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Advancing liposome technology for innovative strategies against malaria

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ABSTRACT

This review discusses the potential of liposomes as drug delivery systems for antimalarial therapies. Malaria continues to be a significant cause of mortality and morbidity, particularly among children and pregnant women. Drug resistance due to patient non-compliance and troublesome side effects remains a significant challenge in antimalarial treatment. Liposomes, as targeted and efficient drug carriers, have garnered attention owing to their ability to address these issues. Liposomes encapsulate hydrophilic and/or hydrophobic drugs, thus providing comprehensive and suitable therapeutic drug delivery.

Moreover, the potential of passive and active drug delivery enables drug concentration in specific target tissues while reducing adverse effects. However, successful liposome formulation is influenced by various factors, including drug physicochemical characteristics and physiological barriers encountered during drug delivery. To overcome these challenges, researchers have explored modifications in liposome nanocarriers to achieve efficient drug loading, controlled release, and system stability. Computational approaches have also been adopted to predict liposome system stability, membrane integrity, and drug-liposome interactions, improving formulation development efficiency. By leveraging computational methods, optimizing liposomal drug delivery systems holds promise for enhancing treatment efficacy and minimizing side effects in malaria therapy. This review consolidates the current understanding and highlights the potential of liposome strategies against malaria.

1. Introduction

Malaria is a contagious parasitic infectious disease that causes substantial morbidity and mortality, particularly in children and pregnant women (Mishra et al., 2017). According to the latest report from the World Health Organization (WHO), approximately 247 million cases and 619 thousand deaths worldwide are due to malaria in 2021 (World Health Organization, 2022). Malaria is caused by the protozoan parasite Plasmodium, transmitted by the infected female Anopheles mosquitoes (Alven and Aderibigbe, 2019). *Plasmodium falciparum* is the most common *Plasmodium* species that causes severe diseases (Saifi, 2013). *Plasmodium falciparum* is endemic in 95 countries on five continents, with approximately 48 % of the world's population, or 2.48 billion people, at risk of malaria infection (Chu and White, 2016).

The life cycle of malaria parasites in humans consists of hepatic and erythrocytic phases. Human infection begins when sporozoites enter the bloodstream via the bite of *the Plasmodium*-infected Anopheles mosquito (Cowman et al., 2016). Subsequently, sporozoites traverse host hepatocytes before invading liver cells (Tavares et al., 2013). During the liver stage, hepatocyte-infected sporozoites rapidly develop into an exoerythrocytic form (EEF), which is surrounded by parasitophorous vacuole membranes inside the hepatocytes (Cowman et al., 2016; Gupta et al., 2012). Some *Plasmodium* species can form hypnozoites that can cause disease relapse (Parhizgar, 2017). The erythrocytic phase begins when merozoites released from hepatic schizonts infect red blood cells and undergo asexual development (Cowman et al., 2016).

The WHO has approved three classes of antimalarial drugs approved by the WHO namely quinolones, artemisinins, and antifolates (Mustapha et al., 2020). Since the 1940s, quinolone-class drugs, such as chloroquine (CQ), primaquine, and amodiaquine, have been used as primary

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therapies (World Health Organization, 2022). The artemisinin and antifolate classes primarily target the erythrocytic phase, whereas primaquine is the only quinolone-class drug that acts during the hepatic phase (Borgheti-Cardoso et al., 2020; Cowman et al., 2016).

The mechanism of action of CQ in the erythrocytic phase is not yet fully understood; however, the current theory suggests that CQ exerts its effects on parasites after entering erythrocytes and parasite membranes owing to its small and lipophilic nature (Coban, 2020). As a protonated, weakly basic drug, CQ has a pH-enhancing effect and can accumulate in parasitic food vacuoles. This leads to the breakdown of host erythrocyte hemoglobin and the release of toxins, such as Fe(III), protoporphyrin IX (FeIIIPPIX), or hematin. Parasites can detoxify these toxins and convert them into a harmless form known as hemozoin. CQ inhibits this detoxification process, accumulating free hematin, which is highly toxic to *Plasmodium*. This buildup of toxic compounds damages the parasitic cell membranes and ultimately causes their mortality (Zhou et al., 2020).

Primaquine (PQ), an 8-aminoquinoline, is effective in killing hypnozoites of *Plasmodium vivax* and *Plasmodium ovale* and displays weak activity in the asexual phase (Ashley et al., 2014). However, PQ risks inducing acute hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Fasinu et al., 2016; Taylor et al., 2019). Because PQ primarily targets the hepatic phase, combining it with antimalarial therapy for the erythrocytic phase is necessary (Chu and White, 2016).

Amodiaquine (AQ) is more effective than CQ in treating malaria caused by *P. falciparum* in Africa, parts of South America, and Oceania (Hoffman et al., 2011). AQ shares a similar structure and mechanism of action with CQ and is effective against *P. falciparum* strains that have developed resistance to CQ (Mustapha et al., 2020; Parhizgar, 2017). However, AQ is not currently used as a prophylactic therapy for malaria because of its severe side effects, including agranulocytosis and hepatitis, which may occur after prolonged use (Mustapha et al., 2020).

Artemisinin, a sesquiterpene lactone derived from *Artemisia annua* L. (Lu et al., 2019), is an antimalarial drug that effectively eliminates *Plasmodium* during both sexual and asexual phases (Cui and Su, 2009). Although its mechanism of action has not been fully elucidated, the opening of the endoperoxide ring by haem, resulting from hemoglobin degradation, is presumed to contribute to its activity by increasing oxidative stress, ultimately leading to parasitic death (Cowman et al., 2016).

Antifolates are antimalarial drugs that inhibit the folate metabolic pathway in *Plasmodium* (Gregson and Plowe, 2005). Two classes of antifolates are known: dihydropteroate synthase (DHPS) inhibitors (class I) and dihydrofolate reductase (DHFR) inhibitors (class II) (Nzila, 2006). Both enzymes play crucial roles in folate biosynthesis in *P. japonica* (Djapa et al., 2007). Folate is essential for protein synthesis and the survival of malarial parasites during the erythrocytic and schizogony stages (Mustapha et al., 2020). Class I antifolates include sulphonamides, whereas class II antifolates include pyrimethamines and cycloguanil (Nzila, 2006).

Some antimalarial drugs are no longer effective due to resistance development (Rahmasari et al., 2022). Resistance to *P. falciparum* typically emerges in areas with low transmission rates, such as Southeast Asia and South America, before spreading to regions with high transmission rates, such as Sub-Saharan Africa (Menard and Dondorp, 2017). Drugs that encounter early resistance include chloroquine, sulphadoxine-pyrimethamine, artemisinin, piperaquine, and mefloquine (Ross and Fidock, 2019). Various factors contribute to the development of drug resistance, including inappropriate therapeutic practices, lack of patient adherence to treatment, and the use of single drugs (World Health Organization, 2022). Patient non-compliance often occurs due to side effects such as nausea, vomiting, and dizziness associated with prolonged treatment (Braga et al., 2015; Galappaththy et al., 2013).

In addition to the issue of resistance, the effectiveness of almost all antimalarial drugs in reaching their target, especially PQ, which acts during the hepatic phase, is still in question. Moreover, most of these drugs require therapeutic regimens with high daily doses, exacerbating the occurrence of side effects (da Silva de Barros et al., 2021). This is because the conventional formulation of Primaquine (PQ) available on the market is oral dosage forms. Oral drug administration exhibits several drawbacks; one is the absorption process in the gastrointestinal tract, where a significant portion of the drug is distributed into the bloodstream in the systemic circulation. Additionally, a first-pass metabolism stage reduces the concentration of the drug reaching the target (Homayun et al., 2019; Patel et al., 2020). Free Primaquine (PQ) in the bloodstream increases the risk of side effects, specifically hemolytic anemia induced by oxidative stress on red blood cells (Srinivasan et al., 2021). Meanwhile, the primary target of Primaquine (PQ) is the liver, which has fenestrae with a diameter of 50–300 nm. Hence, conventional formulations cannot reach this target without a carrier system (Szafranska et al., 2021).

On the other hand, to combat malaria in the erythrocytic phase, antimalarial agents like Chloroquine (CQ) must be able to diffuse across infected erythrocytes and the parasitic membrane. Therefore, small size and lipophilic characteristics are required (Coban, 2020). Furthermore, there is a need for a drug design with the ability to distribute for an extended period within erythrocytes with minimal side effects (Memvanga and Nkanga, 2021). Characteristics of the active ingredient, such as half-life, distribution volume, solubility, and permeability, significantly influence the effectiveness of drugs, especially for preventive malaria therapy. In the hepatic phase of treatment, it is essential to have an active ingredient design capable of achieving high distribution in the liver to prevent the development of sporozoites in the liver into merozoites that infect red blood cells (Louisa et al., 2022).

Meanwhile, for therapy in the erythrocytic phase, a drug design is needed that can be taken up by erythrocytes with minimum side effects (Coban, 2020). Therefore, a carrier is needed to transport targeted antimalarial agents to the liver to address the hepatic phase and facilitate diffusion into erythrocytes to tackle the erythrocytic phase. This design aims to prevent further infection development, particularly the danger of cerebral malaria.

The liver contains fenestrae, transcellular pores 50–300 nm diameter in sinusoidal endothelial cells (LSEC). These fenestrae allow drugs to enter the liver via the bloodstream (Szafranska et al., 2021). However, drugs also encounter the mononuclear phagocytic system (MPS), which includes Kupffer cells that eliminate the drugs (Memvanga and Nkanga, 2021). Hence, a drug delivery system that can penetrate the liver through the fenestrae and evade elimination by the phagocytic system of the liver is required.

In contrast, Kupffer cells are crucial in promoting sporozoite replication in the liver and their development into the erythrocytic stage (Frevert et al., 2006). Research conducted by Pradel et al. (2004) using *in vitro* modeling demonstrated that sporozoites invade the liver through Kupffer cells rather than LSECs. Within Kupffer cells, sporozoites are protected within vacuoles, avoiding colocalization with lysosomal markers and maintaining their structural integrity for several hours, indicating that sporozoites do not undergo respiratory bursts. Targeted drug delivery to Kupffer cells is a potential approach in the future. However, Kupffer cell depletion can increase cytokine expression, potentially leading to cytokine storm syndrome (Zhang et al., 2022).

Liposomes are the most extensively studied nanocarriers used in drug delivery systems. They are spherical lipid vesicles, typically with a particle diameter of 50 –500 nm, composed of one or more lipid bilayers surrounding an aqueous core (Fig. 1). This structure provides optimal protection for hydrophilic and hydrophobic drugs against diffusion and external factors (Kohli et al., 2014; Nsairat et al., 2022). The encapsulation of drugs in liposomes can enhance therapy by controlling their absorption, metabolism, half-life, and toxicity reduction (Khan et al., 2020). Liposomes, as a delivery system for antimalarial therapy to the liver, can decrease the required dosage and minimize toxicity (Gupta et al., 2012). Liposomes with a 125–175 nm diameter can be targeted to

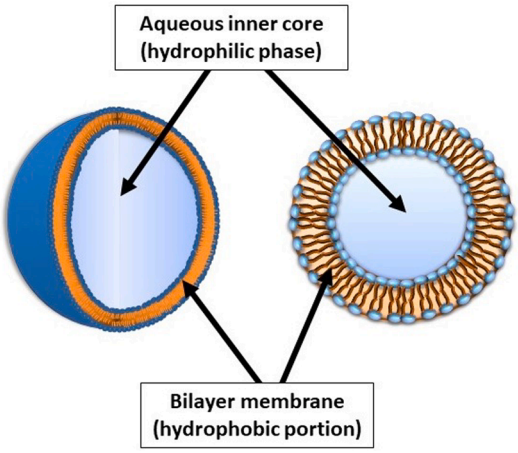

Fig. 1. The schematic figure of liposome.

the liver tissue through intercellular gaps or fenestrae in the liver sinusoids (Miatmoko et al., 2020). Numerous studies have explored encapsulating antimalarial drugs, such as PQ and CQ, in liposomes using various preparation methods (Qiu et al., 2008; Santos-Magalhães and Mosqueira, 2010). However, liposomes containing PQ and CQ often exhibit low entrapment efficiency and slow drug release rates owing to their interactions with liposome bilayer membranes (DMPC and DPPC) (Miatmoko et al., 2021).

Computational methods in drug development offer significant opportunities because non-laboratory simulations can reduce costs and materials and improve processing time efficiency. Developing nanoparticle drug delivery systems involves solubility, loading capability, drug release rate, stability, and drug target recognition. Traditionally, these variables have been determined through trial and error in the laboratory (T et al., 2022). However, computational technology has been utilized in pharmaceutical research, including the development of software that adapts experimental data to mathematical models and provides numerical solutions for drug molecular structures and drug delivery system formulations (Bunker and Róg, 2020).

One computational approach used to study the mechanisms of action of biological macromolecules, including liposomes composed of phospholipids, is Molecular Dynamics (MD) simulation (Parchekani et al., 2022). MD simulations can reveal structural changes and fluctuations in proteins, nucleic acids, and liposomes, providing valuable insights (Parchekani et al., 2022). This method helps study protein stability, nonbinding modifications, protein folding, and other research areas (Bunker and Róg, 2020; Parchekani et al., 2022). To date, MDS has been limited to studying interactions between drugs and target proteins. However, with current technological advancements, its utilization can also be expected to predict the design of drug delivery carriers suitable for the pathophysiological conditions of the target tissue. By predicting the interaction parameters between drugs and carrier matrices or carrier interactions with cell surfaces, we hope to generate optimal drug delivery system formulations even before laboratory production. These predicted results can be tested *in vitro* or *in vivo*, potentially reducing time and cost. For instance, Chen et al. (2020) used coarse-grained MD simulations to compare conventional liposomes' stability with elastic liposomes containing high surfactant concentrations. They analyzed each model's constant diffusion and total vesicle morphological transition time and found that the fusion probability was higher in elastic liposomes, indicating poorer stability. Furthermore, machine learning-based quantitative structure–property relationship (QSPR) modeling can predict suitable drug candidates for remote loading, achieving high intraliposomal drug concentrations (Cern et al., 2014).

This review discusses the potential of liposomes as nanocarriers for malaria drug delivery and the development of liposomes designed using computational approaches to achieve optimal physicochemical characteristics.

2. Designing nanocarrier liposomes for drug delivery

The primary principle in developing drug delivery nanocarriers is efficiently transporting active ingredients to the target site, ensuring treatment efficacy with minimal side effects. (Tewabe et al., 2021). The target tissue's biological conditions heavily influence nanocarriers' effectiveness in delivering active ingredients. In the context of malaria therapy, there are two primary therapy targets: the hepatic phase and the erythrocytic phase, each requiring distinct approaches (Cowman et al., 2016; Tavares et al., 2013). In the hepatic phase, sporozoites invade hepatocytes and rapidly transform into the exoerythrocytic form (EEF), encapsulated within the parasitophorous vacuole membrane inside the hepatocyte. Consequently, targeting therapy becomes challenging when relying solely on free drugs (Cowman et al., 2016; Gupta et al., 2012). On the other hand, during the erythrocytic phase, merozoites infect red blood cells, necessitating free drugs circulating in the bloodstream and capable of diffusing into red blood cells (Coban, 2020). In formulating a drug delivery nanocarrier, various additional components are required to address issues related to the active ingredient and achieve the desired physicochemical characteristics and biological compatibility. However, the nanocarrier formulation process is highly complex due to the influence of various factors on the bioavailability and efficacy of the delivered drug, both from the active ingredient and the composition materials. Therefore, a comprehensive approach is essential, integrating all interrelated physical, chemical, and biological factors.

2.1. The vital role of physicochemical properties of the active pharmaceutical ingredients

In nanocarrier formulation, additional components address issues with the active ingredient without introducing new complications, achieving problem-solving without creating new problems. The characteristics of the active ingredient should be the fundamental consideration in the formulation aspect and manufacturing methods to produce a dosage form that is safe, effective, stable, and acceptable. The characteristics of the drug may influence the design of liposome nanocarriers. Some of these characteristics are as follows:

2.1.1. Solubility and permeability

The bioavailability of a drug is primarily determined by several factors, such as drug absorption, liver metabolism, distribution in the blood, tissues, and organs, and drug excretion from the body. Drug absorption is crucial in determining drug bioavailability and exposure *in vivo* (J. Liu et al., 2020). The drug absorption and bioavailability rates are primarily controlled by two main parameters: solubility and drug permeability (Rao and Babrekar, 2018). For effective absorption, the drug must be dissolved or present in molecular form (Savjani et al., 2012). Additionally, drugs must possess sufficient permeability to cross biological membranes, necessitating various formulation strategies to enhance drug absorption (Padhye et al., 2021). Consequently, log P serves as a critical factor for drugs with a passive absorption mechanism through diffusion in determining the success of drug transport to the systemic circulation (Laksitorini et al., 2014).

Liposomal nanocarriers, composed primarily of major lipid constituents, possess diverse physicochemical properties and biodegradability that can enhance the bioavailability of poorly water-soluble drugs with poor permeability. Liposome lipids are biocompatible and tolerable, which enables them to utilize different absorption pathways and overcome various physiological barriers (Plaza-Oliver et al., 2021; Teixeira et al., 2017). Drug solubility and permeability considerably influence the design of liposomes as nanocarriers. Structurally, liposomes are defined as amphipathic molecules capable of self-arranging into a

spherical phospholipid bilayer, with the hydrophilic head facing the external aqueous environment. This amphiphilic nature makes liposomes ideal carriers for molecules with a broad polarity range (Large et al., 2021). Hydrophilic drugs can be encapsulated in the aqueous phase, whereas hydrophobic drugs can be encapsulated within the lipid tails inside the bilayer membrane (Lee, 2020).

Although liposomes are commonly used as carriers of water-soluble drugs, they also have the potential to enhance drug solubility (Ali et al., 2013). However, the successful incorporation of low-solubility drugs into liposomes is influenced by the physicochemical properties of the drug, the composition of the bilayer, and the preparation (Mohammed et al., 2004). The stability of the liposome bilayer can be improved by incorporating cholesterol, which fills the gaps between the liposome membranes (Ali et al., 2013). Phospholipids commonly used in liposome formulations include soybean phosphatidylcholine (SPC), egg phosphatidylcholine (EPC), dimyristoyl phosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylglycerol (DPPG), dimyristoyl phosphatidylglycerol (DMPG), and distearoylglycerophosphoethanolamine (DSPE) (Memvanga and Nkanga, 2021). A previous study has indicated that the length of the alkyl chain in phospholipids affects the encapsulation rate of lipophilic drugs, with longer lipid chains resulting in higher encapsulation rates (Mohammed et al., 2004).

The lipophilicity of liposome drugs affects their loading efficiency and release rates. Khan et al. (2008) observed the stability of liposomes composed of DSPG:DSPC:cholesterol and DSPG:DPPC:cholesterol at a ratio of 1:4:5, encapsulating highly soluble (fluorescein) and poorly soluble (rhodamine) compounds. The results indicated that liposomes encapsulating rhodamine exhibited 80 % leakage at 25 °C, whereas those encapsulating fluorescein showed only 40 % leakage. As the temperature increased to 37 °C, rhodamine leakage increased to 90 %, whereas fluorescein leakage increased to 50 %. Hydrophobic rhodamine tends to be localized in the hydrophobic bilayer region of liposomes, whereas fluorescein tends to be localized in the aqueous liposomal core. An increase in storage temperature can cause the movement of the phospholipid tails, leading to the expulsion of drugs localized in the hydrophobic bilayer compared to those in the liposomal core.

Zhigaltsev et al. (2005) also demonstrated that more hydrophobic vinblastine and vinorelbine exhibit faster release rates and lower retention percentages compared to the less hydrophobic vincristine in liposomes composed of egg sphingomyelin (ESM) and cholesterol at a 55:45 mol/mol ratio. Increasing the drug-to-lipid ratio in ionophore drug-loading techniques enhances drug retention in liposomes. Furthermore, liposomes loaded with ionophore drugs exhibited higher electron density than those in drug-free control liposomes, suggesting possible drug precipitation within the liposomal interior.

The drug solubility influences the efficiency of drug encapsulation in liposomes. When using passive drug loading methods, such as film hydration, the encapsulation of drugs with high solubility is usually low because a significant amount of the drug remains in the external aqueous medium, with only a small portion being encapsulated (Xu et al., 2012). Active loading methods can effectively encapsulate drugs with high solubility (Sur et al., 2014). Doxorubicin, which has high water solubility, can be loaded using the citrate active loading method onto liposomes, which are mainly composed of phospholipids with long and saturated fatty acid chains, such as DSPC or HSPC, with an encapsulation efficiency close to 100 % and excellent *in vitro* stability (Gubernator, 2011). In the active loading method, the encapsulated drug exists in an uncharged form on the extra liposomal side because of its basic or weakly acidic nature, which allows it to diffuse into the vesicles. Once it reaches the intraliposomal environment, the drug becomes protonated due to the pH difference, resulting in its entrapment within the liposome (Fritze et al., 2006; Sur et al., 2014). Several other liposome preparation methods have been developed to enhance the encapsulation efficiency of drugs with high solubility, including vesicle microencapsulation, reverse phase evaporation, freeze–thaw cycling, and dehydration-rehydration of preformed empty liposomes (Nii and Ishii, 2005).

Antimalarial drugs are comprised of several compounds with varying solubilities and permeabilities. Therefore, information on these drugs' solubility and permeability is crucial to designing liposomes with optimal release rates and encapsulation efficiencies (Table 1).

The design of nanocarriers for the delivery of antimalarial drugs in the hepatocyte phase can be achieved by encapsulating the antimalarial drug into nanoparticles that can selectively accumulate in the liver through passive targeting owing to the presence of fenestrae in the liver (Szafranska et al., 2021). Liposomes can passively deliver antimalarial agents, accumulating in the liver because their size falls within the range of fenestrae pores in the liver (Miatmoko et al., 2020). one limitation of liposomes is the instability of their structure during systemic circulation, which can lead to drug leakage, particularly for drugs with high permeability (Chaves et al., 2022). Hence, a specific design is required to maintain the drug within the system until it reaches its target organ. Conversely, a drug with good permeability is needed to penetrate the erythrocyte membrane to address malaria in the erythrocytic phase, where merozoites invade erythrocytes. However, good drug permeability alone is insufficient, and a nanocarrier is required to selectively release the drug and minimize side effects on other organs. The necessary nanocarrier design is one that, while in circulation, can penetrate the erythrocyte membrane and release the drug (Coban, 2020; Memvanga and Nkanga, 2021). The nanocarrier uptake mechanism selectively recognizes infected blood cells. This is because Plasmodium induces new permeation pathways (NPPs) that enhance the permeability of red blood cells by altering osmotic stability, allowing nanocarriers to enter only infected red blood cells (Baruah et al., 2018; Chaves et al., 2022).

2.1.2. *Pharmacokinetic parameters*

In managing the hepatic phase of malaria, it is imperative to have a drug with pharmacokinetic profiles that demonstrate high permeability, solubility, and volume of distribution to ensure efficient distribution to the liver. Conversely, for treating the erythrocytic phase of malaria, a drug with high permeability and substantial plasma distribution is essential to facilitate the easy penetration of the erythrocytic membrane. However, the currently available conventional formulations still exhibit pharmacokinetic characteristics that do not align with the requirements, as outlined in Table 2. Hence, there is a need for a nanocarrier that can improve the pharmacokinetic characteristics of drugs, thereby enhancing their effectiveness.

The formulation of liposomes depends on the physicochemical properties of the encapsulated drug. It is also strongly influenced by the pharmacokinetic properties of the drug, which must be considered when designing liposomes for effective drug delivery (Drummond et al., 2008). Certain antimalarial drugs, such as artesunate and artemisinin, have short half-lifes of less than 15 min and 2–5 h, respectively. Therefore, they must be administered 2–3 times a day for a minimum of five days to achieve optimal therapeutic outcomes (de Vries and Dien, 1996; Morris et al., 2011; Valissery et al., 2020). Short half-life and high metabolism can result in incomplete parasite clearance, leading to recurrence or resistance (Isacchi et al., 2011). As drug carriers, liposomes possess characteristics such as controlled drug release, minimal side effects, passive targeting, additional immune system-inhibitory effects, and prolonged circulation of drugs in the blood. Consequently, liposomes can enhance the pharmacokinetic parameters of drugs (Hu et al., 2018).

Different liposome compositions can alter the *in vivo* pharmacokinetic parameters, leading to changes in the blood clearance rate, half-life, and biodistribution (Large et al., 2021). The mononuclear phagocytic system (MPS), composed of macrophages and monocytes, is primarily responsible for clearing liposomes from the bloodstream (Betker et al., 2018). In addition to MPS clearance, liposome opsonization by plasma proteins significantly eliminates intravenously injected liposomes (Yan et al., 2005). When exposed to serum or plasma, liposomes

Table 1
Liposome formulation for encapsulating antimalarial drugs.

Antimalarial Drugs	Drug Classification	Liposome composition	Preparation Method	Liposome Characterization	Encapsulation efficiency (%)	Rate of Cumulative Release	Reference
Primaquine	BCS class I (Water solubility: 1.3 g/L; LogP: 2.1)	HSPC:Cholesterol:DSPE-mPEG2000 55:45:5 with drug-to-lipid ratio is 1:10	Thin film method; Active drug loading by transmembrane pH gradient method	%EE %DL Particle size	66. 4 ± 8. 2 % 56 % 163.8 ± 41.4 nm	63 % within 48 h	(Miatmoko et al., 2020)
Chloroquine	BCS class II (Water solubility: 0.14 mg/L; LogP: 2.1)	HSPC:Cholesterol:DSPE-mPEG2000 55:45:5 with drug to lipid ratio is 1:3	Thin film method; Active drug loading by transmembrane pH gradient method	%EE %DL Particle size	60.1 ± 8.27 % 72 % 149 ± 27.4 nm	42 % within 48 h	(Miatmoko et al., 2020)
		SPC and cholesterol with a drug-to-SPC ratio of 1:50	Reverse phase evaporation method; Active drug loading by transmembrane pH gradient method	%EE %DL Particle size	93.6 % NA 110 ± 8 nm	30 % within 6 h	(Qiu et al., 2008)
Artemisinin	BCS class II (Water solubility: 61.83 mg/L; LogP: 2.8)	EPC:cholesterol: PEG2000:artemisinin 5:0.6:0.25:2	Thin film hydration method; Passive drug loading	%EE %DL Particle size	70.0 ± 6. 30 NA 132.6 ± 8.78	NA	(Isacchi et al., 2012)
		Cholesterol:SPC:DSPE-PEG2000-R6 peptide 7:20:1	Thin film hydration method; Passive drug loading	%EE %DL Particle size	>90 % NA 98.8 ± 1.1 nm	50 % within 24 h	(Yu et al., 2021)

quickly adsorb large amounts of protein. Albumin, the most abundant negatively charged serum protein, binds to the surface of positively charged liposomes (Tretiakova et al., 2022). Liposomes that bind to plasma proteins act as an opsonin, promoting specific interactions with receptors on macrophages or hepatocytes, thereby increasing liposome uptake by these cells (Yan et al., 2005). To counteract this, incorporating PEG (polyethylene glycol) into liposome formulations can prolong circulation time, thereby increasing the half-life of liposomes. PEG typically attaches to liposomes via stable amide bonds or ethers that remain intact under physiological conditions. PEG creates a hydrophilic layer around the liposomes, resulting in sterically stable liposomes. This layer delays liposome opsonization and uptake by the MPS (Schöttler et al., 2016; Zalba et al., 2022).

The design of liposomes as nanocarriers should consider the maximum plasma concentration (C_{max}) of the drug, which is proportional to the toxic effects of the drugs, as well as the area under the curve (AUC) of the drug concentration in the plasma, which is proportional to its efficacy. In general, long-circulating nanocarrier systems can improve the AUC of a drug and reduce the required dose because of their excellent selectivity (Santos-Magalhães and Mosqueira, 2010). In the context of malaria treatment, the ability of nanocarriers to remain in the bloodstream for extended periods is a crucial parameter because it can enhance the interaction between drugs and infected red blood cells as well as parasite membranes (Mosqueira et al., 2004).

Several pharmacokinetic studies have been conducted on drugs encapsulated in liposomes, which have shown an increase in pharmacokinetic parameters, such as C_{max}, AUC, and $t_{1/2}$, as well as a decrease in clearance rate compared to non-encapsulated drugs, as presented in Table 3.

2.1.3. Pharmacodynamics profiles

The life cycle of malarial parasites is complex because they involve two hosts, mosquitoes and humans. When occupying a human host, the parasite completes its asexual cycle through the liver and erythrocytes (Valissery et al., 2020). Antimalarial drugs are distinguished by their ability to kill malarial parasites. The 8-aminoquinoline-derived antimalarial drugs, such as primaquine, have unique properties in killing parasites in the pre-erythrocytic, hypnozoite, and adult gametocyte stages of *P. falciparum*; however, they have weak activity against the asexual phase. This compound was active in killing the asexual stages of *P. vivax and P. knowlesi*. Currently, only 8-aminoquinoline has shown significant effects against *P. vivax* or *P. ovale* hypnozoites. Other antimalarial drugs used today can kill parasites in the asexual and sexual stages of sensitive *P. vivax, P. malariae, P. ovale*, and *P. knowlesi*, as well as in the asexual and early gametocyte stages (stages I–III) of sensitive *P. falciparum*. However, they do not kill the adult gametocytes of *P. falciparum* (stage V) (White, 2013).

The success of antimalarial chemotherapy depends on the speed at which the parasite is cleared from human blood (Marquart et al., 2015). Artemisinin, an antimalarial drug, is highly effective and rapid compared to conventional antimalarial drugs (Li and Pybus, 2019). In treating uncomplicated malaria, killing the early stages of the parasite's asexual cycle inside human red blood cells is essential for the patient's recovery; however, the main goal is to reduce parasite multiplication (White, 2013).

The major pharmacodynamic characteristics include lag phase and drug potency. The potency of a drug in antimalarial therapy is characterized by its maximum parasitic concentration (MPC) and maximum inhibitory concentration (MIC). MPC is the maximum concentration of a drug that provides clearance of parasites. When the concentration of antimalarial drugs in blood is below the MPC, the parasite-killing rate decreases (Marquart et al., 2015; White, 2013). Liposome carrier systems with formulations containing hydrophilic and hydrophobic polymers coupled with PEG incorporation on their surfaces can increase the circulation time of liposomes in the blood, thereby increasing the drug's half-life. In addition, nanosized designs can improve drug distribution to target sites such as the liver and erythrocytes for malaria treatment. Liposomes increase the MPC of antimalarial drugs, thereby accelerating parasite eradication and patient healing (Nsairat et al., 2022; Schöttler et al., 2016).

The presence of overexpressed receptors in the area of liver infection, especially in eradicating the hepatocytic phase of malaria, can be utilized for targeted drug delivery (Marwah et al., 2020). ASGPR, also known as "The Ashwell-Morell Receptor," is the first discovered cellular mammalian lectin. ASGPR is expressed basolaterally in hepatocytes (D'Souza and Devarajan, 2015). The interaction between the ASGPR receptor and its specific ligand, arabinogalactan, can be employed as a strategy for targeted drug delivery by modifying liposomes with this ligand (Marwah et al., 2020). The binding mechanism of arabinogalactan involves the interaction between the positive charge of arabinogalactan and the negatively charged liposome, leading to drug dissolution and changes in the lipid transition temperature (Marwah et al., 2020).

For targeted delivery in the eradication of the erythrocytic phase of malaria, it is known that Plasmodium-infected red blood cells (pRBC) undergo structural modifications, including increased permeability and

Table 2
Pharmacokinetic profiles of antimalarial drugs.

Antimalaria Drug	Organ Target	Drug Properties		Reference
		Parameters	**Properties**	
Primaquine (PQ)	Liver	Solubility	66.67 mg/mL in water (soluble)	(Baird and Hoffman, 2004; Nair et al., 2012)
		Permeability	Apparent permeability (Papp) is 177 ± 40 × 10^{-6} cm/s (high permeability)	
		Volume Distribution	3 L/kg (moderate)	
		Half-life	4–9 h	
Chloroquine (CQ)	Erythrocyte	Solubility	250 mg/mL in water (freely soluble)	(Krishna and White, 1996; Verbeeck et al., 2005)
		Permeability	Apparent permeability (Papp) is 2.3 ± 40 × 10^{-6} cm/s (low permeability)	
		Volume Distribution	200–800 L/kg (high)	
		Half-life	1–2 months	
Amodiaquine (ADQ)		Solubility	356.32 mg/mL in water (freely soluble)	(Anyorigiya et al., 2021; Nair et al., 2012)
		Permeability	Apparent permeability (Papp) is 2.3 ± 40 × 10^{-6} cm/s (low permeability)	
		Volume Distribution	38.3 L/kg (high). ADQ showed a high blood-to-plasma ratio of approximately 3:1, but the high concentration of ADQ in the blood is because of the accumulation in the white blood cells and not in the erythrocytes	
		Half-life	47.4 h	(Karbwang and White, 1990; Strauch et al., 2011)
Mefloquine (MFQ)		Solubility	Slightly soluble in water	
		Permeability	Apparent permeability (Papp) is 10 × 10^{-6} cm/s (low permeability)	
		Volume Distribution	20 L/kg (high)	
		Half-life	1–4 h	

overexpression of surface receptors such as glucose transporter 1 (GLUT 1), glucosaminoglycan receptors, and essential protein receptors (Bhide et al., 2023). Several ligands specific to overexpressed receptors, such as human serum albumin (HSA) (Aditi et al., 2016), glucose (Heikham et al., 2015), heparin (Ismail et al., 2019; Marques et al., 2014) and chondroitin sulfate (Bhadra et al., 2006) have been developed for conjugation to the surface of nanoparticles to achieve a targeted effect on pRBC.

3. Physiological barriers become the main challenges in drug delivery

The physiological differences between the liver and erythrocytes pose a challenge in delivering an antimalarial drug system that can eradicate parasites in both phases. The drug must remain stable within the nanocarrier during its journey toward the liver tissue to achieve the treatment target for malaria in the hepatic phase. Conversely, the drug must be promptly released from the carrier to penetrate the erythrocytes and attain the treatment target for malaria in the erythrocytic phase. As nanocarrier drugs, liposomes are susceptible to rapid elimination from the bloodstream, which can reduce their therapeutic efficacy (Sercombe et al., 2015). Interactions between liposomes and the immune system, including opsonization and the reticuloendothelial system (RES), affect liposome survival and activity. Opsonin, a serum protein that binds to liposomes, recognizes liposomes as foreign molecules and triggers their elimination by phagocytes (Ishida et al., 2002). It has been known that complement proteins involved in liposome opsonization cause rapid systemic clearance by MPS cells (Mohamed et al., 2019; Sercombe et al., 2015). The MPS, which consists of dendritic cells, monocytes, and macrophages, plays a vital role in the phagocytosis of pathogens and foreign compounds. This interaction can influence the fate of liposomes in the body and the therapeutic performance of the encapsulated drugs (Mohamed et al., 2019).

The main organs associated with RES include the liver, spleen, kidneys, lungs, bone marrow, and lymph nodes. Among these, the liver exhibits the most remarkable capacity for liposome uptake, followed by the spleen, which can accumulate up to 10 times higher than other RES organs (Tang et al., 2019). Resident macrophages eliminate liposomes in the RES through direct interactions with phagocytic cells. The uptake of liposomes by the RES usually occurs via vesicle opsonization, which involves adsorbing plasma proteins such as immunoglobulins, fibronectin, lipoproteins, and complement proteins on phospholipid membranes. However, *in vitro* studies have shown that liposome clearance by macrophages can occur without liposomes that bind to plasma proteins (Chrai et al., 2002).

Several strategies have been developed to extend the circulation time of liposomes in the bloodstream, one of which involves the conjugation of polyethylene glycol (PEG) to the surface of liposomes. PEG is widely used as a polymeric steric stabilizer. Various methods can be used to coordinate PEG on the liposome surface; however, the most commonly used approach is to link polymers to liposome membranes through cross-linked lipids such as PEG-distearoylphosphatidylethanolamine (DSPE) (Immordino et al., 2006). PEGylation inhibits the binding of opsonin proteins to the liposome surface during circulation, resulting in decreased liposome clearance by mononuclear phagocytic cells in the liver and spleen (Mohamed et al., 2019).

A comparison of the pharmacokinetic profiles of PEGylated and conventional liposomes containing artemisinin showed that the half-life of PEGylated liposomes (2.02 h) was higher than that of conventional liposomes (0.67 h). After 24 h of administration, artemisinin delivered by PEGylated liposomes was still detected at concentrations of 0.007 ± 0.004 μM (Isacchi et al., 2011). To determine the physicochemical properties of PEGylated and conventional liposomes in the serum, phosphatidylcholine/cholesterol PEGylated liposomes and conventional liposome models were compared. In the presence of serum, the zeta potential of the conventional liposomes decreased, whereas that of the PEGylated liposomes remained relatively consistent. This phenomenon may be attributed to the formation of corona proteins around conventional liposomes, which can influence their behavior and interactions with cells in the body (Wolfram et al., 2014).

However, despite the improvement in the pharmacokinetics of encapsulated drugs and the assumed lack of immunogenic and antigenic effects, conjugating PEG to the surface of liposomes may still have implications. Some studies have suggested that PEG can trigger recognition by MPS after repeated administration (Lila et al., 2013; Sercombe et al., 2015). Repeated administration of PEGylated liposomes can lead to the loss of their long-term circulatory properties, resulting in their clearance from the bloodstream (Ishida et al., 2002). This phenomenon is known as “accelerated blood clearance” (ABC). The ABC phenomenon consists

Table 3
Pharmacokinetic profiles of liposomes loading antimalarial drugs.

Antimalaria Drug	Liposome composition	Animal used	Route of administration	Dosage	Pharmacokinetic Profiles			Reference
					Parameter	Free drug	Liposomal drug	
Artemisinin	PEG_{2000}: EPC: cholesterol: artemisinin at a ratio of 0.25:5:0.6:2, respectively	Male CD1 mice (25–30 g)	Intraperitonial (i.p)	10 mg/kg	C_{max} (μM ± SD)	0.25 ± 0.08	1.04 ± 0.01	(Isacchi et al., 2011)
					$AUC_{(0\text{-}20h)}$ $(\mu M/h)^{-1}$	0.132	0.899	
					t $1/2t_{1/2}$ (h)	0.38	2.02	
					CL (mL/h)	63.11	9.20	
Arteether	DPPC: DPBC: Chol: Arteether 1:1:2:1	Adult male New Zealand rabbits (3.38 ± 0.39 kg)	Oral	50 mg/kg	C_{max} (μg/mL)	0.552 ± 0.041	3.03 ± 0.47	(Bayomi et al., 1998)
					T_{max} (min)	117.0 ± 6.71	47.5 ± 6.12	
					t 1/2 $t_{1/2}$ (h)	1.143 ± 0.128	1.083 ± 0.131	
					MRT (h)	2.533 ± 0.069	1.9113 ± 0.096	
					MAT (h)	0.7766	0.1542	
Artelinic acid (Artemisinin derivatitve)	NA	Sprague-Dawley rats age 6–8 weeks, weight 180–220 g)	Intravenous(i.v)	22 μmol/kg	$AUC_{(0\text{-}t)}$ $(mmol\ (L{\cdot}h)^{-1}$	1579.5 ± 486.1	4073.1 ± 1302.6	(Duan, 2019)
					$AUC_{(0\ -\ \infty)}$ $(mmol\ (L{\cdot}h)^{-1}$	1584.1 ± 483.8	4075 ± 1302.0	
					$MRT_{0\text{-}t}$	0.19 ± 0.04	0.26 ± 0.05	
					$CL(L(h{\cdot}kg)^{-1}$	6.45 ± 2.34	2.47 ± 0.72	
					V $(L\ kg^{-1})$	1.82 ± 1.59	1.23 ± 0.50	

of two distinct phases: the induction and effectuation phases. The induction phase occurs when PEGylated liposomes are initially administered while the biological system is still in its "prime" state. The effectuation phase occurs between days 3–7 after the initial dose, where subsequent doses of PEGylated liposomes are rapidly cleared from the systemic circulation (Mohamed et al., 2019).

The administration of a single dose of PEGylated liposomes resulted in shallow hepatic clearance. However, a drastic increase in hepatic clearance was observed when a second injection was administered five days after the first injection. The hepatic clearance increased from 0.0045 to 28.5 mL/h. In contrast, when conventional liposomes were administered as a second dose, the observed hepatic clearance value remained extremely low at 0.011 mL/h (Wang et al., 2007). Research conducted by Shiraishi et al. (2016) demonstrated that injection of PEGylated liposomes can produce PEG-specific IgM and IgG antibodies. These antibodies rapidly eliminate PEGylated liposomes from the bloodstream. Anti-PEG IgM exhibits a strong affinity for carriers with high hydrophobic chain counts, such as liposomes.

Several approaches have been developed to minimize the ABC phenomenon. One approach involves modifying a portion of PEG by altering the bond between PEG and lipid (Lila et al., 2013). Xu et al. (2010) researched and synthesized two PEG-lipid derivatives, PEG-CHMC and PEG-CHEMS, connected by a single ester bond. This chemical bond is expected to be gradually broken by esterases. These results indicate that liposomes prepared using easily cleavable PEG-lipid derivatives can reduce the occurrence of ABC. Furthermore, Shiraishi et al. (2016) suggested a method to prevent the rapid clearance of PEGylated liposomes using many liposomes. This is because the number of anti-PEG IgM molecules in the body is limited (approximately 10^{12} in mouse serum); therefore, many liposomes can evade clearance.

The characteristics of liver tissue significantly influence the uptake of nanoparticles by hepatocytes. The liver is crucial in various metabolic, immunological, and endocrine processes. The blood the liver receives from the intestines and heart circulates through a permeable, discontinuous capillary network called sinusoids to reach the central and hepatic veins. Sinusoids are small blood vessels (with a width of 5–10 μm) that contain fenestrations of sizes ranging from 100-150 nm (Mishra et al., 2013). The presence of these fenestrations can be utilized as entry points for nanoparticles to reach the hepatocytes. Additionally, Kupffer cells, liver-resident macrophages, can be employed as a targeted delivery system for hepatocytes. Kupffer cells specialize in internalizing foreign nanoparticles through multiple receptors, such as scavenger, Toll-like, mannose, and Fc receptors. The mechanisms involved include macropinocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, and other processes. The internalization process is influenced by the characteristics of the delivered nanoparticles, where nanoparticles with a larger size (>200 nm) tend to deposit in the liver more effectively (Colino et al., 2020). Positively charged nanoparticles, in particular, show increased uptake by Kupffer cells compared to neutral nanoparticles (Gustafson et al., 2015). Research conducted by Gad et al. (2012) indicated that AuNPs persist in Kupffer cells in mice for at least 6 months. Incorporating ligands on the surface of nanoparticles can also enhance the specificity of delivery to the liver.

4. Stages of malaria diseases relating to targeted drug delivery

4.1. Hepatic stage

Targeting *Plasmodium* parasites during the hepatic phase is a prophylactic approach for preventing further disease, which the parasite cycle can be seen in Fig. 2. Primaquine (PQ) is the only antimalarial drug with activity against hypnozoites in *P. vivax* (Borgheti-Cardoso et al., 2020). The liver plays vital roles in metabolism, immunology, and endocrine function, with hepatocytes as the primary cell type (Mishra et al., 2013). Sinusoidal liver endothelial cells (LSEC) possess fenestrae with diameters ranging from 50–300 nm that facilitate the entry of drugs from the bloodstream into the liver (Szafranska et al., 2021). Antimalarial therapy targeting the liver using liposomes can potentially reduce the dosage and associated toxicity (Gupta et al., 2012). Liposomes measuring 125–175 nm in size can be effectively concentrated in the liver using fenestrae (Miatmoko et al., 2020).

Several studies have been conducted on antimalarial liposomal formulations to achieve long-term hepatocyte stability. Stensrud et al. (2000) formulated liposomes encapsulating primaquine using a pH gradient method to target liver hepatocytes. The active ingredient must contact physiological targets, such as receptors in liver cells, to achieve

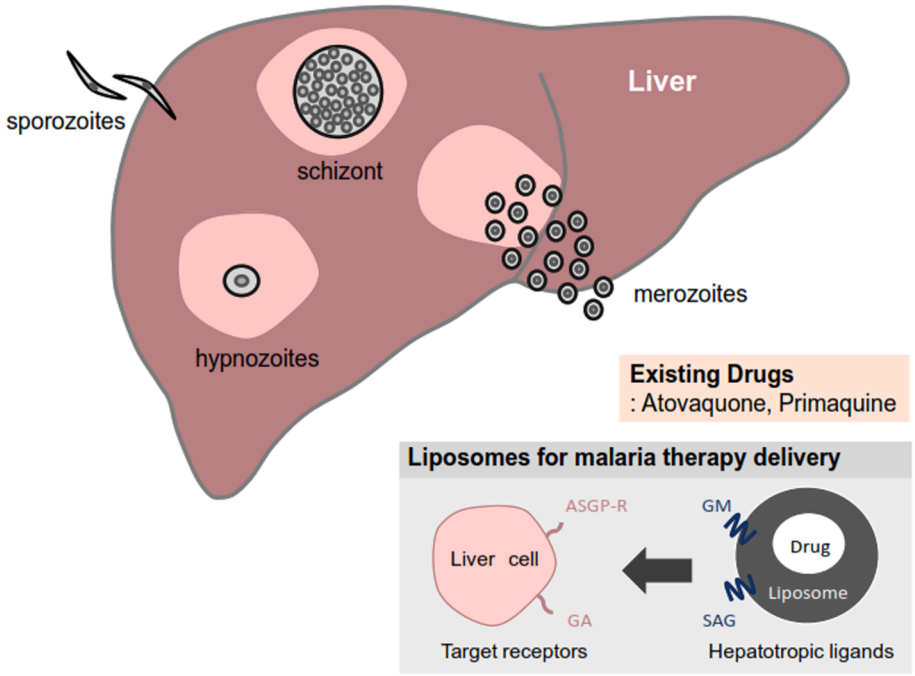

Fig. 2. Malaria parasites in the hepatic stage and some therapeutic strategies for their elimination.

the desired therapeutic effect. The most commonly targeted receptor in hepatocytes is the asialoglycoprotein receptor (ASGP-R), which recognizes carbohydrates, primarily galactose and N-acetylgalactosamine, with varying affinities. ASGP-R vesicles undergo favorable transit to lysosomes; therefore, increasing acidic and oxidative conditions within the organelles after endocytosis must be considered. In addition, the size threshold for colloidal internalization of ASGP-R is typically below 90 nm (Mishra et al., 2013).

Marwah et al. (2020) formulated liposomes specifically targeting hepatocytes using the active ingredient decoquinate, which has potent inhibitory effects against the erythrocytic and hepatic phases of *Plasmodium* infection. The liposomes were formulated by conjugating two types of hepatotropic ligands, arabinogalactan (SAG) and hydrophobically modified glycyrrhetinic acid (GM), which target two specific receptors on hepatocytes, ASGP-R and glycyrrhetinic acid (GA). *In vitro* antimalarial efficacy tests examined the number and area of exoerythrocytic forms (EEF) using fluorescence microscopy on human liver hepatocellular carcinoma (HepG2) cells. Decoquinate-loaded SAG- and

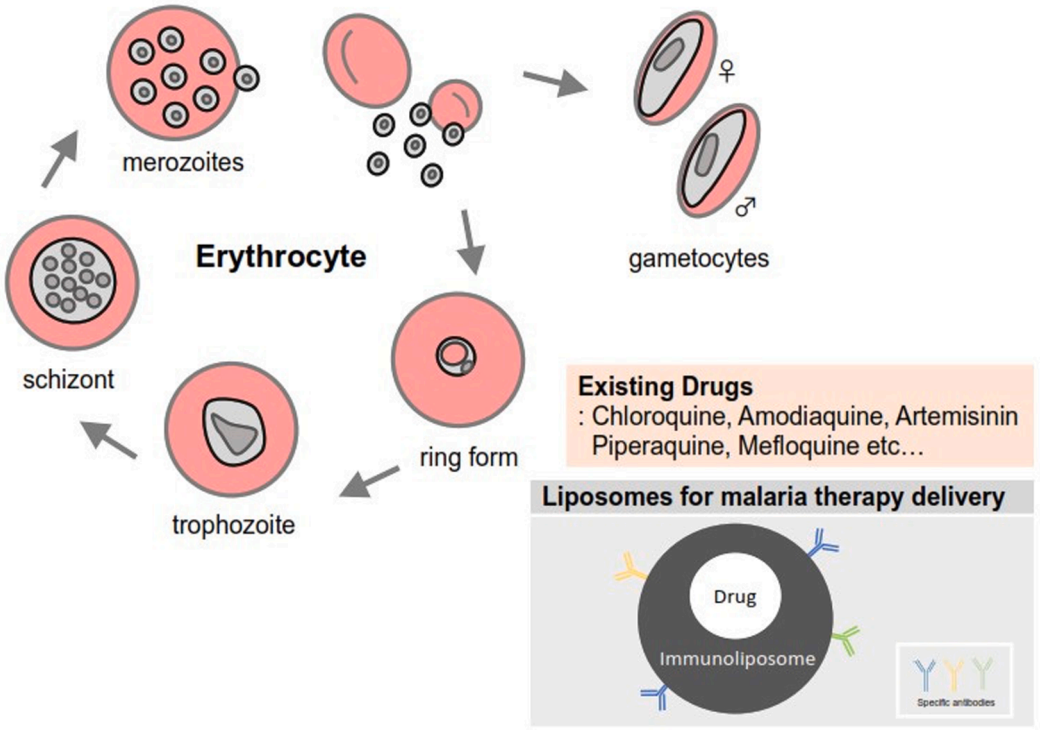

Fig. 3. The erythrocytic malaria stage and some therapeutic strategies for its elimination.

GM-modified liposomes completely abolished EEF formation. Furthermore, liposomes with ligands substantially reduced EEFs at glycyrrhetinic acid receptors.

In addition to utilizing receptor targeting, the natural processing of *Plasmodium* parasite infection in the liver is a highly effective liver-targeting strategy. This specificity is attributed to two main proteins on the surface of sporozoites: circumsporozoite protein (CSP) and thrombospondin-related anonymous proteins. Longmuir et al. (2006) developed a liposomal formulation containing peptides with amino acid sequences derived from the CSP domain region of *P. berghei* that can recognize glycosaminoglycans and directly target hepatocytes.

4.2. Erythrocytic stage

The erythrocytic phase of *Plasmodium* infection commences when the hepatic schizont releases 40,000 merozoites per hepatocyte into the bloodstream within merosomal vesicles (Cowman et al., 2016), as shown in Fig. 3. The bloodstream released-merozoites rapidly invade red blood cells (RBCs) and undergo the ring stage. Subsequently, in the trophozoite stage of development, the parasite undergoes rapid growth, digests hemoglobin, and occupies more than 50 % of the host cell volume. The liberated heme combines with Fe^{3+} from other heme molecules to form insoluble hemozoin. Towards the end of the trophozoite stage, the parasite undergoes multiple divisions before host cell lysis, releasing new merozoites. This rupture of host cells leads to malaria symptoms, such as fever, myalgia, anemia, and potentially fatal cerebral malaria (Santos-Magalhães and Mosqueira, 2010).

In the erythrocytic stage of the malarial parasite infection cycle, antimalarial drugs must be capable of penetrating infected red blood cells. Penetration of RBCs, which lack endocytic pathways, poses a significant challenge. Therefore, a drug must possess hydrophobic properties to enhance its penetrability (Borgheti-Cardoso et al., 2020). Antimalarial drugs must traverse multiple barrier membranes, including the host cell membrane (HCM), parasitophorous vacuolar membrane (PVM), parasite plasma membrane (PPM), and organelle membranes, such as vacuole membranes or endoplasmic reticulum membranes, depending on the drug's site of action (Biagini et al., 2005). Moreover, pH variations within the intracellular compartments of *Plasmodium*-infected RBCs can be leveraged using pH-sensitive liposomal systems for targeted drug delivery (Borgheti-Cardoso et al., 2020). Another strategy proposed by Moles et al. (2017) involved the incorporation of chloroquine into immunoliposomes to combat parasites within RBCs. Immunoliposomes are equipped with specific antibodies on their surface and have demonstrated efficacy in reducing parasite counts in the blood during *in vivo* tests in mice (Moles et al., 2015).

5. Liposome modification for antimalarial drug delivery

The hepatic and erythrocytic phases exhibit distinct characteristics, encompassing the drug's properties and physiological features of the target organs, liver, and erythrocytes. Therefore, careful consideration of these aspects is essential in developing nanocarriers. Nevertheless, the overarching principle of drug delivery is to ensure that the drug reaches the target tissues in significant quantities, remains stable in the systemic circulation during the hepatic phase, and is ready to penetrate erythrocytes during the erythrocytic phase. Combining drugs that serve dual purposes in the hepatic phase and act preventively in the erythrocytic phase can result in a synergistic effect (Miatmoko et al., 2020). Consequently, the nanocarrier formulation is critical in achieving an effective combination of both phases.

5.1. Drug loading

Efficient loading of hydrophobic, ionic, or zwitterionic drugs into liposomes to achieve an appropriate drug-lipid ratio is a critical factor that can significantly impact drug delivery and therapeutic efficacy (Cao et al., 2022; Sheoran et al., 2018; Zucker et al., 2009). The physicochemical characteristics of the encapsulated drug play a crucial role in determining the effectiveness of encapsulation and the method chosen for loading the drug into liposomes (Cao et al., 2022; Sheoran et al., 2018). Drug loading can be achieved through passive encapsulation during liposome manufacturing or active encapsulation after liposome formation (Akbarzadeh et al., 2013).

Hydrophobic drugs can be passively encapsulated within liposomes during vesicle formation, and the use of lipids with longer alkyl chains enhances encapsulation efficiency (Ali et al., 2013). However, the encapsulation of hydrophilic drugs can be achieved through either active or passive methods, with active encapsulation generally resulting in higher efficiency. The composition of the lipid membrane influences the passive encapsulation of hydrophilic drugs as the drug localizes to the lipid bilayer, and the encapsulation efficiency depends on the solubility of the drug within the phospholipid bilayer (Eloy et al., 2014).

Active drug encapsulation involves the ability of a drug to transform into an uncharged species, enabling its diffusion across the membrane and preventing the permeation of liposomes. The degree of ionization of a drug depends on its pKa and the local pH (Zucker et al., 2009). Amphiphatic, alkaline, or weakly acidic drugs meet these requirements (Cern et al., 2012). Active drug loading takes advantage of transmembrane pH gradients, where the external pH of the liposomes maintains the drug in an uncharged state, allowing it to diffuse across the lipid bilayers. Upon entering the liposome, the drug reverts to its charged form owing to the pH difference and becomes trapped within it (Sur et al., 2014). The drug's molecular weight also plays a role in encapsulation; drugs with lower molecular weight exhibit higher efficiency when encapsulated in liposomes (Ali et al., 2013).

Numerous studies have explored the encapsulation of antimalarial drugs in liposomal systems. For instance, the encapsulation of primaquine, which is known for its poor water solubility, was carried out using the thin-film hydration method, resulting in passive loading with encapsulation efficiencies of 10 % (Arica et al., 1995). Another study focused on encapsulating a combination of primaquine and chloroquine using the transmembrane pH gradient method, achieving encapsulation efficiencies of up to 66 % (Miatmoko et al., 2020). However, encapsulating a combination of antimalarial drugs faces challenges; trapped drugs may affect the integrity of the liposomal membrane or intraliposomal environment, consequently influencing the number of loaded drugs (Miatmoko et al., 2021).

5.2. Drug release

The primary mechanism of drug release from liposomal systems is passive, which occurs via diffusion and permeation. Drug release from liposomes is a complex process influenced by the physicochemical properties of both the liposomes and encapsulated drugs. Additionally, external factors such as the release medium, temperature, and pH play a role in drug release. The physicochemical properties that determine drug release from liposomes include the bilayer permeability of the drug, the ionization constant of the drug, its binding to the lipid bilayer, self-association of the drug, and the presence of intraliposomal deposits (Li et al., 2018). Stimulus-responsive liposome carrier modifications and the addition of targeting ligands can be used to achieve control or extension (Cao et al., 2022). These modifications and additions allow for the precise regulation of drug release based on specific triggers or targeting of the desired sites.

In antimalarial therapy, drug release at specific targets, such as hepatocytes and RBCs, can be achieved through passive or active targeting. Passive drug targeting involves modification of the surface of liposomes with PEG to create liposomes with prolonged circulation in the bloodstream. However, active targeting can be accomplished by activating the liposome surface with surface ligands, such as glycolipids, carbohydrates, peptides, antibodies, or proteins, which bind to receptors associated with infected RBCs (Memvanga and Nkanga, 2021). Another

modification is the incorporation of polymers into the liposome bilayer or inside the liposomes, which enhances the liposome structure and allows for controlled drug release. The type of bond between liposomes and the incorporated polymer plays a crucial role in determining the level of control over drug release (Sriwidodo et al., 2022).

The integrity of the liposome membrane, which is determined by its composition, affects the release of drugs from liposomes. The use of fluid-phase phospholipids like phosphatidylcholine (POPC) with a lower transition temperature (T_m) of approximately −2 °C or dipalmitoylphosphatidylcholine (DOPC) with Tm of −20 °C can lead to an increased drug leakage compared to solid-phase phospholipids such as hydrogenated soybean phosphatidylcholine (HSPC) with T_m of 55 °C (Eldin et al., 2016, 2015). Interactions between encapsulated drugs can also affect the integrity of the liposome membrane. Miatmoko et al. (2020) demonstrated a decrease in encapsulation efficiency and drug release rate compared to when each drug was loaded individually. Combining multiple drugs in liposomes using dual-loading techniques allows controlled drug release, which, in turn, affects the biodistribution and metabolism of each drug. Primaquine interacts electrostatically with HSPC, causing irregular acyl chains and increasing membrane fluidity. In contrast, chloroquine interacts with the polar region of dipalmitoylphosphatidylcholine (DPPC), inhibiting the movement of acyl chains and increasing membrane rigidity, resulting in reduced drug release (Miatmoko et al., 2021).

The liposome membrane's integrity also affects encapsulation efficiency, with chloroquine exhibiting higher encapsulation efficiency than primaquine during dual liposome loading. The release test results showed a slower release rate for the combination of primaquine and chloroquine loaded into liposomes than for single drug-loaded liposomes, indicating that chloroquine imparts a stronger rigidifying effect on the liposome bilayer owing to a higher number of drug molecules. This is advantageous for preventing the premature release of primaquine into the systemic circulation before the liposomes reach the hepatocytes. Additionally, the low release rate of chloroquine is essential for its prophylactic effect against the erythrocytic stage of malaria (Miatmoko et al., 2020).

The challenge lies in achieving excellent selectivity for erythrocyte delivery during the erythrocytic phase and ensuring its stability during the hepatic phase. High drug loading within nanoparticles is crucial in both phases, as it determines the extent of penetration into erythrocytes and hepatocytes. Combining the two types of drugs poses a unique challenge because the expected targets differ; the drug should be released into the bloodstream for anti-parasitic action in the erythrocytic phase while remaining stable enough to reach the hepatocytic phase of therapy. Thus, the model for drug loading presents a challenge but can be addressed by improving stability, either through the use of a trapping agent like polyglycolide polymer (Miatmoko et al., 2017) or by employing a prodrug model encapsulated in a liposome (Salmaso et al., 2021). On the other hand, Erythrocytic targeting is suitable for drugs with excellent permeability that can be slowly released with a membrane system whose fluidity is carefully regulated (J. L. Liu et al., 2020).

5.3. Drug administration routes

The drug administration route is crucial to malaria treatment, with distinct requirements for the erythrocytic and hepatic phases. In essence, for erythrocytic penetration, the drug must be present in the bloodstream serum and poised for penetration into erythrocytes. Conversely, the drug must remain stable in the hepatic phase and accumulate in the hepatocytes. Various administration routes present advantages and challenges, adding complexity to malarial drug delivery.

The oral route is preferred for uncomplicated malaria therapy, whereas the parenteral route may be used for severe or complicated cases to prevent cerebral malaria (Santos-Magalhães and Mosqueira, 2010). Oral administration is challenging because liposomes are vulnerable to various detrimental effects from gastric acid, bile salts, and pancreatic lipase in the gastrointestinal tract (GIT). This leads to decreased drug concentration within liposomes due to leakage (He et al., 2019). Oral delivery of liposomes for antimalarial drugs has not yet been commercially utilized because of their rapid excretion from the gastrointestinal tract. Dynamic and repetitive peristaltic movements in the GI tract result in an insufficient residence time for liposomes to undergo drug absorption. Several strategies have been developed to increase the liposome residence time in the GI tract, such as modifying the liposome surface with mucoadhesive polymers, such as cationic chitosan, or with low-molecular-weight PEG (MW 2000) high-density liposomes (Tahara et al., 2018; Yamazoe et al., 2021).

Parenteral routes, though effective, pose inconveniences owing to invasiveness and the need for long-term release regulation. As malaria typically takes time to manifest symptoms, preventive measures are critical to therapeutic success (Schwartz, 2012). While delayed release is challenging to achieve in parenteral models, alternative routes, such as intramuscular, transdermal, or implantation, allow for innovation in design, considering the limited dosage. Limited drug load and the necessity for controlled release further complicate the design, requiring innovative solutions for dosing with burst release for the initial and maintenance doses to preserve therapeutic effects (Jindal et al., 2023).

Currently, antimalarial liposome formulations are being developed for various routes of administration, including parenteral, oral, and transdermal routes. However, most clinically tested liposome formulations are intended for intravenous or intramuscular administration in the form of sterile suspensions or lyophilized powders (Deshmukh, 2023; Hou et al., 2022).

Intravenous administration of antimalarial drugs is necessary in cases of severe malaria with high parasite density during the erythrocytic phase, mainly when oral administration is not feasible (Moles et al., 2017). Liposomes have also been investigated as adjuvants in malaria vaccines when administered intravenously. This strategy involves encapsulating synthetic peptide antigens and vaccines within liposomes to activate MHC class I and II pathways, stimulating cellular immune responses and antibody production (Deshmukh, 2023; Santos-Magalhães and Mosqueira, 2010). The transdermal route of administration provides an alternative for antimalarial drug delivery, offering advantages such as bypassing first-pass metabolism, ease of use, and the ability to discontinue therapy promptly. Cationically active liposome delivery has been explored to overcome the low permeability of antimalarial drugs through the skin (Murambiwa et al., 2011).

5.4. Membrane integrity is the determining factor for a drug's stability

The distinction between drug release targets for eradicating the hepatic and erythrocytic phases necessitates modifications to the integrity of the liposomal membrane. The release of drugs in the erythrocytic and hepatic phases differs because of the distinct targets involved. Consequently, modifications to the liposomal membrane are imperative, especially for a combination drug that targets both the hepatic phase and prevents the formation of the erythrocytic phase. Additionally, variations in drug characteristics can lead to differences in loading, making it challenging to achieve optimal encapsulation and high concentrations within nanoparticles.

Liposome characteristics are strongly influenced by lipid composition, surface charge, and manufacturing methods. The selection of lipid components for the liposomal bilayer determines the integrity of the liposomal membrane, making it more rigid or fluid (Akbarzadeh et al., 2013). This membrane integrity, as described in the drug-release section, affects the drug-release characteristics of liposomes (Ali et al., 2013).

The liposomal membrane consists of a rigid gel phase at low temperatures (between 0 °C and the transition temperature) and a fluid phase above the transition temperature. The modification of the liposomal membrane phase is closely related to the stability of the resulting liposomes (Routledge et al., 2019; Sharma et al., 2014). For instance,

unsaturated phosphatidylcholine species found in natural materials, such as egg and soybean phosphatidylcholine, result in a more permeable and less stable bilayer. In contrast, saturated phospholipids with long acyl chains, such as dipalmitoylphosphatidylcholine, form a more rigid and stable bilayer structure (Akbarzadeh et al., 2013).

Cholesterol, an essential component of liposomal formulations, plays a role in lipid stability and packing, influencing drug release (Magarkar et al., 2014). Incorporating cholesterol into liposome formulations improves their *in vivo* stability and reduces leakage between the lipid bilayers, resulting in controlled drug release (Large et al., 2021). Cholesterol can fill the gaps between liposome membranes, thereby increasing bilayer stability (Ali et al., 2013). Additionally, cyclodextrin can modify the integrity of lipid membranes in liposomes. They can remove lipid components from membranes by forming inclusion complexes, leading to decreased liposomal integrity (Hatzi et al., 2007).

The interactions between drugs and their constituent membranes can also affect membrane integrity. Dual loading of primaquine and chloroquine into PEGylated liposomes causes a decrease in integrity, resulting in a significant release of drugs from the liposomes (Miatmoko et al., 2020). Primaquine has been reported to interact with zwitterionic lipid membranes through an interaction between its charge and the polar region of the lipid bilayer. Primaquine can intercalate into the phospholipid head, disrupting the regularity of the acyl chain and making the lipid bilayer more fluid (Basso et al., 2011).

6. Design and development of liposomes using computational method

Advanced computing technology dominates pharmaceutical research. Computing technology offers numerous benefits in predicting and understanding molecular interactions and in the three-dimensional modeling of systems. Consequently, the drug development process, including the development of liposome systems, has been accelerated. Various computational methods have been employed for liposome system development, including quantitative structure–property relationship (QSPR) and Visual Molecular Dynamics, which will be discussed further.

6.1. Quantitative structure–property relationship (QSPR)

The QSPR is a computational method that assumes a compound's molecular structure contains information that determines its physical, chemical, and biological properties. This method involves the calculation of one or more molecular descriptors and their use in developing a QSPR model. This model aims to predict the properties related to drug formulation requirements, such as release, transportability, and other relevant characteristics. The descriptor parameters were selected based on their influence on the physicochemical properties of molecules, such as logP, Van Der Waals surface area, and pKa. These parameters can be determined using the statistical application QuaSPR-Contingency, designed using the Molecular Operating Environment (MOE) software (Bhatia et al., 2018). The descriptor parameters were then incorporated into a mathematical model trained using experimental data on the activity or properties of known drug molecules. Various machine learning methods, including decision trees, k-nearest neighbor (kNN), linear regression, and support vector machines, can be employed in this process. The resulting model could serve as a computational prediction tool for various drug formulation requirements, ultimately reducing the time and effort required for laboratory-based formulation development (Cern et al., 2014, 2012).

In general, QSPR modeling involves several steps. First, datasets are prepared, which can be sourced from literature or experimental data. Next, independent variables were determined, and molecular descriptors were calculated using the appropriate software. Subsequently, various machine-learning methods used datasets and molecular descriptors to build models. The resulting models were validated using independent data that was not used during the model preparation phase. Also, modeling outcomes can provide insights into the experimental conditions or independent variables significantly influencing liposome systems' formulation outcomes (Bhatia et al., 2018; Cern et al., 2017).

Several studies have been conducted to develop liposomal formulations using QSPR. The QSPR model can predict drug candidates with high loading capacities in liposome manufacturing methods by remote or active loading. Drugs that show a high loading capacity in remote loading methods have specific characteristics that allow them to permeate and diffuse through the lipid bilayer and accumulate in liposomes without permeation and diffusion out of the liposomes. The QSPR model for prediction needs was created by Cern et al. (2012) using several machine learning methods, namely, decision tree, k-nearest neighbor (kNN), Support Vector Machine (SVM), and Iterative Stochastic Elimination (ISE). Sixty drugs and 366 loading experiments were used as datasets to develop the QSPR model. A good drug candidate for remotely loaded liposomes was defined as a drug that provided high encapsulation efficiency at all initial drug-to-lipid ratios. However, a high encapsulation efficiency at a low D/L ratio could be more practical. Thus, in this study, the initial D/L ratio needed in the formulation was predicted to achieve a high loading efficiency.

Moreover, the study tested three drugs predicted to provide high and low loading efficiencies when loaded using remote loading methods at various D/L ratios. The drugs tested included pravastatin, mupirocin, and piroxicam, which are weak acids theoretically suitable for loading using the remote loading method. Pravastatin and mupirocin were predicted to provide a high loading efficiency at a D/L ratio of 0.3, whereas piroxicam was predicted to provide a low loading efficiency at a D/L ratio of 0.3. Proof of the predicted results was experimentally obtained using the calcium acetate gradient method. The prediction results proved correct, with pravastatin and mupirocin providing up to 100 % loading efficiencies at various D/L ratios (ranging from 0.2 to 0.5). Piroxicam only provides a loading efficiency of up to 50 % at various D/L ratios (ranging from 0.05 to 0.42) (Cern et al., 2014).

This study further tested three drugs, pravastatin, mupirocin, and piroxicam, which were predicted to provide high and low loading efficiencies when loaded using remote loading methods at various D/L ratios. All three drugs were amphipathic weak acids, making them theoretically suitable for remote loading. This prediction indicated that pravastatin and mupirocin would exhibit a high loading efficiency at a D/L ratio of 0.3, whereas piroxicam would display a low loading efficiency at the same D/L ratio. The prediction accuracy was experimentally validated using the calcium acetate gradient method. The experimental results confirmed the accuracy of the predictions, with pravastatin and mupirocin achieving loading efficiencies of up to 100 % across various D/L ratios (ranging from 0.2 to 0.5). In contrast, piroxicam only achieved a loading efficiency of up to 50 % across various D/L ratios (ranging from 0.05 to 0.42) (Cern et al., 2014).

In another study conducted by Cern et al. (2017), the QSPR model was employed to identify suitable active ingredients for liposome delivery, emphasizing predicting good stability based on the liposome system's drug loading and drug leakage characteristics. The research revealed that the drug-loading model's most influential descriptors were the active ingredient's partial charge and polar region. In contrast, the log P descriptor impacted the leakage system model the most.

Overall, QSPR models can enhance liposome formulation prediction by bridging experimental measurement with information obtained from molecular simulations. They can also help predict the phase behavior of the liposomal system, serving as a bridge between liposome formulation and *in vitro* or *in vivo* delivery performance.

6.2. Molecular dynamics (MD) simulation

Computational approaches using MD simulations aim to study the mechanisms and behaviors of biological macromolecules, including nucleic acids, membranes, and liposomes. MD simulations allow for

investigating large-scale structure and dynamics of liposomes, such as morphological properties and self-assembly processes, which are critical for understanding their delivery efficiency (Chan et al., 2020). In developing liposome systems, MD simulations offer valuable insights into membrane structural changes, interactions between liposome models and biological molecules, mechanisms of liposome membrane formation, and the behavior of liposomes in the bloodstream (Parchekani et al., 2022). MD simulations provide a dynamic description of the interactions between drugs and proteins. They can also investigate various molecules or macromolecules, such as drug-lipid interactions (Bunker and Róg, 2020).

Before conducting MD simulations, it was necessary to create a liposome structure model that included lipids, drugs, surfactants, ions, and water. The coarse-grained (CG) MARTINI Model is commonly used for liposome simulations. Coarse-grained MD simulations predict the movement of small particles, such as atoms or molecules, using mathematical equations to calculate their interactions and trajectories (Lemaalem et al., 2020). Coarse-grained models combine multiple atoms or atomic groups into larger particles to accelerate the simulation time and estimate the interactions between the particles (Bunker and Róg, 2020). The MARTINI model uses the MARTINI force field, which consists of mathematical parameters derived from experimental data. These parameters describe how molecules interact to form membranes (Arnarez et al., 2015). The coarse-grained MARTINI model is well-suited for simulating membranes because it captures the amphipathic assembly forces acting on the membrane (Lemaalem et al., 2020).

Several studies have used MD to develop liposomal formulations. In a study conducted by Lemaalem et al. (2020), coarse-grained MD based on the MARTINI model was used to investigate the effects of lipopolymer incorporation as a solution to prevent adhesion between liposomes and human liposomes. The research involved simulating a lipid membrane with a grafted polymer model consisting of 15,376 DPPC molecules and various PEG lipopolymer molar fractions (0.005, 0.014, and 0.1), fully hydrated with 112,659 water droplets. The MD simulations were performed using two ensembles: NPHT and NVTE. In the NPHT ensemble, the number of particles (N), pressure (P), and temperature (T) were kept constant during the simulation, and the system remained open, allowing energy and particle exchange with the surrounding environment to maintain a constant density. The NPHT simulations revealed that the distance of the polymer layer increased with higher molar fractions of the lipopolymer, indicating a transition from the mushroom regime to the brush regime. In the NVTE ensembles, the number of particles (N), volume (V), and temperature (T) were kept constant during the simulation, and the system remained confined without exchanging particles or energy with the surrounding environment, thereby allowing the density of the system to change. NVTE simulations were used to analyze the radial distribution function to observe modifications in the molar fraction of lipoproteins and the interactions of hydrophobic chains. In the brush regime, stronger attractive interactions between the hydrophobic chains occur between the bilayers, resulting in a more rigid membrane.

Magarkar et al. (2014) conducted MD simulations to investigate the effects of adding cholesterol at various concentrations and PEG to liposome membranes. This study simulated eight bilayer membranes composed of DSPC, DSPE-PEG2000, and cholesterol at different molar ratios. MD simulations were performed using Gromacs software, and trajectory visualization was conducted using Visual Molecular Dynamics (VMD). Visualization techniques were used to observe the behaviors and interactions of the simulated systems over time. The simulation results revealed that PEG penetrated the lipid bilayer as the cholesterol concentration increased. The DSPC membranes exhibited high rigidity in the absence of cholesterol. However, the addition of PEG disrupts lipid organization by interacting with cholesterol. The PEG molecules entered the membrane at a position parallel to that of cholesterol, and their interactions were mainly observed with the beta face of cholesterol. These interactions lift cholesterol from membranes, resulting in a less stable system.

7. Conclusion

Malaria can cause high mortality and morbidity among children and pregnant women in tropical areas. Drug resistance resulting from patient non-compliance, often due to disruptive side effects, hinders effective antimalarial therapy. To address this challenge, researchers have extensively explored the development of liposomes as drug-delivery systems for the targeted and efficient delivery of antimalarial drugs. Encapsulating drugs in liposomes allows for passive or active drug delivery, enabling drug concentration in target tissues and minimizing side effects compared with conventional drugs. However, liposome formulations are influenced by various factors, including the physicochemical characteristics of the drugs and the physiological barriers liposomes encounter during drug delivery. Therefore, modification of liposome nanocarriers is crucial in achieving efficient drug loading, controlled drug release, and system stability. Numerous studies have demonstrated the modification of liposome formulations, ranging from the selection of constituent lipids to the addition of ligands to the liposome surface, as well as improvements in manufacturing methods. However, the modification process traditionally involves time-consuming trial-and-error experiments in the laboratory.

Consequently, researchers increasingly use computational approaches to predict liposome system stability, membrane integrity, and liposome-drug interactions or ligand conjugation mechanisms. This shift toward computational methods is expected to enhance the efficiency of liposome formulation development in the laboratory. Using computational methods, researchers can predict the stability of liposome systems, assess membrane integrity, and understand the interactions between liposomes and encapsulated drugs or conjugated ligands. The future development of QSAR and MD simulations as modeling for clinical research predicting tools will involve integrating QSAR with biologically based models, extending its reach in chemical space, and modeling toxicity at more refined levels of biological organization. This approach could facilitate the creation of more stable liposomal formulations, enable controlled drug delivery, and enhance the effectiveness of malaria treatment efficiently and cost-effectively. However, experimental validation is still needed to develop validated and predictive QSAR models or MD simulations, which are essential for developing a liposomal formulation.

CRediT authorship contribution statement

Andang Miatmoko: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Rifda Tarimi Octavia:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **Tamasa Araki:** Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. **Takeshi Annoura:** Conceptualization, Data curation, Formal analysis, Resources, Supervision, Validation, Writing – review & editing. **Retno Sari:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval and consent to participate

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