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Formulation Development of Sustained Release Epidural Injection of Analgesic Drug

Mrs. Mirajkar Reshma*, Mrs. Dhavale Kalyani, Dr. Madgulkar Ashwini

Department of Pharmaceutics, AISSMS College of Pharmacy, Near RTO, Kennedy Road, Pune 411001, Maharashtra, India

ABSTRACT

The objective of this work was to formulate and evaluate sustained release epidural injection of analgesic drug diclofenac sodium used in chronic lower back pain. The formulation composed of a thermosensitive polymer Pluronic F127 (20%) and sustained release copolymers HPMC K100M (1%) and HPMC K4M (0.5%) optimized using 3^2 factorial design. The formulation was found to be clear, colorless, sterile, syringeable through 18gauge, forming a stable gel at 37°C with a gel strength of 9.67g/cm. The drug release was found to be 98.13% in 72 hrs. The formulation was found to be stable at refrigerator temperature of 5°C for a month. Thus, a stable parenteral formulation was developed that can be an appropriate and convenient approach for patients requiring frequent parenteral administration, reducing recurrence of dosage and ultimately expanding patient comfort and satisfaction in case of chronic ailments.

Keywords: Diclofenac Sodium, in situ gel, Pluronic F127, Epidural, lower back pain.

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***Address for Correspondence:**

Reshma Mirajkar, Department of Pharmaceutics, AISSMS College of Pharmacy, Near RTO, Kennedy Road, Pune 411001, Maharashtra, India

INTRODUCTION

The lower back supports the weight of the upper body and provides mobility for everyday motions such as bending and twisting. Muscles in the low back are responsible for flexing and rotating the hips while walking, as well as supporting the spinal column. Nerves in the low back supply sensation and power the muscles in the pelvis, legs, and feet. Most acute low back pain results from injury to the muscles, ligaments, joints, or discs. The body also reacts to injury by mobilizing an inflammatory healing response. While inflammation sounds minor, it can cause severe pain.

Symptoms of lower back pain are usually described by type of onset and duration i.e. Acute pain comes on suddenly and lasts for a few days or weeks, and is considered a normal response of the body to injury or tissue damage, Subacute low back pain is usually mechanical in nature (such as a muscle strain or joint pain) but is prolonged and Chronic back pain which is defined as lower back pain that lasts over 3 months, this type of pain is usually severe, does not respond to initial treatments, and requires a thorough medical workup to determine the exact source of the pain. It is also possible for low back pain to develop with no definitive cause. When this happens, the primary focus is on treating the symptoms rather than the cause of the symptoms and the patient's overall health.[1]

Various treatments are available for low back pain such as physical therapy, surgeries, and medications. The medications includes topical such as Aspercreme, Ben-Gay, Oral medications (NSAIDs, Narcotics, Muscle relaxants, steroids, etc) and injections. Epidural steroid injections are common treatment option for many forms of low back pain, however the effects of the injection tend to be temporary, so it need to be used in combination with comprehensive rehabilitation program, providing relief from pain for one week up to one year.[2]

The rationale behind injecting drug into the epidural space adjacent to the spinal nerve is that it will combat the inflammatory response and thus reduce pain. The inflammation can lead to direct neuronal activity, as well as swelling and mechanical compression of the nerve within the intervertebral foramen so to facilitate earlier pain relief and return to full function, for rapid effects of medication on cerebrospinal tissues or meninges, medication can be administered into the epidural space of the spinal cord. This technique avoids absorptive problems otherwise presented by the blood–brain barrier. So far, to our knowledge, for general clinical epidural use there is only one slow-release liposome product of morphine available on market (DepoDur®).[3,4]

Diclofenac is non-steroidal anti-inflammatory drug (NSAID), indicated in the relief of all grades of pain and inflammation associated with a wide range of conditions, including arthritic conditions, acute musculo-skeletal disorders and

other painful conditions resulting from trauma. Usual strength of diclofenac injection is 25 mg per ml. [5]

In-situ forming polymeric formulations are drug delivery systems that is in sol form before administration in the body, but once administered, undergoes gelation, In-situ to form a gel.

It provides accuracy of dose, increase residence time and bioavailability at targeted site, reduction in dosing frequency; prolong duration of action and patient compliance. Side effects such as quick release, less residence time at targeted site of action of conventional injections can be overcomed. A long acting, single dose epidural formulation would improve clinical treatment of various pain states therefore; an injectable epidural in situ gel is being developed. [6]

MATERIALS AND METHOD

MATERIALS

Diclofenac Sodium was obtained as a gift sample from Emcure Pharmaceuticals Pune. PluronicF127 was provided by Ana lab fine chemical, Mumbai. Hydroxy propyl methyl cellulose K100M, K4M (HPMC K100M, HPMC K4M) was provided by Chemica-biochemic-reagents, Ottochemie, Pvt.Ltd. Sodium chloride was provided by LobaChemiePvt. Ltd. Mumbai.

METHOD

1. Optimization study -

A response surface statistical experimental design was used to optimize the effect of different independent factors on dependent variables. The variables were investigated using a 3^2 full factorial designs using Design-Expert Software® 11 (Stat-Ease, Inc., USA)

This design was based on a 3^2 factorial design, three replicates of the central run, leading to 9 sets of possible combinations, allowing each experimental response to be optimized. Different batches were prepared with different independent factors at different levels and responses. The experiments were designed to study effect of three independent variables namely the Syringeability of the formulation through 18 G needle, highest gel strength and % drug release for prolonged period of time i.e. for 3 days.

Table 1: Formulation combination as per the 3^2 full factorial designs

Formulation code	Pluronic F 127 % (W/V)	HPMC K100M % (W/V)	HPMC K4M % (W/V)
F1	20	0.5	0.5
F2	20	1	0.5
F3	20	2	0.5
F4	20	0.5	1
F5	20	1	1
F6	20	2	1
F7	20	0.5	2
F8	20	1	2
F9	20	2	2

The quality of the fitted model was expressed by the coefficient of determination R^2, and its statistical significance was checked by an 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 desirability function and the response surface.[7]

2. Preparation of Formulation

The method of preparation of in situ gel involved slow addition of thermoresponsive polymer i.e. Pluronic F127 with continuous stirring in required quantity of cold water, followed by slow addition of drug in the polymer solution. This solution formed was kept in refrigerator at 5°C for 24 hrs to dissolve completely and form a uniform drug solution. The required quantities of other excipients like copolymer HPMC K100M, HPMC K4M and tonicity adjusting agent i.e. NaCl were added to this solution under continuous stirring to form a homogeneous, clear colourless solution. The formulation was filled aseptically in transparent glass ampoules of 3ml and sterilized by autoclaving at 121°C at 15 psi for 20 min and stored at refrigerator condition.[8, 9, 10, 11]

Evaluation of Formulation

1. Appearance & pH

The optimized formulation was evaluated for visual appearance. The pH of the solution form was measured using standardized digital pH meter (Deluxe pH meter 101/EI) by dipping glass electrode in sufficient volume of 20ml of formulation in beaker.[12]

2. Gelation Temperature

Gelation temperature was determined by modification of Miller and Doravan technique. A 3 ml aliquot of solution was transferred to test tubes immersed in a cryostatic water bath at 20° C and sealed with aluminium foil. The temperature of water circulation bath was increased with increments of 1°C and left to equilibrate for 5 min at each new setting. The samples were examined for gelation which was said to have occurred when the meniscus would no longer move upon tilting through 90°angle.[8, 9, 10]

3. Gel strength

Gel strength was determined by Surimi test using Texture Analyzer (Texture Pro CT V1.4). Formulation (20 ml) was placed in 25 ml beaker and gelled using heating plate at 37°C. The probe (TA 3/100) was allowed to traverse the gel up to 1 cm at a speed of 1mm/s. The load reading was taken in g/cm for gel strength.[13]

4. Viscosity

Viscosity was measured on Brookfield viscometer using RV (spindle no. 21) and T-type helipath spindle (spindle no. S21). Viscosity was analyzed by subjecting the sample at room temperature and 37°C and variable shear stress.[14]

5. Syringeability

Syringiablity of the formulations was assured using 18 to 22 gauge needles. Formulation was withdrawn into identical 5 ml disposable syringes placed with needles to a constant volume (3 ml). The solutions which were easily passed from a particular syringe were termed as pass and the ones which were difficult to pass were termed as fail.[12]

6. Drug content estimation

The quantity of formulation containing about 50mg of Diclofenac Sodium was taken and shaken with 60ml of methanol in a 200-ml volumetric flask and was diluted up to volume with methanol. 5ml of this solution was diluted up to 100ml with methanol was prepared and analyzed by UV spectrophotometer and HPLC. The concentration of the drug present in the formulation was computed from the calibration curve using the equation y = mx+ c.[15]

7. Sterility Testing

Sterility testing was performed as per the IP 2014. The formulation was incubated for not less than 14 days at 30-35˚C (anaerobic condition) in the alternative fluid thioglycolate medium to observe the growth of bacteria & at 20˚C-25˚C (aerobic condition) in soyabean casein digest medium to observe the growth of fungi in formulation. The test was performed using positive and negative controls.[16, 17]

8. *In-vitro* Drug Release Studies

The in vitro drug release studies of in-situ forming gel sustained release injectable of diclofenac sodium were conducted using Orbital Shaking Incubator (REMI model) maintained at 37°C and agitated at 30 rpm under mild agitation for 3 days conditions similar to physiological conditions. This speed was kept slow enough to prevent the formulation gel matrix from breaking. Phosphate buffer (pH 7.4) was used as the dissolution medium. At specific time intervals (1, 24, 48 and 72hrs), 1 ml of aliquots was withdrawn and replaced with a fresh medium to maintain sink conditions. Aliquots withdrawn were filtered, suitably diluted and analyzed using UV spectrophotometer (V-530, Jasco) at 281 nm. The percentage cumulative drug release (% CDR) was calculated using an equation obtained from a calibration curve.[18, 19, 20]

9. Accelerated Stability study

Stability studies were conducted on optimized formulation according to the guidelines of International Conference on Harmonization (ICH). A sufficient quantity of formulations were kept at room temperature i.e. at 25˚C ± 2˚C/60% ± 5% RH, refrigerator i.e. at 5˚C± 3˚C and accelerated conditions(40˚C±5˚C/75±5%RH) for 1 month. After 1month samples were evaluated for appearance, pH, clarity, gelation temperature, % drug content and *in vitro* drug release.[21, 22]

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. The concentration of HPMC K100M and HPMC K 4M were selected as the independent variables whereas Syringeability, Gel strength and Drug release were selected as the dependent variables. The Formulations from F6 to F9 failed to pass through 18 Gauge needle and were not syringeable due to high viscosity though they showed highest gel strength, so they were not taken further for drug release. 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 2 which was analyzed using Design Expert software version 11.0 and polynomial equations were obtained for the same.

Table 2: 3^2experimental design with response

Formulation code	Gel strength (g/cm)	% Drug release (At 72 hrs)
F1	7.28	105.46
F2	9.67	98.13
F3	10.66	84.59
F4	10.17	94.23
F5	9.51	87.43
F6	13.3	-
F7	12.11	-
F8	11.94	-
F9	14.07	-

Response Surface plots:

(1) Gel strength

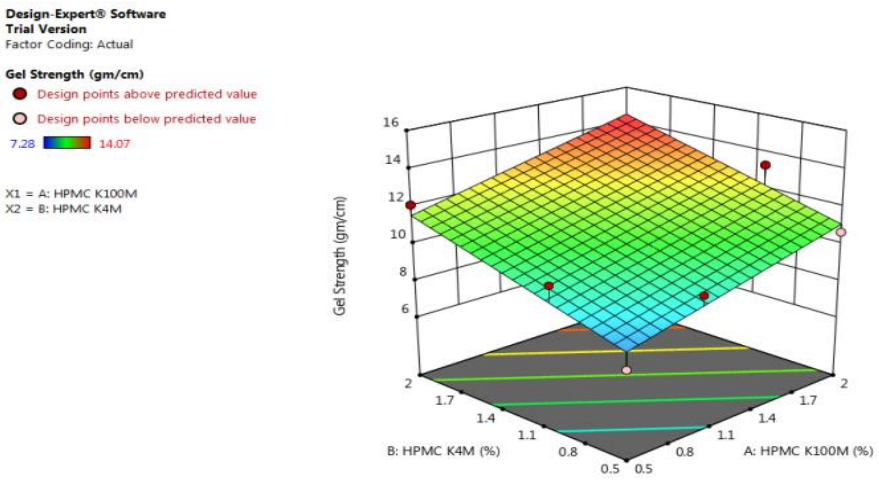

Figure 1: Response surface of gel strength

The optimized batch showed gel strength of 9.67g/cm. This is obtained by synergistic effect of HPMC K100M and HPMC K4M. Pluronic F127 is a non-ionic triblock copolymer having central hydrophobic chain of polyoxypropylene chain and lateral identical hydrophilic polyoxyethylene chains (PEO-PPO-PEO). Pluronic F127 shows thermo-induced changes in micellar properties. They have some important drawbacks that may limit their applicability, such as their weak mechanical strength, not stable and rapid dissolution. The gel must have the sufficient gel strength to withstand the various body shear forces. Alone Pluronic F127 does not impart gel strength. So copolymers HPMC K100M and HPMC K4M were added. Both show the response on gel strength and stability.

As shown in figure 1, response surface plot showed that as the concentration of polymers increases, the gel strength increases.

In the equation, A & B having positive effect on gel strength. The interaction terms A & B have synergistic effect on gel strength. Final Equation of gel strength in terms of Coded Factors

Gel strength= + 11.32+1.46*A+1.68*B.... (1)

Where, A is concentration of HPMC K100M and B is the concentration of HPMC K4M.

(2) Drug Release:

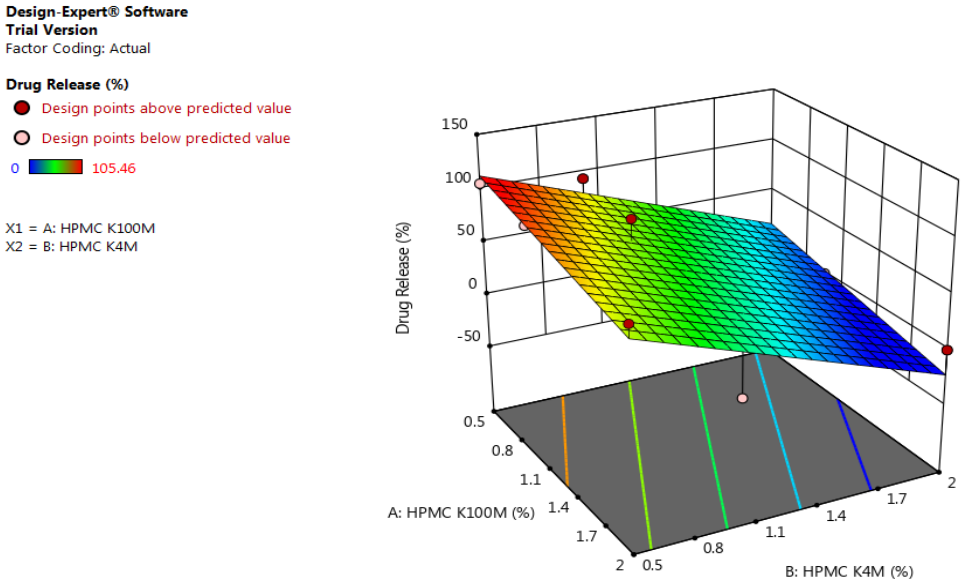

Figure 2: Response surface for Drug Release

As shown in Figure 2, response surface plot showed the combined effect of HPMC K 100M and HPMC K4M on drug release. In order to obtain a sustained release over a 3 days period, it was necessary for the in situ gel formed to have a sufficient strength and stability that was achieved by adding copolymers to the thermoreversible polymer. HPMC K100M was initially added that prolonged the release but showed initial burst release due to lack of uniform and coherent gel. So, another copolymer HPMC K4M was added that reduced the burst release and prolonged the release. 3D response surface plot for drug release showed that with increase in the concentration of both the copolymers release is prolonged.

Final Equation of Drug release in terms of Coded Factors:

Drug release = +44.72-20.06*A-47.72*B... (2)

Where, A is concentration of HPMC K100M and B is the concentration of HPMC K4M. In this case, A and B were found to be significant model terms. The negative sign indicates an antagonistic effect or inverse effect of A and B on the drug release. The negative sign of A and B both polymer is indicating that lower the concentration of polymers, 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 HPMC K 4 M concentration due to high viscosity obtained with K100M than K4M.

Analysis of variance (ANOVA) has been designed to determine the contribution and interaction of each variable to the model. The F distribution is a forecast proportion used by the analysis of the distribution of variances. The value of F would equate to one if they are equal. The F value of the ANOVA is the model mean square (MS) to the corresponding model mean square error. The higher the ratio, the higher the F value and the more likely the variance that the model contributes is significantly higher than a random error.

The conclusion is based on the analysis of variance that the selected design sufficiently represents the statistics formulation of a sustained release parenteral drug delivery system.

The ANOVA analysis of the linear model indicated that the model was significant ($p<0.05$) also endorsed by the large 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 R2	Pred R2	Adequate Precision
Gel strength	20.21	0.0022	0.8277	0.7172	12.4543
% Drug Release	15.30	0.0044	0.7814	0.5927	10.0593

Table 4: Desirability function of optimized formulation

Formulation code	HPMC K100M (%W/V)	HPMC K4M (%W/V)	Desirability
F2	1	0.5	1.000

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

Responses	Experimental value	Predicted value	% Predicted error
Gel strength	9.67	9.14	0.53
% drug release	98.13	99.021	0.891

Appearance and pH

The optimized formulation was colorless, clear and transparent. The clarity of formulation indicates that all the ingredients were dissolved completely showing no visible residue or particulate matter in the formulation. Autoclaving sterilization did not have effect on the stability of formulations. The pH of the solution form was found to be 7.1-7.4 at room temperature which is suitable for epidural administration.

Gelation temperature

The optimized formulation contains 20% pluronic F127 (P407) that showed gelation at the 37°C temperature i.e. body temperature. It was found that with increasing concentration of the polymer, gelation temperature decreased as shown in fig. 3. Pluronic F127 is a triblock copolymer with a central hydrophobic chain of PPO and two identical hydrophilic chains of PEO(PEO-PPO-PEO). In solution form i.e. in water, as the concentration of pluronic F127 increased, micelle formation occurs, followed by aggregation of micelle. The gel phase could occur when the concentration is above the micellar concentration. The hydrogen bonding between PPO chains and water keeps the hydrophobic portions of the pluronic separate when the material is dissolved in cold water. When the temperature is increased, the disruption of hydrogen bonding occurrs and hydrophobic interactions causes gel to be formed.

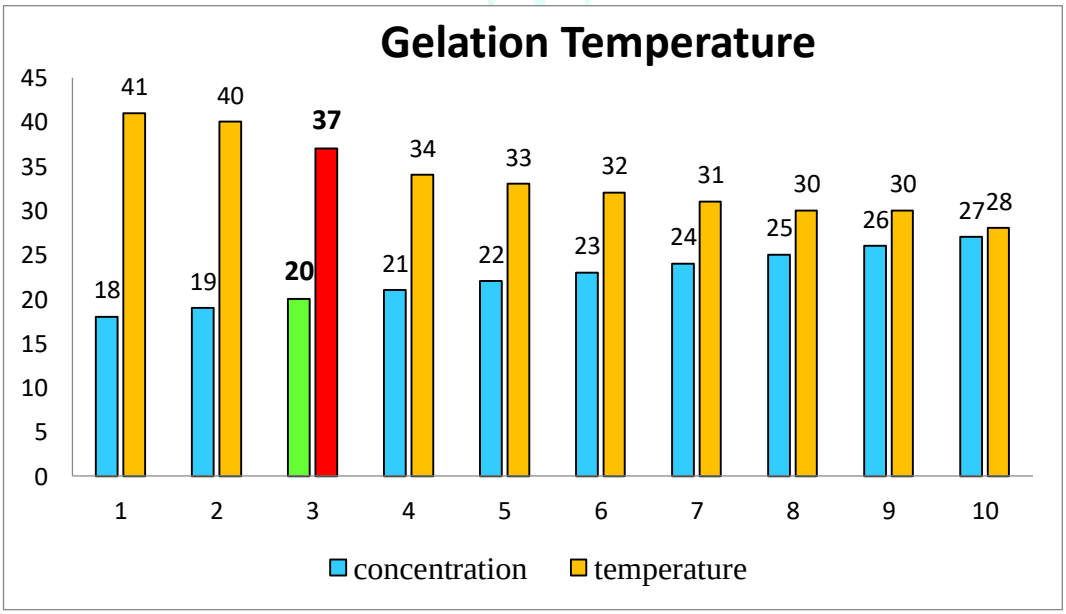

Fig 3: Effect of concentration of PF127 on gelation temp

Gel strength

Gel strength of optimized formulation was found to be 9.67 g/cm which was sufficient to maintain the gel stability over the prolonged time period. Gel strength provides an overview of the mechanical properties of gel. It also signifies the ability of gel to withstand shear forces in the body for prolonged period of time. The Gel strength of formulation was important variable dependent on the concentration of gelling agent i.e. Pluronic F127. Pluronic F127 (20%) reveals that it does not have sufficient gel strength to maintain for prolonged time so copolymers were added that increased the gel strength, by forming hydrophobic interactions with Pluronic F127.

Viscosity

The viscosity of optimized formulation in solution form was found to be 110cP and the viscosity of gel form was found to be 1180cP. To administer formulation easily into the body it should possess optimal viscosity in solution form. This viscosity is sufficient for ease of administration of injection. Further the formulation undergoes phase transition from sol to gel transition upon contact with body temperature. The viscosity of polymer solutions is the result of hydration of polymer chains.

Syringeability

The optimized formulation was easily passed through the needle gauge 18. This needle size was suitable for epidural injection.

Drug content

The percentage drug content in the formulation was calculated and found to be 98.62%, indicating insignificant loss of drug during the formulation.

Sterility testing

Test for bacteria & fungi

The test samples were observed for turbidity after 14 days. It was showing clear solution. So the formulation was found to be free from bacteria and fungi and hence it was found to be sterile that was suitable for parenteral administration. Thus the sterility of the formulation may be attributed to aseptic process of preparation and filling the container and autoclaving at 121°C at 15 psi for 20 min.

Table 6: Observations for bacterial& fungal growth

Sample	Observations		Inference
	Bacteria	Fungi	
Positive control	Turbid	Turbid	Growth
Negative control	Clear	Clear	No growth
Test sample	Clear	Clear	No growth

In vitro Release study

The in vitro release of the optimized formulation F2 was found to be 98.13% after completion of 3 days. The Fig.4 shows the comparative in vitro drug release of all batches. The drug release was regulated by erosion and diffusion mechanism. The formulation got converted into gel form as the formulation was added to the preheated phosphate buffer pH 7.4 at 37^{0}C. The thermoresponsive polymer i.e. pluronic F127 undergoes phase transition from sol-gel and formed barrier for drug release. As the time passes the outer layer became more hydrated and the polymer chains become fully relaxed and leading to erosion from the surface. Simultaneously, The hydrophilic copolymer HPMC K100M and K4M being swellable, polymer started swelling and drug molecules began to move out of gel matrix by diffusion when exposed to the media. As the concentration of HPMC K100M, HPMC K4 M copolymer increases, drug release was retarded.

Accelerated Stability testing

Studies of stability indicated that optimized formulation was physically and chemically stable at ambient temperature i.e. at 25 ° C ± 2 ° C/60 % RH ± 5 % RH and at accelerated conditi ons 40 ° C ± 2 ° C/75 % RH ± 5 %RH and at refrigerator condi tions i.e. at 5 ° C ± 3 ° C for a period of 1 month. From stability studies, it was observed that the formulation of Diclofenac Sodium was stable at selected storage conditions with most suitable storage condition at the refrigerator temperature. It showed that there was no change in colour, gelation temperature but slightly increases in gel strength and slightly decreases in pH, gel strength and in vitro drug release profile at 40°C.

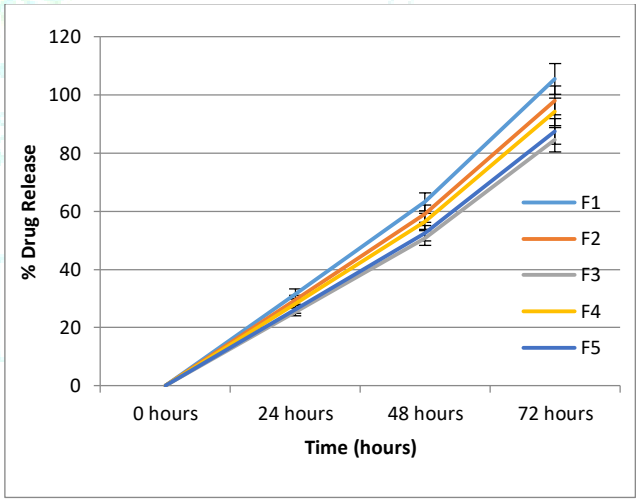

Fig: 4 In-vitro drug release

Table 10: Stability studies results after 1 month

Evaluation parameters	Initial	After storage at room temperature (25±2°C/60±5 % RH)	After storage at Accelerated condition (40±2°C/75±5%)	After storage at Refrigerator temp (5°C±3°C)
Appearance	clear,colorless	clear, colorless	clear, colorless	clear, colorless
pH	7.4	7.3	7.2	7.4
Gelation temperature	37° C	37° C	37° C	37° C
Gel strength (g/cm)	9.67	9.36	9.50	9.17
Drug content	98.62	97.93	96.59	98.12
Syringeability	Passes	Passes	Passes	Passes
In vitro drug release at 72 hrs after 1 month	98.13	97.70	97.81	97.99

CONCLUSION-

Thus a stable and novel formulation of Diclofenac Sodium as a sustained release epidural injection was developed and evaluated for treatment of chronic lower back pain. It is an alternative to the conventional and other sustained release formulations owing to its ability to achieve a prolonged release with single administration of drug to the target site (epidural space),reduced frequency of administration and improved patient compliance. Also the formulation involves less complicated fabrication and ease of administration.

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