BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

# Formulation and Evaluation of Atenolol Topical Gel: A Novel Approach for Penetration Enhancement Formulation

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## ABSTRACT

Transdermal gel has gained more and more importance because the gel based formulations are better percutaneously absorbed than other topical dosage forms. Hypertension is defined by an elevation of the systolic or diastolic threshold or both. Atenolol, a class III molecule is a selective cardio-vascular β-blocker, which block the synthesis of Adenylate Cyclase there by prevents the formation of intra cellular messenger, cyclic AMP responsible for the activation of various protein kinases responsible for phosphorylation. The present work involve in the formulation of Atenolol Transdermal gels by dispersion method with different ratios of polymers for the sustained release of the drug through Stratum Corneum using Di-Methyl Sulphoxide, as penetration enhancer for the treatment of hypertension. All the formulations were evaluated for physico-chemical parameters and all the values were obtained within the limits. The *in-vitro* diffusion studies were carried out against dialysis membrane and *ex-vivo* permeation studies using albino rat abdominal skin by Franz diffusion cells. On hydration, osmotic pressure breaks & drug release follow zero order. Among fifteen formulations, *In-vitro* % Drug Release values of the optimized formulations were figured to be 98.22, 96.42 & 94.62 for F2, F11 & F13 respectively. The drug permeated/$cm^2$ through skin was also determined by flux value for optimized formulations F2, F11 and F13 was 0.073 $gmH^{-1}cm^{-2}$, 0.069 $gmH^{-1}cm^{-2}$ and 0.082 $gmH^{-1}cm^{-2}$ respectively.

**Keywords:** Franz diffusion cell, Di-Methyl Sulphoxide, Dialysis Membrane, Albino Rats.

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Received 02 April 2015, Accepted 17 May 2015

Please cite this article as: Swapna P., *et al.*, Formulation and Evaluation of Atenolol Topical Gel: A Novel Approach for Penetration Enhancement Formulation. British Journal of Medical and Health Research 2014.

## INTRODUCTION

Trans-Dermal Drug Delivery System (TDDS), an alternative to oral, intravascular, subcutaneous & Trans-Mucosal routes that provides a means to sustain release of drug with reduced intensity of action & ultimately reduce the side effects associated with its oral therapy. Various TDD technologies are described including the use of suitable polymers, carriers & penetration enhancers. They may be applied for the treatment of disorders of the skin or to treat diseases of other organs. The functional course of TDDS is mainly confined into four central components namely the skin, the drug candidate, the penetration enhancement method & the design of the dosage form[1,2]. Hypertension is totally asymptomatic; it has been named the "silent killer," as it is the major contributor or risk factor to cardiovascular morbidity & mortality. The success of treating Hypertension has been limited & despite well-established approaches to diagnosis & treatment in many communities fewer than half of all Hypertensive patients have adequately Controlled BP[5]. Atenolol is a Selective Beta Antagonist, having no intrinsic sympathomimetic activity with 40-50% of Bioavailability. The drug undergo Hepatic metabolism (<10%) & Renal Excretion (>90%)[8]. The half life of drug is 6-7H. In treatment of hypertension, angina, acute myocardial infarction, supra ventricular tachycardia & the symptoms of Alcohol Withdrawal[13]. It blocks the synthesis of Adenylate Cyclase inturn prevents the formation of intracellular messenger, cyclic AMP which further activates various protein kinases that control cell function in many ways by causing phosphorylation of various enzymes, carriers & other proteins[3]. Atenolol, a class III molecule, is highly soluble and low permeable with lipophillic character hence suitable as transdermal dosage form. As it has low bioavailability, the rationality of the drug is to enhance its bioavailability and the permeability is enhanced by the addition of penetration enhancers like DMSO.

## MATERIALS AND METHOD

Drug (Atenolol) (Nihal Traders), Carbopol 940 (Neha Chemicals, Hyd), HPMC 15CPS (Neha Chemicals, Hyd), HPMC K-100 (Neha Chemicals, Hyd), Methyl Cellulose (Neha Chemicals, Hyd), DMSO (SD Fine Chemicals, Mumbai),Methyl & Propyl Parabens (Neha Chemicals, Hyd), sod. CMC (Neha Chemicals, Hyd), HPMC (Neha Chemicals, Hyd).

**Preparation of Atenolol Gel using various polymers**

**Methods:** Dispersion Method is applied for the preparation of Trans-Dermal Gel.

**Using individual polymers (F1-F12)**

Different concentrations of Drug: Carbopol 940/ HPMC 15CPS/ HPMC K100/ MC (1:15, 1:25 and 1:35) ratios were prepared. Needful of polymers (1500 mg, 2500 mg and 3500 mg) were soaked overnight. Then the swelled polymer was stirred using mechanical stirrer to

ensure the uniform dispersion. Requisite quantity of Atenolol was added & stirred constantly for uniform dispersion. DMSO, Methyl & Propyl Parabens (0.001 mg) was added as preservative.

**Using combinations of polymers (HPMC 15CPS & sod.CMC, F13)**

Different concentrations of Drug: HPMC 15CPS & sod.CMC (1:15, 1:25, 1:35) ratios were prepared. Needful of HPMC 15CPS & sod.CMC (1500 mg, 2500 mg and 3500 mg) were soaked overnight. Then the swelled polymer was stirred using mechanical stirrer to ensure the uniform dispersion. Requisite quantity of Atenolol was added & stirred constantly for uniform dispersion. DMSO, Methyl & Propyl Parabens (0.001 mg) were added as preservative.

**Using combinations of polymers (HPMC & sod. CMC, F14)**

Different concentrations of Drug: HPMC & sod. CMC (1:15, 1:25, 1:35) ratios were prepared. Needful of HPMC & sod.CMC (1500 mg, 2500 mg and 3500 mg) were soaked overnight. Then the swelled polymer was stirred using mechanical stirrer to ensure the uniform dispersion. Requisite quantity of Atenolol was added & stirred constantly for uniform dispersion. DMSO, Methyl & Propyl Parabens (0.001 mg) were added as preservative.

**Using combinations of polymers (HPMC 15CPS & Carbopol 940, F15)**

Different concentrations of Drug: HPMC 15CPS & Carbopol 940 (1:15, 1:25 and 1:35) ratios were prepared. Needful of HPMC 15CPS & Carbopol 940 (1500 mg, 2500 mg, 3500 mg) were soaked overnight. Then the swelled polymer was stirred using mechanical stirrer to ensure the uniform dispersion of the polymer. Requisite quantity of Atenolol was added & stirred constantly for uniform dispersion. DMSO, Methyl & Propyl Parabens (0.001 mg) was added as preservative.

## RESULTS AND DISCUSSION

**Compatibility studies**

The vials containing samples were observed $2^{nd}$ & $4^{th}$ week & compared with vials kept at 4˚C as control. They were compared for incompatibility like lump formation & colour change. From the results it was observed that there is no change as shown in table 4.

**Drug polymer compatibility studies using Fourier Transform Infrared Spectroscopy[9]**

The IR spectrum of pure drug in Figure 1 was found to be similar to the standard spectrum of Atenolol. The characteristics absorption peaks of Atenolol were obtained at 1793 $cm^{-1}$ indicating C=O stretching of carbonyl group, 2853 $cm^{-1}$ indicating aromatic ring, 3734 $cm^{-1}$ indicating Amines & Alcohols, 1340 $cm^{-1}$ indicating C-O-C (ether) group & 1114 $cm^{-1}$ indicating OH bending**.**

**Drug polymer compatibility studies using Differential Scanning Calorimetry (DSC):**

The resultant Thermo grams generated for the analysis of each of the materials under investigation were presented in Figure 5, 6. The drug exhibited a sharp melting endothermic peak at 145.14°C. No significant thermal shifts were observed for Atenolol, when it was assessed in combination with the other excipients intended for use in gel formulation[9]. Therefore, based on thermal analysis data, it was concluded that Atenolol could be formulated with combinations of the excipients tested, as potential major incompatibilities were not evident. However, more rigorous, long-term stability testing of manufactured dosage forms should also be conducted to rule out real-time long-term dosage form instabilities.

**Skin irritation test**

The test was carried out on animal study protocol was reviewed and approved by the institutional animal ethical committee, St. Peter's Institute of Pharmaceutical Sciences, (SPIPS/AEC-19), Hanamkonda, India using Healthy albino rats. The animals were divided into two groups one for control and other for the formulation. The back skin of the animals was shaved a day before the study is carried out. The study was executed for 4 days. At the end of the study, the animals were observed for any skin irritation like erythema or edema and the score was given as per the irritation[6]. The results of the skin irritation test revealed no irritation from gel formulations F2, F11 & F13 as they produced a score less than 2.

***In vitro* Diffusion Study**

The developed formulations of Atenolol were subjected to *in vitro* dissolution studies using Franz Diffusion Cell Apparatus using dialysis membrane in pH 5.5 acetate buffer up to 6hr in order to simulate the conditions predominant in the Trans-Dermal Delivery[7]. Dialysis membrane is manufactured from natural cellulose reconstituted from cotton inters. It is flexible, transparent and reinforced with porous paper layer with good chemical and $p^H$ resistance ($p^H$ 2-12) and temperature tolerance. During the manufacturing process, membrane is reinforced with a layer of porous paper to increase its wet strength for use in high shear or torque environments, resulting in a lower permeation rate compare to the other membranes. Dialysis membrane does not carry any fixed charge and do not absorb most solutes. Hence it is used in general laboratory dialysis functions like desalting, molecular separation and buffer exchange[17]. The *in vitro* release profiles of all the formulations containing different amount of polymers are shown in Figure 7, 8. Depending on the viscosity parameters and the percentage drug release from the polymers was determined. Hence the formulations prepared with MC, Carbopol 940 & HPMC 15CPS & Na.CMC gave maximum drug release within 1hr. The graphical representation was shown in the figure 9. Drug release from Methyl

Cellulose is depending on two factors viscosity and the molecular weight. Lower the viscosity weaker the gel strength. This suggests the formation of channels with more diameters resulting in the fast drug release. Carbopol 940, on hydration creates osmotic pressure inside the gel. Thus the structure of the gel is deformed guiding to the constant drug release at zero order. Gels formed from the HPMC have desirable fat like functional properties. On hydration each particle swells and macromolecular chains start entangling thus creating diffusional spaces. Lower the entangling more will be the diffusional spaces and faster will be the drug release.

***Ex vivo* permeation studies using albino rat abdominal skin**

**Preparation of rat abdominal skin**

All experiments were conducted according to the protocol approved by the Animal Ethics Committee (AEC). The experiment was deported according to the guidelines of Committee for the purpose of control and supervision of the experiment on animal[15]

**Tissue isolation**

The male Albino rats weighing 150-200 g were sacrificed and the hair on the skin was removed using the depilatories without altering skin properties. The fresh abdominal skin was excised and separated from the underlying tissue. The excised skin was cleared off its subcutaneous fatty substance and stored immediately at -30°C until use to maintain integrity and viability of the skin[16]. *Ex vivo* permeation studies of atenolol transdermal gel was studied through the albino rat abdominal skin membrane as penetration barrier using Franz diffusion cell. The skin was sandwiched between donor and receptor compartments of the diffusion cell

with a diffusional area of 2 $cm^2$ facing stratum corneum towards donor compartment. Receptor compartment with a magnetic bead was filled with acetate buffer pH 5.5[10]. Two compartments were tied with the help of springs so that the skin membrane did not move from its place. The entire setup was ranged over a magnetic stirrer at a speed of 150 rpm and the temperature was maintained at 37˚±0.5°C. Samples of 3 ml were withdrawn through the sampling port at 30 min of intervals for a period of 6hr, concurrently replacing with equal volume by acetate buffer pH 5.5. The samples were analyzed using UV- Visible spectrophotometer at 274 nm[11]. The *in vitro* release profiles of all the formulations containing different amount of polymers are shown in Figure 9.

**Determination of flux (J)**

The flux (J) was determined as the angular coefficient of a curve obtained by plotting the cumulative amount of the permeated drug versus time. The steady state permeation flux was calculated from slope of linear portion of the curv

The flux (J) through the membrane was calculated by using the equation.

$$J = DQ/ A dt$$

Where J is flux (mg $h^{-1}cm^{-2}$);

DQ/dt is the slope obtained from the steady-state portion of the curve and

A is the area of diffusion ($cm^2$)

The drug permeation from optimized formulations F2, F11 and F13 was steady and atenolol could permeate through the skin membrane with a flux of 0.073 gm $H^{-1}$ $cm^{-2}$, 0.069 gm $H^{-1}$ $cm^{-2}$ and 0.082 gm $H^{-1}$ $cm^{-2}$ respectively. It was observed that as the concentration of HPMC was increased the mean cumulative amounts of drug permeated and flux increased considerably. The hydrophilic nature of HPMC seems to have imparted towards increase in penetration of the solvent molecules into the polymeric matrix and disturbed the compactness of polymeric matrix resulting in faster release[12].

**Table 1: Formulation of Transdermal Gel using Carbopol 940 & HPMC 15 CPS**

| **Ingredients** | **Using Carbopol 940 as gelling agent** | | | **Using HPMC 15 cps as gelling agent** | | |
|---|---|---|---|---|---|---|
| **Drug: Polymer** | **F1 (1:1.5)** | **F2 (1:2.5)** | **F3 (1:3.5)** | **F4 (1:1.5)** | **F5 (1:2.5)** | **F6 (1:3.5)** |
| Drug (mg) | 100 | 100 | 100 | 100 | 100 | 100 |
| Carbopol 940 (mg) | 1500 | 2500 | 3500 | - | - | - |
| HPMC 15CPS (mg) | - | - | - | 1500 | 2500 | 3500 |
| DMSO (ml) | 3 | 5 | 7 | 3 | 5 | 7 |
| Glycerine (ml) | 3 | 5 | 7 | 3 | 5 | 7 |
| Water (ml) | Q.s | Q.s | Q.s | Q.s | Q.s | Q.s |
| Methyl/ propyl paraben (mg) | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

**Table 2: Formulation of Transdermal Gel using HPMC K 100 & MC gelling agent**

| **Ingredients** | **Using HPMC K 100 AS Gelling agent** | | | **Using MC as Gelling Agent** | | |
|---|---|---|---|---|---|---|
| **Drug: Polymer** | **F7 (1:1.5)** | **F8 (1:2.5)** | **F9 (1:3.5)** | **F10 (1:1.5)** | **F11 (1:2.5)** | **F12 (1:3.5)** |
| Drug (mg) | 100 | 100 | 100 | 100 | 100 | 100 |
| HPMC K 100 (mg) | 1500 | 2500 | 3500 | - | - | - |
| MC (mg) | - | - | - | 1500 | 2500 | 3500 |
| DMSO (ml) | 3 | 5 | 7 | 3 | 5 | 7 |
| Glycerine (ml) | 3 | 5 | 7 | 3 | 5 | 7 |
| Water (ml) | Q.s | Q.s | Q.s | Q.s | Q.s | Q.s |
| Methyl/ propyl paraben (mg) | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

**Table 3: Formulation of Trans-dermal Gel using ccombination of gelling agent**

| **Ingredients** | **Using Combination Of Gelling Agent** | | |
|---|---|---|---|
| **Drug: polymer ratio** | **F13 (1:1.5)** | **F14 (1:2.5)** | **F15 (1:3.5)** |
| Drug (mg) | 100 | 100 | 100 |
| HPMC 15 CPS (mg) | 750 | - | 1750 |
| Sod.CMC (mg) | 750 | 1250 | - |
| HPMC (mg) | - | 1250 | - |
| Carbopol 940 (mg) | - | - | 1750 |

**Table 4: Drug & Excipient Compatibility Studies**

| **S.No** | **Ingredients** | **Ratio** | **Physical Description** | | |
|---|---|---|---|---|---|
| | | | **Initial** | **55°C (2 W)** | **40±2°C /70±5 % RH(4 W)** |
| 1 | API (Atenolol) | -- | White | No change | No change |
| 2 | API+ Carbopol 940 | 1:1 | Off white | No change | No change |
| 3 | API+HPMC 15CPS | 1:1 | Off white | No change | No change |
| 4 | API+ HPMC | 1:1 | Off white | No change | No change |
| 5 | API+ HPMC K100 | 1:1 | Off white | No change | No change |
| 6 | API+ Na.CMC | 1:1 | Off white | No change | No change |
| 7 | API+Methyl Cellulose | 1:1 | Off white | No change | No change |

**Table 5: Frequency Range of Functional Groups present in Atenolol**

| **Functional Group** | **Frequency ($cm^{-1}$)** |
|---|---|
| C-H Aromatic ring (stretching) | 3017.49 |
| C=O Aromatic (stretching) | 1666 |
| H-N (stretching) | 3198-3071 |
| C-H (stretching) | 2870.16 |
| CH2 (bending) | 2924 |
| O-H (bending) | 3368 |
| O=C-$NH_2$ | 1649 |

**Table 6: Score for skin irritation**

| Score | Description |
|---|---|
| 0 | No irritation. |
| 0.5 | Faint, barely perceptible erythema or slight dryness. |
| 1 | No eruption/ no erythema but definite dryness, may have epidermal fissuring. |
| 1.5 | Well defined erythema or faint erythema with definite dryness, may have epidermal fissuring. |
| 2 | Moderate erythema: may have few papules or erythema in the cracks. |
| 2.5 | Moderate erythema with barely perceptible edema. |
| 3 | Severe erythema may have generalized papules or moderate to severe erythema with slight edema (edges well defines by raising). |
| 3.5 | Moderate to severe erythema with moderate edema (confined to patch area). |
| 4 | Generalized vesicles or Escher formation or moderate to severe erythema |

**Table 7: Evaluations Tests of all the Formulations**

| F.CDE | %DC | Spreadability | pH | Visco. (cps) | Clarity | Extrudability | Homogenesity |
|---|---|---|---|---|---|---|---|
| F1 | 92.66 | 20.83 | 6.06 | 24,620 | +++ | good | +++ |
| F2* | 98.66 | 23.80 | 5.57 | 24,642 | **+++** | excellent | **+++** |
| F3 | 93.33 | 20.83 | 6.30 | 24,645 | ++ | good | ++ |
| F4 | 94.66 | 19.23 | 6.42 | 24,530 | ++ | good | +++ |
| F5 | 93.33 | 20.83 | 6.32 | 24,537 | ++ | excellent | +++ |
| F6 | 94 | 20.83 | 5.36 | 24,541 | ++ | good | ++ |
| F7 | 92 | 17.85 | 5.71 | 32,000 | ++ | good | +++ |
| F8 | 91.33 | 19.23 | 6.21 | 32,220 | ++ | fair | ++ |
| F9 | 94.66 | 18.51 | 6.65 | 32,225 | + | fair | ++ |
| F10 | 96 | 25 | 6.86 | 24,470 | ++ | good | ++ |
| F11* | 98 | 27.77 | 5.48 | 24,456 | **++** | excellent | **+++** |
| F12 | 91.33 | 22.72 | 6.54 | 24,470 | ++ | good | ++ |
| F13* | 98.06 | 23.80 | 5.78 | 24,780 | **++** | good | **+++** |
| F14 | 97.33 | 22.72 | 6.23 | 24,800 | ++ | fair | ++ |
| F15 | 96.93 | 20 | 6.02 | 24,820 | ++ | good | +++ |

**Clarity:** **+++** glasssy, ++ clear, + turbid:

**Extrudability:** +++ excellent, ++ good, + fair.

**F.CDE:** Formulation Code,

**DC:** Drug Content,

**Visco:** Viscosity.

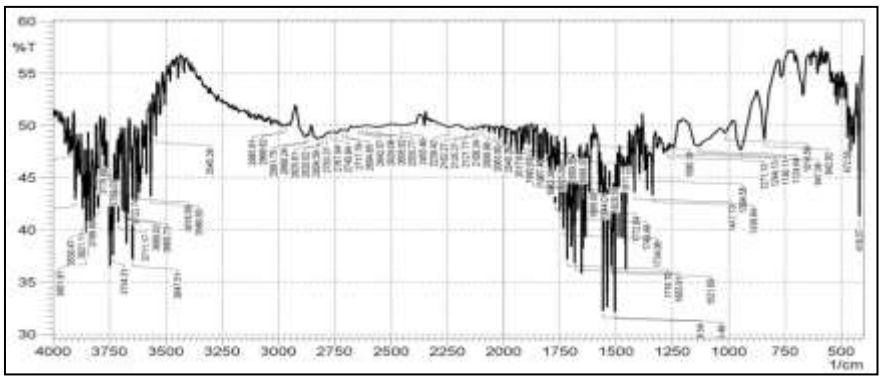


**Figure 1: FT-IR spectra of pure drug (Atenolol)**

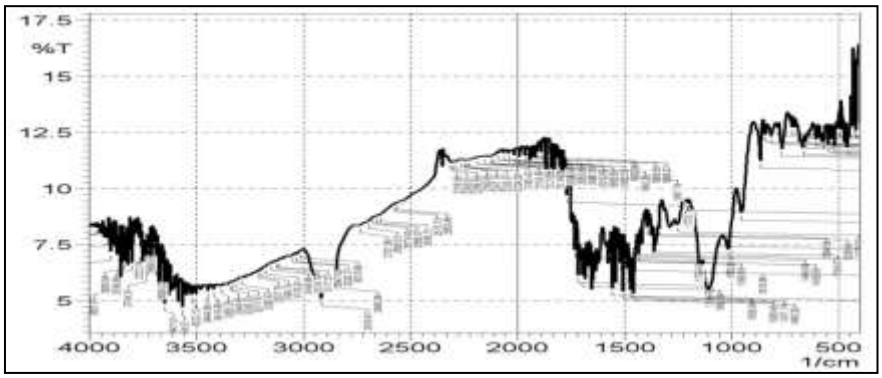


**Figure 2: FT-IR spectra of optimized Formulation (F2)**

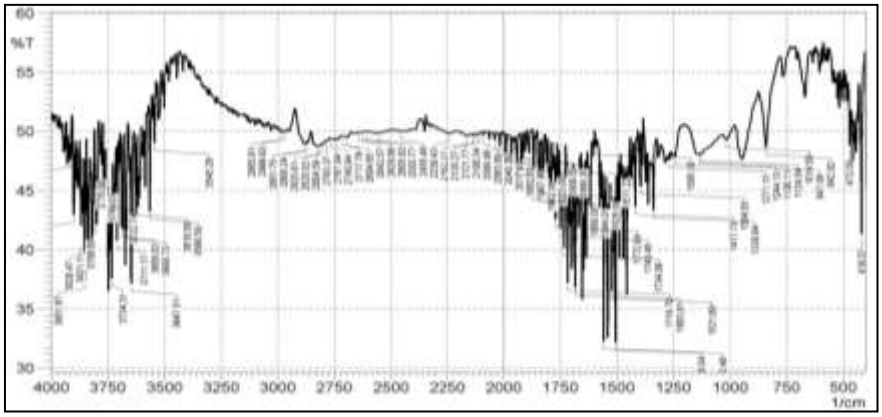


**Figure 3: FT-IR spectra of the optimized Formulation (F11)**

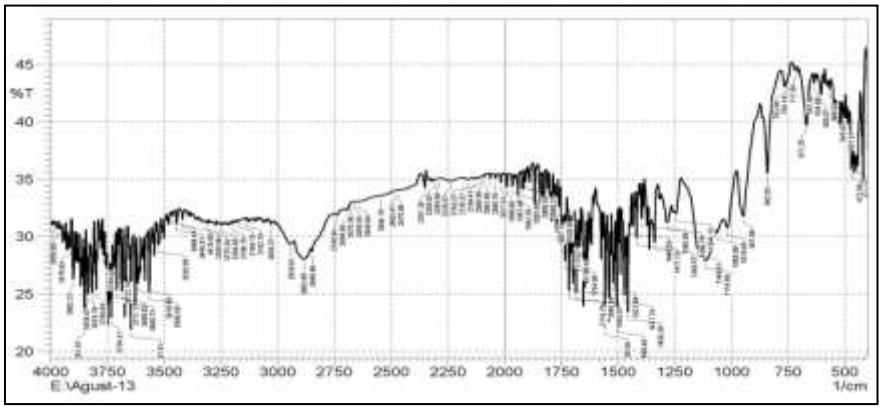


**Figure 4: FT-IR spectra of optimized Formulation (F13)**

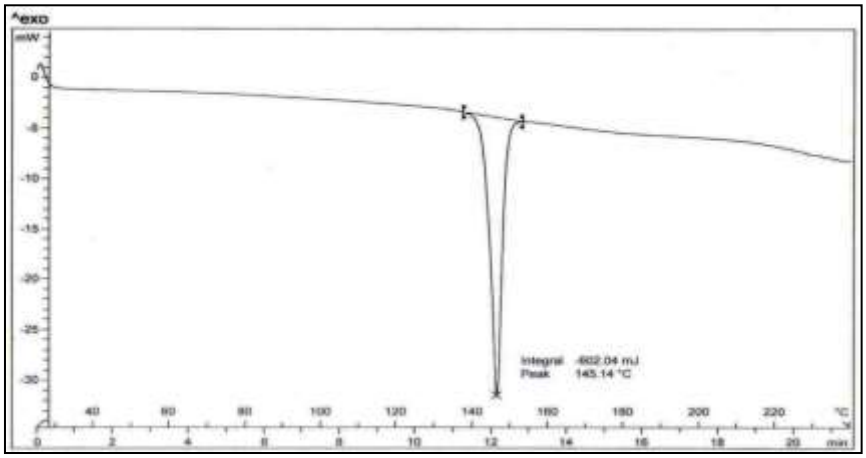


**Figure 5: DSC Thermogram of pure drug (Atenolol)**

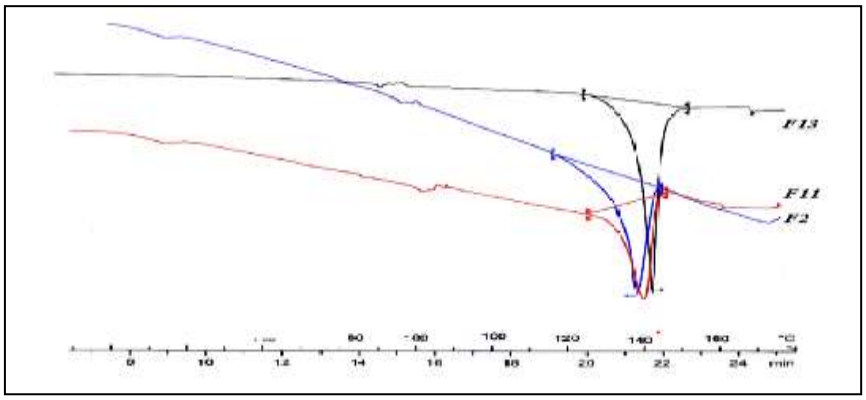


**Figure 6: DSC Thermogram of optimized Formulation (F2), (F11), (F13)**

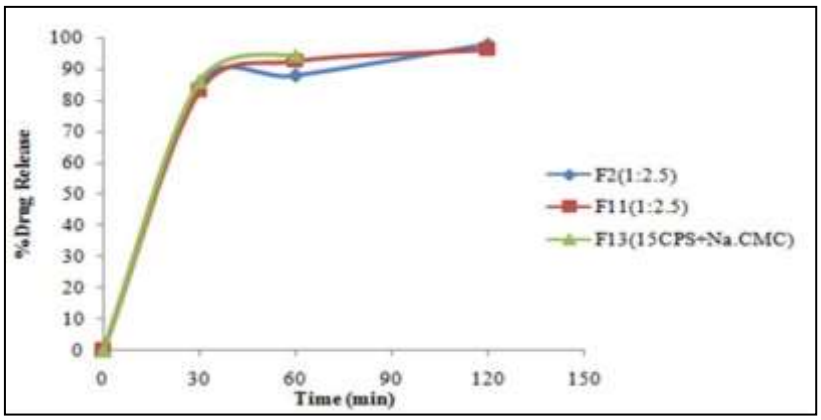


**Figure 7: % Cumulative Drug Release of all the Formulations**

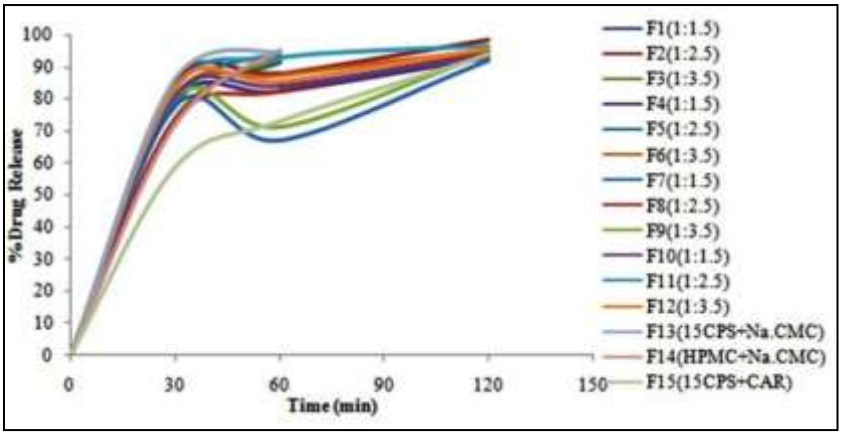


**Figure 8: Cumulative % Drug Release of Optimized Formulations**

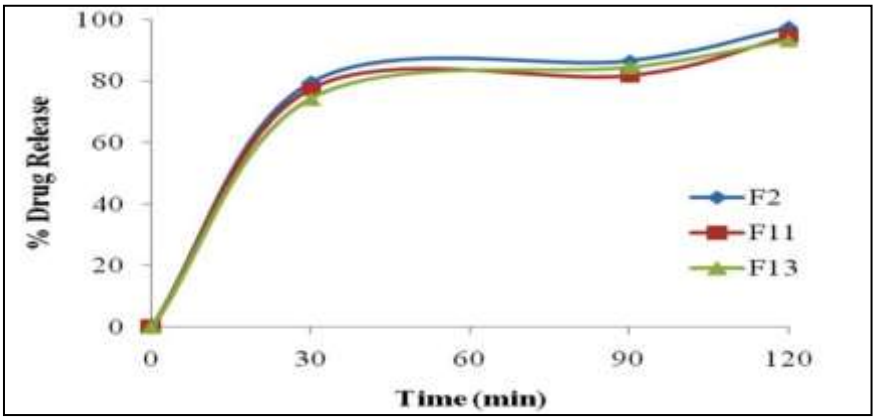


**Figure 9: % Drug Release of Optimized Formulations**

## CONCLUSION

From the experimental findings after formulation & evaluation of Atenolol Gel, it can be concluded that the analytical techniques indicate that the drug sample incurred was pure & does not show any incompatibilities with the excipients. In the present work, Atenolol, a hydrophilic drug has been set about to deliver from gels using various polymers. The polymers used were Carbopol 940 (1:15), methyl cellulose (1:25) & combination of HPMC 15CPS (1:15). The results of the skin irritation test revealed no irritation from gel formulations F2, F11 & F13 as they produced a score less than 2. Based on the viscosity parameters & spread-ability characteristics optimum drug release was obtained when the drug was delivered from Carbopol 940 (F2), Methyl Cellulose (F11), HPMC 15 CPS + Sod.CMC (F13) respectively. *In vitro* % Drug Release values of the optimized formulations were calculated to be 98.22, 96.42 & 94.62 for F2, F11 & F13 respectively. The drug permeation from optimized formulations F2, F11 and F13 was steady and 9.8 mg, 9.6 mg and 9.4 mg of Atenolol could permeate through the skin membrane with a flux of 0.082 gm $H^{-1}$ $cm^{-2}$, 0.073 gm $H^{-1}$ $cm^{-2}$ and 0.069 gm $H^{-1}$ $cm^{-2}$ respectively. Based on the above furnished details, I resolve that Carbopol 940 (F2), Methyl Cellulose (F11), HPMC 15 CPS + Sod.CMC (F13) are the best formulations with maximum release profiles.

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