Formulation optimization of an improved traditional medicine from the stem bark extract of *Mangifera indica* L. using Design of Experiments approach.

Abstract

Introduction: Improved Traditional Medicines (ITMs), a recent concept by the World Health Organization (WHO) was introduced to promote the rational use of herbal medicine for primary health care in developing countries. The WHO together with the African Intellectual Property Organization (AIPO) have categorized these ITMs into 4 categories with respect to the quality of the active ingredient. Category 2 ITM referring to the formulated crude extract with shelf life studies is the quickest and hence most attractive to put in the market. However, this category needs more research in finding a greater variety of acceptable dosage forms. There is a need to account for formulation and process variables in these dosage forms to maintain product properties hence performance of plant extract, ensuring consistent quality. One of the methods to account for formulation and process variables is by using the Design of Experiments (DoE) approach. *Mangifera indica*, our plant of choice for this study is widely used in traditional medicine as an antianemic, stomache and antimalarial.

Objective: The main objective of this work was to optimize the formulation of a category 2 Improved Traditional Medicine containing *Mangifera indica* L. stem bark aqueous extract using Design of Experiments.

Method: *Mangifera indica* L. stem bark was collected and identified at the National herbarium. It was dried, ground and the powder used for extraction using digestion method using water as solvent (at 70°C). Phytochemical screening was done on the extract. The extract then proceeded unto pharmaceutical development. The formulation optimization of *Mangifera indica* aqueous stem bark extract (MIABE) started with the definition of the Quality Target Product Profile (QTPP) that was expected for the final product; which is an orodispersible tablet that will facilitate patient compliance and promote a rapid disintegration. These QTPPs formed the basis of the Critical Quality Attributes (CQAs) which were identified (as hardness, disintegration time and mass uniformity) and used for all experiments. The experimental part was divided into 2 main manufacturing processes; direct compression and wet granulation techniques. Each process was investigated for drug product optimization.

A risk assessment was undertaken to identify the formulation variables that impact product quality. For direct compression, a 3^2 full factorial DoE was used to investigate the effect of superdisintegrant (2-5%) and lubricant level (0.25-5%) on powder flow characteristics. For wet granulation, a 2^2 full factorial DoE was used to investigate the effect of superdisintegrant (2-5%) and binder (5-10%) on flow properties and tablet properties.

Results: A 42.96% yield was obtained by aqueous digestion of *Mangifera indica* L. and phytochemical screening revealed presence of tannins, saponins, phenols, flavonoids and coumarins known to have pharmacological activity. The powder blend was not suitable for direct compression and hence rejected for compression. Appropriate flow was obtained for wet granulation (WG) and so 4 batches of MIABE ODT were produced using WG. Model equations expressing effect of formulation variables were developed for predictability. An optimized formulation made up of 2% superdisintegrant and 6.36% binder was obtained.

Conclusion: Optimization models were developed for the various responses (disintegration time, wetting time and hardness) showing the influence of formulation variables on these responses. Therefore, the formulation optimization of a category 2 ITM containing *Mangifera indica* L. stem extract using Design of Experiment is a suitable approach to save time, money and improve drug product understanding.

Key words: Improved Traditional Medicines, Design of experiments, orodispersible tablets, Quality by Design

INTRODUCTION

Countries in Africa, Asia and Latin America use traditional medicine (TM) to help meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. In China, traditional herbal preparations account for 30%-50% of the total medicinal consumption. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home [1].

In the last decade traditional medicine has become very popular in Cameroon, partly due to the long unsustainable economic situation in the country. The high cost of drugs and increase in drug resistance to common diseases like malaria, bacterial infections and other sexually transmitted diseases has caused the approach to the alternative traditional medicine as an important option for a concerted search for new chemical entities (NCE). WHO in collaboration with the Cameroon Government has put in place a strategic platform for the practice and development of TM in Cameroon [2].

To protect Intellectual Property and ensure proper regulation of these traditional medicines, WHO and the African Intelligence Property Organization (AIPO) developed a categorization system of Improved Traditional Medicines (ITMs) into 4 categories. Category 1 is extemporaneous; Category 2 is the formulated crude extract with improved stability; categories 3 and 4 are more refined extract components [3]. In order to bring ITMs through the pipeline, reduction of early formulation development time and costs is crucial. Approaches that might shorten and improve drug development timeline are much sought-after [4]. In many cases, the value of the design phase is often underestimated in the rush to start development and get products to the market quickly. This can result in much wasted time and valuable resources. The quality of the design activities can strongly influence the success of development of the right product to the market and ultimate return on investment [5].

An ITM formulation is composed of several composition factors and process variables. These factors and variables do not only affect the characteristic properties of the dosage form but also render formulation difficult [6]. This implies the need to account for formulation and process variables to consistently maintain product properties hence properties and performance of extract.One approach to study the effect of formulation and process variables is to use Design of Experiment (DoE), a systematic approach introduced by the International Conference on Harmonization (ICH) and the Food and Drug administration (FDA) in the year 2002 under the canopy of Quality by Design (QbD) [7] . Pharmaceutical development has been brought into a new era. *Mangifera indica* L. (Anacardiaceae) extract was chosen as case study because of its widespread and long term use in traditional medicine as an anti-anaemic, antimalarial and anti-inflammatory agent [8–10]. Pharmacologic research has proven these properties as efficacious and toxicology studies have shown proof of safety. Patented category 2 formulations exist for this extract as capsules, conventional tablets and ointments [11]. The choice of dosage form, orodispersible tablets, is

increasingly gaining popularity due to increased patient compliance [12].The main objective of this work was the optimization of an Improved Traditional Medicine containing *Mangifera indica* aqueous stem bark extract using the Design of Experiments approach.

METHODS

Study type

The study was experimental factorial design based on the Quality by Design approach. The study was from 10th November 2016 to 5th May 2017. The raw material (Mango stem bark) was harvested at Mbangassina (Centre Region, Cameroon) and identified at the National Herbarium, Yaoundé with a voucher number **(**18646/SRFCam). Research took place in the Institute of Medical Research and the Studies of Medicinal Plants (IMPM) Yaoundé, at the phytochemistry laboratory and the Pharmaceutical Technology laboratory (LaboTEP).

Excipients

Talc, Magnesium stearate, gelatine, lactose, corn starch, methyl parahydroxybenzoate, sucrose, crospovidone and povidone were used in the manufacture of the orodispersible tablet and were gift samples provided by IMPM magazine. Strawberry powder was obtained commercially.

Herbal drug processing

Collection of the bark of *Mangifera indica* L. was collected from Mbangassina, in January 2017. It was dried under shade in an aerated room for 2 weeks. It was then ground with an electrical mill and stored in plastic bag.

Extraction

Ground bark was extracted by digestion method at 70°C with water used as solvent. 50kg of water was used for 3kg of dried powder and the process was repeated to increase yield. The extract, together with starch was dried in plates placed in an oven at 70°C to obtain a powder. The dried crude extract and starch was ground and stored in air tight plastic bags till further use. Percentage of extract in starch extract mix after drying was calculated as per equation 2 and percentage yield of extraction as per equation 3.

$$\%\ \textbf{extract in Starch extract mix} = \frac{\textbf{mass of extract in grams}}{(\textbf{starch extract mix})}\textbf{x 100}$$

Equation 1: Percentage of extract in starch-extract mix

$$\%\ \textbf{Yield} = \frac{\textbf{Mass of dry extracts in grams}}{\textbf{Intitial mass of dry unprocessed powder}}\textbf{x 100}$$

Equation 2: Percentage yield of extraction

Phytochemical Analysis

i. Test for alkaloids

1g of extract was mixed with 10ml distilled water in a test tube. To this mixture, 2% sulphuric acid was added. This was later split into 2 different test tubes in equal proportions.

a. **Meyer:** 3 drops of Meyers reagent was added to one of the test tubes. The presence of alkaloids is confirmed by the formation of white precipitates
b. **Draggendorf**: 3 drops of draggendorf reagent were added to the other test tube. Alkaloids are confirmed if formation of a brown precipitate.

ii. Test for phenolic compounds

To 0.5g of extract mixed in 5ml distilled water, a few drops of iron chloride solution were added. A resulting violet, greenish blue or black solution indicates the presence of phenolic compounds.

iii. Test for sterols and triterpenes

In a test tube containing 0.5g extract dissolved in 5ml distilled water, 3 drops of 10% potassium hydroxide is added. The solution was then heated in a water bath for 10minutes. It was left to cool; 3 drops of ether were added. This mixture is agitated, left to rest, and 3 drops of Libermann-Buchard reagent were added. The presence of triterpenes is confirmed by a violet coloration and sterols by a bluish green coloration.

iv. Test for flavonoids

A test tube containing the 0.5g extract and 5ml distilled water solution was heated for 10minutes, a few drops of methanol was added. The resulting solution was then added 3 drops of concentrated hydrochloric acid and a piece of magnesium. The presence of flavonoids is confirmed with effervescence and the brick red coloration.

Test for tannins

A mixture of 0.5g extract and 5ml distilled water was heated in a water bath and filter. To the filtrate was added 3 drops of iron chloride solution 3%. The appearance of a blue, blue black or black coloration indicates the presence of gallic tannins, a dark green indicates the presence of catechins.

Test for saponins:

Foam Test: 0.5g of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins

Quality Target Product Profile

The pharmaceutical development of MIABE ODTs began with the identification of the desired dosage form and performance attributes through the target product profile. *Mangifera indica* ODTs are being developed for clinical trial purposes.

The pharmaceutical target profile for MIABE is an orodispersible tablet that will facilitate patient compliance and promote a rapid onset action. The manufacturing process for the tablet should be robust and reproducible, and should result in a product that meets the appropriate drug product critical quality attributes. The expected quality profile for drug product is shown in (Table 1).

Table 1: Quality Target Product Profile of *Mangifera indica* L. orodispersible tablet

Quality Target Product Profile element		Target	Justification
Dosage form		Tablet	Ease of production
Dosage design		Orodispersible tablets without a score or coating	Increased patient compliance
Route of administration		Oral	Ease of administration
Dosage strength		240mg	Suitable for clinical trial for anti-anaemia properties
Drug product quality attributes	Physical attributes	The colour and shape are acceptable to the patients. No unpleasant odour, no visible defects	Patient compliance
	Content uniformity	USP standards	USP standards
	Disintegration	Less than 3 minutes	European pharmacopoeia standards
Hardness		28- 60N Robust tablet able to transport and handling.	USP standards

Critical Quality Attributes

As discussed above, the QTPP forms the basis for determining the CQAs, critical process parameters (CPPs), and Control Strategy.

From the target product profile, the initial CQAs which were used to define satisfactory quality were identified. The CQAs definition was based on empirical evidence derived from previous experimentation as well as similar experiences with other products. Table 2 indicates which quality attributes were classified as CQAs.

Table 2: Critical Quality Attributes for *Mangifera indica* L. orodispersible tablets

Quality Attributes of Drug Product	Target	CQA	Justification
Physical attributes			
Appearance	Colour and shape acceptable to patients. No visual tablet defects observed.	Yes	Changes in colour, shape and appearance can be an indication of physical and chemical degradation linked to safety and efficacy. Therefore, they are not critical. Target is set to ensure acceptability.
Odour	No unpleasant Odour	No	Neither extract nor the excipients have an unpleasant odour. No organic solvents is used in Tablet manufacturing process; therefore, considered critical
Size and Shape	Round 12mm diameter	No	For comparable ease of swallowing as well as patient acceptance and compliance with treatment regimens
Disintegration	Less than 3mins	Yes	The faster the disintegration the faster rate of dissolution.
Mass uniformity	USP Standards	Yes	Variability in mass uniformity will affect safety and efficacy.

Design of experiments (DoE)

For DoE, two factors three variables (level) (3^2) factorial was used for direct compression which requires 9 experiments. The two factors X1 (level of disintegrant) and X2 (level of lubricant) are represented by –1, 0, and +1, corresponding to the low, middle and high values respectively. These are represented on table 3 below.

Table 3: Coded Design of Experiments for Direct compression

Factor	Level		
	-1 (low)	**0 (medium)**	**1 (high)**
X1 = Disintegrant (crospovidone) %	2	3.5	5

X2 = Lubricant (Magnesium stearate) %	0.25	2.5	5

Table 4 elaborates the design into 9 formulations possible description under superdisintegrant crospovidone and lubricant magnesium stearate (% w/w).

Table 4. 3^2 factorial design used for optimization of orodispersible tablets by direct compression

Formulation	Superdisintegrant Crospovidone	Lubricant Magnesium stearate (%w/w)
F1	-1	1
F2	0	-1
F3	-1	0
F4	1	0
F5	0	1
F6	-1	-1
F7	1	1
F8	1	-1
F9	0	0

Table 5 gives the general formulation of the 9 different formulations under ingredient, function, percentage and quantity (mg).

Table 5. General composition of MIABE ODT by direct compression

Ingredient	Function	Percentages %	Quantity (mg)
MIABE	Active ingredient	60	240
Cornstarch	Superdisintegrant	20	80
Kollidon CL (crospovidone) #	Superdisintegrant	2-5	8.8-22
Magnesium stearate #	Lubricant	0.25-5	1.1-22
Talc	Glidant	1	4.4
Kollidon (Povidone)*	Binder	2	8.8
Aerosil**	Drying agent	2	8.8
Sucrose	Sweetener	5	22
Paraben	Conservative	0.03	0.13
Strawberry flavor	Flavoring agent	0.04	0.18
Lactose	Filler		40.49-74.59

Total		100	440

As per coded value per formulation

*Used in F3-F9 **Used in F1 only

The level provided for each excipient is consistent with previous experience and based on literature. The formulation has a final mass of 440mg. Table 6 gives the details of formulation for all 9 formulations.

Table 6: Detailed formulations for direct compression

Raw materials (%)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
MIABE	60	60	60	60	60	60	60	60	60
Cornstarch	20	20	20	20	20	20	20	20	20
Kollidon CL	2	3.5	2	5	3.5	2	5	5	3.5
Magnesium stearate	5	2.5	2.5	2.5	5	0.25	5	0.25	2.5
Talc	1	1	1	1	1	1	1	1	1
Kollidon	0	0	0	2	2	2	2	2	2
Sucrose	5	5	5	5	5	5	5	5	5
Strawberry powder	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Aerosil	2	0	0	0	0	0	0	0	0
Lactose	4.93	8.18	9.43	4.43	3.43	9.68	1.93	6.68	5.93
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Design of experiments

A 2^2 factorial design was implemented for the optimization of MIABE orodispersible tablet. The dependent response measured were disintegration time, hardness, friability, wetting time and water absorption ratio. Two independent factors, the concentration of crospovidone and concentration of gelatine were set at two different levels. High and low levels of each factor were coded +1 and –1, respectively. Experimental design 2^2 used for optimization of orodispersible tablets by wet granulation is shown in table 7.

Table 7: Experimental design 2^2 used for optimization of orodispersible tablets by wet granulation

Factor	**Level**	
	-1 (low)	**1 (high)**
Disintegrant (crospovidone) %	2	5

Binder (gelatine) %	5	10

Table 8 gives the coded design for the 4 formulations (F10-F13) of wet granulation. The superdisintegrant Crospovidone ranged from -1 to +1.

Table 8: Coded Design of Experiments for wet granulation

Formulation	Superdisintegrant Crospovidone	Binder: gelatine
F10	-1	-1
F11	1	1
F12	1	-1
F13	-1	1

The general formulation of the 4 batches has been represented in table 9.MIABE showed the highest (54.54%), role as active ingredient, while corn starch as superdisintegrant was 18.18%.

Table 9. General formulation for wet granulation

Raw material	Role	Percentages %
MIABE	Active ingredient	54.54
Corn starch	Superdisintegrant	18.18
Crospovidone	Superdisintegrant	2-5
Sucrose	Sweetener	5
Gelatin	Binder	5-10
Aerosil	Drying agent	2
Magnesium Stearate	Lubricant	0.5
Talc	Glidant	1.5
Strawberry powder	Flaovring agent	0.04
Paraben	Conservative	0.05
Microcrystalline cellulose	Filler /disintegrant	Qsp 100

Formulation for wet granulation. The raw material percentages for F10-F13 were of two phases (internal/intragranular phase and external/extragranular phase. The MIABE and corn starch showed no

significant difference for F10-F13.Table 10. below gives the detailed formulation for the 4 batches of wet granulation.

Table 10: Detailed formulation for wet granulation.

Raw material %	F10	F11	F12	F13
Internal /intragranular Phase				
MIABE	54.54	54.54	54.54	54.54
Corn starch	18.18	18.18	18.18	18.18
Gelatin	5	10	5	10
Parabens	0.05	0.05	0.05	0.05
External/extragranular phase				
Kollidon CL	2	5	5	2
Magnesium stearate	0.5	0.5	0.5	0.5
Talc	1.5	1.5	1.5	1.5
Aerosil	2	2	2	2
Sucrose	5	5	5	5
Strawberry powder	0.04	0.04	0.04	0.04
Microcrystalline cellulose	11.19	3.19	8.19	6.19

Technique

For wet granulations, granules were prepared by mixing MIABE, corn-starch, gelatine solution, and paraben solution till the powder became a damp mass known as the internal phase. The damp mass was passed through sieve number 18 and dried in an oven at a temperature of 70°C, until granules were dried properly. Then the dried granules were passed through sieve number 22 and subjected to mixing in of the external phase consisting of crospovidone, magnesium stearate, talc, aerosol, sucrose, strawberry powder, microcrystalline cellulose. It was mixed for 15minutes. The obtained blend was then compressed with punches by using tableting machine set at 7.5N.

Pre-compression evaluation of powder blend

i. Bulk density

Density is defined as weight per unit volume. Bulk density was defined as the mass of the powder divided by the bulk volume. Powder was poured into a graduated cylinder; the volume and weight were measured. It was expressed in g/cm3 and was given by

$$Db = \frac{M}{V0}$$

Equation 3: Bulk density

Db = bulk density, M = Mass of powder, V0 = Bulk volume of the powder.

Tapped density

It was determined by placing a graduated cylinder, containing a known mass of drug-excipient blend on mechanical tapping apparatus. The tapped volume was measured by tapping the powder to constant volume. Tapping was continued, "Until no further change in volume was noted". It is expressed in g/ml and is given by Dt= tapped density, M=Mass of powder, Vt=Tapped volume of the powder

$$Dt = \frac{M}{Vt}$$

Equation 4: Tapped Density

Angle of repose:

It is maximum angle between the surface of a pile of powder and horizontal plane, when powders are allowed to flow freely from a certain height. It can be measured by fixed funnel and cone method. The powder mass is allowed to flow through the funnel kept on a stand at a fixed height. The powder is carefully poured through the funnel on to the surface. Measuring the radius (r) and height (h) of pile repose angle can be measured.

$$\mathbf{Tan\ \theta} = \frac{h}{r}$$

h = height of cone, r = radius of cone

Equation 5: Angle of repose

Carr's index:

The measurement of free flow property of powder is called compressibility, an indication of the ease with which a material can be induced to flow is given by % compressibility. It can be calculated by the following formula

$$C = \frac{(Dt - Db)}{Dt} x\ 100$$

Dt = Tapped density, Db = Untapped bulk density

Equation 6: Carr's index

Hausner's ratio:

It is calculated by using the following formula.

$$H = \frac{Db}{Dt}$$

Dt - tapped density of the powder, Db - Bulk density of the powder.

Equation 7: Hausner's ratio

Swelling index of superdisintegrant:

The study was carried out using 100 ml stoppered graduated cylinder. The initial bulk volume of 10g superdisintegrant (cornstarch, crospovidone) was noted separately. Water was added in sufficient quantity to produce 100 ml of a uniform dispersion. The sediment volume of the swollen mass was measured after 24 hours, stored at room temperature.

The swelling index was then calculated as:

$$\text{Swelling index} = \frac{(V2 - V1)}{V1} \text{x } 100$$

Equation 8: swelling index

Where, V1 and V2 are initial volume of material before hydration and volume of hydrated material, respectively.

Post-compression evaluation of tablets

Tablets were subjected to following evaluation parameters.

i. **Colour and appearance**

For the colour and appearance, the tablets were visually examined.

ii. **Hardness**

The crushing load which is the force required to break the tablet in radial direction was measured using a Schleuniger-2E hardness tester. Ten tablets from each formulation batch were tested randomly and average hardness was calculated. It is given in newton.

iii. **Thickness and Diameter**

The thickness and diameter of tablets was determined using a Vernier caliper. Ten tablets from each type of formulation were used and average values were calculated.

iv. Weight Variation

Weigh individually 20 units taken at random or, for single-dose preparations presented in individual containers, the contents of 20 units, and determine the average mass. Not more than 2 of the individual masses should deviate from the average mass by more than the percentage deviation shown in Table 11 and none deviates by more than twice that percentage.

Table 11: Weight variation according to European Pharmacopeia

Pharmaceutical Form	Average Mass	Percentage deviation
Tablets (uncoated and film coated)	80 mg or less	10
	More than 80 mg and less than 250 mg	7.5
	250 mg or more	5

v. Friability

10 tablets were weighed and placed in the ERWEKA TA friabilator test apparatus, the tablets were exposed to rolling and repeated shocks, resulting from free falls within the apparatus. After 100 revolutions the tablet were de-dusted and weighed again. The friability was determined as:

$$\textbf{Friability} = \frac{(\textbf{Initial tablet weight} - \textbf{Final weight})}{\textbf{Initial weight}} \textbf{x 100}$$

Equation 9: Friability determination

vi. Water absorption ratio

A piece of tissue paper folded twice was placed in small petri dish (10cm diameter) containing 6ml of Phosphate buffer ph. 7.0. A tablet was put on the tissue paper and allowed to wet completely.

The wetted tablet was then reweighed. Water absorption ratio, R was determined using following equation:

$$\textbf{R} = \frac{(\textbf{Wa} - \textbf{Wb})}{\textbf{Wb}} \textbf{x 100}$$

Equation 10: Water absorption ratio

Where Wa=weight of tablet after absorption, Wb=weight of tablet before absorption

Results were presented in terms of mean ± standard error. The test ANOVA (Analysis of Variance) was used to compare multiple samples (Formulations). The test *Posthoc* of Tukey was used to compare the samples two-by-two. The data were analysed using the software IBM/SPSS (Statistical Package for Social Science) 20.0 for Windows and graphs were represented using Microsoft (MS) Excel 2013. The significance level was fixed at $P \leq 0.05$.

A repeated 2 x 2 factorial design with 3 replications was used. The test ANOVA was used to assess the significance of the independent variables in the statistical models. R^2 and adjusted R^2 were used to evaluate the robustness of dependent variables to be explained by the independent variables. The desirability function was used to identify the optimal conditions for the independent variables. Statgraphics Plus 5.1 and Statistica 10.0 were used for developing statistical equations. The significance level was fixed at 0.05.

Ethical considerations

Ethical clearance for this work was obtained from the Institutional Review Board (IRB) of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. Furthermore, we obtained administrative authorisations from the Institute of Medical Research and the Studies of Medicinal Plants (IMPM) to carry out all the required laboratory work and procedures.

RESULTS

A mean percentage yield 42.96% was obtained with water extraction by digestion. Greater percentage yield was observed when the water: drug extract was increased from 12.5:1 (batch 1) to 16.7:1 (batches 2-7). The extraction of *M indica* by digestion is shown in Table 12.

Table 12: Extraction of *Mangifera indica* L. by digestion

Batch	**Starting mass (Kg)**	**Amount of extract (g)**	**Yield (%)**
1	4	1555.9	38.9
2	3	1301.9	43.40
3	3	1294.0	43.13
4	3	1593.6	53.12
5	3	1284.5	42.81
6	3	1070.4	35.68
7	3	1309.5	43.65
Total	22	9409.8	**42.96**

Phytochemical screening

Phytochemical screening provided plants with colour, flavour and natural protection against pests. A phytochemical screening was performed on the *Mangifera indica* stem bark aqueous extract and the results have been shown in table 13. Phytochemical screening showed the absence of alkaloids and triterpenes and detected the presence of active pharmaceutical components such as tannins, saponins, phenols, flavonoids and coumarins.

Table 13: Phytochemical screening of aqueous stem bark extract of *Mangifera indica* L.

Phytochemical	MIABE Mbangassina
Alkaloids	-
Saponins	++
Phytosterols and triterpenes	-
Phenols	+++
Tannins	++++
Flavonoids	+++
Reducing sugars	+++
Coumarins	++

+= Detectable presence ++= slightly abundant +++= abundant ++++ = very abundant

Pre-compression studies

To determine the flow properties of our powder blends, bulk and tapped densities, Carr's index (1), Hausner's ratio (2), and the angle of repose (3) were analysed before compression into tablets. Table 14 shows pre-compression parameters of powder mixture.

Table 14: Pre-compression parameters of powder blends

Method	Formulation	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose (°)	Carr's index	Hausner's ratio
Direct compression	**F1**	0.68	0.88	33.33 ± 0.88^{ab}	23.0	1.29
	F2	0.65	0.90	35.67 ± 0.33^{ab}	27.8	1.38
	F3	0.67	0.94	29.33 ± 0.88^{a}	29.0	1.40
	F4	0.67	0.94	33.00 ± 3.00^{ab}	25.5	1.40

	F5	0.63	0.86	35.33 ± 1.85^{ab}	26.7	1.36
	F6	0.63	0.85	36.67 ± 1.76^{bc}	27.0	1.37
	F7	0.66	0.86	36.00 ± 0.01^{ab}	23.0	1.30
	F8	0.59	0.81	43.67 ± 0.67^{c}	27.0	1.37
	F9	0.56	0.89	36.67 ± 0.67^{bc}	37.0	1.59
Wet granulation	**F10**	0.67	0.80	27.33 ± 0.88^{b}	16.3	1.19
	F11	0.72	0.79	18.63 ± 1.13^{a}	8.9	1.09
	F12	0.63	0.79	28.47 ± 0.78^{b}	20.3	1.25
	F13	0.67	0.76	20.97 ± 2.91^{ab}	11.8	1.13

Legend *: Values carrying the same letter for the same method are not statistically different ($p \geq 0.05$)*

Densities

The bulk density for direct compression ranged from 0.56-0.68g/cm^3 and 0.63-0.67g/cm^3 for wet granulation. Tapped density ranged from 0.81-0.94g/cm^3 for direct compression and 0.76-0.80g/cm^3 for wet granulation technique

Carr's index and Hausner's ratio

For direct compression, we obtained the mean values of Compressibility index (27.33) and Hausner's ratio (1.38). As for wet granulation, we had lower values for both Compressibility index (14.33) and Hausner's ratio (1.17). These are summarised in the tables 15 and figure 1 below:

INTERPRETATION	
Carr's index	**Powder flow**
5-15	Excellent
12-16	Good
18-21	Fair to Passable
23-35	Poor
33-38	Very poor

Table 15: Carr's index Extremely poor interpretation

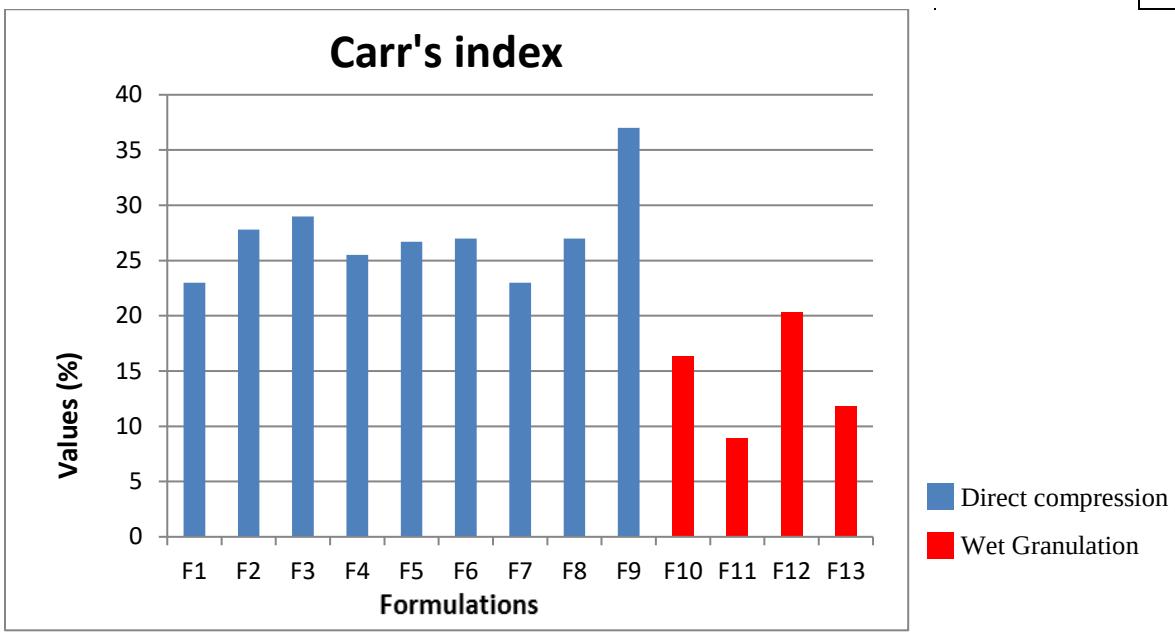


Figure 1: Carr's index values for all formulations

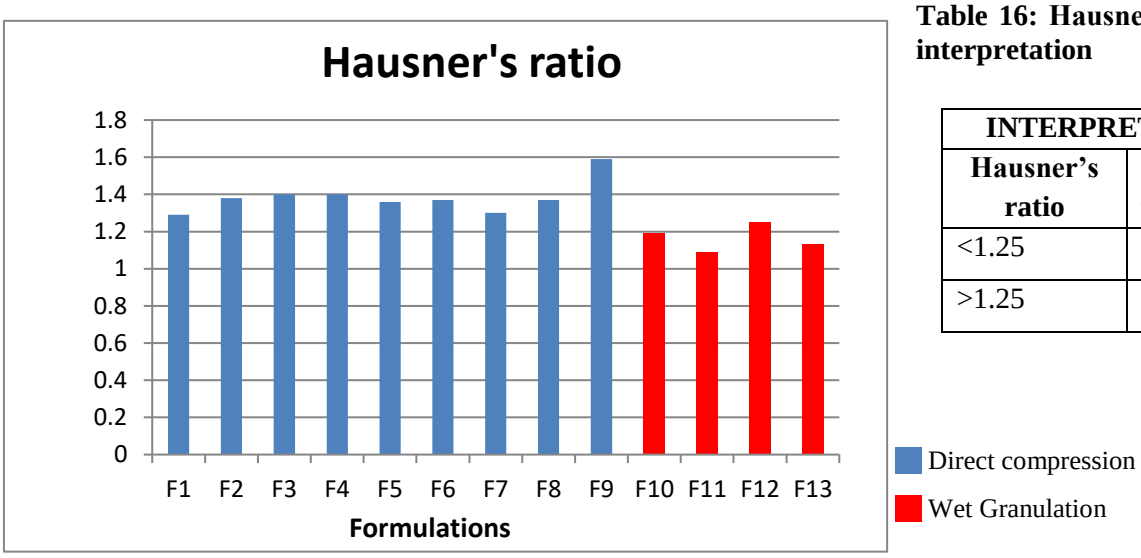


Figure 2. Hausner's ratio values for all formulations

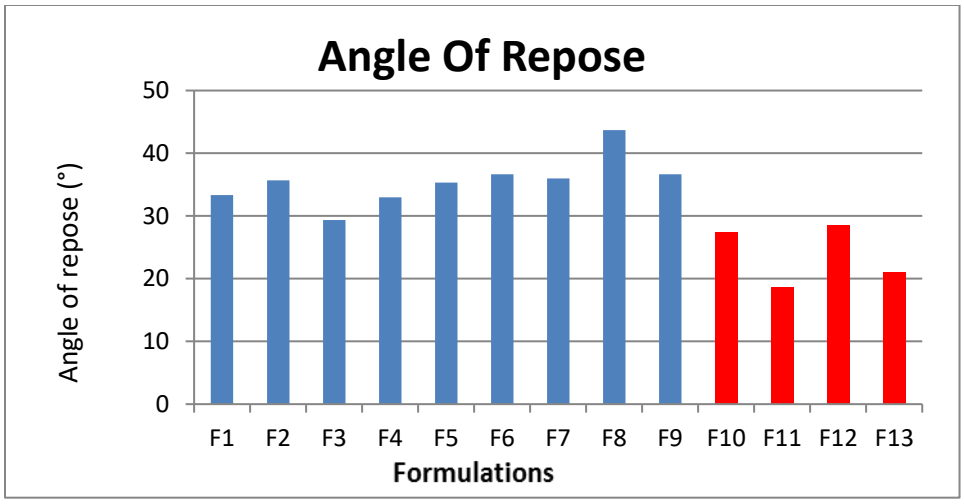
Table 16: Hausner's ratio interpretation

INTERPRETATION	
Hausner's ratio	**Powder flow**
<1.25	Good
>1.25	Poor

Angle of repose

Figure 3 below illustrates angle of repose values obtained for DC and WG. Table 29 helps in the interpretation of these values.

Table 17: Angle of repose interpretation



INTERPRETATION	
Angle of Repose (θ)	**Powder flow**
25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

Direct compression
Wet Granulation

Figure 3: Angle of repose for all formulations

Summary of pre-compression parameters

The figure 4 below shows a summary comparing direct compression and wet granulation technique and the pre-compression parameters of the powder blends.

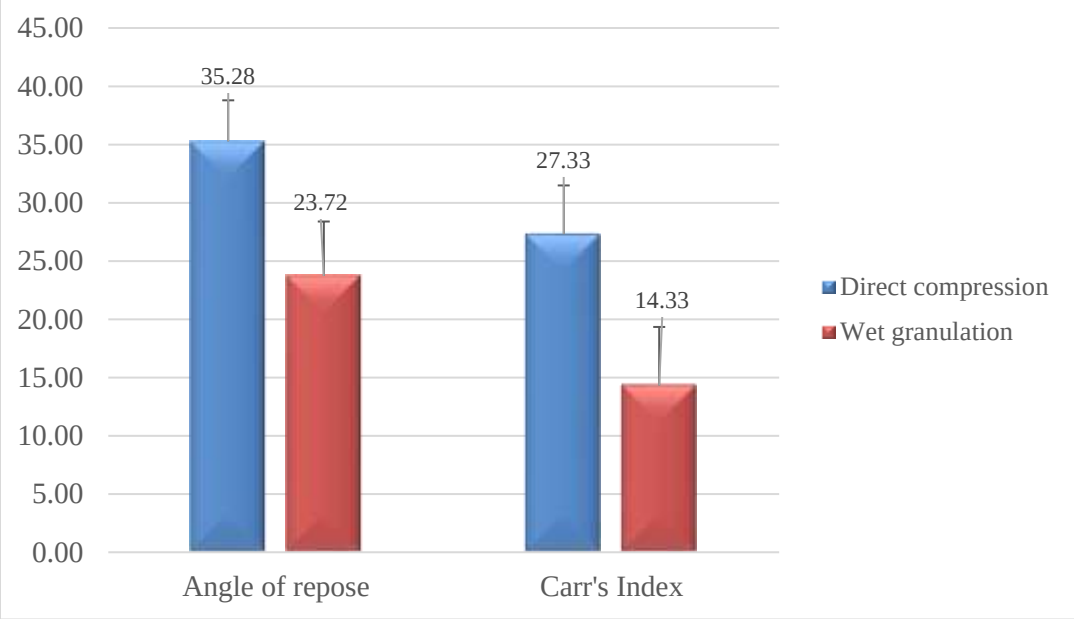


Figure 4: Comparison of Angle of repose and Carr's index for direct compression and wet granulation

Table 18. Comparison of Angle of repose and Carr's index for direct compression and wet granulation

INTERPRETATION

Carr's index	Powder flow
5-15	Excellent
12-16	Good
18-21	Fair
23-35	Poor
33-38	Very poor
>40	Extremely poor

From the pre-compression studies done on the powder blends for both tablet manufacturing processes (direct compression and wet granulation), we made the following observations as detailed in table 18 below. From these results, we observed that the powder blend for wet granulation had better flow characteristics than with direct compression.

Table 19: Observations made from powder flow of formulations

Method	Formulation	Observation
Direct compression	**F1**	Passable and good
	F2	Very poor and poor
	F3	Good and very poor
	F4	Passable and poor
	F5	Very poor and poor
	F6	Very poor and poor
	F7	Very poor and good
	F8	Very poor and poor
	F9	Very poor and very poor
Wet granulation	**F10**	Good and good
	F11	Excellent and excellent
	F12	Good and good
	F13	Good and excellent

Post-compression evaluation

The results of the measurements of various tablet parameters are tabulated in table 20 below:

Table 20: Post-compression evaluation for tablets made by wet granulation

	Superdisintegrant level X1	Binder level X2	Wetting time (s) Y1	Hardness (N) Y2	Disintegration time (s) Y3	Water absorption ratio % Y4
F10	2	5	37.2	26.4	32.5	112.52
F11	5	10	31.6	33.6	128.2	100.58
F12	5	5	19.43	15	22.2	103.86
F13	2	10	66.00	34.8	195.1	93.17
F10	2	5	31.5	30	40.2	110.13
F11	5	10	127.2	36	32.2	85.62
F12	5	5	21.2	12	19.1	104.49
F13	2	10	194.1	36	60	114.52
F10	2	5	33.5	26	40.8	116.19
F11	5	10	129.5	44	31.4	109.77
F12	5	5	23.2	14	19.5	90.28
F13	2	10	196.1	32	73	58.91

Modelling of disintegration time

The ANOVA table partitions the variability in Disintegration time (DT) into separate pieces for each of the effects (table 20). It then tests the statistical significance of each effect by comparing the mean square against an estimate of the experimental error. In this case, 3 effects have P-values less than 0.05, indicating that they are significantly different from zero at the 95,0% confidence level.

Table 21: Analysis of variance for disintegration time

Parameter	Sum of squares	Df	Mean square	F-Ratio	p-Value
Superdisintegrant level	4458.31	1	4458.31	4118.53	0.000
Binder level	54149.8	1	54149.8	50022.88	0.000

Superdisintegrant level x Binder level	2394.19	1	2394.19	2211.72	0.000
Total error	8.66	8	1.08		
Total (corr.)	61010.9	11			

The R-Squared statistic indicates that the model as fitted explains 99,98% of the variability in Disintegration time. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 99,98%.

A two-level experimental design provides sufficient data to fit a polynomial equation for the DT which is in the following form:

y = B0 + B1X1 + B2X2 + B12(X1X2)

Where y represents the experimental response, B0 the intercept and B1 and B2 are the coefficients for the factors X1(crospovidone) and X2 (gelatine), B12 is the interaction effect of X1 and X2. The following regression equation was fitted to the data .

$$\textbf{Disintegration time}(\textbf{Y1}) = \textbf{94.52} - \textbf{19.27}\boldsymbol{X1} + \textbf{67.17X2} - \textbf{14.12}\boldsymbol{X1X2}$$

X1= superdisintegrant amount

X2= binder amount

Equation 11: Model for disintegration time

Optimization of disintegration time

With above equation the lowest disintegration time that can be obtained is 22.2 seconds under the following conditions: superdisintegrant level of 5% and Binder level of 5%. The relationship between the dependent and independent variable was further elucidated by constructing counter plots. The effects of X1 and X2 with their interaction on DT at different levels (low and high level) are displayed in Figure 5; the interaction effect between X1 and X2 are shown in response surface plot in figure 5.

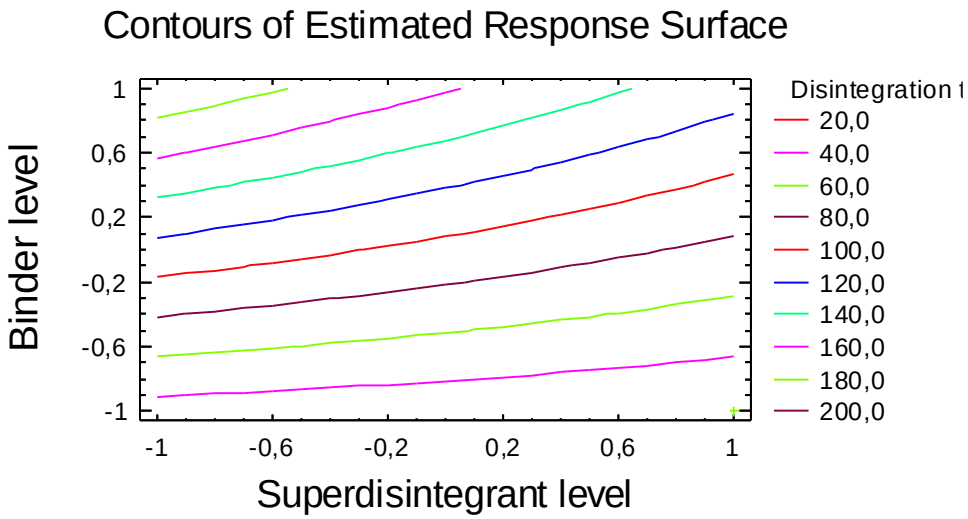


Figure 5: Contour plot for disintegration time

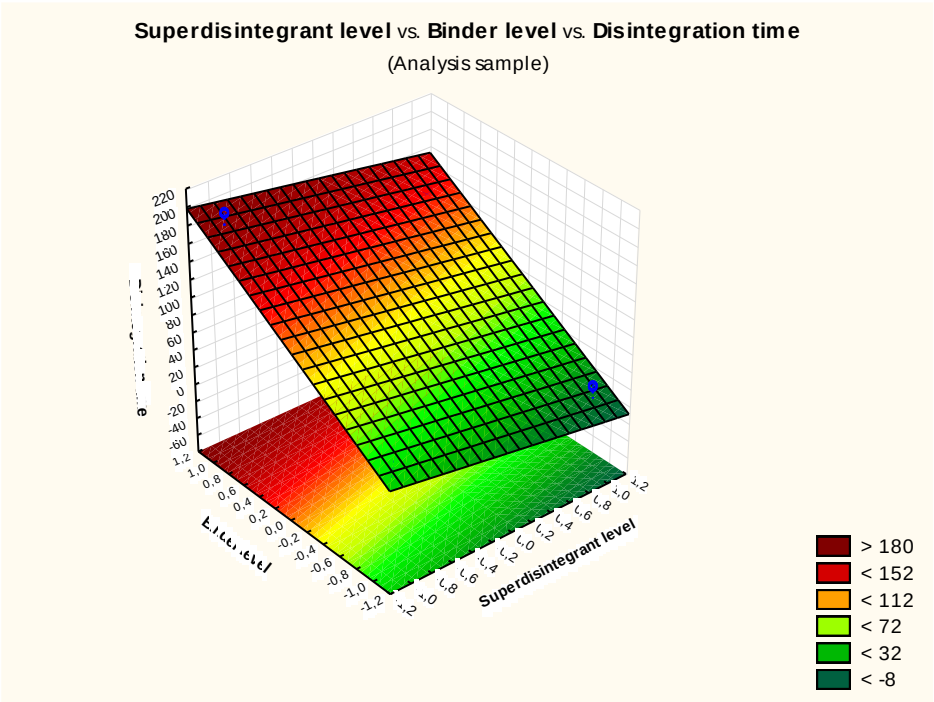


Figure 6: Response Surface plot for disintegration time

Hardness (crushing strength)

As shown in figure 7, 8, F13 (high binder level) showed highest crushing strength of 34.8N and F12 (low binder level) had 15N. Only F11 and F13 had sufficient hardness required of an ODT.

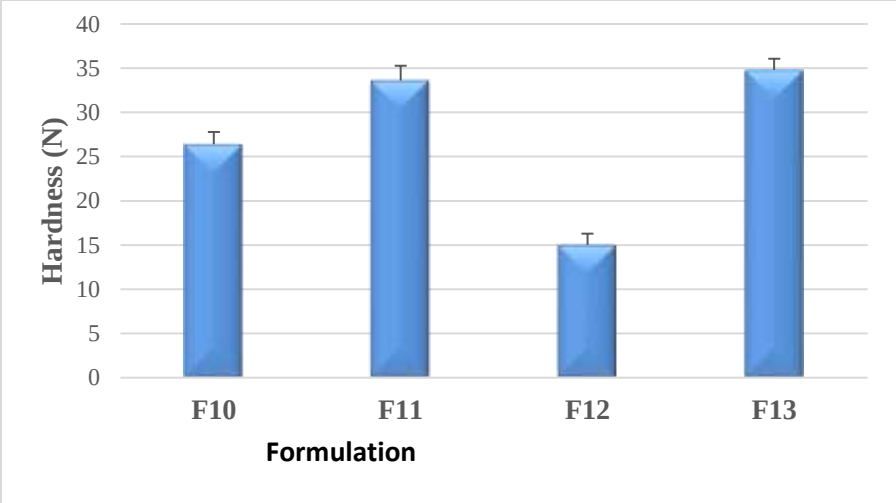


Figure 7: Hardness for tablets made by wet granulation

Modelling of hardness

The ANOVA table partitions the variability of hardness into separate pieces for each of the effects (table 21). It then tests the statistical significance of each effect by comparing the mean square against an estimate of the experimental error. In this case, 3 effects have p-values less than 0.05, indicating that they are significantly different from zero at the 95.0% confidence level.

Table 22: Analysis of variance for hardness

Parameter	Sum of squares	Df	Mean square	F-Ratio	p-Value
Superdisintegrant Level	78.03	1	78.03	7.60	0.025
Binder level	720.75	1	720.75	70.23	0.000
Superdisintegrant level x Binder level	227.07	1	227.07	22.12	0.002
Total error	82.11	8	10.26		
Total (corr.)	1107.96	11			

The R-Squared statistic indicates that the model as fitted explains 92,59% of the variability in Hardness. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 89,81%.

A two-level experimental design provides sufficient data to fit a polynomial equation for the hardness which is in the following form:

$$y = B0 + B1X1 + B2X2 + B12(X1X2)$$

Where y represents the experimental response, B0 the intercept and B1 and B2 are the coefficients for the factors X1(crospovidone) and X2 (gelatine), B12 is the interaction effect of X1 and X2.

The data was fitted to the regression equation

$$\mathbf{Hardness} = \mathbf{28.32} - \mathbf{2.55}X\mathbf{1} + \mathbf{7.75X2} + \mathbf{4.35}X\mathbf{1}X\mathbf{2}$$

X1= superdisintegrant amount

X2= binder amount

Equation 12: Model for hardness

From equation 13, superdisintegrant has a negative coefficient in the equation and hardness a positive coefficient. This implies that the higher superdisintegrant, the lower the hardness and the higher the binder, the lower the hardness. We can note that the coefficient of the binder (7.75) is much higher than the superdisintegrant value (2.55) implying that the binder has an effect of approximately two times that of superdisintegrant. The superdisintegrant effect was antagonistic to binder influence on the disintegration time; this is shown by the reduction of coefficient when combined (interaction effect).

Optimization of hardness

With above equation, the highest hardness that we can obtain is 37.87 N under the following conditions: superdisintegrant level of 5% and Binder level of 10%. The relationship between the dependent and independent variable was further elucidated by constructing contour plots. The effects of X1 and X2 with their interaction on hardness at different levels (low and high level) are displayed in Figure 8; the interaction effect between X1 and X2 are shown in response surface plot figure 9.

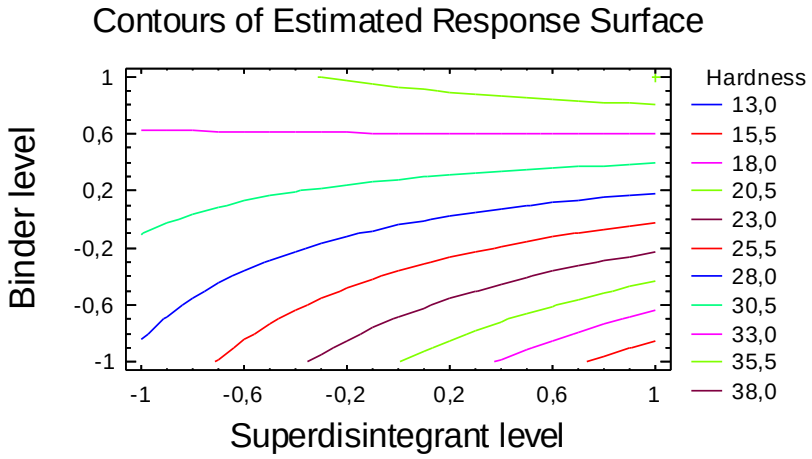


Figure 8: Contour plot for hardness

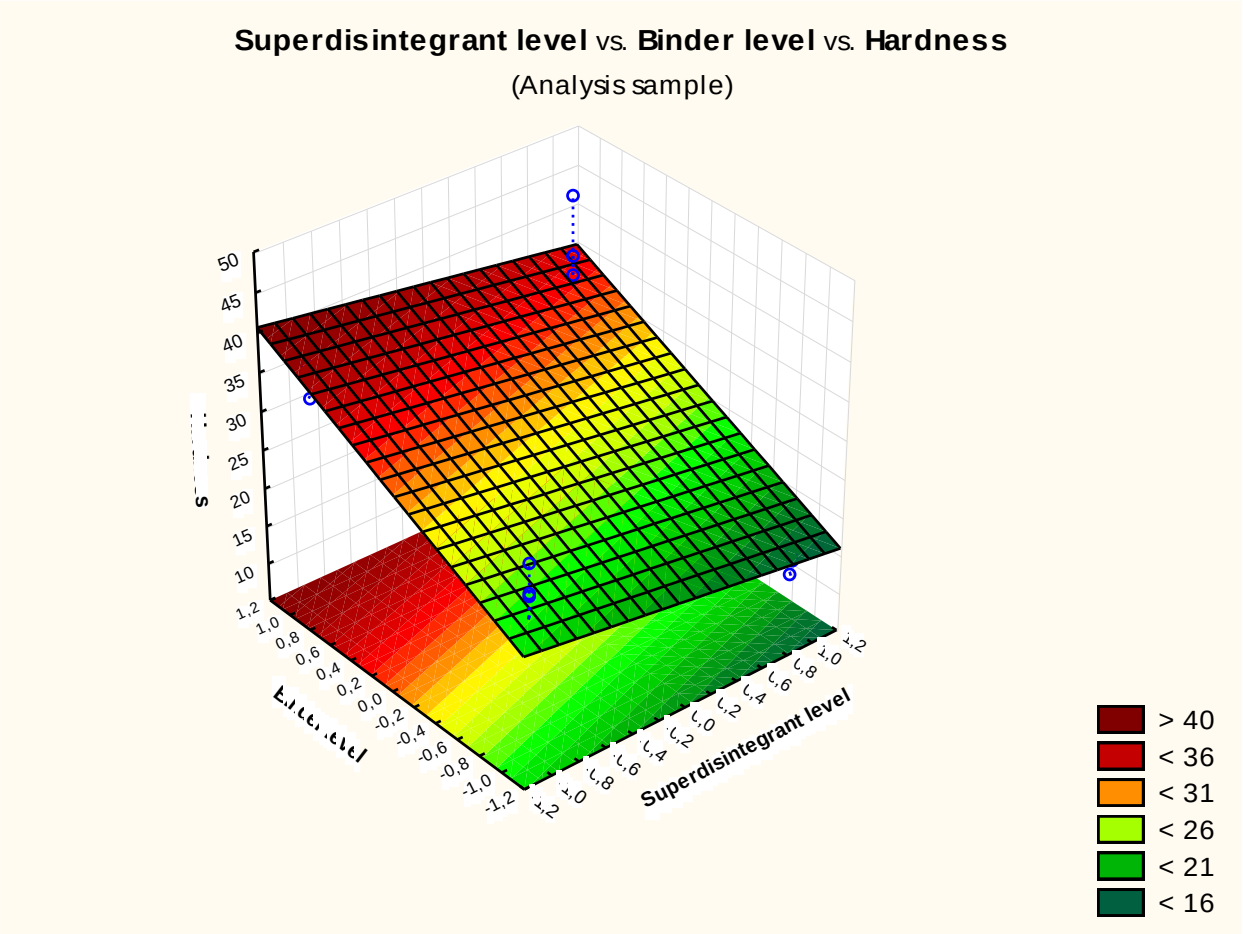


Figure 9: Response surface plot for hardness

Wetting time.

The wetting time for all 4 batches of tablets produced showed highest wetting time for the F13 formulation., as shown in figure 10. F13 (low crospovidone and high binder) showed highest wetting time 66.0s and F12 (High crospovidone and low binder) the lowest with 19.43s. The wetting time of all the tablet formulations was within the range of 19.5-73.0 sec.

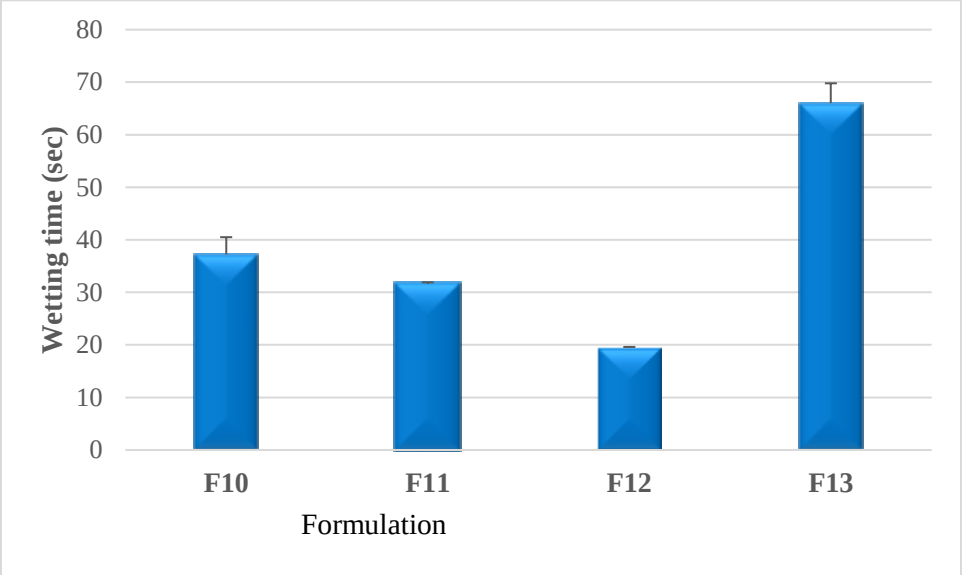


Figure 10: Wetting time values for wet granulation formulations

Modeling of wetting time

The ANOVA table (table 22) partitions the variability in wetting time into separate pieces for each of the effects. It then tests the statistical significance of each effect by comparing the mean square against an estimate of the experimental error. In this case, 3 effects have p-values less than 0.05, indicating that they are significantly different from zero at the 95.0% confidence level.

Table 23. Analysis of variance for wetting time

Parameter	Sum of squares	Df	Mean square	F-Ratio	p-Value
Superdisintegrant level	2240.51	1	2240.51	193.68	0.000
Binder level	1159.74	1	1159.74	100.25	0.000

Superdisintegrant level x Binder level	158.63	1	158.63	13.71	0.006
Total error	92.54	8	11.57		
Total (corr.)	3651.54	11			

The R-Squared statistic indicates that the model as fitted explains 97.46% of the variability in wetting time. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 96.51%. A two-level experimental design provides sufficient data to fit a polynomial equation for the wetting time which is in the following form:

y = B0 + B1X1 + B2X2 + B12(X1X2)

Where y represents the experimental response, B0 the intercept and B1 and B2 are the coefficients for the factors X1 (crospovidone) and X2 (gelatine), B12 is the interaction effect of X1 and X2.

The following regression equation was fitted to the data is:

$$\textbf{Wetting time} = 39.20 - 13.66X1 + 9.83X2 - 3.63X1X2$$

X1= superdisintegrant amount

X2= binder amount

Equation 13: Wetting time model

Optimization of wetting time

With above equation the lowest wetting time that can obtain is 19.34 sec under the following conditions: superdisintegrant level of 5% and Binder level of 5%.

The relationship between the dependent and independent variable was further elucidated by constructing counter plots. The effects of X1 and X2 with their interaction on hardness at different levels (low and high level) are displayed in Figure 22; the interaction effect between X1 and X2 are shown in response surface plot figure 11.

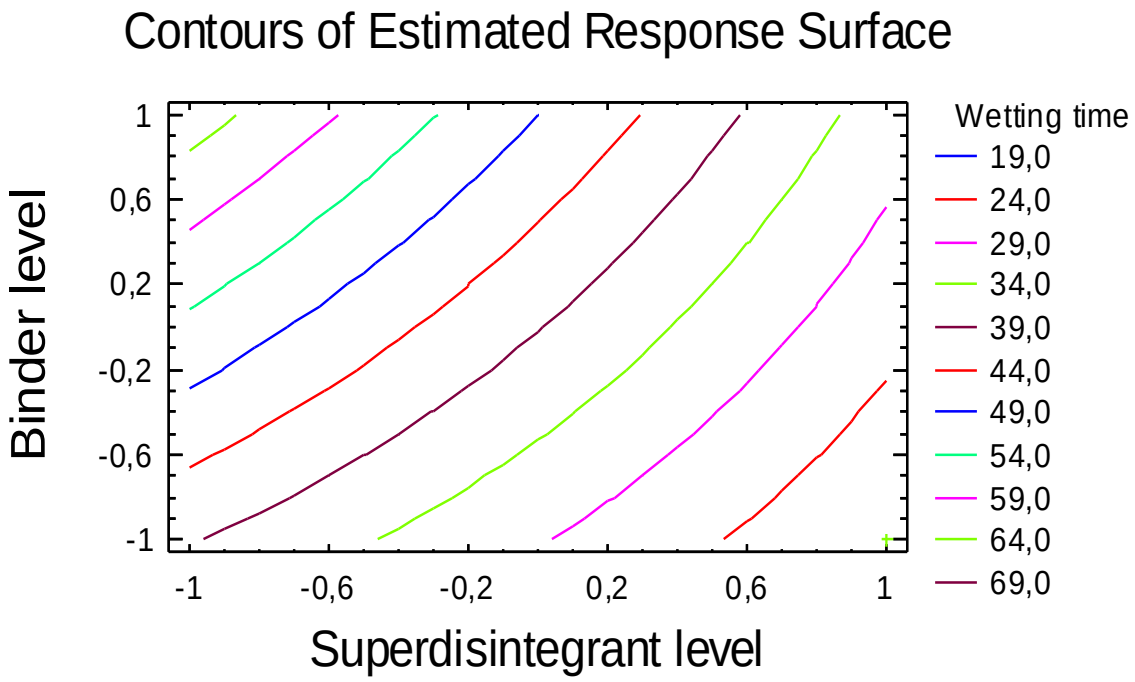


Figure 11: Contour plot for wetting time

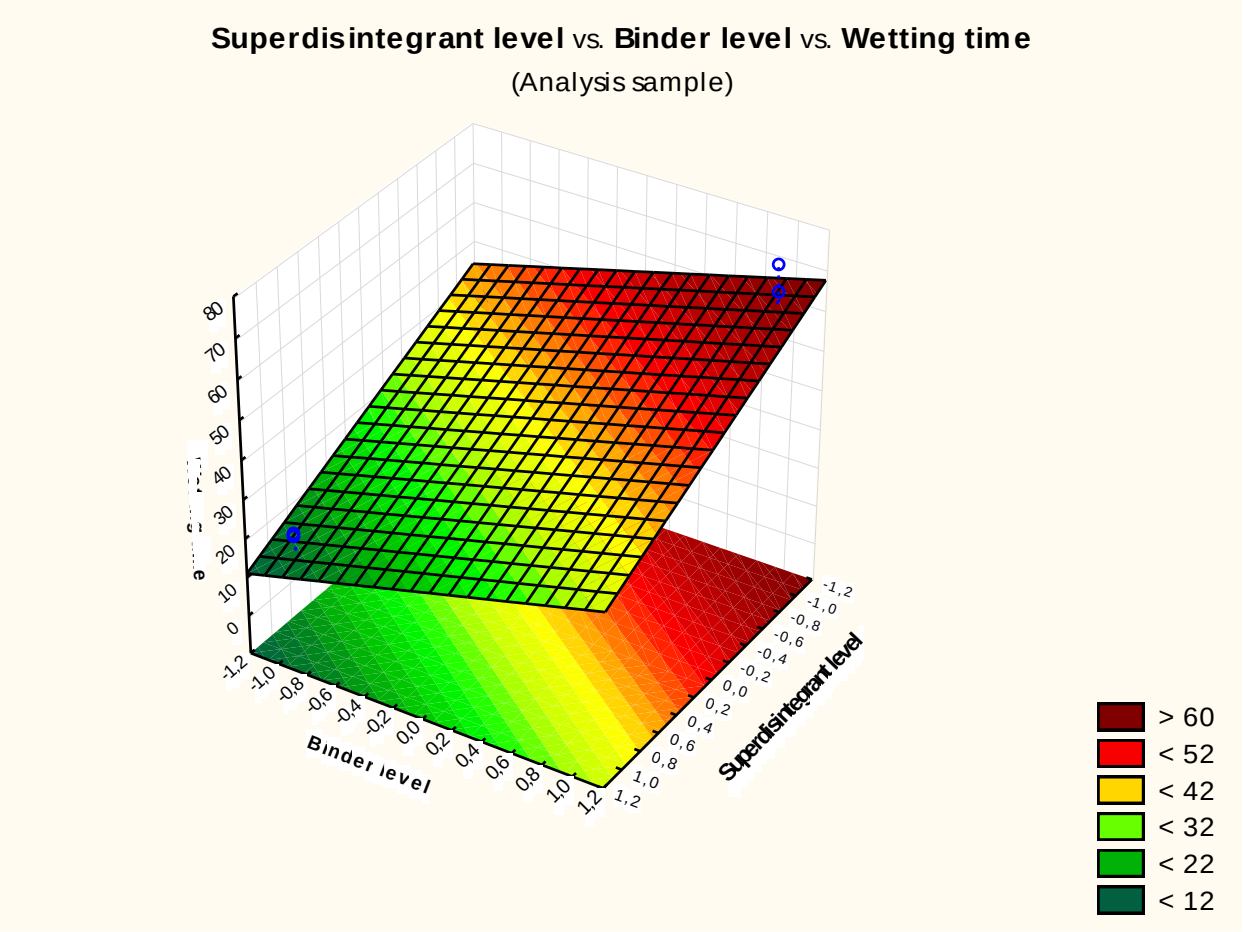


Figure 12: Response surface plot for wetting time

Water absorption ratio

The R-Squared statistic indicates that the model as fitted explains 30.38% of the variability in Water absorption ratio. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 4.27%. The ANOVA table 23 partitions the variability in water absorption ratio into separate pieces for each of the effects. It then tests the statistical significance of each effect by comparing the mean square against an estimate of the experimental error. In this case, 0 effects have P-values less than 0.05, indicating that they are not significantly different from zero at the 95.0% confidence level.

It can therefore be concluded that water absorption ratio in the developed design cannot be modelled.

Table 24: Analysis of variance for water absorption ratio

Parameter	Sum of squares	Df	Mean square	F-Ratio	p-Value
Superdisintegrant level	9.79	1	9.79	0.04	0.85
Binder level	467.50	1	467.50	1.85	0.21
Superdisintegrant level x Binder level	403.45	1	403.45	1.60	0.24
Total error	2018.72	8	252.34		
Total (corr.)	2899.46	11			

Multiple optimization: Desirability function

The summary of the different optimization models and their coefficients is represented in table 24.

Table 25: Summary of models

Response	B0	B1	B2	B12
Disintegration time	94.52	-19.27	67	-14.12
Hardness	28.32	-2.55	7.75	4.35
Wetting time	39.20	-13.66	9.83	-3.63

The following constraints were placed on the dependent variables (table 25)

Table 26: Multiple optimization constraints on the desirability

	Desirability			Weights	
Response	Low	High	Goal	First	Impact
Disintegration time	21,0	196,0	21	0,1	5,0
Hardness	12,0	44,0	Maximize	0,1	5,0
Wetting time	19,0	73,0	50	0,1	5,0

From the constraints placed above, it was possible to reach a global desirability of 0.96 (see figure 13) under the following conditions: superdisintegrant level of **2%** and Binder level of **6.36%.**

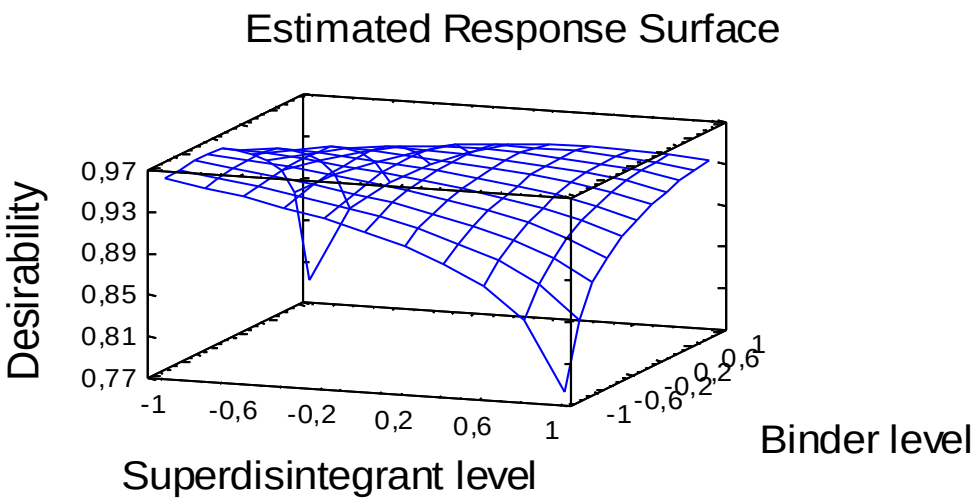


Figure 13: Optimal desirability model.

Table 26 below details the optimized formula based on design of experiments performed in this study.

Table 27: Optimized MIABE ODT formula

Raw material	**Role**	**Percentages %**
MIABE	Active ingredient	54.54
Corn starch	Superdisintegrant	18.18
Crospovidone	Superdisintegrant	2
Sucrose	Sweetener	5
Gelatin	Binder	6.36
Aerosil	Drying agent	2
Magnesium Stearate	Lubricant	0.5
Talc	Glidant	1.5
Strawberry powder	Flaovring agent	0.04
Paraben	Conservative	0.05
Microcrystalline cellulose	Filler /disintegrant	9.83

The predicted optimal values of dependent variables are: disintegration time of **76.7 sec,** Hardness **29.32 N** and wetting time **46.72 sec.**

DISCUSSION

The percentage yield 42.96% proved to be relatively higher as compared to other researchers who used maceration and obtained a yield of 12.3% [6] and 27.3% when rotated to increase yield [13].

The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal[2, 14].

In our study water was chosen; which is a universal solvent and is frequently used to extract plant products; components easily extracted with water include anthocyanins, starches, tannins, saponins, terpenoids, polypetides and lectins [15], most of which were of interest in our study. However, other solvents could further reveal the presence of more phytochemicals.

In conclusion, decoction though a laborious and time-consuming process is a method of choice in the case of heat sensitive material and to increase yield. Distilled water is an acceptable solvent in the extraction process for MIABE in the case of human consumption.

Phytochemical screening

The presence of active pharmaceutical components such as saponins, phenols, tannins, flavonoids and coumarins in our MIABE concurs with findings by other researchers and further support the use of *M. indica* stem bark in herbal medicine to fight diseases [16, 17]. In contrast, similar studies done by Mada S *et al* in Nigeria [18] showed the presence of alkaloids and terpenoids in addition to the phytochemicals mentioned above; they also showed positive for cardiac glycosides, xanthoproteins and anthroquinones. Alkaloids were absent but resins were detected in addition to flavonoids [19-23].

The biological functions of flavonoids include protection against allergies, inflammation, free radical scavenging, platelets aggregation, microbes, ulcers, hepatoxins, viruses and tumors. Mangiferin, catechin and epicatechin are the major phyto-constituents of *M. indica*. These flavonoids are responsible for the antioxidant effects of the mango stem bark and leaves [24]. The mango stem barks contain polyphenols, which have the ability not only to protect the human organism from the attack of oxidative chemical species (OCS) but also are able to reach the target organs and tissues. The antioxidant activity of all those polyphenols is governed by the number and location of these aromatic hydroxyl groups. Phenols protect plants from oxidative damage and perform the same functions for humans. The outstanding phytonutrients feature of phenols is their ability to block specific enzymes that causes inflammations. They also modify the prostaglandin pathways, thereby protecting platelet from clumping [13, 25].

Saponins' natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotics, helping the body to fight infections

and microbial invasion. These compounds also appear to greatly enhance the effectiveness of certain vaccines. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccine and knock out some kinds of tumour cells particularly lung and blood cancers. They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting prospects for saponins are how they inhibit or kill cancer cells. They may also be able to do it without killing normal cells in the process, as is the mode of some cancer fighting drugs. Cancer cells have more cholesterol type compounds on their membranes than normal cells. Saponins therefore bind cholesterol and thus, interfere with cell proliferation [26].

Tannins are reported to exhibit antiviral, antifungal, antibacterial, anti-tumour activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and also used as diuretic. Plant tannins have been recognized for their pharmacological properties and are known to make trees and shrubs a difficult meal for many caterpillars. Tannins have important roles such as been stable and potent antioxidants. Herbs that have tannins as their main component are astringent in nature and are used for treating asthma, pneumonia, and dysentery, thus justifying the use of the plant in traditional medicine practice [13]. In conclusion, the presence of these phytochemicals could explain the use of MIABE in traditional medicine. It should be noted that many factors account for the variability in results such as geographical location, extraction method, season and time of harvest [15].

The various factors that can influence flowability were the object of our pre-compression studies. Bulk density is basically how much a material will compact under various loads and it is an indicator of flow. Generally, a free-flowing powder will show very small change in bulk density from the initial value to its tapped value (consolidation stress). A cohesive or poor flowing powder will generally show a large increase in bulk density (30-50%) from bulk density as tapping increases. Bulk density depends on a number of factors including particle size distribution, true density, particle shape and cohesiveness due to surface forces including moisture. It generally decreases with decreasing particle size and decreases as the particle shape becomes less spherical and more irregular. This explains the lesser values of bulk density obtained direct compression (mean value of 0.64g/cm^3) versus wet granulation (mean value of 0.67g/cm^3).

The smaller the particles, the greater the surface of the powder; this phenomenon increases the friction between particles and subsequently decreases bulk density (case of direct compression powders). Another explanation stems from the fact that lactose used in direct compression is denser and bigger (diameter) than microcrystalline cellulose (MCC) used in wet granulation [7, 15]. This is significant in that small differences in particle size have been observed to make a big difference in flowability of powder and powder mixtures. Increase in lactose from 1.93% in F7 to 6.68% in F8 resulted in considerable decrease in bulk density from 0.66g/cm^3 to 0.56g/cm^3 respectively. High particle density has been observed to favour free flow of powders, therefore an increase in lactose, will consequently improve powder flow

characteristics. Same observation is gotten when comparing F10 and F11 of wet granulation where MCC was used in 11.19% and 3.19% respectively; an increase in bulk density was observed as MCC (filler) was increased. These results are similar to that of other researchers where flow properties are improved as MCC is increased [27]. This means that once the lubricant and the respective excipient were blended, a lubricant film was formed around the excipient particles, easing their rearrangement, sliding and packing in the powder bed. As a consequence, the powder bulk and tap densities increased and porosity decreased, as compared to the un-lubricated materials. Other authors have found similar results, especially when lubricants such as talc and stearic acid are employed. In terms of efficiency, highly hydrophobic lubricants such as magnesium stearate induced the largest volume reduction in most excipients [9].

Research has shown that lactose gives higher volume reductions after tapping than microcrystalline cellulose [21]. Finer particles are expected to give larger values of volume reduction (hence higher tapped density values). MCC is more porous and hence less compressible (consequently possess good flowability and low cohesiveness) than lactose. It is therefore expected that MCC will impart increase flowability to the mixture in which it is included. The increase in bulk density of a powder is related to the cohesivity of a powder. Ratios of the poured to tapped bulk densities are expressed in two ways (Hausner's ratio and Carr's index) to give indices of flowability.

Fillers greatly influence bulk properties of powders and so selection of fillers is very crucial when formulating. Filler such as MCC and lactose could cover up the effect of a poor flowing active ingredient if active ingredient is low dose. The technique also influences the flow properties; with wet granulation creating heavier particles with increased sizes (good flow).

The better flow characteristics displayed by the powder mix for wet granulation as opposed to direct compression is in accord with findings by other teams[12]. Some researchers have however shown some superiority of direct compression over wet granulation. For the purpose of our study, we decided to proceed to tableting of wet granulation powder blends which had better flowability.

Disintegration time the most important parameter that needs to be optimized in the development of orally dispersible tablets is the disintegration time of tablets. In the present study tablets, three formulations out of four (F10, F11, F12) disintegrated under 3 minutes thereby fulfilling the official requirements (<3 min, i.e. 180s) for dispersible tablets (European Pharmacopoeia, 2001). F12 (high superdisintegrant and low binder levels) had the least disintegration time of 22.2seconds and F13 (low superdisintegrant and high binder levels) had the highest disintegration time of 195.1seconds**.**

It was observed that among the various disintegrants used such as Corn starch, Crospovidone, Croscarmellose Sodium and Sodium Starch Glycolate, Crospovidone shows relatively faster disintegration time at same concentration as compared to others Crospovidone is a synthetic homopolymer of cross-linked N-vinyl pyrrolidinone and it is a white, free flowing, compressible powder and hygroscopic in nature. It might be due to high water uptake capacity and low gelling capacity of Crospovidone [17] when compared to others. Difference in swelling may also play a role in disintegrating agent efficiency it causes the tablet to disintegrate quickly. And because of its high crosslink density, it swells rapidly in water without gel formation. Therefore, crospovidone uses a combination of mechanisms to provide rapid disintegration (wicking and swelling).

The optimum concentration of superdisintegrant can be selected according to critical concentration of disintegrant. Below this concentration the tablet disintegration time is inversely proportional to the concentration of superdisintegrant. If the concentration of superdisintegrants incorporated in tablet is above the critical concentration, the disintegration time remains approximately constant or even increases [25]. However, in high dose tablets, research shows that increasing the strength of the super disintegrant Crospovidone did not alter the DT considerably, unless mixed with other disintegrants. Addition of CP alone to the dry extract reduced the DT of the tablet appreciably when compared to the tablets containing lower amount of the dry poly herbal extract [16].

It was observed that further increase in concentration of binder led to the increase in disintegration time. Such delay in disintegration may be because of the tight binding between molecules which ultimately slow down the water uptake by the tablets and thus superdisintegrant do not get sufficient water to swell. Similar results obtained by other researchers [3, 17]. Conventional disintegration tests for conventional tablets may not allow precise measurement of the disintegration time of ODTs because of their fast disintegration. In vitro testing may not always reflect the real in vivo disintegration time of tablets especially as the method of USP uses 900ml of water which is not reflective of saliva in mouth [8]. Modified methods have been developed[25]

Determination of disintegration time appears to be method dependent. Some methods are more discriminating than others. To provide both a standard for and consistency in disintegration testing, it was recommended that applicants use the European Pharmacopeia method for disintegration testing. However,

other methods that can be correlated with or are demonstrated to provide results equivalent to the USP method also can be used and submitted to determine disintegration time (e.g. wetting time)[19]

The hardness of an orodispersible tablet unlike conventional tablets (50-150N) is kept at a low crushing strength (30-50N) to facilitate disintegration in the mouth. F13 (high binder level) showed highest crushing strength of 34.8N and F12 (low binder level) had 15N. Only F11 and F13 had sufficient hardness required of an ODT. Binder level clearly influences crushing strength, this results are similar to that of other studies [24]. Binder level is not the only determinant factor for crushing strength; compression force greatly affects the hardness of a tablet [26] and so crushing strength of F10 and F12 could have been increased by increasing the compression force. However, all tablets were compressed at 7.5N to be able to appropriately evaluate the effect of binder level on crushing strength. More studies should be done to evaluate the effect of compression force on tablet properties.

Superdisintegrant has a negative coefficient in the **equation 13** and binder level a positive coefficient. This implies that the higher superdisintegrant, the lower the hardness and the higher the binder, the higher the hardness. We can note that the coefficient of the binder (7.75) is much higher than the superdisintegrant value (2.55) implying that the binder has an effect of approximately two times that of superdisintegrant. It also worth noting that the superdisintegrant effect is antagonistic to binder influence on the disintegration time; this is shown by the reduction of coefficient when combined (interaction effect, impossible to detect with the OFAT and best guess method).

The measurement of wetting time may be used as another confirmative test for the evaluation of fast dissolving tablets. The wetting volume is important to check minimum volume of water required for wetting of tablet. It is related to the contact angle and gives an insight into the disintegration properties of tablets; a lower wetting time implies a quicker disintegration of the tablet [8].
Superdisintegrant has a negative coefficient in the equation 14 and binder level a positive coefficient. This implies that the higher superdisintegrant, the lower the wetting time and the higher the binder, the higher the wetting time. We can note that the coefficient of the binder (7.75) is much higher than the superdisintegrant value (2.55) implying that the binder has an effect of approximately two times that of superdisintegrant. It also worth noting that the superdisintegrant effect is antagonistic to binder influence on the disintegration time; this is shown by the reduction of coefficient when combined (interaction effect, impossible to detect with the OFAT and best guess method).

CONCLUSION

Based on the results from this study, the following conclusions were reached that the aqueous extraction of *Mangifera indica* L. through digestion had more yield as compared to other extraction methods and the extract contains the following phytochemicals: tannins, saponins, phenols, tannins, flavonoids and coumarins compounds, known by literature to possess pharmacological activities. Direct compression and wet granulation techniques greatly influenced the flow properties of the powder blend with wet granulation showing superiority by producing free flowing granules of MIABE ODTs. Formulation variables, lubricant and diluent amount were also found to play a role in powder flow. Their presence, if well selected could significantly improve powder flow. Superdisintegrant and binder levels were seen to influence significantly the tablet properties (disintegration time, wetting time and hardness) of MIABE ODT with a non-significant influence on the water absorption ratio. The formulation containing 2% of crospovidone, 6.36% of povidone was in the optimum zone and was considered as an optimum formulation. The statistical design has the advantage of performing a small number of experiments and the fitted model from the statistical analysis can be used to predict values of responses at any point inside the experimental space. The design can be successfully used to optimize the wet granulation formulation. In conclusion, in this project, ODTs of *Mangifera indica* L. stem bark extract was successfully prepared using a QbD (DoE) approach, using a wet granulation method. The design and evaluation of the formulations in this study resulted in successful formulation optimization of an Improved Traditional Medicine. DoE proved to be an excellent method to optimize formulations of ITMs, providing several tools that increase a much better understanding of the formulation and manufacturing process. Further studies on this formulation DoE are needed to evaluate the effect of more process variables (compression force and speed) and more formulation variables such as palatability.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.