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Research Article

EVALUATION OF ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITIES OF FLOATING MICROSPHERES OF HERBAL DRUG

Kapil Kumar[1]*, A.K. Rai[2]
[1]Institute of Pharmacy NIMS University, Jaipur, 303121, Rajasthan, India, Global Institute of Pharmaceutical Education and Research, Kashipur, U.K., India
[2]Pranveer Singh Institute of Technology, Kanpur, U.P. India

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*Email: kapil5november@gmail.com

ABSTRACT
The aim of the present studies was to prepare and evaluate floating microspheres of curcumin for anti-inflammatory and anti-arthritic effect. Floating microsphere were prepared using hydroxyl propyl methylcellulose (HPMC), ethyl cellulose (EC), polyvinyl pyrrolidone K30 (PVPK30), eudragit RS 100 polymer in different ratio and dichloromethane and heavy liquid paraffin as solvent by emulsion solvent diffusion method. The floating microspheres were evaluated for flow properties based on parameters such as angle of repose and compressibility index, as well as for various other physicochemical properties including particle size, incorporation efficiency, in-vitro floatability, and in-vitro drug release. The shape and surface morphology of the microspheres were characterised by scanning electron microscopy. Scanning electron microscopy showed pores on the surface and interior of the microspheres. Anti-inflammatory, anti-arthritic effect of formulation C4 and E1 were compared with standard market product Indomethacin. The effect of formulation C4 and E1 was evaluated for acute inflammation in carrageenan induced rat paw edema and for chronic inflammation in complete Freud's adjuvant (CFA) induced arthritis in rats. Further histopathological and radiographic evaluation was performed. It may be concluded that Curcumin microspheres would be promising drug delivery system for oral administration of Curcumin to sustained the drug release. Incorporation of herbal drugs in novel drug delivery system may lead to an excellent result.
Keywords: Curcumin, floating microsphere, HPMC, EC, anti-inflammatory, anti-arthritic activity

INTRODUCTION

In previous time herbal drugs were not attracting scientists for development of novel drug delivery systems due to processing, standardizing, extracting and identification difficulties. But now days with the advancement in the technology, novel drug delivery systems new doors are opened for the development of herbal drug delivery systems[1]. Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Inflammation is a response of the tissue to an injury, infection, irritation of foreign substance. Jain et al. suggested that it is a part of host defense, but when the response becomes too great it may be far worse than the diseases itself and in extreme condition, it may be fatal[1].Inflammation manifests usually in form of painful swelling associated with some changes in skin covering the site. It can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Kumar C explained that prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process[2]. According to Saag et al. the therapy of inflammation deals with the drugs used to treat inflammatory and immune disorders[3]. NSAIDs, glucocorticoids or so called disease modifying drugs such as gold or methotrexate are prescribed for the treatment of rheumatoid arthritis. NSAIDs represent an indispensable place in the treatment of rheumatoid disorders. Administration of NSAIDs to patients with congestive heart failure, renovascular hypertension and cirrhosis of the liver, activated renin-angiotensin system may cause acute renal failure. Long-term use of NSAIDs may cause infertility in women. The prolonged use of NSAIDs could lead to infertility and impotence in males[4]. Since time immemorial, indigenous plants have been a major source of medicine. In folk medicine, they are used, in single or in combined forms for treating different types of inflammatory and arthritic conditions. Prolonged administrations of steroidal and non-steroidal anti-inflammatory drugs are known to be associated for their adverse effects[5]. The floating microspheres beneficially alter the absorption of a drug, thus enhancing its bioavailability. They prolong dosing intervals which will allow to develop once a day formulations and thereby increase patient compliance beyond the level of existing dosage forms by achieving control over gastric residence time[6]. Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. These microspheres are characteristically free flowing powders having a size less than 199 µm and remain buoyant over gastric contents and for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration[7]. Curcumin (*Curcuma longa*) is the active ingredient of the spice turmeric, used in cooking in India and other regions of Asia. It has a long history as an herbal remedy for a variety of diseases and was used in Indian and Chinese traditional medicine as early as 700 AD. The origin of the plant Curcuma longa L., which belongs to Zingiberaceae family is India.

Curcumin is a potent phytomolecule with wide range of biological activity; possess a low absorption[8]. Curcumin was selected fro the studies as it is poorly absorbed in the lower GIT and has short elimination half-life ~0.39 h. The poor bioavailability (< 1%) of the molecule owing to the insolubility at gastric pH and degradation at alkaline pH of

intestine in the human body has severely limited its clinical application. High oral doses (8 g/day) in humans result in Cmax of < 2 μM, and short half life (~28 minutes) limits it's use by oral route[9].

A vast research revealed curcumin has a wide spectrum of therapeutic effects such as anti-inflammatory, antibacterial, antifungal, anticancer, antispasmodic, antioxidant[10].

MATERIALS AND METHODS

Isolation of curcumin from rhizome of *Curcuma longa*

C. longa (Zingiberaceae) rhizome were collected from the agricultural fields. It was cleaned, thoroughly washed with deionised water and was kept for drying in shade at room temperature for 20 days. The thoroughly air dried material was grinded to about 40 -60 mesh size. 40 gm of powder of *C. longa* was taken and magnetically stirred in 1oo ml of dichloromethane and refluxed for 2 hr in a reflux condenser using ethanol as solvent. After filtration, filtrate was concentrated in hot water bath at 50^{0}C. The reddish oily residue was pulverized with 30 ml of hexane and the resulting solid was collected by suction filtration. TLC of Alcoholic extract of drug on silica gel 'G' plate using chloroform: benzene: ethanol (45:45:10) as developing solvent system. Visible light spot are seen at Rf. 0.49, on spraying with boric acid: methanol reagent and heating the plate at 105ºC until the light violet color develops. Comparison was made with the relevant literature and pure curcumin as standard[11-12].

HPMC and Eudragit S 100 were received as a gift samples from Glukem pharmaceuticals (P) Ltd, India. All other chemicals used were of analytical grade.

Preparations of floating microspheres

The floating microspheres were prepared by emulsion solvent diffusion method[13]. Briefly the drug and polymer ratio are used as shown in Table 1 were mixed in ethanol by using blending solvent dichloromethane and heavy liquid paraffin. The slurry was introduced into 250 ml beaker containing 0.2% tween 80 while being stirred at 750 rpm by mechanical stirrer for 1hr at room temperature. The floating microspheres were collected by decantation while the non-floating microspheres were discarded along with polymer residues and washed thrice with n-hexane. The collected microspheres were dried overnight in an oven at 40 ± 2 ^{0}C and stored in a desiccators containing calcium chloride as a desiccant.

Evaluation of floating microspheres

Particle size

The particle size was measured by microscopic technique. In this method suspension of floating microspheres was prepared using castor oil. A drop of suspension was mounted on a slide and observed under optical microscope about 600 particles were measured with the help of the eye piece micrometer. All the microspheres in a field were counted[14].

Bulk density

In this method floating microspheres are transferred to a measuring cylinder and is tapped manually till a constant volume is obtained. This volume is bulk volume and it includes true volume of the powder and the void space among the microspheres[15].

$$\text{Bulk density} = \frac{\text{Mass of microspheres}}{\text{Bulk volume}} \quad \ldots\ldots\ldots (1)$$

Tapped density

In this method floating microspheres were transferred to a measuring cylinder & tapped for 100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density floating microspheres[16].

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}} \quad \ldots\ldots\ldots (2)$$

Carr's (Compressibility) index

This parameter was calculated from bulk density (the ratio of weighed quantity of microspheres to its volume), DP, and tapped density as follows

$$\text{Compressibility index} = \frac{(DT - DP)}{DTx100} \quad \ldots\ldots\ldots (3)$$

Hausner,s Ratio

Hausner,s ratio of microspheres was determined by comparing tapped density to bulk density using the equation.

$$\text{Hausners ratio} = \frac{\text{Bulk density}}{\text{Tapped density}} \quad \ldots\ldots\ldots (4)$$

Values less than 1.25 indicate good flow (= 20% Carr), whereas greater than 1.25 indicates poor flow (= 33% Carr).

Angle of repose

Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method4. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder cone was measured and angle of repose was calculated using the following equation[17].

$$\theta = tan^{-1}\frac{h}{r} \quad \ldots\ldots\ldots (5)$$

Where,

θ - Angle of repose, h - Height of granules above the flat surface, r - radius of the circle formed by the granule heap.

Scanning electron microscopy

Scanning electron microscopy was carried out for formulation C4 and E1. Dry microspheres were placed on an electron microscope brass stub a coated with gold in an ion sputter. Then picture of microsphere were taken by random scanning of the stub. The SEM analysis of the microspheres was carried out by using JEOL – 6360A analytical scanning electron microscope. The microspheres were viewed at an accelerating voltage of 20KV[18].

Sphericity of the microsphere

To determine the Sphericity, the tracings of prepared microspheres (magnification 45x) were taken on a black paper using camera Lucida, (Model -Prism type, Rolex, India) [19]. Circulatory factor (S) was calculated using,

$$s = \frac{p^2}{12.56 X A} \quad \ldots\ldots\ldots (6)$$

Where A is area (cm^2) and, p is the perimeter of the circular tracing.

Drug entrapment efficiency

Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N

HCl. The solution was filtered and the absorbance was measured at 254 nm by spectrophotometer (Shimadzu 1700) against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula:

$$DEE = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

…….. (7)

Yield of microspheres

The prepared microspheres with a size range of 251-µm were collected and weighed. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of Product}} \times 100$$

…….. (8)

In-vitro buoyancy

Microspheres (300mg) were spread over the surface of a USP XXIV dissolution apparatus type II filled with 900 ml of 0.1 N hydrochloric acid containing 0.02% tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 hrs. The floating and the settled portions of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres[20].

$$\text{Buoyancy (\%)} = \frac{W_f}{W_f + W_s} \times 100$$

……. (9)

Where W_f and W_s are the weight of floating and settled microsphere respectively

In vivo floating behavior

Healthy rabbit weighing approximately 2.5 kg was used to assess in-vivo floating behavior. The animals were fasted for 12 h and the first X-ray photographed to ensure absence of radio opaque material in the stomach. The rabbit were made to swallow barium sulphate loaded floating microspheres C4 of curcumin with 100ml of water. During the experiment rabbit were not allowed to eat but water was provided *ad libitum*. At predetermined time intervals the radiograph of the abdomen was taken using an X-ray machine (Medford) [21].

In-vitro drug release studies

A USP basket apparatus has been used to study in vitro drug release from microspheres. In the present study drug release was studied using a modified USP XXIV dissolution apparatus type I (basket) at 100 rpm in distilled water and 0.1 mol L-1 HCL (pH 1.2) as dissolution fluids (900ml) maintained at 37±1^{0}C. Samples withdrawn at periodical intervals and analyzed spectrophotometrically at 254 nm. The volume was replenished with the same amount of fresh medium to maintain the sink condition. All experiments were performed in triplicate. Cumulative percentage drug release was calculated using an equation obtained from a standard curve[22].

Pharmacodynamic Studies

Anti-inflammatory studies of floating microsphere of curcumin

Healthy albino rats of either sex (Wistar strain) weighing 160-190 g were used for present study. The animals were kept in plastic cages with soft bedding (6 per cage). The animals had free access to food and water and were maintained under controlled temperature (27±2^{0}C) and 12 hrs: 12 hrs light and dark cycle. They were allowed to acclimatize for one week before the experiments. Initial body weight of each animal was recorded. Before the experiment, food was withdrawn overnight but adequate water was given to the rats. Due to painful condition imposed on animals the numbers of subjects used were restricted to the minimum six per group that allowed reliable statistical analysis of the results. The animals were divided into five groups of 6 animals each. Control group are given normal saline, standard group was treated with 10mg/kg indomethacin given by I.P. Two test group receive orally, floating microsphere of selected formulation C4 and E1 containing curcumin equivalent to the dose of the drug 30 mg/kg body weight. Another test group receives curcumin 30mg/kg orally. The animals were then injected with 0.1 ml of 1% carrageenan solution in saline in plantar region of left hind paw and the paw volume was measured after 1, 2, 4, 6, 8 hr using water plethysmometer. The right hind paw served as a reference non inflamed paw for comparison[23].

Paw volume: Initial Rat paw volume was measured using plethysmometer (Model 7150, UGO Basile, Italy). The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark in the plethysmometer. Mean changes in paw volume were calculated and % inhibition of paw edema was calculated using formula:

$$\% \text{ Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

…….. (2)

Where, Vc is mean changes in paw volume of control group and Vt is mean changes in paw volume treated group.

Paw thickness: Paw thickness was measured by compressing the joint by rotating the screw of micrometer screw gauge till the pain elicited as indicated by squeaking or leg withdrawal. The distance moved by the screw gauge was recorded and % inhibition of paw thickness was calculated using formula:

$$\% \text{ Inhibition} = \frac{Tc - Tt}{Tc} \times 100$$

…….. (3)

Where, Tc is mean change in paw thickness of arthritis control group and Tt is mean changes in paw thickness of treated group.

Anti-arthritic activity

Freud's adjuvant induced arthritis model was used to assess the anti-arthritic activity in albino rats[24]. Animals were divided into five groups of six animals each. Group I served as control, which received 5% gum acacia suspension, Group II served as reference standard, which received 10 mg/kg body weight IP of indomethacin, and Group III and IV served as test, which received 40 mg/kg floating microsphere of curcumin of batch C4 and E1.Group V received 40 mg/kg curcumin powder orally. Arthritis was induced by injecting 0.05 ml of suspension of killed Mycobacterium tuberculosis bacteria (0.5% w/w) homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0 day), 30 min before adjuvant injection and continued till 20 day. Paw volume and thickness were measured on 5th, 10th, 15th and 20th day with the help of plethysmometer and micrometer screw gauge respectively. The mean changes in injected paw edema with respect to initial paw volume and thickness, were calculated on respective days and percentage inhibition of paw edema with respect to untreated group (control) was calculated.

Radio graphic evaluation: The rats were anaesthetized using ketamine (100 mg/kg, i.p.) and radio graph was recorded on a digital system and seamen's X-ray machine after 20 days[25].

Histopathological studies

The hind paw was amputed above the knee joint and fixed in 10 % formalin solution. The sections were stained with haemtoxylin and eosin and were examined microscopically for histopathological changes

Statistical Analysis

To ascertain drug release mechanism and release rate, the release data were model fitted using PCP Disso V3.0 dissolution software. The models selected were Zero order, Higuchi Matrix, Korsemeyer,s-Peppas. Data was expressed as means ± SEM and analyzed for statistical significance using Student's t test, one-way analysis of variance (ANOVA) followed by Dunnett's test or two-way ANOVA followed by Bonferroni test. <0.05, p<0.01and p<0.001 was considered to be significant

RESULT AND DISCUSSION

The floating microspheres of curcumin were prepared by emulsion solvent diffusion method. The results of the physicochemical characterization are shown in Table 2 and 3. The prepared floating microspheres were found to be discrete, spherical and free flowing. The mean arithmetic diameter varied between 227.12±2.34-294.64±1.82µm. Surface morphology characteristics were studied using SEM (Figure 1). SEM indicated that the prepared microspheres are spherical with smooth surface; distinct pores are evident on the surface of microspheres, which will be responsible for the release. The photomicrographs also showed presence of loose crystals of drug on the surface of few microspheres.

Angle of repose, Hausners ratio, and Carr index were determined to predict flow ability. A higher Hausners ratio indicates greater cohesion between particles while a high Carr index is indicative of the tendency to form bridges. It would be expected that the viscosity of the polymer mixture would increase as polymer concentration rose, resulting in enhanced interfacial tension and hence, formation of larger particles. Angle of repose for cellulose microspheres was between 12.46±0.062-19.45±0.074º, thus indicating good flow property of microspheres. The percentage yield of floating microspheres was found to be in between range of 50.34±0.061-81.69±0.081. Percentage incorporation efficiency was in the range of 74.52±0.014-81.21±0.051. A large proportion of the microspheres remained floating after 12 hrs. Floatation might have been influenced by the low bulk and tapped densities of the microspheres. Average buoyancy in percentage was found to be 70.23±0.145-90.63±0.084%.In-vitro release study shows maximum release for formulation E4, 68.618 %, and minimum for formulation C3 i.e. 33.466 % during the studies of 24 hrs. To ascertain the drug release mechanism and release rate, data of the above formulations were model fitted using PCP Disso V3.0 dissolution software. The models selected were Zero order, Higuchi Matrix, Korsemayer Peppas. The regression coefficient values for all these models are shown in Table 4. In all the cases the best fit model was found to be Peppas with 'n' value between 0.65 to 0.73 suggesting the non fickian (anomalous) release mechanism for the drug i.e., erosion followed by diffusion controlled[26]. Local injection of carrageenan into rat hind paw induces acute inflammatory responses such as edema[27]. The development of the edema induced by carrageenan has been described as a biphasic event. A rapid early phase (up to 2 h) is triggered by the concerted release of histamine, bradykinin, 5-hydroxytryptamine or cyclooxygenase products. And a more sustained late phase (2 to 5 hr) is regulated by neutrophil infiltration and sustained production of arachidonic metabolites (prostanoids) (primarily by cyclooxygenase) or nitric oxide from inducible nitric oxide synthase[28].

Standard indomethacin shows 34.861% mean paw volume percentage inhibition, while C4, E1 and oral curcumin shows 7.462 %, 7.0182 % and 4.5038% respectively (table 5).Mean percentage inhibition of paw thickness by indomethacin was found to be 19.914%, while C4, E1 and oral curcumin shows 6.771%, 6.333% and 4.662% respectively (table 6). Adjuvant-induced arthritis in rats is a well established experimental model that has features similar to the human rheumatoid arthritis. In addition, it is a good chronic inflammatory model for development of potential analgesic and/or anti-inflammatory drugs useful for arthritis treatment. Adjuvant arthritis is characterized by chronic proliferative and inflammatory reactions in synovial membranes, producing pain, disability and eventually destruction of joints[29]. Floating microsphere C4 and E1 shows significant prevention of paw edema, as compared to standard indomethacin 10 mg/kg (table 7 and 8). Radiographic examination of curcumin and CFA injected hind paws of the arthritic control rats on 20 day revealed the severe soft tissue swelling, narrowing of the joint spaces and the subsequent destruction of the bones and cartilages in the knee joint as compared to the normal control. The treatment with floating microsphere C4 and E1 and indomethacin 10 mg/kg (Figure 5) was shown marked reduction of the histological injury of joint tissue sections and most of the histological changes were minimized and found negligible as compared to the arthritis control

CONCLUSION

The use of traditional medicine is expanding to newer horizons and plants still remain as the novel source of structurally important compounds that lead to the development of innovative drugs. In present study Curcumin Floating Microspheres were prepared successfully using emulsion solvent diffusion method. The formulation was found to be efficient with good recovery yield, percentage drug entrapment. The flow properties of all formulations were within the acceptable range and therefore they could be easily filled into capsules dosage form. Floating microsphere C4 and E1 used in this study showed significant reduction of paw edema thickness and volume at 8 hrs or more after carrageenan injection, demonstrated that the proniosomal gel possess fairly good anti-inflammatory activity. Floating microsphere C4 and E1 shows sufficient anti-arthritic activity in CFA induced model for chronic level studies of 20 days. Anti-inflammatory and anti-arthritic activity of both formulations was similarly to indomethacin but not as good as commercial product. All formulation i.e. standard market product indomethacin and floating microsphere C4 and E1 showed potent anti-inflammatory and anti-arthritic activity and the potency of the treatment follows the order

Standard>C4>E1

However, future studies with inclusion of penetration enhancer in floating microsphere formulation may provide herbal formulation comparable to topical NSAIDs. It may be concluded that Curcumin microspheres would be promising drug delivery system for oral administration of Curcumin to sustained the drug release and there is great potential in

development of novel drug delivery system for valuable herbal drugs.

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Table 1: Composition of formulations

Batch code	Curcumin (mg)	Eudragit S100 (mg)	HPMC (mg)	EC (mg)	PVP K30 (mg)	Tween 80 (mg)	Di-chloromethane :ethanol ::1:1 (10 ml)	Heavy Liquid Paraffin (ml)
C1	250	250	-	-	-	-	-	50
C2	250	500	-	-	-	-	-	50
C3	250	750	250	-	-	-	-	50
C4	250	-	250	-	-	-	-	50
E1	250	-	-	-	250	3	10	-
E2	250	-	-	-	500	3	10	-
E3	250	-	-	250	750	-	10	-
E4	250	-	-	250	250	-	10	-

Table 2: Characterization of Floating Microspheres

Batch Code	Particle Size (µ m)	Bulk Density (g/cm3)	Tapped Density (g/cm3)	Hausner,s ratio	Carr Index
C1	241.51±1.34	0.146±0.02	0.215±0.05	1.472±0.0012	32.09±0.005
C2	238.23±1.24	0.139±0.04	0.210±0.08	1.510±0.003	33.80±0.002
C3	227.12±2.34	0.156±0.06	0.198±0.15	1.269±0.003	21.21±0.004
C4	231.37±2.17	0.147±0.07	0.197±0.09	1.340±0.006	25.38±0.001
E1	290.45±1.90	0.164±0.03	0.205±0.04	1.250±0.001	20±0.001
E2	287.58±2.65	0.170±0.07	0.194±0.07	1.141±0.006	12.37±0.002
E3	283.23±2.59	0.167±0.08	0.211±0.05	1.263±0.007	20.85±0.002
E4	294.64±1.82	0.158±0.06	0.204±0.07	1.291±0.005	22.54±0.001

N = 6

Table 3: Evaluation parameters of curcumin floating microspheres

Batch Code	Sphericity	Yield (%)	Drug Entrapment Efficiency (%)	Angle of Repose θ	Buoyancy %
C1	1.01±0.03	50.34±0.061	74.52±0.014	14.67±0.054	70.23±0.145
C2	1.03±0.05	56.41±0.085	78.64±0.021	13.89±0.102	71.46±0.93
C3	1.12±0.1	55.46±0.102	80.37±0.018	12.46±0.062	70.84±0.043
C4	1.08±0.09	60.23±0.043	79.37±0.019	13.78±0.101	74.21±0.085
E1	1.06±0.07	80.63±0.063	78.32±0.059	16.32±0.421	84.57±0.021
E2	1.04±0.08	79.46±0.141	80.56±0.062	17.43±0.081	86.76±0.058
E3	1.02±0.3	80.73±0.127	79.42±0.018	17.28±0.056	89.43±0.091
E4	1.09±0.04	81.69±0.081	81.21±0.051	19.45±0.074	90.63±0.084

N = 6

Table 4: Data showing in-vitro drug release model fitting kinetic parameters of formulations

Batch Code	Cumulative % drug released vs. time (zero order)		Cumulative % drug released vs. square root of time (Higuchi,s)		Log cumulative % drug retained vs. time (Kosermeyer,s peppas)			First order	
	R	K	R	K	Slope (n)	R	K	R	K
C1	0.9151	2.3790	0.9416	10.2571	0.6184	0.9967	7.1554	0.9649	-0.0309
C2	0.9596	1.9203	0.9543	10.3641	0.7018	0.9978	4.5343	0.9847	-0.0235
C3	0.9834	1.5841	0.9632	9.8363	0.8415	0.9960	2.5089	0.9928	-0.0187
C4	0.8747	2.8715	0.9715	9.4781	0.5407	0.9976	10.7404	0.9620	-0.0400
E1	0.9251	2.6492	0.9364	10.4172	0.6236	0.9981	7.8471	0.9800	-0.0358
E2	0.9540	2.0293	0.9644	8.6148	0.7077	0.9977	4.7378	0.9823	-0.0252
E3	0.9712	1.6714	0.9537	10.5321	0.8265	0.9957	2.8006	0.9864	-0.0199
E4	0.8848	3.3750	0.9820	9.8362	0.5721	0.9954	11.5969	0.9790	-0.0514

Table: 5. % Inhibition on carrageenan induced paw edema volume by different treatment

Treatment	Percent inhibition of paw edema volume					Mean of % Inhibition
	1 hr	2hr	4hr	6hr	8hr	
Standard	19.871±0.26	31.395±0.37	41.72±0.42	43.414±0.58	37.837±0.83	34.861
Floating microsphere C4	3.104±0.41	4.147±0.52	8.219±0.39	10.512±0.52	11.328±0.17	7.462
Floating microsphere E1	3.251±0.39	4.371±0.49	7.52±0.41	9.428±0.39	10.521±0.38	7.0182
Curcumin	2.537±0.28	3.372±0.19	4.160±0.26	5.258±0.31	7.192±0.73	4.5038

N = 6, P < 0.05

Table: 6. % Inhibition on carrageenan induced paw thickness by different treatment

Treatment	Percent inhibition of paw thickness					Mean of % Inhibition
	1 hr	2hr	4hr	6hr	8hr	
Standard	12.087±0.47	17.894±0.69	20.618±0.13	23.232±0.28	25.742±0.37	19.914
Floating microsphere C4	3.136±0.48	6.215±0.58	7.241±0.39	8.130±0.58	9.135±0.63	6.771
Floating microsphere E1	3.528±0.41	4.732±0.53	6.821±0.68	7.643±0.31	8.935±0.52	6.333
Curcumin	2.349±0.52	3.572±0.47	5.218±0.52	5.559±0.77	6.614±0.28	4.662

N = 6, P < 0.05

Table: 7. % Inhibition on CFA induced paw edema volume by different treatment

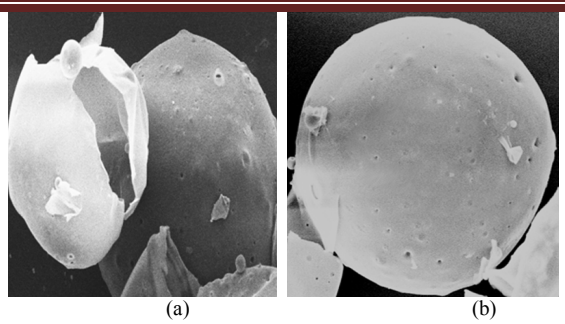
Treatment	Percent inhibition of paw edema volume				Mean of % Inhibition
	5 Day	10 Day	15 Day	20 Day	
Standard	30±0.37	64±0.68	80±0.33	98±0.17	68
Floating microsphere C4	8±0.49	12±0.29	20±0.36	26±0.92	16.5
Floating microsphere E1	7±0.37	10±0.28	17±0.15	25±0.26	14.75
Curcumin	4±0.39	7±0.47	9±0.58	12±0.71	8

N = 6, P < 0.05

Table: 8. % Inhibition on CFA induced paw edema thickness by different treatment

Treatment	Percent inhibition of paw thickness				Mean of % Inhibition
	5 Day	10 Day	15 Day	20 Day	
Standard	42±0.72	58±0.94	75±0.86	92±0.33	66.75
Floating microsphere C4	6±0.18	11±0.26	24±0.39	30±0.44	17.75
Floating microsphere E1	5±0.27	10±0.41	20±0.52	28±0.38	15.75
Curcumin	5±0.58	8±0.29	12±0.40	25±0.66	12.5

N = 6, P < 0.05



(a) (b)

Figure 1: SEM images of floating microspheres (a) batch C3 and (b) BatchE1

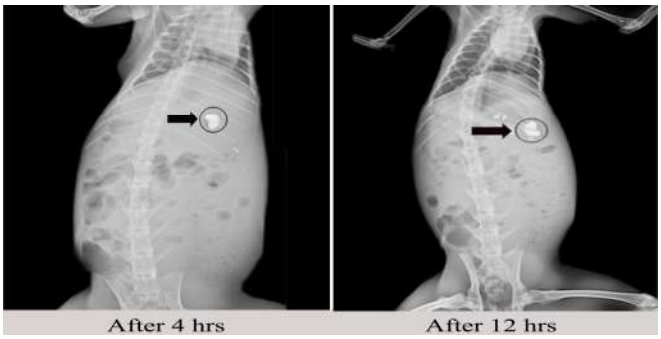


Figure: 2. X-ray photographs showing in-vitro buoyancy of floating microsphere C4 at different time.

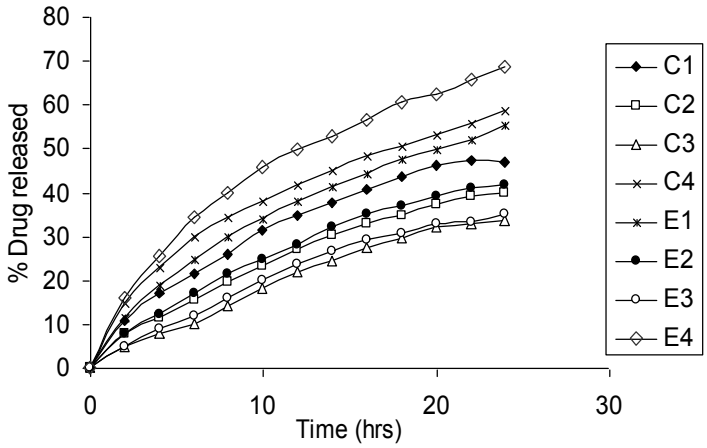
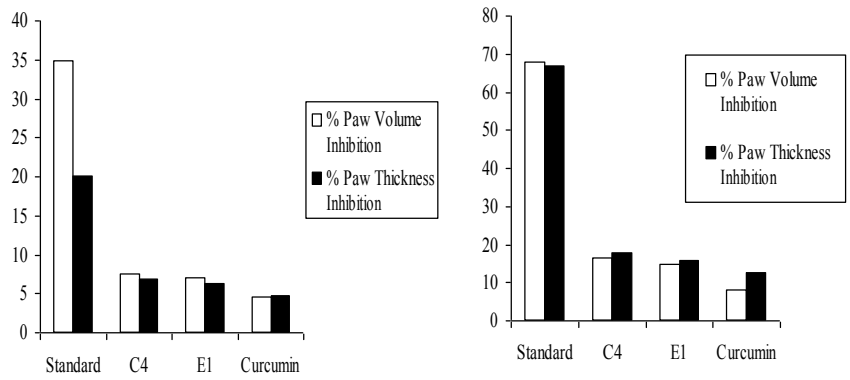


Figure 3: Drug release profiles of Curcumin from floating microspheres



(a) (b)

Figure 4: Mean % edema inhibition by different treatment induced by (a) carrageenan and (b) CFA

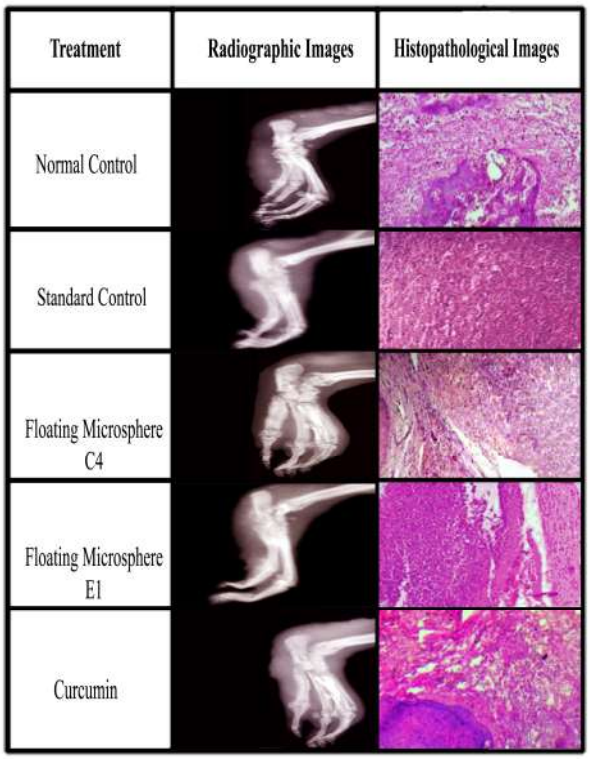


Figure: 5.Effects of different treatment on radiographic evaluation and histopathological changes in CFA induced arthritis in rats

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