Determination Of Dissolution Profile Sprinkle Formulation From Noni Fruit Extract (*Morinda citrifolia* L.)

Nurista Dida Ayuningtyas*, Yohana Inge Sugiarto, Anita Monalisa
Faculty of Allied Health Sciences, Universitas Pelita Harapan, Jakarta, Indonesia
*email: Nurista.ayuningtyas@uph.edu

Abstract

Noni fruit (*Morinda citrifolia* L.) has immunomodulatory properties due to the presence of scopolamine and quercetin compounds. This immunomodulator activity will be utilized in pharmaceutical products formulated into fine granules (sprinkle formulation). In this study, dissolution testing of the formulation will be conducted using a type 2 (paddle) apparatus and a medium with a pH value of 1.2 and 4.5. The formulation was then subjected to physical granule quality testing and dissolution testing. In the dissolution test, the levels of samples taken during the dissolution test were determined using UV-Vis spectrophotometry. The results obtained were the dissolution profile of quercetin compounds in the sprinkle formulation of noni fruit extract *Morinda citrifolia* L. at minute 60 in a pH 1.2 medium was 57.50% ± 1.33 and in a pH 4.5 medium was 86.24% ± 0.69. Statistical analysis of the total flavonoid content (mgQE/gram) in the extract, sprinkle formulation, and the amount of dissolved drug at minute 60 in pH 1.2 and pH 4.5 medium yielded a p-value < 0.05, indicating a significant difference in total flavonoid content among the samples. Conclusion: Sprinkle formulation of noni fruit extract provides significantly different dissolution profiles at pH 1.2 and pH 4.5.

Keywords: quercetin, immunomodulator, granules, dissolution profile

1. INTRODUCTION

Noni fruit (*Morinda citrifolia* L.) is a plant native to Southeast Asia that grows abundantly in Indonesia and has been widely used by the community as a source of herbal medicine (Chanthira Kumar et al., 2022). Research conducted by (Mufidah et al., 2013) shows that noni fruit extract also has the ability to act as an immunomodulator, as indicated by the presence of scopolamine and quercetin compounds that act as mitogens, where at doses of 25 mg/kg BW, 100 mg/kg BW, and 300 mg/kg BW can increase the relative number of CD4+ T cells, CD4+IFN-γ+ T cells, and CD4+CD25+ regulatory T cells. Noni fruit has been developed into a sprinkle formulation in granule form, making it easy for children to consume.

Disolution testing is related to the solubility process of drug products in the body. Solubility is the initial process of active substance absorption into the bodies, which are then dissolved in the gastrointestinal tract and absorbed into the blood, other body fluids, or tissues, then distributed to the drug target to bind and provide therapeutic effects. Preparations witha high solubility rate provide high dissolution rates, so that the dissolution rate affects the absorption rate, which also has an effect on the bioavailability of the preparation (Sagala, 2019). Dissolution testing is important because it provides a dissolution profile that guides drug

formulation and formulation development, quality control during production, confirmation of in vitro bioequivalence between batches, and drug product marketing regulations (Pasupuleti Sunitha et al., 2024). A dissolution test can be performed using a solution medium that has been adjusted in terms of pH, temperature, viscosity, surface tension, and composition to resemble the environment where the drug formulation is absorbed, such as the stomach or intestines of a human (Mali et al., 2014). The dissolution medium can be prepared in various types by varying the pH, surfactant, or enzyme addition. A pH of 1.2–5 typically represents the stomach environment, while a pH of 6–7 represents the intestinal medium (Asare-Addo et al., 2013).

Based on that, a study was carried out on the dissolution test of a sprinkle formulation of noni fruit extract, specifically quercetin as the main active ingredient. Dissolution profile was conducted with variations in medium pH (pH 1.2 and 4.5), which represent the gastrointestinal tract conditions in the presence of food (preprandial) and in the absence of food (postprandial). In another study, *Kaempferia parviflora* extract was found to have low solubility. Preparation of solid dispersions with hydroxypropyl methylcellulose (HPMC) and polyvinyl alcohol-polyethylene glycol grafted copolymer (PVA-co-PEG) polymers can increase its dissolution (Weerapol et al., 2017). An excellent dissolution profile indicates that the drug formulation is capable of dissolving and being absorbed completely in the body, thereby effectively providing therapeutic effects for the body. Thus, the immunomodulatory activity produced by the compound quercetin in noni fruit extract can be absorbed into the body optimally through the sprinkle formulation as an innovative and enjoyable way to maintain and improve children's immune systems.

2. METHOD

Extraction

Dried noni fruit (1 kg) was soaked in 3 liters of 96% ethanol solvent. The maceration process was repeated three times, each using 1 liter of 96% ethanol solvent. Maceration was carried out for 48 hours with frequent stirred. The maceration solution is filtered using filter paper to obtain the liquid extract. The liquid extract is concentrated using a rotary evaporator at 40°C, followed by a water bath at 50°C and an oven at 45°C (Ayuningtyas et al., 2024).

Formulation of Sprinkle Formula

In a previous study, the optimal spray formula was formulated with the following composition: 16.67% extract, 2.70% povidone, 2.29% SSG, 20% mannitol, 3% aspartame, 0.5% Mg.Stearate, and 54.84% lactose (Ayuningtyas et al., 2024). This formula is made into a sprinkle formulation with 2 grams of extract per package and a total of 12 grams per dosage.

Tabel 1. Formula Sprinkle Formulation Noni Extract

Ingredients	F0 (%)	F sample (%)
Noni Extract	0	16.67
Povidone	2.70	2.70
Sodium Starch Glycolate	2.29	2.29
Manitol	20	20
Aspartame	3	3
Mg. Stearate	0.5	0.5
Lactose	71.5	54.83
Ethanole 96%	Qs.	Qs.

The formulation was carried out using the wet granulation method. Povidone, sodium starch glycolate, mannitol, aspartame, and lactose were homogenized. Noni fruit extract and 96% ethanol were added to form a granulable mass. The mixture was filtered with a mesh size of 12 to form granules. The granules were dried at 40°C for 3 hours. The dried granules were filtered with a mesh size of 14. Magnesium stearate was added as a lubricant. The formula was made in an amount of 90 grams, with three replications. The powder formulation was packaged in sachets. Each sachet weighed 6 grams of granules and contained 1 gram of extract (Ayuningtyas et al., 2024).

Uniformity of Dosage Test

The content of each of the 10 sachets of sprinkle formulation was determined using an appropriate analysis method, and then its acceptability value was calculated. In this study, the content of the preparation was determined by calculating the total flavonoid content. Determination of total flavonoid content of the preparation was carried out by weighing 12 grams of the preparation, then dissolving 1.5 grams of sprinkle formulation in 10 mL of 70% ethanol to obtain a concentration of 25,000 ppm (extract). A 0.5 mL sample solution was taken, then 1.5 mL of ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water were added. The sample was incubated for 30 minutes at room temperature. The absorbance of the sample was then measured at the maximum wavelength using a UV-Vis spectrophotometer (Kumar & Pandey, 2013).

Dissolution Test

Dissolution testing was initiated by preparing medium with a pH of 1.2 and 4.5. 900 mL of medium was heat to 37°C and added to a type 2 dissolution apparatus. Each container was filled with one sachet of the formulation and placed in the apparatus, which was then set to rotate at 100 rpm. Samples are collected at 5 mL intervals at minutes 5, 10, 15, 30, 45, and 60.The collected sample solutions are replaced with an equal volume of dissolution medium. Sample results are then measured for total flavonoids using UV-Vis spectrophotometry at the maximum wavelength (Perez-Chauca et al., 2022).

Data Analysis of Dissolution Test

In this study, the dissolution profile of the sprinkle formulation was determined using the following equation:

$$\% \text{ Dissolution} = \frac{Total\ dissolved\ (mg)}{Active\ substance\ content\ (mg)} \times 100\%$$

3. RESULTS AND DISCUSSION

Extraction

Maceration method is based on the flavonoid content in noni fruit. Flavonoid have been identified as compounds that are sensitive to high temperatures and can oxidize at elevated temperatures. These conditions cause flavonoid components to degrade due to the breaking of molecular chain bonds, leading to oxidation reactions that trigger the hydroxyl groups to bond with volatile compounds (Kristiana et al., 2023). The noni fruit extract obtained has a thick texture, a distinctive noni aroma, and a dark brown color. The extract yield was 21.5%.

Formulation of Sprinkle Formula

Sprinkle formulation preparations use the wet granulation method because it takes into consider the characteristics of the active compound, which is an extract with high viscosity and hygroscopic properties (Figure 1). In addition to the characteristics of the extract, the wet granulation method also has the advantage of improving flow properties and compressibility, thereby creating high-quality granules (Zarekar Shivani Gulab et al., 2024).

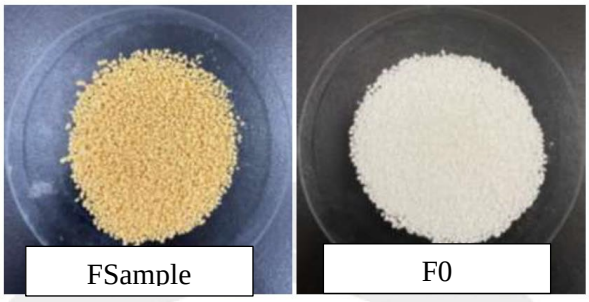

Figure 1. Sprinkle Formulation Noni Extract

Uniformity of Dosage Test

A pharmaceutical product must guarantee the consistency of its active ingredient content within a narrow range that is close to the content stated on the label of each unit of the product in the batch. To ensuring this guarantee, a uniformity test is carried out on the product, which does not apply to products external on the skin such as suspensions, gels, or emulsions. Uniformity of preparations is divided into two types of testing, which are weight uniformity and content uniformity. Sprinkle formulations use content uniformity testing because they are

classified as solid preparations in single-dose containers containing active and inactive ingredients. The results of the content uniformity testing of the triplo sprinkle formulation (Table 2).

Tabel 2. Test Results For Uniformity Of Sprinkle Formulation Content

Sample	Flavonoid Content (%)			% NP
	Rep 1	Rep 2	Rep 3	
FSampel	92.27±3.38	92.28±3.19	91.26±3.20	14.31% ± 0,4263

Uniformity testing was carried out by weighing 12 grams of each preparation 10 times, then taking 1.5 grams to be dissolved in ethanol. The content determination procedure was carried out based on the flavonoid content determination method because the active compound measured was quercetin. The test results after three replications showed that the acceptability value met the requirements because the NP% value was less than or equal to the L1% value (15%) (Vyshnavi et al., 2022). This shows that the active flavonoid compound is evenly distributed in the sprinkle formulation.

Dissolution Test

A dissolution test is an important parameter in testing the bioavailability of pharmaceutical products in vitro because the bioavailability of a drug in the body is related to its dissolution rate.To determine the dissolution rate of a drug, a dissolution test is conducted to measure the amount of active ingredient released from the drug formulation into the medium over a certain period of time. Maximum wavelength differences in pH 1.2 and pH 4.5 buffer media are caused by quercetin undergoing auto-oxidation, which is a non-enzymatic reaction by atmospheric oxygen. This condition is caused by quercetin in an aqueous medium and is influenced by the pH value and the buffering properties of the medium, leading to changes in the hydroxyl group of quercetin, which also acts as an auxochrome group affecting the maximum wavelength of the quercetin compound (Jurasekova et al., 2014) .

Standards of quercetin were prepared at concentrations of 1, 5, 10, 15, and 20 ppm. Standards were prepared to determine the relationship between absorbance and concentration of a test solution using a linear regression equation. The results of the standard curve equation at pH 1.2 are $y = 0.0070x - 0.0011$ with an R^2 value of 0.9999. In a buffer solution at pH 4.5, the equation is $y = 0.0074x + 0.0005$ with an R^2 value of 0.9986. The dissolution test of the sprinkle formulation was carried out using a type 2 test device. Samples of the sprinkle formulation (Fs) and placebo (F0) were placed in tubes and the appropriate dissolution medium

was added. Sampling was carried out at 5, 10, 15, 30, 45, and 60 minutes. Furthermore, reagents were added to the samples and incubated for 30 minutes, then the absorbance was measured at the maximum wavelength. The absorbance values obtained were used to determine the dissolved quercetin content and establish the dissolution profile of the samples (Figure 2).

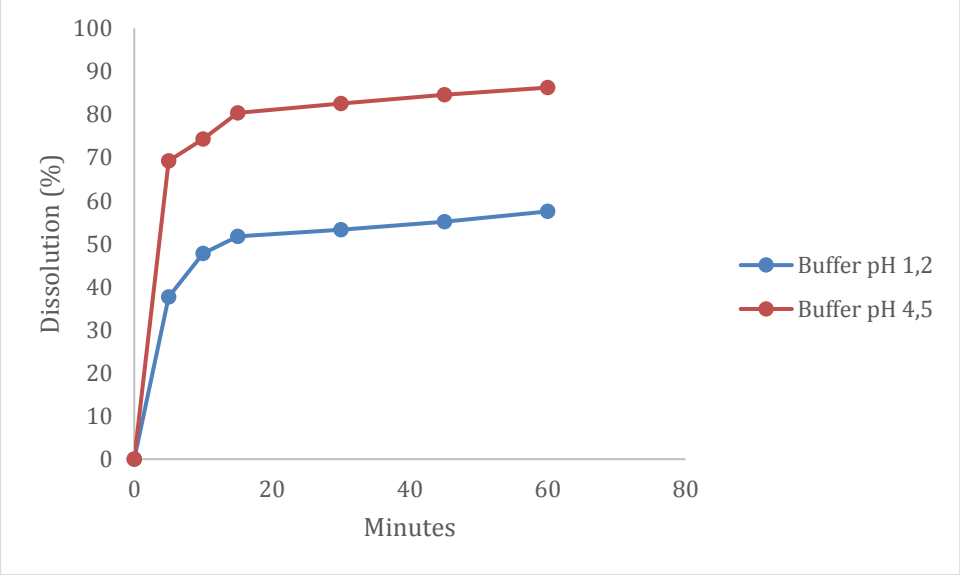

Figure 2. Dissolution Profile at Various pH Levels

A dissolution profile is a description of the release of active compounds that occurs during passage through the gastrointestinal tract per unit of time. Determination of the dissolution profile of a drug product begins with determining the concentration of active compounds dissolved in the dissolution medium per unit of time by measuring the absorbance of the active compounds from the dissolution medium. The initial release of the active ingredient is influenced by the disintegrant used. This study used sodium starch glycolate (SSG). SSG is a modified starch that can absorb large amounts of water. This expansion process causes internal mechanical forces that break the bonds between particles in the tablet, resulting in the immediate release of the active ingredient. A steady state occurs between 20 and 60 minutes, during which the concentration of the active substance tends to remain constant. This occurs because a dynamic equilibrium is achieved between the rate of dissolution of the active substance from the surface of the solid and the rate of diffusion of the dissolved substance into the dissolution medium, so that the concentration gradient in the diffusion layer remains constant (Pasupuleti Sunitha et al., 2024).

Data Analysis of Dissolution Test

Profiles of dissolution of sprinkle formulation in pH 1.2 and pH 4.5 buffer medium were analyzed using IBM SPSS Statistics 26. The data used were the cumulative percentage dissolved ($Qkum$) values shown at 60 minutes for each dissolution medium. The statistical test results obtained were normally distributed and homogeneous data, therefore the test was continued with an independent sample T-test. The results obtained from the independent sample T-test showed a Sig. (2-tailed) value < 0.05, indicating a significant difference between the cumulative percentage of dissolution ($Qkum$) in the pH 1.2 buffer medium and the pH 4.5 buffer medium. The active compound of the sprinkle formulation, quercetin, in a pH 1.2 buffer medium only reached around 37-38% at 5 minutes, in contrast to the

pH 4.5 buffer medium, which had reached > 50%. At 30 minutes, the quercetin that had successfully dissolved in the pH 1.2 buffer medium had already reached approximately 47% of the total quercetin content in the formulation, while at the same time, quercetin had dissolved in the pH 4.5 buffer medium to 81-83%. After 60 minutes of testing, the % $Qkum$ of quercetin in the pH 4.5 buffer medium had dissolved to 86.24%±0.69, while the $Qkum$ value of quercetin in the pH 1.2 buffer medium only reached approximately 57.50%±1.33.

Differences in the dissolution profile of sprinkle formulations in pH 1.2 and pH 4.5 buffer media are influenced by the characteristics of quercetin content. Quercetin is a flavonoid compound from the flavonol subclass, characterized by a molecular structure consisting of two benzene rings (rings A and B) connected by a pyran heterocycle (ring C) and five hydroxyl groups. At low pH, quercetin cannot be ionized, whereas at higher pH, ionization of quercetin occurs. This is influenced by an increase in medium pH, which causes the quercetin compound to become more negatively charged during the release of H+ cations from the molecule, forming a conjugate base and thereby increasing its solubility in water. However, at pH > 7.0, quercetin undergoes autooxidation, which is one of the common biotransformation pathways for quercetin and other flavonols. This reaction is influenced by oxygen and is known as oxidative decarboxylation (Jurasekova et al., 2014).

4. CONCLUSION

Sprinkle formulation of noni fruit extract has the most optimal dissolution profile in a pH 4.5 buffer medium with a dissolution rate of 86.23% ± 0.68 at 60 minutes. Therefore, this preparation as an immune-boosting supplement for children is best consumed when the stomach contains food (postprandial) because the stomach is at a pH of 4.5-5.5.

5. ACKNOWLEDGMENT

This research was funded by the Research and Community Service Institute (LPPM) of Universitas Pelita Harapan through its research grant program. The authors gratefully acknowledge the use of facilities at the Pharmaceutical Technology Laboratory, Faculty of Allied Health Sciences, Universitas Pelita Harapan. The authors also extend their sincere appreciation to all members of the research team for their contributions and commitment throughout the study, as well as to the authors involved in the preparation and finalization of this manuscript.

6. REFERENCE

Asare-Addo, K., Conway, B. R., Larhrib, H., Levina, M., Rajabi-Siahboomi, A. R., Tetteh, J., Boateng, J., & Nokhodchi, A. 2013. The effect of pH and ionic strength of dissolution media on in-vitro release of two model drugs of different solubilities from HPMC matrices. *Colloids and Surfaces B: Biointerfaces*. 111, 384–391. https://doi.org/10.1016/j.colsurfb.2013.06.034.

Ayuningtyas, N., Natalie, C., Monalisa, A., Pramitaningastuti, A. S., & Arianditha P, E. 2024. Optimization of Povidone and Sodium Starch Glycolate Formulas in the Formulation of Solid Sow of Noni Fruit Extract (Morindacitrifolia L.). *International Journal of Pharmaceutical Research and Applications*. 09(05), 1108–1116. https://doi.org/10.35629/4494-090511081116.

Chanthira Kumar, H., Lim, X. Y., Mohkiar, F. H., Suhaimi, S. N., Mohammad Shafie, N., & Chin Tan, T. Y. (2022). Efficacy and Safety of Morinda citrifolia L. (Noni) as a Potential Anticancer Agent. In *Integrative Cancer Therapies* (Vol. 21). SAGE Publications Inc. https://doi.org/10.1177/15347354221132848.

Jurasekova, Z., Domingo, C., Garcia-Ramos, J. V., & Sanchez-Cortes, S. (2014). Effect of pH on the chemical modification of quercetin and structurally related flavonoids characterized by optical (UV-visible and Raman) spectroscopy. *Physical Chemistry Chemical Physics*. 16(25), 12802–12811. https://doi.org/10.1039/c4cp00864b.

Kristiana, M., Fitriyana, F., & Kurnyawaty, N. (2023). Pengaruh Waktu Maserasi terhadap Senyawa Flavanoid dari Umbi Bawang Dayak. Jurnal Teknik Kimia Vokasional *(JIMSI)*. 3(2), 66–71. https://doi.org/10.46964/jimsi.v3i2.547

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. In *The Scientific World Journal*. ScientificWorld Ltd. https://doi.org/10.1155/2013/162750.

Mali, S. S., Hajare, A. A., Nitin, B. B., AdhikraoV, Y., SachinS, M., Rohan, K. A., Ashok, H. A., Sachin, S. S., & Sameer, N. J. 2014. A review on development of biorelevant dissolution medium. A REVIEW ON DEVELOPMENT OF BIORELEVANT

DISSOLUTION MEDIUM. *Journal of Drug Delivery & Therapeutics*. 2, 140. http://jddtonline.info.

Mufidah, Z., Rifa'i, M., Laboratorium, R., Hewan, F., Biologi, J., Matematika, F., Pengetahuan, I., Universitas Brawijaya, A., Malang, J. V., & Timur, J. 2013. Aktivitas Imunomodulator Ekstrak Buah Mengkudu pada Mencit yang Diinfeksi *Staphylococcus aureus*. *Jurnal Veteriner*, 14 (4).

Pasupuleti, S., Shaik, M. P., Siliveru, A. K., Souvik, S., S. Soumya, & Shaistha Tarannum. (2024). A Review on Dissolution Method Development and Validation. *IJPAR JOURNAL*. 13(4), 709–715. https://doi.org/10.61096/ijpar.v13.iss4.2024.709-715.

Perez-Chauca, E., Alva-Plasencia, P. M., & Ferraz, H. G. (2022). Development and validation of ultraviolet spectrophotometric methods for lamivudine and zidovudine quantification in dissolution test. *Journal of Pharmacy and Pharmacognosy Research*. 10(5), 865–874. https://doi.org/10.56499/jppres22.1433_10.5.847

Sagala, R. J. (2019). Review: Metode Peningkatan Kecepatan Disolusi Dikombinasi Dengan Penambahan Surfaktan. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*. 5(1), 84–92. https://doi.org/10.22487/j24428744.2019.v5.i1.12360.

Sharma, S., & Thakkar, M. (2019). *Development and validation of UV-Visible Spectrophotometric method for the estimation of Qercetin in Herbal Dosage forms Development and Validation of UV-Visible Spectrophotometric Method for the Estimation of Quercetin in Herbal Dosage Forms*. https://www.researchgate.net/publication/374264204.

Vyshnavi, K., Sinduja, Y., Adeyemi, P. G., Ebuka, A. D., Srija, M., & Sushma, G. (2022). A Review Article on Dissolution Studies in Novel Drug Delivery System. *Journal of Drug Delivery and Therapeutics*. 12(3), 220–225. https://doi.org/10.22270/jddt.v12i3.5337.

Weerapol, Y., Tubtimsri, S., Jansakul, C., & Sriamornsak, P. (2017). Improved dissolution of Kaempferia parviflora extract for oral administration by preparing solid dispersion via solvent evaporation. *Asian Journal of Pharmaceutical Sciences*.12(2), 124–133. https://doi.org/10.1016/j.ajps.2016.09.005.

Zarekar Shivani Gulab, Raykar Sonali Dattu Zende Gauri Sampat, & Puranik Aboli K and Sanjay J. Ingle. (2024). A review on the wet granulation technique and its modules. *World Journal of Biology Pharmacy and Health Sciences*. 20(2), 113–124. https://doi.org/10.30574/wjbphs.2024.20.2.0836.