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Research Article

FABRICATION AND *IN VITRO* EVALUATION OF NATEGLINIDE-LOADED ETHYL CELLULOSE NANOPARTICLES

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ABSTRACT

Objective: The objective of the present study is to formulate freeze-dried oral sustained release polymeric nanoparticles of nateglinide (NTG) to decrease dosing frequency, increase bioavailability, and minimize side effects.

Methods: NTG-loaded ethyl cellulose (EC) nanoparticles were prepared by solvent evaporation technique and subjected to various studies for characterization, such as particle size, zeta potential, drug loading (DL), entrapment efficiency (EE), scanning electron microscopy, and evaluated for *in vitro* drug release.

Results: From this study, it was observed that the fabricated nanoparticles showed satisfactory results, i.e. particle size with 172 nm, 72.19% EE, −15.81 mV zeta potential, and 14.30% DL. The results of *in vitro* release show that sustained release of NTG from the nanoparticles over the period of 12 hrs and comparable with the immediate release tablets. Furthermore, accelerated stability studies revealed that the formulation is stable as per International Conference on Harmonisation guidelines.

Conclusion: Thus, the nanoparticles formulation could be a promising delivery system for NTG with improved anti-diabetic activity, stability, and bioavailability.

Keywords: Drug delivery, Nanoparticles, Nateglinide, *In vitro* release, Solvent evaporation method.

INTRODUCTION

Nanoparticles are an effective formulation for the delivery of hydrophobic and hydrophilic drugs, since the drugs are protected from possible degradation by enzymes, and it can deliver drugs at a sustained rate providing better efficacy and lower toxicity for treatment of various diseases [1]. Recently, nanoparticles process have been developed and reported for pharmaceutical application to increases the dissolution rate of low-soluble drugs which in turn may leads to significantly increase in bioavailability and also it is essential for pharmaceutical industry as an alternative drug delivery system for the treatment of chronic disease like diabetes mellitus [2].

Diabetes mellitus is a metabolic disorder characterized by high blood glucose level resulting from inadequate secretion or utilization of insulin [3]. Nateglinide (NTG) has been exploited as a new class of an oral anti-diabetic agent used in the management of Type 2 diabetes mellitus. NTG, (-)-N-[(trans-4-isopropylcyclohexane) carbonyl]-D-phenylalanine, is structurally (Fig. 1) unrelated to the oral sulfonylurea insulin secretagogues. NTG is a D-phenylalanine derivative recently approved for the management of Type II diabetes [4,5]. In difference to sulfonylureas, NTG increases pancreatic β-cell sensitivity to ambient glucose without increasing basal insulin secretion after oral administration. It can be used as monotherapy or in combination with metformin or thiazolidinedione. It has a short half-life of 1.5 hrs. and peak plasma concentration extents at 0.5-1.0 hr. It is metabolized by cytochrome P-450 system to an inactive metabolite and eliminated with a half-life of 1.4 hrs [6].

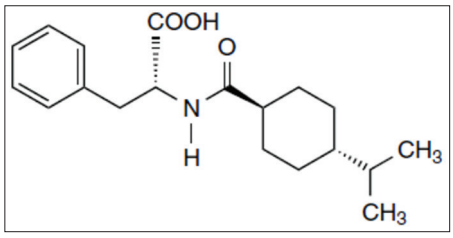

Fig. 1: Chemical structure of nateglinide

The objective of the present study was to formulate and evaluate the freeze-dried oral sustained release polymeric nanoparticles of NTG to decrease dosing frequency, minimize side effects, and increases the bioavailability.

METHODS

Materials

NTG was a gift sample from Glanmark Pharmaceutics Ltd., Mumbai, India. Ethyl cellulose (EC) was from Hi-media Laboratories, Mumbai, India. Polyvinyl alcohol was from Fourrts India Laboratories Pvt. Ltd., Chennai, India. Methanol was from Qualigens Fine Chemicals, Chennai, India. All other chemicals used were of analytical reagent grade.

Methods

Preparation of NTG nanoparticles

The NTG-loaded EC nanoparticles were prepared by the solvent evaporation method. Briefly, 30 mg of NTG and 150 mg of EC were dissolved in 40 ml mixture of methanol and acetone in 1:2 ratio using a vortex shaker to form homogeneous organic phase of NTG and EC and it was added drop by drop into the 60 ml of 1% aqueous phase of polyvinyl alcohol using mechanical stirrer at 1000 rpm for 2 hrs to prepare nano-suspension and thoroughly evaporate the organic phase followed by magnetic stirring for 2 hrs under atmospheric pressure at room temperature. The solution was centrifuged at 15,000 rpm for 15 minutes and after centrifugation the supernatant was removed and the pellets obtained were washed thrice with distilled water and finally freeze-dried to get the powdered nanoparticles. The nanoparticles were prepared in triplicate to get the reproducibility and reliability.

These nanoparticles were characterized by fixing the experimental assay conditions based on the pre-conducted preliminary experimental results, carried out in our previous studies [7,8].

Characterization of nanoparticles

Determination of particle size and zeta potential

Particle size of fabricated nanoparticles was measured by particle size analyzer (MASTERSIZER 2000, MALVERN Instruments, UK) equipped with MAS OPTION particle sizing software. The measurements were made at a fixed angle of 90° for all samples. The samples were suitably diluted with Milli Q water for every measurement. Zeta potential measurements were measured by Malvern zetasizer (MAL 1054413 Zetasizer Version 6.20 Instruments, UK). For zeta potential determination, samples of all formulations were diluted with 0.1 m mol KCl and placed in the electrophoretic cell, where an electric field of about 15 V/cm was applied. The mean hydrodynamic diameter (Dh) and polydispersity index (PI) of the particles were calculated using the cumulative analysis after averaging the three measurements [9].

Scanning electron microscopy (SEM)

SEM analysis of the nanoparticles formulation was performed to evaluate the surface morphology of nanoparticles. Images were taken using JEOL JSM-6701F (Tokyo, Japan) at 3.0 kV with 50,000 magnifications and 100 nm scale bar was used [10].

Chromatographic conditions

NTG estimation was carried out by reverse phase high pressure liquid chromatographic (RP-HPLC) based on the reported method by Madhavi*et al.,* 2008 [11]. An isocratic RP-HPLC with Shimadzu LC-20AD PLC pump and an SPD-M20A photo diode array detector were used. Separation was carried out on a phenomenex C18 column (particle size 5 µm; 150 × 4.6 mm i.d.) using acetonitrile: 10 mM sodium di-hydrogen phosphate (NaH_2PO_4) buffer solution [phosphate-buffered solution (PBS); adjusted to pH 3.0 with H_3PO_4] in the ratio of 50:50, v/v. The flow rate was 1.0 ml/min at 27°C, and the detection was monitored at a wavelength of 210 nm. The injection volume was 20 µl. Acetonitrile was used as diluent.

Determination of drug loading (DL) and entrapment efficiency(EE)

The DL and EE were estimated by RP-HPLC method, following the previously described protocol with slight modification [11]. In briefly, A 10 mg sample of the formulated nanoparticles was dissolved in 10 ml acetonitrile (as common solvent for both the drug and polymer) and from the above solution 20 µl was taken for RP-HPLC analysis. The amount of drug in the solution was calculated using standard graph of NTG in pH 7.4 PBS buffer analyzed by RP-HPLC method [12].

$$\text{Drug loading}\left(\%\frac{w}{w}\right)=\frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles recovered}}\times 100 \quad (1)$$

$$\text{Entrapment efficiency }(\%)=\frac{\text{Mass of drug in nanoparticles}}{\text{Mass of drug added in formulation}}\times 100 \quad (2)$$

Drug release studies

The *in vitro* release of marketed formulation and NTG-loaded EC nanoparticles were carried out using dialysis bag diffusion method. Briefly, 2 mg samples are dispersed in 2 ml of PBS at pH 7.4 and were kept in a dialysis bag (cellulose acetate membrane with molecular weight cut-off value of 10,000) and tightly closed. The dialysis bag was immersed in the compartment containing 50 ml of dissolution medium (PBS pH 7.4), which was stirred in a water bath shaker at 100 rpm and maintained at 37°C±0.5°C. At predetermined time intervals, the requisite quantity (1 mL) of samples were withdrawn and analyzed by RP-HPLC method. Equal quantity of fresh releasing media was added to maintain the definite volume [6].

Stability studies

The fabricated formulation was subjected to accelerated stability studies as per International Conference on Harmonization (ICH) guidelines to evaluate the effect on stress conditions. NTG-loaded EC nanoparticles were packed in 0.044 mm delaminated aluminum foil and subjected to elevated temperature and humidity conditions of 40°C±2°C/75%±5% RH (accelerated), 30°C±2°C/65%±5% RH (intermediate), and also 25°C±2°C/60%±5% RH (long term condition). Samples were withdrawn at the end of 0, 3, and 6 months and evaluated for physical properties of the nanoparticles by particle size, zeta potential, drug content, and drug release rate [7,13].

RESULTS AND DISCUSSION

Preparation of NTG nanoparticles

The NTG-loaded EC nanoparticles were successfully prepared by solvent evaporation technique. This method was simple and reproducible for the preparation of NTG loaded EC nanoparticles. In this method, the drug and polymer were dissolved in organic solvent mixture and the organic phase was mixed slowly to aqueous phase containing surfactant with constant stirring by using mechanical stirrer to form the nanoparticles and to evaporate the organic phase. The solution was centrifuged and sediments were collected and washed with deionized water three times to remove any un-entrapped drugs. The sediment was freeze-dried to get the powdered products.

Determination of particle size and zeta potential

The hydrodynamic diameter of the prepared nanoparticles was found to be ~172 nm (Fig. 2). The PI of the particles was 0.341, which indicate the prepared nanoparticles were mono dispersed.

Stability of the nanoparticles is indicated by zeta potential. No matter what is the charge type, higher the magnitude, higher the stability. Zeta potential of the nanoparticles showed higher stability with a value of −15.6 mV (Fig. 3). Pure EC nanoparticles are expected to have a high

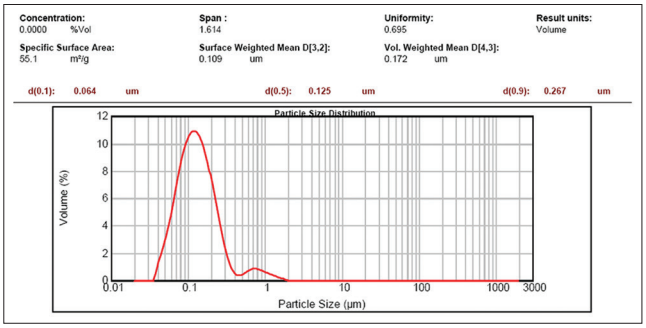

Fig. 2: Particle size distribution of optimized nateglinide-loaded ethyl cellulose nanoparticles formulation

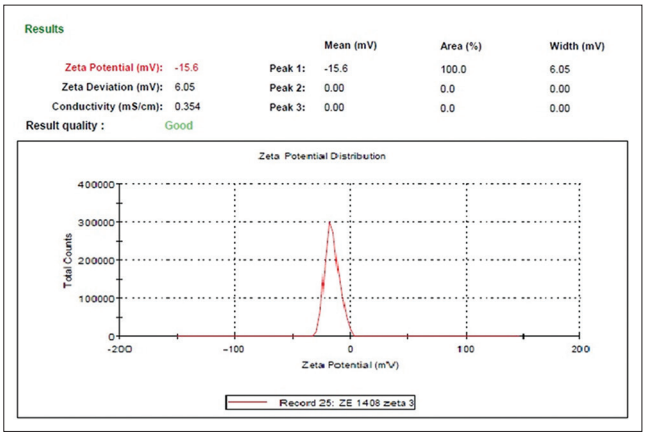

Fig. 3: Zeta potential of optimized nateglinide-loaded ethyl cellulose nanoparticles formulation

negative zeta potential due to the presence of residual stabilizers on the nanoparticles surface. A shield could have been created between the nanoparticles surface and the surrounding medium due to the presence of the residual stabilizer on the nanoparticles surface. In addition, the stabilizers could mask the available charged group found on the particle surface [9].

Determination of DL and drug EE

The drug content and drug EE of the NTG-loaded EC nanoparticles was determined by the RP-HPLC method. The respective values were calculated from a standard curve prepared under the same experimental parameters. The DL and drug EE of the NTG-loaded EC nanoparticles were found to be 14.30%±0.27% and 72.19%±0.24%, respectively, all the measures were carried out in triplicate (n=3).

SEM analysis

SEM analysis was carried out for optimized NTG-loaded EC nanoparticles formulation to examine its shape and surface morphology (Fig. 4). The SEM images showed that smooth surface of the particles with distinct, uniform, and spherical structure and it indicates that the formulation method was efficient. The smooth surface observed in the images reveals complete removal of the solvent from the formulated nanoparticles [14].

Drug release studies

The percentage of drug release from NTG-loaded EC nanoparticles was studied as a function of time in *in vitro* condition. The drug release study was performed by using the dialysis bag diffusion method. The percentage amount of drug released from NTG-loaded EC nanoparticles formulations was depicted in Fig. 5. The formulation shows a significant and sustained release (up to 86.21% in 12 hrs) of NTG in nanoparticles form as compared to the marketed conventional formulation. About 93.42% of the drug was released from the marketed formulation after 1.5 hrs. Thus, it is evident that nanoparticles have a small initial burst and sustained release than that of the marketed formulation. The sustained release nature is thought to be mainly because of the 1:5 ratio of polymer and that of the drug. EE 72.19% obtained was expected due to the ratio 1:5 again.

The release of drug from the polymer matrix was based on diffusion mechanisms. EC is widely used to control the dissolution rate of drugs from sustained release preparations. EC possess plastic and hydrophobic property, drug particles present in the surface of matrix is initially released into the surrounding media generating many pores and cracks which facilitate further release of drug and EC did not change its drug retaining activity due to the change of pH. The increase in drug content in the particles influence the absolute release profiles of the drug, in such a way that, it increases the induction period and the cumulative amount of drug released at any given point of time. The drug content which is closer to the surface of the nanoparticles is responsible for an increased initial burst and the drug in the core of nanoparticles is responsible for a prolonged drug release from the polymer [4].

Stability study

NTG-loaded EC nanoparticles were subjected to stability studies as per ICH guidelines. Stability studies according to ICH guidelines revealed that the fabricated nanoparticles were stable at the end of 6 months in all the test conditions (Table 1). No significant changes in particle size, zeta potential, drug content, and drug release rate were observed after the end of 0, 3, and 6 months and found identical in stability studies.

CONCLUSION

NTG-loaded EC nanoparticles were prepared by solvent evaporation method. By using a mixture of protecting excipients such as EC and polyvinyl alcohol more stable nanoparticles could be prepared. The formulation shows mean particles size distribution of ~172 nm. The SEM images showed a smooth surface of the particles with round structure and the optimum EE of 72.19%±0.24%. The sustained drug release was found to be 86.21% in 12 hrs. Thus, NTG-loaded EC nanoparticles could reduce dosing frequency and sustained release leading to improve patient compliance. These developed nanoparticles can be safer and promising agents for rational drug delivery system for the treatment of disease like type II diabetes mellitus.

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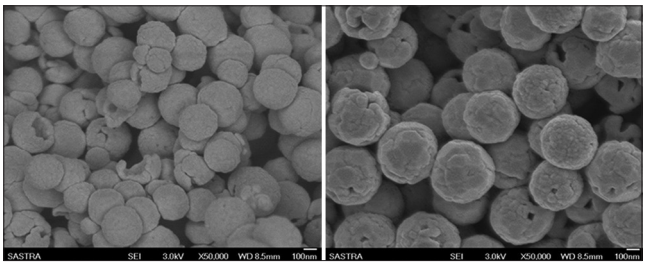

Fig. 4: Scanning electron microscopy images of optimized nateglinide-loaded ethyl cellulose nanoparticles formulation taken at 3.0 kV 50,000 magnifications

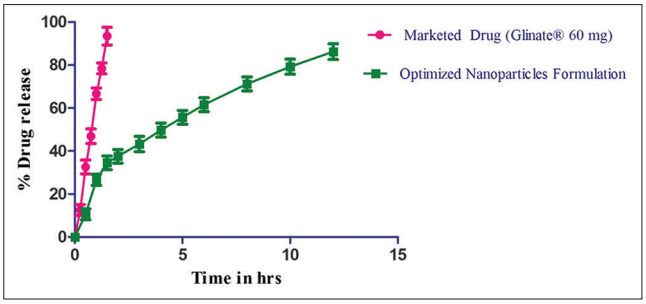

Fig. 5: Comparative cumulative percentage drug release plot for nateglinide marketed tablets (Glinate® 60 mg) and optimized nateglinide -loaded ethyl cellulose nanoparticles formulation

Table 1: Stability studies of optimized NTG-loaded EC nanoparticles formulation

Test duration (months)	Test conditions °C/RH%	Mean particle size (nm)	Zeta potential (mV)	Drug content (%)
0	40±2/75±5			
	30±2/65±5	171±0.52	−15.43±1.20	95.82±0.28
	25±2/60±5			
3	40±2/75±5	173±0.68	−15.31±3.40	95.37±0.29
	30°±2°/65±5	172±0.61	−14.32±4.10	94.73±0.31
	25±2/60±5	172±0.63	−14.21±1.30	94.25±0.23
6	40±2/75±5	174±0.71	−16.09±2.20	94.23±0.27
	30±2/65±5	172±0.63	−15.12±1.30	94.42±0.41
	25±2/60±5	172±0.55	−15.41±2.80	94.08±0.24

NTG: Nateglinide, EC: Ethyl cellulose

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