ORIGINAL ARTICLE

Glyburide nanosuspension: Influence of processing and formulation parameter on solubility and *in vitro* dissolution behavior

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The aim of this study was to formulate and optimize Glyburide (GB) nanosuspension which is poorly water-soluble antidiabetic drug with optimization of the dissolution property and bioavailability by reducing the particle at nano size. The nanosuspension is prepared by Top down technique, i.e. high pressure homogenization. The formulation factors which affects particle size including concentration of surfactant while processing parameters includes homogenization pressure and homogenization cycle. After particle size reduction, we observe that there are increases in the surface energy which requires adequate stabilization by surfactant. In this study, practically water insoluble GB was nanoground and surfactant was employed for their stabilizing effect. *In-vitro* dissolution study revealed that increase in release rate of GB from nanoparticles (NPS) as compare to pure raw GB. Field Emission Scanning Electron Microscope (FE-SEM) study showed the spherical morphology of NPS. Particles size distribution, zeta potential, and crystal form of the formulated nanosuspension were studied by using particle size analyzer and X-ray powder diffraction. The result showed that the drug dissolution rate in nanosuspension formulation is depending upon crystal form, solubility, preparation procedure, and stabilizer employed.

Key words: *Bioavailability, Glyburide, high pressure homogenization, lyophilization, nanosuspension*

INTRODUCTION

Solubility is an important criterion for drug efficacy, independent of route of administration. It also poses a major challenge for pharmaceutical industries, which are developing new pharmaceutical products, since 40% of the active substances being identified are either insoluble or poorly soluble in aqueous media. A limiting factor for *in vivo* performance of poorly water- soluble drugs, following oral administration, is their resistance to being wetted and being dissolved into the fluid in the gastrointestinal tract. Increasing the dissolution rate of poor water soluble drugs is thus important for optimizing bioavailability.[1] The role of solubility enhancement is an attempt to shift the classification of a drug (II disso to eliminate the problem associated with dissolution-limited compounds. Over the last 10 years, nanoparticles (NP) engineering processes have been developed and reported for enhancement of solubility of poorly aqueous soluble drugs. In this approach, poorly water-soluble compounds are formulated in nanometer sized drug particles.[2] According to Muller, NPs are solid colloidal particles ranging in size from 1 nm to 1,000 nm (1 μm). They have the advantage of having an even greater surface area, and being characterized, unlike micronized drugs, by an increase in saturation solubility.

Glyburide (GB) is a second-generation sulfonylurea oral hypoglycemic agent used in the treatment of non-insulin dependent diabetes mellitus. It causes hypoglycemia by stimulating release of insulin from pancreatic β cells and by increasing the sensitivity of peripheral tissue to insulin.[3] It has a history of low bioavailability, which is attributed to poor dissolution. Several attempts for increasing dissolution and

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bioavailability of GB have been made, such as micronization, nanosuspensions, molecular dispersion, and incorporation of surfactants, inclusion complexation with cyclodextrin, crystal modification, and co precipitation.[1,4]

"A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants."

In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and, therefore, improved bioavailability. Nanosized particles can increase solution velocity and saturation solubility because of the vapor pressure effect. The increases in surface area and concentration gradient lead to a much more pronounced increase in the dissolution velocity as compared to a micronized product. Furthermore, the saturation solubility is increased as well. Another possible explanation for the increased saturation solubility is the creation of high energy surfaces when disrupting the more or less ideal drug microcrystal's to nanoparticles. A particle of less than 400 nm is considered to be acceptable for a nanosuspension to be administered intravenously.[4] Nanosuspensions for oral route are mainly characterized by mean particle size *d* (90), zeta potential, crystalline status, dissolution velocity, and saturation solubility. For a physically stable nanosuspension solely stabilized by electrostatic repulsion, a zeta potential of ± 30 mV is required as a minimum.[5,6] In the case of a combined electrostatic and steric stabilization, a rough guide line of ± 30 mV is sufficient.[2] The crystalline structure of nanosuspension is important for drugs existing in different polymorphic forms. This is mainly confirmed by Differential scanning calorimetry (DSC) and X-ray diffraction (X-RD) analysis. Dissolution velocity and saturation solubility are generally performed using official pharmacopeial methods. The stability and robustness of a nanosuspension are mainly governed by various formulation and process variables. Selection of proper steric and electrostatic stabilizer and its optimum quantity plays a major role in formulating a nanosuspension[7] Commonly used steric stabilizer includes hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), povidones (PVP K-30), and pluronics (F68 and F127), whereas electrostatic stabilizer includes polysorbates (Tween-80), sodium lauryl sulfate (SLS).

The aim of this study was to formulate and evaluate nanosuspension containing GB prepared by Top down Technique as High pressure homogenization to achieve a better solubility and dissolution profile with enhanced bioavailability than previous GB.

MATERIALS AND METHODS

Glibenclamide (active pharmaceutical ingredient-API) was obtained from Sanofi Aventis Ankleshwar, (Gujrat) India as gift-sample; SLS was purchased from Himedia Laboratories Pvt. Ltd, Poloxamer 407 from Sigma Aldrich (Steinheim, Germany), PVP-30 from SRL. Pvt. Ltd., Methanol (High Performance Liquid Chromatography-HPLC Grade) and Tween-80 from Merck.

Solubility testing of Glyburide

The solubility of GB in water and in aqueous solutions of different stabilizers was determined by addition of excess of the drug to the solvent, after which the mixture was stirred on a magnetic stirrer at room temperature for 24 h, then filtered and the drug dissolved in was analyzed spectrophotometrically at 300 nm.[8] (Hitachi, ultraviolet (UV)-2900 double beam UV-Visible Spectrophotometer) each sample was analyzed in triplicate.

Preparation of nanosuspensions by top-down technique

The nanosuspension were formulated by using Top down technique, i.e., high pressure homogenization.[9,10] Technique involves the forcing of the suspension under pressure through a valve having a narrow aperture. Before subjecting the drug to the homogenization process, it is essential to form a pre-suspension of micronized drug in a surfactant solution using high-speed mechanical stirrers.

The step involve in the preparation of nanosuspension are as follows:

Formulation of coarse suspension

Coarse suspensions of drug were formulated by using dispersing GB in surfactant solution (Sodium dodecyl sulfate (SDS) in bidistilled water), the mixture was then stirred on mechanical stirrer at 500 rpm and then pass it through high speed homogenizer at 4, 000 rpm for 5 min.

Formulation of nanosuspension

The above formulated coarse suspension were then homogenized at high pressure (5 cycle at 200_bar, 5_cycle at 500 bar and 10 cycle at 800-1,000 bar) using GEA-Nirosoavi Panda plus 2000 (Italy).

Lyophilization of nanosuspension

The obtained liquid nanosuspension formulation (containing GB and surfactant) were frozen at −8°C and then subjected for lyophilization for 48 hrs at −70°C and 50 mmHg using SCANVAC Cool safe instrument (Italy).

Experimental batches

Specifications of Glyburide Nanosuspension formulation (GBS) are described in Table 1.

Characterization of lyophilized nanosuspension

Fourier transform infrared spectroscopy (FT-IR) Study

The compatibility or drug excipient interaction study can be determined by FT-IR Spectrogram.

The pure GB and lyophilized nanosuspension were analyzed by using FT-IR (Shimadzu 8400 Japan) to find out that there is no interaction between drug and excipient.

Determination of drug content in lyophilize powder sample

For determination of drug content in the formulation or lyophilized nanoparticles (containing drug and surfactant), the equivalent weight of the formulation was dissolved in the methanol and stirring the solution on magnetic stirrer at 400 rpm at room temperature until particles dissolved. The solution is then filtered and analyzed at 300nm. Each sample was prepared and analyzed in triplicate.

In vitro dissolution studies

The *in vitro* dissolution studies of GB from the lyophilized nanosuspension was studied using a United States Pharmacopeia (USP) XXIII 8-station dissolution rate test apparatus with a rotating paddle stirrer at 50 rpm and 37 ± 0.5°C in 900 ml phosphate buffer (0.05 M) solution pH 7.5[11] A sample of nanosuspension equivalent to 5 mg GB was used in each test. Sample of dissolution fluid were withdrawn through a filter (0.45 µm. Millipore Millex-HN) at different time intervals and were analyzed at 300 nm using double beam UV spectrophotometer. The drug release experiments were conducted in triplicate.

Scanning electron microscopy

The morphology of pure drug, lyophilized coarse suspension, and lyophilized nanosuspensions were examined by Field Emission Scanning Electron Microscope (FE-SEM-Hitachi S4800). The samples were fixed on a brass stub using carbon double-sided tape. Pictures then taken at an excitation voltage of 15 KV.

X-ray powder diffraction

X-ray powder diffraction analysis was performed on a Bruker D8 advance (Bruker-AXIS, Karlsruhe) controlled by Foxit X-RD commander software. Samples were prepared by spreading powder sample on specimen holder ring from Bruker. All samples were scanned from {3°to 50° at 2θ} at the rate of 1°/min with 0.02° step size and 1.2 s/step at 40 KV and 40 mA at 25°C.

Zeta potential

The zeta potential of nanosuspension was measured using Malvern Zetasizer ZS 200 at 25 ± 0.5°C. Each sample was measured three times. The average values were employed for the calculation of the response surface.

Particle size measurement

The particle size of nanosuspension was measured using Malvern Zetasizer ZS200. The average values were employed for the calculation of the response surface.

RESULTS

Solubility studies of Glyburide

Table 2 shows solubility of GB in different surfactant. In Figure 1 graph shows GB has maximum solubility in the 0.5% SLS.

Fourier transform infrared spectroscopy study

State of drug molecule with hydrophilic surfactant was determined using FT-IR. Figure 2 shows IR spectra of GB and prepared lyophilized nanoparticles. The spectra of both are nearly same and there is no shift of peaks after adsorption of drug on surfactant. The principal peak of GB were obtained at wave number {3457, 3368 cm^{-1}}attributed to –NH stretching-542, 609 cm^{-1} attributed to –Cl bending deformation, 1715 cm^{-1} is due to –C=O bending, 3033, 3067,

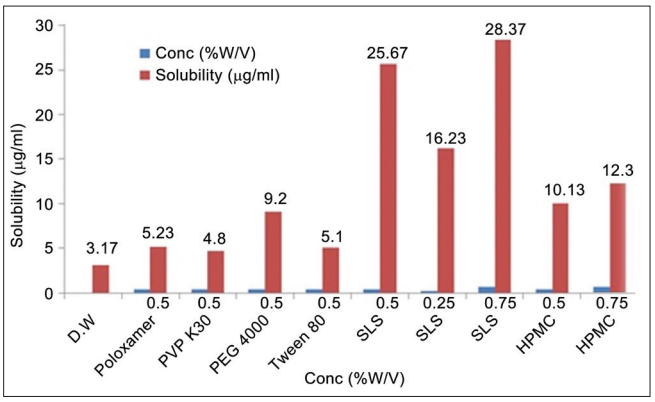

Figure 1: Solubility studies of Glyburide in different surfactant

Table 1: Experimental batches: Specification of GB nanosuspension formulation

Batch name	Drug (mg)	Surfactant (%w/v)	High speed homogenizer (rpm)
GBS1	20	0.5	4,000
GBS2	20	1	4,000
GBS3	20	1.5	4,000
GBS4	30	0.5	4,000
GBS5	30	1	4,000
GBS6	30	1.5	4,000
GBS7	40	0.5	4,000
GBS8	40	1	4,000
GBS9	40	1.5	4,000

GBS: Glyburide nanosuspension

Table 2: Solubility studies of Glyburide

Surfactant	Concentration (w/v) (%)	GB solubility (µg/ml) (mean±SD)
Water		3.16±0.1
Poloxamer	0.5	5.23±0.04
PVP K30	0.5	4.8±0.5
PEG 4000	0.5	9.2±0.08
Tween 80	0.5	5.1±0.2
Sodium dodecyl sulphate	0.5	25.6±0.2
Sodium dodecyl sulphate	0.25	16.23±0.2
Sodium dodecyl sulphate	0.75	28.36±0.2
HPMC	0.5	10.13±0.1
HPMC	0.75	12.3±0.2

HPMC: Hydroxypropylmethyl cellulose, PVP: Povidones, PEG: Polyethylene glycol

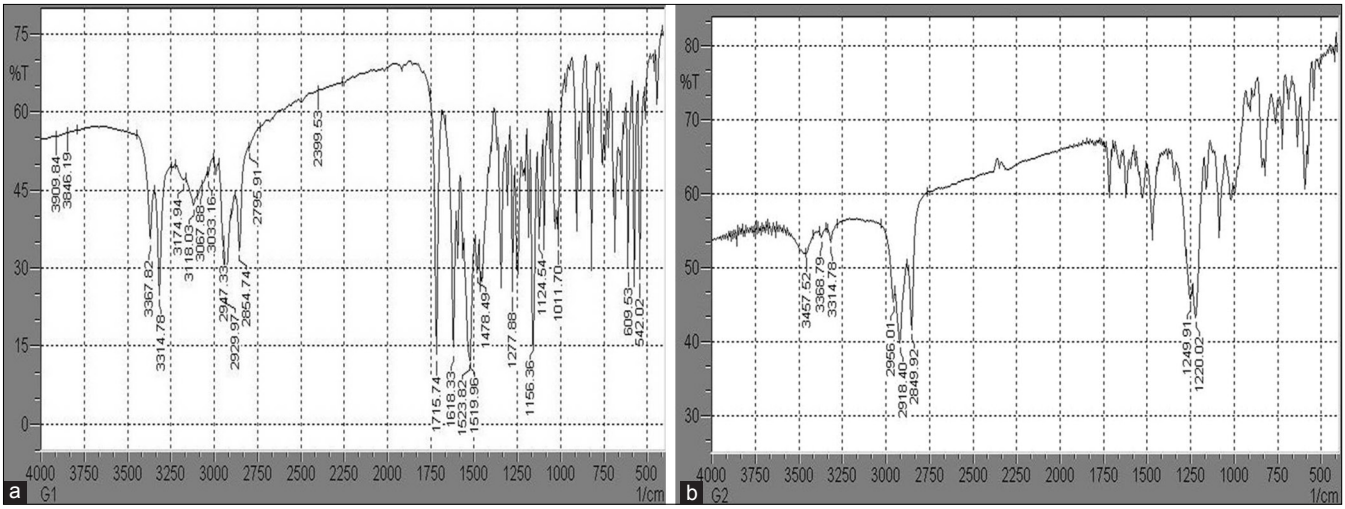

Figure 2: IR spectra of (a) Glyburide and (b) formulation

3118, 3174 is due to aromatic-stretching-2849, 2918, 2956 cm^{-1} attributed to –CH (fundamental) stretching. Thus, from the spectra it was understood that there was no interaction between GB and surfactant used in the formulation.

Drug loading and effect of formulation parameter on particle size

The drug loading of formulated nanosuspensions is described in Table 3.The study showed that GBS5 and GBS9 batches have maximum drug loading (76% and 88%) and physical stability due to small particle size.

In-vitro dissolution studies

The goal of improving the dissolution rate of GB was achieved, by this method *in vitro* dissolution of raw GB and formulated nanosuspensions are described in the Figure 3. The rate of dissolution of raw GB was very low only 2.9% drug was release in 10 min while 21% drug was release in 60 min. The optimized formulation shown improved dissolution rate, since almost 100% drug released in 30 min (GBS9) [Figure 3]. The surfactant is responsible to increase the dissolution rate due to the improved wettability and solubility of drug.

Scanning electron microscopy

The SEM pictures of raw GB (GB-R), lyophilized coarse suspension (GB-Cs), and lyophilized nanosuspension (GB-NS) of the drug are reported in the Figure 4. Micrographs prove a great morphological difference between GB-R, GB-Cs, and GB-NS.

GB-R drug shows regular elongate shape, while coarse suspension crystals are more irregular and more rounded. While GB-NS nanosuspension showed the spherical morphology of the lyophilized nanoparticles and confirmed change in crystals of the coarse suspension after the homogenization. In SEM images of formulation, i.e., GBS5 and GBS9, the scale shows particle size ranges from 300 nm to 500 nm, which was also observed by particle size analysis.

Table 3: Drug loading and effect of formulation parameter and surfactant on particle size

Batch	Drug loading (%)	MPS d (90) nm	Zeta potential (mV)
GBS1	53	295	−32
GBS2	52	255	−35.2
GBS3	48	141	−38
GBS4	30	122	−40.6
GBS5	76	91.2	−42
GBS6	63	105	−44
GBS7	58	105	−38
GBS8	69	78.8	−42
GBS9	88	24.3	−40

GBS: Glyburide nanosuspension

X-ray diffraction analysis and crystallinity of nanoparticles

X-ray diffraction has been used to analyze potential changes in the inner structure of GB, the diffraction pattern of raw GB and GB nanosuspension are given in the Figure 5. The dirrerence in the relative intensities of their peak was observed, it might be attributed due to difference in the crystallinity and molecular conformation of the sample.[10] As analyze by the Foxite XRD pattern processing software the percentage crystallinity of GB was about 85.2% while GB nanoparticles had 76.5%, which was lower than that of raw drug.

Zeta potential and particle size analysis

The particle size distribution of the lyophilized nanosuspension was determined by photon correlation spectroscopy (PCS). The PCS diameter and zeta potential Polydispersity Index (PI) of the formulated nanosuspension is shown in Table 4.

Photon correlation Spectroscopy gives the average diameter and Polydispersity Index as measures of particle size distribution. Table 4 shows the average diameter *d* (90), Polydispersity index and zeta potential of lyophilized nanoparticles (GBS5 and GBS9). The Zeta potential and

particle size distribution of optimize batches (GBS5 and GBS9) are described in Figures 6 and 7. The particle size decreases with increase in homogenization cycle and concentration of surfactant. Particle size and zeta potential is responsible for stability of nanosuspension. For an electrostatically stabilized nanosuspension, a minimum zeta potential of ± 30 mV is required, high value of zeta potential indicating maximum stability which was observed in these formulation (GBS5 and (GBS9). The PI value of same formulation shows broad distribution of the particles and it increases with increasing surfactant concentration.

DISCUSSION

Nanogrinding is a critical process which involves the selection of adequate formulation and process parameter to obtained appropriate particle size reduction and stability of nanosuspension. In this study, we focused on formulation and process parameters. Formulation parameter includes the selection of appropriate excipients such as vehicle surfactant and wetting agent, i.e., the excipients should posses the wetting ability with electrostatic stabilization of the nanoparticles. The initial solubility study of was done in various stabilizers such as PVP, Poloxamer, Tween, HPMC, and SDS and the drug shows highest solubility in SDS. SDS is an cationic surfactant which was found to equally effective for nanogrinding and thus promoting particle size reduction.

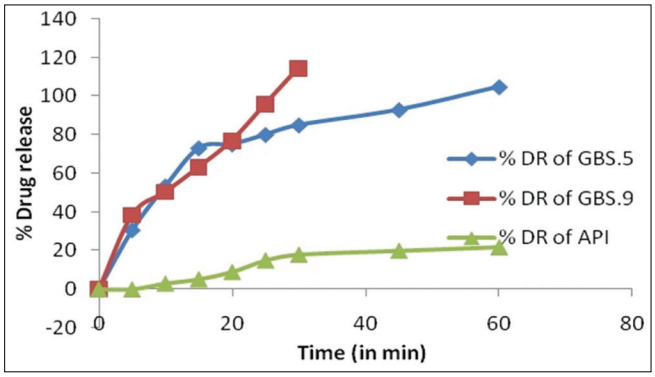

Figure 3: Mean dissolution profile of optimized batches (batch GBS.5, GBS.9) and Glyburide (API)

As described in the result, the formulated nanosuspension, as GBS5 and GBS9, shows the improvement in the dissolution profile compared to pure GB [Figure 3]. It is associated with reduction in particle size and surfactant concentration. The PCS gives the average particle size and PI of the formulated nanosuspension. Table 4 shows the formulated batch of GB nanosuspension, the particles are in nanorange, and having zeta potential more than ± 30 (i.e., −42 for GBS5 and −40 for GBS9), which is responsible for the stability of the nanosuspension. The crystallinity of the nanoparticles differs from that of pure drug particles (API) which was observed in the X-ray diffraction pattern [Figure 5]. It might be attributed due to difference in particle size. FE-SEM study confirms spherical morphology of lyophilized nanoparticles and confirms change in the crystal pattern of coarse suspension after homogenization.

Table 4: Particle size analysis and PI of GBS5 and GBS9

Batch	MPS d (90) (nm)	Zeta potential (mV)	PI
GBS5	91.2	−42	0.44
GBS9	24.3	−40	0.39

GBS: Glyburide nanosuspension, MPS: Mean particle size

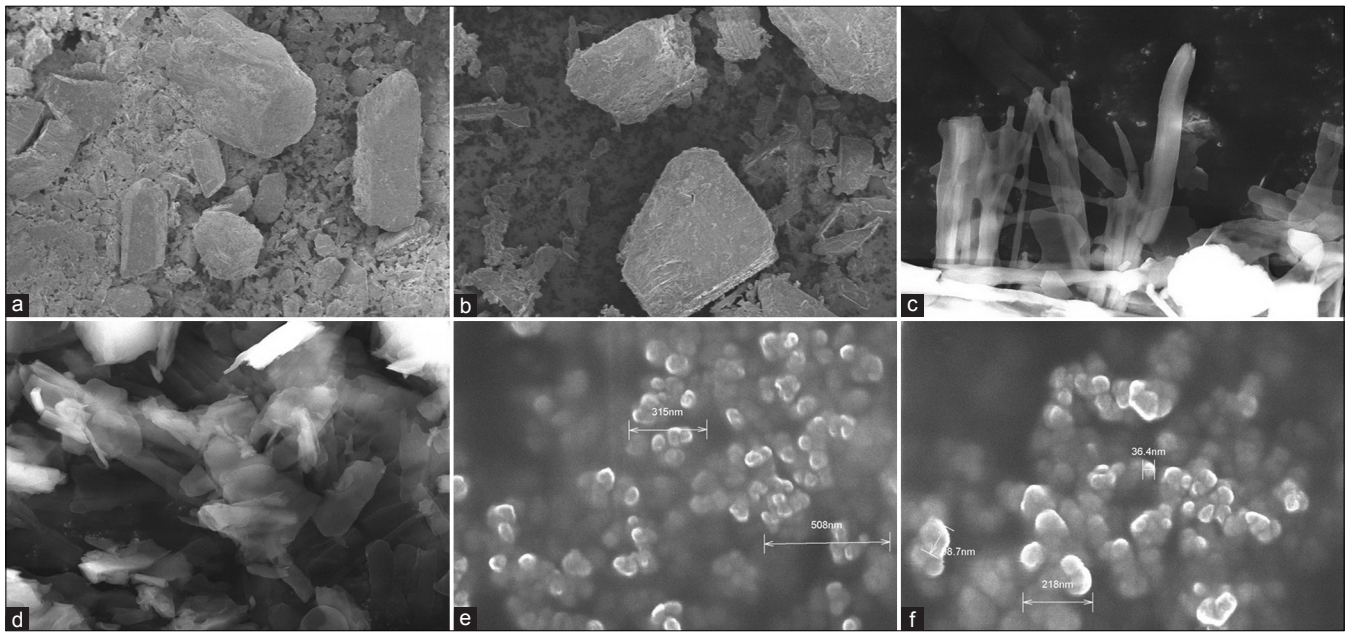

Figure 4: Scanning electron microscopy of (a and b) Raw Glyburide, (c and d) Images of coarse suspension of Glyburide, (e and f) Lyophilizedna nosuspension

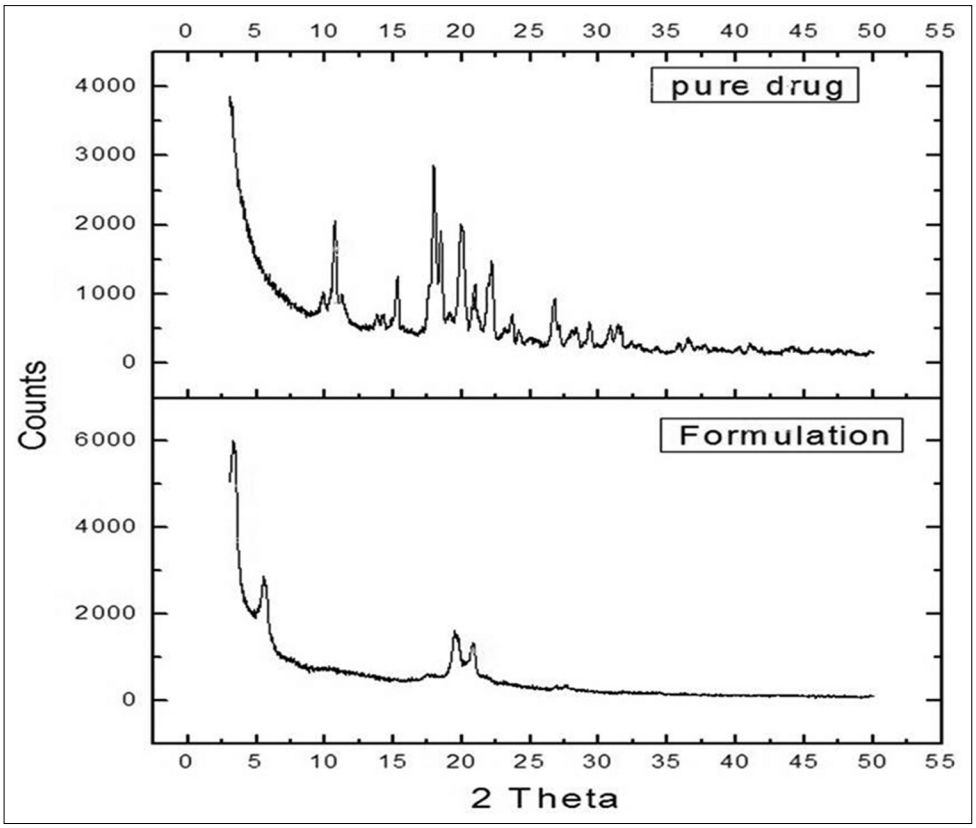

Figure 5: XRD analysis of formulation and pure drug

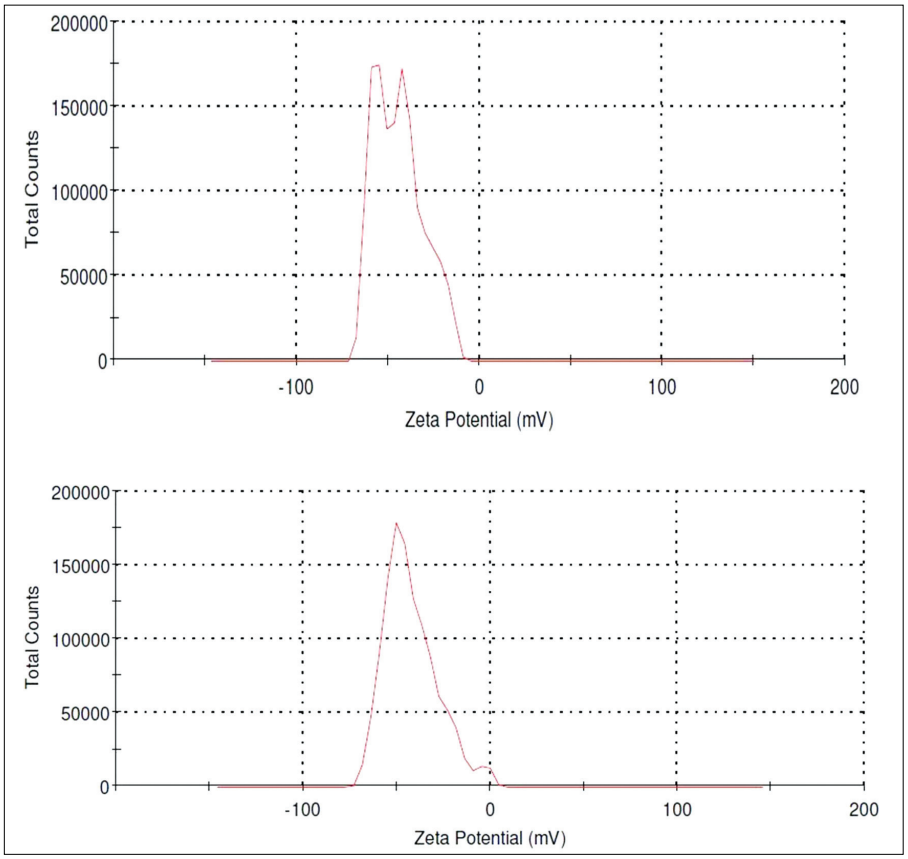

Figure 6: Zeta potential of formulation GBS5 and GBS59

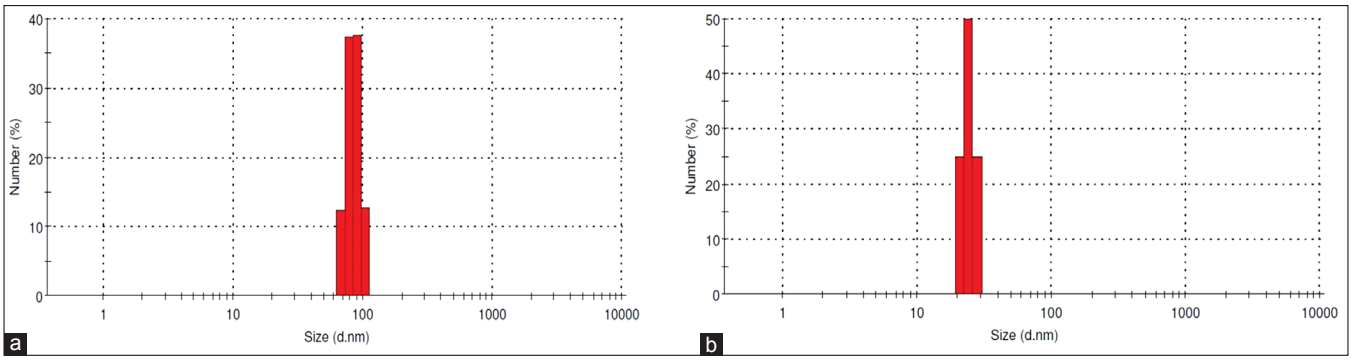

Figure 7: Particle size distribution of (a) GBS5 and (b) GBS9

CONCLUSION

From this study, it was concluded that surfactant concentration and homogenization play an important role in particle size reduction, efficient particle size reduction by nanogrinding requires the use of excipients that provide proper wetting and physical stabilization (Steric and electrostatic) of practically water insoluble drug. This study showed that GB dissolution rate is affected by drug solubility and that depend on the crystalline form. Finally, appropriate particle size reduction and nanosuspension stability of water insoluble drug are important not only for improvement of dissolution behavior and bioavailability of the drug but also for safe use of medicament by different route such as parenteral, oral, topical, etc.

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