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"FORMULATION AND EVALUATION OF DICLOFENAC SODIUM TOPICAL GEL"

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ABSTRACT
Topical gel preparations are intended for skin application or to certain mucosal surfaces for local action or transdermal penetration of medicament or for their emollient or protective action. Topical delivery of drugs can be achieved by incorporating drug into the gel matrix for effective delivery of drugs, thus avoiding first pass metabolism and for increased local action in pain management and skin diseases. NSAID's are non-steroidal drugs having excellent anti-inflammatory and analgesic activity but NSAID's produces GIT ulceration, liver and kidney trouble especially in case of oral administration. In view of adverse drug reaction associated with oral formulations, many NSAID's are increasingly administered by topical route. Hydrophilic polymers like guar gum and carbopol-940 were used in an attempt to develop topical hydrogel formulations of diclofenac sodium. Evaluation tests for visual appearance, pH, viscosity, spreadability, assay, *in vitro* drug release, *ex vivo* drug release were performed. *In vitro* drug release studies were carried out by using USP V dissolution apparatus. The effects of polymer composition on the rate of *ex vivo* drug release from the gel formulations were examined through rat abdominal skin mounting on Franz diffusion cell at 32 ± 0.5°C. No prominent changes in physicochemical properties of formulation were noticed after its exposure to accelerated conditions of temperature (40 ± 2°C) and humidity conditions (75 ± 5% RH). On exposure to accelerated stability conditions, spreadability, p^H and viscosity values were found to be slightly increased but were comparable with initial values and the stability of the formulation was found to be unaffected. The gel formulation, (F8) consisting of 1% w/v guar gum 1% w/v carbopol 940 at 1:1 ratio was found to be suitable for topical application based on *in-vitro* evaluation and *ex-vivo* permeation studies. The optimized formulation, F8 was found to have good patient compliance because of its ease of spreadability and the therapy was found to be improved as, the bioavailability of the drug was enhanced.

KEYWORDS: Diclofenac sodium, guar gum, carbopol 940, topical hydrogel, Franz diffusion cell.

INTRODUCTION

Transdermal drug delivery system

The transdermal drug delivery systems are self-contained, discrete dosage forms which when applied to intact skin deliver the drug through the skin at a controlled rate to the systemic circulation.

At present the most common form of delivery is the oral route. While this has the notable advantage of easy administration, it also has significant drawbacks namely poor bioavailability due to hepatic metabolism (first pass metabolism) and tendency to produce rapid blood level leading to a need for high and / or frequent dosing, which can be both cost prohibitive and inconvenient.

To overcome these difficulties there is a need for development of new drug delivery system ; which will improve the therapeutic efficacy and safety of drug by more precise (*i.e.*, site specific) spatial and temporal placement within the body thereby reducing both size and number of doses. One of the methods most often utilized has been transdermal drug delivery. This delivery transports therapeutic substances through the skin for systemic effect.[1]

The success of transdermal delivery depends on the ability of the drug to permeate the skin in sufficient quantities to achieve its desired therapeutic effects. The skin is very effective as a selective permeation barrier. Percutaneous absorption involves the passage of drug molecule from the skin surface into the stratum corneum

under the influence of a concentration gradient and its subsequent diffusion through the stratum corneum and underlying epidermis through the dermis and into the blood circulation.[2] The skin behaves as a passive barrier to the penetrating molecule. The stratum corneum provides the greatest resistance to the penetration and it is the rate-limiting step in percutaneous absorption.[3]

Advantages of transdermal drug delivery systems

1. Avoids gastrointestinal tract difficulties during absorption caused by enzymes, drug interactions with food, *etc.*
2. Suitable in instances like vomiting/diarrhoea where oral route is not desirable.
3. Avoids first pass *i.e.,* the initial passage of a drug substance through the systemic and portal circulation[4].
4. Maintains constant blood levels for longer period of time.
5. Improves bioavailability.
6. Decreases side or unwanted effects.
7. Decreases gastro intestinal side effects.
8. Improves patient compliance.
9. Decreases the dose to be administered.
10. Easy to discontinue in case of toxic effects.

Limitations of transdermal drug delivery systems

1. Drug with high blood concentration levels cannot be administered.
2. Drug incorporated in the transdermal formulation should be checked for skin irritation.
3. Drug with lower molecular size are only suitable for formulation[5].
4. Limited drug permeability through skin

Rational approach to topical formulation

Topical formulation can be used to manipulate the barrier function of the skin, for example, topical antibiotics and antibacterials help a damaged barrier to ward off infection, sun screening agents and the horny layer protect the viable tissues from U.V. radiation and emollient preparations restore pliability to a desiccated horny layer. Direct drugs to the viable skin tissues without using oral, systematic or other routes of therapy. For example, anaesthetic, anti-inflammatory, antipruritic and antihistaminics drugs are to be delivered to viable epidermis and dermis for localized effect. For skin appendage treatment, for example, antiperspirants, exfolients and depilatories are to be delivered to the skin appendages. Deliver drugs for systematic treatment, for example, transdermal therapeutic systems provide systemic therapy for motion sickness, angina and hypertension.

Topical gel preparations are intended for skin application or to certain mucosal surfaces for local action or percutaneous penetration of medicament or for their emollient or protective action[6]. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system.

Objective of the work

NSAID's are non-steroidal drugs having excellent anti-inflammatory and analgesic activity but NSAID's produces GIT ulceration, liver and kidney trouble especially in case of oral administration. In view of adverse drug reaction associated with oral formulations, many NSAID's are increasingly administered by topical route. Topical delivery of drugs can be achieved by incorporating drug into the gel matrix for effective delivery of drugs, thus avoiding first pass metabolism and for local action in skin diseases and pain management. The chief disadvantage of oral NSAID's administration is not the insufficient bioavailability but rather the serious side effects of the drug. These adverse effects are mainly due to poor agent specificity, resulting from the drug binding to certain (e.g., prostaglandins) receptors. The primary site of such adverse action is the gastrointestinal tract. Orally administered NSAID'S are therefore poorly tolerated and causes stomach ulcerations.

It would be desirable to reach the therapeutic drug concentration in the target tissue while simultaneously keeping the systemic and gastrointestinal agent concentrations as low as possible. Obviously, such a goal can only be achieved by delivering NSAID'S into the body via the route other than the mouth.

In present work attempt was made to formulate and evaluate topical hydrogel drug delivery systems. Attempts were made to modify drug absorption and exposure to improve pharmacokinetics and pharmacodynamics by controlling the rate of drug release from dosage forms. Rate of drug release was controlled by using cross-linking agents[75], gelling or thickening agents. The ultimate aim is to reduce number of applications in order to receive acute and effective dosage forms convenient to patient meeting requirement of steady state blood concentration of drug, leading to better compliance to therapy.

Plan of work

In order to meet objective, the present work was planned in the following manner.

1. Literature survey
2. Selection of drug candidate
3. Selection of drug delivery systems
4. Selection of excipients
5. Preformulation studies

a) Drug authentication studies
b) Drug-Excipients compatibility studies

6. Formulation and development studies

a) Preparation of diclofenac topical hydrogel
b) Analytical method development

c) Preparation of standard calibration curve

7. Evaluation of diclofenac topical hydrogel

a) Visual appearance
b) pH
c) Homogeneity
d) Spreadability
e) Viscosity
f) Assay
g) Extrudability
h) Skin irritation
i) *In-vitro* drug dissolution studies
j) *Ex-vivo* drug diffusion studies

8. To compare with the marketed formulation.

9. Stability studies (Accelerated).

Experimental investigations

Materials

Diclofenac sodium, active pharmaceutical ingredient was procured from local vendor. Carbopol 940 (Loba Chemie Pvt. Ltd., Mumbai, India), Guargum (Qualigens Fine Chemicals, Mumbai, India), Benzalkonium chloride (Prime laboratories, Hyderabad), Isopropyl myristate (Alpha Chemika, Maharashtra, India), sodium hydroxide (Prime laboratories, Hyderabad), potassium dihydrogen phosphate (Alpha Chemika, Maharashtra, India) were procured and used in this investigation.

Instrumentation

Magnetic stirrer: It was used for stirring and mixing of polymeric dispersions.

Brookfield viscometer: Viscosity of the gels was determined using a Brookfield viscometer at 100rpm using spindle no. 7 at 25^{o}C.

Digital pH meter: pH of the formulation was determined by digital pH meter (Digital potentiometer 101).

UV-VISIBLE spectrophotometer: Spectrophotometric analysis of the formulation was carried out by using UV-VISIBLE spectrophotometer (Elico SL150).

Dissolution test USP V apparatus[92]: *In-vitro* dissolution studies of the formulations were carried out by USP V dissolution apparatus.

Franz diffusion cell[93]: *Ex-vivo* drug diffusion studies were carried out by Franz diffusion cell.

Preformulation studies

Drug authentication

Melting point determination[94]

The melting point of a substance is the temperature at which the material changes from a solid to a liquid state. Pure crystalline substances have a clear, sharply defined melting point.

The melting point was determined by using Capillary tube method.

The pharmacopeias regard the capillary method as the standard technique for melting point determination. In this methodology, a thin glass capillary tube containing a compact column of the substance to be determined is introduced into a heated stand (liquid bath or metal block) in close proximity to a high accuracy thermometer. The temperature in the heating stand is ramped at a user-programmable fixed rate until the sample in the tube transitions into the liquid state. While determining a melting point, several observations and the temperatures are recorded. The accuracy of a melting point record is assured by: (a) careful sample preparation, (b) proper instrument setup, and (c) routine calibration of the instrument's temperature scale against certified melting point standards.

Sample preparation

Careless preparation of a sample is the leading cause of inaccurate and irreproducible results in melting point determinations. Any substance being loaded into a melting point capillary must be:

1. Fully dry
2. Homogeneous
3. In powdered form

The primary requirement for good melting point determination is that the sample be in a fine powder form. This makes the heat transfer into the sample more efficient and reproducible, and also enhances the overall reflectivity of the sample for easier automated detection of the melt. Coarse crystalline and non-homogeneous samples must be crushed into a fine powder in a mortar.

To fill a capillary tube with a sample, the open end of the capillary is pressed gently into the substance several times. The powder is then pushed to the bottom of the tube by repeatedly pounding the bottom of the capillary against a hard surface (preferred method). Alternatively, the capillary tube can be dropped onto a table through a glass tube of ≈1 m in length. A sample packing wire can be used at the end to further compact the sample and improve the reproducibility of the measurements.

The temperature at which the material changes from a solid to a liquid state was recorded. Melting point was determined in triplicate and average and standard deviation of the values were noted.

Solubility studies[95]

Solubility is the analytical composition of a saturated solution expressed as a proportion of a designated solute in a designated solvent. The maximum equilibrium amount of solute that can dissolve per amount of solvent is the solubility of that solute in that solvent under the specified conditions. Solubility is one of the characteristic properties of a substance and is used to describe the substance, to indicate a substance's polarity and to help to distinguish it from other substances. The

solubility of diclofenac sodium was determined at room temperature in different solvents.

Fourier transform infra-red (ftir) spectroscopy[96]
FTIR study was carried out

a) To check compatability of drug with excipients.
b) To identify unknown materials.
c) To determine the quality and consistency of a sample.

Infrared spectrum of diclofenac sodium was determined on Fourier Transform Infrared spectrophotometer (8400 S Shimadzu) using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug with excipients in the wavelength region 4000 and 400 cm^{-1}.

Compatibility studies
Compatibility of drug with excipients was determined by carrying out FTIR studies. Samples were prepared and analysed by FT IR spectroscopy for interference of peaks[97] in the spectra. Incompatibility can be determined by presence of extra peaks and by the interference of peaks in the spectra. Three samples were prepared. Sample 1 contains pure drug, diclofenac sodium of 50 mg. Sample 2 was prepared by taking drug and excipient mixture in the ratio of 1:1 [sample 2(a): carbopol-940 + diclofenac sodium; sample 2(b): guar gum + diclofenac sodium]. Sample 3 was the optimized formulation (F-8) which consists of carbopol-940 (0.1g), guar gum (0.1g) and diclofenac sodium (200mg). All the samples were analyzed spectroscopically by FTIR.

Analytical method development
Preparation of phosphate buffer pH 7.4[98]
37.1 mL of 0.2M sodium hydroxide solution and 50 mL of 0.2M potassium dihydrogen phosphate solution were mixed and volume was made up to 200 mL with distilled water to produce phosphate buffer pH 7.4.

Determination of λ_{max} of drug solution[99]
10µg/mL of drug solution was prepared and scanned against phosphate buffer 7.4 as reference solution under wavelength range of 200-400 nm by using UV spectrophotometer. A graph was plotted by taking absorbance on Y-axis and wavelength (λ_{max}) on X-axis. The highest peak on the graph was recorded as λ_{max}.

Preparation of standard stock solution of drug
Weigh accurately 100 mg of diclofenac sodium and dissolve in phosphate buffer pH 7.4. This gives 1000 µg/mL concentration of standard stock solution.

Preparation of working stock solution
From the standard stock solution of the drug, 10 mL of the solution was taken and diluted up to 100 mL with phosphate buffer pH 7.4. This gives 100 µg/mL concentration of working standard stock solution.

Preparation of working dilutions
From the working standard stock solution, 2, 4, 6, 8 and 10 mL were taken and volume was made up to 100 mL with phosphate buffer pH 7.4 to produce 2, 4, 6, 8 and 10 µg/mL concentrations, respectively.

Standard calibration curve.[100]
2, 4, 6, 8 and 10 µg/mL concentrations of drug solutions were scanned against phosphate buffer 7.4 as reference solution at 276 nm under UV spectrophotometer. A graph was plotted by taking absorbance on Y-axis and concentration (µg/mL) on X-axis. This graph yields standard calibration graph of drug solutions. These working dilutions were scanned at 276 nm for their absorbencies by using UV spectrophotometer.

Formulation and development of hydrogel formulation
The polymers used in the formulation of diclofenac sodium topical hydrogel were as follows:

1. Carbopol-940
2. Guar gum

Procedure
The topical hydrogels using different proportions were prepared as follows:

1. 0.1, 0.5, 0.75, 1% w/v concentrations of carbopol-940 colloidal dispersions were prepared using distilled water.
2. 0.1, 0.5, 0.75, 1% w/v concentrations of guar gum colloidal dispersions were prepared using distilled water.
3. After complete dispersion, both the polymer solutions were kept aside for 24 h for complete swelling.
4. Hydrogels were fabricated using different concentrations of polymeric dispersions.
5. Dispersions of polymers were made using magnetic stirrer (500 rpm). After dispersing carbopol-940 in distilled water, colloidal dispersion of guar gum was added to it under magnetic stirring. 1% v/v isopropyl myristate[101] and 0.025% w/v benzalkonium chloride was added[102]. Aqueous drug solution was added to the polymeric dispersion after addition of sodium hydroxide solution.

Finally, the remaining distilled water was added to obtain a homogeneous dispersion of gel under magnetic stirring.

20g of topical hydrogel was formulated containing 200 mg of diclofenac sodium using different proportions of carbopol and guar gum.

Evaluation of gels[103]
Gels were evaluated for their clarity, homogeneity, pH, viscosity, spreadability, skin irritation test, drug content, *in-vitro* diffusion studies and *in-vivo* studies by using standard procedure. All studies were carried out in triplicate and mean values were reported along with standard deviation values.

Visual appearance
The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows: turbid: +, clear: ++, very clear (glassy): +++.

Determination of pH
2.5 g of gel was accurately weighed and dispersed in 25 mL of distilled water. The pH of dispersion was measured by using digital pH meter[104] (Digital potentiometer 101).

Homogeneity
All formulated gels were tested for homogeneity by visual inspection after the gels have been set in the container for their appearance and presence of any aggregate.

Determination of spreadability[105]
The parallel plate method is the most widely used method for determining and quantifying the spreadability of semisolid preparations. The advantages of the method are simplicity and relative lack of expense. Also, the assemblies can be designed and fabricated according to individual requirements to type of data required. On other hand, the method is less precise and sensitive and the data it generates must be manually interpreted and presented.

The linear relationship between viscosity and spreading diameter was independent of the derivative. The spreading capacity of the gel formulations was measured 48 h after preparation by measuring the spreading diameter of 1g of the gel between two 20×20 cm glass plates after 1 minute the mass of the upper plate was standardized at 125 g. Panigrahi used a similar apparatus to assess the spreadabilty of gels. The following equation was used for the purpose:

$$S = mL/T \quad (9)$$

Where:
S, is the spreadabilty of gel formulations
m, is the weight (g) tied on the upper plate,
L, is the length (cm) of the glass plates, and
T, is the time taken for plates to slide the entire length.

PROCEDURE
Two glass slide of 20×20 cm were selected. The gel formulations whose spreadability had to be determined were placed over one of the slides. The other slide was placed upon the top of the gel such that the gel was sandwiched between the two slides in an area occupied by a distance of 60 cm along 100 g weight was placed upon the upper slide so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gels adhering to the slide was scrapped off. The two slides in positioned were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20 g weight was tied to upper slide carefully. The time taken for the upper slide to travel the distance of 6 cm and separate away from the lower slide under the direction of weight was noted. The determinations were carried out in triplicate and the average of three reading is recorded.

Measurement of viscosity[106]
Viscosity of the gels was determined using a Brookfield digital viscometer. It is an instrument used for measuring the viscosity of plastisols and other liquids of thixotropic nature.

The instrument measures the shearing stress on spindle rotating at a definite, constant speed while immersed in the sample. The degree of spindle lag is indicated on a rotating dial. This reading is multiplied by a conversion factor based on spindle size and rotational speed, gives a value for viscosity in centipoises. By taking measurements at different rotational speeds, an indication of the degree of thixotrophy of the sample is obtained.

Assay[107]
The diclofenac sodium hydrogel of 100 mg was dissolved in 50 mL of phosphate buffer pH 7.4. The volumetric flask containing gel solution was shaken for 2 h on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically at wavelength 276 nm. Tests were carried out in triplicate and mean value of the three observed values was noted along with standard deviation value.

Extrudability of hydrogel[108]
The extrudability test was carried out by using Pfizer hardness tester. A 15 g of gel was filled in aluminum tube. The plunger was adjusted to hold the tube properly. The pressure of 1kg/cm^2 was applied for 30 sec. The quantity of gel extruded and the ease with which the gel extrudes out was observed. The procedure was repeated at three equidistance places of the tube. Test was carried out in triplicates.

Skin irritation test[109]
The skin irritation test was performed on properly washed rat skin, by applying 1g gel formulation on 2 square inch area of skin and the skin was covered with cotton. After 24 h observe for any irritation or redness of the skin and report if observed.

***In-vitro* drug dissolution studies[110]**
In-vitro drug release was determined by USP V apparatus (Paddle Over Disc). Dissolution medium used for *in-vitro* drug release was phosphate buffer pH 7.4. Temperature of 32 ± 0.5^{o}C was maintained with paddle rpm of 50. Sink conditions were maintained by replacing the medium with fresh dissolution medium when samples were withdrawn for UV spectrophotometric analysis. 1g of formulated gel was taken and evenly spread over disc of diameter 5.65 cm and carefully kept at the bottom of dissolution flask beneath the paddle.

Aliquots were collected from sampling port at time intervals of 5, 10, 15, 20 & 30 min. Samples were observed for their absorbance in UV spectrophotometer at 276 nm against phosphate buffer pH 7.4 as blank. *In-vitro* dissolution studies were carried out in triplicate and mean value of the three observed values was noted along with standard deviation.

***In-vitro* drug diffusion study.**[111]

Albino rats weighing 135-160 g were used to obtain freshly excised full thick skin. Animal was sacrificed by spinal dislocation. Hairs from abdominal region was removed by means of razor taking care not to damage the epidermal surface. Gel of weight equivalent to 10mg of diclofenac sodium was spread uniformly on the skin surface, such that the gel occupies inner circumference of the glass tube. The skin was tied securely to one end of Franz diffusion cell. Receptor compartment of Franz diffusion cell was filled with phosphate buffer pH 7.4 which was kept under optimum magnetic stirring.

Aliquots were collected from sampling port at time intervals of 5, 10, 15, 20, 30 min. Samples were observed for their absorbance in UV spectrophotometer at 276 nm. *Ex-vivo* permeation studies was carried out in triplicate and the mean value of the three observed values was noted along with standard deviation value.

Accelerated stability studies[112]

Stability is defined as the extent to which a product retains with in specified limits and throughout its period of strong and uses *i.e.*, shelf life. Stability studies were carried out on optimized formulation according to International Conference on Harmonization (ICH) guidelines.

The formulation packed in aluminium tube was subjected to accelerated stability testing for 3 months as per ICH norms at a temperature (40 ± 2°C) and relative humidity 75 ± 5%. Samples were taken at regular time intervals of 1month for over a period of 3months and analyzed for the change in pH, spreadability, drug content and *in-vitro* drug release by procedure stated earlier. Any changes in evaluation parameters, if observed were noted. Tests were carried out in triplicate and mean value of the observed values was noted along with standard deviation.

RESULTS AND DISCUSSION

Preformulation studies

Melting point

Melting point of diclofenac sodium was determined by capillary method and found to be 163°C which correlates with that of standard melting point value of diclofenac sodium.

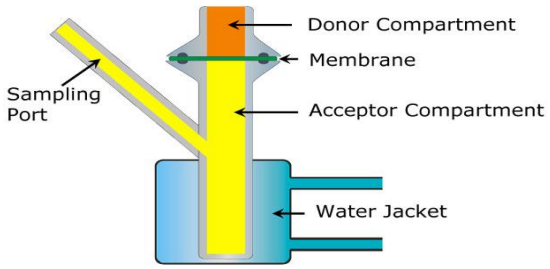

Figure: 6 Franz diffusion cell

Table 1: Determination of melting point

S.No	Trials	Melting point (°C)	Reference standard (°C)
1	1	163	163 (BP)
2	2	163	
3	3	163	

Solubility studies

Solubility studies for diclofenac sodium were carried out at room temperature.

The equilibrium solubility performed in various solvents at the indicated temperature (RT) was shown in the **Table 2.**

Table 2: Solubility of diclofenac sodium

Solvent	Temperature	Solubility (mg/mL)
Deionized water	RT	> 9
Methanol	RT	> 24
Acetone	RT	6
Acetonitrile	RT	<1
Cyclohexane	RT	<1
pH1.1 (HCl)	RT	<1
pH7.4 Phosphate buffer	RT	6

FT IR studies

FT IR studies were carried out on drug, excipients and drug-excipient samples. No new peaks were found and hence compatibility between the drug and the excipients was found.

Peaks were found at the following wavenumbers which are representative of specific functional groups.

Table 3: Determination of FTIR functional groups

S.No	Functional groups	Wave numbers (cm^{-1})
1	C-H (aromatic rings)	3000-3100
2	C=C (aromatic rings)	1500-1600
3	C=O	1690-1760
4	N-H	3300-3500
5	C-N	1180-1360

FTIR spectra of the samples were compared with that of standard spectra of drug and excipients and found to have same peaks at particular wave numbers. No interference of peaks between drug spectra and drug-excipients spectra was seen.

Drug excipient compatability studies

Compatibility between drug and excipients was studied by FTIR spectroscopy and found to have no incompatibility between drug and excipients. The following are the FTIR spectra of drug and drug-excipient mixture (1:1).

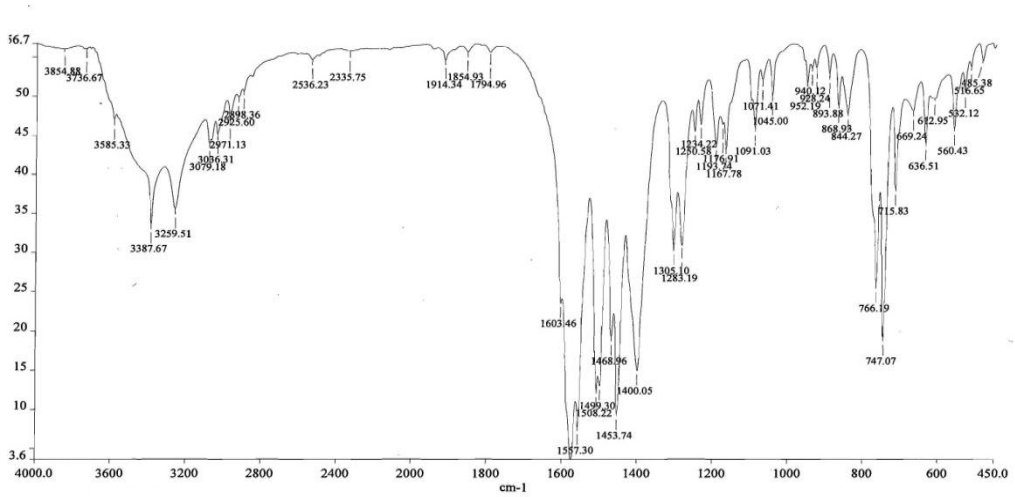

Figure 7: FTIR spectra of diclofenac sodium

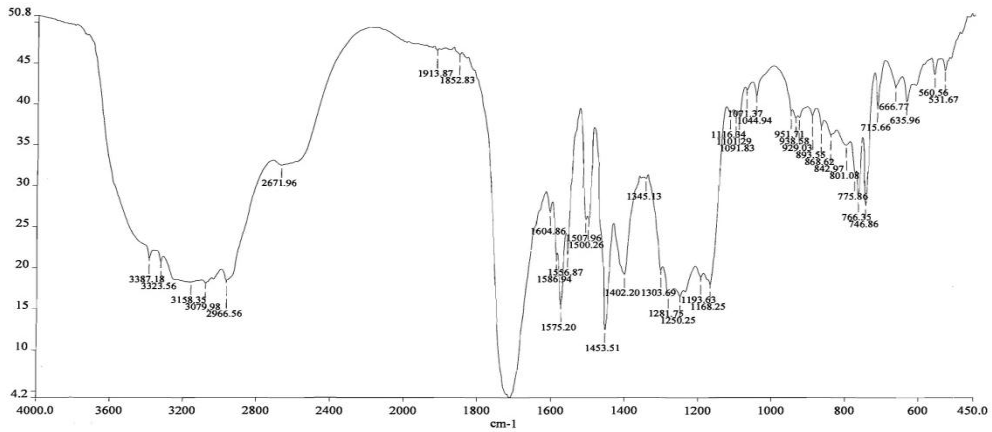

Figure 8: FTIR spectra of diclofenac sodium-carbopol-940 mixture (1:1)

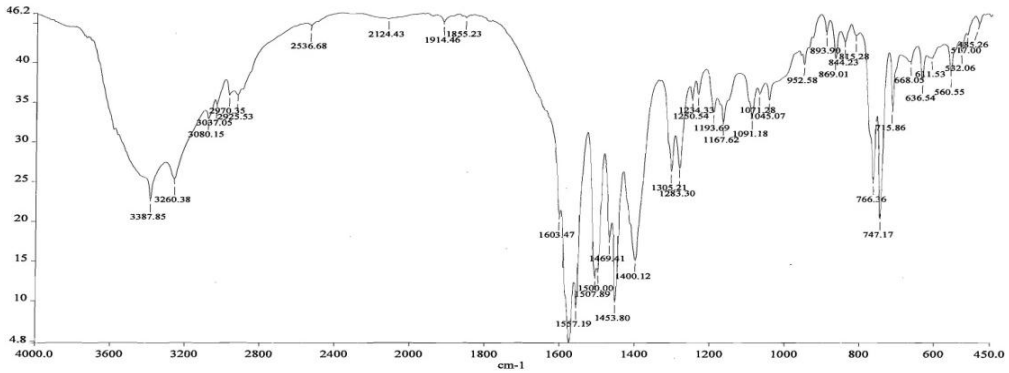

Figure 9: FTIR spectra of diclofenac sodium-guargum mixture (1:1)

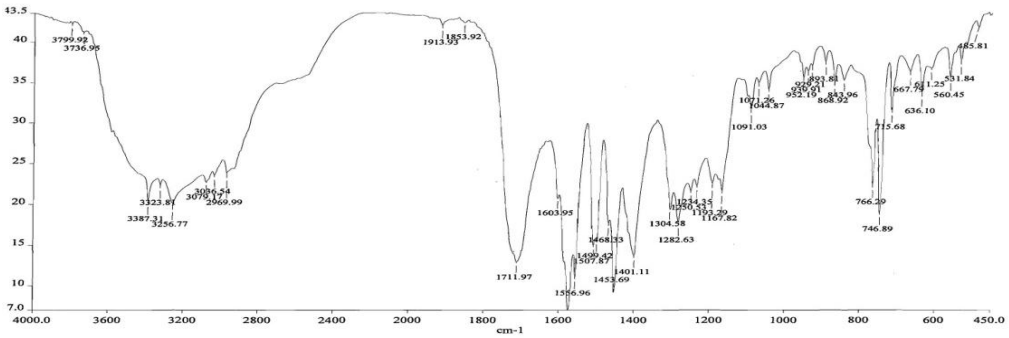

Figure 10: FTIR spectra of optimized formulation

From the FTIR spectra of pure drug and drug-excipient mixture it was found that drug and excipients were compatible with each other as there was no interference of peaks or existence of extra prominent peaks.

Analytical method development

Determination of λ_{max} of drug solution

λ_{max} of diclofenac sodium (10 µg/mL) was determined using UV spectrophotometer against phosphate buffer p^H 7.4 as blank. Only one peak was observed at 276 nm (λ_{max}) of prepared sample. This was considered as λ_{max} of solution and absence of any impurity must be ensured.

Preparation of standard stock solution of drug

Working standard stock solution of diclofenac sodium was prepared as discussed in section **3.3.3.** From working standard stock solution of the drug, working standard stock solution was prepared from which working dilutions of 2, 4, 6, 8 and 10µg/mL were prepared and analyzed for absorbencies at 276 nm against phosphate buffer pH 7.4 as blank.

Standard calibration curve

Linearity of prepared solution was found in the range of 0 to 10µg/mL. From regression analysis value of co-efficient of regression (R^2) was 0.9997. This confers the range selection was satisfactory and follows Beer-Lambert's law.

Table 4: Standard calibration graph of diclofenac sodium

S.No	Concentration (µg/mL)	Absorbance (nm)
1	0	0
2	2	0.098
3	4	0.176
4	6	0.252
5	8	0.320
6	10	0.400

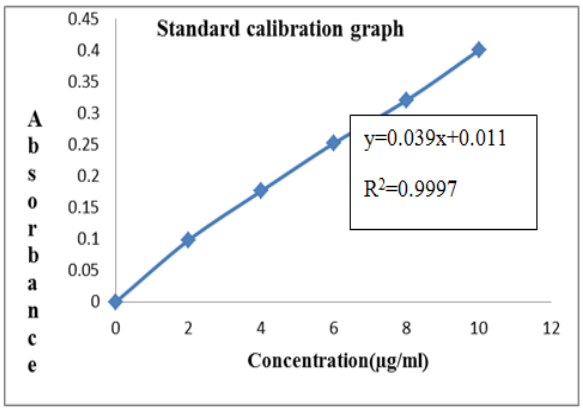

Figure 11: Standard calibration graph of diclofenac sodium

Formulation and development of hydrogel formulation

20 g of topical hydrogel was formulated containing 200 mg of diclofenac sodium using different proportions of carbopol and guar gum.

The topical hydrogels of diclofenac sodium using different proportions of polymers were developed. From the developed gels, gels of better physicochemical properties were selected. Different proportion of polymers used in development of gels is tabulated in **Table 5.**

Table: 5 Optimization of diclofenac sodium topical hydrogels.

Carbopol-940 (%w/v)	uargum (%w/v)				NaOH (xN)
	0.1	0.5	0.75	1	
0.1	+	++	++	+	0.1
0.5	++	+	+++ F3	+++ F6	0.1
0.75	+++ F1	++	+++ F4	+++ F7	0.1
1	+++ F2	++	+++ F5	+++ F8	0.1

+ gelatinous solution.
++ thin, transparent gel.
+++ thick, translucent gel.

From the developed gels, gels of better physicochemical properties were selected. These selected gels were formulated and the composition of these selected formulations (F1-F8) was given in the **Table 6.**

Table: 6 Composition of diclofenac sodium topical hydrogels.

Ingredients (in mg)	FORMULATION CODE							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Diclofenac sodium	1000	1000	1000	1000	1000	1000	1000	1000
Carbopol-940	0.375	0.5	0.25	0.375	0.5	0.25	0.375	0.5
Guargum	0.05	0.05	0.375	0.375	0.375	0.5	0.5	0.5
Isopropyl myristate (mL)	1	1	1	1	1	1	1	1
Benzalkonium Chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Purified water (q.s)	100g	100g	100g	100g	100g	100g	100g	100g

In the present study on topical hydrogel of diclofenac sodium, carbopol 940 and guar gum were used as hydrogel forming polymers with sodium hydroxide as cross linking agent. Isopropyl myristate and benzalkoniumchloride were used as penetration enhancer and preservative respectively.

Evaluation of gels

Gels were evaluated for their clarity, homogeneity, pH, viscosity, spreadability, skin irritation test, drug content, *in-vitro* dissolution studies and *ex-vivo* studies by using standard procedure. All studies were carried out in triplicate and average values were reported.

Physical appearance

The physical appearance of various formulations was determined by visual inspection under black and white background and all formulations (F1-F8) were found to be clear, thick and translucent. All the selected formulations were found to be homogenous without any aggregates or lumps.

Determination of pH

The pH of the selected formulations was determined by digital pH meter as described in the section **3.5.2** and was found to be pH 7.0 for all the selected formulations.

Homogeneity

Homogeneity of all the formulated gels was determined by visual inspection and was found that all the formulations (F1-F8) were homogenous.

Determination of spreadability

Spreadability of the formulated gels was determined and all the gels were found to be easily spreadable and thus shows good patient compliance and good absorption from skin.

Measurement of viscosity

It was observed that, as the polymer concentration increases the viscosity of the gels also increases. This affects the extrudabilty of the gel from the container. Viscosity values of all formulations (F1-F8) were found to be comparable with the viscosity of marketed

formulation. The observed viscosity values were tabulated in the **Table 7 and 8.**

Assay
The % drug content values were determined and tabulated in the **Table 7 and 8**. All the values were found to be in the range of standard value of 98.5-100.5%.

Extrudability of hydrogel
The extrudability test was carried out by using Pfizer hardness tester. The test was carried out as described in section **3.5.7.** All the formulations (F1-F8) were found to be easily extrudable from the container. Easier the gel extrudes out, better the patient compliance.

Skin irritation test
The selected gel formulations were found to show no redness of skin and no skin irritation. The formulation was found to be safe when topically applied.

Table: 7 Evauation of gels of formulation F-1 to F-4

Physical parameters	Formulation code			
	F1	F2	F3	F4
Physical appearance	Thick, translucent	Thick, translucent	Thick, translucent	Thick, translucent
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
pH	7	7	7	7
Assay	98.5 ± 0.2	98.7 ± 0.2	99.2 ± 0.1	99.7 ± 0.1
Viscosity (cps)	8819 ± 2	8850 ± 1.5	8980 ± 1.5	9142 ± 2.5
Spreadability	++	++	++	++

n = 3 ± SD
+ good spreadability
++ better spreadability

Table: 8 Evauation of gels of formulation F-5 to F-8 and marketed formulation

Physical parameters	Formulation code				
	F5	F6	F7	F8	Marketed formulation
Physical appearance	Thick, translucent	Thick, translucent	Thick, translucent	Thick, translucent	Thick, translucent
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous
pH	7	7	7	7	7
Assay	99 ± 0.4	99.4 ± 0.2	99.7 ± 0.1	99.6 ± 0.3	99.7 ± 0.1
Viscosity (cps)	9236 ± 1.5	9344 ± 2	9589 ±2	9636 ± 1.5	9643 ± 1.7
Spreadability	++	++	++	++	++

n = 3 ± SD
+ poor spreadability
++ good spreadability

***In-vitro* drug dissolution studies**
In-vitro drug release was determined by using USP V apparatus (Paddle Over Disc).
The % *in-vitro* drug release can be determined and the values were tabulated in **Table 9 and 10.**

Table 9: *In-vitro* drug dissolution data of gel formulations F-1 to F-5

Time (min)	Cumulative % of drug release				
	F1	F2	F3	F4	F5
5	73.1 ± 0.3	73.6 ± 0.5	51 ± 1	41 ± 0.1	44.9 ± 0.7
10	74.1 ± 0.2	79.7 ± 0.2	57.4 ±0.4	55 ± 0.2	56.2± 0.05
15	76 ± 0.1	80.4 ± 0.4	70.3 ± 0.1	60.4 ± 0.4	64.1 ± 0.2
20	77.7 ± 0.1	80.6 ± 0.2	72.4 ± 0.5	63.7 ± 0.2	68.4 ± 0.5
30	79.8 ± 0.3	81.4 ± 0.6	79.5 ± 0.4	68.1 ±0.2	74.1 ± 0.3

n = 3 ± SD

Table 10: *In-vitro* drug dissolution data of gel formulations F-6 to F-8, drug and marketed formulation

Time (min)	Cumulative % of drug release				
	F6	F7	F8	drug	Marketed formulation
5	49.7 ± 0.2	25.6 ± 0.1	28.4 ± 0.4	93.6 ± 0.3	13.6 ± 0.025
10	54.2 ± 0.3	33.1 ± 0.3	36.1 ± 0.3	99 ± 0.05	25.8 ± 0.3
15	58.5 ± 0.3	39.6 ± 0.4	39.7 ± 0.3	99.8 ± 0.1	34.7 ± 0.3
20	62.8 ± 0.2	49.1 ± 0.5	45.9 ± 0.7	99.8 ± 0.1	40.5 ± 0.6
30	67.5 ± 0.4	51.5 ± 0.6	82.5 ± 0.5	99.9 ± 0.1	45.2 ± 0.2

n = 3 ± SD

From the *in-vitro* drug release data, F8 (1% carbopol 940+1% guar gum) was found to release 82.5 ± 0.5% at the end of 30 min whereas marketed formulation released 45.2 ± 0.2% of the drug at the end of 30 min. F8, among the selected formulations was found to show better *in-vitro* drug release. The polymer composition in F8 confers to the better drug release. A comparative study on *in-vitro* drug release of formulations was depicted in the **Figures 12-22.**

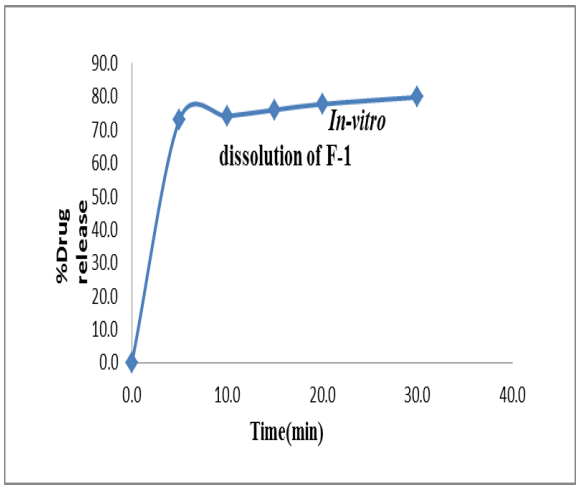

Figure 12: *In-vitro* drug release profile of gel formulation F1

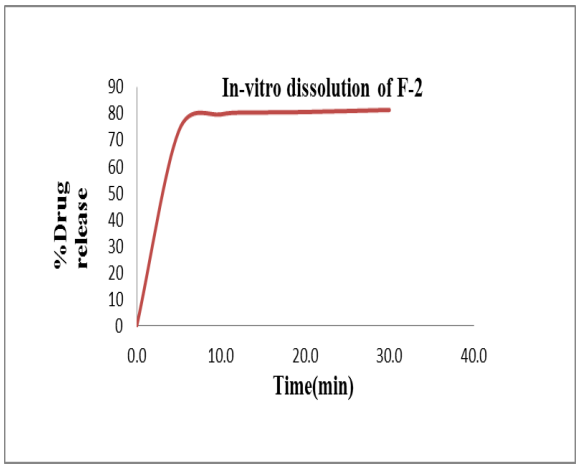

Figure 13: *In-vitro* drug release profile of gel formulation F2

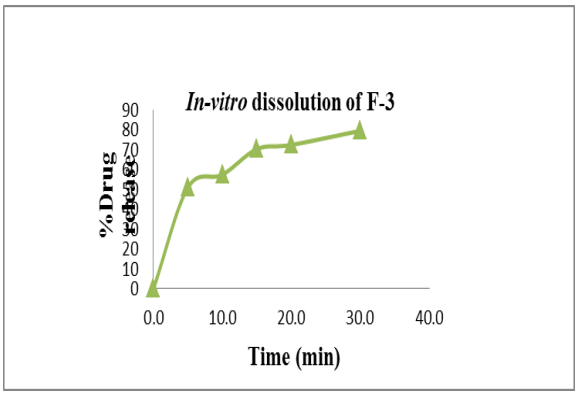

Figure 14: *In-vitro* drug release profile of gel formulation F3

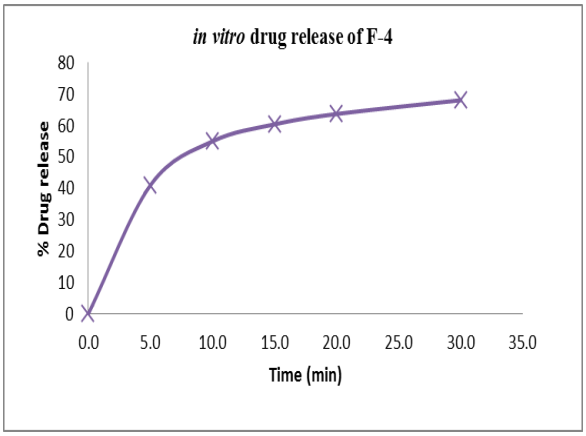

Figure 15: *In-vitro* drug release profile of gel formulation F4

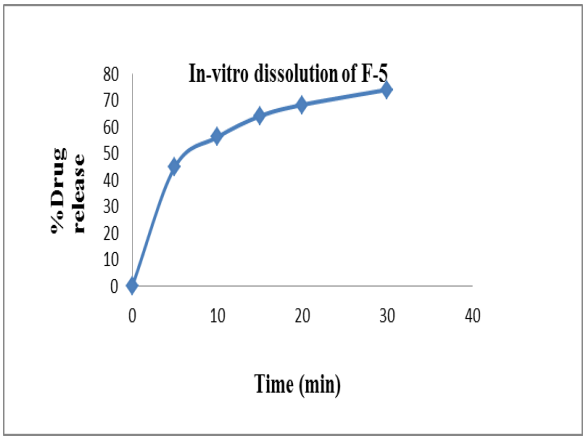

Figure 16: *In-vitro* drug release profile of gel formulation F5

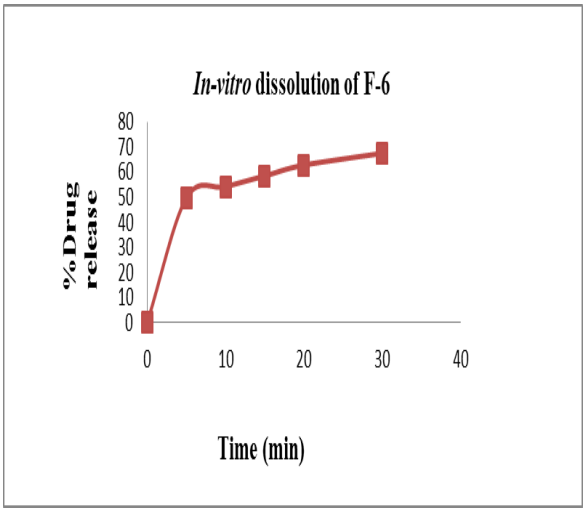

Figure 17: *In-vitro* drug release profile of gel formulation F6

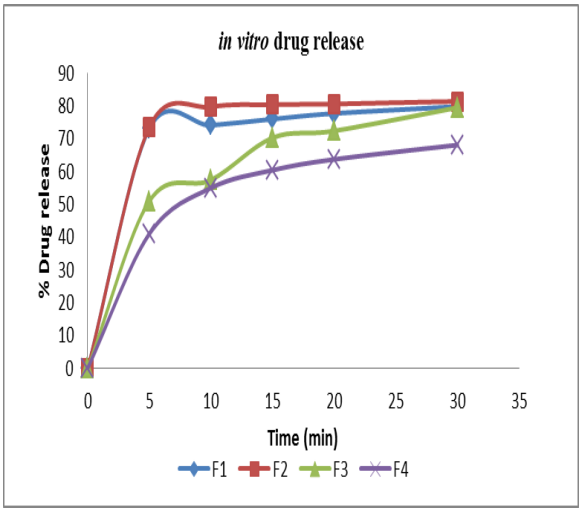

Figure 20: *In-vitro* drug release profile of gel formulation (F1-F4)

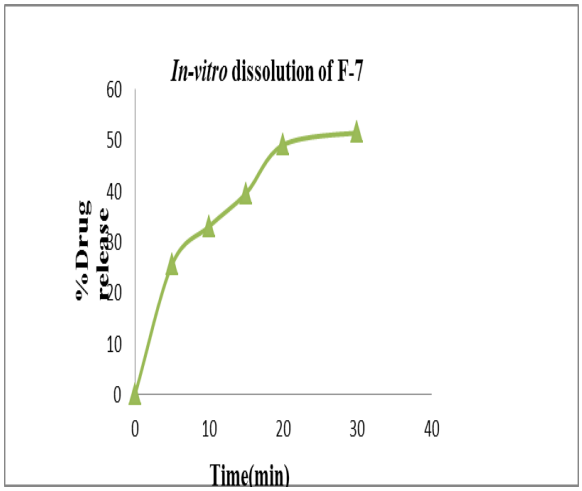

Figure 18: *In-vitro* drug release profile of gel formulation F7

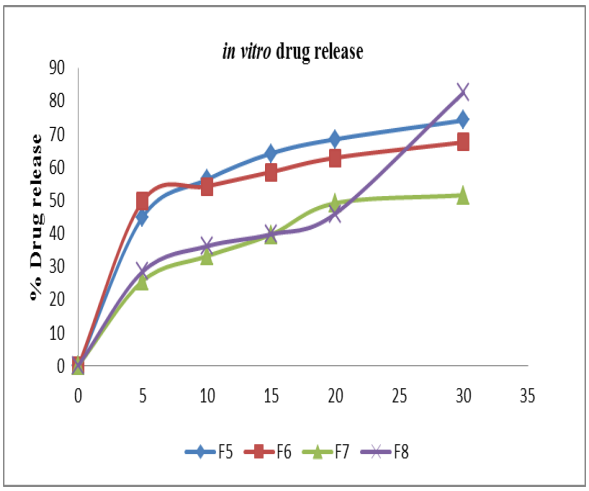

Figure 21: *In-vitro* drug release profile of (F5-F8)

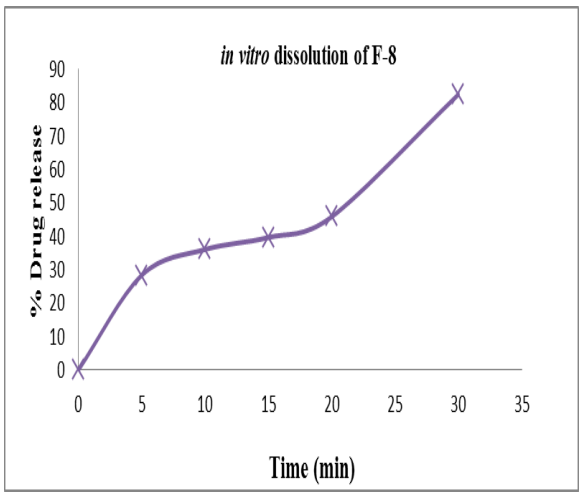

Figure 19: *In-vitro* drug release profile of gel formulation F8

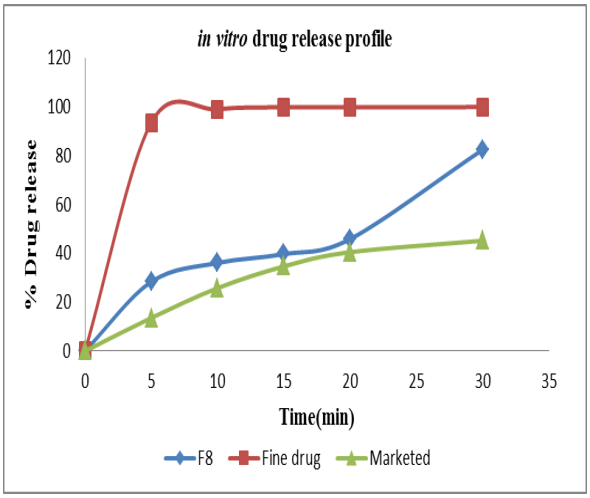

Figure 22: *In-vitro* drug release profile of F8, pure drug & marketed formulation

From the results it was clearly known that F8 shows better drug release when compared to marketed product.

***Ex-vivo* drug diffusion studies**

Aliquots collected at time intervals of 5, 10, 15, 20 and 30 min were observed for their absorbance under UV spectrophotometer at 276 nm. Thus, from the standard calibration graph and its equation, the concentration of the drug was found out.

Thus, from the above equation the % *ex-vivo* drug release can be determined and the values were tabulated in **Table 11 and 12.**

Table 11: *Ex-vivo* drug diffusion data of gels (F1-F5)

Time (min)	Cumulative % of drug diffusivity				
	F1	F2	F3	F4	F5
5	52.1 ± 0.2	52.9 ± 0.1	39.1 ± 0.3	21.3 ± 0.2	21.9 ± 0.4
10	52.9 ± 0.2	58.6 ± 0.4	41.2 ± 0.4	34 ± 0.4	33.9 ± 0.3
15	55 ± 0.1	60 ± 0.8	58.7 ± 0.4	39.5 ± 0.3	42.3 ± 0.5
20	56.9 ± 0.2	60.3 ± 0.5	59.7 ± 0.5	42.5 ± 0.5	50 ± 0.6
30	59.3 ± 0.4	60.4 ± 0.5	61.9 ± 0.4	49.5 ± 0.9	52.3 ± 0.5

n = 3 ± SD

Table: 12: *Ex-vivo* drug diffusion data of gels (F6-F8 and marketed formulation)

Time (min)	Cumulative % of drug diffusivity			
	F6	F7	F8	Marketed formulation
5	28.6 ± 0.5	12.7 ± 0.3	15.7 ± 0.4	12.8 ± 0.4
10	32.4 ± 0.6	19.7 ± 0.5	25.3 ± 0.8	23.4 ± 0.5
15	39.2 ± 0.5	24.5 ± 1.1	32.1 ± 0.9	30.7 ±0.9
20	44.4 ± 0.5	36 ± 0.9	41.2 ± 1	39.1 ± 0.3
30	49.3 ± 0.4	40.7 ± 0.5	64.6 ± 0.2	43.2 ± 0.3

n = 3 ± SD

From the *ex-vivo* drug release data, F8 was found to release 64.6 ± 0.2% at the end of 30 min whereas marketed formulation released 43.2 ± 0.3% of the drug at the end of 30 min. F8, among the selected formulations was found to show better *ex-vivo* drug release. A comparative study on *in-vitro* drug release of formulations was depicted in the **Figures 23-33.**

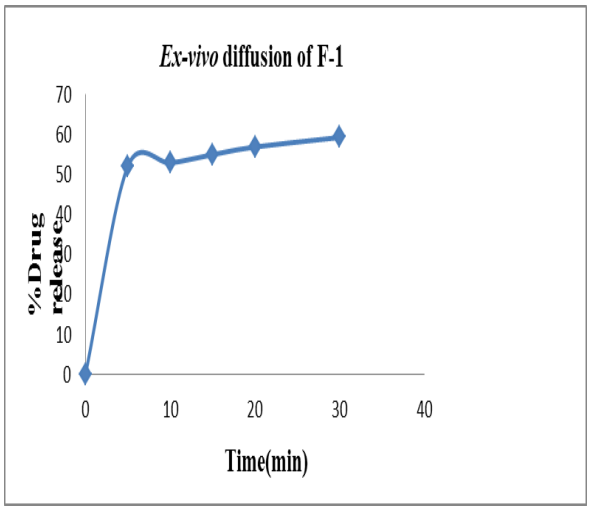

Figure 23: *ex-vivo* drug release profile of formulated gel F1

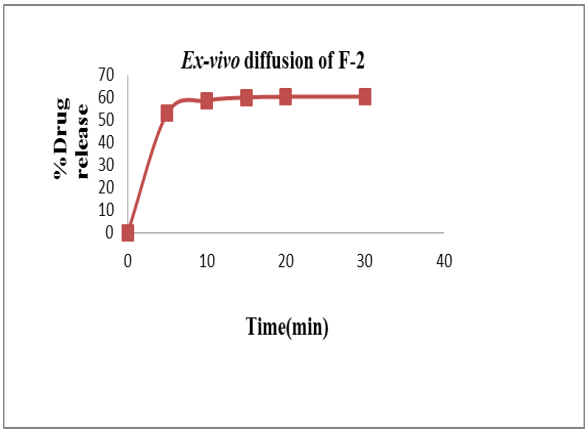

Figure 24: *ex-vivo* drug release profile of formulated gel F2

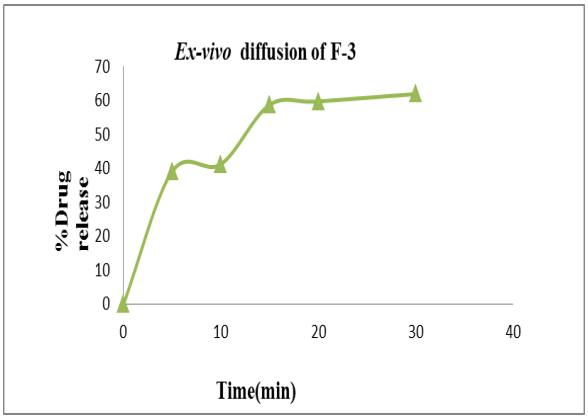

Figure 25: *ex-vivo* drug release profile of formulated gel F3

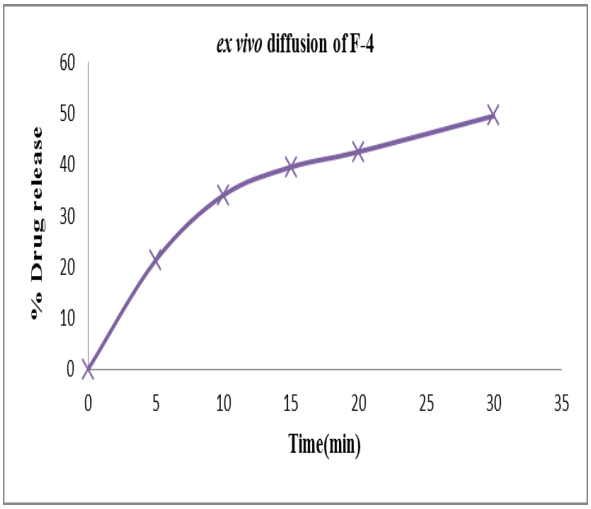

Figure 26: *ex-vivo* drug release profile of formulated gel F4

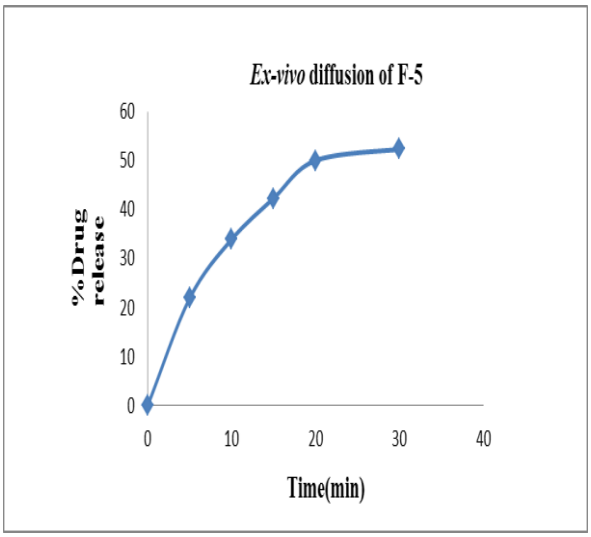

Figure 27: *ex-vivo* drug release profile of formulated gel F5

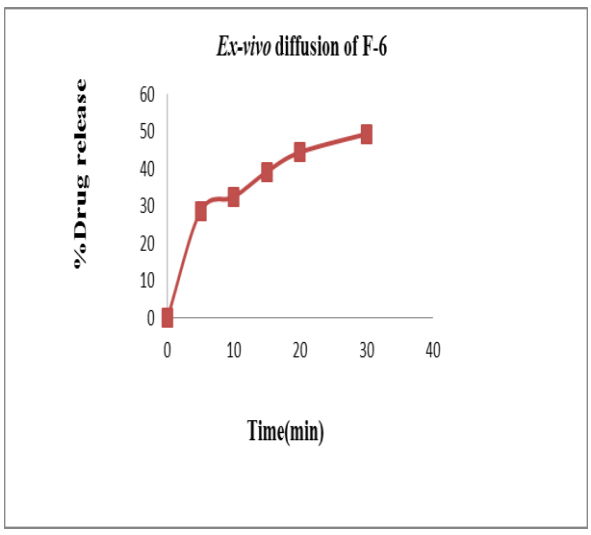

Figure 28: *ex-vivo* drug release profile of formulated gel F6

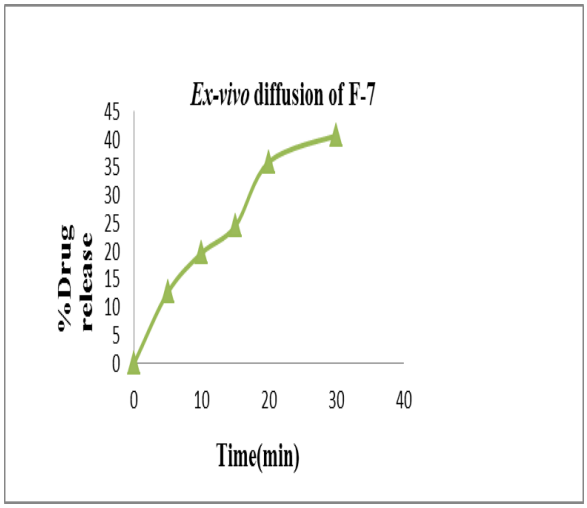

Figure 29: *ex-vivo* drug release profile of formulated gel F7

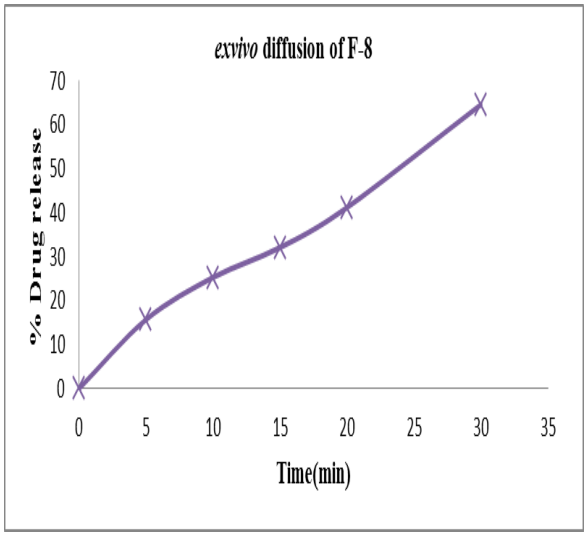

Figure 30: *ex-vivo* drug release profile of formulated gel F8

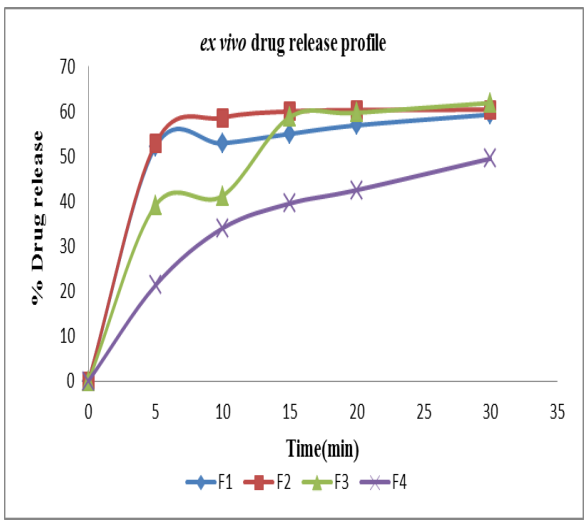

Figure 31: *ex-vivo* drug release profile of formulated gels (F1-F4)

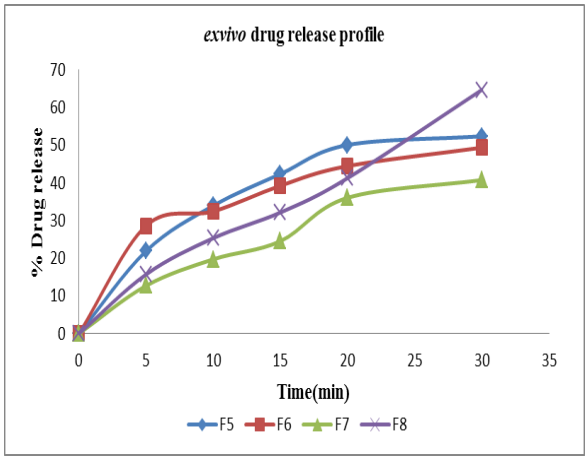

Figure 32: *ex-vivo* drug release profile of formulated gels (F5-F8)

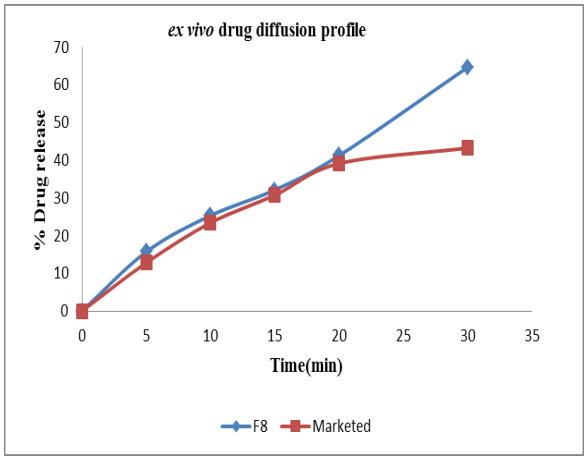

Figure 33: *ex-vivo* drug release profile of F8 and marketed formulation

From the results it was clearly known that F8 shows better drug release when compared to marketed product.

Accelerated stability studies

Significant changes were not noticed. The formulation F8 was found to be stable after exposure to accelerated temperature and humidity conditions for a period of 3 months. No significant changes were seen in physical evaluation parameters and given in the **Table 13.** The *in-vitro* drug release data was given in the **Table 14.**

Table 13: Physical parameters after accelerated stability study of formulation F8

Physical Parameter	Temperature: 40° ±2°C ;Relative humidity (RH): 75 ± 5%RH			
	Initial	After 1 month	After 2 months	After 3 months
pH	7 ±0.07	6.9 ± 0.07	6.9 ± 0.07	6.9 ± 0.07
Assay	99.6 ± 0.1	99.5 ± 0.1	99.4 ± 0.1	99.3 ± 0.1
Viscosity (cps)	9636 ± 1.5	9639 ± 1.2	9645 ± 1.5	9649 ± 2

n = 3 ± SD

Table 14: *In-vitro* drug release data after accelerated stability study of formulation F8

Time (min)	Cumulative % of drug release (mean)			
	Initial	After 1 month	After 2 months	After 3 months
5	28.4	28.2	27.9	27.8
10	36.1	35.9	35.8	35.6
15	39.7	39.5	39.2	38.9
20	45.9	45.6	45.4	45.2
30	82.5	82.1	81.9	81.8

Table 13 and 14 shows no significant changes in physicochemical properties and *in-vitro* drug release profile of optimized formulation even after its exposure to accelerated conditions of temperature (40°C) and humidity conditions (75 ± 5%RH). Hence the developed formulation was found to be stable after subjecting to accelerated stability conditions.

SUMMARY AND CONCLUSION

In present work attempt was made to formulate and evaluate topical hydrogel drug delivery systems. Attempts were made to modify drug absorption and exposure to improve pharmacokinetics and pharmacodynamics by controlling the rate of drug release from dosage forms. Rate of drug release will be controlled by using cross-linking agents, gelling or thickening agents. The ultimate aim is to reduce number of doses in order to receive acute and elegant dosage forms convenient to patient meeting requirement of steady state blood concentration of drug, leading to better compliance to therapy.

Thus, topical hydrogels were formulated by varying proportions of polymers and they were evaluated. The physicochemical properties of the gel formulations were shown in **Table 7 and 8.** From the results it is evident that all gel formulations showed uniform homogeneity and spreadability. The physical appearance of the gel

formulations was white translucent in nature. The drug content of the gel formulations was in the range of 98.7 ± 0.2 to 99.7 ± 0.1, showing content uniformity. The pH of the gel formulations was in the range of 6.9 ± 0.07 to 7 ± 0.1, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time for all formulations. The physicochemical properties of prepared gel formulations were in good agreement with those of a marketed product.

The viscosity of the gel formulations generally reflects its consistency. It is seen that viscosity changes as concentration of polymers changes and among the gel formulations it is seen F-8 formulation (carbopol-940 1%w/v : guar gum 1%w/v) to be having higher viscosity (9636 ± 1.5cps) when compared to other developed gels and is comparable with the marketed formulation (9643 ± 1.7cps).

In-vitro drug release studies were carried out to select appropriate polymer composition for gel formulation having suitable consistency for topical application. In vitro drug release was determined by performing *in-vitro* drug dissolution studies using USP V apparatus (Paddle Over Disc). The cumulative *in-vitro* drug release data is given in the **Table 9 and 10** and found F-8 to release 82.5 ± 0.5 % as marketed product releases 45.2 ± 0.2 %. From the results it is seen that F-8 formulation shows better *in-vitro* drug release when compared with marketed formulation. Based on the physicochemical properties and *in-vitro* drug release, the formulation F-8 was found to be suitable for topical application.

The *ex-vivo* skin permeation profile of diclofenac sodium from gels across rat abdominal skin. The skin permeation profile showed the same pattern as that of the in vitro drug release profile. The % *ex-vivo* cumulative drug release from formulation F8 at the end of 30 min was found to be 64.6 ± 0.2 % as given in **Table 11 and 12** and found to be better when compared with that of marketed formulation whose % *ex-vivo* cumulative drug release was found to be 43.2 ± 0.3 %.

No prominent changes in physicochemical properties of formulation after its exposure to accelerated conditions of temperature (40^{o}C) and humidity conditions (75 ± 5%RH) were seen. Hence the developed formulation was found to be stable even after subjecting to accelerated stability conditions. Diclofenac sodium hydrogel for topical application was formulated using guar gum and carbopol-940 and evaluation tests were performed. The topical delivery of diclofenac sodium from the prepared gel formulations across rat abdominal skin was found to be improved when compared to marketed formulation based on *ex-vivo* permeation studies. Proper selection of polymers and their proportions is a prerequisite for designing and developing a transdermal drug delivery system. The formulated gels showed good homogeneity, good stability and better drug release rates when compared to marketed formulation. The formulation F-8 (optimized formulation) consisting of 1%w/v guargum-1%w/v carbopol-940 was found to be suitable for topical application based upon its evaluation parameters.

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