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Development and Evaluation of Aceclofenac Sodium Nanohydrogel

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ABSTRACT

In present study, the aceclofenac sodium loaded nanohydrogel were prepared by homogenization technique using Carbopol 934 as a polymer. The drug remained intact and stable in the TDDS during storage, with no significant chemical interaction between the drug and the excipients. Physicochemical parameters such as percentage yield, drug content, and viscosity studies were carried out. The prepared nanohydrogels exhibited satisfactory physical characteristics such as percentage yield, SEM, viscosity, IR studies, drug content. Among all the developed formulations, F1 containing 1gm of carbopol 934 showed better release than other nanohydrogel formulations. Aceclofenac sodium shows maximum absorption at wavelength 274 nm in 7.4 pH phosphate buffer using UV spectrophotometer. Standard calibration curve obeyed Beer's law at given concentration range of 2 µg/ml to 10 µg/ml. The value of regression coefficient was found to be 0.9993, which showed a linear relationship between concentration and absorbance. The physical mixture of the drug with polymer gave peaks which corresponded to the parent peaks of the pure drug which confirmed the compatibility between drug and polymer. In the DSC study indicates compatibility between drug and polymer. The release pattern of aceclofenac sodium from different nanohydrogel formulations, *in vitro* diffusion studies, was carried out using Keshry-Chein diffusion cell and pH 7.4 phosphate buffer as receptor medium. The absorption kinetics was subjected to regression analysis. The drug release pattern of F1, F2, and F3 followed first-order kinetics. On the basis of the *in vitro* characterization, it was concluded that aceclofenac sodium is administered transdermally through the nanohydrogel delivery system.

INTRODUCTION:

HYDROGELS[1]

Nanohydrogels are current approaches of hydrogels which are prepared in water by self-aggregation of polymers of natural origin like dextran. These types of hydrogels are formed from natural polysaccharides like dextran, pullulan, or cholesterol-containing polysaccharide.

Nanohydrogels are polymeric networks with the three-dimensional configuration capable of imbibing high amounts of water or biological fluids. These are the swellable polymeric materials, have been widely investigated as the hydrogel carrier for drug delivery systems. These biomaterials have gained attention owing to their peculiar characteristics like swelling in the aqueous medium, pH and temperature sensitivity or sensitivity towards other stimuli, being biocompatible, materials that have been recognized to function as drug protectors, especially for peptides and proteins, from *in vivo* environment etc. Also, these swollen polymers are helpful as targetable carriers for bioactive drugs with tissue and cell specificity. Their affinity to absorb water is attributed to the presence of hydrophilic groups such as –OH, –CONH–, –CONH2–, and $–SO_3H$ in polymers forming hydrogel structures. The contribution of –OH, –CONH–, –CONH2–, and $–SO_3H$ and domains in the network, the polymer is thus hydrated to different degrees (sometimes, more than 90% wt.), depending on the nature of the aqueous environment and polymer composition. While the water content of a hydrogel determines its unique physicochemical characteristics, these structures have some common physical properties resembling that of the living tissues, than any other class of synthetic biomaterials, which is attributed to their high water content, their soft and rubbery consistency, and low interfacial tension with water or biological fluids. Despite their high water absorbing affinity, hydrogels show a swelling behavior instead of being dissolved in the aqueous surrounding environment as a consequence of the critical crosslink present in the hydrogel structure. These crosslinks are two main categories including.

i) Physical and ii) Chemical (tie-points and junctions)

TYPES OF HYDROGELS

pH-sensitive or ion sensitive hydrogels

The pH-sensitive Nanohydrogels respond to changes in pH of the external environment. These gels have ionic groups (which are readily ionizable side groups) attached to impart peculiar characteristics

Temperature sensitive hydrogels

The releases, as well as mechanical characteristics of drug and hydrogels, are altered with the change in the temperature of an external environment.

Glucose-sensitive hydrogels

Glucose-sensitive hydrogels are sugar sensitive and show variability in response depending upon the presence of glucose. Selected pharmaceutical hydrogel system is the cross-linked poly (methacrylamido phenylboronic acids)- acrylamide hydrogel.

PREPARATION OF HYDROGELS

A) Isostatic ultra-high pressure (IUHP)

The nanosuspension of natural biopolymers like starch is subjected to an ultrahigh pressure of 300-700 MPa for 5 or 20 min in a chamber which brings about changes in the morphology of the polymer (i.e. gelatinization of starch molecules occur). It is different from heat-induced gelatinization where a change in ordered state of the polymer occurs. Usually, the temperature within the chamber varies from 40 to 52ºC.

B) Cross-linking method

These cross-linking methods routinely used for the preparation of hydrogels. The characteristics and potential applications of hydrogels of different structures rely not only on the preparation methods but also on the monomers used in the synthesis of hydrogel polymeric networks.

C) Use of water and critical conditions

Use of water and critical conditions of drying Aerogels of carbon has been prepared by

supercritically controlling the drying conditions. The final texture of hydrogel is governed by the molar ratio of resorcinol to sodium carbonate. This method of preparation leads to porous hydrogels with no shrinkage during a drying process.

D) Use of nucleophilic substitution reaction

Nanohydrogels of N-2-dimethylamino ethyl-methacryalmide (DMAEMA), a pH and temperature sensitive nanohydrogel has been prepared by the nucleophilic substitution reaction between methacyloyl chloride and 2-dimethylamino ethylamine. The synthesized nanohydrogel was characterized by its swelling behavior.

E) Use of gelling agents

Where gelling agents like glycophosphate, 1-2 propanediol, glycerol, trehalose, mannitol, etc, have been used in the formation of hydrogels. The gelling agent problem of turbidity and presence of negatively charged moieties which are associated with this method pose problem of interaction with the drug.

F) Use of irradiation and freeze-thawing

However, with irradiation and freeze-thawing method, the nanohydrogels so formed have sufficient mechanical strength and stability but are opaque in appearance with a little swelling capacity.

G) Hydrogel-based drug delivery system

Preparation of hydrogel-based drug product involves either cross-linking of linear polymers or simultaneous polymerization of monofunctional monomers or cross-linking with polyfunctional monomers. The mechanical and physical strength of poorly cross-linked hydrogels can be adequately enhanced by various methods. Polymers from natural, synthetic or semi-synthetic sources can be used for synthesizing hydrogels. Usually, polymers containing hydroxyl, amine, amide, ether, carboxylate, and sulfonate as functional groups in their side chains are used.

Hydrogels for drug delivery systems[2]

Currently, the development of smart hydrogels that can respond to external stimuli such as variations in temperature, pH, and electric fields or hydrogels with controlled

biodegradability has been used in biomedical applications. As such hydrogels are used in a wide range of applications including tissue engineering and regenerative medicine, diagnosis, cell immobilization, bimolecular and cell separation, barriers to regulate the biological adhesion. Hydrogels can be made, in theory, from any water-soluble polymer, encompassing a wide range of chemical compositions and physical properties. The polymer forms aggregates that form a three-dimensional matrix with interconnected pores in which the solvent (usually water or aqueous solutions) and other particles can diffuse. Some hydrogels have a high capacity to contain compounds that can be released in a controlled manner for therapeutic purposes. Porosity hydrogel allows the loading and entrapment of drugs within the polymer matrix and their subsequent release at a rate that depends on the coefficient of diffusion of drug through the gel matrix in the case of hydrophilic drugs. For hydrophobic drugs, the rate of release depends on the rate of degradation of the gel. Besides their biodegradability can be designed to be via enzymatic, hydrolytic, or environmental (pH, temperature or electric fields).

CHARACTERIZATION OF HYDROGELS:

Generally, hydrogels and nanohydrogels are characterized by their morphology, swelling property, and elasticity. Morphology is indicative of their porous structure. Swelling determines the release mechanism of the drug from the swollen polymeric mass while elasticity affects the mechanical strength of the network and determines the stability of these drug carriers. Some of the following important features for characterization of hydrogels:

- **Morphological characterization**

Hydrogels are characterized by morphology which is analyzed by equipment like stereomicroscope. Also, the texture of these biomaterials is analyzed by SEM to ensure that hydrogels, especially of starch, retain their granular structures.

- **X-ray diffraction**

X-ray diffraction used to understand whether the polymers retain their crystalline structure or they get deformed during the processing pressurization process.

- ***In vitro* release study for drugs**

Since hydrogels are the swollen polymeric networks, an interior of which is occupied by drug molecules, therefore, release studies are carried out to understand the mechanism of release over a period of application.

- **FTIR (Fourier Transform Infrared Spectroscopy)**

Any change in the morphology of hydrogels and nanohydrogels changes their IR absorption spectra due to stretching and O-H vibration. Formation of coil or helix which is indicative of crosslinking is evident by the appearance of bands near 1648 cm^{-1}.

Swelling behavior:

The nanohydrogels and hydrogels are allowed to immerse in aqueous medium or medium of specific pH to know the swellability of these polymeric networks. These polymers show the increase in dimensions related to swelling.

Rheology:

Hydrogels are evaluated for viscosity under the constant temperature of usually by using cone using Cone and Plate type viscometer.

MATERIALS AND METHODS

MATERIALS:

All the materials used in the formulations, evaluation and other experiments are listed below. The chemicals used were of laboratory reagent grade and were used as they were procured. The distilled water was used in all experiments.

List of chemicals and reagents used with supplier

Sr. No.	Materials	Manufacturer/ Suppliers
1	Aceclofenac Sodium	Microlabs Pvt. Ltd. Bangalore, (INDIA)
4	Carbopol 934	HiMedia Laboratory Pvt. Ltd. Mumbai (INDIA)
5	Polyethylene Glycol 400	Ozone International, Mumbai.
6	Triethanolamine	Ozone International, Mumbai.
7	Methanol	Nice Chemicals, Kochi

METHODS:

Preformulation studies Aceclofenac Sodium

Preformulation testing study is the first step in the rational development of dosage forms of the drug. It can be defined as an investigation of physical and chemical properties of drug substance, alone and when combined with excipients. The overall objective of the preformulation testing study is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass-produced.

Melting point determination[3]

A melting point of drug sample was determined by taking the small quantity of drug in a capillary tube sealed at one end and was placed in digital melting point apparatus and temperature range at which the drug melts is noted.

Solubility:

The solubility of aceclofenac sodium was determined in different solvents.

Identification of drug sample by IR spectroscopy[4]

FT-IR spectroscopy is a powerful technique, which is used for identification of drug substances the spectral regions for every substance is characteristic for its structures and functional groups it is associated with. The IR spectral analysis identifies the characteristic functions present in substances. To identify given drug sample FT-IR spectra of drug sample was recorded and was compared with standard reference spectra of the drug from literature.

Potassium bromide was mixed with drug and/or polymer and the spectra were taken. The FT-IR spectrum of aceclofenac sodium and compared with FT-IR spectra of aceclofenac sodium with a polymer. The disappearance of aceclofenac sodium peaks or shifting of a peak in any of the spectra was studied.

Differential Scanning Calorimetry (Thermal analysis)[5]

DSC has been one of the most widely used calorimetric techniques employed to characterize the solubility and physical state of a drug in the complex. Thermograms of aceclofenac sodium was recorded using a differential scanning calorimeter and were compared. The

samples (5 mg) were hermetically sealed in flat-bottomed aluminum pans and heated over a temperature range of 100-300 °C at a rate of 10 °k/min using alumina as a reference standard.

UV spectroscopy[6]

Determination of λ_{max}:

Most drugs absorb light UV wavelength (200-400nm) since they contain aromatic double bonds. The solution containing 10µg/ml of aceclofenac sodium was prepared and scanned over the range of 200-400 nm against phosphate buffer pH 7.4 as blank using Shimadzu double beam UV spectrophotometer. The maximum wavelength obtained in the graph was considered as λ_{max} for the pure drug.

Preparation of Blank Nanohydrogels:

To optimize the processing condition, a concentration of excipients, a blank nanohydrogel was prepared. Processing conditions were varied as well as a concentration of excipients was altered so as to get the referred characteristics in the nanohydrogel. From the results obtained for characterization of blank nanohydrogel, a processing condition and concentration of excipients was optimized and subsequently, it was scaled-up for the preparation of aceclofenac sodium loaded nanohydrogel.

Formulation of Drug Incorporated Nanohydrogel:

Accurately weighed quantity of carbopol 934 as polymer with reported pH adjustment technique, an appropriate amount of carbopol 934 was slowly added into beaker containing water under constant stirring, and aceclofenac sodium (1% w/w) was dispersed in propylene glycol in a separate beaker; this mixture was then added to the beaker containing carbopol in water (homogenization at 8000-10000 rpm for 2 hr under at room temperature). The mixture was kept at ambient temperature for 24 hours. A small amount of 0.5% triethanolamine was added and mixed well until the gel was formed. A few drops of 2M sodium hydroxide was added to the gels to adjust the pH between 5-7. This whole system is subjected to further study and the formulations are named as F1, F2, and F3 as shown in Table 1.

Table No.1: Formulation table of nanohydrogels

Formulations	Aceclofenac sodium(mg)	Carbopol 934 (gm)	Propylene glycol (ml)	Water (ml)
F1	100	1	25	25
F2	100	2	25	25
F3	100	3	25	25

EVALUATION OF NANOHYDROGEL:

Compatibility study:

Visual Inspection [7]

About one-week preparation, the dispersions were visually assessed for optical appearance (e.g., color, turbidity).

FT-IR study:

One formulation each from developed nanohydrogel is subjected to FT-IR studies to identify compatibility of a drug with excipient and processing conditions adopted for their scale up.

Differential Scanning Calorimetry (Thermal analysis):

Differential Scanning Calorimetry has been one of the most widely used calorimetric techniques employed to characterize the solubility and physical state of a drug in the complex. Thermograms of aceclofenac sodium and one formulation of aceclofenac sodium loaded nanohydrogel was recorded using a differential scanning calorimeter and were compared. The samples (5 mg) were hermetically sealed in flat-bottomed aluminum pans and heated over a temperature range of 100-300 °C at a rate of 10 °k/min using alumina as a reference standard.

Percentage Yield[8]

The practical percentage yield was calculated from the weight of nanohydrogel recovered from each batch in relation to the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula.

$$\text{Percentage yield (\%)} = \frac{\text{Practical yield}}{\text{Theoretical Yield}} \times 100$$

PARTICLE SIZE ANALYSIS

Particle Size and Surface Morphology[9]

Particle size analysis was done by scanning electron microscopy (SEM). SEM is the most commonly used method for characterizing drug delivery systems, due to simplicity in samples preparation and ease of operation. The three-dimensional information about macro- (0.1-10 mm), meso (1-100 μm) and nanostructure (10000 nm), is often found within the same micrograph. SEM has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface.

Particle size analysis was done by SEM using JEOL JSM-T330A scanning microscope. Cleaned brass specimen studs were used for mounting the samples. Wet solvent paints were applied on these studs and while paints were wet, the pallets were placed on each stud and allowed to dry. Then the sample was observed in scanning electron microscope and photographs were taken.

Viscosity[10]

The viscosity of the prepared formulations was determined at different angular velocities at 25 ˚C using a rotary viscometer (DV- III, Brookfield, USA). The rotation speed was 20rpm, with spin18 #. The average of two readings was used to calculate the viscosity.

Drug Content[11]

Drug-loaded nanohydrogel were mixed with methanol and sonicated for 10 min to obtain a clear solution. Concentrations of a drug were determined spectrophotometrically at λ_{max} 274 nm.

$$\text{Drug content} = \frac{\text{Actual yield}}{\text{theoretical yield}} \times 100$$

Diffusion Studies[12]

Diffusion studies were performed by using a Keshary-Chein diffusion cell with a receptor compartment capacity of 16 ml. The synthetic cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The formulated aceclofenac sodium nanohydrogel formulations of 1gm were placed over the drug release membrane (i.e. in the donor compartment) and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm, the temperature was maintained at 37±0.5˚C by surrounding water in a jacket. The samples of 1ml were withdrawn at the time interval of 1, 2, 3, 4, 6 and 8 hours and analyzed for drug content UV spectrophotometrically at 274nm against blank. The receptor phase was replaced with an equal volume of phosphate buffer pH 7.4 at each time of sample withdrawal. The cumulative amounts of drug from nanohydrogel permeated through synthetic membrane plotted against time.

The diffusion kinetics of the drug aceclofenac sodium was analyzed by graphical method.

Release Kinetics

The data of *in-vitro* study was fitted into three different kinetics models namely zero order, first order and Higuchi's clerical model. The mechanism of drug release is defined statics in terms of co-relation co-efficient the highest values of co-relation co-efficient signify the particular release mechanism.

RESULTS AND DISCUSSION:

Melting Point Determination

The melting point of aceclofenac sodium in literature is 143-146ºC, after estimation, it was found to be in the range of 144.6ºC which indicates the purity of the drug sample.

FT-IR Study

For identification of obtained aceclofenac sodium sample, FT-IR study was conducted. After comparing FT-IR spectra of aceclofenac sodium sample and spectral peaks with the standard

reference spectra, it was confirmed that the obtained sample is aceclofenac sodium. The FT-IR spectrum of obtained aceclofenac sodium shows characteristic absorption peaks as given in Table 2 and depicted in Figure.1

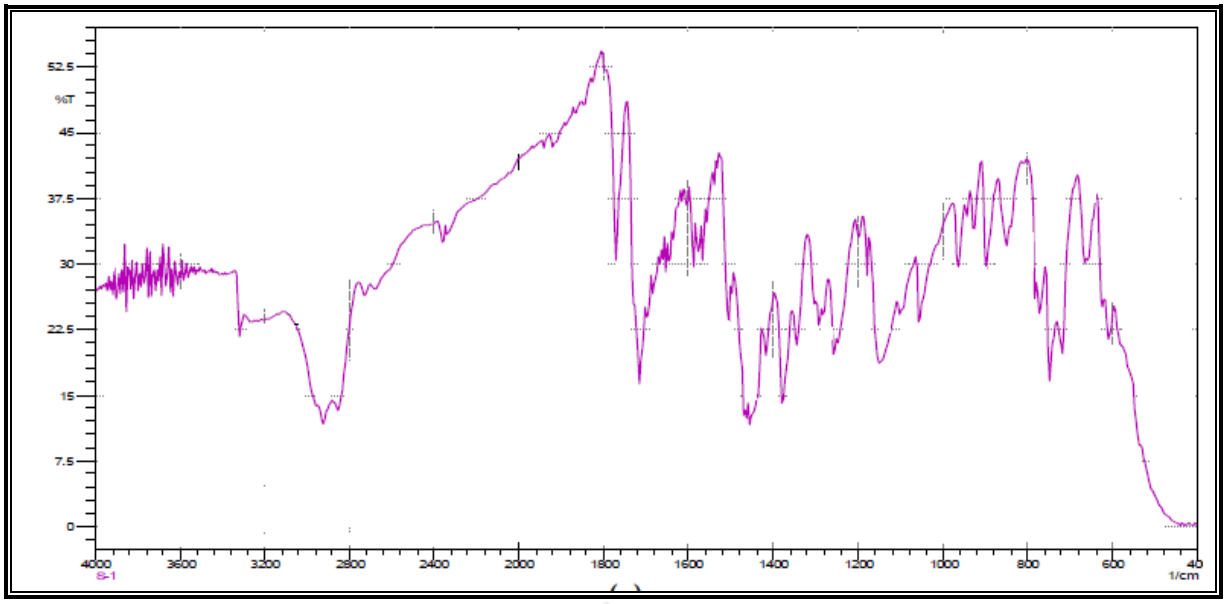

Fig. 1: FT-IR Spectra of Aceclofenac Sodium

Table 2: Peaks (cm^{-1}) and functional groups present in aceclofenac sodium.

Sr. No.	Peaks cm^{-1}	Functional group
1	3400	N-H (str)
2	2900	C-H (str)(Ar)
3	2840	C-H(Str) alkane
4	1680	C=O (Str)
5	1590	C=C(Str)
6	1200	C-O(Str)

Differential Scanning Calorimetry (DSC):

The DSC technique provides a qualitative physicochemical status of drug which is reported in endothermic or exothermic process. The resulted from thermal transition which includes melting, decomposition and outgassing for change in heat capacity. Using DSC analysis of drug, polymer, and excipients the nature of the drug inside the polymer matrix can be assessed and it is shown in Figure 2.

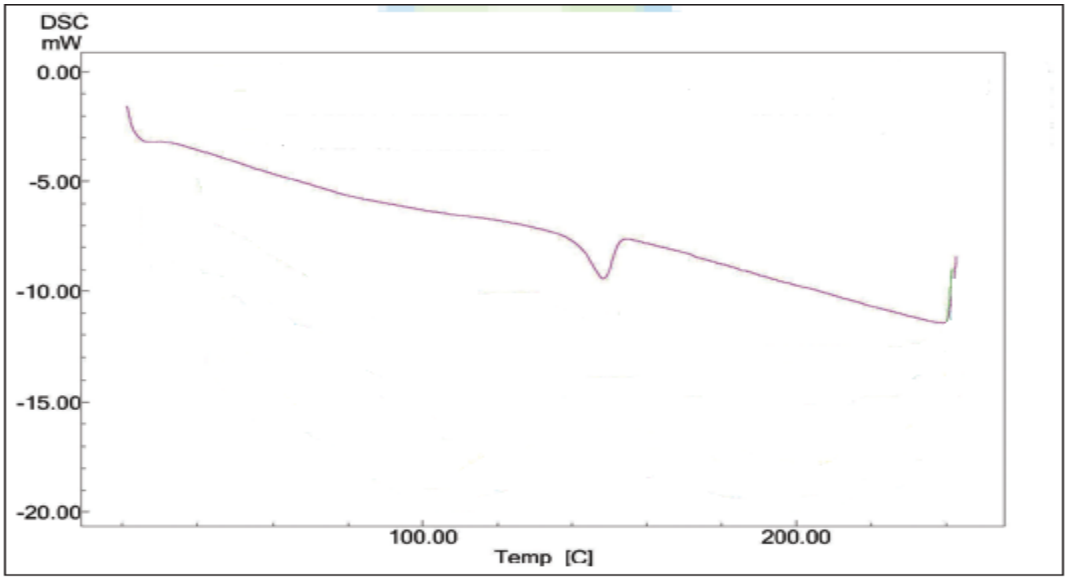

Fig. 2: DSC of Aceclofenac Sodium.

UV spectroscopy:

Determination of λ_{max} of Aceclofenac Sodium:

The λ_{max} of aceclofenac sodium was determined in phosphate buffer pH 7.4, which was scanned between 200-400 nm in the UV spectrophotometer. It was found to be 274 nm. As shown in figure 3 and Table 3.

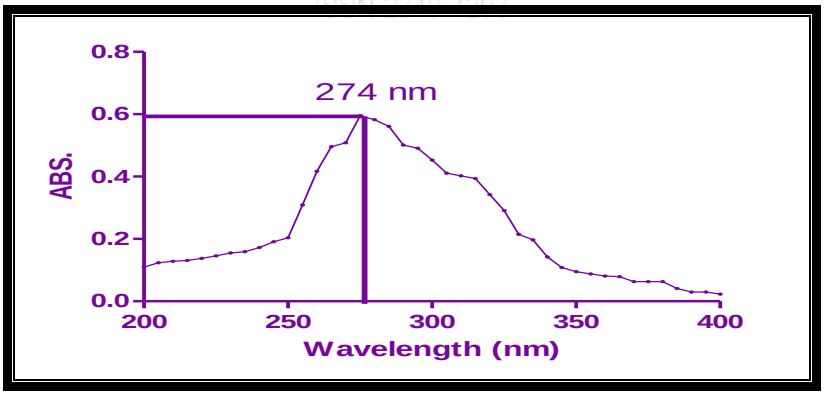

Fig. 3: λ_{max} of Aceclofenac Sodium

Calibration curve for aceclofenac sodium was constructed using phosphate buffer pH-7.4 as a solvent at 274 nm wavelength. The concentration of aceclofenac sodium selected was 2-10µg/ml. and the calibration curve is shown in figure 4.

Table 3: Calibration data of aceclofenac sodium in phosphate buffer pH 7.4

Sr. No.	Concentration(µg/ml)	Absorbance at 274 nm Mean ± standard deviation
1	2	0.056±0.0021
2	4	0.110±0.0025
3	6	0.156±0.0030
4	8	0.215±0.0015
5	10	0.268±0.0026

*Average of three determinations

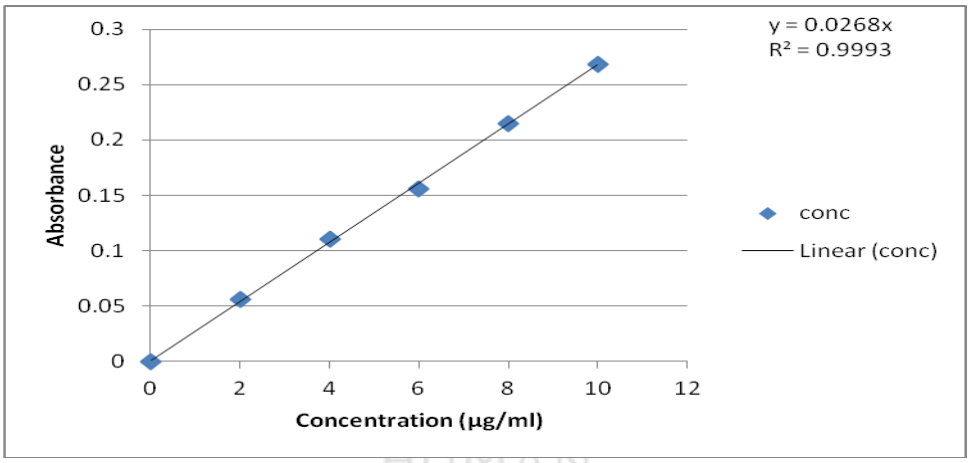

Fig. 4: Calibration Curve of Aceclofenac Sodium in 7.4 pH Phosphate Buffer

A straight line obtained at R^2=0.9993 and the equation of straight line was found to be Y=0.0268x.

Drug - Excipients Compatibility Studies:

FT-IR Study:

To ascertain compatibility between drug and excipients FT-IR study was conducted. The FT-IR spectra of pure aceclofenac sodium and physical mixture of aceclofenac sodium with excipients were recorded and compared. All the characteristic peaks of aceclofenac sodium were retained in the spectra of drug and physical mixture indicating no chemical interaction between drug and excipients and were found to be compatible. The spectra are as shown in Figure 5 and Table 4.

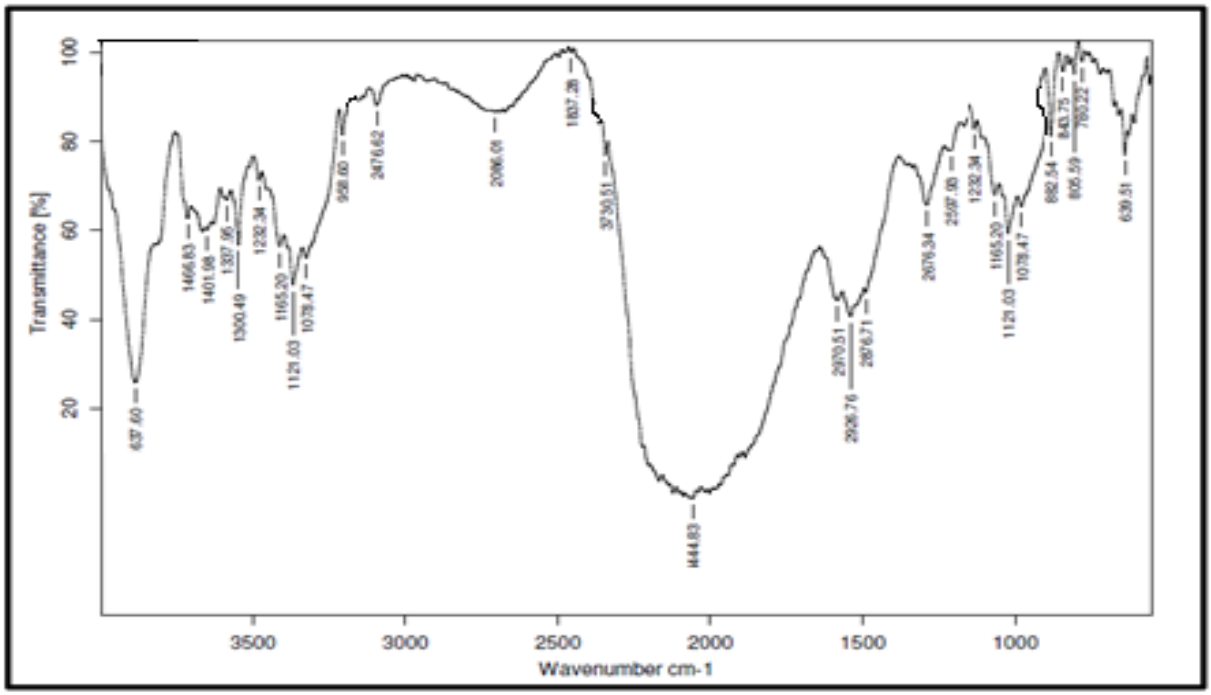

Fig. 5: FT- IR Spectra of Physical Mixture.

Table 4: Spectral peaks of aceclofenac sodium with its excipients physical mixture

Sr. No.	Peaks cm^{-1}	Functional group
1	2926.76	N-H (str)(Ar)
2	2876.71	C-H(Str) alkane
3	1232.34	C-O(Str)
4	2926.76	C-H (Str) (Ar)

Formulations:

Three batches of nanohydrogel were prepared wherein a concentration of gelling agent was changed keeping constant the concentration of all other ingredients including drug. The formed nanohydrogels inspected physically and were found to be transparent. The prepared formulations were filled in the container and kept in a refrigerator until the completion of their evaluation studies.

Evaluation of Nanohydrogel:

Physical Appearance

The prepared nanohydrogels were transparent.

FT-IR of Aceclofenac Sodium Nanohydrogel:

To ascertain compatibility between drug and excipients FT-IR study was conducted. The FT-IR spectra of aceclofenac sodium nanohydrogel were recorded and compared with the spectral peaks of pure aceclofenac sodium. All the characteristic peaks of aceclofenac sodium were retained in the spectra of aceclofenac sodium nanohydrogel indicating no chemical interaction between drug and excipients and were found to be compatible. The spectra are as shown in Figure 6 and the spectral peaks are recorded in Table 5.

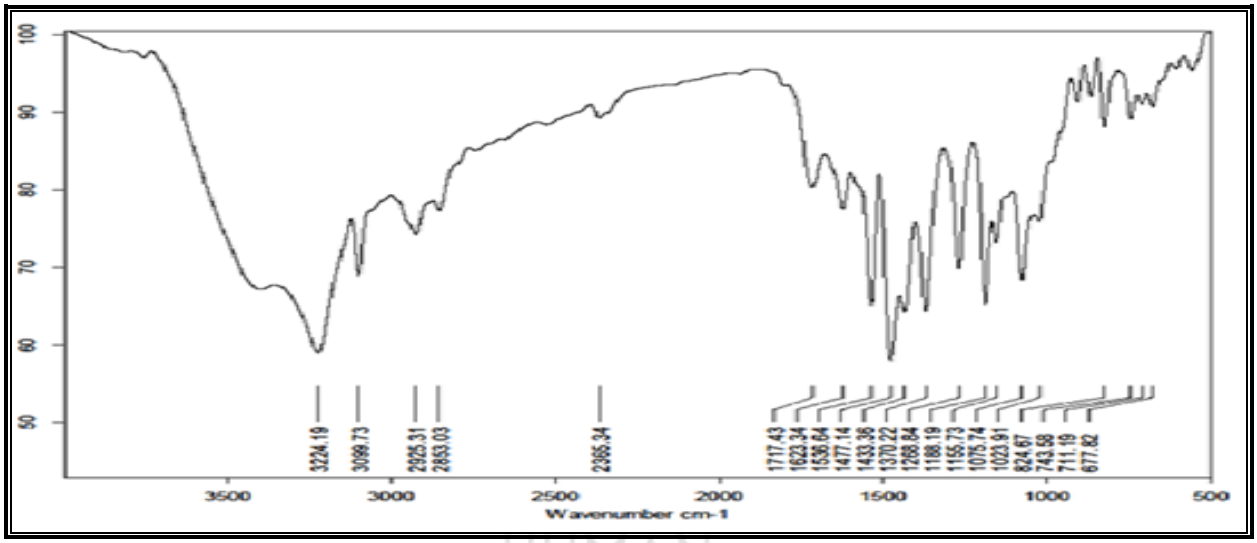

Fig. 6: FT-IR of Aceclofenac Sodium Nanohydrogel

Table 5: Peaks (cm^{-1}) and functional groups present in aceclofenac sodium nanohydrogel

Sr. No.	Peaks cm^{-1}	Functional group
1	3224.19	N-H (str)
2	2925.31	C-H (str)(Ar)
3	2853.03	C-H(Str) alkane
4	1623.34	C=O (Str)
5	1536.64	C=C(Str)
6	1188.19	C-O(Str)

Differential Scanning Calorimetry (DSC) of Aceclofenac Sodium Nanohydrogel:

The DSC thermogram of aceclofenac sodium nanohydrogel was recorded. The endothermic melting peak for pure aceclofenac sodium was found to be 143ºC and endothermic peak of aceclofenac sodium with excipients was found to be 133.82ºC, 220.16ºC, and 290.60ºC, which shows slight change in melting peak of aceclofenac sodium which is attributed to the presence of excipients indicating no chemical interaction of drug with excipients. The DSC studies conclude that the integrity of the aceclofenac sodium is unaffected by the presence of excipients as shown in Figure 7.

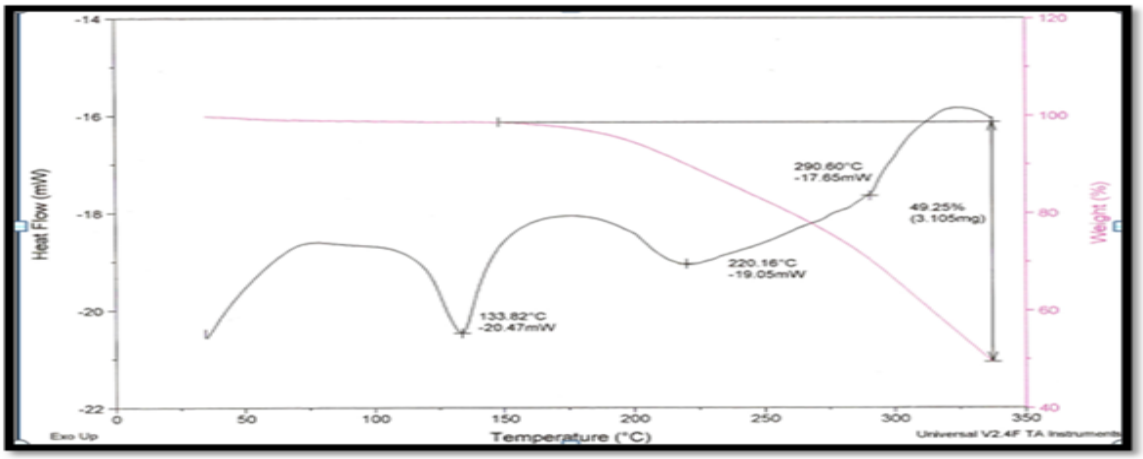

Fig. 7: DSC of Aceclofenac Sodium Nanohydrogel

Percentage Yield:

The percentage yields of three formulations were very high for all nanohydrogel obtained and the percentage yield of nanohydrogel increases with the increase in the polymer concentration. The yields of all formulations are shown in table 6 and displayed in figure 8.

Table 6: Percentage yield of aceclofenac sodium nanohydrogel

Formulation code	**F1**	**F2**	**F3**
Percentage Yield* (%)	78.78±3.2	81.33±3.3	82.11±1.1

*Each value represented as mean ± standard deviation of 3 observations

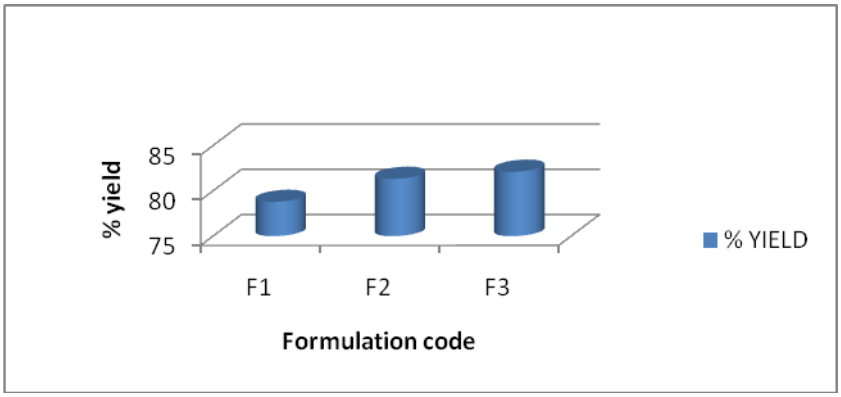

Fig. 8: Percentage Yield of Different Formulations of Nanohydrogel

Drug Content

The cumulative percentage drug released from each formulation in the *in-vitro* release studies was based on the average drug content present in the dispersion. The analyzed drug content has been shown in Table 7 and depicted graphically in Figure 9.

Table No.7: Drug content of nanohydrogel formulations

Formulation code	**F1**	**F2**	**F3**
Percentage of drug content***** (%)	82.10±1.2	85.26±1.0	90.0±0.6

* Each value represents mean ± S.D. of three observations.

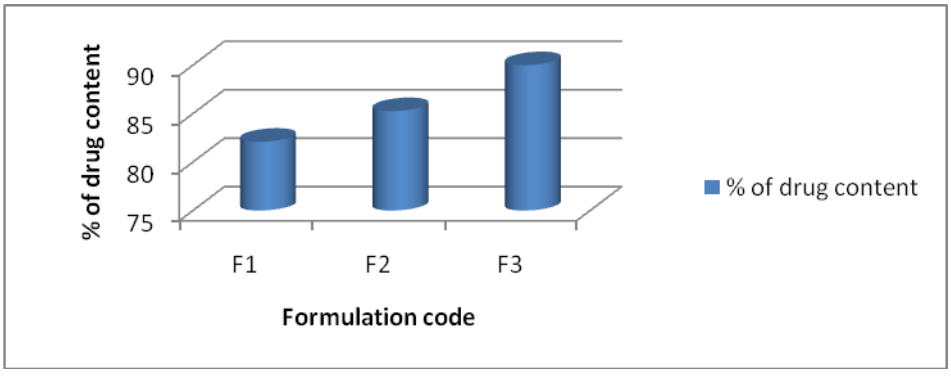

Fig. 9: Drug Content of Aceclofenac Sodium Nanohydrogel Formulations

The percentage drug content was found to increase as the concentration of gelling agent increased, this shows that as the proportion of carbopol increases it results in uniform distribution of aceclofenac sodium in the gel matrix. The percentage drug content was found to be 82.10% to 90.0% for developed nanohydrogels.

Shape and Surface Morphology:

Morphology of nanohydrogel was investigated by scanning electron microscopy (SEM). Nanohydrogels of aceclofenac sodium is three-dimensional network-like structure, where the drug is entrapped in the gel network. The SEM image obtained on drug-loaded nanohydrogel is shown in Figure 10.

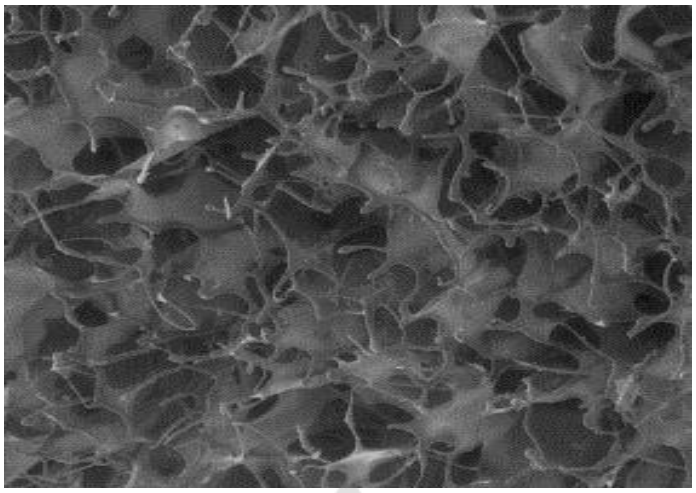

Fig.10: SEM Images of Aceclofenac Sodium Loaded Nanohydrogel Formulation F2

Viscosity Analysis:

The viscosity was directly dependent on the polymeric content of the formulation. The following Table 8 and 9 shows observations of viscosity levels of the formulated aceclofenac sodium nanohydrogels done by Brookfield viscometer and viscosity pattern predicted in Figure 11 and 12

Table 8: The viscosity* of aceclofenac sodium nanohydrogels formulations before gelation

RPM	F1	F2	F3
10	378±0.0	419±0.0	510±0.0
20	230±0.0	269±0.0	301±0.1
30	160±0.1	200±0.0	270±0.0
50	140±0.0	156±0.1	195±0.0
60	110±0.0	130±0.0	153±0.1
100	80±0.0	102±0.1	129±0.0

* Each value represents mean ± S.D. of three observations.

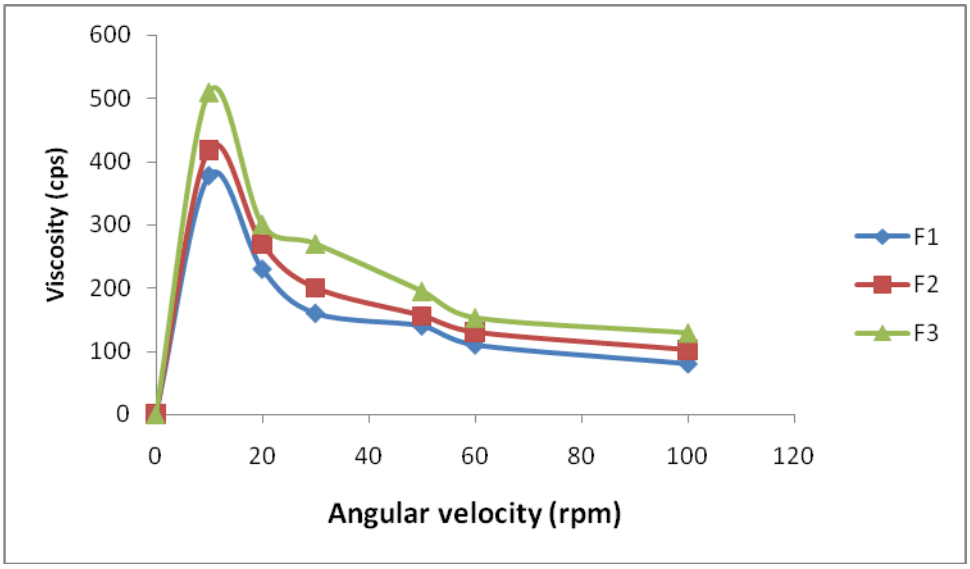

Fig. 11: The Viscosity of Aceclofenac Sodium Nanohydrogel Formulations before Gelation.

By observing the viscosity report of aceclofenac sodium nanohydrogel before gelation, the F1 formulation of aceclofenac sodium nanohydrogel contains lower polymeric concentration and it is having lesser viscosity than the F3 formulation containing higher polymeric concentration indicating that as the concentration of polymer increases in the nanohydrogel formulation the viscosity increases relatively.

Table 9: The viscosity* of aceclofenac sodium nanohydrogel after gelation

RPM	F1	F2	F3
10	32120±0.0	34313±0.0	36721±0.1
20	24320±0.0	25890±0.0	27821±0.0
30	20710±0.1	22321±0.0	24870±0.1
50	15720±0.0	17421±0.0	17891±0.0
60	10170±0.1	11820±0.1	12310±0.0
100	7890±0.0	8321±0.0	9715±0.0

* Each value represents mean ± S.D. of three observations.

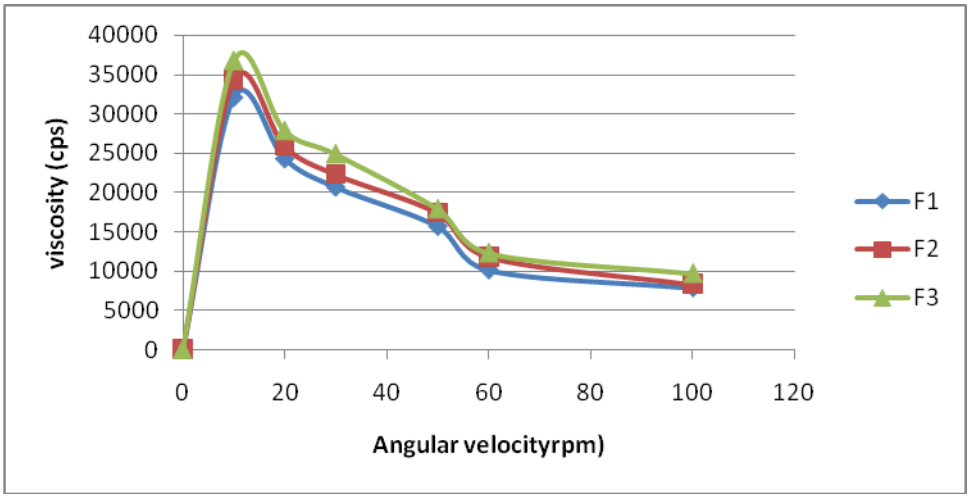

Fig. 12: The Viscosity Plot of Aceclofenac Sodium Nanohydrogel Formulations after Gelation

By observing the viscosity report of aceclofenac sodium nanohydrogel after gelation, the F1 formulation of nanohydrogel contains lower polymer concentration and it is having low viscosity than the F3 formulation containing higher polymeric concentration indicating that increase in polymer concentration increases the viscosity of the formulation.

Diffusion Study

***In vitro* drug release of aceclofenac sodium nanohydrogels using cellophane membrane:**

The *in vitro* release profile is an important tool that predicts in advance how a drug will behave *in vivo*. Release studies are required for predicting the reproducibility of rate and duration of drug release. The percutaneous therapeutic system of aceclofenac sodium nanohydrogel, allows one to control the overall release of the drug via an appropriate choice of a ratio of carbopol.

In vitro, drug release studies of all the formulations of nanohydrogels and polymeric micelles are carried out in phosphate buffer of pH 7.4 using cellophane membrane. The study was performed for 8 hrs and cumulative drug release was calculated at different time intervals.

The formulations F1, F2 and F3 containing different ratios of carbopol have shown the drug release of 76.27%, 73.99% and 68.55% for the nanohydrogel formulation respectively and diffusion study is shown in Table 10,11 and 12.

Table 10: Diffusion study of nanohydrogel F1

Time in min	Sq. Root time	Log time	Absorbance	CDR	Percentage CDR *(%)	Log percent CDR (%)	Percentage Drug retained (%)	Log percent drug retained (%)
0	0	0	0	0	0	0	100	2
60	7.745	0.889	0.066	0.35	17.55±0.31	1.244	82.45	1.916
120	10.954	1.039	0.122	0.688	32.54±0.51	1.512	67.46	1.829
180	13.416	1.127	0.151	0.852	40.23±0.65	1.604	59.77	1.776
240	15.419	1.19	0.181	1.021	48.2±0.49	1.683	51.8	1.714
300	17.32	1.238	0.226	1.276	60.11±0.65	1.778	39.89	1.60
360	18.973	1.278	0.259	1.462	68.92±0.61	1.838	31.08	1.492
420	20.493	1.311	0.277	1.564	73.48±0.69	1.866	26.52	1.423
480	21.908	1.34	0.287	1.62	76.27±0.42	1.882	23.73	1.375

*Each value represented as mean ± Standard Deviation of 3 observations

Table 11: Diffusion study of nanohydrogel F2

Time in min	Sq. Root time	Log time	Absorbance	CDR	Percentage CDR *(%)	Log percent CDR (%)	Percentage Drug retained (%)	Log percent drug retained (%)
0	0	0	0	0	0	0	100	2
60	7.745	0.889	0.054	0.287	14.34±0.26	1.156	85.66	1.932
120	10.954	1.039	0.096	0.541	25.53±0.49	1.407	74.47	1.871
180	13.416	1.127	0.128	0.722	34.2±0.61	1.534	65.8	1.818
240	15.419	1.19	0.165	0.931	43.85±0.43	1.641	56.15	1.749
300	17.32	1.238	0.201	1.134	53.47±0.62	1.728	46.53	1.667
360	18.973	1.278	0.231	1.296	61.35±0.42	1.787	38.65	1.587
420	20.493	1.311	0.253	1.42	67.14±0.86	1.826	32.86	1.516
480	21.908	1.34	0.278	1.56	73.99±0.87	1.869	26.01	1.415

*Each value represented as mean ± Standard Deviation of 3 observations

Table 12: Diffusion study of nanohydrogel F3

Time in min	Sq. Root time	Log time	Absorbance	CDR	Percentage CDR *(%)	Log percent CDR (%)	Percentage Drug retained (%)	Log percent drug retained (%)
0	0	0	0	0	0	0	100	2
60	7.745	0.889	0.05	0.265	13.28±0.22	1.156	86.72	1.938
120	10.954	1.039	0.105	0.592	28.11±0.52	1.407	71.89	1.856
180	13.416	1.127	0.139	0.784	36.96±0.39	1.534	63.04	1.799
240	15.419	1.19	0.164	0.881	43.68±0.67	1.641	56.32	1.75
300	17.32	1.238	0.195	1.094	51.95±0.37	1.728	48.05	1.681
360	18.973	1.278	0.236	1.328	62.77±0.55	1.787	37.23	1.57
420	20.493	1.311	0.243	1.37	64.71±0.69	1.826	35.29	1.547
480	21.908	1.34	0.248	1.39	68.55±0.62	1.869	31.45	1.497

*Each value represented as mean ± Standard Deviation of 3 observations

The data of diffusion study indicates that the drug release profile for nanohydrogel was low and sustained up to 8 hrs, the maximum drug was released from formulation F1. The retard in drug release in formulation F2 and F3 is because of more proportion of gelling agent added, which increases the total path for movement of drug from the gel matrix into diffusion medium.

Release Kinetic Data for Nanohydrogel Formulations:

To find out exact mechanism of drug release from nanohydrogel diffusion study, data were fitted in three different kinetic models and various equations were used, such as zero-order rate equation, first order, and Higuchi's classical diffusion model. Zero-order describes the system where release rate is independent of the concentration of the dissolved species. The first-order equation describes the drug release from the systems where release rate is dependent on the concentration of the dissolving species.

Higuchi's Classical Diffusion model describes the release from the system where a solid drug is dispersed in an insoluble matrix, and the rate of drug release is related to the rate of diffusion. The data obtained from *in vitro* drug release studies were fitted to zero-order, first-order and Higuchi's equations, and is represented in Figure 13, 14 and 15. After performing statistical analysis done for release study data the coefficient of correlation was found to favor for first-order kinetics, Indicating first order type of release.

For studying the release kinetics, all formulations are fitted in the mathematical models. In order to describe the kinetics of the release process of drug in all formulations,

The drug release data obtained from all formulations were plotted in the following modes of data treatment.

Zero Order Kinetics: - Time V/s. % Cumulative Drug Release.

First Order Kinetics: - Time V/s. Log % Cumulative Drug Remaining.

Higuchi Plot: - Square Root Of Time V/s. % Cumulative Drug Released.

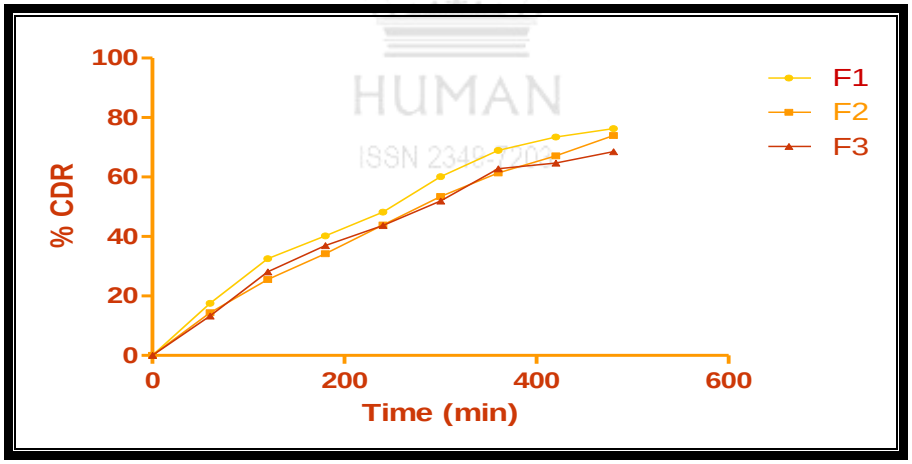

Fig. 13: % Cumulative Drug Release V/s. Time (Zero order kinetic Model)

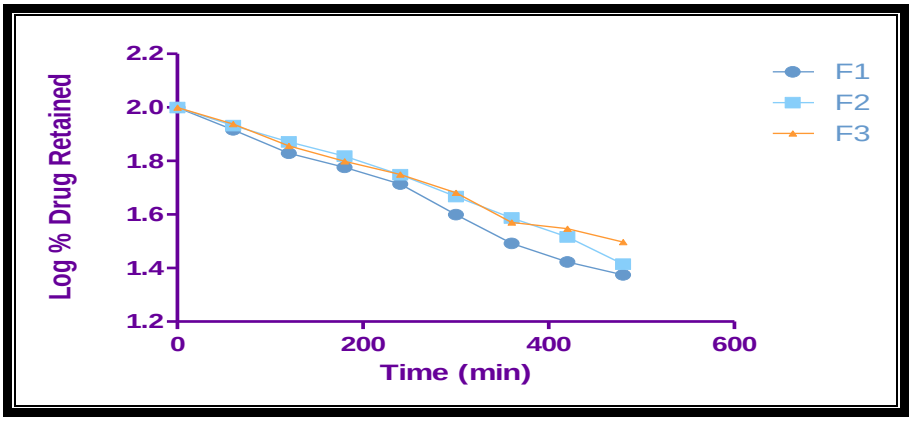

Fig. 14: Log % Cumulative Drug Remaining V/s. Time

(First order kinetic model)

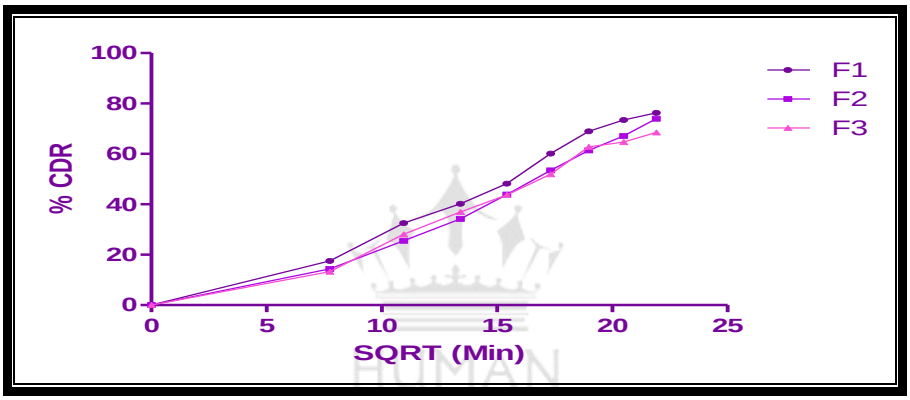

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Fig. 15: % Cumulative Drug Released V/s. Square Root of Time (Higuchi's classical diffusion model)

Table 13: Kinetic modeling of drug release of Aceclofenac Sodium

Formulation code	Zero-order		First order		Higuchi's equation	
	R	Slope	R	Slope	R	Slope
F1	0.9622	0.1571	0.9928	-0.0013	0.9775	3.740
F2	0.9863	0.1515	0.9938	-0.0011	0.9634	3.536
F3	0.9624	0.1424	0.9913	-0.0010	0.9721	3.381

The values for regression coefficient are shown in Table 13 for different kinetic models. From the results, it is seen that the drug release mechanism from the formulation was found

to follow first order kinetics. The diffusional release process is purely defined by the total concentration of the drug present in the developed formulation, indicating that the total drug payload plays an important contributory factor in the release**.**

CONCLUSION:

The aim of this study was to formulate and evaluate aceclofenac sodium nanohydrogel so as to overcome gastrointestinal adverse effects produced by oral administration of the drug and also to provide controlled release of the drug for the longer period of time. A satisfactory attempt was made to develop aceclofenac sodium nanohydrogel using carbopol 934 as polymer and evaluated for *in-vitro* characterization studies.

The following conclusions were drawn from results obtained from aceclofenac sodium nanohydrogel. UV Spectroscopy method was used for the analysis of aceclofenac sodium. The drug showed maximum absorption at wavelength 274 nm in 7.4 pH phosphate buffer. The R^2 value for the standard curve was found to be 0.9993, which showed the linear relationship between drug concentrations and absorbance values.

The preformulation studies involving description, solubility and melting point of the drug were found to be comparable with the standard. Based on the all the above preformulation studies, the drug was suitable for making the nanohydrogel formulation. Drug-polymer compatibility studies by FT-IR and DSC gave confirmation about their purity and showed no chemical interaction between the drug and selected polymer. Three formulations were developed by using carbopol 934 as a polymer and by using homogenization technique.

Developed nanohydrogel possessed the required physicochemical properties such as SEM, viscosity, IR studies, drug content and percentage yield. All the nanohydrogel dispersions are prepared by using the different concentration of polymer and weighing in between 1 to 3 gm. The percent drug content of nanohydrogel was found to be in between 82.10±1.2 to 90.0±0.6 percent of aceclofenac sodium. Percentage yield of nanohydrogel formulations is found to be ranging from 78.78±3.2 to 82.11±1.1 percent of aceclofenac sodium. By observing images of the nanohydrogel by SEM micrographs they were morphologically 3D network-like structure. Before gelation of nanohydrogel F3 formulation exhibited the higher viscosity in the concentration of 3 gm carbopol 934 and lower viscosity in the concentration of 1gm of carbopol 934 in the F1 formulation, and the viscosity after gelation increases relatively. From

the results of the drug content determination, it was seen that there was proper distribution of a drug in the dispersion and the deviations were within the acceptable limits.

In-vitro studies concluded that formulation F1 containing carbopol 934 in the concentration of 1gm showed better release than other nanohydrogel formulations. Kinetic models were used to confirm release mechanism of the formulations. Aceclofenac sodium release from the formulations F1 to F3 followed first-order kinetics. From the above results, it can be concluded that aceclofenac sodium can be delivered in the form of the transdermal route by using nanohydrogel drug delivery system prepared by using carbopol 934 as a polymer. A longer period of time the controlled release can be achieved by using this nanohydrogel preparation and further scale is been advocated for there future utilization.

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