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FORMULATION AND EVALUATION OF DIACEREIN IMPLANTABLE IN SITU GEL FOR THE TREATMENT OF OSTEOARTHRITIS

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ABSTRACT

Osteoarthritis is the most common form of arthritis and the leading cause of disability, with an unmet clinical need for symptomatic treatment. Diacerein inhibits the association with the activity of interleukin 1-β and is useful in the symptomatic treatment of osteoarthritis but also causes diarrhoea when administered orally. Diacerein has rapid clearance due to its short half-life and thus ensures the use of a sustained release formulation for long-term use to improve its patient compliance. Unlike other joint diseases, Osteoarthritis is localized in one or a few joints, which offers a unique opportunity for local Intra-articular treatment without the risk of systemic side effects. To avoid drug-related toxicity and ineffective anti- inflammatory drugs, there is an urgent need for an improved delivery system that increases bioavailability. The potential for Diacerein in-situ gel as an effective treatment for Osteoarthritis was investigated. It is composed of poloxamer 407 as the main polymer, hydroxypropyl methylcellulose K4, and carbopol 934 as the copolymer prepared by the traditional cold method and is characterized by various parameters. All characterization showed satisfactory results. In vitro drug release is performed using phosphate buffer pH 7.4 indicating controlled drug release. In addition, the distribution of Diacerein was even in a sterile and injectable in situ gel.

KEYWORDS: Diacerein, In situ gel, Osteoarthritis, Intra-articular injection, Thermo-responsive gel.

1. INRODUCTION

Osteoarthritis is the most common type of arthritis. It is connected with the crumbling of cartilage in the joints and can occur in nearly any joint in the body. It usually occurs in the joints that carry weight on the hips, knees, and spine. It also affects the fingers, thumb, cervix, and big toes. Osteoarthritis usually does not affect other joints unless previous injury, excessive stress or underlying cartilage disruption is involved. It causes the cartilage in the joint to become stiff and lose elasticity, making them vulnerable to injury. Over time, the cartilage may degrade in some areas, considerably losing its ability to absorb shock. As the cartilage weakens, muscles and ligaments become stretched, causing pain. If the condition worsens, the bones may become inflamed.[1]

NSAIDs are a class of drugs utilized to treat inflammation and pain but their utility become greater the risk of upper gastrointestinal adverse effects and does not alter the underlying pathogenesis of articular diseases thus have very little role in modifying disease course and boost quality of life. Diacerein is a drug used for the treatment of osteoarthritis ata dosage of 50–100 mg twice a day; it retards the synthesis and activity of interleukin 1-β (inflammatory mediator in Osteoarthritis). Diacerein classified as a BCS class II drug with low solubility and high permeability, poor dissolution rate and low oral bioavailability were reported.[2,3] Oral administration of Diacerein results in diarrhoea.[3,4] Diacerein is distinguished by rapid clearance due its shorter half-life and thus justifies the use of sustainedrelease formulation for extended action to enhance its patient compliance. Therefore it is suitable to develop a technique to deliver the drug in a sustained manner.[5]

Unlike other joint ailments, Osteoarthritis is localized in one or a few joints, which offers a unique opportunity for intra-articular local treatment without the risk of adverse systemic side effects. Significant improvements may be made to make intra-articular treatment for the extended duration of action, as it is desirable to limit the number of intra- articular injections per year due to discomfort / pain associated with management, as well as the possibility of infection risk. This highlights the need for the formation of Intra-articular drug delivery that provides continuous drug release from the depot over a period of several weeks. *In-situ* injectable thermo reversible gels may be beneficial in both newly developed Osteoarthritis medications and treatments that are already available by increasing the duration of drug release in the joint space and thereby reducing the required injection frequency.[6, 7]

2. MATERIALS

Diacerein, HPMC K4, Carbopol 934 were gifted by Prim drugs and pharmaceuticals Pvt ltd, Kanchipuram, India. Poloxamer 407 was gifted by Strides, Pondicherry, India. DMSO, benzoyl alcohol, benzalkonium chloride, triethanolamine and other chemicals were purchased from Universal chemicals, Madurai, Tamilnadu, India.

3. METHODS

3.1.Fourier transform infrared (FTIR) spectroscopy

FT-IR studies were carried out to assess the compatibility between drug and excipients using FT-IR spectrophotometer (JASCO-4700). The scanning range and resolution are 400-4000 cm^{-1} and 4 cm^{-1}.

3.2.Determination of standard calibration curve

10mg of diacerein weighed and transferred in a 100ml standard flask. Then dissolved it with 10ml of phosphate buffer pH 7.4 and 10ml of DMSO and made up the volume to 100ml with phosphate buffer 7.4 (stock solution I).10ml of solution taken from the above stock solution I in a 100ml standard flask and made up the volume to 100ml with phosphate buffer 7.4 (stock solution II). 2, 4, 6, 8, 10 ml of solution transferred from stock solution II to a series of 10 ml volumetric flasks. The volume was made up with phosphate buffer pH 7.4. The absorbance of these solutions measured at 258 nm against blank.[20]

3.3.Diacerein *in-situ* gel formulation

Conventional "cold method" was utilized to prepare diacerein *in-situ* gels. The formulations were composed by thermoreversible polymer Poloxamer-407 (20%) and other copolymers such as HK4M (0.5% - 1.5%), Carbopol 394 (0.5 - 1.5%) slowly added and dispersed in cold water (5% – 10) using magnetic stirring. As per the cold method, the mixture was kept at 4 stand for 24 hours to assist the gel formation. Diacerein dissolved inDMSO and ethanol under magnetic stirring and poured into the preformed gel and made up final concentration with cold water. While triethanolamine was added to correct the pH to neutral, 0.99%w/v benzyl alcohol added as antibacterial and benzalkonium chloride (0.001%w/v) as preservative.[8] All the materials used in this formulation were free from bacterial endotoxin and the formulation was carried out under the *UV* light to minimize the microbial contamination. Composition of Diacerein *in-situ* gels were given in table.1.

Table 1: Composition of Diacerein *in-situ* gels.

Formulation code	Diacerein	Poloxamer 407	Carbopol 934	HPMC K4
F1	0.5%	20.0%	0.5%	-
F2	0.5%	20.0%	1.0%	-
F3	0.5%	20.0%	1.5%	-
F4	0.5%	20.0%	-	0.5%
F5	0.5%	20.0%	-	1.0%
F6	0.5%	20.0%	-	1.5%

3.4.Diacerein *in-situ* gel characterization

3.4.1. Gelation temperature

Assessment of gelation temperature was determined by positioned a thin-walled tube in thermostatically controlled water bath and the temperature of which was increased at persistent rate of 2°C/5 minutes with gentle shaking at regular intervals till it was changed into the gel. The temperature (°C) at which it was differentiated from "flow liquid sol" to "no flow solid gel" upon the transposition of tube was considered to be the gelation temperature of the sample.[9,10]

3.4.2. Gelation time

Gelation time is described as the time required for sol to gel transition when placed at gelation temperature. Test tube transposition method was utilized to determine the gelation time which requires the use of thin-walled glass tube (containing 2 ml of Diacerein *in-situ* gel) placed in thermostatically regulated water bath at the gelation temperature (previously determined) with gentle shaking at regular intervals. The time (second) taken for transition to gel was recorded as gelation time which was assessed by flow or no-flow character when the test tube was inverted.[11]

3.4.3. Viscosity

The viscosity of Diacerein *in-situ* gels was determined (n = 3) at 5 ± 1 ° C and at 37 ± 1 ° C using a viscometer (Brookfield DVE RV). Samples were soaked in thermo-regulated a water tank for 10 minutes; viscosity read at 50 rpm with spindle no. 5.[12]

3.4.4. Syringeability

The syringeability test equipment contains a Diacerein *in-situ* gelling solution (5 ml, stored at 5 ± 1 ° C) loaded with 5 ml glass syringe and an 18G needle held in direct support. The time required for the syringeability of a gelling solution (complete removal from the syringe)

under a given pressure (0.5 kg placed in a pan) was recorded.[13]

3.4.5. Drug content

Dissolved 1ml of *in-situ* gel in phosphate buffer of pH 7.4 and made up the volume to 50ml and taken 1ml from the above solution and made up to 10ml with phosphate buffer pH 7.4. Drug content was deduced using UV-visible spectrophotometer (Shimadzu UV-1800) at wavelength 258nm after suitable dilution.[14]

3.4.6. Sterilization

Gamma irradiation is simple, convenient and effective for final sterilization and is recommended by the European Pharmacopoeia.[15] The prepared optimized formulation filled with nitrogen, sealed in borosilicate glass containers was gamma irradiated (ICAR, Bangalore, India -Cobalt-60 sources) with a capacity of 15.76 kGy 25 ℃ for sterilization. Compendial sterility test was performed on sterilized samples to confirm the effectiveness of sterilization.[16]

3.4.7. Sterility test

The effectiveness of the sterilization method for gamma irradiated Diacerein *in-situ* gels was analyzed by incubating the sterile gels into fluid thioglycolate media in aerobic and anaerobic bacteria at 37 ° C ± 1 ° C and soybean casein-digested media for fungi at 25 ° C fora period of 14 days. At the end of the incubation period, the growth of bacteria / fungi is observed.[14]

3.4.8. Bacterial endotoxin test

Bacterial endotoxin testing involves analyzing the sample of diacerein in-situ gel using Limulus Amebocyte Lysate (LAL) reagent. This reagent made from the horse shoe crab. In the presence of pyrogen material, lysate reacts to form clots or colour changes.

3.4.9. In vitro drug release

Phosphate buffer pH 7.4 was used as diffusion medium for this study. Franz diffusion cell was centered on a magnetic field. The recipient compartment was loaded with phosphate buffer pH 7.4 and kept at 37 ± 0.5 ℃. Then prepared goat skin was mounted on the cell carefully so as to avoid the entrapment of air bubble. Intimate contact of the goat skin membrane was ensured with receptor compartment fluid by placing it tightly with clamp or rubber band. The speed of the stirring was kept constant throughout the experiment. 1ml of the diacerein *in-situ* gel was placed on the top of the goat skin membrane inside the donor

compartment. With the help of micropipette sample was withdrawn at predetermined time intervals from the sampling port of the receptor compartment and the same volume of fresh buffer solution was replaced in order to maintain sink condition.

Samples were diluted and analyzed using an UV spectrophotometer for diacerein concentration at 258nm. The cumulative percentage drug release calculated using the calibration curve of diacerein. The invitro drug release profile was plotted and mechanism of drug release from gels was ascertained by fitting release data into various mathematical models.[17]

3.4.10. Release mechanism

The resultant *in vitro* release studies data were fitted into various mathematical models to determine the mechanism of drug release and best-fit model. The drug release mechanism kinetics was also studied using peppas model and higuchi plots.[18, 19]

4. RESULTS AND DISCUSSION

The FT-IR spectra of the pure drug (Diacerein) and formulation F4 & F5 showed that the characteristics peaks of Diacerein were not altered without any change in their position, thereby indicating no chemical interactions between the drug and polymers used (Figure.1 and Table.2). FT-IR study demonstrated the compatibility of Diacerein with Poloxamer-407, HPMC K4 and Carbopol 934.

Table 2: Interpretation of FT-IR spectra of Diacerein and physical mixtures (F2 & F5).

S.NO	Peak observed (cm^{-1})	Interpretation	Formulation F2	Formulation F5
1.	2848.35	-N-H stretching (amine salt)	2880.17	2878.24
2.	2550.40	-S-H stretching (thiol)	2593.79	2597.64
3.	2598.61	-S-H stretching (thiol)	2545.58	2548.47
4.	1592.91	-C-H bending (aromatic)	1593.88	1594.84
5.	1679.69	-C-H bending (aromatic)	1677.77	1676.80
6.	1769.37	-C-H bending (aromatic)	1769.37	1594.84

Solutions of Diacerein in Phosphate buffer pH 7.4 were suitably diluted to give varying concentrations of 2-10µg/ml. The absorbance was measured at 258nm and the values were given in table.3. Calibration curve of diacerein was given in figure.2.

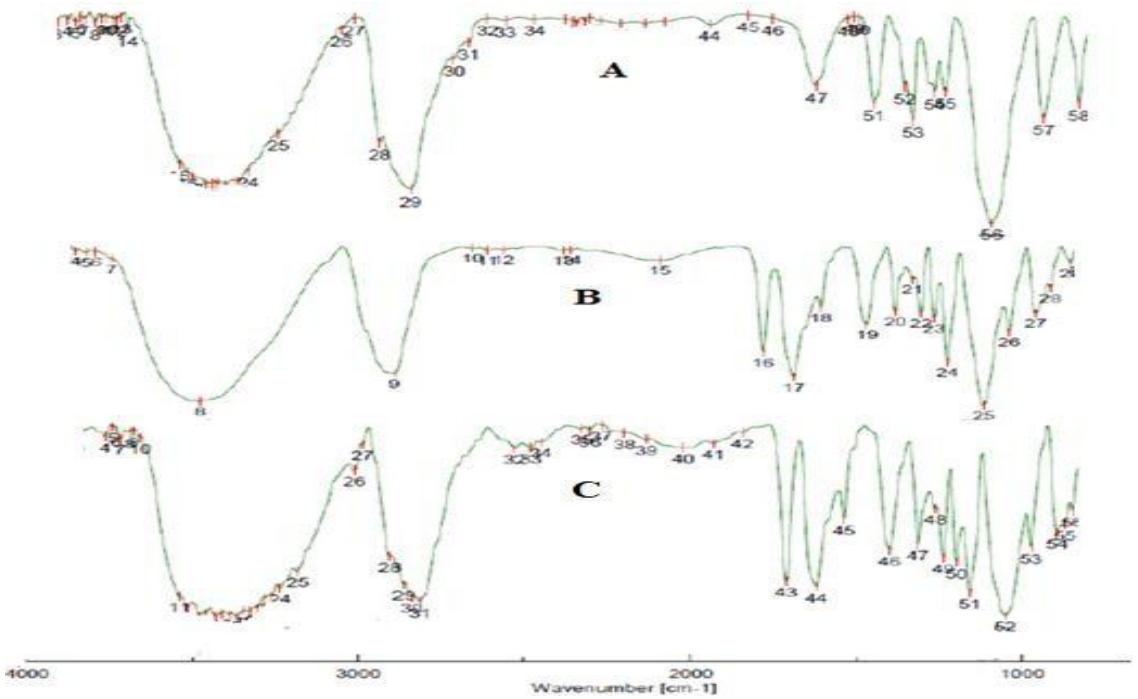

Figure 1: FT-IR spectrum (A- Diacerein, B- Formulation F5, C- Formulation F2).

Table 3: Absorbance of Diacerein in Phosphate buffer pH 7.4.

S.NO.	CONCENTRATION (µg/ml)	ABSORBANCE (nm)
1	2	0.178
2	4	0.357
3	6	0.535
4	8	0.721
5	10	0.916

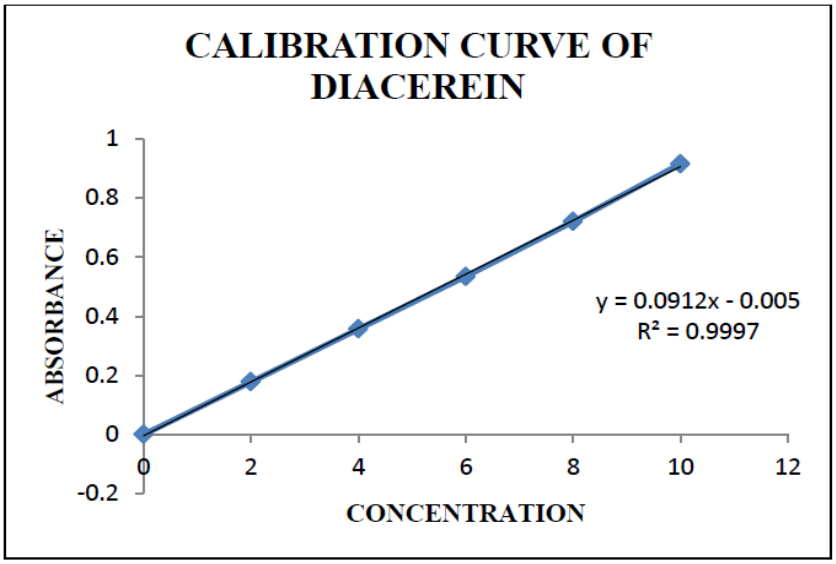

Figure.2: Standard graph of diacerein in phosphate buffer pH 7.4.

Six formulations of diacerein *in-situ* gelling systems were prepared with thermo- sensitive polymer Poloxamer-407 (20%) and other copolymers such as HPMC K4 (0.5- 1.5%), Carbopol 394 (0.5-1.5%) by traditional "cold method" under controlled environment. The drug concentration kept as constant for each formulation (5% w/v). The appearance of the formulations was yellow and clear without any suspended particles and impurities.

Gelation temperature and gelation time were determined for prepared diacerein *in-situ* gels. The prepared diacerein *in-situ* gels showed sol-to-gel transformation. The gelation temperature values ranged from 34.8℃ to 37.9℃ (close to human body temperature). The results showed that the gelation time values were in the range of 58 to 61 seconds. Syringeability was determined for prepared diacerein *in-situ* gels. The length of time to inject the contents of the syringe using a constant force is called the syringeability time. The finding of the syringeability testing revealed that the prepared diacerein *in-situ* gels were conveniently syringeable via 18G needle. It was observed that the syringeablity time reported for gel formulations were within the range of 6 to 8 seconds. The values were showed in table.4.

Viscosity study was carried out for prepared diacerein *in-situ* gels at 5±3℃ storage temperature) and 37±0.5℃ (body temperature). All the formulations exhibited sol state at refrigerator temperature but transformed in to clears stiff gel at body the temperature. The values were showed in table.5.

Table 4: Gelation temperature, Gelling time and Syringeability time of formulations.

S.NO.	FORMULATIONS	GELATION TEMPERATURE (℃)	GELLING TIME (seconds)	SYRINGEABILITY TIME (seconds)
1.	F1	37.9	60	6
2.	F2	36.9	59	7
3.	F3	34.9	58	8
4.	F4	37.2	61	6
5.	F5	35.9	60	7
6.	F6	34.8	58	8
7.	STERILIZED FORMULATION	36.7	60	7

Table 5: Viscosity at storage temperature (5±8℃) and body temperature (37±0.5℃) of formulations.

S.NO.	FORMULATIONS	STORAGE TEMPERATURE (5±8℃) cP	BODY TEMPERATURE (37±0.5℃) Cp
1.	F1	544	26248
2.	F2	621	27449
3.	F3	721	28294
4.	F4	579	28356
5.	F5	678	29997
6.	F6	779	31007
13.	STERILIZED FORMULATION	651	27558

Table 6: Drug content of the formulations.

S.NO.	FORMULATIONS	PERCENTAGE DRUG CONTENT
1.	F1	92.82%
2.	F2	95.30%
3.	F3	91.18%
4.	F4	92.22%
5.	F5	93.98%
6.	F6	90.50%
7.	STERILIZED FORMULATION	95.52%

The drug content of all the formulations estimated by using UV-visible spectrophotometer at 258nm and found to be within the normal range. The values were showed in table.6.

The in vitro drug release studies help in providing important data regarding the performance of a dosage form under in vivo conditions. Sustained diacerein release from formulations up to 24 hours due to gradual degradation of the polymer matrix and chiefly influenced by the combination of polymers resulted in rapid gelation time and lowered gelation temperature. The formulations prepared from poloxamer 407 (20%) and HPMC K4 (1.5%) [F6] and poloxamer 407 (20%) and carbopol (1.5%) [F3] were showed the maximum controlled release of 35.55% and 36.77% respectively followed by formulations prepared from poloxamer 407 (20%) and carbopol 934 (1.0%) [F2] and poloxamer 407 (20%) and HPMC K4 (1.0%) were showed controlled release of 39.19% and 38.97% respectively at 24 hours. The formulations F2, F3, F5 and F6 were selected based on the long-sustained release at 24 hours period of time and the graph is shown in figure.3 Formulation F2 showed the best

controlled release and also possessed the optimum physiochemical behaviour such as gelation temperature, gelation time, syringeability time and viscosity. The cumulative percentage drug release of the prepared Diacerein *in-situ* gels showed in figure.3 and figure.4.

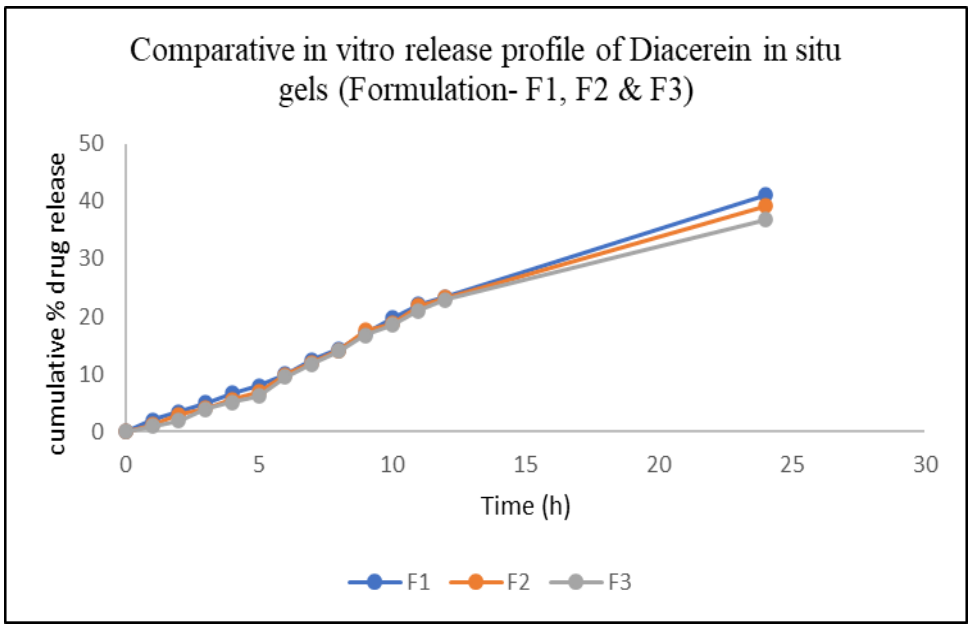

Figure 3: Comparative in vitro release profile of Diacerein *in-situ* gels (Formulation- F1, F2 & F3)

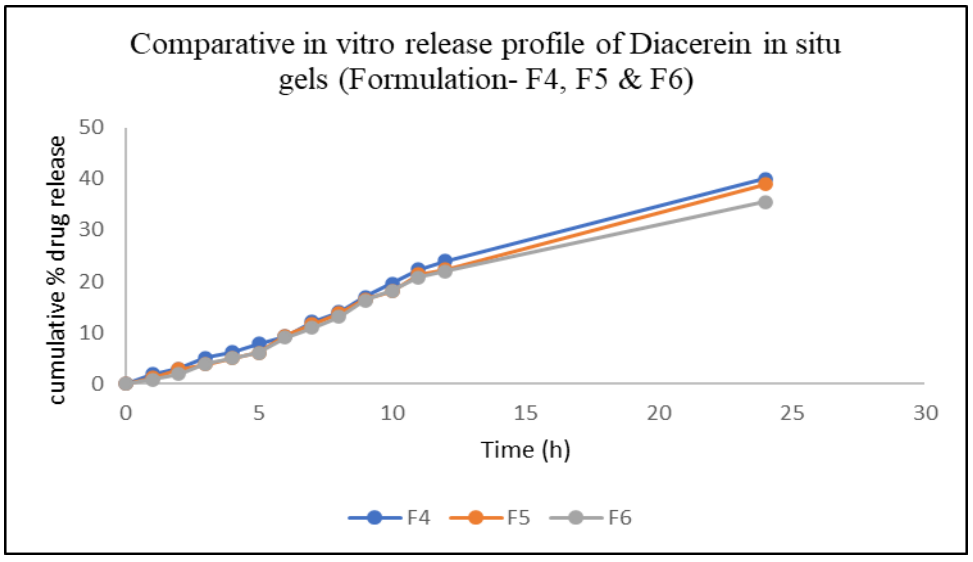

Figure 4: Comparative in vitro release profile of Diacerein *in-situ* gels (Formulation- F1, F2 & F3).

Gamma irradiation sterilization helps in final stage sterilization of the dosage form before its administration into the body. Best formulation F2 was sterilized by gamma irradiation. Among other methods for sterilization, gamma irradiation was preferred for its high ability to

penetrate the product that permits sterilization of even susceptible material without elevation of temperature. However, gamma irradiation may have affected the characterization of the *in-situ* gels In addition, the effect of gamma irradiation on gelation temperature and time, injection time, in vitro drug release and drug content have beenevaluated. The results showed that there were no significant changes in the set parameters between non-irradiated and irradiated formulations.

Sterility is very important requisite for the parenteral formulation. Diacerein *in-situ* gel is designed for parenteral administration to target site. The optimized formulation F2 was sterilized by gamma irradiation and accessed for sterility after the exposure. The microbiological evaluation assures the product‟s sterility and effectiveness of the sterilization method. No turbidity was observed in post incubation period.

Bacterial endotoxin test result showed no coalescence with the LAL reagent. The test confirmed that the diacerein *in-situ* gel was free from bacterial endotoxin material and the gel was suitable for injection.

To determine the release mechanism that provides the best description to the patternof drug release, the in vitro release data were fitted to zero-order, first-order, Higuchi model and korsmeyer-peppas model. The Diacerein *in-situ* gels follow the zero-order kinetics.

Values of n = 1.0 of formulations supported the case II transport (time-independent). The in-vitro drug release data and mathematical model fitting is given in table.7.

Table 7: In vitro drug release data and mathematical model fitting.

S.NO.	Formulation Code	Order of drug release	Mechanism of drug release	‘n’ value
1.	F1	Zero order	Case II transport	1.006
2.	F2	Zero order	Case II transport	1.003
3.	F3	Zero order	Case II transport	1.005
4.	F4	Zero order	Case II transport	1.007
5.	F5	Zero order	Case II transport	1.050
6.	F6	Zero order	Case II transport	1.012

5. CONCLUSION

Diacerein *in-situ* gels were formulated to provide a novel injectable, biodegradable system for the treatment Osteoarthritis. Formulation F-2 was chosen as the optimized formulation on the basis of the longest span of drug release. Also, it has shown optimum physicochemical

parameters such as gelation temperature, gelation time, syringeability time and viscosity. The Diacerein *in-situ* gel remained stable, thermo-responsive and transformed to gel at the body temperature. The optimized *in-situ* gel exhibited controlled release of Diacerein enhancing the treatment modality. The methodology adopted for the *in-situ* gelling system is easy, convenient and cost effective. A novel drug delivery system for prolonged release of diacerein which can reduce dose, dosing frequency, associated side effects, cost of therapy and there by improve patient compliance was developed. This research demonstrated advantages of diacerein *in-situ* gel and may be suitable as an injectable drug delivery system for management of osteoarthritis to overcome the limitations of currently available formulation**.**

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