

EVALUATION OF REPAGLINIDE ENCAPSULATED NANOPARTICLES PREPARED BY SONICATION METHOD

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ABSTRACT

Objective: To develop sustained release repaglinide loaded nanoparticulate system by sonication technique and characterized it.

Method: Emulsification-solvent diffusion technique was used. Drug and polymer at different ratios were dissolved in ethyl acetate and mixed in polyvinyl alcohol/polysorbate-80 aqueous solution by applying sonication energy for different time (5 to 15 min). The obtained nanoparticles were studied for particle size, zeta potential, encapsulation efficiency, *in vitro* release study, etc.

Results: The effects of drug-polymer ratio, sonication time and surfactants on particle size and encapsulation efficiency were studied. The drug-polymer interaction and morphology of particles were studied by FT-IR and Field Emission-Scanning Electron Microscopy (FE-SEM). The selected formulation of dissolution study shows 551.4±3.67 mm size and 85.14±1.89 percent encapsulation efficiency. *In vitro* release was found to be very close to first order kinetic and the mechanism of drug release follows Anomalous (non-Fickian) diffusion.

Conclusion: The sustained release nanoparticles of repaglinide could be reduced dose frequency, decreased side effects and improve patient compliance.

Keywords: Type II Diabetes, Eudragit® RSPO, Sonication, Nanoparticles, *In vitro* release.

INTRODUCTION

Repaglinide is belongs to meglitinide class used to control Type 2 diabetes mellitus. It improves the indicators of glycemic control including glyated hemoglobin, fasting plasma glucose, and postprandial plasma glucose without an associated increase in the incidence of hypoglycemia [1]. It stimulates insulin secretion by blocking ATP-dependent potassium channels of pancreatic β -cell [2]. It rapidly absorbed and metabolized and obtained concentration peak (C_{max}) within one hour after oral administration. Due to its short half life (1 h) required repeated doses may cause different side effects [3]. To avoid the repeated dosing and enhance the bioavailability of repaglinide sustained release nanoparticles were developed. Ammonio methacrylate copolymers are widely used as carrying polymer to sustain the drug release [4,5]. This polymer is non-biodegradable, biocompatible, water insoluble but permeable and pH independent swelling [6,7]. Emulsification-solvent diffusion (ESD) method substantiate that water immiscibility of disperse solvent is not a prerequisite for making emulsion to prepare nanoparticles. The water miscible solvents like ethyl acetate, acetone, methanol, ethanol, ethyl formate, methylethyl ketone or benzyl alcohol, etc. are used successfully to fabricate the drug loaded micro or nanoparticles by this method. The objective of our study was to develop repaglinide loaded sustained release nanoparticles by the ESD method using Ultrasonication and characterizes it. The characterization includes drug-polymer interaction, particle size and zeta potential determination, encapsulation efficiency, drug content, surface morphology and *In vitro* dissolution study.

MATERIALS AND METHOD

Repaglinide was kindly gifted by the Wockhardt Research Centre, Aurangabad, India and Eudragit® RSPO from Evonik Degussa India Pvt. Ltd., Mumbai, India. (MW approx. 32,000 g/mol). Ethyl acetate and polysorbate-80 were purchased from Merck, Mumbai. Polyvinyl alcohol (PVA, MW Approx. 1,25,000) from SD Fine Chem Ltd., Mumbai, India. The experimental work was performed by using triple distilled water filtered with 0.22 μ membrane filter.

Preparation of nanoparticles [8]

Solution of polymer in ethyl acetate containing repaglinide was mixed by using controlled flow rate syringe pump (Infusor, Universal Medical Instruments, India) (3ml/min) with 0.3%, w/v

PVA/polysorbate-80 aqueous solution. The size of the dropping needle was 0.80 x 38 mm. During this mixing the aqueous phase was sonicated using a probe sonicator set at 10 KHz of energy output (CT ChromTech) to produce oil in water emulsion. The organic phase was evaporated under reduced pressure. The obtained nanoparticles were recovered by centrifugation (R243A, Remi) at 18000 rpm for 30 min and washed thrice with distilled water. The washing water was removed by a further centrifugation and nanoparticles were freeze dried (Scanvac, Denmark).

Fourier transform infrared spectroscopy

The samples were homogeneously mixed with potassium bromide and infrared spectrums were traced in the region of 4000-400 cm^{-1} by using an infrared spectrophotometer (IR- 8400, Shimadzu Co. Ltd., Singapore).

Particle size and zeta potential determination

The obtained nanoparticles were discrete in distilled water by sonication and vortex mixing for 30 seconds and the particle size (Z-average mean) and zeta potential were determined by using Nano series Malvern Instruments, UK.

Encapsulation efficiency and drug content determination

Accurately weighed nanoparticles were dissolved in dichloromethane. Then 50 ml phosphate buffer (pH 7.4) solution was added and stirred constantly to extract repaglinide in it after the evaporation of dichloromethane. Removed the precipitated polymer from phosphate buffer by filtration and measured the amount of drug in filtrate using Ultraviolet spectroscopy (U2900, Hitachi, Japan) at 275.5 nm. Encapsulation efficiency (%) and drug content (% w/w) were represented by Eqs. (1) and (2) respectively.

$$\text{Encapsulation Efficiency (EE \%)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of drug used in formulations}} \times 100(1)$$

$$\text{Drug content (\%, w/w)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles recovered}} \times 100(2)$$

Field Emission- Scanning Electron Microscopy

The shape and surface characteristics of nanoparticles were examined and photographed using Field Emission- Scanning Electron Microscopy (FE- SEM) (S4800, Hitachi, Japan). Appropriate samples were mounted on stub, using double sided adhesive carbon

tapes. Samples were gold coated and observed for morphology, at acceleration voltage.

X-ray diffraction analysis

X-ray diffraction of samples was carried out using Model-D8 Advance, Bruker AXS GmbH, Germany diffractometer. An Cu K α source operation (40 kV, 40 mA) was employed. The diffraction pattern was recorded over a 2θ angular range of 3-50° with a step size of 0.02° in 2θ and a 1 Sec counting per step at room temperature.

In vitro release study

Accurately weighed samples were suspended in 100 ml phosphate buffer saline (pH 7.4). The solution was stirred at 50 rpm with temperature adjusted to 37±1 °C. At planned time intervals 5 ml samples were withdrawn and centrifuged at 20,000 rpm for 30 min. Aliquots of supernatant were examined by a UV spectrophotometer at 275.5 nm. The settled nanoparticles in centrifuge tube were re-dispersed in 5 ml fresh phosphate buffer saline (pH 7.4) and returned to the dissolution media [9].

Statistical analysis

The results were evaluated by one-way analysis of variance (ANOVA) using Graphpad Instat® Version 3.06 software, where * $p < 0.05$ was taken to represent a statistically significant difference.

RESULTS AND DISCUSSION

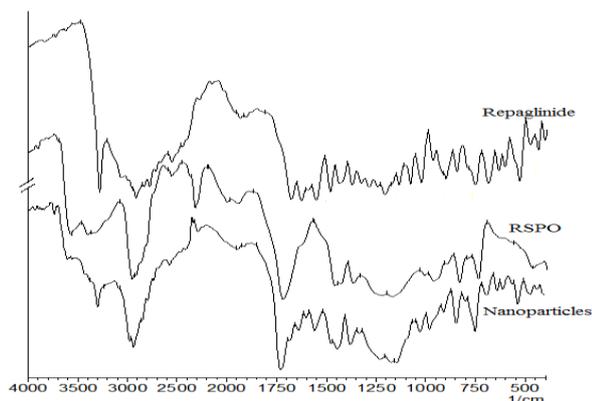


Fig. 1: Infra-red spectrum of drug, sample and their formulation

Drug polymer interaction study

For studying the possible interaction between drug and polymer the obtained FTIR spectrums are shown in Fig. 1. The spectra of pure repaglinide showed peaks at 1220.98 cm⁻¹ (-CH₃ stretching), 1433 cm⁻¹ (C-O stretching), 1689.70 cm⁻¹ (C=O stretching), 2941.54 cm⁻¹ (C-H stretching) and 3308.03 cm⁻¹ (N-H stretching). Similar peaks were observed in repaglinide loaded nanoparticles. Therefore, it concludes no interaction between drug and polymer.

Formulation optimization

The formulation was optimized for further studies, on the basis of particle size and high encapsulation efficiency. Ethyl acetate was used as an organic solvent because both drug and polymer are soluble in it. Due to the high solubility of solvent in water it diffused faster in external phase and precipitate polymer immediately. To decrease the size of emulsion globules before precipitation external energy like sonication was used. Ultrasonicator generates nano-emulsion generally attributed to a mechanism of cavitation [10]. In liquid macroscopic dispersion the ultrasound waves generate cavitation bubbles due to result in a sequence of mechanical depressions and compressions, which tend permanent to implode. Consequently, this shock endows with sufficient energy locally to decrease emulsion droplet size equivalent to nanometric-scaled droplets. Effectiveness of nano-emulsification by sonication with respect to final size of the nano emulsion droplets as well as the time needed to accomplish this size, depends both on the composition of the emulsion and the power device (amplitude). Addition of surfactants was also an important parameter to efficiently reduce droplet sizes. In this oil in water emulsification ultrasonic energy formed unstable interfacial waves at the oil-water interface, which results in the outbreak of large oil droplets into the water phase. Secondly the shock waves of cavitation actions in the close environs of the course oil droplets will cause their interruption into much finer droplets. For viscous fluid (higher drug-polymer ratio) the formation of unstable interfacial waves was compromised. Thus, premixing step produced a coarse emulsion, which can be readily broken up further by cavitation [11,12]. After evaporation of ethyl acetate in next step maximum drug get entrapped in polymeric nanoparticles with surface stabilization. As shown in Table 1 different drug-polymer ratios affect on obtained nanoparticles properties. Both particle size and encapsulation efficiency were directly proportional to the drug-polymer ratios.

Table 1: Effect of drug-polymer ratio on following properties at 15 min sonication time

Formulations	Particle size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Drug content (%)
1:2	371.8±9.31	23.2 ±6.62	71.11±1.59	17.08±0.27
1:4	486.9±6.76	23.6 ±6.21	78.81±1.26	11.74±0.19
1:6	551.4±3.67	23.9 ±8.78	85.14±1.89	9.79±0.18

The reason behind this result is the viscosity of internal phase. As the viscosity of internal phase increased the diffusion rate was also decreased in external aqueous phase or difficult to disperse due to resistance in higher mass transfer and resulted in larger droplets gives more particle size than lower viscous internal phase (* $p < 0.05$) [13]. At lower ratio the polymer may insufficient to encapsulate the maximum amount of drug, therefore drug may leak out before

solidification of nano globules. Inversely in higher ratio polymer may fabricate uniform matrices with drug molecules and encapsulate more drug [14]. So encapsulation efficiency is also more in high drug-polymer ratio. The effect of sonication time on particle size and encapsulation efficiency was also studied (Table 2). The final size of particles depends on globule size throughout the emulsification process.

Table 2: Effect of sonication time on repaglinide loaded nanoparticles prepared by 1:6 ratio

Sonication Time (min)	Particle size (nm)	Encapsulation Efficiency (%)	Drug content (%)
5 min	726.6±15.01	88.92±1.03	10.13±0.14
10 min	633.02±3.62	85.58±2.61	9.84±0.20
15 min	551.4±3.67	85.14±1.89	9.79±0.18

The results revealed that particle size was inversely proportional to the sonication time. The particles prepared with 15 min of

sonication showed a monomodal distribution profile, while the nanoparticles prepared with 10 min and 5 min disturb this profile.

The high energy released in 15 min batch which leads to the rapid distribution of polymeric organic phase as nano droplets of small size. In another two batches of lower time the dispersion of organic phase was insufficient results in large particles (* $p < 0.05$). There was no significant difference in encapsulation efficiency (* $p > 0.05$) of sonication time dependent batches. Two non-ionic

surfactants PVA (formulation X) and polysorbate-80 (formulation Y) of fixed concentration (0.3%, w/v) were incorporated to further optimize the formulation. The obtained results revealed that PVA (0.3%, w/v) is a good surfactant as compared to polysorbate-80 (0.3%, w/v) in terms of particle size and encapsulation efficiency (Table 3).

Table 3: Effect of surfactant on formulations prepared by 1:6 ratio at 15 min sonication time.

Formulations	Particle size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Drug content (%)
X	551.4±3.67	23.9±8.78	85.14±1.89	9.79±0.18
Y	784.4±6.28	23.5±7.97	73.53±0.89	10.44±0.35

X- Formulation using PVA, Y- Formulation using Polysorbate-80

The lower particle size was observed in PVA used formulation because it stabilized the nanoparticles surface more effectively. PVA used formulation also encapsulate more repaglinide than polysorbate-80 used formulations because PVA has greater tendency to migrate towards the surface of nanoparticles [9]. Thus from all obtained results, formulation of 1:6 drug-polymer ratio using PVA (0.3%, w/v) at 15 min sonication time was selected as the final formulation for further study.

Particle size and zeta potential

The average diameter of all obtained nanoparticles was determined and given in Tables 1-3. The nanoparticles prepared by drug-polymer ratio 1:6, sonication time 15 min and PVA as stabilizer have 551.4±3.67 nm sizes with favorable encapsulation efficiency. The zeta potential values of different drug-polymer ratios and surfactants are given in Table 1 and Table 3. There was no significant difference in values. All values are positive because ammonio methacrylate units present in Eudragit polymers [15]. Zeta potential will determine whether the particles within a liquid will tend to aggregate or not. From results it was concluded that the system was stable.

Surface morphology

The obtained nanoparticles were spherical, uniform size, porous and tough in nature (Fig. 2). The porous nature of the nanoparticles may be due to creation of holes since as diffusion of ethyl acetate from nano globules to external aqueous phase before solidification. This porous nature responsible for the controlled release of encapsulated drug from the nanoparticles.

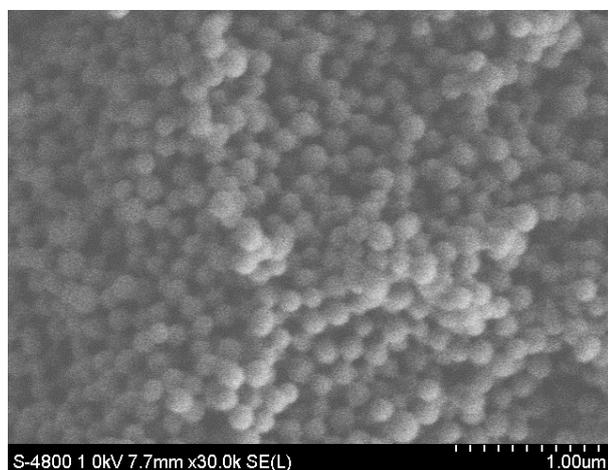


Fig. 2: FE-SEM photograph of repaglinide loaded nanoparticles

Molecular arrangement

Figure 3 illustrates that repaglinide was crystalline in nature due to repeated or pattern arrangement of the molecules. But when repaglinide was coated with polymer the characteristic peaks may overlap by polymer which concluded that the drug was scattered at the molecular level in the matrix [16]. Among the formulations were

prepared by using PVA (formulation X) and polysorbate-80 (formulation Y), PVA used formulation shows less crystalline than polysorbate-80 used formulation. This result revealed that PVA surfactant helps to coat the polymer over drug proficiently than polysorbate-80.

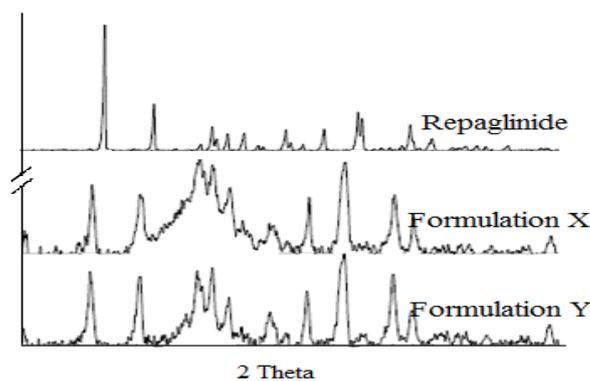


Fig. 3: X-ray diffraction of repaglinide and their formulations

In vitro dissolution profile

The selected formulations were proceeding for dissolution study in phosphate buffer (pH 7.4). Figure 4 shows the *in vitro* release profile of repaglinide loaded nanoparticles prepared by using PVA (formulation X) and polysorbate-80 (formulation Y) at 1:6 drug-polymer ratio. Of these, formulation X was slightly more sustained than formulation Y.

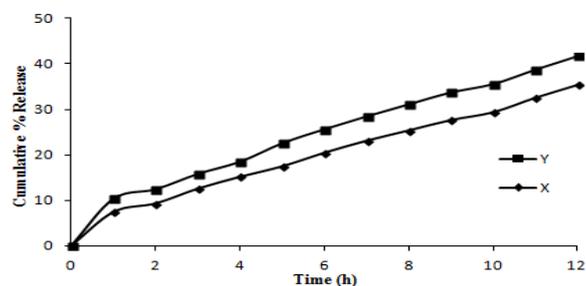


Fig. 4: Drug release profile of selected nanoparticle formulations

In first hour 7.42±0.15% and 10.3±0.04% repaglinide was released from formulation X and Y respectively. This drug may be from the surface of the particle which was adsorbed there and get diffused out into the release medium. The drug which didn't encapsulate in the core of the particles starts to move towards the surface with forced diffusion of ethyl acetate from core to external aqueous phase and settled over there. The difference of 3-5% release between both the formulations was maintained up to 12 hours. The ammonio methacrylate units of Eudragit® RSPO help to make the permeable particle. Therefore from the results it was concluded that both the formulations released repaglinide sustainable due to

the porous nature of the particles. The dissolution medium penetrates to the matrix and dissolves the drug, which then diffused into the exterior liquid. After 12 hours, formulation X and Y released $35.39 \pm 0.17\%$ and $41.68 \pm 0.44\%$ repaglinide respectively. To find out the release kinetics of both the formulations, the release constants were calculated from the slope of appropriate plots, and regression coefficients (R^2) were determined [17,18] (Table 4). From the results it was concluded that *in vitro* release was found to be very close to first order kinetics. The first-order describes the release from systems where the release rate is concentration dependent. This explains after first hour release the drug diffuses comparatively slower rate as the distance for diffusion increased. The zero-order rate describes the systems where the drug release rate is independent of its concentration and Higuchi model describes the drug release from an insoluble matrix as a square root of time dependent process based on Fickian diffusion. As the distance for diffusion increases, drug diffuses at a slower rate, which is also referred to as square root kinetics. The mechanism of drug release was determined by Korsmeyer-Peppas model [19]. The release exponent "n" value is also given in Table 4.

Table 4: Release kinetics of selected formulations

Formulations	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	n
X	0.9859	0.9971	0.9793	0.5985
Y	0.9758	0.9976	0.9822	0.657

X- Formulation using PVA, Y- Formulation using Polysorbate-80

According to the results it was concluded that drug release follows Anomalous (non-Fickian) diffusion because "n" value was in between 0.45 and 0.89. Anomalous diffusion shows the combination of diffusion and erosion mechanisms which signify that the drug release is controlled by several simultaneous processes. Thus it is revealed that PVA is better surfactant as compared to polysorbate-80 for the preparation of repaglinide loaded Eudragit nanoparticles by sonication method.

CONCLUSION

The present research work proposed a novel nanoparticulate formulation technique using sonication. The solvent diffusion rate and sonication energy both help to decrease the size of particles to nano level. Particle size was inversely proportional to the sonication energy. PVA (0.3%, w/v) is better stabilizer than polysorbate-80 (0.3%, w/v) in terms of particle size, encapsulation efficiency and drug release. The release profile was a combination of both diffusion and erosion mechanism, therefore prepared formulation follows sustained release pattern which could be effective in the management of type II diabetes mellitus.

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