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Formulation Development of *In Situ* Sponge Forming Injectable Drug Delivery System of Tramadol Hydrochloride

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<p>Reshma Mirajkar*, Chhavi Singh, Ashwini Madgulkar</p> <p><i>Department of Pharmaceutics, AISSMS College of Pharmacy, Kennedy road, Near RTO, Pune-411001, Maharashtra, India. Phone. No. – 09422760018</i></p> <p>Submission: 7 June 2017 Accepted: 12 June 2017 Published: 25 June 2017</p>		



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ABSTRACT

The overall aim of this work was to formulate sustained parenteral drug delivery system of analgesic drug Tramadol HCL. The study involved the formation of in situ sponge of cross-linked gelatin matrix entrapping the drug and releasing it as a sustained delivery system over a period of 5 days in order to reduce the frequency of administration and to improve patient compliance. The formulation was prepared by the method cryogelation and optimised using gelatin 7% as a sponge-forming polymer, crosslinking agent glutaraldehyde 1.5%, sustained release polymer Hydroxypropyl methylcellulose K100M 1%, cryogelant agent mannitol 4%, tonicity adjuster NaCl solution 0.9% and suspending agent sodium carboxymethylcellulose 1% (NaCMC) to form a dry powder intended to be administered subcutaneously by reconstituting with sterile water for injection. The formulation was evaluated for all prerequisites of parenteral and other parameters like swelling time, swelling index, scanning electron microscopy, sedimentation study, particle size and zeta measurement, *in-vitro* drug release, sterility testing and stability studies. The formulation was found to be sterile, isotonic, having swelling time 17 min, swelling index 87%, particle size 0.8754µm with Zeta potential -5.99 mV. The *in vitro* drug release was found to be 98.59% in Simulated Body Fluid pH 7.2 at 37°C over a prolonged period of 5 days. The formulation was physically and chemically stable at ambient, accelerated and refrigerator temperature for a period of 1 month. Thus, parenteral gelatin sponge formulations can be anticipated as a promising alternative to conventional oral and parenteral dosage forms.

INTRODUCTION

In-situ sponge-forming injectable drug delivery system is an unique technology which consists of nano or microporous matrix loaded with an active agent or drug along with the natural injectable polymers like chitosan, gelatin, sodium alginate, collagen etc. which act as a depot due to formation of in situ sponge on crosslinking with crosslinking agents like glutaraldehyde, formaldehyde etc. [2,4]. Following the injection, the sponge will expand to the original shape and size and start releasing the drug by following diffusion mechanism over prolonged period of time (from few hours to few days) mainly in chronic diseases and thus overcoming the drawbacks of conventional therapy like pain, frequent dosing and patient incompliance [1,6,8,9].

Sponge or scaffolds have been developed using various techniques such as phase separation using porogen (chemical additives that generate pores), fiber bonding, gas foaming, microemulsion formation, lyophilization, via foam formation, and via cryogelation [11,12]. More recently, gelation at a sub-zero temperature known as cryogelation has been used to create sponge with large interconnected pores. The porous polymer is formed in semi-frozen conditions when the major part of the solvent is solidified, forming solvent crystals at temperatures below the normal freezing point, with polymerization taking place in the intercrystalline channels containing the unfrozen solution, while the ice crystals nucleated from the aqueous phase during freezing function as the porogen. Increasing the temperature after completion of polymerization leads to defrosting of the solvent crystals and formation of interconnected voids (macropores) in the polymer structure filled with the solvent [13,14].

Tramadol Hydrochloride (TH), a synthetic opioid of the amino cyclohexanol group, is a centrally acting analgesic with weak opioid agonist properties. TH is mainly used in moderate to severe pain in conditions such as Arthritis pain- osteoarthritis pain, rheumatoid arthritis, Diabetic neuropathy, postoperative neuralgia and Chemotherapy pain etc. The half-life of the drug is about 5.5 hours and the usual oral dosage regimen is 50 to 100 mg every 4 to 6 hours with a maximum dosage of 400 mg/day [7]. So, to reduce the dosing frequency in situ sponge-forming injectable approach was selected.

The objective of the study is to formulate sustained injectable drug delivery system of analgesic drug Tramadol HCL as an in situ forming sponge which consists of cross-linked

gelatin as a sponge-forming polymer releasing the drug over a 5 days period in order to reduce the frequency of administration and to improve patient compliance.

MATERIALS AND METHODS

MATERIALS:

Tramadol Hydrochloride was obtained as a generous gift from Emcure Pharmaceuticals (Pune, India). Gelatin was purchased from SD Fine Chem.(Mumbai, India), HPMC K100M was purchased from Otto Chemie). Glutaraldehyde and Sodium CMC was purchased from LobaChemie (Mumbai, India). All other chemicals and reagents were of the analytical grade.

METHODS:

Optimization study

A two-factor, three levels (3^2) full factorial design was employed for the optimization of Tramadol HCL gelatin matrix. The experimental design was applied to optimize the effect of independent factors such as Gelatin concentration and HPMC concentration on dependent variables i.e. Swelling Time and Drug release. Nine factorial batches were prepared with different polymer and copolymer concentrations. Design-expert software® Version 10.0 (Stat-Ease, Inc. USA) was used to analyze the data and the optimized batch was selected and further subjected to spectral analysis. Design for a factorial experiment is presented in Table 1.

Table 1: Formulation combination as per the 3^2 full factorial designs for Tramadol gelatin matrix.

Formulation Code	Gelatin (% w/v)	HPMC K 100 M (% w/v)
F1	6	1.25
F2	5	1
F3	6	1
F4	7	1.5
F5	6	1.5
F6	5	1.25
F7	7	1
F8	5	1.5
F9	7	1.25

The fitted model quality was expressed by the coefficient of determination R^2 , and its statistical significance was checked by the F-test (analysis of variance) at the 5% significance level. The statistical significance of the regression coefficients was determined by using the t-test (only significant coefficients with p-value < 0.05 are included). The optimum processing conditions were obtained by using graphical and numerical analysis based on the criteria of the response surface and the desirability function. [17]

Part 1: Preparation and Evaluation of Gelatin matrix:

Preparation:

Gelatin was soaked in 100ml of water for 30 min and heated up to get a clear solution at the varying concentration of (1-7%) w/v of gelatin. The crosslinking agent glutaraldehyde (0.1- 2 %) was added along with sustained release polymer HPMC K 100M (1 -2%) followed by the addition of drug Tramadol HCL (500mg) with the cryogelant agent mannitol (4%) and tonicity adjuster (0.9 %) NaCl. This solution was then stirred vigorously for 10 mins at 1200 rpm using overhead stirrer to form a firm foam. This foam was separated and collected on Petri plates and further subjected to process of Cryogelation. The cryogelation process involves freezing mixture solution at sub-zero temperature i.e in the deep freezer at -20°C temperature for 24 h till it forms interconnected polymer matrix mass. This Gelatin matrix is subjected to drying at 70°C in hot air oven for 4 hours. Dried matrix undergoes trituration and later sifted through 60# to obtain fine brown colored powder in aseptic condition. Optimized concentration of suspending agent Sodium CMC (1%) was added, the powder was aseptically filled into previously sterilized amber colored vials 2ml and then were subjected to sterilization by hot air oven at 170°C temperature for 1 hr [20,21].

Evaluation of Gelatin matrix:

Physical appearance:

The physical characteristics and appearance of the gelatin matrix were checked by visual observation using an optical microscope.

Drug Excipient Compatibility study

Compatibility study was carried for pure drug Tramadol HCL and the combination of Tramadol HCL with an excipient in the ratio of 1:1. The spectra were collected in the 400 cm⁻¹

¹ to 4000 cm⁻¹ regions. The FTIR spectroscopic studies were recorded out on Jasco FTIR- 401 (FTIR 8400S, Shimadzu, Japan) at transmittance mode for checking incompatibility between drug and excipients by using KBr pellets method. A small amount of triturated sample was kept in the sample holder and scanned [18,19].

Swelling Time

Swelling time was observed visually for gelatin matrix powder. Dry gelatin matrix powder was added in 2ml distilled water and the time required to swell the powder was determined as swelling time [21,23].

Swelling Index

The extent of swelling was measured in terms of percent weight gain by the gelatin matrix in distilled water. The swelling behavior of all formulations was studied. Swelling index was determined by soaking pre-weighed gelatin matrices in distilled water. Soaked matrices were filtered and blotted to remove excess liquid from the medium at the predetermined time (5, 10, 15 min) and their weight was determined by using weighing balance and % swelling index was calculated by the following equation [20,23].

$$\% \text{ SI} = \{(W_t - W_i) / W_i\} \times 100$$

Where SI = swelling index,

W_t = weight of swollen gelatin matrix at respective time intervals and

W_i = weight of gelatin matrix at time (t) = 0.

Scanning Electron Microscope

Morphological analysis of gelatin matrix powder was carried out by Scanning Electron Microscope (JSM – 6360A, MAKE- JEOL). The sample was previously mounted on a brass stub using double-sided adhesive tape and then was made electrically conductive by coating, with a thin layer of platinum (3–6 nm), for 100s and at 30 W in a vacuum. The stub containing the coated samples was placed in the scanning electron microscope chamber. Scanning electron photomicrograph of the platinum-coated sample was taken at appropriate magnification (1000x & 100x) [20,23].

Particle Size and Zeta Potential Measurements

Formulation powder was suspended in distilled water and sonicated to form a uniform dispersion. The particle size and zeta potential of the formulation were obtained using a particle size analyzer (Malvern Zetasizer ZS 90 UK) [20,22].

Part 2: Preparation and Evaluation of Injectable Suspension

Preparation:

The injectable suspension was prepared by reconstituting the powder formulation. Dry gelatin matrix powder was reconstituted in 2ml of Sterile Water For Injection (SWFI) at the time of administration to form an injectable suspension [15].

Evaluation of Injectable suspension:

Appearance and pH

The appearance of the formulation was checked by visual observation using optical microscope and pH of the injectable suspension was measured using pH meter (Deluxe 101).

Syringeability

It was important to assure syringe ability of formulation prior to the animal study. Syringeability of the formulation was assured using 20 to 26 G needles. All the prepared formulations (2ml) each were withdrawn into 5 ml identical plastic syringes which contain 20 to 26 gauge needles. The solution which was easy to pass from a particular syringe was termed as pass and the one which was difficult to pass was termed as fail [20,22].

Sedimentation Volume

The sedimentation volume F , is the ratio of the equilibrium volume of the sediment V_u , to the total volume of the suspension, V_i . In sedimentation volume study, the suspension was transferred to a stoppered measuring cylinder and was stored at room temp for 24 h. The volume of sediment formed was noted at regular interval of time (5, 15, 30 and 60 mins) [20,21].

$$\text{Sedimentation volume} = \frac{\text{Volume of sediment (V}_u\text{)}}{\text{Total volume of suspension (V}_i\text{)}}$$

Sterility Testing

Sterility testing was carried out as per the IP 2016. The test formulation was incubated for not less than 14 days at 30°-35°C (anaerobic condition) in the Alternative Thioglycolate Medium to find the growth of bacteria and at 20°-25°C (aerobic condition) in Soybean Casein Digest Medium to find the growth of fungi in test formulation. The sterility test was performed using positive and negative controls. As the formulation is the dry powder which will form sponge when it comes in contact with the fluid. Therefore, to confirm the formed sponge is sterile, the bacterial and fungal strains are added along with the test formulation in two test tube respectively and the other test tubes contain only test formulation with media. The bacterial strain used for positive control was *Clostridium sporogenus* whereas the fungal strain used was *Candida albicans*. The above samples were observed visually for any turbidity to assess the growth of bacteria and fungi [26,28].

In-vitro Drug Release Studies



The *in vitro* drug release studies of Tramadol HCL injectable suspension were carried out using Orbital Shaking Incubator (REMI model) to maintain the temperature at 37°C and agitated at 50 rpm for 5 days. The dissolution medium used was Simulated Body Fluid buffer (pH 7.2). 1 ml of aliquots was withdrawn at specific time intervals (1, 24, 48, 72, 96, 120 hrs) and replenished with fresh medium to maintain the sink conditions. Aliquots withdrawn were filtered, suitably diluted and analyzed using UV spectrophotometer (V-530, Jasco) at 271nm [25,27].

Determination of drug release kinetics

To describe the kinetics of the drug release from the sustained release matrix powder, mathematical models such as Zero-order, First-order, Higuchi, Hixon-Crowell, Korsmeyer-peppas models were evaluated by model-dependent (curve fitting) method using PCP Disso V2.08 software (Poona College of Pharmacy, Pune) [25,27].

Drug content estimation

The quantity of formulation powder equivalent to 10mg of Tramadol HCL was taken and diluted with 10ml of methanol (1000 µg/ml) and evaporated to dryness. The residue was diluted up to 10 ml with methanol to get a stock solution of (100µg/ml). From this stock solution, a solution of 50 µg/ml was prepared and analyzed by UV spectrophotometer. The concentration of the drug present in the formulation was computed from the calibration curve using the equation $y = mx + c$ [23].

Accelerated Stability Study

Stability studies were carried out on optimized formulation according to International Conference on Harmonization (ICH) guidelines. Formulations were filled in sterilised vials and subjected to ambient temperature i.e. at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / $60\% \pm 5\%$ RH, at accelerated conditions $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / $75\% \pm 5\%$ RH by storing the samples in the stability chamber and at refrigerator conditions i.e. $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$. Samples were analysed for appearance, pH, swelling time, drug content and *in vitro* drug release [29].

RESULTS AND DISCUSSIONS



Optimization study

The design of experiment (DOE) is an approach in which process variables are first screened and then optimized to determine best settings for the variables. The full factorial design is a quadratic design which requires 3 levels (-1, 0, +1) for each factor. In which the concentration of Gelatin and HPMC K 100M were selected as the independent variables whereas Swelling time and Drug release were selected as the dependent variables. The interactions between the factors were demonstrated using 3-D graphs. The experimental values obtained were compared with those predicted by the mathematical models. The data generated is given in Table 3 which was analyzed using Design Expert software version 10.0 and polynomial equations were obtained for the same.

Table 2: 3² experimental design with response

Formulation Code	Swelling time (mins)	Drug release (%) (Time - 120 hours)
F1	14	88.83
F2	13	92.92
F3	15	95.75
F4	15	87.31
F5	13	82.25
F6	11	81.81
F7	17	98.59
F8	10	78.64
F9	16	90.09

Response Surface plots:

(1) Swelling Time:

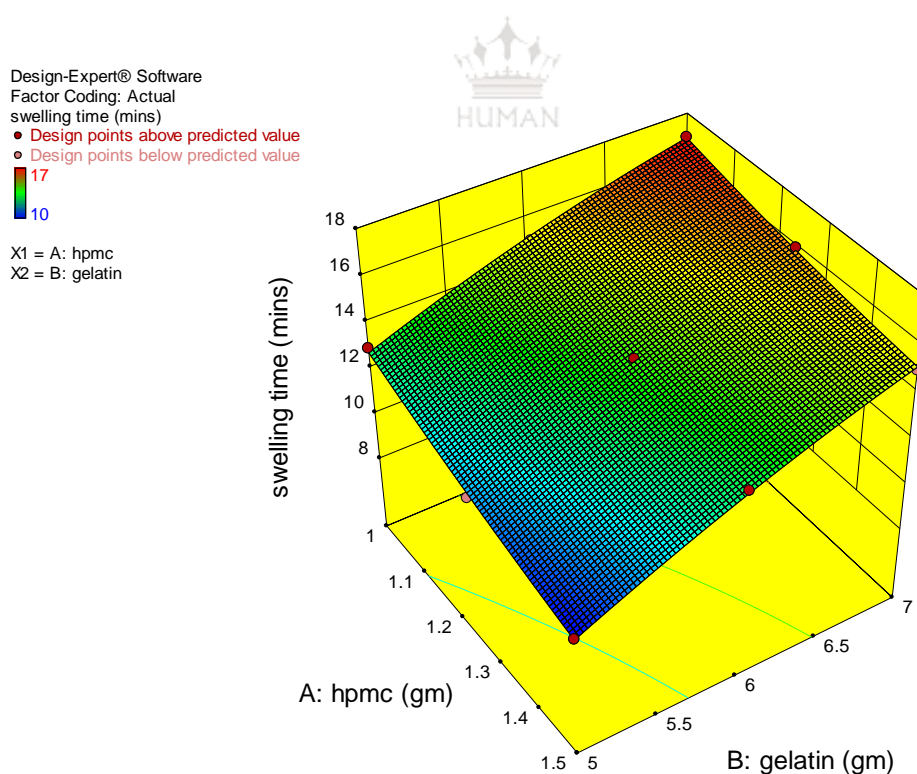


Figure 1: Response surface of swelling time

Swelling time is the time required to swell up the polymer. It is an important parameter in injectable sponge preparation, as the swelling of the polymer before the administration will cause obstruction in injecting the formulation and therefore, the polymer should not swell up immediately at the time of reconstitution and remain suspended till the administration. Swelling time depends on polymer concentration i.e. Gelatin and HPMC K 100 M.

Figure 1 shows the combined effect of gelatin (polymer) and HPMC K 100M (sustain release polymer) on swelling time. HPMC K100 M concentration was selected as an independent variable as it affects the crosslinking density in the polymeric network as well as swelling ability. It was observed that swelling time of polymer decreased as the concentration of sustain release polymer increased. It may be due to fact that cross-linked structure works as the negative force pulling the polymer chains inside, impeding its mobility and hence lowering the swelling time and on the other side increasing the gelatin concentration, swelling time increases.

Final Equation of Swelling time in terms of Coded Factors:

$$\text{Swelling time} = +13.89 - 1.17*A + 2.33*B + 0.25*AB + 0.17*A^2 + 0.33*B^2 \dots\dots\dots (1)$$



Where, A is concentration of HPMC K 100 M and B is concentration of Gelatin.

From the equation, the negative sign of A indicated that as the concentration of HPMC K 100 M increases, swelling time decreases. The positive coefficient of B indicated that as the concentration of gelatin increases, swelling time increases. The high coefficient of the AB term signified the interaction between independent variables and their effect on the response and it can be considered as a positive effect on swelling time. The higher value of a coefficient of B than a coefficient of A indicated that gelatin concentration affected swelling time to a greater extent than HPMC K 100M concentration.

(2) Drug Release:

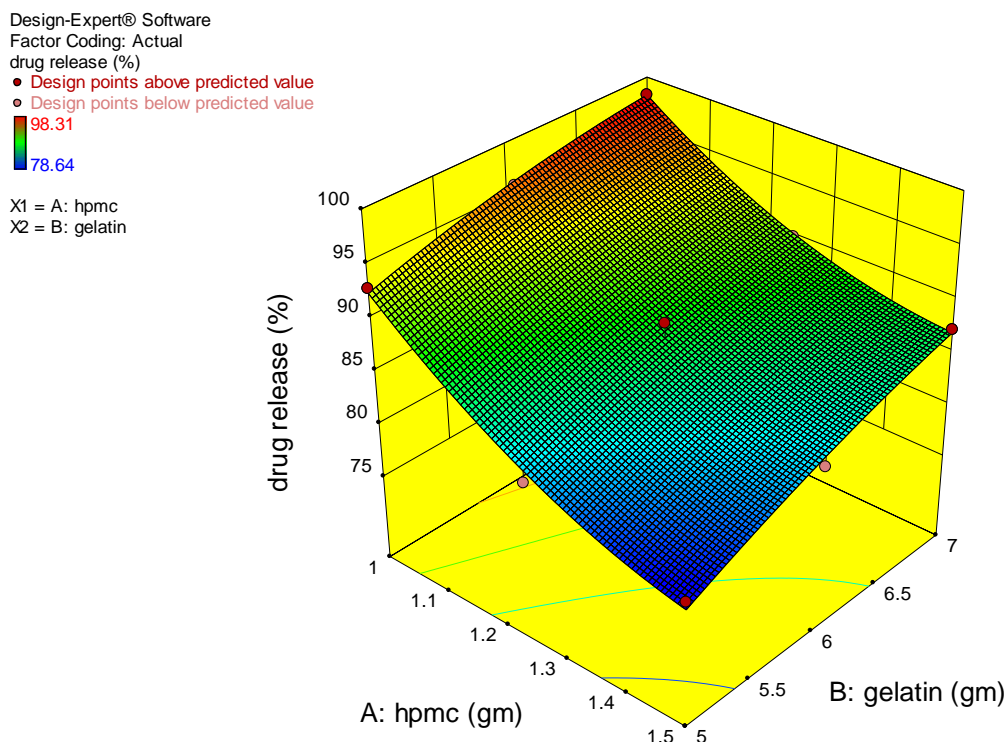


Figure 2. Response surface for Drug Release

Figure 2 shows the combined effect of HPMC K 100M and gelatin on drug release. In order to obtain a sustained release over a 5 day period, it was necessary for the *in situ* sponge formed to have a sufficient strength and stability. Alone gelatin could not impart these parameters so HPMC K 100M was added as a copolymer to achieve sufficient strength, stability, and sustained release. It is observed from the plot that concentration HPMC K 100 M has pronounced effect on drug release because as the concentration of HPMC K100M increases, drug release gets sustain. 3D response surface plot for drug release showed that decreasing the concentration of HPMC K 100M at a higher level of gelatin resulted in a greater degree of drug release.

Final Equation of Drug release in terms of Coded Factors:

$$\text{Drug release} = +87.42 - 6.46*A + 3.72*B + 0.82*AB - 2.29*A^2 + 0.76*B^2 \dots\dots\dots (2)$$

In this case, A and B were found to be significant model terms. The positive coefficient of B indicated that as the concentration of gelatin increased, the drug release also gets increased.

The negative sign of A is indicating that lower the concentration of HPMC K 100M, the higher will be the drug release. The low coefficient of A indicated that HPMC K 100M concentration affected drug release to a greater extent than gelatin concentration. The positive coefficient for term AB for the combined effect of both factors was found to have a positive effect.

In order to find out the contribution of each component and their interaction, Analysis of Variance (ANOVA) was carried. The ANOVA analysis of the quadratic model showed that the model was significant ($p < 0.05$) which was also supported by the high F value and with the adequate Precision (ratio > 4) was observed as shown in Table 3 and validation of the Response Surface Methodology are shown in Table 5.

Table 3: ANOVA studies

Response variables	F value	p-value	Adj R^2	Pred R^2	Adequate Precision
Swelling Time	127.63	0.0011	0.9875	0.9461	33.675
% Drug Release	39.81	0.0061	0.9604	0.8792	18.866

Table 4: Desirability function of optimized formulation

Formulation Code	Gelatin (% w/v)	HPMC K 100M (% w/v)	Desirability
F 7	7	1	0.998

Table 5: Validation of the Response Surface Methodology (RSM)

Responses	Experimental value	Predicted value	%Predicted error
Swelling Time	17	17.482	2.82
% Drug Release	98.59	98.809	0.21

Part 1] Evaluation of Gelatin Matrix:

Physical appearance:

All the formulations were of brown color, brittle matrix powder in appearance. Dry heat sterilization had no effect on physical and chemical properties of the formulation.

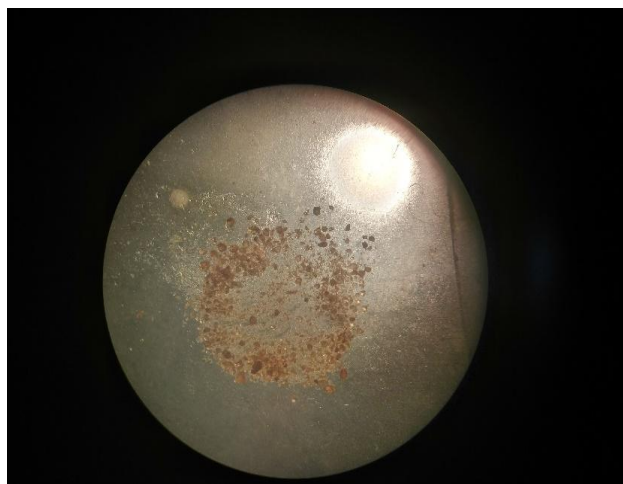


Figure 3: Optical microscopy of gelatin matrix powder

Drug Excipient Compatibility study:

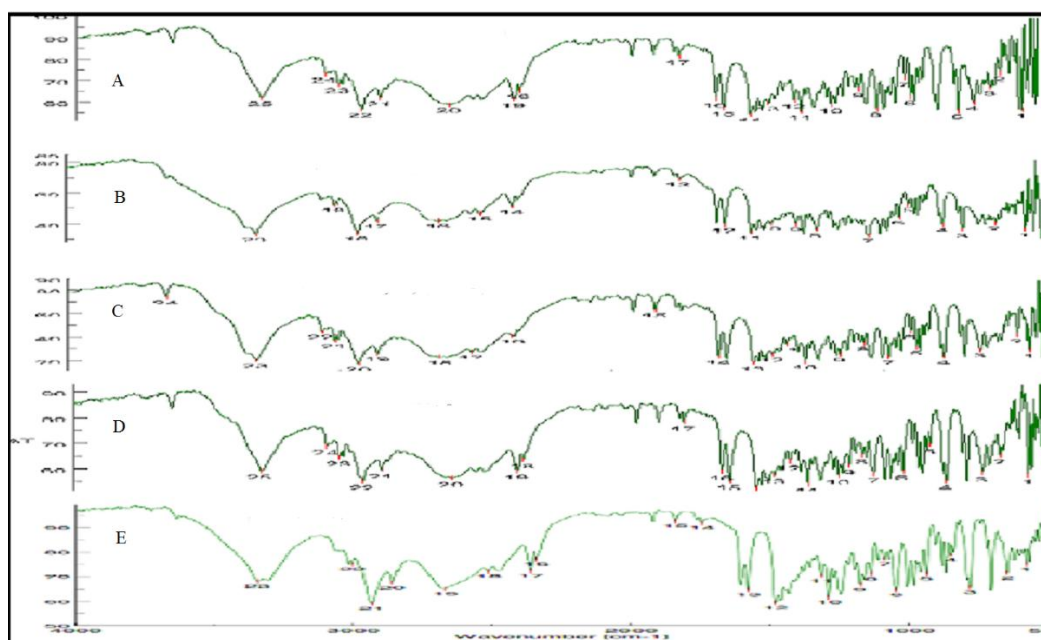


Figure 4: FT-IR Spectra of A) Drug- Tramadol HCL B) Drug + Glutaraldehyde C) Drug + HPMC K100M D) Drug + Gelatin E) Formulation of Tramadol HCL

The characteristic IR absorption peaks of Tramadol HCL and other excipients were at 3305.39 - 3310.85 cm^{-1} due to -OH stretching, 3018.05 - 3065.92 cm^{-1} due to aromatic C-H stretching, 2929.64 - 2950.17 cm^{-1} due to C-H stretching of $-\text{OCH}_3$, 2601.5 - 2861.85 cm^{-1} due to C-H stretching of $-\text{CH}_2$ and $-\text{CH}_3$ groups. 1578.85 - 1607.38 cm^{-1} may be due to C=C ring stretching. 1288 - 1301 cm^{-1} -C-H bending of symmetric and asymmetric of $-\text{CH}_2$ and $-\text{CH}_3$ groups. 1006.66 - 1075.18 cm^{-1} due to -C-O-C group and 702.28- 783.21 cm^{-1} due to substituted benzene ring.

From the results, it is clear that there is no appreciable change in the positions of the characteristic bands of the drug along with the IR spectrum of the optimized formulation. Since there is no change in the drug nature and position of the bands in the physical mixtures and formulation, it can be concluded that the drug maintains its identity without going any physical and chemical interaction with the polymers used.

Swelling time

Swelling time of gelatin matrix of optimized formulation was found to be 17 mins. It is the time required for the matrix powder to swell after reconstitution in sterile water for injection.

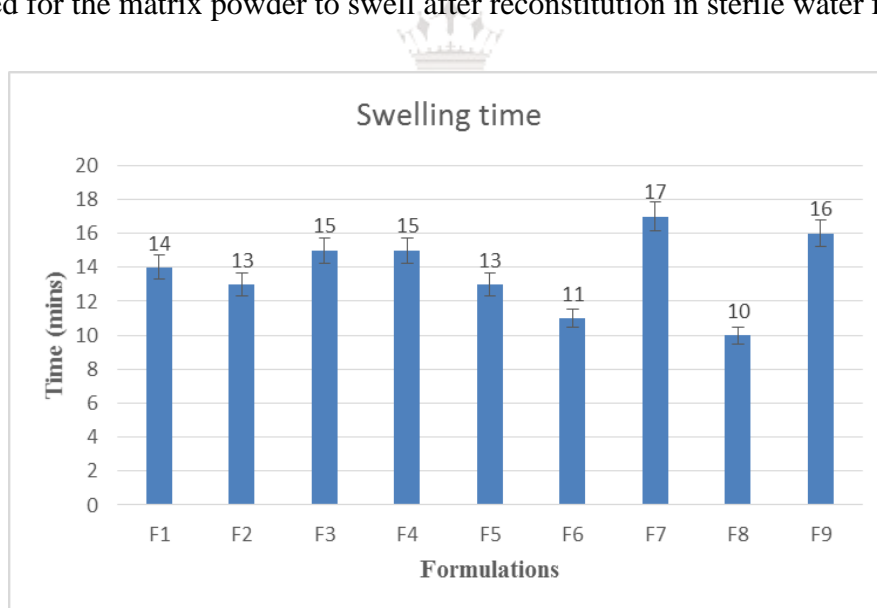


Figure 5: Swelling time of all the formulation

Swelling Index

The prepared gelatin matrices were subjected to the swelling index which ranged from 50% to 88%. Swelling index of the optimized F7 batch was found to be 87%. It represents the

capacity of swelling of the polymer matrix in the body fluid. Gelatin matrix absorbs body fluid in the body and it opens the pores of the matrix to diffuse the drug from the swollen network and sustain the release over 5 days period.

Scanning Electron Microscopy

Scanning Electron Microscopy is used to study the microscopic aspects of the formulation and helps in detecting and analyzing surface fractures, provides information about microstructures and identify crystallinity structures. The gelatin matrix was observed under 100 X and 1000 X magnification respectively. It was evident from the images of SEM (Figure 5a & b.) that gelatin matrix had a very crystalline and rough structure. The pore size was in the range of 10-100 μm . Morphology of gelatin sponge is shown in figure 6.

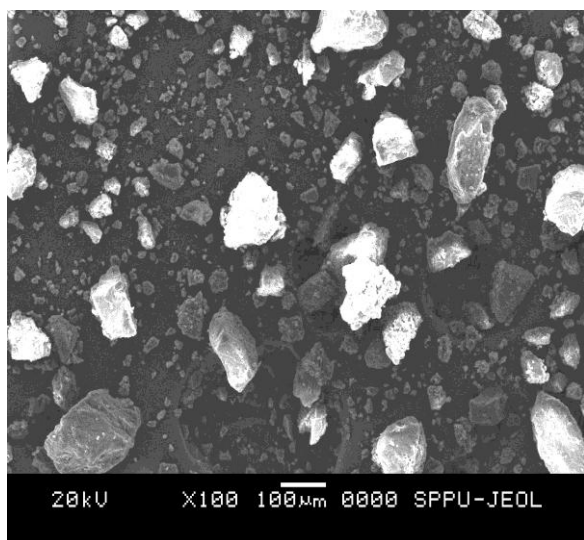


Figure 6a: SEM of formulation at 100 X

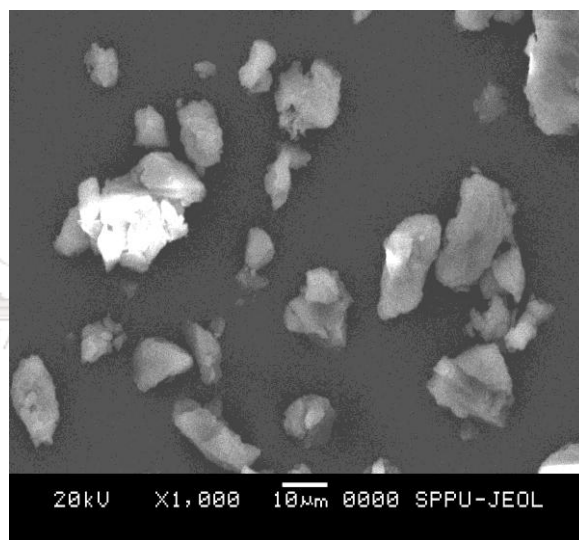


Figure 6b: SEM of formulation at 1000 X

Particle Size and Zeta Potential Measurements:

The particle size of gelatin matrix powder suspension was found to be 0.8754 μm (875.4 d.nm) and Pdi was 0.765. Gelatin suspension has good the particle size that indicates formulation will easily administer in the body and easily passed through blood vessels.

Zeta potential is a measure of surface charge and a key indicator of the stability of the colloidal dispersion. Zeta potential of the formulation was found to be -5.99 mV that significantly show the extent of electrostatic repulsion between all the adjacent, like charged particles in a dispersion. Thus, gelatin suspension has good physical stability.

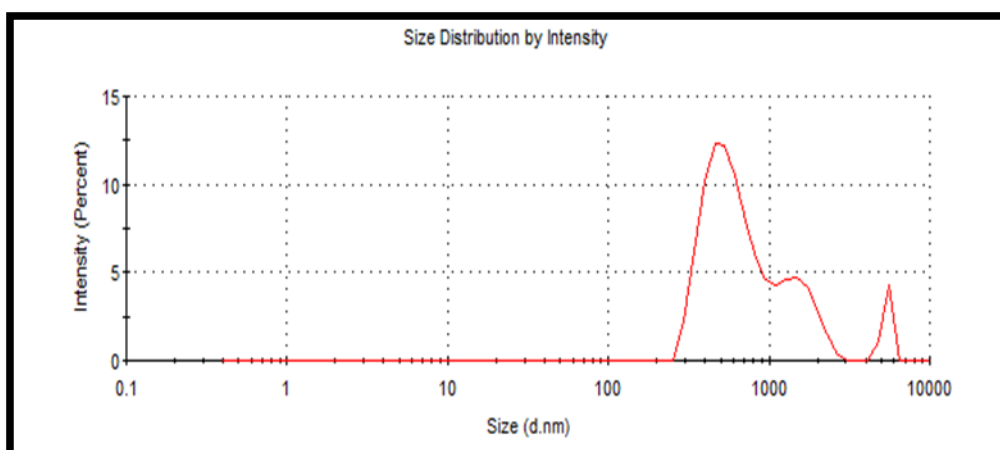


Figure 7: Particle size of the gelatin dry powder matrix

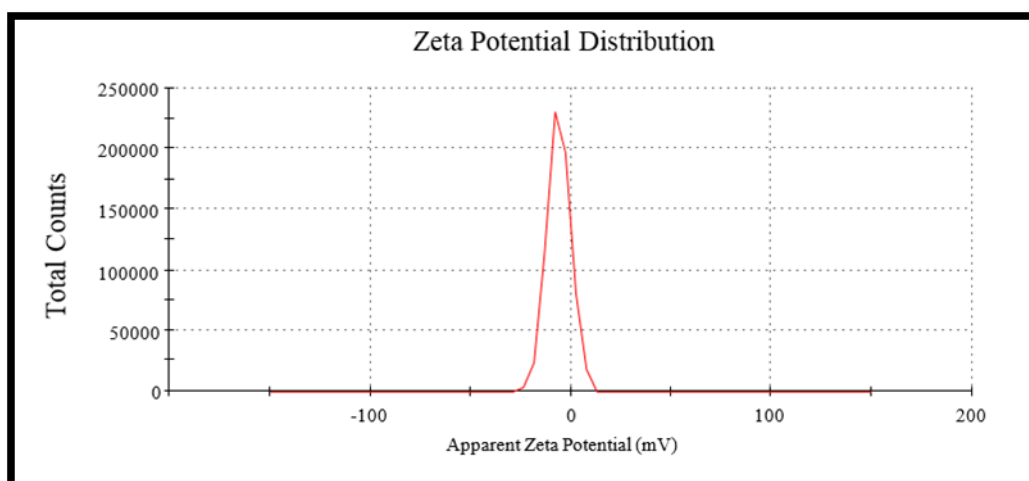


Figure 8: Zeta potential of the gelatin dry powder matrix

Part 2] Evaluation of Injectable suspension:

Appearance and pH:

All the formulations were dispersed suspension system and observed under an optical microscope which is shown in figure 9. The pH of the all the formulations was found in between 6.3-6.6 i.e. in the range tolerated by the subcutaneous tissue. Moreover, the drug was found to be most stable in this pH range. Thus the formulation follows all the prerequisites of the parenteral dosage form.

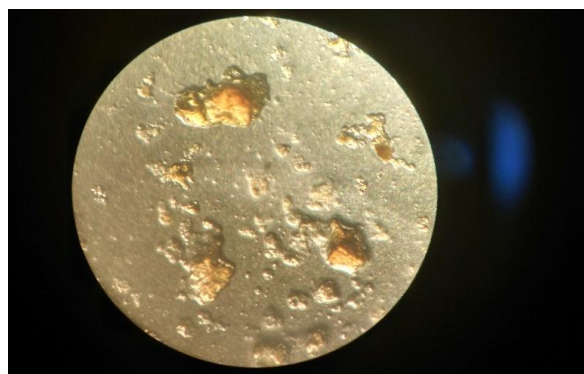


Figure 9: Optical microscopy of gelatin sponge after reconstituted in distilled water.

Syringeability:

The formulation easily passed through the needle gauge 18. This needle size is suitable for subcutaneous injection.

Table 6: Syringeability of optimized formulation

Needle gauge no.	Observation
18	Pass
20	Fail
22	Fail
24	Fail

Sedimentation volume study:

The value of sedimentation volume, F ranges from nearly 0 to 1. The sedimentation volume was found to be constant 0.085 for a period of 60 min and afterward, it was constant. The ultimate height of the solid phase depends on the concentration of solid and the particle size.

Table 7: Sedimentation study of optimized formulation

Time	Sedimentation volume
5 min	0.070
15 min.	0.080
30 min	0.085
60 min	0.085

Sterility testing

Parenteral formulations are sterile preparations. The formulation showed no turbidity after 14 days and thus was found to be free from bacteria and fungi. Hence, it passes the test for sterility as per I.P. The sterility of the formulation may be attributed to the aseptic process of preparation and filling in the pre-sterilised amber color vials and subjected to dry heat sterilization at 170°C temperature for 1hr.

Table 8: Observations of bacterial and fungal growth

Sample	Observations		Inference
	Bacteria	Fungi	
Positive control	Turbid	Turbid	Growth
Negative control	Clear	Clear	No growth
Test sample 1 with bacteria/fungi	Turbid	Turbid	Growth
Test sample 2,3,4	Clear	Clear	No growth

In-vitro Drug Release Studies



In vitro drug release study serve as a comparative tool in formulation and development. In vitro drug release of the gelatin, matrix formulation was regulated by matrix diffusion mechanism. Dry matrix powder when reconstituted with SWFI, it absorbs the water and swells up to form a sponge in which drug is completely diffused. From this swollen network, drug diffusion takes place. To sustain the drug release for 5 days, HPMC K 100M was added and as the concentration of HPMC K100M increases drug release was retarded. The in-vitro release for all the formulation is shown in figure 10. The optimized formulation releases 98.59 % of a drug on the last day which indicated sustenance of the release for 5 days.

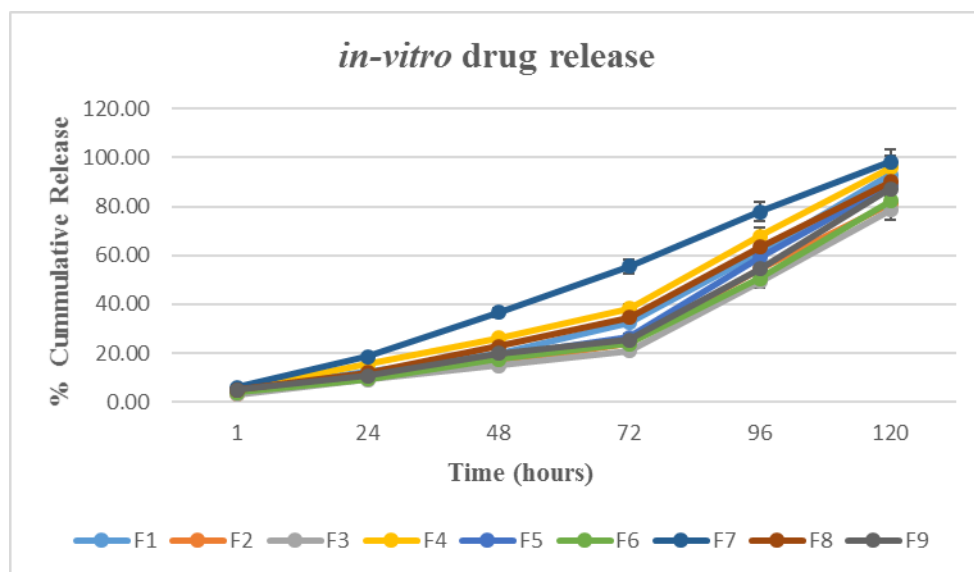


Figure 10: In-vitro drug release of all the formulation

Kinetics and mechanism of drug release

Kinetic results shown in Table 9 reveals that the best fit model for optimized batch was zero-order kinetics as correlation coefficient (r^2) values are higher than other release kinetics. The calculated n values from power law equation for drug release profiles were between 0.5119-0.7523 with a correlation coefficient (r^2) values > 0.93 , suggest that the optimized formulation followed non-Fickian (anomalous) diffusion mechanism for drug release.

Table 9: Model for release kinetics

Formulation F7	Zero order	First order	Korsemeyer-Peppas order	Higuchi order
R^2	0.993	0.918	0.9206	0.897

Drug content estimation

The percentage drug content in the formulation was calculated and found to be 97.85%, indicating insignificant loss of drug during the formulation.

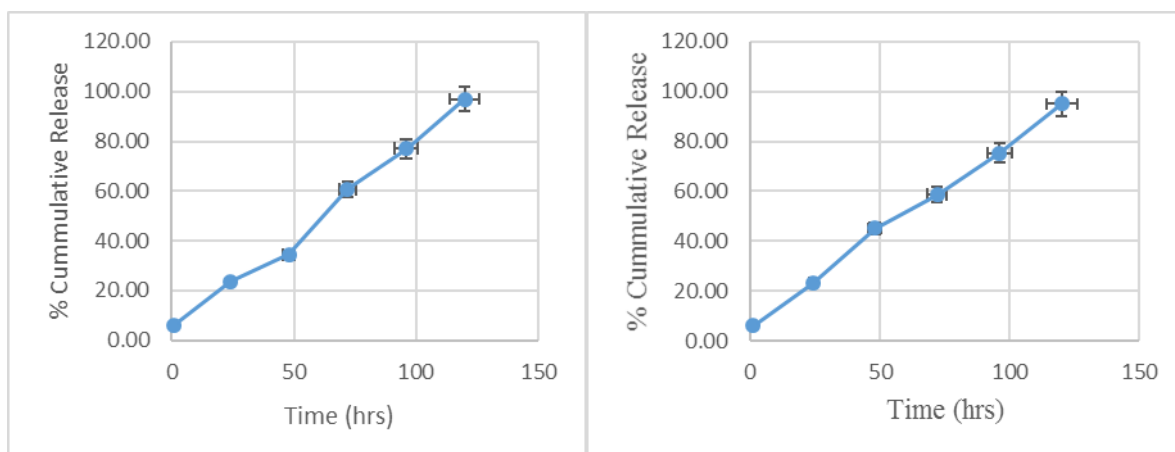
Stability Studies

Stability studies indicate that formulation F7 was physically and chemically stable at ambient temperature i.e at $25^\circ\text{C} \pm 2^\circ\text{C}$ / $60\% \pm 5\%$ RH, accelerated conditions $40^\circ\text{C} \pm 2^\circ\text{C}$ / $75\% \pm$

5% RH and at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for a period of 1 month. From stability studies, it was observed that the formulation of Tramadol HCL was stable at selected storage conditions in amber colored vials. It shows no change in appearance, color, pH, with the minute decrease in swelling time, *in-vitro* drug release and drug content at all the storage conditions. The results from stability studies in Table 10 show that out of 3 storage conditions, refrigerator condition shows the most stable formulation.

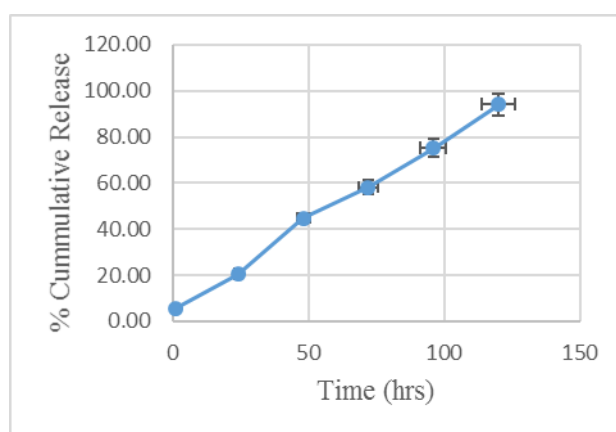
Table 10: Stability studies results after 1 month

Formulation code	Storage condition	Appearance, color, pH		Swelling time (mins)	Drug Release at 120hr after 1 month	Drug content (%)
		Before Reconstitution of gelatin matrix	After Reconstitution of gelatin matrix			
F7	Room temperature($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / 60% RH \pm 5%	Brown powder	Dispersed suspension, 6.5	16	95.10%	97.82
	Accelerated temperature($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / 75% RH \pm 5% RH)	Brown powder	Dispersed suspension, 6.4	15	94.20%	97.80
	Refrigerator ($5^{\circ}\text{C} \pm 3^{\circ}\text{C}$)	Brown powder	Dispersed suspension, 6.5	17	96.95%	97.84



At refrigerator

At room temperature



At 40°C

Figure 11: *Invitro* drug release after 1 month

CONCLUSION

The present work was based on the need to reduce the frequency of administration of Tramadol Hydrochloride by formulating as a sustained-release injectable formulation which is helpful in improving the patient's compliance. Formulation of Tramadol Hydrochloride as an in the situ sponge injectable drug delivery system would be a novel and advantageous over the prevailing tablets and injections that require administration every day. Injectable gelatin sponge formulations can be anticipated as a promising alternative to conventional oral and parenteral dosage forms. Thus, easing the practice of medication with an objective to improve the quality of therapy and save the valuable life of human being.

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