Solid state characterization and effect of PEG 20000 and lecithin on particle size reduction and stability of complexed glibenclamide nanocrystals

B. Sajeev Kumar[1*], R. Saraswathi[2], K. Venkates Kumar[1], T.A Sam[1], S.K Jha[3], and S.A Dhanaraj[1]

[1] Asian Institute of Medicine Science and Technology, Semeling, Bedong, Malaysia
[2] National College of Pharmacy, Kozhikode, Kerala, India
[3] Bengal College of Pharmaceutical Science and Research, West Bengal, India

ABSTRACT

Aims: To formulate and characterize GLB-PEG-LEC NCs (lecithin complexed Glibenclamide nanocrystals) and to analyze the effect of PEG 20000 and lecithin on drug properties, particle size reduction and stability of GLB NCs.

Study design: Precipitated (GLB-PEG) and complexed nanocrystals (GLB-PEG-LEC) of glibenclamide were characterized for particle size, size distribution, zeta potential and stability assessment using photon correlation spectroscopy. The crystallinity was analyzed by X-ray powder diffraction spectroscopy and differential scanning calorimetry. The chemical stability was assessed by means of infrared spectroscopy and surface morphology by scanning electron microscopy.

Place and Duration of Study: Asian Institute of Medicine Science and Technology, Malaysia, between May 2102 and June 2013.

Methodology: GLB-PEG NCs were prepared by precipitation technique using PEG 20000 and complexed by soybean lecithin. The effect of lecithin in particle size reduction, change in crystallinity, stability and surface properties of NCs were analyzed and compared with pure glibenclamide (GLB) and precipitated NCs. The formulations were optimized and its stability was assessed during a 3 month period.

Results: Pure GLB exhibited an average particle size of 1551 nm. The average particle size of precipitated NCs was between 236 - 7000 nm, while that of complexed NCs was between 155 - 842 nm. The particle size of NC was found to decrease, whereas its zeta potential was found to increase after complexation. DSC studies showed no change in crystalline structure. PXRD studies proved that crystallinity was maintained in NCs. SEM analysis showed presence of spherical shape particles with a lipid coat on the surface after complexation. Stability studies revealed no change in particle size during 3 month period. FTIR studies showed the compatability of excipients with the drug.

Conclusion: These results show that lecithin complexed GLB NCs could be utilized as promising carriers in development of various formulations due to its high stability and decreased particle size.

Keywords: Nanocrystals, complexation, lecithin, stability, particle size, precipitation

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

1. INTRODUCTION

Nanotechnology has emerged as a pivotal area of research and it may affect our lives tremendously over the next decade in every field, including medicine and pharmacy [1]. In medicine and pharmaceutics, nanotechnology is used to improve human health at molecular level and is applied in development of nanoparticulate systems [2]. Although nanoformulations are clinically approved in the past decade, major problems like loss in functional properties and poor stability inhibit their wide spread adoption.

The drug delivery efficiency of a nanoformulation depends on various factors like its type, particle size, surface properties and stability of the particles in medium. Ideally a successful nanoformulation should have high drug loading capacity, considerable drug release and better stability [3]. During the process of formulation, the particle size, shape, surface properties and composition of nanocarriers need to be precisely controlled and their effects on pharmacokinetics and pharmacodynamics profiles need to be clearly elucidated [4]. Also, for effective utilization of nanoformulations, its comprehensive structure - function relationships between the nanoparticle structure and pharmacological properties need to be fully established [5]. These characterizations are emerging as new focus for assessing the safety and effectiveness of various nanoformulations.

Nanoformulations include nanocrystals, carbon nanotubes, fullerenes, polymeric micelles, nanosuspensions and nanoemulsions, and they are generally prepared by top down or bottom up approach [6]. Compared to all nanoformulations, nanocrystals are considered to be the least complex and are developed by precipitation or nanonization process. Nanocrystals (NCs) contain 100% drug with no carriers, offer excellent solubility and can solve the issues associated with poor solubility of a drug [7]. Nanonization or nanosizing techniques reduce the particle size and increase the surface area-to volume ratio of drugs thereby offering higher rate of drug dissolution [8]. The particle size reduction process of NCs depends upon the type of polymers, surfactants, stabilizers and the milling method [9].

NCs possess major limitations like crystal growth (aggregation) on contact with fluids or electrolytes, and loss in its functional properties [10]. A strategy to overcome the limitations is to alter the surface properties of NCs by attaching ligands to them or by increasing its stealthiness by complexation [11]. This approach could decrease particle aggregation, improve *in vivo* stability and could provide a more complete and consistent absorption profiles similar to solid lipid nanoparticles (SLN) [12].

During the production process of NCs, real time monitoring of immediate NCs and assurance tests for final product are necessary. This could help in development of a stable formulation and the drugs could be delivered safely and efficiently at a particular site with improved bioavailability. Solid state characterization studies could provide useful information about the properties of NCs. Parameters like particle size, zeta potential, size distribution, surface morphology, crystallinity and aggregation need to be controlled precisely as they may affect the absorption, distribution, metabolism, excretion (ADME) and toxicity of nanoformulation [13]. The above properties can be analyzed using Photon Correlation Spectroscopy, Powder X-ray Diffractometry (PXRD), Differential Scanning Calorimetry (DSC), Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM).

Glibenclamide (GLB) is a second generation oral hypoglycemic agent (BCS Class II drug), with high permeability, low aqueous solubility (~38 μmol L^{-1} at 37 °C) and poor dissolution rate [14,15]. GLB is also a drug of choice for long term therapy for diabetes mellitus and it requires a rapid GI absorption, to prevent a sudden increase in the blood glucose level after food intake [16]. The objective of the present study is to formulate and characterize the properties of GLB NCs by various techniques. NCs were developed by precipitation process

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

using PEG 20000 and stabilized (complexed) by means of soybean lecithin. PEG20000 was used in the study as it is non toxic, applicable to drug carriers and is also used in solubility enhancement studies [17]. The effect of PEG 20000 and lecithin on particle size reduction and change in crystallinity of NCs were also assessed. Solid state characterization studies facilitate in development of a stable formulation with fewer drugs - excipient interactions and enable to design a formulation with improved therapeutic efficiency.

2. MATERIAL AND METHODS

2.1 Materials

Glibenclamide sample was obtained from S.D Biomed (Malaysia). PEG 20000 and soybean lecithin was procured from Sigma Aldrich (Malaysia). Acetone, Tween 80, sodium dodecyl sulphate, polysorbate 80, dichloromethane and methanol were purchased from R and M Chemicals, (Malaysia). Deionized water was obtained from Millipore, MilliQ-Plus. All the other solvents and reagents used were of Anala R grade.

2.2 Methods

2.2.1 Preparation of GLB-PEG NCs

GLB (5% w/w) was dissolved in a solvent mixture of acetone and methanol (2:1). PEG 20000 at different drug-polymer ratio (1:1, 1:2, 1:4, 1:8 and 2:1) was added to the drug solution and stirred using a magnetic stirrer (Erla- EMS H7000) at a temperature not exceeding 60°C. The drug-polymer solution was injected slowly (1 ml/min) into an aqueous phase containing Tween 80 (3% w/v) as stabilizer with mechanical stirring (400 rpm) overnight at room temperature to precipitate NCs. The volume (80 ml) was adjusted to 100 ml using double distilled water. The solution was gently heated (65°C) with magnetic stirring (Erla- EMS H7000, Korea) for 30 min to remove the organic solvent. Later, the contents were centrifuged (Heraeus - Labofuge 200, Germany) at 5000 rpm for 20 min to separate the NCs. The clear supernatant liquid was discarded, and the thick viscous dispersion was collected. The dispersion was further suspended in 15 ml of distilled water and recentrifuged (Hitachi - CT15E, Indonesia) at 20000 rpm for 10 min to remove the impurities and the residual surfactants. The NCs were recovered using a vacuum filter (Kontes Ultra ware - 0.2 µm, USA) and dried in a hot air oven (Memmert - UF110, Germany) at 35°C for 20 min. The procedure was repeated to prepare different batches [18].

2.2.2 Complexation of GLB-PEG NCs

GLB-PEG NCs were complexed using soybean lecithin. 50 mg of dried NCs were accurately weighed and dispersed in 50 ml of phosphate buffer (pH 7.4) in presence of 0.1 % w/v Tween 80 by gentle stirring (Erla - EMS H7000, Korea) for 10 min. Soybean lecithin (2% w/v) previously solubilized in chloroform was gradually added (2 ml/min) to the dispersion and stirred continuously using a magnetic stirrer (Erla - EMS H7000, Korea) at 250 rpm for 30 min at a temperature above its melting point (35°C) so as to obtain a homogenous dispersion. The dispersion was transferred to a shaking incubator (Daiki Scientific - DK-SI 010, Korea) and shaken at 120 rpm for 1 h at 15°C. Later, 5% w/v mannitol was added to the dispersion as a cryoprotectant and shaken for 10 min prior to lyophilization [19].

2.2.3 Freeze Drying

The milky homogenous dispersion “prepared in 2.2.2 above” was subjected to freeze drying in a freeze dryer (Thermo Scientific, USA), with an inbuilt Pirani 501 microprocessor. The samples were lyophilized at a slow freezing temperature (shelf temperature -40 °C at 6 torr and 10^{-1}mbar pressure) for 10 h. The lyophilized products were stored in borosilicate glass vials and placed in a dessicator at room temperature until further use.

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

2.4 Solid State Characterization

2.4.1 Photon Correlation Spectroscopy (PCS)

The mean particle size and polydispersity index (PDI) of precipitated and complexed NCs were measured using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). 2 mg of NC was dispersed in 150 ml of deionized water containing 0.1% w/v of tween 80 and 0.15 mg of sodium dodecyl sulphate (SDS). The dispersion was sonicated using a bath sonicator (Power sonic 410, Lab Tech, Korea) and kept aside for 24 h prior to analysis. 4 µl of each suspension was diluted with 2 ml of deionized water and the samples were pipetted into a disposable polystyrene cuvette. The samples were measured for the mean particle size and PDI at a fixed angle of 90° and at a temperature of 25°C. Each measurement was performed in triplicate. A refractive index of 1.616 and 1.300 were used for the drug and solvent respectively [20].

2.4.2 Zeta potential measurement (ZP)

The zeta potential of precipitated and complexed NCs was measured using the light scattering technique (M3-PALS) in a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Samples were dispersed in deionized water and kept aside for 24 h and were injected into a clear disposable zeta cell after suitable dilution. The zeta cell was checked for presence of air bubbles and if any, was removed by tapping. The average zeta potential was measured for each sample after 3 scans.

2.4.3 X-Ray Powder Diffraction (XRPD)

XRPD diffractograms of pure GLB, polymers, physical mixtures (PM-1:1) and NCs before and after complexation were recorded in X-ray diffractometer (Bruker AXS D8, Germany) with Anton Paar, TTK 450 temperature attachment, using Si (Li) PSD detector. The samples were placed in a glass sample holder and Cu ka radiation was generated at 30 mA and 40 Kv. The samples were scanned from 3° to 70° 2θ with a step size of 0.02° in duplicate [21].

2.4.4 Differential Scanning Calorimetry (DSC)

DSC analysis of pure GLB, polymer, physical mixtures (PM-1:1) and NCs before and after complexation were analyzed in a DSC calorimeter (TA Instruments, Q200, USA), equipped with a liquid nitrogen cooling system. High purity indium was used to calibrate the heat flow and heat capacity of the instruments. About 5 mg of samples were loaded to open aluminum pan, crimped, sealed and further examined at a scanning rate of 10°C / min from 15 to 200°C under nitrogen atmosphere (flow rate 100 ml/min) in room temperature. The analysis was also performed in duplicate [22].

2.4.5 FTIR Analysis

Spectra of pure GLB, PEG 20000, physical mixtures (PM-1:1) and NCs were recorded in FT-IR spectrophotometer (Thermo Nicolet, Avatar 370, USA). 2 mg of sample was mixed with 1% KBr, compressed into a pellet and scanned for 4 seconds at a resolution of 4 cm^{-1} from 4000 to 400 cm^{-1} in duplicate [23].

2.4.6 Scanning Electron Microscopy (SEM)

Morphological evaluation of NCs was performed using a scanning electron microscope (LEO 1530, Gemini, Germany). The samples were mounted to steel stubs (Jeol - 10 mm Dia x 5 mm) using a double sided adhesive tape and sputtered with a thin layer of Au at 20 mA, under $1x10^{-1}$ bar vacuum for 10 min using a sputter coater (EM S550X - Electron microscopy sciences) and was operated at an acceleration voltage of 3 kV [24].

2.5 Stability Studies

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

The optimized formulation (Batch F1) was placed in a clean airtight glass vials and stored at room temperature and 40°C (RH ± 75%) over a period of 3 months. During the storage period, the samples were evaluated for average particle size and any change in spectra [25].

3. RESULTS AND DISCUSSION

3.1 Photon Correlation Spectroscopy

3.1.1 Effect of Polymer on Particle Size Reduction

The particle size analysis data of precipitated and complexed NCs are shown in Table 1. The average particle size of pure GLB was found to be 1551 nm, while that of precipitated NCs (F1-F5) was between 236 - 7000 nm. The particle size was found to increase with an increase in polymer content in precipitated NCs. The complexed NCs were found to have smaller particle sizes than the equivalent uncomplexed systems. The particle size distribution of precipitated NCs was found to be broader, while that of complexed NCs were narrow as the PDI was below 0.5 (except in batch F5). It can be inferred that maximum size reduction was observed in batch F1 with a drug- polymer ratio of 1:1.

3.1.2 Effect of Zeta Potential and Stability of NCs

The zeta potential of pure GLB, precipitated and complexed NCs are compared in Table 1. The zeta potential of precipitated NCs were much lower in comparison to pure GLB (-38.1 mV). A high negative zeta potential value was observed in all samples after complexation. This may be attributed to the presence of lecithin coating on its surface [26, 27].

Table 1. Particle size and zeta potential report of GLB NCs.

Batch	Drug : polymer	Precipitated NCs			Complexed NCs		
		Z.avg (d.nm)	PDI (avg.)	Avg. ZP (mV)	Z.avg (d.nm)	PDI (avg.)	Avg. ZP (mV)
Pure GLB	1:0	1551 ± 0.253	0.417 ± 0.072	-38.1 ± 0.2	-	-	-
F1	1:1	236 ± 0.039	0.369 ± 0.121	-35.1 ± 0.4	155 ± 0.162	0.310 ± 0.067	-51.7 ± 0.1
F2	1:2	5745 ± 0.105	0.610 ± 0.257	-29.0 ± 0.8	710 ± 0.058	0.309 ± 0.133	-45.8 ± 1.3
F3	1:4	7002 ± 0.384	0.417 ± 0.103	-34.3 ± 0.3	842 ± 0.043	0.397 ± 0.151	-48.4 ± 0.7
F4	1:8	5885 ± 0.296	0.520 ± 0.089	-32.3 ± 0.4	227 ± 0.124	0.431 ± 0.132	-58.3 ± 0.5
F5	2:1	3574 ± 0.195	0.957± 0.244	-20.2 ± 1.1	787 ± 0.215	0.878 ± 0.261	-48.0 ± 0.3

± indicates SD (n=3)

3.2 X-Ray Powder Diffraction

The diffraction spectra of pure GLB, physical mixture (PM-1:1), precipitated and complexed NCs are compared in Fig.1A, 1B and 1C respectively. The peak parameters like position, intensity and full width half maximum (FWHM) of NCs are shown in Table 2. Pure GLB spectra showed numerous sharp and narrow intense peaks at 2θ position like 10.85°, 11.65°, 14.696°, 16.09°, 18.82°, 20.84°, 22.92°, 24.42°, 26.19°, 27.52°, 29.11° and 30.08°, and these observations prove its high crystalline nature. It was observed that the characteristic peaks of pure GLB at 11.66°, 20.82° and 30.08° 2θ positions were present in the spectra of all NCs suggesting that crystallinity of GLB was maintained in sample. It was also noticed that the intensity of the peaks were found to reduce slightly in precipitated and complexed NCs. A broad peak with decreased area and sharpness was also noticed in XRPD spectra of samples with high polymer content. The relative intensity value (d-value) was found to decrease initially and became constant indicating that the crystallinity was

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

maintained irrespective of the polymer concentration and complexation. The presence of sharp and narrow peaks in spectra of F5 proved the presence of high amount of drug.

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

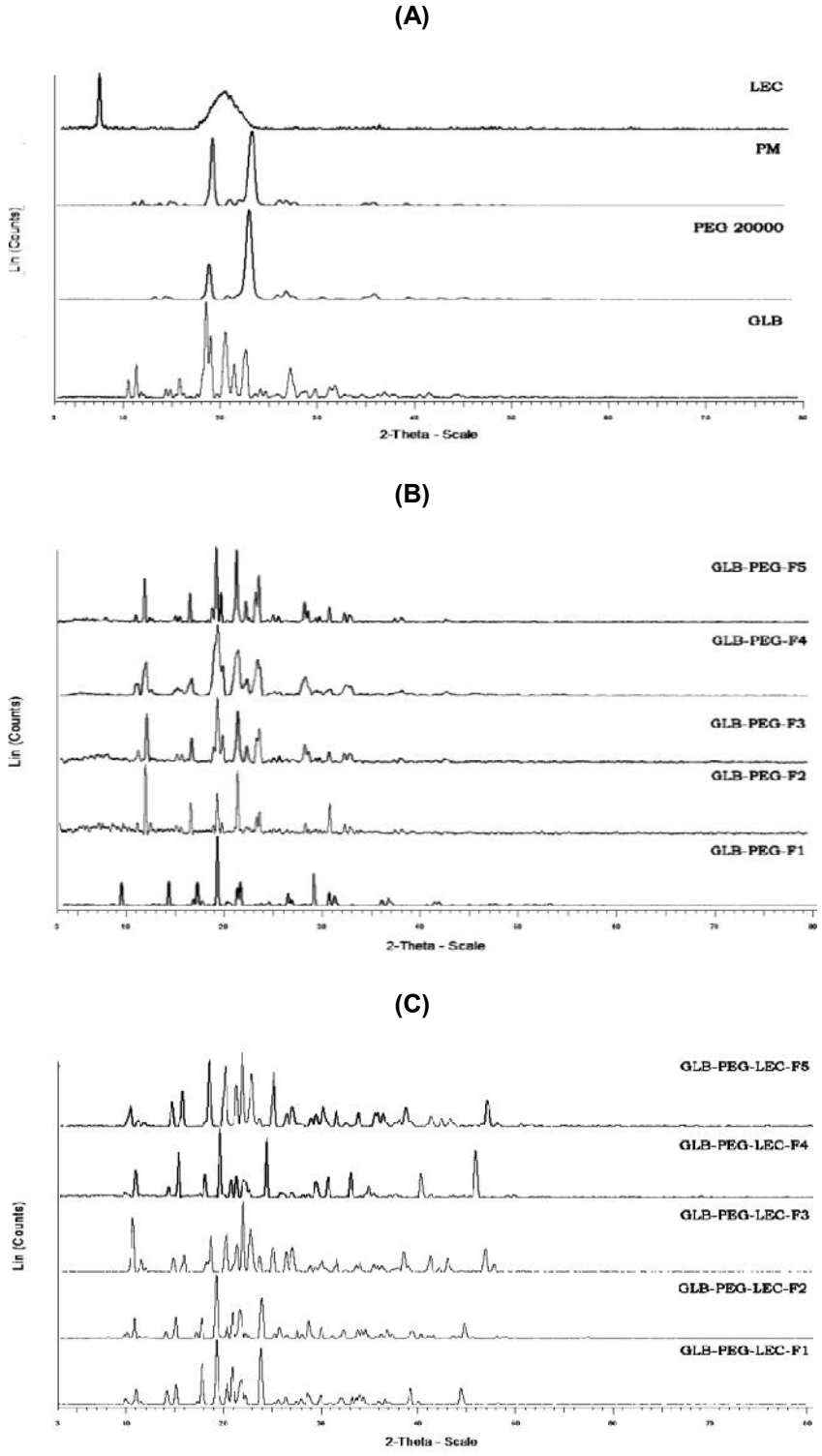


Fig.1. X-Ray Diffraction spectra of pure GLB, PEG 20000, PM (1:1) and Lecithin (A), precipitated GLB NCs (F1-F5) (B), and GLB NCs (F1-F5) after complexation (C) at 2-Theta-scale.

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

Table 2. XRPD peak parameters of GLB and formulations

Batch	Precipitated NCs			Complexed NCs		
	2 θ position	Peak intensity (d)	FWHM (deg)	2 θ position	Peak intensity (d)	FWHM (deg)
Pure GLB	11.66	7.58	-	-	-	-
	20.82	4.26	0.46	-	-	-
	30.08	2.96	-	-	-	-
F1	11.80	7.49	0.19	10.71	8.24	0.22
	21.06	4.21	0.19	20.65	4.29	0.30
	30.37	2.94	0.20	30.62	2.91	-
F2	11.75	7.52	-	10.65	8.29	0.20
	21.03	4.22	0.19	21.35	4.15	0.37
	30.33	2.94	0.19	29.61	3.01	0.24
F3	11.82	7.47	0.23	10.75	8.21	-
	21.10	4.20	0.31	21.40	4.14	0.44
	30.42	2.93	-	30.66	2.91	-
F4	11.84	7.46	-	10.56	8.36	0.24
	21.07	4.21	-	20.56	4.31	0.23
	30.38	2.93	-	29.62	3.0	0.26
F5	11.82	7.48	0.19	11.22	7.87	-
	21.08	4.21	0.22	21.54	4.12	0.36
	30.38	2.93	-	29.70	3.00	0.24

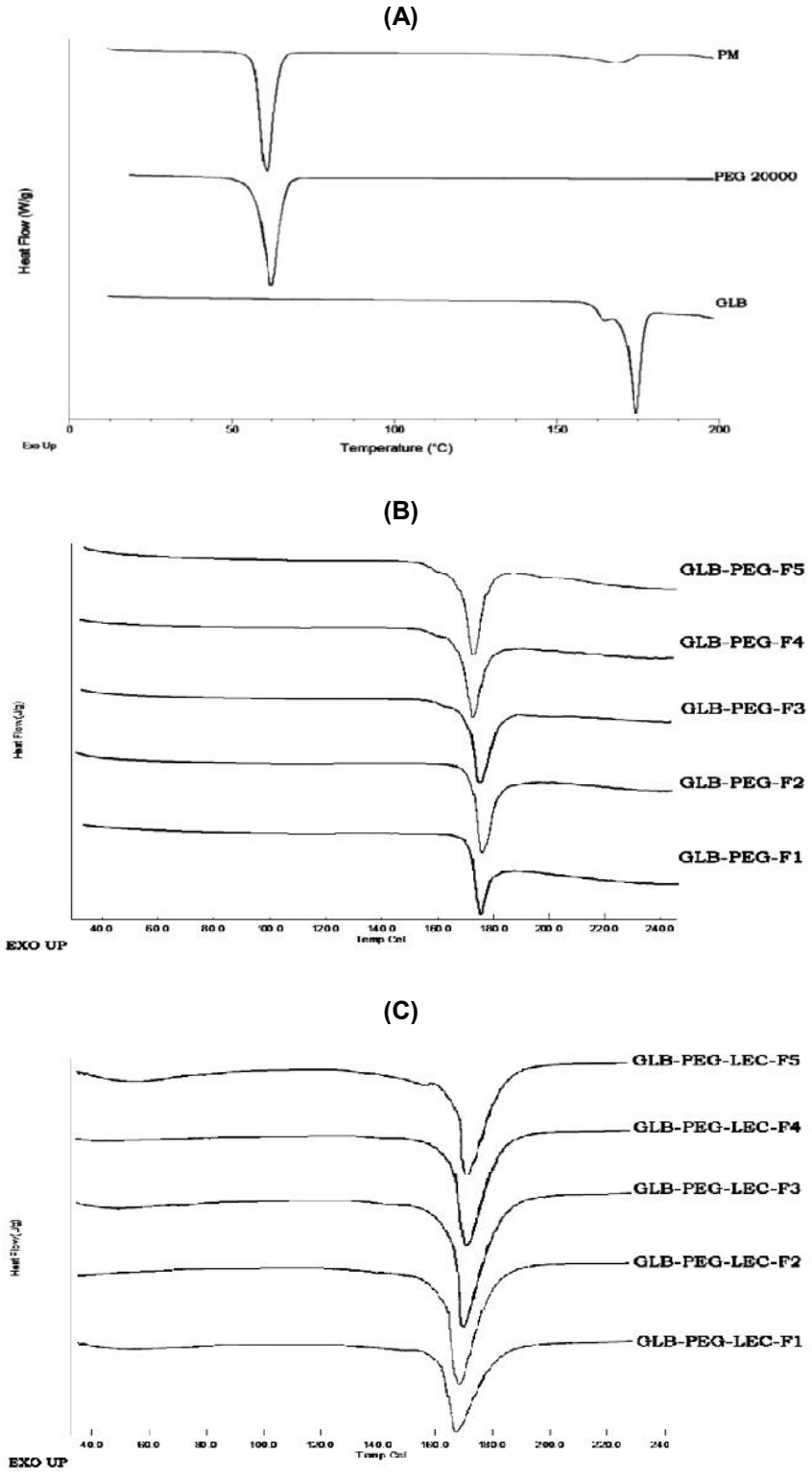
FWHM-Full Width Half Maximum

3.3 Differential Scanning Calorimetry

DSC thermograms of GLB, PEG 20000, physical mixture (PM-1:1), precipitated and complexed NCs are compared in Fig. 2A, 2B and 2C respectively. A sharp endothermic peak at 173.36°C (ΔH = 98.34 J/g) in pure GLB thermogram indicated its high crystallinity.

An endothermic peak (65.24°C) in PEG 20000 thermogram revealed its crystalline nature. Two endothermic peaks (65.68°C and 164.61°C) were observed in the thermogram of physical mixture (1:1) proves the absence of interaction between drug and polymer. The peak temperature (T_M) of precipitated NCs was found to be similar to pure GLB indicating that there was no change in its crystalline structure. It was also observed that the peak temperature (T_M) of complexed NCs was found to reduce (Fig.2C), suggesting crystallinity was maintained with reduced size.

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.



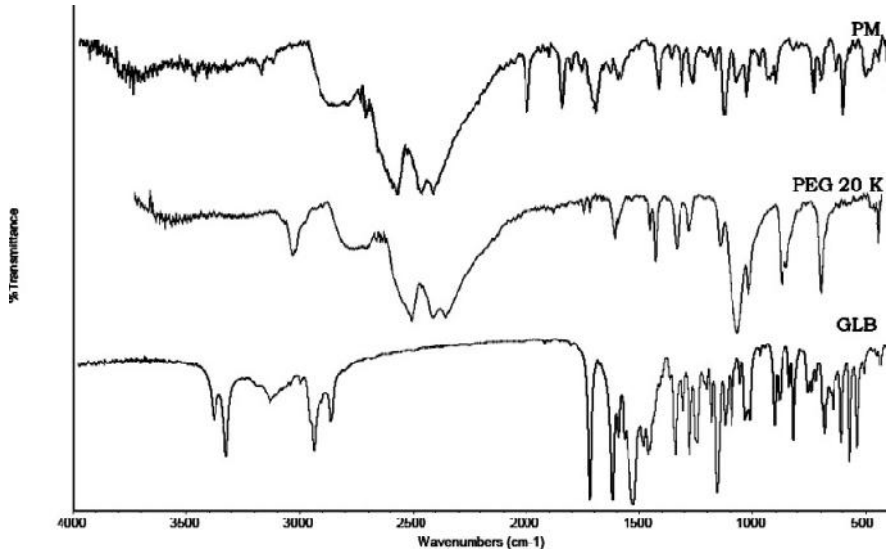
230
231 **Fig.2. DSC thermograms of Pure GLB, PEG 20000 and PM-1:1 (A), precipitated GLB**
232 **NCs (F1-F5) (B), and GLB NCs (F1-F5) after complexation (C).**

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

3.4 FT-IR Analysis

FT-IR spectra of pure GLB, PEG 20000, physical mixture (PM-1:1) and precipitated NCs are compared in Fig.3A and 3B respectively. Pure GLB showed an obvious band at 1715.55 cm^{-1} (carbonyl stretching), two characteristic bands at 1155.96 and 1306.29 cm^{-1} (symmetrical and asymmetrical sulphonyl stretching) and bands at 3315.74 and 3367.82 cm^{-1} (amide stretching) [28]. The presence of characteristic peaks of GLB in all formulations proved the compatibility between drug and polymer.

(A)



(B)

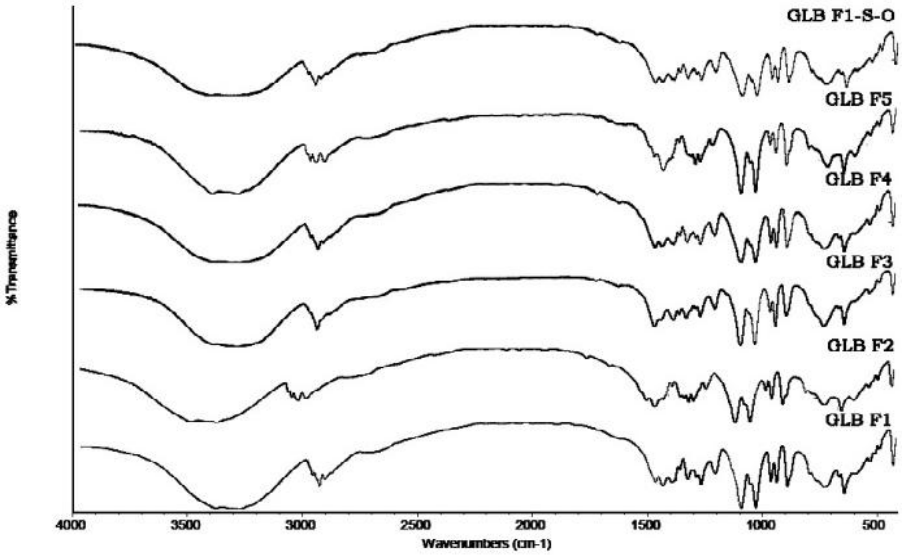


Fig.3. FTIR spectra of pure GLB, PEG 20000 and PM-1:1 (A), and precipitated GLB NCs (F1-F5), and optimized formulation (GLB- F1-S-O) after 3 months of storage (B).

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

3.5 Surface Characteristic Analysis

The SEM images of pure GLB (Fig.4A) showed numerous irregular shape particles with large size (>1.5 µm), whereas precipitated NCs showed uniform prismatic crystals in an agglomerated form with reduced size (Fig.4B). Fig.4C shows aggregated NCs after microscopical examination. Fig.4D shows complexed NCs of spherical shape and smaller size compared to precipitated NCs. A distinct difference in surface morphology was clearly observed between precipitated and complexed NCs. The appearance of a waxy lipid layer on to the surface of complexed NCs showed the presence of lecithin coating.

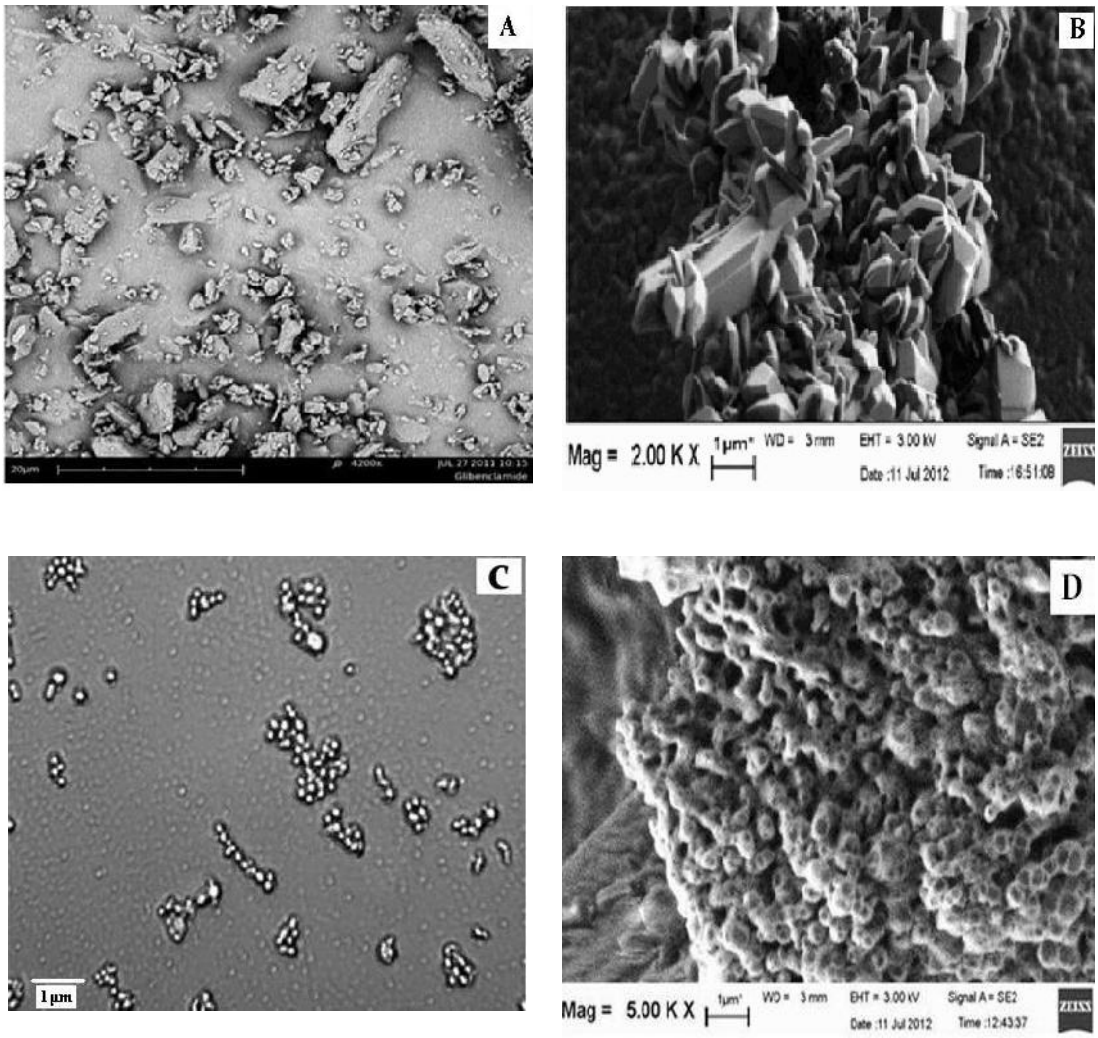


Fig.4. SEM images of pure GLB (A), precipitated F1 NCs (B), aggregated NCs before complexation and after microscopical examination (C) and complexed F1 NCs (D).

3.6 Stability Analysis

The stability data of optimized batch (F1) is given in Table 3. No significant change in particle size and PDI was observed during the storage period. The NCs were stable and less

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

aggregated, and this stability of NCs could be due to the repulsive force associated with the molecules which reduce the particle agglomeration. Stability analysis of optimized batch (F1) was also studied using FTIR. The spectrum was found to possess the characteristic peaks as of pure GLB at specific positions (GLB F1SO Fig.3B). These clearly prove that the chemical identity of GLB was preserved in the samples and the formulation was stable during the study period.

Table 3. Stability data (Particle size and PDI) of optimized complexed NCs Batch F1

Stability conditions	Parameters	Observation (months)			
		0	1	2	3
Room Temperature	Z.avg (d.nm)	155 ± 0.162	155 ± 0.105	156 ± 0.261	156 ± 0.31
	PDI	0.310 ± 0.067	0.332 ± 0.081	0.338 ± 0.013	0.326 ± 0.074
40°C (RH ± 75%)	Z.avg (d.nm)	155 ± 0.225	155 ± 0.058	157 ± 0.043	157 ± 0.084
	PDI	0.283 ± 0.017	0.358 ± 0.021	0.331 ± 0.019	0.375 ± 0.138

± indicates SD (n=3)

4. CONCLUSION

GLB NCs were formulated by precipitation technique and complexed using soybean lecithin. The solid state characterizations of NCs were performed using various techniques and the factors were optimized. Batch F1 was found to be the optimum batch among the samples in terms of smaller particle size and high stability. The particle size was found to decrease after complexation and the NCs were stable due to high zeta potential. The crystallinity of GLB in NCs was not altered on treatment with PEG 20000 and after complexation using lecithin. FTIR studies proved the absence of interaction between drug and excipients. Stability studies on optimized batch (F1) show the NCs were stable for 3 months with no change in particle size and PDI. To conclude, complexed GLBNCs offer enhanced surface properties and stability, and can be effectively used in development of various formulations.

ACKNOWLEDGEMENTS

The authors would like to thank Faculty of Pharmacy and Faculty of Applied Sciences, AIMST University, Malaysia and SAIF-STIC, Cochin, India for their laboratory and instrumentation analysis support.

COMPETING INTEREST

The authors have declared that no competing interest exists.

AUTHORS CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author B.SK formulated and characterized the nanocrystals *in vitro*. Authors R.S and K.VK conducted characterization

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

studies like PCS and DSC. Author T.AS and S.KJ analyzed the data and S.AD coordinated in all aspects of experiments in this study. All authors read and approved the final manuscript.

CONSENT AND ETHICAL APPROVAL

Not applicable

REFERENCES

1. Jens UA, Rainer HM. Nanocrystal technology drug delivery and clinical applications. Int J Nano Med. 2008;3(3):295-299.
2. Tugba G, Gürsoy RN, Öner L. Nanocrystal technology for oral delivery of poorly water soluble drugs. J Pharm Sci. 2009;34(1):55-65.
3. Mohanraj VJ, Chen Y. Nanoparticles - A Review, Trop J Pharm Res. 2006;5(1):561-573.
4. Banu SZ, Nakissa S. Regulatory perspective on the importance of ADME assessment of nanoscale material containing drugs. Adv Drug Deliv Rev. 2009; 61(6):422-427.
5. Huabing C, Chalermchai K, Xiangliang Y, Xueling C, Jinming G. Nanonization strategies for poorly water soluble drugs. Drug Discov Today. 2011;16(7-8):354-360.
6. Marcato PD, Duran N. New aspects of nanopharmaceutical delivery systems. J Nanosci Nanotech. 2008;8(5):2216-2229.
7. Rainer HM, Sven G, Cornelia MK. State of art nanocrystals - special features, production, nanotoxicity aspects and intracellular delivery. Eur J Pharm Biopharm. 2011; 78(1):1-9.
8. Faris NB, Müller RH. Nanocrystals for poorly soluble drugs for oral administration. Topics of PhD thesis and details on technologies, products, IP. New Drugs: 2002;2(2): 20-21.
9. Jaime S, Antoine G, Rainer HM, Jan PM. Nanocrystals: Comparison of the size reduction effectiveness of a novel combinative method with conventional top-down approaches. Eur J Pharm Biopharm. 2012; 81(1):82-90.
10. Sandrine D, Lucie S, Jean LC. Physico-chemical parameters that govern nanoparticles fate also dictate rules for their molecular evolution. Adv Drug Deliv Rev. 2012;64(2): 179-189.
11. Zeng N, Gao X, Hu Q, Song Q, Xia H, Liu Z, et al. Lipid - based liquid crystalline nanoparticles as oral drug delivery vehicles for poorly water - soluble drugs: cellular interaction and *in vivo* absorption. Int J Nanomed. 2012;7(1):3703-3718.
12. Müller RH, Jacobs C. Kayser O. Nanosuspensions as particulate drug formulations in therapy, rationale for development and what we can expect for the future. Adv Drug Deliv Rev. 2001;47(1):3-19.
13. Libo W, Jian Z, Wiwik W. Physical stability of nanoparticles. Adv Drug Deliv Rev. 2011; 63(6):456-469.
14. Lei G, Dianrui Z, Minghui C. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J Nano Res. 2008;10(5): 845-862.
15. Lei Y, Caixia L, Yua L, Jian FC, Haikui Z. Stabilized amorphous glibenclamide nanoparticles by high gravity technique. Mat Chem Phys. 2011;13(1):361-366.
16. Coppack SW, Lant AF, McIntosh CS. Pharmacokinetic and pharmacodynamic studies of glibenclamide in non-insulin dependent diabetes mellitus. Br J Clin Pharmacol. 1990;29 (6):673- 684.
17. Fuminori I, Hiroyuki F, Hiroyuki H, Hiroyoshi K, Kiyoshi K, Kimiko M. Effect of polyethylene glycol on preparation of rifampicin-loaded PLGA microspheres with membrane emulsification technique. Colloids Surf B: Biointerfaces. 2008; 66(1):65-70.

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.

18. Dora CP, Singh SK, Kumar S, Datusalia AK, Deepa A. Development and characterization of nanoparticles of glibenclamide by solvent displacement method. Acta Pharma Drug Res. 2010;67(1):283-290.
19. Elbary A, Salem HF, Maher ME. In vitro and in vivo evaluation of glibenclamide using surface solid dispersion (SSD) approach. Br J Pharmacol Toxicol. 2011; 2 (1): 51-62.
20. Cornelia MK, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur J Pharm Biopharm. 2006;62(1):3-16.
21. Shasha R, Yunmei S. Frank P, Allan ME. Particle size reduction to the nanometer range a promising approach to improve buccal absorption of poorly water soluble drugs. Int J Nano Med. 2011;6(1):1245-1251.
22. Sonke R, Albrecht S. Thomas R, Claudia SL. Thermal degradation of amorphous glibenclamide. Eur J Pharm Biopharm. 2012;83(1):76-86.
23. Corrosion Testing Laboratories. Corrosion Failure Analysis and Material Secetion Specialists. Accessed 2013; April 10, 2013. Available from http://www.corrosionlab.com/facilities/ftir-interpretation.htm
24. Heike B, Tobias U. Characterization of lipid nanoparticles by differential scanning calorimetry, X-ray and neutron scattering. Adv Drug Deliv Rev. 2007;59(6):379- 402.
25. Jane W, Sabine G, Heather O, Lakshmy N, Thomas G, Philip WG, et al. Physicochemical stability of phospholipid-dispersed suspensions of crystalline itraconazole. Eur J Pharm Biopharm. 2008;69(3):1104-1113.
26. Lai F, Sinico C, Ennas G, Marogiu F, Marogiu G, Fadda AM, et al. Dioclofenac nanosuspensions: influence of preparation procedure and crystal form on drug dissolution behaviour. Int J Pharm. 2009;373(1-2):124-132.
27. Barrett ER. Nanosuspensions in drug delivery. Nat Rev. 2004;3(9):785-796.
28. Bhaskar C, Shyam S, Anant P. Preparation and evaluation of glibenclamide polyglycolized glyceride solid dispersions with silicon dioxide by spray drying technique. Eur J Pharm Sci. 2005;26(2):219-230.

ABBREVIATIONS

GLB - Glibenclamide, PEG - Polyethylene glycol, NC - Nanocrystal, LEC - Lecithin, PDI - polydispersity index

* Tel.: +60164563044; fax: + 606044298009.
E-mail address: bsajeev2001@yahoo.com.