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FORMULATION AND EVALUATION OF REPAGLINIDE TRANSDERMAL PATCHES FOR TREATMENT OF DIABETES

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ABSTRACT

The objective of present study was to develop Repaglinide transdermal patches for treatment of diabetesusing Hydroxypropyl methyl cellulose(HPMC), ethyl cellulose in different ratios. Tri-ethyl citratewas used as a plasticizer and methylene chlorideused as a permeation enhancers which were added by solvent casting method. Results revealed that prepared patches showed good physical characteristics, no drug-polymer interaction and no skin irritation was observed. The systems were evaluated for various parameters like weight uniformity, folding endurance, thickness, drugcontent. The systems were evaluated for various in vitro, Ex vivo, in vivo(skin irritation, anti-diabetic activity in rats) parameters. ***In- vitro*** diffusion studies and ***Exvivo*** studies were performed by using Franz diffusion cells. The in vitro release study revealed that Cformulation showed maximum release in 24hrs. From all the formulations, formulation C was selected as best formulation and was stable for 60% RH.

KEYWORDS: Repaglinide, Hydroxypropylmethylcellulose, Ethylcellulose, Transdermal patches.

INTRODUCTION

1. Introduction to Diabetes

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the Pancreas which lowers the blood glucose

level. When the blood glucose elevates (for example, after eating food), insulin is released from the pancreas to normalize the glucose level.[1]

2. Introduction to Transdermal Drug Delivery

Transdermal drug delivery system is defined as self contained, discerete dosage forms which, when applied to the intact skin, deliver the drug, through the skin at controlled rate to the systemic Transdermal drug delivery system is also known as a Transdermal patch or skin patch which delivers a specific dose of medication to the systemic circulation. Transdermal delivery not only provides controlled, constant administration of the drug, but also allows continuous input of drugs with shortbiological half-lives and eliminates pulsed entry into systemic circulation, which often causes undesirable side effects.[2,3]

2.1 Advantages of Transdermal System

1. Transdermal medication delivers a steady infusion of a drug over an extended period of time.
2. Self administration is possible with these systems.
3. They can be used for drugs with narrow therapeutic window.
4. Longer duration of action resulting in a reduction in dosing frequency.
5. Increased convenience to administer drugs which would otherwise requirefrequent dosing.

Repaglinide is an oral insulin secretagogue of the Meglitinide class. It stimulates insulin release by closing ATP–dependent potassium channels in pancreatic cells. Repaglinide is lipophilic drug used for lowering the blood glucose level by stimulating the insulin secretion. It possesses low oral bioavailability (56%) due to hepatic first pass metabolism and has a short biological half-life of ~1h, which makes frequent dosing necessary to maintain the drug within the therapeutic blood level for longer period of time. Since the drug is intended to be taken over a long period of time, transdermal delivery systems may provide a useful drug therapy with regard to patient compliance. Hence, in the present study we plan to formulate transdermal patch of Hydroxypropyl methyl cellulose and Ethyl cellulose encapsulating Repaglinide for transdermal delivery.

3. MATERIALS AND METHODS

Repaglinide was obtained as a gift sample from M/s Torrent Pharmaceutical, Gujarat, India. Triethyl citrate was procured from Hi Media Laboratories Pvt. Ltd, Mumbai. All other reagents and chemicals were of analytical grade.

4. Pre-formulation studies

1. Physical Appearance: It was noted by visual observation

2. Melting Point: A capillary melting point apparatus (Lab-Hosp Corporation, Mumbai) was used to determine the melting point of the drug (Table 4.1).

3. Determination of wavelength maxima (λ_{max}): Accurately weighed 10 mg of Repaglinide was dissolved in small quantity of methanol and the volume was made up to 10 ml of methanol in a 100 ml volumetric flask. Then 1 ml of this stock solution was pipette out into a 10 ml volumetric flask and volume was made up to 10ml with methanol. The sample solution was then scanned between 200-400 nm using Shimadzu 1700 UV-visible spectrophotometer to determine the absorption maxima (λ_{max}) (Fig. 4.1).

4. Infrared Spectroscopy: The infrared spectrum of any compound or drug gives information about the functional groups. It was done by making pellets of the drug in KBr. IR spectra was taken by FTIR spectrophotometer between 500 to 4000 cm^{-1} (Shimadzu 8400, Japan). The obtained IR spectrum with various peaks was interpreted for different functional groups in present compound against reference IR of Repaglinide.[4] Important band frequencies in IR spectra of Repaglinide are shown in Table 4.2 and spectra of reference and sample drug are presented in Figure 4.2 and 4.3.

ANALYTICAL EVALUATION

Standard Curve of Repaglinide in Phosphate Buffer Solution (pH 7.4) at λ_{max} 238 nm

Phosphate buffer solution of pH 7.4 made as per formula given in monograph of Indian Pharmacopoeia. Accurately weighed 10 mg of Repaglinide was dissolved in small quantity of methanol and the volume was made up to 100 ml with phosphate buffer solution pH 7.4. This resulted in preparation of stock solution of concentration 100 µg/ml. From this resultant stock solution aliquots of 1.0, 2.0, 3.0 ….up to 5.0 ml respectively was withdrawn using pipette into a series of 10 ml volumetric flasks of same specification and volume was made up to 10 ml with PBS (pH 7.4) resulting in solutions of concentration10,20,30…50µg/ml respectively. The solutions were then analysed at wavelength maxima (λ_{max}) 238 nm using UV-visible spectrophotometer (Shimadzu 1700, Japan). [5,6] The standard curve was plotted between absorbance and concentration (Fig 4.4, Table 4.5).

Table 4.1

Physical Appearance	**White or almost white powder**	**Fully complied**
Melting point	132°C-135°C	133°C
UV spectroscopy	The UV absorption maxima of Repaglinide in methanol exhibits maximum at 238 nm	Fully complied
IR spectroscopy	Spectrum show relatively broad structure indicating complexity of the molecule.	Fully complied

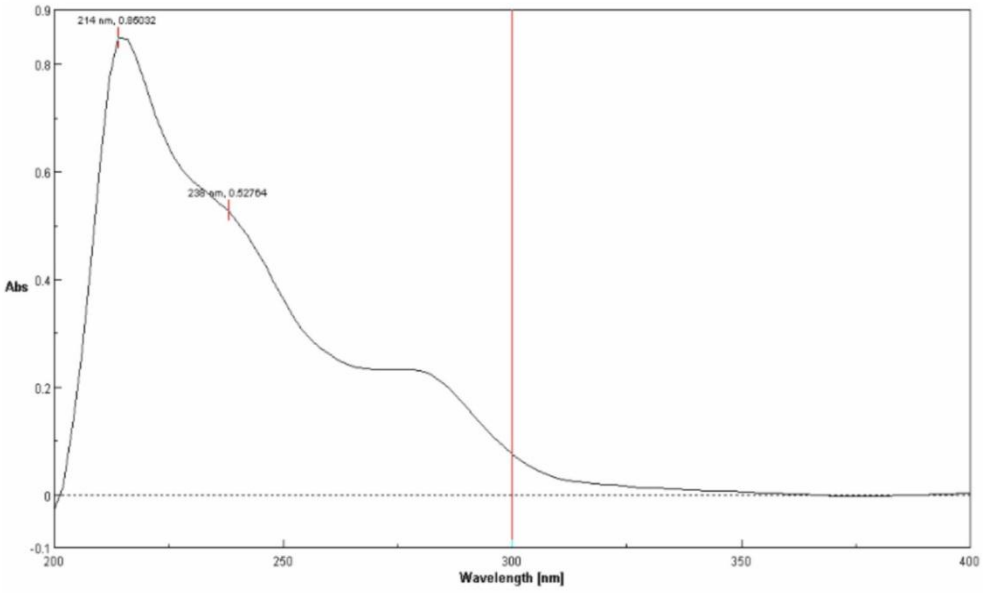


Fig. 4.1: UV scan of Repaglinide in methanol.

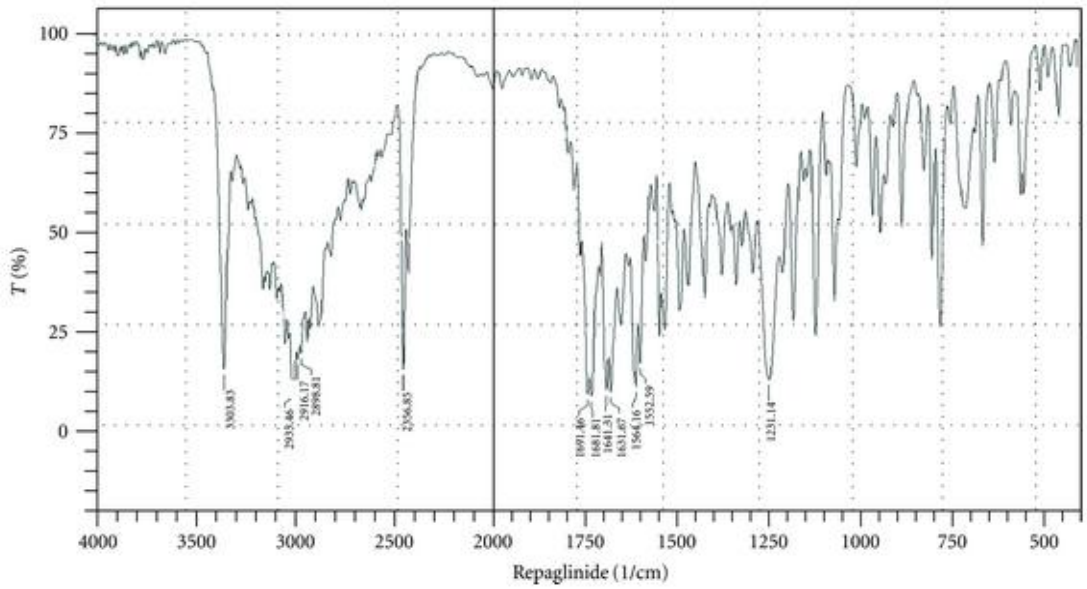


Fig. 4.2: IR spectra of Repaglinide (Reference).

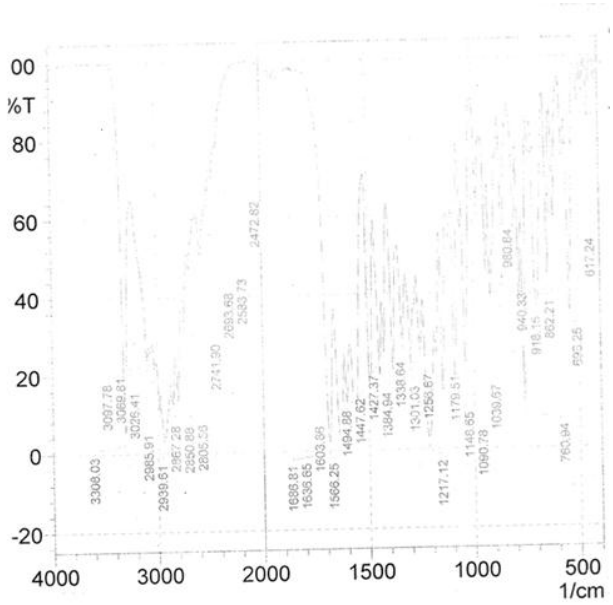
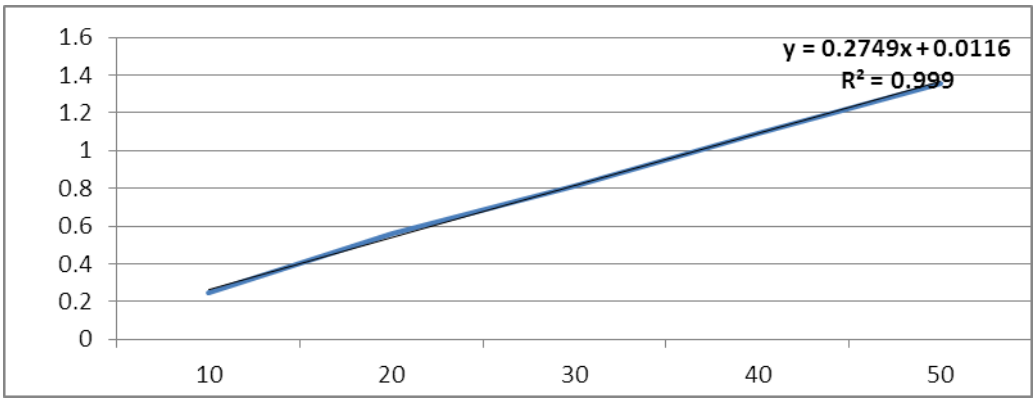


Figure 4.3: IR spectra of Repaglinide (Sample).

S. No.	Concentration (µg/ml)	Absorbance	Statistical Parameter
1.	10	0.2462	**Correlation coefficient** $R^2 = 0.999$ **Straight line equation** y = 0.2749x+0.0116
2.	20	0.5631	
3.	30	0.8104	
4.	40	1.0872	
5.	50	1.3586	

Standard Curve of Repaglinide in Phosphate Buffer Solution (pH 7.4) at λ_{max} 238 nm



5. Development of Transdermal patches

Films composed of different ratios of Hydroxy propyl methyl cellulose and Ethyl cellulose (total polymer weight = 600 mg) were prepared by solvent casting method. HPMC and EC were weighed and dissolved in 10 ml of an equal volume of methylene chloride and methanol (5:5 ratio) to form a 6% w/v solution which was then plasticized with triethyl citrate. Repaglinide (15 mg) was added to the above polymer solution under mild agitation until the

drug dissolves. The resultant solution was poured in a glass Petri dish of 61 cm^2 area, dried at room temperature for 24 hrs and subsequently oven dried at 45° C for 30 min to remove the residual organic solvents. HPMC: EC patches were locked in self-sealable bags and stored in the desicator.[7,8] The composition of different patches is enlisted in Table 5.1.

Table 5.1: Composition of Repaglinide Transdermal Patches.

Formulation Code	Polymeric Blend	Drug (mg)	Ratio (w/w)	Plasticizer Tetra ethyl citrate	Permeation enhancer	Solvent system
A	HPMC:EC	15	5:5	30% TEC	5%	Methylene chloride:Methanol(5:5)
B	HPMC:EC	15	6:4	30% TEC	5%	Methylene chloride:Methanol(5:5)
C	HPMC:EC	15	7:3	30% TEC	5%	Methylene chloride:Methanol(5:5)
D	HPMC:EC	15	8:2	30% TEC	5%	Methylene chloride:Methanol(5:5)
E	HPMC:EC	15	9:1	30% TEC	5%	Methylene chloride:Methanol(5:5)
F	HPMC:EC	15	10:0	30% TEC	5%	Methylene chloride:Methanol(5:5)

6. Evaluation of Transdermal patches[9,10,11]

Folding Endurance: Folding endurance of the film was determined manually by folding a small strip of the film (4x3 cms) at the same place till it breaks. The number of times the film could be folded at the same place without breaking gave the folding endurance value, where the cracking point of the films were considered as the end point. Results are reported in Table 5.2.

Thickness: The thickness of the film was measured using Digital screw gauge micrometer. The thickness was measured at five different points of the film and the average of five readings were calculated. Results are reported in Table 5.2.

Weight uniformity: This was done by weighing five different patches of individual batch of the uniform size at random and calculating the average weight of five. The tests were performed on films which were dried at 60° C for 4hr prior to testing. Results are reported in Table 5.2.

Drug content: Transdermal patches of area 5.0 cm^2 was cut into and taken into 50 ml volumetric flask, 25 ml of phosphate buffer pH 7.4 was added and gently heated to 45° C for

15 min and kept for 24 hrs with occasional shaking. Then the volume was made upto 50 ml again with phosphate buffer pH 7.4 and further dilutions were made from this solution. Similarly, a blank was carried out using a drug free patch. The solutions were filtered and absorbances were read at 238 nm by UV spectrophotometer.[12,13] Results are reported in Table 5.2.

Table 5.2: Evaluation of Repaglinide Transdermal Patches.

Formulation code	Weight (gm)	Folding Endurance*	Thickness (mm)*	Drug Content (%)*
A	0.566	148±13	0.0910±0.0168	95.74±0.5703
B	0.592	156±17	0.0973±0.0283	96.34±1.0079
C	**0.597**	**196±12**	**0.0923±0.0155**	**98.77±0.5972**
D	0.637	187±11	0.1000±0.0174	96.45±0.5950
E	0.612	185±15	0.1037±0.0232	97.27±0.4022
F	0.603	190±10	0.1183±0.0293	96.72±1.270

* Values represents mean ± S.D (n=3)

***In vitro* diffusion study:** A modified Franz diffusion cell was used to study the *in vitro* release profile from prepared formulations. 20 ml of phosphate buffer of pH 7.4 was used as diffusion medium. The transdermal patch was placed in between the donor and receptor compartment in such a way that the drug releasing surface faced towards the receptor compartment. The receptor compartment was filled with the diffusion medium, a small bar magnet was used to stir the diffusion medium at a speed of 60 rpm with the help of magnetic stirrer. The temperature of diffusion medium was maintained and controlled at 37± 1°C by a thermostatic arrangement. An aliquot of 5 ml was withdrawn at predetermined intervals replaced by equal volumes of the diffusion medium, diffusion studied were performed for period of 8 hrs. The drug concentration in the aliquot was determined spectrophotometricallyand calculated with the help of standard calibration curve.

***Ex vivo* drug release:** This study was carried out across the porcine ear skin using a Franz diffusion cell. The transdermal patch was applied on the which epidermal layer of skin acted as donor compartment and the dermal side of skin was facing receptor compartment. The receptor cell contained phosphate buffer of pH 7.4 as the diffusion medium. The medium was magnetically stirred for uniform drug distribution and was maintained at 37±1°C. The samples were withdrawn every hour upto 8 hours and estimated spectrophotometrically after suitable dilutions to determine the amount of drug release.[14,15]

***In vivo* studies**

The word *in vivo* comes from the Latin term "in life" and refers to a medical test, experiment or procedure that is done on living organism, such as a laboratory animal or human. *In vivo* studies are important to evaluate the physiological availability of drug from a designed dosage form.[16]

Skin irritation test

Skin irritation test was performed on two healthy albino rabbits weighing in between 2.0 to 3.5 kg. Aqueous solution of formalin (0.8%) was used as a standard irritant. Drug free polymeric patches of 5.0 cm^2 were used as a test patches. 0.8% of formalin was applied on the left dorsal surface of each rabbit, where as the test patch was placed on the identical site, on the right dorsal surface of the rabbit. Similar process was repeated for application of drug loaded patches. The patches were removed after a period of 24 hours with the help of alcohol swab. The skin was examined for erythema. The values are tabulated in Table 6.1.

Table 6.1: Skin irritation test for drug free and drug loaded transdermal patches.

S. No	Control	Transdermal patch	
		Without drug	with drug
1.	+	_	_

+ = Well defined erythema -= No erythema

Anti-diabetic Activity[17,18]

Studies in normal rats

Male wistar rats weighing between 200-250 g were choosen for the study. Hairs from the neck region of animals was removed using a depilatory cream one day before conducting the experiment. Following an overnight fast rats were divided into 3 groups (n=3). The rats were treated as follows:

Group I (control): Normal

Group II **:** Repaglinide (oral route)

Group III **:** Repaglinide loaded transdermal patches

The patch was applied to the previously shaven area which is in initimate contact with stratum corneum. The top surface of the patch was covered with an aluminium foil and finally with an adhesive tape to keep the patch secured at the site of application. The values are tabulated in Table 6.2.

Studies in diabetic rats

To induce diabetes animals were fasted for 18 hrs and later rendered diabetic by injecting alloxan (150mg/kg), i.p. the blood glucose level were measured after 3 days and animals with blood glucose levels > 250 mg/ dl were selected. The experimental protocol used in normal rats was followed for the assessment of hypoglycemic activity. The values are tabulated in Table 6.3.

Table 6.2: Reduction in blood glucose levels (mg/dl) after oral and transdermal administration of drug loaded transdermal patches in normal rats.

Group	Absolute blood glucose level	2 hr	4 hr	6 hr	8 hr	12 h	24 h
I	85.90±1.565	85.65±1.045	85.30±1.282	84.32±4.305	83.69±1.167	82.69±1.532	82.59±1.319
II	84.10±1.679	84.01±1.906	84.09±1.839	83.96±1.416	79.91±1.726	76.12±0.785	74.24±0.566
III	83.52±1.187	82.55±1.319	82.50±0.780	80.74±1.093	78.00±1.011	75.15±0.840	70.60±0.986

Table 6.3: Reduction in blood glucose levels (mg/dl) after oral and transdermal administration of drug loaded transdermal patch in diabetic rats.

Group	Absolute blood glucose level	2 hr	4 hr	6 hr	8 hr	12 h	24 h
I	376.3±2.090	368.4±2.110	369.4±2.230	370.4±2.103	372.3±2.181	374.2±2.221	381.4±2.238
II	373.0±5.884	360.2±5.992	338.5±5.347	319.5±6.147	300.9±5.366	269.1±6.477	211.5±4.817
III	371.2±7.382	356.7±9.515	290.5±11.89	264.2±7.888	233.3±7.916	206.1±8.391	181.8±7.543

7. Stability studies

Stability studies were conducted for the optimized formulation C.Stability studies were carried out by placing the formulation in amber colored bottles, tightly plugged with cotton and capped. Stability study of the prepared formulation was performed at different temperature and relative humidity like (25^0C/60% RH) and (40^0C/75% RH). The formulation was analyzed for folding endurance and drug content at a time interval of 15, 30, 60, 90 days.[19,20] (Table 7.1, Fig.7.1, Fig 7.2).

Table 7.1: Effect of Storage Conditions [(25^0C/60% RH) and 40^0C/75% RH)] on Folding Endurance, and Drug Content.

S. No.	Parameters	Period (Days)	Storage conditions	
			25^0C/60%RH	40°C/75%RH
1.	Folding endurance	0	205	205
		15	204	201
		30	201	199
		60	201	195
		90	200	194

2.	**Drug content (%)**	0	98.75	98.33
		15	98.75	97.36
		30	98.75	96.33
		60	98.36	94.85
		90	97.52	94.63

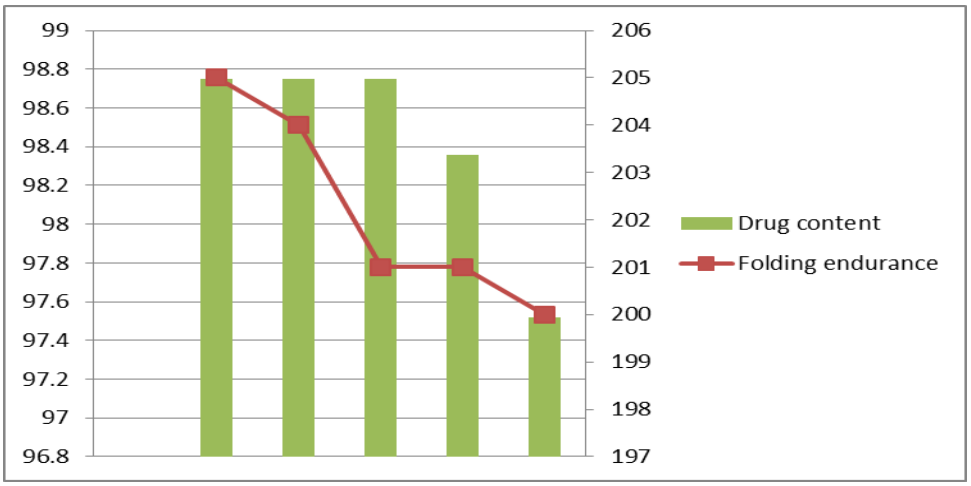


Fig. 7.1: Effect of Storage Conditions (25^{0}C/60% RH) on Folding Endurance and Drug Content.

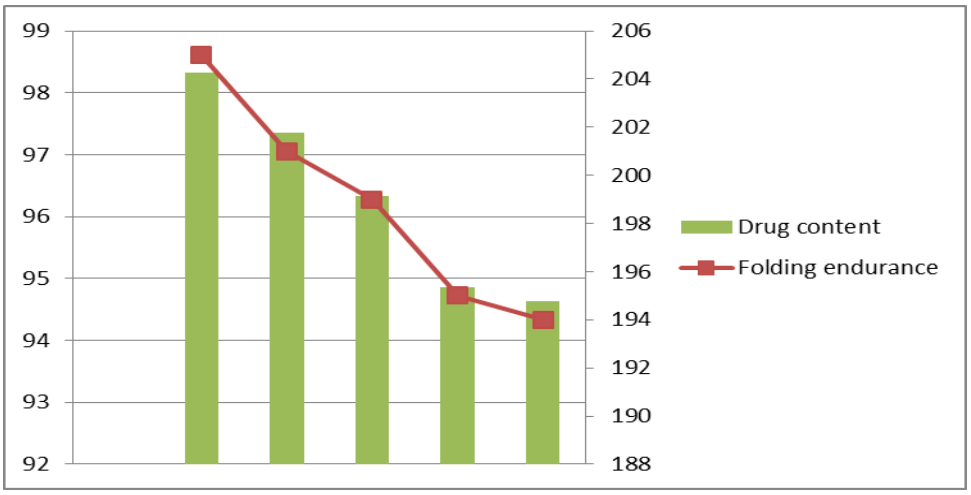


Fig. 7.2: Effect of Storage Conditions (40^{0}C/75% RH) on Folding Endurance and Drug Content.

CONCLUSION

In the present study, an attempt was made to deliver antidiabetic drug, Repaglinide through the transdermal route in the form of transdermal patches. Results revealed that prepared patches showed good physical characteristics, no drugpolymer interaction and no skin irritation was observed. In this study, different matrix type patches were prepared by varying polymer combination and polymer ratios. Transdermal patches were prepared in different

concentration of Hydroxy propyl methyl cellulose and Ethyl cellulose by solvent casting method. The ratio of Hydroxy propyl methyl cellulose and Ethyl cellulose used are 5:5, 6:4, 7:3, 8:2, 9:1, 10:0. Patches casted with triethyl citrate at 30% w/w were found to have good physical properties like flexibility and elasticity. *In vitro* diffusion study were carried out using formulation C by making use of Franz diffusion cell. 76.32±0.54% of drug was released from the formulation after 8 hours. formulation C was selected as best formulation and was stable for 60% RH. *Ex vivo* studies was carried out using porcine ear skin. It was observed that about 68.46±0.53 % of drug was released after 8 hrs. The % of drug release was less from *ex-vivo* studies which may be due to barrier effect exerted by the layer of skin. The optimized evaluation was further evaluated for anti-diabetic activity and skin irritation studies.

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