AMERICAN JOURNAL OF PHARMTECH RESEARCH

Development and Evaluation of Indomethacin Parenteral Delivery of Microspheres for the Treatment of Gout

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ABSTRACT

Gout is a disease caused by the deposition of monosodium urate (MSU) crystals in tissue such as cartilage, synovial membranes, bones and skin which causes inflammation in the synovial tissue. Indomethacin is first line of drug used as NSAID for the treatment of Gout. The aim of this study was to encapsulate Indomethacin in ethyl cellulose microspheres and compare the efficiency of the formulated Indomethacin microspheres with the Marketed formulation. Indomethacin microspheres were prepared by solvent evaporation method. FTIR studies revealed there was no significant interaction between the drug and polymer. Preformulation studies gave satisfactory results. SEM studies showed a spherical smooth microsphere average size of 10.4±3.04. The percentage entrapment efficiency and percentage drug release after 10 hours was found to be 82.97±1.6 % and 52.04±0.58 % respectively. The therapeutic effect of the Indomethacin microspheres was evaluated by the swelling of knee joints, joint range of motion and histologic analysis of MSU induced rat model. The prepared indomethacin microspheres showed effective prolong in the retention time of the drug in the intra articular cavity to 30 d which is more than that of the marketed formulation. Intra- articular injection of Indomethacin microspheres efficiently relieved inflammatory symptoms such as swelling index, joint range motion and suppressed inflammatory cell infiltration than the marketed formulation. Thus intra-articular injection of Indomethacin loaded microspheres proved to be a promising therapeutic method in the treatment of Gout.

Keywords: Gout, indomethacin, ethyl cellulose, microspheres, inta-articular

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Received 10 July 2021, Accepted 9 August 2021

Please cite this article as: Tonse R *et al*., Development and Evaluation of Indomethacin Parenteral Delivery of Microspheres for the Treatment of Gout. American Journal of PharmTech Research 2021.

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and then maintain the desired drug concentration therapy and enhance the therapeutic efficacy of a given drug.

Gout is a disease caused by the deposition of monosodium urate crystals in tissues such as cartilage, synovial membranes, bones and skin. In musculoskeletal system, gout affects both articular and extra-articular structures which results in arthritis, tendinitis and brusitis[1]. Gout can be diagnosed by measurement of joint-swelling, joint range motion and further histologic examination. NSAIDs are the first-line drugs for the treatment of gout. Indomethacin has been the preferred choice as NSAID. The conventional dosage forms of indomethacin causes adverse effects such as gastro-intestinal upsets, nausea and vomiting[2].

Microspheres are small spherical particles with diameters 1µm to 100µm. they are spherical free flowing particles consisting of proteins or synthetic polymers which are biodegradable in nature[3]. Microspheres can be manufactured from various natural and synthetic materials. Microspheres play an important role in improving bioavailability of conventional drugs and minimizing side effects. Parenteral administration of microspheres provides sustained release of drug with less dosing frequency[4]. The present investigation is aimed at microsphere formulation of indomethacin through parenteral controlled delivery of drug in the treatment of gout and compare the efficacy with conventional therapy[5].

MATERIALS AND METHOD

Indomethacin was procured from Yarrow chemicals, Mumbai. Ethyl cellulose and acetone were procured from Oxford lab fine chem Ltd, Mumbai. Liquid paraffin was obtained from nice chemicals Ltd, Bangalore. Petroleum ether was procured from Medilise chemicals, Kannur. Sodium chloride, potassium dihyrogen orthophosphate and sodium hydroxide were procured from Molychem, Mumbai. Potassium chloride and sodium dihydrogen orthophosphate were procured from SD fine chemicals Ltd, Mumbai. MSU crystals were procured from Sigma Ltd, Mumbai.

Formulation of indomethacin microspheres[6]:

Microspheres containing Indomethacin as a core material were prepared by non-aqueous solvent evaporation method. Weighed amount of indomethacin and ethyl cellulose were mixed in acetone at various ratios. The slurry was slowly introduced into a beaker containing 30 ml of liquid paraffin being stirred at 1200 rpm with the help of a mechanical stirrer at room temperature. The solution was continuously stirred for up to 2 hours to allow the solvent to evaporate completely. The microspheres were filtered, collected and washed repeatedly with petroleum ether until free

from oil and then dried for 1 hour at room temperature and stored in desiccators over fused calcium chloride.

Table 1: Different ratios for preparation of the formulations

Sl. no	Formulation code	Indomethacin Concentration(%)	Ethyl cellulose concentration (%)	Stirring speed(rpm)
1.	F1	1	1	1200
2.	F2	1	2	1200
3.	F3	1	3	1200
4.	F4	1	4	1200

Percentage yield[7]:

The percentage yield of all formulations were determined by weighing the microspheres after drying.

Particle size analysis[8]:

Particle sizes of all the formulations were determined by optical microscopy with the help of ocular and stage micrometer. Sizes of around 100 particles were measured, and their average particle size was determined. The mean particle size of all formulations was determined by using the Edmondson's equation.

Bulk density[9]:

The prepared microspheres were weighed and introduced into a graduated measuring cylinder of 10 ml capacity. The volume of the sample was taken and bulk density was calculated.

Tapped density[9]:

The prepared microspheres were weighed and introduced into a graduated measuring cylinder of 10 ml capacity. The initial volume was noted and the cylinder was allowed to fall on to hard surface from a height of 2.5 cm at 2 seconds intervals. Tapping was continued until no further change in volume was noted.

Carr's compressibility index[9]:

The compressibility index was calculated.

Hausner's ratio[9]:

Hausner's ratio of microspheres was determined by comparing the tapped density to the bulk density.

Angle of repose[9]:

It was determined fixed funnel method whose tip was fixed at a constant height (h) of 2cm from the horizontal surface. The microspheres were allowed to freely pass through the funnel until the tip of the pile touches the tip of the funnel. The radius of the base of the pile was measured (r cm) and the angle of repose was determined.

Drug entrapment efficiency[10]:

Microspheres equivalent to 10 mg of Indomethacin were accurately weighed, triturated and digested in 10 ml simulated blood fluid (pH 7.4)and kept overnight for extraction of drug. The homogenate was centrifuged and supernatant was collected. After appropriate dilution of supernatant with same buffer solutions, aliquots were assayed by UV spectrophotometer at λmax 259 nm. Corresponding drug concentrations in the sample was calculated from the standard calibration curve. Efficiency of drug entrapment for each formulation was calculated in terms of percentage drug entrapment.

The theoretical drug content was determined by calculation assuming that that the entire drug present in the solution gets entrapped in microspheres and no loss occurs at any stage of preparation of microspheres.

***In-vitro* drug release studies[11]:**

In vitro drug release was carried out in phosphate buffer saline (PBS) medium (pH 7.4) containing 1% (v/v) sodium lauryl sulfate (SDS). Briefly, weighed amount of microspheres containing 25mg of indomethacin were suspended in 2 ml of PBS and placed in a dialysis bag (Molecular weight cut off: 12,000). The dialysis bag was placed in USP dissolution apparatus type I at a stirring speed of 100 rpm containing 750 ml of the release medium and at 37 °C in a constant temperature water bath. At predetermined time points, 5 ml of supernatant was withdrawn and an equal volume of fresh medium was added. The sample solution was filtered through Whatman's No.1 filter paper and analyzed to determine the amount of drug released using UV spectrophotometer at 259 nm wavelength.

Surface morphology[12]:

Scanning electron microscopy was used to study surface topography, texture and examine the morphology of fractured or sectioned surface of the floating microspheres. The formulations were mounted using a double-sided sticking tape and coated with gold (200 Ao) on the scanning electron microscopy (SEM) sample stab, under reduced pressure (0.001 torr) for 5min using ion sputtering device. The gold-coated samples were observed under the scanning electron microscopy and photomicrographs of suitable magnification were obtained.

Formulation of parenteral microspheres of indomethacin for animal administration[13]:

The prepared indomethacin microspheres (equivalent to 25mg) were dispersed in sterile phosphate buffered saline (PBS) in the form of suspension. Before the injection the suspensions was vortexed vigorously before administration.

Animal selection[13]:

The female Wister rats (220–240 g) were used for the experiment. All animals were maintained on a 12 h day-night cycle and the temperature and humidity were kept at 22– 24 ˚C and 50% RH respectively, with freely available food and water. All possible efforts were made to minimize animal suffering and reduce the number of animals used according to the recommendation of the local and national ethic committees and no animal died during the experiment.

MSU preparation[13]:

MSU crystals were sterilized by heating at 180 ^{0}C for 2 h before experiments. Rats were anesthetized with intra-peritoneal injection at a dose of urethane (1.0 g/kg), and MSU crystals (20mg/mL) were injected into the synovial space of both sides of the knee joint in a volume of 50 uL sterile phosphate buffered saline (PBS) before the injection the suspensions was vortexed vigorously.

Gout animal model[13]:

Rats were allocated randomly to 4 groups, each comprising of two rats. Group I served as a control group treated by vehicle and received an intra-articular (i.a) injection of sterilized PBS in the knee. In group II (Model group), gouty arthritis was induced by MSU crystal and treated by vehicle. Group III MSU crystal-induced rats were treated i.a with Indomethacin microspheres (3mg/kg). Group IV is comprised of MSU crystal induced rats which were treated with marketed formulation of indomethacin administered orally. The freshly prepared parenteral microspheres of Indomethacin were administered i.a at knee joint once a week and marketed formulation were administered orally once a day for 8 days. Before administration, the suspensions were vortexed vigorously. The development of arthritis was assessed by measuring the size of the joint using vernier caliper at 0 h (baseline, before the i.a injection), 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h after the MSU crystal injection. At 24 hours after the induction of gout, the rats were sacrificed by overdose of anesthesia and synovial tissue was gently separated for histopathology examination.

Evaluation parameters of parenteral microspheres containing indomethacin:

Biocompatibility studies of microspheres[14]:

Three female Wister rats were randomly assigned to control group, blank microspheres group and Indomethacin microspheres group. 0.1 ml of normal saline, blank microspheres and Indomethacin microspheres were injected into the right joint of rats, respectively then the outside diameter of the knee joint was measured with a caliper at 1 d, 1 week, and 4 weeks after the injection. The swelling of the knee joint was evaluated by the difference between the knee diameters at different time points after administration and the diameter of the knee before the administration.

In vivo studies:

Measurement of joint swelling[14]:

The swelling of the knee joint was evaluated by the difference between the knee diameter at different time points after administration and the diameter of the knee before the induction of Gout. The outer diameter of the knee was measured with a caliper before induction of Gout on the 1st, 5th, 10th, 20th and 30th d after administration.

Joint range of motion[14]:

Joint range of motion refers to the maximum curvature that can be achieved during joint movement. The joint range of motion was measured with a protractor before and after induction of Gout and at prescribed time points after drug administration.

Histopathology study[14]:

All the rats were sacrificed at 6 weeks after intra-articular administration and the knee joints were removed and fixed with 10% buffered formalin for 7 d, decalcified in 5% (w/v) nitric acid for 3 h and dehydrated. They were then embedded in paraffin and cut into 5 m-thick serial sections. Sections were stained with hematoxylin and eosin (H&E) to observe the general morphology and stained with safranin O & fast green for proteoglycan analysis. The synovial inflammation was evaluated by the scoring of synovial cell proliferation, hypertrophy, inflammatory cell proliferation, infiltration and granulation tissue hyperplasia.

Stability study[15]:

Stability study was carried out as per ICH Guidelines at 25±2ºC/60±5% RH and 40±2ºC/75±5% RH for the optimized formulations of transdermal patches for appearance, percentage drug content, tensile strength and drug release.

RESULTS AND DISCUSSIONS

In the present study, an attempt was made to formulate indomethacin microspheres for parenteral delivery in the treatment of Gout. Formulations were subjected to various parameters such as percentage yield, particle size, tapped density, Hausner's ratio, Carr's index, angle of repose, entrapment efficiency, *in vitro* drug release, *in vivo* animal study in rat model. Stability studies were performed as per ICH-Guidelines. The FTIR spectra of physical mixture were compared with the FTIR of pure drug and optimized formulation F4 and shown in Fig 2, 3 and 4. FTIR spectra of Indomethacin and Ethyl cellulose mixture and optimized formulation also showed identical peaks which indicated that there was no interaction between drug and polymer as depicted in Table 2.

Table 2: Comparison of FT-IR spectra of Indomethacin, Ethyl Cellulose and Best formulation (F4)

Sr. No	Functional Groups	Reported Frequency(cm-1)	Observed Frequency (cm-1)		
			Drug	Drug +Ethyl Cellulose	Best Formulation(F4)
1.	C-Cl	850-550	655.80,831.32	651.94, 837.11	657.73, 835.85
2.	C-O Stretch	1300-900	917.69	916.19	918.12
3.	O-CH3	1470-1350	1359.82	1361.74	1365.39
4.	Aromatic C=C Stretch	1600, 1475	1477.47	1474.65	1478.56
5.	C=O Stretch	1830-1800, 1775-1740	1759.080	1759.080	1759.080
6.	Aromatic C-H Stretch,-COOH (s)	3400-2500	2922.16	2927.31	2920.30

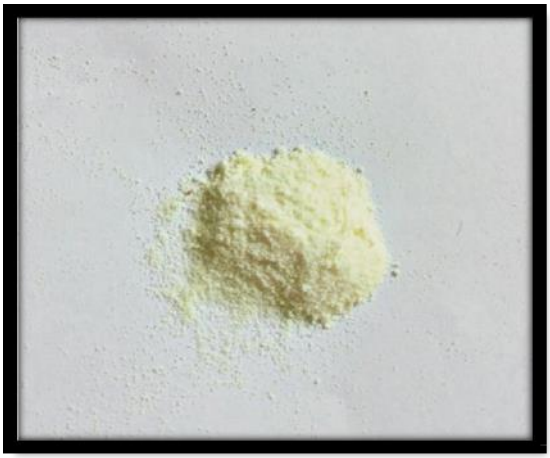

Figure 1: Indomethacin microspheres

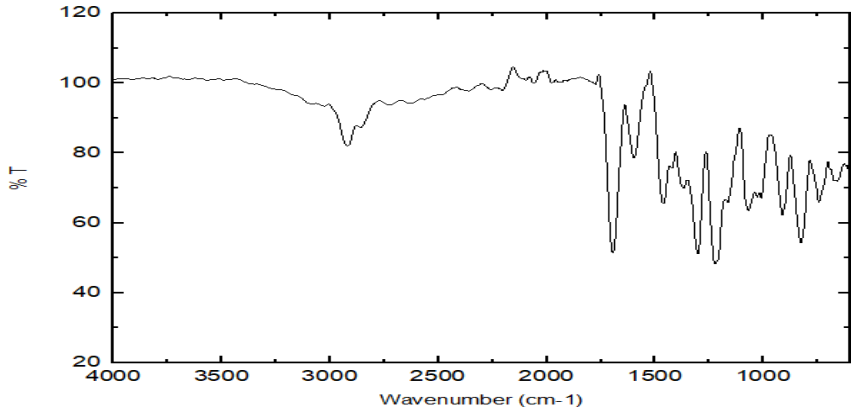

Figure 2: FTIR of indomethacin

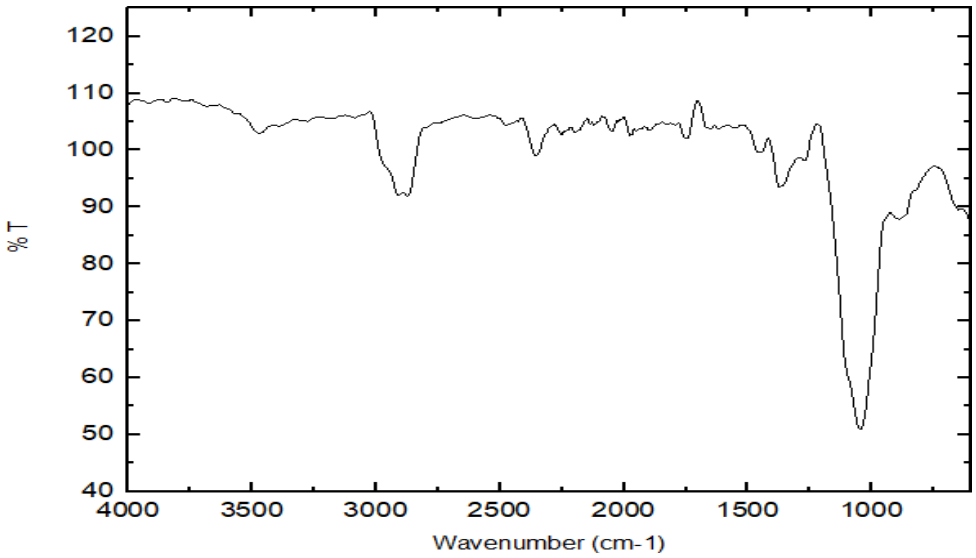

Figure 3: FTIR of indomethacin and ethyl cellulose

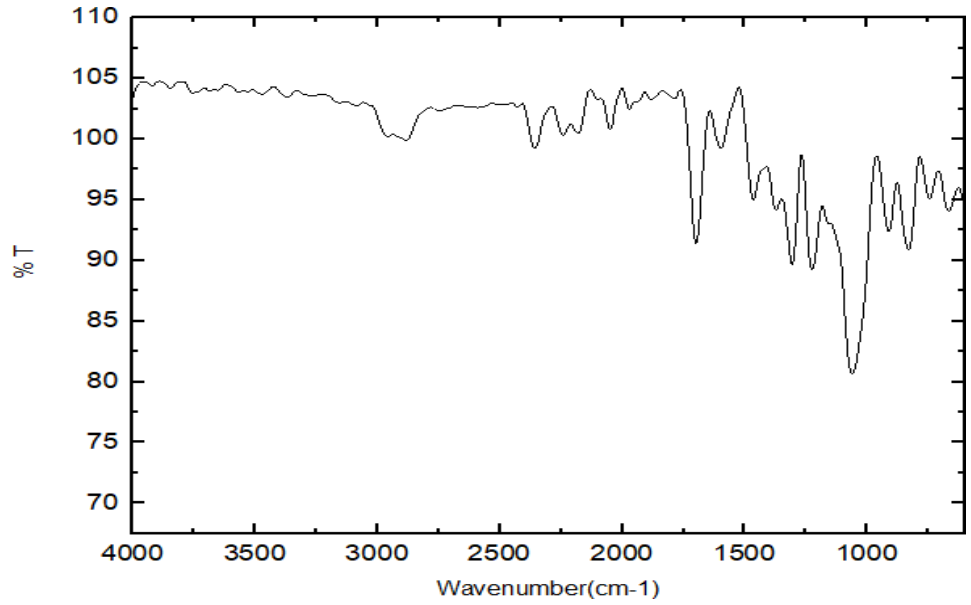

Figure 4: FTIR of best formulation F4

The effect of ethyl cellulose concentration was determined. The percentage yield increased from 42.6 % to 87.6 % with the increase in ethyl cellulose concentration at stirring speed 1200 rpm. As more amount of polymer is available, therefore increase in ethyl cellulose concentration increased the percentage yield. The particle size increased from 5.7±2.6 to 10.4±3.04 µm with increase in concentration of ethyl cellulose at 1200 rpm indicating that as the concentration of ethyl cellulose increases, the particle size increases. Bulk density, Tapped density, Hausner's ratio of formulation F1 to F4 ranges from 0.65±0.84 to 0.62±1.23, 0.66±0.12 to 0.68±0.20, 1.01±0.15 to 1.09±0.14 respectively. The Carr's compressibility index ranges between 1.51±0.45 to 8.82±0.58. The Carr's index and angle of repose values indicated excellent flow properties of microspheres. All the

formulations showed good percentage entrapment efficiency with maximum up to 82.97±1.6%. The percentage entrapment efficiency increased from 57.54±1.1 to 82.97±1.6% with increase in concentration of ethyl cellulose at 1200 rpm indicating that as the concentration of ethyl cellulose increases, percentage entrapment efficiency also increases. The details of percentage yield, particle size, tapped density, Hausner's ratio, Carr's index, angle of repose and entrapment efficiency are shown in Table 3.

Table 3: Evaluation parameters - percentage yield, particle size, tapped density, Hausner's ratio, Carr's index, angle of repose and entrapment efficiency for the formulation batches (F1-F4)

Batch code	% yield	Particle size (µm)	Bulk density	Tapped density	Hausner's ratio	Carr's index	Angle of repose	Entrapment Efficiency (%)
F1	42.6±0.28	5.7±2.6	0.65±0.84	0.66±0.12	1.01±0.15	1.51±0.45	30.52±0.25	57.54±1.1
F2	71.2±0.36	9.3±2.5	0.72±1.54	0.74±0.36	1.02±0.61	2.70±1.25	24.60±0.36	75.69±1.4
F3	78.9±0.45	9.8±4.9	0.56±1.26	0.60±0.14	1.07±0.23	6.66±0.83	20.96±0.86	64.57±1.0
F4	87.6±0.24	10.4±3.04	0.62±1.23	0.68±0.20	1.09±0.14	8.82±0.58	19.42±0.15	82.97±1.6

It was observed that as the concentration of ethyl cellulose was increased, the percentage drug release decreased. The decrease in drug release may be attributed to the increased diffusional path length due to the formation of high density polymer matrix as the concentration of ethyl cellulose is increased. At lower concentration of ethyl cellulose, smaller microspheres were formed which have larger surface area exposed to the dissolution medium giving rise to faster drug release. The details of *in vitro* drug release are shown in Table 4 and depicted graphically in Figure 5.

Table 4: Percentage drug release of formulation batches (F1-F4)

Time(hours)	F1	F2	F3	F4
0	0	0	0	0
1	6.54	10.2	9.98	10.2
2	13.89	11.54	15.6	14.65
3	19.54	21.3	22.5	17.89
4	27.45	26.4	30.4	21.45
5	39.19	35.48	37.64	28.62
6	45.25	42.46	45.61	34.15
7	58.95	51.95	52.64	39.52
8	65.48	58.45	58.45	45.84
9	70.76	62.15	62.54	48.65
10	78.49	72.62	67.38	52.04

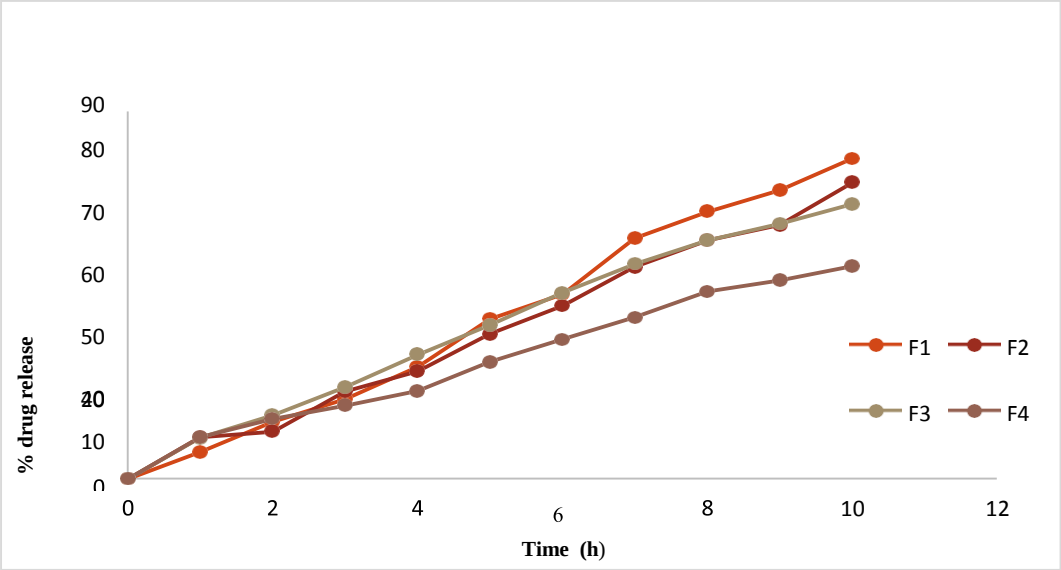

Figure 5: Percentage drug release of formulations (F1-F4)

The morphology of the best microspheres was examined using scanning electron microscopy. The view of the microspheres showed a spherical structure with a smooth surface morphology and within batches exhibited a range of sizes of microspheres. The outer surface of the optimized microspheres was smooth as shown in Figure 6.

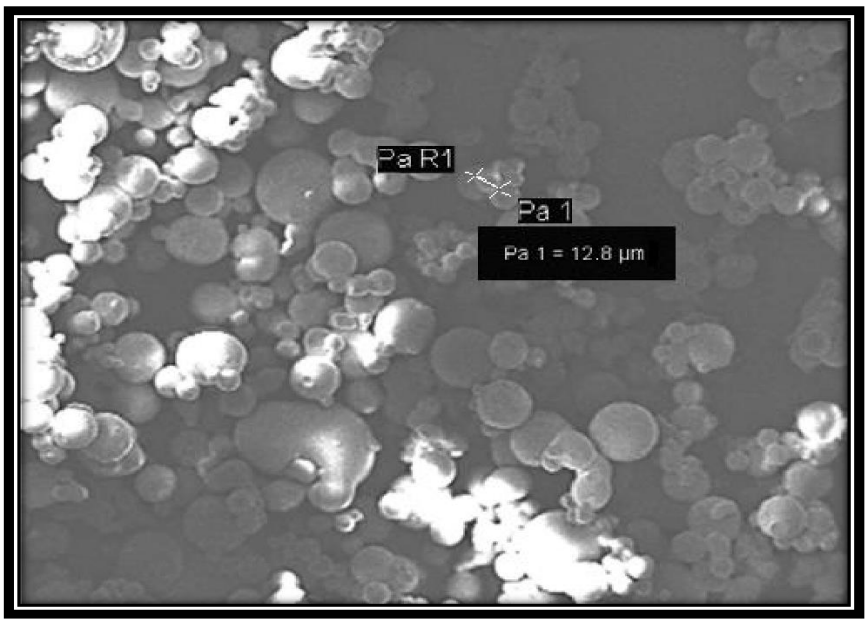

Figure 6: Scanning electron microscopy of best formulation (F4)

The biocompatibility of blank microspheres in rat knee joint was evaluated primarily by histologic examination. Intra-articular injection of saline, blank microspheres and Indomethacin microspheres did not produce inflammatory symptoms. In addition, no joint swelling was observed in the gross morphology observation. These results indicated that blank microspheres injected directly into the joint cavity are biocompatible. The resulting injury may be caused by injection,

since only a minimal and local mild inflammation occurred and it could be healed within a certain period of time.

The progress of gout is often accompanied with joint pain, swelling, stiffness and activity difficulties. The joint swelling and range of joint activity could be used as indicators to evaluate the effect of the drug for treating Gout. In the model group, the diameter of the knee joint after MSU induction was 55.15 ± 1.96 mm, which was significantly higher than that before induction (43.74 ± 3.69 mm) ($P < 0.05$), indicating that the MSU-induced Gout model was successfully established. As shown in Fig 7, the joint swelling of the treated group showed a downward trend, while the untreated model joint remained swollen. On the 10th d, the joint swelling in the marketed formulation treated group was significantly lower than that in the model group ($P < 0.01$). On the 20th d, the joint swelling of Indomethacin microspheres treated group was significantly decreased compared to the model group ($P < 0.05$). The slower onset time of Indomethacin microspheres may be related to its sustained release. Especially, under the condition of this administration regimen, the joint swelling both in the Indomethacin microspheres group and the marketed formulation group was significantly improved when compared to that in the model group on the 30th d after administration ($P < 0.01$), indicating that the single-dose microspheres possessed a long acting effect.

Before the induction of Gout, the joint range of motion was 158.57 ± 7.61°, and it was significantly reduced to 115.78 ± 4.56° after the induction of Gout ($P < 0.01$), indicating the successful establishment of the Gout model. The joint range of motion of the Marketed formulation group was significantly higher than that of the model group on the 5th d after administration, and on the 10^{th} d, the joint range of motion of the Indomethacin microsphere group began to improve significantly ($P < 0.05$). After treated with Indomethacin microspheres and Marketed formulation, the joint range of motion remained significantly improved on the 30th d ($P < 0.01$). The details of joint range of motion is shown in Figure. 6.

Synovial specimens were obtained from the inflamed knee joints in rats at 24 h after injection with MSU crystals. These specimens were then stained with hematoxylin and eosin. MSU crystal significantly increased leukocyte infiltration (primarily neutrophils) of the superficial synovium compared with that in control group. Co-treatment with Indomethacin microspheres and marketed formulation inhibited leukocyte infiltration as shown in Figure 9 (a), 9(b), 9(c) and 9(d). It was compared with control group, the number of leucocytes and neutrophils was significantly elevated in MSU model groups, and in the groups treated with Indomethacin microspheres and marketed formulation the number of leucocytes and neutrophils was significantly reduced

compared with model group ($P < 0.01$). The best formulation which was subjected to stability studies were analyzed for percentage entrapment efficiency and percentage drug release after 10 hours. The results as shown in Table 5 indicated that there were no significant differences found; this indicates that microspheres are fairly stable at storage condition.

Table 5: Characterization of F4 during stability study

Days		Drug Entrapment (%)	% Drug Release after 10hours
0		Before storage	
		60.00±1.6	52.04±0.58
30	At 30 ± 2 °C/	After storage	
	65 ± 5 % RH	59.16±1.3	52.65±0.12
	At 40 ± 2 °C/	58.65±2.6	52.98±0.16
	75 ± 5 % RH		
60	At 30 ± 2 °C/	58.25±4.2	53.18±0.56
	65 ± 5 % RH		
	At 40 ± 2 °C/	57.98±5.1	53.26±0.36
	75 ± 5 % RH		
90	At 30 ± 2 °C/	57.45±6.5	54.25±0.21
	65 ± 5 % RH		
	At 40 ± 2 °C/	57.12±2.5	54.67±0.13
	75 ± 5 % RH		

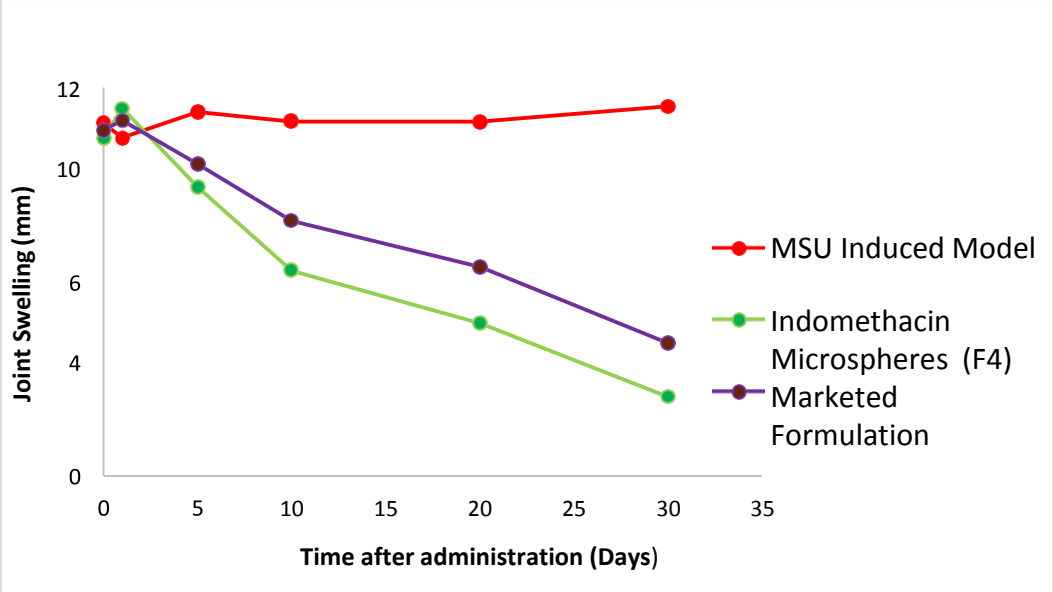

Figure 7: Mean joint swelling in MSU-induced gout rats at different time points after the treatment with Indomethacin microspheres (F4) and marketed formulation

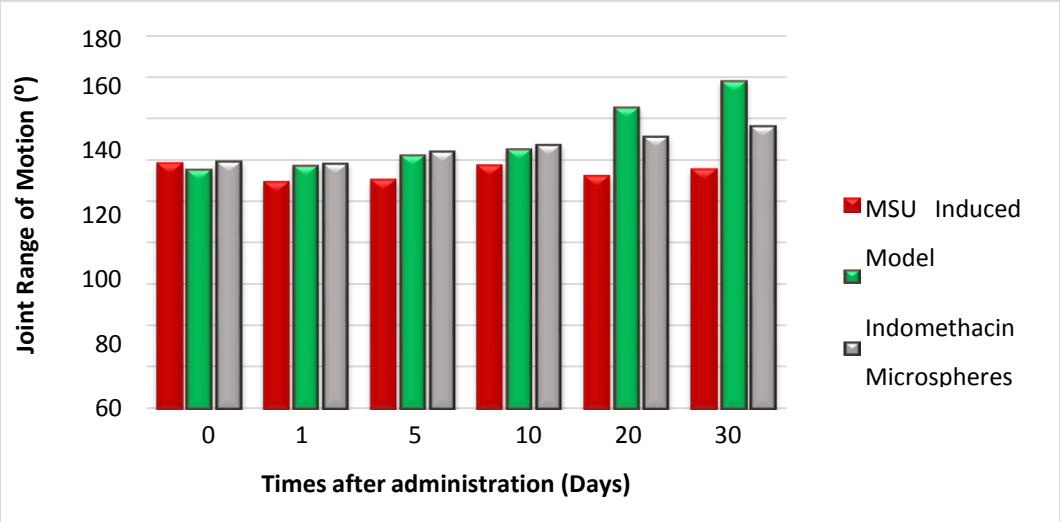

Figure 8: Mean joint range of motion in MSU-induced gout rats at different time points after the treatment with Indomethacin microspheres (F4) and marketed formulation

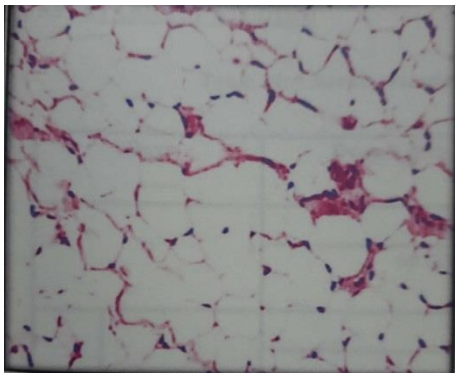

Figure 9.a

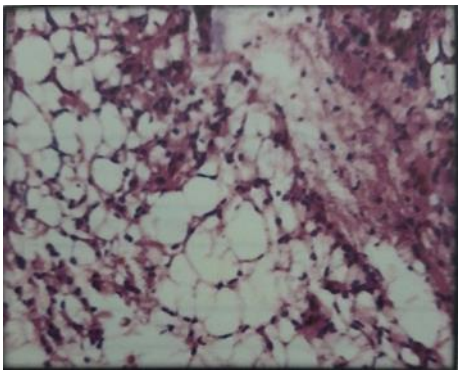

Figure 9.b

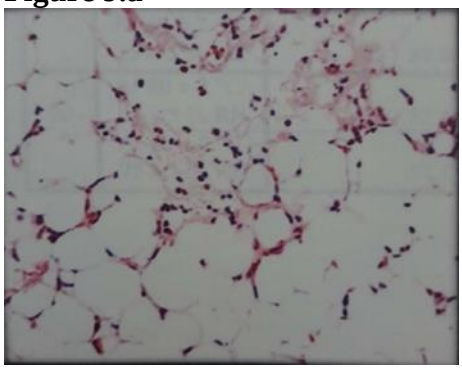

Figure 9.c

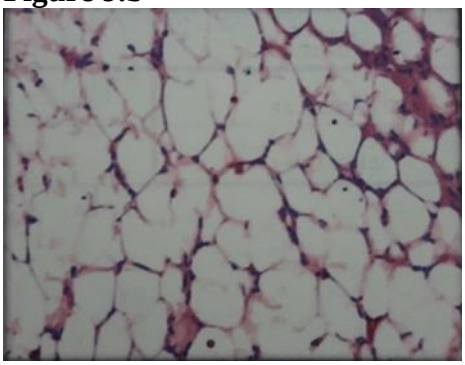

Figure 9.d

Figure 9: Histologic analysis of articular tissue (a) Control group, (b) MSU induced model, (c) Indomethacin microspheres (F4) and (d) Marketed formulation

CONCLUSION

Parenteral drug delivery system containing a suitable drug is a promising anti- inflammatory system for the treatment of Gout. The present study of parenteral Indomethacin microsphere was designed to increase its residence time in the knee joint and compare the efficiency with the conventional delivery system. The delivery system containing indomethacin proved to be an ideal

formulation as it released the drug in sustained fashion for extended period of time by maintaining drug release and retention and thereby creating a scope for improving the bioavailability and better absorption. It was observed that as the concentration of polymer increased, the entrapment efficiency as well as percentage yield increased and drug release decreased. The formulation with polymer concentration of 4% of ethyl cellulose showed better release profile and therefore can be considered as the best formulation. The Indomethacin microspheres in rats with MSU-induced gouty arthritis inhibited joint swelling and suppressed inflammatory cell infiltration. Indomethacin microspheres also showed excellent stability for a period of 3 months. Based on the results it can be concluded that the parenteral drug delivery system containing Indomethacin microspheres has the potential for treating Gout effectively.

ACKNOWLEDGEMENT

The research work was supported by Dr. V. Felix Joe, Professor, Shree Devi College of Pharmacy, Mangalore. The author would like to extend heartfelt gratitude towards Dr. J.V. Kamath, Principal, Shree Devi College of Pharmacy, Mangalore for providing necessary facility to carry out the work with ease and precision.

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