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Formulation optimization of palm kernel oil esters nanoemulsion-loaded with chloramphenicol suitable for meningitis treatment

Siti Hajar Musa[a,*], Mahiran Basri[a,b,*], Hamid Reza Fard Masoumi[a], Roghayeh Abedi Karjiban[a], Emilia Abd Malek[a], Hamidon Basri[c], Ahmad Fuad Shamsuddin[d,e]

[a] Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[b] Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[c] Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[d] Centre for Drug Delivery Research, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
[e] Quality Use of Medicines Research Group, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

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ABSTRACT

Palm kernel oil esters nanoemulsion-loaded with chloramphenicol was optimized using response surface methodology (RSM), a multivariate statistical technique. Effect of independent variables (oil amount, lecithin amount and glycerol amount) toward response variables (particle size, polydispersity index, zeta potential and osmolality) were studied using central composite design (CCD). RSM analysis showed that the experimental data could be fitted into a second-order polynomial model. Chloramphenicol-loaded nanoemulsion was formulated by using high pressure homogenizer. The optimized chloramphenicol-loaded nanoemulsion response values for particle size, PDI, zeta potential and osmolality were 95.33 nm, 0.238, −36.91 mV, and 200 mOsm/kg, respectively. The actual values of the formulated nanoemulsion were in good agreement with the predicted values obtained from RSM. The results showed that the optimized compositions have the potential to be used as a parenteral emulsion to cross blood-brain barrier (BBB) for meningitis treatment.

1. Introduction

Bacterial meningitis is one of the most serious and common infections that can occur in the brain or meninges [1]. The symptoms include fever, nausea, headache, vomiting, neck stiffness, and photophobia. Meningitis can lead to acute inflammation and subsequently brain damage [2]. It carries a high risk of death and severe neurologic sequelae, especially when there is delay in diagnosis and antibiotic administration [3]. Meningitis affects 30% of new-born and young infants and 15–20% of older children [4]. Between 30% and 50% of meningitis survivors will suffer from permanent neurological sequelae [5]. Despite the availability of effective antibiotic treatment, a high mortality rate of up to 30% has been observed [1,6].

Early antibacterial therapy is of utmost importance for treatment. Chloramphenicol is a relatively effective drug for bacterial meningitis and has been used since 1975 [7]. However, the use of chloramphenicol has been administered in increasing dosage in recent years due to the increased incidence of antibiotic resistance. In addition, chloramphenicol is a hydrophobic drug which poorly dissolves in water. Clinically, this kind of hydrophobic drug dissolves in water in the form of a salt. A higher dosage of drug solution will therefore be required to ensure that it can efficiently reach the target cell. A nanoemulsion-based chloramphenicol carrier could improve the solubility of the drug in the dispersed phase and drug penetration into target cells due to its extremely small size. Therefore, a lower dosage would be needed due to more efficient penetration.

The blood-brain barrier (BBB) is a feature that encapsulates the human brain and protects brain from harmful compounds [8]. The BBB is the gate keeper of the brain, and acts as an efflux pump, limiting the entry of many structurally divergent lipophilic molecules, such as peptides and many of the drugs used in psychiatry and neurology for the treatment of the brain [9]. BBB acts as a separator between the brain and its blood supply. The criteria required for a suitable parenteral formulation to cross BBB include: particle size

* Corresponding author. Tel.: +60 3 8946 7266.
E-mail address: mahiran@science.upm.edu.my (M. Basri).

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less than 100 nm, polydispersity index (PDI) less than 0.25, zeta potential greater than 30 mV or lower than −30 mV and osmolality between 285 and 310 mOsm/kg [10–12].

An emulsion is a mixture of two immiscible liquids with one dispersed in the other [13]. The basic components of an emulsion are immiscible water and oil; a surfactant is needed to decrease the interfacial tension and maintain the stability of the emulsion. An emulsion can either be in the form of water-in-oil or oil-in-water [14]. A nanoemulsion is an emulsion with a droplet size ranging from 20 to 200 nm [10]. It has been shown that an emulsion with a very small particle size can provide greater encapsulation efficiency for delivery [15]. Nanoemulsions have special characteristics and can be used for drug delivery due to their extremely small size, biocompatibility, relative stability, ability to solubilize high quantities of hydrophobic compounds, ability to reduce the toxicity of cytotoxic drugs, and ability to protect drugs from hydrolysis and enzymatic degradation under physiological conditions [16].

Nanoemulsions can be produced through low-energy emulsification or high-energy emulsification methods [15]. Low-energy emulsification relies on the spontaneous formation of tiny oil droplets within mixed oil-water-emulsifier systems when the solution or environmental conditions are altered [17]. An alternative method is the fabrication of a nanoemulsion, which is performed using high-energy emulsification methods [18]. The high energy emulsification approach utilizes a mechanical device to generate intense disruptive forces that break up the oil and water phases to produce tiny oil droplets. High energy emulsification is carried out by applying a microfluidic homogenizer, ultrasound homogenizer, or high pressure homogenizer [19].

Multivariate statistical techniques such as response surface methodology (RSM) have been used for the optimization of analytical procedures for many years. [20]. RSM was first developed by Box and collaborators in the 1950s [21]. The RSM technique was inspired by the graphical perspective generated regarding the fitness of a mathematical model [22]. Mathematical and statistical techniques using RSM are in a close relationship with laboratory experiments. Modeling and displacing experimental conditions are conducted by linear or polynomial functions to describe the system under study. RSM has the ability to determine the relationship and the interaction between the independent variable and responses based on the desired criteria. Moreover, a fewer number of experimental trials will be needed to evaluate the interaction if RSM is applied. Thus, optimizing an experimental process becomes less time consuming [23].

In this work, optimization of the composition of a chloramphenicol-loaded nanoemulsion with respect to the amount of oil, lecithin and glycerol was carried out. The four responses consisted of particle size, PDI, zeta potential and osmolality were studied to find the best composition for a chloramphenicol-loaded nanoemulsion carrier.

2. Materials and methods

2.1. Materials

Palm kernel oil esters (PKOEs) were prepared in our research laboratory. Safflower seed oil was purchased from Sigma–Aldrich Chemie GmbH, Germany. Pure soy bean lecithin (Lipoid S75) was purchased from Lipoid GmbH, Ludwigshafen-Germany. Glycerol was purchased from JT Baker, USA. Polysorbate 80 (Tween80) was obtained from Fluka, Sigma–Aldrich Chemie GmbH, Germany. Chloramphenicol was purchased from Euroasias Chemicals Private Limited, India. Water was deionized using a Milli-Q filtration system.

Table 1
Coded level for independent variables used in experimental design for nanoemulsion optimization.

Independent variables	Unit	Coded level				
		−1.68	−1	0	+1	+1.68
Oil amount, x_1	% w/w	0.59	4	9	14	17.41
Lecithin amount, x_2	% w/w	0.18	0.5	1.5	2.5	3.18
Glycerol amount, x_3	% w/w	0.18	0.5	1.5	2.5	3.18

2.2. Determination of the solubility of chloramphenicol in oil

The solubility of chloramphenicol in the mixture of PKOEs with five different types of oil (sesame oil, soybean oil, sunflower oil, safflower seed oil and pine nut oil) at a ratio of 1:1 (w/w) was determined. The drug (1%) was added into the oil containing lecithin (3%). The solution was kept under moderate magnetic stirring for 24 h to reach equilibrium. The sample was then centrifuged at 4500 rpm for 15 min. An aliquot of the supernatant was diluted with methanol. The chloramphenicol content was assayed by ultra-performance liquid chromatography (UPLC).

2.3. Formulation of the nanoemulsion

Emulsions were prepared using an overhead stirrer (IKA® RW 20 Digital, Nara, Japan) at 305–310 rpm. Chloramphenicol (0.5% w/w) was dissolved in the oil phase (PKOEs:safflower seed oil, 1:1 (w/w)) containing lecithin (0.5–2.5%) as the surfactant. Tween 80 (0.75% w/w) was then added into the oil phase as a co-surfactant after the chloramphenicol was completely dissolved. The oil phase was added dropwise into the aqueous phase consisting of glycerol and was continuously stirred to form a coarse emulsion. The mixture was stirred for 1 h and was subjected to further processing through a high pressure homogenizer at 1000 psi for six cycles.

2.4. Selection of the co-surfactant

Co-surfactants including Tween 40, Tween 20, and Cremophor EL were used instead of Tween 80 in the formulation. All formulations were analyzed with respect to the droplet size and PDI.

2.5. RSM experimental design

A three-factor central composite design (CCD) was employed to determine the effect of the amount of oil (4–14%, x_1), lecithin (0.5–2.5%, x_2), and glycerol (0.5–2.5%, x_3) on four response variables: average droplet size (Y_1), PDI (Y_2), zeta potential (Y_3), and osmolality (Y_4) of the nanoemulsion. A total of 20 runs were generated using Design-Expert® 6.0.6 software (Stat ease Inc., Minneapolis, USA). Experiments with three independent variables consisted of right factorial points and five axial points, and six replicates of the center points were carried out [23]. The experiments were carried out in randomized order to minimize the effects of unexplained variability in the actual responses due to extraneous factors [24]. A summary of the independent variables and their coded levels are presented in Table 1. The results at each point based on experimental design (CCD) are presented in Table 2.

2.6. Statistical analysis

Response surface methodology was used to obtain the best formulation for the chloramphenicol-loaded nanoemulsion carrier with respect to the amount of oil (x_1), lecithin (x_2), and glycerol (x_3). The main objective was to determine the composition of the nanoemulsion with the minimum particle size (Y_1) and PDI (Y_2), optimum zeta potential (Y_3), and maximum osmolality