



## **Aspasomes - Revolutionary Nanovesicular Drug Delivery System**

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### **ABSTRACT**

*Aspasomes, also known as bilayer vesicles, are a new type of nanovesicles composed of cholesterol, a negatively charged lipid, and ascorbyl palmitate. By encapsulating pharmaceuticals, these multilayered vesicles improve their stability and enable targeted distribution. Aspasome benefits include increased skin penetration, antioxidant activity, skin retention, innovative treatment, and flexible pharmaceutical encapsulation. However, they could have stability problems, a lengthy shelf life, and difficult preparation methods. Typically, the thin-film hydration method is used to make aspasomes, and then sonication is used to get the right vesicle size. Antioxidants like idebenone, antifungal medications like voriconazole, skin-whitening drugs like magnesium ascorbyl phosphate (MAP), and anti-inflammatory meds like naproxen are all possible aspasome encapsulants. Aspasomes are used in skin whitening and dermatology, antifungal, anti-inflammatory, and antioxidant therapy, as well as in general drug delivery systems. Aspasomal formulations have demonstrated potential in addressing issues such as melasma, improving skin absorption, and minimizing adverse effects. This review article emphasizes the advantages of aspasomes, which include improved drug retention, innovative treatments, increased antioxidant activity, better skin penetration, and heightened therapeutic effectiveness. Nonetheless, they encounter challenges like stability issues, intricate preparation methods, and short shelf life.*

**Keywords:** Ascorbyl palmitate, aspasome, lipid film, vesicles

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### **INTRODUCTION**

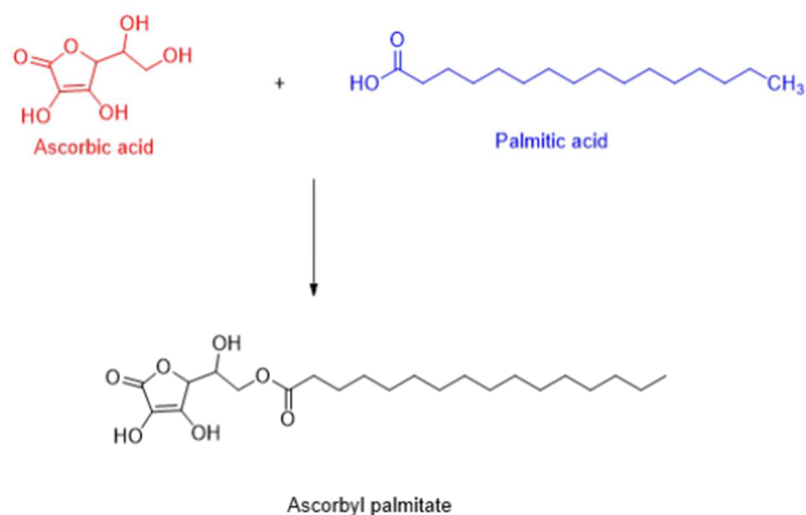
Accurate dose delivery, simplicity of administration, and an extended period of the drug's residence time in contact with the epidermal membrane are all made possible by a novel drug delivery technology [1]. Vesicular drug delivery systems are a practical method for administering medications, particularly for topical conditions. Lipid vesicles, including liposomes and their derivatives such as niosomes, transferosomes, aspasomes, and ethosomes, have been effectively used to treat a variety of topical conditions [2].

Because of their structural and functional similarities, the terms "ascorbyl palmitate" and "liposome" are the roots of the term "aspasome." Aspasomes are unique bilayer vesicles, or nanovesicles, made of ascorbyl palmitate (ASP), cholesterol, and a negatively charged lipid.

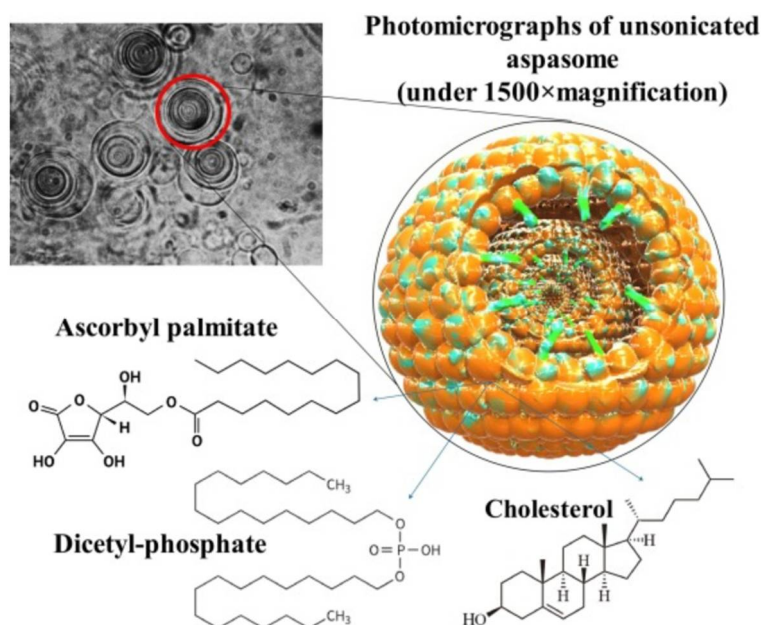
Drug forms that are micro- and nano-particulate have been the subject of extensive research aimed at reducing side effects and the therapeutic dose required for treatment, while also enhancing the therapeutic efficacy of conventional drug forms. In tandem with the benefits offered by the solubility, permeability, and applicability characteristics of nano-sized particles, research on these systems, which seek to improve the patient's quality of life and eliminate negative effects by prolonging the dosing interval at lower doses, is growing daily [3].

These multilayered vesicles are designed to encapsulate drugs, enhancing their stability and facilitating targeted delivery, and are capable of acting as antioxidants. Ascorbyl palmitate can create distinctive micellar structures [4]. However, lipids and cholesterol are necessary for vesicle production.

The modified aspasomes employed lecithin derived from neutral sources in place of dicetyl phosphate. In deeper layers of the skin, these aspasomes showed excellent loading efficiency and drug retention [5]. Moribe et al. confirmed the role of ascorbyl palmitate as the vesicle-forming component and Span 60 acting as a vesicle stabilizer [6].



**Figure. No. 1. Chemical production reaction of Ascorbyl Palmitate**



**Figure. No. 2. Structure of Aspasomes**

Lipids are charge inducers, and cholesterol stabilizes vesicles. Gopinath and colleagues were the first to describe aspasomes in 2004 [7]. They delivered the hydrophilic chemical azidothymidine using dicetyl phosphate, a negatively charged lipid. In 2020, Aboul-Einien et al. published a study on the creation and assessment of modified aspasomes for improved hydrophilic medication transdermal delivery.

#### **Composition of aspasomes:**

Aspasome's composition usually consists of dicetyl phosphate as a charge inducer to help form stable vesicles, cholesterol to improve stability, and ascorbyl palmitate as the main lipid component. Depending on the particular formulation and intended use, these components' molar ratios may change.

#### **Ascorbyl palmitate:**

It is a lipophilic analogue of vitamin C which has been studied because of its ability to associate with cholesterol and negatively charged lipids such as Dicetyl phosphate to form vesicles (aspasomes). Due to their superior antioxidant actions compared to ascorbic acid, aspasomes have shown potential as a drug carrier for diseases associated with reactive oxygen species. Ascorbyl palmitate (AP) is a fat-soluble form of vitamin C which differs from other forms, such as ascorbic acid and palmitic acid, due to esterification by its lipophilic properties with naturally produced fatty acids and its resulting fat solubility, this is primarily between ascorbic acid and palmitic acid creating a fat-soluble form of vitamin C. Used in skin care products: Ascorbyl palmitate can act as an antioxidant, potentially protecting the skin from damage caused by free

radicals. But it could be related to cardiovascular disease and create an overload of the reactive oxygen species, and thus, that said, this could interfere with the cellular signaling, leading to mutations and even cell death. It could also be a poison to the environment. [8]

#### **Cholesterol:**

To enhance the bilayer of these vesicles (aspasomes), sterols such as cholesterol are often included in the formulation of ascorbyl palmitate (AP). At specific cholesterol concentrations, it has been demonstrated that the presence of cholesterol in the aspasome dispersion stabilizes the bilayers and reduces their permeability, resulting in a stable aspasome with slowed Azidothymidine (AZT) permeability. It has been demonstrated that adding cholesterol to AP in the presence of dicetyl phosphate, a negatively charged lipid, results in persistent vesicles known as aspasomes, which have antioxidant qualities better than ascorbic acid and improve AZT's transdermal penetration. The formation of bilayered vesicles requires the liquid crystalline state, and DSC analysis has demonstrated that ascorbyl palmitate, cholesterol, and dicetyl phosphate anhydrous mixtures can form liquid crystals. As the concentration of cholesterol rises, the heat of transition of aspasome dispersion falls, suggesting a stable bilayer structure. When hydrated, the thin layer of ascorbyl palmitate does not form vesicles; nevertheless, when cholesterol is present, vesicles do develop, but they are extremely unstable. Stable vesicles are formed in the presence of cholesterol and dicetyl phosphate, highlighting the importance of cholesterol in the formation and stability of aspasomes.

#### **Dicetyl phosphate:**

Ascorbyl palmitate (AP) and cholesterol associate to produce aspasomes, a type of vesicle, with the assistance of the anionic lipid diethyl phosphate (DCP). DCP is added to the mixture of AP and cholesterol to create a well-formed and stabilized vesicle suspension. It has been shown that the bilayers become stabilized and their permeability decreases by adding DCP to the aspasomes dispersion, resulting in a stable aspasome with delayed AZT permeability at a certain critical level of DCP. The stable vesicles formed when DCP is mixed with AP and cholesterol highlight the importance of this process for the growth and stability of aspasomes. DCP is also introduced into niosomes and various vesicular DDSs to enhance stability and impart a negative charge [9].

#### **Uses of Ascorbyl Palmitate:**

It's an antioxidant and guards against free radicals, which might be damaging your skin. This is good for transdermal medication delivery, which is to say it is lipophilic, and that means that the skin soaks it up better. It can also transport other therapeutic substances by encapsulating and transporting them. It might help skin-whitening or brightening products because it can lighten dark spots.

#### **Uses of Cholesterol:**

It supports the lipid bilayer of the aspasome in preserving its structural integrity.

It affects the aspasome's permeability, which in turn affects how easily medications can be administered.

#### **Uses of Soya Lecithin:**

When combined with ascorbyl palmitate, it forms a bilayer that contributes to the construction of the vesicle.

It stabilises the development of aspasomes and increases the bilayer's stiffness.

#### **Origin and Development:**

Researchers at Kakatiya University's University College of Pharmaceutical Sciences in Warangal, India, first proposed the idea of aspasomes. The researchers investigated ascorbyl palmitate's potential as a bilayer-forming substance for vesicle formation in a 2004 study. When they combined ascorbyl palmitate with dicetyl phosphate and cholesterol, they found that stable vesicles formed, which they called "aspasomes."

#### **Pharmaceutical Properties:**

Aspasomes can deliver drugs continuously because of their stability for up to 18 hours. They have antioxidant action; research indicates that even after being converted to ascorbyl palmitate, the ascorbyl moiety's antioxidant effectiveness is maintained.

#### **Drug delivery efficiency:**

Aspasomes' capacity to boost medication administration through the skin is demonstrated by their increased transdermal penetration when compared to other preparations. Changing the percentage of cholesterol in aspasomes can affect how quickly they release medications.

#### **Pharmaceutical Formulation:**

Important pharmacological attributes that are evaluated during formulation include the prepared aspasomes' size, charge, storage stability, skin deposition ability, and antioxidant qualities. Since active ingredients can be encapsulated within these vesicles, aspasomes are commonly manufactured utilising techniques like thin film hydration.

#### **CLASSIFICATION OF ASPASOMES:**

Aspasome classification is based on the structure, composition, and function of the aspasomes.

##### **1. Based on composition:**

Typical aspasomes are composed of ascorbyl palmitate, cholesterol, and surfactants.

Aspasomes that have been modified: Include additional stabilizers or co-surfactants to enhance drug entrapment and release.

## 2. Based on structure:

We describe aqueous bilayers with a core that can be continually released as multilamellar vesicles (MLV). For rapid drug release, unilayer vesicles, also known as unilamellar vesicles (ULV), are ideal. A few bilayers can be seen in oligolamellar aspasomes, which exhibit intermediate release.

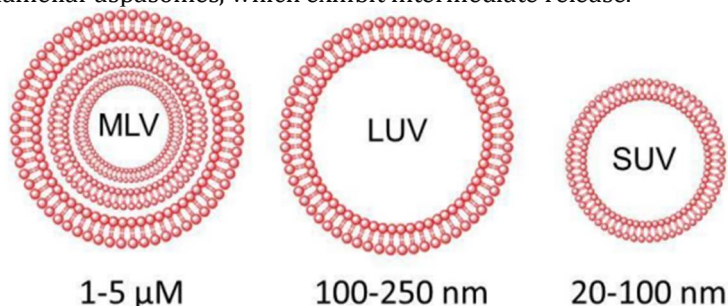


Figure. No. 3. Different sizes of Vesicles

## 3. According to the application:

**Transdermal aspasomes:** Improve the skin's ability to take in topical medications. Ocular aspasomes are used to enhance the bioavailability of drugs in the eye. Improve the solubility and absorption of water-insoluble drugs by utilizing oral aspasomes.

**Parenteral aspasomes:** Created to improve systemic distribution through injection into a muscle or vein.

### Advantages:

1. Their lipophilic characteristics improve the transport of transdermal medications by facilitating greater interaction with the lipid-rich stratum corneum of the skin.
2. Furthermore, aspasomes enable the encapsulation of both hydrophilic and hydrophobic drugs, enhancing the therapeutic efficacy of co-delivered medications [10].
3. Aspasomes may enhance the skin's ability to retain medications, boosting treatment effectiveness.
4. They have shown promise as a novel treatment for melasma and other skin disorders. Additionally, aspasomes offer better protection against oxidative stress due to their higher antioxidant activity compared to ascorbic acid alone.

### Disadvantages:

1. While in storage, aspasomes might encounter stability issues such as fusion, aggregation, and drug leakage, potentially diminishing their effectiveness.
2. Their preparation process is notably intricate because techniques such as thin-film hydration and sonication require significant time and specialized machinery, complicating large-scale manufacturing.
3. Additionally, because of potential instability, aspasome aqueous solutions may have a short shelf life and should be prepared and stored carefully [11, 12].

### TECHNIQUE FOR MAKING ASPASOMES:

The thin-film hydration method is typically used to prepare aspasomes, and sonication is then used to achieve the proper vesicle size.

#### Preparation Steps:

1. Creating a Lipid Blend: Ascorbyl palmitate, cholesterol, and a negatively charged lipid (like dicetyl phosphate) should be dissolved in an organic solvent mixture of methanol and chloroform (typically in a 9:1 ratio). The thin-film hydration method is typically used to prepare aspasomes, and sonication is then used to achieve the proper vesicle size.
2. Thin Lipid Film Formation: Move the lipid solution into a round-bottom flask, then use a rotary evaporator to evaporate the solvent at low pressure at about 50°C. This leaves a thin, dry lipid film on the flask's inner wall.
3. Hydration of Lipid Film: Add an aqueous phase, typically phosphate-buffered saline (PBS) at a pH of 7.4, to the dried lipid film to hydrate it.
4. Vesicle Formation: To transform multilamellar vesicles into tiny unilamellar vesicles, expose the hydrated lipid film to probe sonication for a predetermined amount of time (for example, five minutes at 20 kHz at 4°C).
5. Storage: Until they are used again, keep the prepared aspasomal formulations in vials at a cold temperature [13].

## CHARACTERIZATION OF ASPASOMES:

### Determination of Vesicle Size:

The size of the vesicles was determined using a dynamic light scattering technique. The results were provided as the mean value  $\pm$  standard deviation (SD) of three replicate measurements taken at a temperature of  $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . The size of the vesicles was measured using a particle size analyzer, specifically the Malvern Zetasizer. After diluting the aspasomal formulation with 10 ml of deionised water, a 0.5 ml sample was filtered using a polytetrafluoroethylene (PTFE) syringe filter (Millex® Syringe Filter). The size of the vesicles was assessed using dynamic light scattering. The results were provided as the mean value  $\pm$  standard deviation (SD) of three replicate measurements performed at a temperature of  $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  [14].

### Determination of %Entrapment efficiency:

The percentage of %EE of the VRC-loaded aspasomal dispersion was determined indirectly. A KUBOTA-7000 high-capacity refrigerated centrifuge (Japan) was used to centrifuge 1ml of the freshly made aspasomal dispersion containing VRC from each batch for 30 minutes at 15,000 rpm and  $4^{\circ}\text{C}$ . A 0.1 ml sample was extracted from the supernatant following centrifugation and suitably diluted with 10 ml of methanol. A blank aspasomal dispersion was made similarly, and the resultant solution was filtered through a PTFE syringe filter (Millex® Syringe Filter) before being subjected to spectrophotometric analysis at 256 nm using a SHIMADZU UV-1800 spectrophotometer (Japan). This method was used to determine how well the VRC was encapsulated in the aspasomal formulation [15]. This formula was used to determine the aspasomes' %EE.

$$\%EE = \frac{\text{Total drug added} - \text{Total drug in supernatant}}{\text{Total drug added}} \times 100$$

### Aspasomes morphology:

The surface morphology of the optimised aspasomes was investigated using high-resolution transmission electron microscopy (HR-TEM) fitted with a camera attachment (JSM2100PLUS JEOL, Japan). The aspasomal dispersion was allowed to settle on a copper grid for ten minutes. The materials were thoroughly dried before being stained for image analysis using a 2% w/v uranyl acetate aqueous solution.

### Number of vesicles per cubic millimeter:

This is determined by counting the number of vesicles per cubic millimeter using a hemocytometer. Eighty little squares of the diluted vesicles are counted [16].

### Drug Content:

To evaluate the total drug content, which is encapsulated, 0.2 mL of an aspasome dispersion is dissolved in 25 mL of methanol and stirred on a magnetic stirrer to disrupt the vesicle, and the drug content is measured spectrophotometrically.

### In Vitro Release:

The dialysis bag membrane diffusion method is used to assess the generated Aspasomes formulation's *in vitro* drug release. The formulation will be put into the dialysis bag, which will be kept at  $37^{\circ}\text{C}$  and submerged in a phosphate buffer pH 7.4 solution. To maintain sink conditions, appropriate amounts of the sample are taken out at regular intervals and replenished with an equivalent volume of phosphate buffer solution. The amount of drug released over time will be ascertained by spectrophotometric analysis of the samples using UV-Visible Spectroscopy [17].

### Stability Study:

The Aspasomal gel is made in triplicate and stored at  $25 \pm 2^{\circ}\text{C}/65 \pm 5\%\text{RH}$  and  $40 \pm 2^{\circ}\text{C}/75 \pm 5\%\text{RH}$  for three months. After predetermined amounts of time, samples will be removed and visually inspected for any physical formulation changes.

### Photomicrography:

The creation, arrangement, and nature of the vesicles were confirmed by taking a photomicrograph of the unsonicated aspasomes with a camera mounted over a microscope.

### Size and size distribution:

A dynamic light scattering system (Malvern 4700, Malvern, UK) with a He-Ne laser system and a temperature of  $90^{\circ}$  was utilised to measure the aspasomal particle size and particle size distribution.

### Surface Morphology (SEM):

At  $25 \pm 2^{\circ}\text{C}$ , the Hitachi S-576 Scanning Electron Microscope was used to view the SEM pictures. Appropriate aspasomal dispersion dilutions were prepared and sonicated for this purpose. On the grid, a few formulation drops were placed and left to dry. The photos were shot after drying.

**Zeta potential:**

The formulations' particle sizes were measured using the Malvern Zetasizer 2000. Measurements were made in triplicate using the multimodal mode after the dispersion was combined with PBS pH 7.4. Direct measurements were made of the mean zeta potential and the charge on the vesicles [18].

**Differential scanning calorimetry:**

The thermotropic characteristics and phase transition behaviour of ascorbyl palmitate/cholesterol/DCP anhydrous mixes and ascorbyl palmitate dispersions were investigated using a Differential Scanning Calorimeter (Mettler DSC 821e, Mettler-Toledo, Switzerland). For the analysis, a typical aluminium pan was utilised, and the heating rate was adjusted to 5 °C per minute.

**Considerations:**

1. **Lipid Composition:** Vesicle size, encapsulation effectiveness, and drug release profiles can all be optimized by varying the molar ratios of cholesterol and ascorbyl palmitate.
2. **Hydration Conditions:** To avoid lipid oxidation and degradation, hydration is usually carried out in a nitrogen atmosphere.
3. **Sonication Parameters:** To achieve the appropriate vesicle size and homogeneity, sonication parameters like duration and intensity are essential [19].

**Appropriate Medication for Aspasome Encapsulation:**

1. **Agents that Reduce Inflammation:** One nonsteroidal anti-inflammatory medicine (NSAID) that is frequently used to reduce pain and inflammation is naproxen. In the treatment of skin inflammation, naproxen showed improved therapeutic efficacy when co-loaded into aspasomes with the antioxidant idebenone.
2. **Antioxidants (Idebenone):** A coenzyme Q10 synthetic analogue with strong antioxidant qualities. It has demonstrated enhanced therapeutic efficacy when co-encapsulated with naproxen in aspasomes, indicating the possibility of successful skin anti-inflammatory treatment.
3. **Antifungal Agents (Voriconazole):** A drug that fights infections caused by fungi. The incorporation of voriconazole into aspasomes has demonstrated efficacy in treating fungal infections of the skin by achieving regulated drug release and enhanced antifungal activity against pathogens such as *Candida albicans* [20].
4. **Whitening agents for the skin:** A stable form of vitamin C, magnesium ascorbyl phosphate (MAP), is utilized in anti-aging and skin-whitening solutions. Aspasomes containing MAP have demonstrated promise in the treatment of diseases such as melasma, providing a new, side-effect-free therapeutic option.

**APPLICATIONS:**

Aspasomes are lipid-based vesicular systems that are used in several pharmaceutical applications, most notably in the administration of drugs. These are a few significant aspasome applications.

1. **Antioxidant Therapy:** Aspasomes may help cure oxidative stress-related illnesses because of their inherent antioxidant qualities.
2. **Sustained Release:** They enhance treatment results by enabling the regulated and extended release of antiviral medications [21].
3. **Anti-Inflammatory Treatments:** It has been shown that co-loading antioxidants like idebenone into aspasomes together with anti-inflammatory medications like naproxen increases their therapeutic efficacy in lowering skin inflammation.
4. **Antifungal Applications:** The delivery of antifungal drugs, such as voriconazole, via aspasomes has been studied; this approach offers regulated drug release and enhanced efficacy against fungal infections.
5. **Skin Whitening and Dermatological Applications:** Formulations that include ascorbic acid derivatives into aspasomes have shown promise in the treatment of skin conditions like melasma [22] by leveraging their skin-whitening properties.
6. **General Drug Delivery Systems:** As novel drug carriers, aspasomes have been suggested to enhance targeted delivery, stability, and bioavailability.
7. **Gene Delivery:** Aspasomes can be used in gene therapy as vectors for DNA or RNA delivery. They can prevent nucleic acids from being enzymatically degraded and also increase their uptake in target cells.
8. **Nutraceuticals:** Active ingredients, vitamins, minerals, and polyphenols in nutraceuticals could be encapsulated as aspasomes to increase their stability and bioavailability [23].
9. **Vaccine distribution:** Research has been conducted on the use of aspasomes for vaccine delivery. Responds to the review. There has been research on vaccine deliveries through aspasome. They can prevent their degradation and increase their immunogenicity by surrounding them with adjuvants and antigens. This approach may be a particularly powerful approach to the creation of new vaccine formulations.

10. **Diagnostic imaging:** Aspasomes can also encapsulate imaging drugs applied in MRI, CT, and ultrasound. They display the imaging contrast and specificity, which can be applied for disease diagnosis and monitoring [24, 25].

## CONCLUSION

Aspasomes offer a promising advancement in nanovesicular DDSs with the superior attributes of targeted distribution, optimum cutaneous absorbance, drug stability enhancement, and antioxidant activity. Aspasomes are capable of entrapping various therapeutic agents; hence, they can be designed for dermatological and pharmaceutical applications. Investigations and development of aspasomes may lead to an enhancement of therapeutic efficacy and the use of new systems of topical and systemic drug application.

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## CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

## CONTRIBUTION OF AUTHORS:

Each author made an equal contribution to conceptualization, literature search, compilation, and writing different parts of the review article.

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