Niosomes: An Advanced Delivery System for Natural Antidiabetic Therapeutics

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***Abstract*—Diabetes mellitus is a global epidemic and due to the search of safer and effective therapy, natural products are a focus area, such as flavonoids, alkaloids, polyphenols as they have strong antidiabetic activities having a variety of mechanisms. Their pharmacological efficacies are, however, highly constrained by low oral bioavailability, low aqueous solubility, inadequate permeability, and fast metabolism. Another potential candidate to this challenge is niosomes, self-assembled non-ionic surfactant-based vesicles. This review is an overview of niosomes as a sophisticated carrier in delivering natural antidiabetic agents. Various compositions, structures, and types of niosomes are mentioned and different fabrication methods and their affordability to different compounds are outlined. The strategies that are important in loading, key characterisation parameters and mechanistic benefits to use niosomes to increase bioavailability, targeting and stability are critically evaluated. A critical analysis of preclinical data shows that there are significant changes in their pharmacokinetics and pharmacodynamics of niosome encapsulation of natural products, which are represented by increased bioavailability 3- to 5-fold and better glycemic control, lipid regulation, and improvement of diabetic complications. There is, however, the issue of scalability, regulatory approval, and long-term toxicity. New opportunities are the perspective of smart, stimuli-responsive niosomes, and hybrid systems, as well as co-delivery of drugs and nucleic acids. The final aim is to translate such niosomal natural products into clinical praxis by means of standard guardlines and properly designed human studies. Niosomes are a revolutionary carrier to be able to explore complete therapeutic potential of natural antidiabetic substances and to provide safer and more effective solutions in addressing the challenge of diabetes epidemic all over the world.**

***Index Terms*—Diabetes mellitus, Natural products, Antidiabetic agents, Bioavailability, Niosomes, Nanocarriers, Drug delivery systems**

I. INTRODUCTION

The Global Diabetes Epidemic

Diabetes mellitus (DM) has become an epidemic worldwide and one of the greatest challenges of the 21 st century. The International Diabetes Federation (IDF) has estimated that there were about 537 million adults living with diabetes in the year 2021 and that this number is expected to surge to 643 million by 2030[1]. This disease, which causes long-term hyperglycemia, results in acute complications such as neuropathy, nephropathy, retinopathy and cardiovascular diseases, which cause unprecedented costs and quality-of-life loss to millions of people every year[2]. The treatment of DM and, especially, T2DM necessitates on-going treatment therapy, and thus the necessity of an effective, safe, and endurable treatment system.

Natural Products in Diabetes Management

In the search for safer alternatives to conventional synthetic drugs (e.g., metformin, sulfonylureas, insulin), which can be associated with side effects like hypoglycemia, weight gain, and gastrointestinal distress[3], the range of natural products has created enormous scientific interest. There are extremely diverse phytochemicals reported to exhibit potent antidiabetic properties by acting in many ways, such as the flavonoids (e.g., quercetin), alkaloids (e.g., berberine), polyphenols (e.g., curcumin, resveratrol), and terpenoids in a broad sense [4]. They also are involved in increasing secretion of insulin, increasing insulin sensitivity, delaying breakdown of carbohydrates in the body (by blocking α-glucosidase and alpha-amylase), increasing assimilation of glucose in peripheral tissues, and reducing oxidative stress and inflammation, which are core concepts of DM pathogenesis entanglement. [5].

The Bioavailability Challenge

Although many agents represent important natural antidiabetic candidates based on their responses in preclinical tests, their applications in the clinical setting are greatly limited by their low pharmacokinetic characteristics. Their key limitations are low water-solubility, unfavorable gastrointestinal absorption, hepatic first-pass clearance, and fast systemic clearance, which culminate in a highly poor oral bioavailability [6]. As an example, the recently hyped polyphenol known as curcumin has a bioavailability of arguably less than 1 percent, severely restricting its applications in therapies [7]. Such a bioavailability obstacle makes it necessary to develop the advanced delivery systems which can allow the full pharmacologic potential of these natural therapeutics to be realized.

The Rise of Nanocarriers

NDDS aim at overcoming the problems created by the traditional formulations by introducing innovative systems that offer a solution to these issues. Of these, lipid-based nanocarriers like liposomes, solid lipid nanoparticles (SLNs) and nanoemulsions, have emerged as having the most promise in empowering the solubility, stability and bioavailability, of poorly soluble drugs [8]. The difficulties that have characterized liposomes e.g. chemical instability, oxidative deterioration of phospholipids, high manufacturing cost as well as deviating purity, have led to the search of new, improved substitutes [9].

Scope and Rationale

This review is dedicated to niosomes as vesicular nanocarriers prepared with non-ionic surfactants and cholesterol that offer a stable, cost-effective, and scaleable industry alternative to liposomes [10]. The overall aim of this review is to critically and comprehensively analyze the use of niosomes as superior delivery vehicle of natural antidiabetic therapeutics. We will discuss the structure of the same, different fabrication methods as well as the characterization techniques. We will also review and provide a critical evaluation of the compelling body of preclinical evidence pertaining to their proven capability to increase the bioavailability and therapeutic efficacy of encapsulated phytochemicals. Last but not least, this review will discuss the current translational issues and future outlook on how niosom-based nanomedicines can be applied to diabetes treatment plans.

II. NIOSOMES: COMPOSITION AND STRUCTURE

Basic Architecture

Niosomes are non-ionic liposome-like structures, which are composed of synthetic, non-ionic surfactants; niosomes self-assemble into closed, bilayered structures in an aqueous media. They resemble liposomes in their structure but take the distinction in being made of non-ionic surfactants as the main building blocks of the membrane rather than phospholipids. The bilayer structure enables the simultaneous entrapment of a wide variety of therapeutic agents: hydrophobic drugs can be incorporated as intercalates within the hydrophobic domain of the bilayer; hydrophilic drugs are entrapable in the aqueous core of the particle formed. (Figure 1) [10]. This versatility make them an ideal delivery system for amphiphilic and less soluble natural compounds.

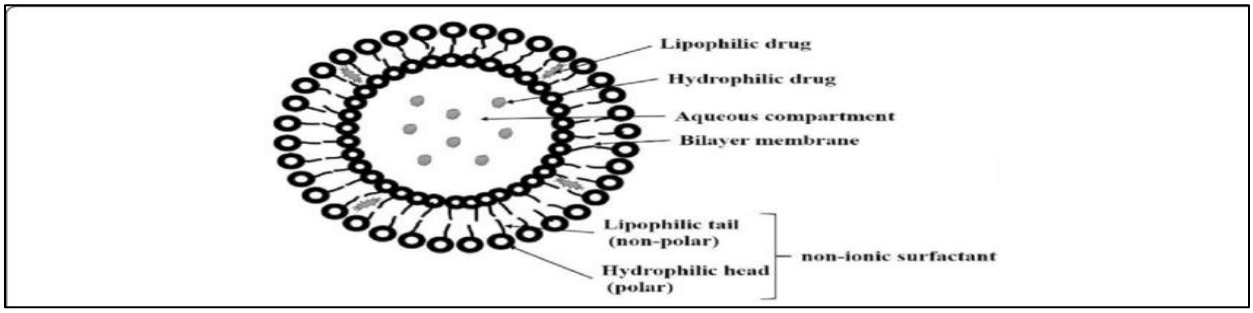

Figure 1. Schematic representation of a niosome structure demonstrating the encapsulation of hydrophilic (in the core) and hydrophobic (within the bilayer) drugs. The membrane is stabilized by cholesterol and may include charge inducers.)

III. KEY COMPONENTS

The formation, stability, and functionality of niosomes are governed by three critical components.

- Non-ionic Surfactants: These constitute the building block of the niosomal membrane. Mainly used are the alkyl ether (Brij), alkyl ester (Span), and alkyl aryl ether (Tween) surfactants. Span 60 (sorbitan monostearate) is especially attractive because of its high phase transition temperature, resulting in formation of stable rigid niosomes with high entrapment efficiency [11]. The main consideration the ability of a surfactant to form vesicles is its Hydrophile-Lipophile Balance (HLB) value. Generally, surfactants which have an HLB range of 4 to 8 are ideally suited to form stable niosomes. Biomimetic fewer HLB vesicles will be larger, more rigid, and preferentially form smaller, more permeable ones except that the addition of cholesterol may be required as well[12].
- Cholesterol: : Cholesterol is also not a trivial excipient; it forms an important part of the niosomal bilayer. It mainly regulates the fluidity and permeability of the membranes. Binding with the hydrophobic tails of the surfactants, cholesterol improves the rigidity of the membrane, and makes the niosomes more difficult to permeate by drugs regularly packaged inside, and also increases the stability of niosomes, suppressing agglomerations and fusions during storage [13]. It simply encloses the membrane and considerably reduces the losses of drugs in it and also yielded a extended time course of release which is critical in maintaining drug levels.
- Charge Inducers: Small additions of charged molecules can have dramatic effects on the physicochemical characteristics and biologic performance of niosomes. The bilayers are charged by insertion of negatively charged molecules such as dicetyl phosphate (DCP) or positively charged molecules such as stearylamine (SA). This surface charge that is measured as zeta potential increases the physical stability of the niosomal dispersion creating a repulsive electrostatic force between vesicles thus preventing aggregation[14]. Besides, mucoadhesion (e.g., due to positively charged mucosal surfaces) can be achieved using surface charge or even targeted delivery.

IV. TYPES OF NIOSOMES

Niosomes may be classified on the basis of size and the number of bilayers as far as they can affect the loading capacity of drugs and circulations between them and biological distribution.

1. Based on Size and Lamellarity:
- Small Unilamellar Vesicles (SUVs): SUV are single-layered vesicles with diameters, typically 10 to 100 nm. Their small sizes are beneficial to penetrate the depth of tissues and to increase circulation time, but they are associated with relatively low aqueous volume and lower capacity to load the drug.
- Large Unilamellar Vesicles (LUVs): LUVs are between 100 to 3000 nm in size and have larger polar core; hence, a better encapsulation of hydrophilic drugs.
- Multilamellar Vesicles (MLVs): These are large vesicles (0.5 to 10 1m) which comprises of multiple concentric phospholipid bilayers. They look like an onion in form. LVs have high encapsulation efficiency of hydrophilic and lipophilic compounds but are associated with low stability and limited in vivo biomedical tool application because of their large size [10].

2. Promosomes: A major breakthrough in niosome technology is the development of promosomes- a free-flowing formulation that contains all the constituents of niosomes in a dry form (surfactant, cholesterol, drug) and the water-soluble carrier (maltodextrin, sorbitol). On simple hydration with warm water immediately prior to use they readily form niosome dispersions. The technique effectively addresses the key physical stability problems of aqueous niosomal suspensions, i.e., aggregation, fusion, and hydrolysis, allowing the extension of shelf-life and industrial scale-up preservation of niosomes[15].

V. FABRICATION METHODS OF NIOSOMES

The fabrication method is a determinant of important niosome attributes, chief among which are size, lamellarity, entrapment efficiency (EE), stability, and, ultimately, scalability to commercial production. Methods include well known laboratory-scale methods to new approaches to technologies that are to be industrialized.

Traditional Methods

- Thin-Film Hydration (TFH) Method: It is also referred to as the hand-shaking method, which is the most used and simply technique of preparation of niosome. It entails the dissolution of the surfactant, cholesterol and medication in an organic solvent (i.e., chloroform or ethanol) in a round-bottomed flask. At reduced pressure, the organic solvent is removed by use of rotary evaporator leaving a thin dry film of the lipid on the inner surface of the flask. The film is then hydrated with aqueous buffer (warmed above the phase transition temperature of the surfactant) with shaking and this results in the spontaneous formation of large, multilamellar vesicles (MLVs) of mostly large size[10]. Though simple and inexpensive, the primary disadvantages are associated with the heterogeneity of vesicle size that usually necessitate subsequent size reduction methods (e.g. sonication or extrusion) and the presence of residual organic solvents.
- Ether Injection Method: In this method, a surfactant-drugs mixture in an immiscible organic liquid (e.g., diethyl ether), is injected into a heated aqueous one (60-70o C) through a needle. An injection is carried out at a steady pace. The spontaneous formation of a one-layered niosome is due to the instant evaporating of the organic solvent when it is washed with the hot, aqueous medium genesis of the single-layered niosomes.[11] . The approach has the benefits of forming relatively uniform vesicles, with no size reduction step necessary. Nevertheless, it has a low encapsulation efficiency when applied to hydrophilic drugs, risks exposure to high temperatures as may degrade thermally sensitive natural compounds, and difficulty in eliminating the organic solvent altogether.
- Reverse Phase Evaporation (REV): Here the drug-surfactant mix is thawed in an organic solvent to form the organic layer. This phase is subsequently blended with a very small amount of water-based buffer (where the hydrophilic drug to be entrenched is present) and sonicated to generate a liquid-oil (l/o) emulsion. The organic solvent is removed under reduced pressure with care, resulting in the reversal of the emulsion and which ends up collapsing, forming niosomes with a high aqueous core in a viscous gel state.[16]. REV is especially beneficial to obtain high encapsulation efficiencies of hydrophilic compounds. The demerits are handling complexities, subjecting the samples to the sonication energy (which has the potential to damage sample properties of some compounds) and scale up difficulty.

Advanced and Scalable Methods

- Microfluidics: This is a new technology which therefore entails fluid control on a micro scale where fluids are handled in microchannels. The blend of the surfactants in an organic solvent (as a water-in-oil blend) and a water-soluble buffer (as an oil-in-water mixture) are pumped through two fluid lines at fixed rates into a microfluidic chip. They combine in a certain mixing architecture, creating a rapid, reproducible nanoprecipitation by the controlled mixing leading to self-assembly of the niosomes to an exceptionally narrow polydispersity (PDI < 0.2) [17]. Microfluidics provides an unprecedented degree of control over the size of the niosome, lamellarity and batch to batch reproducibility, making it ideally suited to clinical translation. Cost of equipments and optimization of flow parameters are present limitations.
- Supercritical Fluid (SCF) Technology: This is an environmentally friendly, solvent-free option that uses supercritical carbon dioxide (scCO 2)as the anti-solvent. The components of the drug and that of the surfactant are dissolved in a solvent, and then it is sprayed into the vessel loaded with scCO2. The scCO 2 extracts the solvent, consequently precipitating the components which upon hydration self-assemble into niosomes [18]. The technique does not use toxic

organic solvents, is well suited to the processing of oil- or heat-labile natural substances, and is scalable. Its complexity and high capital investment turns out to be the inhibitor to its wide usage.

- Transmembrane pH Gradient (Remote Loading): This is not a main formation method but is a strong active loading technique used which is done after formations of niosomes that are empty. It is prepared by synchronizing a certain pH (such as acidic) within the aqueous core of the niosome and carrying them out into an extraneous solvent containing a drug that is base or an acid. A pH difference is created on either side of the membrane The uncharged derivative of the drug freely passes across the bilayer into the interior, where it is ionized and trapped because it cannot diffuse back out again because of its charge state.[19]. This process is remarkably effective in entrapping some natural alkaloids (e.g., berberine) attaining entrapment efficiencies of >90%, which is as high as possible to maximize the number of drugs per unit dose and minimize wastage.

VI. CRITICAL ANALYSIS

Choice of a relevant fabrication process is dependent upon the type of natural compound (hydrophilic/lipophilic, stability), desired vesicle properties, and the intended purpose (lab-scale work 3 vs. commercial manufacturing). The table below entails a comparative analysis of the talked of methods.

Table 1: Critical comparison of niosome fabrication methods.

Method	Principle	Advantages	Disadvantages	Suitability for Natural Compounds
Thin-Film Hydration	Evaporation of organic solvent to leave a thin film then hydration and shaking.	Easy, inexpensive, utilizes normal laboratory equipment, high EE with lipophilic drug candidates	The large size (MLVs), necessitates reduction of size after formation, may have solvent residue.	Good: stagnant, lipophilic compounds (e.g. curcumin). Sensitive molecules may be destroyed in a sonication step.
Ether Injection	Injection of etheric surfactant in hot aqueous phase.	There is no necessity to reduction of size, manufactures SUVs/LUVs	Low EE of hydrophilic drugs, high temperature treatment, ether is hard to eliminate	Unsuitable to thermolabile substances. Care should be taken with solvents
Reverse Phase Evap.	w/o emulsion by formation followed by solvent evaporation and phase inversion.	Large EE on hydrophilic drugs.	Complicated procedure, exposure to the sonication energy, hard to upscale, residue of solvent.	Excellent with hydrophilic extraction. Sonication, and solvents could be harmful to certain sensitive compounds.
Microfluidics	Mixing of organic and aqueous streams in microchannels in a controlled rapid manner.	Excellent control of size, very low PDI, higher reproducibility, scaleable	High initial rates on equipments and it necessitates optimization of parameters.	Very good in all the compounds, so gives better control to protect sensitive molecules. Ideal to cross the preclinical/clinical intersection

Supercritical Fluid	Use of scCO 2 as an anti-solvent to aggregate surfactants and drug	No solvents, low temperatures, high purity, high scalabilit	Very expensive capital cost, process engineering complexity	Best suited to oxygen-sensitive or thermolabile natural antioxidants (e.g. flavonoids). The "green'' standard
Remote Loading	Creation of a PH gradient inside the niosomes to actively entrap ionizable pharmaceuticals following the formation of niosomes	Very large EE (>90%) of specific ionizable drugs, reduces wastage of drugs	It applies only to those drugs that have a particular ionizable functional group (weak bases/acids)	Very appropriate to natural alkaloids (e.g. berberine, Vincristine) but not to non-ionizable compounds

VII. LOADING OF NATURAL ANTIDIABETIC AGENTS INTO NIOSOMES

The effective preparation of the niosomes is dependent upon the effective loading of the therapeutic agent together with the thorough characterization of the resulting nanocarrier. The steps are influential in attaining the desired physicochemical properties, stability, and antidiabetic therapy release profile of the formulation.

Loading Strategies

Two major strategies are employed in the incorporation of natural anti-diabetic agents into niosomes, depending on the nature of the drug involved, i.e. the physicochemical characteristics:

- Passive Loading: This is the largest used where the drug is added in the process of making niosomes. The drug is placed into the blend of initial mixture of the surfactants, cholesterol and the solvent. In the process of vesicles hydration and self-assembly, the drug molecules are entraped by reference to their solubility:

I. Hydrophilic drugs (e.g. many flavonoids e.g., anthocyanins, peptides found in plants) are dissolved in the aqueous hydration medium and become entrapped in the aqueous interior of the niosome.

II. Hydrophobic drugs (e.g., curcumin, resveratrol, berberine in its free (unionized) form) can be co-dissolved in the organic solvent with the lipid components and become intercalated within the hydrophobic bilayer membrane in the process of forming the film[10]. Although passive loading is simpler, it can result in variable and less than optimal entrapment efficiencies because the drug is distributed by its partition coefficient without the force of driving it.

- Active Loading (Remote Loading): It is one method of post formation that applies to weak acidic or basic drugs. Once empty niosomes have been prepared with a difference in pH present in their aqueous core, a pH gradient would be established between the core and the outside of the vesicles. As an example, a poorly soluble base drug (e.g., berberine, an alkaloid) in the neutral, lipophilic form in the outside medium can freely diffuse in and out of the membrane. Once inside the acidic core, the drug is protonated and is charged and therefore impermeable, thus essentially trapping the drug in the aqueous core. [20]. This technique is capable of very high entrapment efficiencies (>90%), has minimal drug loss, and is highly reproducible, advantages that make it ideal with diabetes management specific natural alkaloid drugs.

VIII. KEY CHARACTERIZATION PARAMETERS

Strict characterization is a non-negotiable criterion to ensure successful delivery of niosomes and to predict qualitative or quantitative performance parameters in vitro and in vivo. The parameters that led to the following measures are routine in assessments:

- Particle Size & Polydispersity Index (PDI): Measured predominantly by Dynamic Light Scattering (DLS), the mean particle size (usually reported as Z-average diameter) is a key attribute in determining biological fate. The systemic

administration of smaller vesicles (100-200 nm) is preferred because of better tissue permeation, prolonged circulation time and increased chance of passive targeting [8]. The PDI is a dimensionless parameter of homogeneity of the size distribution. A PDI value <0.3 typically is accepted to be appropriate in a monodisperse, uniform formulation that is essential to reproducible pharmacokinetics.

- Zeta Potential: Electrophoretic Light Scattering can be used to measure the zeta potential that shows the surface charge of the niosomes in suspension. It is the indicator of colloidal stability. A zeta potential that is either strongly positive > +30 mV or strongly negative < -30 mV will produce electrostatic repulsion between the vesicles, and thus guarantee stability (no aggregation) during storage [21]. Moreover, in the case of vehicle surfaces exposed to the oral cavity, charge can be tailored to affect adhesion to the negatively charged mucosal membrane, such as a positive charge to increase odds of increased bioavailability via the mouth.
- Entrapment Efficiency (%EE): This is a decisive parameter to the demands of dosage and efficacy of treatment. It can be obtained by: EE = (Engineered drug in niosomes/the total amount of the drug incorporated into the formula) x100. A high EE is desirable to eliminate drug wastage and to bring down the cost of formulating the formula. Some factors have an effect on the EE:

I. Drug Lipophilicity (Log P): The lipophilicity of the drug tends to indicate higher EE because such drugs are more likely to be more concentrated in the lipid membrane
II. Nature of Surfactant: The HLB value and alkyl chain length of the surfactant alters the stiffness of the bilayer as well as its packing, which can inturn alter drug incorporation.
III. Presence of Cholesterol: Cholesterol makes membrane very rigid and also decreases permeability, which commonly results in an increase of EE by preventing drug leakage.
IV. Fabrication Method: Techniques such as REV and remote loading are to be used to attain high EE of hydrophilic and ionizable drugs, respectively[22].
V. Morphology: TEM and SEM can or should be used to verify that the niosomes are spherical and vesicular and to confirm the particle size data measured by DLS. Transmission electron microscopy, commonly with negative staining (such as with phosphotungstic acid), can give detailed information on the lamellarity of the vesicles (e.g., uni- or multi-lamellar)[23].

- In Vitro Drug Release: In this study, the drug release kinetics is determined in simulated physiological conditions (e.g. PBS pH 7.4, 37 C) using dialysis bags or membrane diffusion method. The ability to maintain a sustained release profile over several hours to multiple days is a very important benefit of niosomes as it allows a reduction in the frequency of administration and/or maintenance of congruent drug levels. Release data is also correlated to different mathematical models (e.g., zero-order, first-order, Higuchi, Korsmeyer-Peppas) in order to uncover the underlying release mechanism (e.g. diffusion, erosion, swell) [24]. Ideal niosomal formulation in antidiabetic therapy must be limited to a concentrated, steady state delivery as opposed to the burst of the free drug.

IX. THE MECHANISTIC EDGE: HOW NIOSOMES ENHANCE THERAPEUTIC OUTCOMES

An important therapeutic advantage of the encapsulation of natural antidiabetic agents within niosomes is the overall benefit of the delivery of the compound in the encapsulated form rather than the free one. This advantage is based on the fact that niosomes have the capability to essentially change the pharmacokinetic and pharmacodynamic profile of the loaded drug via a several complex biological processes.

Enhancing Bioavailability

The major obstacle that niosome technology intends to address is poor oral bioavailability of most natural compounds. It is realized in a complex mechanism:

- Protection from Degradation: The niosomal bilayer is a physical barrier that mediates the protection of the encapsulated drug against gastrointestinal (GI) environmental factors (chemical and enzymatic degradation). That safeguards acid-labile agents against gastric pH and keeps gut luminal enzymes an exposure

away; producing a greater fraction of the intact compound to arrive on absorption site[25].

- Improved Solubilization: Certain promising antidiabetic phytochemicals (i.e., curcumin, resveratrol) are very hydrophobic. Disintegration of theirs in the GI fluids is a rate-limiting step to absorption. Encapsulation in niosomes solubilises these in the hydrophobic membrane or hydrophilic core in a molecularly dispersed form and this is conveniently accessible to absorption thus increasing the effective concentration gradient[26].
- Enhanced Permeability: The Spans or Tweens added in the niosome preparation process are known permeability enhances. They may temporarily impair the tight junctions between the cells of the intestinal wall (paracellular pathway) or liquefy the cellular membranes (transcellular pathway) effectively aiding the drug payload to be absorbed by the body[27]. This is especially advantageous to compounds that exhibit low membrane permeability or high molecular weight compounds.
- Lymphatic Absorption: Niosomes, particularly those prepared using lipids and surfactants can be internalized in the M-cells of the Peyer patches of the intestine thereby entering the lymphatic system. This route avoids the hepatic portal vein and the first-pass disposition in liver system, and hence the drug appears in systemic circulation early, dramatically enhancing its bioavailability [28].

Facilitating Targeted Delivery

Niosomes can be developed to enhance the delivery of drugs to the sites of interest as far as diabetes pathology is concerned.

- Passive Targeting: This is based on type of pathophysiology of diseased tissues. Low-level inflammation and leaking of the microvasculature of tissues such as the liver, pancreas and kidneys may be caused by persistent hyperglycemia in chronic diabetes. This compromised vasculature can allow the leakage and concentration of nanocarriers such as niosomes (100-200 nm diameter) via what is known as the Enhanced Permeability and Retention (EPR) effect [29]. Although there is controversy concerning universality of the EPR effect in human diabetes, it is a possible passive targeting to inflamed tissues.
- Active Targeting: This is a further-developed and precise plan. It is the grafting of niosomes with activating ligands (e.g., antibodies, peptides, vitamins, carbohydrates) that have affinity to over/expressed receptors on the target cells. In diabetes, this may comprise:

I. Liver Targeting: Due to liver being the key to controlling insulin resistance and gluconeogenesis, galactose or lactose ligands can be used to target the hepatocytes via asialoglycoprotein receptors in the liver.

II. Pancreatic Beta-Cell Targeting: the use of ligands such as GLP-1 analogues or specific antibodies to target receptors on the insulin-producing β- cell to enhance their survival and activity.

III. Macrophage Targeting: Diabetes wounds or other inflammatory illnesses can be targeted to macrophages using mannose ligands to achieve active targeting to the inflamed site and reduce undesirable off-target effects and dosage [30].

Improving Stability

Very sensitive to prevailing environmental conditions, most of the naturally occurring antioxidants and polyphenols tend to lose their effectiveness before they are even administered to the patient.

- Sheltering from Light and Oxygen: Some chemicals, e.g. flavonoids and carotenoids are sensitive to photo-oxidation and chemical degradation. The niosomal bilayer creates a sheltered, hydrophobic environment that safeguards these unstable molecules against UV light and molecular oxygen to considerably expand their shelf-life and in-use stability [31].
- Protection from pH Extremes: The inner aqueous layer of a niosome can result in a microenvironment that is dissimilar to the bulk-phase. This may be used to shield pH-sensitive agents against the highly acidic pH of the stomach or the fluctuating pH of the GI tract so that the drug remains stable until release at the desired site. The combination of these properties' bioavailability, site-specific delivery, and

stability, turns these natural and yet pharmaceutically unacceptable antidiabetic agents to promising and efficacious drug candidates.

X. PRECLINICAL EVIDENCE: A CRITICAL REVIEW

Interesting theoretical superiorities of niosomes are backed with the rising preclinical evidence. Critical analysis of the current in vitro and in vivo studies that show improved therapeutic efficacy of natural antidiabetic agents in niosomal systems is provided in this section.

In Vitro Studies

In vitro experiments hold the grounds of greater activity, stability and cellular uptake. A summary of the major findings of some of the studies is presented in the table below.

Table 1: Overview of in-vitro studies of niosomal finalised formulations of natural anti-diabetic compounds.

Natural Compound	Niosome Composition	Cell Line / Model	Key In Vitro Findings	Reference
Curcumin	Span 60, Cholesterol, DCP	L6 myoblast cells	Niosomal curcumin showed significantly higher cellular uptake and glucose uptake stimulation compared to free curcumin.	[32]
Berberine	Span 60, Cholesterol	Caco-2 cell monolayer	Apparent permeability (P	[33]
Quercetin	Brij 52, Cholesterol	α-amylase/α-glucosidase	Niosomal quercetin exhibited superior enzyme inhibition (IC	[34]
Fenugreek Extract	Tween 61, Cholesterol	INS-1 pancreatic β-cells	Niosomal extract significantly enhanced insulin secretion under hyperglycemic conditions and provided better protection against oxidative stress.	[35]
Gymnema sylvestre	Span 40, Cholesterol	L6 myotubes	Niosomal formulation increased glucose utilization by 50% compared to untreated cells and raw extract.	[36]

In Vivo Studies (Animal Models)

The greatest evidence is in in vivo studies done in animals with diabetes in which pharmacokinetic (how the body changed the drug) and the pharmacodynamic (how the drug altered the body) factors are measured

Table 2: Summary of in vivo work on niosomal formulations in diabetic animal models.

Natural Compound	Niosome Composition	Animal Model (Induction)	Key Findings (vs. Free Drug)	Reference
Curcumin	Span 60, Cholesterol	Rats (STZ)	PK: 4.8-fold ↑ in AUC, 3.9-fold ↑ in C_{max}. PD: 2.1-fold greater reduction in blood glucose; significantly improved lipid profile & antioxidant markers (SOD, CAT).	[37]
Berberine	Span 60, Cholesterol, SA	Rats (HFD/STZ)	PK: 3.1-fold ↑ in AUC. PD: 68% vs. 42% reduction in FBG; greater decrease in HbA1c; enhanced hepatic glycogen content	[38]
Quercetin	Brij 30, Cholesterol	Mice (STZ)	PD: Sustained hypoglycemic effect over 24h; niosomes restored pancreatic architecture and insulin levels more effectively.	[39]
Cinnamon Extract	Span 40, Cholesterol	Rats (Alloxan)	PD: Faster and prolonged reduction; significant improvement in insulin sensitivity and liver function enzymes compared to free extract	[40]

Naringenin	Tween 80, Cholesterol	Rats (STZ)	PK: 5.2-fold ↑ in relative bioavailability. PD: Superior renoprotective effects: reduced serum creatinine, BUN, and kidney oxidative stress	[41]

Abbreviations: STZ: Streptozotocin; HFD: High-Fat Diet; PK: Pharmacokinetics; PD: Pharmacodynamics; AUC: Area Under the Curve; C max: Maximum Concentration; FBG: Fasting Blood Glucose; HbA1c: Glycated Hemoglobin; SOD: Superoxide Dismutase; CAT: Catalase; BUN: Blood Urea Nitrogen.

XI. CRITICAL ANALYSIS OF RESULTS

A critical review of the preclinical evidence presents a number of coherent trends and considerations to bear in mind:

- Dramatic Bioavailability Enhancement: The only area where there is complete unanimity among studies is that there is pronounced drug bioavailability enhancement. The comparative 3 to 5-fold jump in AUC of such compounds as curcumin, berberine, and naringenin is revolutionary. This is imminently reflecting upon the improved pharmacodynamic performance, confirming the main supposition that niosomes help to neutralize the main drawback of natural compounds
- Impact of Surfactant Choice: The results indicate tendencies to high-phase transition surfactants of longer alkyl chain. The food additive Sorbitan monostearate (Span 60) is overrepresented in successful papers (e.g., [32] , [33], [37] , [38]). This has been explained by its capacity to yield a stiffer, more stable and less permeable bilayer which improves entrapment efficiency and protects payload more effectively than more fluid surfactants such as Tween 80.
- Beyond Glycemic Control: The observed therapeutic benefit is much more than glycemic reduction alone. The most notable and reproducible effect is the reduction of diabetic complications at a more satisfactory level. Research on naringenin [41] and curcumin [38] demonstrates increased antioxidant and anti-inflammatory response, which results in significant anti-nephropathy and anti-hepatopathy outcomes. This indicates niosomes are highly functional in the delivery of pleiotropic effects of phytochemicals.
- Inconsistencies and Gaps: There are crucial gaps in the results, which are more positive than negative.
- Lack of Standardization: There is lack of standardization of niosome composition (e.g. surfactant: cholesterol ratio or fabrication protocols and animal models (STZ vs PKD vs Alloxan vs HFD). The field is hampered by such a deficiency of a standardized so-called gold standard formulation.
- Limited Long-Term Toxicity Data: The majority of the in vivo findings are acute or sub-acute in nature. It is not well studied how niosome chronic administration influences long-term biodistribution and toxicity of chronic accumulation of the surfactants.
- Mechanistic Depth: Some studies indicate that niosomes are superior, although often without much insight into the underlying molecular mechanism(s) that cause the magnified pharmacodynamics (i.e., which signaling pathway is preferentially upregulated by the niosomes?).

XII. CHALLENGES AND FUTURE PERSPECTIVES

Although the effectiveness of niosomal formulations in the laboratory demonstrations as an antidiabetic agent is valid, some challenges are anticipated in the transfer of niosomal formulations to commercialized drugs. Overcome these obstacles and adopt emerging trends are the key to the best practice in utilizing this technology.

From Bench to Bedside: Translational Hurdles

There are some important hurdles in the path between research and clinical application and they need to be overcome in a stepwise manner.

- Scalable GMP Manufacturing: Many of the fabrication techniques discussed in Section 3 (i.e., thin-film rehydration, ether addition) are optimized to be used in small scale laboratory production and can be cumbersome, inefficient, or expensive to scale up to large scale industrial scale. Techniques: Microfluidics and supercritical fluids S: Great potential but huge capital investment and process optimisation required J: Techniques like microfluidics and supercritical fluid technology have great potential in terms of scalability, yet are very expensive in both capital investment and process optimisation to achieve Good Manufacturing Practice (GMP) standards of reproducibility, quality control and sterility[42]. The main distraction in bridging this valley of death between laboratory scale innovations and industrial level production is the focus of this challenge.
- Regulatory Pathway: The regulatory pathway of nano-phytomedicines, i.e., complex combinations of plant extracts in the form of a novel delivery system, is particularly uncertain. Organizations such as FDA and EMA have no guidelines as to such hybrid products and this has cast a doubt in the minds of those developing them. The key regulatory concerns include:

I. Safety and Toxicity Profiling: Although there is general concern that non-ionic surfactants are considered to be safe, the long-term fate in vivo and chronic toxicity of non-ionic surfactants and their degradation products remain poorly investigated. The effects of repeated-over time dosing, potential tissue accumulation of the surfactants, their immunogenicity, and interactions with organ functions, are all questions that are still inadequately answered[43].

- Characterization: A complex nano carrier that entails the use of a natural product that is naturally variable in itself, presents a major challenge in demonstrating batch-to-batch consistency.
- Sterilization and Storage Stability: Niosome dispersions in aqueous medium are prone to physical instability with time. The problematic areas are:

I. Aggregation and Fusion: The vesicles have the capability to aggregate and fuse which increases the size of the particles and might result in precipitation.

II. Drug Leakage: During storage, the encapsulated drug can leak out of the vesicles and this cuts down entrapment efficiency and efficacy.

III. Hydrolysis/Oxidation: Components may be subject to chemical breakdown.Sterilization procedures such as autoclaving (heat) can degrade the vesicles and some sterilization techniques, such as filtration, may not be feasible in large niosomes. Strategies such as promosome technology (dry, free-flowing powders that can be reconstituted prior to use) provide a solution to such stability issues[15].

XIII. EMERGING TRENDS AND FUTURE DIRECTIONS

In order to overcome these difficulties attention has been paid to the development of second generation niosomes with improved functionality.

- Smart Niosomes (Stimuli-Responsive): Next generation formulations will shift towards smart-designs that release their cargo in response to a given biological stimulus. In the case of diabetes this covers:

I. pH-Responsive Niosomes: Can be prepared to be stable in physiologicalpH but to disintegrate and deliver contents within the more acidic microenvironment of inflamed tissues (often

seen in diabetes) or in endosomal vesicles after cellular internalization.

II. Enzyme-Responsive Niosomes: Designed to be cleaved by enzymes which are overproduced in the diabetic state (e.g. matrix metalloproteinases in locations of vascular injury) thereby resulting in localized release of drugs[44].

III. Hybrid Niosomes: A union of the benefits of niosomes with others: Innate limitations can be countered by combining niosomes with other materials:

- Polymer-Hybridized Niosomes: This is done by covering niosomes with polymers such as chitosan or poloxamers and this can aid mucoadhesion in oral delivery or provide steric stabilization to prolong their circulation
- Bile Salt-Incorporating Niosomes (Bilosomes): The addition of bile salts to the bilayer produces vesicles that are not dissolved by the bile acids in the GI tract which makes their administration to the mouth an excellent delivery mechanism to accomplish antigen-specific immunotherapy in diabetes type 1[45].

- Gene Delivery (Co-Delivery Systems): The co-delivery of antidiabetic drugs and nucleic acids (e.g., siRNA, miRNA) is one of the most promising future directions of niosome applications. As an example, a niosome may be fabricated to carry metformin and siRNA targeting a major enzyme involved in gluconeogenesis (e.g., PEPCK) in a synergistic therapeutic effect that alleviates multiple underlying pathological processes simultaneously[46].
- Clinical Translation: The ultimate goal. Future work must focus on:

I. Standardized Protocols: Design and assure the standardized protocols in formulation, characterization, and long-term maintenance: this will ensure reliability and make it agreeable to licensure.

II. Well-Designed Clinical Trials: Conducting rigorous human trials to definitively demonstrate the better pharmacokinetics, efficacy and safety of niosomal natural products over current standard therapies. This is the crucial phase that has to be achieved so as to move niosomes as a potential preclinical tool to real therapeutic possibility

XIV. CONCLUSION

The extensive discussion provided in this review is conclusive in the development of niosomes as perhaps one of the most modernized and adaptable delivery mechanism that is ready to transform the natural antidiabetes treatment. Due to its bilayered structure, niosomes can overcome arguably the biggest problem with phytochemicals bioavailability. They do this by increasing solubility, protect their payload against degradation, overcome membrane permeability and permit lymphatic absorption therefore achieving a much greater fraction of the administered dose entering the systemic circulation and reach target sites.

By alleviating these major pharmacokinetic deficiencies, niosomal technology offers a potent and game-changing modality to tap the abundant multi-target therapeutic applications of natural products such as curcumin, berberine and quercetin. This strong body of preclinical evidence not only shows a better pharmacokinetic profile but also a better pharmacodynamic outcome such as better glycemic and lipid control as well as improvements in diabetic complications, which are well beyond the capabilities of the free compounds.

Nevertheless, there is a still lengthier distance between bench and bedside. Although the preclinical accomplishments are mind-blowing, clinical uptake will depend on the ability of scientists to address the existing translational issues through dedicated and cooperative beats. The key priorities are designing scalable and GMP-compliant production processes, preclinical toxicity testing, and a long-term follow-up, as well as adapting to the environment of regulatory requirements that is changing fast in regards to nano-phytomedicines. By overcome these obstacles, the vast potential of niosomes can be converted into reality with respect to in vivo study findings into a viable clinical reality and ultimately provide millions of diabetic patients with safer, more effective and natural treatment options.

APPENDIX

None

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