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Development of Orodispersible Film Containing Spray-Dried Rifampicin Nanosuspension

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ABSTRACT

Tuberculosis (TB) is a major global health challenge, with high mortality rates. Conventional anti-TB dosage forms, such as tablets are often unsuitable for patients who struggle with swallowing, leading to poor compliance. Therefore, this study aimed to develop orodispersible film (ODF) containing rifampicin, a widely used anti-TB drug. Rifampicin's low aqueous solubility poses challenges for its incorporation into ODF and affects its therapeutic effectiveness. To address these issues, rifampicin was first prepared as nanosuspension and subsequently dried before being formulated into ODF. Nanosuspension was stabilized using polyvinyl alcohol (PVA), poloxamer 188 (POX), and the combination at various concentrations. Nanosuspension was prepared using solvent-antisolvent precipitation followed by sonication and spray-drying. Spray-dried nanosuspension was characterized, and the optimal formula was used in ODF formulation through the solvent-casting method. Formulations PV2 (PVA 0.4%) and POX1 (POX 0.2%) showed the smallest particle sizes at 306±14.01 nm and 291±7.55 nm, respectively. After reconstitution, PV2 maintained particle size comparably to POX1. Spray-dried PV2 nanosuspension exhibited a 21.48-fold increase in saturated solubility compared to the pure rifampicin, and showed superior drug release, with 79% release versus 58% for the standard rifampicin suspension. ODF containing PV2 showed improved organoleptic properties and enhanced drug dissolution (82% vs 56%) compared to original rifampicin ODF. The formulation of rifampicin into nanosuspension stabilized by PVA and POX, followed by spray-drying, significantly improved solubility and drug release profile. This method also enhanced the organoleptic properties and dissolution of rifampicin in ODF, providing a promising strategy to boost rifampicin's therapeutic efficacy in TB treatment, particularly for pediatric patients.

Keywords: rifampicin, nanosuspension, solvent-antisolvent precipitation, spray-drying, orodispersible film

INTRODUCTION

Tuberculosis (TB) is an infectious disease mainly caused by *Mycobacterium tuberculosis complex* (MBTC) family, which has become a major contributor to death worldwide (Kanabalan et al., 2021). In 2021, data from World Health Organization (WHO) showed an estimated 10.6 million people were infected with TB, and 11% were children. TB cases of children in WHO European Region increased by 10% in 2023 compared to 2022, with under 15 years representing 4.3% of all cases (ECDC, 2025). Similar trends were observed globally, including India, which reported a 38% increase in notified pediatric TB cases from 2020–2024 (Koul, 2025).

Challenges, such as patient non-compliance with treatment regimens and inappropriate drug administration have become major contributors to treatment failures and the rising incidence of multidrug-resistant TB (MDR-TB) (Albanna et al., 2013). Data from WHO estimated that 25,000 to 32,000 children develop MDR-TB annually (WHO,

2020). In 2019, 67,710 cases of MDR-TB were reported among children and adolescents worldwide, leading to 7,061 deaths (Zhong et al., 2025).

An industry survey showed that the systematic development of medicines designed for children has now become a key priority in pediatric studies (Van der Veken et al., 2022). However, current paediatric TB therapeutic strategies still significantly rely on adapted adult dosage forms, and there is a limited study focused on the optimization of pediatric-specific anti-TB drug delivery systems. For this reason, there is an urgent need for a pediatric-friendly dosage form that can play a role in addressing TB outbreak among children (Santoveña-Estévez et al., 2020).

Oral thin film or orodispersible film (ODF) is single or multilayered sheets of suitable materials designed to be placed in the mouth and are rapidly dispersed in less than 1 minute. ODF offers several advantages over other dosage forms, including ease of administration without chewing or water, easy handling, storage, and transport, and flexible dose adjustments. The attributes make oral thin films a promising dosage form for anti-TB drugs, potentially enhancing patient adherence to TB regimens. Developing ODF for pediatric TB treatment could address critical challenges in current treatment, such as inaccurate dosing and poor compliance. By providing a more acceptable and practical alternative, this method has the potential to significantly improve treatment outcomes and reduce the global burden of pediatric TB.

Formulating ODF presents several challenges, including low drug payload due to the limited space available for dispersion. Studies on some drugs in ODF formulation showed only less than 7 mg/cm^2 could be incorporated into film (Khan et al., 2020; Łyszczarz et al., 2020; Sinha et al., 2022; Sjöholm & Sandler, 2019; Thabet et al., 2018). This challenge was more pronounced when the active substance had poor solubility in the solvent used to produce film. The majority of the studies about ODF did not use antibiotics, which was probably due to the high individual dose and low solubility of most antibiotics. Rifampicin, a broad-spectrum antibiotic widely used at the forefront of TB treatment, exhibited low saturated solubility in water, approximately 1.4 mg/mL. Various methods have been explored to enhance the solubility of rifampicin, such as complexation with compounds, including *acyclic cucurbit[n]uri* and cyclodextrin (Liu et al., 2021; Tewes et al., 2008). However, these methods may be less cost-effective due to the need for additional ingredients and processes, potentially increasing production costs.

Nanosuspension formulation provides a promising method to enhance the saturated solubility of poorly soluble drugs and improve the pharmacokinetic profile, potentially increasing the effectiveness compared to conventional preparations (Aisiah & Surini, 2024). This formulation can face challenges, such as particle aggregation, and undergo Ostwald ripening (Wu et al., 2011). Storing nanosuspension in dried form was advisable to mitigate this issue (Aisiah & Surini, 2024). Dried nanosuspension can also serve as a versatile platform for various dosage forms, including tablets, powders for pulmonary delivery, and ODF (Mehanna et al., 2019; Sun et al., 2015). Therefore, this study aimed to develop rifampicin nanosuspension to improve the solubility of rifampicin and facilitate easy formulation into ODF. Based on available information, there are only a few studies on ODF formulation using anti-TB drugs, such as isoniazid and pyrazinamide (Adeleke et al., 2016, 2018). None of these studies used rifampicin, probably due to its solubility issue. Rifampicin nanosuspension was prepared by the solvent-antisolvent method, using polyvinyl alcohol (PVA), Poloxamer 188 (POX), or a combination as stabilizers. A previous study conducted by Mehanna, *et al*., (Mehanna et al., 2019) also prepared rifampicin nanosuspension using the same method. Rifampicin was dissolved in an organic solvent and mixed into an aqueous solvent containing a carbohydrate stabilizer. However, particles produced were in the micrometer range, potentially due to the use of a bath sonicator to aid nanosuspension process.

In this study, a probe sonicator was used to assist in nanosuspension preparation, anticipating the production of rifampicin nanoparticles in the nano-size range to enhance the robustness of the method. Nanosuspension was spray-dried for efficiency, scalability, and suitability for heat-resistant drugs compared to lyophilization (Guan et al., 2022). As the final product, spray-dried nanosuspension was then incorporated into ODF formulation.

MATERIALS AND METHODS

Materials

The materials used in this study included rifampicin (Luohe Nanjiecun Pharmaceutical, China), PVA with 88% degree of hydrolysis (Denka,

Japan), POX EMPROVE® EXPERT (Merck, Germany), Methocel E15 LV (Dow Inc., USA), mannitol (Shijiazhuang Huaxu Pharmaceutical, China), and technical grade methanol (Sucofindo, Indonesia). Other materials included methanol pro analysis (Merck, Germany), hydrochloric acid (Rofa Laboratorium Centre, Indonesia), demineralized water (Brataco, Indonesia), and distilled water (Brataco, Indonesia).

Preparation of rifampicin nanosuspension

Rifampicin nanosuspension (NS) was prepared according to the master formula (Supplementary Table SI), using the solvent-antisolvent precipitation method followed by sonication. A 5% PVA stock solution was prepared by dissolving PVA in demineralized water, heated to 90°C. Meanwhile, POX was dissolved in demineralized water at room temperature. Rifampicin was dissolved in methanol and subsequently added to polymer(s) solution under stirring. The resulting mixture was subjected to rapid sonication using a Probe Sonicator Q500 (QSonica, USA) to obtain rifampicin nanosuspension. The sonication parameters were initially optimized, including amplitude, duration, mode, and probe tip depth, and then the most optimal settings were applied to subsequent formulations.

Spray-drying of rifampicin nanosuspension

A total of 2.4 g of mannitol was added to rifampicin nanosuspension as a matrix-forming agent before spray-drying process. The pre-treated nanosuspension was dried using a Buchi Mini Spray-Dryer B-290. Furthermore, the drying parameters included an inlet temperature of 120°C, air aspiration set at 80%, pump rate maintained at 20%, and an air velocity of approximately 439 L/hour. Continuous sample agitation during drying ensured uniformity and homogeneity throughout the process.

Characterization of nanosuspension and dry nanosuspension

Several evaluation tests were carried out to characterize the produced and dried nanosuspension, including particle size and polydispersity index (PDI) analysis, zeta potential, dispersibility index, and saturated solubility determination. In vitro dissolution and physical stability tests were also carried out, as well as X-ray diffraction.

Particle Size Analysis

Nanosuspension and dry nanosuspension were subjected to comprehensive characterization, including measurement of particle size, PDI, and zeta potential using a Zetasizer Nano ZS instrument (Malvern, United Kingdom). A total of 1 mL of the samples was diluted with 9 mL of demineralized water and vortexed for 1 minute before analysis to measure the properties of nanosuspension. For the dry nanosuspension, 10 mg of the material was dispersed in 10 mL of demineralized water. One drop of the suspension was then diluted with 10 mL of demineralized water and vortexed for 1 minute before the measurement. All measurements were done triplicate with 10 times reading for each sample, at an angle of 173° and temperature of 25°C. The dry nanosuspension was also evaluated using an X-ray diffractometer.

Redispersibility index (RDI)

RDI was calculated by comparing particle size of nanosuspension before spray-dried (D_0) and particle size of nanosuspension after spray-dried and redispersed (D), using Equation (1):

$$RDI = \frac{D}{D_0} \qquad \text{.....(1)}$$

Determination of saturated solubility of rifampicin

A modified saturated solubility determination test was carried out to assess the enhanced saturated solubility of rifampicin based on the methodology described by Mohyeldin *et al*. (M. Mehanna *et al*., 2016). A sample of spray-dried nanosuspension equivalent to 100 mg of rifampicin was added to 10 mL of distilled water and stirred for 24 hours. Subsequently, the sample was centrifuged at 13,000 rpm for 60 minutes at 4°C. The supernatant was then filtered and analyzed using a UV-Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 334 nm. The same procedure was repeated for 100 mg of rifampicin powder.

Dissolution test

The dissolution test was carried out using a USP Apparatus II (paddle type) dissolution tester (Electrolab, USA) in 900 mL of HCl 0.1 N, maintained at 37±0.5°C with a rotation speed of 50 rpm. A dry nanosuspension sample equivalent to 50 mg of rifampicin was dispersed in 10 mL of distilled water and then introduced into the dissolution medium to initiate the test. At

predetermined intervals, 10 mL of aliquots were withdrawn for up to 45 minutes, with fresh dissolution medium added after each sampling to maintain the sink conditions. The samples were then filtered, diluted, and analyzed using a UV-Vis spectrophotometer (Shimadzu, Japan) at the maximum wavelength of 475 nm. The same procedure was carried out on rifampicin powder.

Physical stability study

Rifampicin nanosuspension were stored at 4°C (refrigerator) and 25°C (desiccator with $CaCl_2$). Particle size and PDI were assessed on days 0, 3, 5, 8, 15, 22 and 30. Spray-dried rifampicin nanosuspension was redispersed in water before each measurement.

Preparation of ODF containing spray-dried rifampicin nanosuspension

The optimized nanosuspension formula was scaled up using the same composition and preparation method before ODF development. For film preparation, referring to the formula (Supplementary Table II), 5.5 g of spray-dried rifampicin nanosuspension, containing 410 mg rifampicin, was dissolved in distilled water (mixture 1). Separately, 4 g of 10% (w/w) Methocel E15 LV solution in distilled water was mixed with 800 mg glycerine and stirred at 300 rpm using a magnetic stirrer until homogenous (mixture 2). The product was then added to mixture 1 and stirred at 700 rpm using a magnetic stirrer until uniform. Distilled water was added to the combined mixture, thereby increasing the total volume to 35 g, forming film-casting solution. This solution was cast into a 10x10 cm silicone mold, dried in an oven at 40°C for 48 hours, and manually cut into 3x2 cm films.

Preparation of ODF containing rifampicin powder

A total of 410 mg of rifampicin powder was dissolved in 10 mL of distilled water (mixture 1). In a separate container, 12.3 g of 10% (w/w) PVA stock solution was mixed with 4 g of 10% (w/w) Methocel E15 LV solution in distilled water (mixture 2). Subsequently, 800 mg of glycerin and 2 g of mannitol were added, and then mixture 1 was added continuously into mixture 2. Distilled water was added to adjust the total volume to 35 g to form film-casting solution. This solution was cast into a 10x10 cm silicone mold, dried in an oven at 40°C for 48 hours, and then manually cut into 3x2 cm films.

Characterization of ODF

Physical characterizations

ODF was characterized by organoleptic properties, dimension, weight, and moisture content. The tensile strength was measured using a texture analyzer (Stable Micro System-TA XT Express Enhanced) equipped with a pull probe (A/TG Tensile Grip) and a 5 kg load. For this measurement, ODF was cut into 5x5 cm squares, clamped, and pulled at a 1 mm/sec speed until snapped. The folding endurance of ODF was assessed by repeatedly folding at the same axis until films either broke or reached a maximum of 300 folds.

Determination of Rifampicin Content

A 2x3 cm ODF was dissolved in a 25 mL methanol-water mixture (1:1) in a 50 mL volumetric flask and then sonicated for 10 minutes. Methanol was added to increase the total volume to 50 mL and then filtered under vacuum. A 10.0 mL aliquot of the filtered solution was further diluted with methanol to achieve the desired concentration. The sample solution was measured with a UV-Vis spectrophotometer at λ 475.5 nm. Subsequently, rifampicin content was calculated using a linear regression from the standard calibration curve. A blank solution, prepared with ODF formula without rifampicin, was also measured for comparison.

In vitro disintegration time

The in vitro disintegration time of ODF was assessed using a modified version of a previously published method (Preis et al., 2014). ODF was placed in an 8 cm Petri dish, and 5 mL of demineralized water at 37°C was dripped onto its surface. The time required for ODF to disintegrate was measured with a stopwatch.

In vitro dissolution test

The in vitro dissolution test of rifampicin ODF was conducted using a USP Apparatus I dissolution tester. The test was carried out in 900 mL of 0.1 N HCl medium at 37±0.5 °C, stirred at 100 rpm for 45 minutes. At predetermined time intervals, 10 mL of aliquot was withdrawn and replaced with an equal volume of fresh dissolution medium. The samples were analyzed using a UV-Vis spectrophotometer at λ 466 nm to determine rifampicin concentration.

Accelerated stability study

Rifampicin ODF was stored in a stability chamber at 40±2°C and 75±5% relative humidity in aluminum foil packs. After 1 month, film was evaluated for changes in organoleptic properties, moisture content, rifampicin content, and dissolution profile.

RESULTS AND DISCUSSION

Preparation and characterization of rifampicin nanosuspension

In this study, rifampicin nanosuspension was prepared using the solvent-antisolvent precipitation method, followed by sonication. Methanol and demineralized water were used as solvent and antisolvent, respectively. The most optimum condition for sonication was determined by evaluating particle size of the produced nanosuspension. Each parameter showed an impact on the size of rifampicin particles produced (Figure 1). A decrease in particle size observed when the amplitude was increased from 45% to 50% (Supplementary Figure 1.A) might be due to the cavitation effect, which can reduce the size of drug particles. (Mehanna et al., 2019). Particle size was slightly higher when the amplitude was increased to 55% and 60%, caused by the excess heat produced during the sonication process. This was due to accelerated Brownian motion which can induce particle growth (Tehrani et al., 2019).

Although longer duration of stirring theoretically facilitates faster nucleation rates and leads to smaller particle sizes in terms of sonication duration (Mehanna et al., 2019), this study found no significant difference between 10 and 15 minutes of stirring (Supplementary Figure 1.B). Prolonged sonication may risk excessive heat generation, potentially leading to larger particle sizes. Comparison showed (Supplementary Figure 1.C) that continuous mode could significantly produce smaller particles compared to intermittent mode. This result is consistent with the report of a previous study that pauses during sonication can lead to larger particle sizes (Ruecroft et al., 2005). However, contrary to the other study on sonicator probe depth (Son et al., 2020), the result showed that 1 cm probe tip depth (middle depth) produced the smallest particle size (Supplementary Figure 1.D). This also suggested that an even distribution of ultrasonic waves occurred when the tip was centered in the solution. Therefore, the most optimal sonication conditions were identified as 50% amplitude, 10minutes duration, continuous mode, and a 1 cm probe tip depth, as these conditions consistently produced the smallest particle size. These parameters were then used to produce rifampicin nanosuspension for all formulations.

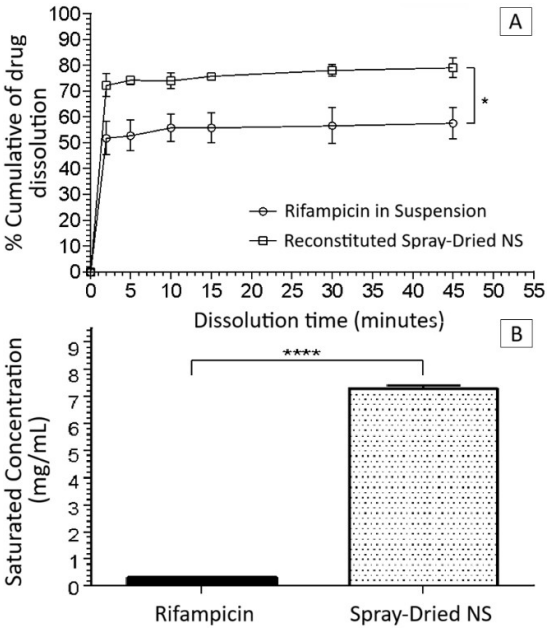

Figure 1. (A) Saturated concentration of rifampicin and spray-dried rifampicin nanosuspension (mean±SD, n=3, unpaired t-test ****p<0.0001) and (B) Dissolution of rifampicin from conventional suspension and nanosuspension in medium HCl 0.1 N (mean±SD, n=3, multiple t-test *p<0.05)

This study developed 13 formulas varying the concentration of PVA and POX as a single or combination (Supplementary Table I) to investigate the effect of polymer concentration. The chosen concentrations of PVA and POX were adapted from the previous studies (Alhamhoom et al., 2023; El-Rafie et al., 2023). In terms of comparing formulations using single PVA as a stabilizer, PV2 (PVA 0.4%) and PV4 (PVA 0.8%) exhibited the smallest particle size at 306±14 nm and 313±37 nm, respectively (Table I). However, PV2 showed a lower PDI compared to PV4 (0.265 vs 0.666), suggesting a more homogenous particle size range when using 0.4% of PVA. Among formulations using single POX, POX2 (POX 0.2%) showed the smallest particle size at 291±7 nm. This result was consistent with the report of Alshweiat (Alshweiat et al., 2018), which used the same polymer for loratadine nanosuspension and observed an increase in particle size with higher POX concentrations. A

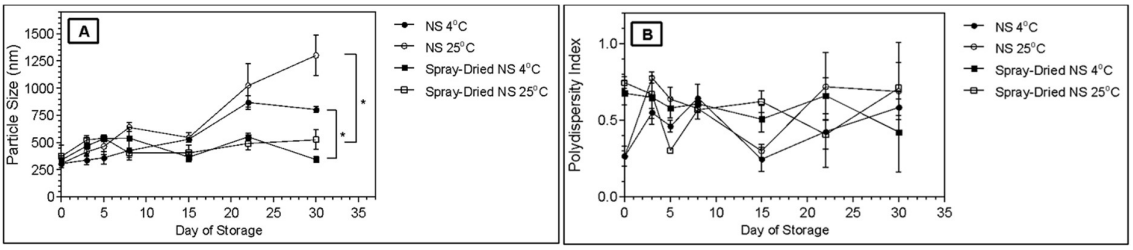

Figure 2. Particle size during storage of nanosuspension in liquid form and dry form. The dry form was reconstituted at each sampling point before the measurement of particle size (mean±SD, n=3)

Table I. Characteristics of rifampicin nanosuspension produced by each formula (mean±SD, n=3)

Formula	Particle size (nm)	PDI	Zeta Potential* (mV)	Spray-Drying Yield (%)	Redispersibility Index
PV1	423 ± 88	0.601 ± 0.143	Not measured	NA	NA
PV2	306 ± 14	0.265 ± 0.064	-23.00 ± 0.10	64.5	0.94
PV3	686 ± 179	0.411 ± 0.118	Not measured	NA	NA
PV4	313 ± 37	0.666 ± 0.030	Not measured	NA	NA
PV5	669 ± 137	0.607 ± 0.104	Not measured	NA	NA
POX1	291 ± 7	0.523 ± 0.079	-25.27 ± 2.85	28.0	0.49
POX2	469 ± 56	0.657 ± 0.141	Not measured	NA	NA
POX3	530 ± 108	0.557 ± 0.266	Not measured	NA	NA
POX4	447 ± 94	0.654 ± 0.059	Not measured	NA	NA
POX5	445 ± 108	0.948 ± 0.013	Not measured	NA	NA
PVX1	471 ± 39	0.553 ± 0.046	-13.93 ± 0.65	54.8	0.96
PVX2	494 ± 40	0.552 ± 0.048	-17.17 ± 1.02	47.2	0.90
PVX3	474 ± 40	0.409 ± 0.154	-17.17 ± 0.83	43.4	0.99

PV refers to the single use of PVA, POX refers to the single use of Poloxamer 188, and PVX refers to the combination use of PVA and Poloxamer 188
*Spray-dried nanosuspension after redispersed in water

minimal amount of polymer suffices to produce nanosuspension with a small particle size (under 350 nm), particularly with amphiphilic polymers such as POX. The high concentration of amphiphilic polymer in solution can induce micelle formation, potentially leading to increased particle sizes as these micelles adsorb onto particle surface.

After evaluating formulations using single PVA and POX, the impact of combining both polymers was examined. Anticipating the creation of small particle rifampicin nanosuspension with reduced amounts of each polymer, the result showed that the combination did not produce smaller particle sizes compared to the individual use of PVA or POX (471, 494, and 474 nm for PVX1, PVX2, and PVX3, respectively). Additionally, PDI of these formulations was higher than PV2 but equal to POX1. This observation may be attributed to the propensity of POX to form micelles in a saturated solution, potentially affecting particle size uniformity.

Zeta potential measurements were conducted to PVA2 and POX1 formulations, representing single-polymer rifampicin nanosuspension, as well as PVX1, PVX2, and PVX3. The results showed that PVA2 and POX1 exhibited zeta potential values exceeding -20 mV, while PVX1, PVX2, and PVX3 had lower values (Table I). This suggests potentially higher physical stability for PVA2 and POX1 compared to the other 3 formulations using polymer combinations. Nanosystems were typically referred to as thermodynamically unstable (Wang et al., 2013), posing a risk of agglomeration or crystal growth when stored in liquid form. This was mitigated using spray-drying method to dry rifampicin nanosuspension formulations PVA2, POX1, PVX1, PVX2, and PVX3, using mannitol as a

filler. The other formulas were excluded since particle size was >500 nm and PDI <0.55. PV2 had the highest yield, accounting for 64.6% (Table I). As the concentration of POX increased from PVX1 to POX1 (0.05% to 0.2%), spray-drying yield decreased. This decline was caused by the melting point of POX, ranging from 52°C to 56°C (Wei et al., 2018) and leading to the adherence of the mixture in spray-drying chamber.

A redispersibility study was conducted to confirm that the dried nanosuspension retained its nanoscale particle size upon redispersion. PV2, PVX1, PVX2, and PVX3 showed negligible changes in particle size and PDI after redispersion, indicating maintained monodispersity (Supplementary Figures 2). However, POX1 showed a significant increase in particle size post-spray-drying, from approximately 200 to 600 nm, with changes in PDI. This study calculated RDI, which was a crucial parameter showing a dried nanosuspension's ability to maintain particle size upon redispersion. PV2, PVX1, PVX2, and PVX3 showed high RDI values (>0.90), suggesting minimal particle agglomeration post-drying. POX1 had a low RDI value of 0.49, potentially due to POX degradation induced by heat above its melting temperature. This condition led to irreversible particle aggregation, as reported by previous studies (Chaubal & Popescu, 2008; Li et al., 2021).

Considering particle size, PDI, zeta potential, spray-drying yield, and RDI, PV2 was the optimal formula for both rifampicin nanosuspension and its dried form (Figure 3). Therefore, PV2 was selected for further investigation in subsequent studies.

Saturated solubility enhancement of rifampicin

A saturated solubility study was conducted to assess the impact of nanosuspension preparation on the aqueous solubility of rifampicin. Remarkable increase in the saturated concentration of rifampicin from 0.339±0.006 mg/mL (pure rifampicin) to 7.293±0.105 mg/mL (spray-dried rifampicin nanosuspension) (Figure 1A), marking a more than 21-fold enhancement and around 3-fold enhancement compared to rifampicin solubility in the literature (2.5 mg/mL, (Supplementary Table III)). This result was consistent with the report of Mehanna *et al*. (2019) where a 50-fold increase in the saturated solubility of rifampicin nanocrystals was observed compared to pure rifampicin powder. In rifampicin nanosuspension formulation PV2, the augmented surface area of drug particles was the primary factor contributing to the saturated solubility.

The saturated solubility of rifampicin in nanosuspension is further evident in the dissolution profile. The release of rifampicin from reconstituted spray-dried nanosuspension reached approximately 79% in 45 minutes (Figure 1B), t. However, pure rifampicin powder dispersed in water had only around 58% of rifampicin release in the same timeframe.

X-ray diffraction test was conducted on pure rifampicin powder, dried nanosuspension, and PVA polymer to confirm the crystal form of rifampicin before and after nanosuspension preparation. The diffractogram (Supplementary Figure 3) showed no alteration in the crystalline form of rifampicin due to the use of PVA as a stabilizer in nanosuspension preparation. The similarity of peaks that the formulation process preserved the crystal structure of rifampicin (Karakucuk et al., 2022). However, the intensity in PV2 formulation was significantly lower compared to pure rifampicin, suggesting a transition from crystalline to semi-crystalline form (Holder & Schaak, 2019).

The presence of PVA and mannitol in the formulations may have contributed to the change in crystallinity. A comparison of the diffractograms of PV2 and PVA showed a similar peak with high intensity at 2θ 19.2°, suggesting that the presence of PVA in the formulation could influence the crystallinity of rifampicin (Gaaz et al., 2015). The use of mannitol might also affect the crystal form in a mixture (Xia et al., 2016). Therefore, the transition to an amorphous state could be another factor contributing to the increased saturated solubility of rifampicin.

Physical stability study of rifampicin nanosuspension

Nanosuspension inherently faces instability due to the presence of Gibbs free energy. This condition prompts particle aggregation and reduces surface area, resulting in increased particle size over time through Ostwald ripening. Therefore, an investigation was conducted to observe rifampicin nanosuspension particle growth when stored in both liquid and dry solid form. Liquid nanosuspension storage showed significant particle growth over time, particularly at elevated temperatures (Figure 2A). By day 30, differences in particle growth between liquid and spray-dried nanosuspension were more remarkable (Figure 2).

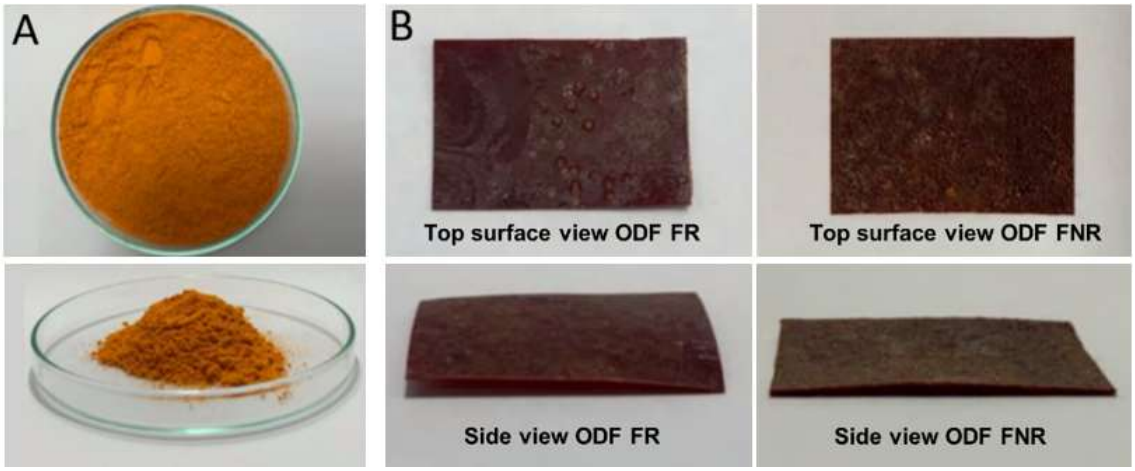

Figure 3. Organoleptic properties of (A) Rifampicin nanosuspension powder after spray-drying process and (B) rifampicin ODF from formula FR and FNR

Table II. Characteristics of rifampicin ODF (mean ± SD, n = 3, **p<0.05, ***p<0.005)

Properties	FR	FNR
Weight (mg)**	292.80 ± 28.21	409.99 ± 30.62
Thickness (mm)***	0.77 ± 0.01	0.87 ± 0.01
Tensile strength (N/mm^2)	1.98 ± 0.28	1.57 ± 0.28
Folding Endurance	Passed	Passed
Moisture content (%)**	7.58 ± 0.40	9.73 ± 0.15
Disintegration time (s)	95.12 ± 9.31	94.90 ± 14.57
Rifampicin content (mg/cm^2)	4.0 ± 0.5	3.6 ± 0.4

FR refers to ODF containing rifampicin original bulk, and FNR refers to ODF containing spray-dried rifampicin nanosuspension.

While particle size of rifampicin nanosuspension in liquid form surged from approximately 250 nm to 1300 nm, spray-dried sample maintained a size below 500 nm. PDI remained consistent regardless of nanosuspension form during storage. These results suggested that spray-dried nanosuspension could uphold physical stability during storage without compromising nanoparticle size and polydispersity.

Preparation and characterization of rifampicin ODF

The optimized formulation of spray-dried rifampicin nanosuspension was successfully incorporated into ODF formulation through a solvent-casting method, leading to a formulation coded as FNR. Each film contained approximately 4.1 mg/cm^2 of rifampicin, equivalent to 24 mg of rifampicin in an individual film, measuring 2x3 cm. Considering a 2-year-old child with a body weight of 10 kg and requires a daily dose of 10 mg/kg rifampicin, approximately 4-5 films are needed. ODF formulation containing rifampicin in its original bulk powder was also prepared with the same amount of rifampicin and designated as FR formulation. A significant advantage of FNR formulation was the enhanced solubility and dispersibility of spray-dried rifampicin nanosuspension, eliminating the need for methanol as a co-solvent, which was required in FR formulation to dissolve rifampicin adequately.

The surface morphology of FNR ODF is smoother and more uniform compared to FR ODF (Figure 3B). This improvement in surface quality suggested better integration and dispersion of rifampicin nanosuspension in the matrix, contributing to its enhanced dissolution profile. Despite the significant differences in weight and

thickness, tensile strength, folding endurance, and disintegration time had no significant difference (Table II). Both formulations maintained comparable structural integrity and flexibility, suggesting that the incorporation of spray-dried nanosuspension did not compromise the mechanical properties of ODF.

The disintegration time for both formulations is still more than 1 minute, which is longer than the desired time for ODF. This prolonged disintegration time could be attributed to the absence of a super disintegrant in the formulation, which was typically used to facilitate the rapid disintegration process of orodispersible preparations.

Rifampicin content in ODF was approximately 4 mg/cm^2 for FR and 3.6 mg/cm^2 for FNR, closely matching the theoretical target of 4.1 mg/cm^2. These results also showed good dispersibility of rifampicin in ODF formulations and suggested that the solvent-casting process effectively achieved the desired dosage levels for both formulations.

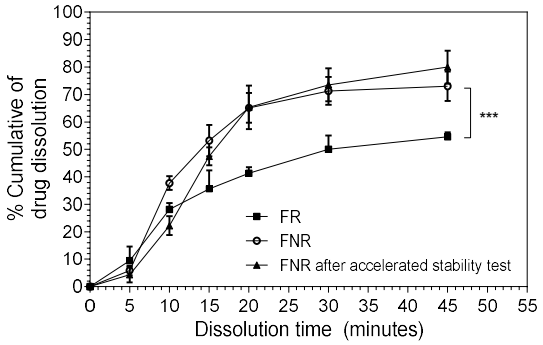

Figure 4. Dissolution of rifampicin from ODF FR, FNR, and FNR after accelerated stability test for 1 month (stability chamber at a temperature of 40±2°C and RH 75±5%) in medium HCl 0.1 N (mean±SD, n=3, t-test ***p<0.001)

In Vitro Dissolution of Rifampicin from ODF

Despite the insignificant difference in the physical characteristics of FR and FNR ODF, a marked discrepancy was observed in rifampicin dissolution profiles. Approximately 70-80% of rifampicin was released from FNR ODF in 45 minutes, compared to only 50% in FR ODF during the same period (Figure 4). This enhanced dissolution performance of FNR ODF might be attributed to the incorporation of spray-dried rifampicin nanosuspension, which significantly improved drug solubility. Despite not achieving the release profile >80%, the value was close to the dissolution requirement standard for rifampicin tablets, as shown in Indonesian Pharmacopeia.

The results showed the importance of dried nanosuspension preparation as a strategy to enhance the dissolution and bioavailability of poorly soluble drugs, such as rifampicin. The improved dissolution profile from FNR ODF suggested that the formulation could provide more efficient drug release, which was critical for the effective management of TB treatment. This method also facilitates ease of administration, specifically for pediatric patients who experience difficulties consuming tablets.

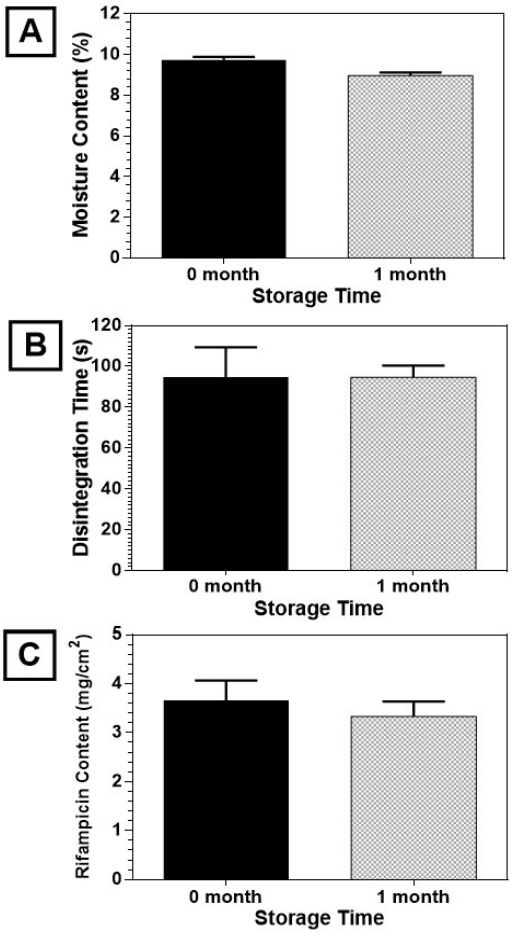

Figure 5. (A) Moisture content, (B) disintegration time, and (C) rifampicin content of ODF FNR after storage in an accelerated condition for 1 month in a stability chamber at a temperature of 40±2°C and RH 75±5%.

Accelerated stability study of ODF

Accelerated stability study was carried out on FDR ODF for 1 month, and the results showed a relatively stable formulation. The dissolution profile of rifampicin from FNR ODF remained relatively constant (Figure 5), confirming the stability of the formulation to maintain its functionality and ensure a good drug release after a particular storage time. Other key parameters, such as moisture content, disintegration time, and rifampicin content showed minor variation between the initial (0-month) and 1-month time points (Figure 5). This result shows that FNR ODF maintains its integrity and does not undergo significant degradation or physical changes during the study period. Ensuring the therapeutic reliability of ODF is crucial, specifically for pediatric patients, where dosage accuracy is also vital.

CONCLUSION

In conclusion, several formulations of rifampicin nanosuspension, stabilized with PVA or POX, were successfully prepared using the solvent-antisolvent preparation method assisted with sonication. This method significantly improved the saturated solubility and dissolution profile of rifampicin compared to the liquid preparation. Physical stability of nanosuspension was further enhanced through spray-drying. Incorporating spray-dried rifampicin nanosuspension in ODF formulation provided better dispersibility, improved appearance, and enhanced dissolution profile. Therefore, spray-dried rifampicin nanosuspension showed promise as a premixed material for preparing ODF containing rifampicin, potentially enhancing TB treatment for pediatric patients. Future studies were expected to apply a more systematic optimization and plan in the formulation using an experimental design (DoE) method.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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