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Research Article

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Formulation and Evaluation of an Injectable *In-Situ* Forming Hydrogel of Dacarbazine as Anticancer Agent

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Abstract Cancer is currently one of the leading causes of death worldwide. Anticancer drugs can be given either by the conventional drug delivery systems including solid dosage forms, Injectable dosage forms and infusions or using the Novel drug delivery systems including targeted drug delivery dosage forms such as liposomes and nanoparticles etc.The objective of present investigation is to formulate and evaluate the *In situ* forming mainly Temperature induced and pH induced gelling Injectable hydrogels of an anticancer drug Dacarbazine using delivery vehicle Chitosan.All selected temperature induced and pH induced Hydrogels showed sustained drug release for a period of 10 hours. Optimized Formulation showed maximum percent drug release. In results it was found that optimized formulations are safe, effective, homogeneous, injectable and stable for delivery of Dacarbazine and this approach represents an attractive technology platform for the delivery of other clinically important Anticancer drugs.

Keywords Anticancer drugs, Dacarbazine, Temperature induced, pH induced, Injectable hydrogels

Introduction

Cancer is the leading cause of deaths worldwide. Every year millions of people died of cancer either due to lack of treatments or due to highly expensive and painful treatment. Conventional dosage forms are subjected to so many limitations. Most of the drug content is released soon after administration, causing drug levels in the body to raise rapidly, peak and then decline sharply, leading to unacceptable side effects at the peaks and inadequate therapy at the troughs [1-5]. Due to the short period of actions, repeated injections are often required, which can lead to exacerbation of side effects and inconvenience. Anticancer drugs can be given either by the conventional drug delivery systems including solid dosage forms, Injectable dosage forms and infusions or by the Novel drug delivery systems including targeted drug delivery dosage forms such as liposomes and nanoparticles etc [6-10].
The smartness of any material is the key to its ability to receive, transmit or process a stimulus, and respond by producing a useful effect. 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 [11-16].

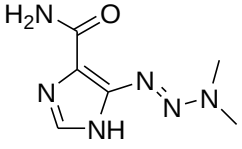

Figure 1: Structure of Dacarbazine

The objective of present investigation is to formulate and evaluate the *In situ* forming mainly Temperature induced and pH induced gelling Injectable hydrogels of an Anticancer drug 'Dacarbazine' which is ananti-metabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid; in order to render it target specific in nature and to maintain its high concentration at the target site for an extended time

period. Dacarbazine, Figure 1, is a synthetic analog of naturally occurring purine precursor 5-amino-1*H*-imidazole-4-carboxamide (AIC) [17-20].

Material and Methods

Materials

Dacarbazine was purchase from Zydus Cadilla, Mumbai, India, Chitosan was purchase from SD Fine Chemical Limited, New Delhi, β-Glycero Phosphate from Central Drug House, New Delhi and Glycerol Mono Oleate from Esteel Chemicals Pvt. Ltd. Ahmadnagar. Equipments and instruments used in Stimuli sensitive Hydrogels Preparation are Electrical balance (Essae Technologies Pvt. Ltd.), Digital pH meter and USP-I Dissolution Apparatus, Model 912 (Elico India Ltd), Brookfield Viscometer (Brookfield Engineering Laboratories Inc., USA), UV-Vis spectrophotometer, UV-1700 (Shimadzu Corporation, Kyoto, Japan) and FTIR (Perkin Elmer) etc.

Methods

Pre-formulation studies

Pre-formulation studies gives the information needed to define the nature of the drug and provide a framework for the drug combination with pharmaceutical excipients in the dosage form [21-23]. In Pre-formulation we have study the melting point using capillary method, Solubility studies in distilled Water, methyl alcohol and acetone., X-ray diffractometry for determine the solid structure of Drug, FT-IR Spectroscopy for compatibility between drug and polymers and the absorbance of the solutions was measured at λ_{max} 258 nm using UV-Vis spectrophotometer. A graph of Concentration vs. Absorbance was plotted for determination of percentage purity of drug.

Method for *In situ* Hydrogel Preparation

In- situ hydrogels were prepared by using Dacarbazine. The delivery vehicle used was Chitosan which has both thermos sensitive and pH sensitive properties. Chitosan solution formulated at physiological pH remains liquid at low temperature and turn into gel when heated; this property of chitosan was used for the preparation of Temperature sensitive Hydrogels. At the same time , acidic solutions of chitosan when exposed to alkaline pH or body biological pH lose this charge and form viscous gels, Hence it can also be used for preparation of pH sensitive Hydrogels [24-26].

For the preparation of Temperature Sensitive Hydrogels, Chitosan solution of desired concentration was prepared 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 pH 7.4. 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, solution were than sterilized by autoclaving at 120 °C for about 30 minutes. The final solutions were mixed an additional 10 min at 4 °C and were filtered by membrane filtration using cellulose membrane. Formulation (TF) design for preparation of Temperature sensitive Hydrogels is reported in Table 1**.**

Table 1: Formulation design for Temperature sensitive Hydrogels

Contents	Quantity (% w/w)				
	TF1	**TF2**	**TF3**	**TF4**	**TF5**
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	QS	QS	QS	QS
	10 gm	10 gm	10 gm	10 gm	10 gm

For the preparation of pH Sensitive Hydrogels, weighed quantity of Drug and Chitosan in desired concentration wasstirred with suitable quantity of 0.33 M Citric acid for 3 hours. This solution was cooled to 4 °C. To cooled Chitosan and Drug solution Glycerol Mono Oleate in desired amount 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 °C and were filtered by membrane filtration using cellulose membrane [27-28]. Prepared

pH sensitive formulations were than sterilized by autoclaving at 120 °C for about 30 minutes. Formulation (PF) design for preparation of pH sensitive Hydrogels is reported in Table 2**.**

Table 2: Formulation design for pH sensitive Hydrogels

Contents	Quantity (% w/w)				
	PF6	**PF7**	**PF8**	**PF9**	**PF10**
Drug	5	5	5	5	5
Chitosan	0.7	0.7	0.5	0.5	0.7
Glycerol mono Oleate	3	4	3	4	7
0.33M Citric acid	QS	QS	QS	QS	QS
Phosphate buffer pH 7.4	QS	QS	QS	QS	QS
	10 gm	10 gm	10 gm	10 gm	10 gm

Evaluation of Prepared formulations

Prepared formulations were evaluated using the parameters of pH**,** Drug content**,** *In vitro* gelation studies, *In vitro* viscosity studies, *in vitro* release studies, Drug Release Kinetics studies**,** Sterility**,** Pyrogens Testing and Stability studies etc.

All the developed Hydrogel formulations were evaluated for pH by using digital pH meter. The drug content of all the formulation was determined by diluting 1 ml of the formulation to 100 ml with pH 7.4 phosphate buffer. Aliquot of 1 ml was withdrawn and further diluted to 10 ml with buffer. Dacarbazine concentration was then determined at 258 nm by using UV-Vis spectrophotometer (UV-1700).The gelling capacity of all the formulation 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 °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 h. 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 7-10 h. 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 h. The viscosity of prepared pH sensitive and Temperature sensitive Hydrogel formulations measured at 10 rpm was used for purposes of determining residence time of drug in the body.

The *in vitro* release of Dacarbazine in all the 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 [29-30]. 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 at 258 nm.

To find out the mechanism of drug release, 60 % drug of release data was first fitted in the Korsmeyer-Pappas model. Where Log of cumulative percent drug released was plotted against Log Time. 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.

For pyrogens testing three rabbits (2.50 kg) were selected. Formulation in a dose of 10 ml/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 h. and rise in temperature was determined. Two formulations (one from each) from Temperature induced and pH induced gelling formulations were subjected to stability studies at ambient humidity conditions at 2 to 8 °C, room temperature (25 °C), 37 °C and 60 °C 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

Melting point of Dacarbazine was found to be in the range of 214-216 ºC. Dacarbazine was found to be freely soluble in water and methanol.Powder X-ray Diffraction Pattern of Dacarbazine is indicate the crystal structure,

reported in figure 2, using UV-Vis spectrophotometer λ_{max} of Dacarbazine was found to be 287 nm. UV spectrum and calibration curve in Phosphate Buffer solution pH 7.4 is reported in Figure 3.

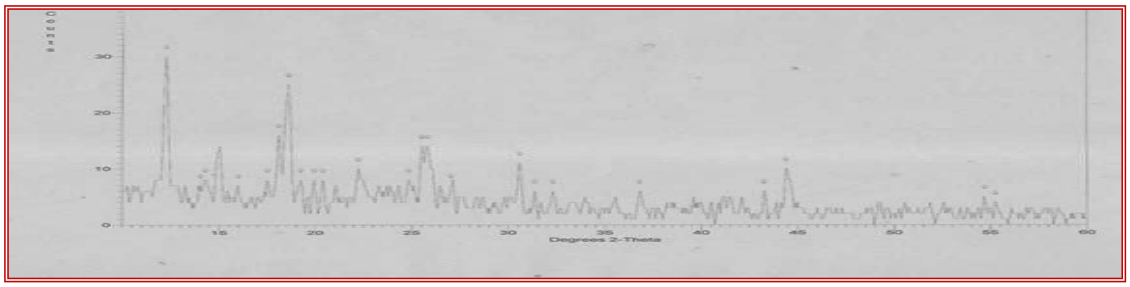

Figure 2: Powder X-ray Diffraction Pattern of Dacarbazine

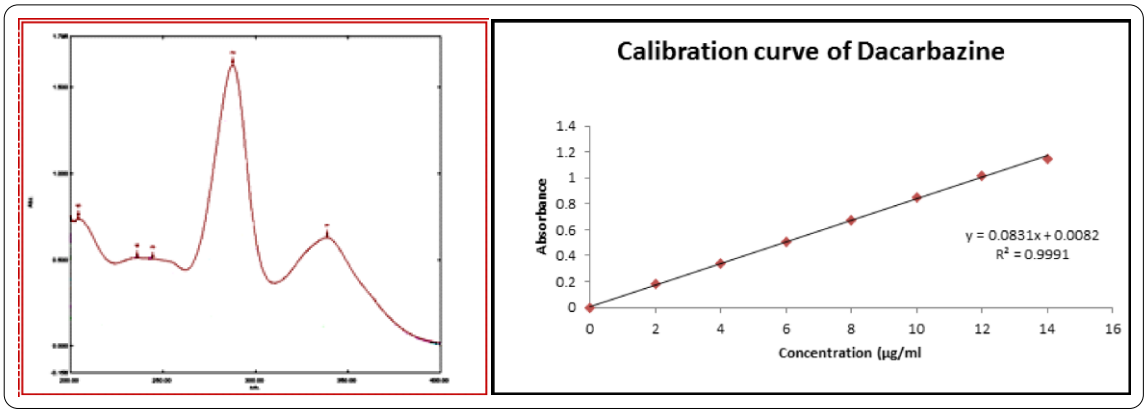

Figure 3: Spectrum diagram and calibration curve of Dacarbazine

There were no Compatibility of pure drug Dacarbazinewas found with the polymers Chitosan, β-Glycerophosphate and Glycerol Monooleate. The individual FT-IR spectra of the pure drug and Physical mixtures of Dacarbazinewith polymers are reported in the Figure 4. The pH of the formulations was found to be satisfactory and was in the range of 6-7.4, Table 3.The drug content was found to be in acceptable range for all the formulations. Percent drug content of Temperature Sensitive formulations TF1,TF2,TF3,TF4 and TF5 was found to be 97.4%,95.05%, 97.3%,97.9% and 95.15% respectively while pH Sensitive formulations PF6, PF7, PF8, PF9 and PF10 was found to be 98.05%,97.25%, 98.1%, 98.35% and 98.85% respectively, Table 3.

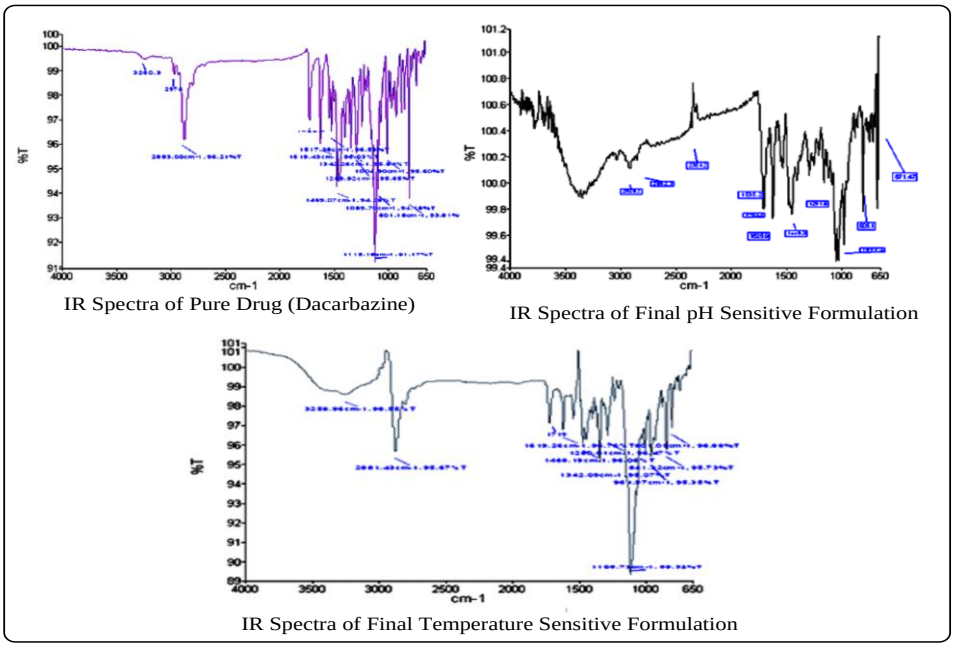

Figure 4: FT-IR spectra of Dacarbazine and Formulation.

Table 3: pH and Percent Drug Content pH Sensitive and Temperature Sensitive Hydrogels

pH Sensitive Formulations	pH	%Drug Content ± S.D	Temperature Sensitive Hydrogels	pH	%Drug Content ± S.D
TF1	7.2	97.4 ± 0.85	PF6	6.1	98.05 ± 0.21
TF2	7.3	95.05 ± 0.21	PF7	6.2	97.25 ± 0.78
TF3	7.0	97.3 ± 0.85	PF8	6.0	98.1 ± 0.14
TF4	7.2	97.9 ± 0.42	PF9	6.2	98.35 ± 0.35
TF5	7.3	95.15± 0.92	PF10	6.1	98.85 ± 0.49

Results of gelling capacity of all Temperature Sensitive and pH Sensitive formulationsare reported in table 4.3D Response surface plots, Fig. 5,of viscosity indicate ofallpH Sensitive Hydrogels that viscosity increased in proportion with viscofying agent both at lower and higher concentration of gelling agent. On the basis of gelling capacity and viscosity PF7, PF8 and PF9 and PF10 showed optimum results within the desired range. Hence, these four pH Sensitive formulations were subjected for further evaluation parameters. Temperature Sensitive Hydrogels, Formulations TF2, TF3 and TF4 showed optimum results for gelling capacity and viscosity Hence three Temperature Sensitive formulations were selected and subjected for further evaluation parameters.

Table 4: *In vitro* Gelling capacity and viscosity studies of Temperature Sensitive Hydrogels and pH sensitive Formulation

Temperature Sensitive Formulation code	Gelling Capacity	Viscosity at 10rpm (Pa-s)	pH sensitive Formulation code	Gelling Capacity Y2	Viscosity at 10rpm (Pa-s)
TF1	++	1.876	PF6	++	2.993
TF2	+++	3.645	PF7	+++	2.856
TF3	+++	3.954	PF8	+++	2.947
TF4	+++	4.382	PF9	+++	3.364
TF5	++	2.205	PF10	+++	3.482

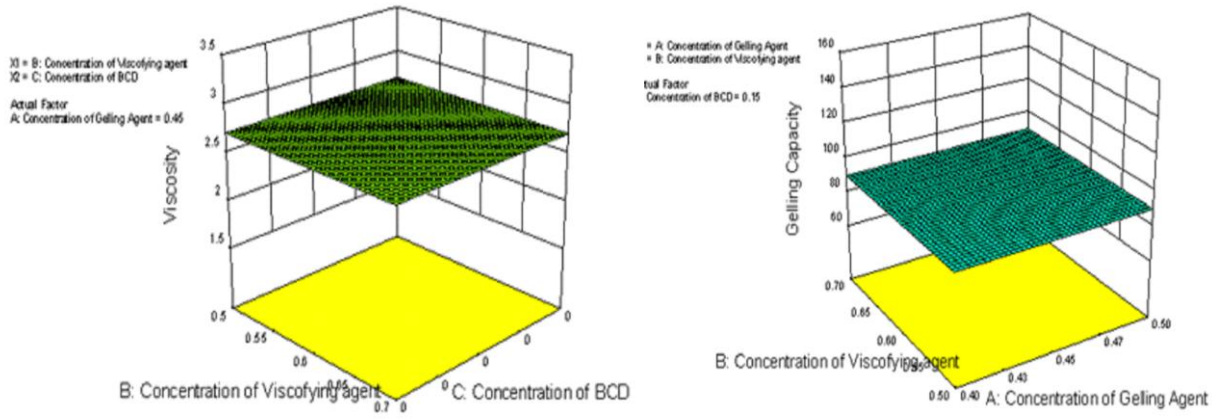

Fig. 5: 3D response surface plot for viscosity and Gelling Capacity in pH Sensitive Hydrogels

In vitro release profile of selected Temperature Sensitive and pH Sensitive formulations of Dacarbazine is reported in Table 5 and Figure 6. It was found that cumulative percent drug release was 79.97 %, 84.31 % and 86.22 % for formulation PF8, PF9 and PF10 and 87.91 %, 88.22 % and 91.19 % for formulation TF2, TF3 and TF4 respectively after 10 hours. The *in vitro* release data indicated that the formulations TF4 and PF10showed better sustained effect than corresponding Temperature Sensitive and pH Sensitive formulations.

Table 5: *In Vitro* Drug Release Profile of Optimized Formulation

Time (h.)	Formulation					
	Mean % cumulative release					
	TF2	TF3	TF4	PF8	PF9	PF10
0.25	10.03	13.95	12.04	16.7	17.44	17.96
0.5	15.11	21.78	15.64	20.4	23.47	21.25
0.75	22.41	28.97	20.4	24.21	29.18	29.82
1	28.87	36.69	28.76	29.93	35.74	36.69
2	35	41.25	37.86	33.52	41.57	41.56
3	40.08	48.34	42.52	40.29	46.33	47.28
4	46.75	53.52	48.97	46.75	52.68	54.68
5	52.36	59.45	56.17	51.83	58.81	59.76
6	59.02	64.63	64.1	55.53	63.36	65.37
7	65.05	70.13	68.97	59.34	69.18	71.19
8	71.19	77.96	75.21	65.48	74.47	76.48
9	79.87	82.72	85.26	68.76	81.77	83.68
10	87.91	88.22	91.19	79.97	84.31	86.22

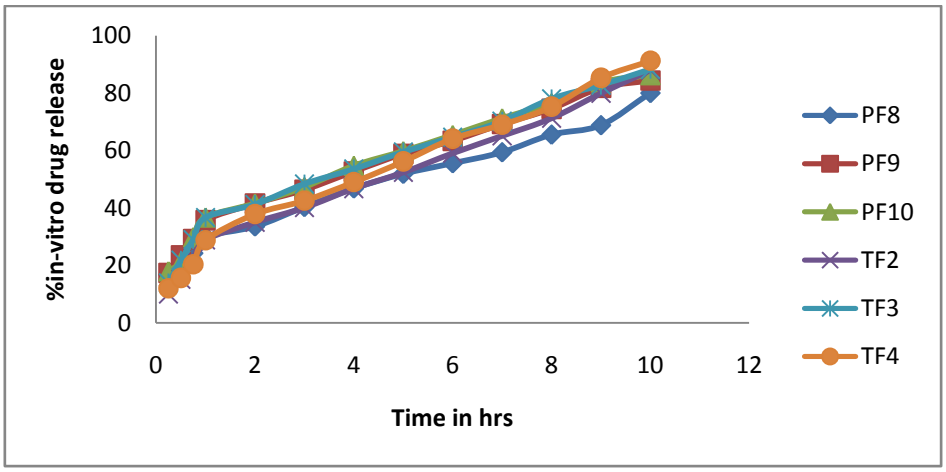

Figure 6: Graph for % in vitro drug release

The regression coefficient (r) and 'n' values of zero order, first order, Higuchi matrix, Pappas and Hixson-Crowell are tabulated in Table 6**,** for all optimized formulations. From the table it is clear that Temperature Sensitive Hydrogels the best fit model was Zero order, while 'n' exponent value for is b/w 0.5 to 0.8 indicating that formulation is released by non fickian diffusion mechanism or by anomalous diffusion in a controlled manner andin the case of pH sensitive Hydrogels the best fit model was Zero order suggesting swelling controlled diffusion.

Table 6: Model fitting for the Release Profile of optimized formulation Using 5 Different Models

Formulaiton	r^2				Korsmayer-Pappas		Best Fit model	Release mechanism
	Hixson Crowell	Zero Order	Higuchi Matrix	First Order	r^2	n		
TF2	0.998	0.952	0.982	0.998	0.978	0.63	Zero	Non Ficknian
TF3	0.997	0.973	0.992	0.998	0.989	0.53	Zero	Non Ficknian
TF4	0.999	0.948	0.982	0.985	0.977	0.597	Zero	Non Ficknian
PF8	0.997	0.974	0.979	0.978	0.983	0.452	Zero	Ficknian
PF9	0.998	0.986	0.991	0.986	0.996	0.451	Zero	Ficknian
PF10	0.998	0.979	0.990	0.991	0.992	0.465	Zero	Ficknian

Results of Pyrogens Testing is reported in table 7, it was found that Temperature Sensitive formulations (less than 0.6). Hence formulations passed the Pyrogens test.

Table 7: Pyrogens testing of Stimuli Sensitive Injectable Formulations

Formulations	S. No. of rabbit	Rise in Temperature (°C)			Average rise in Temperature in the period of three hours
		After 1 h	After 2 h	After 3 h	
Blank	Rabbit	0.1	0.1	0.0	0.06
PF10	Rabbit-A	0.2	0.3	0.4	0.3
	Rabbit-B	0.5	0.7	0.6	0.6
TF4	Rabbit-C	0.3	0.5	0.4	0.4
	Rabbit-D	0.5	0.6	0.4	0.35

From the stability studies it was confirmed that Stimuli Sensitive formulations of Dacarbazine remained most stable at ambient temperature (25 °C) and humidity, Table 8.

Table 8: Stability studies of optimized formulation

Formulations	Storage Conditions	Percent Drug Content (%)		
		7 Days	15 Days	30 Days
TF4	5±3 °C, 60± 5% (Ambient Humidity)	98.97	95.48	92.39
	25±2 °C, 60±5 % (Ambient Humidity)	98.79	96.64	94.28
	37±2 °C, 75±5% Relative Humidity	98.26	95.91	92.75
	60±5 °C	96.59	93.72	82.91
PF10	5±3 °C, 60± 5% (Ambient Humidity)	98.97	95.48	92.39
	25±2 °C, 60±5 % (Ambient Humidity)	98.79	96.64	94.28
	37±2 °C, 75±5% Relative Humidity	98.26	95.91	92.75
	60±5 °C	96.59	93.72	82.91

Conclusions

Preformulation studies showed that there is no interaction b/w drug and excipients to formulate the in-situ forming hydrogels. The drug content of the prepared formulations was within the acceptable range, and ensures dose uniformity. All selected temperature induced and pH induced Hydrogels showed sustained drug release for a period of 10 hours. Formulation TF4 and PF10 showed maximum percent drug release.

The Drug release Kinetics studies it was observed that all the selected temperature induced and pH 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. Results of pyrogens test confirmed that all the selected formulations were sterile and Pyrogens free.All these findings show chitosan/GP and chitosan/GMO gel to be a safe, effective, homogeneous, injectable and stable formulation for delivery of Dacarbazine and this approach represents an attractive technology platform for the delivery of other clinically important Anticancer drugs.

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References

1. Adriana, B., Rochada. (2001). Natural Products in Anticancer Therapy, *Current Opinion in Pharmacology*, 1:364–369
2. Aikawa, K., Mitsutake, N., Uda, H., Tanaka, S., Shimamura, H., Aramaki, Y., & Tsuchiya, S. (1998). Drug release from pH-response polyvinylacetal diethylaminoacetate hydrogel, and application to nasal delivery. *International journal of pharmaceutics*, *168*(2), 181-188.
3. Raff, M., Alberts, B., Lewis, J., Johnson, A., & Roberts, K. (2002). Molecular Biology of the Cell 4th edition.
4. Alexandridis, P., & Lindman, B. (2000). Amphiphilicblock polymers:Self-Assembly and Applications, *Amsterdam*, 448.

5. Andras, G.L., Don, S., & Walter, J.M. (2007). Repositioning of Anticancer Drugs Using Lipoprotein-based Formulations. *Drug Repositioning*, 47-48.
6. Andreas, W., & Gerd, S. (1998). Antitumor Activity of Methotrexate-Albumin Conjugates in Rats Bearing A Walker-256 Carcinoma, *International Journal of Cancer*, 76, 884–890.
7. Anita, L., & Piyun Chao. (2006). Pharmacokinetic and Pharmacodynamics Evaluation of a Novel in Situ Forming Poly(ethylene glycol)-Based Hydrogel For the Controlled Delivery of the Camptothecins, *Journal of Controlled Release, 112*, 333–342
8. Armitage, P., & Doll, R. (1954). The age distribution of cancer and a multi-stage theory of carcinogenesis", *British Journal of Cancer*, *8*, 1-12
9. Aungst, B.J., & Nguyen, N. (1994). Improved oral bioavailability of an HIV protease inhibitor using Gelucire 44/14 and Labrasol vehicles, *Bulletin Technique Gattefosse, 87*, 49–54. .
10. Aungst, B. J. (1993). Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or presystemic metabolism. *Journal of pharmaceutical sciences*, *82*(10), 979-987.
11. Baron, M., & Valin, I. (1990). Bases and techniques de l'oncochirurgie. Rec, *Médicinal. Vétenary Special Cancer*, *11*(166), 999.
12. Bromberg, L. E., & Ron, E. S. (1998). Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. *Advanced drug delivery reviews*, *31*(3), 197-221.
13. Bue, P., Wester, K., SJOstrom, A., Holmberg, A., Nilsson, S., Carlsson, J., ... & Malmstrom, P. U. (1998). Expression of epidermal growth factor receptor in urinary bladder cancer metastases. *The Journal of Urology*, *160*(5), 1937-1938.
14. Cappello, J., Crissman, J. W., Crissman, M., Ferrari, F. A., Textor, G., Wallis, O., ... & Stedronsky, E. R. (1998). In-situ self-assembling protein polymer gel systems for administration, delivery, and release of drugs. *Journal of Controlled Release*, *53*(1), 105-117.
15. Chan, W. C., Maxwell, D. J., Gao, X., Bailey, R. E., Han, M., & Nie, S. (2002). Luminescent quantum dots for multiplexed biological detection and imaging. *Current opinion in biotechnology*, *13*(1), 40-46.
16. Hiemstra, C., Zhong, Z., Van Tomme, S. R., van Steenbergen, M. J., Jacobs, J. J., Den Otter, W., ... & Feijen, J. (2007). In vitro and in vivo protein delivery from in situ forming poly (ethylene glycol)–poly (lactide) hydrogels. *Journal of controlled release*, *119*(3), 320-327.
17. Huynh, C. T., Nguyen, M. K., & Lee, D. S. (2011). Biodegradable pH/temperature-sensitive oligo (β-amino ester urethane) hydrogels for controlled release of doxorubicin. *Acta biomaterialia*, *7*(8), 3123-3130.
18. Conover, C. D., Pendri, A., Lee, C., Gilbert, C. W., Shum, K. L., & Greenwald, R. B. (1996). Camptothecin delivery systems: the antitumor activity of a camptothecin-20-0-polyethylene glycol ester transport form. *Anticancer research*, *17*(5A), 3361-3368.
19. Counsell, R. E., & Pohland, R. C. (1982). Lipoproteins as potential site-specific delivery systems for diagnostic and therapeutic agents. *Journal of medicinal chemistry*, *25*(10), 1115-1120.
20. Curnis, F., Sacchi, A., Borgna, L., Magni, F., Gasparri, A., & Corti, A. (2000). Enhancement of tumor necrosis factor α antitumor immunotherapeutic properties by targeted delivery to aminopeptidase N (CD13). *Nature biotechnology*, *18*(11), 1185-1190.
21. Dagani, R. (1997). Intelligent gels, *Chemistry and Engineering News archive*, *75*, 26–36.
22. Kwon, D. Y., Lee, B. N., Seo, H. W., Kwon, J. S., Lee, B., Han, D. K., ... & Kim, M. S. (2012). Injectable in situ-forming hydrogels for a suppression of drug burst from drug-loaded microcapsules. *Soft Matter*, *8*(29), 7638-7648.
23. Huynh, D. P., Nguyen, M. K., Pi, B. S., Kim, M. S., Chae, S. Y., Lee, K. C., ... & Lee, D. S. (2008). Functionalized injectable hydrogels for controlled insulin delivery. *Biomaterials*, *29*(16), 2527-2534.
24. Horák, D., Švec, F., Adamyan, A., Titova, M., Skuba, N., Voronkova, O., ... & Gumargalieva, K. (1992). Hydrogels in endovascular embolization: V. Antitumour agent methotrexate-containing p [HEMA]. *Biomaterials*, *13*(6), 361-366.
25. Karnofsky, D. A. (1968). Mechanism of action of anticancer drugs at a cellular level. *CA: a cancer journal for clinicians*, *18*(4), 232-234.
26. Dhanikula, A. B., & Panchagnula, R. (1999). Localized paclitaxel delivery. *International Journal of pharmaceutics*, *183*(2), 85-100.
27. Missirlis, D., Kawamura, R., Tirelli, N., & Hubbell, J. A. (2006). Doxorubicin encapsulation and diffusional release from stable, polymeric, hydrogel nanoparticles. *European journal of pharmaceutical sciences*, *29*(2), 120-129.

28. Guo, D. D., Xu, C. X., Quan, J. S., Song, C. K., Jin, H., Kim, D. D., ... & Cho, C. S. (2009). Synergistic anti-tumor activity of paclitaxel-incorporated conjugated linoleic acid-coupled poloxamer thermosensitive hydrogel in vitro and in vivo. *Biomaterials*, *30*(27), 4777-4785.
29. Li, F., Ba, Q., Niu, S., Guo, Y., Duan, Y., Zhao, P., ... & Sun, J. (2012). In-situ forming biodegradable glycol chitosan-based hydrogels: Synthesis, characterization, and chondrocyte culture. *Materials Science and Engineering: C*, *32*(7), 2017-2025.
30. Floyd, A. G. (1999). Top ten considerations in the development of parenteral emulsions. *Pharmaceutical science & technology today*, *2*(4), 134-143.