FORMULATION AND EVALUATION OF ACECLOFENAC LOADED MICROSPONGE

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ABSTRACT

The objective of present work was to prepare microsponges of drug aceclofenac. Preformulation testing is the first step in the rational step in the development of dosage forms of a substance. The drug aceclofenac powder was examined for its organoleptic properties found it was observed that it was off white, bitter, crystalline odorless powder. When tested for its solubility in various solvents, it was determined that drug sample was sparingly soluble in water and soluble in 0.1 HCl, methanol, ethanol and Buffer 6.8 pH etc. melting point observed 150-153^{O}C. The partition coefficient was determined as 0.29 ± 0.06. Aceclofenac solution was scanned in the U.V. range of 200-400 nm using systronics, UV Visible spectrophotometer. The spectrophotometric method of analysis of Aceclofenac at λ_{max} 275.8 nm was found to be reproducible and highly sensitive. The standard curves were prepared in Buffer 6.8 pH at λ_{max} 275.8nm. The IR spectrum of drug substance was authenticated using FT-IR spectroscopy. Drug and polymer compatibility study reveals that there is no interference between drug and polymer thus can be used for further formulation and evaluation purposes. The microsponges containing Aceclofenac were fabricated by quasi-emulsion solvent diffusion method using an inner phase comprising Eudragit S-100 and dibutyl phthalate (1%w/v) dissolved in 7 ml of dichloromethane. Dibutyl phthalate was added to improve the plasticity of the polymer. General parameters of evaluations were observed as Yield (39.66 ± 0.47 to 75.25 ± 0.02), % Drug Content (31.78 ± 0.02 to 45.28 ± 0.01), % Encapsulation Efficiency (91.30 ± 0.04 to 96.56 ± 0.02) and Mean Particle size (98.3 ± 1.02 to 107.6 ± 1.05). SEM Photomicrographs revealed that formulated aceclofenac microsponges were spherical and sponge. In-vitro Release of drug from Aceclofenac microsponges reveals that formulations F-5 and F-6 have release above 90%. Formulated microsponges preparation incorporated in gels in fixed amount. That gel formulations were evaluated and found all formulations were clear, odourless, washable, homogeneous, and free from grittiness and also no phase separation found. Drug stability concerns about drug product safety, efficacy, and quality, found it to appropriate. The percentage drug loss from the formulations was used as a measure of storage stability. It can be concluded that for better stability, the formulations should be stored at low temperature in refrigerator.

KEYWORD: *Aceclofenac, microsponges, Preformulation, quasi-emulsion, solvent diffusion*

INTRODUCTION

Microsponge delivery systems are uniform, spherical, porous polymeric microspheres having myriad of inter connected voids of particle size range 5-300µm. These microsponges have the capacity to entrap a wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens and anti-infective, etc. and then release them onto skin over a time and in response to trigger[1]. Micropores within the spheres comprise a total pore density of approximately 1mL/g, and pore length10ft for extensive drug retention. Further, these porous microspheres with active ingredients can be incorporated in to formulations such as creams, lotions and powders[2]. Microsponges consisting of non-collapsible structures with porous surface through which active ingredients are released in a controlled manner. When applied to the skin, the MDS releases its active ingredient on a time mode and also in response to other stimuli (rubbing, temperature, pH, etc)[3]. MDS technology is being used in cosmetics, over-the-

counter (OTC) skin care, sunscreens and prescription products. Their high degree of cross-linking results in particles that are insoluble, inert and of sufficient strength to stand up to the high shear commonly used in manufacturing of creams, lotions, and powders[4]. Their characteristic feature is the capacity to adsorb or 'load' a high degree of active materials into the particle and on to its surface[5]. Its large capacity for entrapment of actives, up to three times its weight, differentiates microsponge products from other types of dermatological delivery systems[6]. The active payload is protected in the formulation by the microsponge particle; it is delivered to skin via controlled diffusion[7].

Microsponges are mostly used for topical delivery and are used for oral and simultaneous biopharmaceutical delivery[8]. Many patents have reported that due to its high loading capacity and continuous release capacity it can be used as an excipient. This formulation offers many options for developing pharmaceutical and cosmetic products[9]. Microsponges are deliberately able to give a drug active ingredient in a minimal amount efficiently and increase stability, reduce side effects, and modify drug releases. Over the counter products that include the micro-sponge drug delivery system include many moisturizers, special renaissance products and sunscreens[10]. Microsponge is a novel drug delivery system with unique properties such as enhanced product performance and elegancy, extended release, and improved drug release profile, reduced irritation, improved physical, chemical, and thermal stability, which makes it flexible to develop novel product forms. Biodegradable polymers can also be employed for tissue engineering and regulated oral drug administration[11]. It offers a wide range of benefits when it comes to formulation. Alternative drug delivery methods, such as parenteral and pulmonary, must also be developed for MDS. These carrier systems were used in cosmetics because of their elegance[12]. These formulation innovations also open up new avenues for drug delivery. As a result, microsponge-based drug delivery technology is expected to become a viable drug delivery matrix material in the future for a variety of therapeutic applications[13].

MATERIAL AND METHOD

Preformulation Study: Preformulation testing is the first step in the rational step in the development of dosage forms of a substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The drug aceclofenac powder was examined for its organoleptic properties found it was observed that it was off white, bitter, crystalline odorless powder. When tested for its solubility in various solvents, it was determined that drug sample was sparingly soluble in water and soluble in 0.1 HCl, methanol, ethanol and Buffer 6.8 pH etc., melting point, partition coefficient. The standard curves were prepared in Buffer 6.8 pH at λ_{max} 275.8nm. Drug and polymer compatibility study reveals that there is no interference between drug and polymer thus can be used for further formulation and evaluation purposes.

Method of Preparation of Aceclofenac Microsponges

The microsponges containing Aceclofenac were fabricated by quasi-emulsion solvent diffusion method using an inner phase comprising Eudragit S-100 and dibutyl phthalate (1%w/v) dissolved in 7 ml of dichloromethane. Dibutyl phthalate was added to improve the plasticity of the polymer. Further Aceclofenac was put in and dissolved through ultrasonication at 35°C. This mixture was then poured into an aqueous solution of PVA (outer phase) with stirring rate 500 rpm for 60 min. Next on, microsponges were formed due to the removal of dichloromethane from the system by evaporation. Prepared microsponges were then filtered, washed with distilled water and subjected to drying at 40°C for 12 h in hot air oven. Finally, microsponges were weighed to determine production yield. Various formulation batches are prepared as per table below:

Table No. 1: Composition of different batches of microspoge prepared

Ingredient	F-1	F-2	F-3	F-4	F-5	F-6
Aceclofenac:EudragitS-100 (mg)	1:1 100:100	1:2 100:200	1:3 100:300	1:4 100:400	1:5 100:500	1:6 100:600
Dichloromethane (ml)	7	7	7	7	7	7
Dibutyl phthalate (% w/v)	1	1	1	1	1	1
Polyvinyl alcohol (mg)	50	50	50	50	50	50
Water (ml)	100	100	100	100	100	100

Methods of Characterization of Aceclofenac Microsponges

Production yield of microsponges, Actual drug content and encapsulation efficiency were determined. For

morphology and surface topography, prepared microsponges were examined with a scanning electron microscope operating at 5 kV. Using double adhesive tape, samples were mounted on a metal stub and coating with platinum/palladium alloy under vacuum was done. Particle size analysis of prepared microsponges was studied by using particle size analyzer.

***In -vitro* Release Studies:** *In vitro* release was studied using a dialysis bag as a 'donor compartment'. Microsponges containing entrapped Aceclofenac obtained after centrifugation of 2 ml of the formulation were resuspended in 1 ml of buffer pH 6.8 and used for the release study. The dialysis membrane was soaked in warm water for 10 min, one end was sealed with a clip, the Microsponges reparation or drug in solution was pipetted into the bag and the bag was sealed with another closure clip to prevent leakage. The dialysis bag was placed in 50 ml buffer pH 6.8 at 37 ± 2^{o}C. The medium, which acted as the receptor compartment, was stirred at 100 rpm. Samples of medium (5ml) were withdrawn hourly and replaced with fresh buffer and Aceclofenac absorbance at 275.8 nm was measured using buffer pH 6.8 as blank. Results were the mean values of three runs.

Preparation of Aceclofenac microsponge gel

Polymer (Carbopol 940) was soaked in water for 2 h and then dispersed by agitation at approximately 600 rpm with the aid of magnetic stirrer to get a smooth dispersion. The dispersion was allowed to stand for 15 min to expel entrained air. To it the aqueous solution of triethanolamine (2% v/v) was added with slow agitation for adjusting pH to 6.5–7.5. At this stage microsponges containing drug equivalent to 100mg were incorporated into the gel base. Prepared gels were packed in wide mouth glass jar covered with screw capped plastic lid after covering the mouth with aluminum foil and were kept in dark and cool place until use.

Evaluation of Aceclofenac microsponge gel

Visual inspection: The organoleptic properties, such as color, texture, consistency, homogeneity, and physical appearance of gel containing microsponges were checked by visual observation.

Measurement of pH: Gel formulation pH was recorded using digital pH meter. 5 g gel was dispersed in 45 ml distilled water at 27°C and solution pH was measured.

Spreadability studies: One of the requisite qualities for an ideal gel is to pursue excellent spreadability. Spreadability is used to express the extent of the area of skin or affected part to which gel readily spreads. A spreading value significantly affects therapeutic efficacy of the formulation. Expression of spreadability is given in terms of time (in seconds) taken by two slides to slip off from gel placed in between under application of specific load. Better spreadability is indicated by minimum time required for slides separation. Mathematical expression used for spreadability calculation was:

$$S = \frac{M}{L}$$

Where, M = weight (in gm) attached to upper slide, L = length (in cm) of glass slides, T = time (in sec) taken to separate the slides.

Wooden block-glass slides apparatus was used and by applying weight about 20 gm, time for complete separation of the upper slide (movable) from lower slide (fixed) was estimated.

Tube extrudability: One of the properties which an ideal gel should possess is good tube extrudability; so that when slight pressure is applied on tube, the formulation should extrude out uniformly with an ease. The technique adopted for examining gel extrudability was based upon percent quantity of gel extruded from the tube on finger pressure application. More the quantity extruded better the extrudability. Formulations were filled in clean, lacquered, collapsible aluminum tubes with 5 mm nasal tip opening and the pressure was applied on tubes using the first finger and thumb. Afterward tube extrudability was estimated in percentage by measuring the amount of gel extruded through the tip and compared with marketed formulation considering its extrudability as 100%.

Viscosity: The viscosity of the gel formulation was measured with a Brookfield viscometer using 1x model and cone number 01, with an angular velocity of 5 rpm at 25 ºC. An average of five readings was used to calculate

viscosity.

***In-vitro* Permeation Studies:** The In-Vitro permeation studies of gels carried out with Franz diffusion (FD) cell using egg membrane followed by hydration for 30 minutes in buffer pH 6.8 at room temperature to remove the extraneous debris and leachable enzymes. Then excised egg membrane placed between donor and receptor compartments of the FD cell. Gel place in donor compartment and buffer pH 6.8 fill in receptor compartments as media. Temperature of cell was maintained at 37°C ± 0.5°C. The assembly kept on magnetic stirrer and samples withdrawn at time intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h and replaced with equal volume of fresh media. Samples analyzed by UV spectrophotometer at 275.8 nm and calculate cumulative % of drug release.

Stability Studies: Niosomal formulation will selected on the basis of entrapment efficiency *and in vitro* release studies. Stability studies will assessed by keeping niosomal suspension and niosomal gel in sealed glass vials and storing them in two different storage conditions, that is, refrigeration temperature and room temperature for a period of 30 days. The samples withdrawn at different time intervals over a period of one month and the residual content was determined spectrophotometrically.

RESULTS AND DISCUSSION

Preformulation Study: Preformulation testing is the first step in the rational step in the development of dosage forms of a substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The drug aceclofenac powder was examined for its organoleptic properties found it was observed that it was off white, bitter, crystalline odorless powder. When tested for its solubility in various solvents, it was determined that drug sample was sparingly soluble in water and soluble in 0.1 HCl, methanol, ethanol and Buffer 6.8 pH etc. melting point observed 150-153^{O}C. The partition coefficient was determined as 0.29 ± 0.06. Aceclofenac solution was scanned in the U.V. range of 200-400 nm using systronics, UV Visible spectrophotometer. The spectrophotometric method of analysis of Aceclofenac at λ_{max} 275.8 nm was found to be reproducible and highly sensitive. The standard curves were prepared in Buffer 6.8 pH at λ_{max} 275.8nm. The data were regressed to obtain the straight line. The correlation coefficient greater than 0.996 was observed in all the cases, which indicated that, the drug follows Beer-Lambert's law in the concentration range of 2-10 µg/ml. The IR spectrum of drug substance was authenticated using FT-IR spectroscopy. The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted. 3410&3646 (N-H str.), 3264 & 3392 (O-H Str), 2834 & 2822(C=O Str) 1693 & 1672(C-H Str). Drug and polymer compatibility study reveals that there is no interference between drug and polymer thus can be used for further formulation and evaluation purposes.

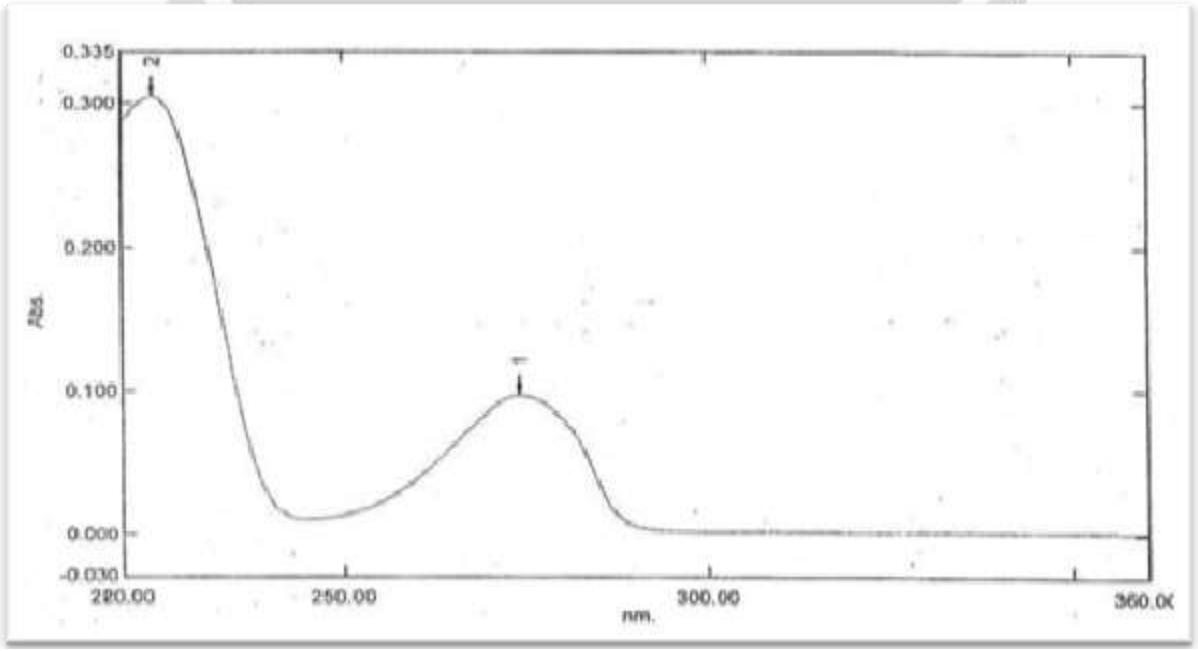

Figure No. 1: UV- spectrometry scanning (200-400 nm) of Aceclofenac

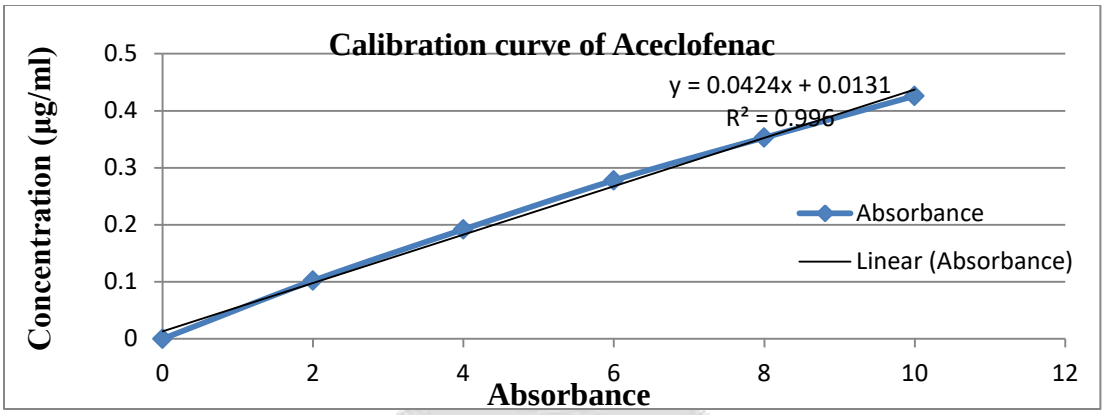

Figure No. 2: Graph showing calibration curve of Aceclofenac

Compatibility studies

(a) Physical compatibility study between drug and excipient

Table No. 2: Physical drug-polymer compatibility studies (7 Days)

S. No.	Drug-Excipient	Initial	Condition			Comments
			CS	RT	Oven	
1.	Drug (Aceclofenac)	White Color	NR	NR	NR	Compatible
2.	Drug +Polymer (1:1)	White Color	NR	NR	NR	Compatible

NR = No Reaction

Table No. 3: Important band frequencies in FT- IR spectrum of Aceclofenac

S. No	Functional Group	Specified Frequency (cm^{-1})	Observed Frequency (cm^{-1})	Specified Frequency (cm^{-1})
1	N-H str.	3700-3400	3410	3646
2	O-H Stretch	3400-3000	3264	3392
3	C-H Stretch	2900-2800	2834	2822
4	C=O Stretch	1700-1650	1693	1672

(b) FT-IR Spectroscopic study for Drug-Excipients Interaction

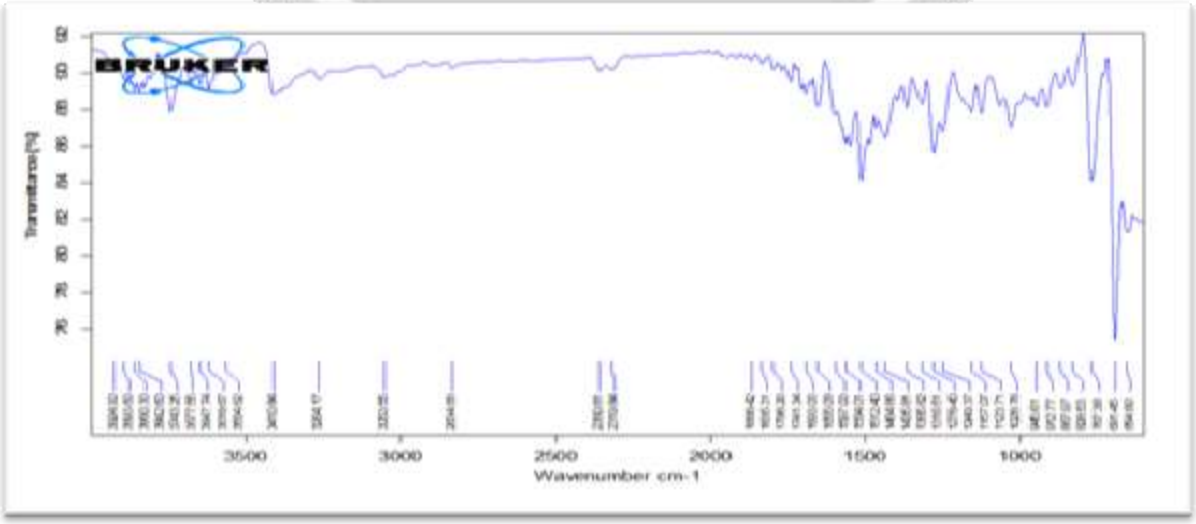

Figure No. 3: FT-IR graph of drug Aceclofenac

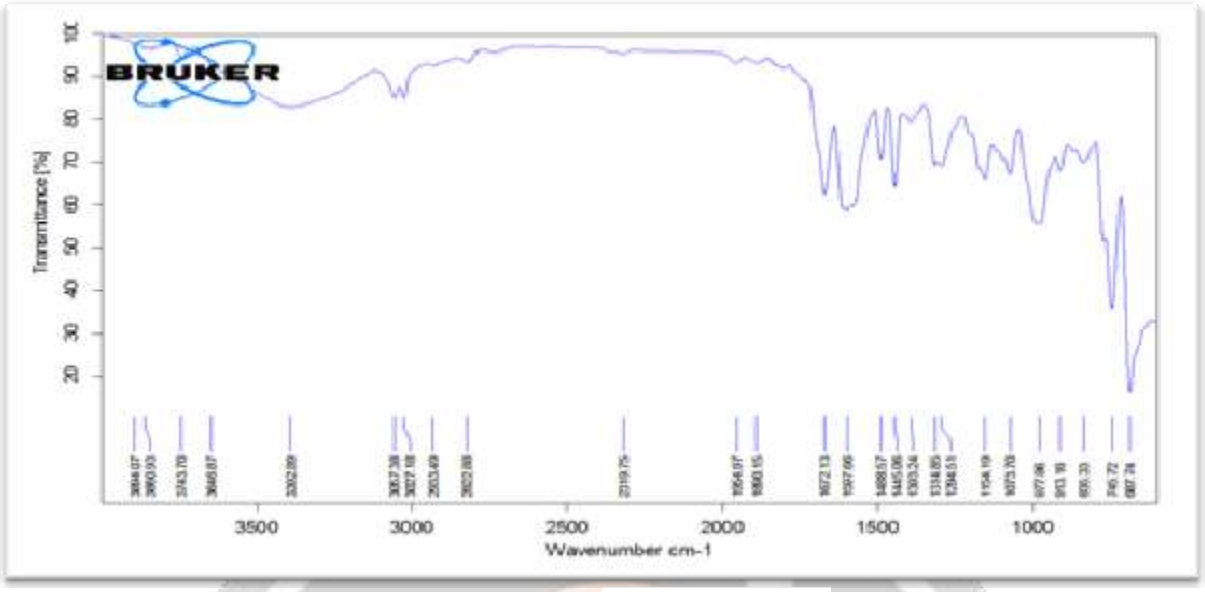

Figure No. 4: FT-IR graph of drug Aceclofenac with all used polymers

Pharmaceutical Characterization of prepared microspoges of Aceclofenac

General parameters of evaluations

Table No. 4: Characterization of prepared microspoges

Batch Code	Drug: Polymer Ratio	Yield (%) ± SD	% Drug Content ± SD	% Encapsulation Efficiency ± SD	Mean Particle size (µm) ± SD
F-1	1:1	73.35 ± 0.02	45.28 ± 0.01	96.56 ± 0.02	98.3 ± 1.02
F-2	1:2	62.76 ± 0.05	36.47 ± 0.02	94.41 ± 0.01	107.6 ± 1.05
F-3	1:3	75.25 ± 0.02	32.50 ± 0.03	93.82 ± 0.02	102.5 ± 1.02
F-4	1:4	39.66 ± 0.47	31.78 ± 0.02	95.50 ± 0.02	106.6 ± 1.47
F-5	1:5	48.10 ± 0.61	35.01 ± 0.01	91.30 ± 0.04	103.1 ± 1.61
F-6	1:6	50.66 ± 0.51	37.15 ± 0.21	92.15 ± 0.04	106.6 ± 1.51

Scanning Electron (SEM)

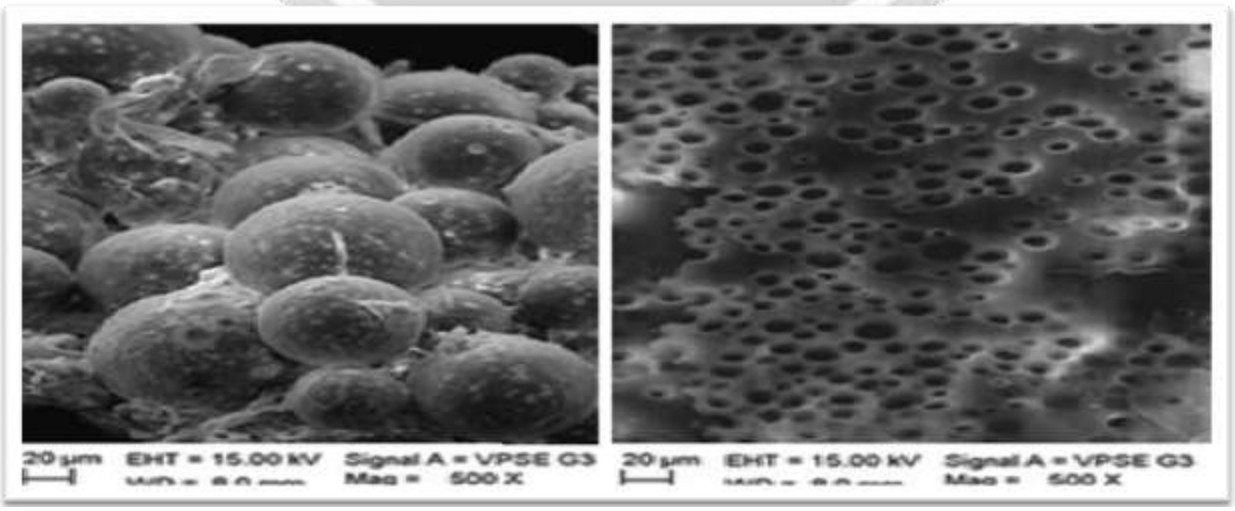

Figure No. 5: SEM photograph of optimized formulation and their porosity

***In-vitro* Release Studies of Aceclofenac microsponges**

Table No. 5: *in-vitro* Release from Aceclofenac microsponges

Time in hours	Cumulative % release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	45.32	40.15	37.32	35.32	25.32	22.12
2	55.32	54.76	47.83	41.82	36.86	33.82
3	66.38	62.23	54.42	47.36	42.42	38.12
4	73.78	69.9	60.48	54.12	48.32	44.14
5	76.68	72.65	64.12	57.82	51.72	47.72
6	77.38	73.73	68.83	62.32	56.82	52.82
7	79.49	74.8	71.12	68.21	62.81	58.64
8	80.62	75.38	74.34	74.14	69.32	65.78
9	82.3	75.7	76.13	79.26	75.21	72.72
10	83.14	76.01	77.38	82.12	80.36	78.76
11	83.57	76.26	78.61	84.46	84.28	83.13
12	84.94	86.32	79.28	88.56	90.32	91.25

*Each value was an average of three determinations

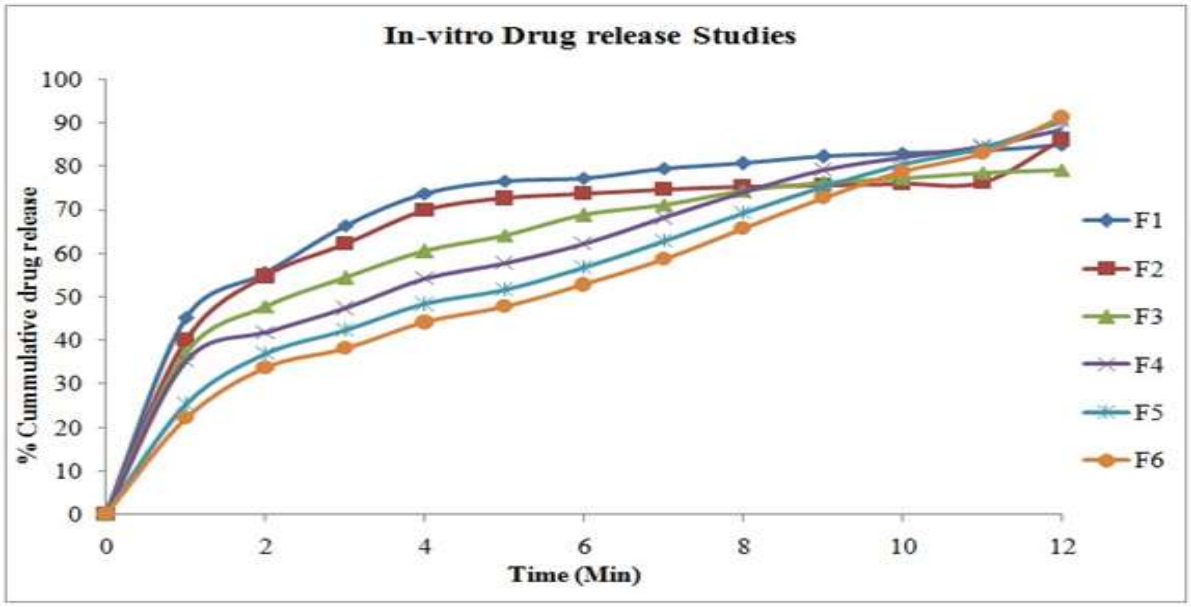

Figure 6: *in-vitro* Release of drug from Aceclofenac microsponges

Preparation of Aceclofenac Microsponge Gel

Table No. 6: Formulation of Aceclofenac microsponge gel

Formulation	GF-1	GF-2	GF-3	GF-4	GF-5	GF-5
Microsponge equivalent to Aceclofenac (mg/ml)	100	100	100	100	100	100
Carbopol-940 (%)	1	1	1	1	1	1
Triethanolamine (%)	2	2	2	2	2	2
Distilled water (ml)	100	100	100	100	100	100

Evaluation of Aceclofenac Microsponge Gels

Table No 7: Evaluation of Aceclofenac microsponge gels

Formulation code	Clarity	Odor	Phase separation	Washability	Homogeneity	Grittiness
GF-1	Clear	No	No	Washable	Yes	No
GF-2	Clear	No	No	Washable	Yes	No
GF-3	Clear	No	No	Washable	Yes	No
GF-4	Clear	No	No	Washable	Yes	No
GF-5	Clear	No	No	Washable	Yes	No
GF-6	Clear	No	No	Washable	Yes	No

Table No. 8: General evaluation of Aceclofenac microsponge gels

Formulation code	pH	Spread ability (cm)	Tube Extrusion	Viscosity(cp)	%Permeation
GF-1	6.5	6.6 ± 0.5	92.3 ± 1.2	110 ± 1.8	78.1 %
GF-2	7.4	7.6 ± 0.0	95.1 ± 1.3	113 ± 2.0	89.4 %
GF-3	7.4	7.6 ± 0.1	98.2 ± 2.1	115 ± 1.2	87.3 %
GF-4	7.2	7.0 ± 0.4	93.1 ± 1.7	100 ± 0.8	91.6 %
GF-5	7.5	7.1 ± 0.3	97.5 ± 0.6	98 ± 2.0	95.9 %
GF-6	7.1	7.3 ± 0.4	96.4 ± 0.2	115 ± 1.9	97.8 %

Stability Studies

Table No 9: Stability of Aceclofenac microsponges on following parameters

Formulation code	Phase separation		pH		Drug content (%)	
	4°C	**40 °C**	**4°C**	**40 °C**	**4°C**	**40 °C**
GF-1	No	No	6.9	7.0	100±1.1	95±2.0
GF-2	No	No	7.4	7.3	100±1.8	99±1.9
GF-3	No	No	7.4	7.2	98±1.9	98±1.8
GF-4	No	No	7.2	7.1	99±0.6	92±1.0
GF-5	Yes	Yes	7.1	7.4	101±1.9	97±2.1
GF-6	Yes	Yes	7.5	7.5	99±1.4	98±1.2

CONCLUSION

From the trial-and-error optimization design, drug loaded Gatifloxacin Niosomes were successfully evaluated. Preformulation study confirms purity of drug and compatibility of drug with excipients using FT-IR. From characterization parameters and stability study, it was concluded that the formulation has acceptable morphology and particle size, no any chemical interaction and was stable at refrigerated condition respectively. From characterization parameters and Drug release study, it was concluded that the formulation has all the parameters are acceptable as sustained release tablet, no any chemical interaction and was stable at refrigerated condition respectively. An extensive investigation is needed with reference to bioavailability and *in-vivo* drug release of batch GF-5 and GF-6. There is a need to develop suitable formulation for commercial exploitation. Thus, the specific objective listed in the plan of work of this thesis were achieved namely design, characterization and release studies of aceclofenac microsponges gels.

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