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Formulation and Evaluation of Transdermal Drug Delivery System of Clopidogrel Bisulfate

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ABSTRACT

Transdermal drug delivery is an alternative route for systemic drug delivery which minimizes the absorption and increases the bioavailability. Orally Clopidogrel bisulfate has a short elimination half-life (7-8 hrs.), low oral bioavailability (50%) undergoes extensive first pass metabolism (85%) and frequent high doses (75 mg) are required to maintain the therapeutic level as a result, dose development toxic effect. The purpose of this research work was to Formulation and evaluation of transdermal drug delivery system of Clopidogrel bisulfate using various polymers such as HPMC, PVP and Ethyl cellulose by solvent evaporation technique for improvement of bioavailability of drug and reducing toxic effects. The prepared formulations were evaluated for different physicochemical characteristics like thickness, folding endurance, drug content, percentage moisture absorption, percentage moisture loss and weight uniformity. The diffusion studies were performed by using modified Franz diffusion cells. The result of diffusion study shows that formulation, F2 (Hydroxy propyl methylcellulose and PVP) showed maximum release of 90.06 % in 24 h, whereas F5 (HPMC and Ethyl cellulose) showed minimum release of 78.24% in 24 h. Based on the drug release and physicochemical values obtained the formulation F2 is considered as an optimized formulation which shows higher percentage of drug release of 90.06 % in 24 h. The developed transdermal patches increase the therapeutic efficacy and reduced toxic effect of Clopidogrel bisulfate.

Key words

Clopidogrel bisulfate, Transdermal patch, Solvent evaporation technique, In-vitro drug release, Penetration enhancer.

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INTRODUCTION

A recent approach to drug delivery is to deliver the drug into systemic circulation at predetermined rate using skin as a site of application. Transdermal patches are delivered the drug through the skin in controlled and predetermined manner in order to increase the therapeutic efficacy of drug and reduced side effect of drug. Controlled drug release can be achieved by transdermal drug delivery systems (TDDS) which can deliver medicines via the skin portal to systemic circulation at a predetermined rate over a prolonged period of time. TDDS has gained a lot of interest during the last decade as it offers many advantages over the conventional dosage forms and oral controlled release delivery systems notably avoidance of hepatic first pass metabolism, less frequency of administration, reduction in gastrointestinal side effects and improves patient compliance.[1, 2]

Transdermal therapeutic systems are defined as a self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug, through the skin at control rate to the systemic circulation. Transdermal formulation maintain drug concentration within the therapeutic window

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for prolong period of time ensuring that drug levels neither fall below the minimum effective concentration nor exceed the maximum effective concentration. An ideal drug to be formulated as transdermal drug delivery should possess several physico-chemical properties, such as short half-life, small molecular size, low dose, low oral bioavailability, etc.[3]

Clopidogrel bisulfate is a Anti platelet drug, undergoes hepatic first pass metabolism and low oral bioavailability (50%).[4] Hence it is suitable for formulation as a transdermal patch. Drug molecules in contact with the skin surface can penetrate by three potential pathways: through the sweat ducts, via the hair follicles and sebaceous glands (collectively called the shunt or appendageal route), or directly across the stratum corneum.[(5)]

MATERIAL AND METHODS

Material

Clopidogrel bisulfate was obtained as a gift samples from Ipca laboratories Ratlam. Poly vinyl pyrolidone, hydroxy propyl methylcellulose and Ethyl cellulose were purchased from LOBA chem. LTD Mumbai (India). PEG 400 was purchased from S.D Fine chemical Ltd. (Mumbai, India). All other laboratory chemicals used in the study were of analytical reagents grade.

Partition coefficient determination

The partition coefficient (log D) is a measurement of lipophilicity of molecules, which can be used to predict its capability to cross biological membrane. The Partition coefficient studies were performed using n-octanol/skin as non aqueous phase and water as aqueous phase. The two phases were mixed in equal quantities and kept for saturation with each other in separating funnel. After mixing the system remain undisturbed for half an hour. About 10 mg of drug added to this solution and was shaken occasionally in separating funnel. After shaken the resulting solution was kept a site for 24 hour.

After 24 hour two phases were separated in a separating funnel. The aqueous phase was filtered through Whatman filter paper, suitably diluted and amount of Clopidogrel bisulfate in aqueous phase was determined by measuring absorbance at 220 nm using UV spectrophotometer (Shimadzu 160). The partition coefficient of Clopidogrel Bisulfate was calculated from the ratio between the concentration of Clopidogrel Bisulfate in organic and aqueous phases from the below mentioned formula. [(7)]

$$D_{O/PBS} = \frac{\text{Concentration of drug in non aqueous phase}}{\text{Concentration of drug in aqueous phase}}$$

Permeation study of pure drug

The *in-vitro* drug permeation studies were carried out by using Franz diffusion cell. The rat skin of abdominal part was cut and hair was removed and clamped between the receptor and donor compartments. The receptor compartment was filled with 15 ml of diffusion medium (Phosphate buffer pH 7.4) through sampling port taking care to remove all the air bubbles. The contents were stirred at 500 rpm. by externally driven, teflon coated small magnetic bead to keep them well mixed. The temperature of the system was maintained at 37.0±2^{0}C. Accurately weighed 5 mg of clopidogrel bisulfate was dissolved in phosphate buffer pH 7.4 and placed in receptor compartment. At suitable time intervals, aliquots (3ml) were collected and suitable diluting the aliquot with phosphate buffer and absorbance was measuring at 220 nm using a double beam UV spectrophotometer (Shimadzu 160). The diffusion medium of the same volume (3ml), which was pre warmed at 37^{0}C, was then replaced into the receptor compartment. Duration of the experiment was 12-24 hours. The amount of drug permeated through skin was calculated from absorbance of aliquots.

Preparation of transdermal patch

Transdermal patches of clopidogrel bisulfate were prepared by solvent casting technique (Table 1). Ethanolic solution of polymer and drug along with polyethylene

glycol (plasticizer) was prepared. The homogenous mixture was poured into plastic mould. The solvent was allowed to evaporate at controlled rate by placing an inverted funnel over the plastic mould. The control of evaporation is necessary for uniform drying of films. The drying was carried out at room temperature for duration of 24 hours. After 24 hours the dry films was removed from plastic mould and stored in desiccators until used. [6,7]

Thickness of the patch

The thickness of the drug loaded patch was measured in different points by using a digital micrometer and determines the average thickness and standard deviation for the same to ensure the thickness of the prepared patch. [8]

Weight uniformity

The prepared patches were dried at 60°c for 4hrs before testing. A specified area of patch was cut in different parts of the patch and weigh in digital balance. The average weight and standard deviation values are to be calculated from the individual weight. [8,9]

Folding endurance

A strip of specific area was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance. [10]

Percentage Moisture content

The prepared films were weighed individually and to be kept in a desiccators containing fused calcium chloride at room temperature for 24 hrs. After 24hrs the films are to be reweighed and determine the percentage moisture content from the below mentioned formula. [8,9,10]

$$\text{Percentage moisture content} = \frac{\text{Initial weight- Final weight}}{\text{Final weight}} \times 100$$

Percentage Moisture uptake

The weighed films were kept in a desiccators at room temperature for 24hrs containing saturated solution of potassium chloride in order to maintain 84% RH. After 24hrs the films are to be reweighed and determine the percentage moisture uptake from the below mentioned formula. [8,9,10]

$$\text{Percentage moisture uptake} = \frac{\text{Final weight- Initial weight}}{\text{Initial weight}} \times 100$$

Water vapor permeability

Glass vials of 5 ml capacity were washed thoroughly and dried to a constant weight in an oven. About 1gm of fused Calcium chloride was taken in the vials & the polymer films were fixed over the brim with the help of an adhesive tape. Then the vials were weighed and stored in a humidity chamber at 85 % RH condition for a period of 24 hours. The vials were removed and weighed at various time intervals like 3, 6, 12, 18 and 24hrs to note down the weight gain.[11]

Drug content

A specified area of patch was dissolved in a suitable solvent in specific volume. Then the solution is to be filtered through a filter medium and analyze the drug contain with the suitable method (UV or HPLC technique). [11,12]

Percentage Elongation break test

The percentage elongation break was determined by noting the length just before the break point, the percentage elongation can be determined from the below mentioned formula: [12]

$$\text{Elongation percentage} = \frac{L1 - L2}{L2} \times 100$$

Where, L1is the final length of each strip and L2 is the initial length of each strip.

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In vitro drug diffusion studies

The in vitro diffusion study was carried out with the abdominal rate skin using Franz diffusion cell. The cylinder consists of two chambers, the donor and the receptor compartment. The donor compartment was open at the top and was exposed to atmosphere. The temperature was maintained at 37 ± 0.5^{o} C and receptor compartment was provided with sampling port. The diffusion medium used was phosphate buffer (pH 7.4).

The diffusion studies were done to get an idea of permeation of drug through barrier from the transdermal system. In vitro studies are also done for TDDS development.

Usually, two types of diffusion cells are used as horizontal and vertical. The Franz and Keshary Chien (K-C) type of diffusion cells are of horizontal type of cells. In this work, K-C type of diffusion cell was used. Diffusion cells generally comprise two compartments, one containing the active component (donor compartment) and the other containing receptor solution (receptor compartment), separated by barrier i.e. albino rate abdominal skin.

The cell consisted of sampling port and temperature maintaining jacket. The outlet and inlet was connected with latex tube so the jacket had stagnant water inside and heat was provided by hot plate. The stainless steel pin was used to stir the receptor solution using magnetic stirrer. The mice abdominal skin was placed on receptor compartment and both compartments held tight by clamps. Phosphate buffer pH 7.4 was used as receptor solution. The volume of diffusion cell was 15 ml and stirred with bent stainless steel pin. The temperature was maintained at 37 ± 2°C with the help of magnetic stirrer. The diffusion was carried out for 24 hours and 1 ml sample was withdrawn at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 hour. The same volume of phosphate buffer pH 7.4 was added to receptor compartment to maintain sink conditions and the samples were analyzed at 220nm in UV spectrophotometer. [8,10]

RESULT AND DISCUSSION

In the present work efforts have been made to prepare transdermal drug delivery system of Clopidogrel bisulphate using the polymers HPMC, EC and PVP, using polyethylene glycol as a plasticizer by solvent casting technique. The selection of polymer combinations produces clear, smooth, uniform, substantive, flexible and desired thickness film for the transdermal drug delivery systems of Clopidogrel bisulphate. The prepared formulation were evaluated for different Physico-chemical characteristics such as Thickness, Folding endurance, Drug Content, Percent moisture absorption, Percentage moisture loss and Weight uniformity. The release characteristics of the formulation were studied in *in-vitro* conditions. *In vitro* dissolution studies were carried out in phosphate buffer (pH 7.4) for 24 hours. The partition coefficient of the clopidogrel bisulfate was found to be 2.1. After 24 hour 68.11 % drug was permeated through skin.

The thickness of the patches varied from 0.125 to 0.246mm. The minimum standard deviation values assumed that the process used for preparing the drug delivery system is capable of giving reproducible result.

As the concentration of PVP and Ethyl cellulose increase, moisture content of patches was also increase. Formulation F1 (3.01±0.057) absorbed highest amount of moisture which also revealed its high hydrophilicity and formulation F6 (1.28±0.042) absorb least amount of moisture.

The folding endurance was measured manually, films were folded 72 times maximum in Formulation F2 and if the film shows any cracks it was taken as end point. The folding endurance was better in F2 formulation.

As the concentration of PVP and Ethyl cellulose increase, moisture uptake of patches was also increase. The highest moisture absorption was found in the formulation F6 and lowest value of moisture absorption was found in the formulation F1.

The drug content uniformity of the prepared formulation have shown that the process used to prepared the transdermal film in this study was capable of giving film with uniform drug content. The result of drug content indicates that drug is uniformly dispersed in formulation.

Water vapour transmission study determines the permeability characteristics of the patches. The result of water vapour transmission study reviled that all the formulation are permeable to water vapour.

In vitro drug release studies were carried out for the different formulations using French diffusion cell. The medicated films showed drug Release study in % cumulative release. The relationship can be established as F2 >F1 > F3>F6>F4>F5Thus, by varying amount of polymer in film, percent release can be varied. Drug-polymer affinity can be major factor that control release of drug from formulation.

Maximum percentage of drug release (i.e.90.06%) was observed with formulation F2 and the minimum (i.e. 78.24%) was found with formulation F5. The addition of hydrophilic components such as PVP in to the formulation tends to enhance its release-rate constants. This outcome can be attributed to the leaching of the soluble component, which leads to the formation of pores and thus a decrease in the mean diffusion path length of drug molecules to release into the dissolution medium. The result is higher dissolution rates. Substances such as PVP act as antinucleating agents that retard the crystallization of a drug. Thus they play a significant role in improving the solubility of a drug in the matrix by sustaining the drug in an amorphous form so that it undergoes rapid solubilization by penetration of the dissolution medium.

TABLE 1: Composition of transdermal patches

Ingradient	**Formulation Batches**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Clopidogrel Bisulfate (mg)	37	37	37	37	37	37
HPMC (mg)	300	300	300	300	300	300
EC (mg)	100	150	200	-	-	-
PVP (mg)	-	-	-	100	150	200
Ethanol (ml)	5	5	5	5	5	5
PEG 400 (ml)	0.4	0.4	0.4	0.4	0.4	0.4

CONCLUSION

The prepared transdermal drug delivery system of Clopidogrel bisulfate using different polymers such as HPMC, EC and PVP had shown good promising results for all the evaluated parameters. Based on the In-vitro drug release and drug content Result, formulation F2 was concluded as an optimized formulation, which shows its higher percentage of drug release.

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TABLE 2: Partition coefficient of drug in PBS 7.4

Partition coefficient of drug	Solvent system	Log D Values
Clopidogrel bisulfate	Phosphate buffer: n-octanol	2.1 ± 0.03

TABLE 3: Partition coefficient of drug in skin

Partition coefficient of drug	Solvent system	Log D Values
Clopidogrel bisulfate	Phosphate buffer: skin	2.4 ± 0.04

TABLE 4: Permeation study of clopidogrel bisulfate in phosphate buffer pH 7.4

S. No.	Time (hrs.)	%Amt. permeation
1	0	0
2	1	7.03
3	2	12.15
4	3	19.27
5	4	25.26
6	5	34.37
7	6	41.97
8	8	48.44
7	10	56.70
8	12	61.24
9	24	68.11

TABLE 5: Physico-chemical properties of prepared formulations

Formulation Code	Thickness (mm) ± S.D	Weight uniformity (mg)	Folding endurance ± S.D	Moisture content (%) ± S.D
F1	0.155 ± 0.0075	96±5.130	45±2.4	3.01±0.057
F2	0.174 ± 0.0079	104±3.06	72± 3.1	2.43±0.038
F3	0. 232± 0.0040	110±2.51	51 ±2.8	2.25±0.073
F4	0.192 ± 0.0090	116±3.05	39 ± 4.4	1.85±0.031
F5	0.148 ± 0.0091	125±2.08	57 ±1.8	1.72±0.053
F6	0.246 ± 0.0079	135±3.51	45 ±2.1	1.28±0.042

TABLE 6: **Physio-chemical properties of prepared formulations**

Formulation code	Moisture uptake ± S.D	Percent elongation break test ± S.D	cumulative drug release (%)	Water vapor transmission test ± S.D
F1	1.32±1.01	75.0%	85.22	0.22 ± 0.024
F2	1.52±1.27	93.3%	90.06	0.40 ± 0.031
F3	1.58±1.21	102.5%	83.92	0.56 ± 0.038
F4	2.05±1.76	84. 17%	80.68	0.36 ± 0.016
F5	2.10±1.83	94.17%	78.24	0.60 ± 0.027
F6	2.51±1.92	114.16%	81.61	0.78 ± 0.041

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TABLE 7: Drug content of prepared formulations

Formulation code	Drug content(%)
F1	89±0.3
F2	92±0.1
F3	85±0.6
F4	86±0.2
F5	83±0.4
F6	90±0.5

TABLE 8: Curve fitting data for the release rate profile of formulation F2

Model	r^2 value
Krosmeyers – peppas	0.9984
Zero order	0.8064
First order	0.1534
Higuchi matrix	0.5154
Hixson Crowel	-0.9548

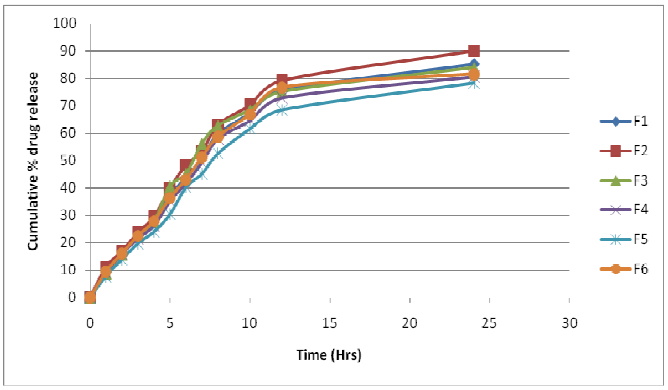

Fig 1. Comparative *In vitro* drug release of clopidogrel bisulfate in TDDS Formulation

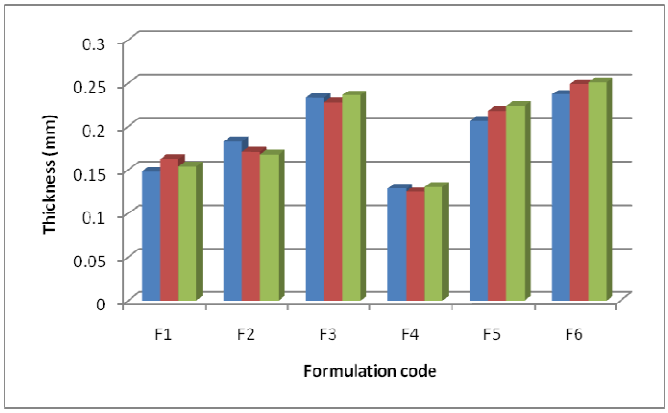

Fig 2. Thickness of various batches.

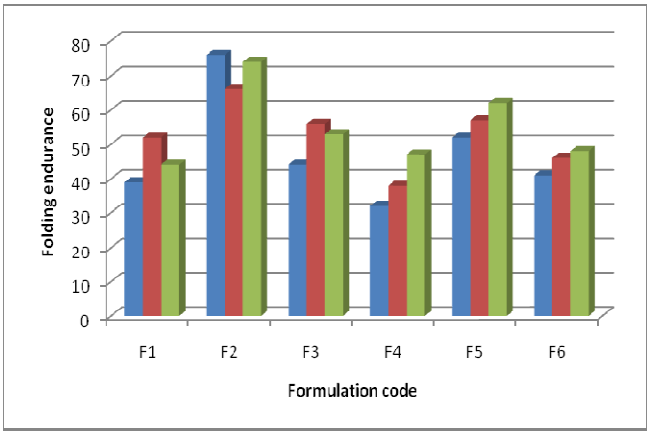

Fig 3. Folding endurance of various batches.

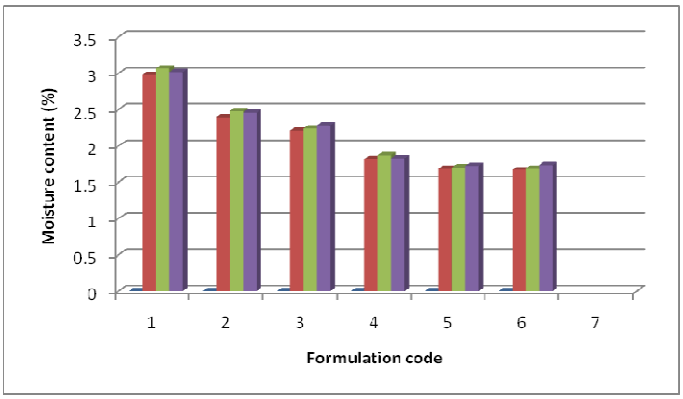

Fig. 4 Moisture content study of various batches.

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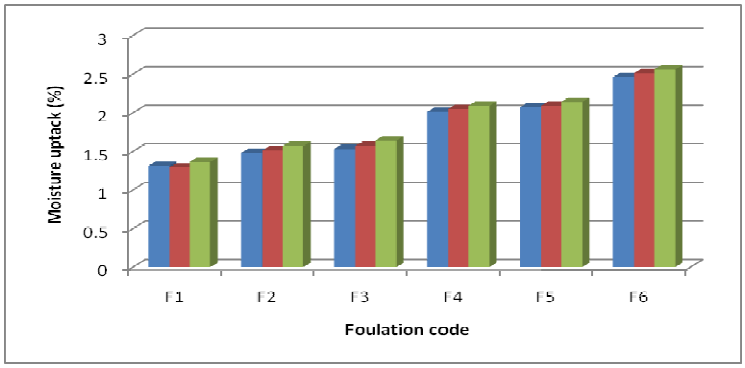

Fig. 5 Moisture uptack study of various batches.

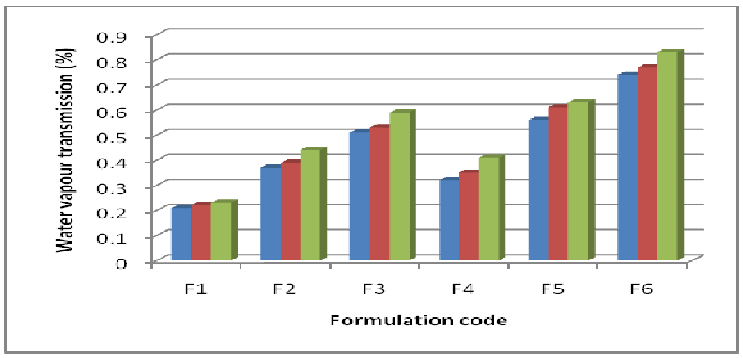

Fig. 6 Water vapour transmission study of formulated batches.

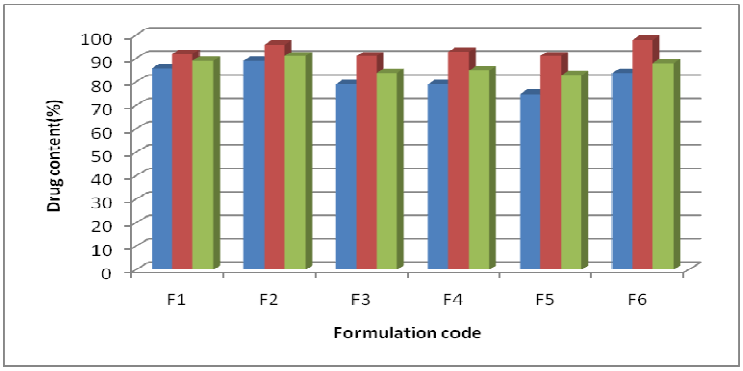

Fig. 7 Drug Content study of formulated batches.

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