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Formulation and Evaluation of an In-Situ Forming Inject able Sustained Release Sponge of Mefenamic Acid

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ABSTRACT

The overall aim of the present study was to develop sustained release parenteral drug delivery system involving formation of an in-situ sponge of anti-rheumatoid drugs. The study involvedformulation of the drug mefenamic acid in a sponge forming gelatin matrix as a dry powder for reconstitution into a suspension to be injected into the body forming a sponge in-situ intended to release the drug over a period of 5 days. The formulation was prepared by the method cryogelation and optimised using gelatin as a sponge forming polymer,crosslinking agent glutaraldehyde, sustained release retardant hydroxypropylmethyl cellulose 1% andsuspending agent sodium carboxymethylcellulose to form a solution intended to be administered subcutaneously. The formulation was evaluated for all prerequisitesof parenteral and other parameters of gelatin matrix like welling index, sedimentation study, syringeability, percentage drug content, swelling time, in-vitro diffusion study, sterility study and stability studies. The formulation was found to sterile, isotonic, having swelling index 90%, swelling time was 10 min. The in vitro diffusion study was found to be over 95.98% in simulated body fluid PH 7.4 at 37°C. Over a prolong period of 5days the formulation was physically and chemically stable at accelerated conditions for period of 1 month.

Key words: In-situ injection, Anti-rheumatic, Cryogelation, Sponge forming gelatin, Sustained release

INTRODUCTION

There are many possible routes of drugadministration such as oral, transdermal and parenteral. Oral route is not effective because of first pass metabolism. Transdermal drug delivery has advantage over oral route which is first-pass effect. But main problem with drug penetration in transdermal drug delivery is stratum corneum. (S.K. Sindhu et al.,2014) The Parenteral route is the most effective and common form of drug delivery system. Parenteral route has more advantages over both oral & transdermal routes. In parenteral drug delivery system, drug reaches to systemic circulation with rapid absorption. But main problem with parenteral route is rapid decline of drug concentration in systemic circulation.

It is used for which the bio-availability in limited by high first pass metabolism effect of otherphysicochemical limitation and for drugs with a narrow therapeutic index. Parenteral drug delivery has different technologies that can reduce the total number ofinjectionsthroughout the drug therapy period. To overcome this problem, Extended-release & Controlled-release drug delivery systems have been developed. The development of injectabledrug delivery system has received considerable attention over the past few years. The reason to receive attention is advantages of new injectable drug delivery system.Development of new sustained release injectable formulation has received considerable attention due to many advantages of these systems such as localized and site-specificaction, prolonged delivery period, decreased drug dosages, reduction of side effects and improved patient comfort and compliance over the conventional parenteral delivery. (Reshma Mirajkar etal.,2016)

Injectable sponge drug delivery system can entrap wide range of drugs and then release them into body over time. It is a unique technology for the novel release of injectable

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agents and consists of nano or micro porous beads loaded with active agent. After the creation of micro beds or matrix, it gets compressed following the compression the sponge gets filled with active agent or medicine and injected into body. Following the injection, the sponge will expand to the original size and shape and start releasing drugs. Sponge or scaffolds have been developed using various techniques such as fiber bonding, gas foaming, microemulsion formation, phase separation, freezedrying, and porogen leaching. More recently, gelation at sub-zero temperatures, known as cryogelation, has been used to create sponge with large interconnected pores. During cryogelation, the reactants are restricted to the unfrozen/semi-frozen phases and form a crosslinked network upon polymerization, while the ice crystals nucleated from the aqueous phase during freezing function as porogens. The melting of the ice crystals at temperature above the freezing temperature gives rise tointerconnected macroporous networks. Injectable sponge drug delivery system which involves use of natural injectable polymers like chitosan, gelatin, sodium alginate, collagen etc. which act as a depot and release the drug in a timely fashion Gelatin is a naturalpolymer which isbiodegradable, nontoxic in nature. Due to its easy process ability and gelation properties, gelatin has been manufactured in a range of shapes including sponges, injectable hydrogels and gelatin microspheres etc. Gelatin sponges or matrices have been utilized for many regional drug delivery systems among the other forms of gelatin.Absorbable gelatin sponge (AGS) was introduced by Correl and Weisman as absorbable hemostatic agent in 1945.(Princely S. et al., 2015, Perry. C et al, 2013)

In the present study, Mefenamic acid injectable sponge in a gelatin matrix system intended for 5days sustained release to be administrated in patient suffering from Rheumatoid arthritis. The formulation could furtherinvestigateto improve the bioavailability of drug and avoid first pass metabolism thereby improve the patient compliance in Rheumatoid arthritis patients.

Materials

Mefenamic acid was obtained as a gift sample from Sun pharma Mumbai. Hydroxy propyl methyl cellulose from Himedia Laboratories Mumbai. All other chemicals used were analytical grade and were used without purification. Double distilled water was used in the study.

Preparation of Gelatin matrix

Gelatin was soaked in 100ml of water for 30 m to get solution at the varying concentration of 1-6% w/v of gelatin. The drug Mefenamic acid was added followed by addition of crosslinker glutaraldehyde (0.1-0.5 %) and then added sustained release polymer HPMC K100M (1-3%). This solution was then stirred vigorously for 10 min at 1000 rpm using overhead stirrer to form firm foam. This foam was separated and spread on petri plates and further subjected to process of cryogelation. The process involved drying of gelatin matrix in deep freezer at -10 to -12 ºC temperature for 24 hrs till it formed a dry porous matrix mass.This dry gelatin matrix mass was sifted through sieve 60 to get fine brown colored powder in aseptic condition. Optimized concentration of suspending agent sodium CMC (1%) was added the powder was aseptically filled in previously sterilized amber colored vials 2ml and then were subjected to sterilization by hot air oven at 140°C temperature for 2 hrs.(Wei,Princely S 2015, Reshma Mirajkar 2016 et al)

Table 1 Formulation Table of Gelatin Matrix

S.No	Formulation	Mefenamic Acid (mg)	Gelatin	Glutaraldehyde	HPMC	Na CMC
1	F1	250	4	0.1	3	1
2	F2	250	5	0.1	3	1
3	F3	250	6	0.1	3	1
4	F4	250	4	0.3	3	1
5	F5	250	5	0.3	3	1
6	F6	250	6	0.3	3	1
7	F7	250	4	0.5	3	1
8	F8	250	5	0.5	3	1
9	F9	250	6	0.5	3	1

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Evaluation of Gelatin matrix

Appearance

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

Drug Excipient Compatibility study

The FTIR spectrum of pure drug and physical mixture in 1:1 ratio was recorded on spectrophotometer for interaction between them by using KBr pellet method.

Swelling Index

Swelling index was determined by soaking pre-weighed pieces (1 x 1 cm) of gelatin matrices in double distilled water. Soaked matrices were removed with blunt forceps and blotted to remove excess liquid from the medium at predetermined time (10, 20, 30 min) and their weight was determined by using digital weighing balance and % swelling index was calculated by the following equation.(Perry, Princesly et al.,2015)

$$\% S = \frac{W2 - W1}{W1} \times 100$$

Where, S is the percentage water adsorption of gelatin matrices at equilibrium.
W1 is the initial weight of gelatin matrix
W2 is the after-immersion weight of the gelatin matrix

Preparation and Characterization of Injectable suspension Preparation

Injectable suspension was prepared by reconstitution of 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.

Evaluation of Injectable suspension

Appearance and pH

The appearance of formulation was checked by visual observation and pH of injectable suspension was measured using pH meter which was previously calibrated using standard buffers of pH 4 & pH 7.

Gelling Capacity

The gelling capacity plays important role for in-situ gelling system. The formulation should have an optimum viscosity must be easy to administered by injection as a liquid which undergo sol-to-gel transition.

Syringeability

Syringeability of the formulation was assured using 21 to 24 G needles. It was important to assure syringeability of formulation prior to animal study. All prepared formulations were withdrawn into identical 5 ml plastic syringes placed with 21to24gauge needles to a constant volume (1 ml). The solutions which were easily passed from a particular syringe were termed as pass. (Patel R, 2010, Alam A et al., 2009)

Sedimentation Volume

In sedimentation study, the suspension was transferred to a stopper measuring cylinder and was stored at room temperature for 24 hrs. The volume of sediment formed was noted at regular interval of time (1, 3, 5, 8 hrs). (Patel 2010, Alam 2009 et al.,)
Sedimentation volume = final volume (Vu)
Original volume (VO)

Swelling Time

Swelling time was observed visually for gelatin matrix. Dry gelatin matrix powder was added in 2ml Simulated body fluid (pH 7.4) Time required to swell was determined.

Particle Size

Formulation was suspended in water and sonicated to form a smooth and uniform dispersion.

Drug content estimation

0.25 ml of the test formulation was diluted with excess of methanol and evaporated to dryness. The residue was diluted up to 100 ml with mobile phase to get a stock solution of 100µg/ml. From this stock solution, a solution of 40µg/ml was prepared and analyzed by UV Spectroscopy. (Bhalerao AV et al., 2013)

Sterility Testing

Sterility testing was carried out as per the IP 2014. The formulation was incubated for not less than 14 days at 30°-35°C in the alternate fluid thioglycolate medium to find the growth of bacteria & at 20°-25°c in Soya bean casein digest medium to find the growth of fungi in formulation. The test was performed using positive and negative controls. (Indian Pharmacopeia 2014)

In-vitro diffusion studies

In-vitro diffusion was determined by Franz diffusion cell. In Franz diffusion cell, cellophane membrane is sandwiched securely between donor and receptor compartment. The receptor compartment is filled with buffer solution, which is continuously stirred and thermostated at 37^{o}C ±1^{o}C throughout the experiment. Before starting the experimentthe donor cell was sealed with paraffin film and covered with aluminum foil to prevent exposure to light. At predetermined time interval 5ml of aliquots are withdrawn and are replaced with an equal volume of fresh buffer to ensure sink condition and drug content can be determined by spectrophotometrically.(Kulwant Singh et al., 2013)

Drug Release Kinetic Data Analysis

The release data obtained from various formulations were studied further for their fitness of data in different the kinetics models like zero, first, Higuchi, Peppas.

In order to understand the kinetics and mechanism of drug release, the result of in-vitro drug release study was fitted with various kinetic equation like zero order as cumulative % release vs time, Higuchi model as log cumulative % drug release vs square root of time.r2 and k values were calculated for the linear curve obtained by regression analysis from the plots.

Zero order kinetics Drug dissolution from pharmaceutics dosage forms that do not disaggrated andrelease the drug slowly, assuming the area does not change and no equilibrium conditions are obtained can be represents by the following equation:

At=A0-k0t

Where, At= amount of drug dissolved in time t. A0 is the initial drug concentration.K0 is zero order rate constant (hr-1) when the data is plotted as cumulative percent drug release versus time, if plot is linear then the data obeys zero-order equal to K0. K0 zero order in units of conc/time and t is time in hours.

First Order Kinetics

Predicted by equation;

Log C = log C0-Kt/2.30

Where, C is the amount of drug remained at time t, C0 is initial amount of drug and K is the first order rate constant (hr-1). When the data is plotted as log cumulative percent drug remaining vs time yields a straight line indicated that the release follow first order kinetics. The constant k can be obtained by multiplying 2.303 with the slope values.

Higuchi model:

Higuchi developed several theoretical models to study the release of water soluble and low soluble drug incorporated in semi solid or solid matrixes.

Mathematical expression wasobtained for drug particles dispersed in a uniform matrix behaving as the diffusion media and equation is

Qt = KH.t1/2

Where, Qt = amount of drug release in time t, KH = Higuchi constant.

Krosmeyer and Peppas release model:

To understand the release mechanism in-vitro data was analyzed by Peppas model. As log cumulative drug release vslog time and exponent n was calculated through the slope of the straight line.

Mt/Mα=btn

Where, Mt is amount of drug release at time t, M is the overall amount of the drug, b, is constant and n is releasing exponent indicative of the drug release mechanism. (Suvakantan dash et al., 2010).

Accelerated Stability study

Stability studies were carried out on optimized formulation according to International Conference on Harmonization (ICH) guidelines. Formulations were filled in vials and subjected to room temperature i.e. at 25°C ± 2°C/60% ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH for 1 month. Samples were analyzed for appearance, pH and in vitro drug release.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR studies conducted on pure drug, polymer and physical mixture of drug with polymer showed that there is no marked interaction between drug and selected polymers. The resultant graphs were showed in fig 2.

Appearance

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

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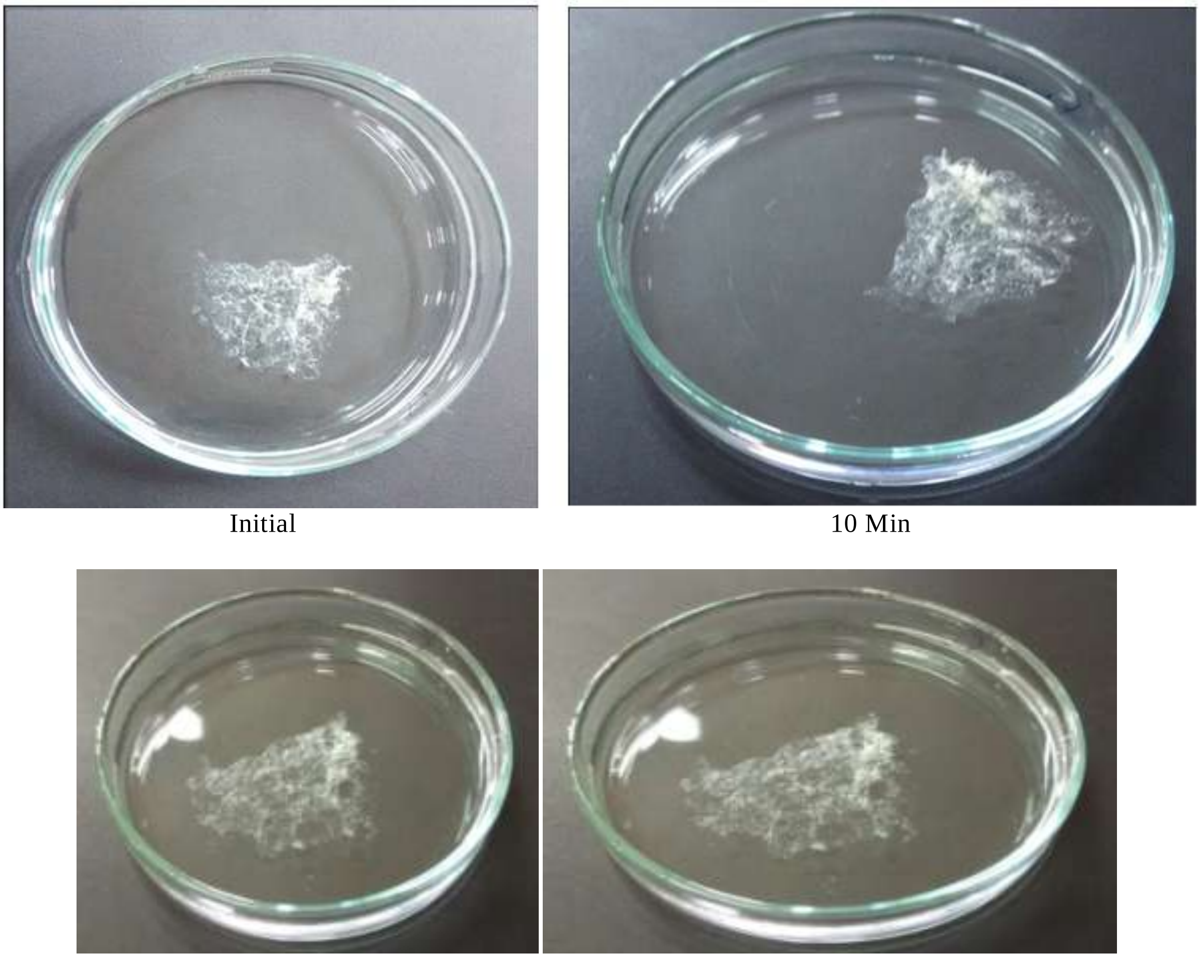
Swelling Index

The prepared matrices were subjected to swelling Index which ranged from 50 % to 90%.Swellingindex of optimized F5 batch was found to be 90%. It represents the capacity of swelling of matrix in blood. In body gelatin matrix absorbs blood or body fluid and it opens the pores of matrix to gives sustained release of drug over 5 days period. As shown in table 25% gelatin concentration has higher swelling property and also gives higher sustained to release of drug.



Initial 10 Min

20 Min 30 Min

Fig 1 Swelling Index

Evaluation of Injectable suspension

Appearance and pH

All the formulations were dispersed suspension system. The developed formulations were inspected visually for clarity in sol and gel form.

pH

All the formulations were showing pH in the range of 6.9-7.1 as shown in Table No 2.The pH values were found to be in the range tolerated by the subcutaneous tissue. Moreover, the drug was found to be most stable in this pH range. This is well in the range for parenteral administered formulation. Therefore, there is no need for adjusting pH**.**

Gelling Capacity

The formulation was transferred in such a way that places at surface of fluid in petri plates and formulation was slowly released from syringe. As solution comes in contact with gelation solution, it was immediately converted into gel sponge like structure. The gelling capacity was evaluated on basis of formed gel and time period for which formed gel remained

as such. The gelling capacity was graded in 3 categories on basis of gelation time and time period for which formed gel remain as such shown in table no.3
+ = gels dispersed rapidly
++ = gelation for few hour
+++ = gelation for an extended period.

Syringeability

Formulations F1 to F6 were easily passed through the needle gauge 21, 22as shown table no 4. This needle size is suitable for subcutaneous injection. But Formulations F7 to F9 were not passed through this type of needles because of high viscosity.

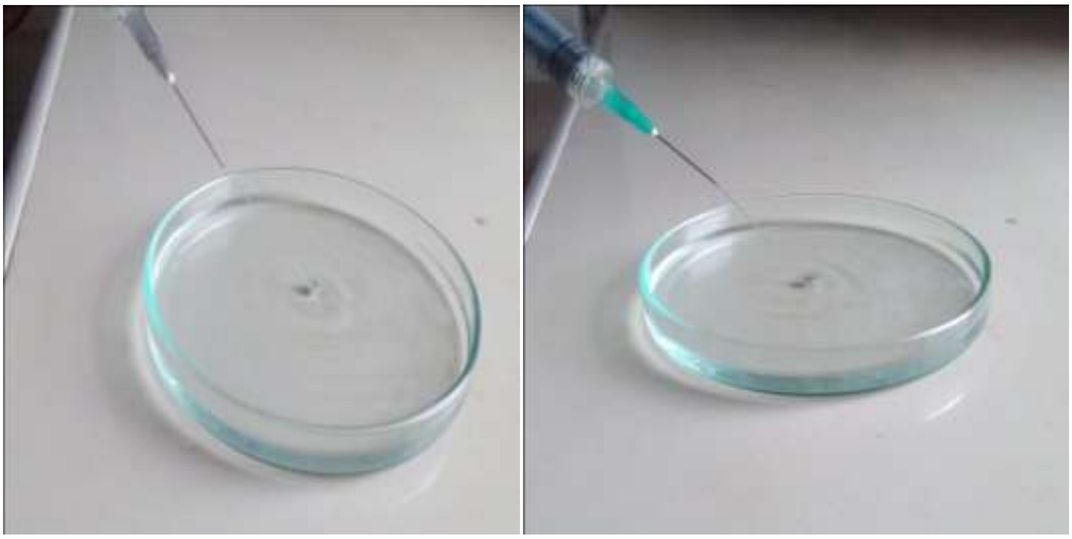


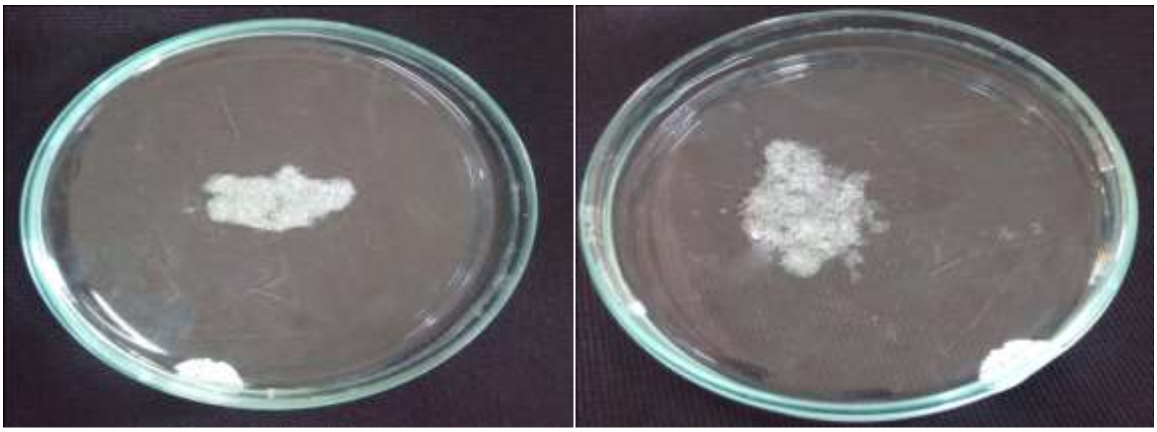
Fig 2 Syringeability

Sedimentation study

All the formulations where the sedimentation volume can have values ranging from less than 1.The sedimentation volume of F5 was found to be constant 0.075 for a period of 15 min. The ultimate height of the solid phase depends on the concentration of solid and the particle size.The result shown in table no:5

Swelling time

Swelling time of gelatin powder matrix was 10 min. It is time required to swell after injecting the reconstituted powder in sterile water for injection and thus formation of sponge in-situ.



Initial **10 Min**

Fig 3 Swelling Time

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Particle Size

Involvement of concentration of polymer and crosslinking agent has effect on both the parameters i.e. swelling index and pore size. As the matrix swells in the media, it forms porous sponge. The pore size of the sponge entraps the drug and slowly releases the drug. Thus 5% of gelatin concentration and 0.3% Concentration of glutaraldehyde (F5) was giving maximum swelling and pore size of the sponge.

% Drug content

The percentage drug content in the formulation was calculated and found to be 95.54 % indicating insignificant loss of drug during the formulations shown in table no.2.

Sterility testing

No turbidity was observed in test samples after 14 days. The formulation 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 aseptic process of preparation and filling the amber color vials and dry heat sterilization at 140°C temperature for 2 hr.

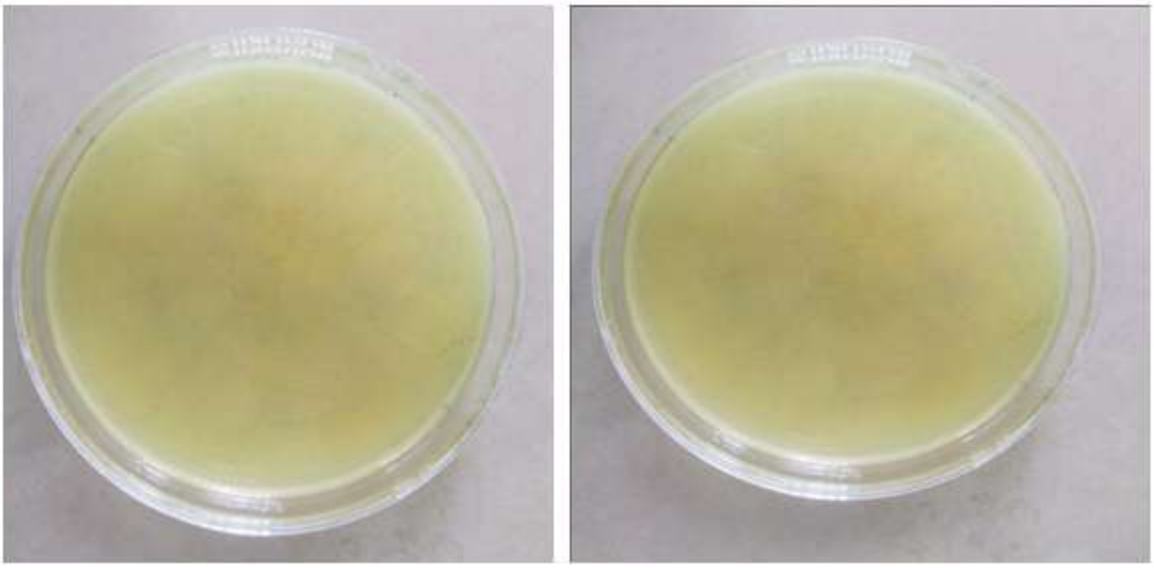

Fig 4 Sterility Test

In vitro diffusion studies

Diffusion study of Final formulation (F4, F5, F6, F7, F8 and F9) was conducted in PBS 7.4 and the results of in-vitro diffusion release study are tabulated in (table no:6) and release kinetics (mathematical model) tabulated in table no:7 The in-vitro diffusion showed that formulation F5 has given percentage cumulative drug release in 5 days 95.98 %, thus is best formulation capable of sustaining the release of drug. From shown table no:7all the values of release rate exponent (n) of Korsmeyer-Peppas release model were within the range of (0.5-1.0). 0.783 to 0.869 in PBS (pH 7.4). Therefore, it can be concluded that drug release in PBS (pH 7.4) were mainly following Anomalous Transport which corresponds to diffusion, erosion and swelling mechanism or mixed order kinetics.

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Fig No:5In-vitro Diffusion Study

Accelerated stability study

Stability studies indicates that formulation F5 was physically and chemically stable at ambient temperature i.e at 25°C ± 2°C/60% RH ± 5% RH and at accelerated conditions 40°C ± 2°C/75% RH ± 5% RH for a period of 1 month. From stability studies it was observed that the formulation of Mefenamic Acid was stable at selected storage conditions in amber colored vials. From table no: 8 shows no change in appearance, color, pH, with negligible decrease in vitro diffusion study.

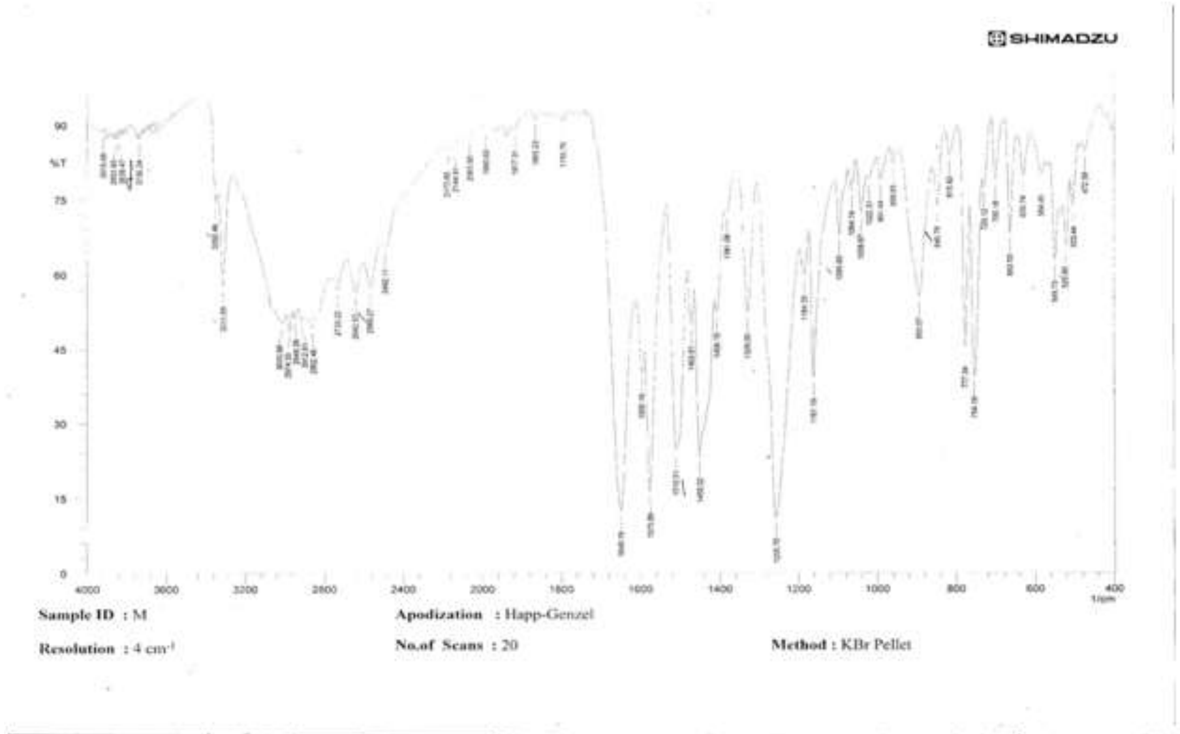

Fig 6 FT-IR Spectrum of Physical mixture

Table No: 2 Swelling Index, pH, % Drug Content

S.NO	FORMULATION	SWELLING INDEX	pH	% Drug Content
1	F1	50	6.9	83.96
2	F2	88	6.9	86.56
3	F3	66	6.9	85.74
4	F4	52	6.9	93.18
5	F5	90	7.0	95.54
6	F6	70	7.0	90.35
7	F7	58	7.1	84.24
8	F8	83	7.1	86.75
9	F9	76	7.1	83.92

Table No:3 Swelling Capacity

S.NO	FORMULATION	SWELLING INDEX
1	F1	++
2	F2	++
3	F3	++
4	F4	+++
5	F5	+++
6	F6	+++
7	F7	+++
8	F8	+++
9	F9	+++

Table No: 4Syringeability

Needle Gauge No	Result
21	Pass
22	Pass
24	Fail

Table No:5 Sedimentation Study

S.NO	FORMULATION	5 min	10 min	15 min
1	F1	0.05	0.08	0.08
2	F2	0.05	0.08	0.08
3	F3	0.05	0.09	0.09
4	F4	0.05	0.08	0.08
5	F5	0.05	0.075	0.075
6	F6	0.05	0.08	0.075
7	F7	0.05	0.08	0.08
8	F8	0.05	0.08	0.08
9	F9	0.05	0.09	0.08

Table No: 6In vitro Diffusion Drug Release

Time	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0
1	1.678	1.753	1.642	1.466	1.632	1.487
6	6.324	7.776	6.672	5.562	4.873	5.013
24	25.623	30.914	19.939	18.465	13.742	12.863
30	32.923	38.534	25.544	23.369	17.368	15.885
48	46.314	57.006	38.522	29.186	23.289	22.637
54	54.742	61.505	45.912	34.901	27.553	25.141
72	66.891	74.06	58.577	45.593	35.515	36.136
78	73.865	77.613	64.308	50.179	39.996	42.174
96	82.9	86.123	75.311	57.55	53.899	53.385
102	87.379	89.045	87.051	60.469	61.706	59.527
120	89.981	95.984	91.025	64.105	69.706	65.865

Table No: 7 Mathematical Models for the Diffusion Data For F4-F9

S.No	Zero Order	First Order	Higuchi Model	Korsmeyer Peppas Model	
	R^2	R^2	R^2	n	R^2
F4	0.975	0.722	0.950	0.869	0.996
F5	0.960	0.689	0.922	0.859	0.994
F6	0.994	0.764	0.986	0.853	0.996
F7	0.985	0.742	0.968	0.807	0.998
F8	0.985	0.816	0.996	0.783	0.982
F9	0.989	0.813	0.997	0.784	0.984

Table No: 8Stability Study

Parameters	Initial	After 30 days stability studies
Appearance	Dry Matrix powder	Dry matrix powder
Color	Yellowish	Yellowish (No change)
Ph	7.0	7.0
In vitro diffusion study	95.984	95.378

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