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# Development and Evaluation of Aceclofenac Gel for Topical Application

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## ABSTRACT

Aceclofenac, a non-steroidal anti-inflammatory drug, has been used in the treatment of rheumatoid arthritis and osteoarthritis. In order to decrease the gas triculcerogenic effects, aceclofenac gels have been developed. This study was conducted to develop agel formulation of aceclofenac using four types of gelling agents: carbopol, Triethanolamine, Ethanol (99.9 %) and Polyethylene glycol. Effect of penetration enhancer (propyleneglycol) on the release has been studied. The gels were evaluated for physical appearance, rheological behavior, drug release and stability. The drug release from all gelling agents through a rat skin for diffusion study was evaluated using Keshary-Chien diffusion cell. All gels showed acceptable physical properties concerning color, homogeneity, consistency, spreadability and pH value. Among all the gel formulations, carbopol showed superior drug release than followed by Ethanol (99.9 %) and Polyethylene glycol and Sodium hydroxide. Drug release decreased within crease in polymer concentration. Drug release was not linearly proportional with the concentration of penetration enhancer or co-solvents. Stability studies showed that the physical appearance, rheological properties, and drug release remained unchanged upon storage for three months at ambient conditions.

**Keywords:** Aceclofenac; topical gel; Carbopol 940, 971, 974; Polyethylene glycol 200, 400, 600; Triethanolamine; Isopropyl alcohol.

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INTRODUCTION

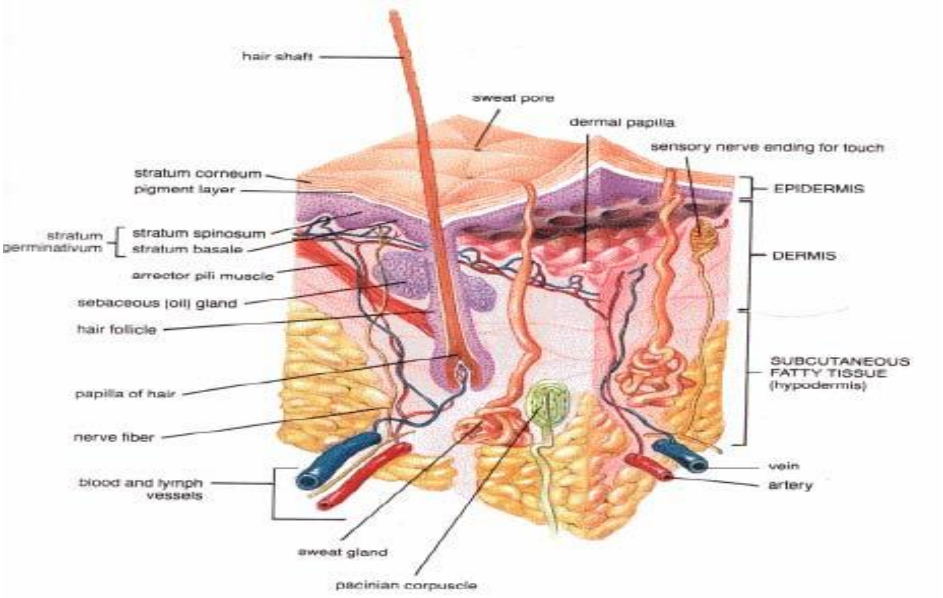



**Figure.1: Histology of skin**

**The Skin**

**Anatomy of skin**

Microscopically, the skin is a multilayered organ, composed of many histological layers (Figure.1). Skin is generally subdivided into three main layers, epidermis, dermis, and hypodermis

**Epidermis**

It can be subdivided into two parts, stratum corneum and the remainder of the epidermis is the so called viable epidermis.

**i) Stratum corneum-**

This is the outermost layer of skin also called as horny layer. It is approximately 10 µm thick when dry but swells to several times this thickness when fully hydrated. The stratum corneum is the principal barrier for penetration.

**ii) Viable epidermis-** This is situated beneath the stratum corneum and varies in thickness from 0.06 mm on the eyelids to 0.8 mm on the palms. Going inwards, it consists of various layers as stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale.

**Dermis**

Dermis is 3 to 5 mm thick layer and is composed of a matrix of connective tissue, which contains blood vessels, lymph vessels, and nerves.

**Hypodermis**

The hypodermis or subcutaneous fat tissue supports the dermis and epidermis. It serves as a fat storage area. For transdermal drug delivery, drug has to penetrate through all these three layers and reach into systemic circulation while in case of topical drug delivery only penetration through stratum corneum is essential and then retention of drug in skin layers is desired.

**Permeation pathways through skin**

Percutaenous absorption involves passive diffusion of the substances through the skin. A molecule may use two diffusional routes to penetrate normal intact skin, the appendageal route and the epidermal route (figure.2).

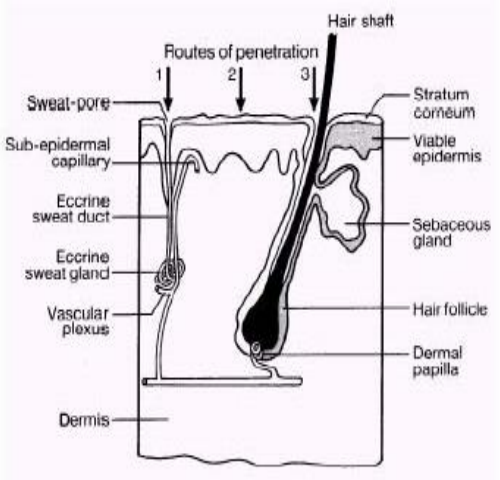



**Figure.2: Routes for drug permeation through skin**

**Appendageal route**

Appendageal route comprises transport via sweat glands and hair follicles with their associated sebaceous glands (shown 1&3 in Figure. 2). These routes circumvent penetration through the stratum corneum and are therefore known as "shunt" routes.

**Epidermal route**

Epidermal route comprises transport through epidermal layer (shown as no.2 in Figure.2).

For drugs, which mainly cross intact horny layer, two potential micro routes of entry exist, the transcellular and intercellular pathways

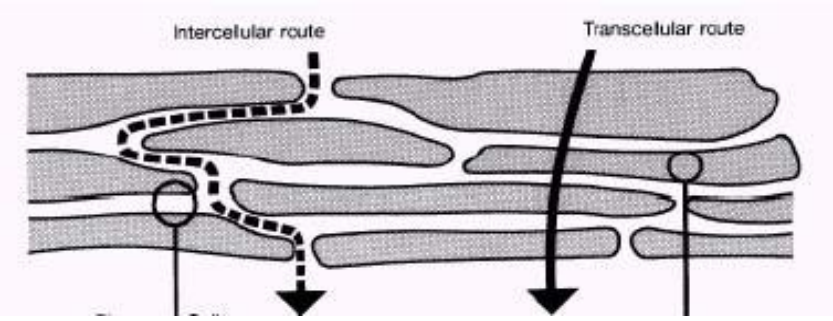



**Figure.3: Epidermal routes for drug permeation.**

**Factors Influencing Skin Permeation**

Skin permeation is a complex process, with a variety of barriers to cross. This is influenced by many factors, which are described as follows:

**Physicochemical factor**

**Diffusion**

Since the stratum corneum is the major barrier to penetration and passive diffusion is the main type of process, Fick's law has been used as a basis for the development of equations for drug absorption, as given by equation (I).

$$J = -D\, dc/dx$$

Where, **J** is the flux, **D** is diffusion coefficient, and is a function of size, shape, and flexibility of diffusing molecule as well as membrane resistance. dc/dx is concentration gradient.

**Additives**

**Buffers**

These can control the hydrogen ion concentration of preparation. According to the pH-partition hypothesis, only un-ionized molecules pass across lipid membranes in significant amounts. Weak bases and weak acids are dissociated to different degrees depending on the pH and pKa or pKb of the diffusion. Since many pharmaceuticals are weak bases or acids, the pH of the vehicle influences degree of ionization and proportion of ionized to unionized forms of the drug. This ratio has effect on partition coefficient of drug between the vehicle and the stratum corneum; in addition, the diffusion coefficients of two forms likely differ. The pH also affects the total solubility of drug, the concentration in the dissolved state in a suspension formulation.

**Surface-active agents**

Surfactants have ability to form micelles, and thus can solubilize the drug. These likely have an effect on skin by decreasing the interfacial tension between topically applied dosage form and stratum corneum, which can increase diffusion of drug. Finally, many surfactants may be used as penetration enhancers.

**Vehicles**

In general, the diffusion coefficient of a pharmaceutical within the vehicle varies inversely with viscosity. The composition of many vehicles changes with time, due to loss of volatile ingredients such as water in emulsions or alcohol in lotion. As a result, the concentration of nonvolatile ingredients increases, leading to change of solubility, activity and perhaps precipitation of the active.

**Pharmaceutical Factors**

Consideration should be given to properties of the drug, which can influence efficacy of the product.

**Polymorphic forms of drug*:*** Different polymorphic forms of a drug have different solubilities and physical stabilities, affecting absorption and shelf life.

**Molecular modification of drug:** This results in a different solubility, a different partition coefficient between the preparation and stratum corneum, and a different diffusion rate.

**Skin factors**

**Hydration of stratum corneum**

Increased hydration diminishes the resistance of the skin. Hydration of stratum corneum allows opening up' of intra and intercellular channels for easier passage of drugs. Additionally, moisture layer provides a medium for dissolution of the drug.

**Skin temperature**

Normally, it is not considered as a important factor in drug absorption although it is known that a rise of 10°C in temperature can produce 2 to 3 fold increase in permeation, depending upon the drug, the method (in vitro or in vivo), temperature rise, and relative humidity.

**Penetration enhancers**

These have direct effect on the skin and include solvents, surfactants, and other chemicals.

**Biological factors**

**Age**

It is assumed that the skin of infants, the young, and the elderly are more permeable than the skin of adults.

**Condition of skin**

Damage to the skin results in increased penetration as compared to the intact skin.

**Site of application**

In general as the thickness of the skin increases diffusion decreases. The skin of the plantar and palmer regions is thick, but the diffusion coefficient is considerably higher than in other regions of the body. The diffusivity is probably influenced by the lipid composition, its concentration in the stratum corneum, and the arrangement and number of cells, temperature of particular skin region.

**Metabolism**

Biotransformation of drugs is possible due to catabolic enzymes that are present in the skin layers and thus bioavailability can be affected.

**Blood circulation**

Since the stratum corneum is the major rate controlling barrier for drug absorption increase in blood flow usually does not promote drug absorption.

**Drug skin binding and reservoir effect of drug.**

The binding of drugs to tissue components can affect a number of parameters in formulation of topical preparations. Formation of depot or reservoir of drug in the skin increases the efficacy of topical formulations.

**Topical drug delivery**

**Introduction**

The delivery of drugs into and through the skin has been an important area of research for many years. Historically topical pharmaceuticals were developed by incorporating new drug compound into the vehicles such as hydrophilic petrolatum. In last two decades there is radical change in the manner in which dermatologicals are formulated, developed and tested. Topical drug delivery means drug administration via the skin for local therapeutic effect on diseased skin. Topical preparations are applied to skin for surface, local, or systemic effects. They may be used for prophylaxis (e.g. sunscreens, astringents, etc.) or for treatment (e.g. inflammation, bacterial infection, viral infection, etc.). The primary goal of topical products is to increase the retention of drugs in the skin rather than penetration through the skin. It differs from the transdermal drug delivery as transdermal products are designed to deliver the drugs through the skin to achieve systemic effects, hence here skin is not the target site. The topical drug products are designed to deliver the drugs into the skin for treating various dermal disorders, and here skin is the target organ. But penetration of drugs through the stratum corneum is essential for both types of deliveries and hence the rate limiting step in percutaneous absorption i.e. permeation through stratum corneum is common for both topical as well as transdermal drug products. Although some medication from topical products may unintentionally reach the systemic circulation, it is usually in sub-therapeutic concentrations, and hence does not produce side effects of any major concern.

**Evaluation of Topical Preparations**

The factors to be evaluated for semisolids at various stages of its development are described below. These must be within prescribed specifications, and all must remain so over the stated lifetime for the product.

1. pH
2. Viscosity
3. Drug content

4. Loss of water or other volatile components
5. Phase distribution
6. Particle size distribution of dispersed phase
7. *In-vitro* drug diffusion

## MATERIALS AND METHOD

**Chemicals, Equipments used for the present study.**

**Chemicals**

The chemicals used for research work were Aceclofenac (Amoli Organics Pvt. Ltd, Mumbai), Carbomer 940, Carbomer 971, Carbomer 974, PEG 200, PEG 400, PEG 600, Triethanolamine, Propylene glycol, Isopropyl alcohol, DMSO, Transcutol, Ethanol 99.9 %, , Methanol, n-octanol, Sodium hydroxide, Potassium dihydrogen phosphate, Picric acid, Cotton Wool. All the chemicals used are AR and LR grades.

**Equipment's**

The equipment's/instruments used for study are UV-visible double beam spectrophotometer, pH meter, Brookfield programmable viscometer with helipath attachment, FTIR spectrophotometer with diffused reflectance spectroscopy (DRS) attachment, Overhead stirrer, Magnetic stirrer with hot plate, Keshary-Chien (K-C) diffusion cell, Melting point apparatus, Mechanical shaker, Hot air oven, Electronic Balance.

**Characterization of Aceclofenac**

**UV- spectrum**

10mg of Aceclofenac was weighed accurately and transferred to 100 ml volumetric flask. Drug was dissolved in methanol and volume was made upto 100 ml with methanol. This was considered as stock solution (100 µg/ml). Further dilutions were made with phosphate buffer (pH 7.4) in the range of 2 to 20 µg/ml. Resulting solutions were scanned in the range of 400 to 200 nm using phosphate buffer (pH 7.4) as a blank with help of UV-visible spectrophotometer &λ max was recorded.

**Standard Curves**

Three different standard curves of Aceclofenac were developed using different solvents. For all standard curves stock solutions of Aceclofenac (100 µg/ml) were prepared in methanol. Further dilutions were made in the range of 2 to 20 µg/ml with phosphate buffer (pH 7.4), n-octanol and distilled water respectively. The absorbance's of resulting solutions were measured at 273 nm using UV-visible spectrophotometer against respective solvent blanks.

**IR Spectrum**

IR spectrum of Aceclofenac was determined using FTIR spectrophotometer with DRS attachment. Baseline correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and KBR was run.

**Melting point**

Melting point of Aceclofenac was determined by capillary method. Drug filled capillary was placed in the melting point apparatus containing silicon oil as a heating medium and the melting point was noted. The stirrer was kept on while recording the melting point to ensure uniform heat transfer.

**Solubility determination of Aceclofenac**

The saturation solubility of Aceclofenac was determined in water and phosphate buffer (pH 7.4). The methods are described below.

**Solubility in water**

Accurately weighed 25 mg of drug was suspended in 25 ml of distilled water in 50 ml volumetric flasks. The flasks were shaken with help of mechanical shaker for 48 hours. The dispersions were then filtered using Whatman filter paper no.41 and aliquotes were diluted suitably with distilled water. The filtrates were analyzed spectrophotometrically at a wavelength of 273.0 nm and the concentration of Aceclofenac in each sample was determined from a previously prepared standard curve. This experiment was performed in triplicates. Solubility in phosphate buffer (pH 7.4): Accurately weighed 25 mg of drug was suspended in 25 ml of phosphate buffer (pH 7.4) in 50 ml volumetric flasks. The flasks were shaken with help of mechanical shaker for 48 hours. The dispersions were then filtered using Whatman filter paper no.41 and aliquotes were diluted suitably with phosphate buffer (pH 7.4). The filtrates were analyzed spectrophotometrically at a wavelength of 273.0 nm and the concentration of Aceclofenac in each sample was determined.

**Determination of Partition coefficient of Aceclofenac**

Partition coefficient of Aceclofenac was determined in n-octanol/phosphate buffer (pH 7.4) system. The method is described below.

**Step-1:** Initially, 250 ml of phosphate buffer (pH 7.4) and 100 ml of n-octanol were mixed in separating funnels were and kept for 24 hrs. for presaturation of the phases with each other. The two phases were then separated from each other and 5 mg of Aceclofenac was suspended in 100ml of aqueous phase and stirred for 1hr with help of magnetic stirrer, This solution was filtered through Whatman filter paper no. 41.

**Step-2:** 5 mg Aceclofenac was suspended in 100 ml of water separately and was stirred well in a

beaker for 1 hr.

**Step-3:** The stirred sample was filtered through Whatman filter paper no.41. This was considered as solution A.

**Aceclofenac and n-octanol**

**Step-4:** The three samples of 5, 10, & 15 ml of filtrate of solution A. were taken and diluted to 25 ml with n-octanol pre-saturated with phosphate buffer pH (7.4).

**Step-5:** These were shaken for 15-20 min and filtered through Whatman filter paper no.41

**Step-6:** These samples were analyzed at wavelength 273.0 nm and the concentration of Aceclofenac in them was determined by using previously prepared standard curve.

**Aceclofenac with Phosphate buffer (7.4)**

**Step-7:** Similarly three samples of 5ml, 10ml, & 15ml of filtrate of solution A were diluted with 25ml of phosphate buffer ( 7.4) pre-saturated with n-octanol. They were shaken for about 15-20 minutes & were filtered through Whatman filter paper no.41 they were analyzed at 273 nm.

**Solubilization of Aceclofenac with cosolvents**

Various cosolvent systems were tried for Solubilization of Aceclofenac. The combinations of ethanol (99.9 %) with different PEG (200,400 and 600)were used for Solubilization of Aceclofenac in order to obtain clear gel.

**Formulation of 0.5%, 1%, 2% (w/w) Aceclofenac gels**

**Method of preparation**

Required quantities of Carbomer 940,971, 974 were soaked in some amount of distilled water for 2 to 3 hrs. Then it was neutralized by adding sufficient quantity of triethanolamine while stirring with help of overhead stirrer (Phase I). Aceclofenac was dissolved in the mixture of ethanol (99.9%), either of the PEG 200,400,600 and propylene glycol (Phase II),Transcutol & DMSO were added in some formulations as penetration enhancers.(Phase II) was added slowly to the (phase I) while stirring with help of overhead stirrer, the remaining quantity of distilled water was then added to make up the final 100gm weight.PH of all formulations was maintained in the range of 6.8  7.4.

**Table 1: Effect of PEG 200, 400, 600 with Carbopol 974**

| Name of ingredient | Weight (gm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ | $F_9$ |
| Aceclofenac | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Ethanol (99.9 %) | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 |
| PEG 200 | 12..10 | 18.15 | 24.20 | - | - | - | - | - | - |
| PEG 400 | - | - | - | 11.20 | 16.81 | 22.40 | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| PEG 600 | - | - | - | - | - | - | 10.82 | 16.20 | 21.63 |
| Propylene glycol | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 |
| Carbomer 974 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Triethanol amine | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| Distilled water (up to 100 gm ) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 2: Effect of PEG 200, 400, 600 with Carbopol 940**

| Name of ingredient | Weight (gm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $F_{10}$ | $F_{11}$ | $F_{12}$ | $F_{13}$ | $F_{14}$ | $F_{15}$ | $F_{16}$ | $F_{17}$ | $F_{18}$ |
| Aceclofenac | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Ethanol (99.9 %) | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 |
| PEG 200 | 12..10 | 18.15 | 24.20 | - | - | - | - | - | - |
| PEG 400 | - | - | - | 11.20 | 16.81 | 22.40 | - | - | - |
| PEG 600 | - | - | - | - | - | - | 10.82 | 16.20 | 21.63 |
| Propylene glycol | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 |
| Carbomer 940 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Triethanol amine | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| Distilled water (up to 100 gm ) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 3: Effect of change in conc. of carbopol 971, 940, and 974 with PEG 400**

| Name of ingredient | Weight (gm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $F_{19}$ | $F_{20}$ | $F_{21}$ | $F_{22}$ | $F_{23}$ | $F_{24}$ | $F_{25}$ | $F_{26}$ | $F_{27}$ |
| Aceclofenac | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Ethanol (99.9 %) | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 | 23.94 |
| PEG 400 | 22.40 | 22.40 | 22.40 | 22.40 | 22.40 | 22.40 | 22.40 | 22.40 | 22.40 |
| Carbopol 974 | 0.5 | 1 | 2 | - | - | - | - | - | - |
| Carbopol 971 | - | - | - | 0.5 | 1 | 2 | - | - | - |
| Carbopol 940 | - | - | - | - | - | - | 0.5 | 1 | 2 |
| Propylene glycol | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 | 15.52 |
| Triethanol amine | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| Distilled water (up to 100 gm ) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Evaluation of gels**

**Appearance**

All formulations were observed for appearance of any colour & transparency of gel.

**pH measurements**

The pH of each gel was measured using a pH meter. The pH meter was calibrated before each use with standard pH 4, 7 and 10 buffer solutions. The pH of gels was adjusted in the range 6.8 to 7.4.

**Drug content**

The quantity of formulation equivalent to 10 mg of Aceclofenac was dissolved in 100 ml methanol. From this solution, 1 ml samples were withdrawn and diluted to 10 ml with phosphate buffer pH 7.4. The samples were analyzed spectrophotometrically at a wavelength

of 273.0 nm. The concentration of Aceclofenac in each sample was determined from a previously prepared standard curve.

**Rheological study**

**Viscosity**

A Brookfield programmable viscometer (RVDV-II+) along with helipath assembly was used to determine viscosity (cp). The spindle used was T-bar spindle no.1 (S91). Sample gel was placed in the weighing bottle, the spindle was dipped into it and helipath was adjusted in such a way that neither it was touching the bottom of container nor did it come out of the sample during the movement of helipath .The spindle was rotated in the gel at various speeds, firstly at increasing rpm and then at decreasing rpm. At each speed, the corresponding viscosity (in cp) and torque (in %) values were noted.

**Spreadability**

The spreadability of the gel was determined using an apparatus described in the literature. This apparatus was fabricated in our laboratory. The apparatus consisted of two glass slides (7.5 × 2.5 cm), one of which was fixed onto the wooden board and the other was movable, tied to a thread which passed over a pully, carrying a weight.

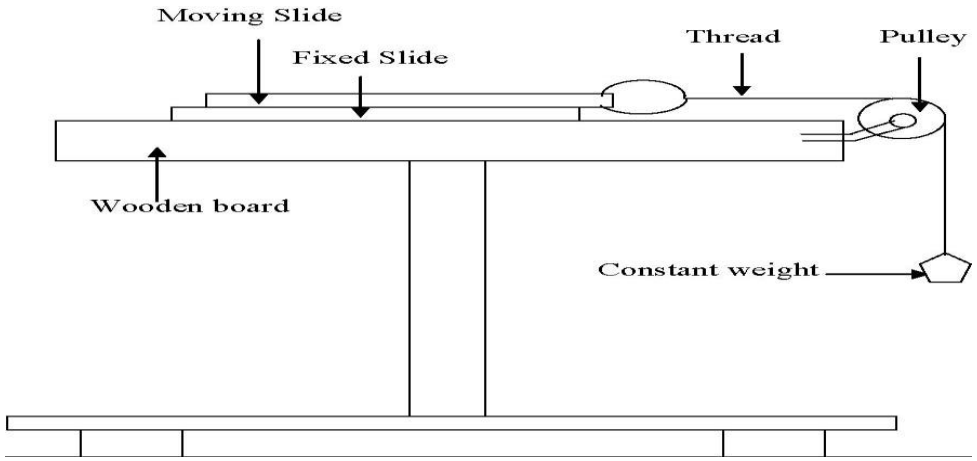



**Figure.4: Schematic representation of apparatus for determination of spreadability of gels.**

Firstly 0.5 gm of gel was placed between the two glass slides. 100 gm weight was allowed to rest on the upper slide for 1 to 2 minutes to expel the entrapped air between the slides and to provide a uniform film of the gel. The weight was removed and the top slide was subjected to a pull of 5 gm. The time necessary for top slide to travel pre-marked 6.5 cm distance was noted. This gave an idea of relative spreadability of the different gels.

***In- vitro* diffusion study**

**Diffusion study with help of Keshary-Chien (K-C) diffusion cell**

A Keshary-Chien (K-C) diffusion cell (Figure.16) with a receiver compartment volume of 27 ml, diameter of 2.6 cm and effective diffusion area of 5.31cm$^2$was used in this study.

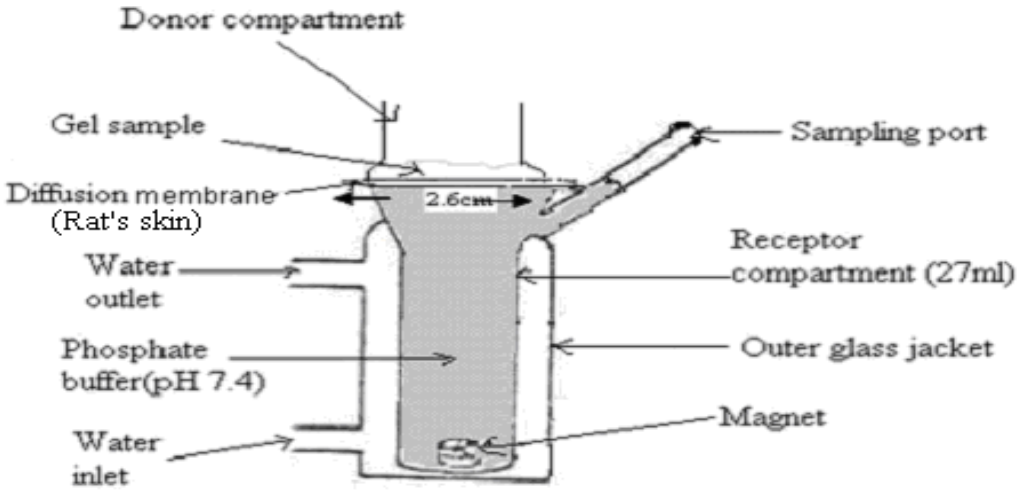



**Figure 5: Schematic representation of Keshary-Chien (K-C) cell used for diffusion study of gels**

Rat skin (Wistar albino, weighing 150-180gm) was used as diffusion membrane. Rat skin was soaked in phosphate buffer (pH 7.4) for 10 minutes before subjecting to diffusion study. The procedure for preparation of rat skin for diffusion study is given in the following section. The skin was positioned between the two cell halves of a glass chamber. The two compartments were held together with a clamp. The lower receptor compartment contained 24ml of phosphate buffer 7.4 and in the upper donor compartment 1gm of gel was spreaded, evenly on the excised rat skin. The receptor phase (phosphate buffer pH 7.4) was continuously stirred with help of magnetic stirrer and was maintained at temperature of 37 ±1 C during the experiments. 1gm of gel was placed in the donor compartment. At 1/2, 1, 2, 3, 4, 5, 6, 7, 8 hrs time intervals, 1 ml (or so) of the sample was withdrawn from the receiver compartment and the same amount of fresh buffer solution was added to maintain the sink conditions in receptor compartment. The care was taken to ensure that no air bubbles were lodged underneath the diffusion membrane during the experiments. The samples were diluted upto 5ml with phosphate buffer pH 7.4 and were analyzed spectrophotometrically at a wavelength of 273.0 nm and the concentration of Aceclofenac in each sample was determined from a previously prepared standard curve. This experiment was carried out for a period of 6 hours and in duplicate.

**Preparation of Rat skin for diffusion studies**

Sacrificed wistar albino male adult rats (150-180gm) were used as a source of skin for ‘in vitro” diffusion studies. The skin from the abdominal region of rat was carefully isolated and the

subcutaneous fat tissue adhering to the skin was removed taking care not to damage the skin. The hairs on the skin were removed with help of sterilized shaving blade and then the separated skin was washed 2 to 3 times with water. Fresh skin was now used for in-vitro diffusion study.

**Stability study**

Selected formulations i.e. one without penetration enhancer [G-PEG400a 974], one with 5% DMSO i.e. [G-PEG 200c 940] and one with transcutol[G-Carbo940 PEG400a] as penetration enhancer i.e. [G-Carbo940 PEG400a] formulations were subjected to stability studies for 3 months. The gels (50gm) were filled in glass weighing bottles and were stored at ambient temperatures, elevated temperature (40 ± 1 $^0$C) and refrigerated temperature (5 ± 1$^0$ C)These samples were tested initially and then at 15 days intervals for a total period of 3 months. These samples were evaluated for appearance, drug content, spreadability, pH and viscosity and observations were noted.

## RESULTS AND DISCUSSION

**Analysis of Aceclofenac using UV- spectrum**

The UV spectrum of Aceclofenac is shown

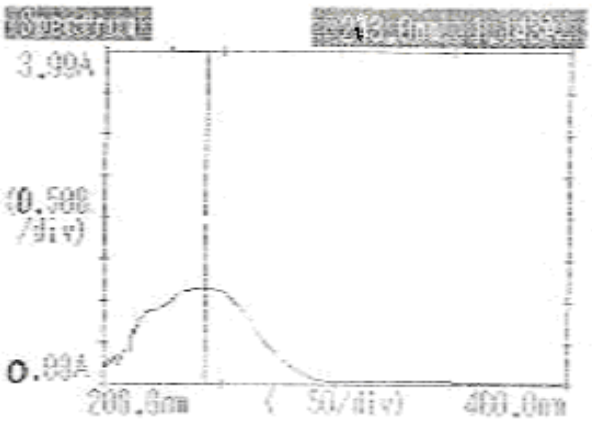


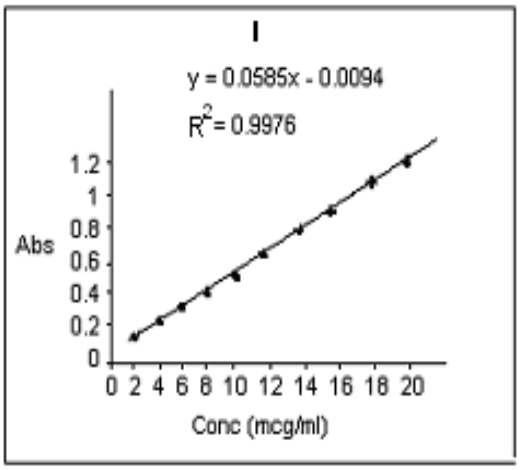

**Figure 6. UV spectrum of Aceclofenac**

The wavelength of maximum absorption (λmax) was found to be 273.0 nm and reported value is 274.0 nm. This wavelength was used for solubility determination, partition coefficient determination, determination of drug content of formulation, and in-vitro drug diffusion studies.

**Standard curves**

The standard curves with regression line equations and $R^2$ values are given below (Figure.18 to 21). Dilutions with phosphate buffer (pH 7.4) shows higher dilution factor as compared with the

other dilution as Dilutions with methanol, Dilutions with n-octanol, Dilutions with water so it is taken for further study such as in vitro drug diffusion.



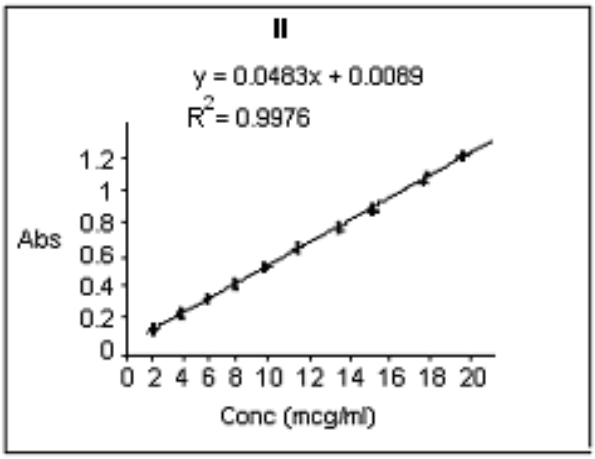
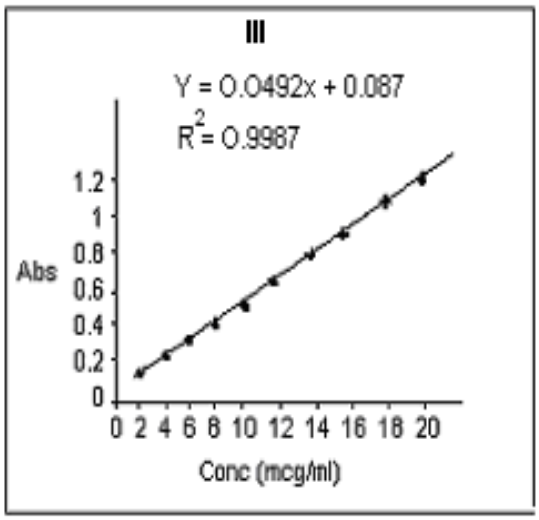




**Figure 7 and 8 : Standard curve in phosphate buffer (pH 7.4) and methanol**



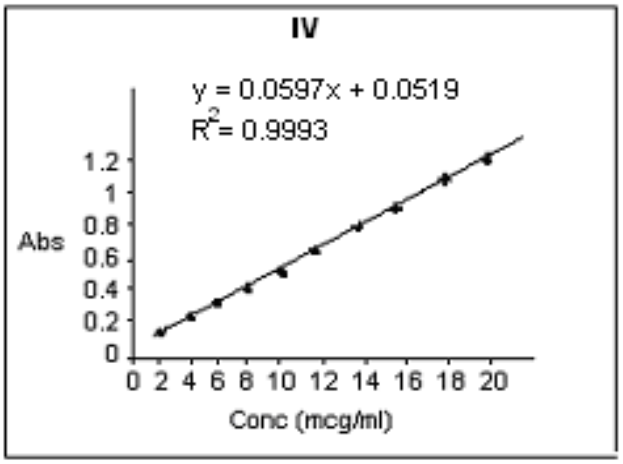




**Figure.9: Standard curve in n-octanol** **Figure.10: Standard curve in water**

Equation I was used for determination of in-vitro drug diffusion and drug content of formulations of Aceclofenac in phosphate buffer (pH 7.4) and Equation II was used for determination of partition coefficient of Aceclofenac in n-octanol/water system. Equation III was used for determination of solubility of Aceclofenac in methanol. Equation IV was used for determination of solubility of Aceclofenac in water.

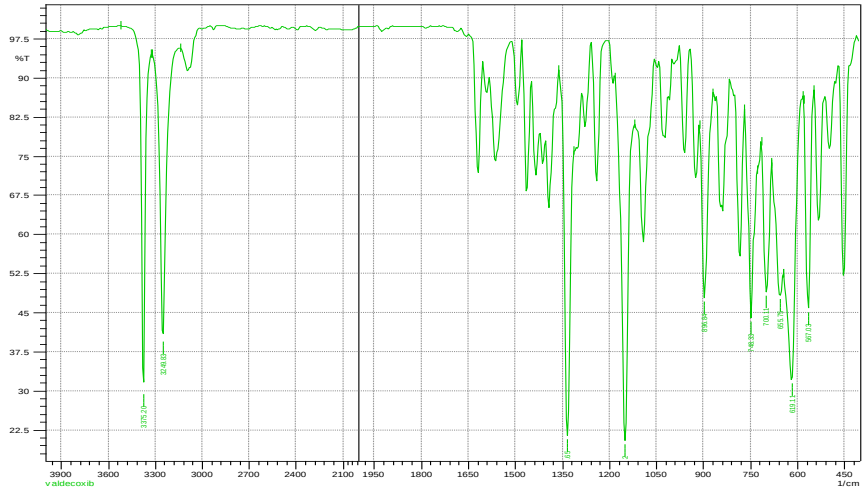



**Figure-11:** The IR spectrum of Aceclofenac

**Interpretation**

Various vibrations due to different groups that were observed in IR spectrum are given in Table 4.

**Table 4: Interpretation of IR spectrum of Aceclofenac.**

| Frequency (Cm-1) | Group | Deformation |
|---|---|---|
| 3377 | N-H | Stretching |
| 3100 -3000 | Aromatic C-H | Stretching |
| 1350-1300 | C-O isoxazolyl | Stretching |
| 1161 | S=O | Stretching |

It shows N-H stretching at 3377 and aromatic C-H Stretching at 3100-3000 Also C-O isoxazolyl shows stretching at 1350-1300 and S=O shows stretching at 1161. Thus the IR spectrum shows major functional groups in given sample of Aceclofenac.

**Melting point**

The melting point of Aceclofenac was found to be 170ºC. This is within specified range of 166 to175ºC.

**Solubility determination of Aceclofenac**

**Saturation Solubility in water**

The saturation solubility of Aceclofenac in water was found to be 11.12 ± 0.87 µg/ml and reported is 11.55 ± 0.95µg/ml in the literature.

**Saturation Solubility in phosphate buffer (pH 7.4)**

The saturation solubility of Aceclofenac in phosphate buffer (pH 7.4) was found to be 16.17 ± 0.82 µg/ml. In the literature, reported value for saturation solubility of Aceclofenac in phosphate buffer (pH 7.4) is 16.8 ± 0.4 µg/ml.

**Partition coefficient determination of Aceclofenac**

The partition coefficient of Aceclofenac in n-octanol/phosphate buffer (pH 7.4) system was found to be 5.84 ± 0.13.

**Solubilization of Aceclofenac**

The combinations of ethanol (99.9 %) with different PEGs and with different carbomer i.e.940, 971 and 974 were found satisfactory. Among these, following combinations in the ratio of 1: 1, 1:1.5& 1:2 were found satisfactory.

**Selected Formulation for further study**

Initially the gel products were prepared i.e. Carbopol 940 as gelling agent with PEG 200, PEG 400 and PEG 600 as cosolvents for drug. The concentration of PEGs was varied from 5%, 10% and 15%.Similarly 9 formulations were made with carbopol 974 as gelling agents PEG 200, 400 and 600 as cosolvents at concentration 5%, 10% and 15%. More 9 formulations were formulated wherein all the three Carbopols i.e. 940, 971 and 974 were combined individually PEG 400 alone. However the % of carbopol was varied as 0.5 % w/w, 1%w/w & 2.0% w/w. All these formulations were evaluated for pH, viscosity, spreadability time, drug content &*in-vitro* diffusion studies using rat skin. Based on the findings of these primary products 3 most suitable from each of above mentioned three schemes were chosen & optimized further. Two penetration enhancers 5% DMSO & 5% Transcutol were added to each of these 9 formulations separately & their *in-vitro* diffusion was compared. Thus the products without addition of penetration enhancers & with penetration enhancers were compared & then finally 3 of them i.e. $F_4$ (without penetration enhancer) $F_{12}$ (5% DMSO) &$F_{25}$ (5%Transcutol) were selected for *in-vivo* testing for skin irritation potential &efficacy of their anti-inflammatory action. Simultaneously these products were subjected to stability studies at refrigerated temp (5 + $1^0$C), ambient temp & elevated temp (40 + $1^0$C).

**Evaluation of gels**

**Table 5 Evaluation of Aceclofenac gel for Appearance, pH, Average % drug content, Viscosity & Spreadability time.**

| Formulation Type | Formulation code | Appearance | pH | Average % drug content (± S.D), of labeled amount | Viscosity (cp) at 5 rpm using T-bar spindle no.1 (S91) | Spreadability Time (in seconds) |
|---|---|---|---|---|---|---|
| Without addition of Penetration enhancer | $F_1$ | Translucent | 7.26 | 98.59 ± 0.77 | 4865 | 42.05 ± 0.30 |
| | $F_4$ | Translucent | 7.02 | 100.99 ± 0.65 | 7095 | 45.68 ± 0.56 |
| | $F_9$ | Translucent | 7.0 | 99.00 ± 0.80 | 5786 | 45.43 ± 0.53 |
| | $F_{12}$ | Translucent | 7.04 | 98.69 ± 1.25 | 6720 | 51.60 ± 0.76 |
| | $F_{14}$ | Translucent | 6.85 | 99.37 ± 0.78 | 5260 | 46.55 ± 0.60 |
| | $F_{18}$ | Translucent | 6.84 | 99.78 ± 1.11 | 5400 | 48.05 ± 0.91 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | $F_{23}$ | Translucent | 6.88 | 101.04 ± 1.43 | 5670 | 54.10 ± 0.33 |
| | $F_{20}$ | Translucent | 6.90 | 102.32 ± 0.58 | 6180 | 55.68 ± 0.48 |
| | $F_{25}$ | Translucent | 7.09 | 100.53 ± 1.20 | 6700 | 58.76 ± 0.50 |
| DMSO as Penetration enhancer | $F_4$ | Translucent | 7.02 | 100.99 ± 0.45 | 6260 | 44.25 ± 0.53 |
| | $F_{12}$ | Translucent | 7.02 | 100.99 ± 0.35 | 7096 | 51.43 ± 0.56 |
| | $F_{25}$ | Translucent | 7.02 | 99.99 ± 0.25 | 7091 | 51.33 ± 0.53 |
| Transcutol as Penetration enhancer | $F_4$ | Translucent | 7.02 | 100.99 ± 0.35 | 6256 | 44.23 ± 0.57 |
| | $F_{12}$ | Translucent | 7.03 | 100.99 ± 0.35 | 7091 | 50.93 ± 0.56 |
| | $F_{25}$ | Translucent | 7.08 | 99.89 ± 0.30 | 7098 | 51.33 ± 0.54 |

**Appearance**

Almost all formulations were found Translucentin appearance.

**pH measurements**

The pH values of all formulations were found in the range 6.8 to 7.3. The usual skin pH range is Hence the gels are satisfactorily complying with pH values needed for topical application

**Drug content**

The drug content of all formulations was found satisfactory

**Viscosity measurements:**

Among the group $F_{25}$ formulation the viscosity was higher than with $F_{20}$& $F_7$ i.e. PEG 400 with any other carbopol has exhibited highest viscosity.

**Spreadability**

Expected to spread easily on the skin areas when applied. The spreadability of the formulation characteristics derived from its more basic property i.e. viscosity. The greater the viscosity the longer will be the time taken for spreading, Thus the spreadability time for more viscous preparations such as $F_4$, $F_{12}$were highest in the given group.

## CONCLUSION

The carbopols 940, 971 and 974 with PEG 200, 400 and 600 in combination make suitable base for Aceclofenac gels. All the products are homogeneous, elegant and retained most of their physical and chemical properties at different conditions. The products containing penetration enhancers are showing better drug diffusion as compared to those containing no penetration enhancer. The products are safe or on-toxic after single application. All the three type of gels i.e. without penetration enhancer & with DMSO & Transcutol as penetration enhancer showing satisfactory anti-inflammatory activity. The *in-vivo* anti-inflammatory activity is quite satisfactory.

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