

Formulation and evaluation of microspheres of anti-inflammatory drug diacerein prepared by ionotropic gelation method

Tathagata Roy ^{1,2} & Tapan Kumar Chatterjee ^{3*}

¹ Department of Pharmaceutical Technology, JIS University, Kolkata- 700109, India. ²Department of Pharmacology, Calcutta Institute of Pharmaceutical Technology & AHS, Uluberia, Howrah- 711316, India. ³Department of Pharmaceutical Technology, JIS University, Kolkata- 700109, India

*Corresponding author: drtkchatterjee@jisuniversity.ac.in

Received: (8/4/2022), Accepted: (7/7/2022)

ABSTRACT

The context of this research was to reduce the untoward effects of diacerein by preparing a microsphere formulation of the same. The ionotropic gelation method was adopted to prepare diacerein microspheres. This method involves the interaction of two ionic components under certain conditions. Among these two ionic components, at least one component must be a polymer. Simple interaction between ionic polymer with oppositely charged ion leads to the formation of microspheres. In this process, sodium alginate and Chitosan were used as polymers, whereas for the crosslinking process, calcium chloride was used. The prepared formulations of diacerein displayed mean particle size ranging from 106-542 μm , while the percentage yield ranged from 49-82. An increased concentration of calcium chloride and sodium alginate increases the particle size. However, an increased chitosan concentration was found to increase the drug entrapment efficiency of the microspheres. Further, phosphate buffer media (pH 6.8) in the USP paddle type dissolution apparatus were used for the percentage drug release study. Results showed that increased chitosan concentration slows the release of drugs from microspheres. We noted that the B3 formulation, which contains sodium alginate: Chitosan (1:3), showed the lowermost rate of drug release. Besides, SEM studies of the same formulation (B3) revealed that microspheres have spherical shapes with rough surfaces. It was observed that the B3 formulation showed a significant result in formulation evaluation studies; hence, it may be further considered an optimized formulation for preclinical evaluation.

Keywords: Diacerein, Microsphere, Chitosan, Sodium alginate.

INTRODUCTION

Significant variations in plasma-drug concentration can be observed in traditional formulations like tablets and capsules. Patients must remember their dosing schedule to avoid this. A patient may occasionally miss a dose. In this case, steady-state plasma concentration cannot be attained due to the fluctuation of plasma drug concentration. Conventional dosage forms also cause unwanted adverse drug reactions and poor therapeutic responses [16]. Aside from adverse drug reactions and poor therapeutic responses, conventional dosage forms reduce patient compliance by increasing dosing frequency [17]. Proper plasma drug concentration must be maintained to achieve the desired pharmacological response. The establishment of the oral sustained or controlled release drug delivery system, a relatively new concept, was prompted by factors such as repetitive drug

administration to the patient and unpredictable drug absorption [1].

Recently, novel drug delivery systems have increasingly supplanted traditional dose forms such as tablets and capsules. Controlled and sustained release formulations are the most extensively employed novel drug delivery systems in recent years [2]. Sustained release drug delivery systems maintain consistent drug plasma levels throughout time by releasing the drug at a predetermined rate, decreasing adverse drug responses, and increasing patient compliance [3].

Microspheres are characterized by small spherical particles ranging from 10 millimeters to a thousand millimeters [19]. Microspheres significantly improve patient compliance by enhancing the absorption of standard drugs while lowering side effects. The main benefit of employing microspheres as a medication delivery technology is the controlled release of the medicament [19]. Microsphere

increases patient compliance by lowering dose frequency and maintaining a consistent medication plasma concentration [20].

Polymeric microspheres are widely used microspheres widely used as the controlled released dosage form. Polymeric microspheres are mainly two types:

1. **Biodegradable polymeric microsphere:** Biodegradable polymeric microspheres are mostly made from natural polymers like starch. Natural polymers degrade quickly and are bio sticky in nature. Because these polymers have a high degree of swelling in aqueous media, they enhance the residence period when they touch the mucous membrane. This results in the production of a gel that sticks to the mucous membrane easily. The polymer concentration primarily controls the rate and extent of drug release from the microsphere.
2. **Synthetic polymeric microsphere:** Synthetic polymers are used to make these microspheres, which are used as fillers, drug delivery vehicles, bulking agents, and embolic particles. The fundamental disadvantage of these microspheres is that they quickly migrate away from the injection site, resulting in embolism and organ damage [21].

Some methods are available by which polymeric microspheres can be produced, like

the single emulsion/ double emulsion technique, Spray drying technique, Emulsion solvent evaporation technique, Phase separation coacervation technique, and; ionotropic technique gelation method, among which we have chosen the ionotropic gelation procedure for microsphere preparation.

There are many applications of polymeric microspheres in drug delivery, including bulking agents and fillers, stress urinary incontinence, and embolization therapy [22].

Diacerein is an anti-inflammatory drug used to treat osteoarthritis. Along with its tremendous potential in the pharmacotherapy of osteoarthritis, Its various pharmacological implementations are frequently investigated, including psoriasis, epidermolysis bullosa, breast cancer, periodontitis, and type 2 diabetes [4]. It belongs to the anthraquinone family of drugs. It is BCS (Biopharmaceutical classification system) class II drug (low solubility, high permeability). Diacerein mainly acts by inhibiting of IL-1 β biosynthetic pathway [14]. Due to inhibiting the IL-1 β biosynthetic pathway, diacerein inhibits IL-1 β mediated inflammatory reactions in previously mentioned inflammatory disorders. Diacerein is a pro-drug. The deacetylation metabolism pathway is transformed into the active metabolite rhein, which also shows anti-inflammatory effects [15] (Figure 1).

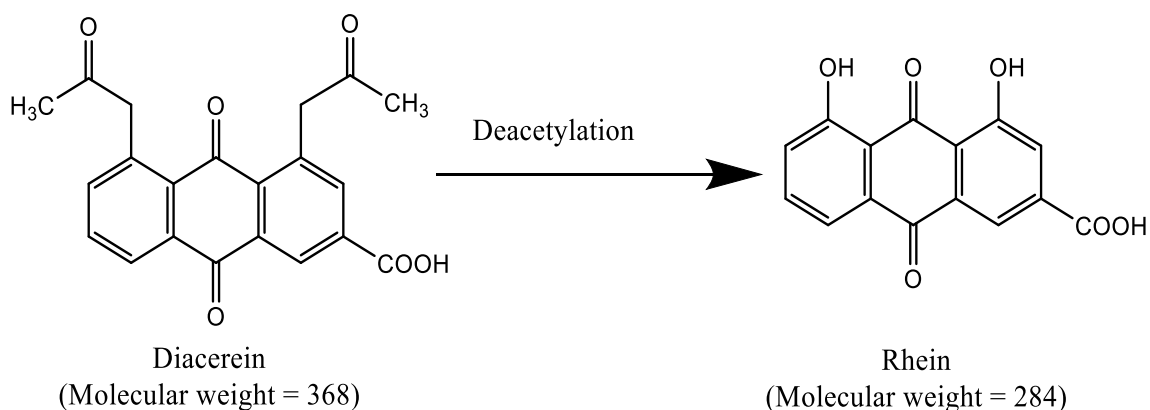


Figure (1): Metabolism of Diacerein.

The main adverse effect of diacerein therapy is soft stool and diarrhea, which mainly reduces patient compliance [18]. However, this drug shows promising results in treating osteoarthritis, rheumatoid arthritis, and; some

other inflammatory disorders. The main rationality behind selecting this drug was to reduce the adverse effect by using a controlled release formulation to improve patient compliance.

The work aims to examine the effect of polymer concentration and calcium chloride on diacerein's release profile and develop sustained release diacerein microspheres to minimize dosing frequency and boost patient compliance.

LITERATURE REVIEW OF THE DIACEREIN FORMULATIONS

Swapna S *et al.* prepared floating microspheres of diacerein by ionic gelation technique. HPMC (K 100M) and sodium alginate were used as polymers, sodium bicarbonate as a gas generating agent, and calcium chloride as a crosslinking agent. The purpose was to maintain the dosage form floating in the stomach for controlled and known drug release and to enhance bioavailability. (wjpls 2018, vol. 4, Issue 7, 117-123).

Gomez-Gaste C *et al.* and others worked on biodegradable PLGA microparticle (MPS) of Rhein for intra-articular administration. The emulsion-solvent evaporation technique was prepared for MPS. (Eur. J. Pharm Sci. 2017 (Jan 1; 96; 390-397).

Soumen M *et al.* prepared diacerein microspheres by Spray Coating (Wurster Method). Ethyl cellulose and hydroxyl propyl methyl cellulose (HPMC) were used as polymers. (JDDT 2019, vol.9, Issue 4, 454-460).

Achint J *et al.* prepared liquid nanoparticles of diacerein. Stearic acid-based nanoparticles were prepared, which showed enhanced bioavailability and reduced toxicity. Soya lecithin and pluronic F68 were used as a surfactant. Citric acid was used to acidify the medium. The particle size of prepared diacerein nanoparticles ranged from 270 ± 2.1 to 510 ± 2.8 nm and had maximum drug entrapment efficiency of 88.1%. Prepared diacerein nanoparticles reduce diarrhea by up to 37%. (Journal of Biomedical Nanotechnology, 2013, Volume 9, Issue 5, 891-900)

Soumen M. *et al.* prepared diacerein microspheres by the solvent evaporation method. Sodium alginate and ethyl cellulose were used as the polymer to prepare microspheres. Three different diacerein microspheres were prepared based on variable concentrations of Sodium alginate and ethyl cellulose. It was noted that entrapment efficiency and particle size increase on increasing polymer concentration, and drug release of all the batches decreases on increasing the polymer concentration. (IJPBS 2016, vol 7, Issue 3, 231-239).

PHYSIOCHEMICAL PROPERTIES OF DIACEREIN

Synonyms: - Diacetylrhein; Discerhein

Chemical Structure: (Figure 2):

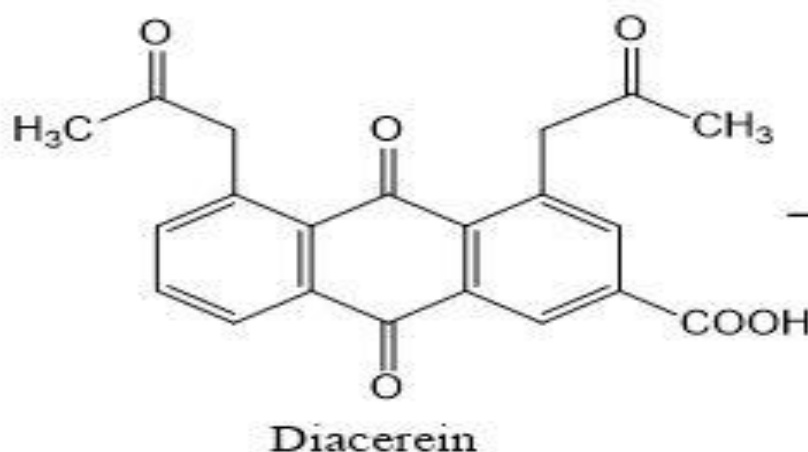


Figure (2): Chemical Structure of diacerein.

IUPAC name: 4,5-bis(acetyloxy)- 9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid.

Solubility Profile: It is practically insoluble in water, 3.197mg/liter at 20^oc [23]. It is soluble in DMSO.

Melting point: 218^oc

Physical Appearance: yellow-colored crystalline powder.

Storage condition: 25-30 ^oC

MATERIALS AND METHODS

Materials

Diacerein was contributed by Emcure Pharmaceutical Ltd, India, as a gift sample. Sodium alginate, Chitosan, and Calcium chloride were procured from Loba Chem Pvt. Ltd.

Instruments Used

1. Magnetic stirrer (REMI 1 MLH).
2. Digital electronic balance (KEROY balance- FB360H).
3. Optical microscope (Olympus, ESAW optscopes classic).

4. Fourier transforms infrared (FTIR) spectroscopy (BRUKER Alpha).
5. UV- Visible spectrophotometer (Shimadzu, UV 1800)
6. Paddle type USP Type II dissolution test apparatus (Veego VDA-8D)
7. SEM Model: JEOL JSM -6360 (JSM 6360 LV)

Preparation of diacerein microspheres

Diacerein microspheres were prepared by the ionotropic gelation method (Fig 2) [5]. The composition of various formulations of microspheres is mentioned in (Table 1).

Table (1): Composition of different formulations of the microsphere.

Formulation Code	Drug (mg)	Calcium chloride (gm)	Sodium Alginate (gm)	Chitosan(gm)
B1	10mg	4	3	1
B2	10mg	4	2	2
B3	10mg	3	1	3
B4	10mg	3	2.5	1.5
B5	10mg	3	1.5	2.5
B6	10mg	5	3.5	0.5
B7	10mg	4	1	3
B8	10mg	5	1	3

In one beaker, a specified proportion of Chitosan was dispersed in 100 ml of 1% acetic acid solution, and this chitosan solution was allowed to swell for 2 hours. In another beaker, a specified proportion of sodium alginate was added to 100 ml of distilled water. This sodium alginate solution was heated on a hot plate until all the sodium alginate get dissolved in water. When the solution temperature became normal, a specified amount of diacerein was added to the sodium alginate solution. In another beaker specified amount of calcium chloride was taken and dissolved in distilled water. Now, calcium chloride solution was mixed with chitosan solution. Sodium alginate and drug solution were then taken in a syringe having a 0.45 mm internal diameter, and from the height of 5 cm, the solution was added dropwise in the beaker containing calcium chloride and chitosan solution. The beaker containing Calcium chloride

and chitosan solution was placed in a magnetic stirrer with a rotation of 400rpm. Resultant microspheres were filtered, washed with distilled water, and; vacuum dried. Prepared microspheres were preserved in a tightly sealed container for their characterization studies.

Evaluation of microsphere

1. % Yield of microspheres

After completely drying the prepared microspheres, the weight of the dried microspheres was taken, and the total drug and polymers taken for each formulation were taken.

The formula used to calculate the percent yield of all manufactured diacerein microsphere formulations is bellowed [6]:

The percentage yield of the prepared microsphere = $\frac{\text{Weight of dried microsphere}}{\text{Weight of total drug and polymer}} \times 100$

2. Swelling index

10mg of prepared diacerein microsphere soaked into 100 ml of freshly prepared phosphate buffer of pH 6.8 for 24 hrs at 37^oc. After 24hrs, microspheres were removed from a phosphate buffer, and the residual buffer was wiped out using filter paper; after that final weight of the microspheres was taken.

The swelling index of the different formulations of the microspheres was estimated by applying the following formula [7]:

$$\text{Swelling index} = (W_t - W_0 / W_0) \times 100$$



Figure (3A, 3B): Optical Microscopy.

The ocular micrometer or eyepiece micrometer was first calibrated to determine particle size. Mainly 2 types of micrometers were used: eyepiece micrometer and stage micrometer. The eyepiece micrometer was kept just below the eyepiece of the microscope, whereas the stage micrometer was placed in the stage of the optical microscope (Olympus optical microscope, ESAW optscopes classic). The following formula calculated the calibration factor of the ocular micrometer:

Calibration factor = number of parts of stage micrometer/number of parts of eyepiece micrometer.

Microspheres were suspended in a suitable liquid; the liquid should not solubilize the microsphere or cause swelling. Samples were put into a microscopic slide, and the diameter of 100 particles was recorded.

Where W_t = weight of microspheres observed at 24 hours; W_0 = initial weight of microspheres.

3. Particle size and shape [26]

The purpose of the test is to determine the uniformity of the prepared microspheres. The average particle size of each formulation was measured using an optical microscope technique (Figure 3A, 3B), by which diameters and shapes of 100 particles were recorded.

4. % Drug entrapment efficiency:

10 mg of the formulated diacerein microsphere were crushed using a mortar and pestle and suspended in 100 ml of freshly prepared phosphate buffer of pH 6.8 in a volumetric flask (100 ml). Continuous stirring of the medium is done by using a magnetic stirrer. The next day, the medium was filtered, and 1 ml of the filtrate solution was diluted by 10 ml phosphate buffer; using a UV spectrophotometer, the absorbance was assessed at 258nm.

% Drug entrapment efficiency was estimated by the equation [9]:

Percent drug entrapment efficiency = Actual drug content / Theoretical drug content $\times 100$

5. Fourier transform infrared (FTIR) spectroscopy

The drug, polymers, and prepared microspheres were investigated using FTIR analysis. Samples were scanned over a range of

4000-500 cm^{-1} on BRUKER Alpha Fourier transform infrared (FTIR) spectroscopy using KBr powder.

6. Drug release studies (In-vitro) [8]

An in-vitro drug release study was performed using paddle type USP Type II dissolution test apparatus at 50 rpm. 900 ml of freshly prepared phosphate buffer of PH 6.8 served as dissolution media. The temperature was adjusted and maintained at $37 \pm 0.5^\circ\text{C}$. At predetermined intervals, 5 ml of each sample was removed and substituted with an equal quantity of freshly prepared medium that was kept at an exact temperature. Collected samples were filtered using No. 41 Whatman filter paper. The filtrate was diluted and measured at 258.2 nm on a Shimadzu, UV 1800 UV visible spectrophotometer.

7. Drug release kinetics mechanism

A variety of kinetic models were used to analyze drug release data from the dissolution test like Zero order release kinetics (Eq. 1), First Order (Eq. 2), Higuchi's square root of time equation (Eq. 3), Korsemeyer and Peppas equation (Eq. 4), $C=K_0t$ (1) [10]

Where K_0 represents zero order rate constant expressed as concentration/ time and t is the time.

$$\text{Log } C = \text{Log } C_0 - K_1 t / 2.303 \quad (2) [11]$$

Where C_0 represents the initial concentration of the drug and K_1 is the first-order constant.

$$Q = K_h t \quad (3) [12]$$

Where K_h represents the constant reflecting the design variables of the system.

$$M_t / M_\infty = K_{kp} t^n \quad (4) [13]$$

Where M_t / M_∞ represents the fraction of drug release, K_{kp} is the release rate constant, n is the diffusion release exponent indicative of the drug release mechanism, and t is the dissolution time.

8. Scanning electron microscopy (SEM Study)

Morphology of prepared diacerein microspheres was studied by SEM using JEISS make (UK) model (JSM 6360 LV) scan sample of DCN microsphere with a focused electron beam to images with information about samples shape and topography. Drug-containing microspheres were placed on conducting stubs, and vacuum coated gold- palladium film using a Gold Sputter Coater (Model Edwards-S 150 B; Mfg. BOC Edwards UK). Images were captured using 17 kV electron intensity in SEM to examine the surface morphology.

RESULTS AND DISCUSSIONS

The study's main objective was to formulate sodium alginate-based microspheres encapsulated with Chitosan that may be used as drug release modifiers in a sustained drug release system. We used the ionotropic gelation method to create microspheres containing diacerein and investigated the impacts of several parameters such as sodium alginate concentration, chitosan concentration, and calcium chloride concentration on drug release rate.

A calibration curve (standard curve) of diacerein

The calibration curve of diacerein in freshly prepared phosphate buffer pH 6.8 was prepared. An increase of concentration was done in a preset manner. For quantitative estimation of the drug Regression equation was calculated and utilized. The correlation coefficient was found to be 0.997.

Data for the calibration curve preparation is shown in (Table 2) and the standard curve of diacerein is given in (Fig 4).

Table (2): Data for the preparation of calibration curve of diacerein (mean \pm SD, n=3).

Concentration (mcg/ml)	Absorbance
0	0
2	0.092 \pm 0.5
4	0.206 \pm 0.04
6	0.282 \pm 0.03
8	0.377 \pm 0.02

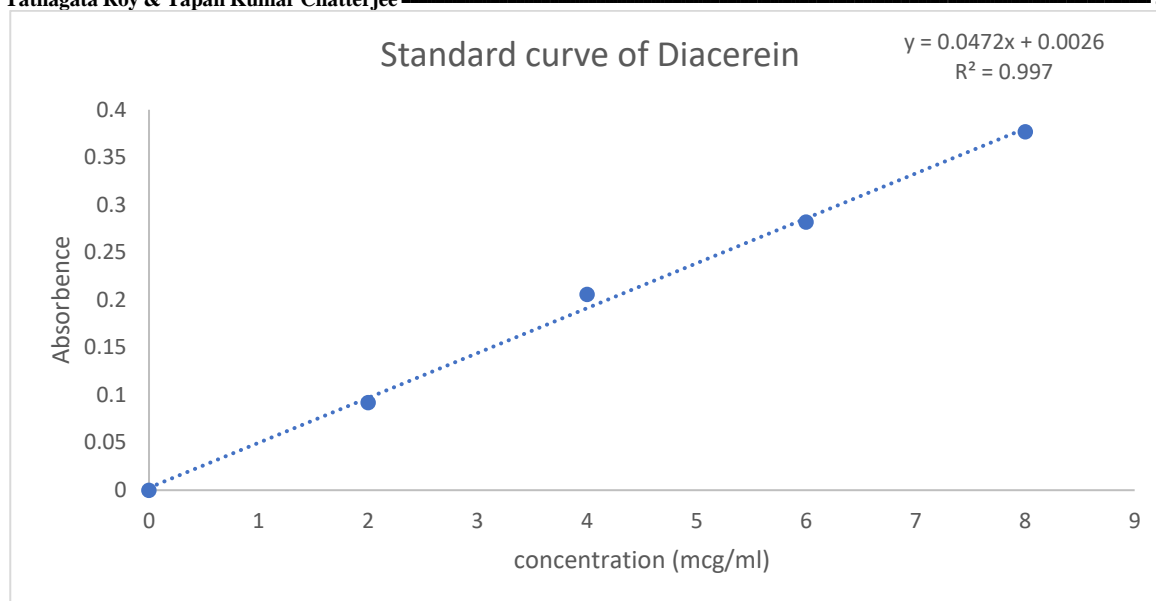


Figure (4): Standard curve of diacerein.

1. Analysis of Particle size of various formulations of the microsphere

Optical microscopy was used to evaluate the mean particle size of several formulations of diacerein microspheres. The findings show that the average particle size of microspheres was increased by enhancing the concentration of calcium chloride and sodium alginate. Visual examination was used to figure out the shape of the microspheres. The particle size of various formulations of microspheres and their shapes are mentioned in (Table 3).

2. % Yield of microspheres

% Yield of different microsphere formulations is shown in table 3. Chitosan concentration played an essential role in % the yield of various formulations of prepared microspheres. Increased chitosan concentration increases the viscosity of the polymer mixture. That is why process loss will be less due to higher viscosity and % yield of microspheres increases.

3. Swelling index of microsphere

A swelling study of all 8 formulations of microspheres was performed in a freshly prepared phosphate buffer of 6.8. Results of the swelling index are mentioned in table 3. Data

of swelling index indicates that the % equilibrium water uptake increases dramatically as the matrix's proportion of crosslinker (calcium chloride) increases. This is because increased calcium chloride concentration causes more substantial crosslinking with sodium alginate. Pore volume and density of the polymeric network also increased [24]. The swelling index of various formulations of prepared microspheres is mentioned in (Table 3).

4. %Drug entrapment efficiency

The amount of diacerein present in the various microsphere formulations was measured using entrapment efficiency determination. % Drug entrapment efficiency showed dependency on the concentration of Chitosan. As the concentration of the Chitosan increased in the formulation, the entrapment efficiency also increased. B3 formulation has a Chitosan: sodium alginate ratio of 3:1 and shows maximum entrapment efficiency (Table 3).

An increase in drug entrapment can be observed as the drug: polymer ratio increases. It happened because the viscosity of the aqueous phase increases as the polymer concentration increases, stabilizing the droplets. This droplets' stabilization prevents diacerein's further outflow from the microspheres [25].

Table (3): % Yield, Swelling index, Particle size, Drug entrapment efficiency of 8 formulations of microsphere (mean \pm SD, n=3).

Formulation Code	% Yield	Swelling index	Particle size (μm)	%Drug entrapment efficiency	Sphericity
B1	56.92 \pm 0.4	78.12 \pm 0.1	539 \pm 0.4	33.6 \pm 0.5	Irregular spherical
B2	65.32 \pm 0.2	60.78 \pm 0.4	537 \pm 0.5	47 \pm 0.3	Spherical
B3	82.78 \pm 0.9	49.08 \pm 0.03	106 \pm 0.5	52 \pm 0.2	Spherical
B4	60.33 \pm 0.5	78.11 \pm 0.09	531 \pm 0.5	38.2 \pm 0.5	Spherical
B5	72.51 \pm 0.7	59.46 \pm 0.29	512 \pm 0.04	41 \pm 0.2	Spherical
B6	52.13 \pm 0.2	82.067 \pm 0.03	542 \pm 0.4	31 \pm 0.2	Irregular spherical
B7	84.37 \pm 0.5	57.017 \pm 0.1	501 \pm 0.5	50 \pm 0.3	Spherical
B8	80.47 \pm 0.5	58.19 \pm 0.5	507 \pm 0.4	49.5 \pm 0.3	Irregular spherical

5. Fourier transform infrared spectroscopy (FTIR)

The results of Diacerein API, Sodium Alginate, Chitosan, and Diacerein microspheres IR spectra are shown in (Fig 5). Diacerein API shows the peak at 1678.55 cm^{-1} (C=O stretching of conjugated ketones), 2938.61 cm^{-1} (C-H stretching aliphatic), and 1688 cm^{-1} (C=O

stretching of Carboxylic Acids). These peaks are available on Diacerein microspheres IR spectra, suggesting no incompatibility between API and Polymers during formulation. The peak at 3438.54 cm^{-1} in Diacerein microspheres is visible, probably due to Sodium Alginate. So, all of this data indicates the stability of diacerein microspheres.

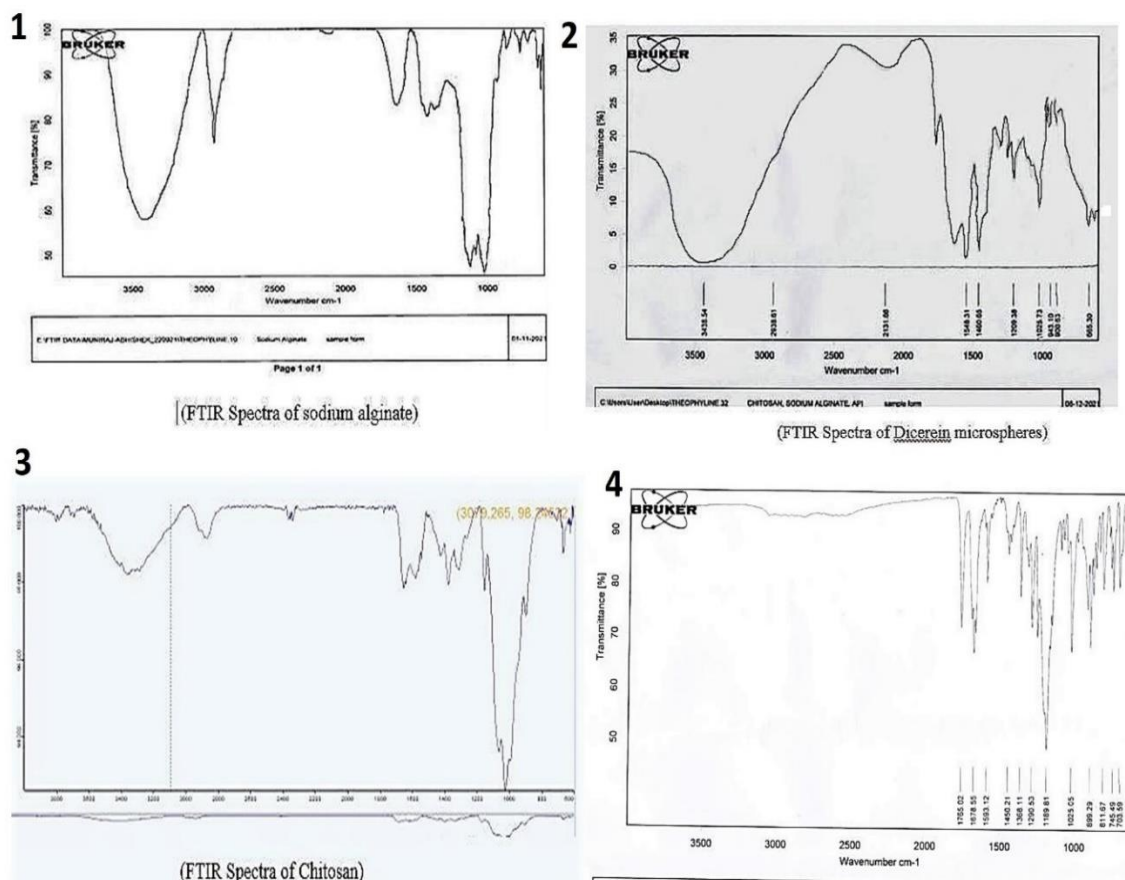


Figure (5): FTIR spectra of chitosan, sodium alginate, diacerein, and prepared microspheres.

6. In-vitro drug release studies

In- vitro, drug release studies of all 8 formulations of the microspheres (B1-B8) divulged that the drug release range was 76%-

98% shown in (Figure 6). Data indicates that increased concentration of Chitosan decreases the drug release rate. Cumulative% drug release of 8 formulations of microspheres is shown in (Table 4).

Table (4): Cumulative% drug release of 8 formulations of microspheres (Mean± S.D, n =3).

Time(min)	B1	B2	B3	B4	B5	B6	B7	B8
0	0	0	0	0	0	0	0	0
30	13.19±0.08	9.81±0.06	16.12±0.51	11.47±0.28	7.79±0.04	16.53±0.02	8.42±0.03	6.3±0.03
60	25.45±0.4	18.7±0.18	18.67±1.27	21.78±0.11	16.79±0.06	28.73±0.03	14.87±0.02	14.2±0.3
120	30.37±0.11	25.67±0.3	26.25±1.06	28.71±0.2	23.86±0.04	32.87±0.02	20.66±0.02	22.04±0.15
180	38.49±1.06	33.39±0.4	31.7±0.72	35.45±0.26	31.26±0.03	41.4±0.4	29.88±0.03	30.01±0.11
240	55.78±0.11	47.75±0.18	38.85±0.9	52.8±0.08	46.84±0.03	52.22±0.03	37.65±0.03	42.48±0.18
300	60.67±0.11	56.43±0.29	44.48±1.85	57.23±0.11	55.85±0.03	63.64±0.03	46.92±0.03	53.34±0.11
360	75.65±0.29	68.6±0.25	54.84±1.03	70.62±0.28	67.91±0.17	78.36±0.38	57.55±0.03	65.82±0.04
420	84.76±0.19	80.68±0.3	61.5±0.64	82.72±0.17	79.46±0.03	85.15±0.03	66.87±0.02	76.64±0.22
480	95.55±0.25	89.58±0.16	76.01±0.43	92.84±0.31	86.26±0.02	98.63±0.03	79.55±0.03	82.32±0.12

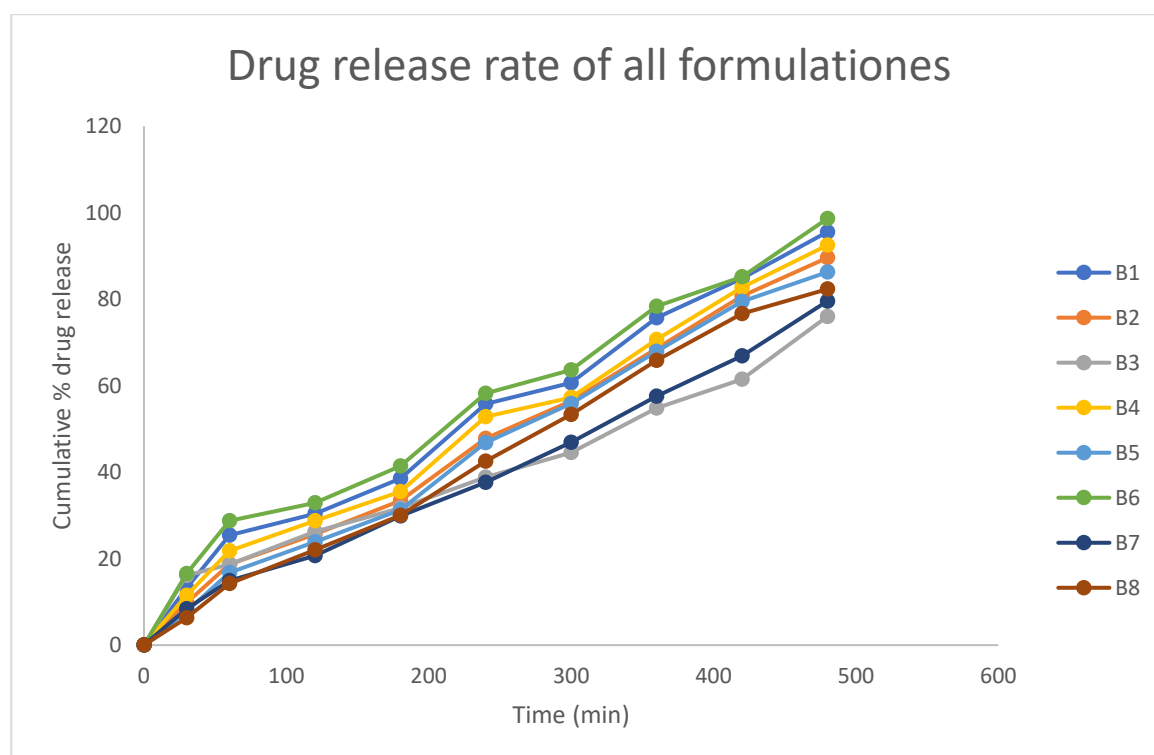


Figure (6): zero order drug release kinetics of all 8 formulations of microspheres.

In-vitro drug release study of 8 formulations of microspheres can be presented in the following order:

$$B3 < B7 < B8 < B5 < B2 < B4 < B1 < B6$$

7. Drug release kinetics mechanism

In-vitro drug release data of 8 formulations of microspheres (B1-B8) were fitted to zero order, first order, Higuchi, Korsmeyer-Peppas model to determine the appropriate drug release kinetics model for every formulation. (Figure 7-Figure 10, table 5).

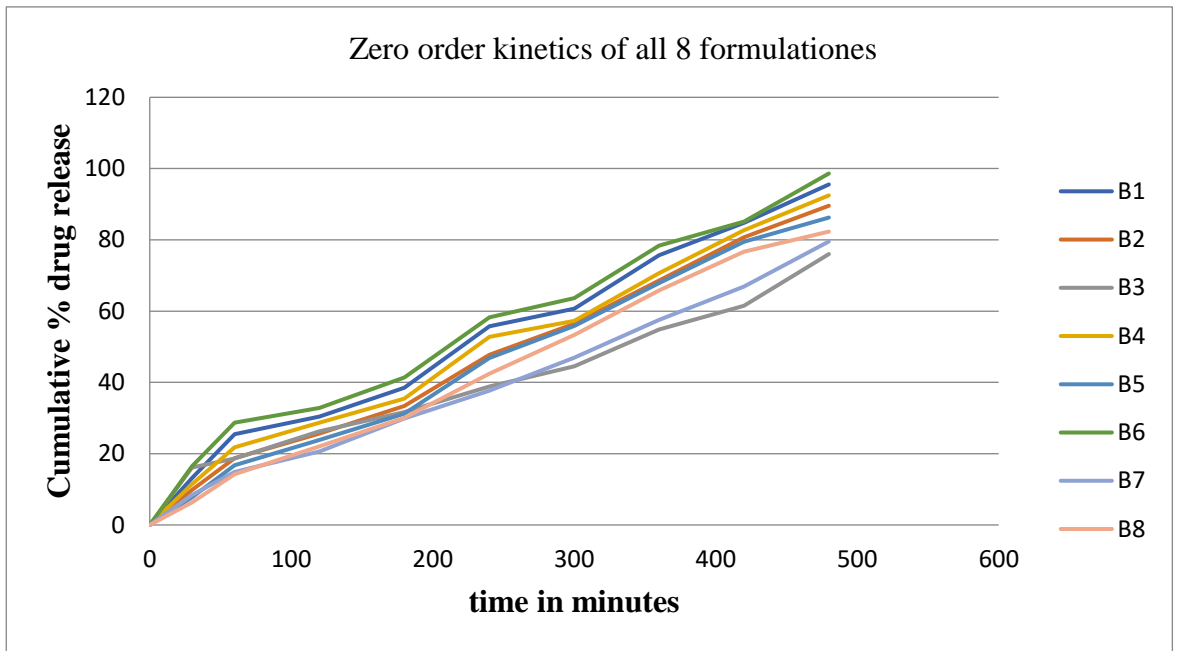


Figure (7): Zero order kinetics model of 8 formulations of microspheres.

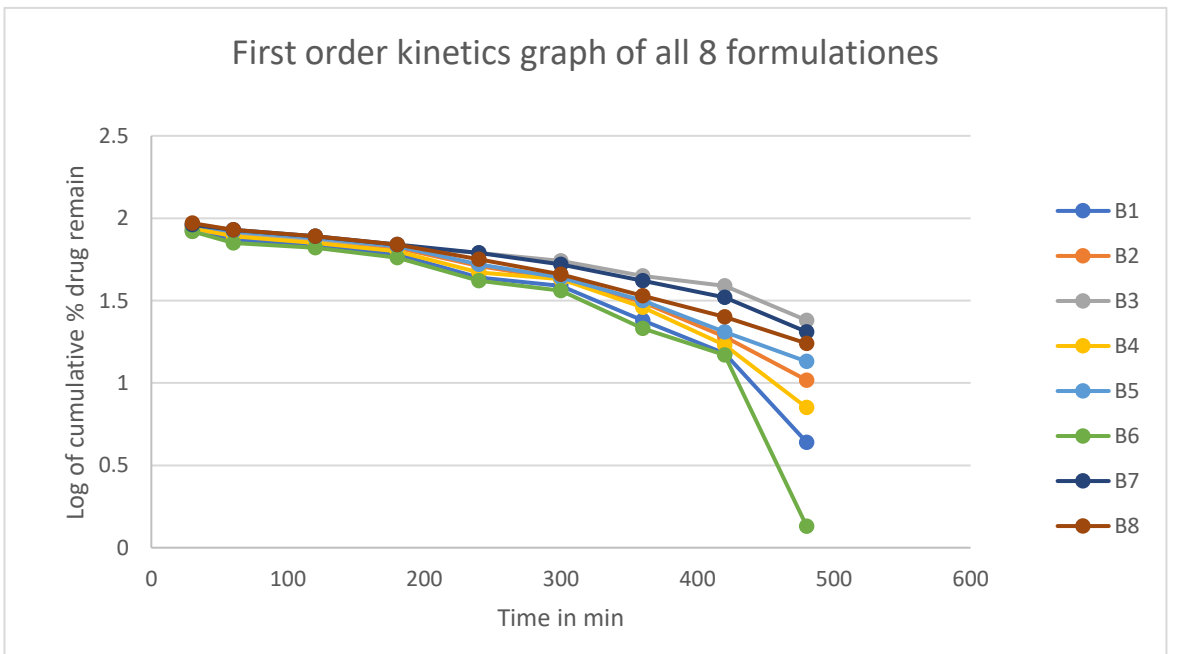


Figure (8): First order kinetics model of 8 formulations of microspheres.

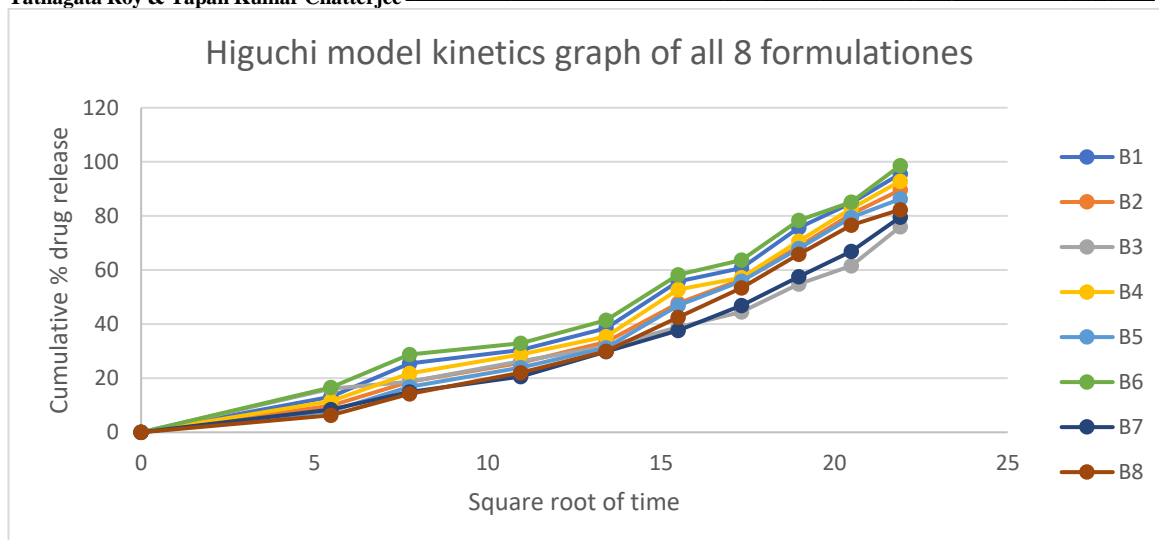


Figure (9): Higuchi kinetics model of 8 formulations of microspheres.

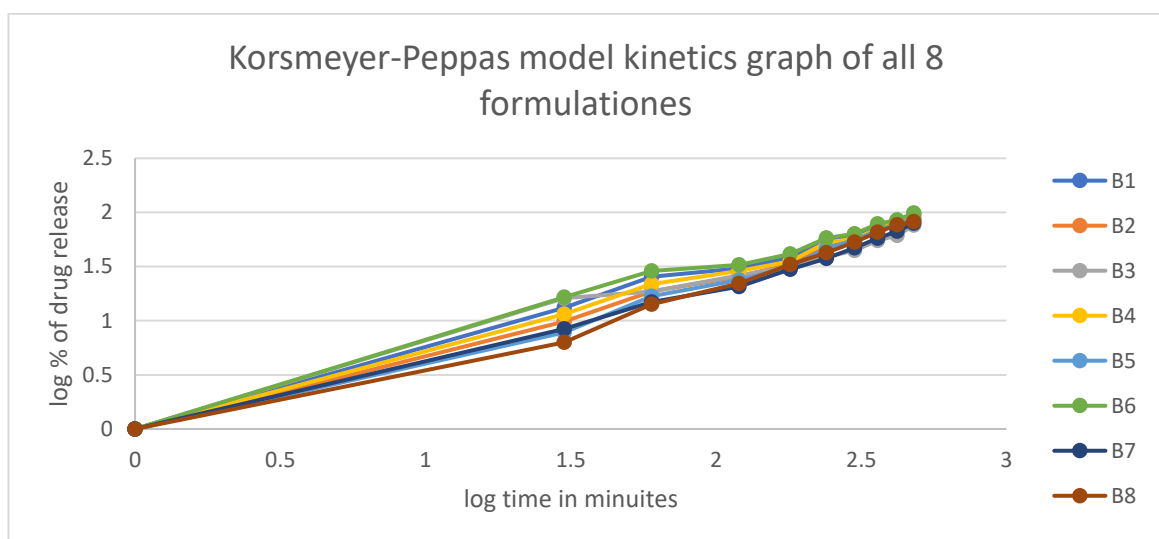


Figure (10): Korsmeyer- Peppas kinetics model of 8 formulations of microspheres.

Table (5): Drug release kinetics mechanism.

Kinetics	R ² value of B1batch	R ² value of B2batch	R ² value of B3batch	R ² value of B4 batch	R ² value of B5batch	R ² value of B6 batch	R ² value of B7 batch	R ² value of B8 batch
Zero-order	0.985	0.9948	0.973	0.989	0.995	0.979	0.994	0.996
First Order	0.861	0.920	0.924	0.886	0.943	0.751	0.935	0.958
Higuchi Model	0.953	0.937	0.941	0.945	0.934	0.96	0.925	0.928
<u>Korsmeyer-peppas Model</u>	0.993	0.9945	0.983	0.995	0.988	0.988	0.991	0.980
Kinetics model followed	<u>Korsmeyer-peppas Model</u>	Zero order	<u>Korsmeyer-peppas Model</u>	<u>Korsmeyer-peppas Model</u>	Zero order	<u>Korsmeyer-peppas Model</u>	Zero order	Zero order

Selection of the optimized formulation

Selection of optimum formulation from 8 formulations of prepared diacerein microspheres was based on size and shape of the

microspheres, % entrapment efficiency, and cumulative % drug release values. From the data of previously mentioned parameters, it can be concluded that the B3 formulation is the optimized formulation.

8. Scanning Electron Microscopy (SEM) Study of Diacerein microspheres

The morphology of B3 formulation microspheres was studied by SEM using JEOL JSM -6360 (JSM 6360 LV) to scan a DCN microsphere with a focused electron beam to images with information about samples shape and topography. Drug-containing

microspheres were placed on conducting stubs and vacuum coated gold-palladium film using a Gold Sputter Coater (Model Edwards-S 150 B; Mfg. BOC Edwards UK). Images were taken using 17 kv electron intensity in SEM to examine the surface morphology. SEM study of B3 formulation indicates microspheres' spherical structure and rough surface (Figure 11).

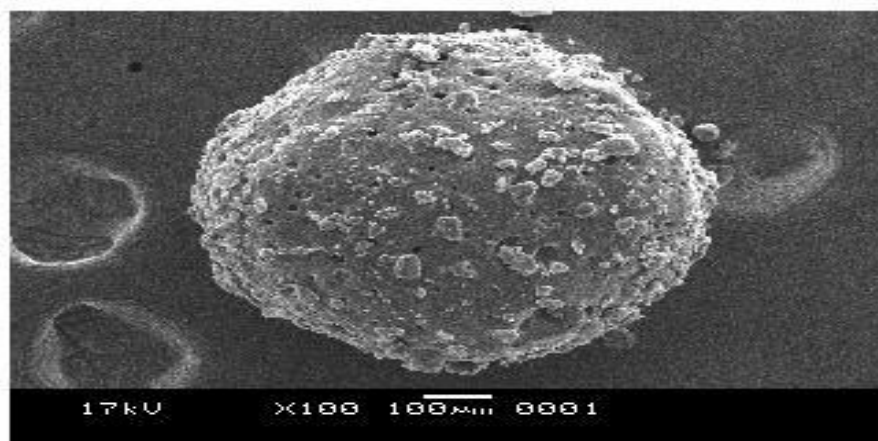


Figure (11): SEM of microspheres B3 formulation microspheres.

CONCLUSION

The ionotropic gelation method was adopted to formulate diacerein microspheres by considering calcium chloride as a cross-linking agent and sodium alginate and Chitosan as polymers. This technique prepared eight different formulations (B1-B8) of microspheres, and different parameters like swelling index, % entrapment efficiency, particle size, shape, % yield, and drug release studies were performed. The results concluded that the B3 formulation was the optimized formulation that gave the best results. Further, an SEM study was carried out for B3 formulation to reveal the microspheres' spherical shape and rough surface. Thus, we can conclude that diacerein microspheres may be suitable as a controlled release dosage form. It releases the entrapped drug sustainably so that diacerein-mediated adverse drug reactions like soft stool and diarrhea can be minimized with improved patient compliance.

Ethics approval and consent to participate

Ethical approval

Not applicable.

Consent for publication

Both the authors have read and approved the manuscript for publication.

Availability of data and materials

Not applicable.

Author's contribution

Tathagata Roy: Involved in conceptualization, writing-original draft, data curation, formal analysis, methodology development, supervision, validation, visualization, and writing review & editing of the manuscript. **Dr. Tapan Kumar Chatterjee:** Involved in conceptualization, formal analysis, and supervision of the research work.

Competing interest

The authors declare that there is no conflict of interest.

FUNDING

None

ACKNOWLEDGMENT

We would like to thank Prof. (Dr). Himangshu Sekhar Maji, HOD, Department of Pharmaceutical Technology, JIS University, for providing the necessary laboratory

facilities to carry out this experimental work, and Prof. (Dr). S.M. Firdous, Professor and HOD, Department of Pharmacology, Calcutta Institute of Pharmaceutical Technology & AHS, Uluberia for encouraging to write this research article.

REFERENCES

- 1) Adepu, S. & Ramakrishna, S. (2021). Controlled drug delivery systems: current status and future directions. *Molecules*. 26(19). 5905.
- 2) Bose, S. Kaur, A. & Sharma, SK. (2013). A review on advances of sustained release drug delivery system. *Int. Res. J. Pharm.* 4. 1-5.
- 3) Kumar, AR. & Aeila, AS. (2019). Sustained release matrix type drug delivery system: An overview. *World J Pharma pharm Sci*. 8(12). 470-80.
- 4) Kaur, D. Kaur, J. & Kamal, SS. (2019). Diacerein, its beneficial impact on chondrocytes and notable new clinical applications. *Brazilian Journal of Pharmaceutical Sciences*. 8. 54.
- 5) Raha, A. Bhattacharjee, S. Mukherjee, P. Paul, M. & Bagchi, A. (2018). Design and characterization of ibuprofen loaded alginate microspheres prepared by ionic gelation method. *Int. J. Pharma. Res. Health. Sci*. 6. 2713-6.
- 6) Rathi, S. Vaghela, S. Shah, R. & Shah, S. (2021). Formulation and Evaluation of Gastroretentive Floating Tablets of Febuxostat. *Research Journal of Pharmacy and Technology*. 14(10). 5359-65.
- 7) Ozoude, CH. Azubuike, CP. Ologunagba, MO. Tonuewa, SS. & Igwilo, CI. (2020). Formulation and development of metformin-loaded microspheres using Khaya senegalensis (Meliaceae) gum as co-polymer. *Future Journal of Pharmaceutical Sciences*. 6(1). 1-1.
- 8) Kulkarni, AR. Soppimath, KS. & Aminabhavi, TM. (1999). Controlled release of diclofenac sodium from sodium alginate beads crosslinked with glutaraldehyde. *Pharmaceutica Acta Helvetiae*. 74(1). 29-36.
- 9) Patil, P. Singh, S. Sarvanan, J. (2018). Preparation and evaluation of microspheres of flurbiprofen. *Int J Pharma Sci Res*. 9(12). 5388-93.
- 10) Narashimhan, B. Mallapragada, SK. & Peppas, NA. (1999). *Release kinetics, data interpretation. Encyclopedia of Controlled Drug Delivery*. John Wiley and Sons, Inc, New York. p. 921.
- 11) Bourne, DW. Banker, GS. & Rhodes, CT. (2002). *Modern Pharmaceutics*. New York: Informa Healthcare. 67-92.
- 12) Higuchi, T. (1961). Rate of release of medicaments from ointment bases containing drugs in suspension. *J Pharm Sci*. 50(10). 874-5.
- 13) Korsmeyer, RW. Gurny, R. Doelker, E. Buri, P. & Peppas, NA. (1983). Mechanisms of potassium chloride release from compressed, hydrophilic, polymeric matrices: effect of entrapped air. *J Pharm Sci*. 72(10). 1189-91.
- 14) Boileau, C. Tat, SK. Pelletier, JP. Cheng, S. Martel-Pelletier, J. (2008). Diacerein inhibits the synthesis of resorptive enzymes and reduces osteoclastic differentiation/survival in osteoarthritic subchondral bone: a possible mechanism for a protective effect against subchondral bone remodelling. *Arthritis research & therapy*. 10(3). 1-0.
- 15) Nicholas, P. Tod, M. Padion, C. & Petitjean, O. (1998). Clinical pharmacokinetics of diacerein. *Clin Pharmacokinet*. 35(5). 347-59.
- 16) Lajoinie, A. Janiaud, P. Henin, E. Gleize, JC. Berlion, C. Nguyen, KA. Nony, P. Gueyffier, F. Maucort-Boulch, D. & Koupaï, BK. (2017). Assessing the effects of solid versus liquid dosage forms of oral medications on adherence and acceptability in children. *The Cochrane Database of Systematic Reviews*. 2017(9).
- 17) RADA, SK. & Kumari, A. (2019). Fast dissolving tablets: waterless patient compliance dosage forms. *Journal of Drug Delivery and Therapeutics*. 9(1). 303-17.
- 18) Pavelka, K. Bruyere, O. Cooper, C. Kanis, JA. Leeb, BF. Maheu, E. Martel-Pelletier,

- J. Monfort, J. Pelletier, JP. Rizzoli, R. & Reginster, JY. (2016). *Diacerein: benefits, risks and place in the management of osteoarthritis*. An opinion-based report from the ESCEO. *Drugs & aging*. 33(2). 75-85.
- 19) Das, MK. Ahmed, AB. & Saha, D. (2019). Microsphere a drug delivery system: A review. *Int J Curr Pharm Res*. 11(4). 34-41.
- 20) Prasad, BS. Gupta, VR. Devanna, N. & Jayasurya, K. (2014). Microspheres as drug delivery system-a review. *J Glob Trends Pharm Sci*. 5. 1961-72.
- 21) Gurung, BD. & Kakar, S. (2020). An overview on microspheres. *Int J Health Clin Res*. 3(1). 11-24.
- 22) Saralidze, K. Koole, LH. & Knetsch, ML. (2010). Polymeric microspheres for medical applications. *Materials*. 3(6). 3537-64.
- 23) Moldovan, F. Pelletier, J. Jolicoeur, FC. Cloutier, JM. & Martel-Pelletier, J. (2000). Diacerhein and rhein reduce the ICE-induced IL-1 β and IL-18 activation in human osteoarthritic cartilage. *Osteoarthritis and Cartilage*. 8(3). 186-96.
- 24) Deshmukh, P. & Barhate, A. (2017). Formulation and evaluation of microspheres of glibenclamide by ionotropic gelation method. *Indo americal journal of Pharmaceutical Research*. 7(9). 471-479.
- 25) Denkbaş, EB. Seyyal, M. & Pişkin, E. (2000). Implantable 5-fluorouracil loaded chitosan scaffolds prepared by wet spinning. *Journal of membrane Science*. 172(1-2). 33-8.
- 26) Lachman, L. Liberman, HA. & Kang, JL. (1991). *The theory and practice of industrial pharmacy*. 3rd ed. Varghese publication house, 296-302.