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Ultrasonic emulsification of parenteral valproic acid-loaded nanoemulsion with response surface methodology and evaluation of its stability

Suk Fei Tan [a], Hamid Reza Fard Masoumi [b], Roghayeh Abedi Karjiban [b], Johnson Stanslas [c], Brian P. Kirby [d], Mahiran Basri [b], Hamidon Bin Basri [a,*]

[a] Neuroscience Cluster, Department of Medicine, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Nanodelivery Group, Department of Chemistry, Faculty of Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[c] Pharmacotherapeutic Unit, Department of Medicine, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[d] School of Pharmacy, Royal College of Surgeons in Ireland, 123 St Stephens Green, Dublin 2, Ireland

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ABSTRACT

Response surface methodology (RSM) was used to optimize the formulation of a nanoemulsion for central delivery following parenteral administration. A mixture of medium-chain triglyceride (MCT) and safflower seed oil (SSO) was determined as a sole phase from the emulsification properties. Similarly, a natural surfactant (lecithin) and non-ionic surfactant (Tween 80) (ratio 1:2) were used in the formulation. A central composite design (CCD) with three-factor at five-levels was used to optimize the processing method of high energy ultrasonicator. Effects of pre-sonication ultrasonic intensity (A), sonication time (B), and temperature (C) were studied on the preparation of nanoemulsion loaded with valproic acid. Influence of the aforementioned specifically the effects of the ultrasonic processing parameters on droplet size and polydispersity index were investigated. From the analysis, it was found that the interaction between ultrasonic intensity and sonication time was the most influential factor on the droplet size of nanoemulsion formulated. Ultrasonic intensity (A) significantly affects the polydispersity index value. With this optimization method, a favorable droplet size of a nanoemulsion with reasonable polydispersity index was able to be formulated within a short sonication time. A valproic acid loaded nanoemulsion can be obtained with 60% power intensity for 15 min at 60 °C. Droplet size of 43.21 ± 0.11 nm with polydispersity index of 0.211 were produced. The drug content was then increased to 1.5%. Stability study of nanoemulsion containing 1.5% of valproic acid had a good stability as there are no significant changes in physicochemical aspects such as droplet size and polydispersity index. With the characteristisation study of pH, viscosity, transmission electron microscope (TEM) and stability assessment study the formulated nanoemulsion has the potential to penetrate blood–brain barrier in the treatment of epilepsy.

1. Introduction

One of the primary concerns in healthcare practice is to treat disease effectively with a minimal incidence of side effect. The main reason why most drug candidates failed in drug discovery pipeline is because of the drug toxicity issues [1]. It is understood that the occurrence of adverse side effects is due to a non-specific *in vivo* distribution nature of the drug. Valproic acid is a drug used for therapy of epilepsy, schizoaffective and bipolar disorder. It is also best known as a major antiepileptic drug for generalized and partial seizures in adults and children [2]. Despite its wide spectrum usage, valproate is also well known for its idiopathic fatal side effects such as hepatotoxicity and teratogenicity [3]. This has limited its use particularly in infant patients and pregnant patients. In addition, valproic acid has a low brain "penetrability" compared to other anti-epileptic drug. This is because valproic acid has a high plasma protein binding and it is ionized at physiological pH 7.4 [4]. In order to achieve therapeutic effect, high doses of valproic acid were required in cerebrospinal fluid (CSF) and brain. Side effects such as weight gain, hair loss, nystagmus, and tremor are prevalent in patient who uses the drug [4]. Toxicity problems arise often due to valproate metabolites which result from oxidative metabolism [5]. The non-specific *in vivo* distribution nature of the drug further aggravates the toxicity profile as it increases the incidence of oxidative metabolism before the drug has reached the therapeutic site. This has dampened the efficacy of the drug as only insignificant amount of drug are able to reaches the site.

* Corresponding author.
E-mail address: hamidon@upm.edu.my (H.B. Basri).

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Over the past decade, nanotechnologies was applied in various aspects such as cosmetic, food technology, pharmaceutical and agricultural pesticide [6–8]. In pharmaceutics discipline, many nanocarriers such as nanoparticles, solid lipid nanoemulsions, nanocapsules and nanoemulsions were designed to encapsulate interested biologically active drug. These nanocarriers encapsulating drugs were intended for treatment with controlled released and targeting properties involving different routes such as oral, parenteral, intranasal, and transdermal [9]. Nanoemulsion is a heterogeneous system in which an immiscible solution is made up of oil and an aqueous phase with either one was made to disperse into another phase. Unlike microemulsion, nanoemulsion are kinetically stable with transparent appearance and a droplet size range between 20 and 200 nm [10]. Nanoemulsion is able to dissolve large amount of lipophilic drug. On top of that, it is able to reduce the degradation of drug by enzyme [11]. In this study, nanoemulsion was used as a nanocarrier for valproic acid which hopefully able to reduce the clearance rate in biological study later.

Formulation of nanoemulsion requires high energy input as energy was dissipated into a large surface area of nanoemulsion during emulsification process [12]. Ultrasound horn system was suggested to be a better alternative processor than the microfluidizer since it is able to give an identical result with less energy input [13]. Other study shows ultrasound emulsification is more superior in terms of droplet size and energy efficient [14]. In despite of its low scale production, there are an increment in use of it in pharmaceutical research and industry [14,15]. Ultrasonic horn was used in this study as it was able to produce smaller nanoemulsion compared to ultrasonic bath [16]. It was found that increment in energy intensity and sonication time can help in the emulsification properties [17]. However excess of energy in ultrasound emulsification might cause instability too as droplets in nanoemulsion can collide and form a larger droplet [18]. Temperature plays an important factor in emulsification as it influences the viscosity of the oil phase. Lower viscosity of oil will ease the emulsification process, thus the oil phase is able to be dispersed evenly and efficiently [19].

Response surface methodology is one of the multivariate statistical techniques that was used in the past for various optimization studies [20]. It involves mathematical models and statistical techniques which are able to determine the relationship and interaction of independent variables on the response variables [21]. Also, less number of experiments were needed with RSM study [22]. In this study, RSM was employed to investigate the optimal processing conditions at low composition of surfactant in valproic acid-encapsulated (VANE). Central composite design was used to study effects of three independent processing variables including power intensity, sonication time, and temperature on the two dependant variables (outcome), namely droplet size and polydispersity index value (PDI). This design allows the development of nanoemulsion to be completed in a reduced number of experiments with desirable outcome under optimal condition. Response surface methodology was used in development of central nervous system (CNS) drug such as chloramphenicol-encapsulated nanoemulsion and levodopa-encapsulated nanoemulsion [23,24]. The ultimate goal of this study is to produce a solvent-free, biocompatible parenteral formulation for the treatment of epilepsy using sonication method.

2. Materials and methods

2.1. Materials

Ultrapure water used in the preparation of all reagents and formulation in the experiment were obtained from a Milli-Q® Plus apparatus, Millipore, Billerica, USA. Tween 80, safflower seed oil, valproic acid (2-propyl pentanoic acid), phosphate buffered saline tablet (PBS), sodium hydroxide (NaOH), and MTT formazan powder were purchased from Sigma–Aldrich Chemie GmbH, Germany. Medium chain triglyceride was obtained from Huls, Germany. Pure soy bean lecithin (Lipoid S75) was purchased from Lipoid GmbH, Ludwigshafen-Germany. Xylitol was obtained from Merck Company, Darmstadt, Germany. HPLC grade of acetonitrile was from J.T.Baker®, USA and phosphoric acid was from Mallinckdt, USA.

2.2. Selection of oil phase

The oil excipients were selected based on the emulsification properties from different types of oil. Other than complex oil, linoleic acid, the only fatty acid oil was also used in the screening of oil phase. Droplet sizes of each of the formulated nanoemulsion from different oil sources were obtained from ZetaSizer NanoZS (Malvern Instr., Malvern, UK).

2.3. Formulation of valproic acid-based nanoemulsion

Valproic acid-based nanoemulsion (VANE) were formulated with medium chain triglyceride and safflower seed oil at 20:80 ratio (MS) as disperse phase and deionized water as continuous phase. 0.5% (w/w) valproic acid was first dissolved into 2% (w/w) MS following by 1% (w/w) lecithin with magnetic stirring, at $1.34 \times g$, 40 °C. This resulting oil phase was then added into aqueous phase containing 2% Tween 80. This coarse emulsion was then further subjected to ultrasonic dispersion by 24 kHz ultrasonic tip processor (UP400S); (Hielscher Ultrasonics GmbH, Teltow, Germany) with a maximum power output of 400 W. The total alpha-tocopherol in this final nanoemulsion was 0.25% (w/w). The ultrasonic processing was stopped for 5 min after each 5 min run. The temperature of the nanoemulsion were monitored by a thermometer and regulated by ice-water bath.

2.4. Experimental design

A three-factor central composite design was used to optimize the ultrasonic processing method including the energy intensity (30–90%, A), sonication time (5–15 min, B), and temperature (40–60 °C, C). Each of the effect of these three parameters was determined on two response variables, namely average droplet size (Y_1) and polydispersity index (Y_2) of VANE. Twenty experimental runs based on the CCD were generated by the Design-Expert® version 6.0.6 software (Stat-Ease Inc., Minneapolis, USA). Three independent variables were run at three levels for each of the individual variable. Therefore this design involves 8 factorial points, 6 axial points and 6 replicates of center points. The central composite design allows us to study the effect of each variables and the interaction between variables on the outcomes independently. All the independent variables and the levels code used are as described in Table 1. The repeatability of the optimal condition model was tested by repeating the center point for six times.

Table 1
Experimental designed established with 3-level-3-factor central composite design (CCD).

Variable	Unit	Coded levels				
Independent variables		−1	0	1	Axial (−α)	Axial (+α)
Ultrasonic intensity (A)	%	30	60	90	20	100
Sonication time (B)	min	5	10	15	3.33	16.67
Temperature (C)	°C	40	50	60	36.67	63.33