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Section C: Drug Design, Delivery & Targeting

Strategies Adopted to Improve Bioavailability of Glibenclamide: Insights on Novel Delivery Systems

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ABSTRACT

Objectives: Diabetes mellitus (DM) is one of the most prevalent chronic diseases worldwide. According to the statistics declared in 2020 by the World Health Organization [WHO], in the year of 1980, 108 million people have been diagnosed with diabetes mellitus worldwide, then, in 2014, that number had increased to 422 million. In 2020, International Diabetes Federation [IDF] has highlighted that; there will be nearly 700 million adults all over the world to be diagnosed with diabetes by the year of 2045. DM is sub-categorized into Type 1 and Type 2 DM. The former is considered as an autoimmune disorder, in which pancreatic β-cells are destructed, impairing insulin production. While Type 2 DM occurs due to loss of cells sensitivity to insulin. Both are associated with a marked increase in the blood glucose level (BGL) and hence, patients need for pharmacological treatment. T2DM is the most common among diabetic patients and its treatment involves the use of different classes like sulphonylureas, miglitinides and thiazolidinediones. Glibenclamide (GB) belongs to sulphonylureas, its low water solubility accompanied with lower bioavailability demands the use of a higher dose to assure proper management of diabetes. However, this is accompanied in some cases by a severe hypoglycemia that might be lethal in some cases. Therefore, finding a proper delivery system that could improve GB solubility is highly recommended. Nano-medicine could be considered as a promising strategy to improve the solubility and hence, bioavailability of GB. This review is exploring and presenting the recent studies performed to either to encapsulate GB into NPs or reduction of its size to the nano-size level, in order to improve its bioavailability. The review also points to the various obstacles facing nano-medicine clinical application and lack of human studies and sufficient long term safety studies suggesting that future work is needed to make a clearer conclusion about the clinical applicability of the nano-delivery of GB. **Methods:** This review reports a collection of research articles that investigated the use of several nano-delivery approaches for the delivery of GB up to 2022, using internationally accepted scientific journals and databases. **Results:** This review shows that the delivery of GB using nano-medicine strategy improves the GB aqueous solubility and hence, improves its oral bioavailability. It also shows that nano-medicine approach could sustain/control the release of GB, therefore, maintaining stable plasma drug concentration and avoiding any undesired fluctuation. **Conclusion:** The results of the collected articles show that the use of the different nano-medicine categories could be promising to overcome the poor bioavailability of GB. The successful clinical application of the liposomal delivery of certain drugs renders liposomes promising carrier for GB, however, because of the several advantages of the niosomes over liposomes, they seem to be a

promising alternative. Despite this, there are still several barriers against the large scale manufacture of nano-medicines including niosomes, also, more research is required to confirm the long term safety of clinical application of GB-loaded niosomes.

Keywords: *Type 2 diabetes mellitus, Glibenclamide, Advanced drug delivery systems, Nano-technology*

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder. It affected around 463 million adults aged between 20 and 79 years in 2019 and is expected to affect 700 million by 2045, as reported by International Diabetes Federation (IDF). In 2019, mortality due to DM was around 4.2 million deaths [1]. DM involves abnormal high blood glucose level (BGL) due to disorders associated with insulin blood level [2]. Insulin is a hormone, secreted by β-cells of islets of langerhans in pancreas and it is responsible for controlling BGL, for instances, after a meal, high BGL triggers the release of insulin to enhance glucose uptake by cells and inhibiting the action of glucagon to produce glucose [3].Therefore, disorders that affect the blood insulin level could be associated with a dramatic increase of BGL and this could lead to damage of blood vessels and consequently, cause a huge damage on the organs they supply. For example, vision problems, nerve damage and kidney disease were reported due to damage of small (micro-vascular complications) blood vessels[4], while cardiovascular disease such as heart diseases and stroke, or poor blood circulation were reported due to damage of large (macro-vascular complications) vessels [4].

DM involves two types; Type 1 DM and Type 2 DM [5]. The former is considered as an auto-immune disorder, in which pancreatic β-cells are damaged, impairing the normal insulin production [5], while Type 2 DM ‘’also known as non-insulin dependent diabetes mellitus’’ occurs as a result of the loss of cells’ sensitivity to insulin (Insulin resistance) [5]. Both are associated with a marked increase in BGL and hence, diabetic patients need for therapeutic management. Type 1 DM is treated with insulin, while Type 2 DM could be treated with other medications that control BGL, as well as life style modification such as controlling diet and regular exercise, however, at late stages it may also be treated with insulin when non-insulin oral anti-diabetic medications are not able to control BGL [6].

Type 2 DM is reported to affect more than 85% of total diabetic population [7]. In this review, we are concerned with non-insulin oral anti-diabetic treatment of Type 2 DM. they involve two classes; (1) The insulin secretagogues (i.e. sulfonylureas and meglitinides), which increase the pancreatic secretion of insulin, they do so by closure of the ATP-sensitive K^+ channels of the pancreatic beta islets cells, which is followed by Ca^{2+} influx through voltage-dependent Ca^{2+} channels, leading to exocytosis of insulin granules [8], and (2) the insulin sensitizers, (i.e., metformin and thiazolidinediones), they work by increasing the cells’ sensitivity to insulin. Insulin sensitizers are also referred to as Peroxisome Proliferator Activated Receptor (PPARs) agonists, because they work by binding to PPARs. PPARs are considered to be the regulators of carbohydrate, lipid and protein metabolism and maintain the glucose homeostasis, activation of these receptors leads to increased expression of several key target genes that are involved in the carbohydrate and lipid metabolism. The expression of these genes ultimately results in lowering the level of fatty acids in systemic circulation and hence, the cells become more dependent on glucose oxidation for energy [9,10].

Glibenclamide (GB) is an oral hypoglycemic drug belongs to sulfonylureas class, it is broadly prescribed by health care providers [11]. Its mechanism of action involves enhancement of insulin release by inhibition of ATP-sensitive potassium channels leading to membrane polarization followed by release of insulin [12]. However, GB belongs to Class 2 of biopharmaceutical system (BCS), i.e. it has a high permeability across the cell membranes but it is poorly soluble in the biological fluids [13]. For a better therapeutic management of diabetes, oral medications should be dissolved efficiently in the biological fluids to be absorbed across the biological membranes to produce a therapeutic plasma concentration that is enough to control high BGL [14]. Consequently, poor solubility and low bioavailability (ranged from 40 to 45%) of GB are considered as the major obstacles encountered its oral use[12–14].

Several strategies were investigated to improve both GB solubility and bioavailability such as particle size reduction e.g. micronization [15], cyclodextrin complex formation [16] and solid dispersion systems[17].
Nano-technology is a cutting-edge science that has been greatly impacted the field of pharmaceuticals and drug delivery. Nano-medicine is the branch of medicine that uses nano-particles (NPs) sized from 1 to 1,000 nm for either therapeutic or diagnostic purposes [18,19]. For example, NPs have been previously applied to treat many diseases such as viral infections [18,20–22], be effective against multidrug resistant bacteria [21,23–25], improve wound healing [26,27], inflammation [28], cancer [29], overcome blood-brain barrier [30,31] and has a potential to be used for diagnostic purposes [32] .

Nano-medicine as a drug delivery system was used to target the drug to a specific site and consequently overcome drug accumulation at off-target tissues and consequently decrease the side effects that might associate drug administration [33] ,[34]; [35]. Moreover, nano-medicine was able to reduce frequency of drug administration and thus, improve the patient's compliance [36]. In addition, nano-medicine was a good strategy to improve the delivery of hydrophilic drugs into cells [37], improve bioavailability of poorly soluble drug, control/sustain drug release [38–40].

In this review, we focused on the different attempts performed using advanced drug delivery systems including both nano- and micro-particles, to improve GB bioavailability. These include polymer nanoparticles (PNPs), solid lipid nanoparticles (SLNPs), Liposomes, Self-nano-emulsifying drug delivery system (SNEDDS), and Core–shell nano-biomaterials as presented (Table1). Moreover, we are presenting different challenges and obstacles facing its clinical translation.

GB as previously discussed, is broadly prescribed for treatment of Type 2 DM; a total of 4598 articles were identified in PubMed by searching for "Glibenclamide for diabetes" on June 13, 2022 (**Figure 1**). Nano-medicine could be applied to improve treatment of DM; a total of 987 articles were found in PubMed by searching for "nanomedicine for diabetes" on June 13, 2022. However, few studies were found regarding the application of nano-medicine to improve the hypoglycemic effect of GB; a total of 24 articles were found in PubMed by searching for "Glibenclamide nanoparticles for diabetes" as key words on June 13, 2022, and after intensive literature research, only 16 were found to investigate the potential of enhancing GB bioavailability using the nano-delivery approach, which will be discussed later in this review article. It is worth noting that, to date no nano-medicine products either containing GB or any other anti-diabetic agent, have been approved by the Food and Drug Administration (FDA) for use to control diabetes. This may be attributed to challenges encountered in their large-scale manufacture and/or many tests that must be performed to assure safety profile of the nano-medicine containing GB.

Polymeric nano-particles, micro-particles and beads

Biodegradable and bio-compatible polymers such as Eudragit, Alginate and Poly (lactic-co-glycolic) acid (PLGA) were reported to encapsulate GB [41]. They were all found to improve GB solubility and hence expected to be accompanied with a better bioavailability. Eudragit L100 was the only polymer investigated in an animal model and was found to have a superior pharmacological activity compared to un-encapsulated GB as presented in **Table1**.

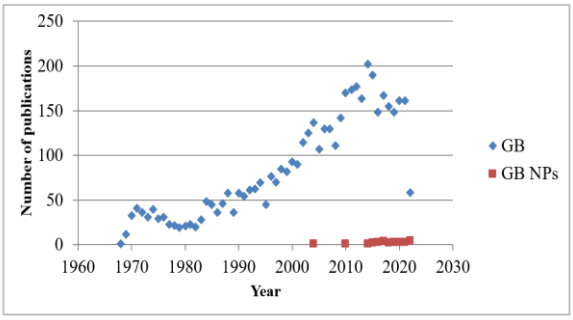

Figure 1. The number of publications (a) Glibenclamide (GB), (b) Glibenclamide nanoparticles (GB NPs) investigating the potential applications of nanotechnology to improve the treatment of diabetes by GB published per year from 1968 to 2022 (data extracted from PubMed using the search term "Glibenclamide for diabetes" and "Glibenclamide nanoparticles for diabetes" on June 13, 2022).

Eudragits are cationic synthetic polymers based on methacrylate subunits that have a positive charge [42]. Dora and his colleagues [43] studied the encapsulation of GB into Eudragit L100 NPs (**Table 1**), they tested the effect of drug: polymer ratio, particle size, and drug loading on the release profile of GB Eudragit NPs. As GB is poorly soluble in water, so; increasing its water solubility would increase its own oral bioavailability. Encapsulation of GB into NPs was associated with a significant change in saturation solubility (The maximum mass of solute dissolved per unit volume of solvent at a given temperature and pressure) in comparison with the pure drug and hence, an improvement of GB bioavailability. It was found that, the increase of polymer ratio was accompanied with a reduction in drug release and this was explained by the increase of diffusion length. Moreover, the particle size was found to dramatically affect the release pattern of GB, where NPs with particle size of 704.67 ± 0.56 nm had the best release pattern due to the large surface area compared to other NPs with a larger size. Furthermore, this formula proven to significantly increase the drug release compared to the marketed product, Daonil β.

In-vivo study on diabetic rabbits using a dose equivalent to 2 mg/kg of GB revealed that the oral application of these NPs significantly decreased SGL ($p < 0.01$), T_{min} (Time required to achieve minimum effective drug concentration) and AUC (area under the curve) compared to pure GB [43].

Khames and his colleagues [44] encapsulated GB into beads made of alginate and amphiphilic alginate derivatives, alginate hexyl amide polymer (AHAP, a derivative form of alginates where a relatively short-chain alkyl group (hexyl) was associated with the alginate polysaccharide backbone through a polar amidic linkage) using ionotropic gelation method. AHAP has

Table 1: New drug delivery systems for Glibenclamide

Nanoparticles	Preparation method	Particle Size ± PDI*	Zeta Potential (mv)	Drug Loading %	Encapsulation %	In-vivo hypoglycemic studies	Ref.
A- Polymeric Nanoparticles, micro-particles and beads							
Eudragit L100 NPs	Solvent displacement method	704.67 to 1632 nm ± 0.37 to 0.56	-27.8 to -15.27	17.47 to 35.85	53.97 to 74.97	The in vivo study was carried out on alloxan-induced diabetic rats, GB nano-suspension significantly reduced Serum Glucose Level (SGL), when compared to plain GB.	43
Eudragit RLPO	Solvent displacement method	95.4 to 154 nm ± NA	NA	NA	50 to 60	NA	73
Alginate	Ionotropic gelation method	680–750 to 760–830 µm	NA	NA	69.57 to 87.31	NA	44
Alginate-hexyl amide polymer (AHAP)	Ionotropic gelation method	475–510 to 510–560 µm	NA	NA	89.46 to 97.82	NA	44
Poly (lactic-co-glycolic acid)	Emulsification solvent evaporation method	532 to 1453 nm	NA	10.28 to 36.73	33.21 to 90.52	NA	74
Core–shell nano-biomaterials made of chemically modified native xanthan polymer	Solvent evaporation method	485.8 to 925.4 nm ± 0.639 to 0.641	−25.9 to −26.6	42.79 to 48.87	NA	Normal healthy rats were used to assess the pharmacodynamics action of GB-micellar formulation. The micellar formulation of GB successfully reduced Blood Glucose Level	62
B- Solid Lipid Nanoparticles							
Solid lipid nanoparticles made of commercial lipids (Precirol® and Compritol®).	Emulsification-solvent evaporation and Hot high-shear. Homogenization, with and without incorporation of lecithin and/or PEG 6000.	104.1 to 105.1 nm ± 0.20 to 0.22	-34 and -35	NA	19.6 to 81	Male wistar rats with Streptozotocin-induced diabetes were used for the in-vivo study. Reduction of the blood glucose level successfully achieved.	49
C- Self – nanoemulsifying drug delivery system							
Self-nanoemulsifying drug delivery system (SNEDDS) made of commercial Cremophor_ RH 40, propanediol and Miglyol_ 812.	Emulsification.	36 to 81 nm ± NA	3.7and 2.7	-3.7 to - 2.7	98	Healthy beagle dogs were used for the study. Oral administration of SNEDDS resulted in 1.5 folds increase of AUC.	59
D- Liposomes							
Liposomes made of hydrogenated soyphosphatidylcholine (HSPC)	Deposition carrier method	232.4 nm ± 0.506	NA	NA	61.78 to 83.79	NA	58

E- Nano-suspension							
Nano-suspension made of PVP K30, and Tween 80	Nano-precipitation method	0.216 to 0.856 nm ± NA	+12 and +13	5 – 10 mg	NA	**In-Vivo study of inhaled GB nano-suspension:** Streptozotocin-induced diabetes in male Albino rats. The blood glucose level was significantly decreased by nearly 60% and such reduction after the use of inhaled GB nano-suspension was higher than after oral use.	65
F- Niosomes							
Niosomes made of Span 20 and Span 80	Modified ether injection technique.	Span 20; 12.94, 12.38 and 12.16 µm Span 80; 8.83, 8.55 and 8.06 µm	NA	NA	Span 20; 73.81to 75.27 Span 80; 67.44 to 69.45	**In-Vivo study of GB niosomal gel:** Streptozotocin-induced diabetic male wistar rats were used for the in-vivo study. Significant lowering of BGL was obtained.	67
Span 40 and Span 60-based niosomes	Modified thin film hydration method.	172 nm ± 0.304	49.9	NA	45% ± 5 to 78% ± 6	**In-Vivo study of inhaled GB niosomal drug delivry system:** Diabetes was induced to male Albino rats with a single dose of intra-peritoneal Streptozotocin. The BGL was considerably reduced.	69
G- Nano-crystal							
GB nano-crystals	Combined bottom-up and top-down technique.	415 nm ± NA	NA	96	NA	**In-Vivo study of GB transdermal system:** Both normal and diabetic rats were used to assess the hypoglycemic effect of GB nano-crystals. Significant reduction in BGL was noticed in both cases.	64
H- Nano-particles							
Zein nanoparticles	Desolvation method.	196 nm ± 0.13	−33.8	4.28	43	NA	71
GB nanoparticles	Nanoprecipitation method.	140 nm ± 0.6 to 459.7 nm ± 0.2	24 and 21.9	NA	NA	NA	72
Methacrylic acid–ethyl acrylate copolymer nanoparticles	Solvent displacement method.	18.98 ± 0.3 nm	−13	NA	44.5	The in vivo study was carried out on Wistar rats which have been rendered diabetic through using streptozotocin . The GB-Loaded nano-particles significantly reduced BGL.	47

PDI: Polydispersity Index

extra advantages over alginates due to its surface-activity that makes it able to potentiate the solubility of the encapsulated drug. The formed beads were able to control the drug release rate, its solubility and hence, improving its bioavailability.

In this study, different concentrations (1, 2, and 3%) of alginate and AHAP were used and this resulted in beads with different sizes and thus, different release patterns. The particle size ranges of AHAP' beads were found to be 510–560 mm, 490–530 mm and 475–510 mm at concentrations 1, 2, and 3% respectively. The particle size range of AHAP' beads was smaller than the particle size range of alginates' beads prepared at the corresponding concentrations; 680–750 mm, 720–800 mm and 760–830 mm respectively. Both alginate and AHAP improved GB solubility compared to the pure form of GB. However, AHAP had a higher solubilizing effect at all polymer concentrations. The highest solubility recorded for alginate and AHAP was 24.6 and 53 µg/ml respectively.

The maximum release percentage recorded for beads of alginate and AHAP after 12 h was 75% and 100%, respectively. The increase of drug release from AHAP's beads was attributed to its surface-activity, where the prepared polymer increased the degree of subdivision of the drug within the bead and decreased the contact angle between the drug particles and the dissolution medium leading to increased drug wettability.

Kumar and his colleagues [45] prepared GB Eudragit RLPO microparticles by solvent evaporation method to sustain its release and overcome its administration frequency. They tested different drug: polymer ratio and the drug content ranged from 24.5 to 47% and the formulations have a particle size ranged from 134 to 180 µm (Table1). The inclusion of GB into Eudragit RLPO micro-particles extended its release in-vitro (phosphate buffer, pH 7.4), however, the authors did not test the formula In-vivo.

PLGA was also used to improve the bioavailability of GB by forming GB PLGA NPs [46]. The effect of drug: polymer ratio on the release of GB, particle size, drug loading percentage was also investigated as presented (Table 1). Similar to the previous studies, an increase of polymer concentration was associated with an increase in particle size; NPs obtained ranged from 532 to 1453 nm. By increasing the drug: polymer ratio (from 1:1 to 2:1), an increase of drug release was observed in vitro at the release media ''hydrochloric acid (0.1M) for the first 2 h and then in phosphate buffer (pH 7.5) from 3 - 72 h'' at 37 ± 0.5 °C. The increase of drug release was attributed to the less amount of the polymer available to coat the drug to retard its release. However, this study did not test the efficacy of NPs *in-vivo*.

From the previous studies, it could be noted that, the increase in polymer content retarded drug release and this was related to the increase of matrix thickness of NPs, thereby increasing the distance that the drug should transverse to reach the surface of the NPs.
Escobar and his colleagues have prepared Nanoparticles made of methacrylic acid–ethyl acrylate copolymer and encapsulated GB within them. The optimum formula has shown nearly 100% release in vitro of GB. An in- vivo study has been conducted using streptozotocin to induce diabetes in wistar rats. The administration of GB-Loaded nanoparticles significantly ($p > 0.5$ between the treated and untreated groups) decreased the blood glucose levels in the tested animals[47].

Solid Lipid nanoparticles (SLNPs) is another approach adopted to improve the bioavailability of GB and sustain its release at the biological system. Similar to other nano-delivery systems (nano-emulsions, micelles, liposomes, and polymeric nano-particles), SLNPs have the merits of protecting the drug against bio-degradation, improve the drug solubility due to the high surface area provided by its nano-size, could control or sustain drug release and hence, could be employed to overcome the short half-life of GB. Moreover, it has a better stability and a less toxicity compared to other nano-delivery systems[48].

Gonçalves and his colleagues [49], investigated Different lipid components; Glyceryl behenate, (Compritol®) and glyceryl palmitostearate (Precirol®) and different preparation methods (the emulsification-solvent evaporation (ESE) and the hot high-shear homogenization (HH)) and they found that ESE produced particle sizes lower than HH. Moreover, SLNPs prepared with glyceryl palmitostearate produced SLNPs of sub-micronized size and presented as diameter nm ± Polydispersity index (PDI), 129 ± 0.23 nm. They examined the stability of the obtained formula under the gastrointestinal condition and they recorded a massive aggregation under the acidic condition of stomach that was associated with a massive loss of loaded GB "around 80% loss" before being delivered to intestine. Coating SLNPs with lecithin (Size ± PDI: 100 ± 0.23) only or in combination with PEG 6000 (Size ± PDI: 105 ± 0.22) succeeded to stabilize the formula against aggregation under acidic condition where the maximum particle size recorded due to aggregation was 160 µm with no loss of loaded GB. The percentage of encapsulation efficiency for SLNPs prepared in presence of PEG 6000 (19.6 ± 4.8) was much lower than those recorded with lecithin coated SLNPs (70.3±5.2). The release study for the latter formula showed a rapid phase of GB release (around 20%) after 30 min followed by a slow release phase where GB was fully released after 24h in a medium containing buffer, pH 7.4. The anti-diabetic activity for SLNPs of high encapsulation percentage was investigated in diabetic male rats and shown to have a

rapid onset of BGL reduction and a significant decrease of BGL compared to un-encapsulated GB. The hypoglycemic action of SLNPs encapsulating GB lasted for 8h. The hypoglycemic activity was explained by SLNPs can facilitate the oral absorption of poorly-water soluble drugs by maintaining a solubilized state of the drugs in the GI tract, and by facilitating the formation of mixed micelles, promoting secretion of endogenous phospholipids and bile [50–52]. In addition, bio-adhesion of SLNPs to the gut wall seems to prolong the residence time of SLNs in the gastro intestinal tract and enhance their intimate contact with the epithelial membranes, which possibly contribute to enhancing oral drug absorption[50,53].The potential muco-penetrating ability of SLNPs is another aspect possibly responsible for the enhanced absorption of the drug [54,55]. Also, the small particle size and the surface characteristics of the nano-particles have an important role [53]. An additional reason for the improved drug absorption from SLNPs could be attributed to the enhancement of lymphatic delivery, lipids can stimulate lipoprotein formation and intestinal lymphatic lipid flux to increase the extent of lymphatic drug uptake [56,57]. However, it is worth noting that the reasons for the improved oral bioavailability of drugs, obtained by lipid-based delivery systems, including SLNPs, as well as the question if the drugs formulated as SLNPs are absorbed as free drug or in the form of SLNPs have not been well clarified yet and thus, further studies should be performed in the future to investigate these aspects.

Liposomes are composed of phospholipids and cholesterol, they are susceptible to oxidation and hydrolysis rendering them less stable, thus, pro-liposomes, the powder form of liposomes ready to be reconstituted upon administration is considered as a better alternative to keep their stability. Sharma and his colleagues [58] prepared GB loaded pro-liposomes containing hydrogenated soyphosphatidylcholine (HSPC), cholesterol and using sorbitol as the carrier in different ratios. The particle size of the optimized formula of pro-liposomes was 232.4 nm. The polydispersity index value was, 0.506 and it reflected a uni-modal size distribution, and lies within the acceptable range considered for pro-liposomal formulations. The percentage of encapsulation efficiency ranged from 66 to 84%. The dissolution study of pro-liposomes containing Glibenclamide in 0.1 N HCl (pH 1.2) was performed, where the dissolution of GB from pro-liposomes was found to be 3 – 3.5 times higher than the pure drug and hence, it is expected to improve the GB bioavailability. This might be due to increased surface area of drug molecules and also the conversion of GB from the crystalline to the amorphous state inside the liposomal carrier. However, this study did not perform in-vivo test to evaluate its anti-diabetic activity.

Self-emulsifying drug delivery systems (SEDDS) is another approach used to enhance the drug solubility and permeability through biological membranes and thus improving the bioavailability of poorly soluble drugs [57,59]. SEDDS are isotropic mixtures of lipids, surfactants, co-surfactants and drug substance that rapidly emulsified under gentle agitation or the motility of gastrointestinal tract forming an oil-in-water (O/W) emulsion[59]. The droplet size of the formed emulsion ranged from 100 to 250 nm (self-micro-emulsifying drug delivery systems, SMEDDS) or lower than 100 nm (self-nano-emulsifying drug delivery systems, SNEDDS). The latter provide a larger surface area for drug release and absorption. The latter approach was adopted to improve the bioavailability of GB after oral administration [59].

Liu and his colleagues [59] developed and optimized SNEDDS of Cremophor RH 40 as surfactant, 1,2-pranpanediol as co-surfactant and Miglyol 812 as the oil phase where the droplet size obtained was around 50 nm and reported to be stable under storage conditions either at 4 or 25 ˚C. The in vitro release pattern of GB from SNEDDS was performed at different release media (PBS with both pH values of 6.8 and 7.4 and water) in comparison to the marketed micronized tablet of GB, Glyburide. It was shown that GB released from the SNEDDS was significantly better and faster than the marketed tablets, where after 5 minutes, SNEDDS released more than 75% in all release media compared to variable release percentage recorded for micronized marketed tablet; 3.7%, 17.3% and 43.2% in water, PBS 6.8 and PBS 7.4, respectively. The latter might be attributed to the higher dissolution properties of GB at higher pH. Contrary to SNEDDS, where its release was not affected by the type of release medium and complete release of drug was recorded after 20 min in all media and this could be explained by increased GB solubilization by the nano-emulsion form due to presence of GB at the molecular level. The efficacy of SNEDDS in-vivo was evaluated by determination of its pharmacokinetic parameters versus the marketed micronized tablet on non-diabetic beagle dogs after a single oral dose of SNEDDS capsules and micronized tablet, each containing 3 mg of GB. Plasma concentrations of GB after administration of SNEDDS were significantly higher than commercial tablets at all investigated time points, which suggests significant increase in the drug absorption. Area under the curve for SNEDDS was 2.5 times more than that recorded for the micronized tablet. The relative bioavailability of SNEDDS formulation was 253% in comparison with micronized tablets. The higher bioavailability of SNEDDS prepared in this study was attributed to nano-size of the droplet that enhanced GB solubility and release in comparison to marketed tablet. Moreover, the component used to formulate of SNEDDS in this study;

Cremophor RH has been reported to inhibit the activity of the efflux [60,61], which might account for the increased uptake of GB in gastrointestinal tract as GB was reported to be excreted outside cells by efflux transporters[61].

Polymer-based micelles have received much attention recently due to availability of many amphiphilic polymers that are degradable and compatible with the biological system as well as the potential for inducing many functional groups on these polymers for drug conjugation purposes[62]. Amphiphilic polymers form core–shell structures[62], and they have a lower hydrophilic polysaccharide, derived from the bacterial coat of Xanthomonas campestris [63]. Xanthan could be modified to form an amphiphilic co-polymer of xanthan by grafting aliphatic C-16 cetyl chain on its backbone. The obtained amphiphilic polymer was used to improve the solubility of GB through formation of micellar dispersion encapsulating GB [63]. The diameter of drug-loaded micelles ranged from 753 to 925 nm, the micelles were stable with zeta potential value range of -26 to -29 mV and showed no signs of aggregation. This micellar system improved the solubility of GB by 122 times more than the pure form of GB.

In vitro drug release from the micellar dispersions were studied in simulated gastric (HCl solution, pH 1.2) and intestinal fluids (phosphate buffer solution, pH 6.8). The release pattern of GB was affected by the pH of the release media; the dissolution efficiency was higher in the alkaline medium than in the acidic one. Around 20% released at the acidic pH and by moving the sample to phosphate buffer pH 6.8, drug release rate was doubled over 30 min and then after, the release rate declined with an overall of 45% of encapsulated drug was released over 8h. This was argued to be due to the presence of carboxylic functional group in the anionic form at alkaline pH and this potentiated the ability of the polymer to absorb water and swell, thus enhancing drug release. The hypoglycemic activity of this formulation was also investigated in diabetic rabbits (diabetes induced by intravenous injection of alloxan (80 mg/kg bodyweight). The micellization of GB was found to have a shorter onset and produced a significant reduction of BGL compared to the dispersion of the pure form of GB where 51% reduction of BGL recorded for GB micelles after 5h versus 34% reduction in case of dispersion of pure GB after 4 h and then BGL started to rise gradually back to their normal level. This was attributed to the improvement of GB solubilization and consequently its bioavailability after micellization.

Nano-crystal formation: GB nano-crystals and micro-crystals were engineered using combined homogenization and precipitation techniques. Poloxamer was added during the precipitation, in order to decrease the surface free energy, and hence increasing stability through preventing aggregation and Ostwald ripening of the generated drug nano- and micro particles. These GB nano- and micro-crystals were then incorporated into chitosan solutions in order to design a transdermal delivery system. The percent Drug Content was found to be 92 and 96 for micro- and nano-GB, respectively. The in vitro release studies revealed that about 85 % of GB was released within 24 hours, while it was 61 % from micro-GB. Inclusion of GB into nano-crystals greatly improved its permeation across the skin, the cumulative permeation values of GB nano-crystals and micro-crystals were found to be 498 and 362 mg/cm^2, respectively, after 24 hours. Also, the calculated flux (which describes the amount of the drug diffused across the skin) across rat skin was found to be 23.14 for nano-GB, compared to 13.64 mg/cm^2/h for the micro-crystals. The in vivo study showed that both GB nanonization and micronization were efficient to lower the blood glucose levels, and also able to maintain higher GB concentration for prolonged time (24 hours) and minimize the incidence of the undesired intense hypoglycemia associated with GB oral therapy.

The in vivo hypoglycemic activity of both nano- and micro GB transdermal formulations relative to orally administered GB was investigated in normal and diabetic rats. Lowering of blood glucose levels was significant in normal rats for all tested formulations. The oral administration of GB induced a marked hypoglycemia, reaching a maximum reduction percentage of 48.71 % after 4 hours, after that, the hypoglycemic action declined rapidly, which can be related to the short half-life of GB, and only a level of reduction of about 8.8 % was noticed after 24 hours. The hypoglycemic action for nano-GB and micro-GB was increased gradually to the levels of 19.6% and10.4, respectively, after 2 hours. Maximum reduction in the blood glucose levels was noticed after 6 hours (33% for the nano-crystals and 24% for the micro-crystals) and then declined gradually till the values of 28.6 % and 20.4 at 24 hours, showing that longer action was obtained in comparison to the oral pure GB. For the diabetic rats, all treated groups showed considerable hypoglycemia when compared to diabetic control group[64].

Nano-suspension: Hashim and colleagues successfully prepared GB nano-suspension to be inhaled using modified nano-precipitation method. The design of expert software were used to predict the effect of three factors ((Polyvinyl pyrolydine (PVP K30) amount, Tween 80 amount and drug loading amount) on three dependent factors (particle size, zeta potential and solubility). Fifteen formulae have been tried on the software and three levels have been selected for the preparation on the nano-suspension, were three different amounts (20, 30and 40 mg) of PVP K30, and Tween 80 (0.1, 0.15, 0.2 ml) and three different drug loading values (5, 7.5 and 10 mg) were used based on the results of the software-based optimization. The release data showed that all nano-suspension formulae improved the

GB solubility. The results shown that increasing the amount of PVP was associated with an increase in GB solubility; this was explained by the fact that PVP could inhibit the crystallization of GB giving systems containing the drug in an amorphous phase.

The in-vivo anti-hyperglycemic action was assessed by measuring the level of plasma glucose in normal healthy male Albino rats. The rats were injected intra-peritoneally with a single dose of Streptozotocin (STZ) 60 mg/kg to induce diabetes. The diabetes induction was confirmed through measuring blood glucose level after three days of the injection; and rats having levels exceeding 300 mg/dl were considered diabetic. Eighteen diabetic rats were divided into 3 groups. The rats were fasted for 16 hrs before the study with free access to water, the they were treated as following, Group I: has been considered as negative control and received 0.5% CMC, Group II: Received oral GB powder (suspended in 0.5% CMC) and Group III: Received GB Nano-suspension via inhalation. Blood samples were collected from the tail vein pre- and post-treatments at (0, 15, 30, 60, 90, 120, 180 and 210 min) and used for glucose level determination. It was found that the blood glucose level was significantly reduced by about 60% and the decrease in blood glucose level after administration of inhaled GB nano-suspension was higher than GB oral route [65].

Niosomes are non-Ionic surfactant-based nano or micro-vesicles, they have either spherical, uni-lamellar, bi-layered, multilamellar and poly-hedral structures and are efficient in encapsulating both hydrophilic and lipophilic drugs [66]. They are obtained by hydration of surfactants in presence of absence of cholesterol. Addition of cholesterol helps in sustaining/controlling the drug release from the vesicular structure[66]. They have several merits over liposomes such as higher stability, lower manufacturing costs and ease of scaling up [66]

Vadlamudi and his colleagues [67] encapsulated GB into niosomal delivery system using Span 20 and Span 80 with Drug: surfactant: cholesterol molar ratio of 1:2:1, 1:3:1. 1:4:1 respectively. The prepared GB niosomes were incorporated into Carbopol 934 and applied topically on diabetic rats to tackle its anti-diabetic effect. GB as previously discussed, suffers from low solubility and first pass metabolism, both together lead to low bioavailability, short half-life and consequently more frequent dosing, as well as other side effects such as; severe and sometimes fatal hypoglycemia. Other side effects encountered after oral therapy include gastric disturbances like nausea, vomiting, anorexia, heart burn and increased appetite [68]. Therefore, it was assumed that transdermal delivery of GB might be able to sustain drug release, control BGL for a long time and overcome the associated side effects of GB [67]. Span 80 gave vesicles of smaller size as presented (Table1) and this was attributed to the lower HLB value of span 80 resulted in decreasing the surface free energy and increased hydrophobicity [67]. The increase in surfactant concentration for both span 20 and span 80 was associated with an increase of particle size. The encapsulation efficiency of span 20 was better than span 80 as presented (**Table 1**) and this was explained by presence of unsaturated alkyl chain in span 80 that made the vesicles more permeable to the drug and thus lower encapsulation efficiency. Therefore, span 80 was associated with a higher release of GB than span 20; The percentage drug release at the end of 8 hours for span 20 was 53.66, 57.28 and 54.79% at molar ratios of 1:2:1, 1:3,1: 1:4:1, respectively compared to 61.28, 55.49 and 56.04 for niosomes prepared with span 80 at the same molar ratios. Topical application of GB niosomal gel was found to decrease the BGL efficiently compared to the marketed tablet [67].

In another study, Abdel-Rashid and her colleagues investigated the anti-hyperglycemic effect of inhaled GB loaded niosomes. Box-Behnken design was used to prepare 15 formulae for the purpose of optimization, where three factors (the drug loading amount, Cholesterol molar ratio and hydrophilic lipophilic balance (HLB) values of the surfactant) were the independent factors, while (the mean niosomal particle size, Zeta potential, PDI, entrapment efficiency and the percentage of GB released after 2 hrs) were the dependent factors, then those 15 niosomal formulae were prepared by a modified thin-film hydration method. It was found that there is a direct proportionality between both the cholesterol: surfactant ratio, the HLB value of the surfactant and the particle size. The results of the percent entrapment efficiency (%EE) revealed that, the increase in the cholesterol content was associated with an n crease in the %EE; this was explained by the fact that cholesterol reduces the niosomal fluidity and hence, provides rigidity to them leading to less-leaky vesicles. The increased cholesterol content also resulted in enhanced release of GB in vitro, which can be related to the possible competition between the cholesterol and GB for the niosomal packing space, and hence causing the drug to be released easily from the niosomal bilayer system. The in-vivo study was carried out using eighteen Albino rats showing hyperglycemia. The animals were divided into three groups (n=6). Group I, was considered to be negative control, where it received 0.5% CMC orally, Group II, was given oral GB powder and Group III was treated with Inhaled GB niosomes. The blood samples were then collected from the tail vein pre- and post- drug treatments at pre-determined time intervals (0, 15, 30, 60, 90, 120, 180 and 210 min). The collected samples were used to measure the blood glucose level.
The results of the in-vivo study showed that the mean percentage reduction in blood glucose level (BGL) of diabetic rats after administration of the inhaled GB

niosomes was nearly two folds that caused by oral GB administration. The results of the in-vivo study suggest the success of systemic GB delivery from niosomes via the lungs[69].

Nano-particles: Zein Nanoparticles, Zein is a protein that was approved by the US-FDA in 1985 as a GRAS (generally recognized as safe). Zein is available and has shown to be suitable for medical applications, including controlled release of drugs, because of its safety and low digestion rate [18]. In one study zein nano-particles were used to incorporate GB. The nanoparticles were prepared by desolvation method that depends on obtaining nano-particles through drop wise addition of an anti-solvent to the aqueous solution of the nano-particle forming material[70]. The release rate of GB was studied relative to the pure drug in simulated gastric fluid (SGF), as well as simulated intestinal fluid (SIF).

The GB release after 2 hours at pH 1.2 was found to be lower than 5% for both, pure drug and GB incorporated zein nanoparticles. Such finding can be related to the impact of the acidic medium on the dissolution of GB that has pKa-value of 5.3. On the other hand, the GB release rate was higher in SIF (pH 6.8). These findings refer to the fact that intestinal conditions are more favorable to dissolve GB. The rate of GB release from zein nano-particles was faster than that of the pure GB; as after 12 hours, more than 90% of GB was released, while the amount of pure GB dissolved was found to slightly exceed 60%, and needed a period of 24 hours in order to release the whole drug amount. This may be attributed to the enhancement of dissolution of GB after incorporation into zein nanoparticles[71].

GB nanoparticles: Arrua and colleagues have prepared surfactant-Free GB nano-particles using only Eudragit RLPO and polyethylene glycol as stabilizers. The resulting GB showed improved saturation solubility in comparison to free GB. Free GB exhibited an aqueous solubility of 20 µg/mL, while GB nano-particles formulated without using PEG showed an increased solubility to 35 µg/mL, this might be due to decreasing the particle size. PEG with different molecular weights has also been used in the formulation of GB nanoparticles, such molecules increased GB solubility to greater extent. It was obvious that increasing the molecular weight of the used PEG increased the solubility proportionally, where GB nanoparticles prepared with PEG 400 and PEG 6000 showed a solubility of 38 µg/mL and 125 µg/mL, respectively. Such findings could be related to the improvement in GB wettability by the hydrophilic PEG molecules. The findings of saturation solubility have been assured with the results of GB release, where only up to 35% of GB has been released from PEG 6000 free GB nano-particles, while the inclusion of PEG 6000 resulted in the release of 99% of GB. No animal study has been conducted in this research work[72].

All the previous studies confirmed that the encapsulation of GB into novel delivery systems could be a promising approach to improve its bioavailability and overcome its associated side effects. However, despite this, to date there are no GB particulate delivery systems clinically approved for treatment of diabetes. This might be related to the different obstacles and challenges such as biological barriers and technical challenges for manufacturing the particulate delivery systems that are slowing the clinical translation of these particulate delivery systems as discussed below.

Challenges face clinical translation of GB nanoparticles.

Nano-medicines could solve many drawbacks associated with many therapeutic agents as discussed earlier. Initially, they were developed to improve the treatment of cancer [75]. More recently, researchers have developed a range of nano-medicine products for the diagnosis and treatment of myriad other diseases. However, the number of nano-medicines currently approved by the FDA or still in the clinical trials is very small compared to the massive volume of research work published, where only 51 nano-medicine products have been approved by the FDA and another 77 are at different stages of clinical trials [75–77].

Biological barriers

We are concerned with formulation of GB NPs for oral application. In this context, we discussed the biological barriers facing the oral route application. Firstly, the potential for particle aggregation at high salt concentrations of biological fluids and the adsorption of proteins onto the surface of NPs, which could also trigger their aggregation [23]. Then after, nano-medicine has to cross the gastrointestinal endothelium to reach the systemic circulation to exert its pharmacological activity, therefore, nano-medicine firstly has to cross the mucus layer covering the epithelium layer and then the intestinal epithelial layer. This mucous has a jelly-like consistency and contains proteins, carbohydrates, antibodies, lipids, cellular debris, bacteria, inorganic salts and water [78];[79]. Mucus is a hydrogel complex, it has a mesh pore size between (10–200 nm), which limits a size threshold beyond which the diffusion of the particles is suppressed[80]. The effect of particle size on the gastrointestinal absorption was previously investigated where Caco-2 and Caco-2/HT-29 cells were treated with spherical, disc-shaped and rod-shaped Polystyrene particles, the rate of NPs uptake was inversely correlated with the particle size [81]. Such effect is not limited to the size, as it was also found that the mucous has an effect on the absorption of the charged entities. One study investigated the effect of the surface charge of NPs; polyethylene glycol-block-polylactic acid (PEG-PLA) nano-particles incorporating differently charged lipid

components (including DOTAP "1,2-dioleoyl-3-trimethylammonium-propane", DSPG "1,2-distearoyl-sn-glycero-3-phospho-(1'-rac-glycerol)" and DOPE "1,2-dioleoyl-sn-glycero-3-phosphoethanolamine", which are cationic, anionic and zwitterionic respectively),while keeping constant size for them all (55 nm), the positive surface charge greatly enhanced the cellular uptake of nanoparticles by both Caco-2 cells in vitro and small intestinal epithelial cells in vivo [82].

It has been stated that particle size and surface charge could markedly affect the crossing of NPs across the previous barriers [83]. Regarding the charge, neutral and positively charged NPs have the ability to penetrate the mucus layer easily [83]. Adding to this, if NPs pass successfully through the mucus layer, they next should cross the epithelium of the gastrointestinal tract [23]. This epithelium is composed of tightly linked cells that are linked together via inter-cellular junctions, which restrict the passage of materials between them [23]. All epithelia are known to reside on a basement membrane that is followed by an underlying connective tissue that contains capillaries, lymph vessels, and lymph follicles [84]. All of these must be crossed firstly before reaching the systemic circulation[84].

Then, within the systemic circulation, opsonin adsorption onto the surface of NPs (formation of protein corona) could facilitate its removal from blood circulation by reticuloendothelial system present in the lymph nodes, liver, and spleen [85]. This could be overcome by attachment of PEG onto the surface of NPs [79]. PEG attachment increased the circulation time of the nano-medicine [79] and it also releases the drug in a sustained and controlled manner for a better management of disease symptoms.

Technical challenges

The production of a pharmaceutical dosage form must be reproducible at the industrial scale [23]. To assure the reproducibility of nano-medicines, additional tests recommended by FDA [23] than those performed for the conventional dosage form are required[86]. For instances, determination of particle size and size distribution, measurement of surface charge, assessment of active ingredients release from formulation, purity, and finally, the stability of the nano-medicine at the biological fluids at the pre-clinical and clinical trials [86]. These properties are crucial for pharmacological effects, as they control the interactions of NPs with cells and other biological components [87]. Size of NPs regulates their circulation and navigation in the circulatory system, penetration across biological membranes, specific localization at certain sites, and also the ability to induce cellular responses [88]; [89]. The NP surface charge has the potential to affect the dissolution, aggregation, and accumulation of NPs [23]. Moreover, to evaluate the pharmacological activity of NPs, the conventional notion of pharmaco-equilibrium theory that relates the therapeutic efficacy to the drug blood concentration that is used in case of conventional dosage forms cannot be simply applied to NPs [86]. Also, higher efficacy to risk ratio must be achieved in comparison to conventional dosage forms [90]. Measuring plasma concentrations of nano-medicine will reflect the nature/number of NPs in circulation, but this solely cannot directly predict the potential pharmacological or toxicological effects [23].

Despite the aforementioned challenges, the nano-delivery approach seems to be promising and a lot of research is directed toward overcoming such challenges in order to produce drug-encapsulated nano-delivery systems on the large scale to be available in the market. For instance, the clinical potential of using liposomes is being researched since the 1980s, where liposomal delivery showed to be useful for improving the therapeutic index of many encapsulated drugs, such as the anti-fungal amphotericin and the chemotherapeutic doxorubicin, where liposomal formulations decreased the toxicity of these drugs in vivo, through modification of the pharmacokinetics and bio-distribution (targeting) to enhance the delivery of the drug to the diseased tissue when compared to the free drug [91].

CONCLUSION

In this review, we have reported several research articles that focused on the improvement of poor GB bioavailability using nano-medicine approach. As described above, the results of investigating different nano-medicine sub-categories (i.e: Polymeric nanoparticles, micro-particles and beads, SLNPs, SEDDs, liposomes, niosomes, nano-crystals and nano-particles) seem to be promising regarding overcoming this problem. Also, it has been mentioned that, although there are still many barriers against the clinical application of the nano-medicine, the success of the clinical application of the liposomal delivery of certain drugs and the commercial and large scale production of these formulations reflect that there is a potential for similar applications using other types of nano-carriers, and as mentioned earlier in this review, niosomes bear several advantages over liposomes, hence their use might be far better than the liposomal counterparts.

From this literature we can conclude that nanotechnology seems to be a promising approach to improve the oral bioavailability of GB, and because liposomal delivery of certain other drugs has been already applied clinically, similar application to GB might be potential. When we consider the several advantages of the niosomal drug delivery system over the liposomal one, we think that niosomal drug delivery of GB may be promising, however, there is still a lack of studies in humans and also the number of studies that evaluate the long term safety of such GB nano-

formulations is limited and hence, future research is strongly required to evaluate these aspects in order to draw a more thorough conclusion about the applicability of this approach on the clinical level.

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Conflict of interest

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