

THERMOSENSITIVE HYDROGEL FOR CONTROLLED DRUG DELIVERY OF ANTICANCER AGENTS

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ABSTRACT

Objective: To formulate and evaluate temperature sensitive controlled release safe Camptothecin for anti cancer drug delivery based on hydrogel with enhance solubility.

Methods: The temperature sensitive hydrogel based on chitosan/ β -Glycerophosphate/HP- β -Cyclodextrin (chitosan/ β -GP/HP- β -CD) was prepared by Crosslinking methods. The formulations were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), gelation time and viscosities (Brookfield DV-II + Pro viscometer) were investigated for controlled release hydrogel formulation. The formulation, containing homogeneously dispersed camptothecin, was studied by MTT assay on tumor cell MCF-7. The effectiveness of treatment was measured in terms of percentage control tumor growth inhibition (TGI).

Results: The hydrogel formulation of camptothecin (CPT) showed good release profile with polymer (chitosan/ β -GP/HP- β -CD) compare to without polymer. This formulation showed good properties in terms of pH, gelation, viscosity and in-vitro release. The gelation temperature and viscosity of the formulation was optimum. It was found that cumulative percentage drug release for formulations prepared TF. TF13, TF14, TF16, TF17, TF19, TF20, TF22, TF23 were 38.97%, 92.56%, 77.17%, 99.87%, 74.61%, 96.15%, 87.43%, 85.64%, 77.43% respectively. And the tumor growth inhibition (TGI) was found that for formulations TF. TF13, TF14, TF16, TF17, TF19, TF20, TF22, TF23 were 9.8%, 22.39%, 13.96%, 26.73%, 13.13%, 25.23%, 16.91%, 15.55%, 13.88% respectively.

Conclusion: The drug delivery vehicle is an in-situ thermogelling formulation, which is based on the natural biopolymer chitosan. Hydrogel is a promising safe and more effective delivery system that can be developed to serve as an alternative to currently used system for anticancer drug delivery.

Keywords: Chitosan, Hydrogel, HP- β -Cyclodextrin, MCF-7, Camptothecin.

INTRODUCTION

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 [1], diagnostics [2], cellular immobilization [3], separation of biomolecules or cells and barrier materials to regulate biological adhesions [4].

Anticancer agents are typically given at their maximum tolerated dose in order to achieve the greatest possible effect on rapidly dividing malignant cells. However, at these doses, the inclusion of treatment free periods is essential to permit the recovery of normal cells. Although such chemotherapy regimens are initially efficacious, the therapeutic responses are often short-lived. During the rest periods, re-growth of the vascular endothelial cells that support the tumor resumes, resulting in more aggressive cancers that are resistant to the cytotoxic drug. Continuous drug infusions have confirmed that constant low blood levels of an anticancer agent can be more effective than intense high dose short schedules. However, continuous infusions for weeks or months may not be feasible due to cost and issues of patient compliance with such inconvenient regimens. An alternative to constant intravenous infusions is implantable depot devices that release drug at the desired rate for prolonged time periods. Polymeric hydrogels are excellent candidates for long-term depot formulations due to their excellent biocompatibility and water permeability [5]. Our own hydrogel depot has the advantage of being injected as a liquid that changes into a solid gel immediately after injection, thereby avoiding the need for surgical implantation.

20(S)-camptothecin (CPT) is a cytotoxic quinoline alkaloid which binds to the complex of the enzyme topoisomerase-I with DNA during replication, and thereby stabilizing it. This prevents DNA re-ligation and therefore causes DNA damage which results in

apoptosis. In this sense, CPT has shown significant antitumor activity in a broad spectrum of human malignancies [6]. CPT holds a pH-dependent equilibrium in aqueous medium between the lactone and the carboxylate forms. The former is essential for anticancer activity, where as the carboxylate is almost inactive [7]. Unfortunately, the clinical application of CPT is hampered by its poor pharmaceutical profile, with extreme aqueous insolubility, low stability of the lactone form at physiological pH, and severe systemic toxicities which included myelosuppression, vomiting, diarrhea, and hemorrhagic cystitis [8-10].

Cyclodextrins (CD) due to their complexation ability and other versatile characteristics, widely used in the field of pharmaceutical industry and in different areas of drug delivery system. Cyclodextrin molecules are relatively large molecules with number of hydrogen donors and acceptors, thus in general they do not permeate lipophilic membrane. CD widely used to enhance the solubility, bioavailability, stability and safety of drug molecules [11]. Cyclodextrins (CD) have lipophilic inner cavities and hydrophilic outer surfaces. They are capable of interacting with a large variety of guest molecules to form non covalent inclusion complexes. Cyclodextrins are cyclic oligosaccharides and contain at least six D-(+) glucopyranose units which are attached by α -(1, 4) glycosidic bonds. They have been widely used for the formulation of drugs having bioavailability problems resulting from poor aqueous solubility, poor stability (hydrolytic or photodegradation, etc.) and severe side effects [12-14]. Amphiphilic cyclodextrins are synthetic derivatives of natural cyclodextrins, obtained by modification of the primary or secondary phase with linear or branched aliphatic chains of varying lengths (C2-C18) linked with different chemical bonds (ester, ether or amide)[15].

Controlled drug-delivery systems are designed to deliver the drugs at desirable times and/or specific sites to achieve the therapeutic objective [16, 17]. Hydrogels are hydrophilic polymer networks that may retain a large amount of water and exhibit a semi-solid morphology. The hydrophilic three-dimension net-work formed by chemical or physical crosslinking can be considered as an ideal candidate for the controlled drug release matrix [18]. During the last

decade, injectable *in situ* gel-forming systems have received increased interest in drug delivery and tissue engineering. These devices can overcome many of the problems associated with polymers or microspheres in that they are both injectable and produce solid biodegradable implants with a range of mechanical characteristics in terms of rigidity and load bearing making them compatible with both soft and hard tissues. Thermo reversible gels can be prepared with naturally occurring polymers. Most natural polymer aqueous solutions form a gel phase when their temperature is lowered. Classic examples of natural polymers exhibiting a sol-gel transition include gelatin and carrageenan, Chitosan. At elevated temperatures; these polymers adopt a random coil conformation in solution [19].

In the present work, we have used a chitosan polymer to formulate a biodegradable and biocompatible formulation for controlled delivery of camptothecin in a slow release manner directly into a tumor cell. In this paper, we report the *in vitro* release characteristics of the camptothecin-polymer hydrogel and the *in vitro* effect of delivering camptothecin in different concentrations to a MFC7 tumor cell. The delivery vehicle used is one of a family of thermosensitive chitosan solutions, formulated at physiological pH, which remain liquid at low temperature and turn into gel when heated. The polymeric matrix used in this study consists of chitosan polymer and β -Glycerophosphate (β -GP). Addition of glycerol-2-phosphate (β -GP) to chitosan solution produces a hydrogel which undergoes sol-gel transition at a temperature close to 37°C, making the formulation a suitable vehicle for drug administration since the hydrogel when implanted into the body, flows to fill voids or cavities and becomes solid at body temperature. These hydrogels are suitable carriers for water-insoluble drugs and they are non-toxic and highly biocompatible. Chitosan is an important natural polymer widely used for medical and pharmaceutical applications.

MATERIALS AND METHODS

Materials

Chitosan (Deacetylation degree DDA = 80%) was obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Camptothecin (CPT) obtained from Coral Drugs, New Delhi and β -Glycerophosphate (β -GP) and HP- β -Cyclodextrin (β -CD) were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Demineralized and double distilled water was used. All chemicals and reagents used were of analytical grade.

Table 1: Formulation table for temperature sensitive hydrogel

Formulation code	CPT (% w/v)	Chitosan (%w/v)	β - GP (%w/v)	HP- β -CD (%w/v)	HCl (M)
TF	0.5	-	-	-	0.1
TF13	0.5	1.0	7.0	0.5	0.1
TF14	0.5	1.0	7.0	1.0	0.1
TF16	0.5	1.5	7.0	0.5	0.1
TF17	0.5	1.5	7.0	1.0	0.1
TF19	0.5	1.0	8.0	0.5	0.1
TF20	0.5	1.0	8.0	1.0	0.1
TF22	0.5	1.5	8.0	0.5	0.1
TF23	0.5	1.5	8.0	1.0	0.1

Fourier Transform Infrared spectroscopy (FTIR)

It was performed, using a Perkin Elmer Spectrum Two spectrophotometer, to understand if there exists some interaction between drug and excipients. The spectra were obtained in the region from 4000 cm^{-1} to 650 cm^{-1} .

X-ray Diffractometry

The crystal X-ray scattering measurements for the obtained sample of Camptothecin and its formulation 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 ($\lambda = 1.541841\text{\AA}$), a voltage of 40 kV and a 100 mA current. Samples were scanned from 0–60° 2 θ for qualitative studies and the scanning rate was 4°/min.

Preparation of an autogelling chitosan solution

Chitosan/ β -GP

Chitosan solutions were prepared in 0.1 M hydrochloric acid at room temperature. The chitosan powders were progressively added to the solvent with stirring and mixture was stirred for a further 3 h. Sterile formulations were obtained by autoclaving (121°C, 20 min) [20]. To 18 ml of cooled chitosan solution, chilled β -GP aqueous solution (sterilized through a 0.22 μm filter) was carefully added drop wise to obtain clear and homogeneous liquid solutions in a final volume of 20 ml. This ratio of Chitosan: β -GP was had a thermogelling temperature of 37°C. The final solutions were mixed an additional 10 min at 4°C. The pH of the final cold solutions was 6.8.

Preparation of chitosan / Cyclodextrin Solution

Homogeneous clear chitosan/hydrochloric acid solutions were prepared then added HP- β -CD at room temperature by homogeneously dispersing the powdered HP- β -CD in chitosan solution under aseptic condition.

Preparation of chitosan/ β -GP/HP- β -CD loaded with camptothecin

Chitosan/ β -GP/HP- β -CD/CPT

Chitosan/ β -GP/HP- β -CD/CPT formulations were prepared at room temperature by homogeneously dispersing the powdered camptothecin in chitosan solutions under aseptic conditions. The β -GP solution was added slowly to the cooled camptothecin/chitosan dispersion under aseptic conditions. The final formulations were prepared according to Table 1.

Physicochemical characterization

Detection of CPT by HPLC

Quantitative analysis was performed on a Shimadzu LC 2010C HT HPLC chromatographic system equipped with an Auto sampler, a solvent module, Detector and a System HP ChemStations system. The column was a reverse-phase RP18 column. The HPLC system was eluted isocratically with methanol: water (63:37; v/v) at room temperature. The flow rate of the mobile phase was 1.0 ml/min and samples were measured at a wavelength of 370 nm. A standard curve was constructed by plotting peak area against concentration. The assay was found to be 98.20 %.

In-vitro Gelation and Viscosity Studies

The two main prerequisites of an *in situ* gelling system are viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity that will allow easy injectable into the body as a liquid (drops), which would undergo a rapid sol-to-gel transition. Additionally, to facilitate sustained release of drug to the tumoral tissue, the gel formed *in situ* should preserve its integrity without dissolving or eroding for a prolonged period of time. Viscosity of injected formulation is an important factor in determining residence time of drug in the injected area. The developed formulations were poured into the small sample adaptor of the Brookfield DV-II + Pro viscometer, RV spindle 6 and the angular velocity increased gradually from 0.5 to 50 rpm. The hierarchy of the angular

velocity was reversed. The average of the three readings was used to calculate the viscosity.

Standard calibration curve of Camptothecin

Accurately weighed 10 mg Camptothecin was dissolved in 100 ml of Phosphate buffer pH 7.4 to get the stock solution of 100 µg/ml. From this stock solution aliquots of 1 ml was withdrawn and get the stock solution of 10 µg/ml. from this stock solution aliquots of 1, 2, 3, 4, 5 & 6 ml were withdrawn and further diluted to 10 ml with buffer to obtain a concentrations range of 1, 2, 3, 4, 5 & 6 µg/ml. The absorbance of the solutions was measured at 285 nm by using UV-Vis spectrophotometer.

In vitro release of CPT from Hydrogel formulations

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its in vivo behavior. All the temperature sensitive and pH sensitive in situ gelling formulations of Camptothecin (CPT) 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.

Approx 1.2 inch length of the dialysis tube was taken and then soaked overnight in the phosphate buffer 7.4 pH. Now the amount of CPT equivalent to 10 mg of drug was calculated and placed in the dialysis tube whose ends were tied with a thread to prevent leakage. The dialysis tube bags were then placed in 100 ml of phosphate buffer 7.4 pH placed in the shaking water bath and maintained at 37°C with a frequency of 50 shakings per minute. Aliquots of 2 ml were withdrawn and filtered and sink condition maintained using phosphate buffer. The filtrate obtained was then suitably diluted 10 times (1 ml filtrate up to 10 ml) and the absorbance taken after scanning. The experiment was carried out in triplicate.

In vitro Cytotoxicity study of CPT formulations

MTT assay

The in vitro cytotoxicity of the CPT formulations was performed on the human breast cancer cell line MCF-7. The concentration of drug was 10 µg/ml used for in vitro studied. Sensitivity of MCF-7 cells to formulations was determined individually by the MTT colorimetric assay. Cells were seeded in a flat-bottomed 96-well plate and incubated for 24 h at 37°C and in 5% CO₂. The cell line was exposed to all formulations mentioned above. The solvent DMSO treated cells served as control. Cells were then treated with MTT reagent (20 µl/well) for 4 h at 37°C and then DMSO (200 µl) was added to each well to dissolve the formazan crystals. The optical density was recorded at 492 nm in a microplate reader. Percentage of residual cell viability was determined as $[1 - (\text{OD of treated cells} / \text{OD of control cells})] \times 100$.

RESULTS

Fourier Transform Infrared spectroscopy (FTIR) studies

The FTIR spectra of drug CPT, Chitosan, β-GP, HP-β-CD and Physical Mixture of CPT are shown in Figure 1. The FTIR studies showed that there no interactions between CPT and Excipients. The main characteristic peaks of CPT are at around 1750, 1460-1600, 1270-1290 cm⁻¹. We can see from the FTIR spectra between mixture of CPT, Chitosan, β-GP and HP-β-CD that no significant differences were shown.

In-vitro Gelation and Viscosity Studies

The gelation temperature and the viscosity of the formulated hydrogel are shown in table 2. The gelation temperature of the hydrogel formulation was in range of 34.6 to 37.4°C and the viscosity of the hydrogels was in range of 2312.0 cP to 5821.6 cP at 20 rpm and 25°C temperature which is effective for the syringeable of the formulation.

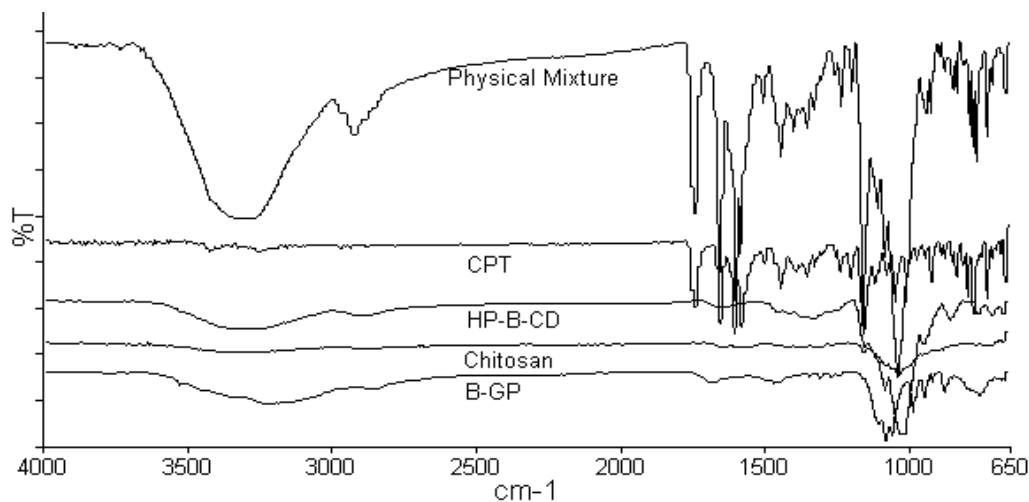


Fig. 1: FTIR Spectra of drug & excipients

Table 2: Gelation time & viscosity of formulation

Formulation code	pH	Gelation Temperature (°C)	Viscosity (cP) (20 rpm, 25°C)
TF	7.12	37.2±0.21	2473.3±16.35
TF13	6.96	38.1±1.42	2312.0±25.51
TF14	7.25	37.4±2.31	2981.2±11.39
TF16	6.91	34.6±0.82	3939.9±09.23
TF17	7.58	36.4±0.42	4548.3±22.85
TF19	7.19	35.6±0.70	2889.8±15.28
TF20	7.38	35.2±1.56	3880.0±28.88
TF22	7.27	36.3±2.43	5673.3±11.46
TF23	7.40	36.9±2.11	5821.6±14.62

The gelation time & viscosity data is (Mean ± SD, n=3) for formulation.

In vitro release of CPT from Hydrogel formulations

Chitosan/ β -GP/HP- β -CD was loaded with camptothecin 0.5% (w/v) and triplicate samples of polymer hydrogels were incubated in phosphate-buffered saline solutions pH 7.4, 37°C. At intervals, the supernatant fractions were removed and the medium replenished to maintain the sink conditions. The amount of drug in the supernatant samples was quantified by UV Spectrophotometer and the cumulative percentage of the loaded drug released in the supernatant fractions was studied versus time as shown in table 3. The amount of drug loaded initially in the polymer was confirmed by extraction of the polymer with methanol to release the residual camptothecin.

The graphical representation between percentage cumulative releases of camptothecin versus time is shown in Fig. 2. 38.97 % of drug was released from formulation code TF which was without HP- β -CD over 8 h. in buffer solution pH 7.4. And 92.56% of the drug was released from formulation code TF13, 77.17% of drug was released from formulation code TF14, 99.87 % of drug was released from formulation code TF16, 74.61% of drug was released from

formulation code TF17, 96.15% of drug was released from formulation code TF19, 87.43% of drug was released from formulation code TF20, 85.64% of drug was released from formulation code TF22 and 77.43% of drug was released from formulation code TF23. The percentage cumulative release of CPT code TF16 > TF19 > TF13 > TF20 > TF22 > TF24 > TF 14 > TF17 > TF from the HP- β -CD contains temperature sensitive hydrogel formulation.

The models fitting for the release profile of formulations by using various models shown in table 4. The transport mechanism of formulation TF and TF23 was found to be super case II transport and the best fit model was Higuchi Matrix. The transport mechanism of formulation TF13, TF14 and TF20 was found to be Non-Fickian diffusion and the best fit model was Zero order. The transport mechanism of formulation TF16 was found to be Fickian diffusion and the best fit model was Higuchi matrix. The transport mechanism of formulation TF17 was found to be Non Fickian diffusion and the best fit model was Hixson Crowell. The transport mechanism of formulation TF19 and TF22 was found to be Fickian diffusion and the best fit model was Zero order release.

Table 3: % cumulative drug release of formulation

Hrs.	TF	TF13	TF14	TF16	TF17	TF19	TF20	TF22	TF23
0.5	00.25±0.21	16.92±2.22	10.51±2.98	47.69±0.24	11.79±1.82	27.17±1.16	13.20±1.44	33.71±0.38	02.05±3.25
1.0	01.02±0.63	24.61±2.33	14.35±0.28	52.82±0.68	16.92±1.28	28.46±3.25	14.74±1.64	38.71±4.44	13.97±1.67
1.5	03.97±1.42	27.56±2.31	21.15±0.18	59.61±2.50	20.89±0.62	33.97±1.67	21.15±0.24	42.94±2.12	21.15±3.22
2.0	08.46±0.94	28.97±3.22	23.46±2.84	61.92±1.40	24.61±0.86	35.00±3.20	25.00±2.33	46.53±3.22	24.23±1.82
2.5	11.66±2.70	32.69±1.12	26.28±1.16	64.74±0.48	31.92±2.12	42.94±1.20	30.12±2.31	49.35±1.12	32.69±4.88
3.0	12.94±0.56	37.17±2.98	29.35±3.25	65.25±0.76	35.76±3.25	50.12±0.88	37.17±1.98	50.00±2.90	39.61±2.46
3.5	16.92±0.68	39.87±1.16	33.46±1.67	78.33±2.50	39.1±1.67	52.69±0.76	39.87±1.66	52.94±3.22	40.89±2.33
4.0	19.48±0.46	41.66±2.80	36.53±3.20	80.12±0.28	42.94±3.20	54.48±1.45	42.05±3.48	54.48±1.24	44.23±2.31
4.5	25.51±1.90	51.02±2.30	40.76±1.20	80.51±0.42	51.02±2.20	63.84±2.22	47.56±4.22	63.84±2.82	51.28±2.98
5.0	25.89±1.20	56.66±4.50	49.74±0.42	83.58±3.22	51.66±1.94	64.10±4.22	51.53±3.60	65.64±3.24	52.17±0.88
5.5	33.46±3.38	62.17±3.40	53.20±0.88	94.23±1.92	55.76±2.78	68.58±0.65	56.53±1.26	67.30±2.42	55.76±4.22
6.0	34.35±2.41	65.12±2.50	58.71±1.14	97.17±0.92	58.71±0.52	71.53±0.66	61.28±4.84	70.25±2.88	59.10±3.36
6.5	35.51±1.46	75.89±2.42	61.79±0.82	98.33±1.82	63.07±0.68	75.89±1.84	63.71±2.32	72.05±0.82	63.71±0.12
7.0	38.07±1.60	80.89±1.35	69.35±0.84	98.71±2.32	64.23±3.22	83.46±1.40	64.23±3.25	77.05±2.33	66.92±2.94
7.5	38.84±0.88	88.07±1.62	70.12±1.32	99.74±1.66	69.61±1.12	95.76±2.24	81.15±1.67	82.94±2.31	72.69±2.70
8.0	38.97±1.22	92.56±1.12	77.17±1.42	99.87±1.48	74.61±1.92	96.15±1.24	87.43±2.64	85.64±2.98	77.43±0.52

The % cumulative drug release data (Mean \pm SD, n=3) for formulations.

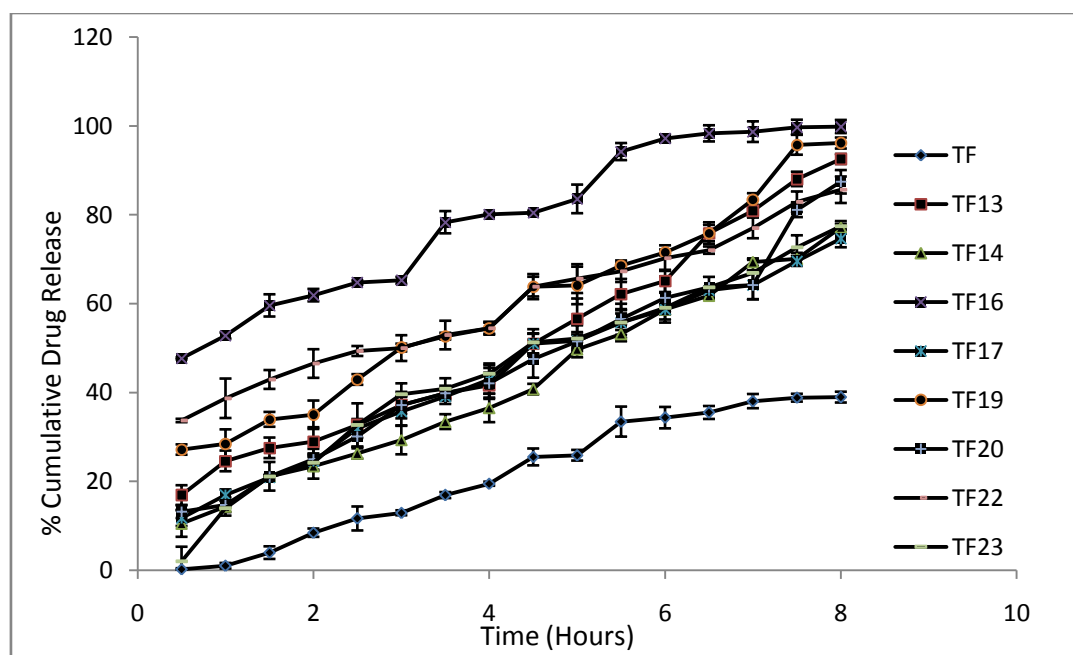


Fig. 2: In-vitro drug release of formulation, the % cumulative drug release data (Mean \pm SD, n=3) for temperature sensitive hydrogel formulations.

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

Code	Zero Order R ²	First Order R ²	Higuchi Matrix R ²	Hixson- Crowell R ²	Korsmeyer- Peppas n	R ²	Transport Mechanism
TF	0.978	0.980	1.000	0.980	1.780	0.953	Super case II transport
TF13	0.977	0.838	0.914	0.903	0.478	0.931	Non -Fickian
TF14	0.990	0.943	0.947	0.965	0.684	0.974	Non -Fickian
TF16	0.959	0.844	0.965	0.947	0.197	0.945	Fickian diffusion
TF17	0.991	0.982	0.985	0.992	0.675	0.991	Non -Fickian
TF19	0.983	0.767	0.942	0.874	0.371	0.929	Fickian diffusion
TF20	0.979	0.846	0.941	0.909	0.655	0.968	Non -Fickian
TF22	0.987	0.931	0.958	0.960	0.234	0.938	Fickian diffusion
TF23	0.970	0.866	0.974	0.928	1.176	0.907	Super case II transport

Statistical Analysis

All observations were presented as Mean \pm SD (standard deviation). The data was analyzed by student's t-test. $P < 0.05$ was considered as significant.

In vitro Cytotoxicity study of CPT formulations

To evaluate its antitumor efficacy camptothecin formulated in chitosan/ β -GP/HP- β -CD was intratumorally using a MCF-7 breast tumor cell model. The MCF-7 tumor has proven to be a useful model for preliminary screening of various compounds

for efficacy because of its reproducible growth, non-immunogenicity in the syngeneic host and low frequency of spontaneous metastases. The effect of the camptothecin containing biodegradable polymer on tumor percentage growth inhibition was examined. The results of these studies are shown in Fig. 3. The hydrogel containing 0.5% (w/v) camptothecin was found to be more effective than without hydrogel delivered camptothecin in delaying percentage growth inhibition. Tumors injected with blank chitosan/ β -GP/HP- β -CD showed no inhibition of growth as untreated tumors, confirming that the hydrogel alone has no effect on the growth of this tumor.

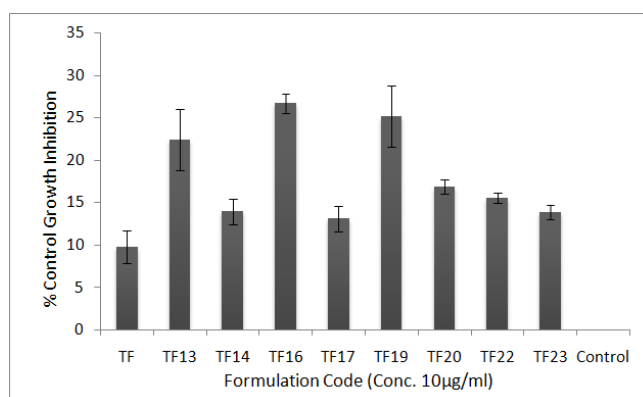


Fig. 3: In-vitro study on MCF-7 Tumor Cell line, % control growth inhibition data (Mean \pm SD, n=3) for temperature sensitive hydrogel formulations.

The greater effectiveness of the hydrogel formulation code TF16 is due to fast release of the drug in the tumor and the exposure of tumor cells to drug concentrations for a period of time which causes more cell death. The formulation TF16 showed 26.73% of tumor growth inhibition. The formulation TF showed 9.8% of tumor growth inhibition, TF13 showed 22.39% of tumor growth inhibition, TF14 showed 13.96% of tumor growth inhibition, TF16 showed 26.73% of tumor growth inhibition, TF17 showed 13.13% of tumor growth inhibition, TF19 showed 25.23% of tumor growth inhibition, TF20 showed 16.91% of tumor growth inhibition, TF22 showed 15.55% of tumor growth inhibition, TF23 showed 13.88% of tumor growth inhibition, and Blank showed no tumor growth inhibition. The percentage tumor growth inhibition of formulation code showed as TF16 > TF19 > TF13 > TF20 > TF22 > TF14 > TF23 > TF17 > TF and no inhibition by blank formulation from the formulation.

DISCUSSION

We selected camptothecin as a model drug for this study, because its insolubility in water makes it difficult to administer systemically by other means and because of the potential applications of camptothecin and the insoluble camptothecin analogues in chemotherapy. Additionally, the pharmacologically important lactone ring of camptothecin and its analogs is unstable in the presence of human serum albumin which results in the conversion of the active drug to the inactive carboxylate form bound to albumin

[21]. This imposes a severe pharmacokinetic limitation on the systemic use of camptothecin and related compounds. An approach to overcoming this and other shortcomings of camptothecin and its analogs, especially their high systemic toxicity is to load it into a delivery system such as a chitosan based formulations which will protect the drug from hydrolysis and control its release over a prolonged period.

There are three primary mechanisms for the loaded drug to be released from hydrogels: swelling, diffusion and degradation. Drug release from chitosan/ β -GP gel with initial water content of 84% (w/w) occurs through the diffusion of water through the polymeric matrix and dissolution of the soluble fraction of the drug. Water is taken up by hydrogels immediately after being exposed to an aqueous media, the rate of water uptake depending on the hydrophilicity of the polymer. In a study of the release of compounds from chitosan/ β -GP/HP- β -CD gels it was found that release occurred largely by diffusion. The physical three-dimensional structure of the hydrogel does not change with time suggesting there is no substantial erosion of the polymer matrix. The third mechanism, which involves degradation of the polymer matrix, would only occur under in vivo conditions as a result of enzyme activity.

It is known that chitosan with block structures and lower degrees of deacetylation (DDA=80%) are more readily biodegraded due to the presence of blocks of glucosamine moieties containing acetyl groups that serve as a substrate for lysozyme [22, 23]. In this study

estimation of % tumor growth inhibition of CPT or chitosan/ β -GP/HP- β -CD/CPT was based on changes in tumor weight following hydrogel formulation. Several published studies describe the effect of formulation of chitosan/ β -GP on the histology of the surrounding tissue. The effect of formulation in normal tissue has been described by Molinaro et al. [24] as a mild non-specific inflammatory reaction.

In the present study the hydrogel was injected into the tumor. A report of the effect of chitosan/ β -GP/HP- β -CD/CPT injected into the MCF-7 tumor described the histology of the injected tumors as showing some degree of necrosis interspersed between viable tumor tissues with necrosis generally decreasing away from the center of the tumor. This pattern was seen for both CPT injected tumors and for those injected with chitosan/ β -GP/HP- β -CD/CPT. In the case of the MCF-7 tumor CPT without excipients appears to have less tumoricidal effect. Chitosan/ β -GP/HP- β -CD/CPT has been shown to activate macrophages for tumoricidal activity in MCF-7. Again, since we found the difference in the % tumor growth inhibition between the CPT and with chitosan/ β -GP/ β -CD/CPT. The effectiveness of the polymer hydrogel in delaying tumor growth clearly demonstrates the importance of this delivery system in maintaining an inhibitory level of drug over a long period of time. The main advantages of the biodegradable polymer implant such as chitosan/ β -GP/HP- β -CD used for the delivery of camptothecin to the mouse tumor are the high intra-tumoral concentrations of drug attainable, low systemic toxicity and the extended period of time over which the drug can be released in the tumor. The dose of camptothecin delivered using the hydrogel was 10 μ g/ml, which is 3 times the mean dose for MCF-7 cell, for the hydrogel the delayed release of the drug and localization in the tumor prevents toxic systemic levels being reached.

CONCLUSION

Local deliveries of chemotherapeutic agent by controlled release polymers are a new strategy with the potential to maximize the anti-tumor 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 camptothecin locally to a mouse tumor model. Growth of tumors treated in this fashion was retarded for significantly longer periods than were tumors treated with systemically administered camptothecin.

The system formulated with camptothecin was found to be stable and the release profiles of a formulation with chitosan, β -GP and HP- β -CD showed all most effective release kinetics. These findings show chitosan/ β -GP/HP- β -CD hydrogel to be a safe, effective, homogeneous, injectable and stable formulation for delivery of camptothecin and this approach represents an attractive technology platform for the delivery of other clinically important hydrophobic 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. Drug release of CPT from the hydrogel was found to be too rapid due to the hydrophilic nature of the drug and the small size of the molecules compared to that of the pore size in the hydrogel.

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