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DEVELOPMENT AND EVALUATION OF CHRONOMODULATED PULSINCAP DRUG DELIVERY SYSTEM OF KETOPROFEN

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Keywords:

Chronotheraputics, Pusincap drug delivery, Solid disperssion, Ketoprofen, Eudrajit RS 100

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ABSTRACT: Chronotherapeutic drug delivery systems have been recognized as potentially beneficial to the chrono-therapy (time optimized) of chronic diseases that display time-dependent symptoms. In pulsincap system, therapy can be optimized by tailoring the dosing schedule based on chrono-biological pattern. The safety and efficacy of the drug is achieved by coordinating the peak plasma concentration with circadian rhythm. People with rheumatoid arthritis, usually experience peak pain in the morning. The absorption kinetics of conventional dosage forms that have night time dosing indication is inadequate in the morning, when the need is more. Ketoprofen is a potent NSAID used to treat RA. In the present study, a modified Pulsincap of Ketoprofen was developed and evaluated. Hard gelatine capsule bodies were treated with formaldehyde and are filled with best formulations of solid dispersion (β-cyclodextrin) for burst release and microsponge (ethyl cellulose) for sustained release after specific lag time attained by hydrogel plug. The filled capsules were completely coated with 5% CAP to prevent variable gastric emptying. The results indicated that formulated Pulsincap of Ketoprofen with solid dispersion, microsponge and HPMC plug administered after meals at 9 pm starts releasing drug after about 7 hrs and provides sufficient plasma concentration in the early morning and thereafter sustain the release from microsponge for more than 24 hrs.

INTRODUCTION: Chrono-pharmaceutics is a branch of pharmaceutics devoted to the design and evaluation of drug delivery systems that release a bioactive agent at a rhythm that ideally matches in real time the biological requirement for a given disease therapy or prevention[1]. The oral controlled-release system shows a typical pattern of drug release in which the drug concentration is maintained in the therapeutic window for a prolonged period, thereby ensuring sustained therapeutic action.

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However, there are certain conditions for which such a release pattern is not suitable. These conditions demand release of drug after a lag time. In other words, it is required that the drug should not be released at all during the initial phase of dosage form administration. Such a release pattern is known as "pulsatile release" [2]. Pulsatile drug delivery system is defined as the rapid and transient release of certain amount of molecules within a short time period immediately after a predetermined off - release period, *i.e.*, lag time.

Here release of drug can be controlled by circadian rhythm, which regulates many body functions in human beings [3]. Methodologies which have been developed and applied to design chronotropic systems for desired Pulsatile drug release are time controlled chronotropic systems, stimuli induced

pulsatile drug delivery systems, externally regulated pulsatile drug delivery systems. Pulsatile drug delivery is related to the term "chrono-pharmaceutics" which is a combination of chronobiology and pharmaceutics. Chronobiology is the study of biological rhythms and their mechanisms [4]. Chrono-therapeutics is the discipline concerned with the delivery of drugs according to the intrinsic activities of a disease over a certain period of time because the biochemical, physiological and pathological variations over a 24 hr period in humans has been occurred.

Pulsincap drug delivery systems are mostly developed in capsule form. The lag time is controlled by a plug, which gets pushed away by swelling or erosion, and the drug is released. When this capsule comes in contact with the dissolution fluid, it swells; and after a lag time, the plug pushes itself outside the capsule and the drug is released rapidly. The lag time can be controlled by manipulating the dimension and the position of the plug [5]. Pulsatile release multiparticulate systems have been developed on the basis of various approaches of designing pulsatile drug delivery like time controlled, stimuli induced or externally regulated pulsatile drug delivery systems. These can be developed in various types of dosage forms like: i. Pellets ii. Granules iii. Microspheres iv. Beads v. Nanoparticles vi. Microsponges. By incorporation into a microsponge carrier system, it is possible to alter the therapeutic index and duration of the activity of drugs.

Solid dispersion technique is used to increase the intrinsic solubility and dissolution and in turns oral bioavailability of poorly water soluble compounds. The term solid dispersion refers to the dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by the melting (fusion), solvent or the melting-solvent method. It has been used for a variety of poorly soluble drugs such as nimesulide, ketoprofen, tenoxicam, nifedipine, nimodipine, ursodeoxycholic acid, carbamazepine, celecoxib and albendazole.

MATERIALS AND METHODS:

Materials: The materials used were laboratory grade Acetyl acetone, cellulose acetate phthalate, Hydroxyl Propyl Methyl Cellulose, Potassium Dihydrogen Orthophosphat, (Central Drug House Pvt. Ltd., New Delhi), Beta Cyclodextrin, Urea, (Chemdyes Corporation, Rajkot) Dichloromethane, Ethyl Cellulose, Eudragit RS100, Ketoprofen (Yarrow Chem Pvt. Ltd., Mumbai).

The instruments used were digital and analytical scales balance, dissolution apparatus type II paddle (Electrolab TDT-06L), UV - spectrophotometer (Shimadzu U - 1800 Japan), Desiccators (Tarsons), DSC Analyzer (Perkin Elmer), FTIR Spectro-photometer (Shimadzu A 213748, Japan), Hot air oven (Kemi KUHS-2), Magnetic stirrer (Remi equipments, Mumbai), Mechanical stirrer (RQ 122, Remi motors, Mumbai), Multi station rotary press (Rinek mini press, Karnavati, Gujarat), Optical microscope (Olympus), Scanning electron microscope (JEOL JSM-6390 England.), X-ray diffractometer (Pan Analytical X'pert PRO, India).

Methodology: Preformulation testing for the identification of drug where done by melting point determination, FTIR-spectroscopy. Physic - chemical properties like colour, order and solubility were determined. Analytical methods including wavelength maxima (λmax), Calibration curve for Ketoprofen 82 were carried out. Compatibility of drug with polymer was determined using FTIR Spectroscopy.

Preparation of Solid Dispersion:

Kneading Method: [5] A mixture of ketoprofen and β-cyclodextrin was wetted with water: methanol (1:1 v/v, 3 ml) solution, and kneaded thoroughly for 30 min in a glass mortar. The paste formed, was dried under vacuum at 60 °C for a day. Pulverized, and passed through sieve no.100 and stored in a desiccators over fused calcium chloride.

Solvent Evaporation Method: The drug and urea in different ratios were dissolved in sufficient volume of methanol with continuous stirring. The solvent was then completely evaporated at 40 – 45°C with continuous stirring to obtain dry granules and were pulverized and passed through sieve no. 60 and stored in airtight container until further use.

Evaluation of Solid Dispersion: [6]

Determination of Percentage Yield: The percentage yield was found out for all the formulations based on the dry weight of the drug

and the polymers taken. The percentage yield was calculated using the equation.

Percentage yield = Practical mass (solid dispersion) × 100 / Theoretical mass (drug + carrier)

Determination of Drug Content: Accurately weighed amount (100 mg) of sample was dissolved in 100 ml of phosphate buffer solution of pH 6.8. The solution was filtered diluted suitably with PBS pH 6.8 and assayed for Ketoprofen content spectrophotometrically at 260 nm.

Determination of Percentage Drug Entrapment:

Percentage drug entrapment = Actual amount of drug in SD × 100 / Theoretical amount of drug in SD

Loading Efficiency:

Loading efficiency = Actual amount of drug in solid dispersion × 100 / Weight taken

Saturation Solubility Study: [7] Known excess of solid dispersion formulations was added to 10 ml of distilled water. Samples were shaken for 24 hours at room temperature in a rotary flask shaker. Samples were then filtered, suitably diluted and analyzed spectrophotometrically at 260 nm.

***In-vitro* Release Study of Solid Dispersion:** [8] The dissolution was carried out in phosphate buffer pH 6.8 and phosphate buffer pH 7.4. Solid dispersion equivalent with 100 mg of drug were spread onto the surface of 900 ml of preheated dissolution medium at 37 °C. Aliquots of 2 ml were withdrawn at regular intervals of time and the same is replaced with fresh dissolution medium each time. The samples obtained were filtered, determined the concentration spectrophotometrically at 260 nm and optimum formula was selected for optimum drug release.

Characterization of optimized formulation of ketoprofen solid dispersion (formulation code: sf5) were done by SEM analysis, X-ray diffraction, DSC.

Preparation of Microsponge: [9] Microsponges were prepared by Quasi-emulsion solvent diffusion method. The organic internal phase consisted of Ethyl cellulose or Eudragit RS100 dissolved in 10ml dichloromethane or ethanol. The calculated amount of Ketoprofen was added gradually with stirring at 40 °C. The resulting solution was then poured in to 0.5% or 0.75% (w/v) of PVA solution in water (external phase of 100ml volume) at room temperature. After emulsification, the mixture was stirred at 4000 rpm for 3 hours at room temperature to remove dichloromethane or ethanol from the reaction flask. The formed microsponges were filtered, washed with distilled water, and dried overnight at room temperature. The prepared microsponges were evaluated for the following parameters:

Particle Size Analysis: The average particle size was determined by using the Edmondson's equation,

$$D\ mean = \Sigma\ nd / \Sigma n$$

Where, n = number or frequency of microsponges observed and d = mean size range.

Determination of Percentage Yield:

Percentage yield = Weight of microsponge obtained × 100 / Weight of polymer and drug fed initially

Determination of Drug Content: Accurately weighed amount (100 mg) of sample was dissolved in 100 ml of phosphate buffer solution of pH 6.8. The solution was filtered diluted suitably with PBS pH 6.8. After appropriate dilution, the amount of drug was detected by a UV Spectrometric method at 260 nm using blank microsponges treated as same manner.

Determination of Percentage Drug Entrapment:

Percentage drug entrapment = Actual amount of drug in microsponge × 100 / Theoretical amount of drug in microsponge

Determination of Loading Efficiency:

Loading efficiency = Actual amout of drug in microsponge × 100 / Weight taken

***In- vitro* Release Study:** The dissolution was carried out in phosphate buffer pH 6.8 and phosphate buffer pH 7.4. Samples of microsponge equivalent with 100 mg of drug were taken and dissolution was carried out in 900 ml of preheated

dissolution medium at 37 °C. Aliquots of 1 ml were withdrawn at regular intervals of time and the same is replaced with fresh dissolution medium each time. The samples obtained were filtered and determined the concentration spectrophoto-metrically at 260 nm.

The best formulation was chosen to study the mechanism of drug release. In order to understand the mechanism of drug release, *in vitro* drug release data were treated to kinetic models such as zero order, first order, Higuchi model and Peppas model.

Preparation of Cross- Linked Gelatine Capsules:

Formaldehyde Treatment: About 100 hard gelatine capsule of size 0 were taken. Their bodies were separated from the caps and placed on a wire mesh. 25 ml of 15% (v/v) formaldehyde was taken in a beaker and kept in an empty glass desiccators and a pinch of potassium permanganate was added to it, to generate formalin vapours. The wire mesh containing the empty bodies of capsule was then exposed to formaldehyde vapours. The caps were not exposed leaving them water-soluble.

The desiccators were tightly closed. The reaction was carried out for 12 hrs after which the bodies were removed and dried at 50 °C for 30 min to ensure completion of reaction between gelatine and formaldehyde vapours. The bodies were then dried at room temperature to facilitate removal of residual formaldehyde. Afterwards the capsule body and the untreated soluble cap were stored in desiccators for further use.

Evaluation of Formaldehyde Treated Capsules:

Physical Tests:

Identification Attributes: The size "0" capsules chosen were opaque with yellow coloured body and red cap. They are lockable type, odourless, soft and sticky when touched with wet hand. After treating with formaldehyde these attributes are checked.

Visual Defects: Among 100 capsules body which were treated with formaldehyde checked for visual defects**.**

Solubility Test for Formaldehyde Treated Capsules: The empty hard gelatine capsules were stirred vigorously in 100 ml of dissolution medium taken in 250 ml beaker, with magnetic stirrer. Dissolution medium used were pH 1.2, buffer pH 6.8 and buffer pH 7.4 the time at which the capsules dissolves or forms a soft mass was noted.

Dimensions: Variation in dimensions between formaldehyde treated and untreated capsules were studied. The length and diameter of capsules were measured before and after formaldehyde treatment using screw gauge.

Chemical Test:

Qualitative Chemical Test for Free Formaldehyde: Standard formaldehyde solution: - Formaldehyde solution (0.002% w/v).

Sample Solution: 25 formaldehyde treated bodies were cut into small pieces and taken into a beaker containing 40 ml distilled water. This was stirred for 1 hr with a magnetic stirrer, to solubilise the free formaldehyde. The solution was then filtered into a 50 ml volumetric flask, washed with distilled water and volume was made up to 50 ml with the washings.

Procedure: To 1 ml of sample solution, 9 ml of water was added. 1 ml of resulting solution was taken into a test tube and mixed with 4 ml of water and 5 ml of acetyl acetone reagent. The test tube was warmed in a water bath at 40 °C and allowed to stand for 40 min. The solution is not more intensely coloured than a reference solution prepared at the same time and in the same manner using 1 ml of a solution containing 0.002% w/v formaldehyde (CH_2O) in place of dilution of the preparation. The comparison should be made examining tubes down their vertical axis.

Hydrogel Plug:

Preparation of Hydrogel Plug: Direct compression method was used to prepare the erodible tablet plug. The compositions of different the erodible tablet plugs used were as shown in **Table 1**. The plug ingredients (HPMC 15 cps and Lactose) were mixed for 10 minutes and compressed using single punch tablet machine with 7 mm concave punch. The diameter of the tablet plug was 7 mm and the weight was kept constant as 200 mg. By varying the amount of HPMC, the effect on lag time was studied.

TABLE 1: COMPOSITION OF DIFFERENT HYDROGEL PLUGS

Formulation Code	HPMC 15 CPS (mg)	Lactose (mg)	Total Weight of Plug (mg)
HP1	140	60	200
HP2	100	100	200
HP3	60	140	200

Evaluations of the Powder Blend:
Bulk Density (Db):

$$\text{Bulk Density, } Db = M / V_0$$

Where, M = mass of the powder sample
V_0 = unsettled volume.

Tapped Density (D_t):

$$\text{Tapped Density, } D_t = M / V_f$$

Where M = mass of the powder sample V_f = Final tapped volume.

Carr's compressibility Index (I):

$$\text{Compressibility Index, } I = (D_t - D_b) / D_t \times 100$$

Hausner Ratio: Hausner ratio is an index of ease of powder flow; it is calculated using the formula,

$$\text{Hausner Ratio} = D_t / D_b$$

Angle of Repose:

$$\tan \Theta = h / r$$

$$\Theta = \tan^{-1} h / r$$

Where, Θ = angle of repose, h = height of the powder cone in cm, r = radius of the powder cone in cm.

Evaluations of hydrogel plug were done by analysing Thickness, diameter using screw gauge. Weight variation test as described in USP, Hardnesss with Pfizer tester, friability with Roche friabilator.

In vitro dissolution studies were carried out using USP dissolution test apparatus II (Paddle type). Capsules were tied with the paddle in order to prevent floating. In order to simulate the pH changes in intestine, dissolution media with 7.4 and 6.8 were sequentially used. When performing experiments, the pH 7.4 medium was first used for three hrs. After 3 hrs (average small intestinal transit time is 3 hrs), the medium was removed and fresh pH 6.8 dissolution medium was added for subsequent hrs. 900 ml of the dissolution medium was used at each time. The release was performed at 37 ± 0.5 °C with a rotation speed of 50 rpm. 2 ml of samples were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through filter paper and analyzed after appropriate dilution using spectrophotometer at corresponding wavelength. Lag time was calculated and recorded.

Preparation of Pulsincap Containing Micro-sponge and Solid:
Dispersion: Optimized formulation of micro-sponge equivalent to 120 mg of drug and solid dispersion equivalent to 50 mg of drug was placed in the formaldehyde treated body of the capsule. The bodies were then plugged with optimized formulation of hydrogel plug to get 5 hrs lag time in intestine. Then join the capsule body and cap and sealed with a small amount of the 5% ethyl cellulose ethanolic solution. The sealed capsules were completely coated with 5% Cellulose Acetate Phthalate (CAP) to prevent variable gastric emptying. The whole system thus produced is modified pulsincap.

Coating of Pulsincap: Dip coating method was used for the development of pulsincap. A 5% w/w solution of Cellulose Acetate Phthalate was prepared by using acetone: ethanol (8: 2) as solvent. The capsules were dipped in 5 % CAP solution and dried in room temperature. Coating was repeated until an expected weight gain of 8 - 12% was obtained.

Optimization of No. of Dips in 5% CAP Solution: The coating of pulsincap was optimized by varying the number of dips from 1 to 6. Minimum number of dips for the capsules to resist disintegration in 0.1 N HCl for a minimum period of 2 hrs is calculated.

Evaluation of Modified Pulsincap:

- The thickness of cellulose acetate phthalate coating was measured using screw gauge and expressed in mm.

- 10 capsules were selected randomly from each batch and weight individually for weight variation.

- *In vitro* dissolution studies of the pulsincap containing optimized formulation of micro-sponge equivalent to 100 mg of drug and solid dispersion equivalent to 50 mg of drug plugged with optimized formulation of hydrogel plug were carried out using USP dissolution test apparatus II (Paddle type). Capsules were tied with the paddle in order to prevent floating. In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used referred to as sequential pH change method. When performing experiments, the pH 1.2 medium was first used for 2 hrs (since the average gastric emptying time is 2 hrs) then removed and the fresh pH 7.4 phosphate buffer saline (PBS) was added. After 3 hrs (average small intestinal transit time is 3 hrs), the medium was removed and fresh pH 6.8 dissolution medium was added for subsequent hrs. 900 ml of the dissolution medium was used at each time. The release was performed at 37 ± 0.5 °C, with a rotation speed of 50 rpm. 2 ml of samples were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through filter paper and analyzed after appropriate dilution using spectrophotometer at corresponding wavelength. Lag time was calculated and recorded.

Stability Studies: The prepared formulations of pulsincap were monitored as per ICH guidelines up to 45 days at accelerating conditions of temperature and relative humidity (40 ± 2 °C/ 75 % ± 5% RH) to check the stability. Samples were withdrawn at predetermined intervals and determined the drug release.

RESULT AND DISCUSSION:

Pre - Formulation Studies:

Identification of Drug:

- **Melting Point:** 92 °C
- **FTIR - Spectroscopy:** The IR spectrum of Ketoprofen is recorded by FTIR spectrometer which was then compared with standard functional group frequencies.

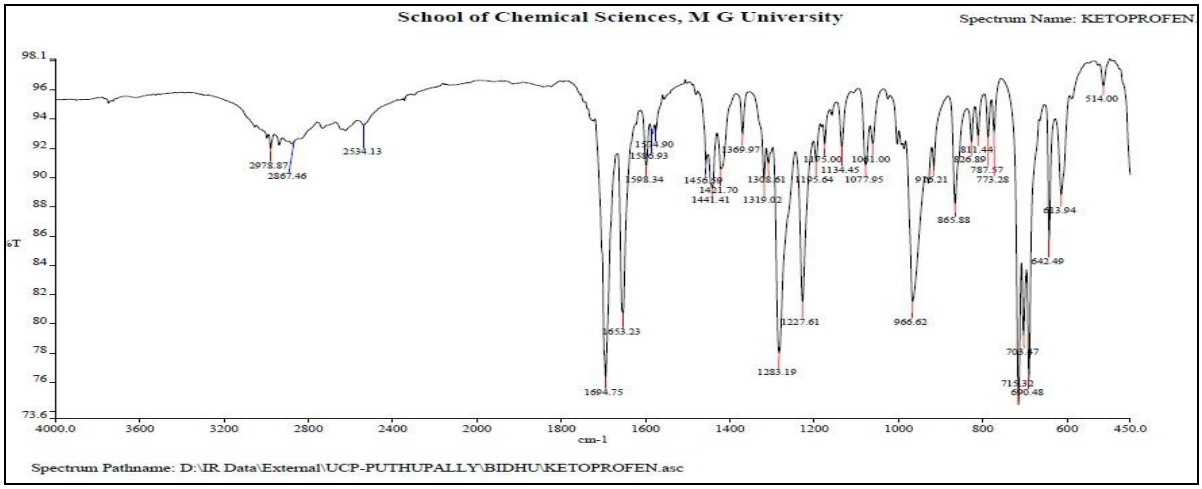

FIG. 1: FTIR SPECTRUM OF KETOPROFEN PURE DRUG

Physico-Chemical Parameter:

Organoleptic Properties:

TABLE 1: ORGANOLEPTIC PROPERTIES

Drug	Nature	Colour	Odour
Ketoprofen	Powder	Colourless	Odourless

Determination of Solubility:

TABLE 2: DETERMINATION OF SOLUBILITY

Solvents	Methanol	Ethanol	Propylene Glycol	Dichloro Methane	Water
Solubility	Readily Soluble	Readily Soluble	Readily Soluble	Readily Soluble	Poorly Soluble

Analytical Methods:

Determination of wavelength maxima (λ_{max}): λ_{max} of Ketoprofen in different media.

TABLE 3: DETERMINATION OF WAVELENGTH MAXIMA (λ_{max})

Media	λ_{max} of Ketoprofen in nm
0.1N HCl pH 1.2	260
PBS pH7.4	260
PBS pH6.8	260

Development of Calibration Curve: Absorbance values of different concentration of Ketoprofen in different media.

TABLE 4: DETERMINATION OF CALIBRATION CURVE

Concentration (µg/mL)	0.1N HCl pH 1.2 Absorbance at 240 nm	PBS pH 7.4 Absorbance at 240 nm	PBS pH 7.4 Absorbance at 240 nm
2	0.125	0.117	0.099
4	0.255	0.250	0.217
6	0.380	0.390	0.353
8	0.515	0.528	0.494
10	0.630	0.684	0.630

Standard Graph of Ketoprofen at pH 1.2, 7.4, 6.8 at 260 nm

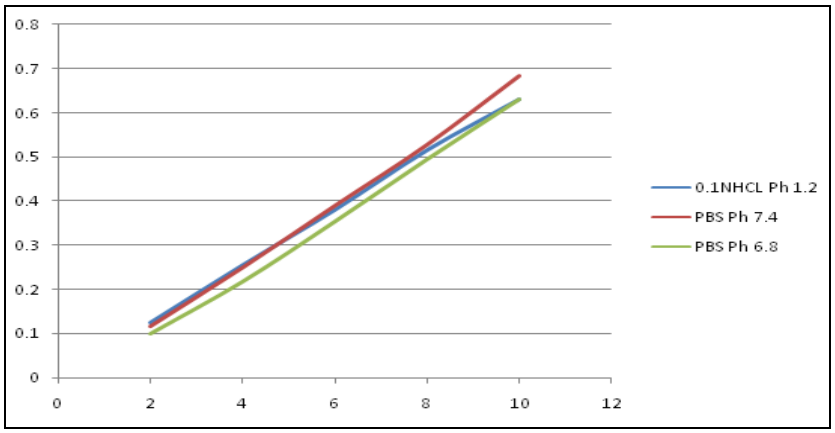

FIG. 2: STANDARD GRAPH OF KETOPROFEN AT pH 1.2, 7.4, 6.8 AT 260 nm

Drug - Polymer Compatibility:

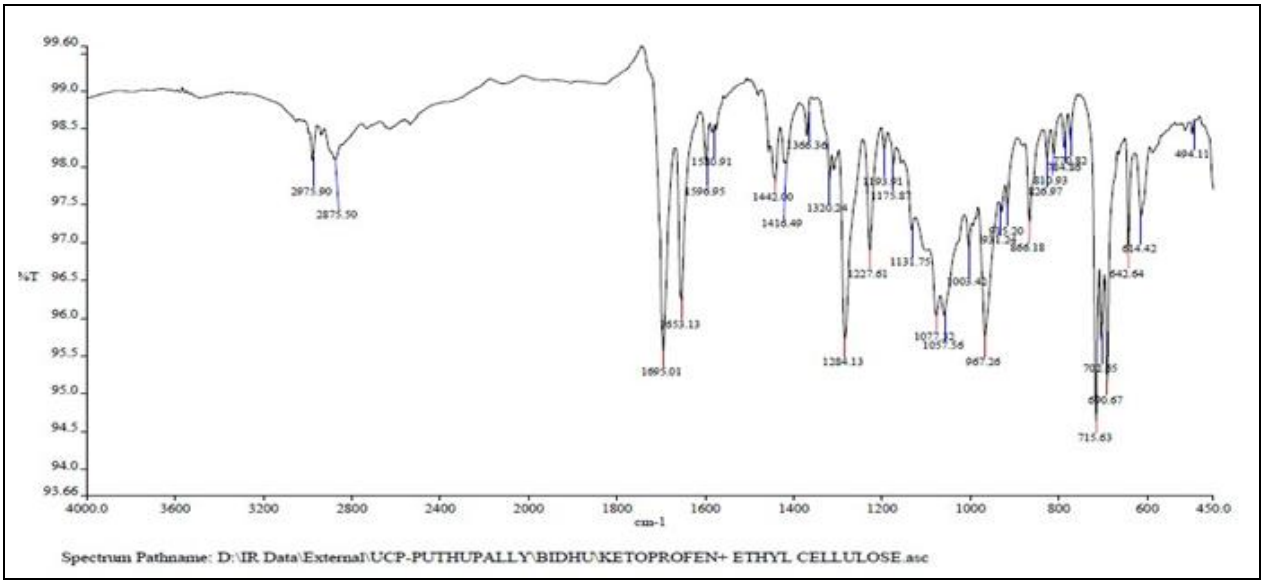

FIG. 3: FTIR SPECTRUM OF KETOPROFEN AND 2-HYDROXYL PROPYL BETA CYCLODEXTRIN MIXTURE

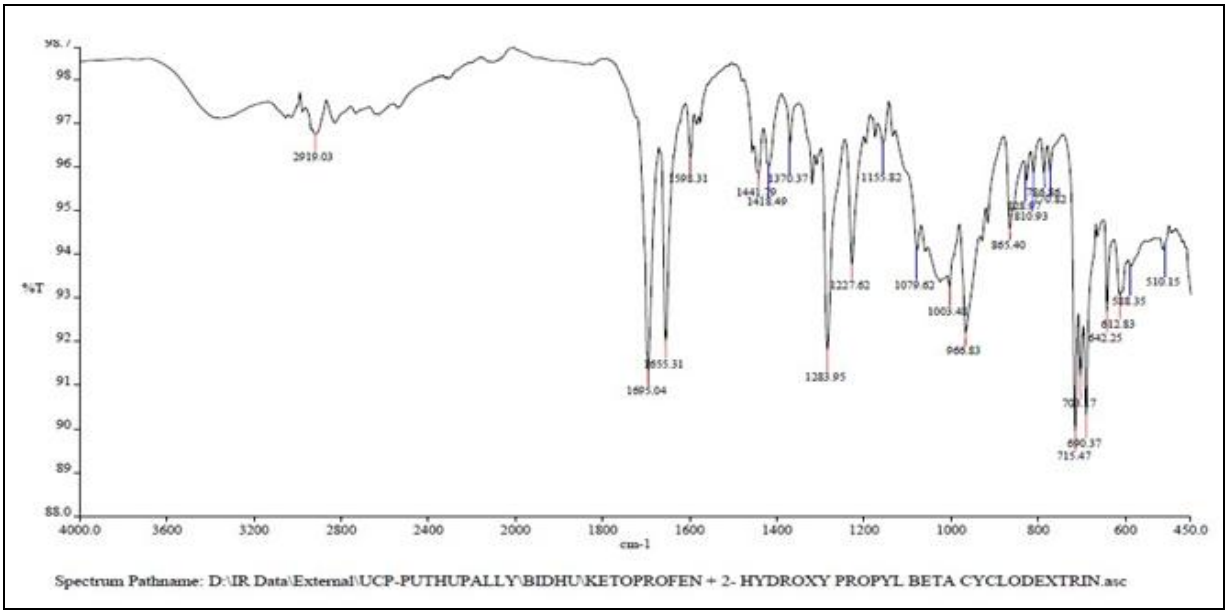

FIG. 4: FTIR SPECTRUM OF KETOPROFEN AND 2-HYDROXYL PROPYL BETA CYCLODEXTRIN MIXTURE

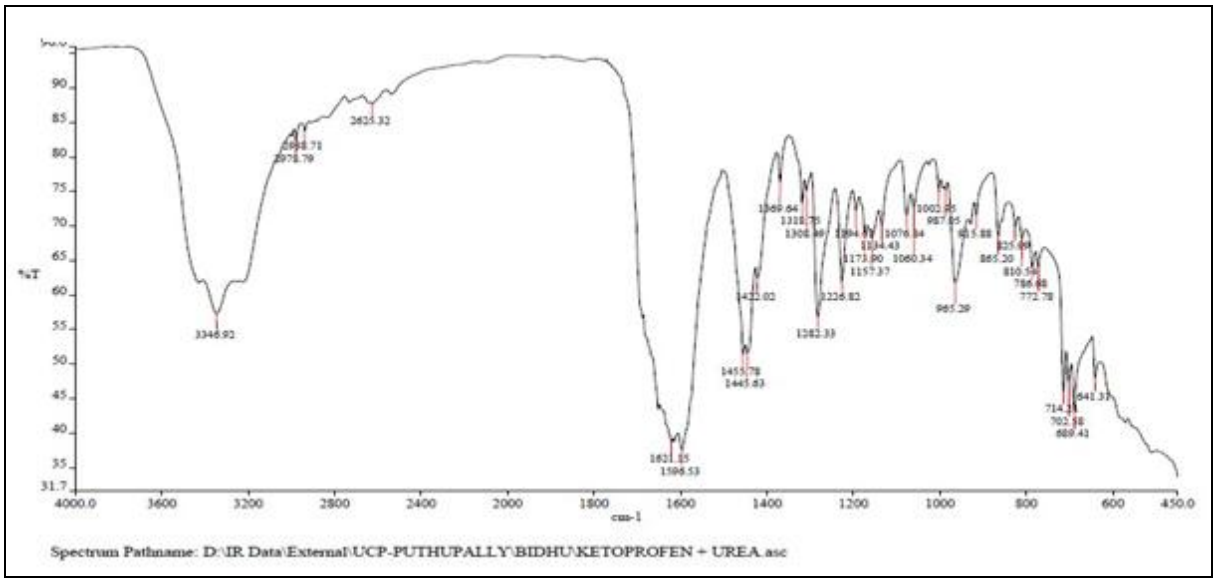

FIG. 5: FTIR SPECTRUM OF KETOPROFEN AND UREA MIXTURE

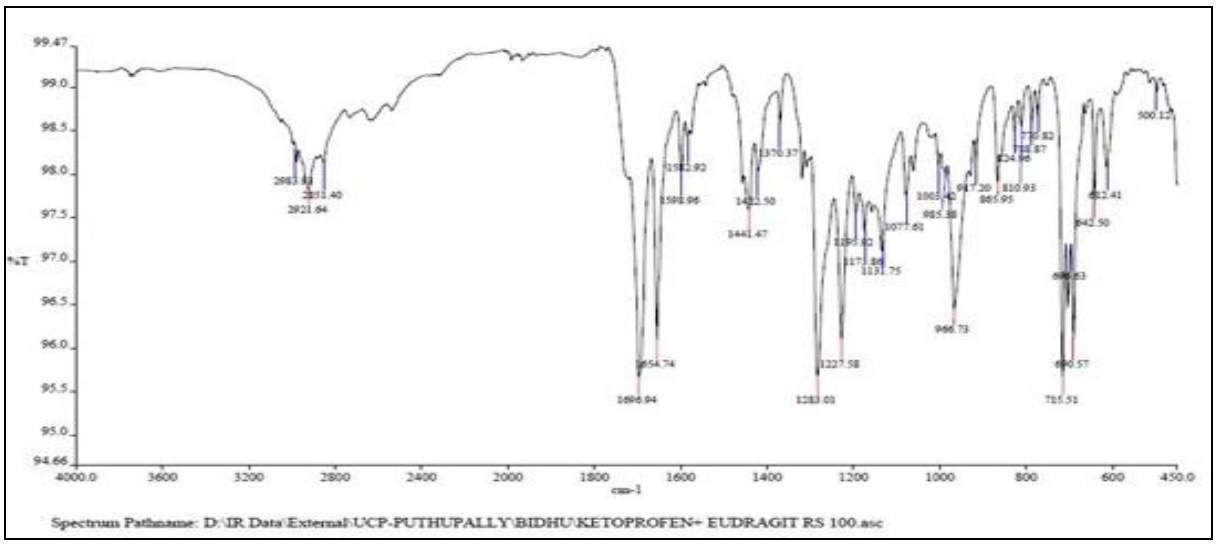

FIG. 6: FTIR SPECTRUM OF KETOPROFEN AND EUDRAGIT RS100 MIXTURE

Solid Dispersion:

Preparation of Solid Dispersion: Ketoprofen solid dispersions were prepared by Kneading as well as Solvent evaporation method by using different polymers like 2- hydroxyl propyl β-cyclodextrin and urea. Prepared formulations were subjected to evaluation.

TABLE 5: SOLID DISPERSION

		Solvent Evaporation			Kneading Method		
		SF1	**SF2**	**SF3**	**SF4**	**SF5**	**SF6**
Ketoprofen		50	50	50	50	50	50
Urea		50	100	150	-	-	-
2-Hydroxyl Propyl Beta Cyclodextrin		-	-	-	50	100	150
Solvent	Ethanol	qs	qs	qs	qs	qs	qs
	WATER	-	-	-	qs	qs	qs

Evaluation of Solid Dispersion:

Determination of Percentage:

TABLE 6: DETERMINATION OF PERCENTAGE

Formulations	Percentage yield (%)
SF1	95.52 ± 1.98
SF2	94.66 ± 1.00
SF3	95.50 ± 0.09
SF4	98.10 ± 0.14
SF5	98.66 ± 0.96
SF6	97.21 ± 2.01

Characterization of Solid Dispersions of ketoprofen:

Determination of Drug Content, Percentage Drug Entrapment and Loading Efficiency: All solid dispersion formulations showed the presence of high drug content and low standard deviations of the results. It indicates that the drug is uniformly dispersed in the powder formulation and solvent evaporation and kneading techniques are highly efficient for the preparation of uniform dispersion.

TABLE 7: CHARACTERIZATION OF SOLID DISPERSION

Sample Code	Drug : Polymer Ratio	Drug Content in 100 mg sd	Percentage Drug Entrapment	Loading Efficiency
SF1	1:1	46.89 ± 0.09	93.78 ± 1.23	46.89 ± 0.09
SF2	1:2	31.23 ± 1.26	93.69 ± 3.01	31.23 ± 1.26
SF3	1:3	23.41 ± 2.89	93.64 ± 2.00	23.41 ± 2.89
SF4	1:1	47.52 ± 2.43	95.04 ± 0.78	47.52 ± 2.43
SF5	1:2	32.17 ± 1.10	96.51 ± 0.58	32.17 ± 1.10
SF6	1:3	24.19 ± 0.67	96.77 ± 0.13	24.19 ± 0.67

Saturation Solubility Study: The results of saturation solubility study showed that β-cyclodextrin is an efficient carrier for solubility enhancement of poorly water soluble drugs. Ketoprofen showed 15 - 17 fold increase in solubility from β - cyclodextrin solid dispersion formulations. The solid dispersion of β-cyclodextrin with drug: carrier ratio 1:2 showed highest solubility. The results of solubility study, namely solubility of pure drug, solubility of drug in solid dispersion sand solubility enhancement ratio are given below.

TABLE 8: SATURATION SOLUBILITY STUDY

Sample Code	Solubility (mg/mL)	Solubility Enhancement Ratio
Pure drug	0.596	-
SF1	4.756	8
SF2	5.993	10
SF3	6.635	11

SF4	8.864	15
SF5	10.108	17
SF6	9.936	17

In-vitro release study of solid dispersion: *In vitro* release rate of Ketoprofen from different formulations of solid dispersion in PBS 6.8 and PBS 7.4 were determined.

TABLE 9: *IN VITRO* DISSOLUTION PROFILE OF KETOPROFEN SOLID DISPERSION IN PHOSPHATE BUFFER pH 7.4

Time	SF1	SF2	SF3	SF4	SF5	SF6
0	0	0	0	0	0	0
15	37.63	34.74	37.89	47.88	44.73	32.61
30	45.81	47.91	50.82	59.23	63.71	58.99
45	56.64	62.17	60.34	68.50	70.60	72.46
60	70.12	72.50	70.4	74.62	79.11	85.16
75	82.29	80.19	84.93	83.13	88.39	93.66
90	95.00	93.17	95.06	97.42	99.27	96.38

TABLE 10: *IN VITRO* DISSOLUTION PROFILE OF KETOPROFEN SOLID DISPERSION IN PHOSPHATE BUFFER pH 6.8

Time	SF1	SF2	SF3	SF4	SF5	SF6
0	0	0	0	0	0	0
15	27.68	32.61	34.17	29.24	40.32	29.66
30	44.62	47.87	53.37	37.58	51.40	40.54
45	54.26	53.84	64.42	57.77	65.54	53.41
60	69.39	61.08	75.05	73.62	71.95	69.11
75	84.69	78.34	88.81	80.75	86.97	85.67
90	95.63	96.61	98.31	92.24	99.46	98.45

Characterization of Optimized Formulation of Ketoprofen Solid Dispersion (Formulation Code: SF5):

SEM Analysis: The shape and surface morphology of pure drug and solid dispersions were as follows.

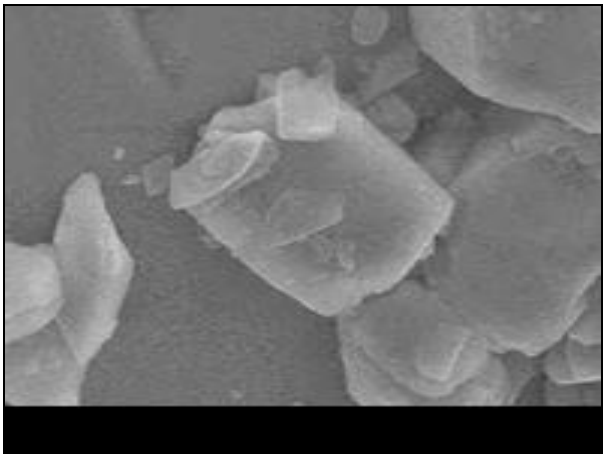

FIG. 7: SEM PHOTOGRAPH OF KETOPROFEN PURE DRUG

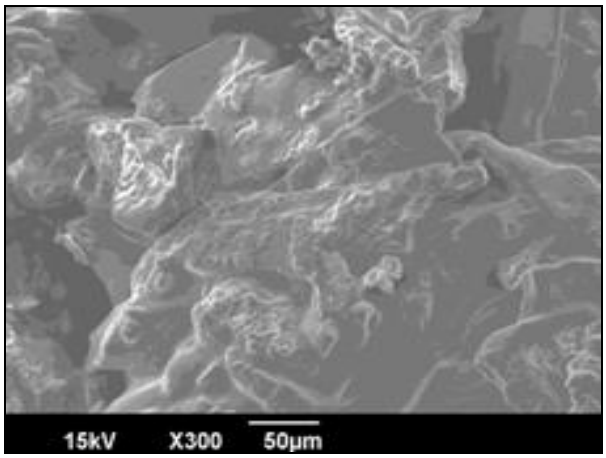

FIG. 8: SEM PHOTOGRAPH OF KETOPROFEN SOLID DISPERSION (SF5)

X-Ray diffractogram of pure Ketoprofen shows numerous peaks which are intense and sharp, shows the crystalline nature of the drug. The diffraction angles are similar as far as both X-ray diffraction patterns are concerned. Only a decrease in reflection intensities can be pointed out for the solid dispersion sample. This suggests that crystallinity of the drug in solid dispersion could be inferior. This amorphous nature is thought to enhance the solubility of ketoprofen. Study shows change in crystal pattern of the drug in the solid dispersion system. This change in crystal pattern accounts for increased solubility of solid dispersions.

DSC: Ketoprofen showed a sharp peak at 90 °C, which is related to the melting point of the pure drug. The DSC curve of the formulation doesn't showed any peaks corresponding to the melting point of pure drug. This shows that the drug Ketoprofen has been molecularly dispersed inside the 2- hydroxyl propyl beta cyclodextrin molecules.

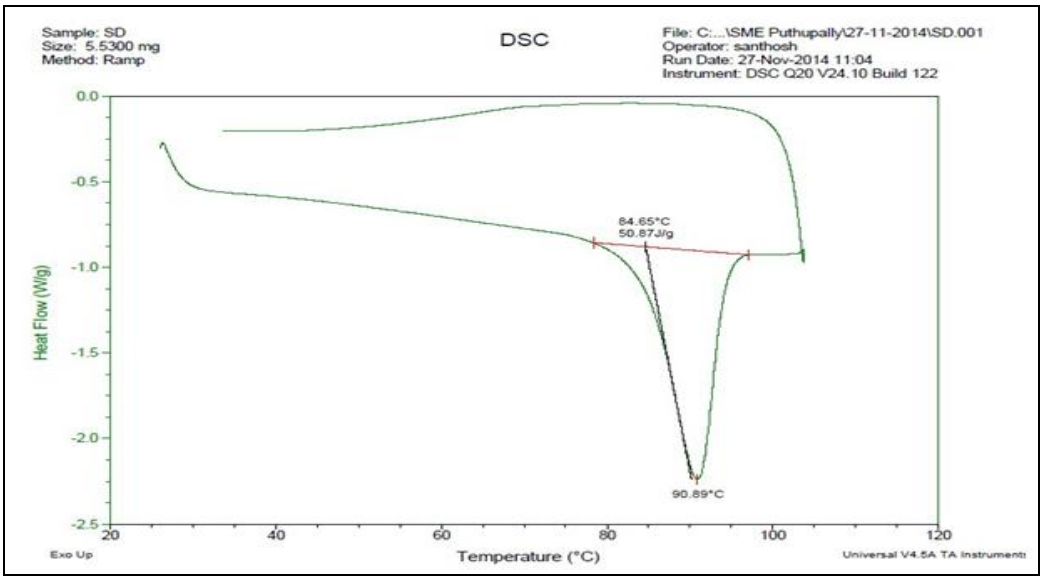

FIG. 9: DSC CURVE OF KETOPROFEN PURE DRUG

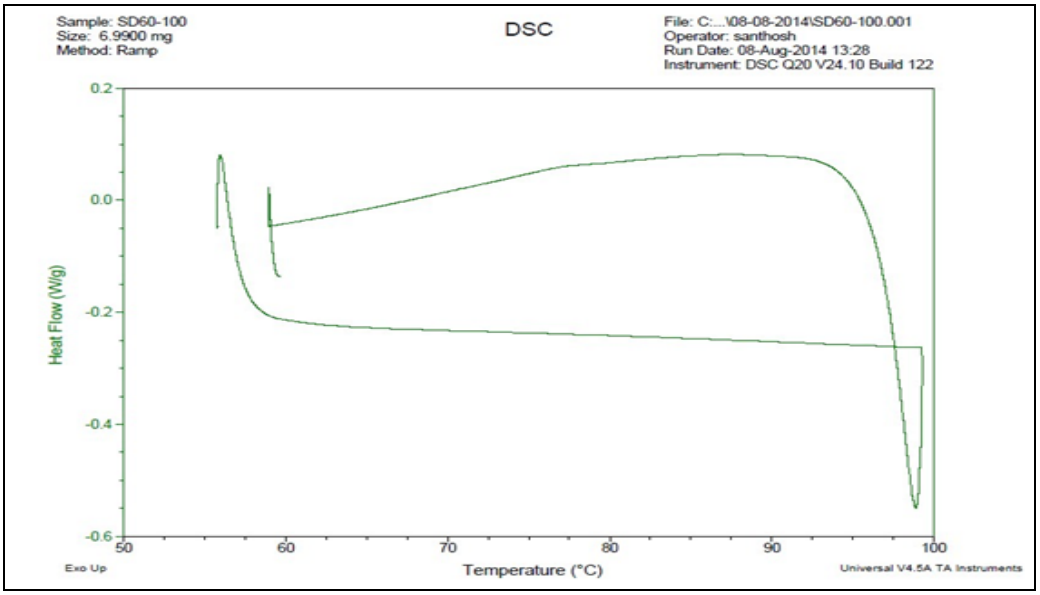

FIG. 10: CURVE OF KETOPROFEN SOLID DISPERSION (SF5)

Preparation of Microsponge:

TABLE 11: COMPOSITION OF DIFFERENT MICROSPONGE FORMULATION

	F1	F2	F3	F4	F5	F6	F7	F8
Internal phase								
Ketoprofen (mg)	120	120	120	120	120	120	120	120
Ethyl Cellulose (mg)	240	240	240	240	-	-	-	-
Eudragit RS 100 (mg)	-	-	-	-	240	240	240	240
Ethanol (Ml)	20	20	10	10	10	10	20	20
Dichloromethane (Ml)	-	-	10	10	10	10	-	-
External Phase								
Polyvinyl alcohol (mg)	50	75	50	75	50	75	50	75
Distilled Water (ml)	100	100	100	100	100	100	100	100

Evaluation of Drug Loaded Microsponges:

TABLE 12: PARTICLE SIZE ANALYSIS

Sample Code	Average Particle Size (µm)
SF1	89.49 ± 0.04
SF2	63.21 ± 0.61
SF3	82.96 ± 1.33
SF4	57.00 ± 1.90
SF5	37.56 ± 0.04
SF6	42.39 ± 1.52
SF7	61.98 ± 0.86
SF8	50.56 ± 0.39

TABLE 13: DETERMINATION OF PERCENTAGE YIELD

Sample Code	Percentage Yield (%)
SF1	91.10 ± 1.00
SF2	94.71 ± 1.73
SF3	91.09 ± 2.98
SF4	85.73 ± 2.09
SF5	84.29 ± 0.86
SF6	82.93 ± 0.69
SF7	80.27 ± 1.82
SF8	80.73 ± 0.42

TABLE 14: DETERMINATION OF DRUG CONTENT, PERCENTAGE DRUG ENTRAPMENT AND LOADING EFFICIENCY

Sample Code	Drug Content in 100 mg Microsponge	Percentage Drug Entrapment	Loading Efficiency
F1	28.46 ± 0.63	85.40 ± 1.90	28.46 ± 0.63
F2	29.51 ± 0.56	88.53 ± 1.70	29.51 ± 0.56
F3	25.23 ± 0.23	75.69 ± 0.71	25.23 ± 0.23
F4	28.15 ± 0.64	84.46 ± 1.94	28.15 ± 0.64
F5	22.20 ± 1.94	66.62 ± 5.84	22.20 ± 1.94
F6	22.93 ± 1.63	68.81 ± 4.89	22.93 ± 1.63
F7	24.18 ± 0.82	72.56 ± 2.47	24.18 ± 0.82
F8	26.69 ± 0.27	80.09 ± 0.81	26.69 ± 0.27

In- vitro Release Study:

TABLE 15: CUMULATIVE PERCENTAGE RELEASE OF DRUG FROM MICROSPONGES INBUFFER pH 7.4

Time (in hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	51.04	26.38	38	35.4	56.56	56.04	28.41	26.31
2	55.82	34.11	54.76	53.4	59.52	60.04	52.66	53.45
3	62.47	38.96	61.66	54.77	72.46	69.56	56.66	55.35
4	67.25	45.2	67.5	61.68	78.06	79.1	64.87	63.04
5	74.44	52.55	69.4	68.57	83.12	85.24	69.41	67.05
6	81.09	58.82	72.38	73.12	87.18	88.22	73.43	71.86
7	86.16	63.09	81.66	76.08	88.58	90.68	77.45	78.24
8	92.05	71.26	85.18	80.11	94.2	93.42	81.23	82.02
11	97.93	85.11	88.7	86.25	99.04	97.98	88.41	89.99
22	102.25	97.98	98.78	93.97	102.82	99.14	94.3	95.08
23	102.89	98.57	99.94	96.98	103.46	99.78	95.45	95.97
24	103.25	99.23	100.84	98.64	104.64	101.72	96.35	97.66

Effect of Physical Parameters on Formulation of Microsponge:

Effect of Internal Phase on the Production of Microsponges: The particle sizes of microsponges were directly proportional to the viscosity of dispersed phase. Hence larger particle size was obtained due to increasing the amount of polymer available per microsponge. With increase in polymer concentration, viscosity also increased and can impede drug mobility in the droplets which was observed as an increase in the EE%. And also the EE% may be improved simply by greater

proportion of polymer with respect to the amount of drug available.

Effect of Polymer Type on the Production of Microsponges: Concerning the polymer type Ethyl cellulose significantly increased the mean particle size when compared to Eudragit RS100. This could probably be due to increasing the viscosity of the dispersed phase containing EC, as the formed globules can be hardly divided into smaller particles, hence larger droplets were formed and the mean particle size was increased. When compared with Eudragit RS100, Ethyl cellulose significantly shows improved drug entrapment efficiency. This may be due to increased viscosity of the internal phase containing the ethyl cellulose polymer, reducing the drug mobility outside the formed droplets, and hence entrapping larger amount of Ketoprofen.

Effect of PVA Concentration on the Production of Microsponges: PVA concentration has a key role to play in the preparation of microsponges. Entrapment efficiency of Ketoprofen increases with increase in PVA concentration due to the non ionic nature of the emulsifier. And also there was a reverse proportionality between the PVA concentration and the mean particle size. This may due to decreasing the surface tension of the continuous phase upon increasing the surfactant concentration which results in reduction of the particlesize. 0.75% PVA solution provided good microsponges.

Effect of Solvent Type: This study reveals that ethanol shows significantly increased entrapped efficiency and particle size. This may be due to the higher boiling point of ethanol (78.4 ºC) compared to dichloromethane (40 ºC). So ethanol would evaporate more slowly than dichloro methane.

Surface Morphology of Microsponge by Scanning Electron Microscopy (SEM): From SEM studies it was found that the samples had porous and almost spherical nature. The pores were induced by the diffusion of the solvent from the surface of the microparticle.

Evaluation of Formaldehyde Treated Capsules:

Physical Tests:

Identification Attributes: The size‘0’capsules chosen were opaque with yellow coloured body and redcap. They are lockable type, odourless, soft and sticky. After treating with formaldehyde, there were no significant changes in the capsule body except for the stickiness. The body of the capsules were hard and non-sticky even when touched with wet hand.

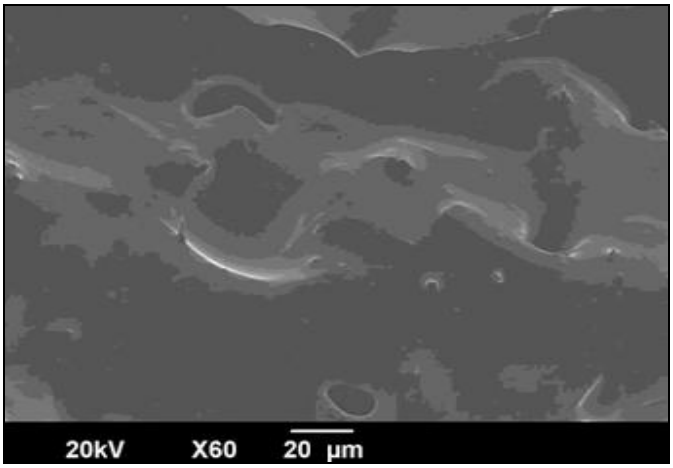

FIG. 11: SEM PHOTOGRAPH OF FORMULATION F2 (WHOLE IMAGE)

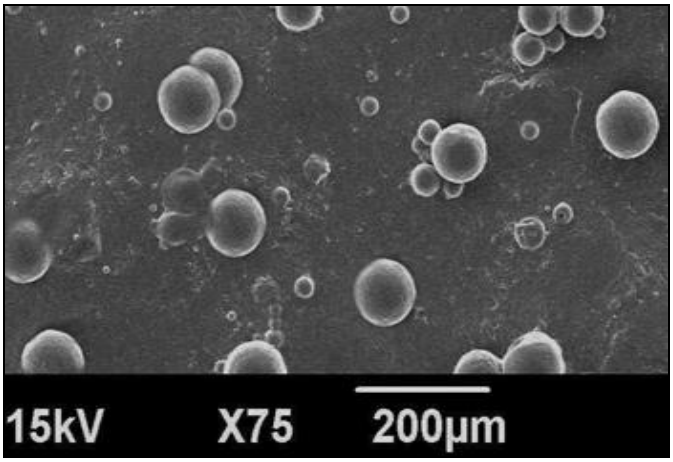

FIG. 12: SEM PHOTOGRAPH OF MICROSPONGE FORMULATION F2 (SURFACE IMAGE)

Visual Defects: Among 25 capsules body which were treated with formaldehyde, 3 capsules were found to be shrunk or distorted into different shapes due to the complete loss of moisture.

Solubility Test for Formaldehyde Treated Capsules: When the capsules were subjected to solubility studies in different buffers for 24 hrs the following observations were made on all the case of normal capsules, both cap and body dissolved within 15 minutes. In the case of formaldehyde treated capsules, only the cap dissolved within 15 min while the body remained intact for about 24 hrs.

Chemical Test:

Qualitative Chemical Test for Free Formaldehyde: The formaldehyde capsules were tested for the presence of free formaldehyde. The sample solution was not more intensely coloured than the reference solution indicating that less than 20 µg of free formaldehyde is present in 25 capsule bodies.

TABLE 16: EVALUATIONS OF THE POWDER BLEND

*Bulk Density	*Tapped Density	*Carr's Index	*Hausner Ratio	*Angle of Repose
0.549 ± 0.051	0.675 ± 0.03	18.66 ± 0.875	1.229 ± 0.02	29.72 ± 0.33

Evaluation of Hydrogel Plug: The plugs were evaluated using various parameters. The appearance of the plugs was elegant. There were no considerable differences in the thickness and diameter from tablet to tablet. The weight variation was found to be within the limit prescribed by the USP. Friability and hardness were also in the permissible limit. The weight variation and friability were found to be within acceptable limits according to USP. There is no considerable difference in the thickness, diameter and hardness of the tablet from batch to batch.

TABLE 17: EVALUATION OF HYDROGEL PLUG

Sample Code	Thickness (mm)	Diameter (mm)	Weight Variation (%)	Hardness (kg/cm^2)	Friability (%)
HP1	4.28 ± 0.09	7.03 ± 0.25	0.85 ± 0.61	3.50 ± 0.44	0.59
HP2	4.20 ± 0.15	7.03 ± 0.01	1.05 ± 0.21	3.79 ± 0.07	0.73
HP3	4.35 ± 0.07	7.04 ± 0.09	0.96 ± 0.56	3.81 ± 0.17	0.69

***In vitro* Dissolution Testing:**

TABLE 18: *IN VITRO* DISSOLUTION TESTING

Time (hrs)	Cumulative Percentage Release		
	HP1	HP2	HP3
0	0.000	0.000	0.000
1	0.587 ± 0.56	0.000	0.000
2	50.729 ± 0.73	2.893 ± 0.05	0.981 ± 0.06
3	82.442 ± 0.06	52.784 ± 0.43	0.996 ± 0.54
4	98.738 ± 1.43	79.751 ± 0.14	2.980 ± 0.09
5	100.091 ± 0.09	99.650 ± 0.74	3.011 ± 0.04
6		100.065 ± 0.5	67.781 ± 0.34
7			98.660 ± 0.43
8			100.358 ± 0.6

The lag time is controlled by a plug, which gets pushed away by swelling or erosion, and the drug is released as a "Pulse" from the insoluble capsule body. A swellable hydrogel plug was used to seal the drug contents into the capsule body. When this capsule came in contact with the dissolution fluid, it swelled; and after a lag time, the plug pushed itself outside the capsule and rapidly released the drug. The lag times observed by different formulation are as follows:

TABLE 19: THE LAG TIME OBSERVED BY DIFFERENT FORMULATION AREAS

Formulation Code	Lag Time (hrs)
HP1	1
HP2	2
HP3	5

Coating of Pulsincap: Dipcoating in 5% w/w solution of Cellulose Acetate Phthalate was used for the development of pulsincap.

Optimization of No. of Dips in 5% CAP Solution: Minimum number of dips for the capsule stores is disintegration in 0.1N HCl for a minimum period of 2 hrs is calculated and it was observed that on fifth dip the capsules resist disintegration in 0.1N HCl for a period of 2 hrs 05 min. Therefore 5 dips were selected for the development of Pulsincap.

TABLE 20: PERCENTAGE WEIGHT GAIN AND TIME TAKEN FOR CAPSULES TO DISINTEGRATE

No. of dips	*Percentage weight gain	Time taken for the capsules to disintegrate
1	1.9%	28 min
2	4.6%	52 min
3	6.9%	1 hour15 min
4	7.5%	1 hour45 min
5	9.1%	2 hour10 min
6	11.3%	3 hour05 min

*Average of triplicate sets

Modified Pulsincap:

Preparation of Modified Pulsincap:

TABLE 21: FORMULATION TABLE FOR MODIFIED PULSINCAP

Solid Dispersion	SF5 (Drug equivalent to 50 mg)
Microsponge	F2 (Drug equivalent to120 mg)
Hydrogel plug	HP3 (HPMC: Lactose = 1:3)
Capsule Shell	Formaldehyde treated capsule body and untreated cap
Coating	5 dips in 5% CAP Solution

Evaluation of Modified Pulsincap:

Thickness and Weight Variation of Pulsincap: The thickness of cellulose acetate phthalate coating and weight variation of pulsincap was determined as shown in **Table 22**.

TABLE 22: EVALUATION OF PULSINCAP

Thickness (mm)	Weight Variation (%)
0.065 ± 0.07	0.528 ± 0.42

In vitro Release Study:

TABLE 23: *IN VITRO* RELEASE STUDY

Time (hrs)	Cumulative percentage release (%)
0	0.00
1	0.00
2	0.00
3	0.00 ± 0.41
4	0.36 ± 0.54
5	1.02 ± 0.40
6	1.96 ± 1.12
7	2.08 ± 0.09
8	52.98 ± 0.03
9	65.34 ± 1.04
12	84.65 ± 0.77
21	96.31 ± 0.35
22	98.98 ± 1.50
23	99.03 ± 0.86
24	100.14 ± 0.05

Stability Studies: The stability of the formulated pulsincap was monitored as per ICH guidelines upto 45 days at accelerated stability conditions of temperature and relative humidity. The comparison of cumulative percentage drug release from pulsincaps on day 1 and on day 45 are given in table and in figure.

TABLE 24: CUMULATIVE PERCENTAGE DRUG RELEASE FROM PULSINCAP IN ACCELERATED STABILITY STUDIES

Time (hrs)	Cumulative percentage release	
	Day 1	Day 45
1	0.00	0.00
2	0.00	0.00
3	0.00	0.00
4	0.00 ± 0.45	0.00 ± 0.417
5	0.38 ± 0.54	0.38 ± 0.50
6	1.22 ± 0.98	1.23 ± 0.04
7	2.01 ± 1.16	1.98 ± 1.14
8	2.16 ± 0.99	2.13 ± 0.09
9	54.98 ± 0.23	54.05 ± 0.93
12	69.34 ± 0.04	68.12 ± 1.14
21	88.65 ± 1.77	87.22 ± 0.97
22	99.91 ± 0.45	99.78 ± 0.35
23	99.92 ± 1.16	99.78 ± 1.26
24	101.94 ± 0.38	101.03 ± 0.86

The dissolution profile showed no great differences in the drug release. The lag time of drug release was also found to be same as that of the first day. This proved that the pulsincap retained the expected stability requirement with regard to the dissolution profile.

SUMMARY AND CONCLUSION: From the present study, it can be concluded that modified pulsincap of Ketoprofen can be prepared for the chrono-therapeutic drug delivery in arthritis. A preformulation study helps in identification of drug and revealed that the drug shows no interactions with polymers used. Solid dispersion of Ketoprofen was prepared for immediate release. Solid dispersion with different drug: polymer ratio were prepared and optimized for the best formulation. F5 which shows complete drug release within 1.5 hrs and is taken as the best formulation Ketoprofen microsponge was prepared for sustained release.

The physical characterization showed that entrapment efficiency (EE %) and mean particle size were increased with increasing polymer fraction in which EC significantly increased the EE% and mean particle size when compared to Eudragit RS100. With increase in PVA concentration, EE% increased and mean particle size decreased. The minimum concentration of PVA required to produce microsponges was found

to be 0.50% w/v. Regarding solvent type, ethanol shows increased EE% and particle size as compared to dichloro methane.

The *in vitro* release studies of micro-sponges in pH 7.4 and pH 6.8 showed a sustained release pattern more than 24 hrs. The particle size was also within the limit. Formulation F2 was found as the best formulation by analysing the data. Drug release mechanism was assumed to be 'diffusion controlled'. From the release exponent data, the diffusional release was found to follow non fickian transport. The gelatine capsule bodies were cross linked by formaldehyde treatment Evaluation of cross linked gelatine capsule bodies done and found to be satisfactory. The Hydrogel plug was prepared with different HPMC - Lactose ratio, HP3 which gave 5 hrs lag time was selected as the optimum and used in pulsincap dosage form.

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CONFLICT OF INTEREST: Nil

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