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Transdermal Patch of Timolol Maleate: Formulation and Evaluation

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Abstract

Transdermal drug delivery system can be described as desecrate and independent type of dosage form deliver the drug by controlled manner when applied to skin. Patches of Timolol Maleate was prepared by solvent casting technique. Timolol maleate is an antihypertensive drug having λ_{max} 294 nm. Solid lipid nanoparticles (SLN) of Timolol maleate was prepared using microwave assisted microencapsulation technique by using stearic acid, tween 20 and PEG 400. Transdermal patches were prepared using different grades of HPMC i.e. HPMC K4M, HPMC K15M, HPMC 50 cps HPMC K100M and cellulose acetate and different percentage of glycerine. Final formulation prepared by HPMC 50 cps. Drug diffusion study performed by using modified KesharyChien Diffusion Cell. Rat skin used as membrane for diffusion. Prepared patches examined for skin irritation and antihypertensive study. Anti- hypertensive activity performed by using BioPack instrument for blood pressure measurement. Stability studies performed at 40°C/75% RH, and 25°C/60% RH conditions. The physical stability of Timolol Maleate based transdermal patches proved unchanged after storage up to 3 months. Final prepared patch having good tensile strength, folding endurance, weight variation and drug diffusion as 91.75% in a period of 24 hrs.

Keywords: Patch, SLN, drug diffusion, anti-hypertensive, Keshary Chien Diffusion Cell**.**

Introduction

The development of a novel delivery system for existing drug molecules not only improves the drugs performance in terms of efficacy and safety but also improves patient compliance and overall therapeutic benefit to a significant extent. Consequently, much effort has been put into the development of strategies that could improve the patient compliance with new modes of application[1]. A drug is administered to eliminate a diseased state in living system to achieve this objective; it should be available in the system at a certain minimal concentration for a specified period of time. During the past 20 years, advances in drug formulations and innovative routes of administration have been made.

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Our understanding of drug transport across tissues has increased. While topical products or drug delivery systems have been used for centuries for the treatment of local skin disorders, the use of the skin as a route for systemic drug delivery is of relatively recent origin. These changes have often resulted in improved patient adherence to the therapeutic regimen and pharmacologic response [2]. The administration of drugs by transdermal route offers the advantage of being relatively painless. The appeal of using the skin as a portal of drug entry lies in ease of access, its huge surface area, and systemic access through underlying circulatory and lymphatic networks and the noninvasive nature of drug delivery. Delivery of drugs through the skin for systemic effect, called transdermal delivery was first used in1981, when Ciba- Geigy marketed Transderm V **(**present day marketed as Transderm Scop**)** to prevent the nausea and vomiting associated with motion sickness.

Throughout the past 2 decades, the transdermal patch has become a proven technology that offers a variety of significant clinical benefits over other dosage forms. It constitutes a new trend in controlled delivery system and has opened new scientific horizon in innovations[3]. The delivery of drugs transdermally **(**through the skin**)** provides several important advantages over traditional oral and intravenous delivery routes. Transdermally delivered drugs avoid the risk and inconvenience of intravenous therapy, usually provide less chance of an overdose or underdose, allow easy termination, and permit both local and systemic treatment effects.

Currently there are 11 drug molecules: fentanyl, nitroglycerin, estradiol, ethinyl estradiol, norethindrone acetate, testosterone, clonidine, nicotine, lidocaine, prilocaine, and scopolamine available on the United State market. Two new, recently approved transdermal patch products **(**a contraceptive patch containing ethinyl estradiol and norelgestromin, and a patch to treat overactive bladder, containing oxybutynin**)** should help to expand the US transdermal market. Hypertension is a common disorder that, if not effectively treated results in a greatly increased probability of coronary thrombosis, strokes and renal failure. Until about 1950, there was no effective treatment, and the development of antihypertensive drugs, which restore healthy life expectancy, has been a major therapeutic success story. Physiology mechanisms that control arterial blood pressure and shows sites at which antihypertensive drugs act. The main systems include the sympathetic nervous system, the renin-angiotensin-aldostrone system and tonically active endothelium-derived autocoids[4].

Carvedilol competitively blocks a1 b1b2 adrenoreceptors and widely used in the management of Hypertension. The low bioavailability (about 20%) due to extensive hepatic first pass metabolism associated with oral route can be avoided by transdermal administration. The drug has a short half life and hence requires more frequent dosing by the oral route. A prolonged duration of action can be possible with a single application of transdermal film. The objective of the proposed study is to formulate transdermal films of Carvedilol and to study the release kinetics of drug with a view to prevent its first pass metabolism and to achieve a controlled drug release with improved bioavailabiity and better patient compliance.

Material and method

Timolol Maleate (TM) was generously gifted by Gangwal Pharmaceuticals, Mumbai, India. Stearic acid was gift sample of Chandri Wax Specialities Private Limited, Mumbai. Tween 20 and PEG 400 was purchased from Fine Chemicals, Canada. HPMCK 100M was gifted by colorcon Asia Pvt. Ltd., All aqueous solutions were prepared using Milli Q/Elix water (Millipore, Moscheim Cedex, France). All other chemicals used were of analytical grade.

Preformulation study

The active material Timolol Maleate (TM) was generously gifted by Gangwal Pharmaceuticals, Mumbai, India and was observed for physical appearance. The absorbance maxima was determined by using UV-Visible Spectrophotometer. Melting point of Timolol maleate was determined by the capillary method using digital melting point apparatus.The calibration curve was prepared in pH 6.4 and 1% Tween 20. It was prepared to carry out the drug release medium in the dissolution study as it mimics the transdermal condition of the body. For the preparation of standard, plot of TM in pH 6.4 and 1% Tween 20 used. In this, the drug was firstly solubilized in 1% Tween 20 and after complete solubilized the volume was made up to 100 ml with buffer solution pH 6.4 in 100 ml volumetric flask to yield the solution of concentration 100 µg/ml. From the above standard stock solution, further aliquots were diluted to get the working standard solutions i.e. 0, 2, 4, 6, 8, 10 µg/ml were made in triplicate to prepare a calibration plot of the drug in pH 6.4 and 1% Tween 20 for drug release study. The absorbance of further aliquots was taken at λ_{max} 294 nm[5]

Formulation of Solid-Lipid Nano Particle of Timolol Maleate

Preparation of Drug Loaded SLN of TM

The SLNs were prepared by a novel microwave-assisted micro-emulsion technique using Discover SP – Microwave Synthesizer (model No.: 909155) with explorer SP (model no. 909505) and 10 ml reaction vials were used to carry out present reactions. The microwave conditions were set during the preparation of SLNs using Synergy™ software. The "Fixed Power Control" method was chosen for preparation of SLNs. This option allows for control and monitoring of the microwave conditions during the preparation of SLNs. The fixed power control option applies the desired maximum power until the temperature set point is reached after which the feedback loop modulates the amount of power to maintain the temperature set point. Accurately weighed quantities of ingredients (i.e. lipid, surfactant and water) were taken in a 30 mL thick walled Pyrex reactor tube, tightly sealed with a septa cap and heated in the microwave system with constant stirring. Here Stearic acid were used for preparing SLN. Tween 20 and PEG 400 was used as a surfactant and cosurfactant

[6]. The microwave temperature set point was 80 - 85ºC (above the melting point of stearic acid) with microwave power not exceeding 20 W. A continuous supply of cooling gas (nitrogen) and the self-tuning capability in the microwave cavity maintained the reactor tube at the temperature set point of 80 - 85ºC for 10 min. The software allows the user to release the reaction product at elevated temperatures. The release temperature was set above the melting point of stearic acid. This process constitutes a single-pot synthesis of an o/w micro-emulsion, which is then cooled to form an SLN. The prepared and optimized TM loaded SLN was subjected for morphological study using optical microscopy. The optimized formulation was evaluated for particle size and size distribution using the Malvern Zetasizer The sample was diluted 100 times with the double distilled water before analysis and stirred as well. Then, the sample was poured into the sample holder and analysed at 25°C temperature and 90°C scattering angle[7].

Percent entrapment efficiency of TM in the optimized SLN was carried out by dispersing the weighed quantity of the formulation in buffer pH of 6.4 (100.0 mL) [8]. Initially, the SLNs were dispersed in buffer pH of 6.4 followed with placing overnight undisturbed. Then, it was completely stirred (15 mins) followed with sonication (probe ultrasonication) to extract the drug. Insoluble matters (if any) were centrifuged at 5500 rpm for 15 mins [9]. The supernatant (0.5 mL) was withdrawn followed with membrane filtering (membrane filter, Millipore Mumbai, India) and diluted with buffer pH of 6.4 (2.5 mL) before assessment. Sample was then analysed using UV-Vis spectrophotometer at λ_{max} (294 nm). The assessment was carried out in triplicate to find mean value. The entrapment efficiency of the drug in the formulations was calculated using the reported formula, % Entrapment efficiency =

(Total amount of drug – free drug in supernatant) / Total amount of drug.

Preparation of SLNs Transdermal Patches

Transdermal patches containing Timolol Maleate were prepared by solvent casting technique employing mercury as substrate. Different formulations were formulated using different grades of HPMC i.e. HPMC K4M, HPMC K15M, HPMC 50 cps HPMC K100M and cellulose acetate, different percentage of glycerine and 100mg of optimized nanoparticles. The prepared solution was poured into glass petri dishes of 25 cm^2 area and dried at room temperature. After 12 h, the patches were cut in 5 cm^2 area and packed in aluminium foil until used. Casting solutions were prepared by dissolving appropriate polymers, plasticizer in suitable vehicle using magnetic stirrer. The mixture was stirred continuously in such a manner that evaporation of solvent was minimum. 100mg of optimized nanoparticles was added slowly to the solution and dissolved by continuous stirring for 30 minutes. For the formulation of films, mercury was used as the backing membrane. Mercury was spread uniformly on a glass Petri dish. The mould was kept on a mercury surface. About 4 ml of the solution was poured on the mercury. The rate of evaporation was controlled by inverting the funnel over the mould. After 2 hours, the dried patches were cut into 2.2 cm diameter, wrapped in aluminium foil and stored over fused calcium chloride in a desiccator at room temperature for further use.

Characterization of Transdermal Patches

Physicochemical Properties of Patches

The patches were evaluated for the following physicochemical properties[10].

Weight Variation

Uniformity of weight was determined by weighing five matrices of each formulation. After each film unit was weighed individually on a digital balance, the average weight of film was taken as the weight of the film[11].

Thickness

The thickness of the patch was measured at five different points using a digital micrometer and average thickness was recorded[12].

Percentage Moisture Absorption

The films were weighed accurately and placed in the desiccator containing 100 ml of saturated solution of aluminium chloride, which maintains 79.50% RH. After 3 days, the films were taken out and weighed. The percentage moisture absorption was calculated using the formula[13].

Percentage of moisture absorption =

$$\frac{\text{Final weight- Initial weight}}{\text{Initial weight}} \times 100$$

Water Vapour Transmission Rate

Glass vials of 1.6 cm and area of 2 cm^2 were used as transmission cells. About 1 g anhydrous calcium chloride was placed in the cells and the respective polymer film was fixed over the brim. The cells were accurately weighed and kept in a closed desiccator containing saturated solution of potassium chloride to maintain a humidity of 84% [14]. The cells were taken out and weighed after 72 h of storage.

Folding Endurance

Folding endurance of the film was determined repeatedly by folding a small strip (2 cm × 2 cm) at the same place until it breaks. The number of times the film can be folded at the same place without breaking gives the value of folding endurance[15].

Tensile Strength

A small film strip (2 cm × 2 cm) was cut on a glass plate with a sharp blade. One end of the film was fixed between adhesive tapes to give support to the film when placed in the film holder. Another end of the film was fixed between the adhesive tapes with a small pin sandwiched between them to keep the strip straight while stretching. A thread was tied to this hook, passed over the pulley and a small pan attached to the other end to hold the weights. To determine tensile strength, the film was pulled by means of a pulley system. Weights were gradually added to the pan to increase the pulling force till the film was broken. The elongation was determined by recording the distance traveled by the pointer before break of the film on the scale[10].

Content Uniformity

The formulations were dissolved in 10 ml phosphate buffer pH 7.4 and stirred for 30 minutes. The resulting solutions were quantitatively transferred to volumetric flasks, and dilute up to 50 ml with pH 7.4 phosphate buffer. The resulting solutions were filtered and analysed for Timolol Maleate content at 294 nm in UV spectrophotometer. The average reading of three patches was taken as the content of drug in one patch[16].

***In-Vitro* permeation study**

A Keshary Chien Cell was used for evaluation drug permeation profiles across cellophane membrane. Phosphate buffer pH 7.4 (50 ml), was used as a receptor medium was filled in the diffusion cell. It was placed on a magnetic stirrer with a Teflon bead placed inside for uniform distribution. The semipermeable membrane was mounted in such a way that it continuously remained in intimate contact with the transdermal patch in the donor compartment. Transdermal patch of 2.5 cm diameter was overlaid on the membrane/skin preparation with good contact and placed in between two halves of the diffusion cell. The top side was covered with the aluminium foil as backing membrane. The receptor fluid was agitated using magnetic stirrer at 60 rpm and temperature was maintained at 37 ±1°C. The amount of drug permeated into the receptor solution was determined by removing 1 ml of sample at hourly intervals for 10, 15, 20 and 24 hour. The withdrawn volume was replaced with an equal volume of fresh receptor. The absorbance noted at 294 nm[10].

Stability Studies of Optimized Transdermal Patches

The samples were stored and tested in accordance with the storage conditions and the valid test method. The samples were taken out of the storage on the planned testing date.

Experimental Animals

Forty five Wistar albino rats in adult size of either sex in weight ranging from 246-255 g were obtained from the Institutional Animal Ethics Committee of Institute of biomedical and industrial research, Jaipur vide Approval Number IAEC/2018-07/04. They were kept in groups and each group consist of nine animals. Each group was kept in clean poly-acrylic cages. It was maintained for 12 hrs day and light cycles at an average of ambient temperature of 25± 2° C and 60±10% relative humidity (RH) [17].

Table 1: The allocation of animals to various groups

S. No.	Groups	Treatment given	No. of Rat
1	Group I	Blank patch, as a control	9
2	Group II	Hypertension induced	9
3	Group III	Hypertension induced + captopril (20 mg/kg/day)	9
4	Group IV	Hypertension induced +Prepared nano-particle formulation of TM	9
5	Group V	Hypertension induced +Timolol maleate nanoparticles loaded Transdermal patch	9

Skin Irritation Study

Study performed on wistar rats for 14 days. Skin irritation study performed as the group assigned. Patches are applied on the backside of hairless skin of rats for 23 ± 1 hr upto 14 days to the same skin site(Table 1).

Table 2: The ranking of skin irritation in animals

Ranking	Criteria
0	No evidence of irritation
1	Minimal erythema, barely perceptible
2	Definite erythema, readily visible; minimal edema or minimal popular response
3	Erythema and papules
4	Definite edemal
5	Erythema, edema, and papules
6	Vesicular eruption
7	Strong reaction spreading beyond test site

After 24 hrs if any type of irritation found then patch should be apply on other site. Each day skin was examine for any type of major and minor skin reactions as mention below scale of 0 to 7 numbers

Evaluation of *in-vivo* Antihypertensive Effect

Diverse models available, for the existent study, the dietary induction of hypertension in Wistar rats was employed using 66% w/v D-Fructose according to methods described by Jena *et al*.[18].. Each rat was educated and familiarized to the restrainer and transducer, for about 15 mins before the experiment. The rat was nonaggressive in a low-stress environment and allowed to enter the holder freely at least 10–15 min prior to obtaining BP measurements. The animal's nose was made to protrude through the front of the nose cone permitting for relaxed breathing and the tail of the animal was fully prolonged to exit through the rear hatch opening of the holder. The rat was warmed but not heated using restrainer, the room temperature was maintained about 32–35.4°C, reduce stress and the blood flow to the tail was enhanced to acquire a BP signal. The rat never had its head bent sideways or its body compressed against the back hatch. The animal's temperature was monitored throughout the experiment as the procedure suggested by Malkoff *et al*., [19].

Data Treatment

From the dissolution data, dissolution rate constant (k), and dissolution half-life ($t_{50\%}$) were calculated. The slope and regression coefficient of % cumulative release versus time (Zero order release kinetics), log % unreleased versus time (First order release kinetics), CBR(Wo)-CBR(Wt) versus time (Hixson–Crowell), % cumulative release versus time$^{0.5}$ (Higuchi release kinetics) and log cumulative % drug release versus log time (Korsmeyer–Peppas) were calculated using MS Excel 2007 computer program[20].

Results and discussion

The preformulation study is a stage of product development during which the physicochemical properties of drug substance are characterized. Timolol maleate absorption maxima were found to be 294 nm. As the observed melting point (200^{0}C) was found to be in similar range as that of reported value (202.5 ^{0}C). The calibration curve of TM in water was found to be linear in the concentration range of 2 mcg/ml to10 mcg/ml with R^2=0.993. The calibration curve of TM in phosphate buffer saline pH 6.4 was found to be linear in the concentration range of 2mcg/ml to10mcg/ml with R^2=0.992.

Formulation of Solid-Lipid Nano Particle of Timolol Maleate

In the microwave-assisted method, preparation of the microemulsion is a "single-pot" step wherein all the composition ingredients are heated together in controlled microwave reaction conditions.

Morphological Assessments

In order to visualize the architecture of the surface of the SLNs of the TM, SEM confirmed uniform surface maintained throughout the preparation.

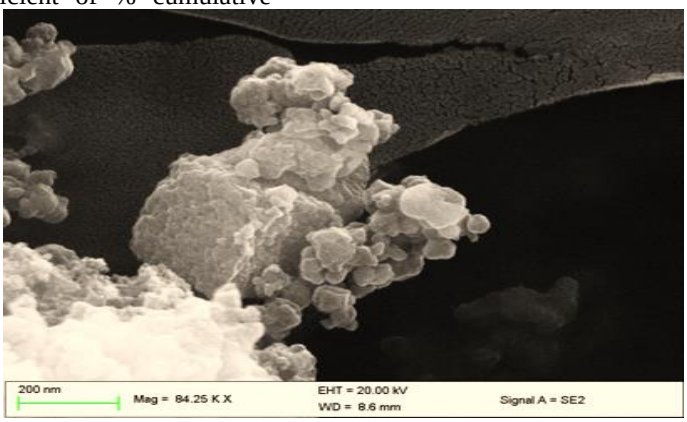

Fig. 1: Scanning electron microscopy (SEM) images of TM loaded SLNs

Particle Size and Distribution Studies

The results of particle size analysis of SLNs confirmed the size of the developed optimized formulation.

Table 3: Particle size distribution and Zeta potential by Malvern Instrument

Formulation	Size Distribution(nm)	Mean Intensity(%)	Zeta potential(mV)
F9	122.6	67.3%	-40.3

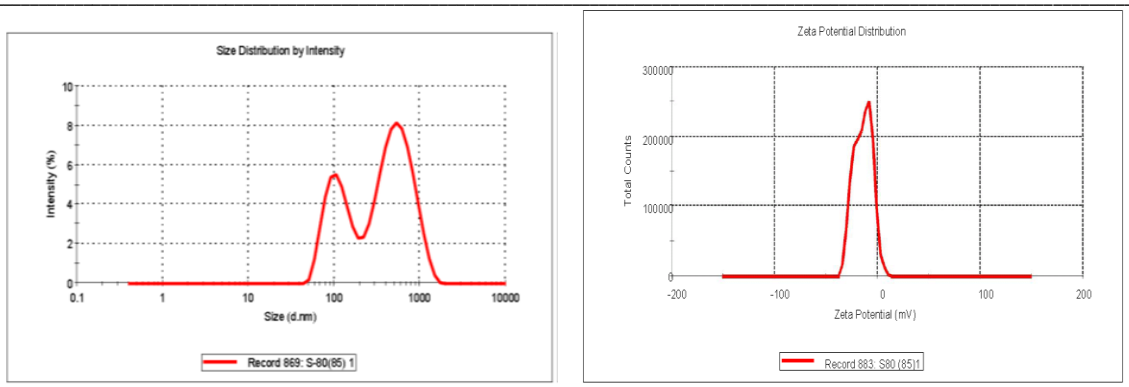

Fig 2: Particle size distribution and Zeta potential of SLNs of TM

Percent Drug Entrapment Efficiency Study

Entrapment efficiency of the SLNs was determined by lysing the SLNs in a pH 6.4 PBS and further with Triton-X-100. Regression equation for the standard plot of TM in pH 6.4 PBS – Y= 0.007X and Dilution Factor = 50. Drug content and encapsulation efficiency are important parameters to evaluate the drug content of SLNs. Drug content of the TM loaded SLNs was found to be 72.5%.

Characterization of Optimized Formulations

Various physical evaluations like thickness uniformity, weight variation, tensile strength, folding endurance of the optimized transdermal patches were performed according to the methods given in the

In-vitro permeation studies

In vitro permeation studies of the optimized formulations were performed using modified Keshary-Chien cell.

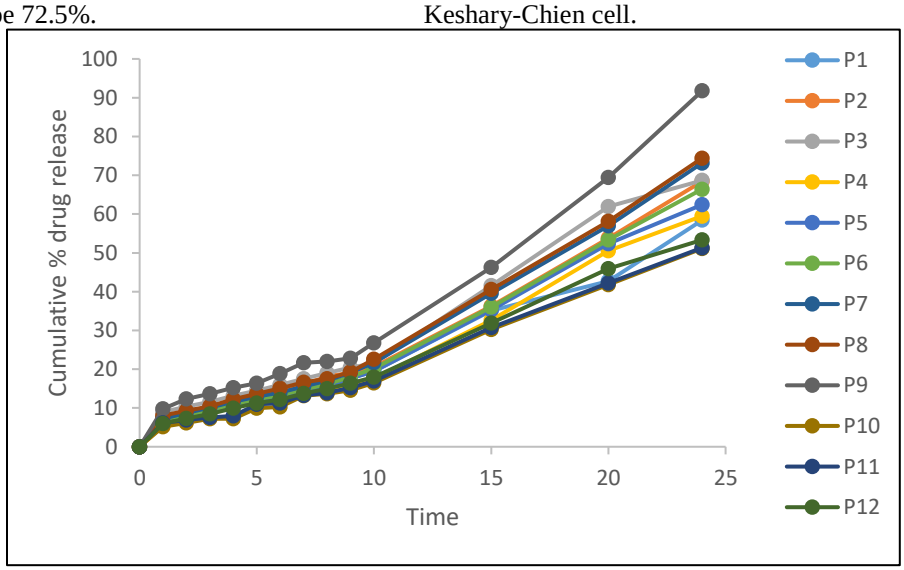

Fig. 3: Plot of Cumulative percent permeated versus time from formulations P1-P12

Table 4: Physicochemical properties of optimized formulations

F.C.	Weight Variation (mg)	%MA	%ML	WVTR*	Thickness (mm)	Tensile Strength (kg/mm^2)	Folding Endurance
P1	44.21±0.12	3.92±0.14	4.93±0.432	0.237±.042	0.58±0.00	0.479±0.004	1583±2.32
P2	43.93±1.35	3.94±0.31	4.78±0.786	0.321±0.045	0.48±0.001	0.434±0.008	1498±2.13
P3	43.22±0.10	3.82±0.13	4.83±0.423	0.217±.043	0.54±0.00	0.429±0.004	1563±1.52
P4	41.63±1.35	3.49±0.33	4.87±0.768	0.312±0.045	0.43±0.001	0.443±0.008	1457±1.84
P5	45.25±0.92	4.01±0.21	5.93±0.291	0.189±0.019	0.51±.001	0.356±0.009	1567±1.75

P6	40.12±1.57	4.98±0.37	6.42±0.385	0.347±0.09	0.63±0.00	0.518±0.004	1520±2.22
P7	43.83±0.46	4.42±0.24	3.86±0.629	0.411±0.065	0.69±0.00	0.464±0.003	1479±1.96
P8	44.93±0.80	3.67±0.49	4.67±0.354	0.284±0.029	0.68±0.001	0.452±0.006	1543±2.23
P9	46.91±0.49	3.30±0.19	3.12±0.344	0.137±.012	0.65±0.002	0.587±0.001	1614±2.32
P10	41.87±0.74	3.11±0.24	3.34±0.621	0.375±0.035	0.42±0.001	0.401±0.005	1321±2.54
P11	40.11±0.10	3.12±0.14	3.93±0.423	0.217±.024	0.48±0.00	0.429±0.004	1383±1.73
P12	38.93±1.53	3.24±0.13	3.87±0.466	0.221±0.025	0.49±0.001	0.443±0.006	1398±1.93

Indicates values are average of three observations.

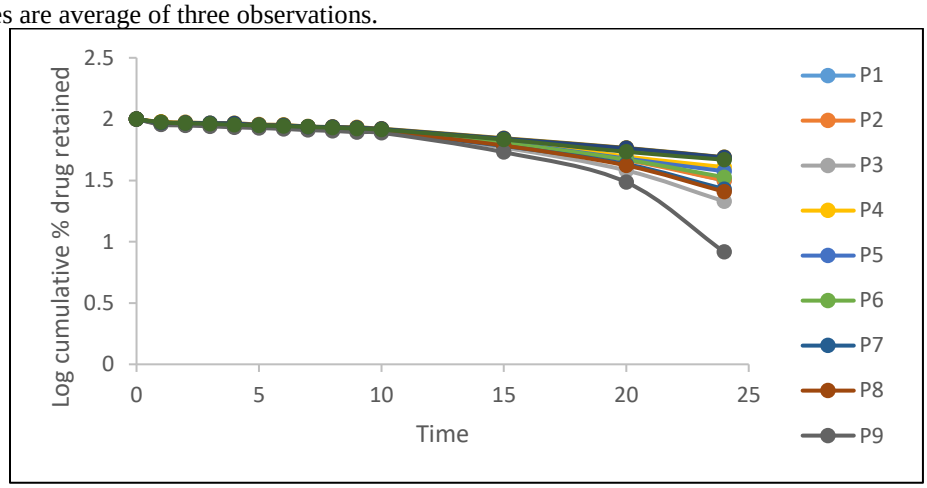

Fig 4: Plot of Log Cumulative Percent Drug Retained Versus Time formulations P1-P12 (First Order Kinetics)

Table 5: *In-Vitro* permeation profile of Timolol Maleate formulation P1 to P12

Time	Cumulative % Drug Permeated											
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
0	0.00± 0.0	0.00± 0.0	0.00± 0.0	0.00± 0.0	0.00± 0.0	0.00± 0.0	0.00± 0.0	0.00± 0.0	0.000. 0	0.00± 0.0	0.00± 0.0	0.00± 0.0
1	7.20± 0.45	7.56± 0.42	8.95± 0.41	5.37± 0.34	6.24± 0.34	6.79± 0.36	7.25± 0.42	8.04± 0.41	9.79± 0.23	5.15± 0.42	6.15± 0.47	5.95± 0.52
2	8.41± 0.34	9.44± 0.42	10.33 ±0.41	7.66± 0.24	8.23± 0.34	8.08± 0.38	8.66± 0.34	9.29± 0.38	12.37 ±0.35	6.12± 0.41	6.90± 0.52	7.29± 0.42
3	9.50± 0.35	9.98± 0.45	11.58 ±0.35	9.08± 0.41	9.22± 0.41	9.50± 0.42	10.12 ±0.34	10.41 ±0.38	13.62 ±0.38	7.23± 0.42	7.53± 0.36	8.50± 0.41
4	11.33 ±0.41	12.43 ±0.38	13.12 ±0.41	10.50 ±0.40	10.66 ±0.42	10.91 ±0.41	11.58 ±0.38	12.29 ±0.40	15.20 ±0.36	7.25± 0.40	7.95± 0.28	9.95± 0.24
5	12.54 ±0.35	13.42 ±0.32	14.50 ±0.32	11.79 ±0.36	11.24 ±0.45	12.33 ±0.41	12.87 ±0.28	13.66 ±0.34	16.41 ±0.32	9.92± 0.49	10.94 ±0.41	11.29 ±0.53
6	13.83 ±0.38	13.89 ±0.34	15.95 ±0.38	12.70 ±0.41	12.98 ±0.29	13.25 ±0.35	14.00 ±0.41	15.04 ±0.24	18.83 ±0.47	10.23 ±0.48	11.24 ±0.36	12.20 ±0.45
7	15.04 ±0.35	15.94 ±0.44	17.50 ±0.35	14.25 ±0.38	14.56 ±0.43	14.83 ±0.41	15.54 ±0.34	16.62 ±0.44	21.66 ±0.34	13.15 ±0.45	13.25 ±0.36	13.70 ±0.32
8	16.29 ±0.41	18.19 ±0.34	19.04 ±0.41	14.88 ±0.48	15.34 ±0.41	16.16 ±0.35	17.04 ±0.41	17.54 ±0.34	21.95 ±0.42	13.66 ±0.35	13.89 ±0.42	14.95 ±0.43
9	17.70 ±0.34	18.98 ±0.41	20.45 ±0.38	15.45 ±0.42	17.58 ±0.41	18.04 ±0.41	19.08 ±0.36	19.25 ±0.42	22.79 ±0.41	14.57 ±0.34	15.25 ±0.42	16.37 ±0.41
10	19.45	20.58	21.50	17.50	19.45	20.16	21.62	22.54	26.75	16.45	16.98	17.95

	±0.42	±0.44	±0.42	±0.43	±0.46	±0.54	±0.42	±0.48	±0.42	±0.47	±0.43	±0.52
15	35.08 ±0.48	36.23 ±0.42	41.58 ±0.48	32.62 ±0.44	35.25 ±0.42	36.04 ±0.46	39.54 ±0.42	40.52 ±0.41	46.25 ±0.48	30.25 ±0.37	30.75 ±0.43	31.87 ±0.48
20	42.66 ±0.42	53.72 ±0.48	61.91 ±0.45	50.50 ±0.48	52.34 ±0.43	53.33 ±0.58	57.00 ±0.54	58.16 ±0.42	69.41 ±0.41	41.75 ±0.46	42.17 ±0.48	45.91 ±0.45
24	58.41 ±0.48	68.42 ±0.43	68.66 ±0.42	59.47 ±0.48	62.41 ±0.54	66.41 ±0.48	73.16 ±0.42	74.41 ±0.43	91.75 ±0.48	51.12 ±0.58	51.31 ±0.42	53.33 ±0.48

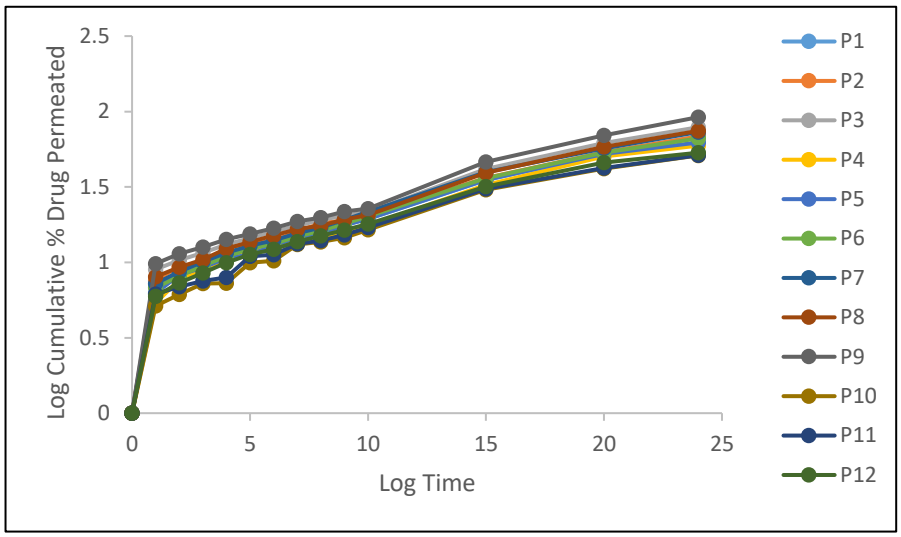

Fig 5: Plot of Log cumulative percent drug permeated versus log time formulations P1-P12 (Korsmeyer peppas model)

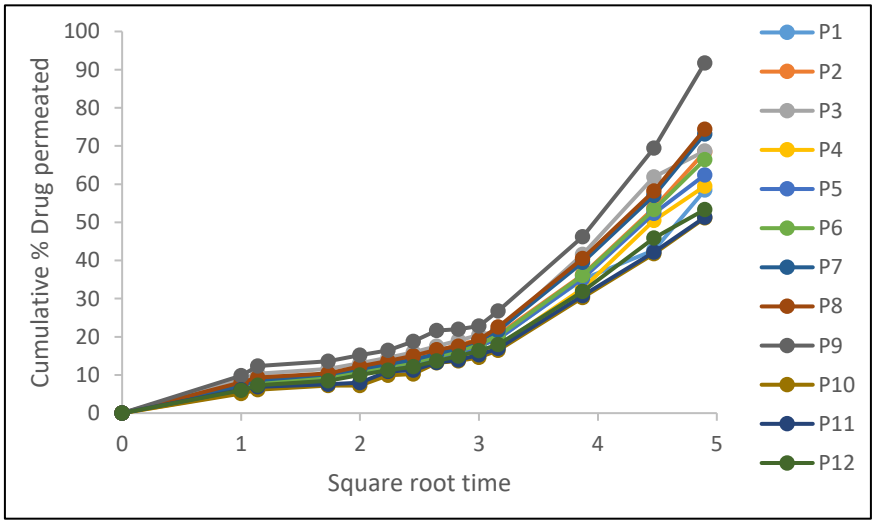

Fig 6: Plot of Cumulative percent drug permeated versus square root of time formulations P1-P12(Higuchi model)

***In-Vivo* Skin Irritation Study**

Results revealed that as hypertension induced rats along with prepared nano-particle formulation of TM is a skin irritant. When rats was treated with SLNs along it produce irritation with minimal erythema after 10 days and definite erythema, readily visible edema was produce after 12 days.Compared with this both the placebo and optimized batch was not show any type of irritation up to 10 days after that there was little erythema found with light redness at the site of application.

***In-Vivo* Anti-hypertensiveStudy**

The dietary induction of hypertension in Wistar rats was employed using 66% w/v D-Fructose according to methods described by Jena *et al*. (2013).

Table 6: Measured Systolic blood pressure of different groups of rat

Sr. No.	Group	0th day	7th day	14th day
1	Control	111±2.11	110±3.25	106±3.77
2	Disease control	108.2±2.25	124.5±3.11	135.5±2.24
3	Standard	109.5±1.75	114.5±2.42*	111.6±3.06*
4	Test I Patch	112.6±2.46	119.4±2.25	118.5±3.60*
5	Test II SLN Patch	110.5±2.35	115.2±3.15	113.6±2.60*

Table 7: Measured Diastolic blood pressure of different groups of rat

Sr. No.	Group	0th day	7th day	14th day
1	Control	77.2±2.50	78.5±2.85	79.6±2.30
2	Disease control	79.2±3.15	89.8±3.47	93.2±3.25
3	Standard	78.6±2.85	82.3±2.11	80.2±3.10*
4	Test I Patch	76.8±2.01	86.2±2.50	84.4±2.78*
5	Test II SLN Patch	78.2±3.22	85.3±3.10	82.5±2.15*

*Significant P‹0.05 as compared to disease control

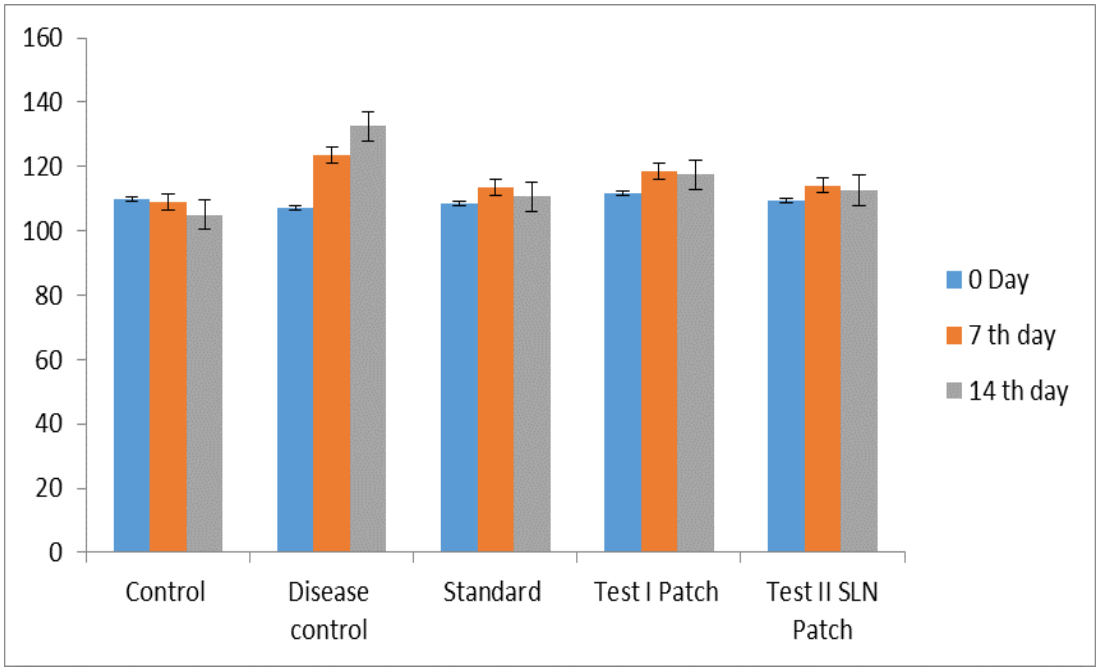

Fig. 7: Graphs showing Systolic blood pressure of rats

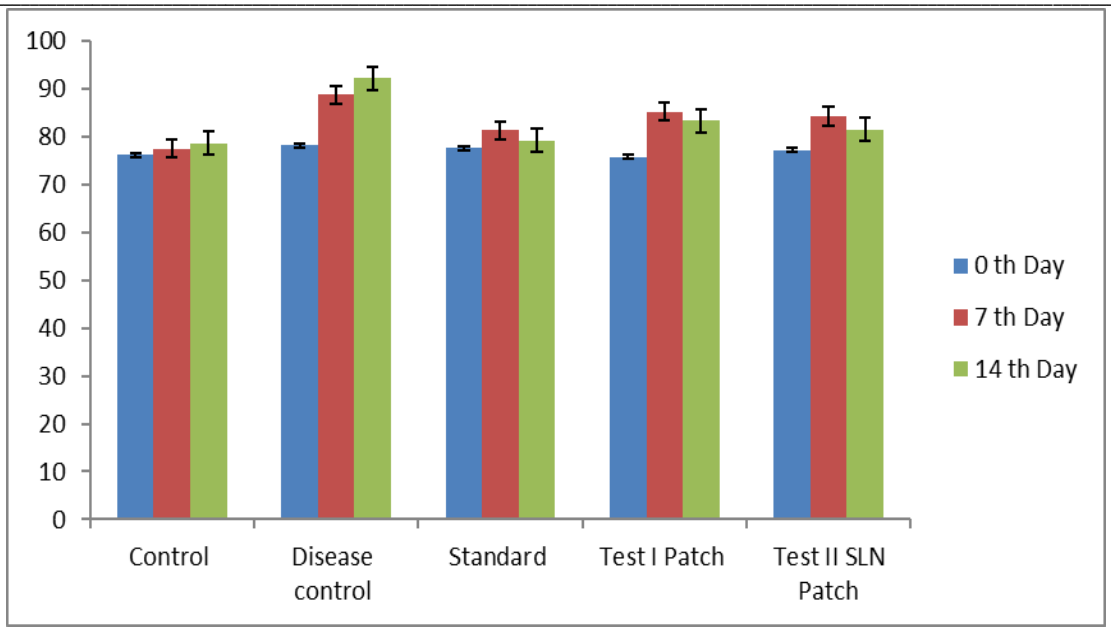

Fig. 8: Graphs showing Diastolic blood pressure of rats

Results revealed that as hypertension induced rats along with prepared nano-particle formulation of TM is a non skin irritant.

Stability Studies

Stability studies of the transdermal patches were carried out at 40 ±2°C and 75 ± 5% RH for three months to assess their potential utility. After storage for three months, the transdermal patches were subjected to any change in physical appearance and drug content studies. Results of the stability study showed that there was no marked difference in physical appearance and drug content of patches before and after storage.

Conclusion

Novel nanoparticle based transdermal formulation was developed for the hypertension. In this system, drug release profiles were controlled by using both natural as well as synthetic polymers. The triple fold release was aimed to target drug delivery to the hypertension patients followed by transdermal drug delivery with preventing the burst release of drugs in the stomach and small intestine. Timolol Maleate was selected due to less skin irritation, low dose, short plasma half life, low molecular weight and its partition coefficient value was in the range. Identification, solubility, partition-coefficient of drug were performed. FTIR technique has been used to study the physical and chemical interactions between drug and excipients used. It has been observed that there was no chemical interaction between drug and polymer used. Calibration curve of Timolol Maleate was prepared in phosphate buffer pH 7.4 at 294 nm using UV spectrophotometer. SLNs formulations were evaluated for different parameter like microscopy, particle size, shape, drug content, *ex vivo* release study, zeta potential etc. All the formulations were optimized to get the best entrapment efficiency. From the above observation it can be concluded that sonication is essential tool for the preparation of SLNs. The selection of an optimized formulation was carefully done after performing preliminary particle characteristics measurements. Use of natural permeation enhancer with various grades of HPMC (50 cps and K100M) and ethyl cellulose in the formulation of transdermal patch of Timolol Maleate that leads to superior properties. HPMC 50 cps has been selected because it was a non-toxic polymer which provides transparent patches with good organoleptic properties. In preliminary studies, drug free transdermal patches were prepared by using different grades of HPMC and ethyl cellulose and concentration of plasticizer was varied. On the basis of physical appearance and uniform thickness, the concentration and grades of HPMC 50 cps were selected. These transdermal patches were evaluated for physical characterization like weight variation, thickness, percentage moisture absorption, percentage moisture loss, water vapour transmission rate, folding endurance, tensile strength and content uniformity. The patches so formed showed satisfactory physicochemical properties. Further, *in vitro* permeation studies were performed by using modified Keshary Chien permeation cell. This study was conducted to investigate the effect of different polymers and type of permeation time profiles from Timolol Maleate patches. Although the drug was

diffused across cellophane membranes but the permeation profile was not satisfactory so it was necessary to add permeation enhancer in these patches. Almond oil was selected as a permeation enhancer. The fabricated final transdermal patches were subjected to *in vitro* permeation studies and the diffusion data obtained were analysed quantitatively to describe kinetics and mechanism of drug permeation from patches. The drug release from the formulation followed zero order drug release kinetic with a significant linear correlation. By employing Korsemeyer-Pepas model, results indicate that anomalous transport ($0.5<n<1.0$) predominated in the optimized formulations indicating swelling controlled drug permeation. By the incorporation of 4 ml of almond oil as permeation enhancer in optimized formulation the drug permeation was increase upto 1.64 fold while the similar result was observed with others. Terpenes present in it enhance diffusion of drug by extracting lipids from stratum corneum which results in reorganization of lipid domain and barrier disruption. Outcomes of the *in-vivo* results have shown that encapsulation of TM by SLNs were successful in the treatment of hypertension without premature release of the drug. Conclusively, the development of a SLNs based transdermal patches method is a good approach for hypertension disease. Stability studies were performed at 40°C/75% RH, and 25°C/60% RH conditions. The physical stability of Timolol Maleate based transdermal patches proved to be unchanged after storage up to 3 months. Transdermal patch evaluated for anti hypertensive study and a skin irritation study. No any marked skin irritation observed before and after applying the patch.

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