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Formulation and Characterization Of Polycarbophil Coated Mucoadhesive Microspheres of Repaglinide

Balaji Maddiboyina[1], Abhay Asthana[1#],Gyati Shilakari Asthana[1],Sima Singh[2], Ramya.M[3], Omprakash Sunnapu[4],Niranjan Kotla[4*]

[1]*Department Pharmaceutical Sciences, M.M. College of Pharmacy, M.M.U., Ambala, Haryana, India*

[2]*Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, India*

[3] *Vagdevi college of pharmacy, Guntur, A.P, India*

[4]*Laboratory of self-assembled biomaterials, Institute for stem cell biology and regenerative medicine, Bangalore, India*

Abstract

Type 2 diabetes mellitus is a heterogeneous disease of polygenic origin and involves both insulin secretion and peripheral insulin resistance. Studies have shown that post-meal hyperglycemic spikes are associated with increased cardiovascular mortality in type 2 diabetes. Over the past decade, a major interest in control of postprandial glucose excursion has emerged as critical parameter and a plethora of new medications that specifically target postprandial hyperglycemia were researched or commercialized. Repaglinide is an anti-diabetic, oral blood-glucose lowering drug of the meglitinide class used in the management of type-II diabetes mellitus. It is the first member of new class of oral hypo-glycaemics designed to normalize the meal time glucose excursions. Repaglinide induces rapid onset short lasting insulin release. Microsphere based carrier systems formulated by using polymer polycarbophil bearing strong mucoadhesive properties and readily biodegradable could be attractive strategy to implement. The purpose of this research work is to formulate polycarbophil coated mucoadhesive microspheres of repaglinide and systematically evaluate its in vitro characteristics for sustained glucose lowering effect and improvement in diabetic condition as compared to immediate release of repaglinide.

Keywords: Repaglinide, Polycarbophil, Mucoadhesive microspheres, Double emulsion solvent evaporation technique.

INTRODUCTION

The effect of a drug can now be reinforced as a result of the development of new release systems. Controlled release consists of techniques that make the active chemical agents available for a target, providing an adequate release rate and duration to produce the desired effect. [1] Adhesion can be defined as the bond produced by contact between a pressure-sensitive adhesive and a surface. The American society of testing and materials has defined it as the state in which two surfaces are held together by interfacial forces, which may consist of valence forces, interlocking action or both. The term "bio-adhesion" is defined as the "attachment of a synthetic or natural macromolecule to mucus and/or an epithelial surface". Adherence of a polymeric material to biological surfaces is known as bio-adhesion or to the mucosal tissue is known as mucoadhesion. [2]

For a material to be bioadhesive, it must interact with mucus, which contains glycoproteins, lipids, inorganic salts and 95% water by mass, making it a highly hydrated system. Mucin is the most important glycoprotein of mucus and is responsible for its structure.The mucin is composed largely of flexible glycoprotein chains, which are crosslinked. The formation of non-covalent bonds such as hydrogen bonds and ionic interactions or physical entanglements between the mucus gel layer and polymers provides a good mucoadhesion. [3]

Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity. Microspheres are the carrier linked drug delivery system in which particle size is ranges from 1-1000 μm range in diameter having a core of drug and entirely outer layers of polymer as coating material. However, the success of these microspheres is limited due to their short residence time at site of absorption. It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane. This can be achieved by coupling bioadhesion characteristics to microspheres and developing "mucoadhesive microspheres". [4]

Mucoadhesive microsphere exhibit a prolonged residence time at the site of application and facilitate an intimate contact with the underlying absorption surface and thus contribute to improved or better therapeutic performance of drug. [5] Mucoadhesive drug delivery systems promises several advantages that arise from localization at a given target site, prolonged residence time at the site of drug absorption and an intensified contact with the mucosa increasing the drug concentration gradient. Hence, uptake and consequently bioavailability of the drug is increased and frequency of dosing reduced with the result that patient compliance is improved. In recent years such Mucoadhesive microspheres have been developed for oral, buccal, nasal, ocular, rectal and vaginal for either systemic or local effects. The principles Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site. [6]

Diabetes mellitus is a major and growing health problem worldwide and an important cause of prolonged ill health and early death. It is a chronic metabolic disorder characterized by a high blood glucose concentration (hyperglycemia) caused by insulin deficiency, and it is often combined with insulin resistance. Repaglinide is an oral blood glucose- lowering drug of the meglitinide class use to treat NIDDM (noninsulin-dependent diabetes mellitus). It lowers blood glucose by stimulating the release of insulin from the pancreas. It has an extremely short half-life of 1 h.[7] Dosage frequency of repaglinide is 0.5 to 4mg in 3 to 4 times in a day.[8] Repaglinide microsphere preparation may be beneficial to the patient since it reduce adverse effects and avoid the hepatic first-pass metabolism. The need for mucoadhesive microspheres of repaglinide is further justified due to the requirement of maintaining fluctuating plasma concentrations for effective management of blood sugar for long period in diabetic patients.

The purpose of the present work was to develop mucoadhesive microspheres of repaglinide was to increases the patient compliance and also sustain the release of drug to increase the bioavailability by using polycarbophil as polymers.

MATERIALS AND METHODS

Repaglinide was received as a gift sample from Torrent Pharmaceutical Ltd., Gujarat, India. Polycarbophil, Dichloromethane, Light liquid paraffin, Tween 80, Span 80 was received as a gift samples from Research laboratories, Hyderabad, India.

Preparation of Mucoadhesive Microspheres

Bioadhesive microspheres were prepared by an oil-in water-in-oil (O/W/O) double-emulsion method.[9] Aqueous polycarbophil solution was prepared and subsequently stored in sealed containers at 48 ºC for 24 h prior to use. Polycarbophil 500 mg was dispersed in 50.0 g of deionized water and mixed by rapid vortexing; pH was adjusted to 7.0 using dilute aqueous sodium hydroxide.

For the first emulsion, Repaglinide dissolved in dichloromethane was emulsified into 50.0 g of aqueous polymer solution. The concentrations and amounts applied are summarized in table1. Addition of 0.15 ml of Tween 80 aided the emulsification process. Silverson homogenizer was used for rapid mixing of the emulsions for 15 min. The first emulsion (25 ml) was added drop wise to 250 ml light liquid paraffin containing 1% Span 80. The resultant double emulsion was stirred at 800 rpm.

The samples were heated to 60-70 ºC to promote evaporation of water. Solid polymer microspheres were subsequently separated from the oil by centrifugation, washed in hexane, and dried in a vacuum oven at 40 ºC for 24 h.

Particle size analysis

Microscopic imaging analysis technique was used for the determination of particle size. Microsphere size and distribution were determined with an AXIOPALN microscope equipped with a computer-controlled image analysis system.

Flow properties

Angle of Repose

The flow characteristics are measured by angle of repose. Flow constrains due to frictional forces between the particles were quantified by angle of repose.

Hausner's ratio & Carr's compressibility index

Hausner ratio is an indirect index of ease of power flow. The compressibility index of the granules was determined by Carr's compressibility index.

Bulk & tapped densities

Bulk density is defined as the mass of a powder divided by the bulk volume. Bulk density of a powder depends primarily on particle size distribution, particle shape, and the tendency of the particles to adhere to one another. The measuring cylinder containing a known mass of blend was tapped for a fixed time. The minimum volume (V_t) occupied in the cylinder to the weight (M) of the blend was measured as tapped density.

Encapsulation efficiency

Encapsulation efficiency of repaglinide was performed by accurately weighing 100 mg of drug loaded bioadhesive microspheres which were added to 100 ml of methanol.[10] The resulting mixture was kept shaking on a mechanical shaker for 24 h. The solution was filtered and 1 ml of this solution was appropriately diluted with methanol and analyzed spectrophotometrically at 247 nm using Shimazdu UV-1700 (UV/VIS double beam spectrophotometer, Kyoto, Japan).

Swelling index

The swelling ability of the microspheres in physiological media was determined by swelling them to their equilibrium. Accurate amounts of microspheres were immersed in a little excess of Phosphate buffer (pH 6.8) and kept for 24 h. [11]

Mucoadhesion test

Mucoadhesion of different microspheres system was assessed using the method reported with little modification. A strip of goat intestinal mucosa was mounted on a glass slide and accurately weighed bioadhesive microspheres in dispersion form was placed on the mucosa of the intestine. This glass slide was incubated for 15 min in a desiccator at 90 % relative humidity to allow the polymer to interact with the membrane and finally placed in the cell that was attached to the outer assembly at an angle 45º. Phosphate buffer saline (pH 6.8), previously warmed to 37 ± 0.5 ºC, was circulated to the cell over the microspheres and membrane at the rate of 1 mL/min. Washings were collected at different time intervals and microspheres were separated by centrifugation followed by drying at 50 ºC. The weight of microspheres washed out was taken and percentage mucoadhesion was calculated. [12]

Scanning electron microscope (SEM)

A scanning electron microscope (ESEM TMP with EDAX, Philips, and Holland) was used to characterize the surface topography of the microscope. The microspheres were placed on a metallic support with a thin adhesive tape and microspheres were coated with gold under vacuum. The surface was scanned and photographs were taken at 30kV accelerating voltage for the drug loaded microspheres.

Drug release study

Dissolution rate was studied by using USP type-II apparatus (USP XXIII Dissolution Test Apparatus at 50 rpm) using 900ml of 1.2 pH buffer for first 2 hrs and remaining 10hrs in phosphate buffer pH (6.8) as dissolution medium. Temperature of the dissolution medium was maintained at 37 ± 0.5°C, aliquot of dissolution medium was withdrawn at time intervals and filtered. The absorbance of filtered solution was measured by UV spectrophotometric method at 247 nm.

Release kinetics

In order to understand the mechanism and kinetics of drug release, the results of the in vitro drug release study were fitted with various kinetic equations namely zero order (% release vs time), first order (log% unreleased vs time), and Higuchi matrix (% release vs square root of time). In order to define a model which will represent a better fit for the formulation, drug release data further analyzed by Peppas equation, $Mt/M\infty=ktn$, where Mt is the amount of drug released at time t and $M\infty$ is the amount released at time ∞, the $Mt/M\infty$ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusion exponent, a measure of the primary mechanism of drug release. Regression co-efficient (r^2) values were calculated for the linear curves obtained by regression analysis of the above plots. [11]

1. Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation.

$Q_t = Q_0 + K_0 t$

Where, Q_t = amount of drug dissolved in time t,

Q_0 = initial amount of drug in the solution,

K_0 = Zero order release constant.

2. First order kinetics

To study the first order release rate kinetics the release rate data were fitted to the following equation.

$\text{Log } Q_t = \log Q_0 + K_1 t /2.303$

Where, Q_t = amount of drug released in time t,

Q_0 = initial amount of drug in the solution,

K_1 = first order release rate constant

3. Higuchi model

Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs in corporate in semisolids and or solid matrices.

Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media.

$Q_t = K_H . t^{1/2}$

Where, Q_t = amount of drug released in time t,

K_H = Higuchi dissolution constant.

4. Krosmeyer and peppas release model

To study this model the release rate data are fitted to the following equation

$M_t / M_\infty = K . t^n$

Where, M_t / M_∞ = fraction of drug release,

K = release constant,

t = release time,

n = Diffusional exponent for the drug release

Stability studies

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light that can estimate and recommend appropriate storage conditions, re-test periods and shelf lives to be established. In the present study, stability studies were carried out at $40^0C \pm 2^0C$ / 75 ± 5 % RH for a specific time period up to 60 days for the selected formulations. [13]

RESULTS AND DISCUSSION

Particle size

The processing variables such as drug to polymer ratio, stirring speed, stabilizer concentration affect the particle size of microspheres. The drug to polymer ratio appeared to influence on particle size distribution of microspheres.

When drug to polymer ratio was increased from 1:1 to 1:6, the proportion of larger particles formed became higher, which may be due to increase in viscosity of the solvent with increase in polymer to drug ratio. The mean particle size ranged from 24.30 to 52.40 µm as shown in Table 2**.** The minimum concentration of span 80 required to form stable emulsion was found to be 1%. Changing the stirring speed during emulsification process seems to influence the mean particle size of the microspheres. When the stirring speed was kept below 800 rpm, the mean particle size of the microspheres was increased and they were larger and aggregated. When the speed was kept above 800 rpm, the size of the microspheres was smaller and irregular in shape.

Flow Properties

The flow property of the prepared formulations was checked by the method, angle of repose, hausner's ratio and carr's index. Acceptable range of angle of repose is 22°60' to 31°58'; carr's index is less than ten and hausner's ratio of 1.0 to 1.11. All the formulations showed an angle of repose, carr's index and hausner's within the range as shown in Table 3 and 4. Formulations F1 to F6 showed an angle of repose in the acceptable range, which indicates a good flow property.

Encapsulation efficiency

The drug entrapment efficiency within microspheres produced using the solvent evaporation method is of fundamental importance as failure to achieve acceptable drug loadings may preclude the use of this method for economic reasons. The entrapment efficiency of various formulations was found to be in the range of 78.9 to 92.7 % as shown in table 5. The low entrapment efficiency may be due to solubility of the drug in the solvent, the drug may be migrated to the processing medium during extraction and evaporation process of dichloromethane.

Swelling index

The most promising approach to achieving gastro retention is that of creating a swelling or expanding system in situ. Figure depicts the percentage swelling of microspheres. It is evident that all prepared batches of microspheres rapidly swelled in phosphate buffer pH 6.8. The high swelling property of polycarbophil (294%, F1) could be attributed to high molecular weight and their ionized ability to uncoil polymer into an extended structure.

Table1. Composition of formulations

Formulation code	Repaglinide (g)	Polycarbophil (g)	Dichloromethane (mL)	Span80 (%)	Liquid Paraffin (mL)	N-hexane(mL)
F1	0.500	0.500	10	1	250	50
F2	0.500	1.000	10	1	250	50
F3	0.500	1.500	10	1	250	50
F4	0.500	2.000	10	1	250	50
F5	0.500	2.500	10	1	250	50
F6	0.500	3.000	10	1	250	50

Table2. Mean particle size

Formulation	Mean particle size(µm)
F1	52.40+1.23
F2	26.30 +1.00
F3	31.43+1.20
F4	34.03+1.01
F5	38.02+0.92
F6	24.30 +1.00

Table3. Flow properties of microspheres

Formulation	Bulk Density (g/cm^3)	Tapped density(g/cm^3)	Carr's Index	Hausner Ratio
F1	0.41 ± 0.02	0.52 ± 0.01	21.15 ± 0.14	1.26 ± 0.02
F2	0.45 ± 0.01	0.52 ± 0.01	13.4 ± 0.21	1.15 ± 0.07
F3	0.16 ± 0.010	0.20 ± 0.02	20 ± 0.16	1.25 ± 0.07
F4	0.16 ± 0.01	0.19 ± 0.01	15.7 ± 0.16	1.18 ± 0.08
F5	0.45 ± 0.01	0.54 ± 0.02	16.6 ± 0.26	1.2 ± 0.06
F6	0.43 ± 0.03	0.52 ± 0.01	17.3 ± 0.21	1.2 ± 0.04

Table4. Angle of repose

Formulation	Angle of repose
F1	24°58'
F2	22°60'
F3	30°60
F4	31°58'
F5	27°48'
F6	29°56'

Table5. Drug entrapment efficiency of microparticles

Formulation	Theoretical content(mg)	Actual content(mg)	Percentage Drug entrapment efficiency
F1	10	9.27	92.7
F2	10	8.43	84.3
F3	10	9.04	90.4
F4	10	8.12	81.2
F5	10	8.91	89.1
F6	10	7.89	78.9

Table6. Percentage mucoadhesion of microspheres

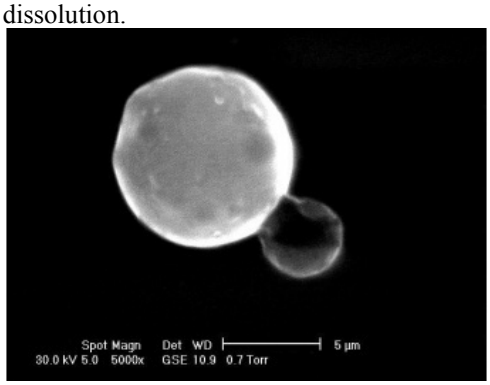
Formulation No.	Percentage Mucoadhesion
F1	74.30
F2	77.21
F3	79.80.
F4	80.12
F5	82.32
F6	84.11

Mucoadhesion

It can be seen that the microspheres had good mucoadhesive properties and could adequately adhere to intestinal mucosa. The results also showed that with change in polymer to drug ratio, the % mucoadhesion also varies. The maximum and prolonged mucoadhesion (84.11%) was observed with the formulation 6 as shown in table 6.

Scanning Electron Microscopy

Surface morphology of microspheres and the morphological changes produced through Polymer degradation can be investigated and documented using scanning electron microscopy (SEM). From SEM study, it was found that microspheres were spherical and rough as shown in Figure. The study of drug loaded microspheres shows the presence of drug particles on the Surface; this may be responsible for an initial burst release of the drug during dissolution.



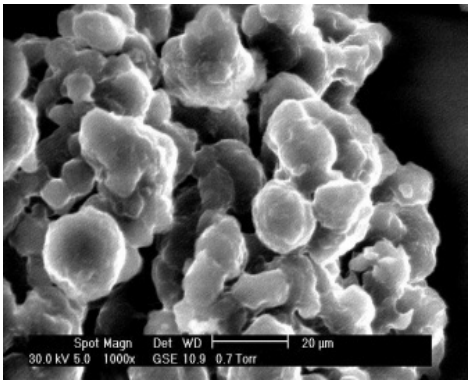


Figure1. SEM photographs of microspheres

***In-vitro* release study**

The release profiles of the formulations appear to be slow release with negligible burst effect. The burst effect corresponds to the release of the drug located on or near surface of the microspheres or release of poorly entrapped drug. The rate of release of drug from the bioadhesive microspheres was slow and found to further decrease with increase in drug to polymer ratio. In order to achieve near to complete release, the formulations were prepared by increasing the concentration of polycarbophil. F1 showed a cumulative release of 92.11% within 12 h as shown in Table 7. Further increasing the concentration of polycarbophil (F4, F5 and F6) the release rate decreased to 71.66%. This decrease in dissolution rate can be explained based on the viscous gel formation by polycarbophil at higher concentration; whereas at lower concentration, easy solubilization of polycarbophil may aid increased dissolution rate. It was observed that the polymeric gel might have act as a barrier to penetration of the medium, thereby suppressing the diffusion of Repaglinide from the swollen polymeric matrix. The slow release may be due to the medium being diffused in the polymer matrix and the drug diffusing out of the microspheres.

Release kinetics

The *in vitro* release profile was analyzed by various kinetic models. The kinetic models used were Higuchi, zero order, first order and Krosmeyer Peppas equations. The release constants were calculated from the slope of the respective plots. Higher correlation was observed in the Higuchi equation. For planer geometry, the value of n=0.5 indicates a Fickian diffusion mechanism, for 0.5<n<1.0, indicates anomalous (non-fickian) transport, and n=1 implies case II (relaxation controlled) transport. In the present systems, the value for n was found to be in the range of 0.469 to 0.802 indicating that the release mechanisms followed fickian diffusion and anomalous (non-fickian) transport as shown in table 8 and 9. The formulation F1 was having n=0.491, indicating that the release mechanism followed is fickian diffusion controlled mechanism.

Table 7. Cumulative percentage drug release

Formulation	Cumulative percentage drug release
F1	92.11
F2	91.11
F3	89.90
F4	81.66
F5	78.66
F6	71.66

Table 8.Values of Correlation-coefficient(r) of Repaglinide

Formulation	First order	Zero order
F1	0.912	0.978
F2	0.948	0.966
F3	0.956	0.972
F4	0.922	0.947
F5	0.934	0.945
F6	0.924	0.957

Table 9. Curve Fitting Data of the Release Profile for Repaglinide

Formulation	Higuchi	Krosmeyer-Peppas	n-values	Mechanism
F1	0.951	0.958	0.491	Fickian
F2	0.946	0.921	0.513	Anomalous
F3	0.948	0.943	0.423	Fickian
F4	0.949	0.911	0.456	Fickian
F5	0.945	0.930	0.527	Anomalous
F6	0.947	0.927	0.482	Fickian

Table 10. Stabilities studies of Repaglinide Mucoadhesive Microspheres

Formulation	Tested after time (in days)	Percentage Drug Entrapment	Cumulative percentage Drug Released
Stored at 25°C/ 60% RH			
F1	30	91.2	91.33
F3	30	87.6	87.88
Stored at 40°C/ 75% RH			
F3	30	90.1	89.55
F6	30	86.2	85.44

Stability studies

In the present study, stability studies were carried out at 40^0C / 75 % RH for a specific time period up to 60 days for the selected formulation. Stabilities studies of Repaglinide Mucoadhesive Microspheres as shown in table 10.

CONCLUSION

In present study, anti-diabetic drug repaglinide loaded mucoadhesive microspheres were prepared by using polymer namely polycarbophil as drug carries. Cross-linked microspheres of polycarbophil loaded with drug were successfully prepared by the emulsification technique. The prepared microspheres were found to be rough; smooth some of them were spherical. Based on these results formulation F1 was considered the best batch for sustained/prolonged release of repaglinide. From this study it is concluded that release of repaglinide drug was slow and extended over a longer period of time depending upon the composition of polymers and drug. In this study drug release was diffusion controlled and followed zero order kinetic. The study also indicated that the amount of drug release decreases with an increase in the polymer concentration.

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