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Research Article

Investigation of Effect of Extensively Used Polymers on Thermoreversible Properties of Pluronic® Tri-Block Polymer

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ABSTRACT

This investigation presents a study on the effect of various polymers on gelling properties of tri-block (Pluronic®) copolymers and increasing the stability parameter of *in situ* gelling system by altering their composition. The tri-block copolymers finds their importance in fabrication of *in situ* gelling system for the delivery of various kinds of drugs, which can be administered by topical, ophthalmic or parenteral routes. Pluronic®, is a category of non-toxic, water soluble, biodegradable poly (ethylene oxide)/poly (propylene oxide)/poly ethylene oxide), tri-block copolymers which have application in formulation of various *in situ* gelling systems. This formulation undergo thermo-reversible gelation, where it exists as a free flowing liquid at low temperature and gels in the range of body temperature to form stable depot in aqueous environment. Gelling system was prepared according to the 'Cold Method' using different concentration of polymers (15% to 20% w/v) and subjected to the determination of gelation temperature (GT), viscosity study and effect of various polymers on the strength of gelation. Overall study on the gelation of system at particular temperature is the important parameter for formulation of *in situ* drug delivery system. It was established that addition of 0.5% w/v of HPMC K4M into gelling system make it stable for forming gel in the range of body temperature whereas methyl cellulose, carbopol 934P, and HPMC E-5 restrict the gel formation.

Key words: Tri-Block Copolymer; Gelation Temperature; Gelling System; Depot.

1. Introduction

The tri-block copolymers have more importance in fabrication of *in situ* gelling system for the delivery of various kinds of drugs, which can be administered by topical, opthalmic or parenteral routes. In situ gelling systems are the liquid formulations generating a solid or semisolid depot after administration (Packhaeuser C.B. *et al.* 2004, Eve Ruel-Garie´py and Jean-Christophe Leroux, 2004). These types of system have wide range of biomedical applications, such as drug delivery, tissue repair and cell encapsulation (Hoffman A. S., 2012, Qiu Y., and Park K., 2012). They are found to be more advantageous than the other traditional form of drug delivery systems. Pluronic® F127 is a category of non-toxic, water soluble, biodegradable tri block copolymer, which composed of polyethylene oxide (PEO) /polypropylene oxide (PPO)/polyethylene oxide (PEO) units. The tri-block copolymers having application in formulation of *in situ* gelling system and these are known for exhibiting the sol-gel transition property under a certain temperature (Gilbert J.C. *et al.*, 1987). The sol-gel transition increases the application of poloxamer in pharmaceutical field. The US FDA guideline has presented Pluronic® F127 as an safe, inactive, low toxic, biodegradable ingredient for different types of preparations because of their chemical inertness (e.g.,

IV, inhalation, oral solution, suspension, ophthalmic or topical formulations) (Rowe R. *et al.,* 2005).

The hydrophobic group of tri-block copolymer can increases the sol-gel transition and minimal the concentration of polymer required for sol-gel transition (Niu G. et al., 2008). The rheological properties of Pluronic® show temperature dependence along with the chemical cross linking or entanglement of structures. As the internal cross linking of gelling system increases the sol-gel transition temperature decreases. Hence, the alteration in the cross linking capacity of copolymers the thermoreversible properties can also be improved. Simultaneously, the chemical cross linking of the tri block copolymer is responsible for the mechanical strength of the gel, lesser the cross linking soften the gel whereas greater the cross linking harder the gel. It means the increase in cross linking will improve the mechanical strength of the copolymers and ultimately the improvement in stability of gelling system whereas the gelation temperature of Pluronic® solution might be altered by addition of various polymeric materials as well as drug addition also shows effect on that (Dumortier G. *et al*., 2006). Thermally induced gelling systems show thermoreversible sol-gel transitions and are characterized by a lower critical solution temperature (LCST). They are liquid at room temperature and produce a gel at or above the LCST (Eliaz R. *et al*., 2000).

Previously we prepared such type of thermoreversible drug delivery system by incorporation of cubosome containing docetaxel trihydrate which is an anticancer agent. The incorporation of the drug-loaded cubosomes into a thermoresponsive gelling system results in a slower and a prolonged drug release. According to the results of this study, a thermoresponsive depot system based on Pluronic® (F127 and F68), containing cubosomal DTX, can be developed for a controlled drug delivery. The cubosome containing thermoresponsive depot formulation was found to be free flowing at ambient temperature and formed a depot gel at body temperature (*i.e.* 37°C) (Rarokar N. *et al.,* 2015). Hence, the cubosome based thermoreversible gel formulation may provide a possible solution to improve therapeutic efficiency of drug. Such systems are more useful for the delivery of cells or biopharmaceuticals that are susceptible to heat or organic solvents (Lee J. et al., 2006).

The aqueous solution of Pluronic® is liquid at room temperature and converted into the semisolid gel at a higher temperature (Gang Wei *et al.* 2002). This thermogelling property of tri-block polymer is applied to prepare thermosesnsitive formulations, however some drawbacks like low mechanical strength and less stability make it unfavourable for application in commercial drug formulations. These problems lead to instability of *in situ* gelling system after administration. These issues can be sort out by altering the composition of gelling system using other copolymers along with the Pluronic® triblock copolymers.

Aim of this study was to investigate the effect of various copolymers on gelling properties of Pluronic® triblock copolymer and increase the stability of *in situ* gelling system by altering their composition. Docetaxel trihydrate (DTX) was used as a model drug for this study and the effect of drug on the mechanical strength of gelling system was also studied.

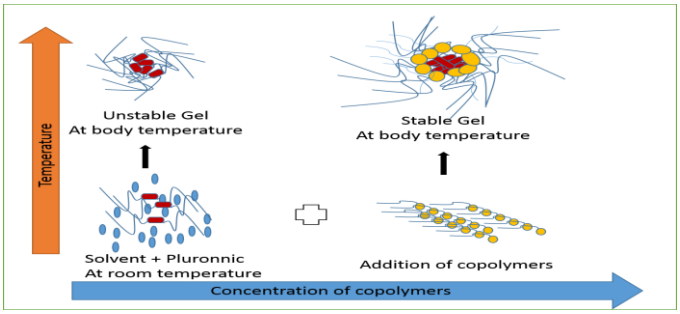


Fig. 1. Effect of copolymers on mechanical strength and stability of gelling system

2. Materials and Methods

2.1. Materials

Docetaxel trihydrate was obtained as a gift sample from Scino Pharmaceuticals Pvt., Taiwan, Pluronic® F127 was supplied by Research Lab Fine Chem Industries, Mumbai (India) and Pluronic® F68 obtained from Himedia Laboratory Pvt. Ltd. Mumbai (India), HPMC K4M was sourced from Otto Chemie Pvt. Ltd., Mumbai (India), HPMC E50, Carbopol 934P and Methyl Cellulose purchased from Loba Chemie Pvt. Ltd., Mumbai (India). All other chemicals used in this study were of analytical grade.

2.2. Preparation of gelling system

Gelling system was prepared according to the 'Cold Method' given in our previous article (Rarokar *et al.*, 2015) with slight modification. Pluronic® F127(15-20%w/v) and Pluronic® F68 (20-40%w/v) were weighed accurately and solubilised in 15 mL of distilled water in a beaker with continuous stirring on magnetic stirrer at a speed of 500 rpm for 2hrs. The temperature of water maintained at 4±2°C. This mixture was then subjected to hydration at 4-8 °C overnight to get uniform glassy solution. The copolymers like methylcellulose (0.3-1.0% w/v), carbopol 934P (0.1-0.3%w/v), HPMC E-50 (0.3-1.0%) and HPMC K4M (0.3-1.0%w/v) was added separately with continuous stirring to prepared different batches. The weighed amount of drug (2%w/v) was dissolve in ethanol to make slurry. The drug slurry then mixed in the above homogeneous mixture. The final volume was made up and pH of the homogeneous mixture was adjusted to 7.0 using triethanolamine, whereas mixture containing carbopol 934P was adjusted to pH 5.8. The prepared gelling system was then subject to different evaluation parameters to study the effect of concentration of different copolymers onto the thermal properties of Pluronic®.

2.3. Drug-Excipient Compatibilty Studies

a. Thermal Analysis

The Differential scanning calorimetry (DSC) analysis of pure drug (DTX) and thier physical mixture with tri-block copolymers were carried out using DSC Q20 (TA Instruments Inc. New Castle. DE). The approximately 5

mg sample wighed into each aluminium pans and was subject to heating at a rate of 10.0 °C/ min from 0 °C to 400 °C. Nitrogen at a flow rate of 40 mL/min was used as a purge gas in DSC analyses. The results were analysed using the universal Analysis software version 4.5A, build 4.5.0.5 (TA Instruments, Inc., New Castle, DE, USA).

b. FTIR Spectroscopy

To determine any possible interactions, the physical mixtures of the drug (DTX) and the tri-polymers were analyzed using the Fourier transformed infrared (FTIR) spectroscopy. Briefly, the samples were dried in a hot air oven at 50°C for 2 h. The samples were compressed under pressure of 10 t/nm2 to prepare circular KBr disks. The samples were scanned in the range of 400 to 4000 cm−1. The shifts in the spectra of the drug in the presence of polymers and other components were investigated to determine physical interactions between the drug and the polymers, if any.

2.4. Viscosity Study of Polymers

The viscosity of homogeneous mixture prepared with Pluronic® tri-block copolymers were measured by using DV-E Brookfield viscometer using small sample adaptor with spindle 63 for 19.1± 0.5°C and LV2 spindle for 37± 0.5°C. The Various concentrations of Pluronic® F127 (15-20%w/v) and Pluronic® F68 (20-40%w/v) previously prepared homogeneous mixtures were poured into adaptor of viscometer and angular viscosity was increase3d gradually from 10 to 100 rpm with time interval of 6 seconds at each speed. Same process was repeated for the reverse speed (100 to 10) with a similar time interval of 6 seconds. The results were calculated for mean± standard deviation(*n*=3). Similarly experiment was repeated for gel at 36.7°C to determined the viscosity by using spindle LV 2.

2.5. Measurement of Gelation Temperature by Tilting Method

The gelation temperature measurement was done by previously reported 'Tilting Method' (Tirnaksiz *et al.*, 2005). The gelation temperature (GT) was recorded for different batches of homogeneous mixture of tri-block copolymer with different concentration with and without a model drug docetaxel trihydrate. Aliquots (1 mL) of prepared formulation were sealed and transferred to test tubes in the water bath at 4 ± 0.5°C then temperature was increased by 2 ± 0.5°C per step until 30 ± 0.5°C and then increment with 1°C in the region of sol-gel transition temperature. Gelation is the temperature that the meniscus would no longer move upon tilting through 90°. The method was very reproducible, giving coefficients of variation of less than 2% (n = 4).

3. Results

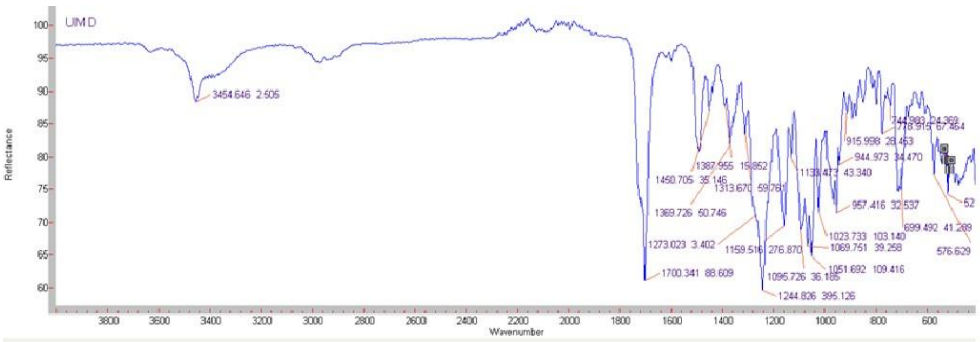
3.1. Drug-Excipient Compatibility Studies

a. Thermal Analysis

The compatibility study of drug-polymer was studied by thermal analysis. DSC thermogram of docetaxel trihydrate exhibits a sharp endothermic peak at 227.82 °C which corresponds to its melting point. This peak of drug was not found in the DSC thermogram of polymer solution containing the drug. It indicates that the drug was uniformly dispersed into the gelling system and did not have any interaction with the other ingredients of gelling system.

b. FTIR Spectroscopy

Drug-polymer interaction was determined by the FTIR analysis, the reports were found to be concurrent with a reference spectrum of docetaxel trihydrate. Docetaxel showed prominent peaks at 711.68, 850.55, 1099.35, 1168.78, 1245.93, 1712.67 and 3456.20 cm^{-1}. The characteristic peaks confirmed the structure of docetaxel trihydrate. These peaks were retained in the other spectra of drug with the tri-block copolymers i.e. Pluronic® F127 / F68 and copolymers Methyl Cellulose, HPMC K4M, Carbopol 934P, HPMC E-50. The IR spectra did not show any shifting in the characteristic peaks of docetaxel as shown in Fig. 2. Hence, the FTIR study indicate that there were no positive evidences of interaction between docetaxel trihydrate and the employed polymers, this prove that drug is stable in presence of tri-block copolymers.



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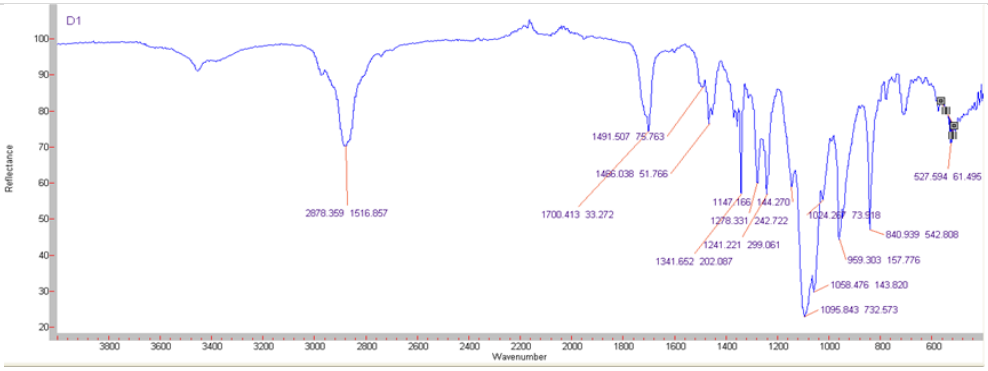
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1341.652 202.087
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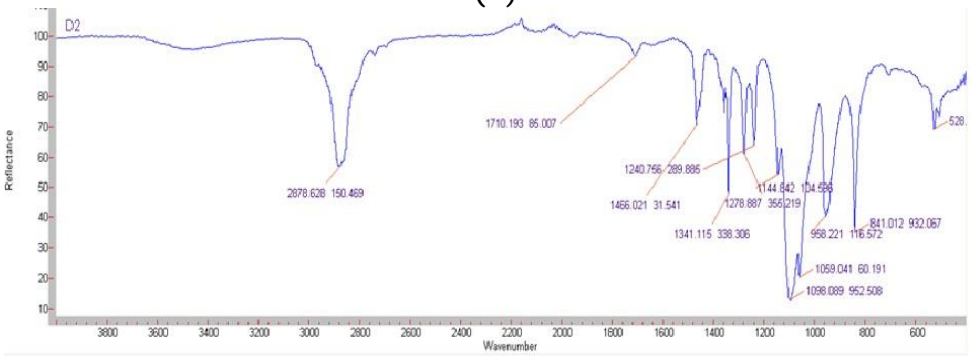
(B)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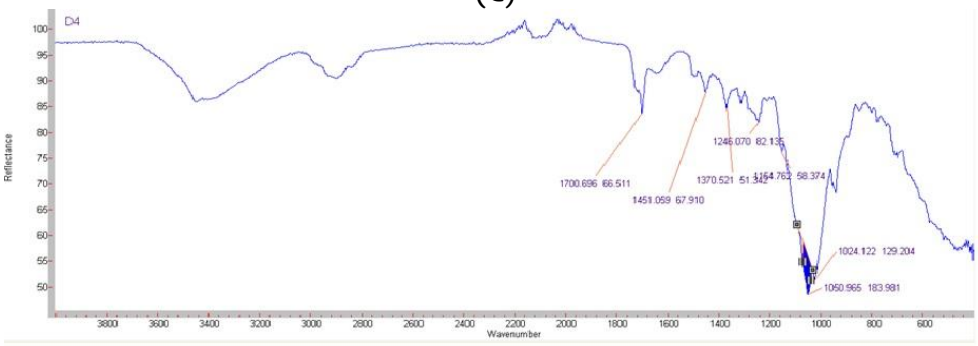
(C)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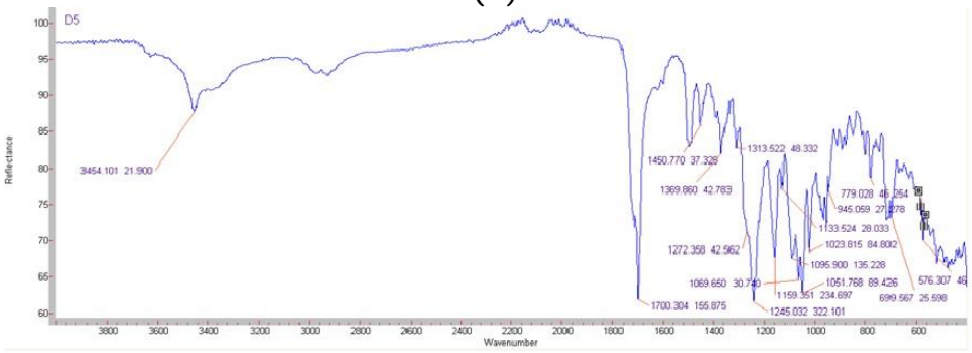
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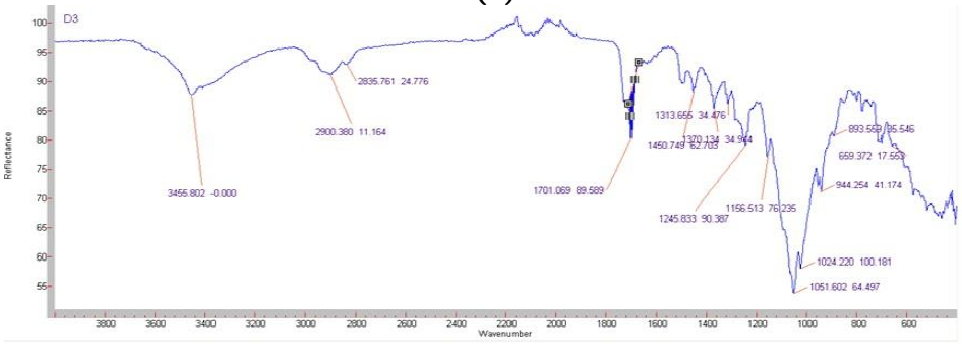
(E)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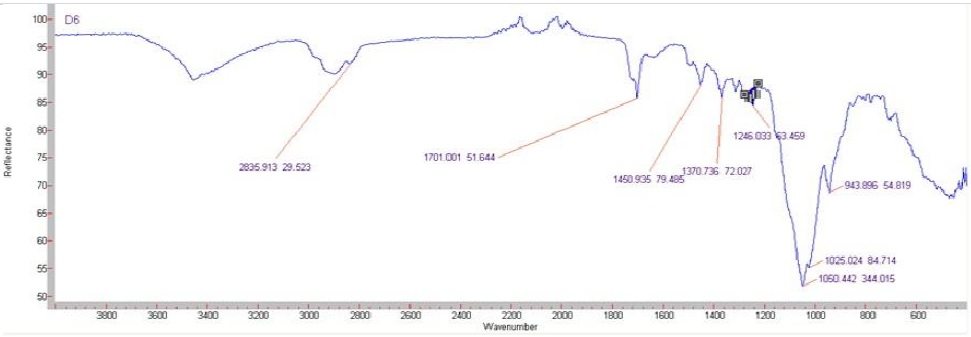

(G)

Fig. 2: FTIR spectra of Docetaxel trihydrate (A), Docetaxel trihydrate + Pluronic® F127 (B) and Docetaxel trihydrate + Pluronic® F68 (C), Docetaxel trihydrate+ HPMC K4M (D), Docetaxel trihydrate + Carbopol 934P (E), Docetaxel trihydrate + Methyl Cellulose (F), Docetaxel trihydrate + HPMC E-50 (G)

3.2. Viscosity Study of Polymer

Results of viscosity study of Pluronic® F127 and F68 in different concentrations at various rpm shown are in the Table 1 and 2 respectively, it can be seen that as the concentration of Pluronic® F127 and F68 increases from 15% to 20%, the viscosity of polymer solutions also increases. Simultaneously, from Fig. 3 and Fig. 4, it becomes clear that the increase in speed of spindle of Brookfield viscometer result into the decrease in viscosity of Pluronic®® F127 and F68 respectively.

Table 1. Viscosity of Pluronic® F127 of Different Concentration at Various RPM

Pluronic® F127 Concentration	15% w/v	16% w/v	17% w/v	18% w/v	19% w/v	20% w/v
RPM	Viscocity (cP)					
10	41.1	38.3	44.2	45.2	50.6	52.8
20	36.5	33.1	41.1	41.1	46.2	48.2
30	31.4	28.1	38.6	39.2	42.1	44.5
50	27.5	22.6	33.2	36.5	38.6	40.3
60	19.6	19.1	26.5	31.7	35.2	37.2
100	12.4	16.6	22.1	25.4	30.6	31.4

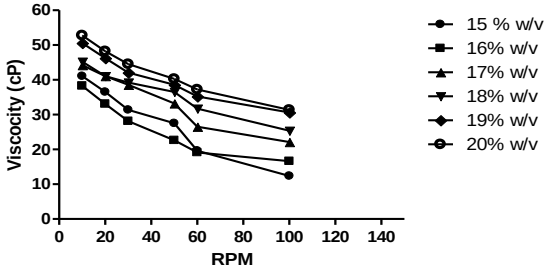

Fig. 3. Viscosity study of different concentration of Pluronic® F127 at various RPM

Table 2. Viscosity of Pluronic® F68 of different concentration at various RPM

Pluronic® F68 Concentration	15% w/v	16% w/v	17% w/v	18% w/v	19% w/v	20% w/v
RPM	Viscocity (cP)					
10	38.2	36.3	44.3	43.2	48.1	50.7
20	34.8	32.5	40.6	39.1	45.6	48.5
30	29.7	26.2	35.2	33.8	40.7	43.3
50	28.5	20.8	32.6	31.6	35.2	41.4
60	18.1	16.3	26.7	27.4	33.3	35.9
100	10.6	12.9	22.8	24.1	28.5	29.8

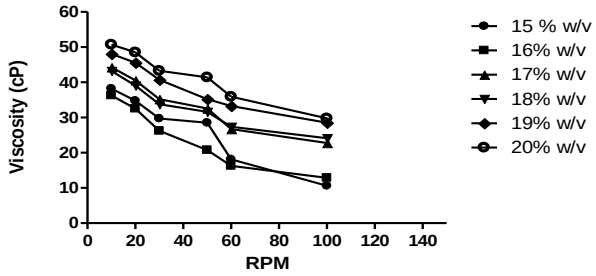

Fig. 4. Viscosity study of different concentration of Pluronic® F68 at various RPM

3.3. Gelation Teperature Study

a. Gelation Temperature of Pluronic ® F127

The GT was recorded for Pluronic® F127 gel alone (GT1) and with drug (GT2). The Table 3 presents that the GT1 and GT2 decreases with increase in polymer concentration from 15- 20 % w/v as depicted in Fig. 5.

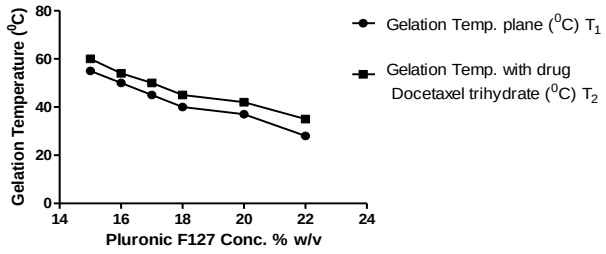

Fig. 5. Determination of GT of different concentration of Pluronic® F127 with and without drug

Table 3. Gelation temperature of Pluronic F127 of various concentrations with and without drug

Sr. No.	Concentration of PF127 (% w/v)	GT (°C) without drug T_1	GT with drug (°C) (DTX) T_2
01.	15	55 ± 0.23	60 ± 0.19
02.	16	50 ± 0.45	54 ±0.33
03.	17	45 ± 0.31	50 ± 0.28
04.	18	40 ± 0.26	45 ± 0.18
05.	19	37 ± 0.34	42 ± 0.31
06.	20	28 ± 0.35	35 ± 0.25

Value are mean±std. dev. (*n*=3)

b. Gelation Temperature of Pluronic ® F68

The Fig. 7 clears that the GT of Pluronic® F68 without drug (GT3) decreases with increase in Pluronic® F68 concentration. While GT of Pluronic® F68 with drug

(GT4) was found to be slightly increased as compared to the Pluronic® F68 alone but decreased with increase in concentration of Pluronic® F68.

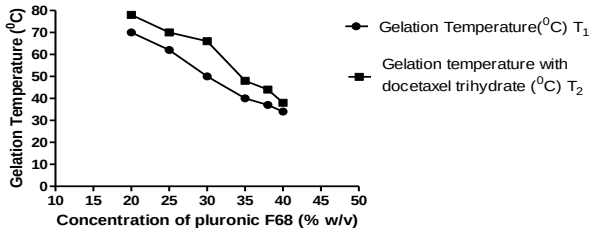

Fig.6. Determination of Gelation temperature of different concentration of Pluronic® F68 with and without drug

Table 4. Gelation temperature of Pluronic F68 of various concentrations with and without drug

Sr. No.	Concentration of PF68 (% w/v)	GT (˚C) without drug T_1	GT with drug (˚C) (DTX) T_2
01.	20	70 ± 0.33	78 ± 0.24
02.	25	62 ± 0.33	70 ± 0.21
03.	30	50 ± 0.33	66 ± 0.33
04.	35	40 ± 0.33	48 ± 0.25
05.	38	37 ± 0.33	44 ± 0.25
06.	40	34 ± 0.33	38 ± 0.33

c. Gelation Temprature of Pluronic ® F127 and Pluronic ® F68 in Combination

Pluronic® mixture of Pluronic® F127 and Pluronic® F68 were selected due to their thermo-gelling property. The GT of gelling system containing 15-18 % w/v concentration of Pluronic® F127 was found to be more by 37 ˚C. Whereas, 20% w/v Pluronic® F127 shows the GT around 25˚C, but it is not easy to administer due to increase in viscosity at the room temperature. The concentration of Pluronic® F68 at 25, 30, 33, 35 % w/v showed GT 63°, 48°, 40°, 37° C respectively. It also exhibit increased viscosity which makes it difficult to administer, so the combination of both the tri-block copolymer was used in various ratio for the preparation of gelling system. The results depicted in Table 5, shows that there was decrease in GT up to 30 ˚C for 18/20 ratio of Pluronic® F127 and Pluronic® F68. This makes the combination more suitable for preparation of gelling system.

Table 5. GT of Pluronic® at various concentrations in combination (Pluronic® F127/ Pluronic® F68) with or without Docetaxel trihydrate.

Sr. No.	Concentration of PF127/PF68 (% w/v)	GT (˚C) without drug	GT with drug Docetaxel trihydrate (˚C)
01.	15/10	45 ± 0.31	50 ± 0.32
02.	15/15	39 ± 0.42	44 ± 0.37
03.	15/20	35 ± 0.35	39 ± 0.41
04.	16/10	40 ± 0.33	44 ± 0.34
05.	16/15	38 ± 0.33	43 ± 0.33
06.	16/20	36 ± 0.31	43 ± 0.33
07.	17/10	38 ± 0.40	42 ± 0.22
08.	17/15	35 ± 0.24	40 ± 0.20
09.	17/20	34 ± 0.33	39 ± 0.23
10.	18/10	35 ± 0.20	38 ± 0.20
11.	18/15	34 ± 0.22	37 ± 0.33
12.	18/20	30 ± 0.33	34 ± 0.32

Value are mean±std. dev. (*n*=3)

3.4. Effect of Various Copolymers on the GT of Pluronic ® F127 and Pluronic ® F68

a. Effect of Methyl Cellulose on the GT of Pluronic ® F127

The effect of concentration of methyl cellulose was investigated on the GT of Pluronic® F127 as depicted in Table 6. At the given concentration of Pluronic® F127 (15-20% w/v), the GT decreases with increase in concentration of methyl cellulose from 0.3-1.0% w/v (Fig.7). The gelling solution prepared by 0.3% w/v methyl cellulose was found to be clear and transparent than 1.0% and 0.5% but have high GT. Whereas 0.5% methyl cellulose gives transparent solution with low GT as compared to the 0.3% and gelling solution containing 1.0% methyl cellulose showed opaque appearance.

Table 6. Effect of Methyl Cellulose concentration on GT of Pluronic® F127

Pluronic® F127 Concentration (% w/v)	Methyl Cellulose Concentration		
	0.3%	0.5%	1.0%
	Gelation temperature (˚C)		
15	52± 0.33	46± 0.33	41± 0.33
16	46± 0.33	40± 0.33	38± 0.33
17	42± 0.33	35± 0.33	32± 0.33
18	35± 0.33	30± 0.33	28± 0.33
20	32± 0.33	26± 0.33	22± 0.33

Values are mean±std. dev. (*n*=3)

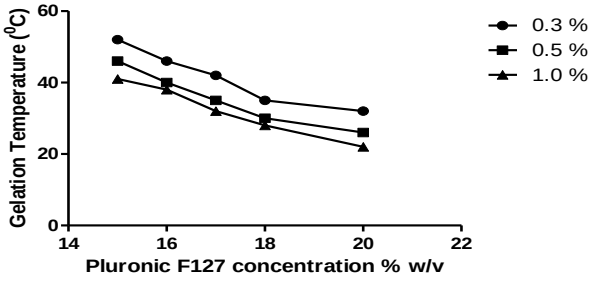

Fig. 7. Effect of various concentration of Methyl cellulose on GT of Pluronic® F127

b. Effect of Methyl Cellulose on the GT of Pluronic ® F68

On determination of effect of methyl cellulose on the GT of Pluronic® F68 having conce-ntration 20-40% w/v, it was found that as the concentration of methyl cellulose increases from 0.3 % w/v to 1.0% w/v the GT of Pluronic® F68 gradually decreases. However, when we compare these results with the GT of Pluronic® without any copolymer it was found that addition of 0.3- 1.0 % w/v concentration of methyl cellulose the GT is decreases by 10˚C.

Table 7. Effect of Methyl Cellulose concentration on GT of Pluronic® F68

Pluronic® F68 Concentration % w/v	Methyl Cellulose Concentration		
	0.3%	0.5%	1.0%
	Gelation temperature (˚C)		
20	68± 0.33	64± 0.33	60± 0.33
25	62± 0.33	58± 0.33	50± 0.33
30	57± 0.33	50± 0.33	45± 0.33
35	51± 0.33	46± 0.33	40± 0.33
40	42± 0.33	35± 0.33	30± 0.33

Values are mean±std. dev. (*n*=3)

c. Effect of Carbopol 934P on the GT of Pluronic ® F127

Carbopol 934P was used as gelling agent that increases the gelling strength of gelling system. The effect of concentration of carbopol 934P on the GT of Pluronic® F127 solution was investigated in the range of 0.1-0.3% w/v as depicted in Table 7. The GT of Pluronic® F127 was found to be decreased with increase in concentration of carbopol 934P as shown in Fig.8. Since, increase in the temperature results into the sol-gel transformation of Pluronic® solution but the presence of carbopol restrict the gel formation after particular temperature and results in to the decreased in viscosity.

Table 8. Effect of Carbopol 934P concentration on GT of Pluronic® F 127.

Pluronic® F127 Concentration % w/v	Carbopol 934P Concentration (% w/v)		
	0.1%	0.2%	0.3%
	Gelation temperature (˚C)		
15	46 ± 0.33	42 ± 0.33	39± 0.33
17	43 ± 0.33	41 ± 0.33	40± 0.33
18	39 ± 0.33	34± 0.33	37± 0.33
20	29 ± 0.33	28± 0.33	26± 0.33
22	26 ± 0.33	24 ± 0.33	22± 0.33

Values are mean±std. dev. (*n*=3)

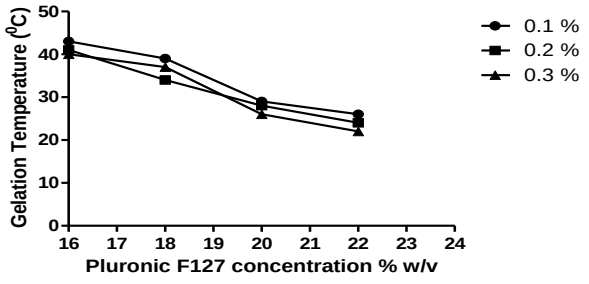

Fig. 8. Effect of various concentration of Carbopol 934P on GT of Pluronic® F127

d. Effect of Carbopol 934P Concentration on GT of Pluronic ® F68

Carbopol 934P in the various concentration from 0.1 to 0.3 % w/v when used as a copolymer with Pluronic® F68 for preparation of gelling system, it was found that as the concentration of carbopol 934P increases from 0.1 to 0.3% w/v the GT of Pluronic® F68 gradually decreases up to certain extent but did not reaches around the normal body temperature. At higher concentration of Pluronic® F68 40% w/v and 0.3% w/v of Carbopol 934P gelling system shows GT about 40˚C and the system was found to be more viscous.

Table 9. Effect of Carbopol 934P concentration on GT of Pluronic® F68

Pluronic® F68 Concentration % w/v	Carbopol 934P Concentration (% w/v)		
	0.1%	0.2%	0.3%
	Gelation temperature (˚C)		
20	65 ± 0.33	64 ± 0.33	58± 0.33
25	60 ± 0.33	60 ± 0.33	55± 0.33
30	58 ± 0.33	55 ± 0.33	52± 0.33
35	55 ± 0.33	50 ± 0.33	45± 0.33
40	50 ± 0.33	45 ± 0.33	40 ± 0.33

Values are mean±std. Dev. (n=3)

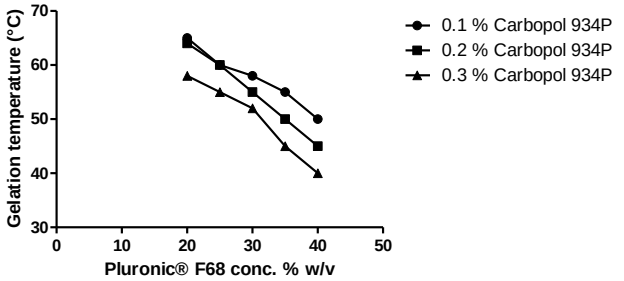

Fig. 9. Effect of various concentration of Carbopol 934P on GT of Pluronic® F68

e. Effect of HPMC K4M on the GT of Pluronic ® F127

The effect of concentration of HPMC K4M on the GT of Pluronic® F127 solution (0.3, 0.5, 1.0% w/v) was investigated as revealed in the Table 8. At the given concentration of Pluronic® F127, the GT was decreased with increased in the concentration of HPMC K4M (Fig. 10). The addition of 0.5%w/v HPMC K4M into the gelling system shows the GT in the range of body temperature and found to be stable at 37 ˚C for more than 6 hour which supports the data retrieved for improvement in gel strength of the gelling system of Pluronic® F127.

Table 10. Effect of HPMC K4M concentration on GT of Pluronic® F127

Pluronic® F127 Concentration % w/v	HPMC K4M Concentration (% w/v)		
	0.3%	0.5%	1.0%
	Gelation temperature (˚C)		
15	46 ± 0.33	37 ± 0.33	30± 0.33
16	44 ± 0.33	35 ± 0.33	28± 0.33
18	40 ± 0.33	26± 0.33	18± 0.33
20	34 ± 0.33	21± 0.33	Below 18
22	29 ± 0.33	18± 0.33	Immediate

Values are mean±std. Dev. (n=3)

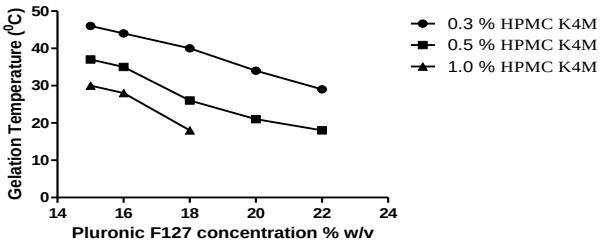

Fig.10. Effect of various concentration of HPMC K4M on GT of Pluronic® F127

f. Effect of HPMC K4M Concentration on the GT of Plurinic ® F68

The study to determine the effect of addition of copolymer on the different concentration of Pluronic® F68 revealed that the addition of HPMC K4M in the concentration of 0.3, 0.5 and 1.0 % w//v to the gelling system prepared by Pluronic® F68 results into the significantly decrease in GT upto the range of body temperature. This study shows that little increase in the concentration of HPMC K4M form stable gelling system with lower GT.

Table 11. Effect of HPMC K4M concentration on GT of Pluronic® F68

Pluronic® F68 concentration % w/v	HPMC K4M concentration (% w/v)		
	0.3%	0.5%	1.0%
	Gelation temperature (˚C)		
20	65 ± 0.33	60 ± 0.33	55 ± 0.33
25	60 ± 0.33	55 ± 0.33	50 ± 0.33
30	55 ± 0.33	50 ± 0.33	45 ± 0.33
35	50 ± 0.33	45 ± 0.33	40 ± 0.33
40	45 ± 0.33	40 ± 0.33	35 ± 0.33

Values are mean±std. dev. (*n*=3)

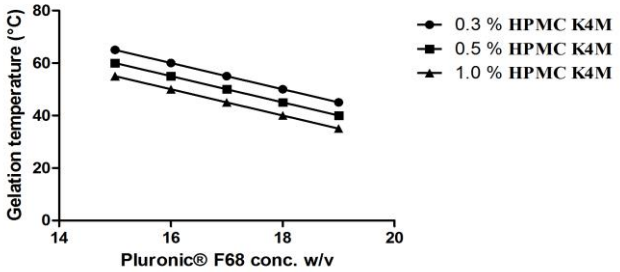

Fig.11. Effect of various concentration of HPMC K4M on GT of Pluronic® F68

g. Effect of HPMC E-50 Concentration on Gelation Temperature of Pluronic ® F127

Results depicted in the Table 12 shows that addition of HPMC E-50 in the concentration of 0.3 to 1.0 % w/v decrease the gelation temperature with increase in concentration but not significantly reach the range of body temperature.

Table 12. Effect of HPMC E-50 concentration on GT of Pluronic® F127

Pluronic® F127 Concentration % w/v	HPMC E-50 Concentration (% w/v)		
	0.3%	0.5%	1.0%
	Gelation temperature (˚C)		
15	60 ± 0.33	55 ± 0.33	50 ± 0.33
16	58 ± 0.33	50 ± 0.33	48 ± 0.33
18	55 ± 0.33	50 ± 0.33	45 ± 0.33
20	50 ± 0.33	40 ± 0.33	40 ± 0.33
22	45 ± 0.33	38 ± 0.33	35 ± 0.33

Values are mean±std. Dev. (n=3)

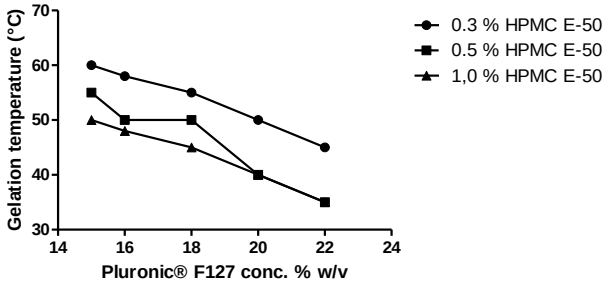

Fig.12. Effect of various concentration of HPMC E-50 on GT of Pluronic® F127

h. Effect of HPMC E-50 Concentration on Gelation Temperature of Pluronic ® F68

The effect of HPMC E-50 on the gelation temperature of Pluronic® F68 was found to be more significant as compared to their effect on Pluronic® F127. The increase in concentration of HPMC E-50 from 0.3 to 1.0% w/v helps to decrease GT of gelling system, at every stage of increasing the concentration of HPMC E-50 the GT was decreased by 5˚C and it reaches 30˚C ().

Table 13. Effect of HPMC E-50 concentration on GT of Pluronic® F68

Pluronic® F68 concentration % w/v	HPMC E-50 concentration (% w/v)		
	0.3%	0.5%	1.0%
	Gelation temperature (˚C)		
20	60 ± 0.33	55 ± 0.33	50 ± 0.33
25	55 ± 0.33	50 ± 0.33	45 ± 0.33
30	50 ± 0.33	45 ± 0.33	40 ± 0.33
35	45 ± 0.33	40 ± 0.33	35 ± 0.33
40	40 ± 0.33	35 ± 0.33	30 ± 0.33

Values are mean±std. Dev. (n=3)

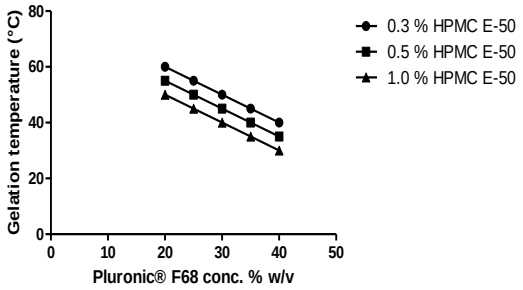

Fig. 13. Effect of various concentration of HPMC E-50 on GT of Pluronic® F68

4. Discussion

The formulation of thermoresponsive gel which forms stable gel at ambient temperature in the range of body temperature is very difficult task. To achieve the desired outcomes the gelation property of tri-block copolymers need to alter with the use of various polymers. The tri-block copolymers find their importance in fabrication of in situ gelling system for delivery of different kind of drugs, which can be administered by topical, ophthalmic or parenteral routs. Pluronic®, is a category of non-toxic, water soluble, biodegradable poly (ethylene oxide)/poly (propylene oxide)/polyethylene oxide), tri-block copolymers. The fabricated in situ gel shows thermo-reversible gelation which confirm by the existence of free flowing liquid at low temperature and undergo gelation to form stable depot at body temperature. The varying concentration of Pluronic® will show effect on viscosity of formulation which results into the change in release of drug from the gelling system. The increased viscosity of polymer solution is also prone to difficulty in the handling and administration of gelling system. However, the gelation temperature of such a formulation is decreases as the concentration of Pluronic® increases but the viscosity still one of the important parameter to fabricate ideal formulation/gelling system.

Though the Pluronic® is tri-block copolymer which having property to form gelling system, it requires other copolymers which may be synthetic, semisynthetic or natural. However the natural polymers are always beneficial for the human biology but because of lack of desired physicochemical properties we cannot use it at every stage of formulation. Whereas synthetic polymers like HPMC, carbopol, methyl cellulose etc. have their own issues regarding biodegradability and biocompatibility so semisynthetic polymers are good option for fabrication of potential drug delivery system. Here in this study we investigate the effect of copolymers on the gelling properties to fabricate ideal thermoreversible gel simultaneously the dependent variables such as gelation time and gelation temperature also studied by altering the concentration of copolymers.

The previous literature shows that when we increase the temperature of polymer solution containing methyl cellulose beyond the critical temperature point (29± 2°C) the viscosity strongly increases and that results into the formation of thermoreversible gel the reason behind it the initiation of aggregation of particles to form pre-gel or loose gel domain showing shear thinning behaviour of the system. The author (Kobayashi et al., 1999) did not discussed about the exact concentration of methylcellulose needed for formation of stable gel.

When we used methyl cellulose as a copolymer or stabilizing agent along with the Pluronic® F127 and F68 the different results were obtained. The results depicted in this study shows that the increase in concentration of methyl cellulose (0.3%w/v to 1.0%w/v) in gelling system prepared with Pluronic® F127 leads to the decrease in gelation temperature by 5°C. It forms opaque solution with increased viscosity and hence it was difficult to inject. This system might be useful for thermoreversible gel but it was not so useful to form injectable gelling system. The Pluronic® F68 shows somewhat different results when it was used in preparation of gelling system and the addition of 0.3-1.0% w/v methyl cellulose in to it results into the decreased in gelation temperature by 10°C.

The surprising results were obtained when the effect of carbopol 934 P on the thermoreversible properties of Pluronic® F127 and F68 was studied. The previous literature did not tell about the effect of carbopol 934P as a copolymer with Pluronics®. When the concentration of gelling system was alter by addition of 0.1-0.3% w/v of carbopol 934P in gelling system prepared by use of Pluronic® F127, gelation temperature continuously goes on decreasing with increase in the concentration of carbopol 934P. The gelation temperature with increase in concentration of carbopol 934P was found to be decreases but upto certain extent later on further addition it did not show any effect on gelation temperature, which may be due to the lack of water absorption sites on the surface of carbopol molecules.

From the results obtained after studying thermal behaviour of Carbopol 934P it was clear that the increase in concentration of carbopol 934P restrict the gel formation and ultimately the sol-gel transition was hampered. This gives the clue to use of carbopol 934P as a stabilizer in fabrication of thermoreversible gel rather than their use to improve gelling strength. Same study was carried out with Pluronic® F68 and carbopol 934P in the concentration of 40% w/v and 0.3% w/v respectively which shows gelation at about 40°C and the system was found to be more viscous. This results proven the potential of Carbopol 934P to affect the sol-gel transition temperature which is exactly against the results depicted in previous literature (Asasutjarit R *et al.,* 2011).

Mondal S. *et al*. were used HPMC E50LV as a copolymer with sodium alginate at the concentration of 1.5% w/w for the formulation of in situ ge and conclude that the use of HPMC E50LV in such a higher concentration gives transparent and clear free flowing gel which is very contraversial to the results obtained in our study (Mondal S. *et al*., 2012). In this study we revealed that the use of 1.0% w/v of HPMC E50 results into the formation of stiff gel which never been free flowing.

The effect of HPMC E-50 on the GT of Pluronic® F68 was found to be more significant as compared to their effect on Pluronic® F127 however it also having physicochemical drawbacks in relation to the thermal properties of the gelling system. The increase in concentration of HPMC E-50 from 0.3 to 1.0% w/v helps to decrease GT of gelling system by 5°C and it reaches 30°C but further increase in the concentration results into the increase in viscosity with opaque appearance. This more viscous solution did not show desired results in comparison with HPMC K4M. Hence, the use of HPMC E-50 as a copolymer was not so advantageous in fabrication of thermoreversible gel formulations.

The investigation of effect of addition of HPMC K4M to the thermoreversible gelling system for altering the gelling properties of Pluronic® F127 and F68 revealed that the 0.5% w/v of HPMC K4M shows better results than the previously added copolymers. The 0.5% w/v of HPMC K4M shows desire gelling temperature. However, other copolymer shows either higher or lower GT. In addition to that the introduction of HPMC K4M in 0.5% w/v concentration results into the formation of stable gel with improved gelling strength i.e. upto 6h the similar results were obtained when gelling system prepared with Pluronic® F68 but having issue with the gelation temperature which was found to be more than the ambient temperature. The slightly increase in the concentration of HPMC K4M tends to form stable gel and lower GT. Hence the ultimate aim of the study was achieved by use of HPMC K4M as a copolymer in combination with Pluronic®.

5. Conclusion

Gelation temperature of the gelling system plays important role in the formulation of thermoresponsive depot system. As these formulations have GT near to the body temperature it opens the door for forming depot after insertion into the body, either in muscle or beneath the skin. So, it is necessary to optimize the gelation temperature of gelling system to form a potential depot system. In this study it was revealed that GT1 and GT2 of Pluronic® F127 decrease with increase in polymer concentration. However, addition of 0.5% w/v of HPMC K4M into gelling system make it stable for forming gel in the range of body temperature whereas carbopol 934P and methyl cellulose restrict the gel formation. Besides the effect of addition of copolymer on the gelation temperature of gelling system, the role of model drug on the GT of system is also one of the major aspects as far as depot formulation is concern. Regardless of the other concentrations of the drug and polymers, the addition of 0.2% w/v docetaxel trihydrate increases the GT of polymer solution by 2-3°C. On the other hand presence of 0.5% w/v of HPMC K4M drops down the GT upto ambient temperature, which is the decisive factor for formation of *in situ* gel or depot system. Hence the prepared gelling system and the results obtained during this study can be utilized for the formulation of a potential depot forming drug delivery system.

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