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Research Article

DEVELOPMENT AND EVALUATION OF NOVEL REPAGLINIDE BIO STRIPS FOR TRANSLABIAL DELIVERY

N.V. Satheesh Madhav, Abhay Pratap Yadav*

Novel drug Delivery Research Laboratory, Faculty of Pharmacy, Dehradun Institute of Technology University, Dehradun (UK), India

*Corresponding Author Email: mpharm_abhay@yahoo.com

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ABSTRACT

The aim of this study was to explore the potentiality of lip skin as a novelistic Translabial drug delivery platform due to its unique histology and by formulating drug loaded bio lip strips using novel bio-excipients which was isolated from the seeds of *zea mays* by simplified economical process and purified by hot dialysis method. Repaglinide loaded bio-lip strips were formulated by using *zea mays* bio-material as a strip former and dextrose as a flexicizer. The formulated strips were subjected for various evaluation parameters like thickness, folding endurance and *in-vitro* and *In-vivo* drug release. Present study revealed that the biopolymer possessing promising strip ability. The Repaglinide release was extended over a period of 24 hrs and by fitting the drug release data into zero order, first order and Higuchi model, it was concluded that drug release from strips followed Higuchi model and the mechanism of the drug release was diffusion and anomalous type. On the basis of above parameters and used concentration of biopolymer in the formulation, F2 was selected as the best formulation. The *in vitro* and *in-vivo* studies have shown the potential of the Repaglinide loaded bio strip for sustained delivery through Translabial route.

Key words: Translabial, Repaglinide, *zea mays*, release kinetics, strips.

INTRODUCTION

The lips or labium oris are two fleshy folds surrounds the orifice of the mouth. They are composed of skin, muscle and mucosa. Lip skin consisting of flat, scale-like cells in three to five cellular layers. The skin of the lips has no hair follicles, no sweat glands and no sebaceous glands. The mucous membrane of the lip is full of capillaries (tiny blood vessels) that are close to the translucent surface, giving it a reddish color. Lip skin composed of stratified squamous epithelium tissue, which only means that the cells are arranged in layers [1]. In this research paper a pioneering attempt was made for using this novelistic platform as systematic delivery of drugs by considering its inbuilt properties. The trans- labial application of drugs provides several benefits; including the avoidance of hepatic first-pass effects, Frequency of administration and the near constant drug delivery over a long period which may reduce systemic side effects[2-5]. However, the skin forms an excellent barrier against drug permeation, due to the rigid lamellar structure of the stratum corneum lipids. Our novel lip drug delivery sidesteps this barrier due to very less layers of stratum corneum. Repaglinide is a non-sulfonyl urea oral hypoglycemic agent. It is used in the management of type II diabetes mellitus[6,7]. It lowers blood glucose by stimulating the release of insulin from the pancreas by closing ATP-dependent potassium channels in the membrane of the beta cells. This depolarizes the beta cells and opening the calcium channels and the resulting calcium influx induces insulin secretion. Repaglinide, a BCS class II compound i.e. poorly soluble but highly permeable, and they exhibit bioavailability that is limited by dissolution rate[8]. Repaglinide having low oral bioavailability (56-63%) due to extensive hepatic first pass metabolism[9] and extremely short half life 1 hour. These properties make it suitable for Translabial delivery. *Zea mays* belong to family poaceae. Seeds of *Zea mays* composed of carbohydrates, sugars, dietary fibers, fat, protein, tryptophan, lucine, lycine and vitamins. It is used as a diuretic, reducing stone formation in kidney and so many medicinal uses.

The aim of our research work was to isolate and characterize *zea mays* biomaterial along with formulation and evaluation of bio lip strip of Repaglinide using natural biomaterial (ZB) and to evaluate its bioadhesitivity and strip forming capability for lip as a site for drug delivery.

MATERIALS AND METHODS

Repaglinide (assigned purity, 99.8%) was a gift sample from M/S Torrent Pharmaceuticals Ltd., Ahmedabad, India. Seeds of *zea mays* seeds were procured from market of Dehradun (Uttarakhand), India. Sodium carboxy methylcellulose (Na-CMC) and Hydroxy propyl methyl cellulose (HPMC) were purchased from Merck Specialties Private Limited, Mumbai, India. All other chemicals and solvents were of analytical grade.

Isolation of *Zea mays* bio material

Zea mays seeds were procured from the local market. 100g of *Zea mays* seeds were treated with 600 ml of double distilled water and stirred with mechanical stirrer at 4000 rpm for 60 minutes. The mixture was subjected for centrifugation (Remi) at 4000rpm for 30 minutes. The supernatant liquid was pooled and treated with twice the volume of acetone and the mixture was kept in a refrigerator for 12 hours. The mixture subjected for centrifugation at 5000 rpm for 30 minutes. The bio material was recovered by discarding the supernatant liquid and was dried in vacuum dessicator for a period of 10 hours. The bio material was purified by hot dialysis method using ORCHID scientific dialysis apparatus for complete removal of impurities like Chlorides and sulfates. The procedure was optimized by repeating the procedure for 6 times and the percentage yield was calculated and reported. The purified bio material was screened through 200# and stored for further research work.

Preparation of Bioadhesive Lipstrip

Accurately 100mg of *Zea mays* biopolymer was weighed and transformed into the mortar, to this 110mg of dextrose was

added and triturated the mixture for a period of 5minutes after that 5mg of Repaglinide was incorporated in geometrical dilution pattern. Further 10ml of double distilled water was incorporated by adding drop by drop to the mixture with constant trituration. The mixture was subjected for magnetic stirring for a period of 10 minutes and sonicated at 400 Hz for 3 cycles of 60seconds each in order to form a colloidal mixture. The colloidal mixture was poured into Petridis having 6cm diameter and subjected for evaporation at room temperature for a period of 10hrs. Dried strips were carefully removed and it was cut into 2X2cm^2 and strips were placed over the adhesive backing membrane. Similarly six formulations (F1-F6) were prepared by varying the concentrations of *zea mays* polymer (Table no.1). Two standard formulations (F7 and F8) were also prepared using Na Alginate and Sodium carboxy methyl cellulose.

Drug- Excipient interaction study

The pure drug along with formulation excipients were subjected to interaction studies. The study was performed by using FT-IR spectroscopy. It was performed by mixing/grinding definite proportions of drug and excipients with a specially purified salt (potassium bromide) finely (to remove scattering effects from large crystals). The powder mixture was then pressed in a mechanical press to form a translucent pellet through which the beam of the spectrometer can pass. The FT-IR peaks were found and reported.

Weight uniformity study

Weight uniform study for all formulated bio-lip strips was performed by taken three randomly selected bio-lip strips from each formulation with surface area 1cm^2 were used. Each strip was weighed individually on electronic balance. The study was performed thrice and average weights were calculated and registered[10,11].

Content uniformity

All formulated bio-lip strips were evaluated for its drug content uniformity. From each formulation the randomly selected strip (1cm^2) was transferred into a 100ml volumetric flask containing 7ml of phosphate buffer of pH7.4 and 1ml of methanol. The flask was stirred for 4 hrs on magnetic stirrer. A blank was prepared by using a drug free patch treated similarly. The solutions were filtered through a 0.45micro meter membrane. The drug content was then determined after proper dilutions by using an UV spectrophotometer (Shimadzu 1800)[10,11].

Folding endurance

Folding endurance for all bio-lip strips containing Repaglinide was performed by using a strip of area 4cm^2 from each formulation. The selected bio-lip strip was subjected to folding endurance by repeatedly folding a strip at the same place until it broke. The number of folding required to break or crack a strip was taken as the folding endurance. This test was repeated thrice and overcomes was noted[12,13].

Swelling index

Swelling study of all formulated bio lip strip was calculated by taken a bio strip from each formulation of size 1 cm^2 .the bio-lip strip was weighed on a pre weighed cover slip. It was kept in a Petri dish and 10 ml of phosphate buffer of pH 7.4 was transferred. After one hour, the cover slip was removed and weighed. The difference in the weights gives the weight increase due to absorption of water and swelling of bio-film. The change in weight was noted after 24 hrs. The procedure was repeated thrice and swelling index(S) was determined by using below formula.

$$\% S = (Xt\neg - Xo / X0)\ 100$$

Where, Xt - weight of the swollen bio strip after time t and
Xo - original weight of bio strip[14]

Percentage moisture absorption (PMA)

Percent moisture absorption study for all formulated bio-lip strips was conducted by taking a 1cm^2 of Repaglinide loaded bio-lip strips. The bio-lip strips were transferred into a watch glass and it was placed in dessicator containing saturated solution of Aluminium chloride and kept a side for 72hrs. At the end the weight gained by the strip was determined. The study was repeated thrice and percentage moisture absorption calculated by using the below mentioned formula[15].

Percentage moisture absorption=
[(final weight – initial weight)/initial weight] X 100

Percentage moisture loss (PML)

Percentage moisture loss study for all formulated bio lip strip was performed by taking three 1cm^2 strips from each formulation. The strips were cut out and weighed accurately and kept in dessicator containing fused anhydrous calcium chloride for 72 hrs. At the end the weight loss by the strips were determined. The study was repeated thrice and percentage moisture loss calculated by using the below mentioned formula and reported[15].

Percentage moisture loss =
[(initial weight – final weight)/ initial weight] X 100

Surface pH study

The surface pH of the bio lip strips containing Repaglinide was determined by using a glass electrode. The bio lip strips was allowed to swell by keeping it in contact with 0.5 ml of distilled water for 1 hour at room temperature. The pH was measured by bringing the electrode in contact with the surface of the bio strip and allowing it to equilibrate for 1 minute. The experiments were performed in triplicate and average values were noted[16].

Water vapor transmission test (WVT)

WVT defined as quantity of moisture transmitted through unit area of strip in unit time. Glass-bottle (length= 5 cm, narrow mouth with internal diameter =0.8 cm) filled with 2 g anhydrous calcium chloride and an adhesive (Feviquick®) spread across its rim, was used in the study. The bio strip was fixed over the adhesive and the assembly was placed in dessicator in which 200 ml of saturated sodium bromide and saturated potassium chloride solution were placed. The desiccator was tightly closed. The weighed bottle was then placed in dessicator and procedure was repeated[17,18].

$$WVT = W / ST$$

W is the increase in weight in 24 h; S is area of strip exposed (cm^2);
T is exposure time.

Skin Irritancy

Primary skin irritation studies were conducted with best two optimized patch in four rabbits. Rabbits were divided into two groups of two animals. Blank strip were applied on the lip of rabbits of group I which served as control and rabbits of group II received medicated strips on their lip. Strips were changed after 6hrs with fresh strips. The study was carried

out for a period of 7 days and application sites were graded for redness, erythematic or irritation visually[19].

In-Vitro Diffusion study

The *In-Vitro* drug diffusion was carried out in the M.S. diffusion apparatus[20]. This was the static method and employed complete replacement of the sample. Dialysis membrane was tied to the terminal portion of the cylindrical donor compartment. A 1cm^2 bio-lip strip was kept above the dialysis membrane in the donor compartment, and the receiver compartment was filled with 13 ml of diffusion medium. The complete sample was withdrawn at different time intervals and the receiver compartment was refilled with 13 ml of fresh medium. The amount of drug released was assessed by measuring the absorbance at 247nm using UV spectrophotometer (Shimadzu 1800).

In-vivo release study

The *in-vivo* release was performed in rabbits for the optimized formulation. The bio-lip strip was applied to the lip of rabbit and blood samples were taken from the ear vein at intervals of 2, 6, 10, 12 and 24hours to determine the concentration of drug in the blood plasma. Plasma was separated immediately by using centrifugation at 3000xg for 10min. The plasma was treated with 5ml methanol of HPLC grade, subjected for sonication for 5 cycles and filtered through membrane filter[16]. The drug content was estimated by injecting the filtrate into the HPLC column using methanol and phosphate buffer of pH 7.4 as mobile phase at a rate of 1.2ml/min. The plasma concentration of Repaglinide at different time intervals was subjected to pharmacokinetic analysis to calculate various parameters like Cmax, Tmax and area under the curve (AUC). The value of Cmax and Tmaxwere were read directly from the arithmetic plot of drug plasma concentration VsTime. The AUC was calculated by using the trapezoidal rule.

Stability studies

Optimized bio lip strip was subjected to stability study. Bio strips were wrapped in Aluminum foil and packed them in glass vials. These strips were kept in an incubator (stability study chamber) maintained at 37±5°C and 75±5%R.H. for six months. The change in appearance, physical characteristics and release behavior of the stored strips were investigated after 1-6 months. The data presented were the mean of three determinants[17].

Table1: Composition of various batches of Repaglinide loaded bio-lip strips

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Repaglinide (mg)	5	5	5	5	5	5	5	5
Zea mays biopolymer (mg)	100 (1%)	200 (2%)	300 (3%)	400 (4%)	500 (5%)	600 (6%)	--	--
Sodium carboxy methyl cellulose (mg)	--	--	--	--	--	--	400 (4%)	--
Sodium alginate (mg)	--	--	--	--	--	--	--	400 (4%)
Dextrose (mg)	110	110	110	110	110	110	110	110
Double distilled water(ml)	10	10	10	10	10	10	10	10

Table 2: Different parameters of the model equations on the in-vitro release kinetics

Formulation Code	Zero order R^2	First order R^2	Higuchi model R^2	Korsmeyer Peppas	
				R^2	n
F1	0.6187	0.7755	0.9290	0.9343	0.6667
F2	0.5677	0.8052	0.9356	0.8945	0.6340
F3	0.6375	0.7892	0.9213	0.8989	0.6104
F4	0.8003	0.9121	0.9160	0.9450	0.8463
F5	0.8540	0.9894	0.9170	0.9827	0.7981
F6	0.7208	0.8510	0.9214	0.9610	0.6796
F7	0.7918	0.9280	0.9157	0.9734	0.6472
F8	0.7062	0.8696	0.9103	0.9539	0.5224

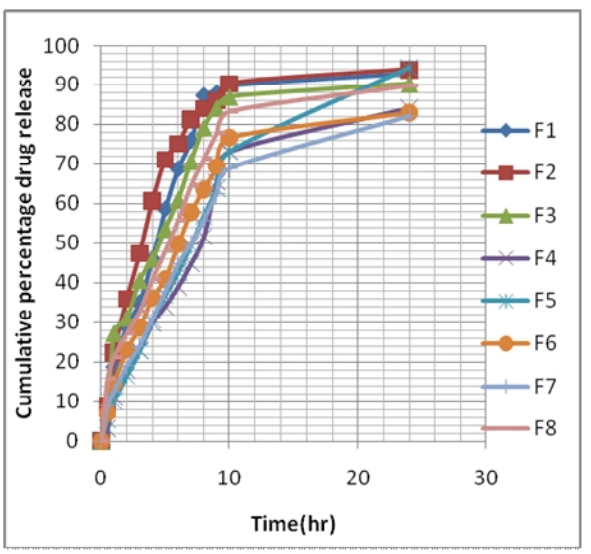

Figure 1: *In-Vitro* release study of Repaglinide

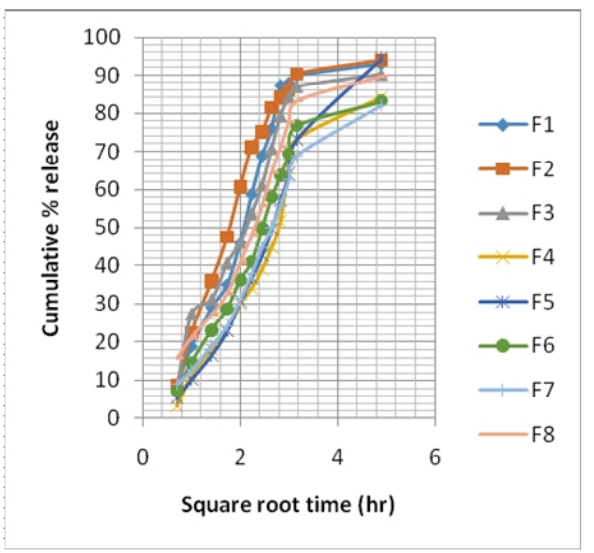

Figure 2: Higuchi plot of Repaglinide loaded bio lip strips

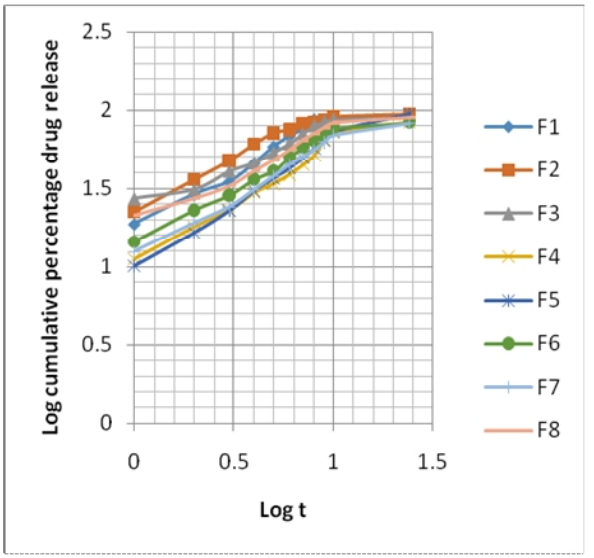

Figure 3: Korsmeyer-Peppas model for *in-vitro* release of Repaglinide loaded Bio lip strips

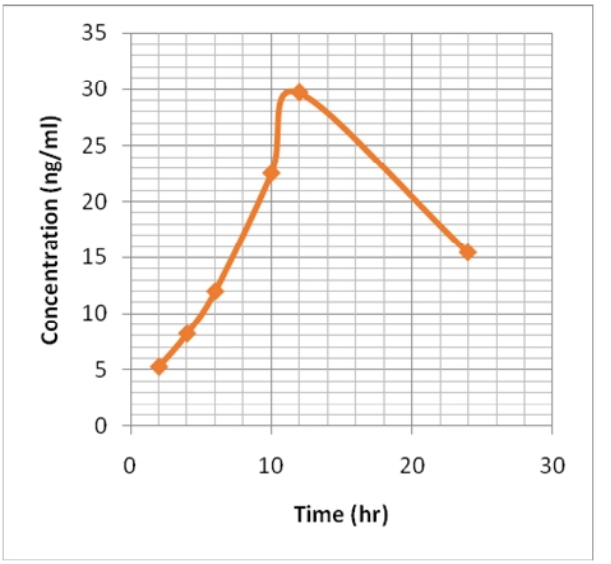

Figure 4: *In-Vivo* release study of Repaglinide

RESULTS AND DISCUSSION

Drug-Excipients Interaction study

FT-IR study revealed that presence of intact Repaglinide functional groups (Ketonic, alcoholic, secondary amine etc.) were shown in the FT-IR spectrum which clearly indicates that the biopolymer is not reacting and compatible with Repaglinide. Based on the two out comings all the biomaterials were used as bio-excipients for designing the bio-lip strips.

Thickness, Swelling index, Surface pH and folding endurance

The average thickness of all prepared bio-lip strips ranged from 0.36±0.02 to 0.52±0.015mm. Weight variation values of all strips (1 cm^2) were found in the range of 22.54±0.38 to 36.53±0.34mg. Thus the proportional gain in weight of strips was observed as the thickness of strips increased. The values were uniform for the strips within the respective group of formulation type. This depicts that the strips cast was uniform. The range of swelling index for bio-strips was found to be 79.81±0.32 to 134.57±0.58 .The swelling index of strips suggests they will cause minimal discomfort when in use. This property of strip has direct influence on release of drug. Surface pH for all formulations was found to range from 6.19±0.24 to 6.66±0.12. Since range of the pH of strip is near to the skin pH. No skin irritation was expected. The folding endurance of strips was found in the range of 99±4.9 to 144±3.8. High folding endurance values for strips indicates high mechanical strength of strips. This is highly desirable because it would not allow easy dislocation of the strips from the site of application or breaking of strip during administration.

Skin irritation, Moisture content, Moisture uptake, WVT and Content uniformity

No skin irritation, redness or erythema was observed during primary skin irritation studies with all formulations. The moisture content of the prepared formulation was low, which could help the formulation remain stable and reduce brittleness during long term storage. Moisture content of the bio-strips ranges from 0.38±0.056 to 1.23±0.098%. Moisture uptake for the bio-strips ranges from 4.35±0.32 to 2.87±0.46 %. The moisture uptake of the formulation was low which protect the formulation from microbial contamination and reduce bulkiness. The range of WVT for bio-strips was found to be 2.71±0.16 to 5.41±0.41. The range of content uniformity for bio-strips was found to be between 90.38±0.28 to 94.56±0.36 %. There was no significant difference in the drug content among the all bio-lipstrips, which indicated uniform dispersion of drug throughout the strips.

In-vitro release

In-vitro release of Repaglinide from different strips is shown in (Figure 08). Formulation F5 showed the maximum release of 94.44% at the end of 24hrs. Formulation F6 showed slower drug release, and showed maximum drug release of 83.03% after 24hrs (Figure 1). The release data of the tested strips were analyzed by BIT-SOFT 1.12: drug release kinetics with model fitting. Coefficients of correlation (R^2) were used to evaluate the accuracy of fit. In-vitro release profile of the formulations did not fit into either zero order kinetics (r^2 = 0.5677 to 0.8450) or first order kinetics (r^2 =0.7755 to 0.9121) except F5 and F7 which follow First order kinetics. However, the release profile of the formulated strips followed Higuchi equation (r^2 =0.9103 to 0.9356), which indicated that the permeation of drugs from the strips were governed by the diffusion mechanism (Table 2). We could not detect any relationship between the drug release profile and polymer composition may be due to release mechanism which governed by diffusion as well as erosion controlled, since our biomaterial is slightly soluble in water. All formulations showed non-Fickian drug release ($0.5<n<1$) (Table 3). Higuchi plot of Repaglinide loaded bio lip strips (Figure 2) represents sustained release of drug. Korsmeyer-Peppas model graph (Figure 3) showed that the drug release mechanism followed by diffusion as well as erosion of biomaterial. One-way analysis of variance applied on the *in-vitro* release obtained by Trans labial strip and they were found to be extremely significantly ($p<0.0001$) different. On the basis of above parameters and used concentration of biopolymer in the formulation, F2 was selected as the best formulation. The *in vitro* studies have shown that this is a

potential drug delivery system for Repaglinide with considerable good stability and release profile.

***In vivo* study**

Translabial administration of F2 bio-lip strip achieved Cmax of Repaglinide to 29.65ng/ml at a tmax of 12 hour. The AUC $_{(0\text{-}24hr)}$ was found to be 430.29ng.h/ml (Figure 4).

Stability study

At the end of stability study, the tested strips showed similar drug content as observed at the beginning of the study. They also showed insignificant difference for in-vitro drug release. All optimized strips showed satisfactory flexibility and elastic properties during and at the end of the accelerated stability period. These all indicated that there were no influences on the chemical and physical stability of the formulation during the test period.

CONCLUSION

In the present study bioadhesive bio-lip strips based on *Zea mays* biomaterial was developed, which released the drug over the required period of time (12 h) which would prevent first-pass metabolism. Thus, an attempt of formulating a stable bioadhesive bio-lip strip of Repaglinide for treatment of diabetes using novel biomaterial was made by optimization technique. Biomaterial showed good strip forming property as well as satisfactory bioadhesion. Thus, this natural biomaterial could be a promising excipient for systemic delivery of drugs through labial route and other transdermal route. The *in vitro* studies have shown that this is a potential drug delivery system for Repaglinide with considerable good stability and release profile. *In vivo* study also confirms these results.

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