Preparation and Characterization of Pharmaceutical Nanococrystals for Drug Delivery

Dylan G. Ramanan[1#], Nadeesh M. Adassooriya[1]

[1]Department of Chemical & Process Engineering, Faculty of Engineering, University of Peradeniya, 20400 Peradeniya, Sri Lanka
[#]Corresponding Author: dylanramanan@gmail.com

1 Introduction

Cocrystals are made up of two or more distinct molecules or ions that are kept together by non-covalent interactions such as pi-pi stacking, H bonds, and Vander Waal forces. The two components in a pharmaceutical cocrystal are the Active Pharmaceutical Ingredient (API), which causes the desired effect of the medicine, and the coformer which accommodates the API. They are commonly employed in the pharmaceutical business to facilitate drug solubility in the human body. Class II and Class IV pharmaceuticals, which account for more than 40% of the market, typically have solubility concerns (Karagianni et al., 2018). The poor solubility issue is caused by the medicines' high lipophilicity, which allows them to be absorbed more quickly into cells with fat membranes than in the blood (Koranne et al., 2019). This influences on how a medicine is absorbed, distributed, and metabolized. In order to increase solubility of drugs, various techniques have been used and one such method is the formation of cocrystals.

Downsizing of cocrystals further will form nanococrystals which are defined to have at least one dimension below 100 nm. Nanococrystals have a very large surface area to volume ratio, which enables it to have enhanced properties to those of cocrystals, such as dissolution, efficacy and targeted bio availability, which are very useful in providing efficient treatment to patients. By tuning the size and changing surface properties of these particles, we are able to control and influence the physical and chemical properties of nanococrystals (Liu et al., 2016).

In this study we are attempting to fabricate a new nanococrystal of Anthranilic acid (AA). There are drugs on the markets which use Anthranilic acid derivatives for antidiabetic and antiviral purposes (Prasher & Sharma, 2021). Maleic acid (MA) was chosen as the coformer due to its functional groups and physical properties, depicting its popularity and significance in various experiments conducted by researchers (Bhandari et al., 2020). In literature, AA has been used as a coformer (Madusanka et al., 2014), but at the moment there are no nanococrystal formulations with AA as the API. A vibratory grinding mill was utilized to perform Dry and Liquid Assisted Grinding (LAG) to synthesize various samples of nanococrystals. Characterization techniques such as FTIR, TGA, PXRD and SEM were performed to confirm the formation of new bonding and to study the nature and properties of the nanococrystal.

2 Methodology

Dry grinding and Liquid Assisted Grinding methods were followed during the synthesis of the nanococrystals. There are several advantages of LAG over dry grinding. The use of solvent during grinding, regulates the temperature when it is done for long durations at high frequency. This helps in particle motion, preventing the formation of aggregates and provides a narrow size distribution of particles formed. Whereas clumps of product and degradation of API can be observed during dry grinding.

A Retsch MM400 vibratory mill was used at a frequency of 30 Hz for 5 minutes. For LAG, solvents such as acetonitrile, methanol, hexane and water were used. Tween 20 was used as a stabilizer in certain trials. The following figure shows the overall methodology that was followed during the research.

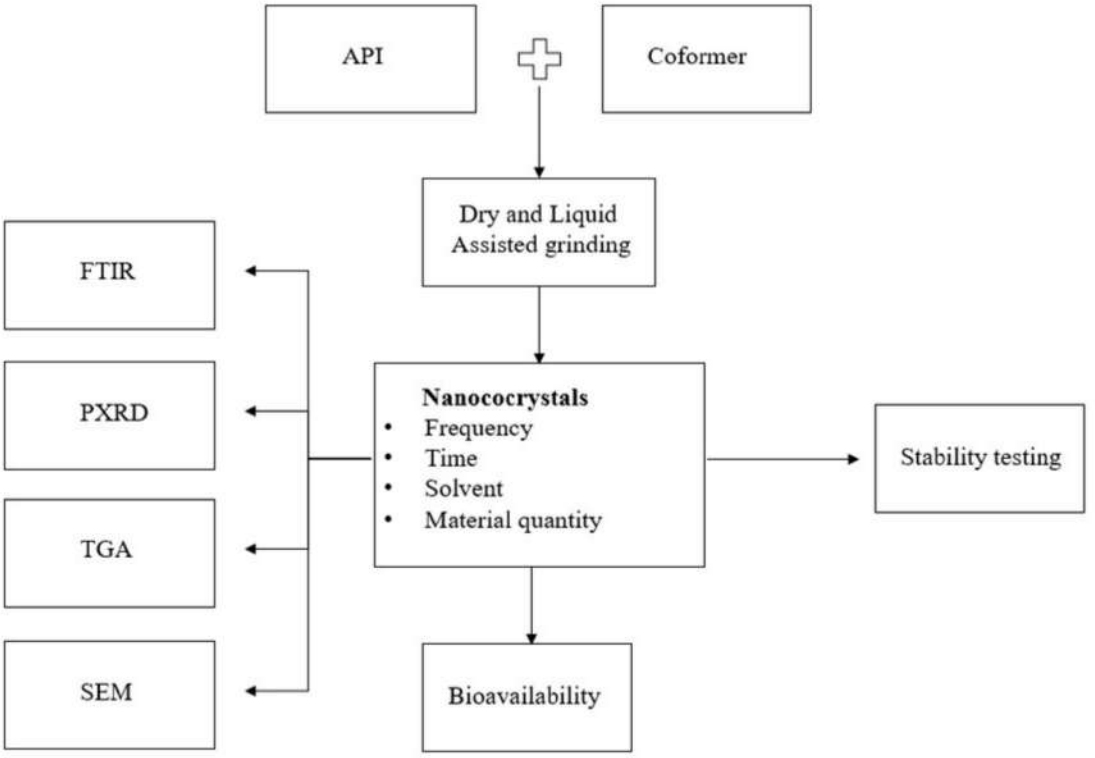

Fig. 1: Methodology of the experiments.

Initially, Anthranilic acid and Maleic acid samples were obtained in their anhydrous forms. To make sure that purity, forms and the overall condition of the samples are optimal, FTIR tests were done and compared with the FTIR spectra from literature. It was concluded that the samples were in the optimum condition to continue with research.

The molar mass of Anthranilic acid is 137.14 g/mol and the molar mass of anhydrous maleic acid is 98.06 g/mol. 200 mg sample sizes were used for each grinding turn. The controllable parameters in the fabrication process are material quantity, solvent and its quantity, frequency of milling (vibrations) and the duration for which grinding is to be done. A 1:1 molar ratio of both Anthranilic acid and maleic acid were weighted using a chemical balance (116.6 mg of Anthranilic acid and 83.4 mg of Maleic acid) and carefully transferred to the milling chambers with two 5 mm stainless steel balls (vibratory mill). The product generated was subjected to several characterization techniques, such as FTIR, PXRD, TGA, and SEM. Additionally, single crystal preparation is underway. This will assist us in determining the structure's nature; stability tests and bioavailability investigations which are now being conducted.

3 Results and Discussion

Characterization techniques such as FTIR (Fourier Transform Infrared), PXRD (Powder X-ray Diffraction), TGA (Thermo Gravimetric Analysis) and SEM (Scanning Electron Microscopy) were used to identify and confirm the formation of the nanococrystals.

FTIR was done for certain combinations of LAG with solvents and dry grinding. From FTIR, we were able to identify the formation of secondary bonds. During the formation of secondary bonding, the peaks of the primary bonds shift by a range of 10 to 100 cm^{-1}. The FTIR spectrum for AA, MA, dry ground sample of AA:MA and LAG sample of AA:MA with hexane is given in Fig. 2(c). The primary bonds which have been denoted in Fig. 2(c) are those of C-O (1200-1300 cm^{-1}) and C=O (1600-1700 cm^{-1}) (Chemistry Database, 2020). From the analysis, we can observe the obvious shifts of the C-O and C=O peaks by a range of 10 to 100 cm^{-1}. This confirms the formation of secondary bonding between Anthranilic acid and Maleic acid during dry grinding and liquid assisted grinding (Fitriani et al., 2022).

PXRD was done to find new peaks that were not originally present in the initial components and to see shifts in some significant peaks. New peaks denote the formation of new crystalline phases, which suggests cocrystallization. From the

PXRD pattern in Fig. 2(b) we can see the formation of 2 new peaks between 5 and 10 degrees which are not present in the starting materials.

TGA weight loss curves proved the absence of the formation of solvates. The sample used for TGA was AA:MA 1:1 with methanol used as the solvent. From Fig. 2(d) we can observe that there is no loss of mass up to 150 °C, which is a higher temperature than the boiling point of solvents used during LAG. If solvates were present, there would have been a loss of mass.

SEM was utilized to study the size of the particles, homogeneity and the surface properties of the crystals formed. It was done for LAG AA:MA 1:1 sample with acetonitrile used as the solvent. From Fig. 2(a), we can identify that the particles have a consistent rectangular shape with sharp edges, indicating the presence of crystal structures. The similarity in the shapes found in the figure, shows the presence of homogeneity in the sample. This shows that 1:1 ratio mixture has allowed majority of both the components to form bonding. Furthermore, we can see particles with at least one dimension below 200 nm.

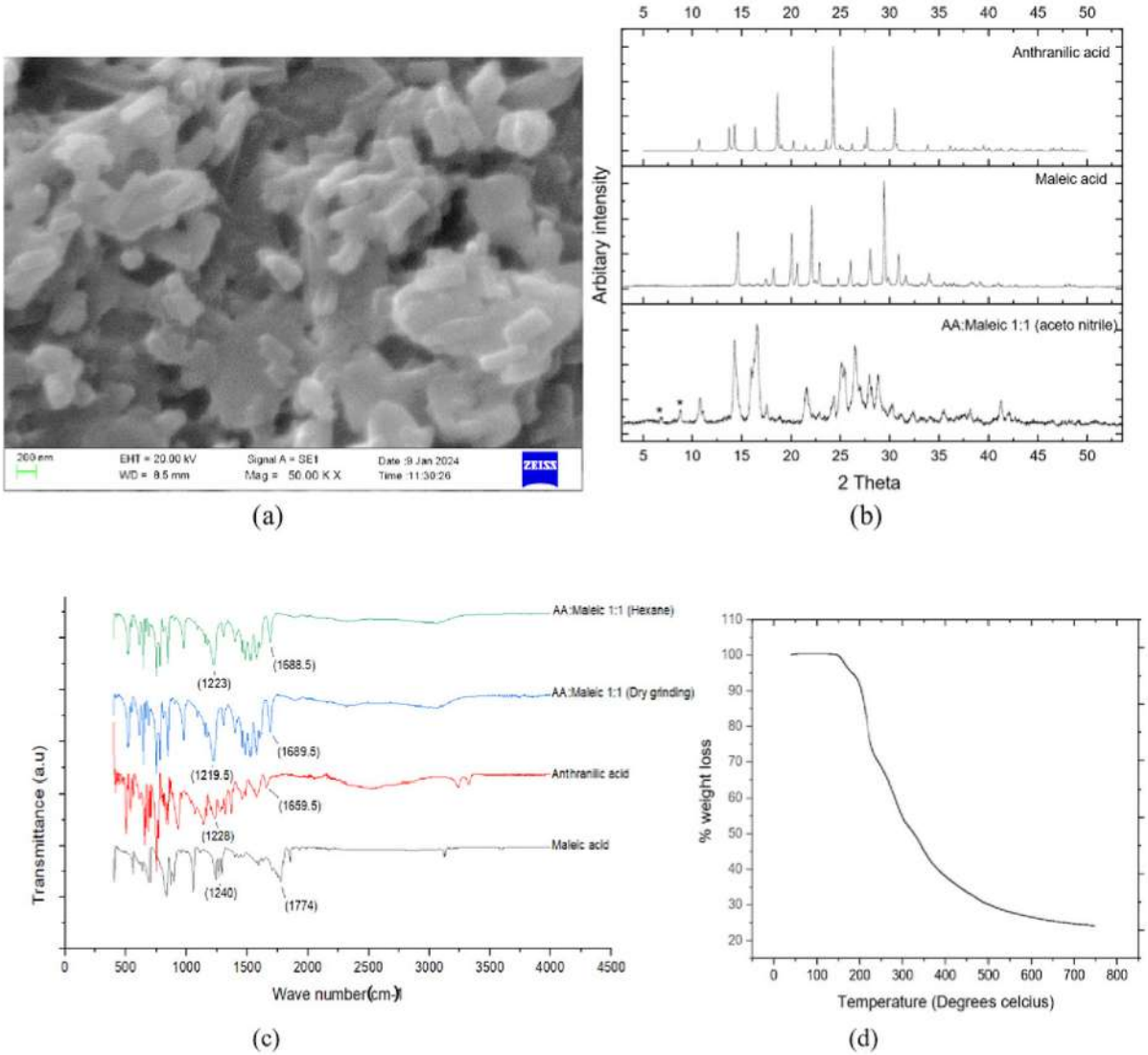

Fig. 2: (a) SEM imaging of LAG AA:MA 1:1 (acetonitrile solvent) cocrystals, (b) PXRD pattern stack of AA, MA and AA:MA cocrystal, (c) FTIR stack of AA, MA, Dry ground AA:MA cocrystal and LAG AA:MA 1:1 (hexane solvent) cocrystals, (d) TGA weight loss curve for LAG AA:MA 1:1 (methanol solvent) cocrystal

Conclusion

From the results obtained it can be stated with confidence that Anthranilic acid and Maleic acid have formed a novel nanococrystal system. The produced crystal was cross checked to see whether it has been reported previously on the Cambridge Crystallographic Data Centre (CCDC), however there were no hits, suggesting its originality. The possibility of a new nanococrystal structure of Anthranilic acid brings many opportunities to tune properties such as solubility, bioavailability, stability of it in pharmaceutical applications which are currently under investigation. Vibratory mill was the equipment used for the synthesis of nanococrystals, while dry grinding and LAG were the techniques used. From the results, it can be confirmed that the equipment is capable of downsizing particles to the nanometer range, while maintaining crystallinity, which was confirmed by the sharp shapes found in Fig. 2(a) and the distinct peaks identified in the PXRD graphs in Fig. 2(b). Bioavailability testing is currently underway, and it will help us establish on how the solubility characteristics will be altered as a result of cocrystallization and nanosizing. The introduction of nanococrystals will transform the way on how low solubility drugs will be produced and administered in a few years' time.

Keywords: Nanococrystals, cocrystals, Anthranilic acid cocrystals, Liquid assisted grinding, characterization techniques

References

Bhandari, J., Kanswami, N., & Lakshmi PK, L. (2020). Nano Co-crystal Engineering Technique to Enhance the Solubility of Ezetimibe. *Journal of Young Pharmacists*, *12*(2s), s10–s15. https://doi.org/10.5530/jyp.2020.12s.40

Chemistry Database. (2020). Infrared spectroscopy absorption Table - OChemOnline. *LibreTexts*, 1–6. https://chem.libretexts.org/@go/page/22645

Fitriani, L., Fitriandi, A. D., Hasanah, U., & Zaini, E. (2022). Nano-Cocrystals of Piperine-Succinic Acid: Physicochemical Characterization and Dissolution Rate Studies. *ChemistrySelect*, *7*(14). https://doi.org/10.1002/SLCT.202104196

Karagianni, A., Malamatari, M., & Kachrimanis, K. (2018). Pharmaceutical cocrystals: New solid phase modification approaches for the formulation of APIs. *Pharmaceutics*, *10*(1), 1–30. https://doi.org/10.3390/pharmaceutics10010018

Koranne, S., Krzyzaniak, J. F., Luthra, S., Arora, K. K., & Suryanarayanan, R. (2019). Role of Coformer and Excipient Properties on the Solid-State Stability of Theophylline Cocrystals [Research-article]. *Crystal Growth and Design*, *19*(2), 868–875. https://doi.org/10.1021/acs.cgd.8b01430

Liu, M., Hong, C., Li, G., Ma, P., & Xie, Y. (2016). The generation of myricetin-nicotinamide nanococrystals by top down and bottom up technologies. *Nanotechnology*, *27*(39). https://doi.org/10.1088/0957-4484/27/39/395601

Madusanka, N., Eddleston, M. D., Arhangelskis, M., & Jones, W. (2014). Polymorphs, hydrates and solvates of a co-crystal of caffeine with anthranilic acid. *Acta Crystallographica Section B: Structural Science, Crystal Engineering and Materials*, *70*(1), 72–80. https://doi.org/10.1107/S2052520613033167

Prasher, P., & Sharma, M. (2021). Medicinal chemistry of anthranilic acid derivatives: A mini review. *Drug Development Research*, *82*(7), 945–958. https://doi.org/10.1002/ddr.21842