WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 12, 1341-1357. Research Article ISSN 2277– 7105

PREPARATION AND CHARACTERIZATION OF SURFACTANT FREE GLIBENCLAMIDE NANOPARTICLES

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Article Received on
29 June 2022,

Revised on 19 July 2022,
Accepted on 09 August 2022

DOI: 10. 20959/wjpr202212-25264

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ABSTRACT

The aim of the present study involved is the Preparation and characterization of surfactant- Free Glibenclamide Nanoparticles. Wherein the polymer used for the Preparation of Glibenclamide Nanoparticles were Eudragit RLPO and PEG with Various molecular weight. Glibenclamide nanoparticles were obtained by nanoprecipitation and evaluated in terms of drug content, encapsulation efficiency, apparent saturation solubility, drug release profile, solid state and storage stability. The particle size, size distribution and zeta potential of the nanoparticles was investigated by using the stirring speed. Results Revealed that by increasing the molecular weight of PEG decreases the drug encapsulation and dissolution rate of the drug increased. Stability studies confirmed that glibenclamide Nanoparticles were stable even after 150 days. The prepared surfactant- free glibenclamide nanoparticles represent a promising alternative to enhance the treatment of non-insulin dependent diabetes.

KEYWORDS: Glibenclamide, Nanoprecepitation, Bottom up Approach, Surfactant Free, Eudragit.

INTRODUCTION

Nanotechnology has raised giant attention over time. Nanoparticle is the fundamental

component of nanotechnology. Particles between 1 and 100nanometres are called Nanoparticles.[1] A nanoparticle shows a unique chemical, physical & biological property at Nano scale[2] The nanoparticles are of different size, shape & structure like cylindrical, tubular, spherical, hollow core, conical, flat & spiral etc.[3] The nanoparticle is the combined name for Nano capsules and Nano spheres.[4] The same chemical can generate a wide variety of nanoparticles.

Diabetes is one of the most prevailing and advancing diseases in the world having affected 6.6% of the world population. Glibenclamide is a second generation sulphonylureas oral hypoglycaemic agent used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). The duration of action of up to 24 hours. The usual initial dose of conventional formulations in type 2 diabetes mellitus is 2.5 to 20 mg taken orally. In the case of micronized preparations of Glibenclamide, the drug is formulated with a smaller particle size, and display enhanced bioavailability. The usual initial dose of such preparation (GlynaseTM) is 1.5 to 3 mg daily.[5-10]

It has a history of low bioavailability, which is attributed to poor dissolution Several attempts for increasing dissolution and bioavailability of GB have been made, such as micronization, molecular dispersion incorporation of surfactants, inclusion complexation with Cyclodextrin, crystal modification, glass formation and coprecipitation Eudragit L100 polymer is commonly used for enteric coating and also for preparation of controlledrelease dosage forms Eudragit L100 is also used in preparation of nanosuspensions for controlled delivery of ketoprofen and also in microparticles as a carrier for enhancing oral bioavailability of carbamazepine.[11-15]

The aim of this study was to formulate and characterize Surfactant free Glibenclamide Nanoparticles prepared by Eudragit RLPO using solvent displacement to achieve a better release profile suitable for *per oral* administration with enhanced efficacy than previous GB delivery.

MATERIALS AND METHODOLOGY

1.1 Development of Calibration Curve

Preparation of Buffer Solution (pH-1.2)[16]

250ml of 0.2Ml Potassium chloride solution (14.911 gm of KCL in 1000ml) and 425ml of0.2N Hydrochloric acid (7.292 gm in 1000ml) were mixed properly and the volume was made up to 1000ml with distilled water.

Preparation of Standard Solution of Glibenclamide[17]

A Solution of 5mg Glibenclamide was prepared by dissolving in 100ml methanol, from which 1ml was withdrawn in separate volumetric flask and diluted to 10ml with Hcl buffer to produce 5µg/ml concentration and absorbance at 302 nm.

Preparation of Working Solution

From standard solution, 0.5, 1, 1.5, 2, 2.5, and 3ml was withdrawn in six 10ml volumetric flasks and diluted to 10ml with HCL buffer pH 1.2 to produce concentration 2.5, 5, 7.5, 10, 12.5 and 15 respectively. The solutions were analyzed by U.V. spectrophotometer at 302nm and results were recorded. The calibration graph was plotted as concentration on X-axis Vs absorbance onY-axis.

1.2 Preparation of Glibenclamide Nanoparticles[18]

An accurate amount of GB (200 mg) and EuRLPO (200 mg) were dissolved in 18 mL of acetone: ethanol (2:1 *v*/*v*). The resulting solution was then injected (1 mL/min) into water (80 mL) containing 200 mg of PEG 400, 1500, 3000 or 6000. The obtained suspension was stirred at 500 rpm or 7000 rpm using magnetic stirrer, for 30 min. The final mixture was stirred (250 rpm) overnight at 20°C to allow organic solvent evaporation. GB NP were collected by ultracentrifugation (20 min at 15000 rpm) using centrifuge. The resulting particles were washed twice with distilled water centrifuged again (after each washing) and the supernatants were collected for further analysis. The solids were freeze-dried for 48 h in refrigerator. Samples were prepared and characterized in triplicate.

1.3 Encapsulation Efficiency & Drug Loading Capacity

For the determination of encapsulation efficiency accurately weighed NPs (10 mg) were added to 10 mL of distilled water and after the equilibrium solubility was attained, clear supernatant after centrifugation was filtered and 1 mL of the filtrate was mixed with 4 mL of methanolic HCl. Resulting sample was analyzed on UV visible spectrophotometer at 300 nm. The encapsulation efficiency was determined using the equation Nanosuspensions were prepared in triplicate, and data are presented as mean ± standard deviation (SD).[19]

The encapsulation efficiency was determined by using the following formula[23]

Encapsulation efficiency (%) = [1-(Drug in supernatant liquid / Total drug added)] * 100

For the determination of drug loading capacity, NPs (5 mg) were dissolved in 5 mL of methanolic HCl and the solution was filtered through 0.2 m filter (Axiva syringe filter). GB concentration in the sample was determined using UV visible spectrophotometer at 300 nm.

The percentage drug loading capacity was determined using the following formula[24]
% Drug loading = (Mass of GB in NP / Mass of NP recovered) *100

1.4 Particle Size, Polydispersity Index, and Zeta Potential

The particle size (Z-average), size distribution (polydispersity index, PDI), and zeta potential (ZP) of the nanosuspension were determined by Zetasizer by Dynamic light scattering (DLS). Lyophilized NPs were first resuspended into a solution of distilled water and Tween® 80 (0.1% *w*/*v*) and measured. Zeta potential, an indicator of surface charge, which determines particle stability in dispersion, was also measured using the principle of electrophoretic mobility in an electric field. The PI which is a dimensionless number indicating the width of the size distribution, was also measured.

1.5 Saturation solubility test

Solubility of GB and freeze dried NPs of GB were obtained by adding an excess (2 g) of the sample to 10 mL of water in a glass container at room temperature. The samples were kept on water bath shaker for 24 h to ensure saturation. After the equilibrium solubility was attained, clear supernatant was filtered and 1 mL of the filtrate was mixed with 4 mL of methanol HCl. Resulting sample was analyzed on UV-vis spectrophotometer at 300 nm.

1.6 DSC characterization[20]

Phase transition behavior of GB loaded freeze dried NPs were analyzed by the differential scanning calorimeter. As a control, the pure GB, Eu RLPO, PEG 400 to 6000 NPs of GB were analyzed by DSC Approximately 5 mg of samples were loaded to aluminium pan, crimped, sealed and analysed at a scanning rate of 10°C/min from 25°C to 200°C under nitrogen atmosphere (flow rate was100 mL/min).

1.7 X-Ray Studies[21]

The XRD studies for analyzing structural nature of Glibenclamide, and nanoparticle formulation of Glibenclamide. The samples were placed in sample cell and spread evenly. The sample cell was placed in X-ray Diffractometer (BRUKER ECO D8). The samples were scanned over the frequency range of 10-80.

1.8 FTIR Studies[22]

To know about the interaction between the drug and carriers used in the formulation, the IR analysis was carried out. The IR spectra of pure Glibenclamide, Solvent displacement ratio of 1:1 and nanoparticles was studied by FTIR It is scanned over the Frequency range of 4000-500 cm-1.

1.9 Morphology

Morphological analysis of NPs in suspension was performed using transmission electron microscopy (Hitachi, H-7500, Japan). Samples of the nanosuspension (5 -10 □L) were dropped onto Formvar-coated copper grids and dried. Digital Micrograph and Soft Imaging Viewer software were used to perform the image capture and analysis, including particle sizing.

1.10 In Vitro Dissolution[23]

In vitro dissolution studies were carried out in 500 mL phosphate buffer (pH 7.5) at 37 ± 0.5°C, using a USP-II dissolution apparatus at 50 rpm. Samples containing 30 mg of GB were added to the dissolution medium and 5 ml of each one was withdrawn in triplicate at 0, 5, 10, 20, 30, 45, 60, 90, 120 and180 min and replaced with fresh media. The samples were passed through a syringe filter (0.2 mm), centrifuged for 30 min at 15000 rpm to separate dissolved GB from NPs, and analysed spectrophotometrically for GB quantification at 300 nm against a suitable blank. Blanks were prepared using the same components and proportions as the NPs, but without the drug and diluted under the same conditions than the NPs samples. All measurements were run in triplicate; data are presented as mean ± SD.

1.11 Stability Studies

To evaluate the stability of drug, Glibenclamide, and the effect of polymer after storing at different Temperature and Relative Humidity for 30 days stability studies were carried out .About 100mg of equivalent of Glibenclamide formulations were taken in well closed containers from ideal batches and stored separately at 400C+ 20C/75% RH + 6% (Accelerated testing) and 300C+ 20C/60% RH + 5% (Alternate testing). From these, sample equivalent to 20 mg of Glibenclamide was removed at the interval if 10, 20, 30 days and analyzed the drug content by spectrophotometrically at 302 nm.[24-26]

RESULTS

1.1 Development of Calibration Curve.

Table 1: Standard Calibration Curve of Glibenclamide.

S.No	Concentrations (µg/ml)	Absorbance at 330nm
1	0	0
2	2	0.197
3	4	0.356
4	6	0.484
5	8	0.622
6	10	0.790

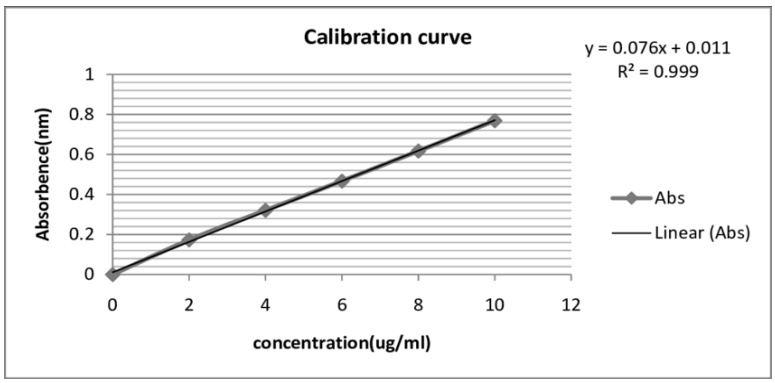

Figure 1: Calibration curve of glibenclamide.

Table 2: Formulation Table of Glibenclamide Nanoparticles.

Samples	Stirring speed	GB(mg)	Eu RLPO (mg)	PEG 400 (mg)	PEG 1500 (mg)	PEG 3000 (mg)	PEG 6000 (mg)
GB	–	200	200	0	0	0	0
1 A	500 rpm	200	200	0	0	0	0
1 B	7000 rpm	200	200	0	0	0	0
2 A	500 rpm	200	200	200	0	0	0
2B	7000 rpm	200	200	200	0	0	0
3 A	500 rpm	200	200	0	200	0	0
3B	7000 rpm	200	200	0	200	0	0
4 A	500 rpm	200	200	0	0	200	0
4B	7000 rpm	200	200	0	0	200	0
5 A	500 rpm	200	200	0	0	0	200
5B	7000 rpm	200	200	0	0	0	200

1.3 Encapsulation Efficiency

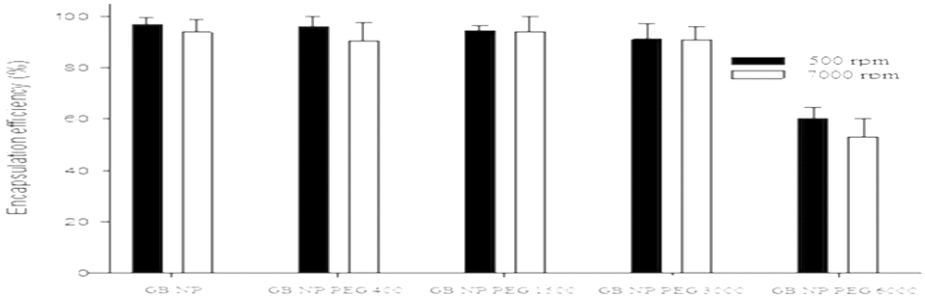

Fig 2: Encapsulation efficiency off Glibenclamide Nanoparticles.

1.4 Particle Size, Polydispersity Index and Zeta Potential.

Table 3: Results of Particle Size, Polydispersity Index and Zeta Potential.

Samples	PS (nm)	PI (nm)	ZP (mV)
1 A	358.6±0.3	0.37±0.06	-11.1
1B	459.7±0.2	0.28±0.08	-21.9
2 A	298.4±0.4	0.15±0.04	-5.74
2B	317.5±0.6	0.14±0.08	-5.79
3 A	225.2±0.7	0.50±0.07	-.14.12
3B	264.1±0.5	0.12±0.04	-12.75
4 A	197.3±0.2	0.22±0.09	-19.45
4B	201.9±0.4	0.13±0.07	-18.54
5 A	140.6±0.1	0.61±0.05	-24.75
5B	178.1±0.4	0.24±0.04	-24.125

1.5 Saturation Solubility

Table 4: Solubility of Nanoparticles.

FORMULATION CODE	Solubility (ug/ml)
Free GB (Water)	20 µg/mL
GB Nanoparticles without PEG	35 µg/mL
PEG 400	38 µg/mL
PEG 1500	58 µg/mL
PEG 3000	93 µg/mL
PEG 6000	125 µg/mL

7.6 DSC characterization

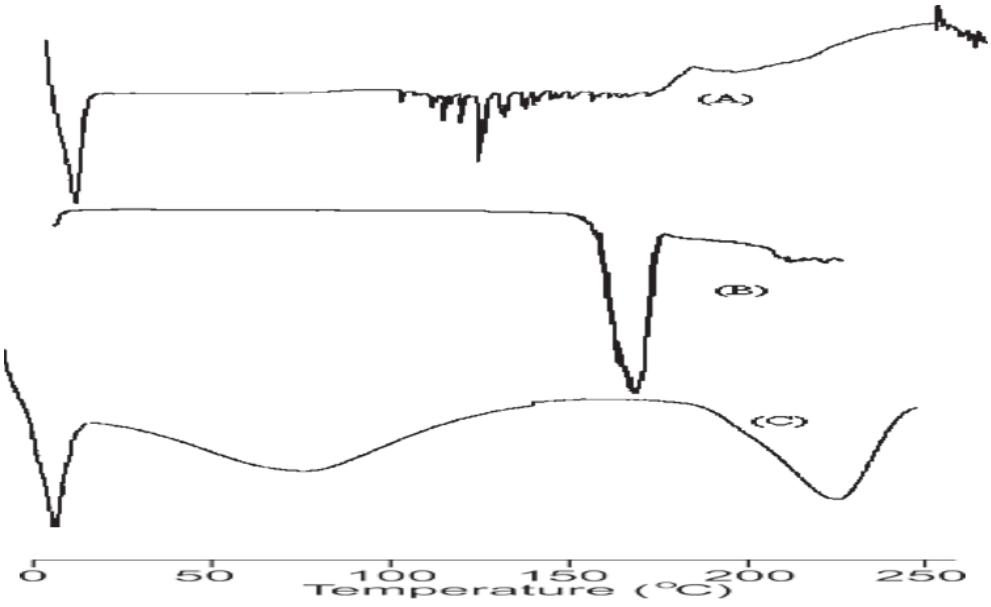

Fig 3: DSC Characterization of (a) pure drug, (b) ER LP0, and (c)Polymer PEG.

1.7 XRD ANALYSIS

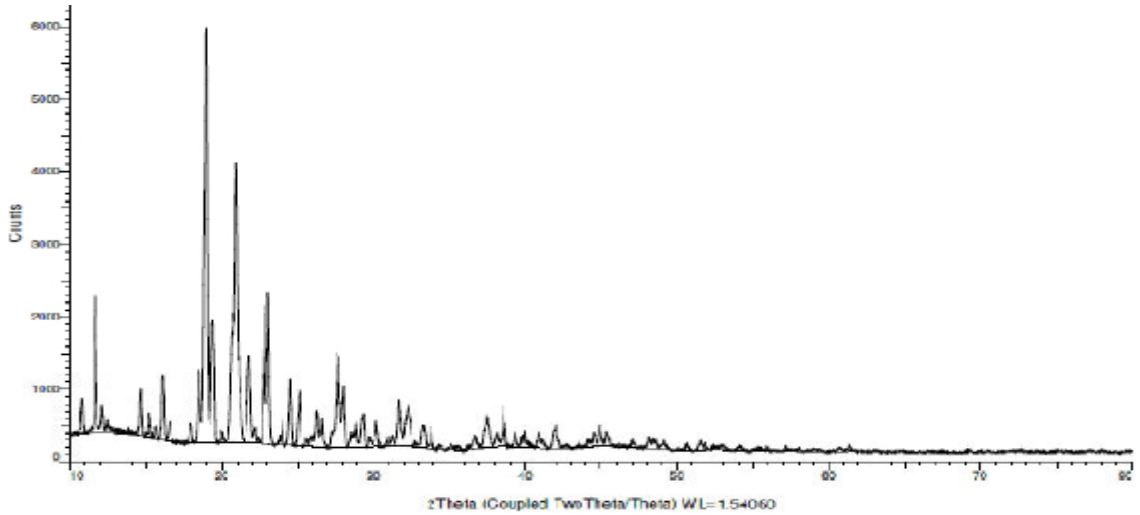

Fig 4: XRD Spectrum of Pure Gliclazide.

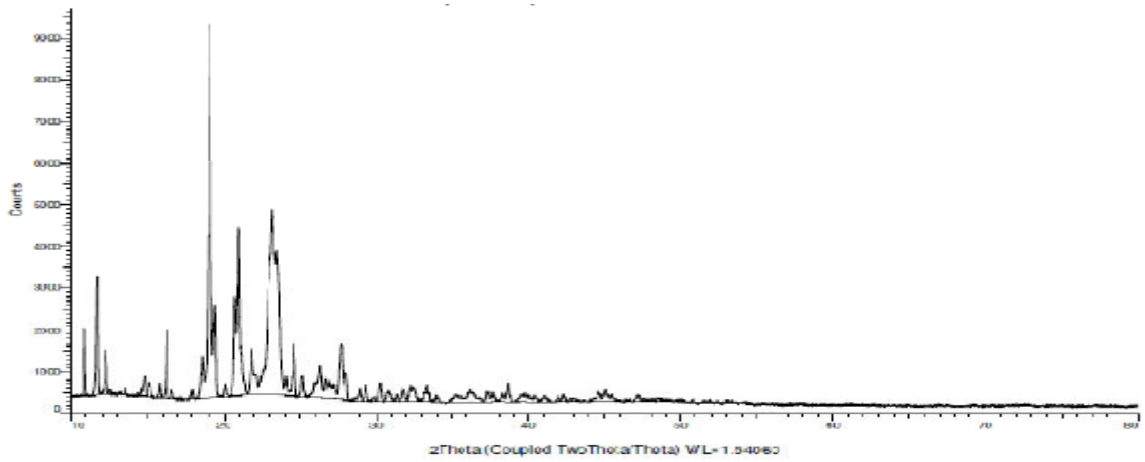

Fig 5: XRD Spectrum of Gliclazide along with the Polymer PEG 6000.

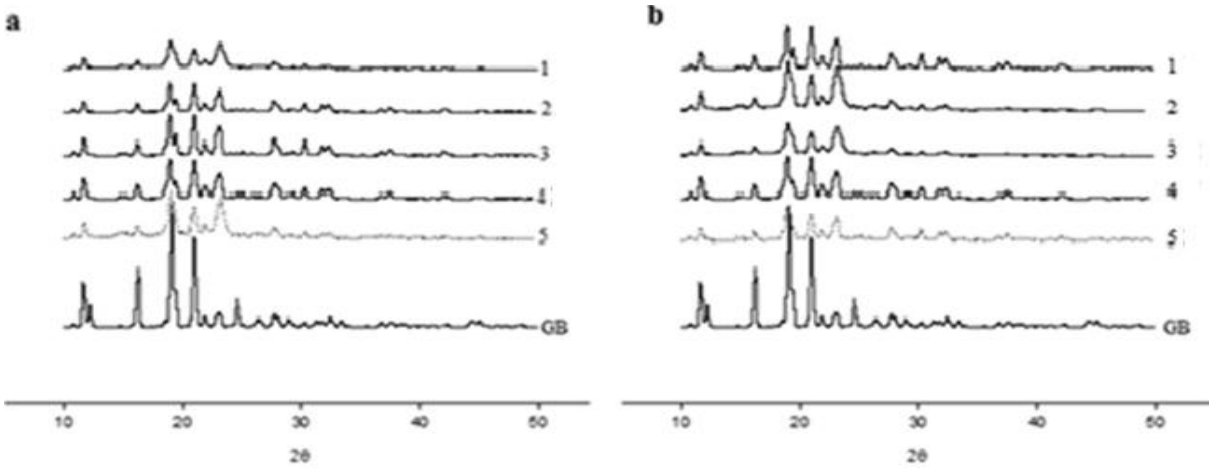

Fig 6: XRD Pattern of Raw Glibenclaimde along with the nanoparticles prepare with various stirring speeds(a) 500 (b) 7000 rpm .

8 FTIR SPECTRA

The Infrared spectral analysis was carried out to the Study the Interaction between the drug and polymer. The IR spectrums as shown in Figures:7 and 8.

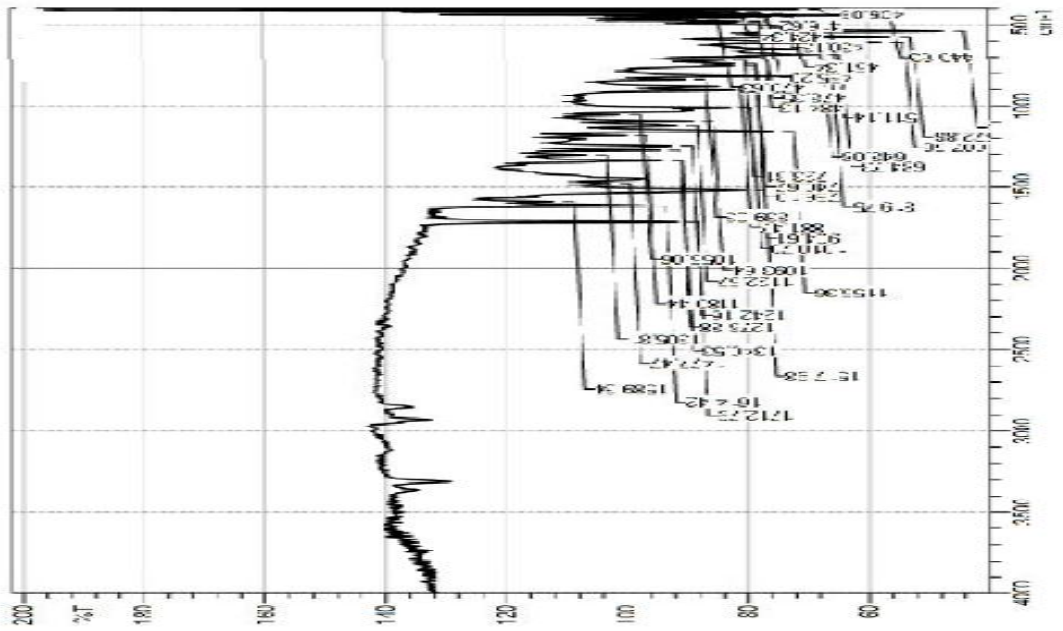

Fig 7: FTIR spectrum of pure drug Glibenclaimde.

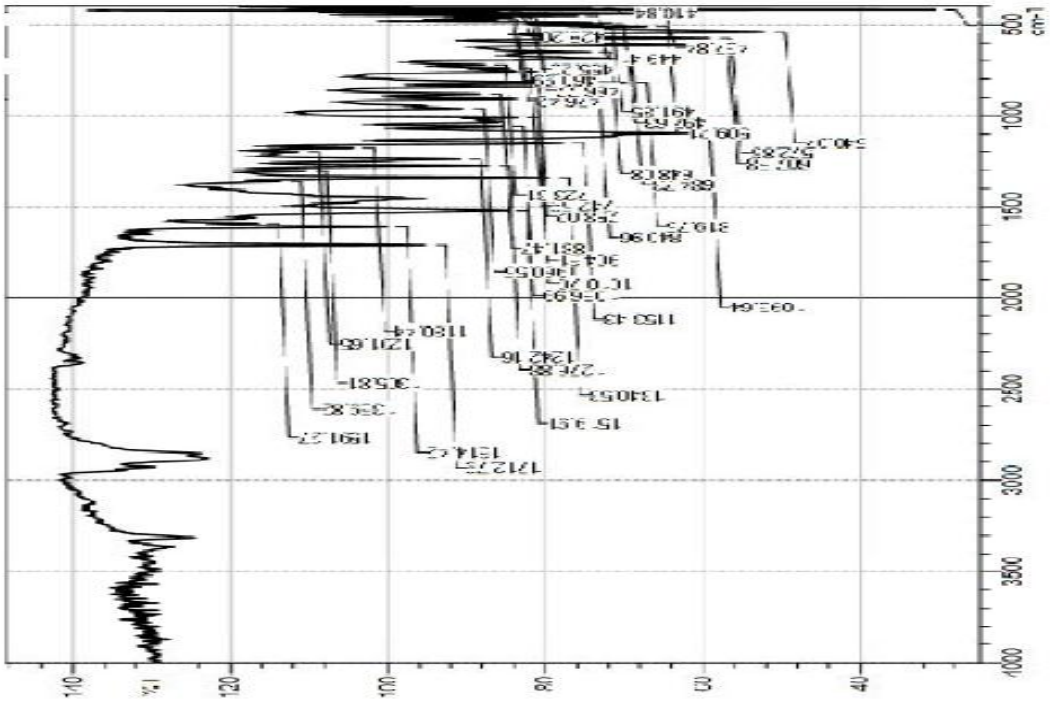

Fig 8: FTIR Spectrum of Glibenclamide along with the Polymer PEG 6000.

Table 5: Interpretation of FTIR peaks present in Glibenclamide.

S.No.	Wave number in formulation (cm^{-1})	Characteristic Wave number range cm^{-1}	Bond nature and bond attributed
1	3447.78	3000-3700	N-H Stretching
2	2956.4	3000-2850cm	S-O Bending
3	1639.47	1600-1700	NH2 deformations
5	2870.17	2700-3300	C-H3asymetrical Stretching
6	1710.23	1600-1900	C-O Stretching
7	1466	1200-1500	O-H Bending
8	1596.55	1500-1800	C=C stretching
9	1348.07	1300-1490	c-c stretching
10	1164.24	1100-1200	C-N stretching
11	645.74	785-540	Cl-bending

1.9 MORPHOLOGY

Particle size of Prepared formulations were analyzed by using compound microscope the obtained values can be followed: The results of particle size of the prepared formulation were shown in Table 6. and figure 9.

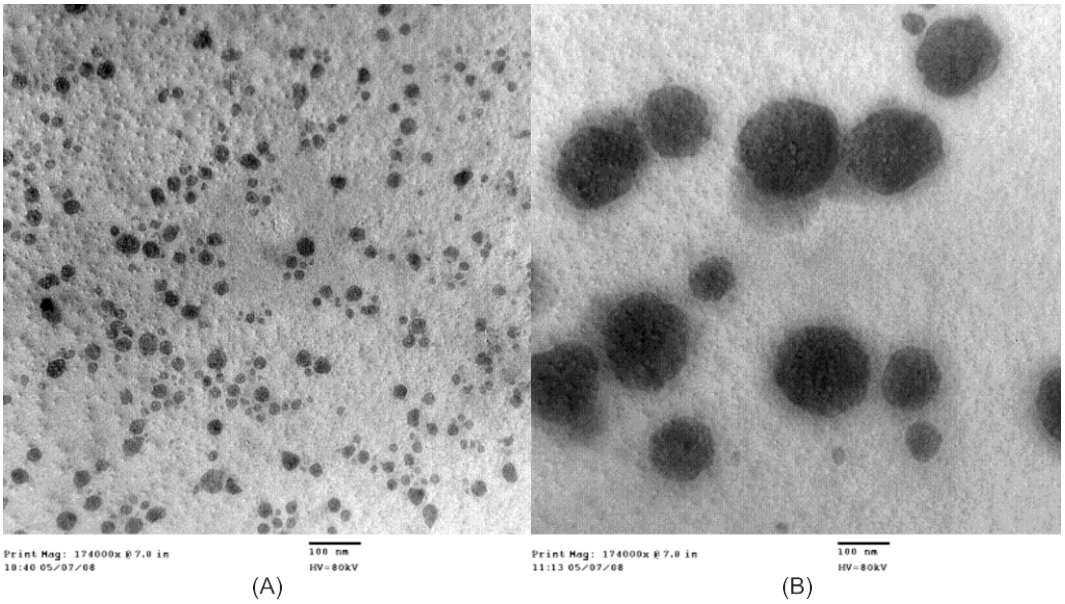

Table 6: Average Particle size of Prepared Nanoparticles.

Formulations	Average particle size(µ)
1A	165.87
1 H	162.53
2 A	153.89
2 H	150.42
3 A	155.56
3 H	149.82
4 A	148.93

4 H	133.34
5 A	120.9
5 B	110.54

1.10 INVITRO- DISSOLUTION

Table 7: Invitro- Dissolution data of prepared Formulations (1A- 5A)

S.No	Time (min)	% of Cumulative drug release					
		GB	1A	2A	3A	4A	5A
1	0	0	0	0	0	0	0
2	10	10.0±0.01	16.65±0.53	14.88±0.24	13.55±0.34	10.45±0.51	15.54±0.51
3	20	15.25±0.12	23.70±0.47	20.68±0.57	18.68±0.41	15.49±0.38	25.32±0.65
4	30	20.54±0.35	36.02±0.80	33.76±0.30	31.69±0.48	32.22±0.49	35.47±0.75
5	45	22.56±0.23	50.64±0.47	51.75±0.49	47.01±0.71	44.19±0.67	42.54±0.32
6	60	25.84±0.63	61.96±0.14	63.70±0.49	58.15±0.91	56.14±0.72	54.96±0.45
7	90	28.98±0.96	68.54±0.45	69.57±0.52	61.74±0.98	65.98±0.85	77.98±0.65
8	120	33.12±0.65	73.02±0.25	72.54±0.32	74.57±0.65	73.54±0.65	89.84±0.58
9	180	35.21±0.57	75.86±0.48	77.54±0.24	79.87±0.99	89.74±0.62	99.47±0.57

Table 8: Invitro- Dissolution data of prepared Formulations (1B- 5B)

S.No	Time (min)		% of Cumulative drug release				
		GB	1B	2B	3B	4B	5B
1	0	12.25±0.52	0	0	0	0	0
2	10	17.15±0.63	16.69±0.53	17.88±0.24	18.55±0.34	19.45±0.51	20.54±0.51
3	20	22.26±0.82	25.70±0.47	27.68±0.57	28.68±0.41	29.49±0.38	29.52±0.65
4	30	24.36±0.93	36.02±0.80	37.76±0.30	39.69±0.48	42.22±0.49	45.47±0.75
5	45	26.35±0.75	52.64±0.47	55.75±0.49	57.01±0.71	60.19±0.67	65.54±0.32
6	60	28.38±0.58	63.96±0.14	64.70±0.49	65.15±0.91	66.14±0.72	69.96±0.45
7	90	32.96±0.94	69.54±0.45	69.57±0.52	70.74±0.98	71.98±0.85	75.98±0.65
8	120	36.89±0.65	75.02±0.25	77.54±0.32	78.57±0.65	79.54±0.65	89.84±0.58
9	180	37.52±0.97	80.86±0.48	83.54±0.24	88.87±0.99	89.74±0.62	99.27±0.57

1.11 Storage Stability

Formulations were stored at 40C+ 20C/75% RH + 6% (Accelerated testing) and 30C+ 20C/60% RH + 5% (Alternate testing). After 30 days of storage, the formulations were observed physically and no color changes occurred. The content of Glibenclamide in all best formulations at various intervals of 10, 20, and 30 days was calculated. The result proved that the percentage of Glibenclamide was not less than 2-3 % in all the formulations as shown in TableNo: 9.

Table 9: Stability Studies of Glibenclamide Nanoparticles.

Formulation code	Temperature(°C)	% of Glibenclamide			
		0 Days	10 Days	20 Days	30 Days
GB	40°C± 2°C/75% RH± 6%	98.98	98.94	98.96	98.76
	30°C± 2°C/60% RH± 5%	98.98	98.99	98.96	98.97
1 A	40°C± 2°C/75% RH± 6%	98.20	98.23	98.21	98.22
	30°C± *2°C!60%* RH±5%	98.20	98.19	98.24	98.21
1B	40°C± 2°C/75% RH± 6%	98.60	98.58	98.63	98.62
	30°C± *2°C!60%* RH±5%	98.60	98.65	98.64	98.58
2A	40°C± 2°C/75% RH± 6%	96.40	96.48	96.44	96.38
	30°C± 2°C/60% RH± 5%	96.40	96.42	96,47	96.47
2B	40°C± 2°C/75% RH± 6%	98.40	98.39	98.43	98.37
	30°C± 2°C/60% RH± 5%	98.40	98.42	98.42	98.39
3A	40°C± 2°C/75% RH± 6%	98.20	98.30	98.17	98.24
	30°C± *2°C!60%* RH±5%	98.20	98.19	98.13	98.24
3B	40°C± 2°C/75% RH± 6%	98.60	98.69	98.57	98.63
	30°C± 2°C/60% RH± 5%	98.60	98.62	98.58	98.64
4A	40°C± 2°C/75% RH± 6%	96.60	96.89	96.80	96.79
	30°C± 2°C/60% RH ± 5%	96.60	96.73	96.79	96.58
4B	40°C± 2°C/75% RH± 6%	98.00	98.12	98.02	97.97
	30°C± *2°C!60%* RH±5%	98.00	98.16	98.09	98.03
5A	40°C± 2°C/75% RH± 6%	98.30	98.35	98,42	98.48
	30°C± *2°C!60%* RH±5%	98.35	98.42	98.42	98.42
5B	40°C± 2°C/75% RH± 6%	98.42	98.52	98.75	98.96
	30°C± *2°C!60%* RH±5%	98.55	98.62	98.78	98.96

DISCUSSION

As a polymeric carrier for NP formulation was selected EuRLPO, a biocompatible copolymer of ethyl and methyl methacrylate and methacrylic acid ester with quaternary ammonium groups. It is not soluble in water but due to its particular polymeric network is capable to swell in contact with gastrointestinal fluids, become permeable and control the drug release. To avoid possible undesirable effects of ionic or non-ionic surfactants, PEG of different MW were evaluated as stabilizers for avoiding or minimizing the growth of drug particles during NP preparation. The composition and details of samples are presented in Table 2.

As seen in Fig. 2, only slight differences in the %EE of the GB NP were found by using two stirring speeds, 500 rpm and 7000 rpm. Regarding the MW of PEG, the % EE dropped to 60% when PEG 6000 was used as stabilizer, while the NP prepared with the other PEG exhibited %EE > 90%. It could be due to the higher solubilizing properties and wettability of PEG 6000, compared to the other PEG used, which could lead to a major solubilization of

GB in the aqueous phase reducing, therefore, the amount of encapsulated drug.

Stabilizers can minimize the interfacial tension between the solid particles and liquid phase Thus, the impact of PEG MW on the GB NP characteristics including Z-average, PDI and ZP was investigated. Results showed that the mean Z- average decreased up to 50% with an increase of the PEG MW. Thus, the addition of PEG 6000 produced particles in the range of 140.6 ± 0.1 nm to 178.1 ± 0.4 nm, whereas in the case of PEG 400, particles were obtained in the range of 298.4 ± 0.4 to 317.5 ± 0.6 nm. For comparison, GB NPs obtained without any stabilizer exhibited a size range between 358.6 ± 0.3 and 459.7 ± 0.2 nm. In this regard, it is postulated that MW of PEG may governs the attraction or repulsion of the newly formed particles . Even though such mechanism is still unclear, PEG of higher MW might produce a major repulsive steric barrier to aggregation by being adsorbed at the surface of the GB particles, minimizing the aggregation phenomena.

From the results it was that the suitability of PEGs to yield smaller particles. As seen in Table 3, slight modifications of GB particle size were detected by increasing the stirring speed from 500 rpm to 7000 rpm.

Free GB exhibited a water solubility of nearly 20 μg/mL, while GB NP formulated without PEG showed a saturation solubility of nearly 35 μg/mL, probably due to the reduction of particle size. In the case of GB nanoformulated with PEG, drug solubility increased even more by increasing the MW of PEG. Thus, NP formulated with PEG 400 and PEG 6000 exhibited a solubil- ity of 38 μg/mL and 125 μg/mL, respectively. Such findings showed that saturation solubility of GB NP stabilized with PEG 6000 was more than six-fold higher than the free drug, most probably due to the reduction of drug particle size after nanoprecipitation. Additionally, the partial reduction of the drug crystallinity and the improvement of drug wettability, particularly by PEG 6000, might also explain the higher solubility of the nanoformulated GB. Also, this finding could be due to the higher hydrophilicity of PEG 6000 in comparison with the other PEG used in this work.

DSC is a fast and reliable method to understand the polymorphic transitions, to screen drug excipient compatibility and providing maximum information about possible interactions. The DSC heating curves were recorded as a plot of enthalpy (m/w) *vs.* temperature (^{O}C). The results are shown in Figure 3.

GB showed melting endotherm at 172.75^{O}C. The thermogram of Eudragit L100 showed three peaks at 52.14, 104.96 and 212.65^{O}C, but sharp peak only at 52.14^{O}C. Thermogram of GB loaded NPs showed not one but multiple endotherms. This indicates the presence of more than one polymorphic modifications.

Results of FTIR Spectra were tabulated in fig 7-8 and table 5. The FTIR spectra of pure Glibenclamide and formulations of glibenclaimde and PEG Polymer in the ratio 1:1 were found to identical. The Absorption peak of following functional group are shown within the rang like Amide group at 1680-1630 cm-1,Aromatic CH group at 3000-2850cm-1Sulfonamide SO group at1050cm-1 and Halogen Cl group at 785-540-1. The above functional groups also present in the Glibenclamide. The IR spectrum of pure Glibenclamide, and nanoparticles of the formulations are shown peaks within the range of functional groups. This indicates that there was no chemical interaction or bonding or decomposition of Glibenclamide employed in the formulations and formulations with various polymers.

Results of invitrpo dissolution studies were shown in table 7- 8. GB release profile was clearly influenced by the presence of PEG. Free GB dissolved up to 35% whereas 99% of GB was dissolved from the PEG 6000-based nanoformulation, within 180 min. According to these findings, it is worth mentioning a fast release of GB from such nanoformulation within 5 min. In addition, the MW of the polymer affected the GB dissolution rate. Thus, following a trend observed in the solubility assay of the nanoformulated GB, there was a remarkable improvement on drug dissolution rate by increasing the MW of PEG from 400 to 6000. It could be due to a reduction of the drug particle size, which leads to an increase in the surface area and, therefore, a higher dissolution performance.

In comparison with PEG 400, the conformation of PEG 6000 in water tends to be a helix with the hydrophobic groups in its interior. As a consequence, a major capacity of interaction with water is postulated. Thus, such different con- formations might lead to a major wettability of GB in presence of PEG of higher MW Such wettability effect may reduce, also, particle aggregation and agglomeration, which result on a higher drug dissolution. Drug release from PEG- based nanosystems exhibited different patterns. PEG of higher MW (3000 and 6000) produced a fast release of GB whereas NP based on PEG of lower MW (400 and 1500) exhibited a sustained drug release profile within the first 90 min. Then, due to the continuous hydration of PEG 400 and 1500, drug release increased to 95%, at

the end of the study (240 min). On the other hand, within the first 90 min only 20% of GB was released from NP prepared without PEG, confirming that the presence of the PEG 3000 and 6000 are crucial if a fast release of GB is needed.

The results of storage stability of all the formulation were shown in table 9. The content of Glibenclamide in all best formulations at various intervals of 10, 20, and 30 days was calculated. The result proved that the percentage of Glibenclamide was not less than 2-3 % in all the formulations.

CONCLUSION

The present work relates to the preparation and characterization of surfactant free glibenclamide nanoparticles. Eudragit RLPO (EuRLPO)-based-NP of GB were prepared by the solvent displacement method using ethanol/water and PEG as sole stabilizers. Glibenclamide nanoparticles were obtained by nanoprecipitation and evaluated in terms of drug content, encapsulation efficiency, apparent saturation solubility, drug release profile, solid state and storage stability. The influence of the MW of PEG on the development of surfactant-free GB NP was systematically evaluated for the first time. The results indicated that increasing the MW of PEG from 400 to 6000 decreased drug encapsulation whereas the aqueous solubility and dissolution rate of the drug increased by increasing the MW of PEG. PEG 6000 produced a fast release of GB while a sustained release was obtained with PEG 400 and 1500, particularly in the first 90 min. The prepared PEG-based nanoformulations showed a particle size in a range of 140– 320 nm and the particle size decreased up to 50% by increasing the MW of the polymer. Storage stability studies confirmed that NP were stable, in terms of particle size, after 120 days at 4 ± 2°C. In conclusion, PEG stabilized NP represent a promising approach for improving the oral delivery and the bioavailability of GB. Taking into considerations I can conclude that the, polyethylene glycol is a useful polymer for stabilizing these surfactant- free glibenclamide nanoparticles.

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