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Formulation and characterization of mucoadhesive microspheres of anti-diabetic drug nateglinide

Dr. Kavitha Rajesh

Assistant Professor, Department of Wellness, Faculty of Health, Sciences, Male', Maldives National University, Maldives

ABSTRACT

Nateglinide is an anti-diabetic, oral blood-glucose lowering drug of the meglitinide class used in the management of type-II diabetes mellitus. The present investigation involves formulation and evaluation of mucoadhesive microspheres with nateglinide as model drug for prolongation of drug release time. An attempt was made to develop microspheres of nateglinide by double emulsion solvent evaporation technique, with a view to deliver the drug at sustained or controlled manner in gastrointestinal tract and consequently into systemic circulation. The mucoadhesive microspheres were formulated by double emulsion solvent evaporation technique using polycarbophil as polymer, the prepared microspheres were evaluated for Flow behavior, Compatibility study, Drug Entrapment Efficiency, In-vitro Dissolution, particle shape and size by Scanning Electron Microscopy and Sieving method.

Keywords: Nateglinide, 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. Adhesion can be defined as the bond produced by contact between a pressure-sensitive adhesive and a surface[1]. 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.

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[2].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].

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

Author for Correspondence:
Dr. Kavitha Rajesh
Assistant Professor, Department of Wellness, Faculty of Health, Sciences, Male',
Maldives National University, Maldives

or better therapeutic performance of drug. 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.

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. Nateglinide is an oral bloodglucose- 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. Dosage frequency of Nateglinide is 0.5 to 4mg in 3 to 4 times in a day. Nateglinide microsphere preparation may be beneficial to the patient since it reduce adverse effects and avoid the hepatic first-pass metabolism. The need for transdermal delivery of Nateglinide is further justified due to the requirement of maintaining unfluctuating 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 Nateglinide which increases the patient compliance and also sustain the release of drug to increase the bioavailability by using Polycarbophil as polymers.

MATERIALS AND METHODS

Nateglinide 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.

Compatibility Studies

To check the compatibility of the drug with various polymers, IR spectra of drugs, Polymers, and combination of the drug and polymers were taken. FTIR of Nateglinide and other polymers were detailed by a KBr disc over wave No. 4000 to 400 cm^{-1}.

Preparation of Mucoadhesive Microspheres[7]

Bioadhesive microspheres were prepared by an oil-in water-in-oil (O/W/O) double-emulsion method (Sandra et al. 2005). Aqueous polymer solution was prepared and subsequently stored in sealed containers at 48 ºC for 24 h prior to use. Polycarbophil (0.500 g) was dispersed in 50.0 g of deionized water and mixed by rapid vortexing; the pH was adjusted to 7 using dilute aqueous sodium hydroxide. Nateglinide was dissolved in dichloromethane.

For the first emulsion, Nateglinide dissolved in dichloromethane was emulsified into 50.0 g of aqueous polymer solution. The concentrations and amounts applied are summarised in Table. The addition of 0.15 ml of Tween 80 aided the emulsification process. A 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

A microscopically imaging analysis technique for determination of particle size distribution was used. Microsphere size and distribution were determined with an AXIOPALN microscope equipped with a computer-controlled image analysis system are shown in tables 2.

FLOW PROPERTIES

Angle of Repose[12]

The flow characteristics are measured by angle of repose. Improper flow is due to Frictional forces between the particles. These forces are quantified by angle of repose. Angle of repose is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. The flow of powder and the angle of repose is depicted in following. By definition:

$$\mathbf{Tan\ \theta = h / r}$$
$$\mathbf{\theta = \tan^{-1}(h / r)}$$

Where, h = height of pile, r = radius of the base of the pile, θ = angle of repose

Bulk densities

Bulk density is defined as the mass of a powder divided by the bulk volume. The bulk density of a powder depends primarily on particle size distribution, particle shape, and the tendency of the particles to adhere to one another.

$$BD = \frac{\text{Weight of the powder}}{\text{Volume of the packing}}$$

Tapped densities

The measuring cylinder containing a known mass of blend was tapped for a fixed time. The minimum volume (V_t) occupied in the cylinder and the weight (M) of the blend was measured. The tapped density (ρ_t) was calculated using the following formula

$$\rho_t = \frac{M}{V_t}$$

Hausner's ratio

Hausner ratio is an indirect index of ease of power flow. It is calculated by the following formula.

$$\text{Hausner ratio} = \frac{\rho_t}{\rho_d}$$

Where ρ_t is tapped density and ρ_d is bulk density. Lower Hausner ratio (<1.25) indicates better flow properties than higher ones (>1.25).

Carr's compressibility index

The compressibility index of the granules was determined by Carr's compressibility index. (%) Carr's Index can be calculated by using the following formula

$$\text{Carr's Index (\%)} = \frac{TD - BD}{TD} \times 100$$

Encapsulation efficiency[10]

Encapsulation efficiency, of nateglinide was performed by accurately weighing 100 mg of drug loaded bioadhesive microspheres were added to 100 ml of methanol. The resulting mixture was kept shaking on a mechanical shaker for 24 h. Then, after the solution was filtered and 1 ml of this solution was appropriately diluted with methanol and analyzed with spectrophoto metrically at 247 nm using a Shimazdu UV-1700 (UV/VIS double beam spectrophotometer, Kyoto, Japan). The drug encapsulation efficiency was calculated using the following formula:

(Practical drug content/ Theoretical Drug content) × 100.

Mucoadhesion[2, 3]

Mucoadhesion of different microspheres system was assessed using the method reported with little modification. A strip of rat 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 by the following formula:

$$\%\ \text{Mucoadhesion} = Wo - Wt / Wo \times 100$$

Where Wo = weight of microspheres applied; Wt = weight of microspheres leached out.

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 and concentration of the drug was determined from standard calibration curve.

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 t), first order (log% unrelease vs t) , 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 analysed 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 diffusional 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.

Stability studies[11]

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 an light and enables recommended storage conditions, re-test periods and shelf lives to be established. In the present study, stability studies were carried out at 40^0C / 75 % RH for a specific time period up to 30 days for the selected formulations.

RESULTS AND DISCUSSION

Compatibility Studies

FTIR Studies: It was clear from the infra spectrum that the drug has no interactions. The characteristic peaks of the IR pure drug spectrum are 3319.34 cm^{-1}, 1770.97 cm^{-1} and 17201.63 cm^{-1}. The IR range, as shown in Figure 1, showed a peaks of 3319,39 cm^{-1}, 1770,77 cm^{-1} and 1715,92 cm^{-1}.This endorses the undisturbed structure of the drug in the formulation. This indicates that the drug is not likely to be incompatible with the polymers used in the formulations.

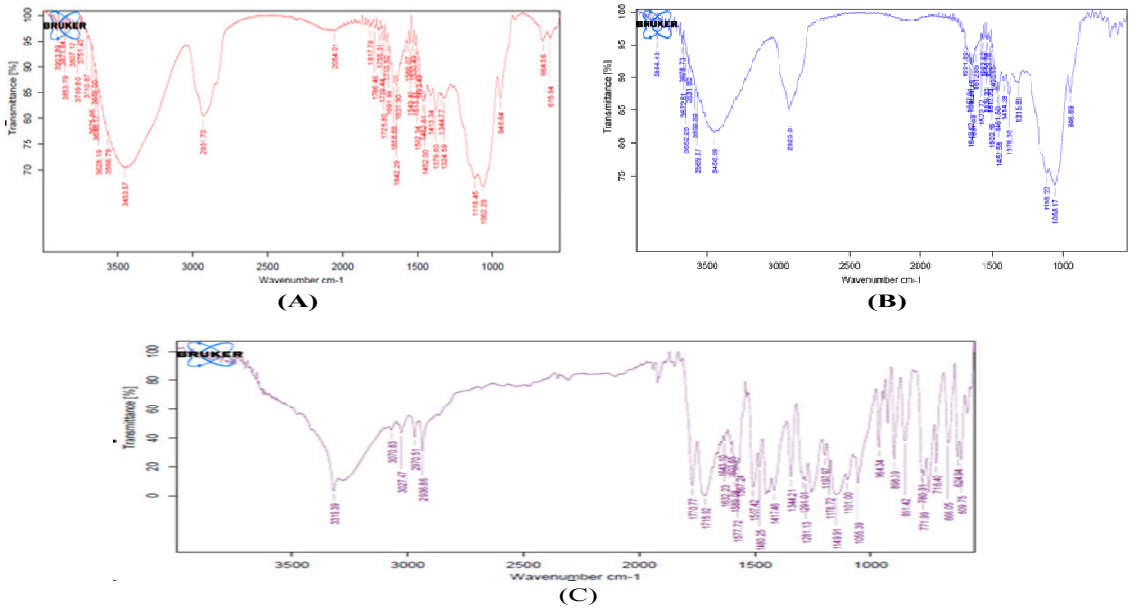

Figure 1: IR spectra of (A)-Nateglinide (B)-Polycarbophil (C)- Physical mixture

Particle size

The processing variables such as drug to polymer ratio, stirring speed, stabilizer concentration affect the particle size of microspheres as revealed in Table 1. 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 22.50 to 53.60 µm. 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 became large and aggregated. When the speed was kept above 800 rpm, the size of the microspheres was smaller and irregular in shape.

Table No. 1: Mean particle size

Formulation no	Mean particle size(µm)
F1	53.60+1.23
F2	25.30 +1.00
F3	30.43+1.20
F4	33.03+1.01
F5	39.02+0.92
F6	22.50 +1.00

*Mean ± SD, (n=3)

Flow Property

The flow property of the prepared formulations was checked by the method, angle of repose. Acceptable range of angle of repose is 22°60' to 31°58'. All the formulations showed an angle of repose within the range as shown in Table2& 3.Formulations F1 to F6 showed an angle of repose in the acceptable range, which indicates a good flow property.

Table 2: Flow properties of microspheres

Formulation	Bulk Density (g/cm^3)	Tapped density (g/cm^3)	Carr's compressibility Index	Hausner Ratio	Angle of Repose
F1	0.41 ± 0.02	0.52 ± 0.01	21.15 ± 0.14	1.26 ± 0.02	24°58'
F2	0.45 ± 0.01	0.52 ± 0.01	13.4 ± 0.21	1.15 ± 0.07	22°60'
F3	0.16 ± 0.010	0.20 ± 0.02	20 ± 0.16	1.25 ± 0.07	30°60
F4	0.16 ± 0.01	0.19 ± 0.01	15.7 ± 0.16	1.18 ± 0.08	31°58'
F5	0.45 ± 0.01	0.54 ± 0.02	16.6 ± 0.26	1.2 ± 0.06	27°48'
F6	0.43 ± 0.03	0.52 ± 0.01	17.3 ± 0.21	1.2 ± 0.04	29°56'

*Mean ± SD, (n=3)

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 76.8 to 93.5 % as shown in Table 3. 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.

Table 3: Drug entrapment Efficiency of Micro particles

Formulation	% Drug entrapment efficiency	% Mucoadhesion
F1	93.5	74.30
F2	84.3	77.21
F3	90.4	79.80.
F4	81.2	80.12

F5	89.1	82.32
F6	76.8	85.17

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 (85.17%) was observed with the formulation 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.

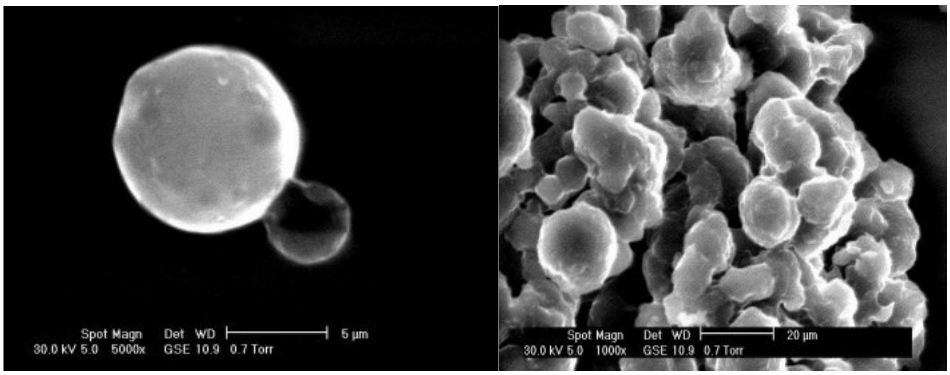

Figure 2: SEM photographs of microspheres.

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 4. 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 nateglinide 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.

Table 4: Cumulative Percentages of Drug release of formulations

S.No	Time (Hrs)	Cumulative Percentage of Drug released					
		F1	F2	F3	F4	F5	F6
1	1	1.00 ±0.01	1.00 ±0.69	1.00 ±0.02	1.00 ±0.06	1.00 ±0.80	1.00 ±0.19
2	2	3.11 ±0.61	2.11 ±0.06	2.11 ±0.83	2.11 ±0.64	2.02±0.07	2.22 ±0.09
3	4	30.44 ±0.65	40.44 ±0.09	30.44 ±0.57	40.55 ±0.18	33.35±0.90	30.55 ±0.39
4	6	50.55 ±0.36	50.66 ±0.89	50.55 ±0.91	50.66 ±0.05	50.46 ±0.79	46.66 ±0.75
5	8	70.77 ±0.41	60.99 ±0.22	70.77 ±0.48	60.88 ±0.78	68.79 ±0.02	50.88 ±0.01

6	10	84.44 ±0.29	81.44 ±0.01	84.44 ±0.55	71.22 ±0.04	71.24 ±0.60	61.22 ±0.61
7	12	92.11 ±0.35	91.11 ±0.16	89.90 ±0.75	81.66 ±0.54	78.66 ±0.78	71.66 ±0.58

*Mean ± SD, (n=3)

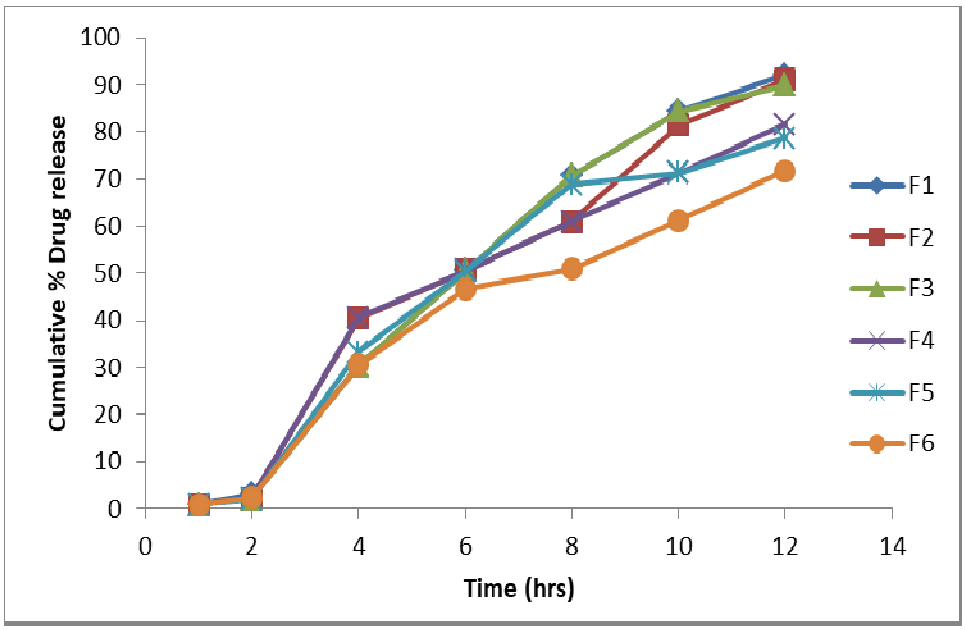

Figure 3: *In vitro* drug release studies

Release kinetics

The in-vitro release profile was analyzed by various kinetic models. The kinetic models used were Higuchi, zero order, first order and KrosmeyerPeppas 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. The formulation F1 was having n=0.491, indicating that the release mechanism followed is fickian diffusion controlled mechanism.

Table 5: Curve Fitting Data of the Release Profile for Nateglinide

Formulation	Zero order	First order	Higuchi	Krosmeyer-Peppas	n-values	Mechanism
F1	0.978	0.912	0.951	0.958	0.491	Fickian
F2	0.966	0.948	0.946	0.921	0.513	Anomalous
F3	0.972	0.956	0.948	0.943	0.423	Fickian
F4	0.947	0.922	0.949	0.911	0.456	Fickian
F5	0.945	0.934	0.945	0.930	0.527	Anomalous
F6	0.957	0.924	0.947	0.927	0.482	Fickian

Stability studies

In the present study, stability studies were carried out at 40^0C / 75 % RH for a specific time period up to 30 days for the selected formulation.

Table 6: Stabilities studies of Nateglinide Mucoadhesive Microspheres

Formulation	Tested after time (in days)	% Drug Entrapment	Cum. % 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

CONCLUSION

Oral controlled release of mucoadhesive microspheres of nateglinide can be achieved by double emulsion solvent evaporation technique using polycarbophil as a polymer. From the study it is evident that a promising sustained release microparticulate drug delivery of nateglinide can be developed. Further in-vivo investigation is required to establish efficacy of these formulations. The study also indicated that the amount of drug release decreases with an increase in the polymer concentration.

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