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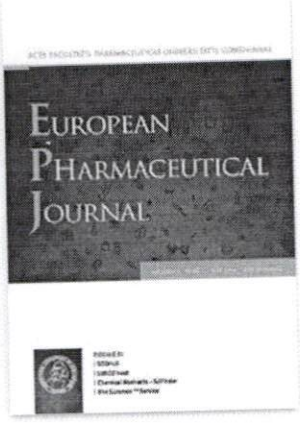

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Dual Loading Of Primaquine And Chloroquine Into Liposome

Original Paper

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Abstract Primaquine (PQ) has long been recognized as the only effective drug in the treatment of hepatic stage malaria. However, severe toxicity limits its therapeutical application. Combining PQ with chloroquine (CQ) has been reported as enhancing the former's efficacy, while simultaneously reducing its toxicity. In this study, the optimal conditions for encapsulating PQ-CQ in liposome, including incubation time, temperature and drug to lipid ratio, were identified. Furthermore, the effect of the loading combination of these two drugs on liposomal characteristics and the drug released from liposome was evaluated. Liposome is composed of HSPC, cholesterol and DSPE-mPEG$_{2000}$ at a molar ratio of 55:40:5 and the drugs were loaded by means of the transmembrane pH gradient method. The particle size, ζ-potential and drug encapsulation efficiency were subsequently evaluated. The results showed that all liposome was produced with a similar particle size and ζ-potential. PQ and CQ could be optimally loaded into liposome by incubating the mixtures at 60°C for 20 minutes at a respective drug to lipid ratio of 1:3 for PQ and CQ. However, compared to single drug loading, dual-loading of PQ+CQ into liposome resulted in lower drug encapsulation and slower drug release. In conclusion, PQ and CQ can be jointly loaded into liposome with differing profiles of encapsulation and drug release.

Keywords *Dual loading – primaquine – chloroquine – liposome – release*

INTRODUCTION

Globally, malaria ranks fourth on a scale of life-threatening infectious diseases (Mishra et al., 2017). Shortly after being bitten by a Plasmodium-infected female Anopheles mosquito, the sporozoite accumulated in its salivary glands enters the liver leading to the hepatic phase of malarial infection. This stage is very important since it represents the starting point of erythrocytic-stage malaria and fatal cerebral malaria (Prudêncio et al., 2006). In addition, the latent phase of hypnozoites in the liver often found in *Plasmodium ovale* and *Plasmodium vivax* infection can cause relapses in about 50–80% of malaria sufferers (Chu and White, 2016).

Primaquine (PQ), recognized as the primary treatment for the hepatic phase of malaria (Longley et al., 2016), is an antimalarial pro-drug compound belonging to the 8-aminoquinoline group that actively works against sporozoites, hypnozoites, asexual phases and gametocytes through inhibition of the metabolic activity of mitochondrial parasites and the production of reactive metabolites, which are toxic to cells (Chu and White, 2016; Marcsisin et al., 2016). PQ constitutes a drug with a short half-life, which is rapidly metabolized by the liver into a carboxylic acid derivative ultimately excreted in the urine. In order to treat malarial infection and prevent relapse, PQ must be administered for a period of 14 days (Karyana et al., 2016). However, although it demonstrates proven efficacy against hepatic phase malaria, PQ can cause methemoglobinemia and hemolysis in patients presenting glucose-6-phosphate dehydrogenase (G6PD) deficiency (Kedar et al., 2014; Marcsisin et al., 2016; Recht et al., 2015). Furthermore, prolonged drug therapy can also induce abdominal cramps, nausea and vomiting (Jong and Nothdurft, 2001). Such side effects can potentially undermine the adherence of patients to the prescribed drug regime resulting in low PQ levels in the blood. It has been known that low doses administered in the cases of high parasitemia can induce drug resistance, which represents a significant problem in the control program relating to malaria (Gonzalez-Ceron et al., 2015).

It has been previously reported that the administering of a single dose of PQ combined with chloroquine (CQ) constitutes an effective method of treating malaria (Gonzalez-Ceron et

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al., 2015). CQ is a 4-aminoquinoline compound frequently employed in managing the erythrocytic stage of malaria (World Health Organization, 2015). The combination of administering CQ tablets for three consecutive days and PQ tablets for 14 days proved effective in treating erythrocytic phase malarial infection and preventing its reoccurrence (Gonzalez-Ceron et al., 2015; World Health Organization, 2015). In addition, the specific metabolite interaction between PQ and CQ reduced the toxicity of the former without compromising its efficacy against parasites (Fasinu et al., 2016). This study demonstrates that CQ can inhibit PQ metabolism by means of CYP2D6, thus reducing the formation of active metabolites, which are toxic to erythrocytes.

Developing an effective anti-malarial treatment, especially one countering hepatic phase infection, which could involve the use of liposome to deliver PQ and CQ is important. Through the encapsulating of a combination of PQ and CQ in liposome, PQ will prove effective in treating acute infections caused by sporozoites and/or malaria relapse during the latent phase of hypnozoites in the liver, while the CQ loaded in liposomes can provide prophylactic therapy for erythrocytic phase infection. During hepatic phase infection, sporozoites are known to specifically attack hepatocytes, rather than other non-parenchymal cells present in the liver. Therefore, the specific form of delivery intended for hepatocytes will prove useful in enhancing the efficacy and decreasing the toxicity of PQ and CQ during the treatment of malaria.

Liposome constitutes a vesicular formation consisting of a phospholipid bilayer surrounding an inner water phase, which provides optimal protection for drugs against diffusion and external factors (Kohli et al., 2014). Liposome with a particle size within a 125–175 nm range can concentrate densely in hepar tissue because of the presence of an intercellular gap or fenestrae within endothelial cells in the liver sinusoid (Baratta et al., 2009). Moreover, PEGylation of liposome can minimize drug clearance from the body and produce drugs that circulate for extended periods in the bloodstream (Barenholz, 2012). Therefore, the drug will largely accumulate in hepatocytes.

The use of liposome as a carrier for PQ and CQ has been widely reported (Qiu et al., 2008; Stela Santos-Magalhães and Carla Furtado Mosqueira, 2009; Stensrud et al., 2000), while, in contrast, no previous research on its application to a combination of both drugs has been conducted. Consequently, in this study, a dual drug loading of PQ and CQ in liposome was prepared. However, it has been reported that PQ interacts strongly with the polar headgroup region of dimyristoylphosphatidylcholine (DMPC) in the membrane bilayer forming the space intercalation between the lipids (Basso et al., 2011). Turning to the results, perturbation in the lipid order occurred, which increased the fluidity of the liposomal membrane. CQ has been reported to rigidify the dipalmitoylphosphatidylcholine (DPPC) liposomal membrane by increasing molecular packing in the lipid (Ghosh et al., 1995). This observation is supported by that of another study incorporating the use of Amodiaquine, a 4-aminoquinoline drug similar in structure to CQ, as the drug model. Amodiaquine demonstrated electrostatic and hydrophobic interactions with DPPC in the headgroup region of the liposomal bilayer, thus increasing the lipid order (Barroso et al., 2015). These contradictory effects of PQ and CQ addition may affect their dual loading and the release of liposome.

It is generally accepted that, in order to achieve high drug accumulation in the target tissue, the drug should be stably encapsulated in liposome during distribution throughout the entire body, either by the use of a sturdy bilayer membrane (Barenholz, 2012; Kokkona et al., 2000) or the formation of drug aggregates in the intraliposomal phase (Barenholz, 2012; Lasic et al., 1992; Miatmoko et al., 2017). This study was aimed to determine the effect of the loading combination of PQ+CQ compared to a single drug, on the physicochemical characteristics and rate of release of PQ and CQ from liposome. PQ and CQ were loaded into liposome consisting of lipid with high rigidity, which was hydrogenated soy phosphatidylcholine (HSPC). It was found that dual loading PQ with CQ affected drug encapsulation efficiency and drug release from liposome.

MATERIALS AND METHODS

Materials

For the purposes of this study, primaquine bisphosphate (PQ) was purchased from Sigma-Aldrich Inc. (Rehovot, Israel), while chloroquine diphosphate (CQ) was a product of Sigma-Aldrich® (Gyeonggi-do, South Korea). Hydrogenated soya phosphatidylcholine (HSPC) and methoxy-(polyethylene-glycol)-distearylphosphatidyl-ethanolamine (mPEG-DSPE, PEG mean molecular weight, 2000) were obtained from NOF Inc. (Tokyo, Japan). The cholesterol constituted a product of Wako Pure Chemical Industries Inc. (Osaka, Japan). Potassium dihydrogen phsophate (KH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were both products of Merck® (Darmstadt, Germany), while Sephadex® G-50 was obtained from Sigma-Aldrich Inc. (Steinhem, Germany). The organic solvents, that is, chloroform and methanol, were products of Merck® (Darmstadt, Germany). Deionized water (Otsuka Inc., Lawang, Indonesia) was used as water solvent. All other chemicals and reagents were of the highest quality available.

Determination of optimal incubation for preparation of liposome

Liposome containing a single drug was generated by using CQ as a drug model to determine optimal conditions for drug loading. Liposome was prepared in accordance with the thin-film method (Miatmoko et al., 2016) at a molar ratio of 55:45:5 for HSPC, cholesterol and DSPE-$mPEG_{2000}$, respectively. Each lipid compound was dissolved in chloroform before

Table 1: Formulation of liposome loading combination of PQ and CQ

Component	Formulation				
	P1C0	P0C1	P1C1	P1C3	P1C5
PQ	1.00 mg	-	1.66 mg	0.83 mg	0.55 mg
CQ	-	3.33 mg	1.66 mg	2.48 mg	2.78 mg
HSPC	5.94 mg	5.94 mg	5.94 mg	5.94 mg	5.94 mg
DSPE-mPEG$_{2000}$	1.94 mg	1.94 mg	1.94 mg	1.94 mg	1.94 mg
Cholesterol	2.13 mg	2.13 mg	2.13 mg	2.13 mg	2.13 mg

Note:
P1C0, weight ratio of PQ:total lipid (1:10); P0C1, weight ratio of CQ:total lipid (1:3); P1C1, weight ratio of PQ:CQ:total lipid (0.5:0.5:3); P1C3, weight ratio of PQ:CQ:total lipid (0.25:0.75:3); P1C5, weight ratio of PQ:CQ:total lipid (0.17:0.83:3)

appropriate quantities were inserted into a round bottom flask. The chloroform was then completely removed by means of a vacuum rotary evaporator in a water bath (Buchi Rotavapor R-3, Flawil, Switzerland) at 60°C, leading to the formation of a thin dry film in the bottom of the flask. This layer was hydrated with citrate buffer at pH 5.0. In order to prepare homogenous liposome suspension, the mixture was vortexed and subjected to sonication in a waterbath sonicator of approximately 15 minutes' duration. The mixture was passed through a polycarbonate membrane with a pore size of 100 nm by means of an extruder (Avanti®, Alabaster, Alabama, US) in order to obtain a homogenous liposome particle size.

The drug loading was conducted by transmembrane pH gradient method, which involved eluting the liposome through a Sephadex® G-50 column with phosphate buffer saline (PBS) at pH 7.4. The CQ solution in aquadest was then added at a drug-lipid ratio of 1:5. The drug-liposome mixtures were incubated at specific temperatures, which were 50°C and 60°C, for various incubation periods of 10, 20 and 30 minutes.

Determination of optimal drug to lipid ratios for the preparation of liposome

In order to determine the optimal drug to lipid ratio for the preparation of liposome, the PQ or CQ was loaded as a single drug component of the liposome. The drug loading was completed by transmembrane pH gradient method, which involved eluting liposome hydrated with citrate buffer pH 5.0 through a Sephadex® G-50 column with phosphate buffer saline (PBS) at pH 7.4. The PQ or CQ solution in aquadest was subsequently added at a pre-determined drug-lipid ratio of 1:3, 1:5 or 1:10. The drug-liposome mixtures were incubated at 60°C for 20 minutes. Separation of the liposomal drug from the free drug was achieved by eluting the mixture through a Sephadex® G-50 column with PBS at pH 7.4.

The concentration of entrapped PQ or CQ was measured with a UV Spectrophotometer (Shimadzu, Kyoto, Japan) at λ= 282 nm or λ= 330 nm after lysing with methanol (50% v/v). The encapsulation efficiency was calculated as follows:

$$Percentage\ of\ drug\ loading = \frac{amount\ of\ drug\ entrapped\ within\ liposome}{total\ \ amount\ of\ drug} \times 100\%$$

Preparation of a liposome loading combination of PQ and CQ

Preparation of a liposome loading combination of PQ and CQ involved processing the lipid components in the manner described above. In order to prepare control liposome containing the drugs, PQ and CQ was added at respective drug:lipid weight ratios of 1:10 and 1:3, while for the liposome loading combination of PQ and CQ, the drugs were added at a weight ratio of 1:3 for total PQ+CQ and lipid, respectively, at a composition shown in Table 1. During the drug loading, the drug-liposome mixtures was incubated at 60°C for 20 minutes.

The entrapped PQ and CQ concentrations were measured with a UV Spectrophotometer (Shimadzu, Kyoto, Japan) using a derivative order 1 method at λ = 280 nm or λ = 346 nm (data unpublished) for PQ and CQ respectively after lysing the liposomal vesicle with methanol (50% v/v).

Determination of particle size and ζ-potential of liposome

In order to determine the particle size and ζ-potential of liposome, the sample was diluted appropriately with deionized water. The average particle size and ζ-potential of the liposomes were then measured using a cumulative method and electrophoretic mobility with a light scattering photometer (Delsa™ Nano C Particle Analyzer, Beckman Coulter Inc., Indianapolis, US) at 25°C.

In vitro drug released from liposome

The *in vitro* study of PQ and CQ released from liposome was conducted by placing a liposome sample in dialysis tubing Spectra Por®7 with a molecular weight cut-off (MWCO) of 3,500 (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA). The dialysis media consisted of 50 mL of PBS at pH 7.4. The study was performed through continuous agitation at a

Table 2: Characteristics of liposome loading CQ prepared at different temperature and period of incubation with drug loaded at a weight ratio of 1:5 for drug and total lipid, respectively

Incubation temperature	Period of incubation	Particle size (nm)*)	Polydispersity Index/PDI*)	ζ-Potential (mV) *)	Entrapment efficiency (%)*)
50°C	10 minutes	121.0 ± 6.5	0.30 ± 0.07	-11.3 ± 4.8	17.9 ± 3.2
	20 minutes	123.1 ± 7.5	0.35 ± 0.11	- 5.6 ± 2.0	21.5 ± 4.6
	30 minutes	126.2 ± 14.6	0.27 ± 0.05	-10.9 ± 6.1	15.0 ± 1.9
60°C	10 minutes	122.9 ± 21.4	0.31 ± 0.04	-16.9 ± 3.7	17.5 ± 2.1
	20 minutes	123.4 ± 19.2	0.32 ± 0.08	-23.5 ± 12.2	18.2 ± 2.2
	30 minutes	140.8 ± 30.5	0.26 ± 0.11	-19.8 ± 5.0	16.5 ± 2.8

*) *Each value represents the mean ± S.D. (n = 3).*

speed of 400 rpm in a water bath at 37°C.

At determined sampling points, approximately 2 mL of aliquots were drawn from the media and replaced with the same volume of PBS at pH 7.4. The PQ and CQ concentration was measured spectrophotometrically using a derivative order 1 method at λ = 280 nm or λ = 346 nm for PQ or CQ respectively. Dilution correction factor was used to calculate the cumulative amount of drug released (Aronson, 1993).

Statistical analysis

The data existed in triplicate and was presented as the mean ± S.D. The statistical analysis consisted of a one-way ANOVA followed by an LSD post-hoc test, which were performed to determine the significance of the difference. A *P* value less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The characteristics of liposomes are significantly influenced by several factors, including: length of the incubation period, temperature during the incubation period and drug-to-lipid ratio (Qiu et al., 2008). Moreover, the quantity of drug released by liposomes depends predominantly on the physicochemical properties of liposome membrane and its encapsulated drugs (Liang, 2010). In this study, liposomes were prepared for the loading of PQ and CQ. Dual loading these drugs affected both encapsulation and the properties of drug release.

The loading of PQ and CQ into liposome involved remote loading of a drug with a pH gradient using citrate buffer pH 5.0 as the intraliposomal phase and PBS pH 7.4 as the outer phase. The first step was to evaluate the effect of temperature and the incubation period by using CQ as a drug model, since – during clinical therapy – it will be at a higher dose than PQ (World Health Organization, 2015), thus limiting the drug loading capacity of liposome. PQ has different properties to CQ (Qiu et al., 2008; Stensrud et al., 2000), thereby probably resulting in contrasting optimal loading conditions. However, these were undetermined by this study. As shown in Table 2, all liposomes were produced with a similar particle size of approximately 100–150 nm, with a slightly negative charge of ζ- and potential of approximately -15 mV. There was no significant difference in particle size or ζ-potential due to the same components of liposome, that is, HSPC, DSPE-mPEG$_{2000}$ and cholesterol (Qiu et al., 2008; Yadav et al., 2011). Moreover, the implementation of this transmembrane pH gradient method meant that only approximately 17–22% of the CQ could be loaded into the liposome. There were no significant differences in the encapsulation efficiency of liposome CQ because of the use of varying temperatures in different incubation periods, as shown in Table 2. For further experiments, the incubation of a drug mixture with liposome will be performed at 60°C for 20 minutes, regarded as the highest transition temperature (T_m) of liposome component, which is HSPC, at 55°C (Chen et al., 2013). However, it can be seen that the encapsulation efficiency of CQ at a drug:lipid ratio of 1:5 was low.

The optimal drug-to-lipid ratio for the entrapment of PQ and CQ in liposome was determined. A previous study reported that CQ was loaded into liposome at a drug-to-lipid mass ratio of 1:80 (Qiu et al., 2008), while PQ was loaded at one of 1:14 (Stensrud et al., 2000). It proved unfeasible to achieve an efficient drug loading at a very low drug-to-lipid ratio. The optimum ratio of 1:5 adopted by other studies of liposome prepared by using the transmembrane pH gradient (Miatmoko et al., 2017) was modified to drug-to-lipid ratios of 1:10 and 1:5. Decreasing the drug-to-lipid ratio enhanced the encapsulation efficiency of PQ in liposome. Compared to liposome PQ prepared at a drug-to-lipid ratio of 1:10, PQ1-L10 demonstrated the highest encapsulation efficiency of 66.4%, as shown in Table 3. In contrast, CQ could be optimally loaded at a high drug-to-lipid ratio of 1:3 (CQ1-L3) with an encapsulation efficiency of 60.1%. It has been reported that the intravesicular loading capacity of liposome is limited and the significant addition of drugs will reduce the pH gradient between the intra- and extravesicular phases, thus reducing drug loading (Qiu et al., 2008). CQ will be protonated into two basic ionization states since it has pKa values of 8.10 and 9.94 (Qiu et al., 2008). On the other hand, PQ is an amphiphatic drug with pKa values of 3.2 and 10.4 (Stensrud et al., 2000). Therefore, it produced a different profile of drug loading in the same transmembrane pH gradient condition due to contrasting amounts of ionized and unionized drug fractions.

Table 3: Characteristics of liposome loading single PQ or CQ prepared by loading drugs at 60oC for 20 minutes

Drug Component	Formulation	Particle size (nm)*)	Polydispersity Index/PDI*)	ζ-Potential (mV) *)	Entrapment efficiency (%)*)
PQ	PQ1-L3	175.8 ± 27.1	0.52 ± 0.33	-16.8 ± 5.2	40.0 ± 3.3
	PQ1-L5	162.1 ± 31.3	0.57 ± 0.25	-19.6 ± 5.4	48.5 ± 3.1
	PQ1-L10	163.8 ± 41.4	0.34 ± 0.05	-17.8 ± 3.9	66.4 ± 8.2
CQ	CQ1-L3	149.1 ± 27.4	0.21 ± 0.02	-22.7 ± 5.3	60.1 ± 7.9
	CQ1-L5	123.4 ± 19.2	0.32 ± 0.08	-23.5 ± 12.2	21.5 ± 4.6
	CQ1-L10	153.1 ± 23.1	0.15 ± 0.04	-22.2 ± 7.9	21.3 ± 9.2

*) *Each value represents the mean ± S.D. (n = 3).*
PQ, primaquine; CQ, chloroquine; L, total lipid of liposome; PQ1-L3, one part of primaquine to 3 parts of total lipid of liposome (w/w)

Based on these results, a 20-minute incubation at 60°C and PQ-to-lipid ratio of 1:10 and CQ to-lipid ratio of 1:3 (w/w) were selected for loading drugs into liposome in further experiments.

In order to prepare a liposome loading combination of PQ and CQ, the liposome was added to PQ and CQ solution at a determined drug weight:lipid ratio, namely; 0.5:0.5:3; 0.25:0.75:3 and 0.13:0.87:3 for PQ:CQ:total lipid, as shown in Table 1. All liposomes were produced with particle sizes ranging from 100 to 175 nm as shown in Fig. 1A with a polydispersity index of approximately 0.20–0.40 (Fig. 1B). These liposomes had slightly negative ζ-potential charges of -9.7 to -22.7 mV (Fig. 1C). Compared to single drug-loaded liposome, combining PQ and CQ into liposome resulted in lower drug encapsulation efficiency (Fig. 1D). The addition of CQ into liposome affected PQ encapsulation, which stood at 72% for the single drug-loaded PQ liposome (P1C0) and 6% for dual drug-loaded liposome (P1C1). Moreover, PQ also influenced liposomal encapsulation of CQ. Compared to single-loaded CQ liposome (P0C1), dual drug loaded-liposome had a lower CQ loading, 56% and 31% for P0C1 and P1C1 liposome, respectively. The PQ-CQ ratio also played an important role in determining liposomal drug encapsulation, which decreases the proportion of CQ to PQ. This resulted in lower encapsulation of PQ as achieved in P1C1 liposome. In contrast, increasing the proportion of CQ to PQ did not produce significant differences in CQ encapsulation. Although these two drugs were encapsulated within an aqueous intraliposomal compartment of the same volume, the addition of PQ and CQ probably affected the permeability of the bilayer during incubation in a contradictory manner. This produced a different optimal drug-to-lipid ratio required for the achieving of impressive encapsulation efficiency. In liposome, drugs can be encapsulated within the hydrophobic bilayer or the hydrophilic aqueous phase, or may interact with the polar headgroup region of the lipid bilayer. The encapsulation efficiency is affected by many factors such as bilayer fluidity (Kulkarni et al., 1995). It has been reported that the positively charged amine of PQ interacts with the polar headgroup region of phosphatidylcholine/PC. In contrast, its quinolone ring indicates Van der Waals interaction with the hydrocarbon core of lipids resulting in fluidizing effects within and pertubation to the bilayer membrane (Basso et al., 2011). On the other hand, the positively charged amine of CQ has been reported as interacting with negative phosphate groups of phosphatidylcholine and producing rigidification of the liposomal membrane (Barroso et al., 2015; Ghosh et al., 1995). However, although dual loading produced low drug encapsulation, PQ could be delivered together with CQ, which may play an important role in drug metabolism in hepatocytes improving therapeutical efficacy of PQ as well as reducing its toxicity.

The *in vitro* drug released from liposomes was evaluated by immersing liposomes in PBS at pH 7.4 (Fig. 2). The results showed that both PQ and CQ were released more gradually from dual drug-loaded (PICI) liposome than from P1C0 and P0C1 liposomes. Approximately 63% of the initial dose of PQ was released from P1C0 liposome over a period of 48 hours, while this figure fell to 44% in the presence of CQ encapsulated in P1C1 liposome. CQ displays a similar profile of liposomal drug release indicating an approximate 50% reduction in the drug released by the P1C1 liposome compared to the single CQ-loaded liposome (P0C1 liposome). These results indicate that the liposome loading combination of PQ and CQ produced slower drug release than single drug-loaded liposome, suggesting that the CQ may produce powerful rigidifying effects on the liposomal bilayer since it contains more numerous drug molecules entrapped within the liposome than does PQ. It would be advantageous to avoid premature PQ release during systemic circulation before the liposome enters the hepatocytes. On the other hand, slow release of CQ would also be important for the prophylactic effect on the erythrocytic stage development.

The dual drug loading of PQ and CQ into liposome, which was composed of HSPC, cholesterol and DSPE-mPEG$_{2000}$, greatly influenced drug encapsulation efficiency and drug release. It is important to produce high drug loading and tailor delivery for deliberate release of the drug in an appropriate manner in order to achieve high accumulation in liver tissue for treatment of hepatic stage malaria. However, further investigation is still required to evaluate PQ interaction with the liposomal membrane in the presence of CQ, pharmacokinetic profiles and activity for further exploration of dual-loaded PQ+CQ liposome as part of malaria therapy.

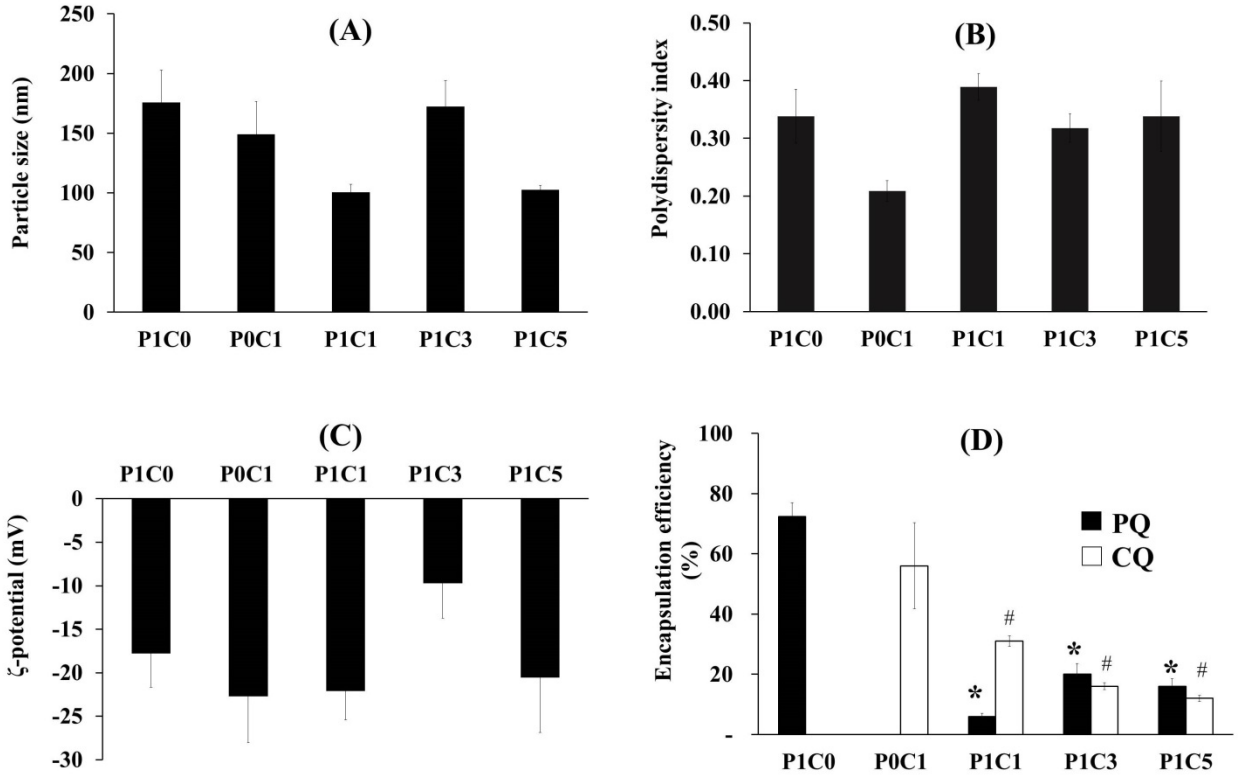

*Figure 1: The characteristics of (A) particle size, (B) polydispersity index, (C) ζ-potential, (D) encapsulation efficiency of liposome encapsulating PQ (black), CQ (white) and the combination of PQ+CQ loaded by incubating the mixtures at 60°C for 20 minutes. Each value represents mean ± S.D. (n=3). *P< 0.05 compared with P1C0. #P< 0.05 compared with P0C1.*

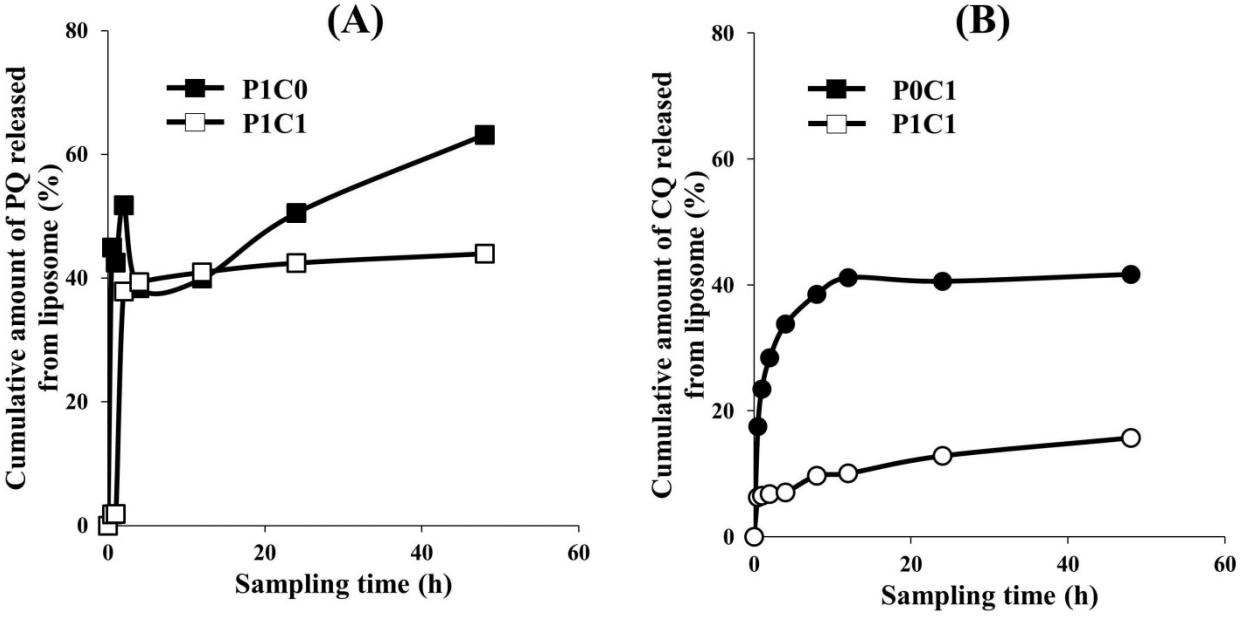

Figure 2: Profiles of release of (A) PQ and (B) CQ from single drug-loaded liposome (P0C1 and P1C0) and dual drug-loaded liposome (P1C1) in phosphate-buffered saline (PBS), pH 7.4 at 37°C.

CONCLUSIONS

In this study, liposomal containing dual drug loading, which consisted of PQ and CQ, was prepared and subsequently evaluated for drug loading and *in vitro* drug release. Nano-sized particles, high encapsulation for the PQ+CQ combination and slow drug release were achieved by combinedly loading PQ and CQ at 1:1 weight ratio. This finding suggested that dual PQ+CQ-loaded liposome could potentially be used for comprehensive malaria therapy involving hepatic and erythrocytic stage malaria.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest or financial interests such as grants, employment, gifts, stock holdings, honoraria, consultancies, expert testimony, patents and royalties, in any product or service mentioned in this article.

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