Formulation and Evaluation of Emulgel Containing Diclofenac Sodium

Rahul Singh[1,2]*, Brijesh Kumar Shukla[2], Brajendra Singh[3], Mohd Arif[4], Kaushlendra Kumar Singh[3], Sajeev Kumar Patel[1], Nazneen Dubey[1]

[1]*Department of Pharmaceutics, Shri Ram Institute of Technology-Pharmacy, Jabalpur (M.P.), India-482002*

[2]*R. K. Singh Hospital & Institute of Medical Sciences, Chitrakoot (U.P.), India- 210205*

[3]*Geetanjali Institute of Pharmacy, Geetanjali University, Manvakhera Udaipur (Rajasthan), India-313001*

[4]*Department of Pharmacy, MJP Rohilkhand University, Bareilly, Uttar Pradesh, India-243006*

**Corresponding author: Rahul Singh; e-mail:* rahulsinghckt2014@gmail.com*; Contact: +91-8175089935*

Received: 06-08-2023	Accepted: 16-08-2023	Published: 18-08-2023

Abstract

Emulgels are a type of topical drug delivery system that combines the properties of emulsions and gels. They are typically composed of an oil phase, a water phase, and a gelling agent. In the present study, three different emulgels of diclofenac sodium were prepared and evaluated for physiochemical properties, *in vitro* drug release and *in vitro* drug permeation. The three emulgels were found better when compared to AGFD in terms of viscosity, pH, spreadability, *in vitro* drug release and *in vitro* drug permeation. In terms of viscosity, the lower viscosity of the emulgels can be correlated to their greater spreadability, which facilitates the easier application and improves the patient compliance. The pH of the emulgels was found to be close to that of the human skin, which minimizes the potential for irritation. The release of diclofenac sodium across a cellulosic membrane was found to be significantly greater for all three emulgels as compared to AGFD. Similarly, the permeation of diclofenac sodium across a human skin-like synthetic membrane was found to be greater for all three emulgels as compared to AGFD. From this study, it can be concluded that emulgels serve as a better vehicle for diclofenac sodium than gels. However, the presence of un dissolved diclofenac sodium suggests the use of a higher proportion of oil phase/solubilizing agents sufficient to fully dissolve the diclofenac sodium. This may significantly alter the properties of the emulgels. Also, the use of a combination of penetration enhancers in F3 resulted in a better permeation as compared to F1 and F2. This suggests that the use of penetration enhancers, which work by different mechanisms, may have been helpful in improving the overall permeation of diclofenac sodium in F3.

Keywords: Diclofenac sodium, Emulgels, Spreadability, pH, *In-vitro* drug release

DOI: 10.48047/ecb/2023.12.si11.028

Introduction

Skin serves as a physical barrier between the body and the surrounding environment. It also poses as a first line of defense against pathogens, prevents loss of water and impedes the entry of chemicals by functioning as a barrier [1]. In addition to serving as a sensory organ, it helps regulate body temperature, provide immunity, protect against ultraviolet rays and synthesize vitamin D. The two main structural layers of the skin are the epidermis and dermis are depicted in **Fig. 1**. The epidermis consists of five strata: corneum, lucidum, granulosum, spinosum and basale [2]. The dermis consists of layers of collagen fibers, elastic fibers, blood and lymph vessels, soft connective tissue and nerve endings [3]. The barrier function of the skin is primarily provided by the stratum corneum (SC) of the epidermis. Keratinocytes, melanocytes, and Langerhans cells are the three types of cells found in the epidermis. Amongst these, the keratinocytes, which originate in the basal layer, are the predominant cells in the epidermis. These cells migrate to the stratum granulosum (SG) and are transformed into corneocytes, which essentially play the role of "Bricks" in the brick and mortar model of the S C. The SG, which is only few cells thick, plays an essential function in the formation of cells, which serve as a barrier [1]. The transformation of keratinocytes to corneocytes occurs in the SG layer by a process called cornification. Cornification is a programmed cell death, which results in enucleation of the keratinocytes followed by disappearance of cytoplasm and release of lipids into the intercellular space [4]. The keratinin termed ate filaments organize to form micro fibrils and transform into a complex scaffold serving as a tight mechanical barrier. The water repellant barrier is formed by the lipids present in the intercellular space, which stack against each other, and provide the "mortar" in the brick and mortar model of the SC [1, 4].

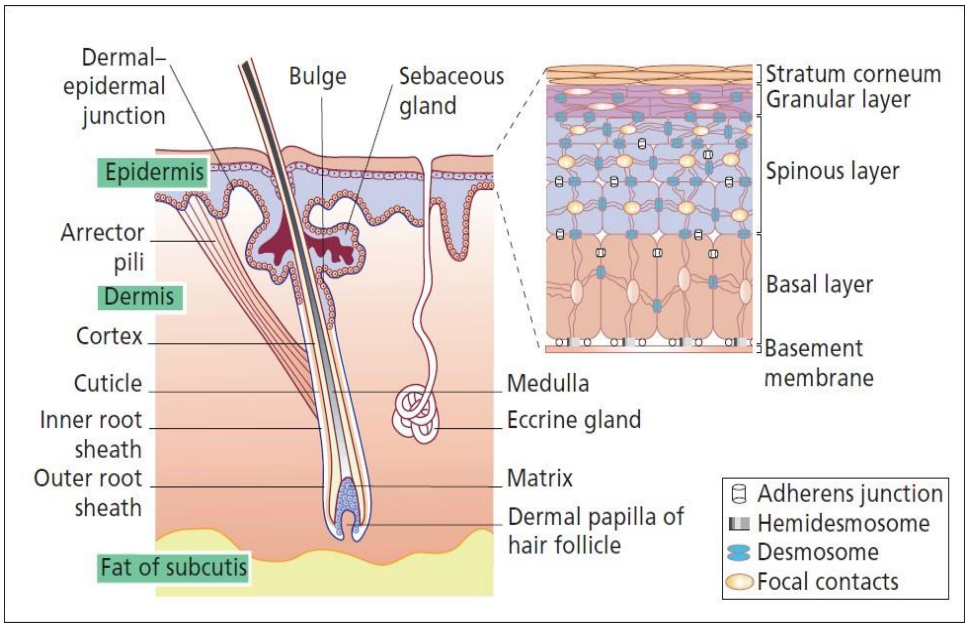

Fig. 1: Schematic illustration of skin and its appendages

The "brick and mortar" mosaic of corneocytes embedded in lipid-rich extracellular environment composed of ceramides, free fatty acids and cholesterol is the basis of the barrier function of the skin. This barrier functions not only to restrict the trans epidermal water loss (TEWL), but also impedes the entry of certain chemicals or penetrants [6]. A full understanding of the penetration barrier function of the SC is imperative to drug delivery via the topical route.

1. Materials and methods

1.1. **Requirements:** Diclofenac sodium was a gift sample from Hema Pharmaceuticals Pvt. Ltd, India. This sample meets the current United States Pharmacopeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) monographs. Carbomer 940 and triethanolamine (Lot no. J9822H3060B) were procured from Making Cosmetics (Snoqualmie, WA). Gransolve® DMI (USP/NF: dimethyl isosorbide, Lot no.: 152320933) was procured from Grant Industries, Inc (Elmwood Park, NJ). Kollicream® IPM (USP/NF: Isopropyl Myristate, Lotno: 0010084422) and Kolliphor ® PS 60 (KPS 60) (USP/NF: polyethylene (20) sorbitan monostearate, Lot No.: 0010149655) were procured from BASF Pharma Solutions (Tarrytown, NY). LexFeel® Natural (LFN) (INCI: heptylundecylenate, Lot No.: DL4189) was procured from InolexInc (Philadelphia, PA). Deionized water supplied at the University of Toledo Health Science Campus was used. Sodium hydroxide, potassium phosphate monobasic, acetonitrile (ACN), triethylamine and orthophosphoric acid were procured from Fischer Scientific

(Hampton, NH). All reagents used were of analytical grade.

1.2.**Pre-formulation study**

Pre-formulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of new drug substances, in order to develop stable, safe and effective dosage forms. These studies are designed to determine the compatibility of initial Excipient with the active substance for a biopharmaceutical, physicochemical and analytical investigation in support of promising experimental formulations.

1.2.1.**Organoleptic parameter:** It is the initial evaluation during Pre-formulation studies which assess the colour, odour and taste of the substance.

1.2.2.**Solubility:** Aqueous solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy. Solubility of drug was determined in different solvents.

1.2.3.**Melting point determination:** The melting point of a substance is a characteristic property that is determined by the intermolecular forces in the solid. Melting point of drug was determined by open capillary method.

1.2.4.**Determination of partition coefficient:** 50 mg of drug was taken in three separating funnels. The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug in phases was calculated by using formula:

$$\mathrm{KPC} = \frac{\text{Concentration of Drug in Oil Phase}}{\text{Concentration of Drug in Water Phase}}$$

1.2.5.**Determination of λ_{max}:** A solution of drug containing the concentration 10 µg/ ml was prepared in ph 7.2 phosphate buffer. The solution was scanned in the range of 200 – 400 nm UV spectrum using Systolic double beam spectrophotometer.

1.2.6.**Standard calibration curve of diclofenac sodium**

1.2.7.**Determination of absorption maximum (λ_{max}) :** 100 mg of Diclofenac sodium was accurately weighted into 100 ml volumetric flask, dissolved in small volume of acetone and volume was made up with ph 7.2 phosphate buffer. Pipette 1ml of this solution into another 10 ml volumetric flask and the volume was made with ph 7.2 phosphate buffer and marked as Stock. The resultant

solution is scanned in the range of (200-400nm) by UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan) to get absorption maximum (λ_{max}).

1.2.8.**Preparation of calibration curve:** Diclofenac sodium standard stock solution (1000µg/ml), 1ml solution was diluted to 10 ml using phosphate buffer (pH 7.4) to get concentrations of 100 µg/ml. from this solution, aliquots of, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml from standard drug solution were diluted to 10 ml with phosphate buffer (pH 7.4) and the absorbance of these solutions was measured spectrophotometrically using phosphate buffer (pH 7.4) as a blank. A standard curve was plotted using concentration on X-axis and the absorbance obtained on Y-axis.

1.3.**Preparation of formulations**

The three emulgel formulations F1, F2 and F3 were comprised of a carbomer 940 gel base and an oil phase with different compositions as mentioned in **Table 1**. Each formulation contained 5% w/w diclofenac sodium as the active ingredient. The two phases were prepared separately as per the procedures detailed below.

Table 1: Composition of F1, F2 and F3 emulgels

	Amount (% w/w)		
Ingredient	**F1**	**F2**	**F3**
0.5% w/w Carbomer 940 gel	78.0	78.0	78.0
Diclofenac sodium	5.0	5.0	5.0
LFN	14.0	5.0	-
DMI	-	9.0	9.0
IPM	-	-	5.0
KPS 60	3.0	3.0	3.0

1.4.**Preparation of 0.5% w/w carbomer 940 gel:** A 0.5% w/w Carbomer 940 gel was prepared by dispersing a pre-weighed amount of carbomer 940 into deionized water using an overhead mixer (IKA RW20 Digital, Wilmington, NC) at 750 rpm for about 30 minutes. The pH of the gel was brought to 6.0 using triethanolamine.

1.5.**Preparation of the emulgels:** An accurately weighed amount of diclofenac sodium was added to LFN, a mixture of DMI and LFN and a mixture of DMI and IPM to prepare the oil phase of F1, F2 and F3, respectively. KPS60 was added to a pre-weighed amount of carbomer gel base and mixed using over head mixer at 750 rpm for about 5 minutes.

1.6.**Evaluation Parameters**

1.6.1. **Differential scanning calorimetry (DSC):** To assess the physical interaction between drug and the utilized polymer (Chitosan) DSC thermograms (Differential Scanning Calorimetry) was performed. The DSC thermograms of drug and mixture of drug-polymer were done with a Perkin

Elmer Precisely (Jade DSC). All Samples for thermal analysis were sealed in aluminum pans (Flat- bottom) and heated over a temperature range of 35 to 400°C at an increment rate of 10°C/min. DSC thermograms of pure drug and the formulation were analyzed.

1.6.2. **Determination of viscosity:** Viscosity of the three formulations F1, F2 and F3 and AGFD was determined using a Discovery HR 3 hybrid rheometer with a peltier plate with 60 mm radius at a shear rate ranging from 0-1000s^{-1} at both 20°C and 32°C in triplicate. Average viscosity values (Pa.s) were reported at both the temperatures.

1.6.3. **Determination of pH:** The pH of the three formulations F1, F2 and F3 and AGFD was determined using a Mettler Toledo Seven Compact pH meter (Billerica, MA). The pH meter was calibrated withstandardbuffersolutionsofpH4,7 and 10 before each run. The electrode was dipped directly into the formulations and the gel product and readings were recorded in triplicate for each sample. Average pH values were reported.

1.6.4. **Determination of drug content:** The drug content of the formulations F1, F2 and F3 was determined by dissolving accurately weighed quantities of each formulation in ACN. The resultant solution was filtered using EMD Millipore membrane filter and serially diluted with ACN to obtain suitable dilution. Drug content was analyzed using the HPLC method developed for diclofenac sodium and determined quantitatively from the calibration curve.

1.6.5. **Determination of spreadability**: Spreadability of formulation F1, F2, F3 and AGFD was determined using TA. XT *Plus* texture analyzer (Texture Technologies Corp., Hamilton, MA) with a TTC spreadability fixture comprising of male and female Perspex 90-degree cones at 25°C. The instrument was calibrated using 5kg load cell before each run. For probe calibration, the male cone was lowered into empty female cone (sample holder) so that the two were practically touching. The starting point was then set at 25.0 mm above the female cone. Probe calibration was done before each run. To determine spreadability, each sample was placed into the female cone and pressed down using metal spatula to eliminate air pockets. The test mode was set to ‘measure force in compression’ and ‘return to start’ option was used. The starting distance of male and female cone was then set to 23 mm to avoid over loading/under loading. The test speed and post-test speed were set to2.0mm/s and 10.0 mm/s, respectively. Data acquisition rate was set to 200 pps. Exponent stable micro systems software (version 6.1.10.0) was used to generate spreadability curves.

1.6.6. **Optical imaging analysis**: Optical imaging analysis was done for F1, F2 and F3

formulations using AmScope MD35 microscope (Irvine, CA) under 10x and 40x magnification. The formulations were suitably diluted with water before placing on glass slide to observe under 40 x magnifications. Am Scope 3.4 software was used to capture images under the 10x and 40x magnification.

1.6.7. ***In vitro* release and *in vitro* permeation studies**: Jacketed Franz diffusion cells (Perme Gear, Hellertown, PA) witha15.0mmorifice diameter and 12.0 mL volume were used to study *in vitro* release and *in vitro* permeation ofF1,F2,F3andAGFD.The cells were placed in a V9-CB stirrer (Perme Gear, Hellertown, PA) connected with a water bath assembly set at 32±0.2°C. For the *in vitro* release, Spectra/Por 2 dialysis membrane of12-14kDamolecularweightcut-off (MWCO) and 25.0 mm diameter was used. For the *in-vitro* permeation, Strat-M® membrane with 25.0 mm diameter was used. For each cell, water from the water bath was circulated through the outer jacket to maintain constant temperature and a magnetic stirrer bar was placed in the receptor chamber. Phosphate buffer (pH 7.4) was used as the receptor medium. An accurately pre-weighed amount of formulations F1, F2, F3 and AGFD were applied onto the membrane through the donor chamber of the cell. The receptor chamber of each cell was occluded with parafilm to prevent evaporation. For each formulation, the *in vitro* release and *in vitro* permeation studies were carried out over the course of 24 hrs and 12 hrs, respectively, with n=4. Samples of 0.3 mL were taken at the start of the study (i.e., at 0 mins) and subsequently at regular time intervals (i.e., 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5,3,4,6,8,10,12,16, and 24 hrs).The sample volume was replaced by an equal volume of fresh phosphate buffer each time. Any air bubbles generated beneath the surface of the membrane were carefully removed by tilting the Franz cell to facilitate bubble escape through the side arm. Flux (*J*) was calculated from slope of the line obtained by plotting the cumulative amount of diclofenac sodium permeated (µg/cm^2) vs. time (hr). Apparent permeability coefficient (*Papp*) was calculated by dividing flux (*J*) with the initial concentration of diclofenac sodium in the donor chamber (*Cdonor*). Average values of flux (*Javg*)and apparent permeability coefficient (*Papp. avg*) were reported. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test for multiple comparisons in SPSS software (version 24). Results were considered to be statistically significant at p-value <0.05.

2. Results and discussion

2.1.**Physical Appearance:** The drug was obtained as a kind gift from Hema Pharmaceuticals Pvt. Ltd, India. The supplied powder of Diclofenac sodium was off white and crystalline in powder.

2.2.**Melting Point:** Melting point of Diclofenac sodium was determined by melting point apparatus (Tempo) and found to be 280 °C.

2.3.**Solubility:** Solubility of Diclofenac sodium was seen in water, ethanol, DMSO and dimethyl formide and was partially soluble in water. Solubility profile of Diclofenac in different solvent is tabulated in **Table 2**.

Table 2: Solubility of Diclofenac sodium in different solvents

S. No.	Solvent	Solubility
1.	Water	++--
2	Ethanol	++- -
3.	DMSO	++++
4.	Dimethyl formaide	++++

Where, + = less soluble, ++ = slightly soluble, ++++ = most soluble

2.4.**Partition coefficient value of Diclofenac sodium:** Partition coefficient is calculated by the ratio of equilibrium concentration of a dissolved substance in a two-phase system. The results for partition coefficient of Diclofenac sodium came out to be 4.9.

2.5.**Standard Curve of Diclofenac sodium Phosphate Buffer Solution (pH 7.4):** All dilutions and measurements were made as above in phosphate buffer solution of pH 7.4 made as per formula (I.P.). The absorbance was taken at λ_{max} 285 nm against a reagent blank (**Fig. 2** and **Table 3**). The standard curve was plotted between absorbance and concentration.

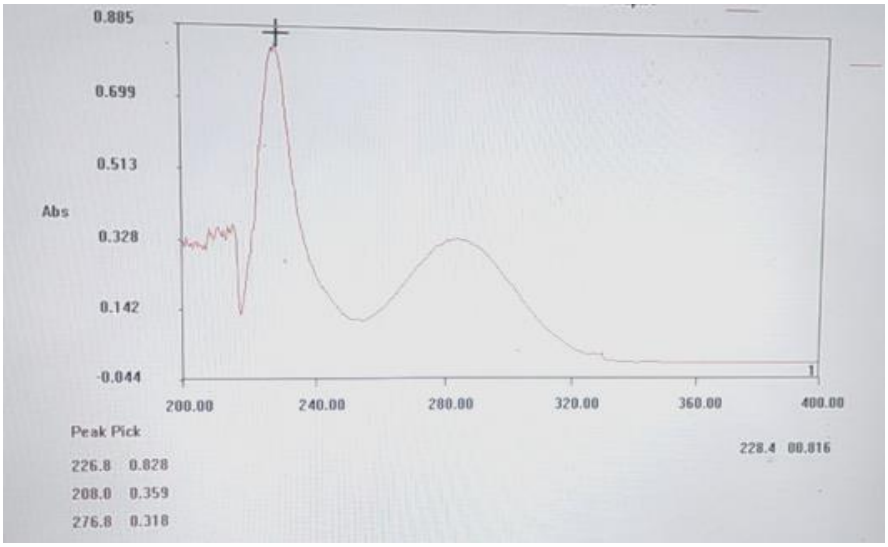

Fig. 2: Standard Curve of Diclofenac sodium Phosphate Buffer Solution (pH 7.4)

Table 3: Standard curve of diclofenac sodium phosphate buffer solution (pH 7.4)

S. No.	Drug Conc. (µg/ml)	Absorbance at 285 nm
1.	10	0.112
2.	20	0.287
3.	30	0.452
4.	40	0.543
5.	50	0.672

2.6. **Differential Scanning Calorimetry:** The DSC thermograms of pure diclofenac sodium, the blank formulations of F1, F2 and F3, drug-loaded formulations of F1, F2 and F3, and AGFD are presented in **Fig. 3**, **Fig. 4**, and **Fig. 5**. The DSC thermogram of diclofenac sodium showed a sharp endothermic peak at 80.48°C, which corresponds to the melting point of diclofenac sodium [5]. The DSC thermograms of the blank and drug-loaded F1, F2, F3, and AGFD showed a characteristic broad melting endotherm. These are indicative of the semi-crystalline nature of the formulation in both the emulgels and the gel [6]. In the light of the results from the optical imaging analysis, it can be concluded that undissolved diclofenac sodium is present in crystalline form in the formulation base. The lack of endotherm of drug can be attributed to the melting and solubilization of drug in the formulation base during the heating process. Such behavior has been characterized in the thermograms of physical mixtures of crystalline drugs and polymers [6]. It is also reported that some transitions in DSC thermograms are missed as these are smaller than the major transitions or due to a faster scan rate.

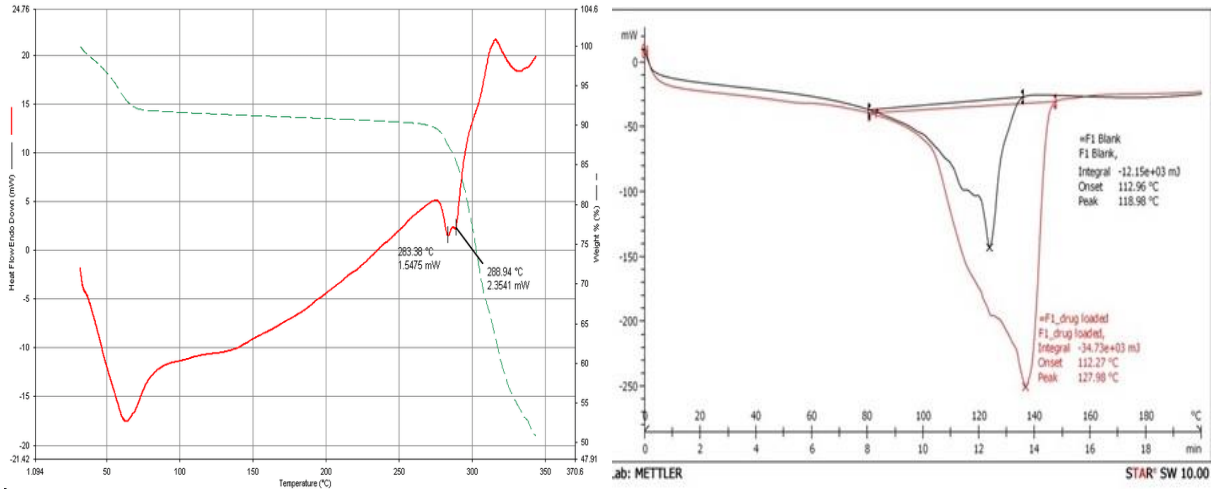

Fig. 3: DSC of pure drug, blank F1 and drug-loaded F1

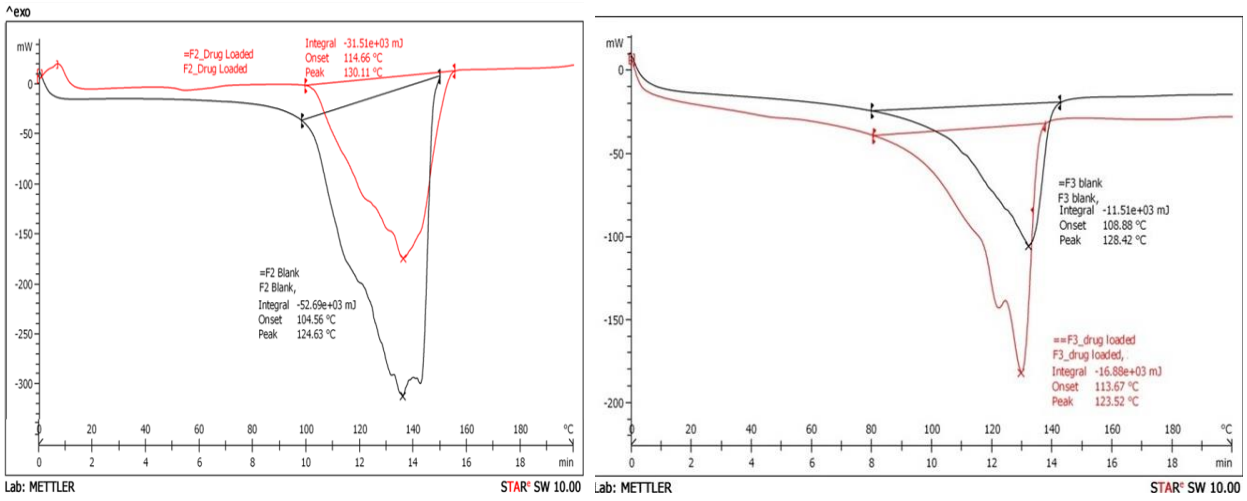

Fig. 4: DSC thermograms of blank F2, drug-loaded F2, blank F3 and drug-loaded F3

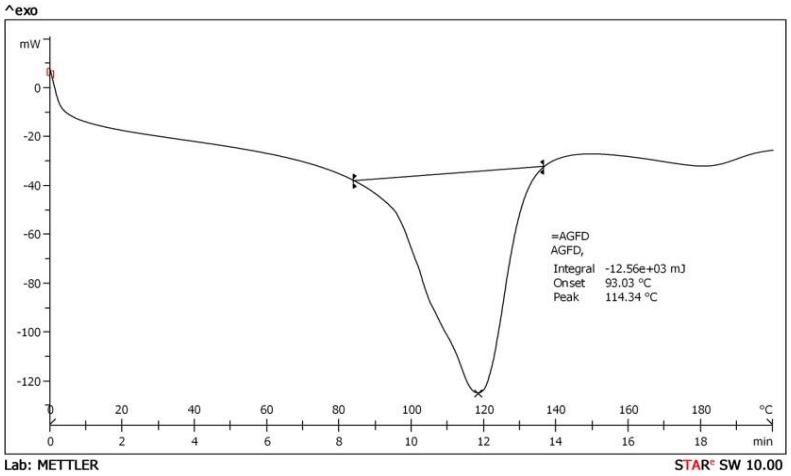

Fig. 5: DSC thermogram of AGFD

2.7. **Viscosity:** All three formulations F1, F2, and F3, and the marketed gel product AGFD exhibited non-Newtonian pseudoplastic behavior as represented in **Fig. 6** and **Fig.7**. Pseudoplastic fluids are also called shear-thinning fluids as these fluids exhibit a reduction in viscosity with an increase in shear rate [7]. It can be observed that all the formulations and AGFD tended to behave like Newtonian fluids at low shear rates [8].

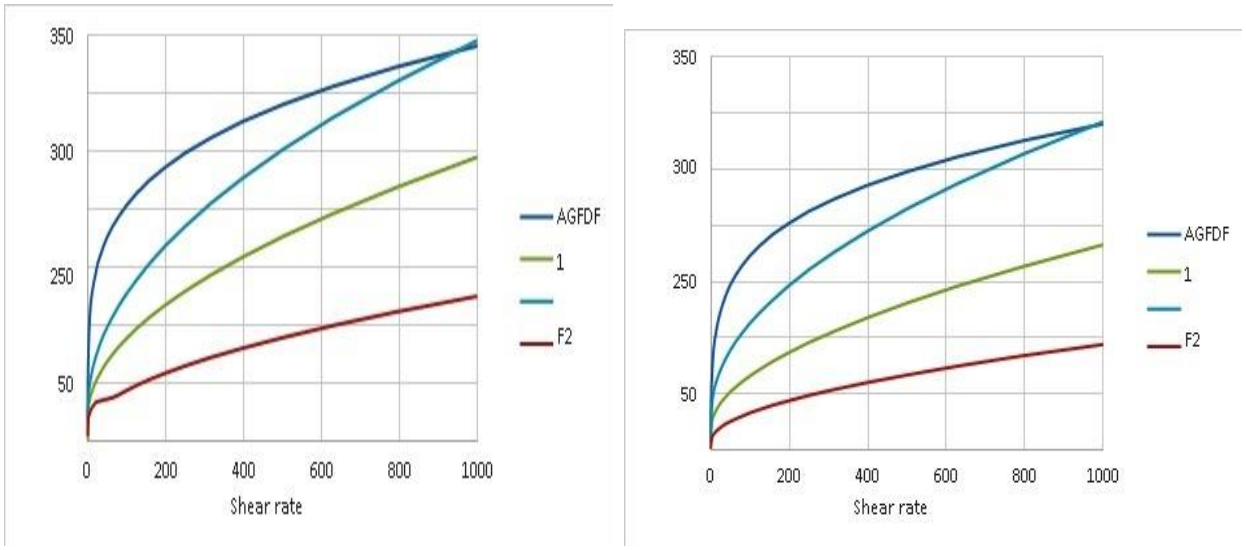

Fig 6: Flow curves of F1, F2, F3 and AGFD at 20°C & 32°C

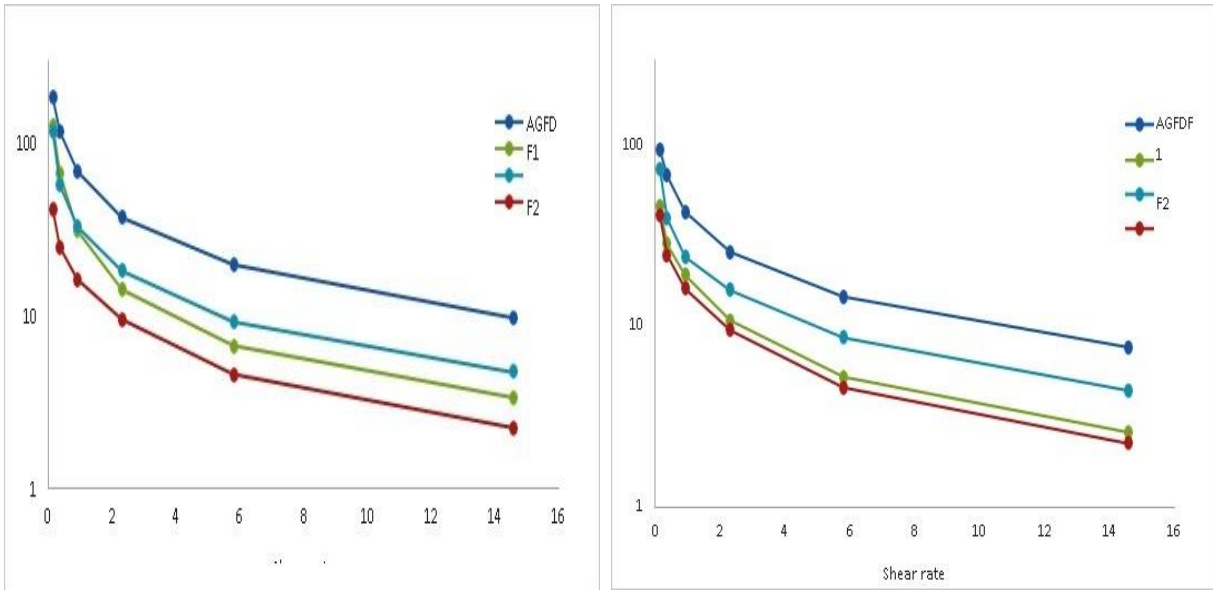

Fig 7: Log viscosity (η) vs. shear rate at 20°C & 32°C

The average viscosity of the emulgel formulations was found to be in the range of 38-141 Pa.s at 10 rpm at 20°C while AGFD displayed a higher average viscosity of 175 Pa.s at 10 rpm at 20°C, which emulates the temperature for storage. Similarly, the emulgel formulations displayed significantly lower average viscosity values ranging from 67-11 Pa.s at 10 rpm at 32°C, while AGFD had an average viscosity value of 93 Pa.s at 10 rpm at 32°C, which is the temperature of human skin. The average viscosity values at 10 rpm at both temperatures are reported in **Table 4**. A comparative profile of average viscosity of F1, F2, F3 and AGFD is presented in **Fig. 6-7**. It is evident that AGFD had a significantly higher average viscosity than F2 and F3. Although lower,

the average viscosity of F1 is comparable to AGFD. The higher viscosity of AGFD can be attributed to a higher amount of gelling agent in the monophasic structure. In contrast, emulgels are biphasic semi-solid formulations with an oil phase, which tends to influence the viscosity. The inter-particle interactions between the oil droplets and the continuous gel phase affect the viscosity of the biphasic system. Primarily, the viscosities of the oil phase components are critical in determining the final viscosity of a formulation [9]. When comparing the emulgels, F1 had the highest viscosity and F3 had the lowest viscosity at 20°C. At 32°C, F2 had the highest viscosity and F3 has the lowest viscosity. These differences may be attributed to the inherent difference in the composition of the oil phase of the emulgels [10]. It can also be observed that the viscosity of all the formulations decreased with an increase in temperature from 20°C to 32°C.

Table 4: Average viscosity of F1, F2, F3 and AGFD at 20°C and 32°C at 10 rpm

Formulation	Average viscosity and SD (Pa.s) at 10 rpm	
	20°C	32°C
F1	140.63 ± 0.06	42.57 ± 0.09
F2	100.89 ± 0.09	66.89 ± 0.07
F3	37.88 ± 0.02	10.93 ± 0.03
AGFD	174.95 ± 0.07	92.96 ± 0.05

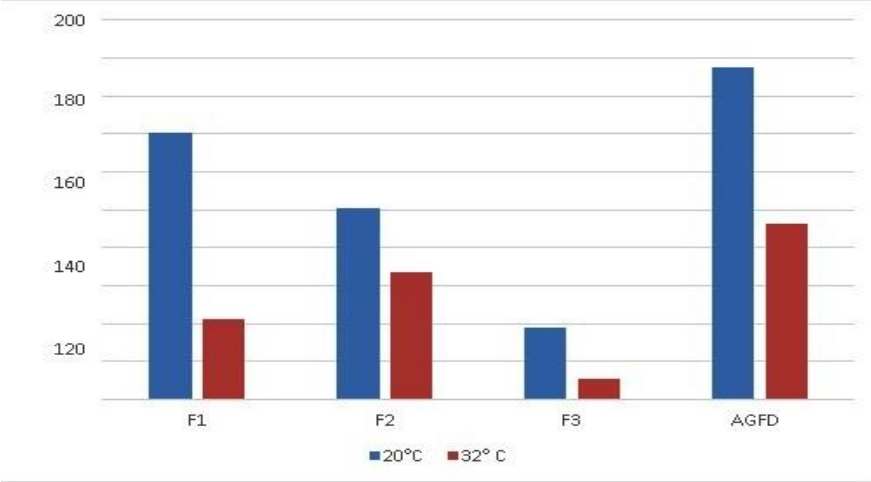

Fig. 8: Average viscosity of F1, F2, F3 and AGFD at 20°C and 32°C

2.8.**pH:** The average pH of the formulations was found to be in range of 5.48 to 5.65, while the average pH of AGFD was found to be 6.22 as represented in **Table 5**. The pH of the skin ranges from 4 to 6 [11], depending on the gender, age and body part. The pH range of the emulgel formulations was considered optimal for topical delivery as there is a minimal potency for causing irritation to the skin [10]. However, the pH of the formulation also plays an important

role in determining the ratio of ionized to unionized species of a drug [11]. The pK_α of diclofenac sodium is 4.91 [11] and at a pH range of 5.48 to 5.65, the percent of unionized diclofenac sodium ranges from 21.2 to 15.4 %, respectively.

Table 5: Range of pH of F1, F2, F3 and AGFD (values displayed as average ± SD)

Formulation	pH range
F1	5.65 ± 0.02
F2	5.48 ± 0.01
F3	5.63 ± 0.01
AGFD	6.22 ± 0.01

2.9. **Drug content:** The average drug content of all three formulations was found to be within the optimal range of 90-110 % with a RSD value of 1.72 %. As per the USP 40-NF 35, the assay values for content uniformity of topical semi- solid dosage form should range from 90-110 % of the stated amount of drug with a RSD of not more than 6 % (**Table 6**) [11]. The values for F1, F2 and F3 are indicative of an acceptable content uniformity.

Table 6: Average drug content values (± RSD)

Formulation	Average drug content (%)
F1	97.59 ± 1.82
F2	97.75 ± 1.47
F3	96.50 1.85

2.10. **Spreadability:** The above table summarizes the mean maximum positive force and means positive area obtained from the texture analysis of F1, F2, F3 and AGFD. The texture analysis curves are presented in **Table 7** and **Fig. 8**. Spreadability, as a textural property, refers to the ease of spreading a product from the point of application to the adjacent areas. It is often one of the most important attributes of topical dosage forms that affect patient acceptability, the ease of application and clinical efficacy [2]. Firmness, expressed as the maximum force required obtaining a given deformation, is highly correlated to spreadability. From the texture analysis curve in **Fig. 8**, it can be observed that AGFD has the maximum value for force and positive area under the curve, followed by F2>F1>F3.

Table 7: Texture analysis parameters of F1, F2, F3 and AGFD

Formulation	Maximum positive force (g)	Positive area under the curve (g.s)
F1	79.61	59.83
F2	109.58	90.14
F3	60.97	44.14
AGFD	214.99	189.81

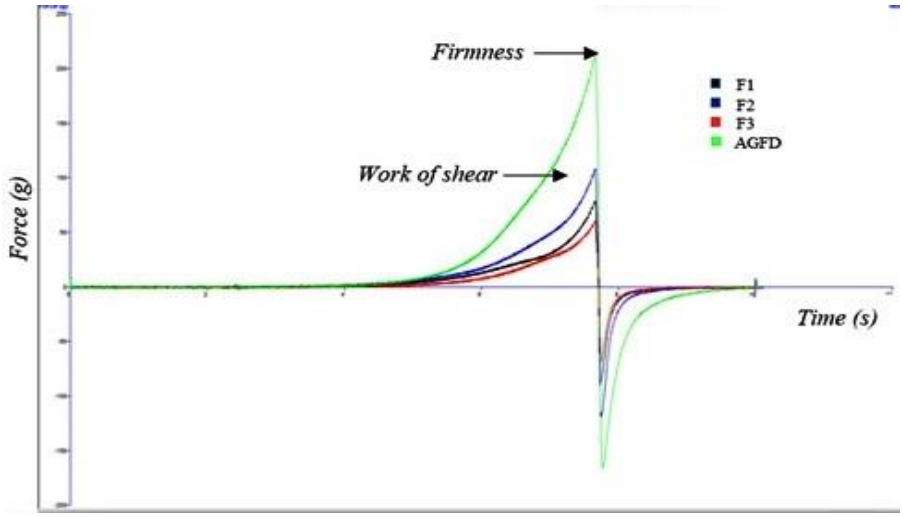

Fig 8: Texture analysis curves of F1, F2, F3 and AGFD

2.11. **Optical imaging analysis:** The images of F1, F2 and F3 captured under 10X and 40X magnification are presented in **Fig. 9**. The presence of insoluble drug crystalsis evident in all formulations F1, F2 and F3 under the 10x magnification.

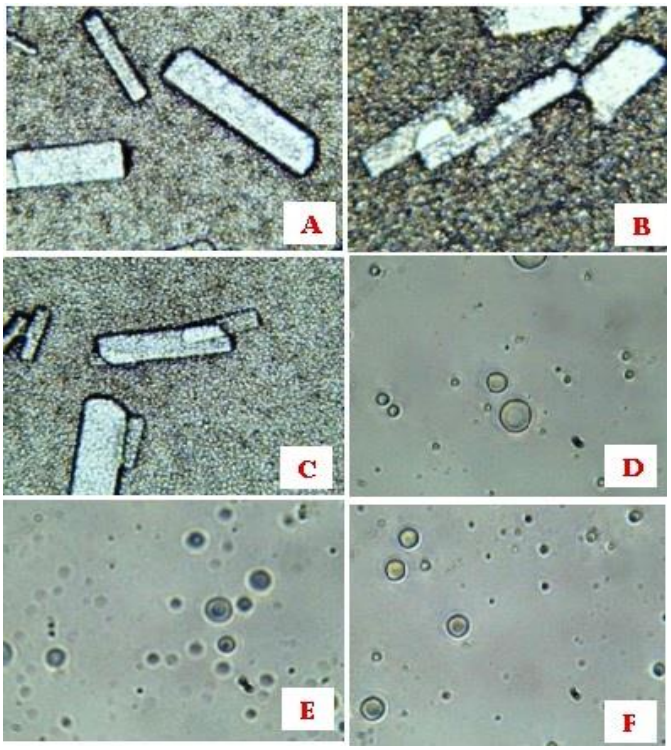

Fig. 9: Optical Imaging Analysis F1under 10X (A), F2 under 10X (B), F3 under 10X (C), F1 (diluted) Under 40X (D), F2 (diluted) Under 40X (E), F3 (diluted) Under 40X (F)

This can be attributed to the low solubility of diclofenac sodium in the continuous phase of the emulgel, which is aqueous in nature. As the drug partitions between the two phases of the

emulgel system, it precipitates as an insoluble fraction in the aqueous gel phase. However, the presence of drug in the crystalline form of diclofenac sodium in the emulgels is not confirmed by DSC thermograms. This may be due to undetectable amount of crystalline drug in DSC samples. It is also reported that some transitions in DSC thermograms are missed as these are smaller than the major transitions or due to a faster scan rate. The presence of insoluble drug crystals may pose an impediment to diffusion of the drug into the skin layers. A drug should be soluble in the vehicle to diffuse readily into the skin. The use of anti-nucleating polymers, such as hydroxyl propyl methyl cellulose (HPMC) or poly vinyl pyrrolidone (PVP) has been associated with the prevention of crystallization in supersaturated systems. Under the 40x magnification, the oil droplets of the biphasic emulgels can be clearly observed. The oil phase of the emulgels serves as a reservoir for lipophilic drugs. Due to the complex gel structure, emulgel systems also facilitate a sustained release of drugs making them suitable NSAID-carrying vehicles for the management of chronic musculoskeletal pain.

3.12. *In vitro* release and *in vitro* permeation of diclofenac sodium

The average percent release of diclofenac sodium from F1, F2, F3 and AGFD at 0.5, 6 and 24 hrs is summarized in **Table 8**, which shows the release profile of diclofenac sodium from F1, F2, F3 and AGFD.

Table 8: Average release of diclofenac sodium from F1, F2, F3 and AGFD (±SD) (* indicates statistically significant figures at $p < 0.05$)

Formulation	Average release (%) 0.5 hr	6 hrs	24 hrs
F1	13.6 ± 0.4	86.4 ± 0.6	98.1 ± 0.5
F2	13.0 ± 0.1	77.7 ± 0.5	91.9 ± 1.0
F3	12.5 ± 0.3*	59.7 ± 3.5*	76.1 ± 2.9*
AGFD	7.3 ± 0.7*	32.0 ± 9.8*	35.0 ± 8.1*

A comparative representation of the percent release of diclofenac sodium from F1, F2, F3 and AGFD is shown in **Fig. 10** and **Fig. 11**. The release data of F1, F2, F3 and AGFD were fitted to different kinetic models and were found to follow first-order release kinetics with an R^2 value of 0.9911. While no statistically significant difference was found in the percent release of diclofenac sodium from F1 and F2, F1 and F2 showed highest release followed by F3 and AGFD.

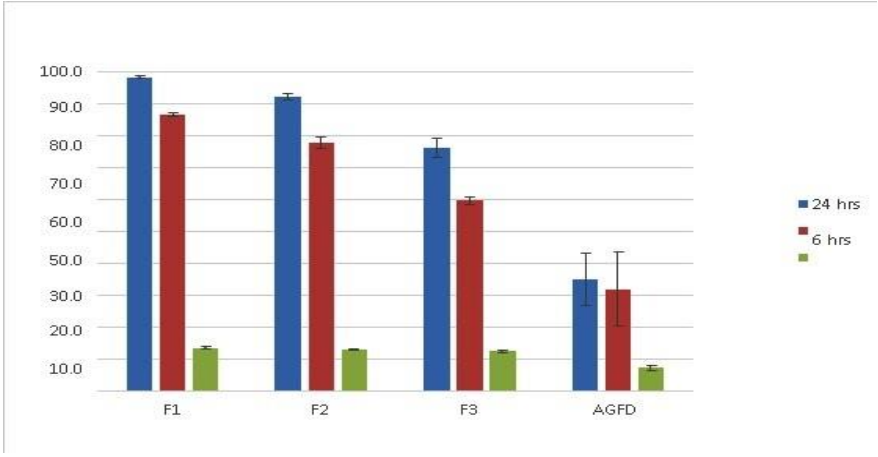

Fig. 10: Average release (%) of diclofenac sodium from F1, F2, F3 and AGFD

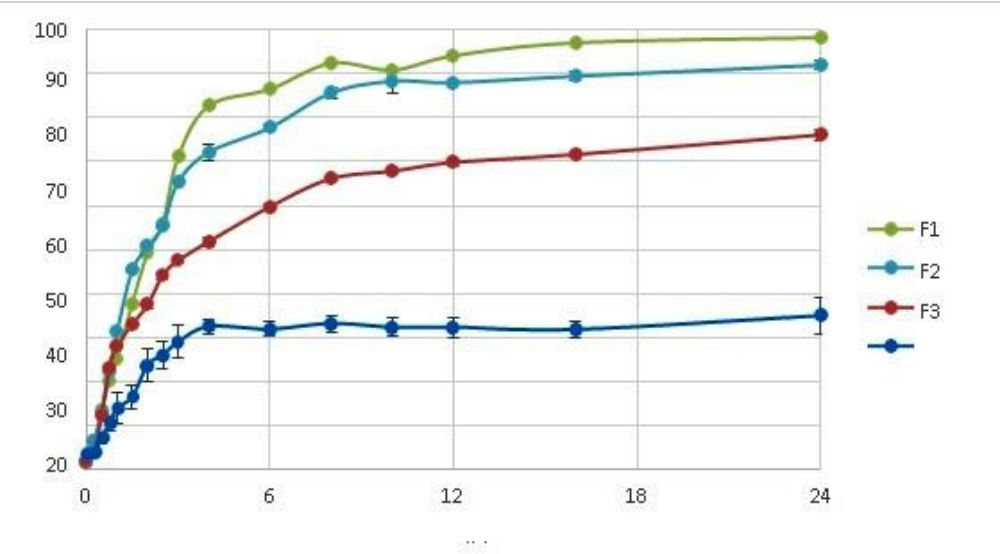

Fig. 11: Release profile of diclofenac sodium from F1, F2, F3 and AGFD

All three formulations showed a significantly higher release than AGFD. The fraction of the dissolved drug in the vehicle, the nature of vehicle, the pH of the formulation and the receptor medium, and the log P of the drug are important factors affecting the release of the drug from the vehicle. While F1 contained no penetration enhancer, F2 contained DMI, F3 contained both DMI and IPM, and AGFD contained propylene glycol and isopropyl alcohol as mentioned on the product label. In addition to the differences in the composition, the inherent differences in the structure of emulgel and gel should be considered as well to understand the release trend. The aqueous nature of the gel may not allow faster diffusion of lipophilic drugs like diclofenac sodium whereas the biphasic emulgels with an inner oil phase may offer better diffusion of such molecules. Thus, the effective amount of diclofenac sodium available for release across the

membrane may be higher in the emulgels as compared to AGFD. Furthermore, F1, which did not contain DMI, andF2, which contained DMI, showed a comparable percent release of diclofenac sodium over 24 hr. It is important to note that DMI mainly acts by altering the polarity of the SC and IPM increases the fluidity of the lipids present in the SC. Because the cellulosic membrane used in the release study is not rate-limiting to drug release, the penetration enhancement effect of DMI and IPM is irrelevant to the release of diclofenac sodium.

2.12. ***In vitro* permeation of diclofenac sodium:** The average percent permeation of diclofenac sodium from F1, F2, F3 and AGFD at 2, 6 and 12 hrs is represented in **Fig. 12** and **Fig. 13**. Formulation F3 showed the highest permeation over 12hrs, while no significant difference was found in the permeation of F1 and F2. All three emulgel formulations showed a significantly higher permeation than AGFD.

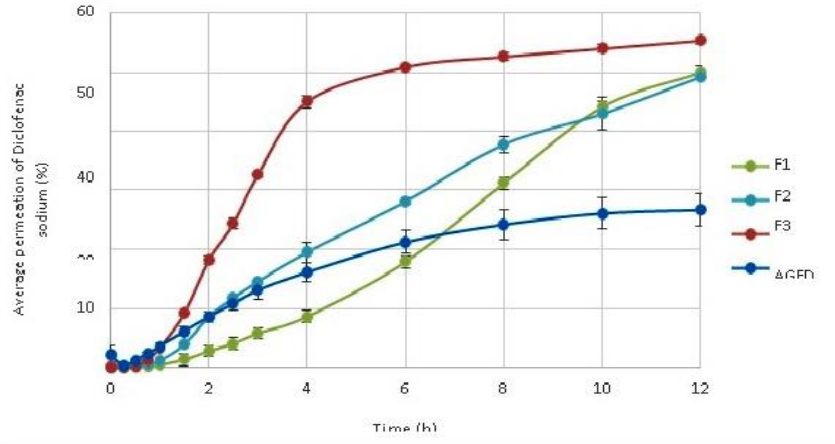

Fig. 12: Average permeation (%) of diclofenac sodium from F1, F2, F3 and AGFD

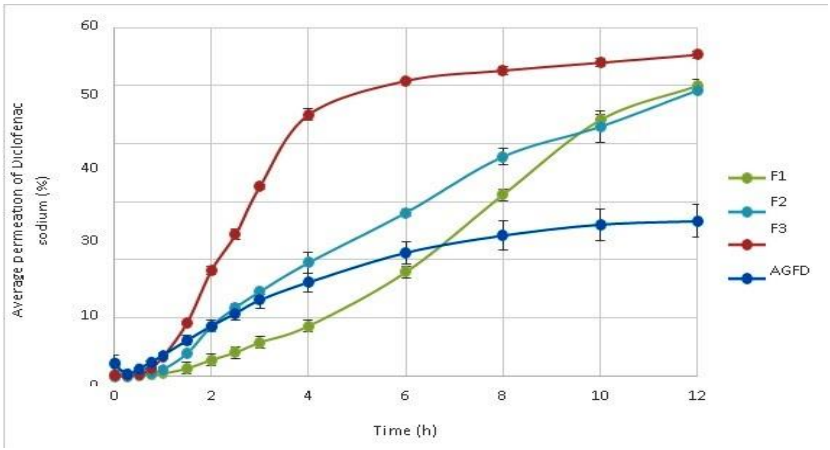

Fig. 13: Permeation profile of diclofenac sodium from F1, F2, F3 and AGFD

It was observed that F3 and F2 showed a higher sustained permeation as compared to F1 and

AGFD. Also, F3 showed a faster permeation than F1, F2 and AGFD. **Table 9** and **Table 10** summarizes the apparent permeability coefficient (P_{app}), steady- state flux (J_{ss}) and lag time ($tlag$) of F1, F2, F3 and AGFD. It was observed that F3 exhibited the shortest lag time followed by AGFD<F2<F1. Thus, it can be concluded that the emulgel F3 is better suited for a sustained release of the drug over a longer period as compared to AGFD. Interestingly, F3 showed the highest permeation over first the 2 hours followed by F1>AGFD>F2. This indicates that emulgels with suitable penetration enhancers may show a faster permeation as well as a sustained release over a longer period of time. It should be noted that F3 contained both DMI and IPM, while F2 contained only DMI, F1 contained no penetration enhancer and AGFD contained propylene glycol and isopropyl alcohol. Thus, it is imperative to use right penetration enhancers to achieve the desired rate of permeation of the active moiety. As mentioned previously in section 1.8, certain penetration enhancers may function as penetration retardants, or show little to no effect on penetration of an active moiety depending on the vehicle characteristics. In this study, using a combination of penetration enhancers, i.e., DMI and IPM, was found to be more effective at increasing the permeation than using a single penetration enhancer, i.e., DMI. The different mechanisms of penetration enhancement of these two chemicals should also be considered. It should also be noted that Strat-M® membrane, which is composed of multiple layers of polyester sulfone, acts as a skin-mimicking artificial membrane. A high degree of positive correlation has been observed between the permeability coefficients obtained through Strat-M® and human/pig ear skin, especially for molecules with molecular weight between 155 and 288, and log P value between -0.90 and 3.53. However, the mechanism of rate-limitation of permeation of an active moiety through Strat-M® remains unknown. Thus, the effect of certain penetration enhancers may not be same when tested on human skin for different active moieties.

Table 9: Average permeation of diclofenac sodium from F1, F2, F3 and AGFD (±SD)

(*indicates statistically significant figures at $p < 0.05$)

	Average permeation (%)		
Formulation	**2 hrs**	**6 hrs**	**12 hrs**
F1	2.7 ± 0.1	17.9 ± 0.9	50.0 ± 1.1
F2	8.6 ± 0.2	28.1 ± 0.5	49.2 ± 0.3
F3	18.1 ± 0.5*	50.8 ± 1.5*	69.8 ± 1.5*
AGFD	8.6 ± 0.9*	16.1 ± 9.8*	26.7 ± 2.8*

Table 10: Apparent permeability coefficient (P_{app}), steady-state flux (J_{ss}) and lag time (t_{lag}) of F1, F2, F3 and AGFD

Formulation	Apparent permeability coefficient (P_{app}) (cm hr^{-1})	Steady-state flux (J_{ss}) (µg hr^{-1} cm^{-2})	Lag time (t_{lag}) (hr)
F1	0.007225 ± 0.00004	361.25 ± 2.16	2.75 ± 0.04
F2	0.003127 ± 0.00007	156.35 ± 3.40	1.57 ± 0.16
F3	0.007922 ± 0.00013	396.11 ± 6.33	0.78 ± 0.02
AGFD	0.003213 ± 0.00010	160.66 ± 4.75	0.80 ± 0.04

3. Conclusion

In the present study, three different emulgels of diclofenac sodium were prepared and evaluated for physiochemical properties, *in vitro* drug release and *in vitro* drug permeation. The oil phase of the emulgel formulations was designed using FFE software. The three emulgels were compared to AGFD in terms of viscosity, pH, spreadability, *in vitro* drug release and *in vitro* drug permeation. All three emulgels were found to be better than AGFD in terms of spreadability, *in vitro* drug release and *in vitro* drug permeation. In terms of viscosity, the lower viscosity of the emulgels can be correlated to their greater spreadability, which facilitates the easier application and improves the patient compliance. The pH of the emulgels was found to be close to that of the human skin, which minimizes the potential for irritation. The role of the formulation pH in determining the fraction of drug that is present in an ionized state was also studied. The release of diclofenac sodium across a cellulosic membrane was found to be significantly greater for all three emulgels as compared to AGFD. Similarly, the permeation of diclofenac sodium across a human skin-like synthetic membrane was found to be greater for all three emulgels as compared to AGFD. From this study, it can be concluded that emulgels serve as a better vehicle for diclofenac sodium than gels. However, the presence of un dissolved diclofenac sodium suggests the use of a higher proportion of oil phase/solubilizing agents sufficient to fully dissolve the diclofenac sodium. This may significantly alter the properties of the emulgels. Also, the use of a combination of penetration enhancers in F3 resulted in a better permeation as compared to F1 and F2. This suggests that the use of penetration enhancers, which work by different mechanisms, may have been helpful in improving the overall permeation of diclofenac sodium in F3.

Acknowledgments

Mr. Rahul Singh is grateful to the Honorable management of Department of Pharmaceutics, Shri Ram Institute of Technology-Pharmacy, Jabalpur (M.P.) for providing all the facilities to complete this study.

Authors' contributions

R. S, N.D., and S.K.P. conceptualized the present research. Data collection was done by R.S., and B.K.S. Writing of original draft was done by R.S., M.A., B.S., and K.K.S Review and editing was performed by B.K.S., and R.S. under the supervision of N.D.

Data availability: Not applicable.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Code availability: Not applicable.

4. References

[1] Tanwar YS, Jain AK. Formulation and evaluation of topical diclofenac sodium gel using different gelling agent. AJPRHC 2011;4:1-6.

[2] Shah D, Londhe V. Optimization and characterization of levamisole-loaded chitosan nanoparticles by ionic gelation method using 2(3) factorial design by Minitab 15. Therapeutic delivery 2011;2(2):171-9.

[3] Turk J. Hasçicek C, Bediz-Ölçer A, Gönül N. Preparation and evaluation of different gel formulations for transdermal delivery of meloxicam Pharm Sci 2009;6:177-86.

[4] Sanna V, Peana AT, Moretti MDL. Development of new topical formulations of diphenhydramine hydrochloride: *in vitro* diffusion and *in vivo* preliminary studies. International Journal of PharmTech Research 2010;2:863-9.

[5] Colombo P. Principi di tecnologie farmaceutiche. CEA 2004.

[6] Kaur J, Srinivasan KK, Joseph A, Gupta A, Singh Y, Srinivas KS, et al. Development and validation of stability indicating method for the quantitative determination of

venlafaxine hydrochloride in extended release formulation using high performance liquid chromatography. Journal of pharmacy & bioallied sciences 2010;2(1):22-6.

[7] Bucur L, Istudor V. Hîrjău V, Elaeagnus angustifolia L. Flower soft extract valorification in a dermatological preparation note 2 Dermatological preparation rheologic control Farmacia 2009;57:309-14.

[8] Stozkowska W. Effect of various vehicles on diclofenac sodium and indomethacin pharmaceutical availability. Acta poloniae pharmaceutica 2002;59(4):253-8.

[9] Durrësi S. Teknologjia farmaceutike Tirane 2008.

[10] Ahuja N, Saini V, Rasayan J. Formulation and evaluation of diclofenac sodium gel by using natural polymer. Chem Vol No3 2008;1:564-6.

[11] Aejaz A, Azmail K, Sanaullah S, Mohsin AA. Formulation and in vitro evaluation of aceclofenac solid dispersion incorporated gels. International Journal of Applied Pharmaceutics 2010;2:7-12.

[12] Nayak P, Kumar R. Retromastoid-sub occipital: A novel approach to cerebello pontine angle in acoustic neuroma surgery-our experience in 21 cases. Journal of neurosciences in rural practice 2011;2(1):23-6.