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Formulation and *In-Vitro* Characterization of Floating Drug Delivery System of Nateglinide

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ABSTRACT

The present work is aimed at formulation and evaluation of floating drug delivery system of nateglinide for the management of diabetes. Hollow microspheres (microballoons), loaded with nateglinide in their outer polymer shells were prepared by using emulsion solvent diffusion method. The prepared formulations were evaluated for their surface morphology by scanning electron microscopy (SEM), micromeretic properties, % drug entrapment efficiency, % buoyancy and *in-vitro* drug release studies. The prepared microspheres exhibited prolonged drug release (24 h) and remained buoyant for >12 h. The mean particle size increased and the drug release rate decreased at higher polymer concentrations. No significant effect of the stirring rate during preparation on drug release was observed. The release pattern of nateglinide in simulated gastric fluid from all floating microspheres showed their sustained action.

Keywords: microspheres, nateglinide, buoyancy, *in-vitro* drug release

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INTRODUCTION

Diabetes is the fourth leading cause of death by disease and a person dies from diabetes related causes in every 10 seconds. It is estimated that in 2000 there were approximately 150 million individuals with the disease and this number is likely to double by 2025[1,2]. Oral route remains so far the most convenient route of administration mainly because of its ease of administration, patient compliance, and flexibility of formulation. Various attempts have been made to prolong the retention of drugs in stomach. One such method is the preparation of a device that remains buoyant in the stomach contents due to its lower density than the gastric fluids[3,4].

Multiple-unit floating polymeric drug delivery systems such as floating microspheres offer advantages of retaining the dosage form in the upper part of GIT for prolonged period and thereby releasing the drug in a controlled manner. Such floating devices show more reproducible release profiles in comparison to all-or-nothing emptying nature or dose-dumping phenomenon associated with single-unit system[5,6,7]. At present, hollow microspheres are considered to be one of the most promising buoyant systems, because they combine the advantages of multiple unit systems and good floating properties. These systems are also called “microballoons” due to their low-density core[8].

Nateglinide, an anti-diabetic drug belongs to the meglitinide category. It has short half life of 1.5 hr. It helps to lower blood glucose levels by blocking ATP-dependent potassium channels in pancreatic beta cells, which in turn, stimulates insulin secretion. Similar to repaglinide, nateglinide also has a rapid onset and short duration of action. It has been used alone or in combination with other medications to treat patient with type 2 diabetes[9]. Nateglinide is a novel D-phenylalanine derivative that inhibits ATP-sensitive K+ channels in pancreatic beta-cells in the presence of glucose and thereby stimulates the prandial release of insulin[10]. Nateglinide is an insulinotropic agent that restores the physiological pattern of insulin secretion lost in T2DM in a transient and glucose-sensitive manner and thus can control glucose mealtime excursions. Nateglinide can be used in monotherapy, in order to control excessive mealtime glucose spikes early in the development of diabetes, or in combination with other agents that have complementary modes of action, such as metformin or glitazones, thus providing better overall chronic glycemic control by reducing Hb_{A1c} [11].

Eudragit RS 100 is copolymer of ethyl acrylate, methyl methacrylate and a low content of a methacrylic acid ester with quaternary ammonium groups (trimethylammonioethyl methacrylate chloride)[12]. The present study is aimed at preparation of nateglinide microspheres by a novel

emulsion solvent evaporation technique and its evaluation in order to achieve an extended release in the upper GIT, which may result in reduction in dosing frequency thereby improving patient compliance.

MATERIALS AND METHODS

Materials

Nateglinide was obtained as a gift sample from Dr. Reddy Lab, Hyderabad. Eudragit RS 100 was obtained as a gift sample from Dr. Reddy Lab, Hyderabad. Dichloromethane (DCM) was procured from Central Drug House Ltd., New Delhi**.** All the other reagents were of analytical grade and were used as received.

Emulsion solvent evaporation technique

Microballoons with an internal hollow structure were prepared by emulsion solvent diffusion method with slight modification using the method established by Kawashima[5], using Eudragit RS 100 as polymer. Drug and polymer in ratio of 1:1 and 1:2 were dissolved in 2:1 mixture of solvent system of dichloromethane (DCM) and acetonitrile. This clear solution was then poured slowly in a thin stream into the aqueous solution of 0.5% w/v polyvinyl alcohol (PVA). The emulsion was continuously stirred for 1 hour at speed of 300 rpm using mechanical stirrer (Remi, India), equipped with propeller at 40^{o}C. Microballoons were collected by filtration, washed with petroleum ether and dried at room temperature for 24 hours. The prepared formulations are given in Table 1.

Table 1: Various formulation of microballoons of nateglinide

Formulation code	Drug: Polymer	Dichloromethane: Acetonitrile	Conc. PVA (%w/v)	Stirring speed (rpm)
F1	1:1	2:1	0.5	300
F2	1:2	2:1	0.5	300
F3	1:1	2:1	0.5	400
F4	1:2	2:1	0.5	400
F5	1:1	2:1	1.0	300
F6	1:2	2:1	1.0	300
F7	1:1	2:1	1.0	400
F8	1:2	2:1	1.0	400

Morphology and Particle size

The surface morphology of microspheres was studied using Scanning Electron Microscope (SEM LEO 430, Leo Electron Microscopy Ltd., England). For determination of surface characteristics all the microspheres were coated uniformly with gold palladium by using sputter coater for 5 to 7 minutes, after fixing the sample in individual steps. All samples of microspheres were then randomly examined for surface morphology at different magnification ranges. Particle

size of the microcapsules was evaluated using optical microscopy method[13]. Approximately 100 microspheres were counted for particle size determination using a calibrated optical microscope (Magnus MLX-DX). The experiments were done in triplicate (n=3).

Micromeretic properties

Accurately weighed microspheres were poured gently through a glass funnel into a graduated cylinder exactly to 10 ml mark. Initial volume was noted. Bulk density and tapped density were noted using tapping method using 10 ml measuring cylinder. Angle of repose of prepared microspheres (**n**=3) was determined by fixed funnel standing method. The granules were allowed to flow through funnel orifice on a plane paper kept on the horizontal surface to form a pile of granules. Angle of repose (θ), Hausner's ratio (H) and Carr's index (% C) were calculated to study the flow properties of microspheres by using following formulas[14].

$$\theta = \tan^{-1}\frac{h}{r}$$

where, h is height and r is radius of the pile, respectively.

$$H = \frac{Dt}{Db}$$

$$\%\,C = \frac{Dt - Db}{Dt} \times 100$$

where, Dt is tapped and Db is bulk density, respectively.

Drug entrapment efficiency

Dried microballoons (120 mg) containing drug were taken, crushed by trituration and suspended in a minimal amount of dichloromethane (10 ml) for dissolving the coat shell of the microspheres. The suspension was suitably diluted with 0.1N HCl and filtered to separate the shell fragments. Drug entrapment efficiency was analyzed after suitable dilution spectrophotometrically by UV at 210 nm (Shimadzu Pharmspec UV-1700, Japan). The entrapment efficiency of microspheres (n=3) were calculated by using following formulas[15].

$$\%\,EE = \frac{Dcal}{Dth} \times 100$$

where, Dcal is the calculated drug content and Dth is the theoretical drug content, respectively.

Percentage buoyancy

Microparticles (50 mg) were placed in simulated gastric fluid (pH 1.2, 100 ml) containing Tween 20 (0.02% w/v). Tween was used to impart wetting effect of the natural surfactants such as phospholipids in the GIT. The mixture was stirred at 100 rpm using a magnetic stirrer. After 12

h, the layer of buoyant particles was pipetted and the floating particles were separated by filtration. Particles in the sinking particulate layer were separated by filtration. The particles were dried at 40°C overnight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of the floating particles to the sum of floating and sinking particles[16].

$$\% \text{ buoyancy} = \frac{W_f}{W_f + W_s} \times 100$$

where, W_f and W_S are the weights of the floating and settled microballoons, respectively. All the determinations were made in triplicate (n=3).

In vitro drug release

A USP XXIII basket type dissolution apparatus was used to study in-vitro drug release from microballoons. A weighed amount of microballoons equivalent to 100 mg drug was filled into a hard gelatin capsule (No. 0) and placed in the basket of dissolution rate apparatus. 900 ml of simulated gastric fluid (SGF) pH 1.2 containing 0.02 % w/v tween 20 was used as dissolution fluid. Dissolution medium was maintained at temperature 37±0.5°C at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release study. Five milliliter samples were withdrawn at each 1 hour interval, and analyzed spectrophotometrically at wavelength of 210 nm (Shimadzu Pharmspec UV-1700, Japan). This volume was replenished with the same amount of fresh dissolution fluid each time to maintain the sink condition[17].

Statistical analysis

The results were expressed in mean ± S.D. One way ANOVA (Analysis of Variance) was performed for studying the statistical significance using Minitab 15 software. Values of $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Morphology and Particle size

Scanning electron microscopy revealed pores on the microsphere as well as a hollow microsphere interior (Figure 1). It was observed that pores were formed due to drug release. It may be said that due to the blasting of drug, the drugs were released through this bigger pore and the controlled release of drug may be through the smaller pore.

The mean particle size was found to be in the range of 140.35±5.23 (F1 with drug: polymer concentration, 1:1) to 174.73±6.83 µm (F6 with drug: polymer concentration, 1:2) (Table 2). The mean particle size of microballoons significantly increased with increasing polymer

concentration (P<0.05). This may be due to increase in viscosity of the medium at a higher polymer concentration resulting in enhanced interfacial tension. Shearing efficiency was also diminished at higher viscosities[18].

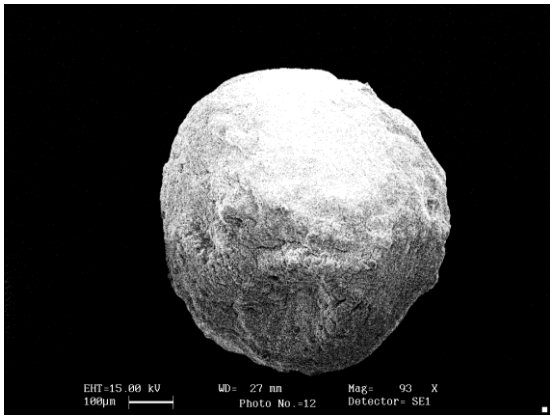

Figure 1: SEM photograph showing surface morphology of microballoons of nateglinide

Table 2: Angle of repose, Carr's index, Hausner's Ratio and Particle Size (µm)

Formulation	Angle of repose	Carr's index	Hausner's Ratio	Particle Size(µm)
F1	26.50±0.03	8.12±1.02	0.95±0.02	141.20±5.87
F2	28.30±0.04	10.26±2.64	1.16±0.07	155.50±6.18
F3	26.03±0.02	7.62±1.01	1.00±0.05	140.35±5.23
F4	27.60±0.02	8.58±1.52	0.98±0.03	150.46±6.00
F5	31.20±0.05	14.06±3.24	1.15±0.06	168.24±6.42
F6	32.40±0.03	16.00±3.86	1.14±0.05	170.45±6.52
F7	30.00±0.04	12.50±2.84	1.18±0.07	160.65±6.21
F8	34.06±0.05	17.24±4.02	1.20 ±0.06	174.73±6.83

Values are given in mean±S.D. (n=3)

As the stirring speed was increased from 300 rpm to 400 rpm, the particle size decreased from 192.02±7.85µm to 145.42±5.36 µm and buoyancy of microballoons decreased from 91.24±3.24% to 86.13±2.49%. This might be due to decrease in the size of cavity present inside the microballoons. It was observed that at stirring speed of 400 rpm enough shear force was not produced to from stable emulsion and particles formed were irregular in shape and drug entrapment was low due to reduction in shearing stress. This resulted in increased leaching of drug from matrix cavity to external phase[12].

The type and concentration of emulsifier play a key role in the preparation of microballoons. In case of 0.5% w/v emulsifier concentration, the particle size was found to increase significantly (P<0.05). This might be due to low emulsifier concentration was not sufficient to reduce the surface charge and particles were aggregated resulting in an increase in particle size. At higher concentration of PVA i.e. 1.0 % w/v stable emulsion was formed which resulted in decreased

entrapment efficiency and particle size. It might be due to excessive PVA concentration which resulted in reduction in surface charge so that the particles were not aggregated due to sufficient pressure of emulsifier on the outer surface of particles and formation of micelles thus particle size was found to be smaller with low entrapment efficiency because of the leaching of the drug[19].

Micromeritic properties

For the prepared formulations angle of repose (26.50±0.03 to 34.06±0.05), Carr's index (8.12-17.24 %) and Hausner's ratio (0.95-1.20), confirmed good flow properties of the microspheres (Table 2).

Drug entrapment efficiency

The drug entrapment efficiency of all formulations was found to be between 60-80% (Figure 2). Formulation F8 had maximum drug entrapment efficiency. An increase in polymer conc. resulted in formation of larger microspheres entrapping greater amount of drug[20].

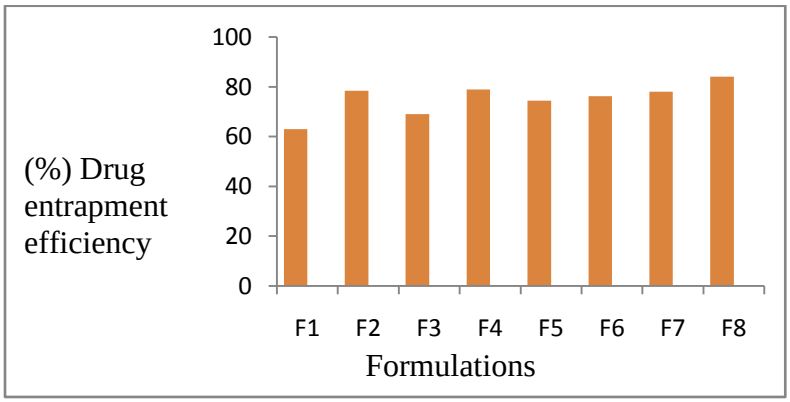

Figure 2: Graph showing percentage drug entrapment efficiency of the prepared formulations

Percentage buoyancy

The percentage buoyancy of floating microspheres was greater than 86% for all the formulations (Figure 3). In the floatation test, more than 75% microspheres remained floating at the end of 12 h. Smaller the microspheres lesser was the floating ability and faster was the release rate of the drug form the microspheres, while larger the size, floating ability was found to be more and sustained was the release of drug. The good buoyancy behavior of the microspheres may be attributed to the hollow nature of the microspheres. Formulation F5 gave the best floating ability (93%) in SGF. Tween 20 (0.02% w/v), added to SGF, counteracted the downward pull at the liquid surface by lowering surface tension, because the relatively high surface tension of simulated gastric fluid causes the highest decrease of surface area at the air-fluid interface[13].

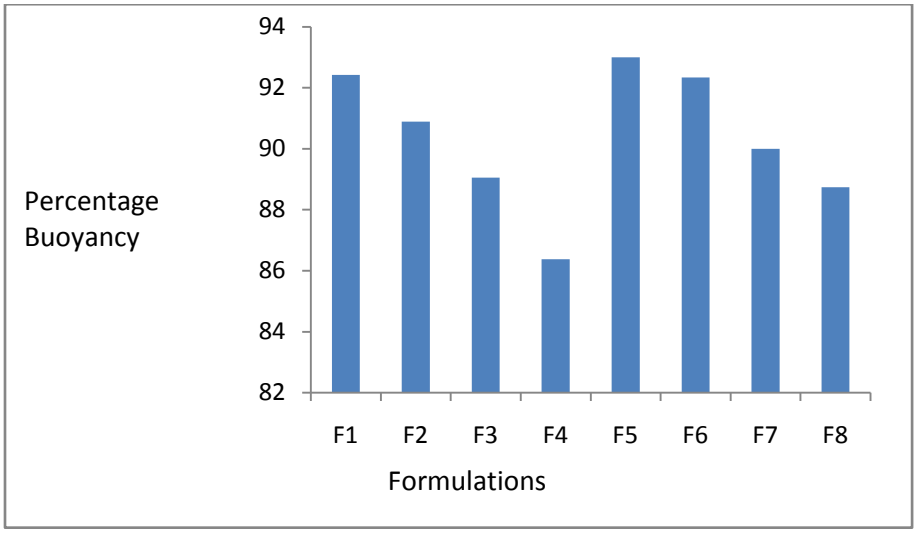

Figure 3: Graph showing percentage buoyancy of the prepared formulations

In vitro drug release

The in-vitro release studies of nateglinide were performed in simulated gastric fluid (pH 1.2) for 24 h. The cumulative release of nateglinide significantly decreased with increasing Eudragit RS 100 concentration. The increased density of polymer matrix at higher concentration resulted in an increased diffusion pathlength. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release[14]. However, increase in emulsifier concentration in the formulations showed non significant results in the drug release rate ($p>0.05$). Nateglinide release was higher in the case of microspheres prepared at a higher stirring speed but the difference in drug release was not statistically significant ($p>0.05$). The in-vitro release data is depicted in Figure 4.

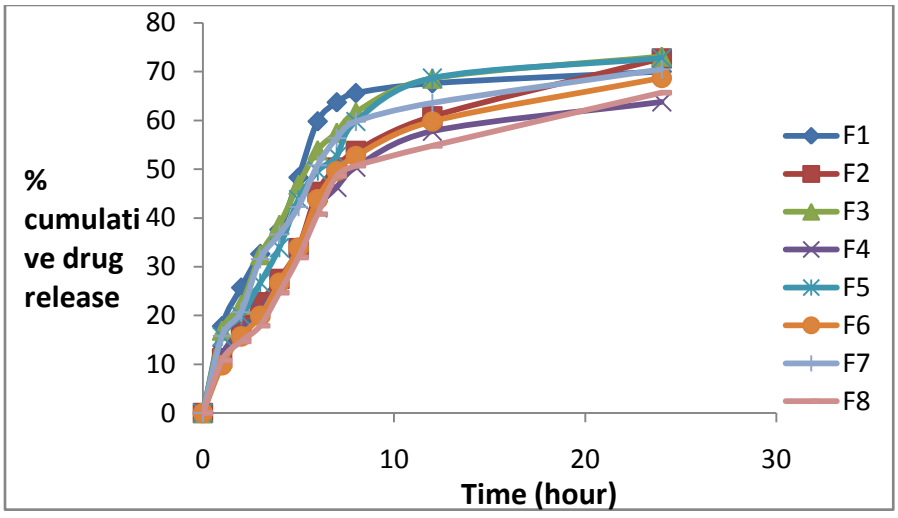

Figure 4: Graph showing % cumulative drug release Vs time of the prepared formulations

CONCLUSION

The present formulation study of nateglinide was performed in an attempt to prepare a floating drug delivery system consisting of a floating multiple-unit system. The performance of these formulations was evaluated and the effect of various formulation variables was studied. The excellent buoyant ability and suitable drug release pattern could possibly be advantageous in preparing an intragastric floating and sustained release microspheric preparation.

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