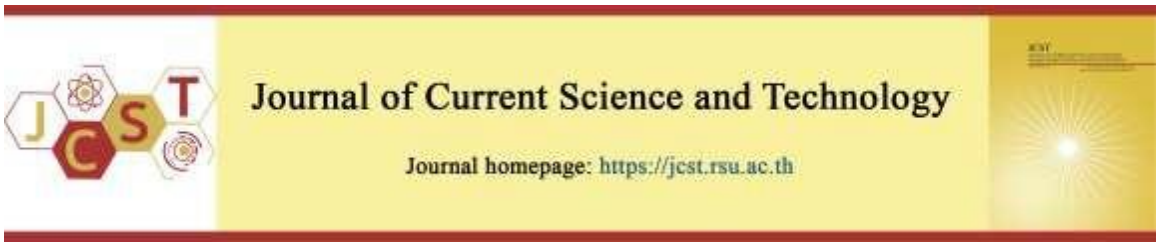
Journal of Current Science and Technology, May-August 2023 Vol. 13 No. 2, pp. 500-516

ISSN 2630-0656 (Online)

Cite this article: Rukari, T., Pingale, P., & Upasani, C. (2022, May). Vesicular drug delivery systems for the fungal infections' treatment through topical application-a systemic review. *Journal of Current Science and Technology, 13*(2), 500-516. https://doi.org/10.59796/jcst.V13N2.2023.1856




# Vesicular drug delivery systems for the fungal infections' treatment through topical application-a systemic review

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Received 17 June 2022; Revised 12 December 2022; Accepted 10 January 2023;
Published online 15 July 2023

**Abstract**

In this systemic review, we tried to explore and summarize the Vesicular Drug Delivery Systems (VDDSs) which can exclusively used for topical applications in the treatment of fungal infections. The specific algorithm is developed for this systemic review, exclusion criteria for the review were set and results were excluded which were meets the exclusion criteria. To build a review, Google Scholar® and PubMed® are two databases that are targeted so that we can collect the number of freely available full-text articles and can minimize the risk of bias (unavailability of full-text articles, for this study). By adopting the algorithm, searched articles were studied thoroughly and we found that thirteen types of VDDS were used by the researchers to treat skin infections caused by fungi in the last five years. From this current systemic review, we found a numbers of VDDSs are available for skin fungal infection, but all are not suitable for topical applications because of some drawbacks associated with some VDDS such as instability (Emulsomes), high cost of manufacturing (Sphingosomes), low transfer efficiency (Aquasomes), etc. As per the data analysed, we can say that; Liposomes, Ethosomes, Niosomes, Bilosomes, Cubosomes, and Ufasomes are the choices of researchers as VDDS in the therapy of different fungal diseases. Upcoming researchers can focus on these VDDSs in the treatment of infections spread on the skin by the fungi.

***Keywords*: *bilosomes; cubosomes; ethosomes; liposomes; niosomes; sphingosomes; ufasomes.***

## 1. Introduction

Nowadays a thoughtful public health concern has added to the disease called fungal infections. In the recent era of the pandemic, we extensively agreed that the rate of recurrence of fungal infections in patients with other diseases including Covid-19 is related to severe fungal infections such as mycosis and even leads to death (Reddy, Padmavathi, & Nancharaiah, 2022). As per the Leading International Fungal Education (LIFE) organization, two million species of fungi are found on earth, and out of that approximately six hundred have caused disease.

Maximum infections are instigated because of Candida, Aspergillus Trichophyton, and Cryptococcus-like species. The majority of serious fungal infections are 'hidden', as a result of other health issues such as cancer, asthma, transplantation, AIDS/HIV, and other chronic diseases (LIFE, 2022). Almost a billion people are calculated to possess fungal infections. Though the epidemiology of fungal diseases has changed significantly over the last few decades, the majority of serious fungal diseases are because of fungal pathogens and the cases remain the same (Bongomin, Gago, Oladele, & Denning, 2017). Fungal infections are both dangerous and invasive because the infection can spread from the subcutaneous to the epidermis region.

Because of the rise in the number of fungal infections as well as the inability or delayed curative action of existing medications, it's important to adopt a novel approach that overcomes the present obstacles (Bhattacharya, 2021). To achieve targeted and controlled drug delivery, a novel drug delivery system has proven as the most satisfying as well as approachable, but there is a lack of concise information, which summarizes the VDDSs for the use of the antifungal agent for the treatment of topical fungal infections (Verma et al., 2019; Chaudhari et al., 2022).

The rationale for the review is to explore and find out the VDDSs which can be used for topical applications in the treatment of fungal infection.

### 1.1 Vesicular drug delivery system (VDDS)- Novel approaches as a targeted delivery of drugs

Vesicles, as a carrier system, are now the preferred vehicle for drug delivery. These vesicles from biological origin were first conveyed in 1965 by Bingham. Vesicles are extremely organised structures composed of singular or multiple lipid bilayers formed after exposure of some amphiphilic building blocks to water (Jadhav, Morey, Karpe, & Kadam, 2012). Based on the composition, the vehicles are grouped as lipoidal bio carriers and no lipoidal bio carriers. They are comprehensively summarized in the form of advantages and disadvantages in Table 1

**Table 1** Comprehensive summary of types of VDDS with their Merits and Demerits (Arundhasree, Aiswarya, Kumar, Kumar, & Nair, 2021; Witika et al., 2021; Myneni, Radha, Soujanya, 2021)

| Name of the VDDS | Explanation | Merits | Demerits |
|---|---|---|---|
| **i. Lipoidal Biocarriers** | | | |
| **Liposome** | • Liposomes are the vesicles made up of colloidal bilayer and surrounded completely by an aqueous section.<br>• The bilayer membrane composed primarily from synthetic or natural lipids. | • Specific drug targeting.<br>• Deliver both low and high molecular weight drugs.<br>• Decreases toxicity.<br>• Prevents toxic drugs and their metabolites from reaching sensitive tissues.<br>• Helps in the removal of toxicity associated with certain drugs.<br>• Assists in resolving drug stability.<br>• Increases drug circulation.<br>• Hydrophilic as well as hydrophobic drugs encapsulation is possible.<br>• Improve bioavailability.<br>• Improve drug pharmacokinetics. | • Expensive and high production cost.<br>• Short self-life.<br>• Low encapsulation efficacy, especially for hydrophilic drug.<br>• Burst release of drug because liposomes leaking.<br>• Sometimes an external trigger system is required for drug transport across biological membrane. |

| Name of the VDDS | Explanation | Merits | Demerits |
|---|---|---|---|
| **Emulsomes** | • Emulsomes contains fats as well as triglycerides in the interior core, which are stabilised as an o/w type of emulsion with lecithin in high concertation.<br>• They also show the properties of both emulsion and liposomes. | • It has liposome and emulsion characteristics.<br>• For delivery of the drugs via topical, mouth, parenteral, rectal, ocular, and intranasal routes.<br>• Improve biodegradability, biocompatibility, entrapment efficiency and GIT stability.<br>• Low absorption in the body through systemic path.<br>• Increase drug levels at wounded tissues.<br>• Reduce toxicity.<br>• Improve Pharmacological activity.<br>• Prevent drug instability in gastric environment.<br>• Economical production when compared to other commercial lipid formulation. | • Low drug loading capacity.<br>• Parenteral administration is restricted, concerning their side effects.<br>• Higher oil content resulting in thermodynamic instability. |
| **Enzymosome** | • It is nanoscale vesicular delivery method for drugs specially for therapeutic proteins like enzymes and adopting a technique such as polymeric carriers. Enzymosomes are formed when enzymes and lipids form a compound. | • The antibody release on the target location.<br>• Improve encapsulation and stability.<br>• Increase therapeutic impact and less adverse effects.<br>• Maintaining the integrity of enzymatic activity. | |
| **Sphingosomes** | • These are bi - layer vesicles with a circular, colloidal aquatic compartment entirely surrounded inside a mebrane of bilayer made mostly of natural or synthetic sphingolipids. | • Encapsulation provided more stability.<br>• Reduce drug toxicity.<br>• Selective passive medication targeting for tumour cells.<br>• Oral, subcutaneous, intravenous, intramuscular and transdermal drug delivery is possible.<br>• Better medication retention.<br>• Design flexibility and specific sites targeting.<br>• Improve therapeutic index.<br>• Increase pharmacokinetics profile. | • Limited entrapment efficiency.<br>• Highly expensive. |
| **Ethosomes** | • Because of the high ethanol content, these lipid carriers have a high permeability and drug delivery capabilities. | • Optimum encapsulation efficacy.<br>• Deliver both low and high molecular weight drugs.<br>• Simple and Cost-effective method of preparation.<br>• Greater elasticity and deformability than liposomes. | • Skin irritation due to high ethanol concentration. |

| Name of the VDDS | Explanation | Merits | Demerits |
|---|---|---|---|
| **Transferosomes** | • Transferosomes are stress-responsive, and vesicles having ultra-deformable nature and has aqueous core at the centre enclosed by a complex bilayer of lipids.<br>• Chemically composed of a natural amphiphilic lipid vesicle. | • Use for topical as well as systematic drug delivery.<br>• Permeate the skin through minute and microscopic pores.<br>• Deliver both low and high molecular weight drugs.<br>• Depot formation delivery can be possible. | • Purity of phospholipid.<br>• Expensive.<br>• Chemically unstable. |
| **Pharmacosomes** | • Pharmacosomes are drug carriers, with 'pharmacon' referring to the drug and ‘soma' to the carrier. Pharmacosomes are a new form of vesicular drug delivery system that differs from prior drug delivery systems in several ways. | • There is no spillage of the medication.<br>• Improve bioavailability of weakly water-soluble or lipophilic.<br>• Maximum entrapment efficiency. | |
| **ii. Non-lipoidal bio carriers** | | | |
| **Aquasomes** | • The solid nanocrystalline core of these three-layered self-assembled nanostructures is coated with an oligomeric film that facilitates in the absorption for biologically active therapeutic compounds regardless of their modification.<br>• "Water bodies" is another term for aquasomes. | • Physical properties are like colloids.<br>• Reduce Reticuloendothelial clearance and environmental degradation.<br>• Biodegradable.<br>• Preservation of the bioactive molecules i.e., biochemical stability and structural integrity.<br>• Absorb topically hence pharmacological/biological activity occurs instantly. | • Expensive.<br>• Low transfer efficiency. |
| **Niosomes** | • Niosomes are quite recent vesicular drug delivery system, which contains unilamellar or may be vesicles multilamellar nature.<br>• These vesicles are developed from surfactant from non-ionic origin, such as alcohol/diaIkyl polyglycerol, is introduced to cholesterol after it has been hydrated with aqueous media. | • More stable than liposomes.<br>• Osmotically active.<br>• Not require special storage and handling.<br>• Use as a depot system.<br>• Entrap drugs from broad solubility range.<br>• Increase bioavailability in topical, oral, and parenteral forms.<br>• More flexible structural designing<br>• Minimize drug clearance and improve therapeutic efficacy. | • Encapsulated drug may get hydrolysed and thus reduces shelf-life. |
| **Bilosomes** | • Bilosomes are a specialised new delivery vehicle that protects vaccinations from being broken down in the stomach, | • Effective even with minor quantities of antigen.<br>• Boost the efficacy of antigens that are ineffective when injected. | |

| Name of the VDDS | Explanation | Merits | Demerits |
|---|---|---|---|
| | allowing vaccines to be administered orally rather than intravenously.<br>• Composition of bilosomes is non-ionic surfactant and bile salt. | • Effective and safe alternative for traditional delivery of vaccines.<br>• More user acceptance and compliance.<br>• Remove refrigeration. | |
| **iii. Some other biocarriers of VDDS** | | | |
| **Colloidosomes** | • Colloidosomes are microcapsule shells having hollow nature made out of coagulated or fused particles at interface of the emulsion droplet.<br>• These are vesicular DDS that are designed to carry vitamins, proteins, and other dietary supplements. | • Easy formulation.<br>• High mechanical strength.<br>• Allow sustained or controlled release of drug.<br>• Control drug permeability.<br>• More flexibility and compact size | • During the transfer of colloidosomes from organic to aquatic media, a considerable proportion is lost.<br>• Low yield value. |
| **Cubosome** | • These are self-assembled, thermodynamically stable liquid crystalline phases in the presence of a polar solvent. In addition to structural symmetry, such crystalline phases with equal lengths have enough molecular orientation. | • Preparation is simple.<br>• Biodegradable.<br>• Ideal for mucosal and topical drug delivery systems.<br>• Targeted release capability.<br>• Allow encapsulation of amphiphilic or hydrophobic or hydrophilic drugs. | • Limited yield.<br>• Expensive. |
| **Ufasomes** | • Ufasomes have a lipid carrier that attaches to the surface of the skin surface and stimulates exchange of the lipid across outermost layers i.e., stratum corneum. | • Less expensive than the liposomes.<br>• More effective trapping of hydrophobic and hydrophilic medicines.<br>• Better stability than liposomes. | • Fluctuate drug level.<br>• Low Bioavailability.<br>• Poor deeper penetration. |

### 1.2 Vesicular Drug Delivery Systems for the topical fungal infections

As discussed earlier in the introduction, due to immunosuppressant treatment of some chronic diseases, people are more susceptible to skin fungal infections. The treatment available for fungal infections is as follows:

1. Azoles like itraconazole, ketoconazole posaconazole and fluconazole prevent ergosterol production (Garg et al., 2020).

2. Antifungal medicine is equivalent to Terbinafines and Morpholines that block the change of lanosterol into ergosterol (Garg et al., 2020).

3. Liposomes and solid lipid nanoparticles made up of squalene epoxidase are lipidic and may help the permeability of the drug through the skin (Garg et al., 2020).

4. Antibiotics containing polyenes, such as Amphotericin B in combination with Nystatin, combine with ergosterol which modifies the permeability of fungal cell membranes, resulting in cellular contents leakage and cell death (Garg et al., 2020).

5. The key components for the integrity of the cell wall of fungi are glucans. In fungal cells, glucan synthase adds glucose monomers to pre-existing glucan, helping to maintain the integrity of the cell. The diminishing activity of glucan synthase promotes the lysis of cells by weakening the cell membrane. Flucytosine, for example, inhibits the

nucleic acid synthesis and converts 5-fluorouracil into 5-fluorouridylic acid via a cascade including UMP pyrophosphorylase and cytosine deaminase. Additionally, phosphorylation of 5-fluorouridylic acid and its incorporation into mRNA, inhibit protein production of fungi and result in fungal cell lysis (Garg et al., 2020).

For this comprehensive review, it was decided to go systemically, hence, to do a systemic review is the output of discussion. For the systemic review, PRISMA 2020 (Page et al., 2021) decided to use where quantities of articles will be more than 10, which will help in smoothly sorting-out relevant articles from found data.

## 2. Objectives

The objectives of the systemic review were:

1. To explore the Vesicular Drug Delivery Systems used, by running a search query into the databases.

2. To study the research articles and find out the Vesicular Drug Delivery Systems for the treatment of skin fungal infections.

3. To summarize various approaches researched in the last five years i.e., from 2017-2022 in the treatment of infections from fungi to the skin.

## 3. Methodology

The specific algorithm was developed to fulfill the objectives of this systemic review, and exclusion criteria for the review were set as shown in Figure 1 and results were excluded for the drugs which are not from the anti-fungal class. Book chapters and review articles were also excluded from the study. To build a review Google Scholar® and PubMed® are two databases that are targeted, so that we can collect the number of freely available full-text articles and we can minimize the risk of bias (unavailability of full-text articles, for study). The search strategy, selection process, and filters used for excluded results can be overviewed in the following steps.

### 3.1 Steps followed in Systematic Review are as follows:

1. Google Scholar® and PubMed® are two databases targeted for the collection of articles for this study.

2. To run a search, a specific algorithm or query of keywords are developed, and results are filtered. This algorithm or queries of keywords are summarized in Table 2.

3. After running a first search on both databases, the number of articles were found. A further filter is applied to sort articles for the last five years i.e., 2017- 2022 in Table 3 (The same query is run by replacing the name of VDDS).

4. From the collected data, duplicate results are removed with the help of Rayyan®– Intelligent Systematic Review application.

5. Further sorting of the articles was also done as per the PRISMA using Rayyan®– Intelligent Systematic Review application itself and the results which do not match with the vesicular drug delivery system are excluded.

6. The selected articles are processed for retrieval and the articles which are failed for retrieval are excluded in this step.

These systematically processed, eligible articles were included in the study.

**Table 2** Specific algorithm or queries of keywords were developed to filter the results.

| Sr. No. | Name of Database used in the study | Specific algorithm or query of keywords to run a search* |
|---|---|---|
| **1.** | Google Scholar® | allintitle: liposome topical OR fungal OR fungus OR antifungal -review |
| **2.** | PubMed® | (((((((liposome) AND (topical)) AND (fungal)) AND (fungus)) AND (antifungal)) AND (formulation)) AND (preparation)) AND (evaluation) |

* While running a search only name of Vesicular Drug Delivery is changed each time, else rest of the command remains the same to have equity in the search.

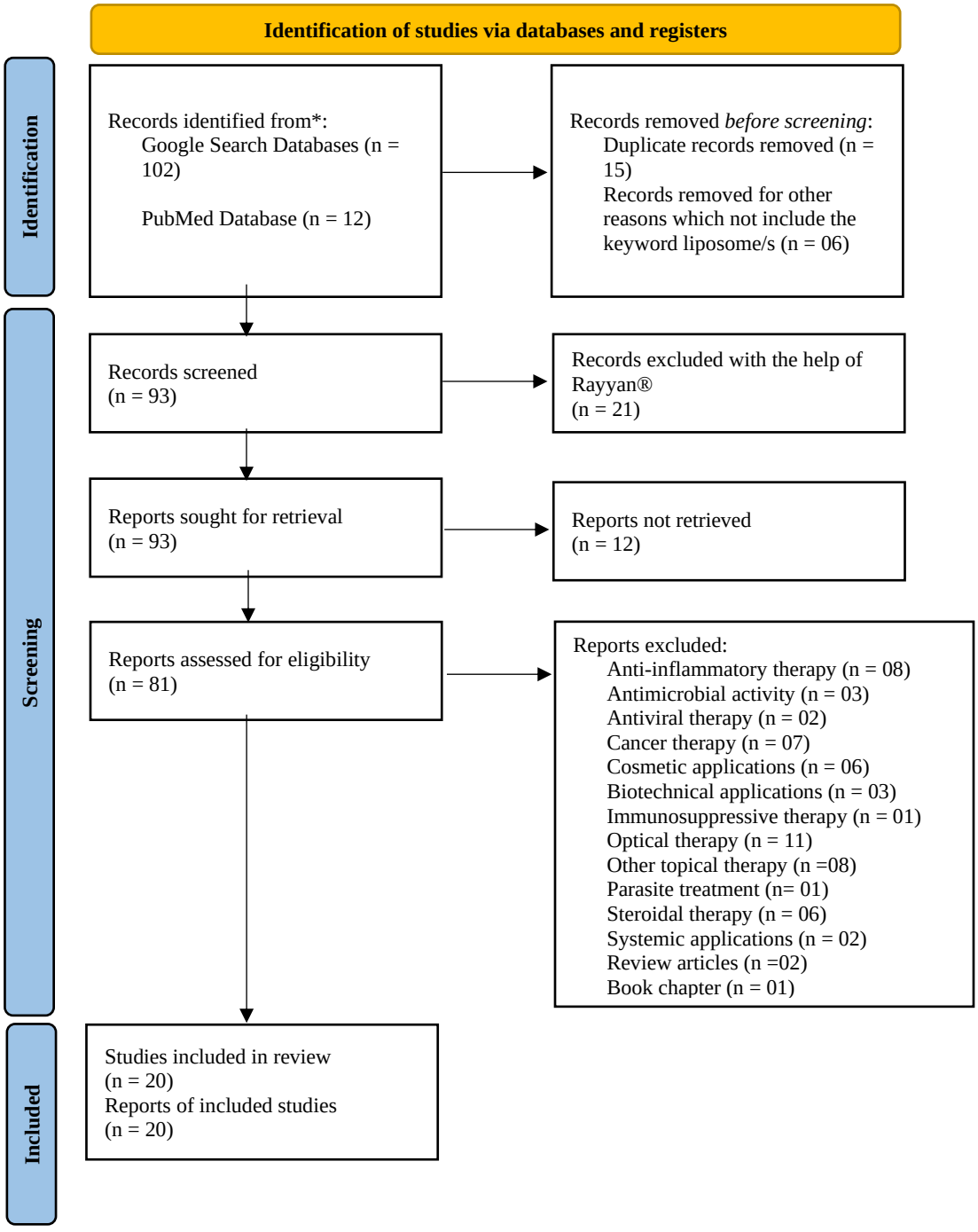


**Figure 1** PRISMA 2020 flow diagram for systematic reviews for use of Liposomes in infections spread from fungus on the skin for the past five years.

## 4. Results

By adopting the algorithm, searched articles we have found about 627 articles found the databases we targeted. As shown in Table 3, in this research we tried to summarize about thirteen types of VDDS used for the treatment of infections on the skin due to fungi. To conclude our study, we applied the filter in search engines for the last five years of research activities. In the present study, we came to know that out of the thirteen VDDS we searched across the databases; Research carried out on Liposomes in the last five years when compared with the number of articles found and retrieved from Google Scholar® is about 24.05% and from PubMed® 66.66%. Similarly, it was found that Sphingosomes (Google Scholar®-50%), Ethosomes (Google Scholar®- 42.59 % and PubMed®-50%), Transferosomes (Google Scholar®-41.66%), Niosomes (Google Scholar®-62.66% and PubMed®-66.66%), Bilosomes (Google Scholar®- 100%), Cubosomes (Google Scholar®-66.66 and PubMed®-66.66%) and Ufosomes or Ufasomes (Google Scholar®-50%) are available from different researchers (Table 3). Liposomes are still preferred by many researchers and are a basic type of all VDDS, we tried to summarize all the details about the Liposomes in this systemic review, and some research outputs are summarized for Liposomes in Table 4.

**Table 3** Summary of vesicular drug delivery system exclusively available for therapy by topical application of fungal infection after running specific algorithm or query in the targeted database.

| Sr. No. | Name of the database used | Name of the vesicular drug delivery system searched in the database (Keyword) | Number of articles found in the first search | Number of research found in the last 5 years | % of research in last five years compared with the number of articles found |
|---|---|---|---|---|---|
| **1.** | Google Scholar® | Liposome/s | 424 | 102 | 24.05660377 |
| | PubMed® | | 18 | 12 | 66.66666667 |
| **2.** | Google Scholar® | Emulsome/s | 02 | 01[# Occular] | 50 |
| | PubMed® | | 00 | 00 | 00 |
| **3.** | Google Scholar® | Enzymosome/s | 00 | 00 | 00 |
| | PubMed® | | 00 | 00 | 00 |
| **4.** | Google Scholar® | Sphingosome/s | 00 | 00 | 00 |
| | PubMed® | | 01 | 01 | 100 |
| **5.** | Google Scholar® | Ethosome/s | 54 | 23 | 42.59259259 |
| | PubMed® | | 02 | 01 | 50 |
| **6.** | Google Scholar® | Transferosome/s | 12 | 05[$] | 41.66 |
| | PubMed® | | 00 | 00 | 00 |
| **7.** | Google Scholar® | Pharmacosome/s | 00 | 00 | 00 |
| | PubMed® | | 00 | 00 | 00 |
| **8.** | Google Scholar® | Aquasome/s | 00 | 00 | 00 |
| | PubMed® | | 00 | 00 | 00 |
| **9.** | Google Scholar® | Niosome/s | 75 | 47 | 62.66666667 |
| | PubMed® | | 18 | 12 | 66.66666667 |
| **10.** | Google Scholar® | Bilosome/s | 01 | 01 | 100 |
| | PubMed® | | 00 | 00 | 00 |
| **11.** | Google Scholar® | Colloidosome/s | 00 | 00 | 00 |

| Sr. No. | Name of the database used | Name of the vesicular drug delivery system searched in the database (Keyword) | Number of articles found in the first search | Number of research found in the last 5 years | % of research in last five years compared with the number of articles found |
|---|---|---|---|---|---|
| | PubMed® | | 00 | 00 | 00 |
| 12. | Google Scholar® | Cubosome/s | 15 | 10 | 66.66666667 |
| | PubMed® | | 01 | 01 | 100 |
| 13. | Google Scholar® | Ufasome/s | 04 | 02 | 50 |
| | PubMed® | | 00 | 00 | 00 |
| **(# ocular = not included in the study because of not matches the category) ($ = not included in the study as no antifungal work was found for topical applications)** | | | | | |

**Table 4** Study on the use of Liposomes in infections spread from fungus on the skin for the past five years.

| Sr. No. | Species used in research | The antifungal agents used in research | Research Output | Ref. |
|---|---|---|---|---|
| 1. | *Candida albicans and Cryptococcus neoformans* | Amphotericin B | These findings also show that extracellular vesicles may be able to cross through the cell walls and transfer soluble and membrane-bound effectors. | (Walker et al., 2018) |
| 2. | - | Tacrolimus | Because lipid nanocarriers and skin have structural similarities, these vesicles would effectively target skin tissues and treat psoriasis with no or minimal adverse effects. | (Jindal, Awasthi, Singhare, & Kulkarni, 2020) |
| 3. | - | Methotrexate | Methotrexate-entrapped deformable liposomes could be a future topical option for treating human psoriasis with reduced toxicity, and they deserve more research. | (Bahramizadeh et al., 2019) |
| 4. | *Aspergillus niger and Candida tropicalis* | Ketoconazole | The findings revealed that the gel of ketoconazole (liposomes), in combination with an extract of neem, had a high possibility for treating seborrheic dermatitis and giving synergetic effect. | (Dave, Sharma, Yadav, & Agarwal, 2017) |
| 5. | *Aspergillus fumigatus mycelium* | Rapamycin | Rapamycin can minimise fungal keratitis fungal burden and drastically limit MCP-1 protein and mRNA expression. | (Zhang et al., 2019) |
| 6. | *Aspergillus flavus* | Voriconazole | The formulation with reduced side effects of the pure medication, reduced drug toxicity, and improved drug bioavailability and stability. | (Hassanpour et al., 2020) |
| 7. | - | Ibrutinib with Curcumin | Topical treatment with liposomal gel effectively reduced psoriasis-related acanthosis lesions, according to histological analysis. | (Jain et al., 2022) |
| 8. | *Candida albicans* | Voriconazole | Synthetic liposomal Voriconazole could be an effective antifungal against Candida albicans. Furthermore, the so-called formulation can overcome azole resistance to a degree. More in vivo study is needed in this area, though. | (Hassanpour et al., 2021) |
| 9. | *Candida tropicalis, Candida krusei and Candida albicans* | Farnesol and fluconazole | Liposomes containing farnesol have the potential to be exploited in the development of antifungal drugs. | (Bezerra et al., 2020) |
| 10. | *Leishmania mexicana* | Sodium stibogluconate and ketoconazole | A promising therapy approach for cutaneous leishmaniasis has been discovered: sodium stibogluconate and ketoconazole co-loaded nano-elastic liposomes. | (Dar, Khalid, Varikuti, Satoskar, & Khan, 2020) |

| Sr. No. | Species used in research | The antifungal agents used in research | Research Output | Ref. |
|---|---|---|---|---|
| **11.** | *Candida albicans, Cryptococcus neoformans, and Aspergillus fumigatus* | Liposomes coated with Dectin-2 and loaded with amphotericin B | Candida albicans, Candida neoformans, and Candida fumigatus linked 50 to 150 times stronger to Dectin-2-coated liposomes containing amphotericin B than untargeted liposomes, killing the fungi by an order of magnitude. Most antifungal drugs may be significantly enhanced. | (Ambati et al., 2019a) |
| **12.** | *Trichophyton rubrum/ Dermatophytosis* | Itraconazole | In the case of dermatophytosis, the proposed formulation could be a promising and quick alternative to traditional antifungal therapy. | (Kumar, & Goindi, 2021) |
| **13.** | *Rhizopus delemar* | Dectin-1 and amphotericin B; | DEC1-AmB-loaded Liposomes, which delivered sub-micromolar AmB concentrations, were an order of magnitude more effective than AmB- Loded Liposomes at inhibiting or/and killing R. delemar. Mucormycosis treatment could be improved with targeted antifungal drug-loaded liposomes. | (Choudhury, Ambati, Lewis, Meagher 2022) |
| **14.** | *Candida albicans* | AmB/AmB-α cyclodextrin complex | Double loaded liposomes were used to evaluate improved antibacterial effectiveness, least inhibitory concentration, and minimum fungicidal concentration against Candida albicans. | (Mutlu-Agardan, Yilmaz, Kaynak Onurdag, & Celebi, 2021) |
| **15.** | *Candida tropicalis, Candida krusei and Candida albicans* | Nerolidol and Fluconazole | According to the findings of this study, nerolidol shows antifungal activity against Candida tropicalis and Candida albicans as well as increases the power and the effects of fluconazole only in the liposomal form | (Fonseca Bezerra et al., 2020) |
| **16.** | *Aspergillosis* | Voriconazole | The entrapment efficiency of long-circulating liposomes was determined to be 75-85%. Pegylated liposomes successfully demonstrated extended circulation action. | (Patel, Modiya, Shinde, & Patel, 2018) |
| **17.** | *A. fumigatus* | Amphotericin B | Dectin-1- Amphotericin B-LLs effectively reduced A. fumigatus growth and viability more than untargeted control liposomes providing the same quantities of AmB, effectively boosting the effective dose of AmB. | (Ambati et al., 2019b) |
| **18.** | Candida species | Essential oil of *Artemisia annua* | liposomes contain the essential oil of Artemisia annua loaded founded significantly effective than free essential oil against Candida species, according to the findings. | (Risaliti et al., 2019) |
| **19.** | - | Fluconazole | Fluconazole liposomal gel extends the duration of medication release, lowering drug application frequency and enhancing patient compliance. | (Hemanth, Kumar, Goudanavar, & Sagar, 2021) |
| **20.** | *A. fumigatus* | Dectin-2 and Amphotericin B | Dectin-2-targeted amphotericin B administration to A. fumigatus produced much better efficacy than untargeted antifungal formulations. | (Ambati et al., 2021) |

**4.1 Liposome**

Liposomes are the first medication delivery using phospholipid-based nanocarrier systems, and they are initially identified in the 1980s. Liposomes have the capacity to bring either lipophilic or hydrophilic active molecules due to their unique structural features. (Bongomin et al., 2017; Tansathien et al., 2020). Phospholipid liposomes are ultra-flexible, amphiphilic, and protect the medication entrapped in them. Liposomes can be adsorbed on the surface of the skin or reach deeper layers. These are major benchmarks for topical drug administration as because of the capacity to modify the biological distribution characteristics. (Garg et al., 2020).

4.1.1 *Method of preparation* (Akbarzadeh et al., 2013)

Four basic steps are involved-

1. Withdraw the solvent used and lipid film is formed and further dried.
2. Dispersion in an aqueous medium.
3. Purification of formulated liposomes.
4. Analysis and finalization of the results.

Practically liposomes can be prepared by either of the methods, passive loading techniques, or active loading techniques.

Passive loading is again subdivided into:
A. Mechanical dispersion.
B. Solvent dispersion.
C. Removal of non-encapsulated material or detergent.

**A. Mechanical dispersion method**

Available several techniques for liposome preparations by this method, it involves.

I. Sonication.
II. Extrusion or use of French pressure cell.
III. Freeze-thawed method.
IV. Handshaking or Lipid film hydration or non-hand shaking or freeze drying.
V. Micro-emulsification.
VI. Membrane extrusion
VII. Dried reconstituted vesicles

**I. Sonication** (Akbarzadeh et al., 2013)

The most utilised procedure for SUV preparation is sonication. MLVs are sonicated with a bath or probe sonicator in an inert atmosphere. The drawbacks of the approach are, comprise low encapsulation efficacy, the risk of chemical degradation, the presence of metal contamination of phospholipids due to the tip of the probe, and the existence of MLV alongside the SUV. There are two alternatives here as well:

**a. Sonication of the probe:** The sonicator's tips are entirely immersed in the dispersion. The energy input is particularly high in lipid dispersion for this method. The container must be immersed in an ice bath or water bath because the generation of energy towards the tip creates localised heat. During sonication, more than 5% of the lipids can be de-esterified in of one hour. The utilisation of the probe sonicator made of titanium results in the peeling of titanium which pollutes the solution.

**b. Bath Sonication:** Dispersion is in a cylinder. Temperature control of the lipid dispersion is generally easier than with sonication by direct dispersion with the use of a tip. Sonicated material may be kept separate from the probe units in a sterile vessel or an atmosphere having in inert environment.

**II. Extrusion or French pressure cell** (Akbarzadeh et al., 2013)

In this method, MLV is extruded through a tiny aperture. The proteins that do not appear to be as haughty as they do during sonication, which is an important element. The SUVs generated by sonication or detergent removal tend to recall encapsulated solutes substantially longer than the French press vesicle, which is an interesting observation.

Handling unstable materials with caution is part of the process. The method has some merits compared with sonication. Formulated liposomes are greater than that of sonicated SUVs. The disadvantages of this method are that the high temperature is difficult to achieve and the modest quantities of working (as the maximum is about 50 mL).

**III. Freeze-thawed liposomes** (Akbarzadeh et al., 2013)

After frozen down, the SUVs are immediately thawed. Dispossession of aggregated materials into LUV happened because of quick sonication. Unilamellar vesicles are created when SUV fuses during the freezing as well as thawing actions. Synthesis is greatly reduced with increasing concertation of phospholipid and the ionic strength of the medium. Encapsulation efficacies ranging from 20% to 30% have been attained.

**IV. Handshaking or non-hand shanking or Lipid film hydration** (Mulla, Thorat, Rayate, & Nitalikar, 2019)

In this method, lipids are caste like stacks of film from its organic solution using a handshaking or with the use of the flash rotary evaporator under reduced pressure may. When lipids are hydrated, they possess swelling properties and peel off from the circular bottom flask resulting in the formation of MLVs and LUVs, respectively. Swelling and

dispersion of lipids are happening because of the mechanical energy which is provided by hand shaking or by exposure of the film to a water stream saturated with nitrogen for 15 minutes or by manual agitation and then swelling is carried out in aqueous medium without shaking also known as non-shaken vesicles.

**V. Micro-emulsification** (Mulla et al., 2019)

The "Micro Fluidizer" turns concentrated lipid dispersion into micro MLVs. Lipids can be added to fluidizers in the form of a slurry (which contains un-hydrated lipids in an organic medium) or as a dispersion of big MLVs. The microfluidizer pumps the fluid via a 5-um aperture at extremely high pressure (10,000 psi or 600-700 bar). The fluid is then forced via micro channels, resulting in the collision of two streams of fluid at right angles at high speeds, resulting in efficient energy transfer. The collected fluid can be cycled through the interaction chamber and pumped until spherical vesicles form. After a single pass, vesicles are shrunk to between 0.1 and 0.2um in diameter.

**VI. Membrane Extrusion Liposomes** (Mulla et al., 2019)

It works with both LUVs and MLVs. This process produces liposomes called membrane filter extrusion liposomes. A high lipid concentration can be used to collect the 30% volume. This method may catch 1-2 liters of lipids per mole. It's because they're simple to make, with random vesicle diameter selection, reproducibility from batch to batch, and no solvent or surfactant contamination.

**VII. Dried reconstituted vesicles** (Mulla et al., 2019)

Liposomes obtained this way are often "uni or oligo lamellar," with a diameter of 1.0 mm or less. This process begins by freeze-drying an empty SUV dispersion and then rehydration is carried out in a water entrapment of material.

**B. Solvent dispersion method** (Akbarzadeh et al., 2013; Mulla et al., 2019)

**I. Ether injection or solvent vaporization**

The mixture of lipids in an ether-methanol or diethyl ether mixture is gradually introduced at $55^0$C to $65^0$C into an aqueous solution of the substance to be encapsulated or under reduced pressure. Formulation of liposomes is created after the removal of ether from a solution under a vacuum. Formulated vesicles are heterogeneous in nature having a size of about 70 to 200 nm, at last, the drug molecules to be encapsulated are exposed to organic solvents at high temperature.

**II. Ethanol injection**

A considerable buffer quantity is injected rapidly with a lipid solution of ethanol resulting in the formation of MLVs in real-time. The heterogeneity of the population (30 to 110 nm), the dilute nature of liposomes, due to the formation of azeotrope it is difficult to remove all ethanol quantity, and the high possibility of inactivation of biologically active macromolecules in the presence of even minute concentration of ethanol are all disadvantages of the method.

**III. Reverse phase evaporation method**

For the very first-time liposomes synthesis is done with a high aqueous space: lipid ratio and the entrapment efficiency is increased significantly. Inverted micelles are developed in this method by sonicating the mixture of an organic phase containing amphiphilic molecules with a buffered aqueous phase containing the water-soluble drug molecules to be encapsulated. Due to the gradual withdrawal of organic solvents, synthesized inverted micelles are transformed into the viscous gel. The gel state collapses at a critical stage in the process, disrupting a portion of the inverted micelles.

The formation of liposomes occurs after the addition of extra phospholipid's environment, which helps in the formation of a complete bilayer round to a residual micelle. Hand-shaken liposomes and multilamellar liposomes have a four-fold larger aqueous volume-to-lipid ratio than reverse-phase evaporation liposomes.

To summarise, brief sonication is used to shape a bi-phase structure consisting of phospholipids in an organic solvent e.g., diethyl ether or isopropyl ether, or a combination of chloroform and isopropyl ether with an aqueous buffer. The creation of a thick gel happens when solvents are detached at decreasing pressure. The formation of liposomes is carried out by evaporating residual solvent in a rotating evaporator at

low pressure. The efficiency of encapsulation may be increased by about 65% with the use of 0.01 M NaCl as a low ionic strength medium. By using this method small-sized or large-sized macromolecules can be encapsulated. The primary drawback is that the encapsulated components are exposed to organic solvents and are only temporarily enclosed.

**C. Removal of non-encapsulated material or Detergent removal** (Akbarzadeh et al., 2013; Mulla et al., 2019)

It can be done by following methods-

**I. Dialysis**

Detergent-free buffers known as equilibrium dialysis are the key step in this process. LipoPrep made by Diachema AG, Switzerland can be used for this purpose. The micelles become phospholipid-rich when the detergent is removed, and they eventually join to produce LUVs.

**II. Mixed micelles absorption for removal of Detergent like alkyl glycoside, Triton X-100 cholate etc.**

Absorption of detergent is accomplished with shaking of mixed micelle solution and beaded organic polystyrene adsorbers. Bio-beads SM2 make by Bio-Rad Laboratories, Inc., Hercules, USA and XAD-2 beads make of SERVA Electrophoresis GmbH, Heidelberg, Germany.

**III. Gel-permeation chromatography**

Pre-treatment is essential because the inflated polysaccharide beads absorb considerable amounts of amphiphilic lipids. Empty liposome solutions are used to pre-saturate the gel filtration column with lipids during pre- treatment. At low flow rates, liposomes and detergent monomers can be easily separated.

4.1.2 Evaluation parameter for prepared liposomes (Mulla et al., 2019)

Prepared liposomes can be characterized for their various aspects such as physical characterization, chemical characterization, and biological characterization. They are summarized as follows:

**A. Physical characterization** (Bolla et al., 2019)

**I. Vesicle shape and surface morphology-** Characterized by Freeze-fracture electron microscopy or TEM that is Transmission Electron Microscopy, etc.

**II. Size distribution and Mean vesicle size-** Techniques available to determine vesicle size and distributions are gel permeation, gel exclusion, Zeta Potential Analysis, Dynamic light scattering, laser light scattering, etc.

**III. Surface charge -** Can be analysed by Free-flow electrophoresis.

**IV. pH and Electrical potential on the surface -** It can be determined by using pH-sensitive probes and Zeta potential analyser.

**V. Lamellarity-** 31P-NMR, Freeze-fracture electron microscopy and small-angle X- ray scattering can be used.

**VI. Phase behaviour-** Is identified by Freeze- fracture electron microscopy, Differential Scanning Calorimetry or DSC.

**VII. Drug entrapment efficiency -** Is done by radiolabelling, Minicolumn centrifugation technique or ion exchange Chromatography, etc.

**B. Chemical characterization**

**I. Phospholipid concentration-** The concentration of phospholipid can be determined by Stewart assay, Barlett assay, or HPLC.

**II. Concentration of Cholesterol-** The concertation can be determined by HPLC and Cholesterol oxidase assay.

**III. Phospholipid peroxidation-** Determined by GLC, Iodometric, and UV absorbance.

**C. Biological characterization**

**I. Sterility-** Is determined by cultures of Aerobic bacteria or anaerobic bacteria that can be used.

**II. Microbial enumeration-** Generally, microbial enumeration is a microbial limit test for skin topical preparation. Sterility and pyrogen tests are optionally used for ophthalmic preparations.

**III. Toxicity study in Animals-** Pathology, histology, and observing rate of survival for determination of toxicity.

4.1.3 Applications of Liposomes (Myneni et al., 2021; Akbarzadeh et al., 2013; Mulla et al., 2019)

- Doxorubicin Liposomes, Amphotericin B

Liposomes, Paclitaxel Liposomes, Cytarabine Liposomes, Irinotecan Liposomes, and Cisplatin Liposomes are some of the liposomes that are already in the market.

- Liposomes are also used to convey genes.
- Genetic (DNA) vaccination, gene, and antisense therapy are possible by using the Liposomes.
- Antifungal or Antimicrobial lung therapies and liposomes as an antiviral or anti-HIV action, liposome medicines, and liposome biological response modifiers are the other applications of the Liposomes Vesicular Drug Delivery system.
- Liposomes as a carrier of immunomodulators, Liposomal vaccines, Liposomes as an immunological (vaccine) adjuvant.
- Liposomes tumor treatment is gaining the attention of researchers because of target specificity.
- Artificial blood substitutes can be prepared by using liposomes.
- Cosmetics and dermatology applications are other applications of liposomes.
- Liposomes are used to immobilise enzymes and in bioreactor technology.
- Antisense oligonucleotide treatment is possible with the use of liposomes.
- Liposomes as the vehicles for the delivery of drugs, sustained or controlled release of the drug, enhancement in drug stability, alteration in biodistribution of drug or alteration in pharmacokinetics properties of drugs, treatment covering enzyme replacement, lysosomal storage diseases, etc.
- Liposomes are also used in Immunodiagnostic therapy.
- Liposomes act as Vehicles for macromolecules such as cytokines or genes, as well as carriers of tiny cytotoxic chemicals.
- Liposomes also act as carriers for radiopharmaceuticals and radio diagnostics.
- Liposomes have immunological applications.

## 5. Discussion

In this systemic review, we explore various Vesicular Drug Delivery Systems (a total of thirteen types; as shown in Table 3), and fund that, much more research has been carried since form last five years despite of pandemic situation, for the treatments of several the tropical diseases or disorders. Throughout the analysis, we determined that there is vast research and development carried out by researchers in various therapeutic areas such as anti-inflammatory therapy, antimicrobial activity, antiviral therapy, cancer therapy, cosmetic applications, biotechnical applications, immunosuppressive therapy, optical therapy, other topical therapy, parasite treatment, steroidal therapy, systemic applications, etc.

From the Novel Vesicular Drug Delivery Systems we studied, we tried to summarize Liposomes as the VDDS in this systemic review as it is preferred by many researchers as it is the basic type of VDDS, which has been used for topical applications on the skin for fungus infection treatment. As per the data analysed- Sphingosomes, Ethosomes, Niosomes, Bilosomes, Cubosomes, and Ufosomes or Ufasomes are the choices of researchers as VDDS in the therapy of different fungal diseases, because of certain advantages associated with these types of VDDS, such as improved bioavailability, improved drug pharmacokinetics, improved encapsulation, and stability, etc.

There are various methods for the preparation of Liposomes including the Mechanical dispersion method (Sonication, Extrusion or use of French pressure cell, Freeze-thawed method, Handshaking or Lipid film hydration or non-hand shaking or freeze drying, Micro-emulsification, Membrane extrusion, Dried reconstituted vesicles) and Solvent dispersion method (Ether injection or solvent vaporization, Ethanol injection, Reverse phase evaporation). Some of these methods can be used for the preparation of other VDDS with slight modifications. Prepared VDDS are subjected to physical, chemical, and biological characterization.

Nowadays, Novel Vesicular Drug Delivery Systems are gaining attention for the treatment of number of the therapies including gene and antisense therapy, Antifungal or Antimicrobial lung therapies, tumor treatment, Cosmetics and dermatology applications, Immunodiagnostic therapy, etc.

We would like to conclude the limitation of this systemic review is, we could only summarize Liposomes. To bridge this gap, we like to appeal to upcoming researchers who are having their target area as anti-fungal drug delivery for topical applications and can focus on VDDS such as Sphingosomes, Ethosomes, Niosomes, Bilosomes, Cubosomes, and

Ufosomes or Ufasomes.

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