Review Article

A Review on Resveratrol-loaded Liposome and Its Characterisation

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Abstract

Resveratrol (3,5,4′-trihydroxystilbene) is a stilbene-type natural polyphenol found in a variety of plant species, including grapes, berries, peanuts, and red wine. Many studies have demonstrated that resveratrol as a nutraceutical supplement, exhibits numerous pharmacological properties such as antioxidant, anti-inflammatory, cardio-protective, neuroprotective, chemo-preventive, and anti-aging. Despite its health-beneficial effects, resveratrol has extremely low water solubility, high absorption, and rapid metabolism that consequently reduces its oral bioavailability. Hence, there have been a growing interest in a liposome as a carrier to optimise its limitations over the past few years. The purpose of this research is to review the potential of liposomes as a suitable carrier to optimise resveratrol limitations and evaluate characteristics pertaining to resveratrol-loaded liposomes based on the articles that have been published. The method for literature search is based on three search strategies: 1) database searches (PubMed free search engine from the United States National Library of Medicine and the Science Direct browser from Elsevier); 2) web-based searches (Google Scholar); and 3) citation searches. The selected articles were restricted from 2016 to 2021 in English, where 107 articles were chosen. This review summarises the findings of liposomes incorporating resveratrol in recently published articles and highlights the potential of liposomes in improving resveratrol limitations, consequently, promoting its pharmacological effects. Therefore, characteristics such as particle size, morphology, *in-vitro* drug release, entrapment efficiency, and stability test, were also evaluated. Resveratrol-loaded liposomes provide excellent solubility, stability, improved drug release, and high entrapment efficiency; thus, it may increase oral bioavailability and efficacy, both of which would promote its pharmacological effects.

Keywords: Resveratrol, bioavailability, liposome, liposome characterisations

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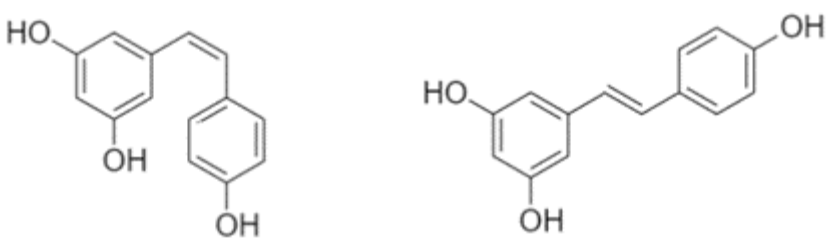
1.0 Introduction

Nutraceutical supplements have recently gained attention from consumers due to their purported beneficial effects, including enhancing health, preventing diseases, or supporting the overall function of the human body (1) and raising awareness among consumers to have healthy lifestyles (2). Nutraceutical is a modern term defined as a dietary health-promoting supplement including food or part of it (3). Some consumers believe that nutraceutical supplement consumption may treat or at least prevent disease in more 'mild' or natural ways than over-the-counter drugs (4).

Resveratrol, or trans-3,5,4′-trihydroxy-stilbene is a naturally occurring polyphenol compound that belongs to the stilbene group (5,6). It has been detected in more than 70 plant species and synthesised in those plants as phytoalexin due to mechanical injury (7) and response to biotic and abiotic stress (8), including pathogen attack (infectious) (9), ozone stress, or ultraviolet (UV) light exposure (7). Resveratrol also exists in human diets, including red wine, peanuts, berries of *Vaccinium* species (10) and other berries (blueberries, bilberries, and cranberries), as well as in chocolate and other cocoa products (11–13).

Their chemical structure consists of two phenol rings linked by an ethylene bridge, and they exist in two isomeric forms: cis- and trans-resveratrol (Fig. I) (7). The trans-isomer is the most stable and displays the best pharmacological properties compared with cis-isomer due to the presence of 4′-hydroxystyryl group in the former's structure (14).

Resveratrol has a wide range of pharmacological properties, including antioxidant, anti-inflammatory, cardio-protective, neuroprotective, chemo-preventive, and anti-aging activities (Fig. II) (7,9,15). Resveratrol potential in improving human health outcomes has attracted a growing interest by scientists in the nutraceutical field over the past few years (16), especially after the alleged "French paradox" (17). The "French paradox" refers to low incidence of mortality rate and improved cardio-protective effects with moderate intake of high concentration red wine despite a high-fat diet in the French population (18). Although resveratrol is very popular due to many favourable results *in-vitro*, the drug's future becomes questionable because of its unfavourable pharmacokinetic properties once administered *in-vivo* and the limited physicochemical characteristics of its natural compound (19). The main problems that limit the effectiveness of resveratrol pharmacological properties are extremely low water solubility and high absorption with rapid metabolism, which consequently reduce their oral bioavailability (20).



cis-resveratrol trans-resveratrol

Figure 1: Chemical Structure of Resveratrol (cis- and trans-isomer).

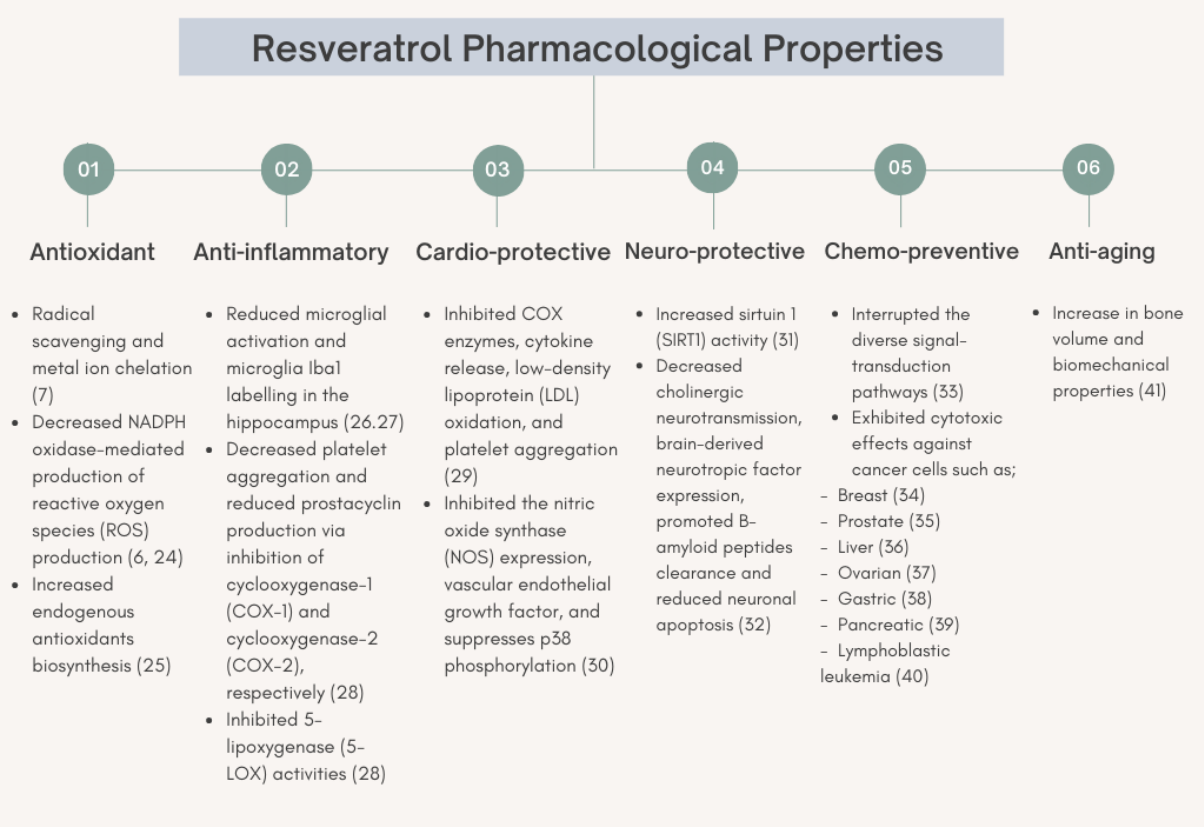


Figure 2: The pharmacological properties of resveratrol and the mechanism(s).

Classified as a Biopharmaceutical Classification System (BCS) Class II drug, with high permeability but poor water solubility (4,39,40), resveratrol is a fat-soluble compound, and is "practically insoluble" in water based on the European Pharmacopeia definition (41) with a water solubility of less than 0.05 mg/ml (20). It undergoes rapid and extensive metabolism into its metabolites (glucuronide, sulfate, or free form) with high binding properties to plasma proteins such as albumin and lipoprotein (20), thus, increasing its elimination and resulting in a low systemic concentration. As a result, the oral bioavailability of resveratrol is extremely low at less than 1%, making it impossible to achieve the pharmacological effects after oral administration (40).

Most of the resveratrol supplements available in the market currently are in the conventional dosage form, such as "21st Century Resveratrol Red Wine Extract" (21st Century HealthCare, Inc.) for antioxidant support and "NOW Foods Natural Resveratrol" (NOW Health Group, Inc.) for cardiovascular support, which both can be obtained in the capsule dosage form. However, due to limitations in the physicochemical and pharmacokinetics of resveratrol when administered in such dosage forms, formulation scientists are constantly developing new drug delivery technologies to overcome the limitations. Among the latest technologies, resveratrol-loaded liposomes have been developed as one of the strategies to enhance the pharmacological properties by overcoming their limitations (15,42,43). One of the supplement examples is "Nature's Essentials Resveratrol Trans-Formula 500 mg" (Nature's Essentials, Inc.) for age defense and DNA revitalisation. The product utilises liposomal plus cyclodextrin technology to stabilise and increase the bioavailability of trans-resveratrol for

better absorption in the intestine and cells. Several other nanotechnology-based carrier formulations have also been developed, such as polymeric nanoparticles (44,45), polymeric micelles (46), lecithin-based nano-emulsions (47), and cyclodextrins (48,49) as a promising carrier for delivery of resveratrol.

The purpose of the study is to review the potential of the liposome as a suitable carrier to optimise resveratrol limitations and evaluate the characteristics pertaining to the resveratrol-loaded liposome based on the article that has been published.

2.0 Methods of Literature Research

All relevant articles were identified using three search strategies: 1) database searches; 2) web-based searches; and 3) citation searches. The primary academic databases are the PubMed free search engine from the United States National Library of Medicine and the Science Direct browser from Elsevier. Concurrently, Google Scholar is a web-based search tool. The relevant articles were limited to the English language and from 2016 to 2021 using search keywords "resveratrol", "bioavailability", "liposome drug delivery system", and "liposome characterisation". Additionally, some of the individual citations in the reference lists of the selected articles were then manually searched on Google Scholar for the related citations. The title and abstract of the articles were reviewed to determine if the study involves resveratrol-loaded liposomes and its characterisations. Accordingly, 107 articles were chosen for this review.

3.0 Liposome as carrier for delivery of resveratrol

For centuries, the oral route of administration for drug delivery has been the preferred route due to its efficacy, safety, compliance, and cost-saving (50). The oral route has several disadvantages for certain drug compounds, such as low solubility, inefficient absorption, rapid metabolism, or poor permeability. Hence, formulation scientists are continuously developing technologies for new drug delivery. Liposomes are known as versatile assemblies and a promising approach in oral drug delivery system, among those technologies (51).

Liposomes are bilayer membranes formed via the self-assembling of natural, non-toxic phospholipid, and cholesterol (16,52,53). They consist of the aqueous compartment and the lipid bilayer to entrap hydrophilic, hydrophobic, and amphiphilic compounds (54,55). Liposomes are mainly composed of phospholipids (51) and can form natural and synthetic liposomes (56). The liposome appears to be spherical with a size ranging from nanometre to micrometre (57). Due to their hydrophobic and hydrophilic characteristics, liposomes are an excellent system for drug delivery. Their advantages include an increased efficacy and therapeutic index of the drug, increased stability due to drug encapsulation, the liposomes are non-toxic, completely bio-degradable, provide a sustained-release drug delivery, and flexible to couple with site-specific ligands to achieve active targeting (58). Nevertheless, they have several disadvantages such as phospholipids components in liposomes tend to undergo oxidation and hydrolysis process, drug leakage and fusion of the vesicle, high production cost, and short half-life leading to less stable liposomes (59,60).

Phospholipids are the primary building blocks of liposomes. The common sources of natural phospholipid are animal tissues (bovine brain and egg yolk), vegetable oils (soya bean, corn, cottonseed, sunflower, and rapeseed) (51), and plant oils (olive oil). The most widely-used phospholipids in liposome formulations are phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol, among others (61). Phospholipids aid in overcoming poor solubility and low bioavailability of various active compounds (62). The cholesterol incorpo-

rated in the lipid membrane helps increase liposome stability and reduce membrane permeability (56). There are various classes of liposomes reported, and they are classified based on size, the number of bilayers, composition, and methods of preparation.

The main target of an ideal liposome preparation is to achieve efficient drug entrapment, low particle size distribution, and long-term stability, especially during storage. Generally, there are four primary stages involved in the preparation of liposomes (51,53): drying of lipid via organic solvent evaporation; the lipid dispersion in an aqueous media; liposome purification and analysis of the final product.

One of the critical methods is the liposome preparation and loading of the hydrophilic or hydrophobic drugs. The methods are divided into two, which are active loading and passive loading techniques. Active loading, also known as gradient loading, refers to methods of incorporating drugs into vesicles after the liposome preparation, whereas passive loading technique involves incorporating drugs during the liposome formation (60). Passive loading technique includes three different methods such as mechanical dispersion methods, solvent dispersion methods, and methods based on fusion or size transformation of the prepared vesicle (51,63).

3.1 Mechanical methods

The mechanical dispersion method is divided into three types: thin-film hydration, sonication or ultrasonication, and French press cell (extrusion). The thin-film hydration method is simple, thus the most common and widely used method for liposome preparation. In this technique, the phospholipid is dissolved in an organic solvent such as chloroform or dichloro-methane, ethanol, or chloroform-methanol mixture. Later, the organic solvent is removed by film dispersion under vacuum at temperatures 45-60°C (55,56). After complete evaporation of the organic solvents, the lipid film is hydrated using an appropriate buffer (distilled water, phosphate buffer, or normal saline water) to form a liposome (63). The lipids undergo swelling and hydration to form a liposome. The disadvantages of this procedure are the large amount of organic solvent use may cause the presence of organic solvent residue and the procedure can also be time-consuming (57).

Sonication or ultrasonication method is the most widely used method for SUV preparation. The aqueous dispersion of phospholipids (MLVs) is sonicated with energy using probe sonicator or bath sonicator under passive atmosphere, yielding SUVs with smaller diameters (63,64). The released energy generates high pressure and is responsible for particles collisions (57). The main drawback of this method is that it is not suitable for large-scale production, and liposomes can be damaged due to overheating during the process (60).

Furthermore, in French pressure cell or extrusion method, the aqueous dispersion of phospholipids (MLVs) undergoes extrusion through the filter of extruder apparatus under high pressure to form SUVs (57). Several parameters such as applied pressure, numbers of cycles, and pore size of the filter may influence the diameter of liposomes. The method is simple, fast, and reproducible but difficult to control high temperature during the process and may result in clogging of the filter pores (65).

3.2 Solvent dispersion methods

Solvent dispersion methods include ether injection and ethanol injection methods. In ether injection method, phospholipids mixture is dissolved in an organic solvent (diethyl ether, chloroform, methanol, or ethanol) and slowly injected into an aqueous solution at 55°C to 65°C under reduced pressure. This is followed by removal of the organic solvent to create the liposomes (55). The main disadvantages of ether injection methods are the exposure of compounds to be encapsulated to high

temperatures and obtaining heterogeneous vesicles (56).

While the ethanol injection method requires an ethanol-lipid solution that is rapidly injected in a huge volume of the buffer, such as preheated distilled water under stirring conditions to form the liposomes (55), this method offers many advantages: fast, simple, reproducible, and easy to scale up (56). However, the drawbacks are difficulty in removing the solvent (ethanol) and the heterogeneous population of the obtained vesicles (63).

3.3 Fusion or size transformation of prepared vesicle methods

In freeze-thawed extrusion methods, the liposomes formed by the thin-film method are vortexed with the compounds to be encapsulated until the entire lipid film is suspended. The liposome formed will freeze in dry ice or acetone, be thawed in warm water, and vortexed again. After repeating the steps for two cycles, the sample is extruded three times. The freeze-thaw step is repeated about six cycles. This was followed by six cycles of freeze-thaw and another eight extrusions (55,64). The freeze-thawed extrusion method is frequently used in liposomes preparation because of its high entrapment efficiency (55). Nevertheless, the method is time-consuming and may result in high product loss (55).

Another type of fusion or size transformation of prepared vesicle methods is dehydration-rehydration. In this technique, the empty buffer containing liposomes is mixed with the compounds to be encapsulated and dried using freeze-drying, vacuum, or under a stream of nitrogen (55). As the vesicle becomes more concentrated during dehydration, they are rehydrated, forming larger vesicles (55).

4.0 The advantages of liposome as carrier for delivery of resveratrol

Nanotechnology-based carriers to encapsulate bioactive natural compounds, such as polyphenol (resveratrol), have many advantages, such as improving bioavailability and therapeutic activities. Liposomes could also improve polyphenol solubility and stability, thereby increasing their bioavailability and efficacy (66). Besides, polyphenol interaction with the liposome lipid bilayer helps increase the entrapment efficiency and drug release rate of polyphenol. Incorporating cationic cholesterol in the liposome formulation causes trans-resveratrol to encapsulate deeply into the lipid bilayer (66).

Moreover, the low solubility and poor bioavailability of resveratrol could be improved by incorporating it into the liposomal formulation (62). For example, the chitosan-liposomal formulation helps solubilise resveratrol, and the chitosan-coated liposomes may facilitate optimal vaginal drug delivery with prolonged release, thus enhancing resveratrol antioxidant and anti-inflammatory properties for vaginal inflammation (62).

Besides, resveratrol encapsulation in different types of liposomes via membrane-binding fluorescent probes influences the binding of resveratrol to the lipid cell membrane and influencing membrane properties and functions while enhancing the pharmacological properties of resveratrol via its location within the lipid cell membrane (resveratrol dehydrated the cell membrane due to the affinity of hydroxy groups in resveratrol's structure to the aqueous surrounding the lipid membrane) (67).

The resveratrol-loaded liposomes were improved and more effective in a study on the anticonvulsant activity than free resveratrol, resulting in a reduction in epileptic seizures compared to free resveratrol (68). The liposomal formulation also allows for sufficient release, increased resveratrol stability and biological activity against oxidative damage, and reduced cytotoxicity; thus, liposomal formulation can result in better antioxidant and anticonvulsant effects (68).

Based on the liposome preparation methods, most research articles adopted the thin-film hydration methods to form the resveratrol-loaded liposomes, followed by sonification or extrusion methods. The comparison between thin-film hydration method, ethanol injection, and inverse evaporation method to prepare resveratrol-loaded temperature-sensitive gel liposomes illustrated that the thin-film dispersion method was more suitable for resveratrol as a lipophilic drug (highest entrapment efficiency. The other two methods showed a low entrapment efficiency and large particle size distribution, resulting in unstable liposomes. Nevertheless, several factors may contribute to the quality of the liposomal preparation, such as the ratio of drug to phospholipid, hydration temperature, ultrasonic input, and sonification duration. Despite the methods, the results indicated that resveratrol-loaded temperature-sensitive gel liposomes provide a long-lasting and stable drug release, which could promote the repair of injured sciatic nerves in rats (69).

5.0 Characterisation of resveratrol-loaded liposome

5.1 Particle size analysis of liposome

Physicochemical properties like the particle size and size distribution are the fundamental interest in the liposome drug delivery system because they can affect the vesicle's *in-vitro* characteristics such as the amount of drug that can be taken in, aggregation, and sedimentation behaviour (70). Particle size is also an important parameter, as it is associated with stability, encapsulation efficiency, drug release, bio-distribution, mucoadhesive, and cellular uptake (71), as well as the key to a successful formulation. The polydispersity index (PDI) is used for particle size distribution and the particle size degree of non-uniformity (50). PDI value below 0.3 is considered within the acceptable limit and indicates the homogeneity of the liposome formulation.

The particle size and size distribution of liposomes can be monitored and controlled by various procedures, including sonication, extrusion, and homogenisation (50,51). The most frequent techniques used to determine the particle size of liposomes are the dynamic light scattering (DLS) technique, also known as photon correlation spectroscopy (PCS), static light scattering technique, and electron microscopy (72). Among all techniques, PCS has the advantages of yielding an accurate result and is fast (takes 2–5 minutes), simple, requires low sample volume, and is fully automated measurement (72). Hence, this method is regularly used to measure the particle size of lipid-based formulations (50). Besides particle size, the PDI index can also be observed via PCS (72). However, PCS does not provide information involving the morphology and liposome shape, is not suitable to analyse particles larger than 6 µ, and presumes any particle aggregation of several vesicles as a single particle (73). PCS measures the intensity of light scattered by a particle with Brownian motion, which is the random collisions between suspended drugs and solvent molecules (51). Smaller liposomes have a greater surface area that promotes the rate of drug release (74) and lower size distribution that is more stable and unfavourable to aggregate or physical instability at storage conditions (75). In contrast, a bigger particle size may result in rapid uptake by the reticuloendothelial system causes rapid drug clearance and a shorter half-life (76).

The particle size of liposomes is directly related to the location and orientation of the compound to be encapsulated in the liposomal membrane (52). Resveratrol's exact location in the liposome membrane is still unclear, but since it is a lipophilic drug, some studies have stated that resveratrol is located within the lipid bilayer region and dominantly located in the outer headgroup region or the headgroup region facing the aqueous compartment (52,77,78). Resveratrol interaction with the outer

headgroups region promotes the expansion of the headgroup layer thickness and causes the tail region's propagation compared to the unloaded liposome bilayer (77). In other words, resveratrol encapsulation results in a change of tail region packing and increases the tail region thickness (52). Therefore, the drug's location and orientation may result in the particle size of the drug-loaded liposome being more significant than the unloaded liposome.

Both unloaded liposomes and resveratrol-loaded liposomes showed a homogenous size distribution with an average diameter estimated between 100 nm to 200 nm (9,61). A comparison between resveratrol encapsulated lipid nanocarriers (R-nano), and resveratrol encapsulated liposome (R-lipo) in the browning activities of white adipocytes for obese treatment reported that the average particle size of R-nano and R-lipo was approximately 140 nm and 110 nm, respectively (79). The nanocarrier tissue distribution is size-dependent, where a nanocarrier size of around 100 nm was said to be distributed successfully into the adipose tissues of obese mice (79). Besides, R-nano and R-lipo showed low PDI values, which is less than 0.2, indicating a higher level of homogeneity. Both R-nano and R-lipo significantly increased resveratrol cellular uptake to enhance the browning of white adipocytes, resulting in thermogenic and fat-burning activities, leading to weight loss in the obese population (79).

Resveratrol-loaded liposome size prepared using the thin-film hydration method and determined by DLS varied between 103.3 nm to 134.2 nm, with the PDI index showing an effective size distribution and homogeneity (80). Within the size range, resveratrol-loaded liposomes significantly enhance the antioxidant effect as the preparation can scavenge or reduce ROS production in the extracellular and intracellular compartments during spontaneous oxidative (80). In addition, ultra-deformable liposomes co-loaded with resveratrol and psoralen for the treatment of chronic skin disease have a particle size analysis using the DLS technique ranging from 120 nm to 130 nm (all below 150 nm) and a PDI value ranging from 0.20 to 0.27 with only a slight difference was observed after the drug loading (80).

5.2 Particle shape and morphology

The morphology and shape of liposomes can be determined using transmission electron microscopy (TEM) or scanning electron microscope (SEM) (72). The shape of the liposome may affect drug encapsulation efficiency, drug loading, cellular uptake, and receptor targeting and binding (75). TEM is the most frequent method of direct visualisation of the liposomal structure because it allows the practical analysis of individual particle's shape and morphology and provides a critical assessment of the liposome population (81,82). Under TEM methods, a technique like negative staining using heavy metal salts (uranyl acetate or phosphotungstic acid) is required to enhance the appearance of black liposomal spots and provide higher magnification against a dark-stained background (81). Nevertheless, the method's limitations are time-consuming, and the pretreatment of the TEM sample might change the shape and morphology, such as vesicle shrinkage or swelling (82).

The morphology of resveratrol-loaded liposomes and the image reveal that the shape was almost spherical, uniform with a smooth surface, and well separated (83,84). There is also no liposomal particle aggregation during the observation because of the particle surface's negative charges, allowing the particles to repel each other (82). Evaluation under TEM with 1.5% phosphotungstic acid (PTA) negative staining confirmed the uniformity of particle size distribution and surface morphology of resveratrol-loaded liposome (42).

Evaluation of the resveratrol-loaded cationic liposome in targeting the hepatocellular carcinoma, the morphological examination using TEM of the formed resveratrol-loaded liposome showed a

spherical shape and a uniform size distribution. The surface of the liposome was also found to be smooth, which indicated the stability of the prepared liposome. Another explanation is the spontaneous formation of the bilayer when the hydrophobic nature of lipids is exposed to an aqueous environment causing a hydrophilic layer to be arranged on the outer liposome surface (76).

The particle diameter observed via TEM shows a low diameter size distribution range and is almost similar to the diameter detected using the PCS method (85–87). The reason is that the PCS method evaluates the hydrodynamic size of liposome particles, namely the inorganic core and hydration layer around the particle. In contrast, size measured by TEM does not include the hydration layer as it is not present (86). TEM was usually taken after freeze-drying of the particle, thus voiding these hydration layers (87). Nevertheless, the cryoprotectant is vital as the absence of cryoprotectant may reduce the roundness of resveratrol-loaded liposomes. The freeze-dried liposomes encapsulating drugs are an essential step because of lipid oxidation, drug leakage, and increased liposome size due to the formation of aggregates or vesicle fusion (88). Therefore, cryo or lyoprotectants must be added to the liposomal formulation to improve the functional properties and stability of the liposome after the freeze-drying process (89). Examples of cryoprotectants that provide the ability to act as integrity membrane protectants are carbohydrates or specifically saccharides such as sucrose, lactose, glucose, mannitol, and trehalose. Some analysis indicated that saccharides effectively protect the integrity of liposomes compared to liposomes that are not incorporated with cryoprotectants (90) and maintained a similar size, morphology, and concentration as the initial formulation with minimal drug leakage (91).

5.3 In-vitro drug release study

Dissolution testing is not only a quality control test but also to determine the formulation's performance (50). Its advantages are that it is a cost-effective and time-saving tool to evaluate the bioavailability of drugs and allows the development of *in-vitro* and *in-vivo* correlation (92). *Dissolution* is defined as a dynamic process in which material is transferred from solid to solution per unit time. The dissolution process involves two steps (92): molecules are released from the surface to the dissolution media; and the drug diffused into the solvent from a high drug concentration region to a low drug concentration.

Several current methods are used in *in-vitro* release tests, including sample and separate, continuous flow, dialysis membrane, or combination methods (93). Among all the methods, the dialysis method is the most popular and preferred because of its ease of set-up and sampling, and its simple technique for studying drug release, especially for dosage forms like emulsions, liposomes, nanospheres, and nanosuspensions (93). Nevertheless, the downside of the dialysis method is that incorrect seal, leakage of media, or dosage form may occur during the set-up, and drugs that tend to bind to the dialysis membrane cannot be used in the test.

On the other hand, the dissolution test indicates the performance of the formulation and its bioavailability. Thus, it is essential for the test to simulate the gastrointestinal tract environment closely (92). Generally, to stimulate the environment, the media consists of substances that are present in the gastrointestinal fluids can be added, especially poorly soluble or weakly acidic drugs that are favourably dissolved and absorbed in the small intestine (50). Thus, simulated intestinal fluid or simulated intestinal fluid are the proposed media for BCS class I and II drugs.

Dialysis membrane or dialysis bag method with medium using water (47), phosphate buffer solution pH 5.5 or pH 7.4 (76,84), receptor fluid (15), and simulated gastric fluid (94) are usually used to evaluate the release profile of resveratrol. Water is an excellent medium but has a low buffer

capacity. Hence, a simulated gastric fluid or phosphate buffer solution with a final pH of around 5.5 can be helpful in the study (50).

Resveratrol molecules appeared to be released in a biphasic way, which included initial burst release and followed by sustained release (84). The initial burst effect may be due to resveratrol particles mainly distributed on the outer headgroup surface. In contrast, the sustained release is because resveratrol is also located in the deeper region of the lipid bilayer, which takes time to diffuse out from the membrane into the medium (83). The biphasic effect is considered advantageous because the initial burst rapidly releases a sufficient amount of resveratrol from the liposome to exert initial therapeutic properties. In contrast, the sustained release of the remaining resveratrol may maintain the therapeutic dosage without the need for repeated administration (95).

Furthermore, dissolution studies showed that, weather resveratrol in the simulated gastric fluid or phosphate buffer solution, the initial burst occurred in the first 15 minutes, followed by even and almost equilibrium release profile for subsequent five hours (96). Hence, resveratrol release from the liposome and diffusion through the membrane was slower, with the highest concentration after more than 200 minutes (15). Contrarily, in the case of pure resveratrol, the drug dissolution was nearly completed for about 30 minutes (94), and its release amount was also less (96). Thus, the release rate for resveratrol-loaded liposomes in the simulated gastric fluid or phosphate buffer solution is much slower than pure resveratrol.

Additionally, in a study conducted to evaluate the synergistic therapeutic potential of phytochemical agents and chemotherapeutic agents for head and neck cancers using resveratrol and 5-fluorouracil, respectively, co-encapsulated into a single PEGylated nanoliposome, the results showed that the location of the drugs in the liposome membrane influences their release profile. Resveratrol located in the hydrophobic region exhibits a slower and steady release, whereas 5-fluorouracil at the polar head group shows a rapid release in the phosphate buffer solution at pH 7.4 and simulated saliva medium at pH 6.8. Thus, resveratrol release from liposomes is characterised by an initial burst released due to resveratrol at the outer headgroup region, followed by sustained release (97). Besides, resveratrol was also co-encapsulated with paclitaxel in a PEGylated liposome as the carrier of combination therapy for breast cancer cells (98). In the first 24 hours, the release of resveratrol and paclitaxel in phosphate buffer solution at pH 7.4 was faster, followed by sustained release patterns (98). This evidence demonstrates that resveratrol co-encapsulated with 5-fluorouracil or paclitaxel in a liposome improves the efficacy of the drugs against cancer cells.

5.4 Entrapment efficiency

The entrapment efficiency determination is a crucial parameter and one of the vital aspects to concluding the justification of developing liposome-based delivery (56). Entrapment efficiency is an expression of the amount of drug incorporated into the liposome and is typically defined as the percentage of drug encapsulated into the liposome relative to the amount of drug (99). Increasing drug entrapment efficiency can reduce costs and indicates the enhancement of drug bioavailability and efficacy (51) because a sufficient amount is delivered for the therapeutic effects.

Free drugs need to be separated from the liposome vesicles to determine the entrapment efficiency (60). Usually, the separation of drugs from vesicles is via separation techniques such as centrifugation, ultracentrifugation, or dialysis bag (100). Then, the extraction of the compound is done by dispersing it in an organic solvent such as chloroform, methanol, chloroform-methanol mixture, ethanol, or phosphate buffer saline. Later, the solution will undergo a sonication process that will cause the vesicle to lysis to allow for the

solubilisation of the drug into an organic solvent. The free drug in the supernatant can be analysed using analytical methods, including UV-vis spectrometry at maximum peak of drug or chromatographic method, or gel permeation chromatography (56).

Transferrin-targeted liposomes loaded with resveratrol (Tf-RES-L) prepared using thin-film hydration and resveratrol loaded passively into liposomes showed entrapment efficiency of about 70% to 75% of the initial resveratrol amount and indicated an excellent entrapment efficiency. The results showed a favourable therapeutic response on the cancer cell compared to the free resveratrol and non-targeted transferrin liposome. Mice treated with Tf-RES-L demonstrated an effective treatment for tumour growth inhibition and improved mice survival by about 60% (42).

Besides, the entrapment efficiency is usually affected by the lipid ratio to the drug (lipid/drug) during the liposome preparation (60), for example, in a study on resveratrol-loaded peptide and sucrose liposomes (PSL) to treat breast cancer. The results revealed that resveratrol-loaded PSL was stable and had excellent entrapment efficiency where at a lipid/drug ratio of 1:1 was less than 70% (43). Whereas increasing the lipid concentration at the ratio of 5:1 and 10:1 caused the entrapment efficiency more than 90% (43). Thus, the resveratrol-loaded PSL can inhibit tumour growth by inducing breast tumour apoptosis after being evaluated in mice bearing breast cancer (43).

In addition, resveratrol-loaded liposomes with different lipid and cholesterol ratios may also affect entrapment efficiency. The entrapment efficiency of resveratrol is said to be increased if the concentration of lipid (soy lecithin) increases by about 78%. Considering that lipophilic drug-like resveratrol tends to dissolve quickly in the lipid, resulting in a higher entrapment efficiency (76).

5.5 Stability test

The stability of liposomes can be classified into three inter-related categories: chemical, physical, and biological stabilities (51). The physical and chemical stability tend to affect the liposome-shelf life, namely size distribution, entrapment efficiency, and compound degradation. Physical stability tests can be conducted via visual inspection or observation of color changes and sedimentation of liposome formulations or microscopic observation via TEM to evaluate the particle size and shape (51). Next, a chemical stability test can be conducted by studying the composition of lipid (hydrolysis or peroxidation) and drug degradation via liquid, chromatography, or spectroscopy.

Liposomal preparation generally does not have a long-term stability standard in the formulation if stored as aqueous suspension (51). Since liposomes exist in a liquid form, it may lead to stability problems, including particle fusion or aggregation, sedimentation, and drug leakage from the lipid bilayer (87). Thus, removing water will avoid lipid hydrolysis as it will improve stability and increase shelf life.

Freeze-drying is commonly adapted for drying to avoid these problems. The three steps involved in the drying process are freezing, ice sublimation, and, lastly, desorption of unfrozen water (51). Nevertheless, physical vesicle changes such as size alteration or loss of an encapsulated compound may occur during the stages. Therefore, the addition of suitable cryoprotectants in the formulation using sugar such as sucrose, glucose, mannitol, lactose, or trehalose can overcome these disadvantages. The cryoprotectants (sucrose, lactose, and trehalose) that allowed minimising the mean diameter changes after freeze-drying and the effectiveness increased with increasing concentrations (45).

Sucrose is usually used as a cryoprotectant to prepare liposomes of the hydrophobic drug (resveratrol) (51). The dried liposomes exhibited high encapsulation efficiency of hydrophobic drugs with small particle size and high storage stability at

4°C for 60 days. Resveratrol-loaded liposomes showed good stability at 4°C for a month and room temperature based on particle size, zeta potential changes, and UV absorbance resveratrol (42). Even though the particle size increased gradually during the storage period, the changes were less than 200 nm, meaning the particles were stable (101). Besides, there is no significant difference in particle size, zeta potential, and entrapment efficiency after three months of storage at 4°C (76). That indicates the stability of the resveratrol-loaded liposome formulation. Besides, zeta potential can determine the electrostatic repulsion between the particles and prevents particles aggregation, influencing physical stability (15). The results showed only a slight difference in mean particle size (less than 12% differences) and a negative zeta potential (no particle aggregation) during three weeks of storage at 4°C, indicating good stability (15).

6.0 Limitation and Future Perspective

Despite various pharmacological properties, resveratrol displays physico-chemical and pharmacokinetic limitations leading to poor bioavailability. Nano-technology has become the promising delivery system for several polyphenols, including resveratrol, to enhance and improve their limitations. This review focused on the literature review of the liposomal drug delivery system approach to promote resveratrol bioavailability. However, the development of the therapeutic potential of resveratrol-loaded liposomes must be applied and confirmed *in-vivo* for rodents or non-human primates to investigate whether the bioavailability and other limitations can be overcome (41).

Oral administration of liposomal resveratrol formulations may provide a positive result. However, only the conventional dosage form such as tablets and capsules are mentioned in the clinical studies to evaluate bioavailability. Thus, future studies may focus on exploring the bioavailability of resveratrol for other dosage forms in the millimetre to micrometre range and colloidal carriers in the nanometre range with a different route of administration. The conventional dosage form alone is probably unable to improve the physicochemical and pharmacokinetics limitations of resveratrol. For example, the development of resveratrol-loaded liposome formulation by targeting brain and nasal administration to prevent and treat neurological disorders such as Parkinson's disease and Alzheimer's disease (102).

Apart from that, the interaction of resveratrol with different types of lipid membranes has been investigated (67). However, some controversies have arisen because some studies have showed that resveratrol might increase membrane fluidity or induce stiffness in several liposomal models (103–105).

The design of a prodrug that is potentially absorbed better is also currently being developed. Frequently, a prodrug approach for parent compounds with problems associated with poor aqueous stability and extensive metabolism that affects their bioavailability and chemical instability is a successful strategy to improve the compound's pharmacokinetics (106). The transformation of the labile chemical group into a more stable compound can result in better distribution and improve bioavailability compared to the parent compound. For example, PEG-resveratrol conjugates containing succinyl or acetyl group and several amino acids as linkers showed excellent water solubility (900 mg/ml) higher than parent resveratrol as well as controlled release and consequently improved bioavailability (107). Nevertheless, prodrug formulation might be challenging and complicated due to many parameters involved during the development process, and further *in-vivo* studies are needed to validate the results.

7.0 Conclusion

Resveratrol can be regarded as an active compound possessing numerous pharmaco-logical properties, including antioxidant,

anti-inflammatory, analgesic, cardio-protective, neuroprotective, chemo-preventive, and anti-aging activities. Hence, they have become a promising subject in the nutraceutical field. However, the pharmacological effects of resveratrol are restricted due to its extremely low water solubility, high absorption with rapid and extensive metabolism, and chemical instability. Consequently, the oral bio-availability of resveratrol is extremely low, and it is not possible to achieve therapeutic concentrations. These limitations can be resolved by developing a nanotechnology delivery system, and thus, most of the published articles indicated that the resveratrol-loaded liposome delivery system was a successful strategy, as it demonstrated remarkable advantages compared with non-encapsulated resveratrol. The limitations of resveratrol were significantly improved with the liposomal drug delivery system. Based on the literature review, resveratrol-loaded liposomes have a particle size below 200 nm with an almost spherical shape, and the particle size is almost similar to the diameter detected when using the PCS method. Concurrently, a PDI value below 0.3 indicated homogeneity of the liposomal formulation and was found stable during the stability test. Moreover, the formulation of resveratrol liposomes exhibited high entrapment efficiency, and *in-vitro* drug release studies have shown an initial burst effect, followed by a sustained release pattern. Based on the results from the published articles, liposomes are suitable as carriers for resveratrol, thus improving its limitations and enhancing its pharmacological properties. Nevertheless, future *in-vivo* studies are required to confirm the potential of resveratrol-loaded liposomes to overcome its limitations.

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Conflict of Interest

The authors declare no conflicts of interest in the present work.

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