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NASAL DRUG DELIVERY OF ANTI-DIABETIC DRUG REPAGLINIDE USING DEGRADABLE STARCH MICROSPHERES

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ABSTRACT

Repaglinide is widely used as a hypoglycemic drug for the treatment of chronic type 2 diabetes mellitus. The objective of the present study was to improve the bioavailability of repaglinide by nasal delivery using degradable starch microspheres. Degradable starch microspheres were prepared by an emulsion polymerization method and formaldehyde was used as a cross linking agent. The formulations were characterized for their encapsulation efficiency, surface morphology, particle size and *ex vivo* drug release pattern. The relative bioavailability in rats was studied in lead formulations. The repaglinide microspheres formulations showed significant increase in bioavailability.

INTRODUCTION: Repaglinide, a novel insulin secretagogue is used for the management [1, 2] of type 2 diabetes mellitus. Its oral bioavailability [3] is less than 50% due to first pass metabolism. Nasal drug delivery approach has been selected to improve the bioavailability. Nasal drug delivery is increasingly important as an alternative to the oral and parenteral route for systemic drug delivery. The nasal cavity as a site for the systemic absorption of numerous drugs has the advantages which include relatively large surface area, highly vascularized nature epithelium with high total blood flow per cm^3 for rapid absorption.

Avoidance of hepatic first pass metabolism [4] owing to a porous endothelial basement membrane for the direct transport of absorbed substances into the systemic circulation is another important advantage. Several products [5] for nasal delivery are now commercially available. The mucoadhesion in the design of drug delivery systems is to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with underlying absorption surface to improve and enhance the bioavailability. Thus, mucoadhesive microspheres can adhere [6] on to the nasal mucosa for reasonably prolonged period preventing rapid nasal clearance.

Starch is widely used for microsphere preparation because it is biodegradable, biocompatible and non toxic. Starch microspheres are not only bio degradable but also show a high degree of swelling when in contact with aqueous media. They form a gel like system which prolongs residence time in the nose. The increased absorption of drugs [7] administered with degradable starch microspheres is due to effect on tight junctions between the epithelial cells. Moreover, starch microspheres do not stimulate [8] an albumin-like antigen response *in vivo*.

The objective of this study was to develop an intranasal delivery system of repaglinide using degradable starch mucoadhesive microspheres, which would enhance nasal residence time, absorption of drug across nasal-mucosal membrane and enhance the bioavailability.

MATERIALS AND METHODS: Repaglinide, was received as gift sample from Dr. Reddy's Laboratories (Hyderabad, India).All other materials used in the study purchased from commercial sources were: extra pure soluble starch (Merck Specialties), arachis oil (V.V.V. & Sons) formaldehyde (S.D. Fine Chemicals), diethyl ether (Merck Specialties), acetone (Paxmy Specialty Chemicals).

Preparation of degradable starch microspheres:

Emulsion polymerization technique: Degradable starch microspheres (DSM) containing repaglinide were prepared by emulsion polymerization method [9, 10]. The compositions for formulations F1-F10 are represented in **Table 1**. 10 % starch dispersion was prepared by dispersing accurately weighed extra pure soluble starch in distilled water. The accurately weighed amount of drug was dispersed in 50 ml of arachis oil. The starch solution was added drop wise into continuously stirred (1500 rpm) drug dispersion in arachis oil at 25°C. The emulsion thus formed was stirred for 10 min and then cooled in an ice bath.

TABLE 1: COMPOSITION OF FORMULATIONS (F1 - F10)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Repaglinide (mg)	200	200	200	200	200	333	333	333	333	333
Soluble starch (%)	10	10	10	10	10	10	10	10	10	10
Arachis oil (ml)	50	50	50	50	50	50	50	50	50	50
Formaldehyde (ml)	-	5	10	15	0	-	5	10	15	20
Diethyl ether (ml)	300	295	290	285	280	300	295	290	285	280

The starch microspheres were then resuspended in 100 ml of diethyl ether containing formaldehyde as the cross-linking agent. The suspension was stirred (1500 rpm) for 30 min at 25^{o}C and the supernatant was decanted. The resulting microspheres were washed with acetone, filtered and air dried. Formaldehyde as cross-linking agent was used in different concentrations in each formulation. Formaldehyde was not added in formulations F1 and F6. In case of formulations F1 to F5 the drug and polymer ratio was 1:50. While in case of F6 to F10 the drug and polymer ratio was 1:30. The same procedure was repeated for all the formulations F1-F10. Repaglinide free (empty) microspheres were prepared following the same procedure as for drug loaded microspheres omitting repaglinide.

Characterization of Starch Microspheres:

Particle size analysis: Particle size analysis was performed on the prepared microspheres suspended in ethanol and sonicated for 5 min. The sample was analyzed by Malvern Multisizer 2000 laser diffraction spectrometer, UK.

Scanning Electron Microscopy (SEM): Particle shape and surface morphology [11] of the microspheres were examined by scanning electron microscopy (JEOL JSM-5610LV). Samples of microspheres were dusted onto double sided tape on an aluminum stub. The stub ware then coated with gold using a cold sputter coated to a thickness of 400 A^{o}. The samples were imaged using a 25 kV electron beam.

Percentage yield value of Microspheres: The percentage yield values [12] was calculated from the ratio of total final weight of the degradable starch microspheres of each formulation to total weight of the drug and polymer used for the preparations.

$$\% \text{ yield} = \frac{\text{Total weight of the degradable starch microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

Encapsulation Efficiency: For the determination of total drug content of the microspheres, the drug quantities on the surface and entrapped inside the matrix structure of microspheres were determined separately [13, 14]. In order to determine the drug amount on the surface, microspheres (100 mg) were weighed accurately and mixed with 50 ml of methanol. This mixture was sonicated in an ultrasonic bath for 5 min and then centrifuged (REMI R-24) at 3000 rpm for 10 min. The supernatant was filtered through a 0.45 mm filter and the absorbance was measured on a UV Spectrophotometer (Shimadzu Model 1601) at 281nm wavelength.

The residue was mixed with 100 ml methanol followed by agitation with a magnetic stirrer for 12 h to dissolve the polymer and to extract the drug. Samples were centrifuged (REMI R-24) at 5000 rpm for 10 min and the absorbance of the supernatant absorbance was measured on a UV Spectrophotometer (Shimadzu Model 1601) at 281nm after filtration through a 0.45 µm filter The amount of drug associated with each phase of the microspheres was determined using a calibration curve constructed over the range 20-120 µg/ ml.

***Ex vivo* release studies on sheep nasal mucosa** [15, 16, 17]: The *ex vivo* permeation study was carried out by following the procedure described by Steffen Lang *et al*. Tissue with nasal mucosa was excised from the noses of freshly slaughtered sheep. After removing the skin, tissue containing nasal mucosa was cut off with a sharp knife from the frontal part of the nasal conch (conchae nasals dorsales) above the *os incisivum* starting from the incisura nasoincisiva.

The excised tissue was stored on ice during transport to the laboratory. At no more than 30 min after the excision, the mucosa was separated from the underlying cartilage by blunt stripping using a pair of tweezers. Samples were taken and inserted into the Franz diffusion chambers, the apical side of

the tissue typically facing the donor compartment. Franz diffusion cells with an area of 1.01 cm^2 and a final volume of 15 ml were used to evaluate the progesterone release profiles from gel formulations in a closed system. 100 mg of microspheres were placed on the upper side of the nasal mucosa. The donor half cell was then carefully placed on top of the receptor half cell and clamped.

The donor and the receiver compartment containing phosphate buffer (pH 6.2), used as the diffusion medium were kept in intimate contact and the temperature was maintained at 37^oC. The whole assembly was kept on a magnetic stirrer and stirred continuously. The samples were withdrawn at definite time intervals and equal amount of the phosphate buffer was replaced. The samples were filtered and the absorbance was measured at 281 nm using UV- spectrophotometer.

The cumulative amount of repaglinide permeated per unit area was plotted against time, and the slope of the linear portion of the plot (mg/min) was used as the steady state flux (J_{ss}). The apparent nasal permeability coefficient K_p (cm/s) was calculated using the equation $K_p = J_{ss} \times 1/ [60 \times C_v]$ in which J_{ss} is steady state flux (mg/cm^2min), C_v is the total donor concentration of the formulation (mg), 60 is the conversion of minutes to seconds.

Bioavailability Study: *In vivo* evaluation studies [18, 19, 20] for repaglinide degradable starch microspheres were performed on normal healthy Wistar rats weighing 250 to 300 g each. The approval of the Institutional Animal Ethics Committee was obtained. The study was conducted in accordance with standard institutional guidelines. Four groups of wistar rats (6 in each group) were fasted (with water) for 15-17 hours before the experiment. After overnight fasting the blood samples were collected from the tail vein of the rats and the blood glucose level of rats were determined. The test samples were given immediately after the collection of initial blood samples. The Group-I was the control, Group-II (standard) was fed orally with a marketed tablet equivalent to 1 mg of repaglinide. The Group-III and Group-IV were administered with repaglinide degradable starch microspheres equivalent to 1mg of repaglinide of formulation F5 and F10 respectively. The formulations F5 and F10 were administered through nostril with a polyethylene tube, PE90.

The test microspheres were put in the tube and weighed. It was introduced into nasal cavity by blowing air from a syringe. All the groups were orally administered 2 g/kg glucose and the blood samples were collected in tail vein of the rats at 0, 15, 30, 45 and 60 min interval. The blood glucose level for the control, standard and test samples was determined using the Ascensia Entrust Glucometer. (Bayer Company, USA). The percentage reduction of blood glucose level (D %) was measured.

Data analysis: The plasma glucose concentration of each rat before nasal administration was taken as the baseline level and the changes in plasma glucose concentrations (percentage of baseline level) at different times after dosing were calculated and plotted against time. The area under the plasma glucose level versus time curve (AUC) was obtained according to the trapezoidal rule and the percentage reduction in blood glucose level (D %) was calculated using the following equation.

$$D\ (\%) = \frac{AUC_c - AUC_s}{AUC_c} \times 100\ \%$$

Where AUC_c represents the AUC of non treated animals and AUC_s represents the AUC of formulations.

RESULTS AND DISCUSSION: The degradable starch microspheres of repaglinide were prepared by emulsion polymerization method with two different mixing ratios of repaglinide/polymer at 1:30 and 1:50. Blank microspheres were also prepared for comparison. In this method, acetone was efficiently

used to remove oil in the microspheres because oil retained on the microspheres caused aggregation and changed the morphological properties of the microspheres. Formaldehyde was used as the cross linking agent in this study. In order to optimize the preparation of microspheres, formulation variables such as drug and polymer ratios as well as different concentrations of cross linking agent were employed. The percentage yield values of the microspheres are represented in **Table 2**. The yield values of the microspheres were in the range of 85% to 89%.

TABLE 2: CHARACTERIZATION OF FORMUALTATIONS F1- F10

Formulation	Particle size (µm)	% Yield	% drug content	% drug on surface	% drug entrapped
F1	118.0	89.5	12.6	6.8	5.8
F2	69.2	87.0	23.8	5.7	25.9
F3	59.5	87.8	25.3	5.0	29.5
F4	48.9	88.6	26.2	4.4	31.3
F5	46.7	88.0	29.8	3.1	34.5
F6	124.2	86.0	14.6	8.7	5.9
F7	64.0	86.8	32.5	6.6	18.1
F8	57 .7	86.5	32.9	3.4	20.3
F9	49.4	86.0	33.7	2.4	21.8
F10	51.6	85.3	36.7	2.2	26.7

Particle size [21, 22] of the degradable starch microspheres of repaglinide are represented in Table 2. A size range of 40- 100 µm was suitable for intranasal administration because particles smaller than 40µm have a tendency to reach the respiratory tract directly after inspiration without impacting the nasal mucosa [23]. Particle size of the degradable starch microspheres of repaglinide ranged from 46µm to 137 µm in F1-F10. Formulations F2- F5 and F7, F7- F10 had particle size between 40- 100µm which are ideal for nasal delivery.

Fig. 1- 5 presents morphology of empty microspheres; F-1, F-5, F-6 and F-10 respectively. SEM analysis of the samples revealed that all the microspheres exhibited spherical shape and smooth appearance while empty microspheres had a crumpled appearance.

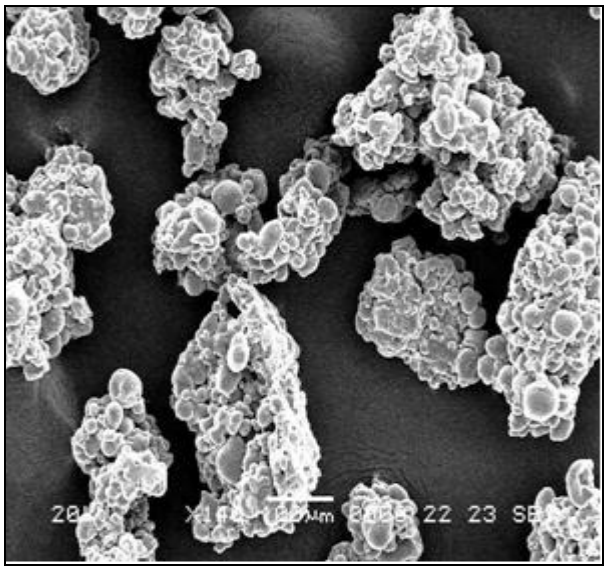

FIG. 1: SCANNING ELECTRON MICROSCOPY PHOTOGRAPH OF EMPTY MICROSPHERES

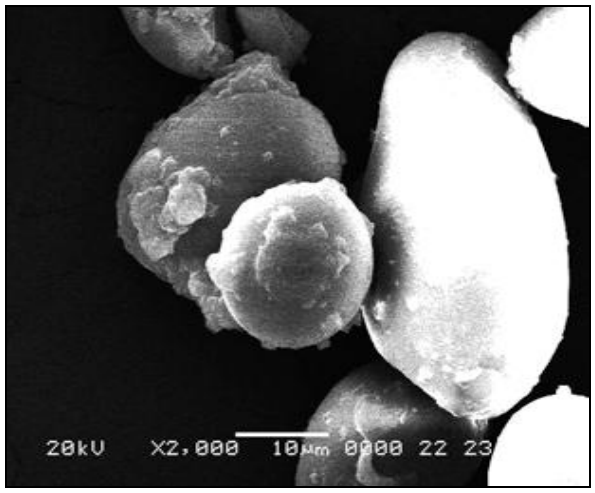

FIG. 2: SCANNING ELECTRON MICROSCOPY PHOTOGRAPH OF F-1

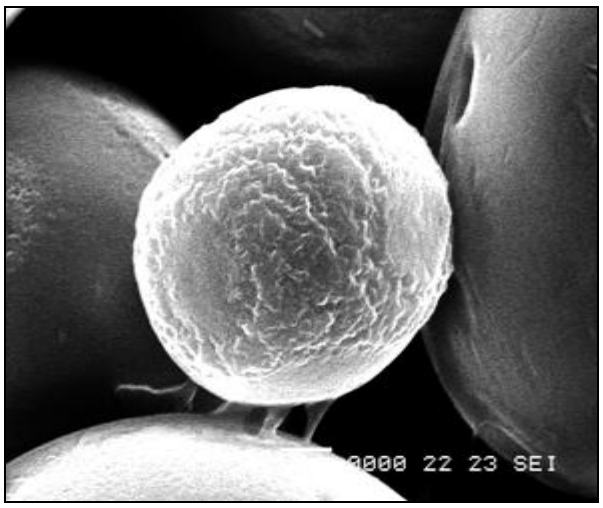

FIG. 3: SCANNING ELECTRON MICROSCOPY PHOTOGRAPH OF F-5

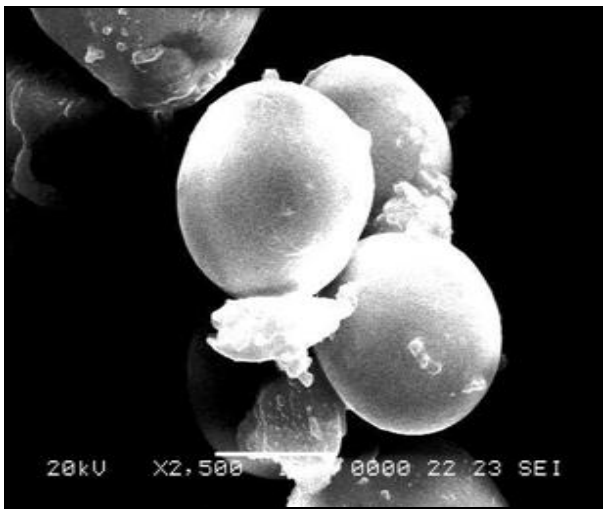

FIG. 4: SCANNING ELECTRON MICROSCOPY PHOTOGRAPH OF F-6

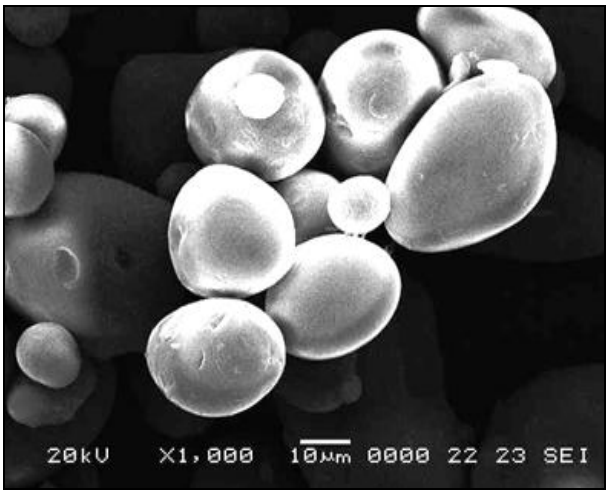

FIG. 5: SCANNING ELECTRON MICROSCOPY PHOTOGRAPH OF F-10

Incorporation of repaglinide into starch microspheres can be influenced by factors such as the method of preparation, drug and polymer concentrations, drug and polymer binding, physicochemical characteristics of the drug, stabilization method and size of the microspheres [23]. In this study, encapsulation of repaglinide in degradable starch microspheres was investigated as a function of formaldehyde concentrations (0, 5, 10, 15 and 20 ml) and drug polymer ratios (1: 30 and 1: 50) by keeping all other parameters, that is, method of preparation, stirring rate, oil phase and stabilization period (30 min) constant.

The encapsulation efficiency is represented in Table 2. The encapsulation efficiency was observed in the range from 9.6% to 36.7%. With increase in the formaldehyde concentrations and drug and polymer ratio drug loading was increased. The amount of drug on the surface and entrapped in the microspheres was also evaluated. The amount of drug present on the surface of the microspheres ranged from 2.2% to 8.7% while the drug entrapped in the microspheres ranged from 5.8% to 34.5%.The maximum encapsulation efficiency when drug and polymer ratio is 1:30 is for F5 at 34.5% (F-5) and when drug and polymer ratio is 1:50 is for F10 at 26.7% (F-10).

Repaglinide permeation from the degradable starch microspheres in different formation were carried out in sheep mucosa. The results are represented in **Fig. 6**. The permeation flux and permeability coefficient determination for each of the formulations are shown in **Table 3**. The permeation of repaglinide from microspheres was complete during the course of the experiment (6 h). The drug permeation amount at 6 h was found to be 72% for F-1, 84% for F-2, 84% for F-3, 86% for F-4, 87% for F-5, 75% for F-6, 88 % for F-7, 92% for F-8, and 96% for F-9, 98% for F-10.

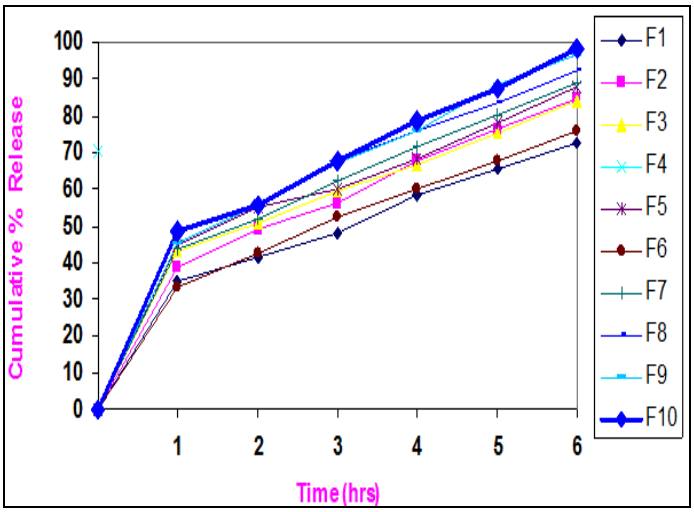

FIG. 6: *EX-VIVO* PERMEATION PROFILE OF REPAGLINIDE IN FORMULATIONS F1- F10

TABLE 3: FLUX AND PERMEAILITY CO-EFFICIENT

Sample	J_{ss} (mg/cm^2.min)	K_p (cm/s)
F1	0.03	0.55× 10^{-5}
F2	0.08	1.48× 10^{-5}
F3	0.10	1.85× 10^{-5}
F4	0.14	2.51× 10^{-5}
F5	0.16	2.96× 10^{-5}
F6	0.04	0.74× 10^{-5}
F7	0.09	1.67× 10^{-5}
F8	0.16	2.96× 10^{-5}
F9	0.19	3.56× 10^{-5}
F10	0.23	4.25 × 10^{-5}

J_{ss} is the steady state flux in mg/cm^{2}min; K_p is the apparent nasal permeability co-efficient in cm/s

The *in vivo* bioavailability study was performed in rats to determine pharmacokinetic parameters for the optimized formulations F-5 and F-10 as well as to compare with marketed tablet of Repaglinide [20]. The results are shown in **Table 4**. In this study C_{min} (% of base line) for marketed tablet was found to be 58.2% as against 54.76% for F-5 and 47.93% for F-10. Similarly T_{min} (Time to reach C_{min} after nasal administration) for marketed tablet, F-5 and F-10 was 30min respectively. The total decrease in plasma glucose level (D %) calculated for marketed tablet was 27.3% as against 38.1% for F-5 and 42.2% for F-10. The *in vivo* study revealed that the bioavailability of repaglinide in intranasal administration is more than oral administration.

TABLE 4: PHARMACOKINETIC PARAMETERS

Formulation	T_{min} (min)	C_{max}	$AUC_{(0-t)}$	D%
Non treated	30	102.2	7459	-
Oral tablet	30	87.0	5421	27.3
F5	59	87.8	4619	38.1
F10	48	88.6	4312	42.2

CONCLUSION: The study concludes that the formulation F-10 is the best formulation in drug and polymer ratio 1:50. The repaglinide degradable starch microspheres improved the bioavailability of the drug by intranasal administration. On the basis of the results of this study it is inferred that the degradable starch microspheres with drug and polymer ratio 1:50 of formulation F-10 appear to have a potential for development of nasal drug delivery of repaglinide. The *in vivo* study demonstrated significant hypoglycemic activity of the repaglinide degradable starch microspheres. The developed degradable starch microspheres of repaglinide are found to be more effective which is the need of day in pharmaceutical industry as an alternative drug delivery system for a highly prevalent and chronic disease likes type II diabetes mellitus.

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