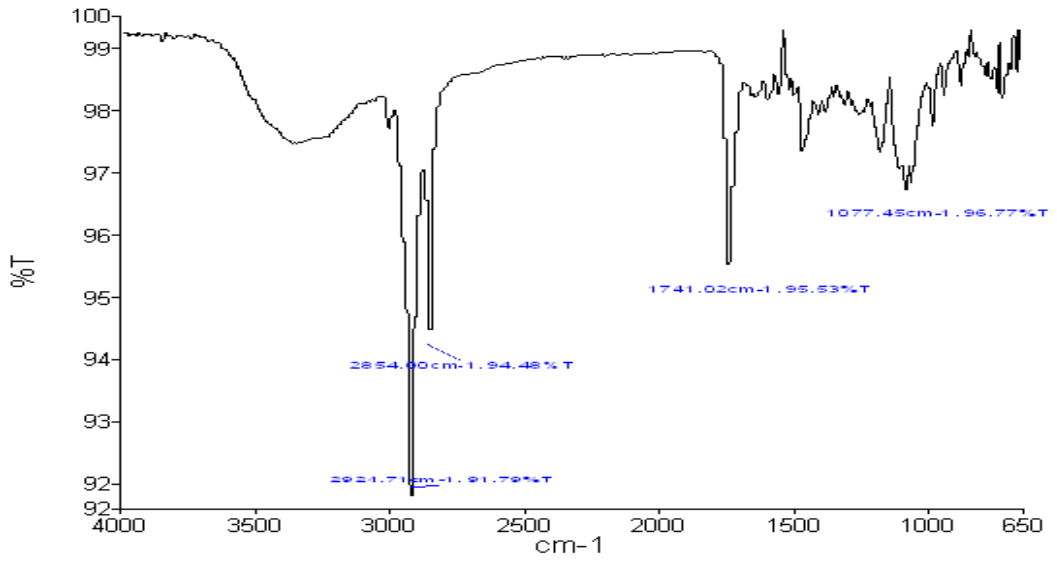

Fig 3: IR Spectra of Physical Mixture of Temperature sensitive Formulation

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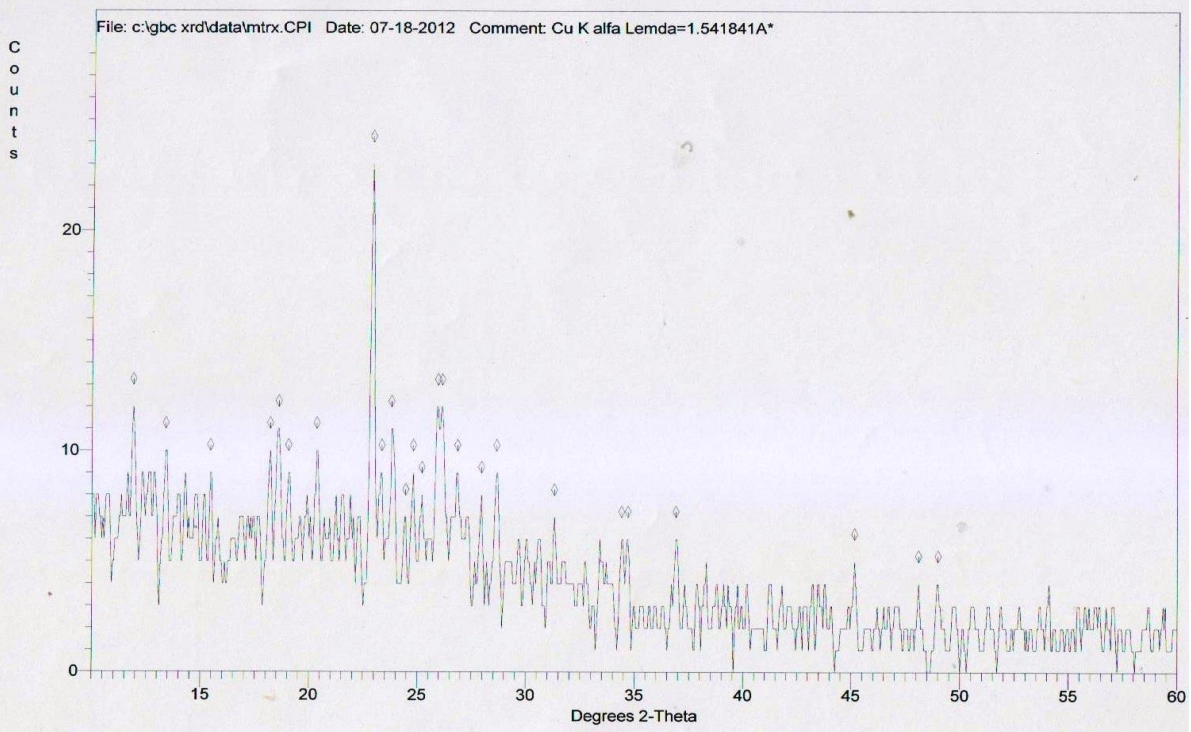

Fig 4: Powder X-ray Diffraction Pattern of Methotrexate

Powder X-Ray Diffractometry

The PXRD Pattern for the Methotrexate is shown in Fig. 4. In the X-ray diffraction spectrum, Methotrexate exhibited several strong characteristic crystalline peaks b/w 2θ =7^0 to 27^0 confirming t the highly crystalline nature of drug.

***In-vitro* Gelation Studies**

The main prerequisite of an *in situ* gelling system is gelling capacity (speed and extent of gelation). The formulation should have an optimum gelling capacity, so that after injection at site of action, it would undergo a rapid sol-to-gel transition and would preserve its integrity without dissolving or eroding for a prolonged period of time.

Table 2: Appearance, pH and Gelling capacity for Temperature sensitive Hydrogels

Formulation	Appearance	Clarity	pH	Gelling capacity
$F1_T$	Yellow	Clear	6.8	++
$F2_T$	Yellow	Clear	6.6	+++
$F3_T$	Yellow	Clear	6.5	+++
$F4_T$	Yellow	Clear	7.1	+++
$F5_T$	Yellow	Clear	6.7	+++

Table 2 shows the gelling capacity of all Temperature induced gelling formulations. All the formulations showed instantaneous gelation when contacted with Phosphate Buffer pH 7.4. However, the nature of the gel formed depended on the concentration of polymers used. Formulation $F1_T$ showed immediate gelation and remained for few hours, where as the formulation $F2_T$, $F3_T$, $F4_T$ and $F5_T$ showed immediate gelation and remained for extended period.

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Viscosity Studies of Liquid Formulations and Gelled Formulations

An Injectable formulation must have an optimum viscosity that will allow easy injection into the body as a liquid, which would undergo a rapid sol-to-gel transition. Viscosity of gelled formulation is also an important factor in determining residence time of drug in the body. **Table 3** shows the viscosity of all Temperature induced gelling formulations in liquid form and in gelled form at 10 rpm respectively. In case of Temperature induced gelling formulations viscosity of liquid as well as gelled formulations is found to increase in direct proportion of concentration of β-GP used. But formulation $F5_T$ showed highest viscosity, it may be due to greater concentration of chitosan. Figure 5 showed the graphical representation of viscosities.

Table 3: Rheological Study of Temperature Induced gelling systems

Temperature sensitive Formulations	Viscosity of Liquid Formulations at 10 rpm (Pa-S)	Viscosity of Gelled Formulations at 10 rpm (Pa-S)
$F1_T$	1.342	28.352
$F2_T$	2.474	31.583
$F3_T$	2.893	35.422
$F4_T$	3.269	39.042
$F5_T$	4.586	56.376

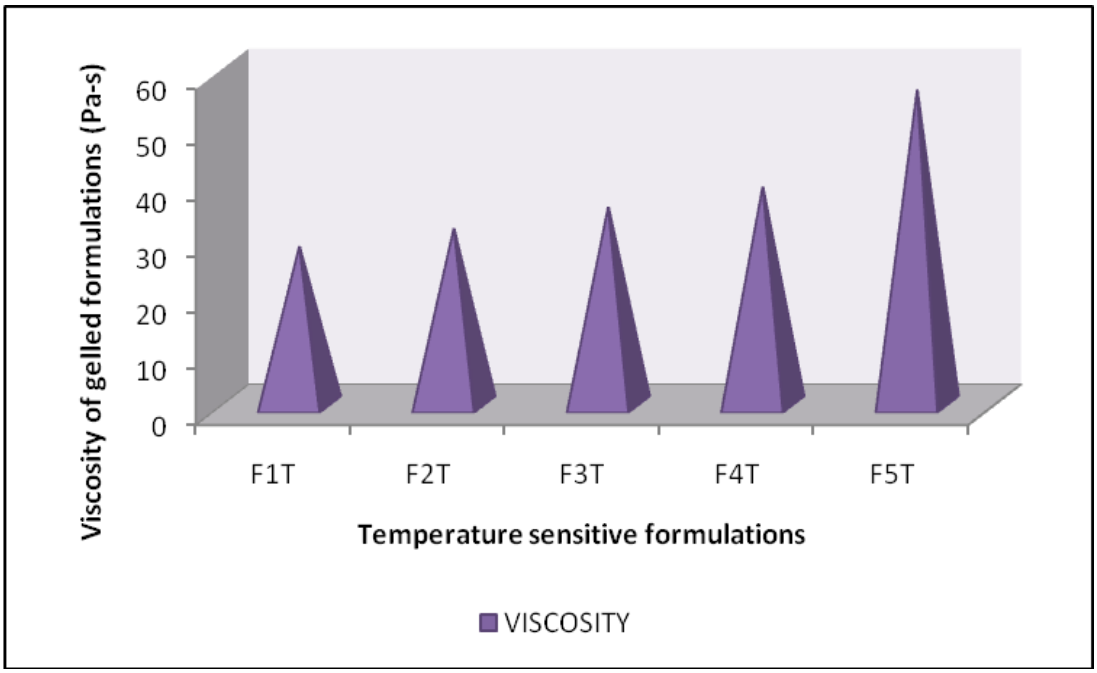

Fig 5: Viscosity of Formulations in gelled form (at 10 rpm)

In Vitro Release Studies:

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its *in vivo* behaviour. All the temperature sensitive *in situ gelling* formulations of Methotrexate were subjected to *in vitro* release studies. These *in vitro* release studies were carried out using Potassium Phosphate buffer of pH 7.4 as the dissolution medium.

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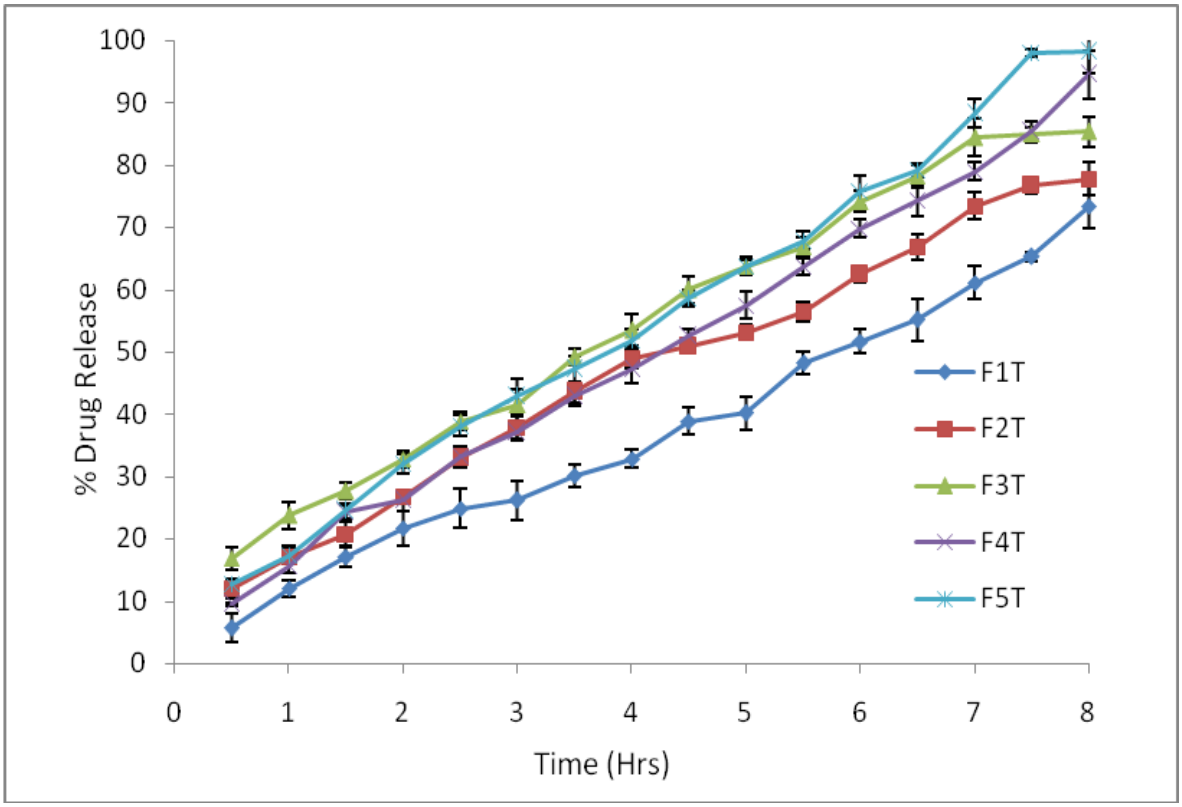

Fig 6: *In-Vitro* Drug Release Profile of Drug from formulations (n=3)

Figure 6 shows the drug release data obtained for temperature sensitive formulations. It was found that cumulative percent drug release was 73.39%, 77.74 %, 85.3%, 94.48%, and 98.10% for formulation $F1_T$, $F2_T$, $F3_T$, $F4_T$ and $F5_T$ respectively after 8 hours. The results clearly showed that the gels have the ability to retain drug for prolonged period of time (8 hour) and that premature drug release will not occur.

Drug Release Kinetics Studies:

Plots of zero order, first order, Higuchi matrix, Peppas and Hixson Crowell for all Temperature sensitive formulations are calculated. The regression coefficient (r) and 'n' values of zero order, first order, Higuchi matrix, Peppas and Hixson-Crowell are tabulated in **Table 4** for all Temperature induced gelling formulations.

Table 4: Model fitting for the release profile of formulations by using 5 different models

Formulation	Zero Order	First Order	Higuchi Matrix	Korsmeyer-Peppas		Hixson-Crowell	Best Fit Model	Transport Mechanism
	R^2	R^2	R^2	R^2	n	R^2		
$F1_T$	0.991	0.971	0.983	0.988	0.693	0.987	Zero	Non Ficknian
$F2_T$	0.991	0.962	0.980	0.982	0.583	0.983	Zero	Non Ficknian
$F3_T$	0.996	0.839	0.968	0.989	0.801	0.928	Zero	Non Ficknian
$F4_T$	0.991	0.935	0.950	0.989	0.847	0.960	Zero	Non Ficknian
$F5_T$	0.994	0.752	0.968	0.991	0.725	0.886	Zero	Non Ficknian

From the table, it is clear that in case of Temperature induced gelling formulations , the best fit model was Zero order suggesting swelling controlled diffusion. While 'n' exponent value is b/w 0.5 to 0.89 indicating that formulation is released by Non-fickian diffusion mechanism or by anamolous diffusion in a controlled manner.

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Sterility Test

There was no appearance of turbidity and hence no evidence of microbial growth when the formulations were incubated for 15 days at 37^{0}C temperature and 75±5% relative humidity in Liquid Nutrient broth media. The preparations being examined therefore passed the test for sterility.

Pyrogen Testing:

Table 5 shows the results of Pyrogen Testing. From the results it was found that the average rise in temperature for individual rabbits was 0.5, 0.35 and 0.4 respectively for $F2_T$ formulation while 0.3, 0.45 and 0.5 respectively for $F4_T$ formulations (less than 0.6). Hence formulations passed the Pyrogen test.

Table 5: Pyrogen testing of In-situ gelling Formulations

Formulations	Sr. No. of rabbit	Rise in Temperature (^{0}C)			Average rise in Temperature
		After 1hr	After 2hr	After 3hr	
$F2_T$	**Rabbit-1**	0.4	0.4	0.2	0.5
	Rabbit-2	0.3	0.2	0.2	0.35
	Rabbit-3	0.5	0.2	0.1	0.4
$F4_T$	**Rabbit-1**	0.3	0.2	0.1	0.3
	Rabbit-2	0.2	0.3	0.4	0.45
	Rabbit-3	0.3	0.2	0.5	0.5

Stability Studies

Two randomly selected formulations from temperature induced gelling systems were subjected to stability studies conditions at 5°C, room temperature (25°C) with ambient humidity, 40^{0}C with 75±5% relative humidity for a period of one month, and evaluated for percent drug content. The observations are shown in **Table 6**. Both temperatures induced gelling formulations showed slight decrease in drug content at 25^{0}C and at 40^{0}C after 30 days of storage whereas at 5^{0}C showed significant decrease in the drug content after 30 days of storage. From the stability studies it was confirmed that *in-situ gel forming* formulations of Methotrexate are stable at ambient temperature and humidity.

Table 6: Stability studies: Characterization of In-situ gelling Formulations

Formulations	Storage Conditions	Percent Drug Content (%)		
		7 Days	15 Days	30 Days
$F2_T$	5±3^{0}C	98.34	96.96	95.83
	25±2^{0}C / 60±5% RH	97.81	94.72	93.67
	40±2^{0}C / 75±5% RH	95.98	92.80	91.26
$F4_T$	5±3^{0}C	97.83	95.38	94.19
	25±2^{0}C / 60±5% RH	97.75	94.95	93.88
	40±2^{0}C / 75±5% RH	97.66	94.34	92.25

CONCLUSION

Local delivery of chemotherapeutic agents by in-situ forming Injectible Hydrogels using controlled-release polymers is a new strategy with the potential to maximize the anticancer effect of a drug and reduce systemic toxicity. In this study, we have demonstrated the effectiveness of using the biodegradable chitosan polymer to deliver high doses of Methotrexate locally to cancer.

The primary objective of this research work was to Prepare and evaluate the temperature induced and pH induced gelling injectible hydrogels of Methotrexate for cancer drug delivery. For the preparation of Temperature induced gelling Hydrogels Chitosan and β-Glycero Phosphate were used. Preformulation studies of Methotrexate and polymers were carried. Preformulation studies included solubility, X-Ray Diffractometry and FTIR etc. Preformulation studies showed that there is no interaction b/w drug and excipients to formulate the in-situ forming hydrogels.

The prepared temperature induced gelling injectible hydrogels were evaluated for different parameters like pH, appearance, drug content*, in vitro* gelation studies, *in vitro* viscosity studies of liquid and gelled formulations, *in vitro* release studies, sterility test, Pyrogen testing and stability studies. The appearance and clarity of all the prepared formulations was found satisfactory. The pH of all formulations was also found to be satisfactory in the range of 5.8- 7.2.

In vitro gelation studies and In vitro viscosity studies of liquid formulations and gelled formulations concluded that temperature induced formulations $F1_T$, $F2_T$, $F3_T$, $F4_T$ and $F5_T$ showed optimum Gelling capacity and Viscosity. All selected temperature induced Hydrogels showed sustained drug release for a period of 8 hour. Formulation $F5_T$ and $F2_T$ showed maximum percent drug release. By the Drug release Kinetics studies it was observed that all the selected temperature induced gelling hydrogel formulations followed the zero order drug release and 'n' value b/w 0.5 to 0.89, suggesting swelling controlled diffusion and Non Fickian transport mechanism. From the stability studies it was confirmed that temperature induced gelling hydrogel formulations of Methotrexate remained more stable at ambient temperature and humidity.

All these findings show chitosan/GP gel to be a safe, effective, homogeneous, injectable and stable formulation for delivery of Methotrexate and this approach represents an attractive technology platform for the delivery of other clinically important Anticancer drugs. The mechanism of gelation, which does not involve covalent cross-linkers, organic solvent or detergents, combined with a controllable residence time, renders this injectable biomaterial uniquely compatible with sensitive chemotherapeutic agents. However some more further studies needed to confirm the in-vivo bioavailability of these hydrogels.

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REFERENCES

[1] Dhanikula AB, Panchagnula R. International Journal of Pharmaceutics 1999; 183: 85-100.
[2] Jemal A, Bray F, Center J. CA: A Cancer Journal for Clinicians 2011; 61**:** 69-90.
[3] Spandidos DA, Anderson M. A Journal of patholology 1989; 157: 1-10.
[4] Dagani R. Chemistry and Engineering News archive 1997; 75: 26-36.
[5] Kost J. In Encyclopaedia of Controlled Drug Delivery 1999; 445-459.
[6] Qiu Y, Park K. Adv Drug Deliv Rev 2001; 53: 321-329.
[7] Franzesi GT, Ni B, Ling Y, Khademhosseini AA. Journal of American Chemical Society 2006; 128: 15064-15065.
[8] Gupta P, Vermani K, Garg S. Drug Discovery Today 2002; 7: 569-79.
[9] Cabral H. Nakanishi M. Kumagai M. Pharm Res 2009; 26: 82-92.
[10] Raymond C. Handbook of Pharmaceutical Excipients, 6th Edition, Pharmaceutical Press 2009; 110: 514-564.
[11] Azab AK, Orkin B. Journal of Control Release 2006; 111: 281-289.
[12] Reddy C, Maheswara FS. International Journal of Innovative Pharmaceutical Research 2011; 2: 131-138.
[13] Kushwaha SKS, Rai AK, Singh S. Inter J Pharm Tech Res 2010; 2: 2271-2282.
[14] Ruel-Gariépy E, Shive M, Bichara A, Berrada M, Le Garrec D, Chenite A, Leroux JC. Eur J Pharm Biopharm. 2004; 57: 53-63.