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PARENTERAL FORMULATION OF NANOEMULSION LOADED WITH CHLORAMPHENICOL FOR MENINGITIS TREATMENT

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By

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PARENTERAL FORMULATION OF NANOEMULSION LOADED WITH CHLORAMPHENICOL FOR MENINGITIS TREATMENT

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Chair : Professor Mahiran Basri, PhD
Faculty : Science

Meningitis is a disease caused by the inflammation of the brain due to bacterial infection. The most common causative agent in bacterial meningitis is *Streptococcus pneumonia.* The bacterial infection occurs in the brain which is protected by a barrier known as blood-brain barrier (BBB). This barrier consists of a very selective membrane which only allows small particle with lipophilic characteristic to pass through. In the current medication treatment, third generation cephalosporin has been used widely in treating this disease. However, this family of drug (cephalosporin) is no longer effective towards the meningitis bacteria due to the drug resistance problem. Traditional antibiotic such as chloramphenicol has caught medical community's attention back to overcome the problem. Delivery of chloramphenicol could be improved via nanoemulsion system with nano-size droplet range.

From the solubility study, it was found that palm kernel oil esters (PKOEs) and safflower seed oil (1:1 ratio) was the best combination in solubilizing chloramphenicol compared to other oil mixture. Lecithin was best mixed with Tween 80 as co-surfactant in the emulsion system, resulting in smaller particle size (131.5 nm) and low polydispersity index (0.135) reading. Methods of emulsification such as spontaneous method (stirring process), spontaneous with high shear homogenizer, spontaneous with ultrasound homogenizer and spontaneous with high pressure homogenizer were studied. With respect to the particle size and polydispersity index, combination of spontaneous method with high pressure homogenizer gave the best particle size and polydispersity index which was 70.65 nm and 0.154, respectively.

Response Surface Methodology (RSM) was used as a tool in optimization process. Relation between independent variables (oil, lecithin and glycerol amount) and response variables (particle size, zeta potential and osmolality)

were analysed. From three dimension (3D) analysis models, it was observed that particle size, zeta potential and osmolality value of nanoemulsion was largely influenced by the amount of oil, lecithin and glycerol, respectively. RSM has suggested composition with 4% oil, 2.50% lecithin and 2.25% glycerol which resulted with nanoemulsion system with 95.33 nm of particle size, 0.238 of polydispersity index, -36.91 mV of zeta potential and 200 mOsm/kg of osmolality. These actual values show good agreement with the predicted values; 86.96 nm (particle size), 0.234 (polydispersity index), -33.47 mV (zeta potential), 198 mOsm/kg (osmolality).

Dosage optimization showed that stable nano-antibiotic emulsion system was successfully achieved up to 3 mg/ml of chloramphenicol. Three formulations with varied loading dosage (1, 2 and 3 mg/ml) were found to be stable at 4ºC. Transmission Electron Microscopy (TEM) of negatively stained nanoemulsion showed that the oil droplets were in a spherical shape and uniform in size. Toxicity analysis shows that nanoemulsion entrapment has successfully reduced the toxicity effect of chloramphenicol towards 3T3 cells. *In-vitro* study showed a good penetration of chloramphenicol through the cellulose acetate membrane which resulted a zero order release mechanism.

Stability evaluation based on the particle size for three months showed that the particle size remain in a nano-size with less than 5% of size changes. Emulsion entrapment study shows no chloramphenicol leakage from the oil droplet. Drug assay through 3 months of storage showed a consistent drug loading in the emulsion with less than 10% of drug lost. In conclusion, the nanoemulsion formulation is a promising vehicle for the delivery of chloramphenicol parenterally (intravenous route).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperlun untuk Ijazah Master Sains

FORMULASI PARENTERAL BAGI NANOEMULSI YANG MENGANDUNGI *CHLORAMPHENICO*L UNTUK PENYAKIT RADANG LAPISAN OTAK

Oleh

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Radang lapisan otak adalah penyakit yang disebabkan oleh jangkitan bakteria di dalam otak. Ejen utama penyebab radang lapisan otak ialah disebabkan oleh jangkitan bakteria *Streptococcus pneumonia.* Jangkitan bakteria terhadap otak berlaku di bahagian yang dilindungi oleh halangan darah-otak (BBB).Halangan ini terdiri daripada membran yang sangat peka dan hanya akan membenarkan zarah lipofilik yang sangat kecil melepasinya. Kaedah perubatan terkini banyak menggunakan *cephalosporin* generasi ketiga bagi mengubati penyakit ini. Walau bagaimanapun, kumpulan dadah ini tidak lagi efektif terhadap bakteria radang lapisan otak disebakan oleh masalah rintangan dadah. Perubatan tradisional seperti kloramfenikol telah mendapat tumpuan semula dari komuniti perubatan bagi mengatasi masalah ini. Penghantaran kloramfenikol masih boleh ditingkatkan melalui sistem nanoemulsi yang mempunyai titisan kecil bersaiz-nano.

Daripada kajian kelarutan, didapati ester minyak kernel kelapa sawit (PKOEs) dan minyak biji bunga kesumba (nisbah 1:1) adalah kombinasi yang terbaik bagi melarutkan kloramfenikol berbanding campuran minyak yang lain. Kombinasi lesitin adalah baik bersama dengan Tween 80 sebagai ko-surfaktan di dalam sistem emulsi yang menghasilkan saiz zarah kecil (131.5 nm) dan bacaan indeks polisebaran yang rendah (0.135). Kaedah emulsi seperti kaedah secara spontan (proses pengacauan), kaedah spontan berserta pengacau mericih tinggi, kaedah spontan berserta pengacau ultra-bunyi dan kaedah spontan berserta pengacau bertekanan tinggi telah dikaji. Kombinasi kaedah spontan berserta pengacauan bertekanan tinggi menghasilkan saiz zarah dan indeks polisebaran yang terbaik iaitu masing masing dengan nilai 70.65 nm dan 0,154.

Kaedah Gerakbalas Permukaan (RSM) digunakan sebagai alat bagi proses pengoptimuman. Hubungan antara pembolehubah bebas (jumlah minyak, lesitin dan gliserol) dengan pembolehubah tindak balas (saiz zarah, keupayaan zeta dan osmolaliti) dikaji. Berdasarkan kepada kajian model tiga dimensi (3D), didapati nilai bacaan saiz zarah, keupayaan zeta dan osmolaliti masing masing adalah sangat besar dipengaruhi oleh jumlah minyak, jumlah lesitin dan jumlah gliserol. RSM mencadangkan komposisi 4% minyak, 2.5% lesitin dan 2.25% gliserol yang menghasilkan nanoemulsi bersaiz zarah 95.33 nm, bacaan indeks polisebaran 0.238, bacaan keupayaan zeta -36.91 mV dan bacaan osmolaliti 200 mOsm/kg. Bacaan sebenar bagi semua nilai ini adalah hampir bersamaan dengan nilai jangkaan; 86.96 nm (saiz zarah), 0.234 (indeks polisebaran), -33.47 mV (keupayaan zeta), 198 mOsm/kg (osmolaliti).

Pengoptimuman dos kloramfenikol menunjukkan sistem emulsi antibiotik-nano berjaya dicapai sehingga 3 mg/mL. Tiga formulasi yang berbeza mengikut dos muatan (1, 2 dan 3 mg/mL) didapati stabil pada suhu 4ºC. Penghantaran Elektron Mikroskopi (TEM) nanoemulsi berwarna negatif menunjukkan titik kecil minyak berbentuk sfera dan bersaiz seragam. Analisis toksik menunjukkan perangkap nanoemulsi telah berjaya mengurangkan kesan ketoksikan kloramfenikol terhadap sel 3T3. Kajian secara *in-vitro* menunjukkan penembusan kloramfenikol yang baik melalui membran selulosa acetat yang menghasilkan mekanisme pelepasan tahap sifar.

Penilaian terhadap kestabilan berdasarkan saiz zarah selama tiga bulan menunjukkan saiz zarah masih kekal berada di dalam lingkungan saiz-nano dengan kurang daripada 5% perubahan. Kajian perangkap emulsi menunjukkan tiada kebocoran kloramfenikol daripada butiran minyak. Pemerhatian terhadap muatan dadah sepanjang penyimpanan selama 3 bulan menunjukkan muatan dadah adalah konsisten dengan kehilangan kurang daripada 10%. Kesimpulannya, formulasi nanoemulsi menjanjikan penghantaran kloramfenikol secara parenteral (laluan intravena) yang baik.

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

BBB	Blood-brain barrier
CNS	Central nervous system
IV	Intravenous
W/O	Water-in-oil
O/W	Oil-in-water
PDI	Polydispersity index
PKOEs	Palm kernel oil esters
PIT	Phase inversion temperature
PIC	Phase inversion composition
HLB	Hydrophilic-lipophilic balance
RSM	Response surface methodology
CCD	Central composite design
PBS	Phosphate buffer solution
UPLC	Ultra Performance Liquid Chromatography
HPLC	High Performance Liquid Chromatography
ELSD	Evaporative Light Scattering Detection
TEM	Transmission Electron Microscopy

LIST OF UNITS

nm	Nanometer
mV	Milivolt
mOsm/kg	Miliosmole per kilogram
cP	Centipoise
ºC	Degree celcius
%	Percentage
w/w	Weight per weight
mg	Milligram
µg	Microgram
mL	Mililitre
µL	Microlitre
mm	Milimetre
µm	Micrometre
cm	Centimetre
Da	Dalton
rpm	Rotation per minute
m	Month
min	Minute
h	Hour

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Human brain is one of the most important organ that play its main role in controlling the whole body system's activity. The brain has more protection compared to other human's organ as a way to make sure that the body systems can function. The feature that encapsulates human's brain is called blood-brain barrier (BBB) which protects the brain from any harmful compounds (Sandoval and Witt, 2008). BBB is recognized as a gate keeper of the brain, an efflux pump, limiting the entrance of many structurally divergent lipophilic molecules, such as peptides and many of the drugs used in psychiatry and neurology (De Klerk *et al.*, 2010). BBB acts as a separator between brain and its blood supply. Thus, this barrier limits many potential therapeutic to reach the central nervous system (Scherrmann, 2002).

Even though BBB is a very selective membrane, but it is still possible for viruses or bacteria to be spread through blood and get into the cerebrospinal fluid, hence causing infection to the brain. One of disease that is caused by the infection of the brain is meningitis. Meningitis is an inflammation of brain caused by either virul meningitis (virus) or bacterial meningitis (bacteria) (Kleine *et al.*, 2003). Statistically, meningitis occurs in 30% of new born and very young infants and 15% to 20% of older children. Between 30%-50% of meningitis survivors will suffer from permanent neurological sequelae (Fitch and Van De Beek, 2007). Despite the availability of effective antibiotic treatment, high mortality rate up to 30% has been observed (Mishal *et al.*, 2008; Weisfelt *et al.*, 2006). Individual that is infected by the meningitis bacteria could also suffer brain damage. Bacterial meningitis also has been recognized as one of the main causes that lead to deafness, seizure disorder and behavioral problems in children (Bonadio, 1997).

1.2 Problem statements

Chloramphenicol is an effective drug towards bacterial infection and is used for meningitis treatment since 1975 (Lewis *et al.*, 1998). However, the use of chloramphenicol has been increasing in the dosage administered in recent years due to the increase incidence in antibiotic resistance. In addition, chloramphenicol is a hydrophobic drug which hardly dissolves in water. Clinically, this kind of hydrophobic drug is used in a salt form as chloramphenicol succinate to be dissolved in water. Due to the less efficient carrier, larger dosage of chloramphenicol is required per injection to ensure it could reach the target cell. Therefore, higher dosages give higher toxicity problem towards cells and also worsen the side effects.

1.3 Scope of study

Nanoemulsion loaded with chloramphenicol was formulated using a mixture of oils and surfactants. Screening of these two compositions and selection on the method of emulsification used were carried out. Optimization was carried out by using Response Surface Methodology (RSM) as a tool with respect to the nanoemulsion's composition. The suggested optimized composition was then been modified due to its stability and osmolality value problems. Characterization was carried out by using Transmission Electron Microscopy (TEM), in-vitro of chloramphenicol release and the mechanism of kinetic study. Evaluation on the stability of chloramphenicol-loaded nanoemulsion was carried out after 3 months of storage at 4 ºC. Stability evaluation was done with respect to the particle size, pH of nanoemulsion, osmolality value, encapsulation efficiency of the droplets and drug assay analysis.

1.4 Objectives

The main objective of this study is to develop parenteral nanoemulsion loaded with chloramphenicol for meningitis treatment. In order to successfully achieve the main objective, following specific objectives were carried out:

i) To formulate and optimize the compositions of chloramphenicol-loaded nanoemulsion suitable for parenteral applications
ii) To characterize the physicochemical properties of the nanoemulsion.
iii) To evaluate chloramphenicol-loaded nanoemulsion release potential using *in-vitro* approach
iv) To evaluate the stability of nanoemulsion.

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