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Research Article

DEVELOPMENT AND ASSESSMENT OF DIACEREIN MICROSPHERES WITH CONTROLLED RELEASE PROPERTIES UTILIZING EGG ALBUMIN

Anjaneyulu Vinukonda[1], Saroj Yadav[2], Sonali Rastogi[3], C. Vanajakshi[4], Neeli Rose Beck[5], Nina Varghese[6], Satish Kumar Pradhan[7] and Sudhir S Hunge[8]*

[1]Scientist- formulation and process development, Strides Pharma Inc New York -USA-10977.

[2]School of Medical &Allied sciences, K.R. Mangalam University, Sohna, Gurgaon.

[3]Department of Pharmacy, Venkateshwara group of institutions, NH- 58, Delhi- Roorkee, Bye Pass Road, Jatoli, Meerut (U.P.), India Pin Code: 250002.

[4]Department of Pharmacy, Jawaharlal Nehru Technological University, oil technological research institute (OTRI), Ananthapuramu, Andhra Pradesh.

[5]Department of Pharmacy, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh.

[6]AIMST University, 08100 Bedong, Kedah, Malaysia.

[7]Sarada Devi Institute of Medical Sciences, Sarakana, Balianta, Bhubaneswar-752100, Odisha, India.

[8]Chintamani College of Science Pombhurna Dist Chandrapur, Gondwana University, Gadchiroli.

***Corresponding author:**

Dr. Sudhir S Hunge,

Associate Professor,

Chintamani College Of Science Pombhurna Dist Chandrapur,

Gondwana University, Gadchiroli.

Email: sudhir@chintamani.edu.in

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ABSTARCT

Diacerein is a one-of-a-kind drug that was created to help osteoarthritis by reducing inflammation and pain and lowering body temperature. The study's main goal was to create and test Diacerein microspheres with controlled release qualities, with egg albumin as the main ingredient. Spreading under control The dicerein nanoparticles were made using a chemical cross-linking method that used different amounts of a natural polymer, like egg albumin. The shape, drug content, entrapment effectiveness, and anti-inflammatory action in vitro of the microspheres that were made were all tested. Including microspheres of F3 in a formulation led to the best drug release, entrapment efficiency, amount of in vitro drug release, and enough anti-inflammatory action.

KEYWORDS: Diacerein, anti-inflammatory, analgesic, antipyretic, microspheres egg albumin.

INTRODUCTION

The novel drug delivery system seeks to efficiently administer the correct dosage of medication to the specific site in the body and sustain the desired concentration of medication (Kattamuri, *et al.,* 2012). The drug delivery method must ensure that the medication is introduced into the body consistently and at a steady rate for a specific duration. The oral route is the most prevalent and convenient means of administering drugs (Panditi and Vinukonda, 2011; Bharti et al., 2012). Due to the greater flexibility in creating dose forms for the oral route compared to other routes, the pharmaceutical industry has focused more on developing drug delivery solutions for this route (Putikam, *et al.,* 2012). In the last twenty years, there has been a fast advancement in drug delivery technology, resulting in the creation of many innovative ways for delivering drugs orally (Akki, *et al.,* 2022). Microspheres are small spherical particles that usually have a size ranging from 1μm to 1000μm (1mm). Microparticles are synonymous with microspheres (Saisri, *et al.,* 2021). These are spherical, buoyant particles consisting of synthetic polymers and proteins that spontaneously degrade over time (Namadeva, *et al.,* 2024). The microsphere plays a crucial function in reducing the adverse effects and enhancing the bioavailability of conventional drugs (RANI, *et al.,* 2023).

Egg albumin can serve as a means to physically connect and secure proteins together. Furthermore, it allows for the controlled or aided release of specific drugs that are incorporated into the polymer matrix (Rajkhowa, *et al.,* 2022). The development of albumin-based microspheres was conceived to enhance the bioavailability of drugs and prolong their therapeutic effect (Gupta, *et al.,* 2022). This suggests a reduction in the frequency of drug administration (Shandilya, *et al.,* 2022). The presence of albumin in solid tumors is the driving force behind the development of medicine delivery systems that use albumin to specifically target cancerous growths (Upadhayay, *et al.,* 2022). Albumin has been employed as a means to transport drugs to tumors (Rajkhowa, *et al.,* 2022). Given that the synovium of rheumatoid arthritis patients shares certain traits with tumors, the same albumin-based delivery methods can be used to transport pharmaceuticals to the inflamed joints (Sethi, *et al.,* 2023). There is a suggestion that the administration of drugs intravenously, together with albumin, helps them to enter the arthritic areas more effectively (Sarkar, *et al.,* 2023). Studies have demonstrated that the presence of albumin significantly prolongs the half-life of medications in the bloodstream (Surana, *et al.,* 2024).

One approach to improve the targeting of the formulation to the affected areas on rats' paws is to prolong its presence in the bloodstream by reducing its absorption through the reticuloendothelial system (Surana, *et al.,* 2024). If the concentration of the drug in the arthritic joint was higher and its distribution to other organs was reduced, the adverse effects would be alleviated (Khairnar, *et al.,* 2024). If only the swollen joints of rheumatoid arthritis patients were targeted for medication, the amount of medication needed to control the condition might be reduced, potentially leading to the disappearance or reduction in severity of unwanted effects (Sonawane, *et al.,* 2024 and Tiwari, *et al.,* 2024).

MATERIALS AND METHODS

Formulation of Microspheres of Diacerein:

Cross-linking chemicals were used to make microspheres. Using this method, different amounts of albumin were mixed with 15 milliliters of pure water. After that, 0.5% Tween 80 was added, and the whole thing was left to sit overnight (Kasimedu, 2023). After that, the drug was added to the albumin solutions that had already been mentioned and stirred for ten to fifteen minutes. Ratios of drug to polymer were used in the preparation process. After that, 60°C was applied to a jar that held 160 ml of liquid paraffin mixed with 2% span 80 to help the mixture mix. Next, the drug-polymer solutions were added slowly by injecting them one

drop at a time. Following that, the mixture was mixed very hard at a speed of 250 turns per minute for 30 minutes (Medabalimi, *et al.,* 2022; Tiwari et al., 2023a; Tiwari et al., 2023b). After being stirred around in a glutaraldehyde solution for three hours, the microspheres became stronger. Following this, five milliliters of n-hexane were added. Using decantation, the microspheres were gathered. They were then washed with petroleum ether and left to dry at room temperature (Medabalimi, *et al.,* 2022 and Radhika, *et al.,* 2021). The specifics of the method are shown in Table 1.

Table 1: Formulation of Microspheres of Diacerein

Sr. No.	**Formulation code**	**Drug (mg)**	**Polymer (g)**	**Drug: polymer Ratio**	**Light Liquid Paraffin (ml.)**	**Span 80**	**Glutaraldehyde (ml)**
1	F1	1000	1000	1:1	160	2.0%	30
2	F2	1000	2000	1:2	160	2.0%	30
3	F3	1000	3000	1:3	160	2.0%	30
4	F4	1000	4000	1:4	160	2.0%	30
5	F5	1000	5000	1:5	160	2.0%	30

Calibration Curve for Diacerein:

Standard Graph Preparation:

A 100.0 ml volumetric jar was filled all the way to the top with 0.1 mg of dialerein. With 100 ml of phosphate buffer solution at a pH of 6.8, a single volumetric flask was filled to the top. This was done after the medicine was dissolved in 10 ml of DMSO. Following this, ten milliliters of the fluid were moved to a different flask. From this secondary stock, 1 to 6 milliliters were taken and mixed with 100 milliliters of phosphate buffer (pH 6.8) to make amounts of 1 to 6 micrograms per milliliter. A UV spectrophotometer was used to measure the intensity at 260 nm (Saravanakumar, *et al.,* 2021).

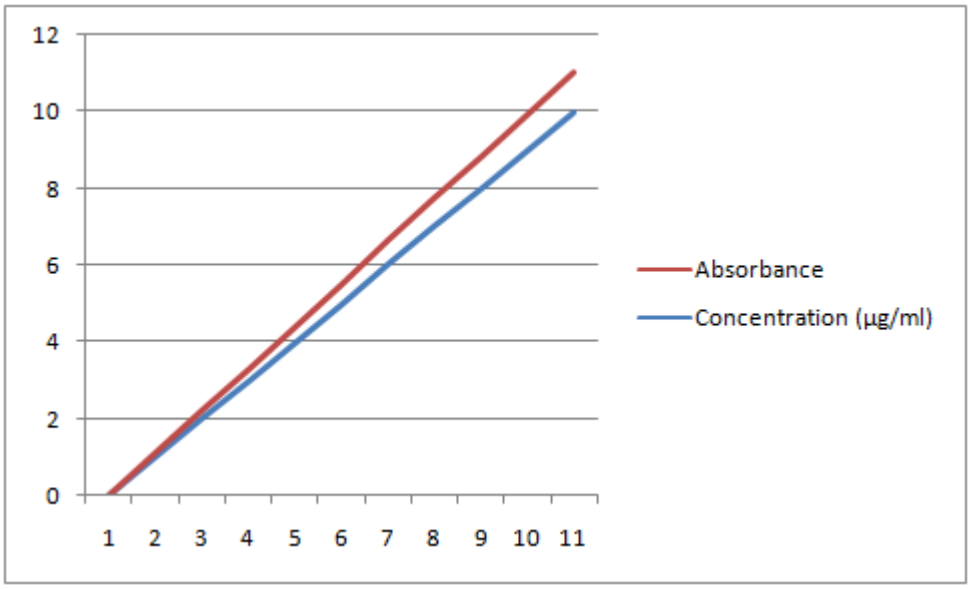

Figure 1: Diacerein calibration curve

MicrosphereFormulations *In Vitro* Drug Release Study:

For in vitro drug release studies, the UPS test equipment I, specifically the basket type, was used. The dissolution tests were done in a certain way: the spin speed was 75 turns per minute, and the temperature was 37 ± 0.5 ºC. As the dissolution medium, 900 cc of phosphate buffer solution with a pH of 6.8 was used in the tests (Khulbe, *et al.*, 2023). There were set times throughout the day when 10 milliliters of a sample were taken out. The original volume of the sample was replaced with a similar amount of fresh medium.A weaker solution is made by mixing 4 milliliters of filtrate with 100 milliliters of 6.8 phosphate buffers. Spectrophotometry at a range of 260 nm was used to find the concentration (Saravanakumar, *et al.*, 2021 and Saravanakumar, *et al.*, 2020; Tiwari and Pathak, 2023; Tiwari et al., 2022).

***In Vitro* evaluation of Anti-Inflammatory activity MicrosphereFormulations:**

A 1 ml sample of the sample solution was taken every hour during the in-vitro drug release experiment and then used for an in-vitro anti-inflammatory test. A control was used, which was the same amount of pure water (Kumari, *et al.*, 2019). One milliliter of a solution with 1% cow albumin in distilled water was added to each reaction mixture. To bring the pH down to 6.3, a small amount of 0.1 N HCl was also used. The samples were first put somewhere dark and then heated to 37oC for 30 minutes. After that, the samples were given 5 minutes of exposure to a temperature of 57oC. A spectrophotometer with a frequency of 660 nm was used to measure the turbidity of each sample after the reaction tubes were cooled with tap water (Tiwari *et al.*, 2021).

RESULTS

Pre-formulation Evalaution:

Preformulation investigations primarily investigate the physiochemical properties of the drug and assess its compatibility with other excipients.

Scanning Electron Microscopy (SEM) Study:

The surface morphology and shape of the optimized microspheres (F3) were examined using scanning electron microscopy (SEM). The microspheres were shown to be approximately spherical (Figure 2).

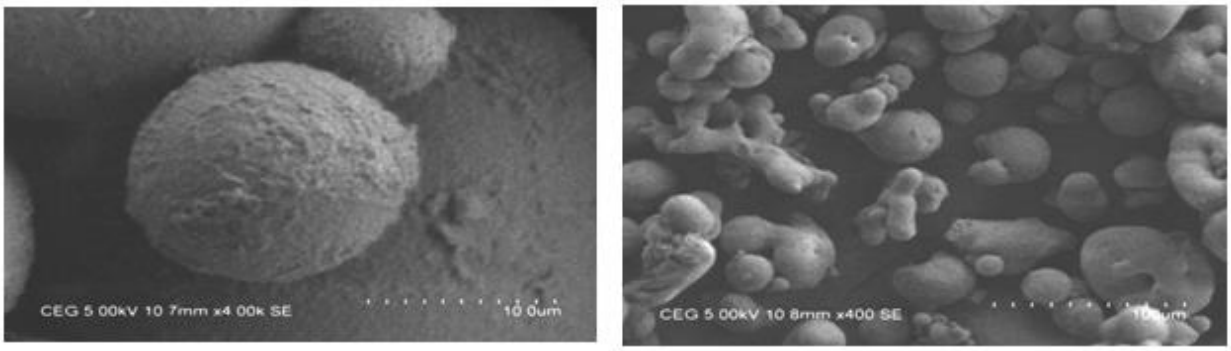

Figure 2: Scanning Electron Microscopy of F3

Evaluation of Microspheres

Table 2: Average Particle Size and Percentage Yield of formulation

Formulation	**Average Particle Size(µm)**	**Percentage yield (%)**
F1	55.53	97.40
F2	80.38	97.30
F3	116.68	99.20
F4	106.21	98.50
F5	121.49	97.10

Table 3: Entrapment Efficiency and Drug Content of formulation

Formulation	**Entrapment Efficiency (%)**	**Drug content (%)**
F1	56.45	95.90

F2	76.35	96.80
F3	95.60	99.30
F4	91.40	97.90
F5	83.70	98.10

The particle size distribution is thought to be between 55.53 µm and 121.49 µm based on the information given so far. This means that the amount of medicine to polymer is directly linked to the particle size getting bigger. When the concentration of polymers goes up, the viscosity of the polymer solution also goes up (Tiwari *et al.*, 2020). This makes the interfacial strain higher. In turn, this makes stirring less efficient, which causes particle sizes to get bigger. Finding out the real yield and percentage yield was done after the microspheres were made. It turned out that the yield was between 97.10% and 99.20%. It was found that the capture efficiency of each microsphere ranges from 56.55% to 95.60%. With a reported figure of 99.30%, formulation F3 has the best entrapment efficiency. Once the microspheres were made, the amount of drug inside them was checked. The number of hits was between 95.70% and 99.30%. Formulation F3 has 99.30% of the drug, which is the biggest amount (Table 3).

In Vitro Drug Release Study

Table 4: *In vitro* drug release study of capsule F3

Sr. No	**Time (Hrs)**	**% Drug release**
1	1	2.90 ±0.172
2	2	8.61 ±0.290
3	4	11.31 ±0.222
4	6	16.56 ±0.272
5	8	23.75 ±0.390
6	10	26.61 ±0.352
7	12	32.65 ±0.322
8	14	36.81 ±0.236
9	16	41.20 ±1.822
10	18	44.41 ±0.490
11	20	46.91 ±0.910

12	22	54.31 ±0.290
13	24	56.84 ±0.511

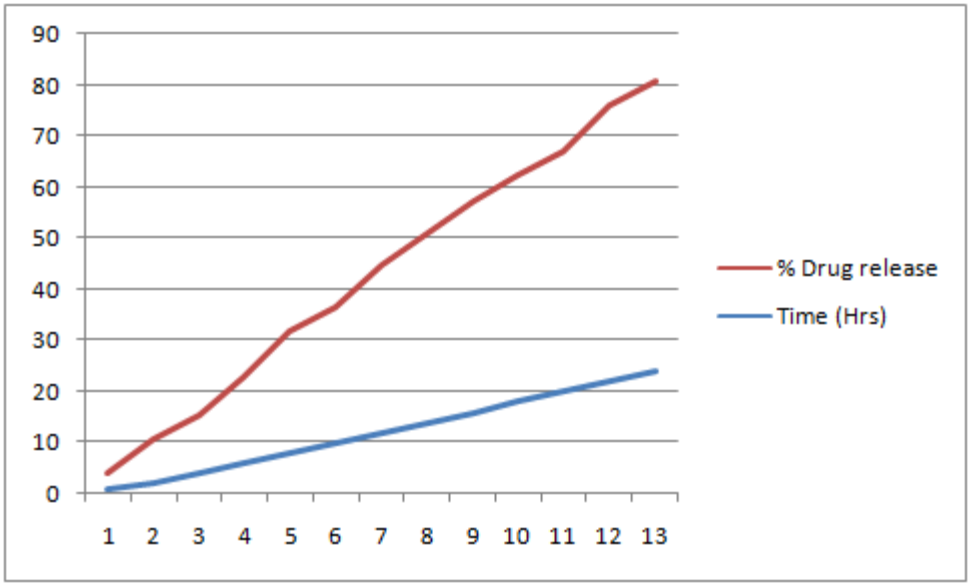

Figure 3: *In vitro* drug release study of F3

When comparing formulation F3 to the others, the in vitro drug release results showed a higher drug release of 56.84% after 24 hours. Thus, formulation F3 was chosen as the optimal formulation.

***In Vitro* evaluation of Anti-Inflammatory activity MicrosphereFormulations:**

The in vitro drug release data for formulation F3 demonstrated a higher drug release of 56.84% after 24 hours when compared to the other formulations. The best formulation was thus determined to be F3 (Table 5).

Table 5: *In vitro* anti inflammatory activity of F3Formulation and Pure drug

Time	**F3 Formulation**	**Pure drug**
1	0	000
2	0.85	44.49
4	0.61	53.71
6	1.91	57.85
8	5.11	63.62
10	11.23	68.44

12	15.23	73.44
14	18.45	81.15
16	23.56	84.56
18	25.26	85.61
20	28.57	88.92
22	31.69	-
24	35.69	-

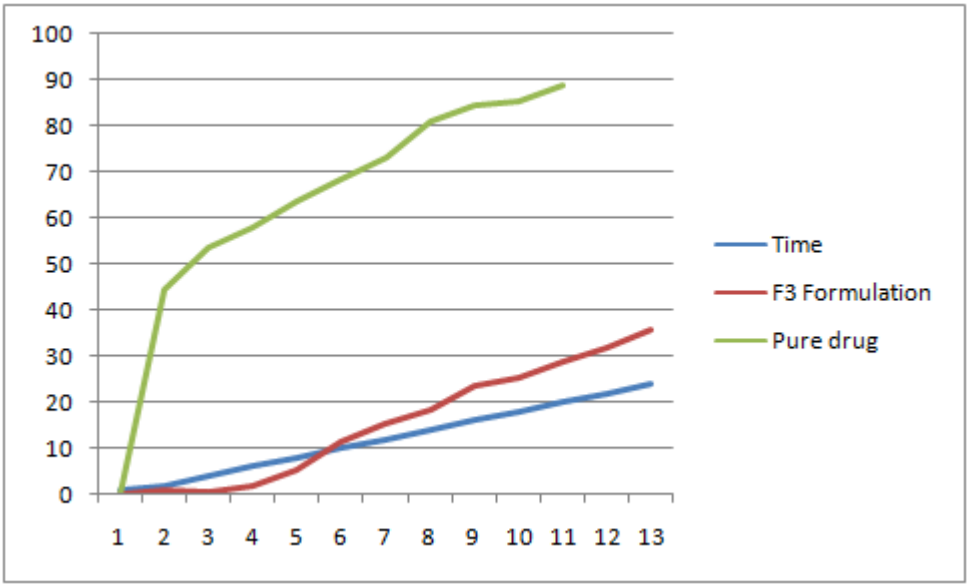

Figure 4: F3 and Pure medication exhibit in vitro anti-inflammatory effect

DISCUSSION:

Because dialerein has a short half-life and isn't easily dissolved, MDDS chose to give the medicine in stages. The chemical cross-linking method is used to make microspheres of diacetein. In this experiment, egg albumin, a natural polymer, was mixed in five different ways to make the F1 through F5 formulas. We tested how adding polymer and more egg albumin affected microspheres by putting all of the mixtures through a number of different tests. SEM was used to look at the shape of the optimized formulation. It showed that the microspheres had a shape that was very close to a sphere, which was thought to be suitable (Tiwari *et al.*, 2020; Tiwari *et al.*, 2021). The studies on the average particle size were done by looking at them under a microscope. Changing the amount of medicine to polymer led to a range of 55.53 µm to 121.49 µm for all formulations. The entrapment rates for all of the formulas were between 97.10% and 99.20%. With a 99.20% success rate, formulation F3 was

the best at trapping. It was time for the in vitro release of all formulations. Because of this, F3 was chosen as the best combination. During the production process, the average particle diameter, drug concentration, and polymer proportion all went up. The albumin denaturation method showed that the improved version F3 lowered inflammation by about 889.92% in just 24 hours. These results make it clear that F3 also has a good amount of anti-inflammatory activity. The drugs and the F3 microsphere were looked into in a preliminary way. They found that the flow of the pure medicine was fine, but the flow of the microspheres was terrific. After being looked at, it was decided that the capsules' post-formulation factors met the necessary standards (Tiwari *et al.*, 2021).

CONCLUSION

It was possible to make a controlled microsphere of diacetein by chemically cross-linking it with egg albumin, a natural polymer. Changing the quantity of the polymer changed how much of the drug was released. It was judged that the evaluation standards, which included entrapment efficiency, in vitro anti-inflammatory activity research, and drug release investigations, were met. Based on the comparison study with the pure DCN drug, it was seen that the F3 microsphere version had controlled release for 24 hours. The study's findings showed that using egg albumin, a natural polymer, is a good way to make controlled droplets loaded with DCN. These particles make it easy and effective to take drugs to treat osteoarthritis by mouth.

DECLARATIONS

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

Funding:

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