Enhancement of glipizide dissolution rate through nanoparticles: Formulation and *In vitro* evaluation

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Abstract

The objective of the present investigation was to enhance the solubility of practically insoluble glipizide by preparing its nanoparticles. The glipizide nanoparticles were prepared by anti-solvent precipitation method using various drug-to-stabilizers ratio. The nanoparticles of glipizide were evaluated for particle size, zeta potential, saturation solubility, and dissolution behavior. Glipizide nanoparticles showed particle size of 425.6 nm and zeta potential of −16.2 mV. Saturation solubility of pure glipizide and nanosuspension were 0.19±0.006mg/20ml and 4.3±0.18 mg/20ml respectively, showing more than 22.63 times increase in solubility. Differential scanning calorimetry (DSC) showed that crystalline state of glipizide remained unchanged in glipizide nanosuspension. In glipizide nanosuspension, 59.57±0.63% of the drug released within 10min and almost 100±0.2% within 60min, while micronized suspension of glipizide showed only 8.91±0.58% release at the end of 5min and 18.21±0.25% release in 60 min. From the results, it was concluded that significant enhancement in solubility of glipizide in phosphate buffer (pH 6.8) thus enhancement in dissolution of it when formulated as drug nanoparticles.

Key words: Glipizide; Nanosuspension; Solubility; Dissolution.

1. Introduction

An improvement of oral bioavailability of poor water-soluble drugs remains one of the most challenging aspects of drug development. The techniques that have commonly been used to overcome drawbacks associated with poorly water-soluble drugs, in general includes micronization, salt formation, use of surfactant, use of pro-drug and more. However, all these techniques have potential limitations. Drug nanoparticles have been reported to improve solubility and in-vitro dissolution rate of poorly water-soluble drugs[1-3].

Over the last 8-10 years, nanoparticle engineering processes have been developed and reported for pharmaceutical applications. Nanosuspension engineering processes currently used are precipitation, pearl milling and high pressure homogenization, either in water or in mixtures of water and water-miscible liquids or non-aqueous media. In this work, particle size reduction in nanometer was achieved through antisolvent precipitation. It is a straightforward technique, rapid and easy to perform at laboratory scale. Drug solution is then emulsified in an aqueous solution containing a surfactant or to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring[4-6].

Glipizide is oral hypoglycemic agent that is, 100 times more potent than Tolbutamide, which is used for treatment of type II diabetes mellitus. It is practically insoluble in water; because of its poor aqueous solubility (classified as BCS class II drug), conventional glipizide dosage form show absorption problem, and its dissolution is considered to be a rate determining step in its absorption from gastrointestinal

tract. During high blood glucose level conditions, an antidiabetic drug should show quick and high oral bioavailability, which can be achieved by high aqueous solubility[7-10].

The present study was performed with an objective of formulating glipizide nanoparticles that may enhance solubility and dissolution rate. Glipizide nanoparticles were characterized in terms of saturation solubility, particle size, zeta potential, and dissolution behavior. Morphology and thermal behavior of the micronized glipizide and nanoparticles were examined by microscopy and differential scanning calorimetry (DSC), respectively. Also, FTIR was used to investigate the drug chemical structure. Finally, drug release of micronized suspension and drug nanoparticles were studied by dialysis bag method in phosphate buffer (pH 6.8) media using different ratio of drug to stabilizers.

2. Materials and methods

2.1 *Materials*

Glipizide was obtained as a gift sample from Cadila Pharmaceutical Ltd (Ahmedabad, India). Hydroxy Propyl Methyl Cellulose (HPMC-5cps) and acetone of analytical grade were procured from high purity laboratory chemicals (Mumbai, India). Hydroxy Propyl Methyl Cellulose E-15 (HPMC-E15) of analytical grade was procured from S.D Fines chemicals (Mumbai, India). Poly Vinyl Alcohol (PVA) of analytical grade was procured from Crystal Chemicals (Himmatnagar, India). Poly Vinyl Pyrolidone K-30 (PVPK-30) of analytical grade was procured from Oxford laboratory (Mumbai, India). Distilled water was used throughout the study.

2.2 *Preparation of micronized suspension of glipizide*

The required amount (5 mg) of glipizide was dispersed in 20 ml of the stabilizer solution using a magnetic stirrer to form a micronized suspension of the drug.

2.2 Preparation of glipizide nanoparticles

Glipizide nanoparticles were prepared by the anti-solvent precipitation method. Glipizide was dissolved in acetone (1.5ml) using sonicator (PCI, Mumbai) for 15 min at room temperature. This organic phase was added by means of a syringe into 20 ml phosphate buffer (pH 6.8) containing different amount of stabilizer (HPMC-E15, HPMC-5cps, PVPK-30, and PVA) maintained at a temperature of 30–40°C (Table1). These were subsequently stirred for 3 hr to allow the volatile solvent to evaporate (Remi, High speed stirrer, India.).[11]

2.3 Saturation solubility analysis

Saturation solubility studies were performed according to the method reported according to literature[12]. The solubility of glipizide in phosphate buffer (pH 6.8), and in solutions of different stabilizers (HPMC E-15, HPMC 5cps, PVPK-30, and PVA or PVP K-30) was determined by addition of an excess of the drug to the phosphate buffer (pH 6.8). The contents were stirred on magnetic stirrer (Remi, India) at 25°C for 24 h at 300 rpm. After reaching equilibrium, samples were filtered through a 0.45μm membrane filter, suitably diluted with phosphate buffer (pH 6.8) and analyzed for drug content at the λmax of 276 nm using a spectrophotometer (Shimazdu-1700, Kyoto, Japan). The individual values for three replicated were measured and their mean values are reported.

2.4 Particle size of analysis

Particle size analysis of the nanosuspension formulations was performed by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, UK). This technique is yield the mean particle diameter. The formulations were added drop-

wise to the sample dispersion unit containing water/buffer as a dispersant. A refractive index value of 1.5 was used for particle size analysis.

2.5 Zeta potential analysis

A zeta potential for optimized nanosuspension in distilled water was determined using Zetasizer 3000 (Malvern Instruments, UK). The measurements were repeated three times and average value was calculated. Samples were diluted in a similar fashion to that described above for the particle size distribution.

2.6 Photo microscopy and scanning electron microscopy

The surface morphology of the glipizide micronized particles and optimized nanoparticles (F_1) was visualized by laboratory microscope (Labomade, India). Also, optimized nanoparticle surface appearance was analyzed by scanning electron microscopy (SEM).

2.7 Differential scanning colorimetry

DSC scans of the powdered samples glipizide, and optimized nanoparticles dispersion (F_1) were recorded using DSC-Shimadzu 60 (Shimadzu Co., Kyoto, Japan) with TDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 20°C/min under dry air flow (100 ml/min) between 50^0C-275^0C. Aluminum pans and lids were used for all samples.

2.8 Fourier Transform Infrared spectroscopy

Fourier–transform infrared (FTIR) spectra of moisture free powdered samples (glipizide, and F_1) were obtained using a spectrophotometer (FTIR-8300, Shimadzu Co., Kyoto, Japan) using potassium bromide (KBr) pellets (2 mg sample in 200 mg KBr). The scanning range was kept 750–4000 cm^{-1}.

2.9 Drug release study

Dialysis bag membrane (Cap; 3.63ml/cm) method was used to study in-vitro release of drug from the prepared glipizide nanosuspension and micronized glipizide suspesion. A 0.25 mg/ml nanosuspension, equivalent to 5mg glipizide, was filled in the dialysis bag having 10cm in length and 21.5mm diameter. The dialysis bag was placed in a 100 ml glass beaker, containing 50 ml of 6.8 pH phosphate buffer solution on magnetic stirring. 5ml of dialyzate was withdrawn for the drug content analysis and was replaced by equal volume of fresh medium. The dialyzate was then subject to the UV analysis against the blank (6.8 pH buffer solution). Percent cumulative release of glipizide was calculated based on the standard UV calibration curve at 276 nm.

2.10 Accelerated stability study of optimized batch

Transparent sealed vials (20ml) of freshly prepared glipizide nanosuspension (F_1) were placed in stability chamber maintained at room temperature[13]. The nanoparticles subjected to stability tests were analyzed over 1 month period for physical appearance and particle size with a frequency of 1 week sampling.

3. Results and Discussion

Anti-solvent precipitation method has been employed to produce nanosuspension of glipizide. Drug-to-stabilizer ratio was contributed much towards the change in particle size in nanosuspension preparation. Nanosuspension of glipizide was prepared as per shown in table 1. F_1–F_9 formulations were containing drug-to-stabilizer ratio 1:1 and 1:2 for HPMC-E15, HPMC 5 cps, PVPK-30, and PVA and without stabilizers. Amount of organic solvent was kept constant for all batches. Bluish white transparent nanosuspension was successfully prepared which was compared with hazy micronized suspension of glipizide (Fig. 1.).

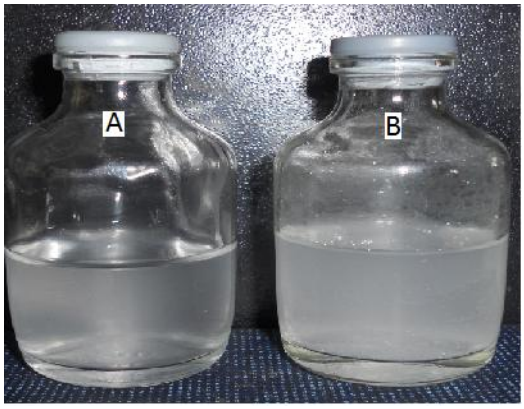
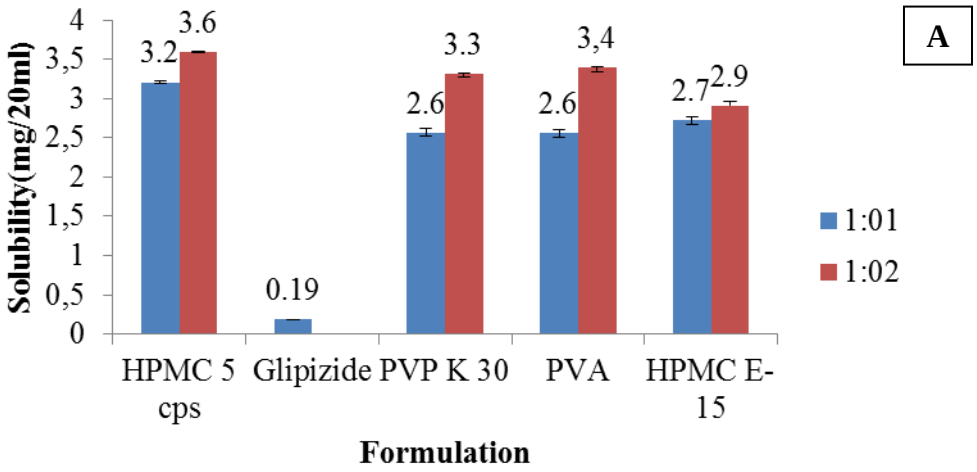


Fig. 1. Bluish white transparent nanosuspension (batch F_1)(A), Micronized suspension of glipizide (batch F_9)(B).

3.1. Saturation solubility

Glipizide is a very hydrophobic compound and disperses poorly in water and phosphate buffer (pH 6.8). Solubility of glipizide is 0.19mg/20ml at room temperature clearly indicate that it is poorly soluble in buffer. Thus, it is challenging to enhance the dispersion of glipizide particles in buffer solution. The saturation solubility data of drug, and micronized suspension showed that the solubility of glipizide increased in presence of HPMC 5cps, PVPK-30, PVA, and HPMC E-15 (Fig. 2A). These could be attributed that all selected stabilizers were enhanced solubility of glipizide up to certain extent in micronized suspension. Saturation solubility of glipizide nanosuspension (F_1), its micronized suspension and pure glipizide at room temperature in phosphate buffer (pH 6.8) was 4.3±0.033gm/20mL; 2.7mg/20ml and 0.19±0.001 gm/20mL respectively (Fig. 2B). Thus, saturation solubility of nanosuspension was 22.63 times that of pure drug. The average particle size of glipizide nanoparticles was below 1µm, thus, theoretically it will help in improving saturation solubility and thus bioavailability (Fig. 3). Thus, saturation solubility will increase if the particle size of the nanoparticles is reduced below particular limit. Thus, nanonization might leads to increase in the saturation solubility. An enhancement of solubility of glipizide due to particle size reduction can be expected to enhance dissolution velocity and justifying the objective of work.



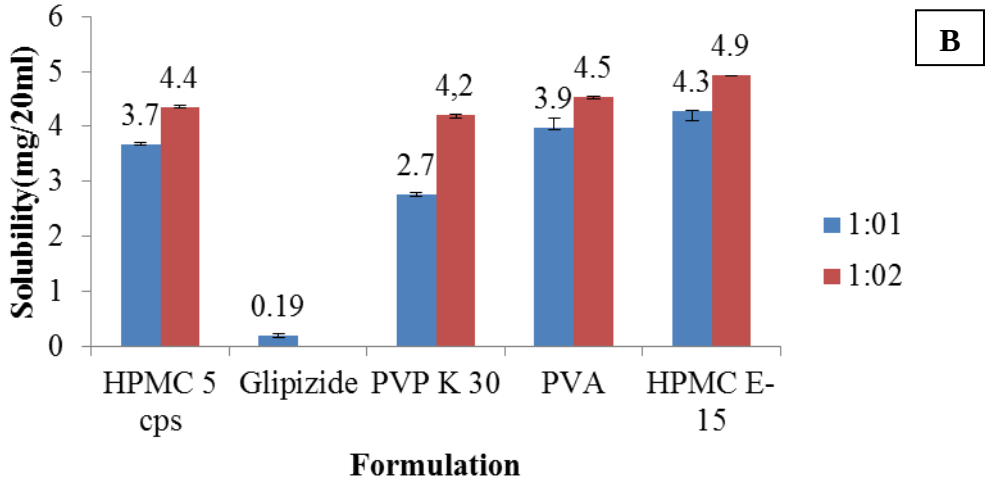


Fig. i. Solubility analysis of glipizide micronized suspensions obtained in drug-to stabilizer in a ratio of 1:1 and 1:2.(A), Solubility analysis of glipizide nanosuspensions obtained in drug-to stabilizer in a ratio of 1:1 and 1:2(B).

3.2. Particle size analysis

Four stabilizers (HPMC 5 cps, PVPK-30, PVA, and HPMC-E15) were tested for their stabilization potential. Important function of stabilizer is that they can form a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual nanoparticles. Also, stabilizer type and concentration play an important role in creating a stable formulation. It must be capable of wetting the surface of the drug particles and providing a steric or ionic barrier. Too little stabilizer induces agglomeration or aggregation and too much stabilizer promotes Oswald's ripening.

Initially, screening of formulations was designed with different concentrations of commonly used well known stabilizers (HPMC-5cps, PVPK-30, PVA, and HPMC E-15). These are efficient steric stabilizers forming adsorption layers on drug nanoparticles. It was noticed that in formulation F_1-F_8, particle size was increased because of when drug-to-stabilizers ratio was shifted from 1:1 to 1:2. Therefore, higher drug-to-stabilizer ratio was created formation of thick adsorption layer onto particles. This was leading to formation of aggregation of particles (Fig.3). For PVA, when drug to stabilizer ratio was shifted from 1:1 to 1:2, more variation in particle size (246.9 nm for 1:1 ratio and 863.8 nm for 1:2 ratio) was identified compare to HPMC-E15.

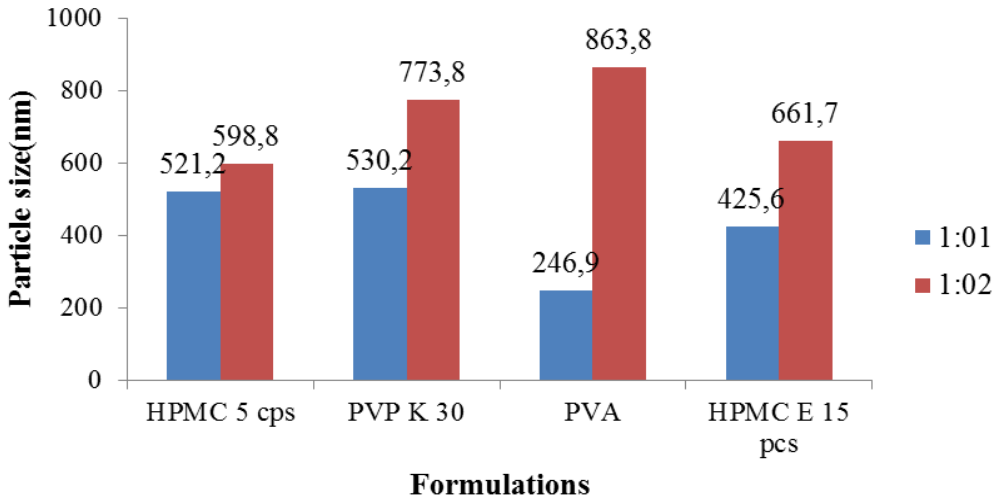


Fig. ii. Mean particle size of glipizide nanosuspensions obtained in drug-to stabilizer ratio of 1:1 and 1:2.

When drug-to-stabilizer ratio was 1:1 for HPMC-E15, it was markedly improved stabilization of nanosuspension. The mechanism of the adsorption of HPMC-E15 is likely by the formation of steric barriers. Steric barriers are produced when the adsorbed stabilizer extends its chain to the water phase, which helps maintaining the distance between closely approaching solid particles.

3.3. Zeta potential analysis

Colloidal systems must preserve their characteristics like particle size and colloidal stability to get advantage of these systems. Technically, colloidal stability is measured in the form of zeta potential. The zeta potential is an important physicochemical characteristic of the nanoparticles. From literature search, zeta potential values in the −15 mV to −30 mV are common for well-stabilized nanoparticles. Zeta potential of glipizide nanosuspension (F_1) was found to be -16.2 mV (Fig.4). Thus, it was concluded that the systems had sufficient stability.

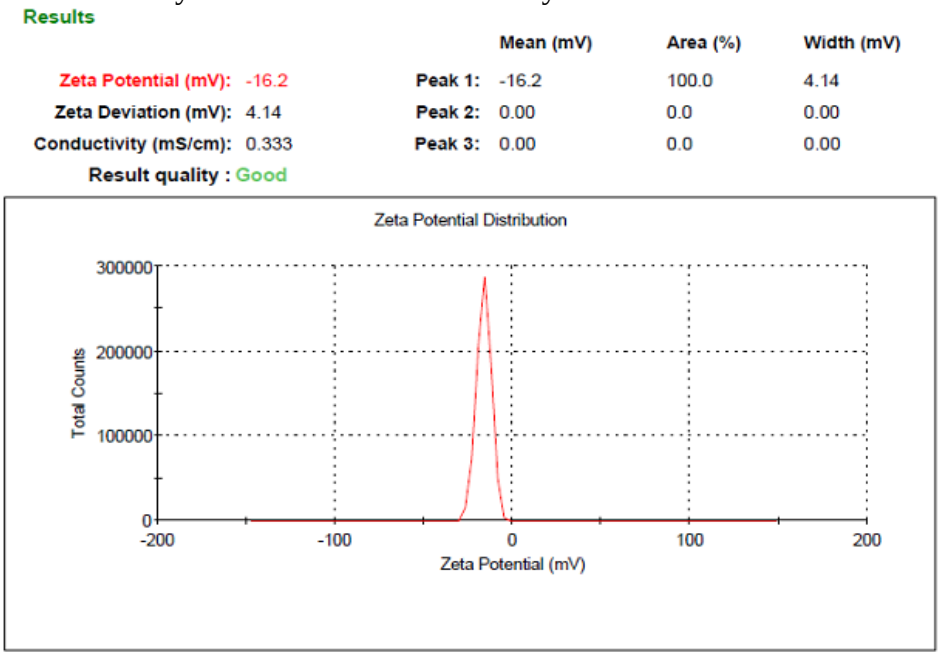


Fig. iii. Zeta potential graph of optimized glipizide nanosuspension (F_1).

3.4. Photo Microscopy and scanning electron microscopy

Morphology of micronized suspension of pure glipizide and glipizide nanosuspension (F_1) is shown in Fig. 5. It could be seen that micronized suspension of glipizide particles exhibited irregular shape and a broad size distribution is shown in Fig. 5(B). Photo microscopy and SEM study revealed that nanosuspension with HPMC E-15 (F_1) shows spherical shape particles with nanometric in size. It can be clearly observed in SEM that these agglomerates or particle assemblies are composed of a large number of individual nanoparticles size of around 600 nm (Fig.5(A) and 5(C)).

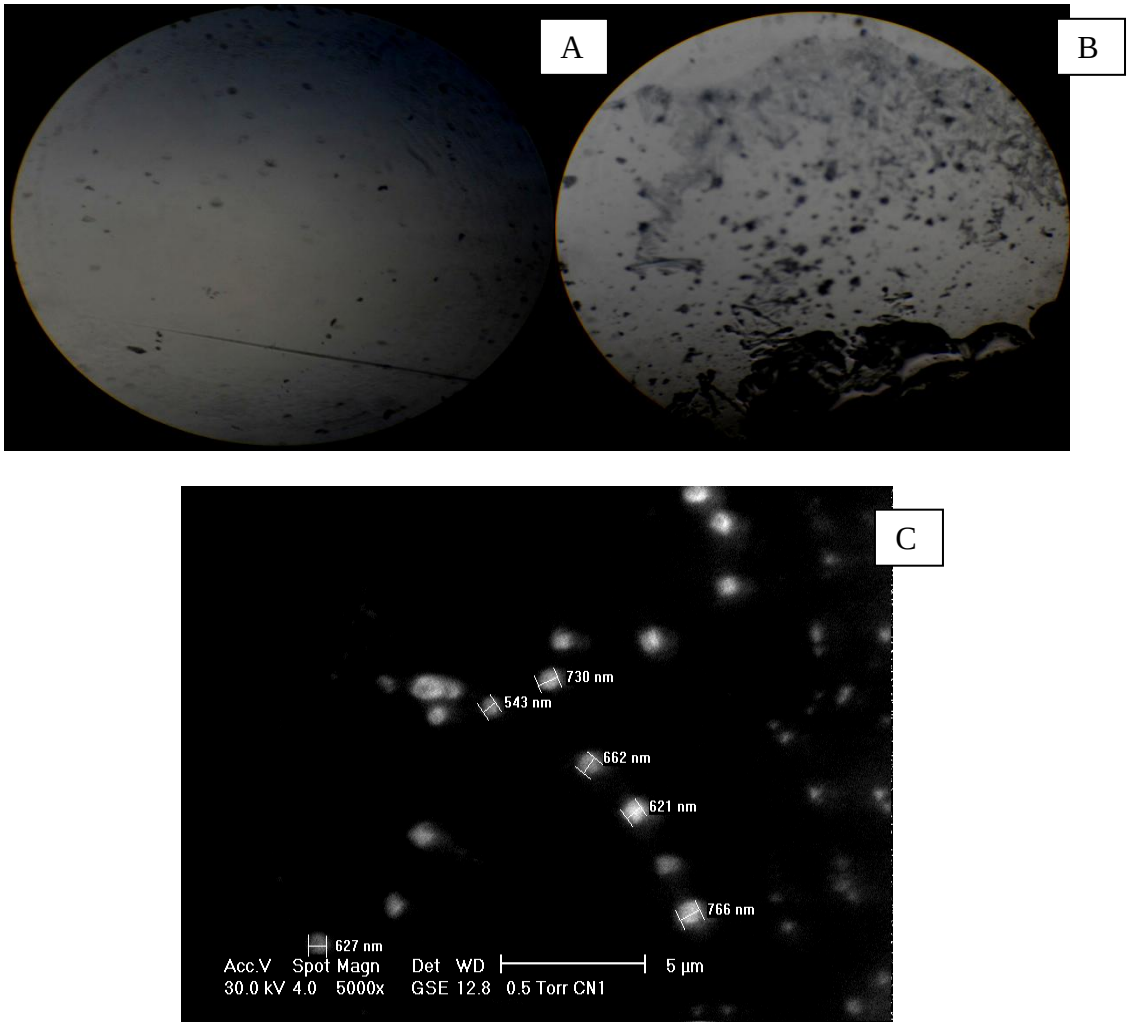


Fig. 5. Photomicrograph of glipizide loaded nanoparticle (A) (F_1), Micronized glipizide suspension (F_9) (B), SEM of formulation F1(C).

3.5. Differential scanning colorimetry

The DSC study was performed to study the effect of nanosizing on solid state of glipizide. The DSC thermograms of glipizide pure powder and nanosuspension (F_1) are shown in Fig. 6. The DSC thermogram of pure glipizide powder showed a sharp endothermic peak at 216.6°C which is due to melting of the drug (Fig 6(A)). After being precipitated as nanoparticles, two peaks are identified. Formulation (F1) was shown endothermic peaks 75°C and 201.8°C due to melting of HPMC-E15 and presence of glipizide respectively. Thermal data shown that melting point of drug was decreased, indicating reduced crystallinity (Fig 6(B)). This phenomenon can be explained by the fact that under fast nucleation rate, the drug solute lacks sufficient

time to incorporate into the growing crystal lattice accurately to form perfect crystals. This again demonstrates the reduced crystallinity of submicron glipizide particles compared to pure drug.

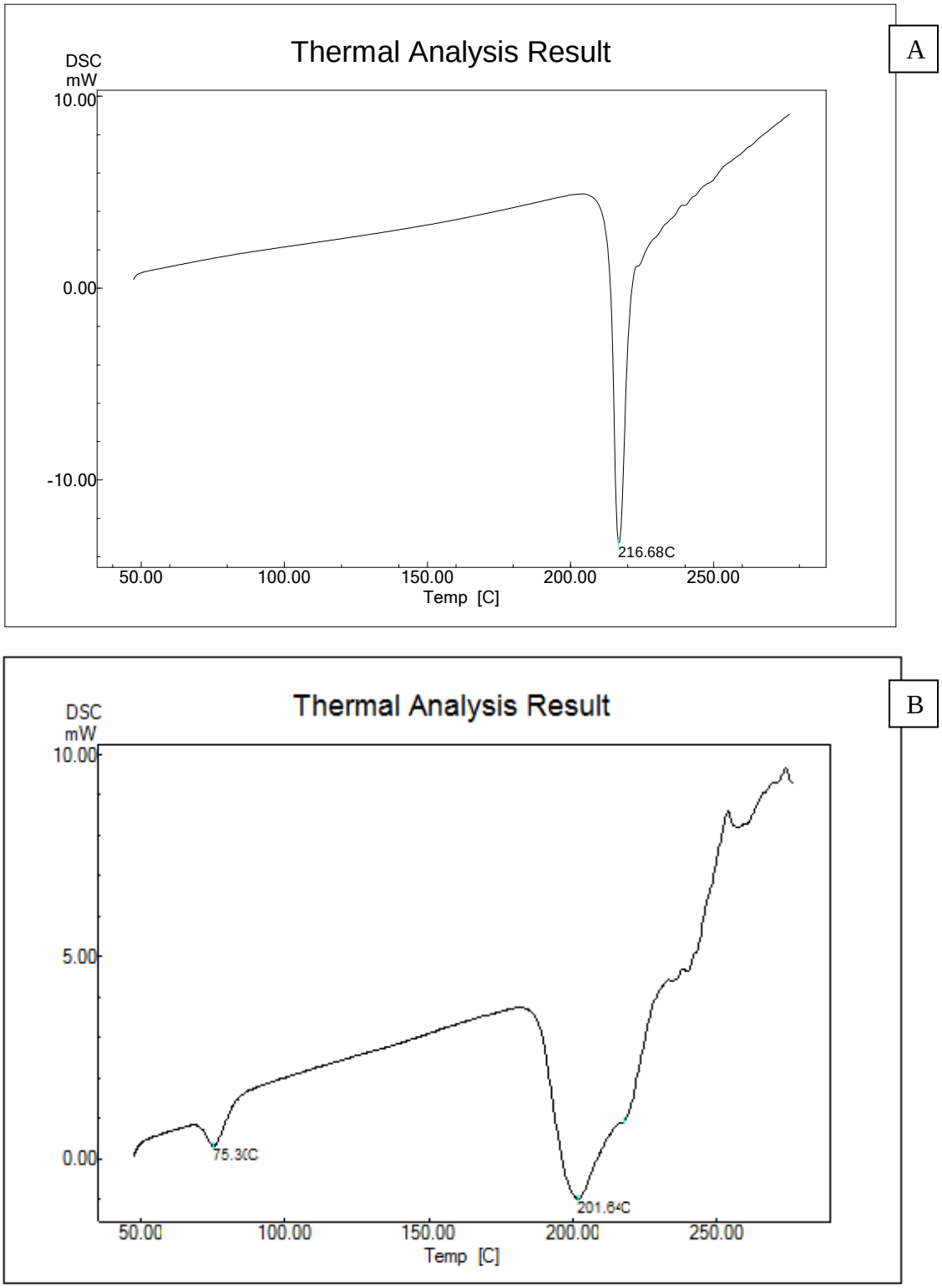


Fig.6. DSC thermogram of pure glipizide powder (A) DSC thermogram of glipizide nanoparticles (F_1) (B).

3.6. Fourier Transform Infrared spectroscopy

The spectra of glipizide nanoparticles were compared with pure glipizide spectra to determine any possible molecular interaction of glipizide after nanoparticles formed. The infrared spectra of glipizide nanoparticles and pure glipizide are shown in Fig 7. Peak of glipizide for C=O at 1600 cm^{-1} which is nearer to aromatic ring and another major peak for C=O at 1687.5 cm^{-1} which is nearer to aliphatic ring. When nanoparticle of glipizide and pure glipizide analyzed using FTIR, the C=O was unaffected at 1600 and 1687.5 cm^{-1}. The absorption bands observed for formulation of glipizide nanoparticles indicated were closed to absorption bands for glipizide. The FTIR spectrum shows that there were no significant changes in chemical integrity of drug and also indicates that interaction between glipizide and HPMC-E15 are compatible to each other.

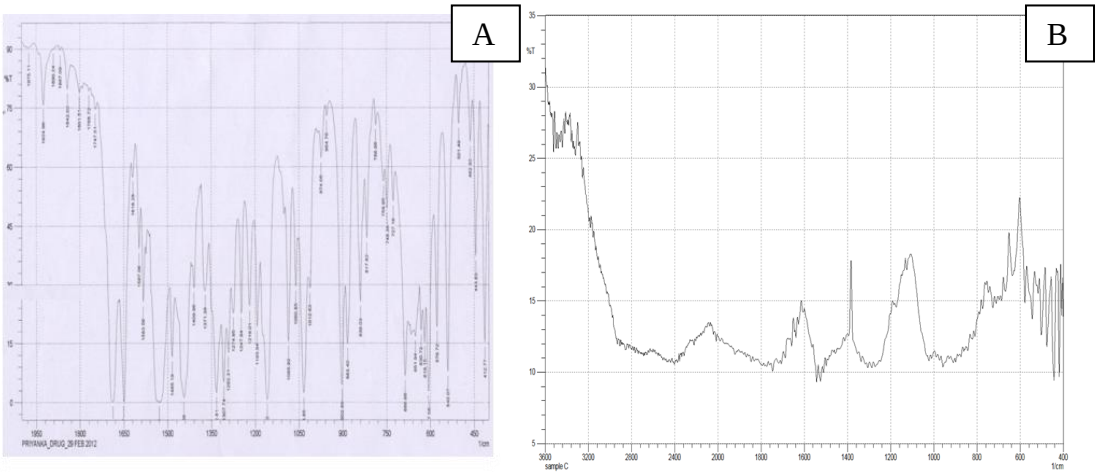


Fig. 7. FTIR spectrum of glipizide (A) and glipizide nanoparticles (F_1)(B).

3.7. Drug release study

The most important feature of nanoparticles is the increase in the dissolution velocity, not only because of increase in surface area but also because of increase in saturation solubility. When the dissolution profile for nanosuspension with the different stabilizers (HPMC-5 cps, PVP K-30, PVA, and HPMC-E15) was compared with micronized suspension of pure glipizide, improved dissolution was observed. In case of HPMC-E15, nanosuspension 59.57±0.63% of the drug released within 10min and about 100±0.2% within 60min, while micronized suspension of glipizide showed only 8.91±0.58% release at the end of 10min and 18.21±0.25% release in 60 min. Thus, there was improvement in dissolution rate containing HPMC-E15 nanosuspension (F_1) compared to micronized suspension of glipizide. The particle size reduction from 5156±5.03nm to 421.6±1.02nm was responsible for this improvement as the excipients in both cases are same. This dissolution rate of the nanoparticles were distinctly superior compared to plain drug, which might be attributed to increase in saturation solubility and decrease in diffusional distance for nanoparticles, showing complete dissolution within short time period. This could be due to the increased surface area of the drug.

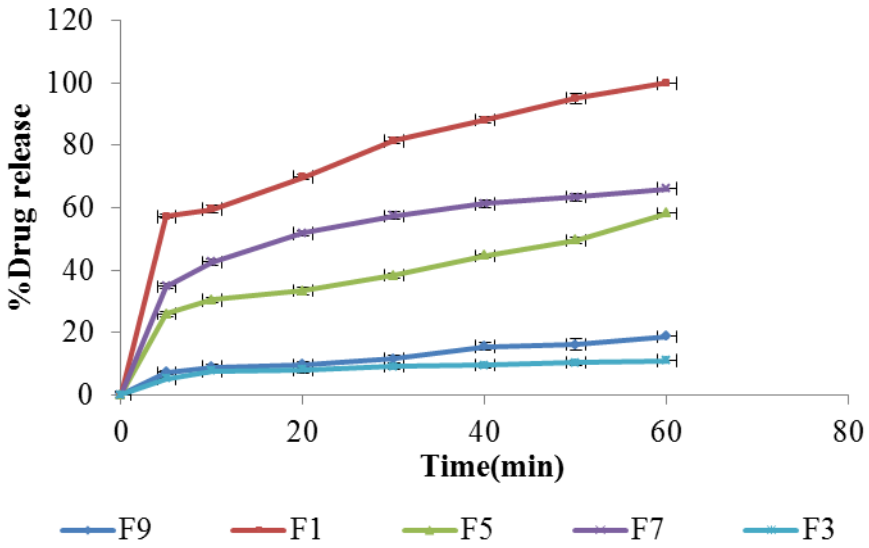


Fig. 8. *In-vitro* drug release profile of formulations F_1, F_3, F_5, F_7, F_9.

MDT reflects the time for the drug to dissolve and is the first statistical moment for the cumulative dissolution process that provides an accurate drug release rate. It is accurate expression for drug release rate. A higher MDT value indicates greater drug retarding ability.

From table 3, it was evident that onset of dissolution of pure glipizide was very low (F9; $DP_{10\ min}$ value 8.91% and $t_{50\%}$ >>2 h). It was shown that formulation F_1 has very high $DP_{10\ min}$ with lowest $T_{50\%}$ (59.6%, and 5 min respectively). MDT value of pure glipizide (F_9) is high (12.56 min). This value decreased to a greater extent after preparing its nanosuspensions. F_1 showed lowest MDT value (8.51 min). Also, MDT values of formulation F_1 was lower than other prepared nanosuspensions.

3.8. Accelerated stability study of optimized batch

Particle size of glipizide nanosuspension formulation was measured after 1st, 2nd, 3rd, and 4th week. The results are shown in Table 4. The glipizide nanosuspension was exhibited bluish white color for 1 month storage period at room temperature. This appearance of nanosuspension was already observed in freshly prepared nanosuspension. This was indicated that no change in appearance of nanosuspension was identified after one month. Particle size of nanoparticles increased from 425.6 nm to 485.2 nm after 4th week at room temperature. The crystal growth could be explained by Ostwald ripening. The sediment was fully redispersable, but only after gentle shaking for several minutes. Thus, the glipizide nanosuspension (F_1) was showed significant variation in particle size under room temperature. These can be minimizing by change in storage condition or using lyophilization approach to convert liquid formulation into solid product for enhancement of life of nanoparticulate dosage form.

4. Conclusion

The present investigation focuses on formulation strategy of glipizide nanoparticles and investigates the improvement of solubility and dissolution rate. Different stabilizers in various ratio and anti-solvent precipitation method were effective in nanosizing the drug. The nanosuspension showed improved solubility and dissolution compared to micronized suspension. DSC results revealed the unchanged crystalline nature of glipizide in the nanosuspension form. Saturation solubility of glipizide was

increased 22.63 times than that of pure drug by nanosuspension formulation. Formulation containing HPMC-E15 in ratio of 1:1 showed significant improvement in decrease in particle size and higher saturation solubility leads to increased dissolution rate. The nanosuspension formulation was stable for one month at room temperature condition. This study results revealed that a significant enhancement in solubility of glipizide in phosphate buffer (pH 6.8) using nanosuspension approach.

References

1. Seedher N, Bhatia S. (2003). "Solubility enhancement of Cox-2 inhibitors using various solvent systems". AAPS Pharm SciTech. 4(3): 1-8.
2. Kocbek P, Baumgartner S, Kristl J. (2006). "Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs". International Journal of Pharmaceutics. 312: 179–186.
3. Jacobs C, Kayser O, Muller RH. (2000). "Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide". International Journal of Pharma ceutics. 196: 161–164.
4. Trotta M, Gallarete M, Pattarino F, Morel S. (2001). "Emulsions containing partially water-miscible solvents for the preparation of drug nanosuspensions". Journal of Control. Release. 76: 119–128.
5. Liversidge GG, Conzentino P. (1995). "Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats". International Journal of Pharmaceutics.125: 309–313.
6. Kayser, O, Olbrich, C, Yardley V. (2003). "Formulation of amphotericin B as nanosuspension for oral administration". International Journal of Pharmaceutics. 254 : 73–75.
7. Aly AM, Qato MK, Ahmad MO. (2003). "Enhancement of the dissolution rate and bioavailability of glipizide through cyclodextrin inclusion complex". Pharma Tech, 7 : 54-62
8. Mehramizi A, Alijani B Pourfarzib M. (2007). "Solid carriers for improved solubility of glipizide in osmotically controlled oral drug delivery system". Drug Development and Industrial Pharmacy. 33: 812-23.
9. Imasankar K, Babu GV, Babu PS, Prasad CD. (2002). "Studies on the solid dispersion systems of glipizide". Indian Journal of Pharmaceutical Sciences. 64: 433-439.
10. Modi A, Tayade P. (2006). "Enhancement of dissolution profile by solid dispersion (kneading) technique". AAPS PharmsciTech. 7: 68.
11. Dong Yuancai, Wai Kiong Ng, Shoucang Shen, Sanggu Kim, Reginald BH. (2009). "Preparation and characterization of spironolactone nanoparticles by antisolvent precipitation". International Journal of Pharmaceutics. 375(1-2): 84–88.
12. Sucheta D. Bhise. "Ternary Solid Dispersions of Fenofibrate with Poloxamer 188 and TPGS for Enhancement of Solubility and Bioavailability". (2011). International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(2): 583-595.
13. Chetan Detroja, Sandip Chavhan , Krutika Sawant. (2011). "Enhanced Antihypertensive Activity of Candesartan Cilexetil Nanosuspension: Formulation, Characterization and Pharmacodynamic Study". Scientia Pharmaceutica, 79(3): 635–65.

Table 1. Composition of glipizide nanosuspensions.

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Glipizide (mg)	5	5	5	5	5	5	5	5	5
Acetone (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
HPMC-E15(mg)	5	10	---	---	---	---	---	---	---
HPMC-5cps(mg)	---	---	5	10				---	---
PVPK-30(mg)	---	---	---		5	10			---
PVA(mg)	---	---	---				5	10	---
Phosphate buffer (pH 6.8) (ml)	20	20	20	20	20	20	20	20	---

Table 2. Physicochemical characterization of different of drug to stabilizers ratio for glipizide nanosuspensions.

Batch code	Stabilizer	Drug to stabilizer ratio	Particle size(nm)	PDI*	% Drug release after 10 min**	% Drug release after 60 min***
F_1	HPMC 5 cps	1:1	521.2	0.774	7.62±0.01	10.97±0.54
F_2	HPMC 5 cps	1:2	598.8	0.304	---	---
F_3	PVPK-30	1:1	530.2	0.778	30.40±0.02	58.16±0.50
F_4	PVPK-30	1:2	776.8	0.780	---	---
F_5	PVA	1:1	246.9	0.224	42.62±0.01	66.17±0.53
F_6	PVA	1:2	863.8	0.799	----	---
F_7	HPMC E15	1:1	425.6	0.583	59.57±0.63	100±0.17
F_8	HPMC E 15	1:2	681.7	0.521	---	---
F_9	Without stabilizer	----	5156	1.000	8.91±0.02	18.71±0.25

* is indicate polydispersivity index of particle size distribution. ** and *** are indicating drug release after 10 and 60 min in respective dissolution fluid.

Table 3. % Drug dissolved within 10 minutes ($DP_{10\ min}$), time to dissolve 50% drug ($t_{50\%}$) and mean dissolution time (MDT) from pure glipizide, its nanosuspensions.

Formulation code	MDT	DP_{10} (min)	$T_{50\%}$ (min)
F_1	8.51	59.7	5min
F_3	8.85	7.62	>>2 h
F_5	12.21	30.40	60min
F_7	7.78	42.62	20min
F_9	12.56	8.91	>>2 h

Table 4 Results of stability study for Formulation F_1.

Time	**1st week**	**2nd week**	**3rd week**	**4th week**
Appearance	No change	No change	No change	No change
Particle size(nm)	430	442	466	485