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Design and Evaluation of Aceclofenac Nanoclay for Improved Oral Bioavailability

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Abstract :

In this studies the significant effect of nanoclay on improving the dissolution rate and bioavailability of Aceclofenac has been demonstrated by preparation of nanoclay. The pure drug with different concentration of clay(0.5-1%)were characterized in terms of solubility, drug content, particle size, thermal behaviour (DSC), morphology(SEM), in vitro performance was assessed by pharmacokinetic studies. The particle size of the prepared nanoclay was drastically reduced during the formulation process .The DSC showed a there is no chemical interaction between drug, clay, physical mixture. The dissolution studies demonstrated a formulation increase in the dissolution rate in comparison with pure drug. *In vivo* studies revealed that the optimized formulation provided a rapid pharmaceutical response in rats besides exhibiting improved pharmacokinetics parameters in rats.

Keywords: Aceclofenac, Nanoclay, Drug content, in vivo, Montmorillonite, Release kinetics.

INTRODUCTION:

Oral route is the most preferable and acceptable route of drug administration because of low cost and ease of administration which leads to high patient compliance. But lipophilic drugs shows low oral bioavailability due to some reasons like poor aqueous solubility, high first pass metabolism, ect. Oral drug delivery is continuously looking into newer platforms due to realization of the factors like poor drug solubility and/or absorption, rapid metabolism, high fluctuation in the drug plasma level and variability due to food effect which are playing major role in disappointing in vivo results leading to failure of the conventional delivery system. Since the last decade, the oral drug delivery has taken a new dimension with the increasing application of lipid as a carrier for the delivery of poorly water soluble, lipophilic drugs [1].

Nanoclay are minerals which have a high aspect ratio and with at least one dimension of the particles in the nanometer range. The most important factor is the aspect ratio of the clay minerals.The clays having a platy structure and a thickness of less than one nanometer are the clays of choice .The length and width of the choice clays are in the micron range. Aspect ratios of the choice clays are 300:1 to 1500:1 range [2,3].

In order to modify drug release, a very interesting possibility is to use clay mineral–polymer composites. Although clay minerals and polymers were frequently used in their pure form as single drug carriers, this type of DDS often did not meet all the requirements [4]. The preparation of polymer-layered silicate composite offered the possibility of improving the properties of each single component: those of the clay mineral particles alone (stability of the clay mineral dispersions and changes in its ion exchange behaviour) and, more frequently, those of the polymer (mechanical properties, swelling capacity, film forming abilities, rheological properties, bio adhesion or cellular uptake) (Viseras et al.2008). Montmorillonite (MMT) belongs to the smectite group, composed of silica tetrahedral sheets layered between alumina octahedral sheets at a ratio of 2:1, respectively (Patel et al., 2006).

MATERIALS AND METHODS

Materials:

Aceclofenac was obtained from Fourts india Ltd,Chennai. MMT, TPGS [alpha tocopherol glycol 1000 succinate, Pluronic F-127 were purchased from Sigma-Aldrich, Mumbai. Ethanol and potassium Dihydrogen phosphate were purchased from Qualigen Fine Chemicals, Mumbai. The solvents for analysis used were of HPLC grade. All other chemicals used were of analytical grade.

Preformulation study:

Preformulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of a new drug substance, in order to develop stable, safe and effective dosage forms. Ideally, the Preformulation phase begins early in the discovery process such that appropriate physical, chemical data is available to aid in the selection of new chemical entities that enter the development process. During this evaluation possible interaction with various inert ingredients intended for use in final dosage form are also considered [5].

Solubility studies:

The solubility of Aceclofenac was determined in various solvents by adding an excess amount of drug to 10 ml of solvents in conical flasks. The flasks were kept at 25± 0.5 ^{o}C in isothermal shaker for 24 hours to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 4000rpm for 15 min. The supernatant was taken and filtered through 0.45-µm membrane filter. The concentration of Aceclofenac was determined in the supernatant after suitable dilution by using UV-Visible Spectrometer at 276nm. [6]

Compatibility studies:

Fourier Transform Infrared Spectroscopy (FTIR):

I.R spectroscopy can be used to investigate and predict any physiochemical interaction between different components in a formulation and therefore it can be applied to the selection of suitable chemical compatible excipients while selecting the ingredients, we would chose, those which are stable, compatible, cosmetically and therapeutically acceptable [7]. Infrared spectra matching approach was used for detection of any possible chemical interaction between the drug, clay and surfactants. A physical mixture of drug, lipid and surfactants was prepared and mixed with suitable quantity of potassium bromide. This mixture was compressed to form a transparent pellet using a hydraulic press at 15 tons pressure. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer (FTIR 8400 S, Shimadzu). The IR spectrum of the physical mixture was compared with those of pure drug, lipid and surfactants and peak matching was done to detect any appearance or disappearance of peaks.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric analysis was used to characterize the thermal behaviour of the drug, lipids and their physical mixtures. Sample was crimped in standard aluminium pans and heated from 20 to 400 ºC at a heating rate of 10 ºC/min under constant purging of dry nitrogen at 30ml/min. An empty pan, sealed in the same way as the sample, was used as a reference. DSC thermograms were obtained using an automatic

thermal analyzer system. Temperature calibration was performed using Indium calibration reference standard [8].

Development of calibration curve:

Standard stock solution was prepared by dissolving 50 mg of drug in 50 mL of ethanol to get concentration of 1 mg/mL . 10 mL of stock solutions were further diluted to 100 mL with phosphate buffer (pH-6.8,prepared as per I.P.) to get a working solution of concentration 100 µg/mL of Aceclofenac , respectively. The solutions were further diluted with phosphate buffer and scanned in the wavelength range 276 nm. The drugs obey Beer's law within the concentration range of 10-50 µg/mL for Aceclofenac. Standard curve between concentration and absorbance was plotted.

Preparation of Aceclofenac /Montmorillonite nanoclay

In the preparation of the AC/MMT, 1 g of MMT was dispersed in 30 ml of distilled water with vigorous stirring for 0.5 h at a room temperature. Various amounts of AC were dissolved in 150 ml of distilled water (pH value adjusted to 8.5 with H_3PO_4). Then, the two solutions were mixed together and kept at80 ◦C with vigorous stirring for 3 h. After centrifugation, the sediment was washed with deionized water, and then further dried in a vacuum oven at 80 ◦C for 24h.

Preparation of drug loaded batches:

Drug loaded nanoclay were prepared MMT as polymers and Aceclofenac as drug, F-127 was used as hydrophilic surfactant.

Composition of drug loaded batches were given in table 1.

Evaluation of nanoclay

Particle size and zeta potential

Particle size and zeta potential of the solid nanoclay were measured by photon correlation spectroscopy using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK), which works on the Mie theory. All size and zeta potential measurements were carried out at 25^{o}C using disposable polystyrene cells and disposable plain folded capillary zeta cells, respectively, after appropriate dilution with original dispersion preparation medium [9].

Polydispersity Index:

Polydispersity was determined according to the equation, [10]

Polydispersity = D (0.9)-D (0.1)/ D (0.5)

Where,

D (0.9) corresponds to particle size immediately above 90% of the sample.

D (0.5) corresponds to particle size immediately above 50% of the sample.

D (0.1) corresponds to particle size immediately above 10% of the sample. **(Muller R.H*et. al.*, 2008)**

Intercalation efficiency and Drug content

Accurately weighed amount of drug was added to accurately weighed nanoclay in a suitable solvent .the solution was stirred for 30 minutes. The solution was then centrifuged. The supernant layer was taken and measured in UV spectrophotometer. The absorbance obtained were extra plotted in the calibration graph to get the concentration. And the concentration is kept in formula to get loading and entrapment efficiency.

%IE = [(Drug added - "unintercalated drug")/Drug added] *100

%LC = [intercalated Drug/nanoclay weight] * 100

Morphological Study (Scanning electron microscopy):

External morphology of nanoparticles was determined using Scanning Electron Microscopy (SEM) [11]. Samples were diluted with ultrapurified water to obtain a suitable concentration. Then the samples were spread on a sample holder and dried using vacuum. They were subsequently coated with gold (JFC 1200 fine coater, Japan) and examined by a Scanning Electron Microscopy (SEM).

***In vitro* release studies:**

The release of aceclofenac from the nanoclay was studied under sink conditions. Aceclofenac loaded nanoclay equivalent to 40mg were suspended in 1ml of dissolution media (Phosphate buffer6 .8) and put in dialysis bags (MWCO 12000, HiMedia). The dialysis bags were placed in 100mL of dissolution medium and stirred under magnetic stirring at 37^{o}C. 5 ml Aliquots of the dissolution medium were withdrawn at each time interval and the same volume of fresh dissolution medium was added to maintain a constant volume. Samples withdrawn from pH 6.8 phosphate buffer were analyzed for aceclofenac content spectrophotometrically against solvent blank [12].

Release kinetics

Invitro dissolution has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of bioequivalence. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where f_t is the function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. To compare dissolution profiles between two drug products model dependent (curve fitting), statistic analysis and model independent methods can be used **(Costa P., et al., 2001).**In order to elucidate mode and mechanism of drug release, the *invitro* data was transformed and interpreted at graphical interface constructed using various kinetic models. The zero order release Eq. (1) describes the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems, matrix tablets with low soluble drugs, coated forms, osmotic systems etc., where the drug release is independent of concentration [13].

$$Q_t = Q_o + K_o t \quad (1)$$

where, Q_t is the amount of drug released in time t, Q_o is the initial amount of the drug in the solution and K_o is the zero order release constant

The first order Eq. (2) describes the release from the system where release is concentration dependent e.g. pharmaceutical dosage forms containing water soluble drugs in porous matrices.

$$\log Q_t = \log Q_o + K_1 t / 2.303 \quad (2)$$

where Q_t is the amount of drug released in time t, Q is the initial amount of drug in the solution and K is the first s order release constant.

Higuchi described the release of drug from insoluble matrix as a square root of time

$$Q_t = K_H \sqrt{t} \quad (3)$$

where, Q_t is the amount of drug released in time t, K_H is Higuchi's dissolution constant.

Haemocompatability studies

Blood samples of healthy human volunteers were obtained from blood bank of government hospital, Ooty in evacuated siliconized glass tube containing sodiumcitrate. Red blood cells were separated by centrifugation at 1500 rpm for 10 min and then washed 3 times with saline. Stock solution of erythrocytes in Saline water was prepared such that the cell count was 1×108 cells/ml. Equalvolumes of RBC suspension and nanoparticles dispersion were suspended in amicrocentrifuge tube such that the final concentrations of nanoparticle dispersion and nanoparticles were 150- 1000 µg/ml and incubated separately at 37 ºC for 1 h. 1% Triton X and Saline water were used as positive and negative controls respectively. After 1 h the tubes were centrifuged at 1500 rpm for 10 min and the hemoglobin released in the supernatant was detected by UV absorbance at 276 nm. All measurements were performed in triplicate (n=3) and the SD was calculated. The percent haemolysis was calculated by the formula;

$$\%\text{haemolysis} = \frac{\text{ABS Sample} - \text{ABS 0\%}}{\text{ABS 100\%} - \text{ABS 0\%}}$$

Where Abs sample is the absorbance of supernatant of erythrocyte and nanoparticles suspension.

Abs0% is the absorbance of supernatant of erythrocyte and PBSsuspension.

Abs100% is the absorbance of supernatant of erythrocyte and Triton X [13].

In vitro and in vivo studies

Determination of mitochondrial synthesis by MTT assay [15]

i. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0x105 cells/ml using DMEM medium containing 10% FBS.

ii. To each well of a 96 well microtitre plate, 100µl of the diluted cell suspension (approximately 10,000 cells/well) was added.

iii. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100 [illegible] cell at different concentrations were prepared in maintenance media were added per well to the partial monolayer in microtitre plates. The plates were then incubated at 37^0C for 48 hrs in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours.

iv. After 48 hours, the sample solutions in the wells were discarded and 20ml of MTT (2mg/ml) in MEM-PR (MEM without phenol red) was added to each well.

v. The plates were gently shaken and incubated for 3 hours at 37ºC in 5% CO_2 atmosphere.

vi. The supernatant was removed and 50ml of iso-propanol was added and the plates were gently shaken to solubilize the formed formazan.

vii. The absorbance was measured using a microplate reader at a wavelength of 540nm.

The percentage growth inhibition was calculated using the following formula and concentration of drug or test samples needed to inhibit cell growth by 50% values were generated from the dose-response curves for each cell line.

$$\% \text{ Cell viability} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

HPLC Bioanalytical method

Reverse phase HPLC method is the most popular mode for analytical and preparative separations of the compounds in chemical, biological, pharmaceutical and food samples. In reversed phase mode, the stationary phase is non polar and the mobile phase is polar. The polar compounds gets eluted first in this mode and non polar compounds are retained for longer time. In present study, methods for the estimation of Aceclofenac present in the blood plasma samples were developed and validated. For the estimation of Aceclofenac in blood plasma, the chromatographic variables, namely pH, solvent strength, solvent ratio, flow rate, addition of peak modifiers in mobile phase, nature of the stationary phase, detection wavelength and internal standard were studied and optimized for the separation and retention of the drug. The following are the optimized chromatographic conditions, preparation of standard and sample solutions and the methods used for the estimation of Aceclofenac in plasma.

Chromatographic conditions: Shimadzu gradient HPLC system was used with following configurations:

- LC-20 AD Solvent delivery system (Pump).
- Manual Injector 25µl (Rheodyne)
- SPD-20A UV detector
- isocratic data station

Stationary phase: Hypersil BDS C18 (250cm x 4.6 mm i.d., 5µ)

Mobile phase: Acetonitrile: 25mM Potassium Dihydrogen Orthophosphate (pH-3.25)

Mobile phase ratio: 60:40

Flow rate: 1 ml/min

Sample volume: 20 µl

Detection:276 nm

Data station: isocratic Solutions

The mobile phase was filtered through 0.22µ nylon membrane and degassed using ultrasonicator. All the experiments were carried out at room temperature.

Preparation of Aceclofenacstandard stock solution

10mg of Aceclofenac was transferred into a 10mL volumetric flask and the volume was made upto the mark with mobile phase to give 1mg/mL (1000µg/mL) solution. From this stock solution, 10mL of 100µg/mL solution was prepared and again from this solution 10mL of 10µg/mL was prepared.

Preparation of analytical calibration curve solutions

From the standard stock solution 0.25-2µg/ml standard solutions were prepared and stored below 8°C until further analysis.

Preparation of blank plasma

Blank plasma (0.2 mL) was transferred into 2.0 ml centrifuge tube and 0.2 mL of precipitating agent (10% perchloric acid) were added. Finally made upto 2mL with the mobile phase. The resulting solution was vortexed for 5 minutes and centrifuged at 4000 rpm for 10 minutes. The supernatant layer was separated and analyzed.

Preparation of bioanalytical calibration curve samples

0.2 mL of Aceclofenac solutions were transferred to 2.0 mL centrifuge tube respectively, to this 0.2 mL of plasma, 0.2 mL of precipitating agent were added. The resulting solution was vortexed for 5 minutes and centrifuged at 4000 rpm for 10 minutes. The supernatant layer was separated and analyzed

Preparation of plasma samples

Plasma samples (0.2 mL) obtained from study subjects was transferred into 2.0 mL centrifuge tube and 0.2 mL of precipitating agent was added. The resulting solution was vortexed for 5 minutes and centrifuged at 4000 rpm for 10 minutes. The supernatant layer was separated and analyzed.

Method of analysis:

The plasma concentration–time data was analyzed by one compartmental model, using Kinetica software (5.0, Thermo scientific). Pharmacokinetic parameters like total area under the curve(AUC)0–8 h, terminal phase half life (t1/2), peak plasma concentration(Cmax) and time to reach the maximum plasma concentration (Tmax)were determined. The relative bioavailability of Aceclofenac loaded Nano clay after oral administration was calculated in comparison to Aceclofenac suspension.

In vivo oral bioavailability studies:

Male Wistar rats weighing 200-250g were used for oral bioavailability studies. All animal experiments were approved by Institutional Animal Ethical Committee, J.S.S. College of Pharmacy, Ooty (Proposal no. JSSCP/IAEC/M.PHARM/PH.CEUTICS/06/2016-17). All the rats were fasted for 12 h before the experiments but had free access to water.

GROUPINGS:

Group 1- Control group

Group 2- pure drug of Aceclofenac

Group 3- F1 Nano clay formulation treated group

Prior to oral drug administration,four group of rats (total no's = 6) were fasted overnight (>12 h). For com-parison of pharmacokinetic parameters, pure drug of Aceclofenac, optimized formulation of nano clay F1 was given to the rats via oral gavage (10 mg/kg). Blood samples (0.5 ml) were collected from the retro orbital plexus at time points 0.5, 1, 1.5, 2, 4, 6 and 8 h after

dosing. Immediately plasma was harvested by centrifugation at 3500rpm for 5 min and transferred to a fresh Eppendorf tubes containing 30µl of heparin and frozen to −20◦C prior to analysis **(Musmade et al., 2007; Mutalik et al., 2007).**

RESULTS AND DISCUSSION

Preformulation studies

Solubility Studies:

The saturation solubility of Acelofenac was determined in different solvents viz. Phosphate buffer pH 1.4, pH 6.8 and distilled water. The results are given in Table 8. Aceclofenac was found to be highly soluble in phosphate buffer pH 6.8. The solubility in phosphate buffer pH 1.4 and distilled water was found to be 24.35µg/mL and 12.85µg/mL respectively.

Solubility profile of Aceclofenac in different media were given in table 2.

Melting point

Melting point of Aceclofenac observed at 147^{o}C and theoretical data at (149-153^{o}C).

Compatibility Studies

FT-IR Studies

The spectra obtained from IR studies at wavelength from 400 1/cm to 4000 1/cm were shown in Fig 1.

After interpretation of the above spectra it was confirmed that there was no major shifting, loss or appearance of functional peaks between the spectra of drug, polymer, physical mixture of drug and polymer(3615 cm^{-1}, 3440 cm^{-1}, 2440 cm^{-1}, 1648 cm^{-1}, 1038 cm^{-1}).From the spectra it was concluded that the drug was entrapped into the polymer matrix without any chemical interaction. From the IR study it was concluded that, the selected polymer MMT and PVA were found to be compatible in entrapping the selected drug Aceclofenac.

DSC Studies

It was concluded that the drug was entrapped into the polymer matrix without any chemical interaction. From the DSC study it was concluded that, the polymers were found to be compatible in entrapping the selected drug Aceclofenac. The melting of the mixture proved that the crystalline form of the drug is converted to amorphous form and hence more solubility.

DSC of drug Aceclofenac, MMT, PVA and physical mixture were shown in figure 2.

Development of calibration curve

Calibration curve of the drug was developed to found out the linearity between concentration of drug in the solution and its optical density. It was concluded that the perfect linearity between the concentration and absorbance was observed when the concentration range was from 10µg/mL to 50µg/mL. Tables and Figures show the calibration of Aceclofenac using phosphate buffer pH 6.8.

Concentration and absorbance given in table 3.

Calibration curve of Aceclofenac in phosphate buffer pH 6.8 (λ_{max} = 276nm) were shown in figure 3.

Evaluation of MMT nanoclay

Zeta Potential and size report and polydispersity

Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e. the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate.

Zeta Potential report of F1 were shown in figure 4.

Batch code	Concentration of drug(mg)	Concentration of clay(gm)	Concentration of f-127(%)
F1	50	1	-
F2	50	0.5	-
F3	50	1	1
F4	50	0.5	1
F5	100	1	-
F6	100	0.5	-
F7	100	1	1
F8	100	0.5	1

TABLE 1: Composition of drug loaded batches

Solvent	Solubility
Phosphate buffer pH 1.4	24.35 µg/mL
Phosphate buffer pH 6.8	55.46 µg/Ml
Distilled water	12.85.µg/mL

TABLE 2: Solubility profile of Aceclofenac in different media

S.No	Concentration (µg/ml)	Absorbance
1	**10**	**0.115**
2	**20**	**0.172**
3	**30**	**0.314**
4	**40**	**0.443**
5	**50**	**0.587**

TABLE 3: Concentration and absorbance

Formulation	Intercalation Efficiency(%)	Drug loading(%)
F1	78	11.94
F2	12	1.7
F3	22	3.6
F4	54	15.1
F5	53	7.5
F6	45	9
F7	10	0.9
F8	20	1.5

TABLE 4: Drug loading and Intercalation efficiency of drug loaded batches prepared with different concentration of drugs in different concentration.

TIME (Hours)	CUMULATIVE PERCENT RELEASE (%)	
	F1	Pure drug
0	0	0
0.5	8.28	33.56
1	16.45	65.42
2	33.25	85.46
4	46.48	95.21
6	57.17	
8	63.85	
12	76.42	
24	89.83	

TABLE 5: Comparative *in vitro* release data for MMT nanoclay formulations compared to pure drug in Phosphate buffer pH 6.8

Sr. No.	Models	R^2 values	Slope values
1	Zero order	0.778	3.581
2	First order	0.971	-0.010
3	Higuchi	0.959	20.183
4	Hixson crowell	0.920	-0.102
5	Korsemayer-peppas	0.611	-0.689

TABLE 6: R^2 values and slope values for applied values

Batches	% Haemolysis		
	125µg/ml	500µg/ml	1000µg/ml
F1	0.011±0.001	0.015±0.001	0.022±0.001

TABLE 7: %Haemolysis

Concentration(µg/ml)	Peak Area
0.3	4267340
0.6	7010633
1.2	9179178
1.8	11238678
2.4	13196251
3	16595313
6	33090627

TABLE 8: Bioanalytical calibration curve of Aceclofenac

Parameter	Aceclofenac	F1
C_{max} (µg/ml)	2.19 ± 0.73	3.12 ± 0.98
T_{max} (h)	1.583 ± 0.083	4.833 ± 0.166
$t_{1/2}$ (h)	2.14	3.45
$AUC_{0\rightarrow t}$ (µg h/ml)	10.90 ± 0.374	56.93 ± 0.905
$AUC_{0\rightarrow \infty}$ (µg h/ml)	9.11 ± 7.54	15.03 ± 7.57

TABLE 9: Pharmacokinetic parameters of Aceclofenac after oral administration (mean ± S.D.) (* p <0.05)

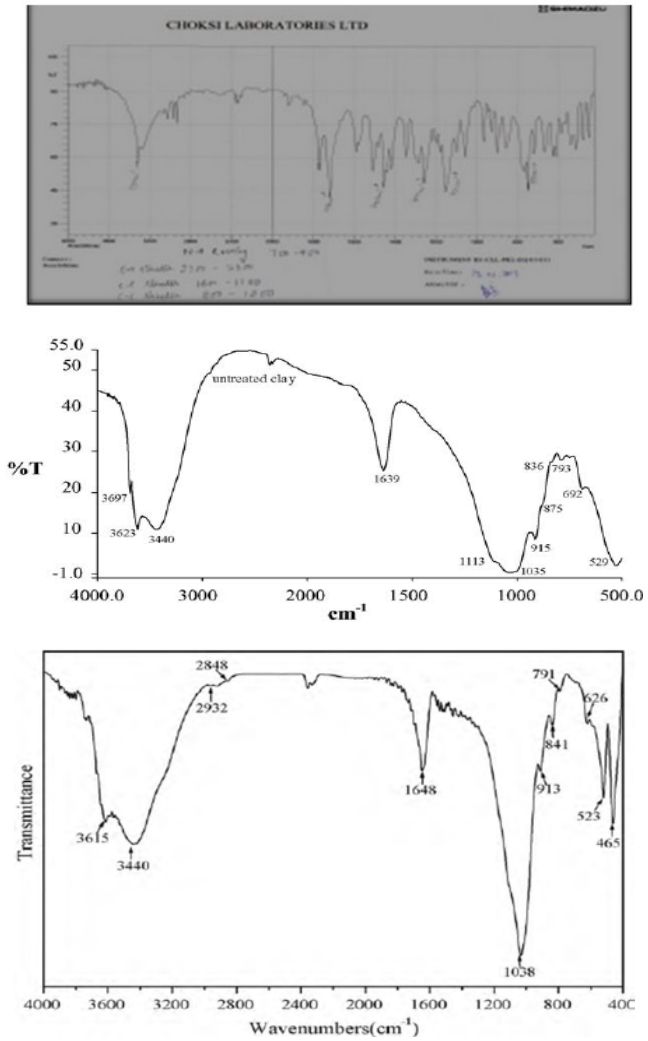

FIGURE 1: (a) IR Spectra of Aceclofenac
(b) IR Spectra of MMT
(c) IR Spectra of Mixture.

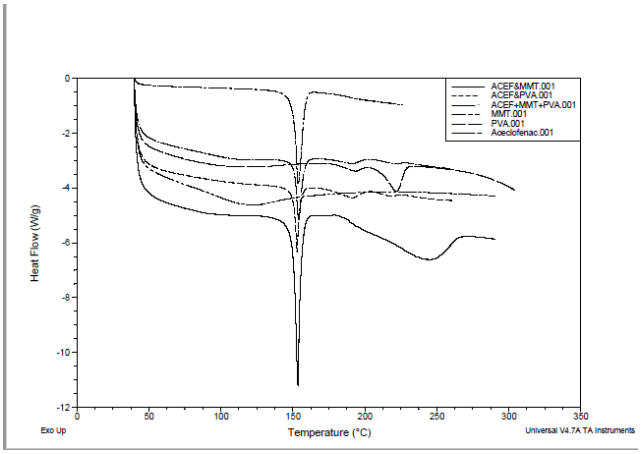

FIGURE 2: DSC of drug Aceclofenac, MMT, PVA and physical mixture.

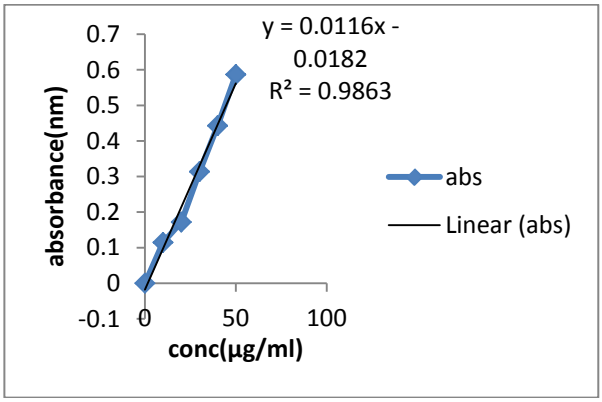

FIGURE 3: Calibration curve of Aceclofenac in Phosphate buffer pH 6.8

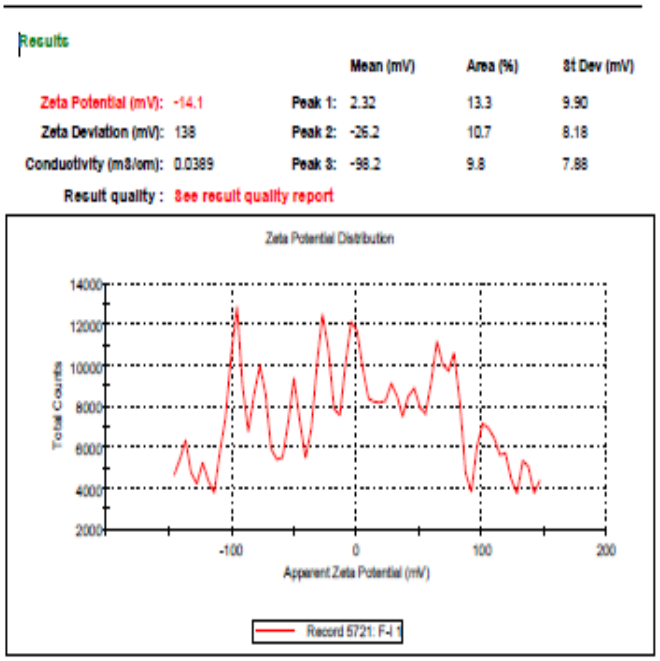

FIGURE 4: Zeta Potential report of F1

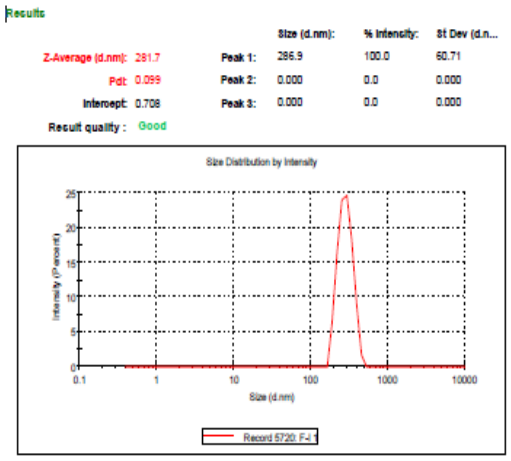

FIGURE 5: Size report of F1

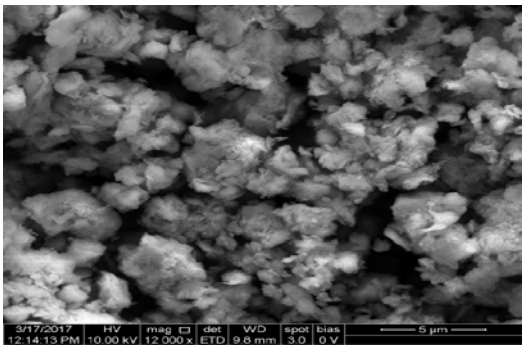

FIGURE6: Scanning electron micrograph (SEM) of F1 Nano clay

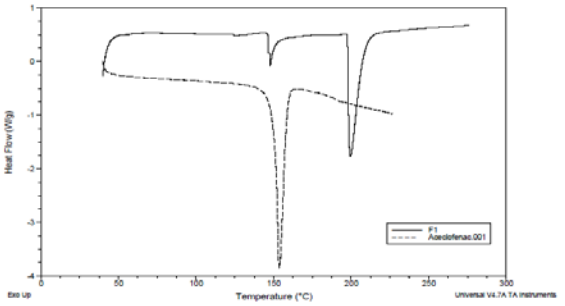

FIGURE 7: DSC after formulation

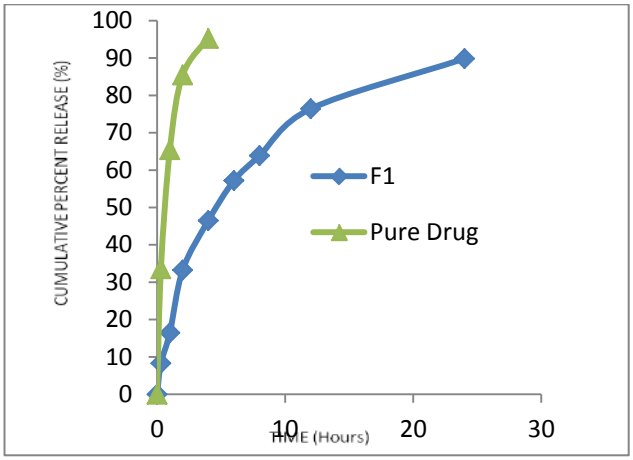

FIGURE 8: Comparative *In vitro* release profile in Phosphate buffer Ph 6.8 TIME (Hours)

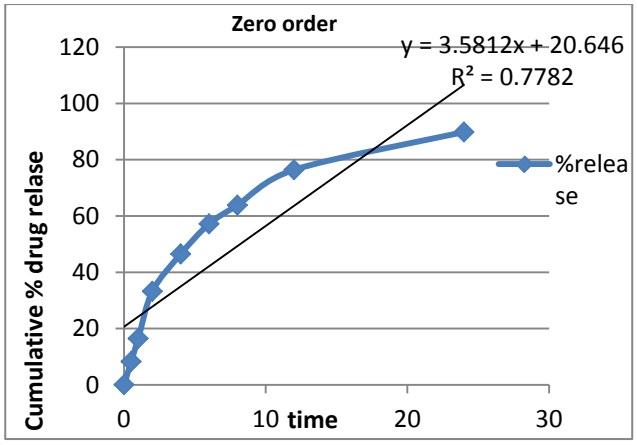

TABLE 9: Zero order kinetics of F1 batch

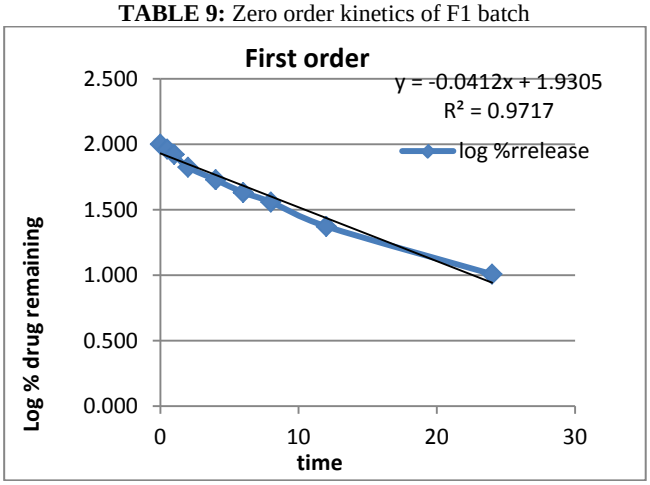

FIGURE 10: First order kinetics of F1 batch

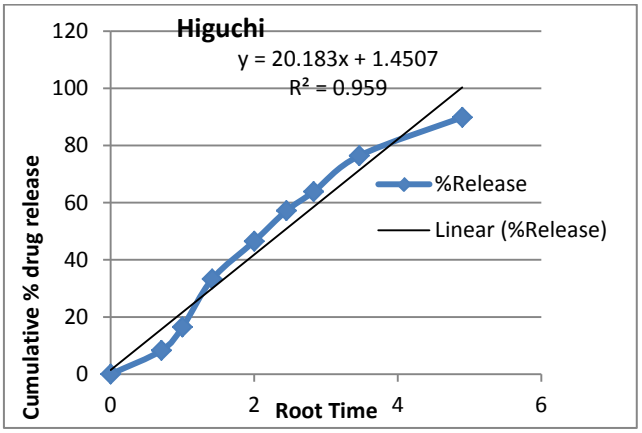

FIGURE 11: Higuchi model of F1 batch

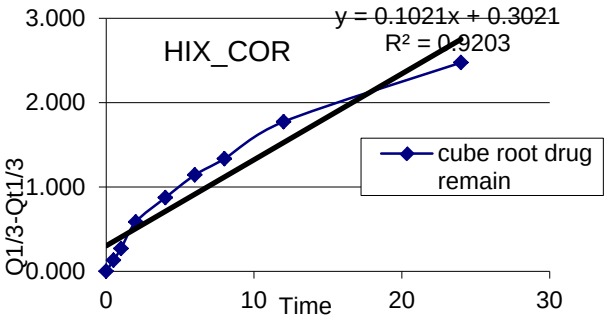

FIGURE 12: Hixson crowells of F1 batch

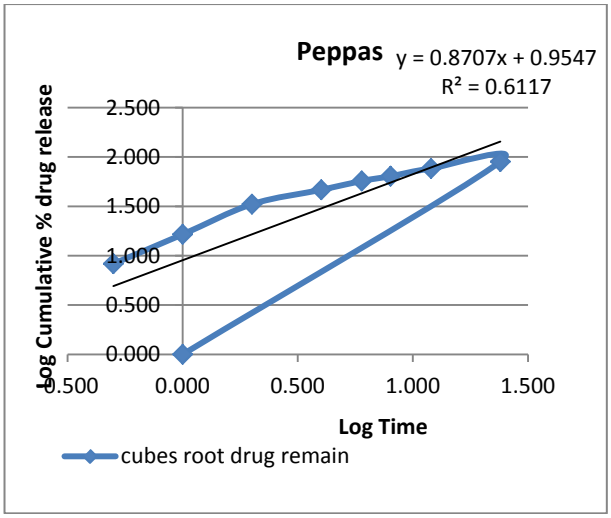

FIGURE 13: Korsemayer-peppas model of F1 batch

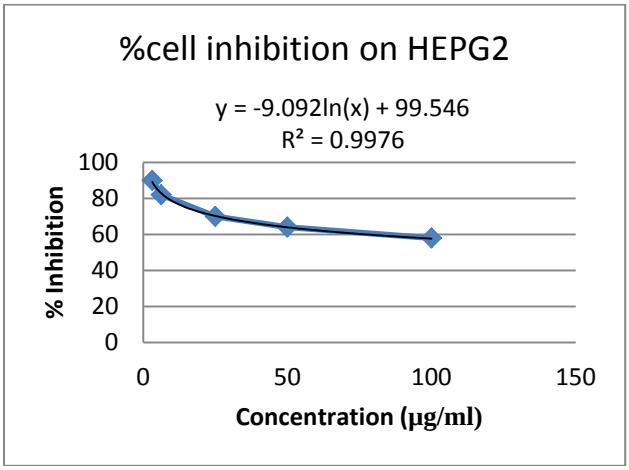

FIGURE 14: %cell inhibition for F1

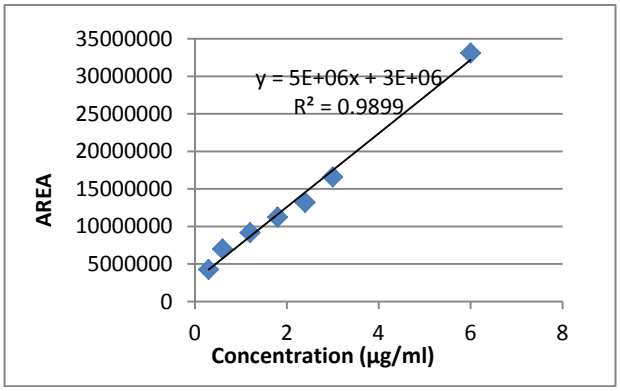

FIGURE15: Bioanalytical calibration curve of Aceclofenac

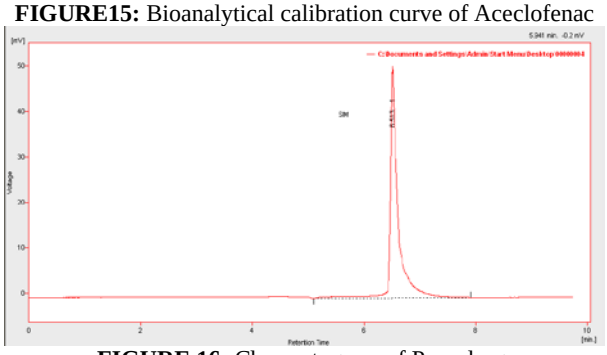

FIGURE 16: Chromatogram of Pure drug.

Polydispersity

The polydispersity index (PI) is the measure of size distribution of the nanoparticle formulation. PI was measured using Malvern zetasizer. PI values range from 0.000 to 1.000 i.e. monodisperse to very broad particle size distribution. PI values of all the formulations indicate that particle size distribution was unimodal. The optimized F1 batch having least particle size (286.9nm) had a PI of 0.09.

Size report of F1 were shown in figure 5.

Intercalation efficiency and Drug loading

Drug loading and Intercalation efficiency of drug loaded batches prepared with different concentration of drugs in different concentration were given in table 4.

Scanning electron microscopy (SEM)

The External morphological studies (SEM) revealed that maximum nanoparticles were nearly spherical in shape. The nanoparticlsize observed by SEM correlated well with the particle size measured by zeta sizer (Malver instrument).

Scanning electron micrograph (SEM) of F1 Nano clay were shown in figure 6.

DSC After formulation

DSC after formulation were shown in figure 7.

***In vitro* Release studies**

In vitro dissolution studies were carried out in Phosphate buffer pH 6.8. The release profiles indicate that Nanoclay formulations showed a retarded release of the drug from the MMT polymer when compared with plain Aceclofenac solution (AC-SOL). The *in vitro* release data and graph of nanoclay formulations and AC-SOL in Phosphate buffer pH 6.8 were shown in Table 5 and Figure 8. It was observed that AC-SOL showed 95.21 release in 4 h compared to 46.48 release for formulation F1 at the end of same time (p <0.05). This is due to fact that there is no barrier for diffusion at dialysis membrane interface for Aceclofenac molecules. Hence, higher release was observed in case of AC-SOL. But, formulation F1 released the drug in a sustained manner.

Release kinetics

In order to elucidate mode and mechanism of drug release, the *invitro* data was transformed and interpreted at graphical interface constructed using various kinetic models. The *in vitro* release data obtained for F1 formulation, in phosphate buffer pH 6.8, was fitted into various kinetic models. The results were given in Table 6.

The dissolution kinetics of optimized batch was applied to various dissolution models such as Zero order, First order, Higuchi, Korsemayer-peppas and Hixson crowell.

The best fitted model gives the highest R^2 value and least slope value. Thus, first order model fits best for the dissolution data of the optimized batch as it showed the highest value for R^2.

Zero order kinetics, First order kinetics, Higuchi model, Hixson crowells and Korsemayer-peppas model of F1 batch were shown in figure 9,10,11,12 and 13 respectively.

Haemocompatability studies

Nanoclay were subjected to rigorous blood biocompatibility tests. Erythrocyte-induced haemolysis *in vitro* can be considered to be a simple and reliable measure for estimating the membrane damage caused *in vivo*. Percent haemolysis was determined spectrophotometricly, detecting plasma free haemoglobin derivatives after incubating the particles with blood and then separating the undamaged cells by centrifugation. Typically less than 5% haemolysis is considered acceptable for blood biocompatibility. The concentration of 125-1000 µg/ml was subjected to determined percentage haemolysis. In this 1% triton X 100 was used as positive control and Saline water was as a negative control. Positive control showed percentage haemolysis of 3.714±0.65 where as negative control of 0.023±0.001. On increasing the concentration from 150µg/ml to 1000µg/ml, there was no significant increase in % haemolysis. The results indicated

that the Aceclofenac nanoclay were haemocompatible and did not produce any toxic effects.
%Haemolysis were given in table 7.

MTT Assay

The percentage growth inhibition was calculated using the following formula and concentration of drug or test samples needed to inhibit cell growth by 50% values were generated from the dose-response curves for each cell line.

$$\% \text{ Cell viability} = 100 - \frac{Mean\ OD\ of\ individual\ test\ group}{Mean\ OD\ of\ control\ group} \times 100$$

The IC 50 value of the formulation F1 is about 245mg/ml.
%cell inhibition for F1 were shown in figure 14.

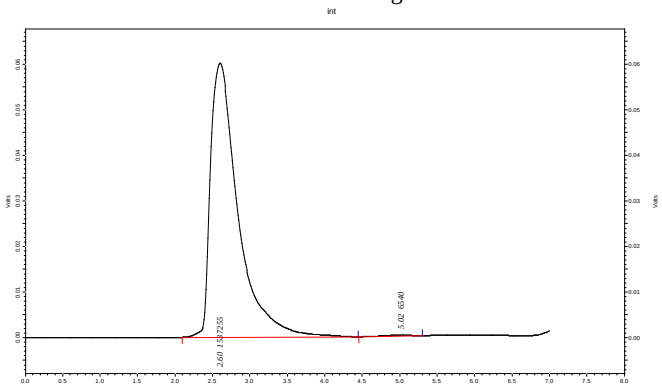

FIGURE 17: Chromatogram of Plasma

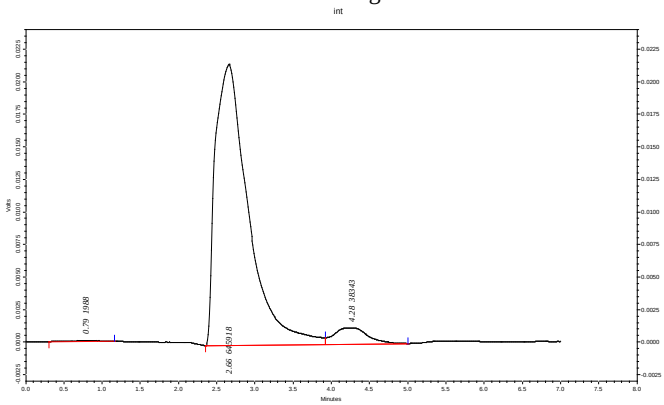

FIGURE 18: Chromatogram of F1

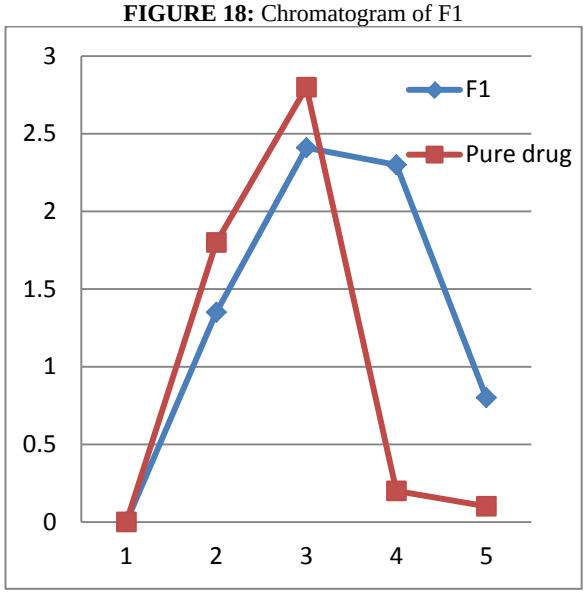

FIGURE 19: Concentration time profile after oral administration of F1 and Aceclofenac suspension

Bioanalytical method development and analysis

Bioanalytical calibration curve of Aceclofenac was prepared in mobile phase and peak area was calculated. The results were given in Table 8 and Figure 15.
The chromatograms of Aceclofenac showed stable baseline. The regression equation in the range of 0.5-2µg/ml was as follows: R^2 = 0.989.
The concentration of Aceclofenac was determined in plasma samples separated at different time intervals by HPLC analysis. The concentration of plasma samples was determined from the area of the chromatographic peak using the calibration graph. Chromatograms of pure drug, blank plasma and F1 were shown in figure 16,17,18 respectively.

Data Analysis

Pharmacokinetic parameters of Aceclofenac after oral administration are shown in Table 16. Peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the individual plasma-concentration time profiles. The area under the concentration- time curve from time zero to time t ($AUC_{0\rightarrow t}$) was calculated using the trapezoidal method. The area under the curve (AUC) determines the bioavailability of the drug for the given the same dose in the formulation. The area under the total plasma concentration–time curve from time zero to infinity was calculated by:

$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow t} + C_t/K_e$

where, C_t is the Aceclofenac concentration observed at last time, and K_e is the apparent elimination rate constant obtained from the terminal slope of the individual plasma concentration–time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The relative bioavailability (F_r) at the same dose was calculated as:

$Fr = AUC_{SLN,\ 0\rightarrow t} / AUC_{SOL,\ 0\rightarrow t}$

The mean residence time (MRT) was estimated from $MRT = AUMC_{0\rightarrow\infty}/AUC_{0\rightarrow\infty}$

Pharmacokinetic parameters of Aceclofenac after oral administration (mean ± S.D.) (* p <0.05) were given in table table 9.

Figure 19 shows the concentration-time curve of F1 and Aceclofenac suspension (AC-SUSP). It is evident that there was increased absorption of Aceclofenac . The C_{max} value of Aceclofenac in nanoclay (2.19 ± 0.73) was significant (p < 0.05) than that observed with F1 (3.12 ± 0.98). Twenty-four hours after oral administration the Aceclofenac plasma concentration was still more than 0.5µg/mL. The $AUC_{0\rightarrow t}$ values of Aceclofenac after oral administration of F1 were 56.93 -fold higher than those obtained with AC-SUSP.

CONCLUSION

To conclude, the preparation of nano clay with MMT was suitable for producing MMT Nanoclay. Lipophilic drugs like Aceclofenac can be successfully incorporated into the clay. The formulated MMT nano clay showed a significant increase in oral bioavailability compared to pure drug suspension. When drug releases from the clay surface and promoted or raises by the weak bonding and due to hydrophilic properties of the clay may increase the dissolution leads to enhanced bioavailability. The dose of the Aceclofenac Nanoclay needs to be corrected in accordance with increased bioavailability, to minimize its dose related adverse effects. Nanoclay provided sustained release of the drugs, and these systems are the preferred drug carriers for lipophilic drugs to overcome the oral bioavailability problem of drug.

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