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## RESEARCH ARTICLE

# Formulation & Characterization of Chitosan based Nanoparticles of an Antidiabetic drug (Voglibose)

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**ABSTRACT:**

The aim of the present research is to develop novel drug delivery system as nanoparticles of a drug meant for lowering post-prandial blood glucose levels in people with diabetes mellitus using Volgibose. Voglibose Loaded Chitosan Nanoparticles as active pharmaceutical ingredient with varying concentration of polymer (chitosan), Poloxamer 188 were obtained successfully using evaporation (single emulsion) technique. These formulations were optimized for various parameters like stirring speed and stabilizer studied etc then characterize for particle size, product yield, drug loading efficiency and physical characterization. Scanning electron microscopy (SEM) images showed the nanoparticles were in size range of 330 to 651 nm and the distribution of particle sizes are found to be monodispersed as the polydispersity index lies below 0 to 1 (0.234 to 0.780) in all the formulations.. The physical characterization showed that nanoparticles formulation coded by NP6 showed a better loading efficiency and production yield. The in vitro study showed the slow release of the drug from the nanoparticles and improved permeability compared with pure drug. From the various release kinetic models the formulation NP6 obey the zero order and first order kinetics, followed by diffusion and erosion mechanism. Formulation NP6 showed stable formulation containing drug content of 96.05% studied for 6 months.

**KEYWORDS:** Telmisartan Nanoparticles; diabetes mellitus; Scanning electron microscopy; polydispersity index.

## INTRODUCTION:

Controlled drug delivery technique presents front line part of today's developed technique, in this includes many scientific approaches, serving for individual care. The drug deliverance technique having abundant advantages than existing conventional type of dosage, it involves enhanced effectiveness, minimized poisoning, enhanced consumer conformity also ease. [1] This type of drug deliverance technique utilizes micro molecules, for caring drugs. As the varieties of forms for dosage are invented like microparticle as well as nanoparticles shown more significance [1-3].

An ideal and advanced oral drug delivery system is that, which exactly controls speed, time as well as site of release of medicament separately of normal physiological variables such as gastrointestinal tract pH, digestive condition of the gastrointestinal tract, peristalsis movement and circadian rhythm. Advance in polymer science and technology outcome in pick up the pace research and developmental activity in the design of drug delivery devices.[4-6.]

Therapeutic effectiveness of a drug depends upon the bioavailability and eventually upon the solubility of drug substances. Solubility is prerequisite to achieve desired concentration of drug in systemic circulation, drug absorption and pharmacological response.[7-9] Oral route of drug administration is the uncomplicated and easiest approach of administration of drugs as it offers good patient compliance, convenience, accurate dosing, easy production, and greater stability. [10-12]

Voglibose is an alpha-glucosidase inhibitor used for lowering post-prandial blood glucose levels in people with diabetes mellitus.[12-13] Alpha-glucosidase inhibitors are saccharides that act as competitive inhibitors of enzymes needed to digest carbohydrates: specifically alpha-glucosidase enzymes in the brush border of the small intestines.[14] The membrane-bound intestinal alpha-glucosidases hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine.[4-6]

Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effect of these drugs therapies is to decrease current blood glucose levels: the long-term effect is a small reduction in hemoglobinA1c level.[15-16]

Chitosan is a widely available, mucoadhesive polymer that is able to increase cellular permeability and improve the bioavailability of orally administrated protein drug. Chitosan

Nano particles bio-compatible and non-toxicity of material make it attractive as a neutral agent for delivery of active agent[17-20].

## METERIAL AND METHOD:

### Material:

Voglibose is obtained as gift sample from Theon Pharmaceuticals, Haryana, India and other excipients used in this work was obtained as gift samples from Loba Chemical Private Limited Mumbai, India and Sigma, Mumbai, India.

### Method:

The nanoparticles of Voglibose were prepared with various ratios of drug and Chitosan polymer as described in table 1 using the solvent evaporation (single emulsion) technique with slight modification (Jain, 2000). The Voglibose was dissolved in the mixture of methanol and acetone (10:90 v/v) containing the chitosan polymer at room temperature. The resultant primary solution was added with a constant flow rate (0.5 ml/min) into water containing the poloxamer-188 (aqueous phase). During this process, the mixture was homogenized using a probe sonicator, at various agitation speeds in an ice bath. The formed oil- in-water (O/W) emulsion was kept at room temperature for 24 h under gentle stirring to evaporate the organic solvents. The obtained nanosuspensions were centrifuged at 40,000 rpm, 4oC for 20 min Ultracentrifuge, Remi India). The pellets were collected and washed at least three times with double distilled water to remove un-entrapped drugs. The recovered nanoparticulate suspension was freeze dried using laboratory lyophilizer maintained at 80 °C and <10 mm mercury pressure for 2 days to get powdered nanoparticles and kept at freeze for further use[11, 13-16].

For optimization of processing parameters agitation speed was varied from 5000, 10,000, 15,000 and 17,000 rpm while keeping the other parameters constant (for further experiment the agitation speed which produced the lowest particle size with highest entrapment efficiency will be chosen);

The various weight ratios of drug and polymer were taken for the nanoparticle formulation keeping the agitation speed and solvent mixture (methanol: acetone = 10:90 v/v) constant[21].

**Table 1. Formulation of loaded Voglibose Loaded Chitosan nanoparticles [16-18]**

| S. no | Formulation | Drug : Polymer Ratio | Wt. of Drug (mg) | Wt. of Polymer | Vol. of organic phase (ml) | Vol. of aqueous phase (ml) | Agitation speed (rpm) |
|---|---|---|---|---|---|---|---|
| 1 | NP1 | 1:1 | 20 | 20 | 10 | 20 | 5000 |
| 2 | NP2 | 1:2 | 20 | 40 | 10 | 30 | 10,000 |
| 3 | NP3 | 1:2 | 20 | 40 | 10 | 50 | 15,000 |
| 4 | NP4 | 1:2 | 20 | 40 | 10 | 20 | 17,000 |
| 5 | NP5 | 1:3 | 20 | 60 | 10 | 30 | 15,000 |
| 6 | NP6 | 1:4 | 20 | 80 | 10 | 20 | 15,000 |
| 7 | NP7 | 1:5 | 20 | 100 | 10 | 30 | 15,000 |
| 8 | NP8 | 1:6 | 20 | 120 | 10 | 20 | 15,000 |

## CHARACTERIZATION OF NANOPARTICLES

- **Determination of particle size and Zeta potential**

Particle size analyses were performed by Zetasizer 3000. The measurements were carried out at a fixed angle of 90o. The freeze dried powdered samples were suspended in Milli- Q water (1mg/ml) at room temperature (25 $^{o}$C) and sonicated for 30 sec in an ice bath before measurement to prevent clumping.[23]

- **Determination of Production Yield and Loading Efficiency**

The production yield of the nanoparticles was determined by calculating accurately the initial weight of the raw materials and the last weight of the nanoparticles obtained. The loading efficiency (%) of the nanoparticles can be calculated according to the following equation:

$$\text{EE (\% w/w)} = \frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the drug added}} \times 100$$

$$\text{DL (\% w/w)} = \frac{\text{Weight of the drug in nanoparticle}}{\text{Weight of the polymer and drug added}} \times 100$$

- **Scanning electron microscopy (SEM)**

The particle shape and surface morphology of drug loaded nanoparticles were examined by scanning electron microscopy (SEM). Lyophilized and completely moisture free samples were consigned on aluminium stubs using adhesive tapes and coated with gold using sputter coater and observed for morphology at an acceleration voltage of 20 kV at high vacuum.

- **Transmission electron microscopy (TEM)**

Morphology of the particles was also examined using transmission electron microscope. A sample of particle suspension was diluted with 3% w/v phosphotungstic acid adjusted to pH 7.5 with potassium hydroxide corresponding to a 1:1 ratio before examination. One drop of sample was placed for 1 minute on a copper grid coated with a formvar carbon film. The excess of sample was wicked away with the aid of filter paper. The sample was then ready for analysis by TEM.

***In vitro* evaluation**

- **Drug release study**

The in vitro drug release study of voglibose nanoparticles was carried out by using bottle method. The prepared nanoparticles and pure drug (each containing 5 mg voglibose) were suspended in glass bottles containing 100 ml of phosphate buffer pH 6.8 (simulated intestinal fluid). Glass bottles were placed in beaker and kept in incubator shaker throughout the study (37 $^{o}$C, 50 rpm). At specified time intervals (1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h) 10 ml samples were collected and centrifuged at 13,800 rpm for 30 min. The supernatants were collected for analysis and the precipitate resuspended in 10 ml of fresh phosphate buffer. The supernatant was lyophilized for 24 h and the obtained dry powder was dissolved in methanol (so that only drug can be dissolved) and analyzed by UV spectroscopy at λmax 229 nm. All the measurements were carried out in triplicate. [24-25]

- **Drug Release Kinetic Studies**

The drug dissolution data were subjected to release kinetic studies to find out means of drug release from the prepared nanoparticles. The in vitro drug release data were analyzed by various mathematical models to determine the kinetics and the drug release mechanism from the developed nanoparticle formulation. The drug release data were fitted with mathematical models including zero order kinetic [Eq. (1)], first order kinetic [Eq. (2)], Higuchi kinetic [Eq. (3)] and Korsmeyer-Peppas model [Eq. (4)].

$$Q_t = K_0 t \quad (1)$$

$$\ln Q_t = \ln Q_t - K_1 t \quad (2)$$

$$Q_t = K_h t^{1/2} \quad (3)$$

$$M_t / M_\alpha = K_p t^n \quad (4)$$

The plots were made: Qt vs. t (zero order kinetic),

ln ($Q_0$ - Qt) vs. t (first order kinetic) and

Qt vs. t1/2 (Higuchi model),

- **Stability study**

The lyophilized voglibose nanoparticle formulation was kept in glass vials and stability study was carried out in three different storage conditions [ICH Q1A (R2)]. The nanoparticles were evaluated at intervals 0, 3 and 6 months for intermediate study and accelerated study. During stability testing samples were evaluated for physical appearance and drug content.[25-26]

## RESULTS AND DISCUSSION

The voglibose loaded nanoparticles were prepared by solvent evaporation technique using different ratios of drug and polymer, various agitation speed and different organic phase and aqueous phase ratios. This method is comparatively easy to prepare the nanoparticles than the other technique due to high drug entrapment efficiency, narrow particle size distribution and high batch to batch reproducibility.

### Particle size and Zeta potential measurement

The particle size is an important parameter as it has a direct effect on the stability, cellular uptake, drug release and biodistribution. The mean particle sizes of the prepared nanoparticles as measured by the Malvern zetasizer were in size range of 330 to 651 nm and the distribution of particle sizes are found to be monodispersed as the polydispersity index lies below 0 to 1 (0.234 to 0.780) in all the formulations. There were no noticeable differences between the sizes of nanoparticles obtained with different drug polymer ratio.

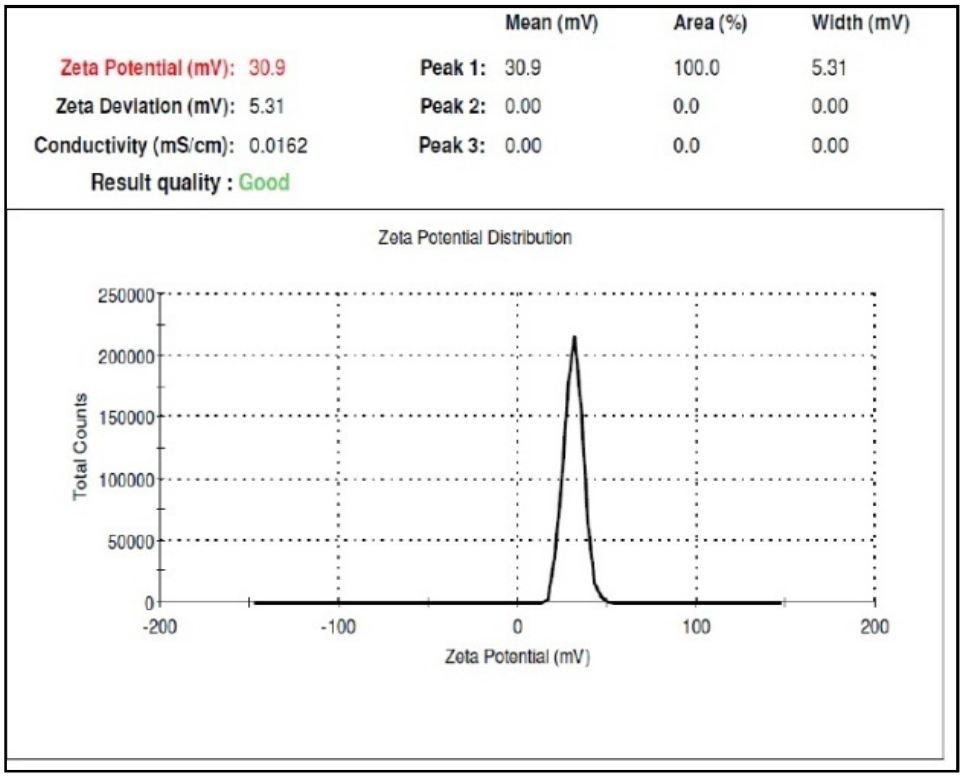


**Fig.1 Zeta potential of Formulation NP6**

**Drug entrapment efficiency and drug loading**

The percentage of voglibose entrapment in the formulation was found to be good. The decreased drug entrapment with increasing drug loading enhances the drug leakage in the organic phase lead to drug loss due to the formation of channels in the polymer structure through which drug can easily escape to the outer phase.

Although the formulation NP4 has high drug content and small particle size than other formulations, this formulation was not selected for further studies due to low drug entrapment (61.45%). The nanoparticles formulation (NP6) prepared with drug- polymer ratio of 1:4 with the agitation speed of 15,000 rpm shows the good entrapment efficiency (89.23%) and smaller particle size of 393 nm. However, based on the particle size and entrapment efficiency the formulation NP6 was selected as best formulation for the further studies.

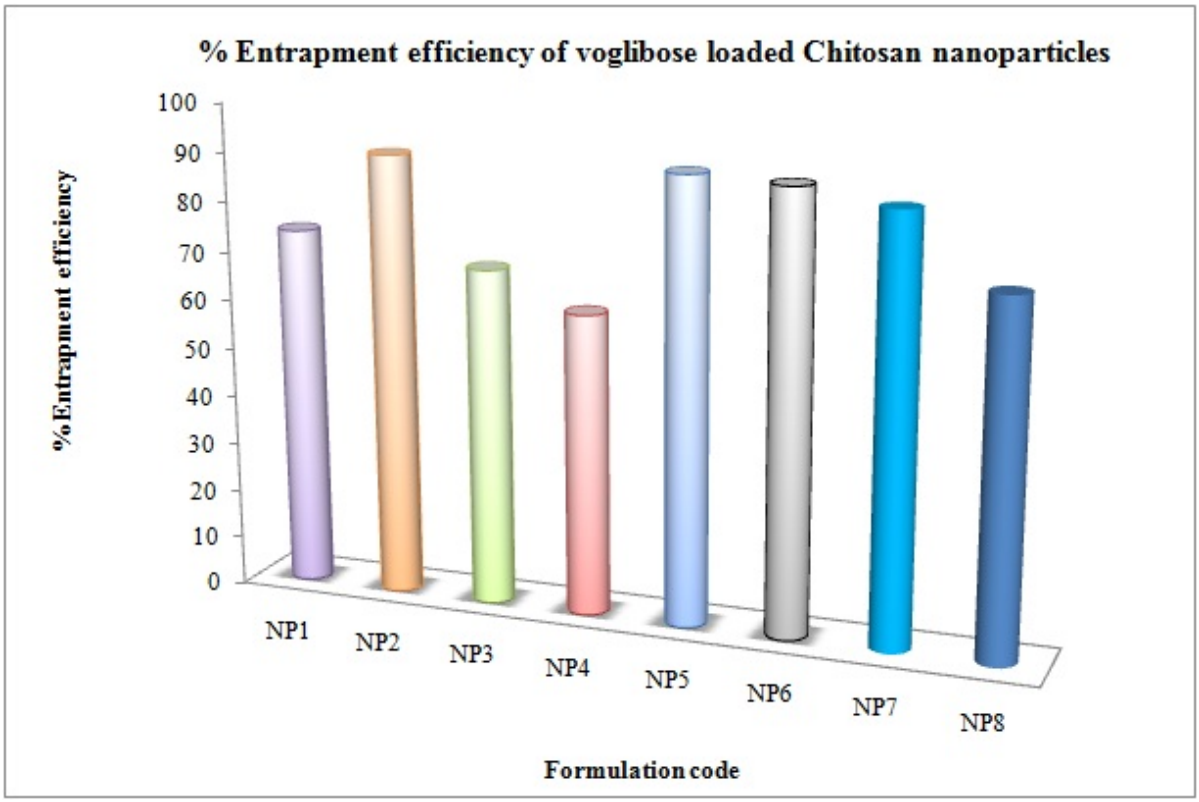


**Fig.2. Entrapment efficiency of voglibose loaded Chitosan nanoparticles**

**Surface morphological properties of Voglibose loaded nanoparticles (NP6)**

The surface morphology and shape of the voglibose loaded nanoparticles (NP6) was measured using scanning electron microscopy. The SEM image of nanoparticles revealed that the particles are of spherical in shape with relative smooth surface.

The Transmission electron microscopy showed the spherical particles with smooth surface which was in conformity with the SEM and Zetasizer data for particle size. Magnification of single particle showed the internal core drug inside the polymer and also confirmed the spherical particles with smooth surface.

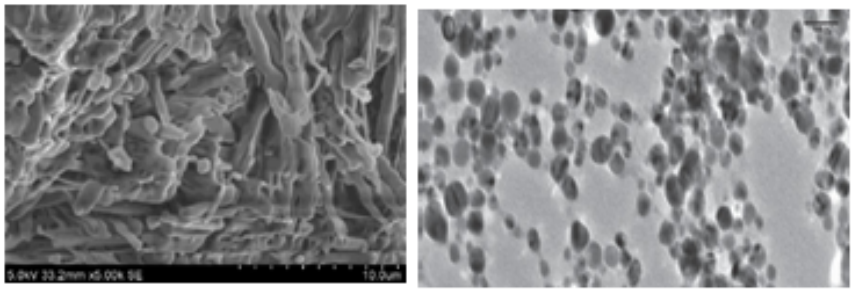


**Fig.3: (A) SEM (B) TEM of formulation NP6 formulation**

### *In vitro* drug release study

The drug release from the dosage form is the indication of dissolution. Dissolution is the rate limiting step in drug absorption. The goal of *in vitro* drug release study (dissolution testing) is to provide a possible prediction of or correlation with the product's *in vivo* bioavailability. The *in vitro* drug release rate was influenced by the drug-polymer composition. However, it seems that a complex phenomenon may occur between the drug and polymer, including entrapment of the drug in the polymer and the adsorption of drug on the surface of the polymer matrix as a result of electrostatic adhesion (Douglas et al., 1987).

Drug release profile from pure drug powder, prepared nanoparticles in phosphate buffer pH 6.8. The voglibose loaded chitosan nanoparticles show slower drug release in comparison with pure drug powder.

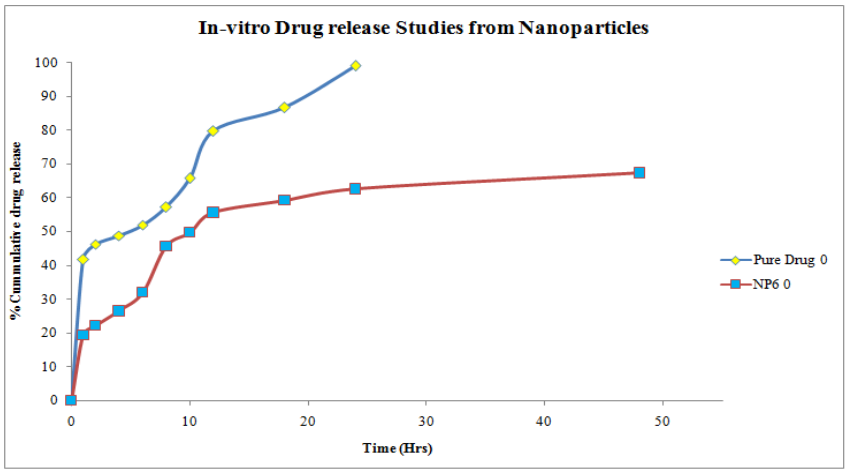


**Fig.4. *In-Vitro* drug release from drug loaded nanoparticles**

### Drug release kinetics:

The drug release data are plotted in various kinetic models, viz. zero order, first order, Higuchi and Korsmeyer-Peppas.

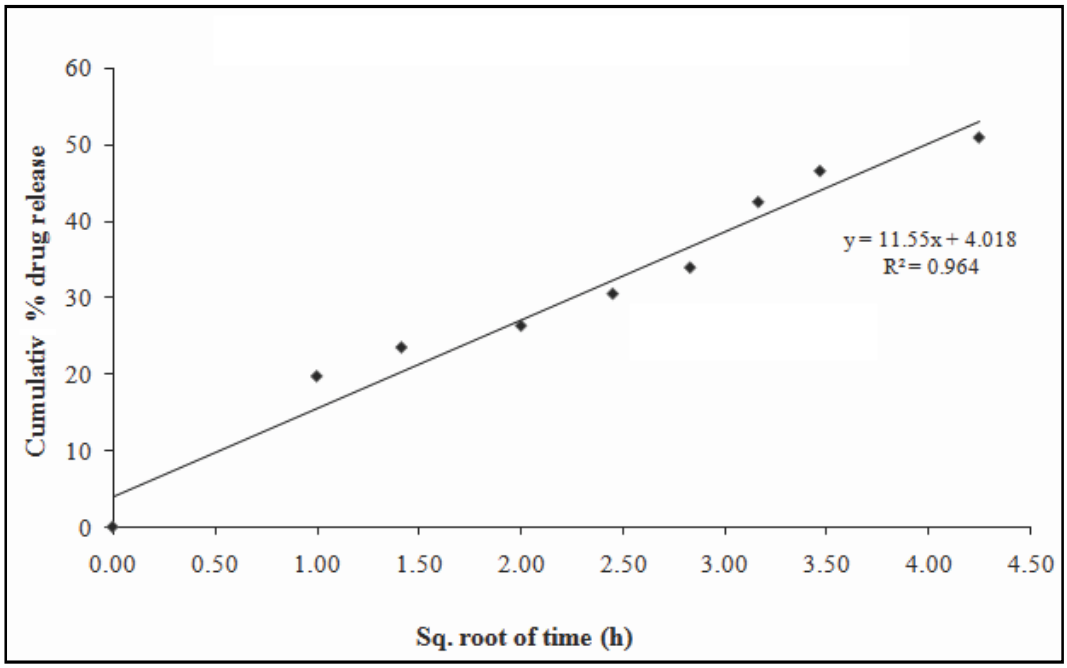


**Fig. 5. Drug release from NP6 in phosphate buffer pH 6.8 – Higuchi kinetic**

Model fitting of the drug release data into zero and first order model indicate that the drug release followed first order kinetics during 1 to 18 h and zero order during 18 to 96 h. Drug release from the nanoparticles also obeyed Higuchi as well as Peppas models, indicating that the drug release was by diffusion mechanism.

**Stability studies**

Nanoparticles formulation NP6 were observed for any change in appearance or colour for the period of 6 months for both Intermediate storage condition (30 °C/65% RH) and Accelerated storage condition (40 °C/75% RH). There was no change in appearance in formulation throughout the period of study. The stability of drug was further confirmed by spectral data and there was no significant change in drug content observed.

**CONCLUSION**

From the above results it revealed that NP6 batch is best suitable for preparation of chiosan nanoparticles and an antidiabetic drug voglibose have bioavailability and frequent dosing issues. For maintaining the required therapeutic level of the drug throughout the treatment period it was attempted to develop a number of stable polymeric nanoparticle formulations of voglobose with chitosan using simple solvent evaporation technique.

Study shows that drug polymer combinations influences the drug entrapment, drug loading and drug release from the polymeric systems. The developed voglibose loaded chitosan based nanoparticulate systems have improved *in vitro* (drug release and stability study) performance compared to pure drug. The stability studies show no remarkable

difference in drug potency in various storage conditions confirming the stability of the nanoparticulate system. Thus, the promising nanoparticulate systems of Voglibose may be further explored to assess their suitability *in vivo* studies (toxicity, bioavailability and antidiabetic) and after successful results can be studied further their suitability in human beings.

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