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Formulation & optimization of the transdermal film of 5-FU with *in-vitro* and *ex-vivo* study using ethyl cellulose and two grades of hydroxy propyl methyl cellulose

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ABSTRACT

5-Fluorouracil (5-FU) is widely used as an anti-cancer drug, but causes severe side effects. Controlled release systems could be useful to keep the concentration of 5-FU at a low level, so that the side effects can be reduced. The purpose of this research study was to formulate transdermal film loaded with 5-Fluorouracil by solution casting method using ethyl cellulose, HPMC E15 and HPMC K4M in various combinations and to investigate the effect of different polymer on drug permeation and other physicochemical characteristics of the film. The ex-vivo permeation study was carried out with rat skin along with in-vitro study using dialysis membrane and both revealed that HPMC E15can exhibit more sustained formulation than HPMC K4M.The hydrophilic polymer alone was incapable to form the film. Further, it was observed that the cumulative % release of the drug from the formulation was retarded with the addition of the hydrophobic polymer EC and increased on addition of the hydrophilic polymer. Again, between two grades of HPMC i.e., HPMC E15 and HPMC K4M, the polymer E15 provided slower rate of release than HPMC K4M. The formulation FF1 with EC showed lowest moisture up take (1.9%) and also lowest % of release (56.76%) in 7 hr than the other formulations. Further, formulation FF2 with combination of HPMC K4M and EC showed highest moisture up take (18.78%) and also highest % of release (96.53%) of drug in7 hr and reduced in respect of moisture uptake and rate of release with the admixture of HPMC E15 and we also observed that there exist a decrease in order of the above with the increase of the concentration of the same polymer HPMC E15. Stability study at intermediate and accelerated conditions according to the ICH (Q1A) specifications showed that all the formulations were found to be very stable. Hence, transdermal film of 5-FU thus formulated could be a promising alternative dosage form in cancer chemotherapy.

Keywords: Controlled release, Hydrophilic, Hydrophobic, *In-vitro & Ex-vivo.*

INTRODUCTION

Transdermal drug delivery system is the modern delivery system to deliver the drug by by-passing the first-pass metabolism problem. It is used to deliver the drug through the skin to systemic circulation. Currently more than 35 TDDS products are approved in United States for the wide variety of condition like hypertension, angina, motion sickness, severe pain, local pain control and nicotine dependence etc. [1]. TDDS provides the benefit of sustained delivery of the drugs with short half life, reduces the dosing frequency, increase the bioavailability of the poor orally bio available drug, decrease the side effects as a result increase the patient compliance [2]. The non- invasive character of TDDS makes it accessible to a wide range of patient population & a highly acceptable option for drug dosing. 5-FU is an antimetabolite with promising antineoplastic activity against several premalignant & malignant

condition of the skin. It is also shown to be active against a variety of solid tumors including those in breast, colon, rectum & cervix [3, 4]. Transdermal delivery of 5-FU may overcome certain limitations associated with oral & parenteral administration. Its oral administration shows significant variation in oral bioavailability, ranging between 0-80% [5] and in case of parenteral administration the main problem is rapid elimination of the drug with apparent half life of 8-20 min for that reason we have targeted to formulate transdermal film using 5-Fluorouracil as the model drug.

Here, we have attempted to optimize the suitable ratio of the hydrophobic & hydrophilic polymer to formulate the film by studying the permeation of the drug through dialysis membrane (*in-vitro*) and also through rat skin (*ex-vivo*).

MATERIAL AND METHODS

5-Fluorouracil was purchased from Sigma Aldrich. Ethyl cellulose, HMPC E15, HMPC K4M, oleic acid and glycerin were also purchased from Loba Chemie Pvt. Ltd, Mumbai. Dialysis membrane from HIMEDIA Lab. All other solvents and chemicals used were of analytical grade.

Animals:
Healthy Wistar rats were received from departmental approved animal house and used for the study. They were acclimatized to laboratory conditions for one week before commencement of experiment. They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. All procedures followed here for the study were thoroughly reviewed and approved by the Institute's Animal Ethical Committee, (Reg. No. BCRCP/IAEC/3/2012).

Method for Preparation of the Transdermal Film:
Transdermal film of 5-FU was prepared by solvent casting method. Here the polymeric solution was prepared by dissolving EC, HPMC K4M, and HPMC E15 in 20 ml mixture of acetone and chloroform (3:1) stirred for 10 min for complete dispersion of the polymers. Then 20 mg of 5-FU was added to it and stirred again for 10 min for complete dispersion. Then 10% glycerin and 5% oleic acid were added to it. After stirring for 5 min the solution was poured on a 1.5 cm diameter Petridis. Then the solvent was allowed to evaporate under ambient temperature (32°C, 45%RH) for 24 hr. The prepared film was then scraped for evaluation.

Pre Formulation Study:
First a polymer combination was selected to produce the film without the drug. The combination of the formulations is shown in table1. We have denoted the formulations with PF.

Table 1. : Pre formulation composition

Pre formulation code	Ethyl cellulose (mg)	HPMC K4M (mg)	HPMC E15M (mg)	GLYCERIN (% w/v)	OLEIC ACID (%w/v)	Result
PF1	500	--	--	10	5	Film formed
PF2	250	250	--	10	5	Film formed
PF3	250	--	250	10	5	Film formed
PF4	250	125	25	10	5	Film formed
PF5	250	100	50	10	5	Film formed
PF6	250	75	75	10	5	Film formed
PF7	250	50	100	10	5	Film formed
PF8	250	125	25	10	5	Film formed
PF9	250	--	150	10	5	Film formed
PF10	--	300	---	10	5	Film not formed
PF11	--	--	300	10	5	Film not formed

From pre formulation study, following eight formulations were selected as the final formulations.

IR STUDY:
Before going to the final formulations first IR study of the physical mixture of the drug, EC, HPMC K4M and HPMC E15 was performed to investigate the compatibility.

Final Formulations:
After the pre formulation study and after conforming that there is no drug-excipient interaction, the following formulations were prepared (table 2).

Table 2:- Final formulations

CODE	DRUG (mg)	EC (mg)	HPMC K4M (mg)	HPMC E15 (mg)	GLYCERIN (% w/v)	OLEIC ACID (% w/v)
FF1	20	500	----	-----	10	5
FF2	20	250	250	----	10	5
FF3	20	250	----	250	10	5
FF4	20	250	175	75	10	5
FF5	20	250	150	100	10	5
FF6	20	250	125	125	10	5
FF7	20	250	100	150	10	5
FF8	20	250	75	175	10	5

EVALUATION:

Physicochemical evaluation:

The prepared films were evaluated for their physical appearance, uniformity of thickness, weight variation, folding endurance, drug content, moisture content, moisture uptake, flatness, *in-vitro* release studies & *ex-vivo* diffusion study across the rat abdominal skin.

Weight variation:

Weight variation was determined by individually weighing 6 randomly selected films and calculating the average weight and standard deviation. The individual weight should not deviate significantly from the average weight [6].

Thickness of the film:

Screw gauge was used to determine thickness of the films. It was placed at three different positions by keeping the film in between two glass slides of known thickness and average thickness was calculated and the values are given in table-4.

Folding endurance

The folding endurance was measured manually. A strip of film having an area of $2cm^2$ was cut evenly and repeatedly folded at the same place till it broke/cracked. The number of times the film could be folded at the same place without breaking/cracking gave the exact value of folding endurance and the results are reported in table-4[7, 9].

Moisture content:

The prepared film were weighed individually and kept in a desiccator containing silica gel at room temperature for 24 h. The films were weighed again after a specified interval until they show a constant weight. The percent moisture content was calculated using following formula [8].

$$\% \text{ Moisture content} = \frac{\text{Initial weight - Final weight}}{\text{Final weight}} \text{ x } 100$$

Moisture Uptake:

Weighed films were kept in a desiccator at room temperature for 24 h. These were then taken out and exposed to75.3% relative humidity using saturated solution of sodium chloride in a desiccator until a constant weight is achieved. Percent moisture uptake is calculated as given below [8].

$$\% \text{ Moisture uptake} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \text{ x } 100$$

Flatness:

A transdermal film should possess a smooth surface and should not constrict with time. This can be demonstrated with flatness study. For flatness determination, one strip is cut from the centre and two from each side of film. The length of each strip is measured and variation in length is measured by determining percent constriction. Zero percent constriction is equivalent to 100 percent flatness.

$$\% \text{ constriction} = \frac{I_1 - I_2}{I_1} \text{ X } 100$$

I_2 = Final length of each strip

I_1 = Initial length of each strip

Biopharmaceutical evaluation

In vitro release study

The in-vitro release study was done by the Keshary Chien diffusion Cell method. In this method transdermal film was placed in between receptor and donor compartment of the diffusion cell. The transdermal system faces the receptor compartment in which receptor fluid *i.e.,* buffer was placed. The whole assembly was kept on magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred with 600 rpm throughout the experiment using magnetic beads. The temperature of receptor compartment was maintained 37 ± 2°C. At predetermined time intervals, the 5ml receptor fluid was removed for analysis and was replaced with an equal volume of phosphate buffer pH 7.4. The concentration of drug was determined spectrophotometrically at 266 nm wavelength with suitable dilution [10, 11].

In-vitro diffusion study

In-vitro permeation studies were carried out using modified Keshary Chien diffusion cell. The dialysis membrane was previously soaked for 24 hours in distilled water. The film were adhered to the barrier membrane (dialysis membrane, which was dipped in phosphate buffer, pH7.4for over night) and the membrane was tied firmly to the donor compartment of the Keshary Chien diffusion cell, the receptor compartment of which was filled with 57 ml phosphate buffer. The donor compartment was lowered to the receptor compartment in such a way that the dialysis membrane just touches the media of the receptor compartment. The total setup was placed on a magnetic stirrer. The temperature of receptor compartment was maintained 37 ± 2°C. The content of the diffusion cell was stirred using a Teflon coated bead at a constant speed (600 rpm). Samples were withdrawn (5 ml) at predetermined time intervals and replaced with same amount of phosphate buffer to maintain the sink condition. After suitable dilution, the samples were analyzed for drug content using UV-VIS 1800 spectrophotometer (SHIMADZU) at wavelength of 266 nm. The permeation study was carried out for 7 hours [12].

Ex-vivo diffusion study:

Preparation of Skin

Rats were kept in a controlled conditions. They were sacrificed using anaesthetic ether. The hair of test animals was carefully removed and the full thickness of skin was removed from the abdominal region. The epidermis was prepared surgically, washed with distilled water and used for *ex vivo* permeability studies.

Ex vivo Skin Permeation Studies

The *ex vivo* skin permeation studies were carried out using Keshary Chien diffusion cell with a diffusional area of 3.73 cm^2. Rat's abdominal skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The receiver phase was filled 57 ml of phosphate buffer pH 7.4 and stirred at 600 rpm on a magnetic stirrer. The stratum corneum side of the skin was kept in intimate contact with the film and over that placed a backing membrane. The whole assembly was kept in a water bath at 37 ± 0.5 °C. Samples (1 ml) were collected at predetermined time intervals and replaced with fresh buffer. The concentration of drug was determined by U.V. spectrophotometrically at 266 nm with suitable dilution if necessary. Cumulative percentage drug permeated was calculated and plotted against time. Flux was determined directly as the slope of the curve between the steady state values of the amount of drug permeated (mg/ cm^2) v/s time (hours) and permeability coefficients were deduced by dividing the flux by the initial drug load (mg /cm^2) [13]

Stability Study:

The stability study of the formulations was done according to ICH guide line Q1A specifications in stability chamber. We performed the stability study in intermediate & accelerated conditions in closed containers (Table 3). In both cases sampling were done 3 times i.e., 0, 3 & 6 months and performed test for the drug content uniformity.

Table 3:- Stability study conditions

Study	Storage condition	Minimum time period covered by data at submission	Sampling interval
Intermediate	30°C ± 2° /65% RH ± 5%	6 months	0 , 3 & 6 months
Accelerated	40°C ± 2° /75% RH ± 5%	6 months	0 , 3 & 6 months

RESULTS AND DISCUSSION

The transdermal drug delivery system of 5-FU was prepared using the polymers EC, HPMC E15 and, HPMC K4M, using glycerin as a plasticizer by solvent casting technique. The prepared films were evaluated for physicochemical parameters with *in-vitro and ex- vivo* drug permeation studies. The selection of polymer combinations produces clear, smooth, uniform, substantive, flexible and desired thickness film for the transdermal drug delivery systems of 5-FU.

Characteristics of the formulation were studied in *in-vitro* and ex vivo conditions. Both permeation studies were carried out in phosphate buffer (pH 7.4) for 7 hours, in order to find out the diffusion mechanism which predominately influences the drug permeation from the membrane. The drug content uniformity and the mass uniformity of the prepared formulations have shown that the process used to prepare the films in this study was capable of giving films with uniform drug content and the thickness of the films varied from 0.0855±0.005 to 0.134±0.003 mm.

Table 4:- Physico Chemical parameters of the formulations

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Parameters	FF1	FF2	FF3	FF4	FF5	FF6	FF7	FF8
Moisture Content (%)	1.3± 0.83	12.7±0.45	10.7±0.63	9.8±0.57	7± 0.79	6.2±0.57	4.5±1.12	3.56±0.58
Moisture Uptake (%)	1.9 ±0.48	18.78±0.63	15±0.35	9.9±0.65	9±0.42	8.54±0.45	6.8±.57	4.97±0.34
Thickness (mm)	0.134±0.003	0.0855±.0005	0.128±0.008	0.097±.0007	0.105±.001	0.112±.078	0.119±0.002	0.123±.001
Folding Endurance	72±1.67	144.5±3.2	139±2.55	133.56±0.15	123.78±0.94	112.75±0.18	102± 1.01	98±1.14
Wt Variation (%)	1.034±0.002	1.023±0.63	1.29±0.56	1.034±.002	1.036±.007	1.059±.0009	1.027±.065	1.051±.002
Drug Content (%)	98.07	97.98	99.67	96.25	98.01	97.11	98.92	95.60
Flatness	98	100	100	100	100	100	100	100

The percentage moisture uptake and percentage moisture content of the formulations increases with addition of the HPMC with EC. The formulation FF2 with 1:1 EC & HPMC K4M showed highest % of moisture content and the moisture content decreased with increase in the concentration of HPMC E15. This proved that the moisture content increased due to the admixture of HPMC. Between the two grades of HPMC we observed that HPMC K4M showed more hydrophilic nature than HPMC E15.

The folding endurance of the films varied from 72±1.67 to 144.5± 3.2 folds. From the results obtained it was found that the folding endurance decreased with the addition of the HPMC. Again,increased concentration of the HPMC K4M provided higher folding endurance than HPMC E15 with equal amount of EC, this may be due to the higher moisture holding capacity of the polymer K4M.

Now, to understand the release profile, first we made *in-vitro* release study using dialysis membrane. From the Cumulative % release in 7 hr (Table 5), we observed that the formulation FF1 showed very slow release of 56.76% in 7hr. The FF2 showed highest % release of 96.53 %. The FF1 showed lowest % release may be due to the release retarding effect of the EC and FF2 showed 96.53 % release due to the combination of HPMC K4M & EC. Again, if we study the formulation FF3 which is 1:1 combination of EC & HPMC E15 we observe that it released the formulation slower than FF2 but higher than the rate of FF1and for the formulations of FF4 to FF8 we prepared the formulations in combination of three polymer EC, HPMC K4M with HPMC E15 and we observed as the concentration of HPMC E15 increased with a decrease in concentration of HPMC K4M the % release of the drug through dialysis membrane decreased accordingly. That means in between two grades of HPMC, E15 provides more sustained release than K4M.

Table 5:- *In-vitro* percentage cumulative permeation of 5-Fluorouracil through Dialysis membrane from the formulations in 5 hr

Formulation code	FF1	FF2	FF3	FF4	FF5	FF6	FF7	FF8
Cumulative% Release	56.76	96.53	63.58	90.41	83.89	76.29	73.15	69.10

To understand the nature of release we fitted the drug release data to Zero order, First order & Higuchi model and tabulated the R^2 Value in each case.

We observed that all formulation showed highest linearity to the Higuchi model.

Table 6: - Regression co-efficient of the model equations on the *in -vitro* diffusion kinetics

Order of reaction	FF1	FF2	FF3	FF4	FF5	FF6	FF7	FF8
Zero Order	0.957	0.53	0.887	0.8283	0.8208	0.8642	0.8843	0.9476
First Order	0.996	0.948	0.988	0.9867	0.9648	0.9713	0.9725	0.99
Higuchi plot	0.952	0.956	0.991	0.9823	0.9782	0.9912	0.993	0.994

From the cumulative % release data we discarded FF1, FF2 and FF4 for further study. FF1 was discarded because it provided very slow release .FF2 & FF 4, the release rate was faster which is undesirable in case of a transdermal film formulation. So we had selected FF3, FF5, FF6, and FF7& FF8 for *ex-vivo* skin permeation study.

***Ex-vivo* Skin Permeation Studies**

From the result of the *ex-vivo* permeation study (table 7)we observed that release was slower through the rat skin than the dialysis membrane, this is probably because of the nature of compact rigidity of the rat skin structure but the order is same as observed in *in-vitro* release that is FF5>FF6>FF7>FF8>FF3.

Table 7 : *Ex-vivo* Percentage cumulative permeation & flux of 5-Fluorouracil through the Rat skin from the formulations in 7 hr

Formulation code	FF3	FF5	FF6	FF7	FF8
Cumulative% Release	56.56	77.24	71.93	63.72	60.14
Flux	0.0292	0.0274	0.0258	0.0229	0.0219

To understand the nature of release we fitted the release profile to zero order, first order and Higuchi equation and tabulated the R^2value (table 8)[14]. From the table we observed that all the formulations showed highest linearity to the Higuchi equation.

Table 8: Regression co-efficient of the model equations on the e*x-vivo* diffusion kinetics

Formulation Code	FF3	FF5	FF6	FF7	FF8
Zero Order	0.8870	0.7690	0.8120	0.8460	0.8600
First Order	0.9880	0.9810	0.984	0.9755	0.9860
Higuchi plot	0.9910	0.9925	0.9954	0.9850	0.9950

Table 9: Table of 'n' values of Korsmeyer-Peppas model of the *Ex-vivo release* of the drug through Rat Skin

Korsmeyer- Peppa's	FF3	FF5	FF6	FF7	FF8
'n' value	0.582	0.512	0.5172	0.5139	0.6052

To understand the release mechanism we fitted the R^2 values to the Korsmeyer-Peppas Model to find out the 'n' values (table 9).

Then we observed that all formulations showed non-fickian release mechanism. Therefore, the release of drug 5-FU takes place by diffusion and polymeric chain erosion.

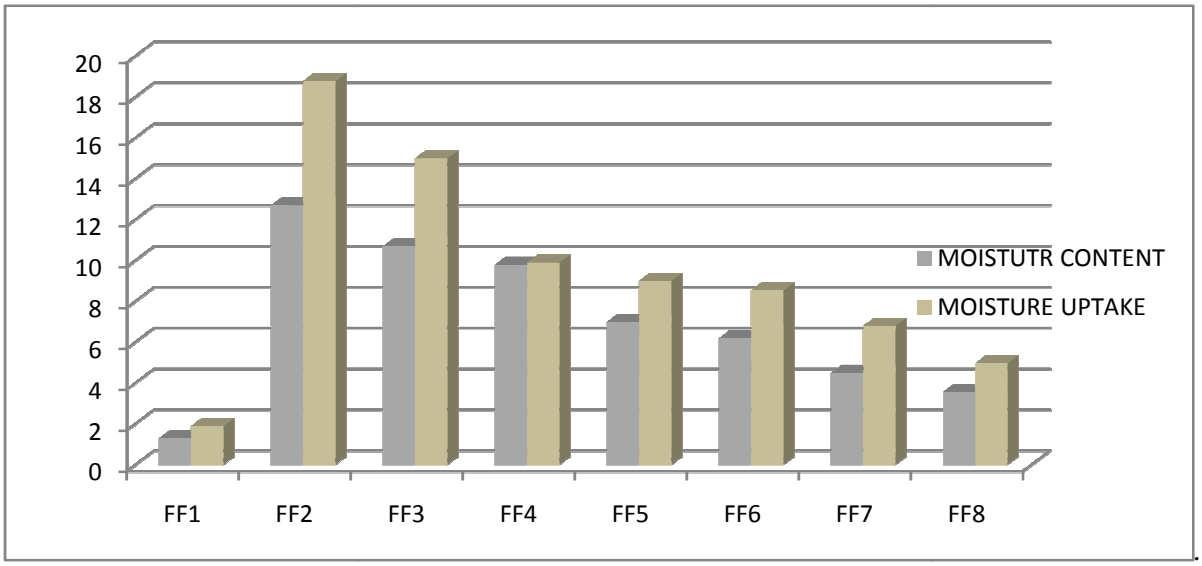

Fig 1:-Graphical representation of the moisture content and moisture uptake of the formulations

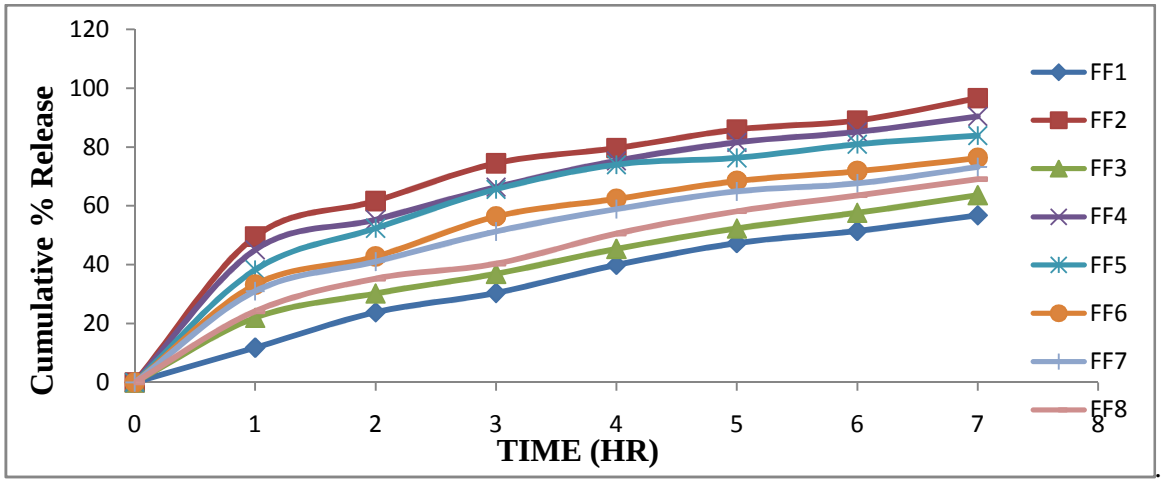

Fig 2:- *In-vitro* cumulative % release vs time (hr) graph of the formulations through dialysis membrane in 7hr

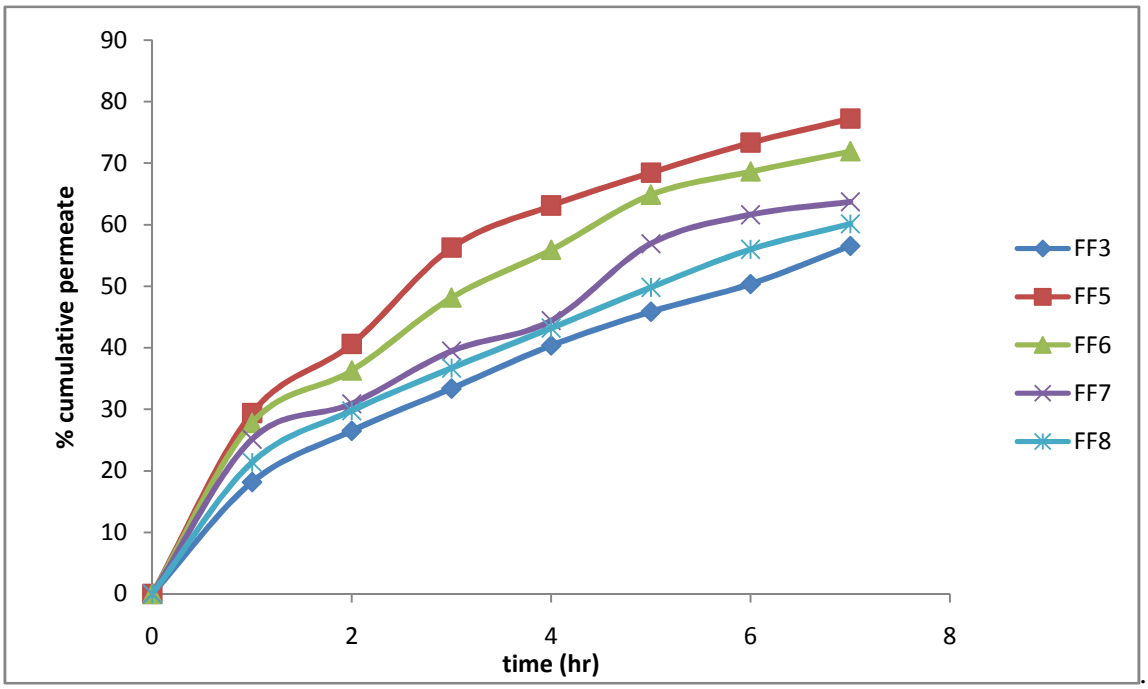

Fig 3:- Zero order plot of the optimized formulations through Rat skin in 7hr

Table 10- Table of stability study data of the formulations at two different conditions.

Code	% Drug content	
	Intermediate condition	Accelarated condition
FF1	99.67	98.40
FF2	99.10	98.30
FF3	98.07	98.12
FF4	98.05	97.98
FF5	98.04	97.98
FF6	98.00	97.90

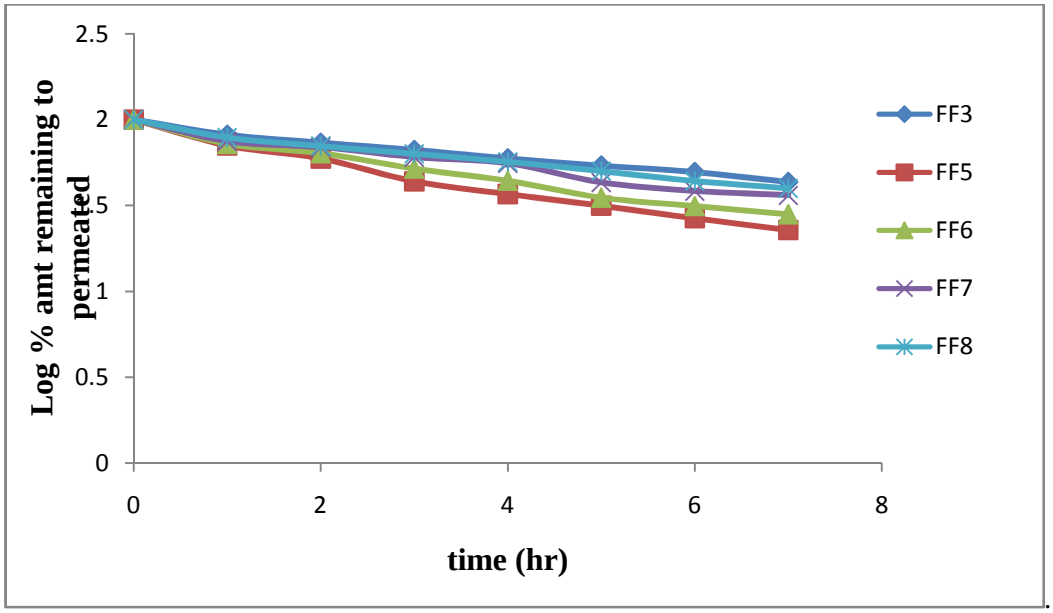

Fig 4:- First order plot of the optimized formulations through Rat skin in 7hr

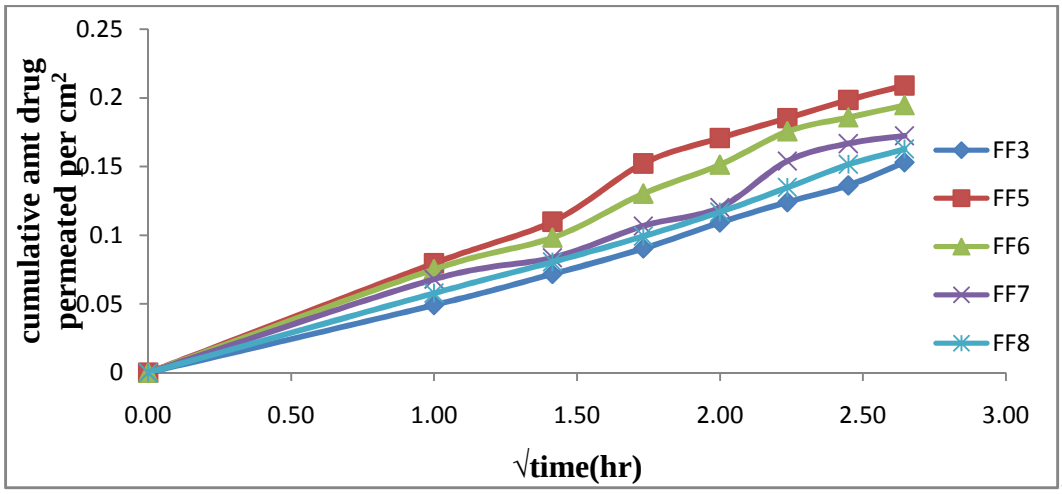

Fig 5:- Higuchi plot of the optimized formulations through Rat skin in 7hr

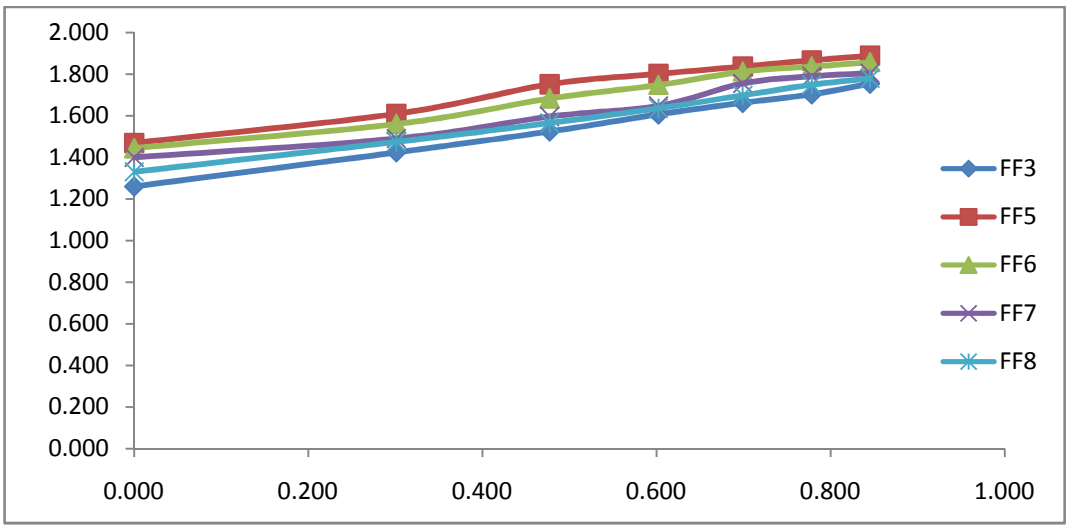

Fig 6:- Korsmeyer Peppas plot of the optimized formulations through Rat skin in 7hr

CONCLUSION

From the above study we can conclude that the transdermal film of 5- FU can be produced using EC, HPMC K4M, and HPMC E15. The formulations showed that between two grades of the HPMC, HPMC E15 showed more sustained action than the HPMC K4M. Further, the formulation with only EC showed release with very slow rate & only HPMC is incapable to form the film. From the stability study data we can see that the formulations are stable in accelerated as well as intermediate conditions. Hence, transdermal film of 5-FU thus formulated could be a promising alternative dosage form in cancer chemotherapy.

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