Research Paper

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

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 crystallanity, compatibility and surface morphology were analyzed using differential scanning calorimetry, powder x-ray spectroscopy, infrared spectroscopy and 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 also assessed for 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 spherical shape particles resembling micelles 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 for drug delivery due to its high stability and lower particle size.

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

List of Abbreviations

NCs: Nanocrystals

NPs: Nanoparticles

GLB: Glibenclamide

PEG: Polyethylene glycol

LEC: Lecithin

PDI: Polydispersity index

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 a number of nanoformulations are available and clinically approved in the past decade, its limitations like comprehensive structure-function relationships between particle structure and its pharmacological properties inhibit their wide spread adoption [3]. The size, shape, composition, surface properties of nanocarriers, effects on pharmacokinetics and pharmacodynamics profiles need to be clearly elucidated. These characterizations are emerging as new focus for assessing the safety and efficiency of various nanoformulations [4].

The drug delivery efficiency of a nanoformulation depends on variety of factors like the type of formulation, particle size, surface properties and stability of particles in medium. Ideally a successful nanoformulation should have high drug loading capacity, considerable drug release, and polymer degradation [5].

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 increase 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 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 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).

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 Glibenclamide

NCs (GLB) by various techniques. NCs were developed by precipitation process using PEG 20000 and stabilized (complexed) by means of soybean lecithin. The effect of PEG 20000 and lecithin on particle size reduction and change in crystallanity 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. MATERIALS 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 was dissolved in a solvent mixture of acetone and methanol (2:1). PEG 20000 was added to the drug solution and stirred at a temperature not exceeding 60°C. The drug-polymer solution was injected slowly 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 of dispersion was adjusted to 100 ml using double distilled water. The solution was then gently heated with magnetic stirring for 30 min to remove the organic solvent. Later, the contents were centrifuged (5000 rpm) for 20 min to separate the NCs. The clear supernatant liquid was discarded, and the thick viscous dispersion was collected and further redispersed in 15 ml of

distilled water and recentrifuged (20000 rpm) for 10 min to remove the impurities and the residual surfactants. The NCs were recovered using a vacuum filter (0.2 μm) and dried in a hot air oven at 35°C for 20 min. The procedure was repeated to prepare different batches [17].

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 for 10 min. Soybean lecithin (2% w/v) previously solubilized in chloroform was gradually added to the dispersion and stirred continuously using a magnetic stirrer at 250 rpm for 30 min at a temperature above its melting point so as to obtain a homogenous dispersion. The dispersion was transferred to a shaking incubator at 120 rpm for 1 h at 15°C. Later, 5% w/v mannitol was added to the dispersion and shaken for 10 min prior to lyophilization [18].

2.2.3 Freeze Drying

The milky homogenous dispersion 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}m bar pressure) for 10 h. The lyophilized products were stored in borosilicate glass vials and placed in a dessicator until further use.

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 optimum volume was considered by positioning the cell to a marker line, drawn on to the instrument panel. The samples were measured for the mean particle size and PDI at a fixed angle of 90° and at a temperature of 25°C after 5 runs. A refractive index of 1.616 and 1.300 were used for the drug and solvent respectively [19].

2.4.2 Zeta potential measurement (ZP)

The zeta potential of precipitated and complexed NCs were 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 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

154 samples were placed in a glass sample holder and Cu ka radiation was generated at 30 mA and
155 40 Kv. The samples were scanned from 3° to 70° 2θ with a step size of 0.02° [20].
156
157 *2.4.4 Differential Scanning Calorimetry (DSC)*
158 DSC analysis of pure GLB, polymer, physical mixtures (PM-1:1) and NCs before and
159 after complexation were analyzed in a DSC calorimeter (TA Instruments, Q200, USA), equipped
160 with a liquid nitrogen cooling system. About 5 mg of samples were loaded to aluminum pan,
161 crimped, sealed and further examined at a scanning rate of 10°C / min from 15 to 200°C under
162 nitrogen atmosphere (flow rate 100 ml/min) in room temperature. High purity indium was used
163 to calibrate the heat flow and heat capacity of the instruments [21].
164
165 *2.4.5 FTIR Analysis*
166 Spectra of pure GLB, PEG 20000, physical mixtures (PM-1:1) and NCs were recorded in
167 FT-IR spectrophotometer (Thermo Nicolet, Avatar 370, USA). The samples were compressed
168 into a pellet using KBr and scanned for 4 seconds at a resolution of 4 cm^{-1} from 4000 to 400 cm^{-1}
169 [22].
170
171 *2.4.6 Scanning Electron Microscopy (SEM)*
172 Morphological evaluation of NCs was performed using a scanning electron microscope
173 (LEO 1530, Gemini, Germany). The samples were mounted to steel stubs (Jeol - 10 mm Dia x 5
174 mm) using a double sided adhesive tape and sputtered with a thin layer of Au at 20 mA, under
175 $1x10^{-1}$ bar vacuum for 10 min using a sputter coater (EM S550X - Electron microscopy sciences)
176 and was operated at an acceleration voltage of 3 kV [23].

2.5 Stability Studies

The optimized formulation (Batch F1) was placed in a clean airtight glass vials and stored at room temperature and 37°C, RH = 75% over a period of 3 months. During the storage period, the samples were evaluated for average particle size [24].

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 - 7002 nm. The particle size was found to increase with an increase in polymer content in precipitated NCs. The complexed NCs possessed an average particle size between 155 nm and 842 nm and were found to decrease in all samples (F1 to F5).

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. 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 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 by lecithin. The stability of NCs is related to the charge imparted by lecithin that makes the drug particle get disassociated within the system. The presence of high negative charge may be correlated to the

existence of number of carboxyl groups on the polymeric chain extremities and formation of a barrier between the particle surface and surrounding medium [25, 26].

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	0.417	-38.1 ± 0.2	-	-	-
F1	1:1	236	0.369	-35.1 ± 0.4	155	0.310	-51.7 ± 0.1
F2	1:2	5745	0.610	-29.0 ± 0.8	710	0.309	-45.8 ± 1.3
F3	1:4	7002	0.417	-34.3 ± 0.3	842	0.397	-48.4 ± 0.7
F4	1:8	5885	0.520	-32.3 ± 0.4	227	0.431	-58.3 ± 0.5
F5	2:1	3574	0.957	-20.2 ± 1.1	787	0.878	-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 all NCs exhibited a similar characteristic diffraction pattern as that of pure GLB specifically at 11.66°, 20.82° and 30.08° 2θ positions revealing the absence of interaction between drug and polymer. Moreover, the intensity of the peaks was also slightly reduced in precipitated and complexed NCs. It was

also noticed the base of the peak was broadened, sharpness was found to decrease and peak area lowered with increase in polymer content in samples. The relative intensity values (d-value) decreased initially and became constant indicating that the crystallanity was 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.

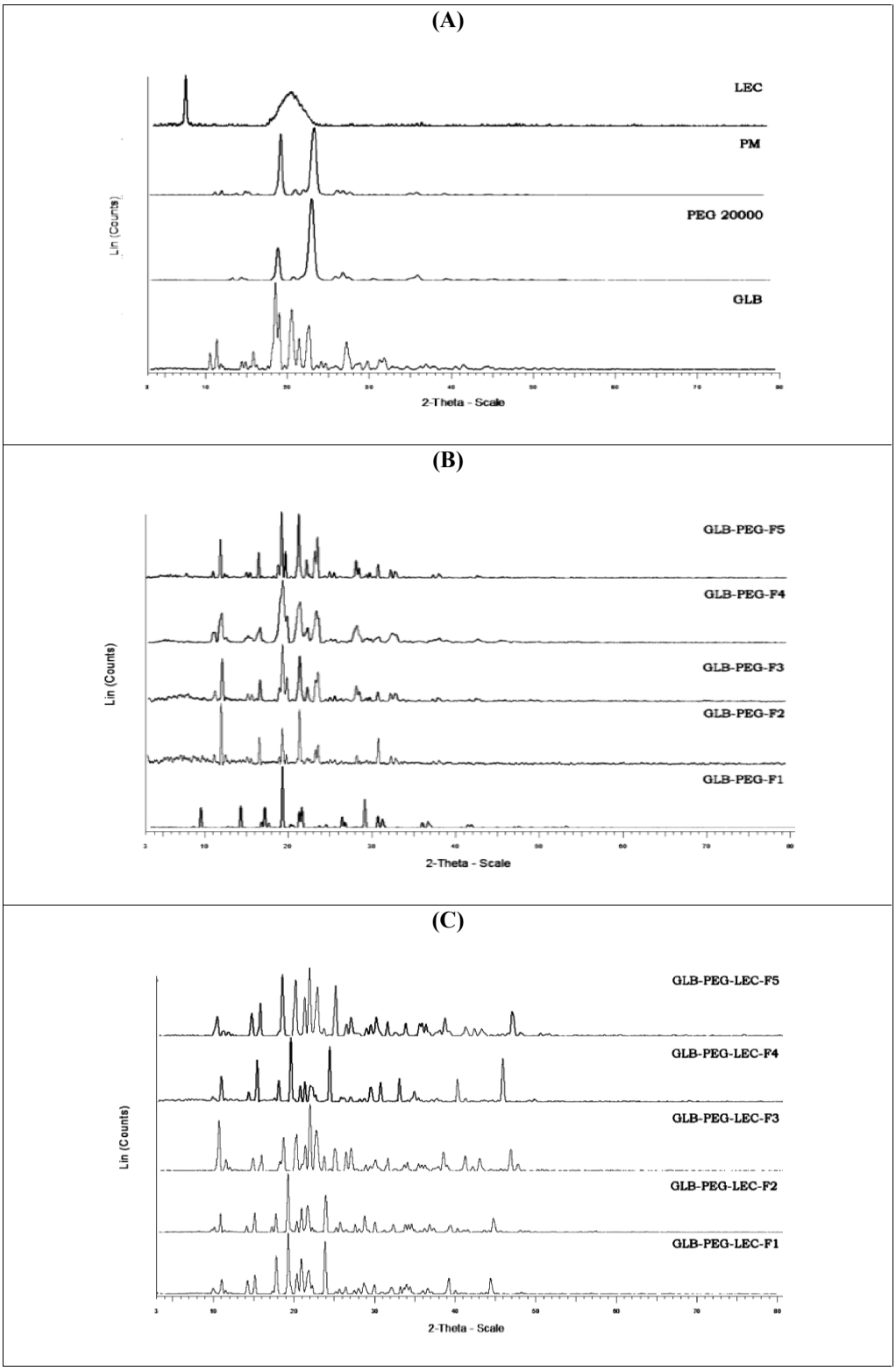


Fig.1. X-Ray Diffraction spectra of pure GLB, PEG 20000, PM (1:1) and Lecithin (A), precipitated GLB NCs (F1-F5) (B), and GLB NCs (F1-F5) after complexation (C) at 2-Theta-scale.

237 **Table 2. XRPD peak parameters of GLB and formulations**

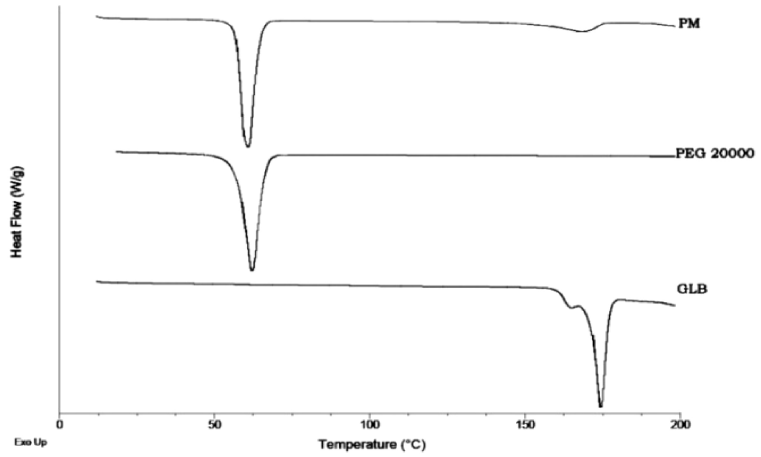
Batch	Precipitated NCs			Complexed NCs		
	2 θ position	Peak intensity (d)	FWHM (deg)	2 θ position	Peak intensity (d)	FWHM (deg)
Pure	11.66	7.58	-	-	-	-
GLB	20.82	4.26	0.46	-	-	-
	30.08	2.96	-	-	-	-
	11.80	7.49	0.19	10.71	8.24	0.22
F1	21.06	4.21	0.19	20.65	4.29	0.30
	30.37	2.94	0.20	30.62	2.91	-
	11.75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

238 FWHM-Full Width Half Maximum

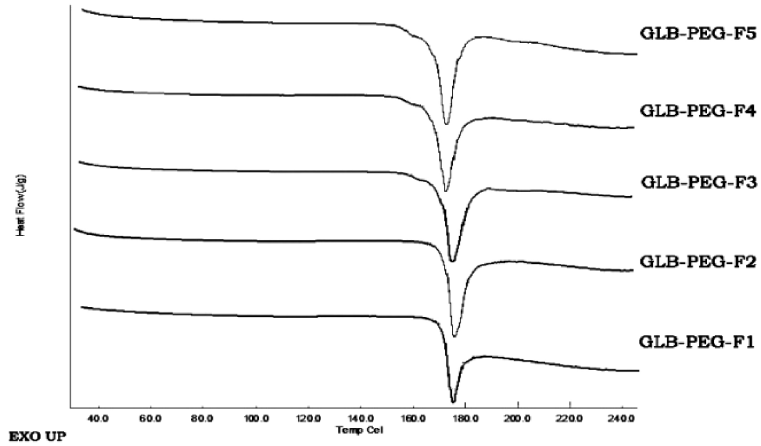
239 *3.3 Differential Scanning Calorimetry*

240 DSC thermograms of GLB, PEG 20000, physical mixture (PM-1:1), precipitated and
241 complexed NCs are compared in Fig. 2A, 2B and 2C respectively. A sharp endothermic peak at
242 173.36°C (ΔH = 98.34 J/g) in pure GLB thermogram indicated its high crystallinity and a broad
243 endothermic peak at 65.24°C in thermogram of PEG 20000 revealed its amorphous nature. Two
244 endothermic peaks (65.68°C and 164.61°C) observed in the thermogram of physical mixture
245 (1:1) proves the absence of interaction between drug and polymer. The glass transition
246 temperature (Tg) of endothermic peaks of precipitated NCs were found to be similar to pure
247 GLB indicating that there was no change in crystalline structure. The glass transition temperature
248 of complexed NCs showed a narrow change in peak (Fig.2C), indicating crystallanity was
249 maintained but with reduced size.

(A)



(B)



(C)

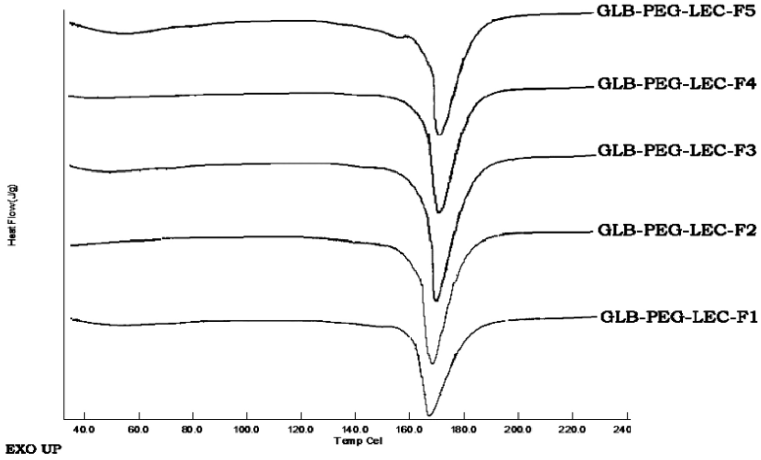


Fig.2. DSC thermograms of Pure GLB, PEG 20000 and PM-1:1 (A), precipitated GLB NCs (F1-F5) (B), and GLB NCs (F1-F5) after complexation (C).

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) [27]. The presence of characteristic peaks of GLB in all formulations proved the compatibility between drug and polymer.

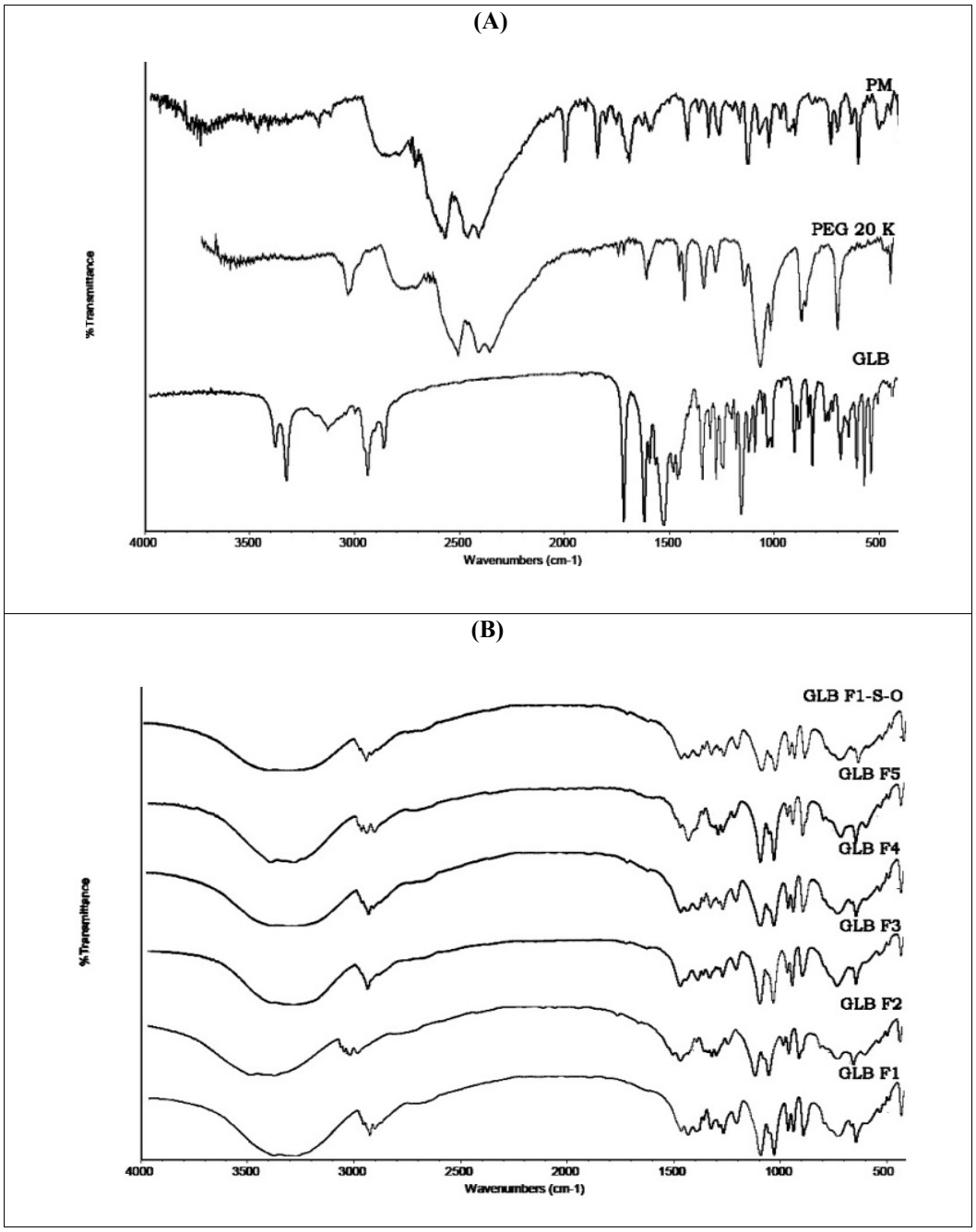


Fig.3. FTIR spectra of pure GLB, PEG 20000 and PM-1:1 (A), and precipitated GLB NCs (F1-F5) and optimized formulation (GLB- F1-S-O) after 3 months of storage (B).

282 *3.5 Surface Characteristic Analysis*

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

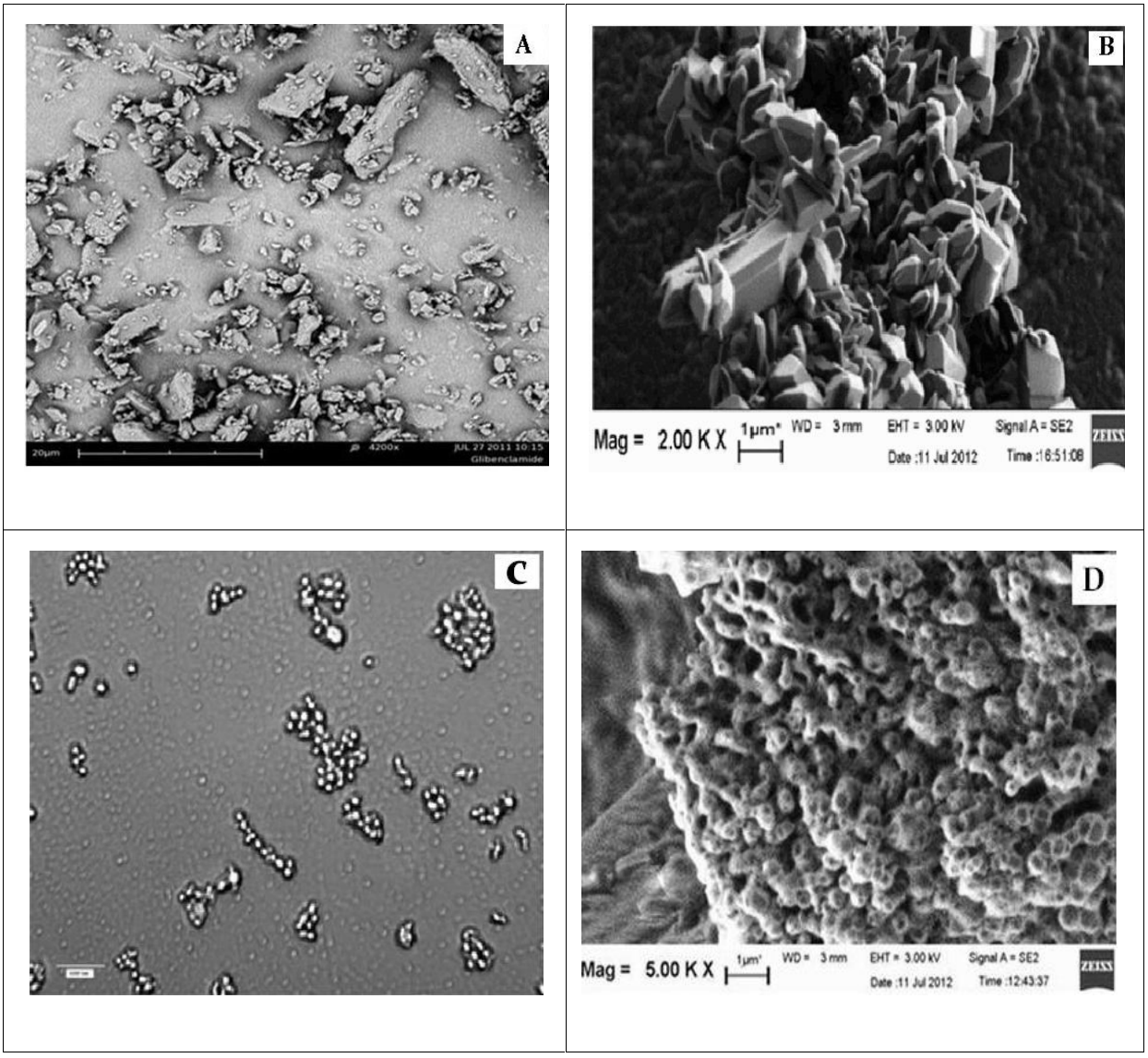


Fig.4. SEM images of pure GLB (A), precipitated F1 NCs (B), aggregated NCs before complexation and after microscopical examination (C) and complexed F1 NCs (D).

3.6 Stability Analysis

The stability data of optimized batch (F1) is given in Table 3. No significant change in particle size was observed during the storage period. The NCs were stable and less 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 batch F1 was also studied using FTIR and the spectra was found to possess the characteristic peaks as of pure GLB at specific positions (GLB F1 SO, 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 analysis) of optimized batch (F1)

Stability conditions	Observation (months)			
	0	**1**	**2**	**3**
Room Temperature	155	155	156	156
40°C (RH = 75%)	155	155	157	157

Values represent Z.avg (d.nm)

4. CONCLUSION

GLB NCs were formulated by precipitation technique and complexed using soybean lecithin. The solid state characterizations of NCs were performed 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 was stable due to high zeta potential. The crystallanity of GLB in NCs was not altered on treatment with PEG 20000 and after complexation. FTIR studies proved the absence of interaction between drug and excipients. Stability studies show the NCs were stable for 3 months with no change in particle size. These complexed NCs offer enhanced surface properties and could solve the stability issues both *in vitro* and *in vivo.* They can be utilized as promising carriers for drug delivery due to its high stability and lower particle size, and can also be effectively used in development of various formulations.

CONSENT and ETHICAL APPROVAL

Not applicable

COMPETING INTEREST

The authors have declared that no competing interest exists.

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