Trop J Nat Prod Res, January 2024; 8(1):5801-5805 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Preparation and Characterization of Usnic Acid Nanocrystals with the Wet Grinding Method Using a Planetary Ball Mill

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ARTICLE INFO

Article history:
Received 07 October 2023
Revised 10 January 2024
Accepted 11 January 2024
Published online 01 February 2024

ABSTRACT

Usnic acid is a potential active pharmaceutical ingredient derived from *Usnea sp*. It has a low bioavailability because of poor aqueous solubility. Particle size reduction by nanosizing has been applied to improve the solubility of active pharmaceutical ingredients which is achieved by preparation of nanocrystals. This study aimed to prepare usnic acid nanocrystals by employing a wet grinding method with a planetary ball mill and using Poloxamer 188 as a stabilizer. The solid state properties of the resulting usnic acid nanocrystals were characterized with a particle size analyzer, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and dissolution rate profile. Usnic acid nanocrystals had a particle size of 358.77 ± 21.18 nm, evenly distributed with a polydispersity index of 0.591±0.02 and a zeta potential of −24.4 ± 0.1. The DSC thermogram showed a sharp endothermic peak at 193.413°C. The XRD pattern contained a high-intensity crystalline peak at 2Θ=10.213°. The dissolution rate of the usnic acid nanocrystals was 2.7 fold faster than that of the intact usnic acid. This study concluded that nanocrystals of usnic acid have improved physicochemical properties and a significantly increased dissolution rate.

***Keywords*:** usnic acid, Poloxamer 188, nanocrystal, wet grinding, planetary ball mill.

Introduction

The aqueous solubility, dissolution properties and membrane permeability of active pharmaceutical ingredients (API) are major factors in determining oral bioavailability of the API. Approaches for solving this issue are crucial in drug formulation because an increasing number of recently produced drug candidates exhibit poor physicochemical properties.

Usnic acid is a secondary metabolite derived from *Usnea sp*. It has many pharmacological properties: antibacterial, antiviral, anti-inflammatory, analgesic, antiprotozoal, and insecticidal.[1] Unfortunately, the potential of usnic acid as an API candidate has been limited by its low solubility in water.[2] Some modifications of usnic acid particles have been developed to improve its solubility, including solid dispersion with polyvinylpyrrolidone (PVP K-30), solid dispersion with hydroxy propyl methyl cellulose (HPMC 2910), inclusion complex with beta cyclodextrin (β–CD), and hydroxy propyl beta cyclodextrin (HP β–CD).[3,4]

Nanocrystals are a cutting-edge approach to enhancing drug dissolution rates and solubility. Nanoscale particles typically range from 10 to 1000 nanometers in size, offering several advantages for pharmaceutical applications.

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Citation: Wahyuni R, Lucida H, Revilla G, Ismed F, Zaini E. Preparation and Characterization of Usnic Acid Nanocrystals with the Wet Grinding Method Using a Planetary Ball Mill. Trop J Nat Prod Res. 2024; 8(1):5801-5805. http://www.doi.org/10.26538/tjnpr/v8i1.13

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

When drug particles are reduced to nanoscale dimensions, they exhibit a significantly higher surface area-to-volume ratio, allowing faster dissolution kinetics due to greater contact between the drug and the surrounding solvent.[6] This work aimed to extend nanocrystal formulation to improve the solubility and dissolution rate profile of usnic acid and to characterization its physicochemical properties.

Materials and Methods

Usnic acid was isolated from *Usnea sp* with soxhletation method using hexane:ethyl acetat (5:1) Poloxamer 188 was purchased from Sigma Aldrich (USA). Chloroform was obtained from Merck (Germany).

Preparation of usnic acid nanocrystals

Nanocrystals of usnic acid and Poloxamer 188 were prepared using the wet grinding method in a planetary ball mill PM 100 (Retsch, Germany) with a combination 30 beads of 5- and 20 beads of 10-mm diameter grinding beads made from zirconium oxide. Suspensions of usnic acid (1% w/w), Poloxamer 188 (0.5% w/w) and double-distilled water were ground for 6 hours at 400 rpm. The nanosuspensions were dried using a freeze dryer (Christ Alpha 1-2 LD Plus, Germany) at −50°C for 8 hours.

Particle size analysis, zeta potential, and polydispersity index

The particle size of the nanocrystal formulation and the polydispersity index (PI) were determined with dynamic light scattering (DLS) using a particle size analyzer (PSA, Horiba, Japan). The nanocrystals were diluted with 100 mL of double-distilled water. The measurements were performed in triplicate. The zeta potential was determined from the electrophoretic mobility with the Zetasizer instrument (Horiba, Japan).

Thermal analysis

The thermal characteristics of the samples were analyzed using differential scanning calorimetry (DSC) (Setaram type EVO 131, France). Intact usnic acid, Poloxamer 188, and the nanocrystals (30 mg

of each) were sealed in aluminum crucibles, heated at a constant rate of 10 °C/min from 30 to 300 °C, and a blank pan was used as a standard reference.[7]

X-ray diffraction
X-ray diffraction (XRD) analysis was conducted using an X-ray diffractometer (PANanalytical X'Pert, Philips, The Netherlands) with a Cu metal target and Kα filter. The voltage was 40 kV. Radiation of 40 mA was spread over the crystal region. The XRD patterns were obtained at diffraction angles between 5° and 35° at room temperature. The sample area was purged with nitrogen gas to maintain an inert atmosphere.[7]

Fourier transform infrared (FTIR) analysis
Fourier transform infrared (FTIR) spectra of usnic acid, Poloxamer 188, and the nanocrystals (1:100 sample:KBr) were collected using an FTIR spectrophotometer (Perkin Elmer, USA) at room temperature. The spectra were recorded from 450 to 4000 cm^{-1}.

Particle surface morphology
The surface morphology of the nanoparticles was investigated using a scanning electron microscopy (SEM) instrument (Hitachi S-3400N, Japan). A small amount of the sample was placed in a holder, coated with a thin layer of palladium-gold compound under vacuum, and then observed at 2000× magnification. The analysis was conducted at a voltage of 10 kV and a current of 12 mA.

Solubility of intact usnic acid and nanocrystals
The same amount of intact usnic acid and nanocrystals were placed in an Erlenmeyer flask with 100 mL of double-distilled water. The Erlenmeyer flask was then shaken for 24 h at room temperature. After reaching equilibrium, the solution was passed through a 0.45 µm Whatman filter paper. The amount of usnic acid dissolved was measured with a UV-Vis spectrophotometer (Shimadzu 1800, Japan) at 291.60 nm.

In vitro dissolution rate profile
The dissolution rate profiles of intact usnic acid and usnic acid nanocrystals were obtained using a dissolution tester (Copley Scientific type NE4-COPD, UK) by the paddle method at 100 rpm, 37 ± 0.5°C for 60 minutes in medium pH 7.4 phosphate buffer. The amount of usnic acid dissolved was measured with a UV-Vis spectrophotometer (Shimadzu 1800, Japan) at 288.60 nm.

Results and Discussion

Particle size, zeta potential, and polydispersity index
The usnic acid nanocrystals were successfully prepared using the wet grinding method with a particle size of 358.77 ± 21.18 nm. The large energy and very high shear force between the grinding medium and grinding ball obtained during the grinding process served as input energy for breaking the particles to nano-size.[8] Poloxamer 188 had a low molecular weight that contributed in reducing particle size than a stabilizer with high molecular weight. Its low molecular weight facilitated its adsorption to the surface of particle.[9] The nanocrystals showed a narrow size distribution with a PI value of 0.591±0.02 (Figure 1). The PI describes the uniformity of the particle size distribution. A value > 0.7 indicates a broad particle size distribution.[9] The zeta potential of the nanocrystals was found to be −24.5±0.1 (Figure 2), confirming a good physical stability of the nanocrystals. The repulsive energy between the particles could enhance the physical stability of the nanocrystals. The high value of the zeta potential (>+30/−30 mV) indicates a high repulsion energy. The hydrophilic chains of poloxamer 188 acted as steric barrier that surround the drug particle avoid an aggregation.[7,9,10,11]

XRD results
XRD analyzes the degree of crystallinity based on the total scattering and crystalline region scattering. The diffractogram of intact usnic acid showed a very sharp and narrow diffraction peak at 2Θ=10.2131°, with a very high intensity of 22653.1600. The diffraction pattern of Poloxamer 188 showed two sharp and narrow peaks at 19.0791° and 23.2131°, with intensities of 7857.9940 and 8688.3580, respectively. This indicates that usnic acid and Poloxamer 188 were in crystalline form. The XRD pattern of nanocrystalline usnic acid exhibited a superimposition of the peaks of usnic acid and Poloxamer 188, indicating no change in the chemical structure of usnic acid and Poloxamer 188 after the wet milling process. The peak intensities significantly decreased at 2Θ=10.2131, 19.0791, and 23.2131 to 3618.5860, 2127.6830, and 2468.8090, respectively. The XRD patterns are presented in Figure 3. The decreased intensity of the peaks occurred due to a reduction in the size of the usnic acid particles and the nanocrystal formulation.[7] The diffractogram confirmed the specific characteristics of nanocrystals, as demonstarted by the previous resaerch of dextran-coated iron oxide nanoparticles verified that the obtained nanoparticle showed a sharp peaks that confirmed a good crystallized structur and the peak broadening observed the reduction of particle size.[8]

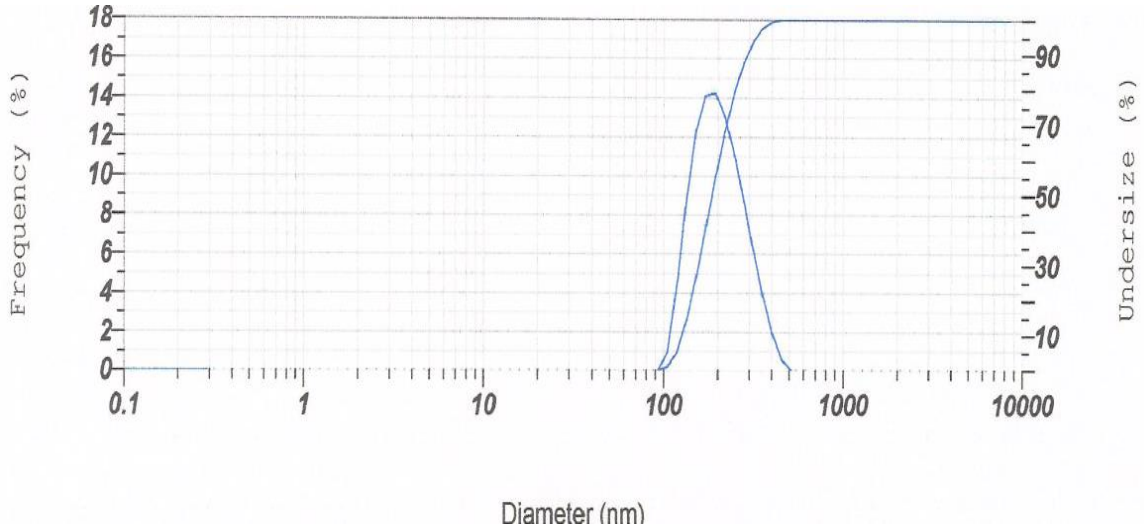

Figure 1: Particle size distribution curve and particle size cumulatif curve of nanocrystal

The crystal size of the particles could be determined from the XRD pattern using the Debye–Scherrer equation: $L = K\lambda / \beta\cos\theta$; L: crystal size, K: costanta 0.9, λ: wave length (nm), β: full width at maximum height FWMH (rad), θ:diffraction angle (rad).[13,14] The calculations using the Debye–Scherrer formula showed the crystal sizes of the intact usnic acid, Poloxamer 188, and the nanocrystals were 26.2710, 15.1248, and 25.5740 nm, respectively. The nanocrystals were only one nanometer smaller than intact usnic acid. This occurred because the stabilizer agent surrounded the surface of the core compound, increasing the diameter.

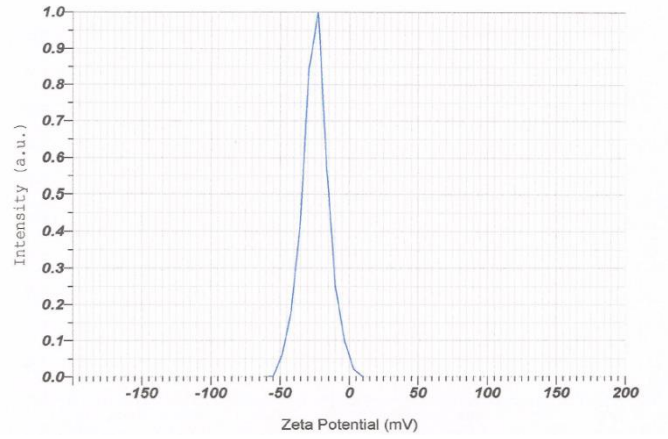

Figure 2: Zeta potential of nanocrystal

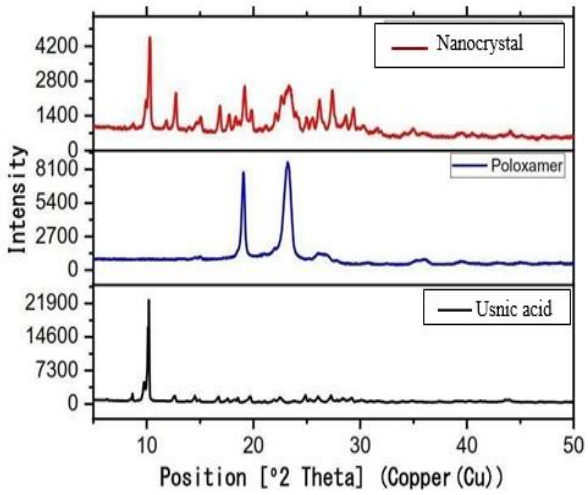

Figure 3: Diffractogram of usnic acid, Poxamer 188 and nanocrystal

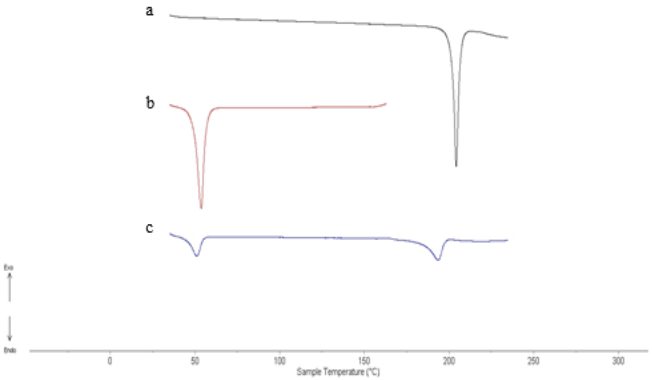

Figure 4: Differential scanning calorimetry thermogram of (a) intact usnic acid, (b) Poloxamer 188 and (c) nanocrystal

The degree of crystallinity is the amount of crystals in a particle. It can be measured by comparing the area of the crystal fraction and the area of the amorphous fraction of the curve in the XRD pattern.[13] The XRD patterns in this study confirmed that the nanocrystal formulation had the highest percentage of crystals (51.11383%) compared to intact usnic acid and Poloxamer 188 (40.94804% and 41.43362%). It can be concluded that Poloxamer 188 had already surrounded the surface of the usnic acid crystal so that the nanocrystalline state was preserved. The stability of the nanocrystals will further increase if the formulation can maintain the crystalline state of the drug.[7]

DSC results

DSC is used to obtain the thermodynamic characteristics of a solid material. Phase transformations occur during exposure to chemical potential, i.e., temperature and pressure, as seen in DSC thermograms obtained in this study. A phase transition is characterized by heat absorption or heat release during isothermal changes of substances.[16,17] DSC analysis showed a sharp endothermic peak of intact usnic acid at 204.2°C, indicating the melting point of usnic acid with a heat enthalpy of 90.596 J/g. Poloxamer 188 had a sharp endothermic peak at 53.77°C with an enthalpy of fusion of 124.549 J/g. The nanocrystal thermogram showed two sharp endothermic peaks at 51.009°C and 193.413°C with enthalpies of fusion of 31.068 J/g and 61.522 J/g, respectively. The thermograms are shown in Figure 4.

Intact Usnic acid showed a single sharp endothermic peak at 204.2°C. This peak shifted approximately 11°C lower, to 193.413°C, in the nanocrystal thermogram, indicating a reduction of usnic acid particle size.[18,19,20] The Particle Size Analysis results supported this finding. There was no glass transition temperature in the nanocrystal thermogram, which indicated the crystalline state of usnic acid and confirmed that the nanosizing process did not cause polymorphism changes within the usnic acid crystals.[7,19] The sharp peaks in the nanocrystal thermogram confirmed the XRD results that showed the solid state of usnic acid was crystalline. The heat enthalpy of usnic acid decreased from 90.596 J/g for intact usnic acid to 61.522 J/g for the nanocrystals, indicating the decreased crystallinity of the drug. These results suggest that the nanocrystals needed less energy to melt than the intact usnic acid.[21]

FTIR spectroscopy results

The FTIR spectra of intact usnic acid, Poloxamer 188, and usnic acid nanocrystals are shown in Figure 5. The usnic acid spectra showed peaks at 3401.87 (OH), 2918.17 (CH), 1692.61 (C=O), 1612.69 (C=C aromatic), and 1144.12 cm^{-1} (C-O-C). Poloxamer 188 had peaks at 3501.94 (OH), 2741.43 (C-O-C), and 1060.33 cm^{-1} (C-O-C). The nanocrystals of usnic acid had peaks at 3436.16 (OH), 2916.61 (CH), 1692.31 (C=O), 1632.10 (C=C aromatic), and 1144.21 cm^{-1} (C-O-C). Each peak in an FTIR spectrum represents a bond within a chemical substance. Collecting spectra can indicate whether any chemical interaction has occurred among active pharmaceutical ingredients and excipients. Usnic acid and Poloxamer 188 peaks were detected in the spectrum of the nanocrystals produced in this study. No changes in the characteristic bands were found in the nanocrystalline formulation.

Particle surface morphology

The SEM images of intact usnic acid showed a prismatic crystal morphology. In addition, the nanocrystalline formulation of usnic acid contains prismatic crystals with rough surface morphology. Photomicrographs of usnic acid and the nanocrystals at 2000× magnification are presented in Figure 6. As determined by SEM, the surface morphology of usnic acid is crystalline and prismatic, both in intact usnic acid and the nanocrystals. These results agreed with the DSC thermogram, which demonstrated the crystalline nature of usnic acid. Flaky crystals of Poloxamer 188 were adsorbed on the surface of usnic acid in the nanocrystals, confirming that the wet grinding process successfully prepared usnic acid nanocrystals stabilized with Poloxamer 188.

In vitro dissolution rate profile

The dissolution rate profiles of intact usnic acid and nanocrystals (Figure 7) showed that the amount of usnic acid dissolved in 60 minutes

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

was 41.971%± 1.879% for intact usnic acid and 88.262%±3.740% for the nanocrystals respectively. The dissolution efficiency increased 2.75 times for the nanocrystals compared to the intact usnic acid. Within the first 5 minutes, almost 50% of the usnic acid had dissolved from the nanocrystalline formulation, while only approximately 10% had dissolved from the intact usnic acid. Other studies have reported an increase in dissolution rate of drugs from nanocrystalline formulations.[7,20,21,22] Based on the Noyes–Whitney equation, dC/dt = kS(Cs-Ct), dC/dt: concentration of drug dissolved over the time, k: dissolution rate constant, S: surface area, Cs: concentration of drug dissloved, Ct: concentration of drug in the bulk fluid, the dissolution rate is proportional to the surface area. Reducing the particle size will increase the effective surface area and reduce the thickness of the diffusion layer. This increases the saturation solubility and dissolution rate.[18,23] These results also confirmed the DSC analysis, which showed that a lower melting point temperature could increase the dissolution rate.[20]

Conclusion

Usnic acid nanocrystals stabilized with Poloxamer 188 were successfully prepared with the wet grinding technique. This formulation improved the physicochemical characteristics and markedly enhanced the dissolution rate profile of usnic acid.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

This research was funded by the Ministry of Education and Culture Republic of Indonesia 2019 through Doctoral Research Grant no. B/87/E3/RA.00/2020.

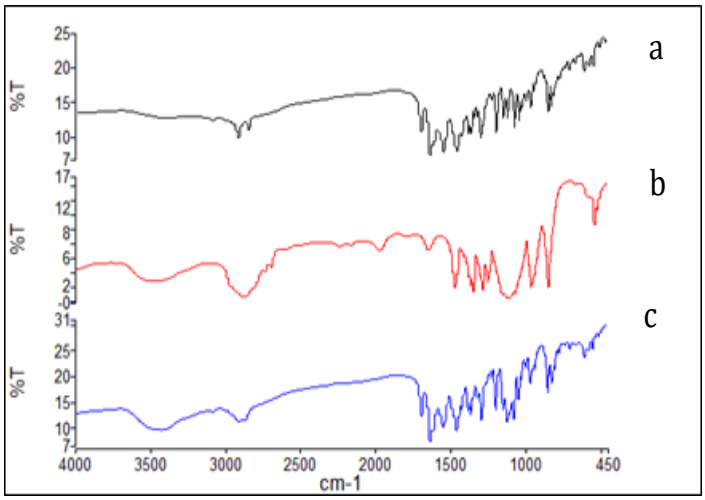

Figure 5: FT-IR spectra of (a) intact usnic acid, (b) Poloxamer 188 and (c) nanocrystal

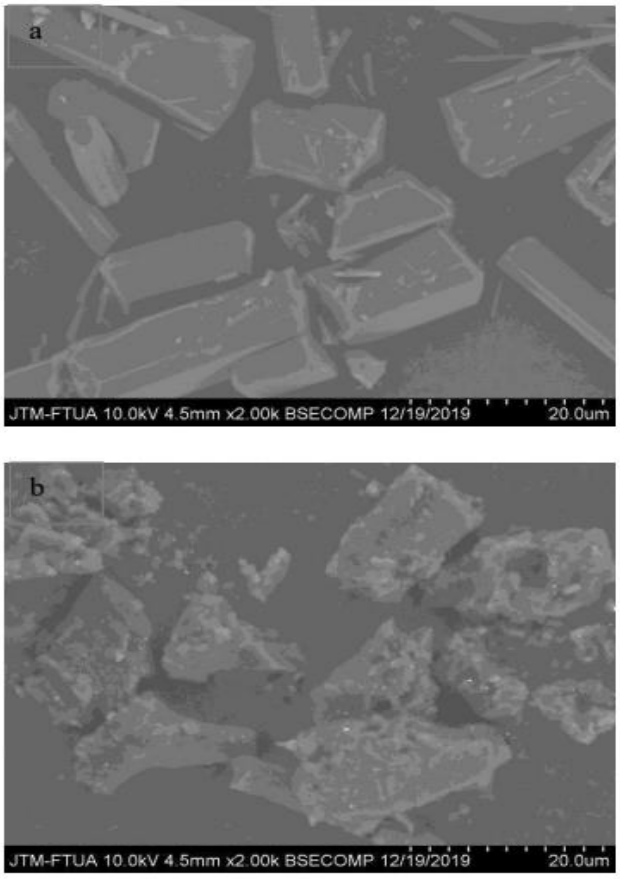

Figure 6: Micrographs of (A) intact usnic acid and, (B) usnic acid nanocrystal at a 2000× magnification

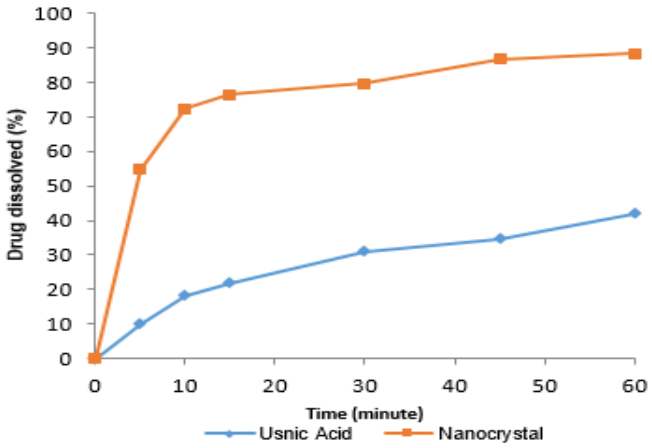

Figure 7: The dissolution profile of intact usnic acid and nanocrystal in medium phosphate buffer pH 7.4

References

1. Ingolfsdottir K. Usnic acid. Phytochemistry. 2002;61(7):729–36.
2. Jin J, Rao Y, Bian X, Yang G. Solubility of (+)-usnic acid in water, ethanol, acetone, ethyl acetate and. J Solut Chem. 2013;42(5):1018–27.
3. Fitriani L, Rismawati E, Umar S, Zaini E. Solid dispersion of usnic acid-PVP K30 and evaluation of antioxidant activity. RJC. 2018;11(4):1643–8.
4. Fitriani L, Afriyanti I, Afriyani A, Ismed F, Zaini E. Solid dispersion of usnic acid–HPMC 2910 prepared by spray

drying and freeze drying techniques. Orient J Chem. 2018;34(4):2083–8.
5. Zaini E, Nisak RK, Utami RDWI, Fitriani L, Ismed F. Effect of milling on physicochemical properties of usnic acid isolated from *Usnea sp*. Orient J Chem. 2017;33(6):3031–6.
6. Kayaert P, Mooter G Van Den. Is the amorphous fraction of a dried nanosuspension caused by milling or by drying? A case study with Naproxen and Cinnarizine. Eur J Pharm Biopharm. 2012;81:650–6.
7. Shinde A, Jadhav N, More H, Heena N. Design and characterisation of lopinavir nanocrystals for solubility and dissolution enhancement. Pharm Sci Asia. 2019;46(3):193–205.
8. Can HK, Kavlak S, ParviziKhosroshahi S, Guner A. Preparation, characterization and dynamical mechanical properties of dextran-coated iron oxide nanoparticles (DIONPs). Artificial cells, nanomedicine, and biothechnology. 2017;46(2):421-431.
9. Sathali AH, M G, Formulation and Evaluation of Paliperidone Nanocrystals. BioMedRX. 2013;1(5):422-438.
10. Peltonen L, Hirvonen J. Pharmaceutical nanocrystals by nanomilling : critical process parameters, particle fracturing and stabilization methods. JPP. 2010;62:1569–79.
11. Ozturk AA, Yenilmez E, Arslan R, Senel B, Yazan Y. Dexketoprofen trometamol-loaded Kollidon® SR and Eudragit® RS 100 D polymeric nanoparticles: Formulation and *in vitro-in vivo* evaluation. Lat Am J Pharm. 2017;36(11):2153–65.
12. Jain AK, Thareja S. Pharmaceutical nanocarriers used for drug delivery. Artif Cells, Nanomedicine, Biotechnol. 2019;47(1):524–39.
13. Monshi A, Foroughi MR, Monshi MR. Modified Scherrer equation to estimate more accurately nano-crystallite size Using XRD. WJNSE. 2012;2012(2):154–60.
14. Sumadiyasa M, Manuaba IBS. Penentuan Ukuran Kristal Menggunakan Formula Scherrer, Williamson-Hull Plot, dan Ukuran Partikel dengan SEM. Bul Fis. 2018;1(1):28–34.
15. Selvia N, Dahlan K. Sintesis dan karakterisasi tricalcium phosphate berbasis cangkang kerang ranga pada variasi suhu sintering. J Biofisika. 2012;8(1):42–53.
16. Zaini E, Azhari D, Fitriani L. Identification and characterization of solid binary system of quercetin-nicotinamide. Orient J Chem. 2016;32(3):1–6.
17. Leyva-Porras C, Cruz-Alcantar P, Espinosa-Solís V, Martínez-Guerra E. Application of differential scanning calorimetry (DSC) and modulated differential scanning calorimetry (MDSC) in food and drug Industries. Polymers (Basel). 2020;12(5):1–21.
18. Lai SL, Guo JY, Petrova V, Ramanath G, Allen LH. Size-dependent melting properties of small tin particles: Nanocalorimetric measurements. Phys Rev Lett. 1996;77(1):99–102.
19. Quan P, Xia D, Piao H, Piao H, Shi K, Jia Y, Cui F,. Nitrendipine nanocrystals: Its preparation, characterization, and in vitro – in vivo evaluation. AAPS PharmSciTech. 2011;12(4):1136–43.
20. Shinde AJ, Sankpal MS, Salokhe S V, More HN, Chitranagari N. Design and characterization of niternedipine nanocrystals for solubility and dissolution enhancement. Int J Pharm Sci Rev Res. 2020;62(11):66–72.
21. Dwichandra O, Putra D, Yonemochi E, Uekusa H. Isostructural multicomponent gliclazide crystals with improved solubility. Cryst Growth Des. 2016;1–23.
22. Du B, Shen G, Wang D, Pang L, Chen Z, Liu Z. Development and characterization of glimepiride nanocrystal formulation and evaluation of its pharmacokinetic in rats. Drug Deliv. 2013;20(1):25–33.
23. Lotfy NS, Borg TM, Mohamed EA. The promising role of chitosan–Poloxamer 188 nanocrystals in improving diosmin dissolution and therapeutic efficacy against ferrous sulfate-induced hepatic injury in rats. Pharmaceutics. 2021;13(2087):2–28.