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DESIGN AND DEVELOPMENT OF HYDROXYPROPYL METHYLCELLULOSE (HPMC) BASED POLYMERIC FILM OF ENALAPRIL MALEATE

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ABSTRACT: The present investigation was aimed to evaluate the possibility of using different concentrations and polymeric grades of hydroxypropyl methylcellulose (K4M, K15M and K100M) for the development of transdermal delivery of enalapril maleate, an antihypertensive drug. Matrix films were evaluated for their physicochemical characterization followed by *in vitro* evaluation. The Thickness and weight of patch increase with the increase in polymeric grade and content. Fourier transforms infrared spectroscopy and differential scanning calorimetry results confirmed that there is no interaction between drug and polymer used. The *in vitro* drug release followed Higuchi kinetics($r = o.9852$: $P < 0.001$) as its coefficient of correlation values predominates over first order kinetics. The *in vitro* Dissolution profiles by using rat skin and human skin showed significant difference. Comparison of skin permeation rate between hairless rat and human cadaver skin was done by using valia-chien glass diffusion cells. Surface topography was studied by scanning electron microscopy. A quantitatively good correlation was found between percent of drug absorbed from the transdermal patches. Out of the various formulations made, the selected formulations are better in their *in vitro* Dissolution thus holds potential for transdermal delivery.

Keywords: Transdermal drug delivery, Enalapril Maleate, Franz diffusion cell, Acetone, Hydroxypropyl Methylcellulose.

INTRODUCTION

The benefits of using transdermal drug delivery include improved systemic bioavailability resulting from bypassing the first hepatic metabolism.Varibles due to oral administration, such as pH, the presence of food or enzymes, and transit times can all be eliminated. The aim in the development of new transdermal drug delivery devices is to obtain a controlled, predictable, and reproducible release of the drug into blood stream of the patient .the transdermal device act as a drug reservoir and controls the rate of drug transfer. when the transdermal drug flux is controlled by the device instead of by the skin ,delivery of the drug is more reproducible ,leading to smaller inter and intrasubject variations because the drug release from the device can be controlled accurately than the permeability of the the skin.

The angiotensin converting enzyme inhibitors have become the first line therapy in treating hypertensive patients. The advantage of ACE inhibitors over other antihypertensive medication includes preventing coronary heart failure ,renal failure of type _2diabetic patients and etc.Most ACE inhibitors are bipeptides that are too hydrophilic to dissolve and penetrate through the lipid layers.Enalapril was selected among the ACE inhibitors due to molecular size ,therapeutic dosage ,and the overall lipophilicity of the drug molecules.prodrug of enalapril is also exhibited a significantly higher transdermal penetration rate.

An appropriate selection of the polymer matrix is necessary in order to develop successful transdermal drug delivery system .the objective of the present study was to design and develop a transdermal patch of enalapril maleate employing varying concentrations of HPMC K4M, K15M, K100M with plasticizer glycerin.

MATERIAL AND METHOD

Materials:

Enalapril Maleate as gift sample was obtained from Wokchardt pharma limited Aurangabad, India. And

hydroxypropyl methylcellulose (HPMC) K4M, K15M, K100M were gift from microlabs pvt .limited Bangalore, India and All other chemicals and reagents used in the study were of analytical grade.

Animals and Human Cadaver Skin Used in the Research Work:
Rats of Wistar strain were collected from the Animal house, The Nandha College of Pharmacy for the present studies.
All experimentation was carried out according to the Institutional Animal Ethical Committee (IAEC).
Human cadaver skin was obtained as a gift sample from the Government Hospital, Erode.

Preparation of transdermal films [2]:
The solvent casting technique was used to formulate the HPMC patches containing different grades of HPMC .K4M, K15M, K100M using varying concentrations individually, keeping drug concentration constant. The drug polymer ratios used were 1:2, 1:3, and 1:4. the required amount of drug :polymer ratios were dispersed separately in a casting solvent (acetone: distilled water in 9:1 ratio)and polymeric dispersion was sonicated for 2 minutes ,to remove entrapped air bubbles .These two were then mixed and glycerin (150% w/w of polymer weight) was incorporated as plasticizer .The polymeric dispersion was poured into a glass mould(5cm×5cm) fabricated in the laboratory . To control the rate of evaporation of solvent, the mould was covered with a funnel of suitable size and the casting solvent was allowed to evaporate overnight to obtain the dried films .The films were cut into small patches containing equivalent of 5mg of the drug per patch .backing membrane was then glued and the films were stored between sheets of wax paper in desiccator until further evaluations.

characterization of transdermal patches:
Physical appearance:
All the transdermal patches were visually inspected for color, clarity, flexibility and smoothness

Film folding endurance [3]:
This was determined by repeatedly folding the patches until it shows any crack or brake. The number of times the film could be folded without breaking/cracking gave the value of folding endurance. Five randomly selected patches for e ach formulation were tested.

Thickness [4]:
The thickness of each patch was measured at the different sites using screw gauge and the average thickness was calculated. Percentage deviation from mean thickness was determined.

Uniformity of weight [4]:
The film was cut into 10 patches of 1cm^{2}each and their average weight was calculated. Percentage deviation from average weight for each patch was also determined.

Moisture content [5]:
The film was weighed and kept in a desiccators containing calcium chloride at 40^{o}C in a drier for at least 24 h or more until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight and was reported in terms of percentage (by weight) moisture content**.**

Accelerated stability study [6,7]:
The optimized patches were subjected to stability studies to evaluate any change in the performance when exposed to accelerated conditions of environment during storage, handling transport and use. The patches were packed in the aluminum foil and kept at 40± 2^{o}C and 75 ±5% RH as per ICH guidelines. **(ICH Q1A [R2] Stability testing new drugs substances and products)**

Fourier transform infrared spectroscopic studies:
A IR prestige -21 FTIR (shimadzu, Japan) spectrometer equipped with attenuated total reflectance (ATR) accessory was used to obtain the infrared spectra of drug matrix as well as placebo films. Analysis of pure drug ,polymers and their physical mixture (1:1 ratio) was carried out using FTIR all the powder samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture .

Differential scanning calorimetric studies:
Thermal analysis was carried out using differential scanning calorimeter (Q10, TA instruments, waters Inc., USA) with a liquid nitrogen cooling accessory. The analysis was performed under purge of dry nitrogen gas (50 cc min^{-1}).high purity indium was used to calibrate the heat flow and heat capacity of the instrument .Sample (2.5-5mg) placed in the aluminum crucible cell was firmly crimped with the lid to provide an adequate seal .The sample was heated at ambient temperature to 25°C at preprogrammed heating rate of 10°C min^{-1}.
All the samples were analyzed in the same manner, in case of two components (150µm) in equal weight ratios were prepared in a glass mortar and pestle.

Evaluation of transdermal patches:
Skin irritation test [8]:
The optimized transdermal formulation was evaluated for skin irritation studies on 12 rats (grouped in 2 and

each group having 6 rats). The hairs of the dorsal portion were removed physically with the help of sharp surgical scissors and the skin was washed properly one day prior to use. Group one was supplied with control formulation and group second was supplied with medicated formulation.
Medicated formulation was secured on experimental side using an adhesive tape and non-medicated patch was adhered on the control side of rats. These were covered with occlusive covering to approximate the condition of use. The patches were removed after 7days and each of the area was observed for any sign of erythema or edema.

Drug content [4]:
An accurately cut patch of 1cm^2 area was taken and added to a beaker containing 1 ml phosphate buffer solution of pH 7.2 The beaker was kept 24 hours with occasion shaking. The sample was analyzed drug content using UV spectrophotometer 207nm.This study was performed for 3 times for a single patch.

Permeation studies across Rat abdominal skin [4,10]:
The rat abdominal skin was excised. The hairy and underlying tissue was removed. The membrane was washed thoroughly with distilled water and saline solution. It was soaked in the saline solution overnight. It was washed several times before use. The rat skin was then cut into appropriate size and mounted at the junction between donor and receptor chamber of diffusion. The matrix formulation to be tested was cut into 1 cm^2 patches and was placed over the optimized skin. It was then covered with aluminum foil as the occlusive backing. The donor compartment was clamped over it. With the help of springs, making sure that there were no bubbles in the receptor compartment. The whole system was sandwiched between the donor and the receptor compartments and secured with a clamp, with the receptor compartment containing phosphate buffer solution of pH 7.2.The agitation speed of 50 rpm and temperature of 37±1^{0}C were maintained during the experiment. Samples of 3 ml were withdrawn at predetermined time interval upto 24 hour. The samples were then analyzed for drug content using UV double beam spectrophotometer at 207 nm.

Preparation of Cadaver Skin [11,12]:
Freshly excised human cadaver epidermis (from the chest region of a male cadaver of age 46 years within 24–48 hr post-mortem) isolated by heat separation method was used as the barrier (Berner et al., 1989). In brief, the full-thickness (thickness 0.2 ± 0.03 cm) skin was exposed to 60 ^{o}C water for 80 s and the epidermis was peeled away from the dermis. The sample was cut of appropriate size and kept in saline solution overnight, the following day it was washed several times before use

Permeation studies across Cadaver Skin [11,13]:
The matrix formulation to be tested was cut into 1 cm^2 patch and was placed over the skin. The patch was placed on the stratum corneum side of the epidermis and slight pressure was applied to adhere the patch on the surface uniformly. The whole system was sandwiched between the donor and the receptor compartments and secured with a clamp, with the receptor compartment containing phosphate buffer solution of pH 7.2.The agitation speed of 50 rpm and temperature of 37±1^{0}C were maintained during the experiment. .Samples of 3ml was withdrawn at predetermined time intervals. The samples were then for drug content analyzed using UV double beam spectrophotometer at 207 nm.

***In vitro* dissolution studies [2]:**
The *in vitro* dissolution study of each selected transdermal patch was determined on USP dissolution apparatus equipped with fractional collector (TDT-08L, Electrolab, India)
A Cygnus ´ sandwich patch holder ,a slightly modified form of FDA´S sandwich patch holder was used to ensure patch to patch reproducibility of trandermal film the dissolution vessel contained 500ml of phosphate buffer 7.2 pH maintained at 32±0.5ºC(the skin surface temperature) paddle speed set at 50 rpm. Patch assembly was carefully placed at the bottom of the vessel and was centered using a glass rod. 5 ml sample was withdrawn at 1hour time intervals until completion of drug release .The withdrawn sample was replaced with 5ml of fresh media

The withdrawn samples were analyzed for drug content by measuring absorbance at 207 nm using UV-visible spectrophotometer (UV-1700 pharmaSpec, shimadzu, Japan).
The three such determinations were carried out for each formulation. The content of enalapril meleate was calculated from the standard curve. The *in vitro* dissolution profiles, namely, cumulative drug release, dissolution rate constant, dissolution half life
($t_{50\%}$) were calculated.

In *vitro drug* release studies [9]:
The *in vitro* evaluation was carried out in the modified Franz diffusion cell.

Design of Modified Franz Diffusion Cell

Franz diffusion cell consists of an upper donor compartment and the lower receptor compartment, surrounded by water jacket for circulation of water to maintain the temperature inside at 32 ± 1^{0}C.The uniformity of solution in the receptor phase was maintained by stirring at high speed of 100 rpm (approx) using a tiny magnetic bead the volume of receptor compartment was maintained at 60 ml and the diffusional surface are of 0.785 cm^2.The receptor compartment was provided with the sampling port on

one side, to withdraw sample at the predetermined time intervals for estimation of drug content by UV spectrophotometer.

Experimental conditions

The receptor medium was phosphate buffer solution of pH 7.2, temperature of the receptor medium was maintained at 32 ± 1^0C, throughout the experiment using water jacket. The donor compartment was in contact with ambient condition of atmosphere.

Scanning electron microscopy:

SEM was done for the Enalapril maleate film and cadaver skin (after permeation studies) to assess the surface topography of the formation. The sample were deposited on the aluminum hold and sputtered with gold. The samples were further coated uniformly with gold after fixing them individually. The SEM photography was taken on LEO 435 VP SE at the required magnification at room temperature. A working distance of about 11 mm was maintained and the accelerated voltage used was 5KV with secondary electron image as detector.

RESULTS AND DISCUSSION

Matrix dispersion type transdermal films of enalapril maleate were prepared using varying ratio of drug: polymer (HPMC K4M, K15M, and K100M) to get the desired drug release profile. The results for skin irritation study were shown in fig- 1 and fig-2. The thickness, weight variation and drug content values of the formulation made are shown in table-2.Irrespective of the grade and concentration of the HPMC used, the drug content per patch was found within 4.894 to 4.970mg per patch but the thickness and weight of the patch increased with the increase in polymer content .Folding endurance values of matrix films found more than 250 indicating good strength and elasticity, which is explained by the linear nature of the cellulose structure. The surface pH of all formulations was in the range of 5.5-6.0, the pH range of skin and hence no skin irritation was expected. After the comparison of skin permeation rate of enalapril maleate between hairless rat and human cadaver skin it showed that permeation rate was more in rat skin which is shown in table-3 and fig-3.Amongst the various formulations made, using different concentration and grades of HPMC, formulation (F9) was selected on basis of drug content and release pattern.

***In Vitro* Dissolution:**

From the in vitro dissolution profile data, kinetics of drug release was found for zero order (k_o), first order (k_1) and higuchi type (k_h) release kinetics.korsmeyer-peppas semi empirical model were also employed, in order to better characterize the drug release behavior.

$M_t/M_\infty = k\, t^n$

Where M_t/M_∞ is the fractional release of drug in time t, k is constant incorporating structural an geometric characteristics of the controlled release device, and n (the release exponent) is a parameter indicative of mechanism of drug release.

The coefficient of correlation of each of these release kinetics were calculated and compared (Table.4). The data revealed that the release pattern of formulations are best fitted for Higuchi kinetics equation as the formulation coefficient of correlation values predominates over zero order and first order release kinetics. This complies with higuchi's equation for drug release from a matrix; a slow and controlled release was observed, indicating that the drug release mechanism was by diffusion, as proposed by higuchi. Based on korsmeyer-peppas semi-empirical model, the best fitting was obtained with $n \leq 0.5$, indicating a fickian release mechanism. In swellable systems, factor affecting the release kinetics are liquid diffusion rate and polymeric chain relaxation rate. When the liquid diffusion rate is slower than the relaxation rate of the polymeric chains, the diffusion is Fickian: whereas when the relaxation process is very slow as compared to the diffusion, the case II transport occurs on the basis of these consideration it is clear that patches released the drug by diffusion-dominated mechanism.

The observed initial burst release in formulations maybe accounted to the direct exposure of the matrix films to the dissolution media. Initial burst release was higher in matrix films, formulated using low viscosity grade polymer (HPMCK4M), compared to higher viscosity grade polymer (HPMC K15M and K100M). Due to initial burst effects, low dissolution half life ($t_{50\%}$) was found in case of transdermal films formulated with HPMC K4M . $t_{50\%}$ values increases linearly with increase in concentration and viscosity grade of polymer.

In vitro drug release study showed that the maximum drug release of prepared formulation was found to be 108.13% upto 24 hours which is shown in fig.4

The surface topography of the formulations was studied by scanning electron microscopy which is shown in fig 5 and fig 6.

TABLE.1 FORMULATION CODE FOR THE POLYMER MATRIX

SR.NO	COMPOSITION	
	ENALAPRIL+ HPMC GRADES	**ACETONE: WATER(9:1)**
	ENP+HPMC(K4M)	
F1	1:2	10
F2	1:3	10
F3	1:4	10
	ENP+HPMC(K15M)	
F4	1:2	10
F5	1:3	10
F6	1:4	10
	ENP+HPMC(K100M)	
F7	1:2	10
F8	1:3	10
F9	1:4	10

TABLE 2: PHYSICAL CHARACTERISTICS OF ENALAPRIL MALEATE TRANSDERMAL PATCHES

Sr. No	Composition	Thickness(mg)	Weight(mg)	Content(mg)
	ENP:K4M			
1	1:2	6.24	36.65	4.932
2	1:3	6.42	45.42	4.970
3	1:4	110.12	55.85	4.964
	ENP:K15M			
4	1:2	68.50		
5	1:3	112.60	39.56	4.908
6	1:4	156.81	47.51	4.916
	ENP:K100M		59.27	4.984
7	1:2	78.90		
8	1:3	136.80		
9	1:4	181.90	40.82	4.926
			48.35	4.948
			62.32	4.968

TABLE 3: COMPARATIVE % CUMULATIVE DRUG RELEASE FOR OF F9 FORMULATION IN CADAVER SKIN AND RAT SKIN

Sr.No	Time in hrs	Cadaver skin	Rat skin
1	1	15.813166	28.16
2	3	25.895502	39.42
3	5	35.461314	46.57
4	8	49.771075	59.62
5	12	58.768420	68.23
6	14	72.082644	78.46
7	18	80.967450	82.64
8	22	92.908122	98.64
9	24	100.771420	108.13

TABLE 4: DISSOLUTION CHARACTERISTICS OF THE ENALAPRIL MALEATE FROM DIFFERENT TRANSDERMAL FILMS

Sr.No	Formulation	Zero order		First order		Higuchi matrix		K-P	
		$K_o(h^{-1})$	r	$K_1(h^{-1})$	r	$K_H(h^{-1/2})$	r	n	$t_{50\%}$
	ENP:K4M								
1	1:2	11.186	0.755	0.886	0.931	22.238	0.972	0.324	0.52
2	1:3	10.485	0.682	0.742	0.956	20.210	0.956	0.332	0.81
3	1:4	09.214	0.838	0.581	0.948	19.712	0.981	0.341	1.12
	ENP:K15M								
4	1:2	09.143	0.811	0.757	0.942	19.587	0.977	0.416	0.90
5	1:3	07.887	0.828	0.645	0.958	18.185	0.985	0.427	1.23
6	1:4	07.182	0.861	0.505	0.963	17.757	0.989	0.432	1.26
	ENP:K100M								
7	1:2	07.831	0.828	0.724	0.961	18.011	0.992	0.430	1.32
8	1:3	06.938	0.844	0.617	0.968	17.017	0.991	0.441	1.45
9	1:4	06.165	0.851	0.458	0.968	15.940	0.984	0.450	1.86

Values in the parentheses indicate $K_{o=}$ zero order; $K_{1=}$first order; and $K_{H=}$ higuchi type dissolution rate constant-p korsmeyer-peppas exponential (n) values

SKIN IRRITATION STUDIES

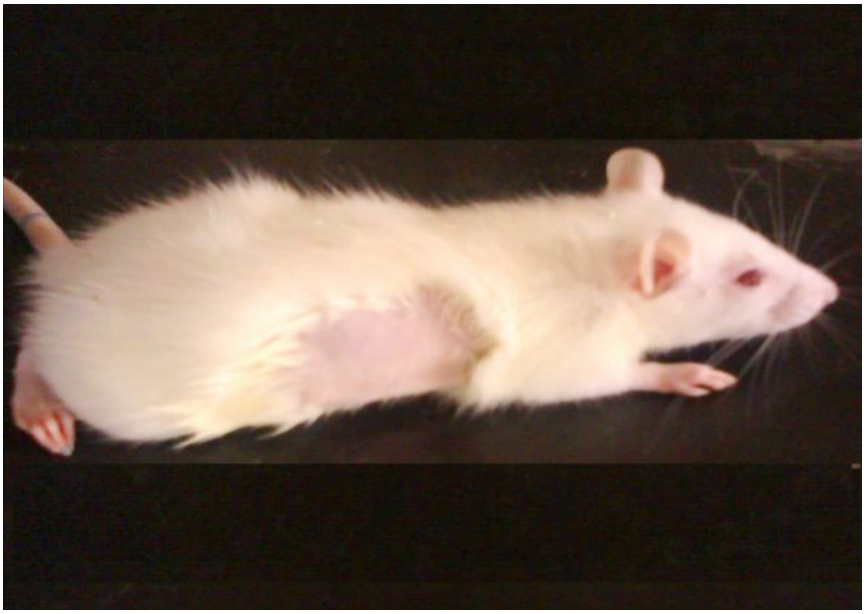

Fig - 1: Photo grap of rat before application of patch

Fig-2: Photo grap of rat after removal of patch

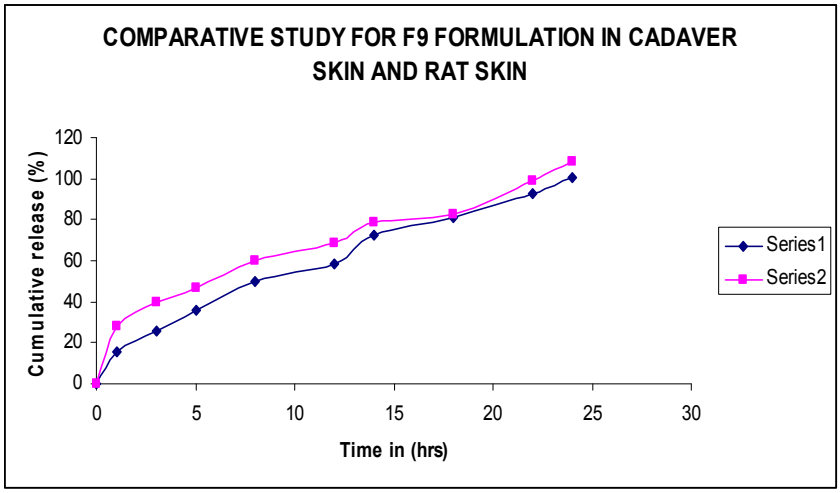

Fig.3: Comparative % Cumulative Drug Release for of F-9 Formulation in Cadaver skin and Rat skin

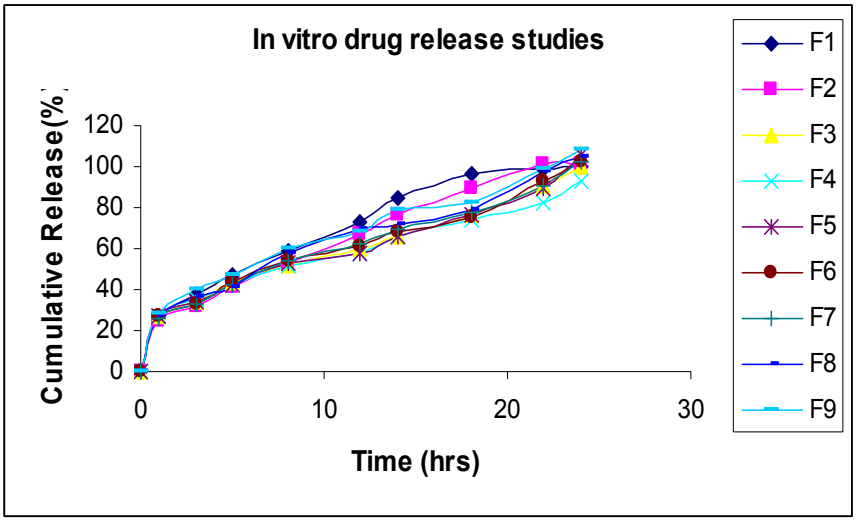

Fig 4: In Vitro Drug Release Studies of Prepared Formulation

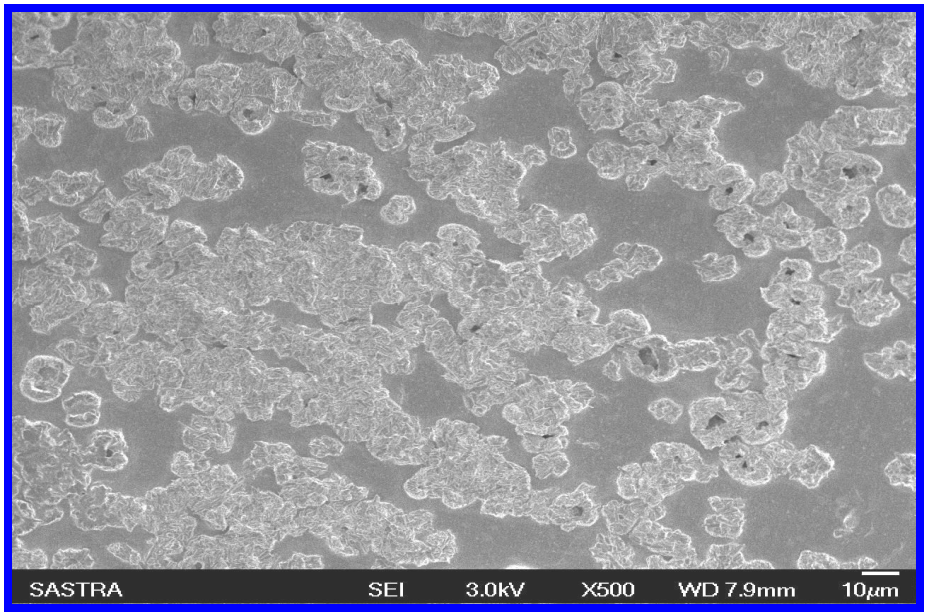

Fig.- 5:Shows the SEM photograph of HPMC(K100M) +Enalapril Maleate films

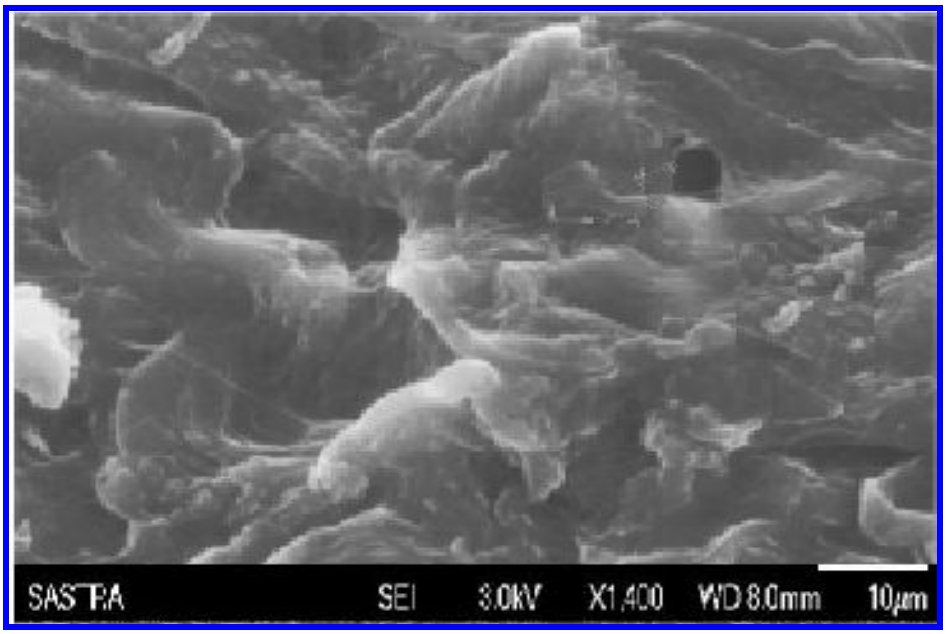

Fig.- 6: Shows the SEM photograph of cadaver skin after permeation studies

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