

## Antioxidant Properties and Effects of Formulation Variables on *Ceiba pentandra* Microspheres

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

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### Abstract

**Background:** New drug delivery techniques have improved therapeutic and physicochemical properties of herbal medicines.

**Objective:** Extracts of *Ceiba pentandra* (CP) leaf have been evaluated for antioxidant properties with the aim of formulating them into microspheres and studying release of flavonoid from the dosage form.

**Materials and Methods:** Microspheres were prepared from extracts of CP using ionotropic gelation method. Formulation parameters were varied to produce A1 to A8. Microspheres were evaluated for size, shape, yield, micrometrics, swelling, entrapment efficiency, and flavonoid release characteristics. Factorial experimentation design was used to determine the individual and interactive effects of the formulation variables on entrapment efficiency and flavonoid release. Results were analysed using ANOVA at  $p \leq 0.05$ .

**Results:** The extract contained flavonoids. Microspheres were discrete, porous, spherical, with good flow. Microspheres swelled moderately and eroded within 3 hours. Entrapment efficiencies ranged from 4.17 to 19.2 %. All Formulations released 80 % of flavonoid within 6 hours, except A2, A3 and A8 which sustained flavonoid release for 24 – 26 hours. Concentration of polymer had the highest positive effect, while stirring speed had the highest negative effect on microsphere entrapment efficiency. Effect of curing time on entrapment efficiency was positive but insignificant. Polymer concentration and curing time had negative effects on flavonoid release, while stirring speed showed a positive effect.

**Conclusion:** Extracts of *Ceiba pentandra* leaves possess antioxidant constituents and could be formulated into microspheres of good physicochemical properties. Concentrations of polymer and curing time are important parameters that should be considered in the formulation of CP into microspheres with sustained release potentials.

**Keywords:** *Ceiba pentandra*, Microspheres, Flavonoids, Entrapment efficiency

### INTRODUCTION

In recent times plants have assumed greater significance in healthcare delivery; no more as lead components for the synthesis of new drugs but as centre stage for prevention, amelioration and management of diseases; the stance that plant drugs are safe, cheap and readily available has played no small part in this trend. Plant actives usually have

shorter half-lives requiring repeated administrations to elicit their activity, and therefore necessitates their formulation as novel drug delivery systems (Arehwoh, 2016). Hence, novel drug delivery techniques have been attempted on herbal medicines to further improve their therapeutic potential with the purpose of achieving sustained release, increasing

patient compliance and improving efficacy of the plant drug (Eraga *et al.*, 2015).

The plant *Ceiba pentandra* is a very large, deciduous tree up to 60 metres tall, with roots spreading quite horizontally, 10 metres or longer, in the upper 40-80 cm of the soil (Elumalai *et al.*, 2012). It belongs to the family Malvaceae and is well known as the silk cotton tree (Ueda *et al.*, 2002). Folk medicine in Northern Nigeria uses the plant to treat hypertension, headache, dizziness, fever, peptic ulcers, leprosy and diabetes (Aloke *et al.*, 2010; Sarkiyayi *et al.*, 2009; Odoemena *et al.*, 2004; Olusola *et al.*, 2003). In India and Malaysia, it is used for the treatment of bowel ailment and diarrhoea (Elumalai *et al.*, 2012). Previous works on *C. Pentandra* indicate the presence of significant amount of phenolic compounds, alkaloids, flavonoids, tannins, saponins, phytate, oxalate, trypsin inhibitor, and hemagglutinin (Friday *et al.*, 2011). Also a number of sesquiterpenoids naphthoquinones (Rao *et al.*, 1993); two newisoflavones, pentandrin and pentandrin 5'-O- $\beta$ -D-glucoside (Ngounou *et al.*, 2000) have been isolated from *C. Pentandra*.

Flavonoids are polyphenolic compounds whose main dietary sources are fruits and vegetables. One of the most important properties of flavonoid is their capacity to act as antioxidants (Arora *et al.*, 2005). Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage (Weiss *et al.*, 2003). Relevant literatures

suggest the ability of flavonoids to scavenge hydroxyl radicals, superoxide anions and lipids peroxy radicals, and hence are considered powerful antioxidants (Alan *et al.*, 1996). These natural antioxidants, flavonoids inclusive, achieve this by stimulating endogenous antioxidants to neutralize oxidative stress (Arora *et al.*, 2005)

Drug delivery systems control the performance of a drug by affecting its concentration, location, and duration of exposure in the body region where pharmacological action is desired. Microspheres as a drug delivery system are small spherical particles, with diameters in the micrometer range (typically 1  $\mu$ m to 1000  $\mu$ m). Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are preferably biodegradable in nature and ideally having a particle size less than 200  $\mu$ m (Svenson, 2004; Agusundaram *et al.*, 2009). Microspheres offer several advantages over conventional dosage forms; these include improved drug solubility, targeted delivery, sustained release, reduced side effects, and better patient compliance (Okunlola *et al.*, 2020). Very few attempts have been made to formulate *C. Pentandra* into a dosage form and no relevant literature exists on its formulation into a novel drug delivery system.

This study therefore aims to evaluate the antioxidant properties of the extract of *Ceiba pentandra* leaves, formulate the extract into microsphere drug delivery system and determine the release of flavonoid from the dosage form design.

## METHODOLOGY

### Materials and Methods

#### Materials:

Leaves of *Ceiba pentandra* were obtained from Kwara State, Nigeria and authenticated at the Department of Botany, University of Ibadan. Sodium alginate (Carl Roth GmbH & Co, Germany), Calcium chloride (Alfa Aesar GmbH & Co, Karlsruhe, Germany), Quercetin (Sigma-Aldrich), Aluminium chloride, Potassium acetate and methanol were purchased from Mekz Global Ltd (Lagos, Nigeria). All reagents used were of Analar grade.

#### Methods

##### *Preparation of Extracts of Ceiba pentandra Leaf*

Fresh leaves of *Ceiba pentandra* (CP) were collected from the tree at Rore, in Irepodun Local Government

Area of Kwara State, Nigeria. The leaves were dried in-doors and then milled, using a domestic blender. Powder was kept in airtight containers. Extraction was carried out using the method of Nep *et al.*, 2016. Approximately 400 g powder was macerated in 4 L of distilled water containing sodium metabisulphite (0.1% w/v) for 30 minutes at 30 °C. The produced mucilage was collected by filtration, using a muslin cloth and then precipitated with 2 volumes of 96 % v/v ethanol. The obtained precipitate was then filtered using a 200  $\mu$ m sieve and oven dried (40 °C for 24 h) to obtain the extract.

##### *Phytochemical screening of CP Extracts*

The extracts were screened for presence of various secondary metabolites, using standard procedures (Manjulika *et al.*, 2014)

**Preparation of CP loaded Microspheres**

Batches (A1 – A8) of microsphere formulations were prepared from CP extracts using the ionotropic gelation method. Dispersions of sodium alginate were prepared by weighing required amounts of Sodium alginate, dissolving them in boiled water and stirring to form fine slurry. The extract (1 g) was added to the sodium alginate dispersions and stirred continuously until uniform mixing was achieved. A 10% dispersion of Calcium chloride in water was made. The mixture of sodium alginate and CP extract dispersion was extruded into a beaker containing the 10% Calcium chloride dispersion through a precision device (21G needle and syringe) to ensure uniform microsphere size. The magnetic stirrer was maintained at the different revolutions per minute as required. Microspheres formed were then left to cure for the required durations before being collected by sieving, washed with water and then dried in a hot air oven at 40<sup>0</sup>c for 24 hours.

**Microspheres Characterization**

Percentage Yield:

Percentage yield of CP loaded microspheres were obtained using the method adopted from Sengel *et al*, 2006 and calculated by the formula below

$$\text{Percentage yield} = \frac{\text{Weight of dried microspheres}}{\text{Total weight of extract and polymer}} \times 100 \dots\dots\dots(1)$$

**Size and Morphology:**

The size and surface morphology, and characteristics of the different batches of microspheres prepared were obtained using scanning electron microscopy (SEM) (Vegan 3 Tescan) at an accelerating voltage of 25 KV after the extracts had been coated slightly with a layer of carbon to aid proper surface and cross visualization and to avoid excess charging.

**Micromeritic properties:**

The bulk densities of the batches of microspheres prepared were determined by pouring 2 g of each batch through a funnel at an angle of 45°C into a 25 mL cylinder and the volume, without any tapping, was noted as V<sub>o</sub>. Triplicate determinations were taken.

For Tapped density determinations, 2 g each of microsphere batches were poured into a 25mL cylinder. The microspheres within the cylinder were

then subjected to a standardized thirty-eight (38) taps per minute. The final volume, V<sub>38</sub>, occupied by the microspheres was recorded. The procedure was done in triplicates. The bulk and tapped densities were calculated as ratio of weight to volume.

Hausner’s ratio of the microspheres were calculated as a ratio of the tapped densities to the bulk densities of the microspheres i.e :

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \dots\dots\dots (2)$$

The Carr’s compressibility index was also determined from the equation below

$$\text{Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \dots\dots\dots (3)$$

**Angle of repose determinations:**

A clean glass funnel was clamped to a retort stand such that the perpendicular height of the tip of funnel was 4 cm from the flat surface with a clean sheet of paper. About 2 g of microspheres were poured through the funnel with opening closed with a cotton wool to temporarily block the flow of the microspheres. The cotton wool was removed, and heap of microspheres was formed. The height was determined with the aid of a divider and recorded as H (cm), and the radius of the base of the heap obtained from the diameter and denoted as R. Determinations of angle of repose was done in triplicates and calculated using the formula below:

$$\text{Tan } \alpha = \frac{H}{R} \dots\dots\dots (4)$$

Where α= angle of repose, H =height of microsphere heap, R = radius of heap base.

**Swellability studies:**

Microspheres (100 mg) was soaked in 20 mL mixture of methanol and water. The microspheres were then removed, and excess buffer was wiped using a dry filter paper and their final weight was determined. The swollen microspheres were handled carefully in order to avoid any loss of mass due to erosion. The weight of the microspheres was determined after 3h. Swellability was calculated using the formula below:

$$\text{Swelling index (\%)} = \frac{c}{l} \times 100 \dots\dots\dots (6)$$

Where C is the weight gain, and I is the initial weight of the microspheres

**Fourier Transform Infra-red Spectroscopy of Microspheres:**

FTIR spectroscopy of different batches of microspheres was obtained on an IR spectrophotometer (Perkin Elmer, 2000, USA) using KBr disk (about, 2mg samples in 200mg KBr). The scanning was done in the range 400-4000cm<sup>-1</sup>

**Microsphere's Entrapment Studies:**

**Content Estimation**

The determination of the wavelength of maximum absorbance was done according to the method of Chang *et al.*, 2002. Absorbance values of wavelengths scans of 1 mg/mL concentrations of CP extracts complexed with Aluminium chloride at 350 nm, 390 nm, 400 nm, 415 nm, 420 nm and 430 nm were obtained.

A quercetin calibration curve was made using the aluminium chloride colorimetric method modified from the procedure reported by Woisky and Salatino (1998). Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a UV-160A spectrophotometer (Kyoto, Japan)

The colorimetric method of aluminium chloride was used to determine the amount of quercetin-like flavonoids present in the extract (Akabay, *et al.*, 2003). Volume, 5 ml of the extract was reacted with five drops of freshly prepared aluminium chloride, and 0.1 mL of 1M Potassium acetate and left for 30 minutes. The resultant mixture was then analysed spectrophotometrically at 415 nm to determine the quercetin like flavonoid content using the equation obtained from the calibration curve of pure quercetin. Triplicate determinations of absorbances were obtained from the spectrophotometer.

**Entrapment Efficiency**

For entrapment efficiency determinations, 100 mg of each batch of microspheres were crushed in a mortar and suspended in 100 mL mixture of methanol and

water (2:98 %v/v). The resultant dispersion was agitated for 30 minutes for complete mixing and filtered, five drops of freshly prepared Aluminium chloride solution was added to 5 mL of the filtrate, and 0.1 mL of 1M Potassium acetate added to obtain a yellow coloration and analysed spectrophotometrically at 415 nm to determine the flavonoid content. All readings were taken in triplicates. The concentration of flavonoids was determined using the equation from the standard calibration plot obtained from pure quercetin and entrapment efficiency (E) was calculated from the formula below.

$$E (\%) = \frac{A}{T} \times 100 \dots\dots\dots(7)$$

Where A and T are the actual and theoretical flavonoid contents of the extract respectively.

**Release studies**

A citrate buffer was prepared by dissolving 4.2 g of citric acid in 2 L of distilled water. 1 M Sodium hydroxide (NaOH) was used to adjust the pH to 2.0 or 6.0 as needed while gently stirring the solution. In-vitro release studies were carried out using a rotating basket BP basket II (Veego digital dissolution tester, India) method for the various batches of the microspheres. A dissolution medium of 900 mL containing citrate buffer pH maintained at 37°C with a basket revolution of 50 rpm was used. A 5 mL volume was withdrawn at various intervals and replaced with an equivalent volume to maintain sink conditions throughout the experiment. The samples were filtered and diluted with an equal volume of 0.1 M HCl. This was continued for 120 minutes and then in citrate buffer at pH 6.0 for a further 360 minutes. The absorbance of the resulting solutions was measured at absorbance of 415 nm after adding 5 drops of aluminium chloride solution and 0.5 mL of Potassium acetate. The amount of flavonoids released at each time interval was determined spectrophotometrically using the equation from the standard calibration plot obtained from pure quercetin. A minimum of triplicate determinations were carried out for all experiments and the results were recorded as mean ±SD.

**Factorial Experiment**

Minitab® software version 20 was used to assess the effect of the formulation variables on the characteristics of CP extract loaded microspheres. The studied independent variables were:

(a) Sodium alginate concentration at a high level of 2 w/v% and a low level of 0.5 w/v%

(b) Stirring speed at a high level of 800 rpm and a low level of 300 rpm

(c) Curing time at a high level of 180 minutes and a low level of 60 minutes

Effects of these independent variables on entrapment efficiency and drug release at 360 minutes were

determined using a 2<sup>3</sup> factorial experimental design (Ayorinde and Itiola, 2010, Ayorinde *et al.*, 2017).

### Statistical Analysis

All experiments were carried out in triplicate and the values were expressed as mean and with a standard deviation. Statistical data analyses were conducted using Minitab® version 20, ANOVA and data generated were assessed for statistical significance using a  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Phytochemical Properties

Phytochemicals found in *C. pentandra* extracts include carbohydrates, flavonoids, cardiac glycosides, alkaloids, coumarins, saponins, tanins, steroids (Table 1). The presence of flavonoids in the extract indicates antioxidant potential of the plant. (Friday *et al.*, 2011; Kubmarawa *et al.*, 2007; Akaneme *et al.*, 2008; and Sule *et al.*, 2009).

### Antioxidant Properties

The DPPH method of antioxidation capacity of a plant predicts the ability of the plant substance to

reduce the DPPH radical to hydrazine (Baliyan *et al.*, 2022).

The IC<sub>50</sub>, a concentration depicting a 50% reduction of DPPH to hydrazine is a widely used maker for establishing the antioxidant potential of many plant extracts. Table 2 shows the IC<sub>50</sub> of CP extract and the reference standard (rutin), at various concentrations as read off on a graph of percentage inhibition versus extract concentration. Aqueous extract of CP obtained from maceration showed a two-fold antioxidation capacity compared to rutin.

**Table 1: Phytochemical constituents of *Ceiba pentandra* Leaf Aqueous Extract**

Phytochemicals	Result
Saponin	+
Tannin	+
Flavonoid	+
Steroid	+
Phlobatannin	-
Terpenoid	+
Coumarin	+
Emodin	-
Anthraquinone	-
Anthocyanin	-
Alkaloid	+
Cardiac Glycosides	+
Charcones	-
Phenols	-
Carbohydrates	+

**Table 2: Comparative Antioxidant Potentials of the Extract and Rutin**

Extract Concentration ( $\mu\text{g/mL}$ )	Percentage Inhibition (%)	
	Extract	Rutin
400	75.61 $\pm$ 0.04	74.13 $\pm$ 0.12
300	74.33 $\pm$ 1.07	70.80 $\pm$ 0.22
200	73.23 $\pm$ 0.14	69.70 $\pm$ 0.26
100	71.6 $\pm$ 0.05	67.72 $\pm$ 0.43
50	68.3 $\pm$ 0.05	61.1 $\pm$ 0.20

Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside, is a flavonol, abundantly found in plants, such as passion flower, buckwheat, tea, and apple. It is a vital nutritional component of food stuff. Rutin, also called as rutoside, quercetin-3-rutinoside, and sophorin is a citrus flavonoid glycoside found in buckwheat. The name 'rutin' comes from the plant *Ruta graveolens*, which also contains rutin. Chemically it is a glycoside comprising of flavonolic aglycone quercetin along with disaccharide rutinose. It has demonstrated a number of pharmacological activities, including antioxidant, antihypertensive, antidiabetic, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities (Javed *et al.*, 2012; Richetti *et al.*, 2011; Nassiri *et al.*, 2010).

Results therefore showed that aqueous extract of CP contained higher proportion of flavonoid than rutin (Table 2) and hence would exhibit higher and better antioxidation properties. Flavonoids possess well known antioxidant potentials for amelioration of a wide range of diseases by complementing endogenous antioxidants to neutralize oxidative stress by scavenging free radicals (Hegarty *et al.*, 2000, Liu *et al.*, 2018).

#### ***Micromeritic properties of microspheres***

Microspheres prepared had good micromeritic properties. Values of angle of repose, Hausner's ratio and Carr's compressibility index can be seen in Table 3. Angles of repose and Carr's index give an insight into the flowability and ease of handling of microspheres and powders. Interparticulate properties like size, shape, porosity, bulk density and, moisture content go a long way in determining ease of handling of powders and flowability (Ayorinde *et al.*, 2004). All the batches of microsphere prepared gave Hausner's ration of  $\leq 1.20$  and Carr's index of 12.1 – 16.4. The angle of repose for the microspheres ranged from 25 to 33°. These values are suggestive of very good flow properties. This flow property of the microspheres may be attributed to their very low moisture content and spherical shapes. Moisture and particle shape has been reported to affect flow of pharmaceutical materials (Ayorinde *et al.*, 2016).

Bulk and tapped densities reveal how closely packed particles are and the ease with which powders will form compact masses upon compression (Itiola *et al.*, 2005). Microspheres had higher tapped densities (0.55 - 0.70) than bulk densities (0.48 – 0.62) indicating the presence of interparticulate voids in the microspheres. Flowability is an important consideration in tableting and capsule filling imparting content uniformity and weight variation considerably.

**Table 3: Physicochemical Properties of the Microspheres**

Microsphere Batches	Swellability (%)	Entrapment Efficiencies	Hausner's Ratio	Angle of Repose	Carr's Index
A1	8.7 ± 0.6	15.1 ± 0.01	1.18	33	16.4
A2	6.8 ± 1.1	19.2 ± 0.1	1.16	32	13.6
A3	4.9 ± 0.5	11.3 ± 0.03	1.14	29	12.1
A4	7.2 ± 0.4	15.4 ± 0.01	1.14	25	12.7
A5	3.5 ± 0.9	7.9 ± 0.1	1.17	28	14.5
A6	2.9 ± 0.7	5.4 ± 0.03	1.20	30	16.1
A7	3.2 ± 0.5	6.1 ± 0.02	1.19	31	15.4
A8	2.4 ± 0.5	4.7 ± 0.1	1.13	29	11.4

### Other Physicochemical Properties

#### Swelling

The relationship between the release of a drug from a polymer matrix and swelling is vital to the design of a delivery system that meets its objectives of timed and targeted delivery. The microspheres absorbed water and attained their maximum sizes within 3 hours, after which the microspheres began to disintegrate by leaching, erosion or both processes (Aulton, 2007).

The swelling index ranged from 2.4 to 8.7% (Table 3) with the swelling index decreasing as the concentration of sodium alginate increased. Swelling properties of microspheres have been reported to depend on the type and concentration of natural gum used in the formulation (Odeku *et al.*, 2013). Swelling in microspheres also depends to a large extent on the particle sizes of the beads formed. Water absorption into smaller particles have been shown to be faster due to the shorter distance between the surface and the centre of the particles (Ayorinde *et al.*, 2017); this is probably attributed to the high swelling index observed generally in the formulations, due to their small particle size.

An initial rapid swell of the microsphere was observed in the first 30 minutes of contact with water. Swelling is an important first step in the breakdown and disintegration of microspheres and is an indication of the materials' ability to take in water, swell, and in turn liberate entrapped drug. In swelling, wetting of matrix surface is usually the first step that occurs; thereafter, liquid may then penetrate into the matrix and swelling behaviour of microspheres helps to predict the drug release profile (Quan *et al.*, 2008).

Studies of HPMC-matrix suggests that increase in polymer concentration is directly proportional to an increase in swelling rate, however, the reverse may

be obtainable in very high concentrations as they may slow down water uptake (Wan *et al.*, 1993).

#### Microspheres Size and Morphology

Sizes of formulated microspheres ranged from 100-850 micrometer. Morphology and surface characteristics of microspheres formed were obtained with a Scanning Electron Microscopy (SEM). SEM images revealed discrete porous and spherical microspheres (Fig. 1). Polymer concentrations used had a significant effect on the size and sphericity of the microspheres; formulations with higher concentrations of sodium alginate were more spherical, with this rank order among the formulations: A4 > A3 > A2 > A1 > A5 > A6 > A7 > A8. Particle shape is an important consideration in pharmaceutical operations influencing flow properties, uniform packing, friability and even particle solubility. Spherical particles flow better, ensures reduced friability and uniform packing, and improves therapeutic benefits due to enhanced solubility. The rate of solvent removal also exerts an influence on the morphology of beads (Abbrera *et al.*, 2010). According to a study, the effect of the rate of solvent removal on particle morphology is as a result of surface recession rate of the droplet being dried. This surface recession rate is a function of the evaporation rate. The receding droplet surface causes a diffusional flux of the solutes away from the surface towards the centre of the droplet (Vehring *et al.*, 2007).

#### FT-IR Spectroscopy

The FT-IR spectra of the extracts are similar with the spectra of the microspheres. This suggests that formulating the extracts into microsphere drug delivery system did not alter any of the functional groups (Figures 2A and 2B). No major shifting of

functional groups was noted; only new peaks characteristic of the polymers used were seen in spectra of the microspheres which further suggest that there were no extract-polymer interactions. Strong absorptions formed in the region of 1600 - 1450  $\text{cm}^{-1}$  indicating the presence of aromatic rings continue to abound even in the final preparations. Also, broad absorption peaks at wavelengths of 3379 and 3309  $\text{cm}^{-1}$  indicate an amine group. Literature studies reveal similar functional groups in barks of *C. pentandra* (Kumar *et al.*, 2017).

### **Entrapment Efficiency**

Entrapment efficiency is a measure of the amount of a drug contained in the microspheres, in relation to the initial quantity of drug used in the formulation. A reasonable drug entrapment is central to the sufficient distribution of drugs to tissues. The ability of the formulated microspheres to encapsulate *C. pentandra* extracts was assessed by measuring the flavonoid contents of the formulations (Table 3).

Entrapment efficiency of the microspheres was poor, it ranged from 4.70 to 19.20 %. Formulations with high concentrations of sodium alginate and calcium chloride (A2, A3 and A4) had better entrapment than other formulations. This could be attributed to the greater availability of active calcium binding sites in the polymeric chains and therefore the higher degree of cross linking (Umesh *et al.*, 2013). The concentration of cross-linking agent (Calcium chloride) used in the formulation of microspheres is an important factor that can affect the entrapment efficiency. The higher the concentration of cross linker used the better the entrapment efficiency (Petel *et al.*, 2004).

Another parameter which could affect the entrapment efficiency is the stirring rate. The stirring speed used had an inverse relationship with the flavonoid entrapment; decrease in entrapment efficiency of the flavonoid was obtained as the stirring speed increased. Previous study suggested that an increase in stirring rate would lead to a decreased mean particle size of microspheres formed and the formation of hollow spheres which in turn could lead to a reduction in entrapment efficiency (Kulkarni *et al.*, 2012). The reduction in flavonoid entrapment as the stirring speed increased was probably due to the decreasing particles size.

### **Release studies**

Studies of drug release from a dosage form are important quality assurance parameters. Drug release

from micro particulate systems involves mass transfer of drug from regions of higher concentration to regions of lower concentrations by various processes. These processes include dissolution, dispersion, swelling, erosion mechanisms either alone or in combinations (Aulton, 2007).

However, difficulties abound in modeling drug release data as there is a wide diversity in the physical form of micro/nano particles or capsules with respect to size, shape, arrangement of the core and the coat; properties of core such as solubility, diffusibility, partition coefficient, properties of coat; such as porosity, tortuosity, thickness, crystallinity, inertness etc. Also, there are problems in translating kinetics of drug release from "micro" products of perfect geometry to various irregular micro systems. The particle size of microspheres has a profound effect on drug release; smaller sized microspheres have been reported to offer better drug release profiles as a result of increased area-to-volume ratio of the particles (Ayorinde *et al.*, 2017)

The calibration curve (Figure 3) obtained displayed high linearity as observed from the regression coefficient of not less than 0.99, and therefore renders subsequent determinations from the calibration curve equation valid.

In accordance with the United States Food and Drug administration, there should be at least 80% release of the active pharmaceutical ingredients from the modified release form in twenty-four hours. A suitable extended release dosage form will not only enhance therapeutic efficiency and patient compliance due to reduced dosing frequency, but also produce more desirable blood levels and lower incidence of adverse effects.

Result of dissolution from all batches of *C. pentandra* microspheres are shown in Figure 4. In all the batches, cumulative percentage of drug released over 8 hours ranges from 76.30 to 89.10 % in a gradual release for the first 3-5 hours. Batch A1 microspheres release more of the flavonoids than the other batches but did not release 50% of the flavonoid until the 5<sup>th</sup> hour; this suggests that this batch did not exhibit a sustained release profile.

On the other hand, microspheres of Batches A2 and A3 had slightly lower percent drug release than A1 and shows a better sustained release effects over a longer period. Batch A8 microspheres also showed a release profile that is similar to that of the Batch A1, however, the release of flavonoid from Batch A8 was sustained, a profile similar to Batches A2 and A3.



Interestingly, none of the formulations show the undesirable initial burst release that is typical of most modified release preparations (Ayorinde *et al.*, 2017).

All batches except A2 had compendial standards in terms of releasing 80% of its drug content in the duration of the dissolution testing. The differences in dissolution times could be attributed to the varying polymer concentration employed in the different batches.

**Factorial Experiments**

Results of the factorial experimental designs are presented in Tables 4, individual effects of the variables and 5, interactive effects of the variables. The effects of independent process variables; concentration of sodium alginate (C), stirring speed (S), and curing time (T) were evaluated on two microsphere performance parameters (entrapment efficiency and percentage flavonoid release). The statistical modulation which utilizes a 2<sup>3</sup> factorial experimental design was employed (Ayorinde and Itiola, 2010). The basis of the experiment was to use a two-level factor (low and high) with the three variables.

The individual and interaction effects provide a clear indication of the qualitative effects of the variables studied on the entrapment efficiency and percentage drug release. A result of zero indicates no interaction between the variables. The amount, by which any value departs from zero, whether positive or negative, gives a quantitative measure of the effect of the variable involved in the response parameter or the

extent of the interaction between two variables. A positive result shows that the values of the parameter have increased while a negative result indicates that the value of the parameter has decreased (Ayorinde and Itiola, 2010; Ayorinde and Odeniyi, 2021).

The ranking of the individual effects on entrapment efficiencies was X1>X3>X2 while the ranking on percentage drug release was X2>X3>X1 (Table 4 and Figure 5). This ranking shows that concentration of sodium alginate has the greatest positive effect on the entrapment efficiency; changing the concentration of sodium alginate from a lower (0.5 g) to a higher (2 g) value increased the entrapment efficiency. On the other hand, curing time had the most negative effect on the entrapment efficiencies; in other words, an increase in the curing time during the formulation of microspheres led to lower entrapment efficiency. The stirring speed also has a positive but almost insignificant (p > 0.005) effect on the entrapment efficiency.

The effect of the variables on percentage release at 360 minutes had the following ranking: X2>X3>X1. All three variables had a negative effect on the percentage drug release, although, to varying extents. Curing time had the most significant negative effect on percentage drug release while concentration of sodium alginate had the least negative effect. The percentage release at 360 minutes was used for the factorial experiment because the microspheres prepared are intended for sustained drug delivery. Therefore, this factorial design gives an insight into which variables are more important for a sustained release formulation.

**Table 4: Individual Effects of Concentration of Sodium Alginate C, Curring Time T and Stirring Speed S on Entrapment Efficiency (EE) and Percentage Release**

Variables	Formulation Codes	Independent Coefficient	
		Entrapment Efficiency (EE)	% Release
C	X <sub>1</sub>	4.475	-0.735
T	X <sub>2</sub>	-5.575	4.785
S	X <sub>3</sub>	0.275	-2.625

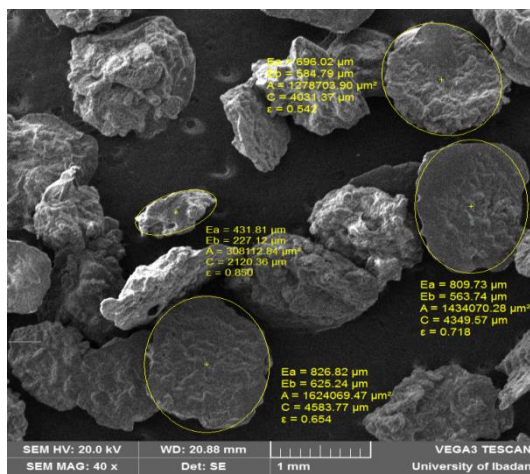
**Table 5: Interactive Effects of Concentration of Sodium Alginate C, Curring Time T and Stirring Speed S on Entrapment Efficiency and Percentage Release**

Variables	Formulation Codes	Independent Coefficient	
		Entrapment Efficiency (EE)	% Release
C-T	X <sub>1</sub> X <sub>2</sub>	-4.525	1.815
C-S	X <sub>1</sub> X <sub>3</sub>	2.525	1.175
T-S	X <sub>2</sub> X <sub>3</sub>	-1.225	-0.075

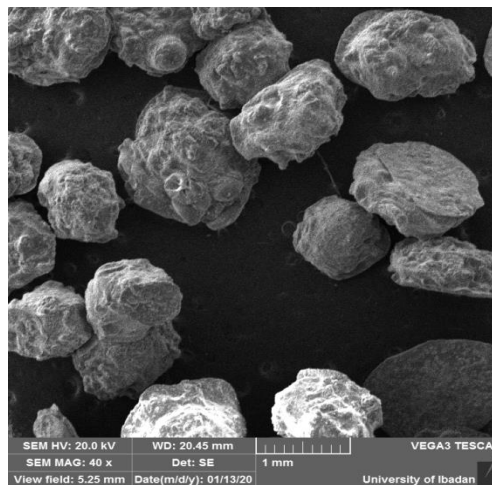
The interaction coefficient values are shown in Table 5 and the effects are presented on contour plots in Figure 6; they indicate the effects of the variables in combination. The ranking for the interaction effects on entrapment efficiency was X1X3>X1X2>X2X3. This result shows that the interaction between the concentration of sodium alginate and stirring speed had a positive effect on the entrapment efficiency of microspheres. All other interactions had negative effects on the entrapment efficiency, with an interaction between concentration of sodium alginate

and curing time notably having the strongest negative effect.

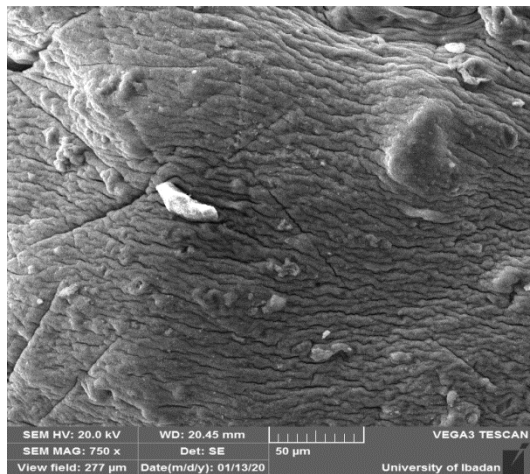
Furthermore, the percentage drug release at 360 minutes was mostly influenced positively by the interaction between concentration of sodium alginate and curing time. An interaction between concentration of sodium alginate and stirring speed also had a positive effect on percentage drug release at 360 minutes. However, the interaction between curing time and stirring speed had a negative effect on percentage drug release at 360 minutes.



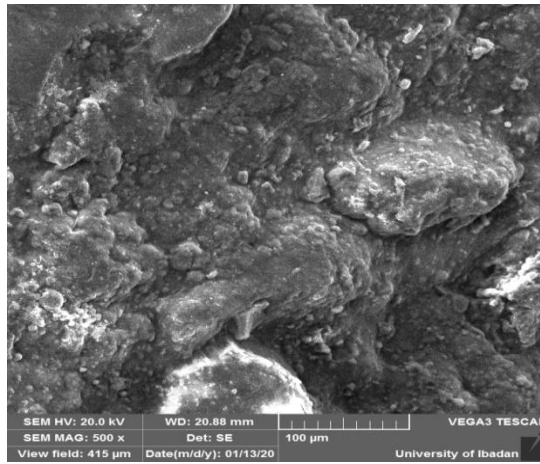
A



B



C



D

Figure 1:  
 Scanning electron micrograph of A1 microspheres (40 x magnifications)  
 Scanning electron micrograph of A2 microspheres (40 x magnifications)  
 Scanning electron micrograph of A3 microspheres (750 x magnifications)  
 Scanning electron micrograph of A4 microspheres (500 x magnifications)

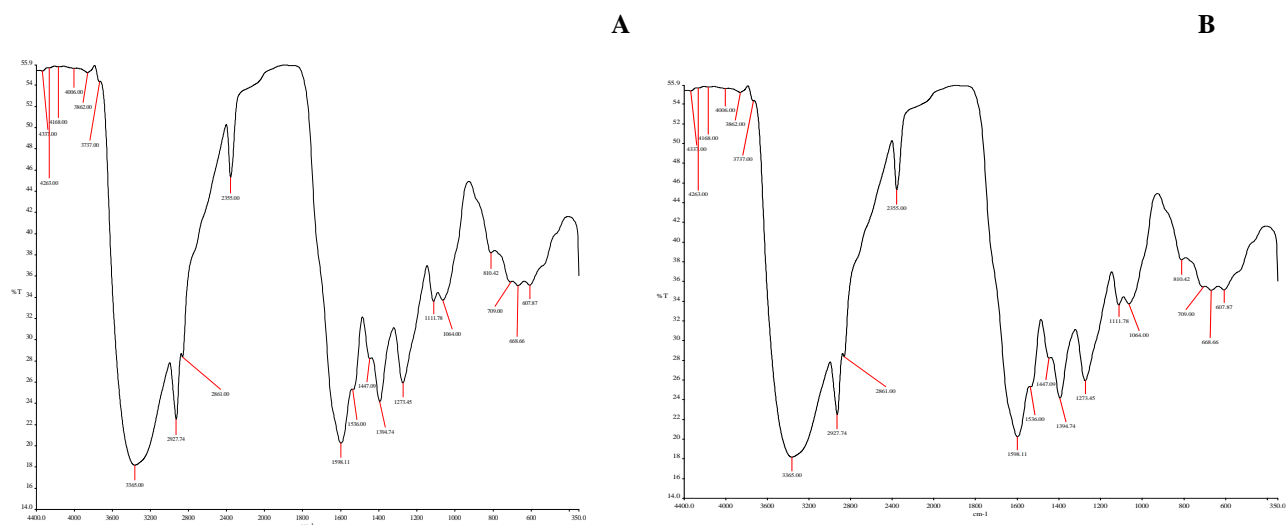


Figure 2: FT-IR Spectra of *Ceiba pentandra* Leaf Extract (A) and Microspheres (B)

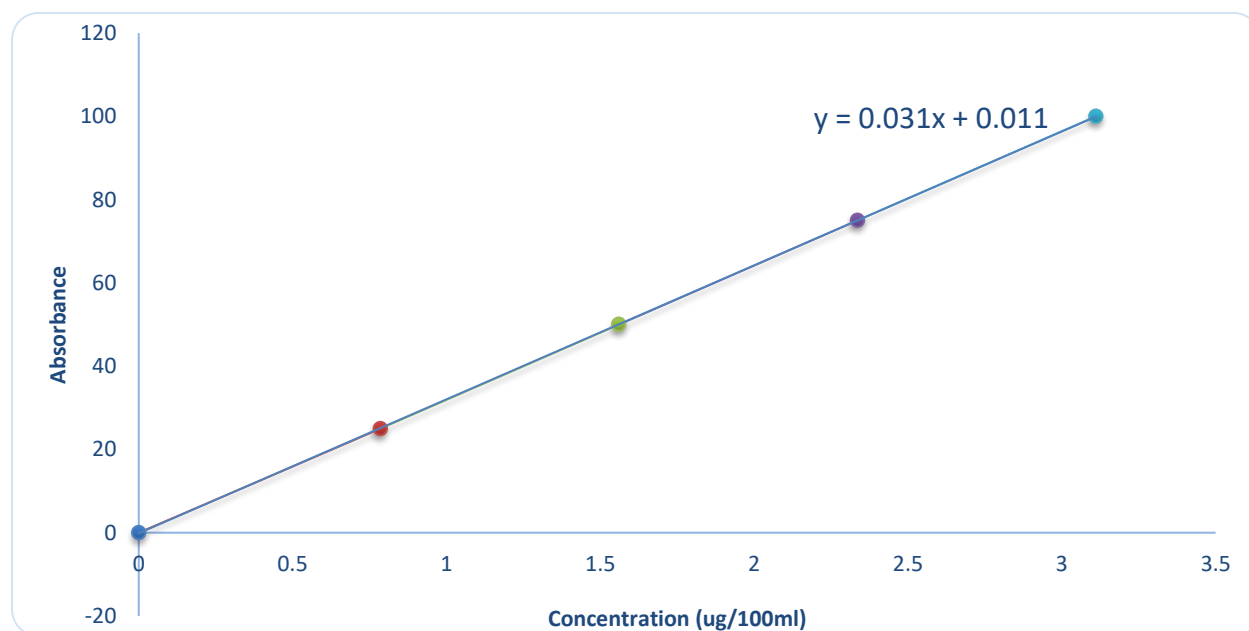


Figure 3: Standard Calibration Curve of Quercetin

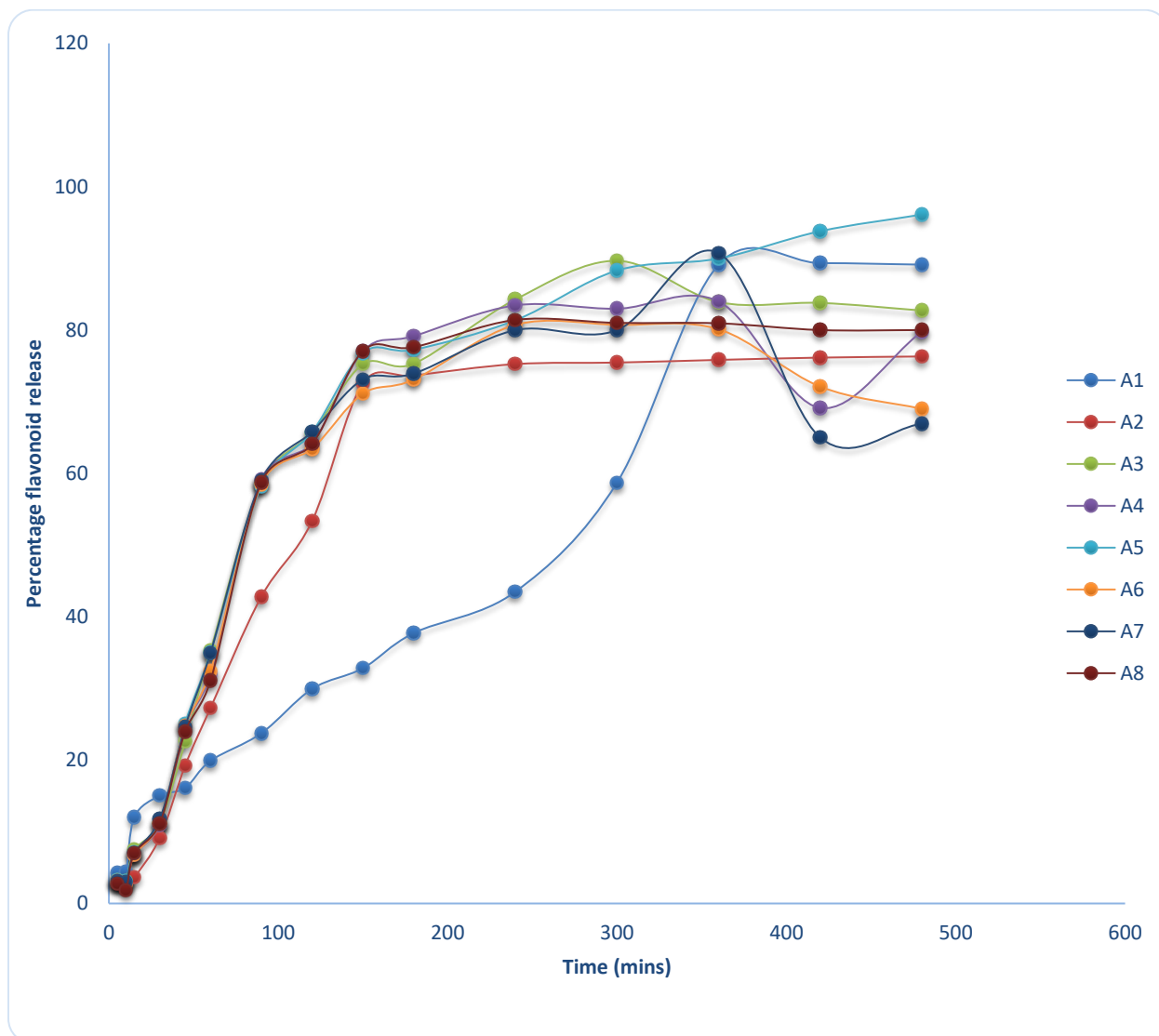
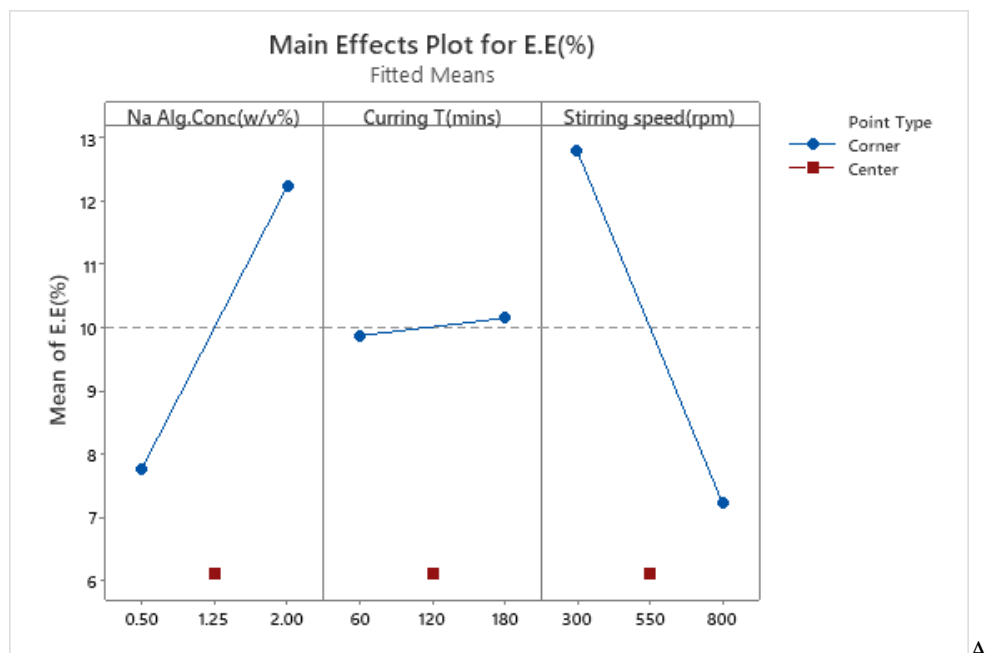


Figure 4: Plots of Flavonoid Release from Microspheres



A

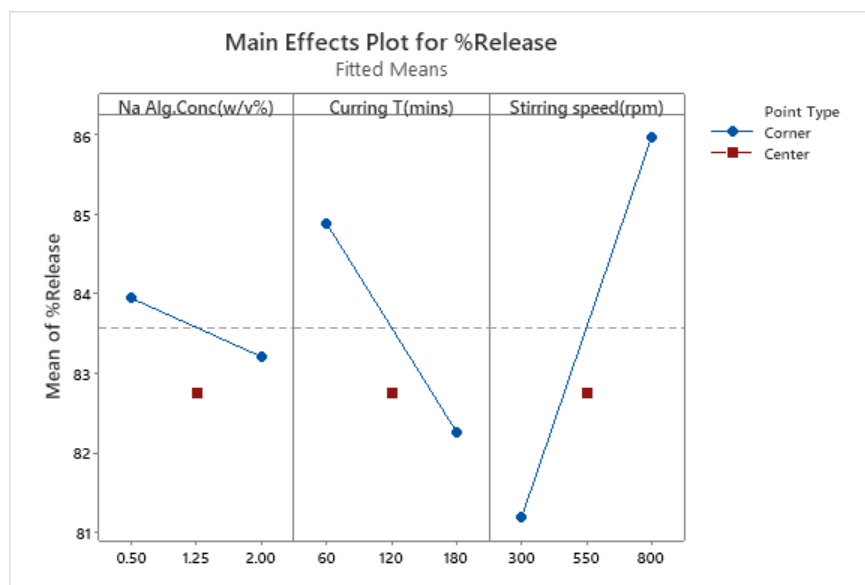
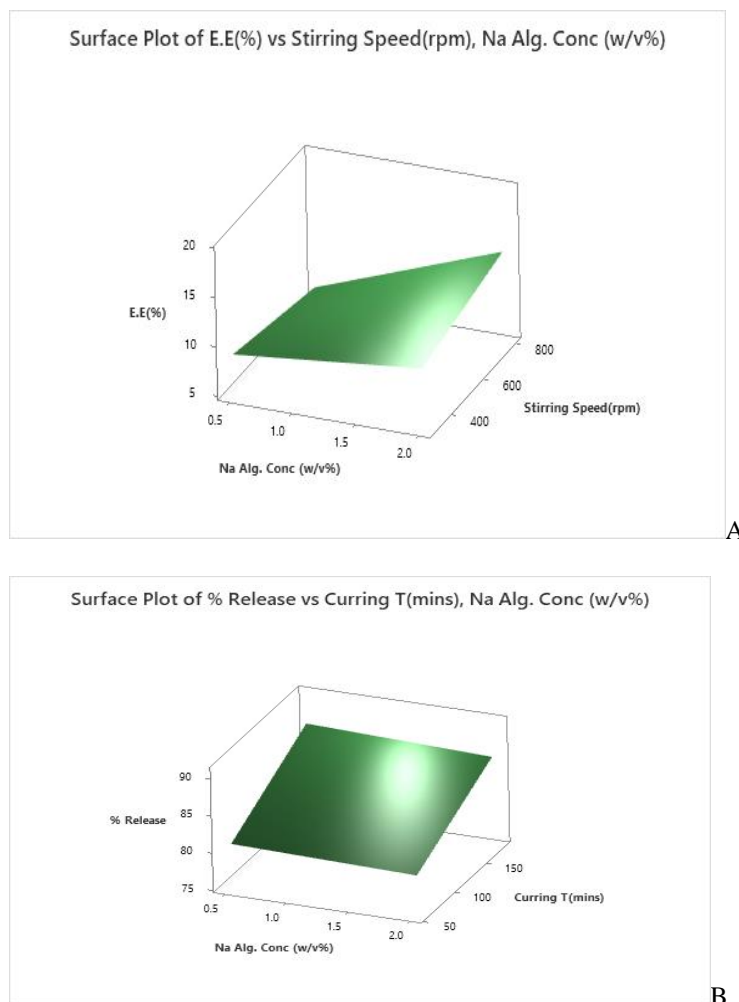


Figure 5: Main effect plot of formulation variables on Entrapment Efficiency (A) and Flavonoid Release (B)



**Figure 6: Surface plot of interactive effects of formulation variables on entrapment efficiency (A) and Flavonoid Release (B)**

## CONCLUSION

The aqueous extract of the leaves of *Ceiba pentandra* contained flavonoids with good antioxidant potentials. The extract was suitable for the formulation of microsphere drug delivery system with good physicochemical properties and flavonoid release profiles. Microspheres of *Ceiba pentandra* leaves could be formulated to achieve both

immediate and sustained release profiles, depending on the formulation variables. Concentrations of sodium alginate and curing times are important parameters that should be considered in the formulation of *Ceiba pentandra* extracts into microspheres.

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Conflict of Interest: None declared

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