

DESIGN AND EVALUATION OF INSULIN LOADED BIO-FILMS FOR TRANS SOFT-PALATAL TO BRAIN DELIVERY

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ABSTRACT:

The current objective of our study was the formulation and evaluation of insulin loaded trans-soft palatal bio-films for treating alzheimer's disease. A novel bio-film former was isolated from the fruit pulp of *Artocarpus heterophyllus* by our previously published method. The bio-films were aimed at delivery through the soft palate for brain targeting. The isolated bio-material was screened for filmability by forming films of the isolated bio-material by solvent casting method and mucoadhesivity by shear stress method. Six(FJ1-FJ6) formulations were prepared by varying the polymer: drug ratios(1:1,1:2,1:3,1:4,1:5,1:6) by solvent casting method. Insulin was used as the model drug as research reveals its stimulating effect on cognition. The formulated films showed uniform surface ph, thickness, weight variation and acceptable content uniformity, folding endurance, moisture uptake, moisture content, tensile strength. Mucoadhesion studies were performed by M.S. apparatus and Park and Robinson method. The formulation FJ3 was found to be the best formulation on the basis of mucoadhesion and *in-vitro* diffusion study. It showed mucoadhesion of more than 6 hours, t50% and t80% of more than 6hours and 10 hours respectively. A smart conclusion was drawn that the isolated bio-polymer can be used as an bio-film former for formulating various pharmaceutical preparations.

KEY WORDS: *Artocarpus heterophyllus*, insulin, bio-material, Alzheimers disease.

be easily fabricated into a number of pharmaceutical dosage forms and hence be used as novel drug delivery carriers. Jackfruit (*Artocarpus Heterophyllus*) belongs to the family moraceae, it contains morin, carotenoids, provitamin A. It is used medicinally as a laxative, tonic and demulcent. The manner in which abnormalities of insulin metabolism contribute to disorders of aging, and in particular to the pathogenesis of Alzheimer's disease has been a topic of recent interest. It has been observed that with direct intracerebroventricular administration of insulin in rodents, as well as with intravenous insulin administration in humans, which induces the transport of insulin into the CNS across the blood brain barrier (BBB) there has been an improvement in cognition. More recently the facilitation of memory in patients with Alzheimer's with intranasal insulin administration has been studied. A number of mechanisms may contribute to insulin-mediated memory facilitation, insulin receptors are present in key brain regions, such as hippocampus, entorhinal cortex, and frontal cortex. Insulin modulates levels of neurotransmitters such as acetylcholine, norepinephrine, and dopamine that play important roles in cognition. Insulin also affects membrane potentials, neuronal physiology, and long-term potentiation, all of which influence the synaptic remodeling processes thought to underlie memory formation. Hence it can be concluded that insulin is useful in the treatment of Alzheimer's disease with possible ways of brain targeting. In the current research we aim at targeting insulin into the brain via the trans soft palatal route by formulation of bio-films using a novel bio-polymeric film former from the unripened fruit pulp of *Artocarpus heterophyllus*.

INTRODUCTION

Biopolymers are polymers that are generated from renewable natural sources, are often biodegradable, and not toxic to produce. They offer the advantages of being bio-degradable, non-toxic and ability to bind with a number of drugs. They can

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Historically, oral transmucosal drug delivery has received intensive interest since ancient times for the most widely utilized route of administration for the systemic delivery of drugs. In more recent years many drugs have been shown to achieve a better systemic bioavailability by self medication through the oromucosal route than by oral administration.[1]

offers several advantages for controlled drug delivery for extended periods of time. The mucosa is well supplied with both vascular and lymphatic drainage and first-pass metabolism in the liver and pre-systemic elimination in the gastrointestinal tract are avoided. The area is well suited for a retentive device and appears to be acceptable to the patient.

TABLE NO.1 FORMULATIONS PREPARED

Formulation	FJ1	FJ2	FJ3	FJ4	FJ5	FJ6
Insulin(mg)	10	10	10	10	10	10
Jackfruit polymer(mg)	100	200	300	400	500	600
Dextrose(mg)	100	100	100	100	100	100
Distilled water(ml)	10	10	10	10	10	10

Among the various transmucosal sites available, soft palatal mucosa was also found to be the most convenient and easily accessible novel site for the delivery of therapeutic agents for systemic delivery as retentive dosage forms, because it has abundant vascularization, rapid cellular recovery time after exposure to stress, Smooth surface of the soft palate and its good flexibility are prerequisites to prevent mechanical irritation and local discomfort. Soft palatal medication delivers steady infusion of drugs over an extended period of times because of the function of soft palate is to cover the glottis while swallowing, it is more fitted for sustained and controlled drug delivery also due to the presence of immobile mucosa and lack of permeability in composition with sublingual mucosa. Even though the sublingual mucosa is relatively more permeable than the buccal mucosa but it is not suitable for an oral transmucosal delivery system because it lacks an expanse of smooth muscle and is constantly washed by a considerable amount of saliva making it difficult for device placement. Because of the high permeability and the rich blood supply, the sublingual route is capable of producing a rapid onset of action making it appropriate for drugs with short delivery period requirements with infrequent dosing regimen.[1] While buccal drug delivery has the low flux due to less permeability which results in low drug bioavailability and other drawbacks include salivary dilution of the drug, and the inability to localize the drug within a specific site (buccal, sublingual, or gingival) of the oral cavity. Therefore soft palatal drug delivery is feasible approach for correcting salivary dilution and achieve absorption site localization is to retain the drug on the mucosa using a bioadhesive system. The palatal mucosa

With the right dosage form design and formulation, the permeability and the local environment of the mucosa can be controlled and manipulated in order to accommodate drug permeation. Palatal drug delivery is a promising area for continued research with the aim of systemic delivery of orally inefficient drugs as well as a feasible and attractive alternative for non-invasive delivery of potent peptide and protein drug molecules[1].

MATERIALS AND METHODS

The drug insulin was obtained from the local market, novo nordisk insulin(40IU/ML) was used for the experiment. Jackfruit was procured from the local market. All other reagents used were of highest purity and analytical grade. Double distilled water was used throughout the experimental work.

Bio-material extraction

The bio-material was isolated from the fruits of artocarpus heetrophyllus from our previously published method. The isolated bio-material was subjected for physico-chemical characterization and spectral analysis.[2]

Formulation of insulin loaded bio-films:

The insulin loaded bio-films for trans soft-palatal drug delivery were prepared by solvent casting method using the bio-polymers and standard polymers in different ratios. The polymeric solutions were prepared in double distilled water with constant stirring. The polymeric solutions were filtered through a nylon gauze to remove debris and suspended particles. Dextrose was used as a plasticizer for the formation of films.[2] The resultant solution was left overnight at room

temperature to ensure a clear, bubble-free solution. The solution was poured into a glass petri dish having 6 cm diameter. 50mg/sqcm equivalent of insulin was added to each film. The backing membrane of ethyl cellulose was prepared for each

drying. The backing membrane was prepared and the bilaminate filme/patches were obtained.[3,4]

EVALUATION OF FORMULATED FILMS

The formulated films/patches were evaluated for

TABLE NO.2 A EVALUATION PARAMETERS

Formulation	Surface pH± S.D	Folding endurance± S.D	Weight uniformity mg± S.D	Content uniformity % ± S.D	Moisture content % ± S.D	Moisture uptake %± S.D	VTR g/cm ² /hr ± S.D
FJ1	6.50±.1	11± 2	21.42±.371	91.1± .65	1.21± .095	3.9± .02	7.47± 0.27
FJ2	6.53±.2	23± 1.1	34.3±.926	90.1± .35	1.41± .06	4.3± .15	7.98± 0.458
FJ3	6.36±.37	50± 8.6	41.4±1.07	95.4± .08	0.98± .065	3.8± .1	8.53± 0.32
FJ4	6.37±.2	74± 5.2	44.3±1.81	97.4± .05	0.85± .17	4.3± .35	8.17± 0.60
FJ5	6.83±.15	195± 8.4	48.9±.459	92.33± .074	0.87± .15	4.7± .07	8.43± 0.45
FJ6	6.80±.3	227± 6.6	56.3±.53	91.7± .1	1.13± .42	4.89± .056	8.67± 0.55

of the films. The dummy patch without drug was also prepared. The Petri dishes were kept on leveled surface and covered by inverted funnel to allow

the following evaluation parameters (table no. 5a and 5b)

TABLE NO.2 b EVALUATION PARAMETERS

Formulation	TENSILE STRENGTH g ± S.D	THICKNESS mm ± S.D	Mucretion time(rotating cylinder method) hr ± S.D	Mucretentive force (park and robinson method) g ± S.D	Mucretion time(M.S. APPARATUS) hr ± S.D
FJ1	74.07±4.3	0.37±0.05	3.5±0.1	3.51±0.64	5±0.25
FJ2	103.29±2.6	0.46±0.02	3.5±0.1	6.92±0.45	5±0.3
FJ3	145.13±2.4	0.54±0.04	4.5±0.2	9.54±0.92	6.5±0.65
FJ4	183.73±1.7	0.58±0.03	8±0.12	15.19±0.71	6±0.55
FJ5	209.52±1.5	0.64±0.05	8.4±0.15	18.63±0.53	8.6±0.5
FJ6	223.67±1.4	0.69±0.05	8.7±0.15	21.46±0.61	8.5±.35

controlled evaporation of solvent at room temperature till a flexible film was formed.[2] Dried films were carefully removed, checked for any imperfections or air bubbles and cut into patches of 1sqcm in diameter by using fabricated punch. The patch containing 50 mg of insulin was packed in aluminum foil and stored in an airtight glass container to maintain the integrity and elasticity of the patches.[2-4] (table no.4)

Preparation of backing membrane:

The backing membrane was prepared by dissolving ethyl cellulose in mixture of methylene chloride:ethanol(80:20),the formed films were coated with the ethyl cellulose solution by spreading the solution onto one side of the film and kept for air

Thickness:

The thickness of six randomly selected patches from every batch was determined using a standard digital micrometer. The average thickness was determined and reported with appropriate standard deviation. [3,4]

Weight uniformity study:

Weight uniformity of patch determined by taking weight of ten patches of sizes 1square cm diameter from every batch and weigh individually on electronic balance. The average weights were then calculated.[4]

Surface pH study

The surface pH of the patches was determined in order to investigate the possibility of any side effects

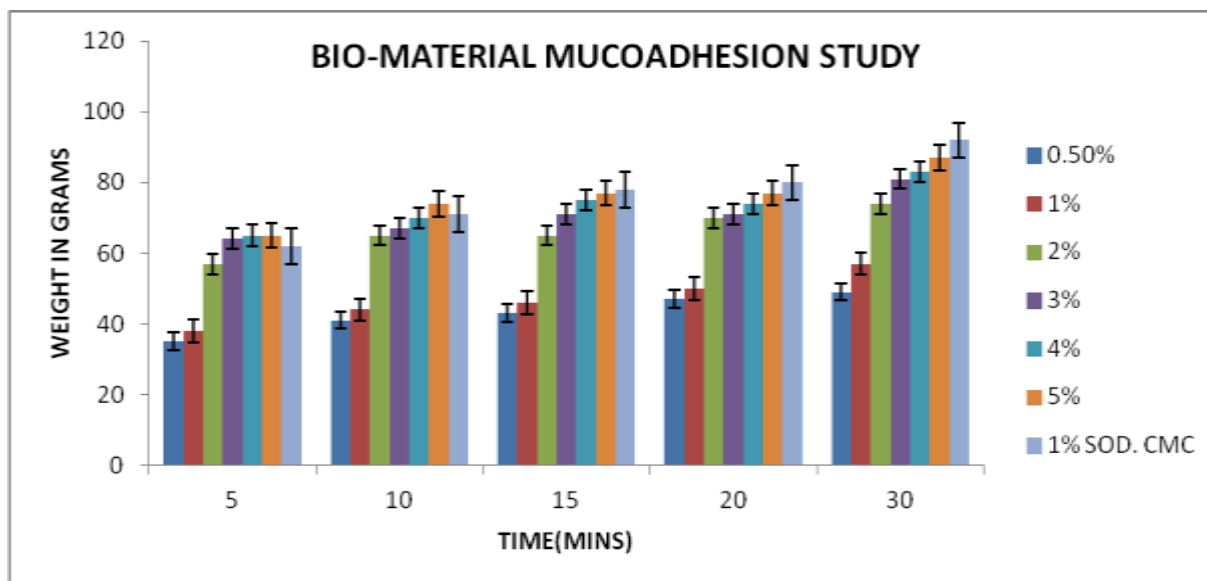


Fig no. 1: mucoadhesivity of the bio-material by shear stress method

in vivo. As an acidic or alkaline pH may cause irritation to the soft palatal mucosa, it was determined to keep the surface pH as close to neutral as possible. The method of Bottenberg et. al. was used to determine the pH, A combined glass electrode was used for this purpose. The patch was allowed to swell by keeping it in contact with 1 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 patch and allowing it to equilibrate for 1 minute. The experiments were performed in triplicate, and average values were reported.[5,6]

Content uniformity

Drug content uniformity was determined by dissolving the patch (1 sqcm in diameter) from each batch by homogenization in 100 ml of an isotonic phosphate buffer (pH 7.34) for 24 hr under occasional shaking. The 5 ml solution was taken and diluted with isotonic phosphate buffer pH 7.34 up to 20 ml, and the resulting solution was filtered through a 0.45 mm Whatman filter paper. The drug content was then determined after proper dilution using a UVspectrophotometer.[5-8]

Folding endurance

Folding endurance of the patch was determined by repeatedly folding one patch at the same place till it broke or folded upto 300 times manually, which was considered satisfactory to reveal good patch properties. The number of times of patch could be

folded at the same place without breaking gave the value of the folding endurance. This test was done on randomly selected three patches from each.[8,9]

TABLE NO. 3 *IN-VIVO* EFFECT ON COGNITION STUDY IN RATS

Time after administration	Time to complete rectangular maze task(sec) (test group)	Time to complete rectangular maze task(sec) (control group)
0	86±2	88±2
10mins	85±1	89±2
30mins	86±2	88±2
60mins	85±3	87±2
4 hrs	84±2	89±3
8hrs	81±1	89±2
24hrs	76±2	88±3
36hrs	74±3	89±2
48hrs	75±2	88±1

Swelling percentage study

Swelling study of prepared patch was calculated by function of weight and area increase due to swelling, which was measured for each formulation as follows. Weight increase due to swelling: A patch of size (1 x 1 cm²) diameter from every batch was

weighed on a preweighed cover slip. It was kept in a petridish and 10 ml of phosphate buffer, pH 7.34 was added. 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 patch. The study was conducted for 24 hours. The percentage swelling ratios was calculated from the average of three measurements using the following equation[2,5]:

$$\% S = (X_t - X_o / X_o) * 100$$

Where, X_t - weight or area of the swollen patch after time t and X_o - is the original patch weight or area at zero time.

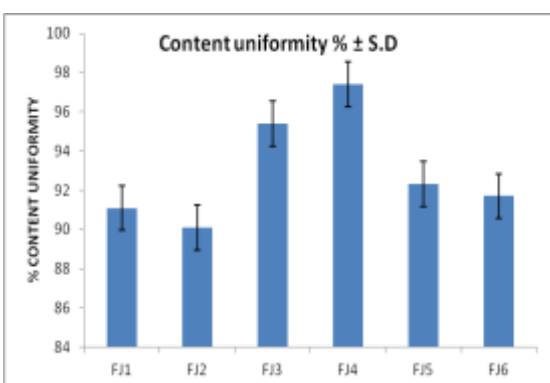


FIG. NO. 2 Content Uniformity of Formulations

Tensile strength

A tensile strength study of patch is total weight, which is necessary to break or rupture the dosage form and this was done by a device has rectangular frame with two plates made up of Plexiglas's 18, 19. The one plate is in front and is movable part of device and can be pulled by loading weights on the string, which is connected to movable part. The 1x1 cm² patch equivalent to 50 mg drug from each formulation was fixed between the stationary and movable plate. The force needed to fracture the film was determined by measuring the total weight loaded in the string. The weight corresponds to break the patches were taken as tensile strength. The following equation was used to calculate the tensile strength (TS).[9-13]

$$TS \text{ (g/cm}^2\text{)} = \text{Force at break (g)} / \text{Initial crosssectional area of patch.}$$

Vapour transmission test (VTR)

Vapour transmission method was employed for the determination of vapor transmission from the

patch. 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 patch was fixed over the adhesive and the assembly was placed in a constant humidity chamber, prepared using saturated solution of ammonium chloride and maintained at 37 ± 2 °C. The difference in weight after 24 hr was calculated 18. The experiments were carried out in triplicate and vapor transmission rate was obtained as follow:[13,15]

$$VTR = (\text{Amount of moisture transmitted}) / (\text{Area} \times \text{Time})$$

Percentage moisture absorption (PMA)

The percentage moisture absorption test was carried out to check the physical stability of the buccal films at high humid conditions. In the present study the moisture absorption capacity of the films were determined as follows. Three 1cm diameter films were cut out and weighed accurately then the films were placed in desiccator containing saturated solution of aluminium chloride, keeping the humidity inside the desiccator at 79.5 %. After 3 days the films were removed, weighed and percentage moisture absorption was calculated. Average percentage moisture absorption of three films was found.[16]

$$\text{Percentage moisture absorption} = [(\text{Final weight} - \text{Initial weight}) / \text{initial weight}] \times 100$$

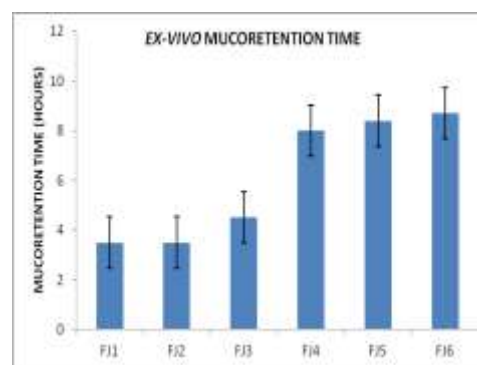


FIG. NO. 3 Ex-Vivo MucoRetention Time of Formulations

Percentage moisture loss (PML)

Percentage moisture loss test was also carried to check the integrity of films at dry condition. Three 1cm diameter films were cut out and weighed accurately and kept in desiccator's containing fused

anhydrous calcium chloride. After 72 hours the films were removed, weighed. Average percentage moisture loss of three films was found out.[16]

$$\text{Percentage moisture loss} = \frac{[(\text{Initial weight} - \text{Final weight})/\text{initial weight}] \times 100}{}$$

membrane. The dissolution apparatus was adjusted at 37°C and the basket rotated at 50 rpm. The time necessary for complete erosion or detachment of the patch from the mucosal surface was recorded (mean of triplicate

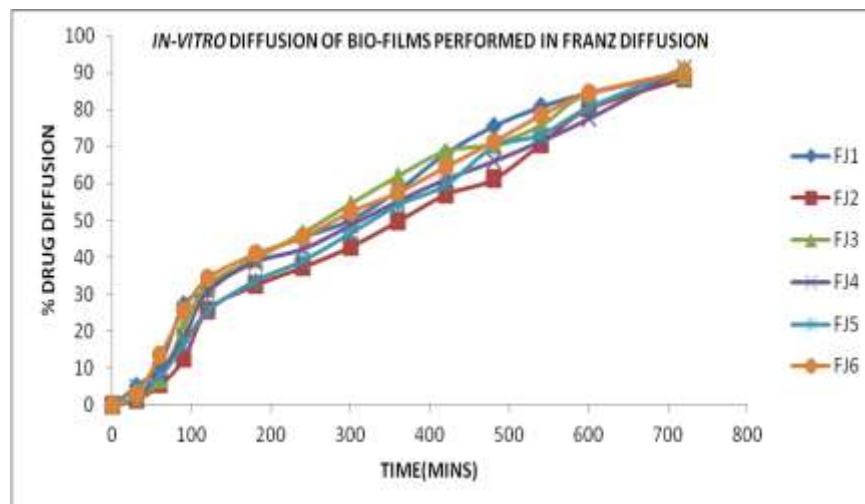


FIG. NO. 4 *In-Vitro* Diffusion Study

Mucoretentivity studies:

Mucoadhesive nature of the bio-polymers:

The mucoadhesive property of almond and jackfruit polymers were determined in vitro by the shear stress method. The biomaterial was subjected to a shear stress study for in vitro assessment of its adhesive strength in terms of weight required for breaking adhesive bonds between polymer and glass plate in a specified contact time of 5, 10, 20, or 30 min period with concentrations of 0.5%, 1%, 2%, 3%, or 5% w/v of the natural mucoadhesive extract and compared with the standard polymer NaCMC 1%.[2-5]

1. Mucoretention time:

By rotating cylinder method

The in-vitro residence time was determined using USP dissolution apparatus. The salivary pH buffer was placed as the dissolution fluid. The Disintegration medium was composed of 800 ml salivary pH buffer maintained at 37°C. A segment of goat soft palate mucosa, 3 cm long, was glued to the dissolution basket and the patches were hydrated from one surface using 15 µl IPB and then the hydrated surface was brought into contact with the mucosal

determinations).[1,4]

By M.S. Apparatus

Novel Madhav-Shankar Mucoretentive Study Apparatus is a novel self designed apparatus by Madhav & Shankar 2009, it provides a unique platform for mounting the tissue for the mucoretentive study of the dosage device and it produced reproducible data. The apparatus assembled as shown in the figure no.1. The study can be conducted by placing a patch 1mm diameter, 3 cm away from the narrow open end with the help of a loop. The ringer solution is then allowed to pass at a rate of 5ml/min. The solution continuously allowed to flow until dislodgement of bioplate. The time of dislodgement of bioplate is registered.[1]

2. Mucoretention force

By park and robinson method

With the Park and Robinson method, the patches were placed in contact with the goat soft palate and placed in a modified physical balance. The goat soft palate was placed on the top of a glass vial with smooth surface on top of which was placed the patch, sandwiched between two layers of the palatal mucosa. The

other end was attached to a weight for balancing. The weight was added to the left pan until the point of detachment of the patch from the mucosa. The force required to detach the bioplate from the mucosal surface was determined and compared with that of the standard polymers sodium carboxymethyl cellulose (NaCMC) and hydroxypropyl methyl cellulose (HPMC). The average of 6 readings was registered.

each group one for control other as the test group. All the rats were applied the insulin loaded patches in the soft palate. Cognition enhancement effect was estimated by the rectangular maze. The observations were recorded at 0, 10, 30, 60 mins followed by 4 hr, 8 hr, 24 hr and 48 hours.

Stability studies:

Stability studies were subjected to

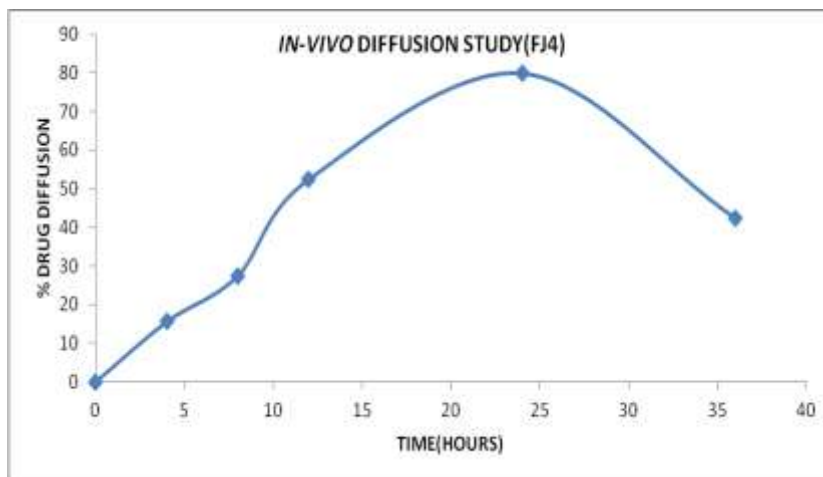


FIG. NO. 5 In-Vivo Diffusion Study Effect on Cognition Study

In-vitro release study

The in-vitro release study was performed by using the Franz diffusion cell at the salivary pH. The release was carried out for all the patches and compared with the standard polymeric patches. The results were applied for statistical analysis.

In-vivo release study

The in-vivo release was performed in rabbits for the optimized formulation (FJ4). The bio-film was applied to the rabbit soft palate and blood samples from the ear vein were taken at intervals of 4, 8, 12, 24 and 36 hours to determine the concentration of insulin in the blood. The estimation was done by HPLC method using RP-HPLC C-18 column and mobile phase of acetonitrile:water(70:30), It had a retention time of 8 mins and wavelength maximum at 271nm.

In-vivo estimation of effect on cognition in rats

The in-vivo effect on cognition was studied in albino rats by taking two groups six rats in

accelerated stability studies where the representative samples were stored at various temperatures. i.e. room temperature, 37°C, 45°C and 60°C.

RESULTS AND DISCUSSIONS

The bio-material was isolated from the fruit pulp of *Artocarpus heterophyllus* and patches loaded with insulin were formulated. The patches from all the batches were translucent and flexible without any sign of crack. The weight of patches of almond bio-polymer ranges from , jackfruit bio-films ranges from and that of standard polymeric films ranges from 21.42±0.371mg to 56.3 ±0.53mg. The thickness of formulated patches ranges from 0.37±0.03 to 0.69±0.05mm. The surface pH of patches was ranges from 6.5 ±0.1to 6.8± 0.3, which indicates no risk of mucosal damage or irritation. For all the patches it was found to be in the range 91.1% ±0.65 to 97.4% ±0.05. The results of content uniformity indicate that the drug was uniformly dispersed in patches. The folding endurance for most patches ranges from, 11 ± 2 to 227 ±6.6 indicating the high flexibility of the patches. The bio-films show very less amount of

moisture ranging from $0.85\% \pm 0.01$ to $1.41\% \pm 0.06$. This shows the low moisture content of the bio-films hence are stable. All the bio-polymeric films show moisture absorption in the range $3.8\% \pm 0.03$ to $4.9\% \pm 0.23$. The tensile strength of drug loaded patches in the range of 74.07 g to 223.23g which is sufficient to withstand any undue pressure or strain. The swelling index of the patches increases on increasing the polymer content. It became constant after 6 hours. Swelling studies reveal the drug release through the patches is by swelling followed by erosion. Results indicate that all the patches were permeable to water vapour. The *ex-vivo* mucoadhesion study in the rotating cylinder method revealed that the patches with ratio 1:4,1:5,1:6 (FJ4-FJ6) had a mucoadhesion of more than 8 hours showing excellent mucoadhesion of the bio-polymers. The mucoadhesive strength is due to the physical interpenetration, swelling of the polymers and the mucus of the goat soft palate. The mucoadhesion force ranges from 3.10-27.16 gms. Different kinetics models viz, zero order, first order and Higuchi's equation were applied to study the release kinetics. All the bio-films showed zero order pattern of release. The plot of the optimized formulations FJ4, FJ5 displayed r^2 values of 0.977, 0.959 respectively and $t_{80\%}$ values of 10.5hrs, 10.1hrs, respectively with controlled drug release. The optimized formulations were found to be linear as indicated by the Higuchi's plot showed diffusion based drug release. It was found that the insulin release in the blood followed a linear pattern and it reached a peak plasma concentration at 24 hours and then the concentration declined showing the elimination phase. It can be concluded that the bio-films depicted zero order release and had sustained and controlled release. The results of the rectangular maze study showed significant increase in the cognition of the rats. The results depicted an increased level of cognition as the time taken to complete the rectangular maze task by the rats decreased significantly after 24 hours of administration. The results showed the increase in cognition after the administration of insulin to the animals as compared to the control group that did not show any increase in the cognition levels. Thus it can be concluded that the insulin is delivered via the soft palate to the brain and it improves cognition. Accelerated stability studies where the representative samples were stored at various temperatures. i.e. room temperature, 37°C , 45°C and 60°C showed that there was no considerable change

in the formulation after one month. (table no.2a, 2b, table no.3) (fig. no.1-5)

CONCLUSION

The isolated biomaterials are biocompatible, biodegradable because of their edibility with devoid of toxicity. They displayed significant mucoadhesivity and mucoadhesiveness. Novel inbuilt mucoadhesive and mucoadhesive properties which were assessed and confirmed by *in-vitro* and *ex-vivo* studies. The soft palatal route has potential strengths like smooth surface, good flexibility, promising accessibility, reasonable patient acceptance and compliance. The potentialities of the soft palate made us to enlighten this as a novel transmucosal platform for delivering APIs by formulating biofilms. The *in-vivo* study depicts the use of insulin via the soft palate for brain targeting as there was no effect or increase in cognition in the control group, this shows that insulin has an effect on the increase in the cognition and soft palatal drug delivery is a potential route for brain drug delivery.

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