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FORMULATION AND DEVELOPMENT OF INDOMETHACIN POLYMERIC SUSTAIN RELEASE MICROSPONGES

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Abstract:

The goal of the present study was to develop and evaluate polymeric microsponges for sustained release of Indomethacin were prepared by quasi emulsion solvent diffusion method.Prepeared microsponge was studied for Effect of drug polymer ratio on active drug content, particle size and entrapment efficiency were studied. Drug polymer ratio greatly affects properties (entrapment efficiency, active drug content, particle size) of microsponges. Indomethacin microsponges showed highest actual drug content, entrapment efficiency and smaller particle size, so 3:1 ratio of drug and polymer was selected for optimization study. The microsponges were characterized by FTIR, DSC and SEM studies followed by determination of total drug content and entrapment efficiency. Optimization study was carried out by taking internal phase volume, stirring rate, emulsifier concentration as independent variables and their effects on entrapment efficiency, particle size were studied. Morphology of obtained micro sponges was revealed by scanning electron microscope and was found to be porous and spherical. Optimized formulation of microsponge was evaluated for drug content, pH, viscosity and in vitro drug release. Release of drug was found to be sustained through microsponge as compared to marketed product and pure drug. Drug deposition was found to be satisfactory. Prepared polymeric microsponges could be a potential for sustained release drug delivery system in pain & inflammatory therapy.

Keywords: *Microsponge, Optimization, Indomethacin, Quasi-emulsion solvent diffusion technique*

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INTRODUCTION:

Microsponges are extremely non-collapsible, cross-linked, porous, polymeric microspheres having particle size range from 5 to 300 µm that can entrap wide range of active ingredients that are mostly used for prolonged topical administration [1]. Microsponges are designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, reduce side effects, and modify drug release profiles. [2] Microsponges have unique dissolution and compression properties due to their sponge-like texture.[3] They are highly effective, stable, non-irritant, nontoxic, non-allergic, non-mutagenic and also minimum side effects with improved patient compliance [4]. Various polymers like Eudragit RS100, ethylcellulose,polystyrene, PHEMA, etc. have been utilized in forming microsponges. Further, these active microsponges can be incorporated into formulations, such as capsules, gel and powders, and share a broad package of benefits. [5,6]. The microsponges have demonstrated their use in cosmetics and pharmaceuticals viz. antifungal vaginal gel, in augmented arthritis therapy, as silver sulfadiazine-loaded microsponge gel for burn wounds, in gastroretentive delivery, as matrix tablet and in colon-specific drug delivery system, etc. [7-10] Indomethacin having widely used in treatment of Rheumatoid Arthritis, spondylitis, acute gout, dysmenorrhea, Osteoarthritis, arthritic gout, exertion headaches, fever and pain associated with malignant diseases. [11] Among the NSAID's, Indomethacin is the drug having short biological half-life (2 to 3 hours), degradation in the upper part of GIT and possess side effect like GI irritation. Also the usual dosage regimen is 25 to 100 mg, three times a day. From the study, it was evident that modified release dosage form of indomethacin was required to be formulated to minimize the side effects like GI irritation. Hence, in the present work an attempt was made to develop sustained release microsponges with use of synthetic polymer for their sustaining effect.[12] The present study was designed with the objective to enhance the dissolution and thus the release rate of the drug and bioadhesive potential of the preparation. The organic internal Inner phase was prepared by dissolving the polymer in ethanol under ultrasonication at 35 0C for 15 minutes. and Outer phase was prepared by dissolving PVA in distilled water and the process was carried out at room temperature. [13,14] The formulations of microsponge containing indomethacin were prepared by keeping the quantity of drug constant and decreasing the concentration of Eudragit RS 100 successively. Out of these, the batches having particle size within the range and spherical shape in appropriate manner were selected for the further studies. [14,15] the total drug content, production yield, mean particle size and entrapment efficiency were calculated.

MATERIALS AND METHODS:

Materials:

Indomethacin is obtained as gift samples from Themis Laboratories Ltd, Mumbai, Eudragit RS 100, and PVA, were obtained as gift samples from Wockhardt Research Centre (Aurangabad, India), all other ingredients used in this study were of analytical grade and purchased from Research Lab Fine Chemicals Ltd., Poona, India. All other chemicals were of reagent grades and used as procured.

Methods:

Preparation of indomethacin micro sponges:

Indomethacin microsponges were prepared by quasi emulsion solvent diffusion method.[16] Inner phase was prepared by dissolving the polymer in ethanol. Then the drug was added to solution and dissolved under ultrasonication at 35 ^{0}C for 15 minutes. Outer phase was prepared by dissolving PVA in distilled water and the process was carried out at room temperature. Then Inner phase was then poured into outer phase at room temperature. After emulsification, the mixture was continuously stirred at 500 rpm for two hours.

After the formation of microsponges the mixture is filtered to separate the microsponges. The product was washed and dried in oven at 40 ^{0}C till constant weight and stored in air tight container.[17] The formulations of microsponge containing indomethacin were prepared by keeping the quantity of drug constant and decreasing the concentration of Eudragit RS 100 successively. Initially primary batches were prepared in the ratio of 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1 and 11:1 (Table 1). Out of these, the batches having particle size within the range and spherical shape in appropriate manner were selected for the further studies.

Table No. 1: Preliminary batches of microsponges

Batch Code	Drug: Eudragit Ratio	PVA (mg)	Size and Shape	Avg. Particle Size (µm)
P1	1:1	50	Large, Irregular	467.36
P2	2:1	50	Average, spherical	332.14
P3	3:1	50	Small, spherical	158.45
P4	4:1	50	Small, spherical	126.58
P5	5:1	50	Small, spherical	95.48
P6	6:1	50	Small, Irregular	23.34
P7	7:1	50	Aggregated, Irregular	-

Optimization of Quasi-emulsion Solvent Diffusion Method:

From the obtained results of preliminary batches, microsponges prepared by drug polymer ratio 3:1, 4:1 and 5:1 gives small and spherical microsponges. For the optimization of quasi-emulsion solvent diffusion method and its process parameters one of the preliminary batch P3 (3:1) was selected and optimized.

Table No. 2: Effect of stirring speed (rpm) on indomethacin microsponges

Sr. No.	Batch	Stirring Speed (rpm)	Percentage Yield (%)	Particle Size (µm)	Particle Shape
1	3:1 ratio	250	78.80	84.42	Spherical
2		500	78.34	89.14	Spherical
3		750	71.58	87.32	Spherical

Table No. 3: Effect of solvent on indomethacin microsponges

Sr. No.	Batch	Amount of Solvent (ml)	Percentage Yield (%)	Particle Size (µm)	Particle Shape
1	3:1 ratio	5	77.48	83.57	Spherical
2		10	79.39	88.37	Spherical
3		15	78.16	82.18	Spherical

Table No. 4: Effect of propeller

Sr. No.	Batch	Type of Propeller	Percentage Yield (%)	Particle Size (µm)	Particle Shape
1	3:1 ratio	Two Blade	73.34	89.38	Spherical
2		Three Blade	79.58	90.37	Spherical

Table No. 5: Effect of stirring time

Sr. No.	Batch	Stirring Time (min)	Percentage Yield (%)	Particle Size (µm)	Particle Shape
1	3:1 ratio	30	-	-	-
2		60	76.47	90.34	Spherical
3		120	76.62	89.23	Spherical

Characterization of micro sponges:

Particle size analysis:

The diameters of 270-300 microsponges were measured by using stage micrometer from each batch (F1 to F15) and particle size was determined.

Micromeritic Properties of Micro sponges:

Angle of repose:

The angle of repose for the each formulation was determined by the funnel method. The microsponges were allowed to flow out of the funnel orifice on a plane paper kept on the horizontal surface. This forms a pile of microsponges on the paper.

Bulk density and Tapped density: Bulk density of all batches of microsponges was determined by pouring gently 2 gm of sample through a glass funnel into a 10 ml graduated cylinder. The volume occupied by the sample was recorded.

Compressibility index: The values of compressibility index are shown in Table 6. These were found in between 17.24 to 22.02 suggesting the acceptable range of particles and this was further supported by values of angle of repose.

Hausner's ratio: It was ranged from1.172 to 1.218, i.e. all the preparation showed that they had good flow properties.

Table 6. Micromeritic Properties of Microsponges:

Batch No.	Angle of Repose* (ɵ)	Bulk Density* (g/ml)	Tapped Density* (g/ml)	Carr's Compresibiliy Index* (%)	Hausner's Ratio*
F1	21.14±0.835	0.509±0.047	0.617±0.058	21.21±0.034	1.212±0.046
F2	20.74±0.742	0.513±0.038	0.623±0.047	22.02±0.045	1.214±0.035
F3	20.36±0.649	0.507±0.032	0.618±0.042	21.89±0.027	1.218±0.047
F4	21.32±0.567	0.513±0.043	0.619±0.053	20.66±0.037	1.206±0.037
F5	20.43±0.654	0.517±0.031	0.627±0.039	21.76±0.029	1.212±0.028
F6	19.87±0.638	0.494±0.039	0.591±0.061	19.87±0.031	1.196±0.048
F7	19.67±0.758	0.497±0.027	0.596±0.049	19.67±0.043	1.199±0.039
F8	18.93±0.498	0.489±0.049	0.591±0.069	18.93±0.049	1.208±0.049
F9	20.07±0.549	0.493±0.041	0.594±0.042	20.07±0.019	1.204±0.041
F10	20.13±0.535	0.499±0.029	0.603±0.051	20.13±0.021	1.208±0.033
F11	19.17±0.589	0.475±0.026	0.561±0.063	18.10±0.024	1.181±0.062
F12	19.63±0.749	0.479±0.033	0.564±0.037	17.74±0.033	1.177±0.057
F13	18.86±0.639	0.483±0.023	0.569±0.056	17.80±0.039	1.178±0.045
F14	18.63±0.793	0.481±0.028	0.566±0.038	17.67±0.042	1.179±0.027
F15	19.06±0.673	0.487±0.031	0.571±0.064	17.24±0.035	1.172±0.031

(* Represents mean ± S.D) (n =3)

Total drug content and entrapment efficiency:
The weight of microsponges equivalent to 40 mg of indomethacin was transferred to a 200 ml volumetric flask, to this 100 ml of a mixture of equal volumes of methanol and pH 7.5 phosphate buffer was added and sonicated until the contents were dispersed. The mixture was diluted with the methanol and pH 7.5 phosphate buffer mixture (1:1) to volume, mixed, and centrifuged. A portion of the clear solution was diluted quantitatively and stepwise if necessary, with the methanol and pH 7.5 phosphate buffer mixture (1:1) to obtain a solution containing about 25 µg of indomethacin per ml.Concomitantly determined the absorbances of this solution and a Standard solution of USP Indomethacin RS, in the methanol and pH 7.5 phosphate buffer mixture (1:1) having a known concentration of about 25 µg per mL in 1 cm cells at the wavelength of maximum absorbance at about 318 nm with a spectrophotometer, using the methanol and pH 7.5 phosphate buffer mixture as the blank. [18]

Table No. 7: Study of particle size, particle shape and drug content

Sr. no.	Total Days	Particle Shape	Avg. Particle Size	Drug Content
1	0	Spherical	54.28±13.4	74.41±0.02
2	15	Spherical	53.83±12.5	74.25±0.02
3	30	Spherical	53.73±13.2	74.37±0.03
4	45	Spherical	53.83±12.4	74.42±0.03
5	60	Spherical	53.71±12.3	74.26±0.02

Stability study:
The stability studies of Indomethacin microsponges were carried out in accelerated conditions as per ICH guidelines. The microsponge formulations were kept at 40 °C±2 °C and 70%±4% RH for 2 months. After 2 months, microsponges were analyzed for physical appearance, in vitro drug release and FTIR spectroscopy.

Evaluation and Characterization of Microsponges:
***In-vitro* study:**
The dissolution studies on microsponge formulations (equivalent to 40 mg of drug) were performed. Accurately weighted quantity of microsponges equivalent to 40 mg of indomethacin were taken in muslin cloth and was kept in basket. During dissolution study, 10 ml aliquot was withdrawn at different time intervals of 1, 2, 3---12 hrs and same was replaced with equal volume of fresh medium. The withdrawn sample was filtered through Whatman filter paper No.42 and absorbances were measured at 318nm (USP 30 NF 25, 2007). The experiment was performed in triplicate.

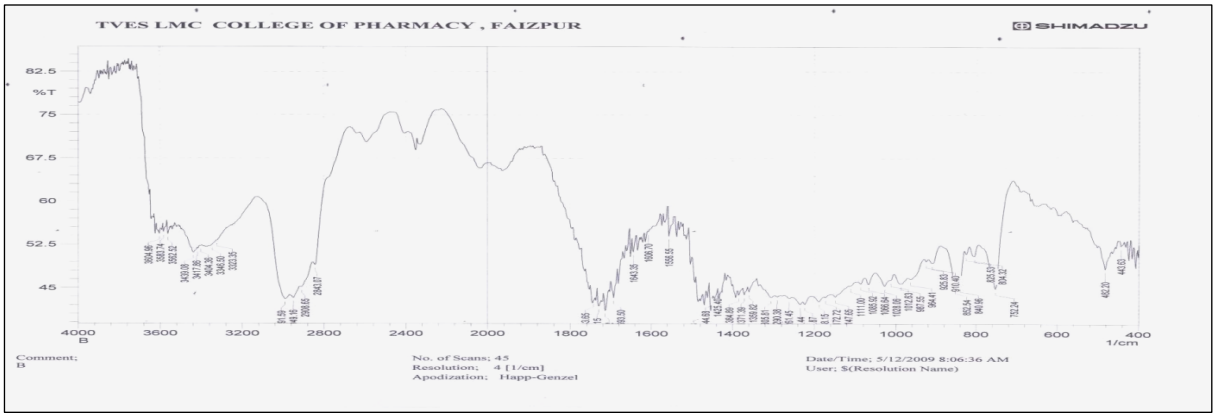

Figure No. 1: FT-IR spectra Indomethacin

Fourier transform infrared spectroscopy (FTIR):
Indomethacin, polyvinyl alcohol and Indomethacin microsponges samples were subjected to Fourier transform infrared spectroscopy using KBr pellets in a Fourier transform infrared spectrophotometer (Perkin Elmer spectrum BX II) in the range from 4000 to 400 cm−1. [19-20]

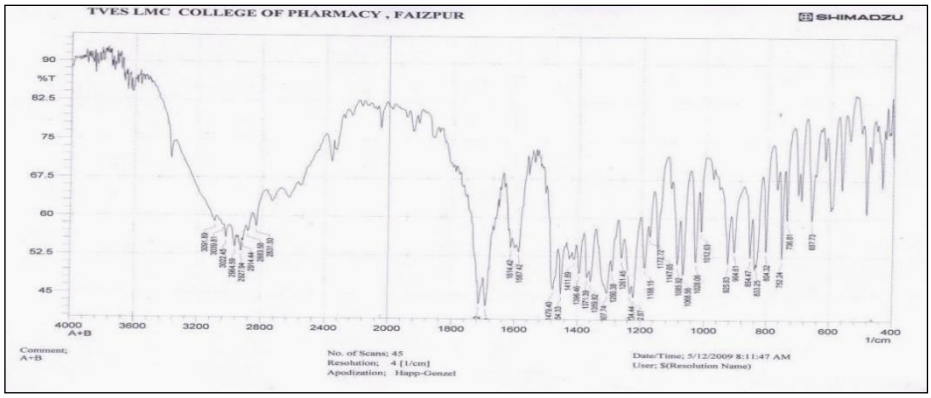

Figure No. 2: FT-IR of spectra physical mixture

Differential Scanning Calorimetry(DSC)::

DSC analysis of Indomethacin and Indomethacin microsponges was carried out by heating the samples from 30 to 300 °C at a heating rate of 10 °C per min using DSC (SDT, Q600, TA instruments, USA). [21]

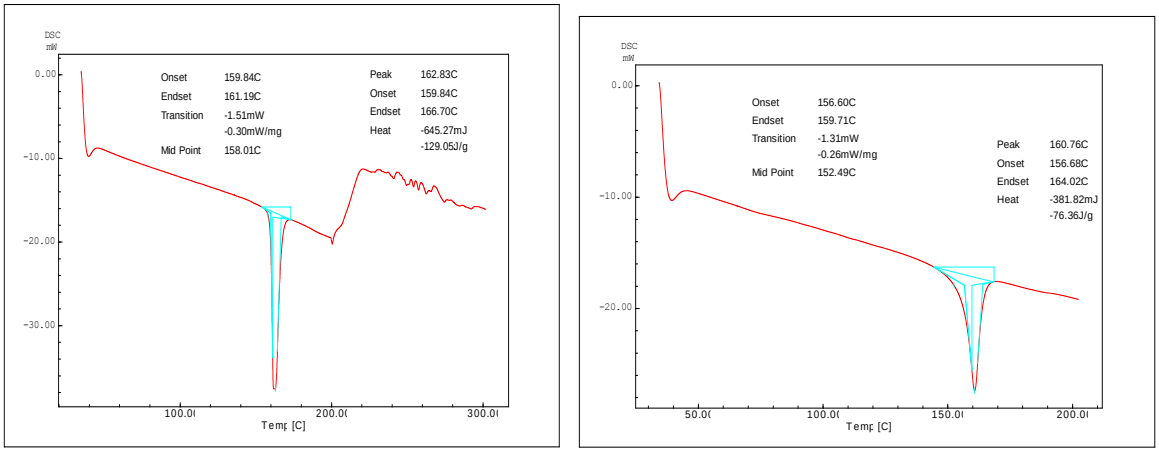

Figure No. 3: DSC thermogram of microsponge formulation

Scanning Electron Microscopy:

The shape and surface of the Indomethacin microsponges were examined using SEM (SEM, Environmental Scanning Electron Microscope model FEI Quanta 200F with Oxford-EDS system (IE 250 × Max 80, The Netherlands) after coating. Prior to observation, the samples were mounted on metal grids, using double-sided adhesive tape and coated with gold under vacuum.

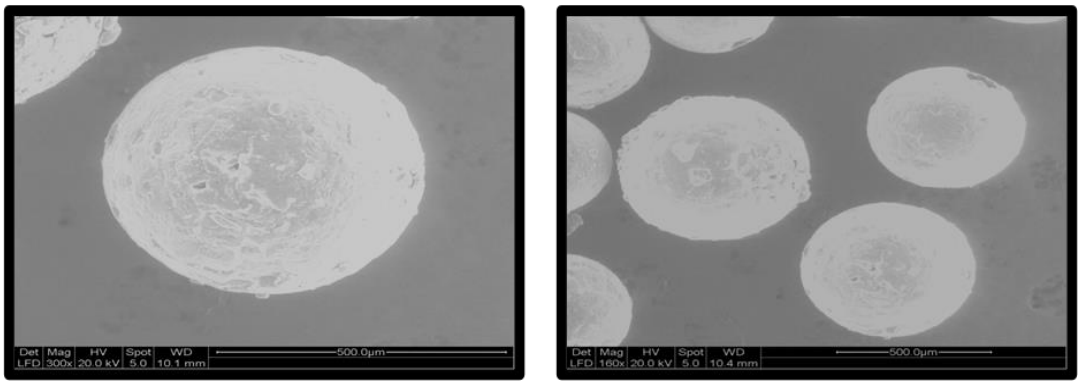

Figure No.4: SEM photograph of Indomethacin microsponges Whole image of microsponge, External surface, Internal surface,

X-ray difraction (XRD) study:

The Indomethacin EC and Indomethacin microsponges powder samples were scanned using an X-ray difractometer (Minifex 2, Rigaku, Japan) from 0° to 50° difraction angle (2θ) range under the following measurement conditions: source, nickel fltered CuKα radiation; voltage 35 kV; current 25 mA; scan speed 0.05 min−1, division slit 1.25°, receiving slit 0.3 mm.

The X-Ray diffraction pattern of indomethacin exhibited sharp, highly intense and less diffused peaks indicating the crystalline nature of drug. The pure drug showed diffraction peaks at 2Θ degree of 18.8, 21.00, 25.8 and 28.6. It can be concluded that the crystalline nature of pure drug indomethacin remain unaffected till the completion of process of microsponge formation which was also supported by DSC results of indomethacin microsponges.

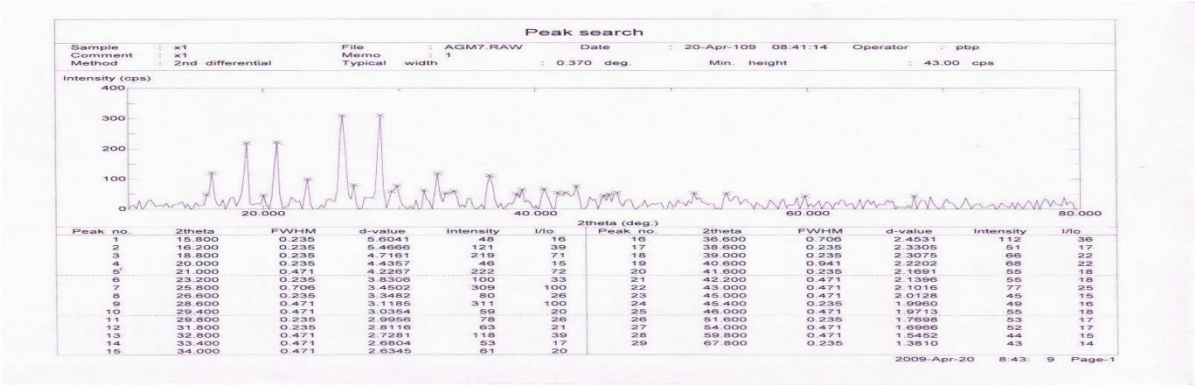

Figure No. 5: XRD Spectrum of Indomethacin

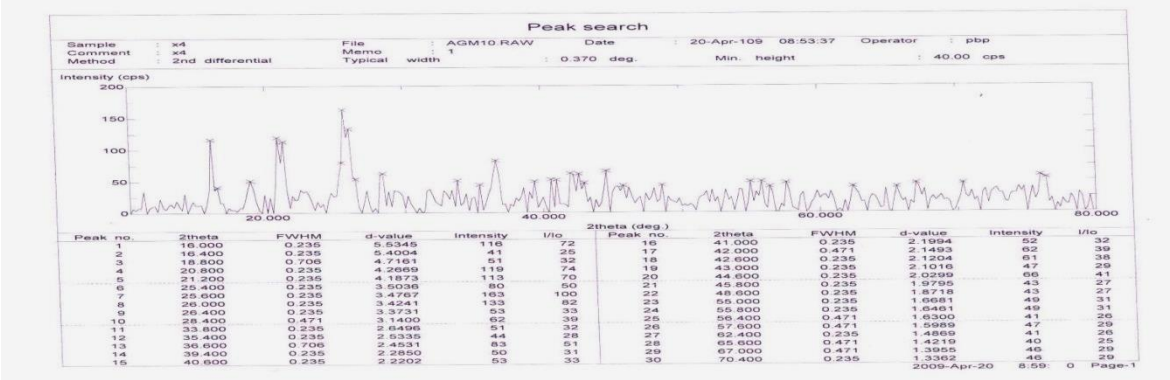

Figure No. 6: XRD Spectrum of microsponge formulation

RESULTS AND DISCUSSION:

Microsponges could not be obtained with the drug polymer ratio 11:1, 10:1 and free indomethacin crystals were seen in the investigation done by optical microscope. Microsponges prepared by 7:1, 8:1, and 9:1 showed aggregation and irregular size while 1:1 and 2:1 showed large and irregular sized microsponges. Microsponges prepared by 3:1, 4:1 and 5:1 ratio gives small and spherical microsponges. For the optimization of quasi-emulsion solvent diffusion method and its process parameters one of the preliminary batch P3 (3:1) was selected and optimized. In this study, for the optimization of process parameters and formulations prepared by quasi-emulsion solvent diffusion method, the effect inner phase solvent amount (8, 10 and 12), effect of propeller (two blade stirrer and three blade stirrer), stirring speed (250, 500 and 750) and stirring time (30, 60 and 120 minutes) on the formation of microsponges were investigated.

Micromeritic Properties of Microsponges studied as all the formulations showed angle of repose value (Θ) in between 18.63 to 21.32 and these lower values for angle of repose (< 30) indicated good flow properties of blends. Lower the angle of repose, lower the

frictional forces existing within the particulate mass and hence better is the flow properties.The values for bulk density were found from 0.475 to 0.517 while the values for tapped density were found from 0.561 to 0.627.Bulk and tapped density values of blends were found to be high which indicates that there is no excessive air voids and hence these mass do not pose any problem during compression**.** These values further correlate with compressibility index. These were found in between 17.24 to 22.02 suggesting the acceptable range of particles and this was further supported by values of angle of repose.Hausner's ratio It was ranged from1.172 to 1.218, i.e., all the preparation showed that they had good flow properties *In-vitro* dissolution study indicated that the release of indomethacin varied according to the concentration of matrix forming polymer.

The release of drug from formulations containing varying concentration of Eudragit RS 100 was inversely proportional i.e. 0.666< 0.500< 0.400 (gm.). The formulation batches F1 to F5 Eudragit RS 100 (0.666 gm.) and changing concentration of PVA (30-70 mg) percentage cumulative drug release i.e. upto 88.698. F1> F2> F3> F4> F5. Batches F6 to F10 Eudragit RS 100 (0.500 gm.), PVA (30-70 mg) % Drug Release up to 93.673 F6> F7> F8> F9> F10. Batches F11 to F15 Eudragit RS 100 (0.400 gm.), PVA (30-70 mg) % cumulative drug release up to 95.533 F11> F12> F13> F14> F15. The marketed sustained release indomethacin capsule (INDOCAP) showed sustained release of Indomethacin for 12 hours and showed maximum percentage cumulative drug release i.e. upto 94.913. The drug release data of all the formulations were fitted into different mathematical models namely zero order, first order, Higuchi model, Hixson-Crowell model and Peppas model. The rate constants and R^2 values for zero order, first order, Higuchi, Hixson-crowell and "n" value for Peppas model of all the microsponge batches are given in Table.6 All the formulations of microsponges showed Higuchi kinetics. The best fitted model was found to be Higuchi kinetics model. The FT-IR spectrum of pure drug, Indomethacin, Eudragit RS 100 and PVA were taken separately. The spectrums of physical mixtures of Indomethacin and Eudragit RS 100 and Indomethacin and PVA and spectrums of microsponges containing the same were taken to find out any interaction. From the above interpretation it is observed that all the characteristic peaks shown by indomethacin was appeared in physical mixtures as well as in microsponge formulations without any remarkable change in their position, so it is concluded that there was no chemical interaction between drug and polymers. According DSC to the thermogram of pure drug indomethacin which presented a sharp endothermic peak at 162.83^0C. Eudragit RS 100 no peak upto 300 ^{0}C At last the thermogram of microsponge formulation was studied which showed endothermic peak at the same point like above The thermograms of physical mixture and microsponge formulation showed that drug was in its crystalline form and also there was no interaction between drug and polymers. The scanning electron microscopic photographs of Indomethacin microsponges formulated using various drug. SEM photographs showed discrete, spherical microsponges.. The microsponge batches F11, F12, F13, F14 and F15 were compared with marketed product by performing t-test. It was found that there was no significant difference between marketed product and microsponge batches (i.e. $p > 0.05$). The drug release pattern of marketed product and microsponge formulation F11 was found similar Hence, from the study it can be concluded that the changes in particle size and morphology of the microsponge systems have a big impact on different crucial properties such as porosity, drug release and kinetics of drug release. Also the careful control of the process parameters, microsponge with desirable can be produced. With this kind of formulation, the undesirable side effects and presystemic metabolism of the drug can be eliminated and a sustained effect can be obtained.

CONCLUSION:

Micro sponges were revealed by scanning electron microscope and were found to be porous and spherical. Optimized formulation of microsponge was evaluated for drug content, pH, viscosity and in vitro drug release. Therefore, Indomethacin microsponges prepared in thus study are promising as being more useful than conventional formulation in therapy. Finally it can be concluded that the objective of this study is achieved. .In future microsponges can be used to prepare suitable dosage form and its *in-vivo* absorption studies in animals/ humans can be carried out to know the bioavailability from sustained release formulation.

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