RESEARCH ARTICLE

Amphiphilic Polymer for Formulation of Ethanol Extract of *Moringa oleifera* Leaves as Solid Dispersion: Formulation, Evaluation, and Stability Studies

Harith J. M. Alsammarraie[1*], Nurzalina A. K. Khan[2], Roziahanim Mahmud[3]

[1]*Department of Applied Chemistry, Faculty of Applied Sciences, University of Samarra, Iraq*
[2]*Discipline of Pharmaceutical Technology, School of Pharmaceutical Sciences, USM, Malaysia*
[3]*Discipline of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, USM, Penang, Malaysia*

Received: 04th December, 2020; Revised: 12th April, 2021; Accepted: 01st August, 2021; Available Online: 25th September, 2021

ABSTRACT

Objective: *Moringa oleifera* Lam. has many reported ethnopharmacological uses, including treating arthritis, joint problems, and pain. Like other medicinal plants, using crude or processed plants are accompanied by several drawbacks like dose-to-dose variability of concentration of active ingredient(s), fluctuation in pharmacological activity and uncertainty of chemical stability, shelf life, and suitability for human consumption. The current study aimed to formulate the ethanol extract of *M. oleifera* leaves into a standardized solid dispersion oral dosage form that is stable and can elicit high pharmacological activity.

Methods: Different types of polymers, as an adsorbent and as matrices, were tested, and different solid dispersion manufacturing methods were evaluated. The formulated *Moringa* solid dispersion was then evaluated for organoleptic properties and physical characteristics, *in vitro* dissolution test, compatibility, thermal behaviour, drug content, heavy metal tests, microbial limit tests, and accelerated and long term stability studies.

Results: 95% ethanol extract of *M. oleifera* leaves was successfully formulated as standardized self-emulsifying solid dispersion with a mixture of polymers include an amphiphilic polymer, gelucire 50/13, and a crystal growth inhibitor HPMC utilising simple and low-cost techniques.

Conclusion: To the best of our knowledge, this work was the first to use gelucire 50/13 in the formulation of a standardized botanical pharmaceutical dosage form based on whole crude plant extract.

Keywords: Gelucire 50/13, HPMC, *Moringa oleifera,* Solid dispersion.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.47

How to cite this article: Alsammarraie HJM, Khan NAK, Mahmud R. Amphiphilic Polymer for Formulation of Ethanol Extract of *Moringa oleifera* Leaves as Solid Dispersion: Formulation, Evaluation, and Stability Studies. International Journal of Drug Delivery Technology. 2021;11(3):926-936.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Up to 69% of the United States population, 90% of the British population and 71% of the Canadian population consume natural products as dietary supplements for treatment and prevention of diseases, and for avoidance of side effects associated with modern medications.[1] The interest in botanical products has been regenerated because of the discovery of many novel natural bioactive phytochemicals.[2] However, the therapeutic potential of these phytochemicals are often limited by their poor solubility, low bioavailability and product instability.[3] One of the available approaches that can solve most of these problems is solid dispersion. Sekiguchi and Obi first introduced the fundamental of solid dispersion. They proposed a solid dispersion approach to reduce particle size, enhance dissolution rate, and increase absorption by forming the eutectic mixture.[4] The term solid dispersion was coined by,[5] which defined solid dispersion as "dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by fusion, solvent or melting-solvent method". Vasconcelos and co-workers[6] classified solid dispersion into three generations. First-generation of solid dispersions were prepared as eutectic mixture using crystalline carriers like urea and sugars. This generation of solid dispersion have the disadvantage of a slow drug release rate due to the formation of thermodynamic stable crystalline solid dispersions. Second-generation solid dispersions were prepared using an amorphous carrier, usually polymers, and the drug was molecularly dispersed. Natural product polymers, like hydroxypropyl methylcellulose (HPMC),

**Author for Correspondence: ph_harith75@yahoo.com*

and fully synthetic polymers, like polyvinyl pyrrolidone and PEG, were used as carriers. Third generation solid dispersions were prepared using carriers with self-emulsifying properties or surface activity such as gelucire and inulin or a mixture of amorphous polymers and surfactants such as HPMC and polysorbates. Third-generation solid dispersions achieved the highest rate of bioavailability, avoiding drug recrystallization and providing excellent stability.[7] Nowadays, most matrices used to prepare solid dispersions are a mixture of more than one polymer and/or surfactant.

One of the medicinal plant with versatile traditional uses for variety of diseases is *Moringa oleifera* Lam. Among the claimed traditional uses of *M. oleifera* are the treatment of arthritis, joint problems and pain.[8] Martínez-González *et al.*[9] and Mahdi *et al.*[10] reported the significant effects of ethanol extract of *M. oleifera* leaves against carrageenan-induced paw oedema, collagen-induced arthritis, CFA-induced immunological rheumatoid arthritis and anti-nociceptive activity. This reported finding promotes for the formulation of 95% ethanol extract of *M. oleifera* leaves as a standardized oral dosage form.

In this study, a 95% ethanol extract will be formulated as a solid dosage form, namely, solid dispersion, which will be evaluated for their physical and chemical characteristics and subjected to *in vitro* dissolution test.

MATERIALS AND METHODS

Materials

Avicel PH 101, Avicel 200, Hydroxypropyl methyl-cellulose (HPMC) Methocel K100M CR, Poloxamer 407, Gum Arabi,c and polyvinylpyrrolidone K12.5 were purchased from Colorcon Ltd, New Hampshire, USA. Gelucire 50/13 was purchased from Gattefosse, Cedex, France. β-Cyclodextrin, Magnesium stearate, Sodium lauryl sulphate (SDS), and Tween 20, 40, and 80 were purchased from Sigma-Aldrich, St., MO, USA. Lactose monohydrate and Polyethylene glycol (PEG) 2000, 4000, and 6000 were purchased from Merck, Darmstadt, Germany. Polyplasdone XL-10 from ISP Technologies Inc., Singapore. Sodium saccharin from CK Chemical Sdn. Bhd., Selangor, Malaysia. Electrolab ETD-1020 Tap Density Tester, Globe- Pharma, Ireland; VK 7000 Dissolution apparatus, North Carolina, USA; Fourier-transform infrared spectrometer, Thermo Nicolet Nexus 470, USA; Miniflex II powder X-ray diffractometry (PXRD), Rangaku, Japan; Pyris6 DSC Differential scanning calorimeter, Perkin Elmer, Netherlands, and Shimadzu HPLC equipped with a double LC-20AD pump, SPD-20A UV-Visible detector, and SIL-20AHT auto-sampler, Shimadzu Corporation, Kyoto, Japan.

Selection of Suitable Dosage Form

A suitable dosage form was selected based on the data collected from the pharmacological activity[10] and preformulation studies.[11] Such data include minimum effective dose, physical and chemical properties like organoleptic properties, solubility profile, swelling index, pH, partition coefficient, and stress stability.

Selection of Adsorbent

Different adsorbents include lactose monohydrate, maize starch, gum Arabic, β-cyclodextrin, Avicel PH101, Avicel PH200, PEG 2000, PEG 4000, PEG 6000, polyvinylpyrrolidone K-12.5 and polyplasdone XL-10 were tested for converting the 95% ethanol extract of *M. oleifera* leaves from a greasy sticky mass to a powder form. The adsorbent(s) which show an acceptable result was then evaluated at different ratios of adsorbent-to-extract. The best adsorbent in terms of flowability and rigidity of the obtained particles with the lowest quantity of adsorbent was selected for further product development steps.

Selection of Surfactant

Surfactant was selected based on visual inspection of solubilization activity as described in.[12] Several types of anionic, non-ionic, and amphiphilic surfactants were tested and evaluated for their ability to improve the solubility of the *Moringa* extract.

Selection of Solid Dispersion Matrix

In general, highly water-soluble carriers are used to prepare solid dispersions. However, water-insoluble carriers are currently used to prepare modified-release solid dispersions.[13] Different types of carriers, carrier mixtures and carrier-to-drug ratios were examined.

Selection of Manufacturing Method

Different manufacturing methods including melt, solvent, evaporation, melt-solvent, and kneading methods, were examined. The selection of manufacturing method depended on the type of carrier(s) used and the extract-to-carrier ratio.

Selection of Other Excipients

Other excipients such as flavour, sweetener, anti-adhesive and lubricant were added as necessary, depending on the organoleptic characteristics of the crude extract and the properties of the prepared solid dispersion granules. These excipients were added to improve the quality and palatability of the final product and more patient compliance and tolerability.

Evaluation and Optimisation of Formulated Dosage Form

Evaluation of prepared dosage form was as described by.[12] The involved tests are to evaluate organoleptic properties, physical characteristics, powder flow-ability, bulk density, tapped density, Carr's compressibility index, Hausner ratio, drug-excipients compatibility, palatability, *in vitro* dissolution test and drug content.

(a) Organoleptic Properties and Physical Characteristics

Organoleptic properties of formulated *Moringa* solid dispersion (*Moringa*-SD) include appearance, colour, odour, and taste. In addition, physical characteristics include particle size distribution using sieves method, loss on drying at 65 °C, pH of 1% (w/v) solution at 25 °C and time of reconstitution was determined as described by.[12]

(b) Angle of Repose

A stainless-steel funnel with 10 mm orifice diameter and 111 mm length from the top to the end of the orifice was fixed at 4

cm from the bench to the funnel orifice. A powder sample of 5 g was charged in the funnel and allowed to fall. The height (h) and the width (w) of the pile were measured. The results were considered valid only when asymmetric cone was obtained. The procedure was repeated in triplicate. Angle of repose (θ) was calculated as follows:

$$\theta = tan^{-1} (h / 0.5w)$$

(c) Bulk Density and Tapped Density

A powder sample of 10 g was first screened through No. 18 sieve then transferred into a 25 mL graduated cylinder with 0.5 ml marks. The cylinder was gently and manually tapped two times on a tabletop surface, and the volume was measured.

Bulk density was calculated as follows:

$$\rho bulk = \frac{W}{V_b}$$

Where, w is the weight of the sample and V_b is the volume occupied by bulk powder.

The cylinder is then, fixed to tap density tester and subjected to 500, 750, and 1250 taps at a rate of 250 taps/min. After each specified number of taps, the tapped volume was measured. The tapped density was calculated as follows:

$$\rho tapped = \frac{W}{V_t}$$

Where w is the weight of the sample and V_t is the volume occupied by powder after tapping.

(d) Carr's Compressibility Index and Hausner's Ratio

The results of bulk and tapped densities were used to calculate Carr's compressibility index (*CI*) and Hausner's ratio. These parameters help in the estimation of flow properties and compressibility of the powder.

$$CI = \frac{\rho tapped - \rho bulk}{\rho tapped} \times 100$$

$$Hausner's\ ratio = \frac{\rho tapped}{\rho bulk}$$

(e) In-vitro Drug Dissolution and Drug Release Profile

In-vitro drug dissolution and drug release were determined using apparatus II (paddle apparatus). The dissolution medium was 500 ml distilled water at temperature 37°C ± 0.5 and a paddle speed of 100 revolutions per mins. Six dosage units were individually placed in each of the 6 vessels of the dissolution apparatus. Aliquots of dissolution medium (3 ml) from each of the 6 vessels were withdrawn at times 5, 15, 30, 45, 60, 90, 120, and 180 mins, combined and filtered with syringe filter 0.45 mm to get a pooled sample and used as test sample as recommended by USP37-NF30. After each sample withdrawal, an equal volume of pre-heated to 37 °C fresh distilled water was added to the dissolution medium. The samples were analyzed for drug concentration using HPLC-UV method described by.[11] Percent cumulative drug release was calculated and plotted against time. The graphical analysis method was used to compare the dissolution pattern of different formulas and reference markers concentration at each time point to compare the extent of dissolution. For characterization of drug release profile, percent dissolution efficiency (%DE) was used. DE is "the area under the dissolution curve between two specified time points expressed as a percentage of the curve at maximum dissolution, y_{100}, over the same period".[14] DE can be calculated using the following equation:

$$DE\% = \left[\frac{\int_{t1}^{t2} y \times dt}{y100 \times (t2 - t1)} \right] \times 100$$

Where y is the percent dissolved reference marker at a specified time and Y_{100} is the maximum dissolution percent.

(f) Compatibility of Excipients

Fourier transform-infra red (FT-IR) spectroscopy for *Moringa* extract, physical mixture and formulated *Moringa*-SD evaluated drug-excipients compatibility. A 1% mixture of *Moringa* extract, physical mixture or *Moringa*-SD with IR grade potassium chloride was prepared as disc and analyzed by FT-IR spectroscopy with single reflection ATR attachment. The samples were analyzed in IR range from 400 to 3000 cm^{-1}.

(g) Differential Scanning Calorimetry (DSC)

Thermal analysis of the formulated *Moringa*-SD was done to evaluate the thermal behavior, melting point, and solid-state character using DSC. About 8-10 mg of *Moringa*-SD were placed in a standard thermal aluminum pan with a comparable lid (Perkin Elmer, USA) and heated from 0 to 200°C at a 10°C/min heating rate under the heating rate pure helium atmosphere at a flow rate of 20 mL/min. An empty aluminum pan and lid were used as a reference.

(h) Powder X-ray Diffractometry (PXRD)

PXRD pattern of optimized formulated *Moringa*-SD was determined by powder X-ray diffractometer at 15 mA, 30 KV and angle speed of 4°/min over the range of 2θ from 5 to 45°, using Cu Kα radiation wavelength of 1.5406 Å.

(i) Drug Content

Drug content was determined using an HPLC-UV method described by[11]. For the test solution, an accurately weighed portion of solid dispersion equivalent to 100 mg of *Moringa* extract was dissolved in 10 ml of 50% methanol, sonicated, and filtered using a syringe filter 0.45 mm to obtain a solution of 10 mg/mL. A mixed standards solution was prepared by dissolving 1 mg each of the three reference markers, i.e., crypto-chlorogenic acid, iso-quercetin, and astragalin together in 10 ml of 50% methanol, sonicated, and filtered using a syringe filter 0.45 mm to obtain a solution of 100 µg/mL. The drug content of *Moringa*-SD was calculated using the plotted standard calibration curve. The mean % drug content was calculated as an average of three determinations.

(j) Heavy Metals

Heavy metals analysis was conducted as described by British Pharmacopeia (BP) 2013, appendix IID. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) technique was used to determine cadmium, arsenic, lead, and mercury levels.

(h) Microbial Limit

Microbial limit test for quantitative estimation of a total number of aerobic microorganisms, total yeasts and molds, bile-tolerated Gram-negative bacteria, *Salmonella sp., Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* were performed according to BP 2013, appendix XVIB.

STABILITY STUDY

Accelerated and long-term stability studies of formulated *Moringa*-SD were conducted as recommended by[17]. An accelerated stability study was conducted for 6 months at 40 ± 2°C and 75% ± 5% RH with testing frequencies of 0, 3, and 6 months. A long-term stability study was conducted for 12 months at 30 ± 2°C and 65% ± 5% RH with testing frequencies of 0, 3, 6, 9 and 12 months. The samples of formulated *Moringa*-SD were stored in separated aliquots for each testing frequency to avoid unnecessary exposure of the sample to air and humidity. At each testing frequency, the sample was evaluated for appearance, odor, taste, pH, loss on drying, reconstitution time, *in vitro* dissolution, and drug content by using HPLC–UV method. Three phytochemicals, namely, crypto-chlorogenic acid, iso-quercetin and astragalin, were used as markers compounds to evaluate chemical stability. Variations in the marker compounds concentration of ± 10% during the proposed shelf life were considered acceptable.[15-17]

DATA ANALYSIS

Data were expressed as mean ± SEM, and the statistical difference between means was statistically analyzed by IBM-SPSS version 20 software using one-way ANOVA followed by post hoc Dunnett-t test (2-tailed) at different variance levels.

RESULTS AND DISCUSSION

Selection of Adsorbent

The sticky, greasy texture of the 95% ethanol extract of *Moringa* leaves requires the use of a suitable adsorbent to convert it into non-sticky, freely flowable powder. The preliminary test of miscibility of the *Moringa* extract with different solid dispersion matrices revealed the need to add an adsorbent to avoid the formation of a sticky soft mass. The adsorbent to be tested was selected based on certain criteria, including non-alkaline amorphous nature, increased the product's solubility, availability as a very fine or micronized powder, and a high safety index. Various adsorbents were tested at the ratio of 1:1 to convert the *Moringa* extract into powder form (Table 1). The four adsorbents that showed promising results were Avicel PH101, Polyplasdone XL-10, PVP-K12.5, and gum Arabic.

Different ratios of adsorbents-to-*Moringa* extract were tested (i.e. 0.5:1, 0.6:1 and 0.75:1) to determine the lowest convenient ratio. The prepared mixtures were evaluated for their flow properties, and bulk and tapped densities (Table 2). Based on the angle of repose and density results , Polyplasdone XL-10 was selected as the adsorbent to be applied at an adsorbent-to-*Moringa* extract ratio of 0.6:1

Polyplasdone XL-10 is a white to creamy white free-flowable powder that is insoluble in water and all other common solvents with an average particle size of 25–40 µm.[18] Polyplasdone XL-10 has a high swelling capacity in water without forming a gel, high capillary activity, high porosity, rapid water intake and moisture absorption, large specific surface area, and good shelf stability.[19,20] Insolubility in water and non-gelling properties are the most important prerequisites for a well-performing solubilizer. If a polymer has any solubility in the medium, the initial stages of the water interaction will yield an intractable coating on the particle. This can partially or completely block the small pores of the particle, hindering or even wholly stopping water penetration into the narrow channels. Polyplasdone XL-10 provides favorable properties over other tested adsorbents due to its ability to improve flowability and compressibility, in addition

Table 1: Types of natural and synthetic materials tested as adsorbent for the 95% ethanol extract of *Moringa* leaf extract at a 1:1 ratio.

Adsorbent	*Comments*
Avicel PH 200	Good shape, coarse granules, dark green colour and long drying time requirement
Avicel PH101	Good shape and green-coloured granules
Lactose monohydrate	Sticky, soft, very dark-green-coloured mass and cannot be shaped as granules
Starch (maize)	Soft, light-green-coloured mass and cannot be shaped as granules
Polyplasdone XL-10	Good shape and green granules
β-Cyclodextrin	Sticky, soft dark green mass and cannot be shaped as granules
PVP-K12.5	Good shape, green granules and hygroscopic during storage
Gum Arabic	Good shape and very dark green granules.

Table 2: Evaluation of the lowest adsorbent-to-*Moringa* extract ratio for flowability and density per absorbent.

Absorbent	*Lowest Ratio*	*Angle of repose*	*Bulk density*	*Tapped density*	*Hausner's ratio*
Avicel PH101	0.6:1	30.191	0.3230	0.3488	1.0788
Polyplasdone XL-10	0.6:1	25.442	0.3018	0.3471	1.1500
Gum Arabic	0.6:1	33.690	0.4046	0.4563	1.1277
PVP-K12.5	0.75:1	37.364	0.3448	0.3726	1.0806

to its small particle size, being non-ionic, and ability to improve drug release rate.

Selection of Surfactant

Different types of surfactants, anionic and non-ionic, (Poloxamer 407, sodium lauryl sulphate, Tween 20, Tween 40, Tween 80, Span 20 and Span 40) were tested. Tween 20 (sorbitan monolaurate) showed the best solubility improvement of the *Moringa* extract. Different concentrations of Tween 20 were then tested to determine the lowest useful concentration, which has a value of 1% (w/w). In addition to its surfactant effect, Tween 20 can also improve bioavailability by acting as a P-glycoprotein inhibitor.[21]

Selection of Solid Dispersion Matrix and Manufacturing Method

Different polymers and manufacturing methods were tested (Table 3) to select the most suitable solid dispersion matrix and manufacturing method. The formula SD6 showed acceptable non-sticking granules with an angle of repose of (25.442). Accordingly, formula SD6 was selected for the next step of product development. The selection of polymer to be used as solid dispersion matrix also depends on certain properties of the polymer. In that, the polymer should have surfactant activity, allow an easy dispersion of *Moringa* extract, improve the prepared granules' flow, and produce hard and regular-shaped granules. Gelucire 50/13 is an amphiphilic, water-dispersible surfactant composed of well-characterized PEG-esters, a small glyceride fraction, and free polyethylene glycol (PEG).[22] It can self-emulsify on contact with aqueous media forming a fine dispersion i.e., micro-emulsion.[23] It increases the solubility, enhances the wettability, facilitates absorption, and improves the bioavailability of the drug.[23,24] Being an amphiphilic compound provides an advantage of minimizing the possibility of drug-excipient incompatibility.

In addition, different methods for manufacturing of solid dispersion were tested. Hot-melt method was not feasible due to the wide variation in the melting temperature of the formulation's compositions and the phytoconstituents in the extract. In particular, some of the phytoconstituents will be charred before the others melt. Hot-melt extrusion was also not applicable due to the sticky nature of the extract that will adhere to the machine wall. The solvent evaporation method was also unsuitable because no common safe solvent can dissolve all the formula's components, especially Polyplasdone XL-10. Accordingly, two solid dispersion preparation methods, namely kneading and melt-solvent, were combined and used to prepare *Moringa*-SD. These methods provide the advantages of avoiding the exposure of the active phytoconstituents to unnecessary high heat and organic solvent.

Optimizing Drug Release Rate of Prepared *Moringa* Solid Dispersion

In vitro dissolution test was conducted to evaluate the drug release profile of the prepared *Moringa*-SD. The results showed an improvement in the percent cumulative drug release between the prepared *Moringa*-SD6 and that of the crude *Moringa* extract (Figure 1). The dissolution of the

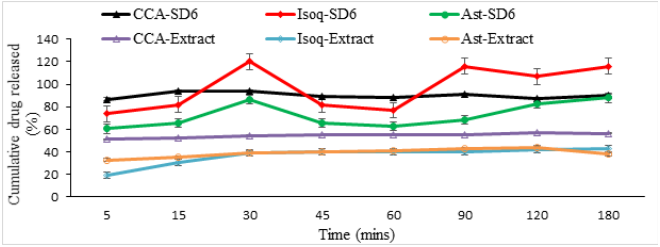

Figure 1: *In vitro* drug release profile and dissolution rate of three reference markers present in 95% ethanol extract of *Moringa* leaf and formulated *Moringa* solid dispersion (SD6). Ast= astragalin; CCA= cryptochlorogenic acid and Isoq= isoquercetin.

Table 3: Different types of materials and manufacturing methods used in formulating a solid dispersion matrix for the 95% ethanol extract of *Moringa* leaf.

Formula	*ME*	*Polyplasdone XL-10*	*G 50/13*	*PEG2000*	*PEG6000*	*PVP-K12.5*	*Gum Arabic*	*Manufacturing method*	*Comment*
SD1	3000	-	3000	1500	-	-	-	Melt-solvent method	Nonshaped, sticky, soft mass
SD2	3000	-	3000	-	-	-	1800	Kneading method	Nonshaped, sticky, soft mass
SD3	3000		3000	-	-	1800	-	Kneading method	Softening granules with diminishing shape
SD4	3000	-	1500	-	-	1800	-	Kneading and hot melt method	Granules with diminishing shape during drying
SD5	3000	1800	3000	-	-	-	-	Melt-solvent method	Granules with good shape but softened during drying
SD6	3000	1800	1500	-	-	-	-	Kneading–hot- melt method	Good-shaped granules
SD7	3000	1500	1500	-	-	-	-	Kneading–hot- melt method	Good-shaped but friable granules.
SD8	3000	-	1500	-	3000	-	-	Melt-solvent method	Nonshaped, sticky, soft mass
SD9	3000	2250	1500	-	3000	-	-	Melt-solvent and kneading method	Friable granules with diminishing shape during drying

- ME = *Moringa* extract; G 50/13= Gelucire 50/13; PEG = polyethylene glycol and PVP = poly vinyl pyrrolidine

marker compounds i.e. crypto-chlorogenic acid, isoquercetin, and astragalin in the prepared *Moringa*-SD6 increased by 1.61, 1.91 and 1.52 folds, respectively, compared with those of the *Moringa* crude extract. Table 4 displays the %DE of *Moringa*-SD6 and indicates the improvement in the drug release rate. The improvement of solubility possibility is related to the reduction of particle size, solubilization effect resulting from the high carrier concentration in the diffusion layer surrounding the solid dispersion, and/or modifications of the physical state of the drug, e.g., conversion of crystalline into a more soluble amorphous form.[5,25] It is quite often that a solid dispersion follows more than one mechanism to increase solubility.[26]

Meanwhile, the drug release profile illustrates the inconsistency in the drug release pattern, possibly because of the precipitation of the marker compounds or phase retardation of the formula.[27] The re-crystallisation or precipitation of a drug involves two stages, namely, nucleation and crystal growth. Such a process can be prevented using a polymer with anti-nucleation and phase retardation activity to form a solid dispersion matrix. HPMC exhibits such activity, that is, it can prevent agglomeration and inhibit the crystal growth of the drug and matrix.[28] HPMC dose-dependently inhibits crystal growth by repressing the nucleation of drugs, especially those rich in hydrogen-bond acceptors.[29]

Different quantities (500, 750, and 1000 mg/dose unit) of HPMC were added to the formula SD6 to optimize the drug release rate and rigidity of the *Moringa*-SD particles. About 30 mg of Tween 20 was added to 3000 mg of the *Moringa* extract. The mixture was then kneaded with 1800 mg of Polyplasdone-XL10. In three separate 250 mL beakers, 1500 mg of Gelucire 50/13 was mixed with 500, 750 or 1000 mg of HPMC pre-dissolved in distilled water. The mixture was melted in a water bath at 60°C and homogenized. While keeping the Gelucire–HPMC mixture hot, the *Moringa* extract was combined with Polyplasdone-XL10 and homogenized using an ultra-speed homogenizer (IKA ULTRA-TURRAX® T18, China) for 10 min. The homogenized mixture was quickly cooled down by spreading it over a stainless steel tray and transferred into a freezer. The cool mass was then dried in a drying oven at 45 °C for 24 h. The solidified mass was screened through sieve No. 35. The prepared *Moringa*-SDs (SD10, SD11 and SD12) were stored in tightly closed amber glass bottles. Regular-shaped, green-colored rigid granules of *Moringa*-SD were obtained. The angle of repose, bulk density, tapped density, Hausner's ratio, Carr's index, and reconstitution time was evaluated (Table 5).

Table 4: Percent dissolution efficiency of three reference markers in MoringaSD6 and 95% ethanol extract of *Moringa* leaf.

	%DE at 45 min (Mean ± SD) (n=6)		
Sample	*Cryptochlorogenic acid*	*Isoquercetin*	*Astragalin*
SD6	74.64 ± 1.393	57.81 ± 1.512	56.02 ± 1.846
Moringa extract	45.66 ± 2.044	25.66±1.965	34.25±2.176

The *in vitro* dissolution and drug release profile of the prepared *Moringa*-SDs were evaluated (Figure 2). *Moringa*-SD10 containing 500 mg of HPMC showed an inconsistent drug release pattern, reflecting insufficient amounts of HPMC added to prevent the precipitation or recrystallization of the reference markers. *Moringa*-SD11 formulated with 750 mg of HPMC achieved a more consistent drug release pattern, faster drug release rate, and greater dissolution extent than *Moringa*-SD10 and *Moringa*-SD12. *Moringa*-SD12 formulated with 1000 mg HPMC showed a consistent drug release pattern but a slow drug release rate and low extent of dissolution, possibly because of the high viscosity of the dissolution medium, as suggested by the modified Noyes–Whitney equation.

The same observation on the effect of HPMC on the solubility of silymarin solid dispersion prepared using HPMC was reported by[30]. The authors mentioned that the aqueous solubility of silymarin gets increased by 2.7-fold after adding 3% HPMC but decreased as the concentration of HPMC was further increased to 5%. It could be due to the increase in the viscosity of the solution. Accordingly, *Moringa*-SD11 was selected for further product development studies.

Not only the extent of dissolution but also the rate of dissolution are important, as described by the Noyes–Whitney equation, which could lead to low bioavailability and effectiveness. The effects of HPMC addition on optimizing the drug release rate, dissolution extent, and drug release consistency were evident (Figure 3). The cumulative drug release was significantly different between *Moringa*-SD11 and *Moringa*-SD6 (without HPMC, $P < 0.05$). The dissolution of crypto-chlorogenic acid, isoquercetin, and astragalin in the

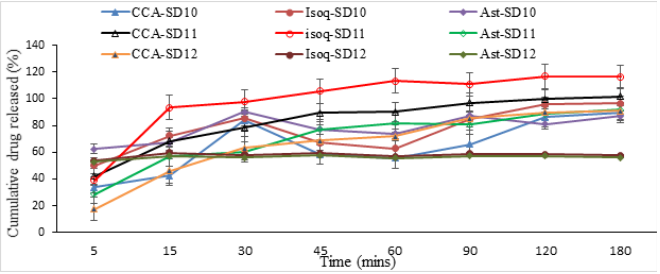

Figure 2: *In vitro* drug release profile and dissolution rate of three reference markers present in formulated *Moringa* solid dispersion SD10, SD11 and SD12. Ast= astragalin; CCA= cryptochlorogenic acid and Isoq= isoquercetin.

Table 5: Effect of adding different quantities of HPMC on the flowability, aqueous solubility and reconstitution time of the prepared *Moringa*-SD.

Formula	*Angle of repose*	*Bulk density*	*Tapped density*	*Hausner ratio*	*Carr's index*	*Aqueous solubility*	*Reconstitution time (s)*
SD10	21.516	0.3108	0.3424	1.1016	9.2289	3ml	35± 5
SD11	34.114	0.3115	0.3571	1.1463	12.7695	3 ml	40± 5
SD12	25.114	0.3184	0.3436	1.0791	7.3341	3.4ml	50± 5

prepared *Moringa*-SD11 increased by 1.01-, 1.47- and 1.43-folds, respectively, compared with that in *Moringa*-SD6. Other researchers like[31] who formulated *Kaempferia parviflora*, and[32], who formulated Osthole, a coumarin derivative, reported the same results regarding the improvement of dissolution rate and drug release profile after HPMC addition.

Organoleptic Properties and Physical Characteristics

Organoleptic properties of the formulated *Moringa*-SD include appearance, color, odor, and taste were observed (Table 6). Particle size distribution was little bit wide distribution range due to using of manual sieving and screening method. Loss on drying was within the acceptance limit of USP37-NF32. The powder flowability tests include angle of repose, Hausner's ratio, and Carr's index, indicating good flowability characters.

Fourier Transform-Infrared (FT-IR) Spectroscopy of *Moringa* Solid Dispersion

FT-IR spectra of 95% ethanol extract of *Moringa* leaves, physical mixture, and formulated *Moringa*-SD11 showed compatibility of the used excipients and *Moringa* extract (Figure 4). All major peaks of *Moringa* extract, physical mixture, and formulated *Moringa*-SD were present. The spectra did not show an additional peak, shift, or disappearance of characteristic peaks. This result confirms the absence of any chemical or physical interaction between *Moringa* extract and polymers used hereby.

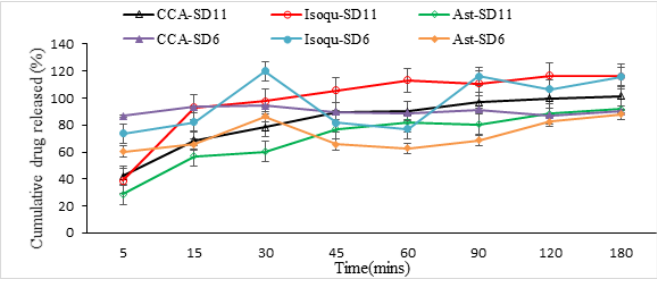

Figure 3: Effects of adding HPMC on *in vitro* drug release profile and dissolution rate of three reference markers present in formulated *Moringa* solid dispersion SD6 (without HPMC) and SD11. Ast= astragalin; CCA= cryptochlorogenic acid and Isoq= isoquercetin.

Table 6: Organoleptic properties and physical characteristics of formulated 95% ethanol extract of *Moringa* leaves as solid dispersion.

Parameter	*Result*
Appearance	Coarse granules not stick or aggregate with acceptable shape
Colour	Dark green
Odour	Faint lemon odour
Taste	Sweet with slight pungent taste
Particle size distribution:	2.6%: 700 µm 80.4%: 500 µm 12.6%: 350 µm 3.5%: 210 µm
Loss on drying (%) at 65 °C	0.642
pH of 5% aqueous solution at 25°C	4.36
Time of reconstitution	30 – 40 second
Angle of repose	34.114
Bulk density	0.3115
Tapped density	0.3571
Hausner's ratio	1.1463
Carr's index	12.76%

Differential Scanning Calorimetry

Thermal analysis of the crude *M. oleifera* extract, physical mixture, solid dispersion matrix, and formula SD11 was conducted, and the results are displayed in Figure 5. A change in the heat of enthalpy (ΔH) and a melting point of the formulated solid dispersion compared to crude *M. oleifera* extract, physical mixture and matrix were detected. The change in the heat of enthalpy is usually occurred due to physical transformation. The decrease in the ΔH means a low amount of energy required to solubilize the drug due to the conversion of the compound from the crystalline state to the more water-soluble amorphous state. The change in the melting point

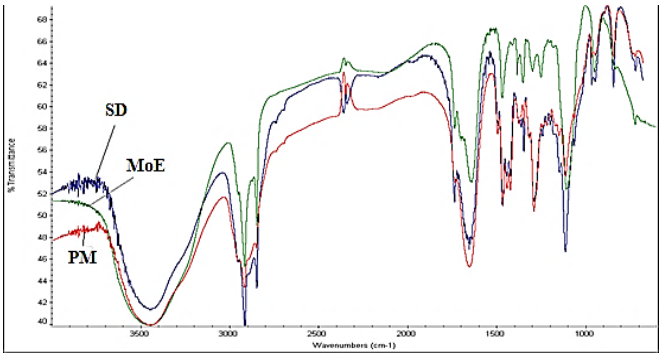

Figure 4: FT-IR spectra for *Moringa* extract (--MoE), physical mixture (--PM) and formulated *Moringa* solid dispersion (--SD).

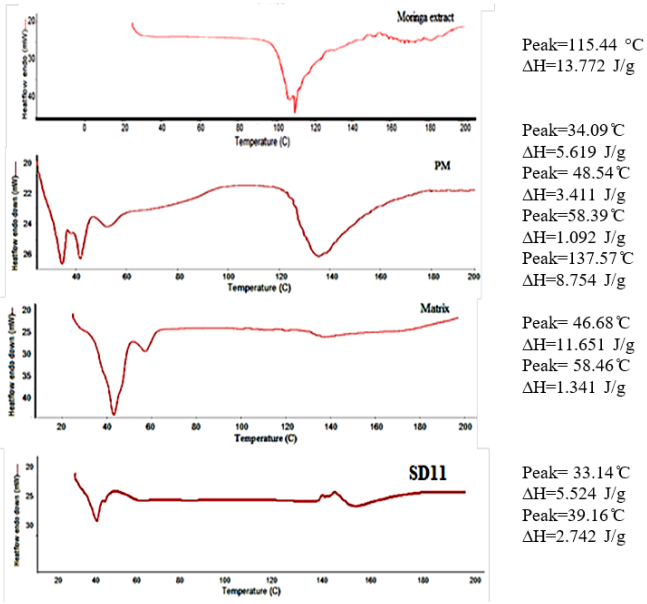

Figure 5: DSC thermogram of crude *Moringa* extract, physical mixture (PM) of the formula, prepared matrix (without *Moringa* extract) and formulated solid dispersion of *Moringa* extract (SD11) with their melting points and peak area.

It indicates a change in crystal nature or formation of amorphous material. Non-appearance of *M. oleifera* peak indicating that *M. oleifera* extract was homogenously dispersed in the matrix and was amorphous in the formulation.

Powder X-Ray Diffraction (PXRD)

Powder X-ray diffraction (PXRD) for crude *Moringa* extract, physical mixture, and formula SD11 were shown in Figure 6. The PXRD pattern of *Moringa* extract showed multi-sharp and highly intense peaks at 2θ degree of 9.58, 19.21, 21.18, and 22.86. These peaks indicate the crystal nature of phytochemicals present in *Moringa* extract. Peaks intensity of formula SD11 was noticeably decrease compared to *Moringa* extract. In addition, %crystallinity and produced area of formula SD11 were decreased, while % amorphous was increased indicating conversion in crystal state into the amorphous state.

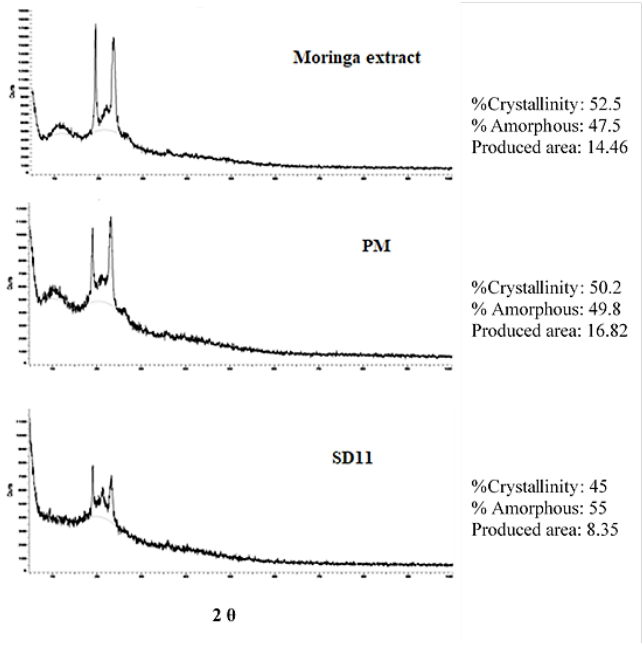

Figure 6: Powder X-ray diffractogram for *Moringa* extract, physical mixture (PM) and formulated *Moringa* solid dispersion (SD11).

Table 7: Heavy metals tests to detect arsenic, cadmium, lead and mercury levels in the formulated *Moringa* solid dispersion (SD11).

Heavy metal	*Result*	*Limit*	*Method*
Arsenic	ND	< 0.1 mg/kg	BP 2013, Appendix IID, ICP-AES
Cadmium	ND	< 0.1 mg/kg	BP 2013, Appendix IID, ICP-AES
Lead	ND	< 0.1 mg/kg	BP 2013, Appendix IID, ICP-AES
Mercury	ND	< 0.1 mg/kg	BP 2013, Appendix IID, ICP-AES

*ND: not detected and BP: British Pharmacopoeia.

Drug Content of Optimised *Moringa* Solid Dispersion

To ensure the quality of the formula, SD11 drug content was determined using HPLC-UV method. The percent drug content for crypto-chlorogenic acid, isoquercetin, and astragalin were 101.521 ± 1.653, 103.094 ± 1.442, and 97.184 ± 1.089% is equivalent to 3.598, 20.275, and 5.599 mg/g dry extract, respectively.

Heavy Metals Tests of *Moringa* Solid Dispersion

Determination of heavy metals including arsenic, cadmium, lead and mercury in the formula SD11 was described in British Pharmacopoeia (2013). The results revealed a non-detectable level of tested heavy metals (Table 7). These results confirm the suitability and safety of formulated *Moringa* solid dispersion.

Microbial Limit Tests of *Moringa* Solid Dispersion

Table 8 showed microbial limit tests for the formula SD11. *Moringa* solid dispersion showed the presence of aerobic microbes. The presence of aerobic microbial could be due to a trace amount of water in the formula. In addition, the examined sample of formula SD11 was prepared in a non-sterilized open laboratory area and non-sterile equipment.

Stability Studies

(a) Accelerated Stability Study

Samples of formula SD11 were stored at 40 ± 2°C and 75% ± 5% RH for 6 months. The organoleptic properties were evaluated at the beginning of the study and after 3 and 6 months of storage under accelerated conditions. In particular, no changes were observed in the appearance, colour, odour, taste and loss on drying of the formulated *Moringa*-SD stored for 6 months under accelerated conditions (Table 9). The change in pH of the aqueous solution of the formula SD11 was below 5% and considered not significant as per the ICH guidelines. pH is an important parameter that determines the quality and stability of pharmaceutical products. Many chemical reactions are affected by and/or affect pH.In addition to unchanged organoleptic properties, the minor change in pH reflects the chemical stability of the product. However, depending only on the evaluation of organoleptic properties and physical characteristics is insufficient to verify the product's shelf life.

Table 8: Microbial limit tests for the formulated *Moringa* solid dispersion.

Microbial type	*Result*	*Limit*	*Method*
Total aerobic microbial count (TAMC)	50	< 10 CFU/g	BP 2013, Appendix XVIB
Total combined yeasts/ moulds count (TYMC)	ND	< 10 CFU/g	BP 2013, Appendix XVIB
Bile-tolerant gram-negative bacteria	ND	< 10 PN/g	BP 2013, Appendix XVIB
Salmonella sp.	Absent	in 10 g	BP 2013, Appendix XVIB
Escherichia coli	Absent	in 1 g	BP 2013, Appendix XVIB
Staphylococcus aureus	Absent	in 1 g	BP 2013, Appendix XVIB
Pseudomonas aeruginosa	Absent	in 1 g	BP 2013, Appendix XVIB

*ND: not detected and BP: British Pharmacopoeia.

Table 9: Organoleptic characteristics, physical properties and percent drug content changes for *Moringa* solid dispersion and *Moringa* granules stored in accelerated stability conditions at 40 ± 2°C and 75 ± 5% RH for 6 months.

	Moringa solid dispersion		
Parameter	*Time 0*	*3 months*	*6 months*
Appearance	Coarse granules not stick to container wall or aggregate with acceptable shape	NOC	NOC
Colour	Dark green	NOC	NOC
Odour	Faint lemon	NOC	Less lemon odour
Taste	Sweet with slight pungent taste	NOC	NOC
pH of 5% (w/v) aqueous solution	4.36	4.41	4.47
Loss on drying (%) at 65 °C	0.462	0.503	0.511
Time of reconstitution	35–40 second	35–40 second	35–40 second
Drug content (%)			
Cryptochlorogenic acid	102.003	100.709	97.811
Isoquercetin	106.281	103.360	101.586
Astragalin	101.764	100.709	98.199

NOC: No observed change

Table 10: Organoleptic characteristics, physical properties and percent drug content changes for Moringa solid dispersion and Moringa granules stored in long-term stability conditions at 30 ± 2°C and 65 ± 5% RH for 12 months.

	Moringa solid dispersion				
	Test frequency				
Parameter	*Initial*	*3 months*	*6 months*	*9 months*	*12 months*
Appearance	Coarse granules not stick to container wall or aggregate with acceptable shape	NOC	NOC	NOC	NOC
Colour	Dark green	NOC	NOC	NOC	NOC
Odour	Faint lemon odour	NOC	NOC	Less lemon odour	Loess lemon odour
Taste	Sweet with slight pungent taste	NOC	NOC	NOC	NOC
pH of 1% (w/v) aqueous solution at 25°C	4.28	4.24	4.31	4.35	4.33
Loss on drying (%)	0.521	0.53	0.498	0.525	0.516
Time of reconstitution	35 – 40 second	35 - 40	35 - 40	35 – 40	35 - 40
Drug content (%)					
Cryptochlorogenic acid	100.206	99.605	98.243	96.822	95.643
Isoquercetin	101.424	99.119	99.124	98.138	96.827
Astragalin	103.45	103.24	101.639	99.886	99.361

NOC: No observed change

The chemical stability of the marker compounds should be evaluated to evaluate the quality of the standardized herbal formulation.

The drug content was plotted against time, and their relationship was used to construct a regression line. The time at which the regression line intersects the lower acceptable limit, i.e. 90% of the initial drug content, is considered the shelf life (data not presented). The constructed regression lines for crypto-chlorogenic acid, isoquercetin, and astragalin in formula SD11 intersected the lower limit at 18, 21 and 20 months, respectively, considered their shelf lives. As recommended by[35] for a dosage form containing more than one active ingredient, the dosage form is the shelf life of the active ingredient with a shorter one. Accordingly, for formula SD11 the estimated shelf life was 18 months.

(b) Long-term stability study

Samples of formula SD11 were stored at 30 ± 2°C and 65% ± 5% RH for 12 months. Samples were taken and evaluated for their organoleptic properties, physical properties, and chemical potency at testing frequencies of 0, 3, 6, 9, and 12 months (Table 12). The organoleptic properties and physical characteristics of the formula SD11 did not show any significant change. No change was observed in the appearance, color, odor, taste, and drying loss of the formula SD11. Regression line intersects the lower acceptable limit (90% drug content) is considered the shelf life (data not presented). The constructed regression lines for crypto-chlorogenic acid, isoquercetin and astragalin in formula SD11 intersected the lower limit at 27, 32 and 36 months, respectively, considered their shelf lives.

Stability studies of medicinal herbal products is a challenging task. The complexity of phytoconstituents, natural variation in reference marker(s) content and uncertainty of the therapeutically active phytochemical are additional factors that should be considered. Due to these factors, in addition to low concentration of reference marker in herbal products, it is recommended to consider a variation limit of ± 10%.[16]

CONCLUSION

The complexity of phytoconstituents and the indecision about the phytochemical(s) responsible for bioactivity are the most challenging problems in formulating and evaluating herbal products. The formulation of *Moringa* extracts as a solid dispersion effectively improved their physical and chemical properties, particularly flowability, crystallinity degree, aqueous solubility, drug release profile and stability under stress conditions.

Solid dispersion technique effectively resolves problems associated with 95% ethanol extract of *Moringa* leaves. DSC and PXRD confirmed a reduction in the degree of crystallinity. To prepare *Moringa* solid dispersion, a hybrid of two preparation methods, i.e. kneading and melt-solvent methods, was used. Gelucire50/13 was successfully improved solubility and drug release rate of 95% ethanol extract of *Moringa* leaves. In addition to that, HPMC was able to inhibit crystal growth and reprecipitation of dissolved phytochemicals. The favorable amount of HPMC required to improve drug release profile and dissolution extent was 750 mg/ single dose unit.

ACKNOWLEDGEMENTS

The authors would like to express their thanks and gratitude to all the staff of Discipline of Pharmaceutical Technology, School of Pharmaceutical Sciences, University of Sains Malaysia (USM) for their friends and support and kindly help in providing all the necessary chemicals, reagents and equipment.

REFERENCES

1. Kulhari Alpana, Sheorayan A, Bajar S, Sarkar S, Chaudhury A, Rajwant K. K. Investigation of heavy metals in frequently utilized medicinal plants collected from environmentally diverse locations of north western India. SpringerPlus, 2013; 2 (1): 676. DOI: 10.1186/2193-1801-2-676
2. Monera PTG, Jani ZT, Maponga CC, Mudzengi J, Morse GD, Nhachi CFB. Quality and labeling information of Moringa oleifera products marketed for HIV-infected people in Zimbabwe. Journal of public health in Africa 2016; 7 (2): 84. DOI: 10.4081/jphia.2016.618
3. Pahwa R, KatariaU, Rana AC, Rao R, Sanju N. (2015), 'Solid dispersion technology: recent advancements in the delivery of various phytoconstituents. Int J Pharm Sci Res 2015; 6 (2), 510-520. DOI:dx.doi.org/10.13040/IJPSR.0975-8232.6(2).510-20
4. Sekiguchi K, Obi N. Studies on absorption of eutectic mixture: a comparison of the behavior of eutectic mixture of sulfathiazole and that of ordinary sulfathiazole in man. Chem Pharm Bull 1961; 9 (11): 866-72.
5. Chiou WL, Riegelman S. Pharmaceutical applications of solid dispersion systems. J Pharm Sci 1971; 60 (9): 1281-302.
6. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug discovery today 2007; 12 (23-24): 1068-75.
7. Kanaujia P, Poovizhi P, Ng WK, Tan RBH. Amorphous formulations for dissolution and bioavailability enhancement of poorly soluble APIs. Powder Technology 2015; 285: 2-15. https://pubag.nal.usda.gov/catalog/6034003
8. Kesharwani S, Prasad P, Roy A, Sahu RK. (2014), 'An overview on phytochemistry and pharmacological explorations of Moringa oleifera. UK Journal of Pharmaceutical and Biosciences 2014; 2 (1): 34-41. DOI: 10.20510/ukjpb/2/i1/91151
9. Martínez-González LC, Martínez L, Martínez-Ortiz EJ, González-Trujano ME, Déciga-Campos M, Ventura-Martínez R, et al. *Moringa oleifera*, a species with potential analgesic and anti-inflammatory activities. Biomed Pharmaco 2017; 87: 482-88. https://doi.org/10.1016/j.biopha.2016.12.107
10. Mahdi HJ, Khan NAK, Asmawi MZ, Mahmud R, Murugaiyah V. In vivo anti-arthritic and anti-nociceptive effects of ethanol extract of *Moringa oleifera* leaves on complete Freund's adjuvant (CFA)-induced arthritis in rats. Integ Med Res 2018; 7 (1): 85-94. https://doi.org/10.1016/j.imr.2017.11.002
11. Alsammarraie HJM, Khan NAK, Asmawi MZ, Mahmud R, Murugaiyah V. Preformulation, stress stability studies and HPLC-UV method development and validation for 95 % ethanol extract of Moringa oleifera Lam. Leaves, Bulletin of Faculty of Pharmacy, Cairo University 2019; 57(2): 114-126. DOI: 10.21608/BFPC.2020.101083
12. The United States Pharmacopeia and National Formulary (USP 37-NF32). General tests and assays, Physical tests and determenations, <621> Chromatography, Section 4.USP The united states pharmacopoeial convention Inc. 2014.
13. Dutta S. A review on solid dispersion. International Journal of Current Research In Health And Biological Sciences 2016; 1 (1): 1-10.
14. Kassaye L, Genete G. Evaluation and comparison of in-vitro dissolution profiles for different brands of amoxicillin capsules. African Health Sciences 2013; 13 (2): 369-75. DOI: 10.4314/ahs.v13i2.25
15. Bhagwat GB, Kadam SS, Hivarale M. The accelerated stability study of constalax churna-an ayurvedic formulation. Int J Ayur Pharma Res 2017; 5(7): 85- 90.
16. Sachan AK, Kumar A. Stability testing of herbal products. J Chem Pharm Res 2015; 7(12): 511-514.
17. ASEAN guideline, ACCSQ-PPWG. (2005). ASEAN Guideline on stability study of drug product. Paper presented at the Proceedings of the 9th ACCSQ-PPWG Meeting, Manila, Philippines. https://ww2.fda.gov.ph/attachments/article/95567/1 ASEAN
18. Srinivasan R, Vinod KK, Lakshmana G, D. Rajesh DK, Yedu KM. Comparative Study of Natural and Synthetic Superdisintegrants in the Formulation of Oral Disintegrating Tablets. International Journal of Chemistry and Pharmaceutical Sciences 2015, 3(2): 1513–1518.http://pharmaresearchlibrary.com/wp-content/uploads/2015/03/IJCPS2423.pdf
19. Ramírez DG, Robles LV. Contrasting the crospovidones functionality as excipients for direct compression. Brazilian J Pharm Sci 2015; 51(1): 155-171.
20. Balasubramianam J, Bee T. The influence of superdisintegrant choice on the rate of drug dissolution. Pharmaceutical Technology Europe 2009; 21(9): 4-14.
21. Srivalli KMR, Lakshmi PK. Overview of P-glycoprotein inhibitors: a rational outlook. Brazilian J Pharm Sci 2012; 48 (3): 353-67. https://doi.org/10.1590/S1984-82502012000300002

22. Vasvári G,,Csontos B, Sovány T, Regdon G, Bényei A, Váradi J, Sinka D. Development and characterisation of modified release hard gelatin capsules, based on In Situ lipid matrix formation. AAPS PharmSciTech 2018; 19 (7), 3165-3176. https://doi.org/10.1208/s12249-018-1146-5.
23. Panigrahi, Kahnu Charan, Patra, Ch Niranjan, Jena, Goutam Kumar, Ghose, Debashish, Jena, Jayashree, Panda, Santosh Kumar, & Sahu, Manoranjan. (2018). Gelucire: A versatile polymer for modified release drug delivery system. Future J Pharm Sci 2018; 4(1): 102 -108. https://doi.org/10.1016/j.fjps.2017.11.001
24. Gaber DM, Nafee N, Abdallah OY. Myricetin solid lipid nanoparticles: Stability assurance from system preparation to site of action. European J Pharm Sci 2017; 109: 569 -580. https://doi.org/10.1016/j.ejps.2017.08.007
25. Corrigan, OI. Mechanisms of dissolution of fast release solid dispersions. Drug Devel Ind pharma 1985; 11 (2-3): 697-724. DOI:10.3109/03639048509056896
26. Sapkal S, Babhulkar M, Rathi A, Mehetre G, Narkhede M. An overview on the mechanisms of solubility and dissolution rate enhancement in solid dispersion. Int J PharmTech Res 2013; 5: 31-39. http://sphinxsai.com/2013/janmar/pharmpdf/PT=05(31-39)JM13.pdf
27. Qian F, Wang J, Hartley R, Tao J, Haddadin R, Mathias N, et al. Solution behavior of PVP-VA and HPMC-AS-based amorphous solid dispersions and their bioavailability implications. Pharm Res 2012; 29 (10): 2766-76. https://link.springer.com/article/10.1007/s11095-012-0695-7
28. Kapsi SG, Ayres JW. Processing factors in development of solid solution formulation of itraconazole for enhancement of drug dissolution and bioavailability. Int J Pharm 2001; 229 (1): 193-203. https://doi.org/10.1016/S0378-5173(01)00867-5
29. Huang Y, Dai WG. Fundamental aspects of solid dispersion technology for poorly soluble drugs. Acta Pharmaceutica Sinica B 2014; 4(1): 18-25. https://doi.org/10.1016/j.apsb.2013.11.001
30. Sonali D, Tejal S, Vaishali T, Tejal G. Silymarin-solid dispersions: characterization and influence of preparation methods on dissolution. Acta Pharmaceutica 2010; 60 (4): 427-43. DOI: 10.2478/v10007-010-0038-3
31. Weerapol Y, Tubtimsri S, Jansakul C, Sriamornsak P. Improved dissolution of Kaempferia parviflora extract for oral administration by preparing solid dispersion via solvent evaporation. Asian J Pharm Sci 2017; 12 (2): 124-33. https://doi.org/10.1016/j.ajps.2016.09.005
32. Yun F, Kang A, Shan J, Zhao X, Bi X, Li J, et al. Preparation of osthole-polymer solid dispersions by hot-melt extrusion for dissolution and bioavailability enhancement. Int J Pharm 2014 465 (1-2), 436-43. https://doi.org/10.1016/j.ijpharm.2014.02.040
33. Mahapatra AK, Murthy PN, Biswal S, Abikesh PK, Pradhan SP. Dissolution enhancement and physicochemical characterization of valsartan in solid dispersions with β-CD, HP β-CD, and PVP K-30. Dissolution Technologies 2011; 18: 39-45. DOI: doi.org/10.14227/DT180111P39
34. Bankoti K, Rana, MS, Bharadwaj MK. Accelerated stability study of herbal capsules. IOSR Journal of Pharmacy 2012; 2(5), 1-6. http://www.iosrphr.org/papers/v2i5/Part_4/A0250106.pdf
35. ICH-Q2(R1), Harmonized Tripartite Guideline. (2005). Validation of analytical procedures: text and methodology. Q2 (R1).