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OPTIMIZATION OF NANOEMULSION FORMULATION CONTAINING PHENYTOIN

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OPTIMIZATION OF NANOEMULSION FORMULATION CONTAINING PHENYTOIN

By

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Faculty : Science

Epilepsy is a Central Nervous System (CNS) disease caused by abnormal neuron signalling causing repeated seizures. CNS is protected by a barrier known as blood-brain barrier (BBB). The selective membrane on the BBB which only allow small lipophilic particle to pass through complicates the penetration of antiepileptic drugs. Phenytoin is a classic antiepileptic drug (AED) listed on the World Health Organization's List of Essential Medicines, which exhibits potent therapeutic efficacy in controlling seizure attacks thus signifying its importance in a basic healthcare system. However, due to poor solubility and bioavailability of phenytoin, large dosage had to be given in its salt form (phenytoin sodium) to reach therapeutic concentration with numerous side effects. Hence, incorporating phenytoin in a nanocarrier would increase its bioavailability while minimizing the dosage required with reduced side effects.

From the formulation study, it was discovered that phenytoin could only be retained in alkali solution. Therefore to address the solubility issue of phenytoin, a two-step method based on the pH solubility profile of phenytoin was used to formulate phenytoin into the nanoemulsion. First step involves the formulation of blank nanoemulsion (without phenytoin) through magnetic stirring followed by high shear homogenization. Second step involves the incorporation of phenytoin onto the nanocarrier by adjusting pH of blank nanoemulsion to alkali condition. Incorporation of phenytoin was confirmed through Transmission Electron Microscopy (TEM) which showed the position of phenytoin on the surface of oil droplets.

Mixture Experimental Design (MED) and Artificial Neural Network (ANN) were employed to optimize the composition of phenytoin-loaded nanoemulsion. Effects of isopropyl myristate (3.00-6.00%, wt%), Tween 80:85 (1.50-3.00% wt%), glycerol (4.00-7.00%, wt%), and water (84.00-91.50%, wt%) on the droplet size of nanoemulsion were investigated. The optimum formulation obtained from the mathematical model with desirable criteria were 3.00% isopropyl myristate, 2.04% Tween 80:85, 7.00% glycerol and 87.96% water. Based on the optimum formulation from MED, the predicted response value for droplet size was 99.58 nm, while the predicted value from ANN was 98.27 nm, which showed in excellent agreement with the actual value obtained from the experiment which was 98.69 nm.

Toxicity comparison between Dilantin (parenteral phenytoin sodium) and phenytoin-loaded nanoemulsion showed that the nanoemulsion to be four times less toxic compared to Dilantin towards Vero (kidney) cells. Stability evaluation based on the droplet size for three months showed that the droplet size remained in nano-size with less than 5% change. Entrapment study showed that more than 95% of phenytoin was encapsulated throughout 3 months storage. In conclusion, the nanoemulsion formulation is a safer and promising vehicle for the delivery of phenytoin through intravenous route.

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

OPTIMISASI BAGI NANOEMULSI FORMULASI YANG MENGANDUNGI *FENITOIN*

Oleh

MAVIN YAP CHONG YANG

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Epilepsi adalah penyakit Sistem Saraf Pusat (SSP) yang disebabkan oleh isyarat neuron yang tidak normal menyebabkan serangan sawan yang berulangan. SSP dilindungi oleh penghalang yang dikenali sebagai halangan darah-otak (BBB). Membran selektif di BBB yang hanya membenarkan zarah lipofilik kecil meretas penembusan ubat-ubatan antiepileptik. Fenitoin adalah ubat antiepileptik klasik (AED) yang tersenarai dalam Senarai Ubat-Ubatan Penting Organisasi Pertubuhan Kesihatan Sedunia, yang memperlihatkan keberkesanan terapeutik yang kuat dalam mengawal serangan sawan,justeru menandakan kepentingannya dalam sistem penjagaan kesihatan asas. Walau bagaimanapun, disebabkan keterlarutan yang kurang baik dan bioavailabiliti fenitoin, dos yang besar perlu diberikan dalam bentuk sebatian garam (fenitoin natrium) untuk mencapai kepekatan terapeutik yang mencukupi dengan banyak kesan sampingan. Oleh itu, penghantaran fenitoin melalui nanoemulsi boleh meningkatkan keberkesananya sementara mengurangkan dos yang diperlukan sekaligus mengurangkan kesan sampingan**.**

Daripada kajian perumusan, didapati bahawa fenitoin hanya boleh dilarut dalam larutan alkali. Oleh itu, untuk menangani isu kelarutan fenitoin, kaedah dua langkah berdasarkan profil kelarutan pH fenitoin digunakan untuk merumuskan phenytoin ke dalam nanoemulsi. Langkah pertama melibatkan perumusan nanoemulsi kosong (tanpa fenitoin) melalui pengacauan spontan diikuti oleh homogenisasi ricih yang tinggi. Langkah kedua melibatkan penambahan fenitoin ke dalam nanoemulsi dengan menyesuaikan pH nanoemulsi kosong kepada pH alkali. Pembentukan fenitoin telah disahkan melalui Transmission Electron Microscopy (TEM) yang menunjukkan kedudukan fenitoin pada permukaan titisan minyak.

Reka bentuk Eksperimen Campuran (MED) dan Rangkaian Neural Buatan (ANN) telah digunakan untuk mengoptimumkan komposisi nanoemulsi yang dimuatkan fenitoin. Kesan isopropil myristate (3.00-6.00%, wt%), Tween 80:85 (1.50-3.00%, wt%), gliserol (4.00-7.00%, wt%), dan air (84.00-91.50%, wt%) pada saiz titisan nanoemulsi telah diselidiki. Perumusan optimum yang diperoleh daripada model matematik dengan kriteria yang diinginkan adalah 3.00% isopropil myristate, 2.04% Tween 80:85, 7.00% gliserol dan 87.96% air. Berdasarkan rumusan optimum dari MED, nilai tindak balas yang diramalkan untuk saiz titisan adalah 99.58 nm, manakala nilai ramalan dari ANN adalah
98.27 nm, yang menunjukkan persetujuan yang sangat baik dengan nilai sebenar yang diperoleh dari eksperimen iaitu 98.69 nm.

Perbandingan analisis toksik antara *Dilantin* (fenitoin natrium parenteral) dan nanoemulsi fenitoin menunjukkan bahawa *Dilantin* adalah empat kali lebih toksik berbanding nanoemulsi terhadap sel-sel Vero (buah pinggang). Penilaian kestabilan berdasarkan saiz titisan selama tiga bulan menunjukkan bahawa saiz titisan kekal dalam ukuran nano dengan perubahan kurang daripada 5%. Kajian pengkapsulan dadah menunjukkan bahawa lebih daripada 95% fenitoin terkandung dalam rumusan dalam simpanan selama 3 bulan. Sebagai kesimpulan, perumusan nanoemulsi menjanjikan penghantaran fenitoin secara parenteral yang lebih efektif.

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LIST OF ABBREVIATIONS

AED	Antiepileptic drug
AMT	Adsorptive-mediated transcytosis
ANN	Artificial Neural Network
BBB	Blood-brain barrier
BBP	Batch back propagation
CNS	Central nervous system
DLS	Dynamic light scattering
GA	Genetic algorithm
HLB	Hydrophilic-lipophilic balance
HPLC	High Performance Liquid Chromatography
IBP	Incremental back propagation
IC50	50% inhibition concentration
IV	Intravenous
LM	Levenberg-Marguardt
MED	Mixture Experimental Design
NVU	Neurovascular unit
O/W	oil-in-water
PDI	Polydispersity index
PIE	Phase inversion emulsification
QP	Quick propagation
RMSE	Root mean square error
RMT	Receptor-mediated transcytosis
RSE	Residual standard error
SAR	Structure activity relationship
TEM	Transmission Electron Microscopy
W/O	Water-in-oil

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Epilepsy is a chronic central nervous system (CNS) disease whereby abnormal electrical activity is present in the brain resulting in seizures (Bennewitz and Saltzman, 2009). Up to date, there is no cure for epilepsy thus epileptic patients are prescribed with antiepileptic drugs (AED) or anticonvulsant drugs. AEDs were successful in suppressing seizures in majority of patients as many as 60-70% (Schmidt, 2009). However, in 20-30 % of patients, epilepsy is drug resistant, in other words epilepsy cases in some patients are uncontrollable (Zhang *et al*., 2012). The purpose of using AEDs is to control seizures as quickly as possible with minimal side effects yet several hypotheses had been postulated as to why antiepileptic drugs failed to suppress seizures.

Among the hypotheses postulated are, the disease itself is too severe, incorrect administration of drugs, and finally the hypotheses which many researchers believe to be the key in treating epilepsy is that drugs administrated are not delivered efficiently to the target side (Zhang *et al*., 2012; Schmidt, 2009). This can be attributed to the role of Blood-Brain Barrier (BBB), a selective membrane protecting the brain from foreign molecules such as AEDs. BBB limits the therapeutic potential of AEDs resulting in uncontrollable seizure attacks. Patients with drug-resistant epilepsy will have serious health problems such as, injury, depression, anxiety, and to the highest extent resulting in mortality (McCagh *et al*., 2009; Smeets *et al*., 2007).

1.2 Problem Statement

A number of AEDs have been introduced over the years ever since the first antiepileptic drug, bromide, to phenobarbital and phenytoin which are still in use in the modern world (Zhang *et al*., 2012). Challenges of phenytoin (PHT) as modern AEDs is the difficulty of PHT to penetrate the BBB which results in poor efficacy. Moreover, PHT is a hydrophobic drug with poor water solubility. Due to the abscence of an efficient carrier, parenteral administration of this hydrophobic drug had to be given in its salt form and in high dosage in order to increase PHT's bioavailability in the mortor cortex of the brain. High PHT dosage will cause numerous side effects and higher toxicity towards the brain cells, hence the significance of introducing an efficient nanocarrier for PHT.

1.3 Scope of Study

Phenytoin-loaded nanoemulsion was formulated using a mixture of oils and surfactants. Screening on the compositions and selection on the emulsification method were carried out. Encapsulation method for PHT was developed. Stability of the encapsulated PHT was evaluated before optimization. Optimization was then carried out by employing Mixture Experimental Design (MED) and Artificial Neural Network (ANN) as a tool with respect to the compositions of phenytoin-loaded nanoemulsion. Morphology of the nanoemulsion was observed through Transmission Electron Microscopy (TEM). Toxicity of the nanoemulsion towards normal kidney cells were evaluated using MTT assay. Evaluation on the stability of optimized phenytoin-loaded nanoemulsion was carried out after 3 months of storage at 4°C, 25°C, and 45°C. Evaluation was done with respect to the droplet size, pH, encapsulation efficiency, and phase separation.

1.4 Objectives

The main objective of this research is to develop parenteral nanoemulsion loaded with PHT. In order to succesfully achieve the main objective, following specific objectives were carried out:

I. To formulate and optimize the compositions of PHT-loaded nanoemulsion suitable for parenteral administration
II. To characterize the physicochemical properties of PHT-loaded nanoemulsion
III. To evaluate the toxicity and stability of PHT-loaded nanoemulsion
IV. To evaluate the *in-vitro* release potential of PHT-loaded nanoemulsion

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APPENDICES

Table A1: Stability of phenytoin-loaded nanoemulsion for 3 months storage at 25°C with respect to droplet size

Storage (25°C)	Droplet size (nm)			Standard deviation
	Reading 1	Reading 2	Reading 3	
Fresh	98.70	99.30	98.10	± 0.60
1 month	99.80	100.50	99.10	± 0.70
2 month	100.80	101.50	100.20	± 0.65
3 month	102.00	101.80	100.90	± 0.59

Table A2: Stability of phenytoin-loaded nanoemulsion for 3 months storage at 25°C with respect to pH

Storage (25°C)	Droplet size (nm)			Standard deviation
	Reading 1	Reading 2	Reading 3	
Fresh	10.3	10.4	10.4	± 0.05
1 month	9.8	9.9	9.8	± 0.06
2 month	9.6	9.7	9.6	± 0.06
3 month	9.3	9.3	9.4	± 0.06

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