Pharmaciana
Vol.14, No.3, Nov 2024, Page. 356-369
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v14i3.28892

Optimization of self-nanoemulsifying drug delivery system of rifampicin for nebulization using cinnamon oil as oil phase

I Komang Aan Adi Ricardo, Dewa Ayu Arimurni*
Sekolah Tinggi Farmasi Mahaganesha
Jl. Tukad Barito Timur no. 57, Renon, Denpasar-Bali, Indonesia

Submitted: 28-03-2024 ***Reviewed: 13-04-2024*** ***Accepted: 02-08-2024***

ABSTRACT

Lung delivery can overcome the problems related to the effectiveness of tuberculosis treatment by increasing the drug concentration at the target site. Rifampicin as the first-line antibiotic for tuberculosis has low water solubility and is unstable in gastric which hinders its effectiveness. Self-nanoemulsifying drug delivery system (SNEDDS) is a strategy known to improve the solubility and stability of such drugs. This study aimed to obtain the optimum formula of rifampicin SNEDDS intended for lung nebulization using essential oil as an oil phase. Several essential oils are known to have effective antibacterial on *Mycobacterium tuberculosis*. However, a high capability to solubilize the drug is required for SNEDDS formulation. Cinnamon oil, tween 80, and transcutol P were chosen as SNEDDS components for optimization using a D-optimal mixture based on the physicochemical characteristics. The optimum formula comprised 12.65% cinnamon oil, 75.00% tween 80, and 12.35% transcutol P which dispersed easily to form a highly transparent emulsion in normal saline under 1 minute. Upon dilution with saline, the optimal SNEDDS can produce a homogenous nanometer droplet (169.2±19.771 nm, PDI of 0.258±0.070) with acceptable pH for lung administration. It also has a viscosity similar to water (0.94±0.01 cP) which allows it to be nebulized easily (aerosol output rate of 0.14±0.02 g/min). Although the diluted SNEDDS has a zeta potential of -2.533±0.268 mV, it was stable for up to 4 hours during the nebulization. These results indicate the potential of cinnamon oil-based rifampicin SNEDDS to be an alternative in the pulmonary delivery of rifampicin via nebulization.

Keywords: SNEDDS, rifampicin, cinnamon oil, nebulizer, D-optimal mixture design

Corresponding author:
Dewa Ayu Arimurni
Sekolah Tinggi Farmasi Mahaganesha
Jl. Tukad Barito Timur no. 57, Renon, Denpasar-Bali, Indonesia
Email: dewaayuarimurni@gmail.com

INTRODUCTION

Tuberculosis is a disease caused by *Mycobacterium tuberculosis* that mainly affects the lungs (Kemenkes RI, 2020). According to the Global Tuberculosis Report 2023, tuberculosis was the world's second-highest cause of death from a single infectious agent in 2022. There were 7.5 million new tuberculosis cases diagnosed and reported, with the total number of deaths reaching 1.30 million cases (WHO, 2023). Tuberculosis is a curable disease but can be fatal if not treated properly. Nowadays, the recommended treatment for pulmonary tuberculosis includes the daily oral administration of four first-line drugs (rifampicin, isoniazid, pyrazinamide, and ethambutol) for two months in the initial phase, followed by rifampicin and isoniazid for four months in the continuation phase (Kemenkes RI, 2020). Nevertheless, the efficacy of these regimens is restricted by several issues, such as constraints of drug dose, undesirable drug effects, and low patient compliance from the lengthy treatment duration that can cause multi-drug-resistant tuberculosis (Shah et al., 2017).

Rifampicin, a semisynthetic derivative of rifamycin, has a bactericidal activity on mycobacteria by inhibiting RNA synthesis (Katzung, 2018). Although rifampicin is a first-line drug for tuberculosis treatment, high oral dosages of rifampicin may be restricted due to the increased risk of systemic toxicities such as hepatic toxicity and gastrointestinal discomforts (Somasundaram et al., 2014). Delivering rifampicin directly to the lungs is one of the promising approaches to improve the effectiveness of anti-tubercular therapy with a smaller dose and to reduce resistance by maximizing drug concentration at the target site (Khadka et al., 2023). The lung provides a vast surface area, which when paired with small particles/droplets from an inhaled aerosol results in fast systemic absorption. The lung has a lesser amount of efflux transporters and lower enzymatic activity than the oral route which increases stability and concentration of the drug in the target site. There have been reports of several types of drug delivery systems for rifampicin inhalation, including microparticle systems (Parikh et al., 2014), micellar systems (Grotz et al., 2019), and solid lipid nanoparticles (SLN) (Maretti et al., 2019). However, more research is required before the clinical application.

According to the AARC (2023), three types of aerosol generators are commonly used for inhaled drug delivery which include nebulizers and pressurized metered-dose inhalers (pMDI) to deliver liquid medications, and dry powder inhalers (DPI) for micronized powder medications. pMDI requires the patient's ability to use this type of inhaler and improper operation reduces the effectiveness of drug therapy. Meanwhile, dry powder formulations require high energy to produce and sometimes face problems such as poor powder flowability and high agglomeration due to high cohesivity between particles leading to poor aerosolization (Shah et al., 2017). Unlike pMDI, a nebulizer does not require active cooperation from the patient, although it takes a long time to administer the drug due to the higher amount/volume of drug that needs to be administered. The drug delivered via nebulization could be in a dispersed form such as suspension or solution that is easier to produce (Wang et al., 2024).

Based on the Biopharmaceutics Classification System (BCS), rifampicin is classified into BCS Class II, which means the drug has high permeability and low solubility (Tsume et al., 2014). One of the strategies that can be used to improve the solubility of rifampicin is to formulate it into a self-nano emulsifying drug delivery system (SNEDDS) dosage form. SNEDDS is a mixture of oil, surfactant, co-surfactant, and drugs that form oil-in-water nanoemulsion under gentle agitation followed by the addition of aqueous media (Divate et al., 2021). In addition to enhancing the solubility of hydrophobic drugs, SNEDDS can also improve the physical and chemical stability of the preparation as it is formulated without water, making it suitable for rifampicin which is unstable to water (Priani et al., 2017). To make it suitable for nebulization, SNEDDS can be diluted/reconstituted using an aqueous vehicle such as water for injection or normal saline to produce nanoemulsion. Nanoemulsion produced by SNEDDS is known to have a superior inhalation and aerosolization performance than other formulations such as nanoparticles, solid lipid nanoparticles, liposomes, and micelles due to its similar viscosity to water (Elbardisy et al., 2022). Therefore, SNEDDS was chosen as a delivery system to deliver rifampicin via nebulization to the lungs. Oil is a key component in SNEDDS. The oil phase contributes to the increased solubility of hydrophobic drugs. Furthermore, several oils are known a high antibacterial effect on *Mycobacterium tuberculosis* such as cinnamon oil, clove oil, and peppermint oil

(Gautam et al., 2023; Sawicki et al., 2018; Vidya Raj et al., 2022). Therefore, selecting oil components that have a high capability to solubilize rifampicin is important. The oil phase also influenced other SNEDDS component's selection. In recent years, the D-optimal mixture design has emerged as a preferred statistical experimental design and modeling method in pharmaceutical formulation studies. This method was applied when the independent variables are the components of a mixture, and the dependent variable is the function of the proportion of each component. D-optimal mixture design allows investigation of the interrelations between the mixture components and response with the least number of experiments (Carneiro et al., 2020). Therefore, components of SNEDDS need to be screened and optimized by a D-optimal mixture design approach to reduce the cost, unpredictability result, and time-consuming process (Sopyan et al., 2022).

Based on the background above, SNEDDS is likely an ideal way to improve the pharmacological effect of rifampicin. This study aimed to obtain the optimum formula of rifampicin SNEDDS targeted to lungs using various concentrations of selected essential oil, surfactant, and co-surfactant by applying the D-optimal mixture design and evaluating its physicochemical characteristics for lung administration.

MATERIALS AND METHODS

Materials

Rifampicin was purchased from PT. Phapros Tbk, cinnamon oil, clove oil, and peppermint oil were purchased from PT. Darjeeling Sembrani Aroma, tween 80 (Brataco), transcutol P (Moellhausen), normal saline (Otsuka), aquadest (Brataco), methanol p.a (Merck).

Methods

Solubility study of rifampicin

Saturation solubility of rifampicin was tested in several oils, including cinnamon oil, clove oil, and peppermint oil. An excess quantity of rifampicin was put into a microtube that each contained 1 mL of different oil. The mixtures were vortexed using a vortex mixer (OHAUS) and sonicated with a water bath sonicator (GT SONIC) for 10 minutes each to ensure appropriate mixing and solubilization of the rifampicin in the oil. Then, mixtures were centrifuged at 6,000 rpm for 10 minutes to separate the undissolved drug using a mini centrifuge (OHAUS Frontier 5306). Aliquots of supernatant were diluted with methanol and rifampicin dissolved in oils was quantified with a UV-visible spectrophotometer (Thermofisher Genesys 10) at 475 nm. Meanwhile, the surfactant (tween 80) and co-surfactant (transcutol P) employed in this study are chosen based on previous research conducted by (Mantena et al., 2015).

GC-MS analysis of selected oil

The volatile components of the selected oil were analyzed by GC-MS (Shimadzu QP2010SE with GC-2020 plus) equipped with InertCap 5MS/Sil column (30 m x 0.32 mm I.D., 0.5 µm) after being diluted to 1% acetone. The operating conditions were helium as the carrier gas, 3 mL/min of flow rate, 1:5 split ratio, 44.5 kPa pressure, and 200℃ ion source temperature. Column temperature was programmed at 50℃ for 5 minutes, increased to 250℃ at a rate of 10℃/min, and maintained for 10 minutes. The mass range was set from 45-500 amu!.

Optimization of rifampicin SNEDDS

Formula optimization was performed by entering the lower and upper limit values of rifampicin SNEDDS composition as shown in Table 1. Lower and upper limit values of the factors were determined by preliminary experiments (data was not shown). Values of other experimental conditions such as the amount of rifampicin, stirring rate, stirring type, and sonication time were kept constant. D-optimal mixture design was then used to analyze the effect of each composition on a physicochemical characteristic of the resulted SNEDDS such as emulsification time, percent transmittance, viscosity, pH, and aerosol output rate in Design Expert® Version 13 software.

Table 1. The lower and upper limit of rifampicin SNEDDS composition

Composition	Lower limit (%)	Upper limit (%)
Cinnamon oil	5	20
Tween 80	75	90
Transcutol P	5	20

Preparation of rifampicin SNEDDS

Oil phase, surfactant, co-surfactant, and drug (rifampicin 100 mg/mL) were mixed with a magnetic stirrer (IKA C-MAG HS 7) at 1,200 rpm for 90 minutes and sonicated for 15 minutes. The mixtures were allowed to stand at room temperature for 24 hours to reach equilibrium.

SNEDDS characterizations

Organoleptic

The organoleptic test for rifampicin SNEDDS preparation includes observation of the color, smell, and the presence or absence of phase separation (Aisy et al., 2021).

Emulsification time

An amount of 1 mL of the SNEDDS was diluted with 100 mL of normal saline (1:100 dilution) under continuous stirring (100 rpm) at ambient temperature. The time required to form a nanoemulsion (a clear-transparent mixture) was expressed as the emulsification time (Winarti, 2016).

Percent transmittance

The SNEDDS mixture was diluted 1:50 with normal saline using a magnetic stirrer. The percent transmittance was measured at 650 nm using a UV-visible spectrophotometer (Thermofisher Genesys 10) with normal saline as the blank (Sahumena et al., 2019).

Viscosity

The rifampicin SNEDDS formula was diluted at a 1:100 ratio with normal saline. The nanoemulsion formed was evaluated using an Ostwald viscometer at 25℃. Record the time it takes for the emulsion to pass the first and second lines (Fithri et al., 2017).

pH

Initially, the sample was diluted 100 times with normal saline to form a nanoemulsion. The pH meter (HANNA Hi 2211) electrodes were then inserted into the emulsion, and the resulting value was recorded (Annisa et al., 2023).

Aerosol output rate

The medicine compartment of the OneMed nebulizer 405A was loaded with five milliliters of nanoemulsion made from 1:100 dilution of SNEDDS with normal saline. The aerosol output rate (g/min) was measured by dividing the weight (gram) of the drug nebulized by the nebulization time using equation 1 (Shah et al., 2017). $W_{initial}$ is the weight of the nebulizer cup containing the sample before nebulization, W_{left} is the weight of the nebulizer cup after nebulization, and t is the time needed to nebulize the sample to dryness (Asmawi et al., 2023).

$$\text{aerosol output rate (g/min)} = \frac{[(\text{Winitial} - \text{Wleft})]}{(\text{t})} \text{ x } 100 \qquad (1)$$

Globule size and zeta potential analysis

The rifampicin SNEDDS optimum formula was diluted at a 1:100 scale with normal saline and then put into a particle size analyzer/PSA (Malvern zetasizer nano ZS series). The evaluation was conducted at a temperature of 25℃ (Shah et al., 2017).

Centrifugation test

The optimum formula was diluted 1:100 with normal saline then centrifuged at 6,000 rpm for 30 minutes. Signs of drug precipitation and phase separation were visually evaluated (Rehman et al., 2022).

Freeze-thaw test

The optimum formula was kept under freezing temperature for 24 hours, then thawed at room temperature for 24 hours (1 cycle). After 6 cycles, the formula is tested for significant changes in physicochemical parameters (emulsification time, percent transmittance, viscosity, pH, and aerosol output rate) and compared with the condition before the freeze-thaw cycle (Hajrin et al., 2024).

Nanoemulsion stability test

The optimum formula was reconstituted by diluting with normal saline at a ratio of 1:50 and then stored at a cold temperature. The nanoemulsion was then observed every 1 hour for any sign of phase separation and its percent transmittance.

Data Analysis

The D-optimal mixture design approach was employed to obtain the optimum concentration of selected oil phase, surfactant, and co-surfactant as independent variables in rifampicin SNEDDS formulation on dependent variables or responses including emulsification time, percent transmittance, viscosity, pH, and aerosol output rate. The correlation between each SNEDDS composition and the response will be explained by the best-fitted model given by the software. Design Expert also displays the mathematical equation of the model to describe the contribution of each SNEDDS composition to the response. Furthermore, the composition of the selected oil, surfactant, and co-surfactant in the optimized formula was determined through numerical optimization. In this method, a desired goal was set for each dependent variable (in range, minimize, maximize, or targeted). Then, the optimization option was utilized to choose the optimal formula determined by the desirability value. The solution with the highest desirability value is selected as the optimum formula (Beandrade, 2018). A paired sample t-test was performed to analyze the mean difference of each response before and after the freeze-thaw test. If the p-value <0.05, the data were significantly different (Purnomo & Syamsul, 2017).

RESULT AND DISCUSSION

Solubility study of rifampicin

Drug solubility in oil, surfactant, and co-surfactant is an important aspect of SNEDDS formulation. The solubility of rifampicin in several oils is presented in Figure 1. Oil with a higher drug solubilization capacity has a greater potential for drug loading. In this study, rifampicin shows the highest solubility in cinnamon oil (176.56±10.65 mg/mL) compared to other oils, thus cinnamon oil was chosen as the oil phase. According to the research conducted by Mantena et al. (2015) rifampicin has a higher solubility in tween 80 (86.84±1.84 mg/mL) and transcutol P (80.46±2.93 mg/mL), among other surfactants and co-surfactants used. Therefore, cinnamon oil, tween 80, and transcutol P were selected for further study.

Identification of oil components

Cinnamon oil, the selected oil phase in this study was analyzed for its chemical compounds using gas chromatography-mass spectrometry (GC-MS). In our research, the main compounds of cinnamon oil were cinnamaldehyde (73.82%), caryophyllene (16.64%), and p-Cymene (5.04%). These compounds exhibit anti-*Mycobacterium tuberculosis* action, with the minimum inhibitory concentration (MIC) value of cinnamaldehyde (3.12 µg/mL), caryophyllene (100.00 µg/mL), and p-Cymene (91.66 µg/mL) (Andrade-Ochoa et al., 2015).

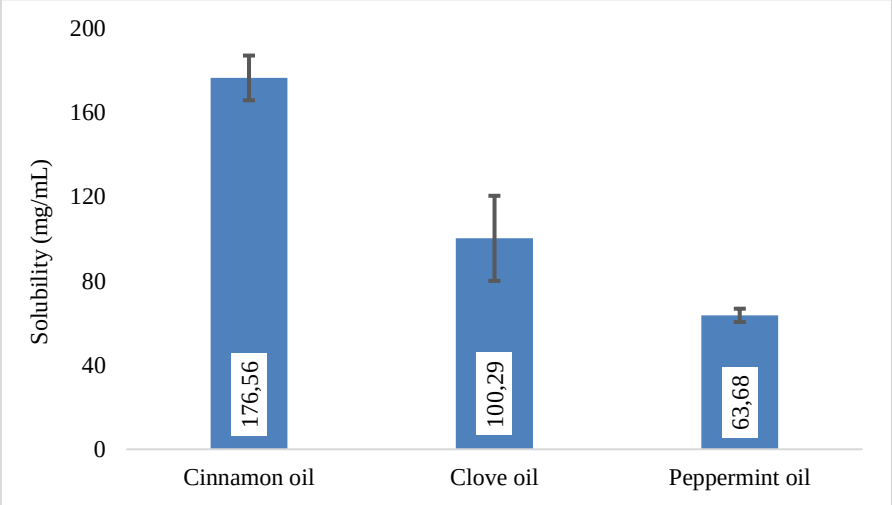
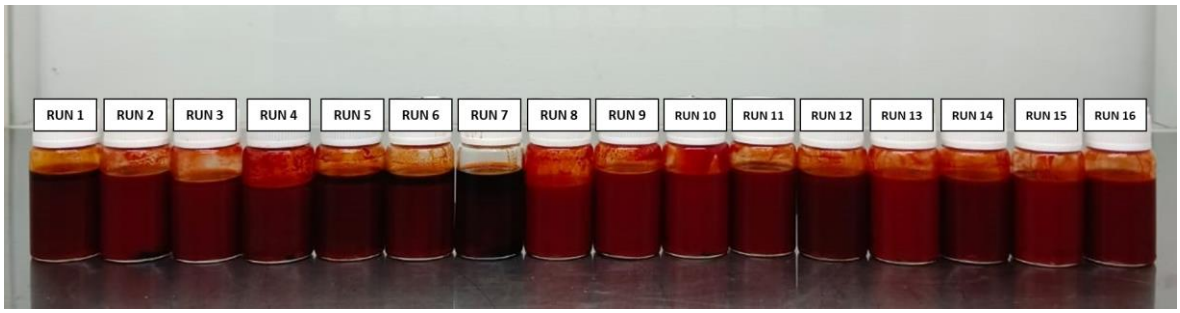


Figure 1. The solubility of rifampicin in several oils

SNEDDS characterizations

The rifampicin SNEDDS composition of each experimental run is displayed in Table 2. Under the D-optimal mixture design, a total of 16 experimental runs were to be performed (with 5 replication points and 5 lack-of-fits points). The model was built using Design Expert® Version 13 software. Figure 2 shows that all experimental runs produced a red-brown, distinctive aroma of rifampicin SNEDDS, and there is an absence of phase separation.



Run 1 (11.25% CIN : 81.25% T80 : 7.5% TC), Run 2 (12.5% CIN : 75% T80 : 12.5% TC), Run 3=Run 10 (5% CIN : 75% T80 : 20% TC), Run 4=Run 6 (5% CIN : 90% T80 : 5% TC), Run 5=Run 12 (20% CIN : 75% T80 : 5% TC), Run 7=Run 13 (5% CIN : 82.5% T80 : 12.5% TC), Run 8=Run 14 (12.5% CIN : 82.5% T80 : 5% TC), Run 9 (15% CIN : 77.5% T80 : 7.5% TC), Run 11 (11.25% CIN : 77.5% T80 : 11.25% TC), Run 15 (7.5% CIN : 77.5% T80 : 15% TC), Run 16 (7.5% CIN : 85% T80 : 7.5% TC)
CIN= cinnamon oil, T80= tween 80, TC= transcutol P

Figure 2. The visual appearance of rifampicin SNEDDS

As presented in Table 2 the emulsification times of all experimental runs ranged from 37.33 to 49.33 seconds. This indicates that the prepared SNEDDS can be emulsified spontaneously and meet the criteria for a good SNEDDS (emulsification time <1 minute) (Reddy & Gubbiyappa, 2022). Percent transmittance refers to the quantity of light that can pass through a material represented as a percentage. This parameter can indicate whether the dispersed droplets are in the nano range or not (Nuari et al., 2021; Wiwiek et al., 2017). If the droplet size gets smaller, the percent transmittance value will be higher (close to 100%), and vice versa. In this study, the percent transmittance value of all formulations ranged

from 18.09 to 92.14%, this suggests that not all the droplets of the resulting SNEDDS formulations have reached nanosized. Viscosity is one of the aspects that influence the nebulizer output. The higher the viscosity of drugs, the flow rate would decrease (Hailu et al., 2020). The rifampicin SNEDDS formulation has a viscosity ranging from 0.93 to 0.95 cP at a temperature of 25℃ indicating good flowability and suitability for pulmonary administration (viscosity <3.9 cP) (Arbain et al., 2018). The pH is a crucial factor in determining the tolerance of nebulized drugs. All formulations met the criteria for lung administration (pH 4.5-8.7), with pH values ranging from 5.69 to 6.40 (Shah et al., 2017). The aerosol output rate is the mass rate of drugs exiting the aerosol generator. This is an important parameter to ensure therapeutic comfort in nebulization (Shah et al., 2017). The higher the aerosol output rate value, the less time is required for nebulization. In this study, the aerosol output rate value of all formulations ranged from 0.12 to 0.15 g/min.

Table 2. D-optimal mixture design runs for rifampicin SNEDDS composition and physicochemical characteristic result

RUN	SNEDDS compositions			Characteristics				
	CIN (%)	T80 (%)	TC (%)	ET (sec)	%T (%)	Viscosity (cP)	pH	AOR (g/min)
1	11.25	81.25	7.5	49.00	65.72	0.95	6.40	0.14
2	12.5	75	12.5	40.00	87.06	0.93	6.02	0.15
3	5	75	20	37.33	37.25	0.93	5.81	0.14
4	5	90	5	49.33	89.95	0.93	5.92	0.13
5	20	75	5	46.67	89.02	0.93	5.69	0.13
6	5	90	5	44.33	88.35	0.93	5.96	0.13
7	5	82.5	12.5	43.00	87.79	0.93	5.83	0.13
8	12.5	82.5	5	48.00	91.92	0.93	5.83	0.12
9	15	77.5	7.5	43.67	80.44	0.93	5.95	0.15
10	5	75	20	38.67	18.09	0.93	6.01	0.14
11	11.25	77.5	11.25	45.00	52.88	0.93	5.99	0.15
12	20	75	5	46.67	92.14	0.94	5.81	0.15
13	5	82.5	12.5	46.67	72.62	0.93	6.14	0.14
14	12.5	82.5	5	39.67	77.05	0.93	5.82	0.13
15	7.5	77.5	15	40.33	76.65	0.94	5.87	0.15
16	7.5	85	7.5	43.00	51.03	0.93	5.71	0.13

CIN= cinnamon oil, T80= tween 80, TC= transcutol P, ET= emulsification time, %T= percent transmittance, AOR= aerosol output rate

Design Expert® Version 13 software was used to analyze the resulting data in Table 2 by choosing a suitable regression model for each response (emulsification time, percent transmittance, viscosity, pH, and aerosol output rate) to describe the influence of each SNEDDS composition. Table 3 shows that the best-fitting models for explaining the influence of independent variables on observed responses are the linear model for emulsification time, the special cubic model for percent transmittance, and the quadratic model for aerosol output rate due to the model's p-value <0.05 (significant), and non-significant lack of fit (p>0.05) (Akbar et al., 2022; Astuti et al., 2017). Meanwhile, no model in the software can explain the relationship between the SNEDDS composition on viscosity and pH. This indicates that the viscosity and pH parameters on rifampicin SNEDDS are not influenced by variations in the independent variables. A mathematical equation that explains the quantitative effects of each SNEDDS composition and their interaction on the observed response is presented in Table 3. The positive sign of the coefficient represents the synergistic effect, whereas the negative sign represents an antagonistic effect of independent variables on the dependent variables. The larger the coefficient value of the factor suggests the more influential the factor is toward the response, and vice versa (Yadav et al., 2020).

Numerical optimization of rifampicin SNEDDS

Based on the ideal criteria of rifampicin SNEDDS formulation targeted to the lungs, the optimal formula should be able to quickly form nanoemulsions with a clear appearance, have a good flow property, pH ranging from 4.5 to 8.7, and have a high aerosol output rate. Therefore, to optimize the composition of cinnamon oil, tween 80, and transcutol P, the goals for percent transmittance and aerosol output rate were set to maximize, the pH was set to in range (5.69-6.40), while emulsification time and viscosity were set to minimize. Based on these criteria, Design Expert® Version 13 creates three solution formulas. The solution with the highest desirability value (0.856) which contained 12.65% cinnamon oil, 75.00% tween 80, and 12.35% transcutol P was selected as the optimum formula and will be used for further investigation. Three replicates of the optimum formula were prepared and characterized similarly to the previous 16 experimental runs with the addition of other physicochemical characteristics tests including globule size, zeta potential, and stability studies. Then, the optimum formula was confirmed by comparing the predicted values provided by the software with the actual values.

Table 3. Determination of the mixture model and mathematical equation generated by Design Expert®

Response (Y)	Mixture model	Mathematical equation	Lack of fit
Emulsification time	Linear (p=0.0130)	Y=0.420699A+0.498805B-0.040405C	Not significant (p=0.7330)
Percent transmittance	Special cubic (p=0.0095)	Y=-96.06273A-1.01839B-136.99257C +1.35201AB+22.34251AC+1.84426BC -0.290683ABC	Not significant (p=0.1188)
Aerosol output rate	Quadratic (p=0.0244)	Y=0.009406A+0.001392B-0.002607C -0.000122AB+0.000295AC+0.000038BC	Not significant (p=0.7451)
Viscosity		N/A*	
pH		N/A*	

Cinnamon oil (A), Tween 80 (B), Transcutol P (C), *N/A= not available

Table 4 showed that the actual values for all responses were within the 95% prediction interval (PI) range, suggesting that the software can reliably predict the values of all responses in the optimum formula. The optimum formula produced SNEDDS with good characteristics includes forming nanoemulsion spontaneously with a clear appearance, having a pH within the range of pH values for nebulized drugs, and easily coming out of the aerosol generator due to its low viscosity. In SNEDDS formulation, the emulsion droplet size is an important factor. A smaller droplet size is favored because it provides a larger surface area for the absorption of drugs (Avachat & Patel, 2015).

As shown in Table 5, the rifampicin SNEDDS optimum formula had a droplet size of 169.2±19.771 nm, meeting the SNEDDS formulation criteria of <200 nm (Fitria et al., 2021). In addition, this particle size also meets the criteria for drug delivery to reach the pulmonary alveolus, where *Mycobacterium tuberculosis* resides (Costa et al., 2016). Polydispersity index (PDI) is a homogeneity parameter of particle size with a value range between 0.0 and 1.0. The smaller the PDI value, the more homogeneous the droplet size in a formulation, and vice versa. The optimum formula had a PDI value of 0.258±0.070 which indicates that the formula is monodispersed (PDI value ≤0.5) (Darusman et al., 2023). Zeta potential is a crucial aspect in determining the stability of SNEDDS. A good zeta potential value for SNEDDS formulation is higher than ±30 mV. In this system, the repulsive force between particles is high, preventing particle aggregation (Syukri et al., 2020). The optimum formula had a zeta potential value of -2.533±0.268 mV. A negative value may be due to hydroxyl groups present in the structure of

the surfactant and cosurfactant used in this study. These negatively charged particles can avoid the mucus layer interaction (biological barrier for pulmonary drug delivery) because the mucus has a negative charge, while positively charged particles will interact with mucus, be entrapped then cleared by the mucociliary clearance mechanism. Thus positively charged particles may have a lower deposition in the deeper lung area (Vu et al., 2024). To be deposited in the lower part of the lungs (alveolar and terminal bronchiolus) which has no mucus, the repulsion from mucus is needed to change the airway direction of the aerosolized particles, making it easier to reach the deeper lung and deposited under sedimentation/Brownian motion mechanism (Majid et al., 2012). Low zeta potential values can result from normal saline that is used to dilute the SNEDDS in this study. Normal saline contains many electrolytes that can disrupt the surface charge of SNEDDS droplets. This occurs because counter ions in the diffusion layer can be repelled into the inner layer, decreasing the zeta potential (Lu et al., 2018). Normal saline is often used to reconstitute nebulized solution to provide a dosage form with suitable tonicity for lung administration. Moreover, the droplets produced by SNEDDS with tween 80 as surfactant are often stabilized by the steric repulsion due to long polyoxyethylene head groups of the tween 80 molecules which cause them to have a low to zero zeta potential (Suryani et al., 2019)

Table 4. Results of model confirmation and physicochemical characteristics of the optimum formula of rifampicin SNEDDS before and after freeze-thaw test

Response	Prediction value	95% PI	Before stability test	After stability test	p-value
Emulsification time (seconds)	42.234	37.95-46.52	40.67±1.53	42.67±1.53	0.321
Percent transmittance (%)	92.100	60.42-123.77	93.11±0.25	92.51±0.02	0.055
Aerosol output rate (g/min)	0.157	0.14-0.17	0.14±0.02	0.14±0.01	0.667
Viscosity (cP)	0.932	0.93-0.94	0.94±0.01	0.93±0.02	0.423
pH	5.923	5.69-6.16	5.91±0.01	5.90±0.02	0.270

Table 5. The results of globule size and zeta potential analysis of the optimum formula

Characterization	Value
Droplet size	169.2±19.771 nm
PDI	0.258±0.070
Zeta potential	-2.533±0.268 mV

Stability is one of the important aspects to consider while developing pharmaceutical preparations, including SNEDDS. The instability of drug products could affect the therapeutic efficacy and cause a toxic effect (Pratiwi et al., 2018). Based on Table 4, no statistically significant changes were noticed in each response after the freeze-thaw stability test (p-value>0.05). These findings show that the optimum formula is thermodynamically stable. Furthermore, results from the centrifugation test showed no signs of drug precipitation and phase separation, meaning the SNEDDS is capable of producing is kinetically stable nanoemulsion.

Another parameter that can be used to evaluate the stability of reconstituted SNEDDS preparations is percent transmittance. The good percent transmittance value for nanoemulsion is >80% which shows that the size of oil droplets which encapsulate the drug is still in the nanometer range <100 nm (Syukri et al., 2020). Figure 3 showed that reconstitution of rifampicin SNEDDS with normal saline which was stored at a cold temperature was stable for up to 4 hours with no visible drug precipitation even after centrifugated at 6000 rpm for 30 minutes, although the transmittance percentage of the resulting nanoemulsion fell to 80%. However, this shows that the reconstituted SNEDDS is still safe to use, as

the duration of nebulization is generally < 30 minutes. Therefore, to ensure the effectiveness and safety of treatment, this liquid is recommended to be used immediately after reconstitution.

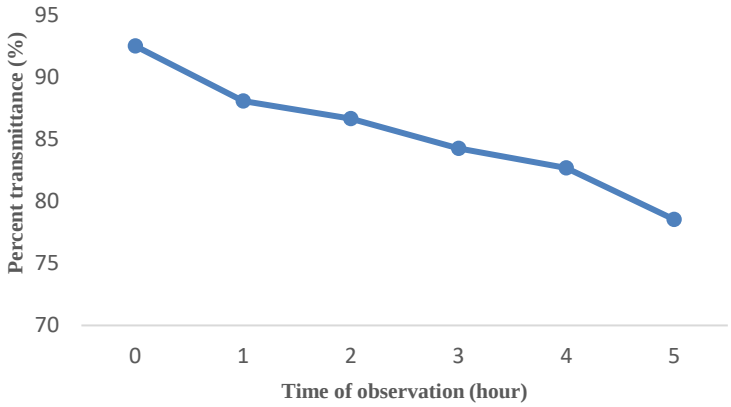


Figure 3. The stability profile of reconstituted rifampicin SNEDDS in normal saline

CONCLUSION

The optimum formula of rifampicin SNEDDS consisted of 12.65% cinnamon oil, 75.00% tween 80, and 12.35% transcutol P. This formula produces a stable preparation with good physicochemical characteristics of SNEDDS. These results indicate the potential of cinnamon oil-based rifampicin SNEDDS to be an alternative in the pulmonary delivery of rifampicin via nebulization and need to be further evaluated to prove its effectiveness.

ACKNOWLEDGEMENT

We acknowledge support by Sekolah Tinggi Farmasi Mahaganesha for providing facilities used in this study.

REFERENCES

AARC. (2023). *Pulmonary disease aerosol delivery devices: a guide for physicians, nurses, pharmacists, and other health care professionals* (4th ed.). American Association for Respiratory Care.

Aisy, Z. H. R., Puspita, O. E., & Shalas, A. F. (2021). Optimasi formula nanoemulsi nifedipin dengan metode self-nanoemulsifying drug delivery system (SNEDDS). *Pharmaceutical Journal of Indonesia*, *6*(2), 85–95. https://doi.org/10.21776/ub.pji.2021.006.02.3

Akbar, N. D., Nugroho, A. K., & Martono, S. (2022). Artikel review: optimasi formulasi SNEDDS dengan simplex lattice design dan box behnken design. *Jurnal Ilmiah Farmako Bahari*, *13*(1), 90. https://doi.org/10.52434/jfb.v13i1.1216

Andrade-Ochoa, S., Nevárez-Moorillón, G. V., Sánchez-Torres, L. E., Villanueva-García, M., Sánchez-Ramírez, B. E., Rodríguez-Valdez, L. M., & Rivera-Chavira, B. E. (2015). Quantitative structure-activity relationship of molecules constituent of different essential oils with antimycobacterial activity against Mycobacterium tuberculosis and Mycobacterium bovis. *BMC Complementary and Alternative Medicine*, *15*(1), 332. https://doi.org/10.1186/s12906-015-0858-2

Annisa, R., Mutiah, R., Yuwono, M., & Hendradi, E. (2023). The development formulation of eleutherine palmifolia extract-loaded self nanoemulsifying drug delivery system (SNEDDS) using D-optimal mixture design approach. *International Journal of Applied Pharmaceutics*, *15*(5), 269–276. https://doi.org/10.22159/ijap.2023v15i5.47645

Arbain, N. H., Basri, M., Salim, N., Wui, W. T., & Abdul Rahman, M. B. (2018). Development and characterization of aerosol nanoemulsion system encapsulating low water soluble quercetin for lung cancer treatment. *Materials Today: Proceedings*, *5*, S137–S142. https://doi.org/10.1016/j.matpr.2018.08.055

Asmawi, A. A., Salim, N., Abdulmalek, E., & Abdul Rahman, M. B. (2023). Size-controlled preparation of docetaxel- and curcumin-loaded nanoemulsions for potential pulmonary delivery. *Pharmaceutics*, *15*(2), 652. https://doi.org/10.3390/pharmaceutics15020652

Astuti, I. Y., Marchaban, M., Martien, R., & Nugroho, A. E. (2017). Design and optimization of self nano-emulsifying drug delivery system containing a new anti-inflamatory agent pentagamavunon-0. *Indonesian Journal of Chemistry*, *17*(3), 365. https://doi.org/10.22146/ijc.22640

Avachat, A. M., & Patel, V. G. (2015). Self nanoemulsifying drug delivery system of stabilized ellagic acid–phospholipid complex with improved dissolution and permeability. *Saudi Pharmaceutical Journal*, *23*(3), 276–289. https://doi.org/10.1016/j.jsps.2014.11.001

Beandrade, M. U. (2018). Formulasi dan karakterisasi SNEDDS ekstrak Jinten Hitam (Nigella Sativa) dengan fase minyak ikan Hiu Cucut botol (Centrophorus Sp) serta uji aktivitas imunostimulan. *JPSCR : Journal of Pharmaceutical Science and Clinical Research*, *3*(1), 50. https://doi.org/10.20961/jpscr.v3i1.15506

Carneiro, A. F., Carneiro, C. N., de N Pires, L., Teixeira, L. S. G., Azcarate, S. M., & de S Dias, F. (2020). D-optimal mixture design for the optimization of extraction induced by emulsion breaking for multielemental determination in edible vegetable oils by microwave-induced plasma optical emission spectrometry. *Talanta*, *219*, 121218. https://doi.org/10.1016/j.talanta.2020.121218

Costa, A., Pinheiro, M., Magalhães, J., Ribeiro, R., Seabra, V., Reis, S., & Sarmento, B. (2016). The formulation of nanomedicines for treating tuberculosis. *Advanced Drug Delivery Reviews*, *102*, 102–115. https://doi.org/10.1016/j.addr.2016.04.012

Darusman, F., Dwiatama, A., & Priani, S. E. (2023). Formulasi dan karakterisasi self-nanoemulsifying drug delivery System (SNEDDS) esomeprazol magnesium trihidrat. *Jurnal Sains Farmasi & Klinis*, *10*(1), 10. https://doi.org/10.25077/jsfk.10.1.10-20.2023

Divate, M. P., Bawkar, S. U., Chakole, R. D., & Charde, M. S. (2021). Self nano-emulsifying drug delivery system: a review. *Journal of Advanced Scientific Research*, *12*(3 Suppl 2), 1–12. https://doi.org/10.55218/JASR.s2202112301

Elbardisy, B., Boraie, N., & Galal, S. (2022). Tadalafil nanoemulsion mists for treatment of pediatric pulmonary hypertension via nebulization. *Pharmaceutics*, *14*, 2717. https://doi.org/10.3390/pharmaceutics14122717

Fithri, N. A., Mardiyanto, M., Novita, R. P., & Andrean, V. (2017). Furosemide self nano emulsifying drug delivery system (SNEDDS) formulation comprising of capryol-90, polysorbate-80, and peg-400 with simplex-lattice-design. *Science and Technology Indonesia*, *2*(4), 85–88. https://doi.org/10.26554/sti.2017.2.4.85-88

Fitria, A., Hanifah, S., Chabib, L., Uno, A. M., Munawwarah, H., Atsil, N., Pohara, H. A., Weuanggi, D. A., & Syukri, Y. (2021). Design and characterization of propolis extract loaded self-nano emulsifying drug delivery system as immunostimulant. *Saudi Pharmaceutical Journal*, *29*, 625–634.

Gautam, S., Qureshi, K. A., Jameel Pasha, S. B., Dhanasekaran, S., Aspatwar, A., Parkkila, S., Alanazi, S., Atiya, A., Khan, M. M. U., & Venugopal, D. (2023). Medicinal plants as therapeutic alternatives to combat mycobacterium tuberculosis: a comprehensive review. *Antibiotics*, *12*(3), 541. https://doi.org/10.3390/antibiotics12030541

Grotz, E., Tateosian, N. L., Salgueiro, J., Bernabeu, E., Gonzalez, L., Manca, M. L., Amiano, N., Valenti, D., Manconi, M., García, V., Moretton, M. A., & Chiappetta, D. A. (2019). Pulmonary delivery of rifampicin-loaded Soluplus Micelles against Mycobacterium tuberculosis. *Journal of Drug Delivery Science and Technology*, *53*, 101170.https://doi.org/ 10.1016/j.jddst.2019.101170

Hailu, N., Postema, M., Krejcar, O., & Assefa, D. (2020). Nebulization criteria and quantification. *Fluids*, *5*(2), 91. https://doi.org/10.3390/fluids5020091

Hajrin, W., Subaidah, W. A., & Juliantoni, Y. (2024). Formulasi dan karakterisasi nanoemulsi dari ekstrak biji buah Makasar (Brucea javanica (L) Merr). *Indonesian Journal of Pharmaceutical Science and Technology*, *11*(1), 117–125.

Katzung, B. G. (2018). *Basic & Clinical Phamacology* (14th ed.). McGraw-Hill Education

Kemenkes RI. (2020). *Pedoman Nasional Pelayanan Kedokteran Tata Laksana Tuberkulosis*. Kementerian Kesehatan Republik Indonesia

Khadka, P., Dummer, J., Hill, P. C., Katare, R., & Das, S. C. (2023). A review of formulations and preclinical studies of inhaled rifampicin for its clinical translation. *Drug Delivery and Translational Research*, *13*, 1246–1271. https://doi.org/10.1007/s13346-022-01238-y

Lu, Y., Qi, J., & Wu, W. (2018). Lipid nanoparticles: In vitro and in vivo approaches in drug delivery and targeting. In *Drug Targeting and Stimuli Sensitive Drug Delivery Systems* (pp. 749–783). Elsevier. https://doi.org/10.1016/B978-0-12-813689-8.00020-3

Majid, H., Madl, P., Hofmann, W., & Alam, K. (2012). Implementation of charged particles deposition in stochastic lung model and calculation of enhanced deposition. *Aerosol Science and Technology*, *46*(5), 547–554. https://doi.org/10.1080/02786826.2011.645957

Mantena, A. D., Mantena, A. D., & Nerella, A. (2015). Formulation, optimization and in vitro evaluation of rifampicin Nanoemulsions. *International Journal of Pharmaceutical Sciences and Drug Research*, *7*(6), 451–455.

Maretti, E., Rustichelli, C., Gualtieri, M. L., Costantino, L., Siligardi, C., Miselli, P., Buttini, F., Montecchi, M., Leo, E., Truzzi, E., & Iannuccelli, V. (2019). The impact of lipid corona on rifampicin intramacrophagic transport using inhaled solid lipid nanoparticles surface-decorated with a mannosylated surfactant. *Pharmaceutics*, *11*, 508. https://doi.org/ 10.3390/pharmaceutics11100508

Nuari, Y. R., Wahyuningsih, I., & Prabawati, S. (2021). Self-Nanoemulsifying drug delivery system (SNEDDS) of piroxicam. *Pharmaciana*, *11*(2), 185. https://doi.org/10.12928/pharmaciana.v11i2.20973

Parikh, R., Patel, L., & Dalwadi, S. (2014). Microparticles of Rifampicin: comparison of pulmonary route with oral route for drug uptake by alveolar macrophages, phagocytosis activity and toxicity study in Albino Rats. *Drug Delivery*, *21*(6), 406–411. https://doi.org/10.3109/10717544.2013.851302

Pratiwi, L., Fudholi, A., Martien, R., & Pramono, S. (2018). Physical and chemical stability test of SNEDDS (Self-nanoemulsifying Drug Delivery System) and nanoemulsion ethyl acetate fraction of garcinia mangostana L. *Majalah Obat Tradisional*, *23*(2), 84. https://doi.org/10.22146/mot.28533

Priani, S. E., Nurrayyan, & Darusman, F. (2017). Formulation self nano emulsifying drug delivery system glimepiride using oleic acid as oil phase. *Pharmaciana*, *7*(2), 267–276. http://dx.doi.org/10.12928/pharmaciana.v7i2.7387

Purnomo, H., & Syamsul, E. S. (2017). *Statistika farmasi (aplikasi praktis dengan SPSS)*.

Reddy, M. R., & Gubbiyappa, K. S. (2022). Formulation development, optimization and characterization of Pemigatinib-loaded supersaturable self-nanoemulsifying drug delivery systems. *Future Journal of Pharmaceutical Sciences*, *8*(1), 45. https://doi.org/10.1186/s43094-022-00434-4

Rehman, F. U., Farid, A., Shah, S. U., Dar, M. J., Rehman, A. U., Ahmed, N., Rashid, S. A., Shaukat, I., Shah, M., Albadrani, G. M., Kamel, M., Altyar, A. E., Abdel-Daim, M. M., & Shah, K. U. (2022). Self-emulsifying drug delivery systems (SEDDS): measuring energy dynamics to determine thermodynamic and kinetic stability. *Pharmaceuticals*, *15*(9), 1064. https://doi.org/10.3390/ph15091064

Sahumena, M. H., Suryani, S., & Rahmadani, N. (2019). Formulasi self-nanoemulsifiying drug delivery system (SNEDDS) asam mefenamat menggunakan VCO dengan kombinasi surfaktan tween dan span. *Journal Syifa Sciences and Clinical Research*, *1*(2), 37–46. https://doi.org/10.37311/jsscr.v1i2.2660

Sawicki, R., Golus, J., Przekora, A., Ludwiczuk, A., Sieniawska, E., & Ginalska, G. (2018). Antimycobacterial activity of Cinnamaldehyde in a Mycobacterium tuberculosis(H37Ra) Model. *Molecules*, *23*(9), 2381. https://doi.org/10.3390/molecules23092381

Shah, K., Chan, L. W., & Wong, T. W. (2017). Critical physicochemical and biological attributes of nanoemulsions for pulmonary delivery of rifampicin by nebulization technique in tuberculosis treatment. *Drug Delivery*, *24*(1), 1631–1647. https://doi.org/10.1080/10717544.2017.1384298

Somasundaram, S., Ram, A., & Sankaranarayanan, L. (2014). Isoniazid and Rifampicin as Therapeutic Regimen in the Current Era: A Review. *Journal of Tuberculosis Research*, *2*, 40–51. https://doi.org/10.4236/jtr.2014.21005

Sopyan, I., Gozali, D., Sriwidodo, & Guntina, R. K. (2022). Design-expert software (DOE): an application tool for optimization in pharmaceutical preparations formulation. *International Journal of Applied Pharmaceutics*, 55–63. https://doi.org/10.22159/ijap.2022v14i4.45144

Suryani, Sahumena, M. H., Alfiandi, Putrawansya, L. R. P., Mallarangeng, A. N. T. A., Aswan, M., & Ruslin. (2019). The self-nanoemulsifying Drug delivery systems formulation of mefenamic acid. *Asian Journal of Pharmaceutics*, *13*(4), 287.

Syukri, Y., Nugroho, B. H., & Istanti, I. (2020). Penggunaan D-optimal mixture design untuk optimasi dan formulasi self-nano emulsifying drug delivery system (SNEEDS) asam mefenamat. *Jurnal Sains Farmasi & Klinis*, *7*(3), 180. https://doi.org/10.25077/jsfk.7.3.180-187.2020

Tsume, Y., Mudie, D. M., Langguth, P., Amidon, G. E., & Amidon, G. L. (2014). The biopharmaceutics classification system: subclasses for in Vivo Predictive Dissolution (IPD) methodology and IVIVC. *European Journal of Pharmaceutical Sciences*, *57*, 152–163. https://doi.org/10.1016/j.ejps.2014.01.009

Vidya Raj, C. K., Venugopal, J., Muthaiah, M., Chadha, V. K., Brammacharry, U., Swappna, M., Sangeetha, A. V., Dhandapani, S. P., Kareedhi, V. R., Calivarathan, L., Karthick, M., & Jayapal, K. (2022). In-vitro anti-Mycobacterium tuberculosis effect of Eugenol. *Indian Journal of Tuberculosis*, *69*(4), 647–654. https://doi.org/10.1016/j.ijtb.2021.09.016

Vu, H.-D., Vu, T.-H., Mai, N. L., Pande, D. C., Dao, D. V., Rehm, B. H. A., Nguyen, N.-T., Grant, G. D., Tran, C.-D., Zhu, Y., & Dau, V. T. (2024). In-flight electro-neutralisation electrospray for pulmonary drug delivery. *Nano Today*, *55*, 102217. https://doi.org/10.1016/j.nantod.2024.102217

Wang, B., Wang, L., Yang, Q., Zhang, Y., Qinglai, T., Yang, X., Xiao, Z., Lei, L., & Li, S. (2024). Pulmonary inhalation for disease treatment: basic research and clinical translations. *Materials Today Bio*, *25*, 100966.https:// doi.org.10.1016/j.mtbio.2024.100966

WHO. (2023). *Global Tuberculosis Report 2023*. World Health Organization

Winarti, L. (2016). Formulation of Self-Nanoemulsifying Drug Delivery System of Bovine Serum Albumin using HLB (Hydrophilic-Lipophilic Balance) Approach. *Indonesian Journal of Pharmacy*, *27*(3), 117. https://doi.org/10.14499/indonesianjpharm27iss3pp117

Wiwiek, I. A., Martodihardjo, S., . S., . J., Ngurah Budiana, I. G. M., & . M. (2017). Preparation and in-vitro characterization of Self-Nano emulsifying system of C- Phenylcalix-[4]-Resorcinaryl Octacinnamate and C-Methylcalix-[4]-Resorcinaryl Octabenzoate as ultraviolet absorbers. *Bali Medical Journal*, *6*(3), 569. https://doi.org/10.15562/bmj.v6i3.699

Yadav, P., Rastogi, V., & Verma, A. (2020). Application of Box–Behnken design and desirability function in the development and optimization of self-nanoemulsifying drug delivery system for enhanced dissolution of ezetimibe. *Future Journal of Pharmaceutical Sciences*, *6*(1), 7. https://doi.org/10.1186/s43094-020-00023-3