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Research Article

Sustained Release Mebendazole Microcapsules Prepared with *Prosopis africana* Peel Powder (PAPP) Hydrogel

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ABSTRACT

Sustained-release microcapsules of mebendazole were prepared using *Prosopis africana* peel powder (PAPP), a novel biopolymer, in the ratios of 1:1, 1:2 and 1:3 by the emulsification method. The microcapsules were evaluated for particle size, morphology, encapsulation efficiency, thermal property, *in vitro* drug release and larvicidal activity on 20 active mosquito larvae. The results show that particle size and encapsulation efficiency (EE%) of the mostly irregularly shaped microcapsules were independent of polymer concentrations, yet the 1:3 (inner coat) and 1:2 (outer coat) microcapsules gave the highest EE% of 72 and 81 % respectively. Thermograms of the microcapsules generally revealed lower melting temperatures suggesting total disappearance of mebendazole peak, indicating that mebendazole was molecularly dispersed in the polymeric microcapsules. The *in vitro* release study indicates that drug release in simulated intestinal fluid (SIF, pH 7.2) followed a pattern: 1:1>1:2>1:3 (for the inner coat microcapsule) and 1:1>1:3>1:2 (for the outer coat) better than in simulated gastric fluid (SGF, pH 1.2). Microcapsules (1:3 outer coat) recorded 65% death of mosquito larvae in 24 h while mebendazole recorded 100 % death in 24 h; microcapsules made with the inner coat recorded death of 50 % of the mosquito larva in 24 h. The larvicidal activity of mebendazole was sustained for up to 3 days in all the formulations unlike the reference standard. This study suggests that PAPP could be employed in sustained delivery of mebendazole for improved patient compliance thus reducing the frequency of drug administration with associated side effects of mebendazole.

INTRODUCTION

In many resource poor countries, there is a preponderance of cases of infestation with intestinal worms especially in young children. Although these worm infections do not cause significant morbidity and mortality, when compared with many other parasitic infections, it has contributed to the prevalence of malnutrition, anaemia, diarrhoea, eosinophilia, pneumonia and poor health in children [1, 2]. Parasitic helminthes also affect millions of livestock resulting in considerable economic losses in domestic and farmyard animals. Over the years, helminthiasis had been controlled by the administration of synthetic anthelmintics, which are drugs that expel parasitic worms (helminths) from the intestinal tract or tissues of the body by either stunning or killing them [3-5].

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Among the various available antihelmintics can be found the benzimidazoles, and within this class of medicines, mebendazole (MBZ) is of paramount importance because of its proven efficacy [6-8]. Mebendazole [(5-benzoyl-1H-benzimidazole-2-yl)- carbamic acid methyl ester, MBZ] is a potent, orally active, broad-spectrum antihelmintic employed in the treatment of a wide range of worm infestations such as ascariasis, uncinariasis, among others [9, 10]. Several studies indicate that Mebendazole exerts its action by inhibiting glucose uptake in the parasite, resulting to immobilization and subsequent death of worms [8, 11]. Recent studies indicate that Mebendazole exists in a variety of physical forms [12]. These solid phases have been extensively studied. Findings indicate that the drug exists in three polymorphic forms (A, B, and C) and these polymorphs display marked differences in their solubility and therapeutic effects [13, 14]. MBZ belongs to class II of the biopharmaceutics classification systems (BCS)

and has been shown to have limited aqueous solubility, and high doses of the drug are required for the treatment of helminthic infections with the added disadvantage of a high incidence of adverse effects such as anaemia and liver damage [13]. In order to obviate the limitations associated with MBZ administration, it has been necessary to explore other means of administering the drug.

In recent times, a lot of effort has been exerted in the field of drug delivery to formulate novel drug delivery platforms that could have a positive impact on the efficacy of drug release both *in vitro* and *in vivo* [15, 16]. Microencapsulation, a processing technique which emerged in the 1950s, has been the focus of intense pharmaceutical research and it is estimated that more than 65 % of all sustained release systems utilize some form of microencapsulation [17]. The popularity of this technique hinges on the wide range of applications attainable [18-21]. In recent times, microencapsulation has emerged as a veritable mode of delivering numerous drugs in a manner that improves their performance [17]. To this end, numerous drugs have been microencapsulated [21-23] and this makes for more efficient drug delivery because it enhances the ability of the drug to interact with the body [15]. Additionally, the active ingredient is normally enclosed in a particle of very small dimension [16]. The developed microcapsules or microparticles may be engineered to achieve controlled and sustained release of drug in the body. Several methods are available for the preparation of microcapsules and microparticles, including air suspension method, pan coating, multi-orifice centrifugal processing, coacervation, solvent evaporation as well as spray drying and spray congealing techniques [21, 22]. Microencapsulation is controlled by many factors such as the drug-polymer ratio, the choice of the coating material, the concentration of the microencapsulating agent and the processing parameters [23].

Prosopis africana (Fam. Mimosaceae), a perennial leguminous woody tree of about 70 ft high mostly found growing in the savanna regions of West Africa [24], has very hard stem that is used in different parts of Nigeria for making boats, pestle and wooden gong; its fermented seeds are used as food condiment/flavouring agent [25]; its young leaves and shoots are folders highly sought after towards the end of the dry season [26]; various parts of the tree have been used for the treatment of various ailments such as bronchitis, dermatitis, gonorrhoea, dysentry, malaria, rheumatism, sore throat, fevers, skin diseases, headache, toothache, stomach cramps, skin diseases, and as a dressing for wounds or cuts [24, 27]; the gum from the seed mesocarp possesses coagulant property against contaminated aqua system [28] and drug delivery properties [29-31], and has been used by our research team in pharmaceutical formulations such as tablets and suspensions [32-34]. In this work, we employ the outer and inner bark of *Prosopis africana* peel powder (PAPP) of prosopis seed as novel polymeric material for the formulation of hydrogel microcapsules with the goal of bringing about a reduction in the particle size of mebendazole that will come in contact with the absorption sites in the body. It is envisaged that the reduction of drug particle size will cumulate in enhanced specific surface area and concomitantly result in improved drug dissolution rate. This would widen the horizon of application of PAPP in drug formulation, specifically as a novel microencapsulating material for mebendazole, which has earlier been delivered as microparticles using low-substituted hydroxypropylcellulose and as microencapsulated targeted drug delivery system by previous researchers [35, 36].

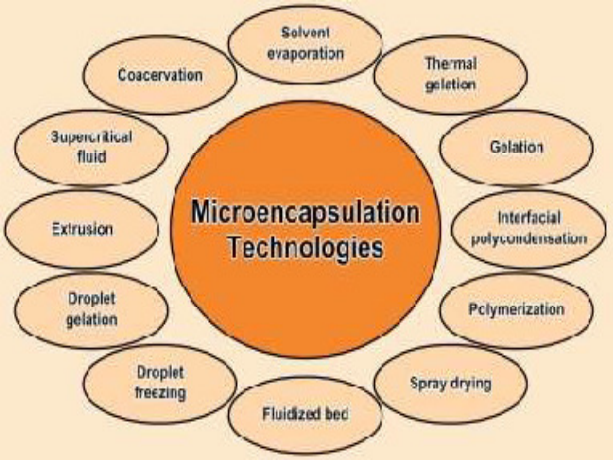


Figure 1: Methods of microencapsulation. [22]

Therefore, this present study was conceived with the objective of developing a novel microparticulate drug delivery system that will effectively deliver mebendazole in a consistently safe and efficacious manner. The mebendazole microcapsules were prepared using outer bark and inner bark of *Prosopis africana* peel powder (PAPP) of prosopis seed by emulsification-coarcervation method and the capability of the novel biopolymers in sustaining the release of mebendazole was ascertained, as

Table 1: Quantities of ingredients used for preparation of mebendazole microcapsules

Batch	Ratio of drug: polymer (g)	Tween® 80 (ml)	Magnessium stearate (mg)	Acetone (ml)	Canola oil (ml)	Water (ml)
Microcapsules from inner seed coat						
A	1:1	2.0	50.0	50.0	5.0	50.0
B	1:2	2.0	50.0	50.0	5.0	50.0
C	1:3	2.0	50.0	50.0	5.0	50.0
Microcapsules from outer seed coat						
D	1:1	2.0	50.0	50.0	5.0	50.0
E	1:2	2.0	50.0	50.0	5.0	50.0
F	1:3	2.0	50.0	50.0	5.0	50.0

Keys: batch A means microcapsule 1:1 PAPP inner coat, batch B means microcapsule 1:2 PAPP inner coat, batch C means microcapsule 1:3 PAPP inner coat, batch D means microcapsule 1:1 PAPP outer coat, batch E means microcapsule 1:1 PAPP outer coat, while batch F means microcapsule 1:1 PAPP outer coat; batches A-C are microcapsules prepared with inner bark of PAPP while batches D-F are microcapsules prepared with outer bark of PAPP.

this would lead to reduction in frequency of dosing as well as improving patient compliance. In addition, the *in vitro* larvicidal activity of the mebendazole microcapsules was determined.

MATERIALS AND METHODS

Materials

The following materials were used as procured from their suppliers without further purification: Canola oil (Nigeria), Magnesium steareate (Merck), Tween® 80 (Germany) Sodium hydroxide (Wharfedale laboratories, Otley, UK), Mebendazole pure powder (Figure 2) (Hetero Drugs limited, India), Acetone (BASF, Germany) and distilled water (Lion water, Nsukka, Nigeria). All other reagents and solvents were of analytical grade and were used as supplied.

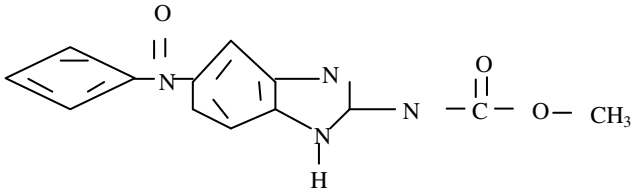


Figure 2: Chemical structure of Mebendazole.

Preparation of the polymeric material

Graded quantities (1, 2, and 3 g) of the inner seed coat of PAPP was weighed out and dispersed in 50 ml of distilled water in a 250 ml beaker using magnetic stirrer. These different polymer dispersions represent different wall ratios. A 50 mg quantity of magnesium stearate and 2 ml of Tween® 80 were also incorporated into the polymer solution. The above procedure was repeated for the outer seed coat powder.

Preparation of microcapsules

The microcapsules were prepared using the emulsification-coacervation method, which was recently employed by our research team to prepare insulin microspheres [37]. *Prosopis africana* peel powder served as the polymer wall material, A 50 mg quantity of magnesium steareate served as the lubricant, Tween® 80 acted as the anti- aggregation agent. Three batches of microcapsules were prepared using different polymer/ drug ratio as shown in Table 1. A 1 g quantity of drug was weighed and dissolved in 5 ml of Canola oil, the mixture of drug/oil was poured into the 50 ml of the polymer solution formed above and stirred for 10 min using a magnetic stirrer plate at a speed of 120 rpm in order to obtain an O/W emulsion. The O/W emulsion was further mixed with a high speed homogeniser Ultra-Turrax at 10, 000 rpm for 5 min to break down the emulsion into smaller droplets. 1 % sodium hydroxide solution was added gradually to the emulsion until a pH of 7.5 was attained so as to allow for complete precipitation. The precipitate formed was washed with acetone and spun down using a centrifuge (Medifield equipment and Scientific Limited, UK) at a speed of 1,000 rpm in order to remove unwanted materials including excess polymer and unencapsulated oil. The microcapsules formed were then dried at ambient room temperature. The above procedure was repeated for the outer seed coat powder.

Determination of percentage yield

The formed microcapsules were recovered and weighed accurately. The yield of microcapsules was determined by comparing the whole weight of formed microcapsules against the combined

weight of the copolymer and drug using the equation below:

$$Yield\ (\%) = \frac{Total\ weight\ of\ microcapsules\ obtained}{Total\ weight\ of\ the\ polymer\ and\ the\ drug\ used}\ X\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Morphology and particle size analysis

The size and morphology of the microcapsules were analyzed by computerized image analysis using samples mounted on a glass slide (Marinfield, Germany). The sample (0.01 g) was dispersed in little quantity of liquid paraffin and smeared on the slide using a glass rod. It was then covered with a cover slip and viewed with a photomicroscope (Hund®, Weltzlar, Germany) attached with a digital camera at a magnification of 400 x. With the aid of the software in the photomicroscope, the particle morphologies were observed and photomicrographs taken. The sizes of the particles were measured (n = 30) and average taken.

Encapsulation efficiency

A quantity of each batch of the microcapsules containing theoretically 0.1 g of the drug was accurately weighed using electronic balance (Seiko, Japan), transferred to a beaker containing 1 ml volume of formic acid and stirred to dissolve the microcapsules. To this solution was added 9 ml of water and the resulting solution was thoroughly mixed, centrifuged at 4000 rpm for 10 minutes, filtered and analyzed spectrophotometrically at a wavelength of 310 nm using a UV spectrophotometer. A mixture of formic acid and diluted water in a ratio of 1:9 served as the blank. Encapsulation efficiency (%) was calculated using the following formula:

$$Encapsulation\ efficiency\ (\%) = \frac{Actual\ drug\ loading}{Theoretical\ drug\ loading}\ X\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ (2)$$

Differential scanning calorimetry (DSC) analysis

Differential scanning calorimetry (DSC) analysis was carried out in other to assess the thermal behaviour of the various samples. Thermal analysis was conducted for mebendazole pure powder, polymer samples (inner and outer barks of PAPP) and microcapsule formulations. The sample weight of 2 mg placed in a perforated aluminium pan of a differential scanning calorimeter (NETZCH DSC 204 FI Germany) operated at a heating rate of 10 ^{0}C/min over a temperature range of 30 – 360 ^{0}C under an inert nitrogen atmosphere with flow rate of 20 ml/min.

***In vitro* release of mebendazole from microcapsules**

Mebendazole release from the microcapsules was evaluated in both simulated intestinal fluid (SIF, pH 7.2) and simulated gastric fluid (SGF, pH 1.2) at 37 ± 1 °C using an established method based on magnetic stirrer plate [37]. The polycarbonate dialysis membrane (pore size 0.22 mm) used as release barrier was pre-treated by soaking it in the dissolution medium for 24 h prior to the commencement of each release experiment. In each case, approximately 300 mg microcapsules were placed in dialysis membrane containing 5 ml release medium, suspended in a 250 ml beaker containing 200 ml of the release medium and tied with paddle of dissolution apparatus. Agitation of the fluid system (100 rpm) was done with a magnetic stirrer (Remi Instruments, Mumbai, India). At predetermined time intervals, 5 ml samples were withdrawn and replaced with each release medium to maintain a sink condition. The mebandazole content of the withdrawn samples was determined using spectrophotometer at 295 nm and the release profiles plotted.

Kinetic analysis of *in vitro* release profiles

The dissolution data for the microcapsules were analyzed to determine the *in vitro* release kinetic models and mechanisms. Four kinetic models including the zero-order, first-order, Higuchi square root and Hixson-Crowell cube root models were applied to process the release data to find out the equation with the best fit using the equations 3-6 below [19]:

$$Q = K_1 t \quad \ldots\ldots\ldots\ldots\ (3)$$

$$Q = 100(1 - e^{-K_2 t}) \quad \ldots\ldots\ldots\ldots\ (4)$$

$$Q = K_3 (t)^{1/2} \quad \ldots\ldots\ldots\ldots\ (5)$$

$$Q = 100^{1/3} - K_4 t \quad \ldots\ldots\ldots\ldots\ (6)$$

Where Q is the release percentage at time, t. K_1, K_2, K_3, and K_4 are the rate constants of zero-order, first-order, Higuchi, and Hixson-Crowell models, respectively.

Evaluation of larvicidal activity of microcapsules

This test was conducted following established methods with slight modification [38 - 41]. Larvae of *Anopheles gambiae* Giles S.S used in bioassays were obtained from a colony maintained at the International Centre of Insect Physiology and Ecology (ICIPE) Insect Mass Rearing Unit, Department of Physiology, University of Nigeria, Enugu Campus. The larvae were fed on Tetramin® fish food (Terta GmbH, Germany) or biscuit crisps at about 1mg per beaker every 24-h and the water temperature was maintained at 28 ± 2 °C throughout larval development. Larvicidal and insect growth regulatory (IGR) activities were conducted in accordance to the World Health Organization method [42]. Batches of twenty freshly moulted late 3rd and early 4th instar larvae of *Anopheles gambiae* Giles S.S were transferred by means of dropper to glass beakers containing 100 ml of tap water. Appropriate volume of stock solution where the microcapsule formulation or mebendazole pure powder were dissolved in 5% dimethylsuphoxide (DMSO) was added to 100 ml water in the glass beakers to obtain different solutions containing same amount of mebendazole. Twenty active mosquito larvae were carefully incorporated into each of the 100 ml solution in a beaker. Each beaker was covered and the set up was then allowed to stand for 24, 48 and 72 h respectively after which the number of dead or immobile larvae was counted under a microscope (at 4 × magnification). The percent mortality (Mc %) was determined using Abott's formula given in equation 7 for corrected mortality [43].

$$Mc\ (\%) = \frac{Mce - Mt}{100 - Mt}\ X\ 100 \quad \ldots\ldots\ldots\ldots \quad (7)$$

Where Mce is the mortality obtained during the test and Mt the mortality registered in the negative control dishes. It is considered that when the mortality rate in the latter dishes is less than 5%, Mc = Mce [44]. The counting was carried out at intervals of 24 h for 3 days and three replicates were set up for each solution.. The bioassay room was kept at a temperature of 30 °C, an average humidity of 78 % and a photo period of 12 h of light and 12 h of darkness. Mebendazole was used as positive control in the larval test since the larva is structurally close to adult which is the main target of this synthetic drug. The same concentration as microcapsule formulations was used in the study.

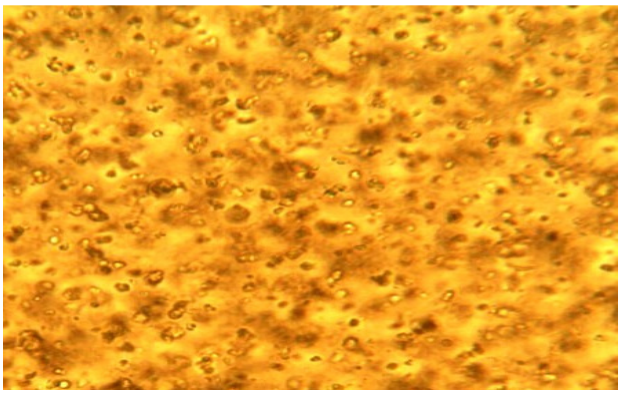
Statistical analysis

All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean ± SD. Student's t test was performed on the data sets generated using Statistical Package for Social Sciences (SPSS). Differences were considered significant for p values ≤ 0.05.

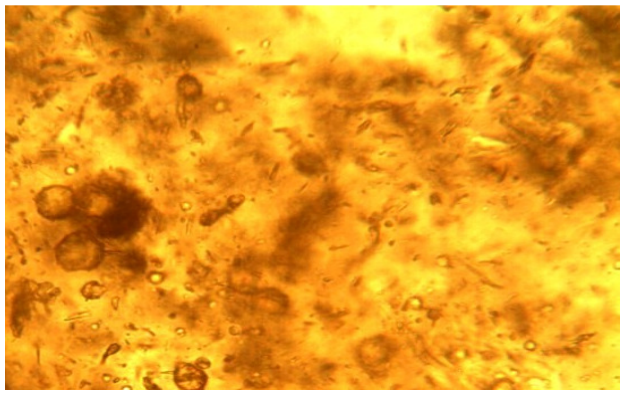
RESULTS AND DISCUSSION

Worm infestations continue to be one of the most prevalent diseases and serious public health problems worldwide. The major control strategy adopted against helminths or worm parasites has been the use of anthelminthics, which are drugs used to eradicate or reduce the number of helmintic or worm parasites in the intestinal tract or tissues of human and other animals [5]. However, the current development of resistance to most commercially available anthelmintics for example mebendazole and the associated side effects clearly suggests that novel drug delivery carriers could be designed to surmount this problem. In this study, we employ the outer and inner bark of *Prosopis africana* peel powder (PAPP) of prosopis seed as novel polymeric material for the formulation of hydrogel microcapsules of mebendazole for sustained drug delivery and to circumvent the adverse effects associated with high doses of this drug [12 - 14].

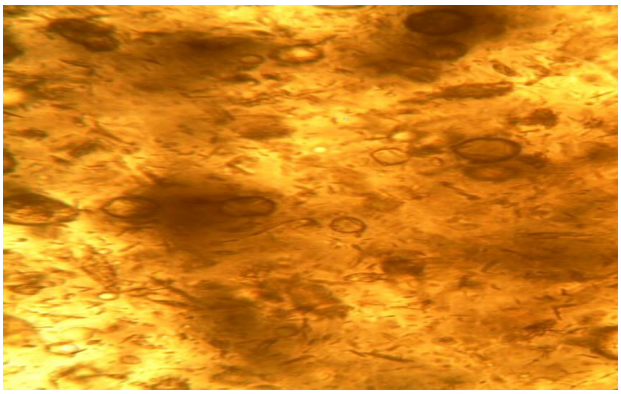
The percentage yields of the microcapsules were high, 72.5 % for batch A microcapsules formulated with drug and PAPP inner coat in ratio 1:1, 81.3 % for batch B microcapsule formulated with drug and PAPP inner coat in ratio 1:2, 68.0 % for batch C microcapsules formulated with drug and PAPP inner coat in ratio 1:3, 64.5 % for batch D microcapsules formulated with drug and PAPP outer coat in ratio 1:1, 59.8 % for batch E microcapsules formulated with drug and PAPP outer coat in ratio 1:2, and 77.6 % for batch F microcapsules formulated with drug and PAPP outer coat in ratio 1:3. The implication is that the formulation technique adopted (emulsification-coacervation) was reliable. There was no evidence of correlation between the proportion of polymer used in the formulation of the microspheres and the microcapsules yield. The percentage loss recorded could be attributed to losses during the filtration, transferring or drying, consistent with previous studies which employed this method of microencapsulation [37, 45].



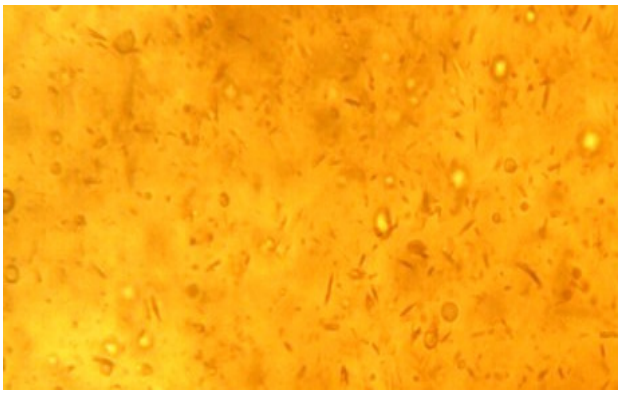
(a)Batch A



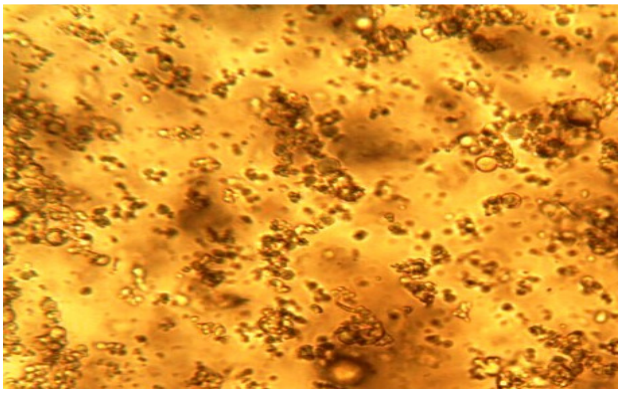
(b) Batch B



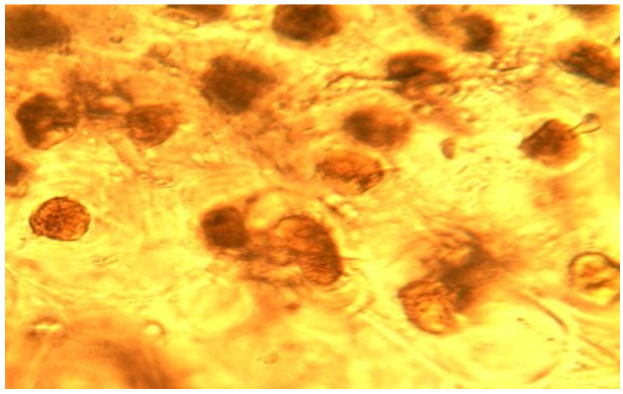
(c) Batch C



(d) Batch D



(e) Batch E



(f) Batch F

Figure 3: Photomicrograph of mebendazole-loaded microcapsules containing various amounts of PAPP: (a) batch A, (b) batch B, (c) batch C, (d) batch D, (d) batch E, (f) batch F.

Keys: batch A means microcapsule 1:1 PAPP inner coat, batch B means microcapsule 1:2 PAPP inner coat, batch C means microcapsule 1:3 PAPP inner coat, batch D means microcapsule 1:1 PAPP outer coat, batch E means microcapsule 1:1 PAPP outer coat, while batch F means microcapsule 1:1 PAPP outer coat; batches A-C are microcapsules prepared with inner bark of PAPP while batches D-F are microcapsules prepared with outer bark of PAPP.

The photomicrographs of the microcapsules (presented in Figure 3) indicate that discrete, mostly irregular and brownish microcapsules were obtained. The mean particle sizes of the various batches of the microcapsules are depicted in Figure 4. The mean particle size (n = 30) of the microcapsules based on the inner bark of PAPP ranged from 11.20 ± 1.90 µm to 32.90 ± 2.30 µm whereas the mean particle size of microcapsules based on the outer bark of PAPP ranged from 19.00 ± 1.20 µm to 33.40 ± 1.40 µm. Thus microcapsules containing lowest amount of PAPP had the smallest mean particle size while microcapsules containing highest amount of PAPP possessed the largest mean particle size. The sizes of the microcapsules were all within the micrometer range, indicating that the production process was able to achieve the intended end-point, consistent with previous reports.[45]

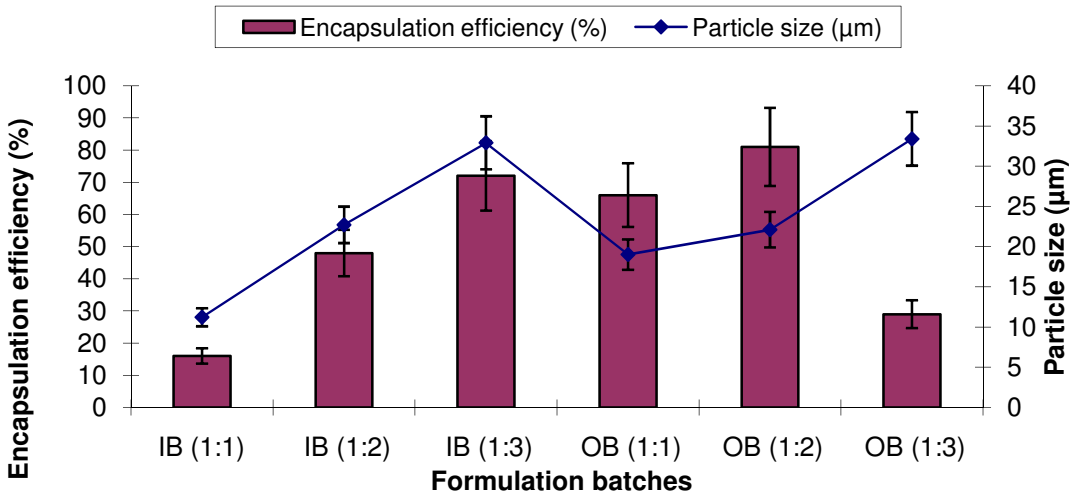


Figure 4: Encapsulation efficieny and particle size of the mebandazole microcapsules prepared with PAPP.

Keys: IB (1:1) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:1, IB (1:2) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:2, IB (1:3) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:3, OB (1:1) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:1, OB (1:2) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:2 while OB (1:3) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:3.

It is discernible from Figure 4 that the average size of the microcapsules increased with an increase in the proportion of the PAPP employed. Particle size of microcapsules is an essential factor that affects drug release and pharmacokinetics [13, 14, 21 – 23]. For microcapsules engineered for parenteral administration, large particles would find it difficult to pass through the syringe. However, the microcapsules evaluated in this study are intended for oral administration and particle size will influence only the rate of drug release and subsequent pharmacokinetics [37].

Figure 4 shows that drug encapsulation efficiency generally increased with increasing amount of inner bark of PAPP in the formulation, although there was no correlation between the encapsulation efficiency of microcapsules and the concentration of the outer bark of PAPP. Thus, microcapsules prepared with least amount of inner bark of PAPP [batch IB (1:1)] entrapped the highest amount of mebendazole (16.21 ± 0.98) whereas microcapsules formulated with highest amount of inner bark of PAPP [batch IB (1:3)] entrapped the greatest amount of mebendazole (72.56 ± 1.70). For microcapsules based on the outer bark of PAPP, mebendazole microcapsules containing drug and polymer in ratio 1:2 entrapped the greatest amount of mebendazole in comparison with the rest of the microcapsule batches. The drug encapsulation efficiency is an important variable for assessing the drug loading capacity of microspheres and their drug release profiles, thus suggesting the amount of drug that would be available at the site of absorption. This parameter is dependent on the process of preparation, physicochemical properties of drug, and formulation variables [13, 14, 21 – 23]. It is also highly influenced by type of polymer, polymer concentration and solvent used to dissolve the drug and polymer [15, 19]. Overall, Figure 4 indicates that microcapsules prepared with drug and inner bark of PAPP at 1:3 ratio had the highest encapsulation efficiency and the highest particle size, those prepared with drug and inner bark of PAPP at 1:1 ratio had the lowest particle size and the lowest encapsulation efficiency, while microcapsules based on drug and inner bark of PAPP at 1:2 ratio had intermediate encapsulation efficiency and particle size. Furthermore, among the outer bark-based formulations, microcapsules prepared with drug and PAPP at 1:2 ratio had the highest encapsulation efficiency while microcapsules prepared with drug and PAPP at 1:3 ratio had the lowest encapsulation efficiency. These findings seem to concur with that of other workers on microencapsulation [16 – 18]. Both the particle size and encapsulation efficiency would influence drug release from the microcapsules, as has been observed in previous studies [19, 34].

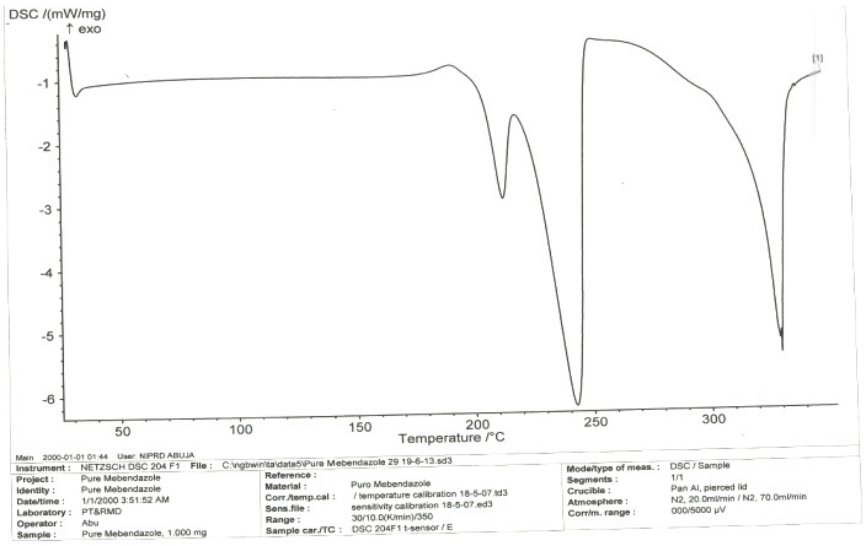
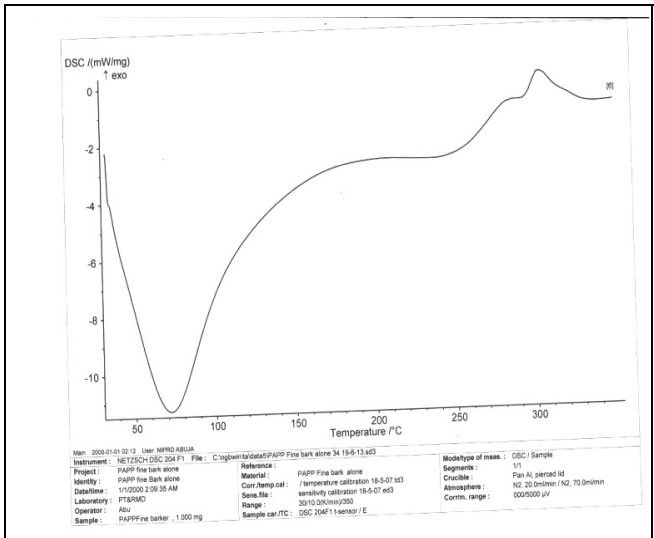
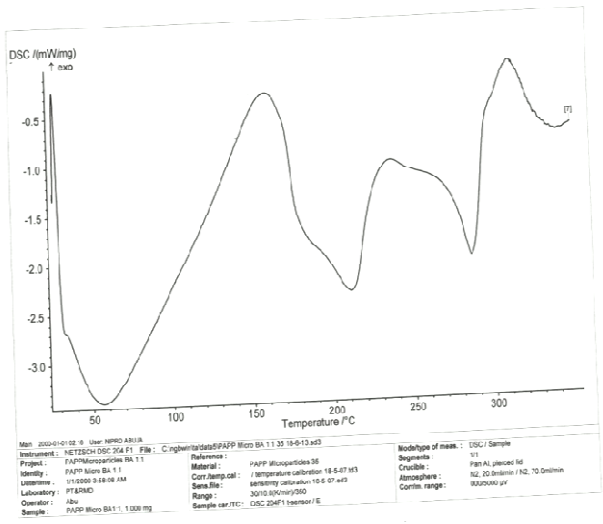


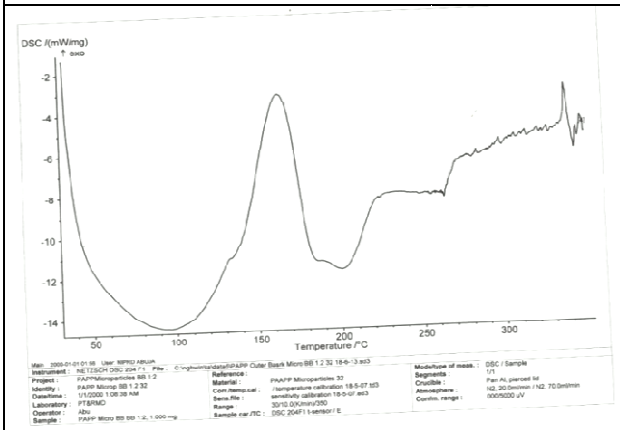
Figure 5A: DSC thermograms of mebendazole pure powder.



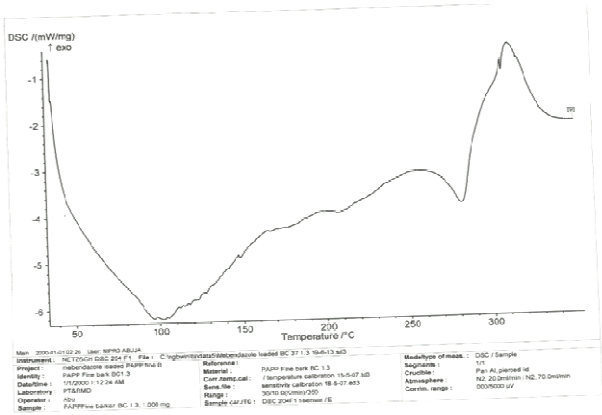
PAPP inner coat alone



Microcapsules from PAPP inner coat 1:1

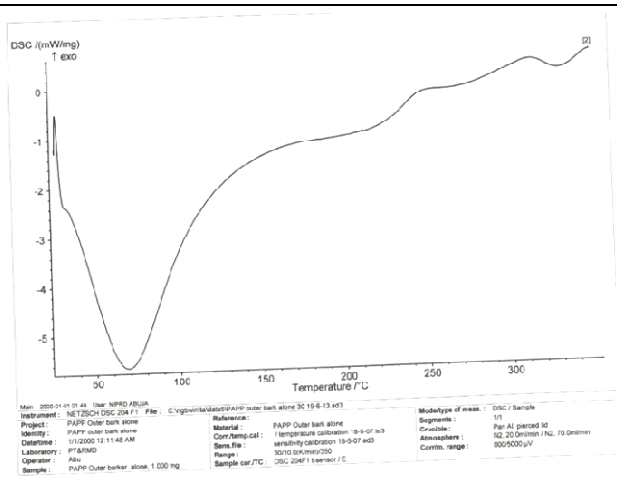


Microcapsules from PAPP inner coat 1:2

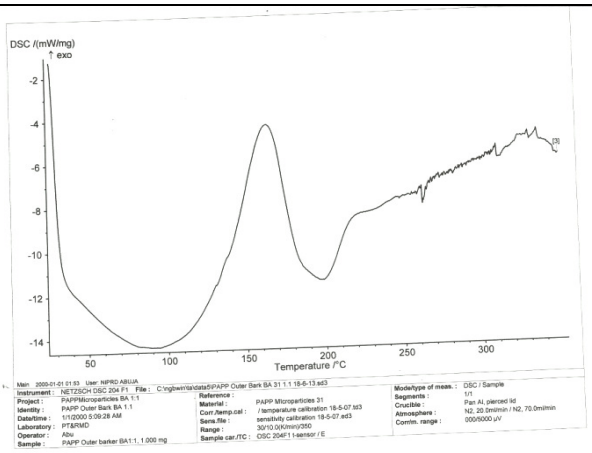


Microcapsules from PAPP inner coat 1:3

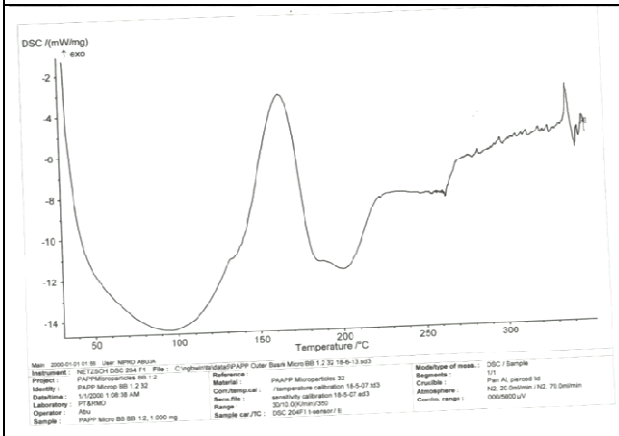
Figure 5B: DSC thermograms of microcapsules based on inner bark of PAPP.



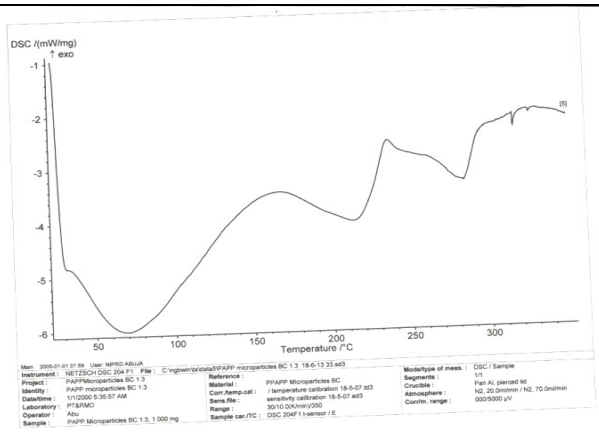
PAPP outer coat alone



Microcapsules from PAPP outer coat 1:1



Microcapsules from PAPP outer coat 1:2



Microcapsules from PAPP outer coat 1:3

Figure 5C: DSC thermograms of microcapsules based on outer bark of PAPP.

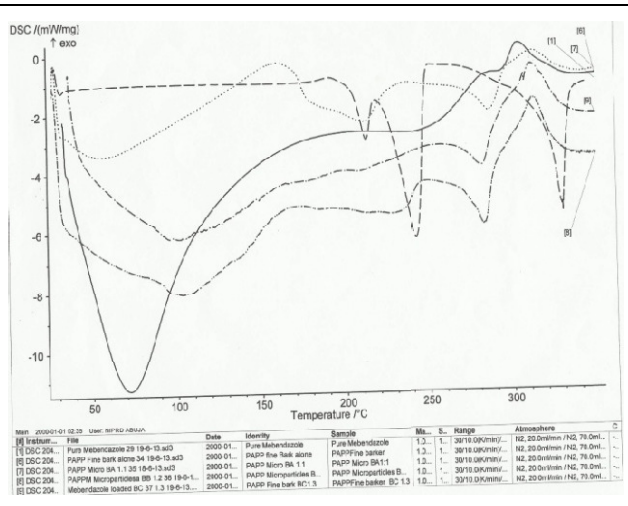


Figure 5D: Overlay of DSC thermograms of microcapsules prepared with PAPP inner bark.

Keys: BA (1:1) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:1, BB (1:2) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:2, BC (1:3) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:3.

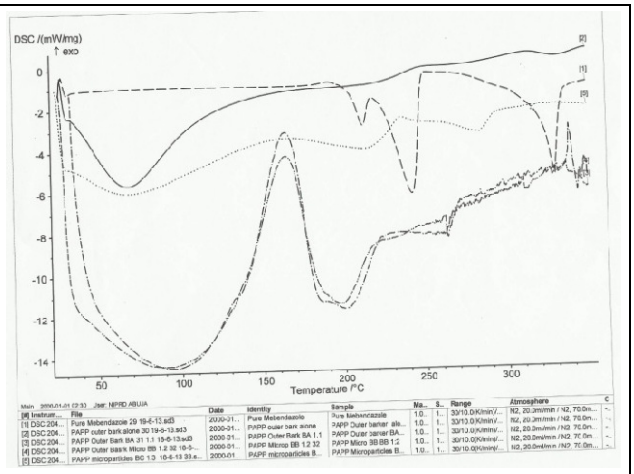


Figure 5E: Overlay of DSC thermograms of microcapsules prepared with PAPP outer bark.

Keys: BA (1:1) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:1, BB (1:2) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:2 while BC (1:3) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:3.

Figures 5A-E show the DSC thermograms of mebendazole, inner bark of PAPP, outer bark of PAPP and the microcapsules while Table 2 presents the thermal properties of mebendazole, inner bark of PAPP, outer bark of PAPP and the formulations. The DSC thermograms of pure mebendazole (Fgure 5A) showed three melting peaks at 213.2 ^{0}C, 242 ^{0}C and 330.1 ^{0}C with corresponding enthalpies of -2.946 -mw/ mg, -6.249 -mW /mg and -5.42 -mW/mg. At 330.1 ^{0}C, it became most stable having the enthalpy of -5.462 -mW/mg. The PAPP inner coat gave two melting peaks at 71.8 ^{0}C and 304.9 ^{0}C with corresponding enthalpies of -11.3 and 0.2071 -mW/mg (Figure 5B). When mebendazole was loaded to the inner coat polymer, the DSC traces of the formulation showed that 1:1 of the microcapsules gave three melting peaks at 56 ^{0}C, 211.3 ^{0}C and 285 ^{0}C with corresponding enthalpies of -3.399, -2.343 and -2.025 -mw/mg (Figure 5B). It also had a maximum area of -104.5. The 1:2 of the inner coat gave only one melting peaks at 103.5 ^{0}C with corresponding enthalpy of -8.073 -mW/mg and area of -2584 J/g. From the result, inner coat 1:1 with the highest enthalpy shows high crystalline arrangement while 1:2 and 1:3 had low enthalpy suggesting less crystallinity and greater possibility of retention of entrapped drug over time. PAPP outer bark showed one melting peak at 68.8 ^{0}C with enthalpy of -5.666-mw/mg and area of-1095 J/g (Figure 5C). The 1:1 mebendazole microcapsules formulated with the 1:1 outer coat polymer gave two melting peaks of 94.3 ^{0}C and 198 ^{0}C with corresponding enthalpies of -14.41 and -11.63 -mW/mg and areas of -6965 and -919.1 J/g respectively. The 1:2 formulation gave two melting peaks at 103.5 ^{0}C and 287.7 ^{0}C with corresponding enthalpies of -8.073 and -5879 -mW/mg and areas of -2584 and -199.1J/g respectively. The 1:3 of the formulation gave three melting peaks of 69.3 ^{0}C, 214.2 ^{0}C and 284.5 ^{0}C (Figure 5C). The presence of multiple melting temperatures could be due to presence of unstable entities or polymorphic forms of the test drug mebendazole.

From above, the thermogram obtained for the pure drug exhibited three melting peaks at 213.2 ^{0} C, 242 ^{0}C and 330.1 ^{0}C respectively with corresponding enthalpies of -2.946 -mw/ mg, -6.249 -mW /mg and -5.42 mW/mg which corresponds to the melting points of the three possible polymorphic forms of the drug [9, 10, 14]. Our findings appear to agree with those of previous researchers [6 – 8]. However, these peaks were not observed in the thermograms obtained for the drug loaded microcapsules. This observation suggests that the incorporated drug was widely dispersed within the matrix derived from *Prosopis africana* peel. In other words, since higher melting point values indicate more ordered crsytal structures [37, 45], it follows that the formulations are less crystalline than mebendazole and that the drug is solubilized in the microcapsules. More so, the physicochemical compatibility of the drug and the polymer studied by differential scanning calorimetry suggested absence of any incompatibility. The results revealed the compatibility of mebendazole and the polymer (PAPP) as well as the stability of the drug in the microcapsules. This was because the formulations (microcapsules) gave lower melting point values than mebendazole. By implication, mebendazole existed in amorphous state in the formulations and also was properly solubilized in the microcapsules [18, 35, 36].

Figures 6A-D represents the *in vitro* release profile of the various formulations in SIF and SGF. It could be seen that the 1:1 inner coat formulations had the highest release followed by the 1:2 and 1:3 in SIF and SGF while 1:1 of the outer coat formulation had the highest release followed by 1:3 and 1:2 in SIF and SGF. The *in vitro* release study indicates that drug release in SIF followed a pattern: 1:1>1:2>1:3 (for the inner coat microcapsule) and 1:1>1:3>1:2 (for the outer coat) better than in SGF. Generally, drug release is affected by the nature or design of the delivery system and the medium used in the release study. Factors such as pH, gastrointestinal motility, agitation, viscosity, temperature of the medium, the stirring speed of the apparatus used in the release study, drug concentration, polymer concentration, among others, could affect the *in vitro* release of drugs significantly [15 – 18]. In this study, the release of mebendazole from the microcapsules in SIF and SGF indicate that drug release was sustained for up to 12 h. Additionally, it was observed that samples containing lower concentrations of the PAPP dissolved faster than those with higher levels of polymer content. This implies that MBZ release is retarded by high polymer concentration. Additionally, quantities of drug released in SIF appear to be higher than values obtained in SGF.

Table 2: Thermal properties of formulations

Sl. No	Composition	Onset temp (°C)	Peak (°C)	Enthalpy (-mW/ mg)	Area (J/g)	Onset (°C)	Peak (°C)	Enthalpy (-mW/ mg)	Area (J/g)	Onset (°C)	Peak (°C)	Enthalpy (-mW/ mg)	Area (J/g)
1	Pure mebendazole	210	213.2	-2.946	-78.71	241	242.7	-6.249	-444.7	330	330.1	-5.462	-459.7
2	PAPP inner coat	71	71.8	-11.3	-2895	300	304.9	0.2071	69.36	-			-
3	PAPP inner coat BA 1:1	56	56.4	-3.399	-1420	210	211.3	-2.343	-395.6	285	286.5	-2.025	-104.5
4	PAPP inner coat BB 1:2	103	103.5	-8.073	-2584			-					-
5	PAPP inner coat BC 1.3	102	102.8	-6.233	-1444	270	282.1	-3.885	-71.64				-
6	PAPP outer coat	68	68.8	-5.666	-1095								-
7	PAPP outer coat BA 1:1	94	94.3	-14.41	-6965	198	198.7	-11.63	-919.1				-
8	PAPP outer outer BB 1:2	103	103.5	-8.073	-2584	287	287.7	-58.79	-199.1				-
9	PAPP outer coat BC 1:3	69	69.3	-6.018	-949.7	214	214.2	-4.067	-216.4	284	284.5	-3.374	-153.6

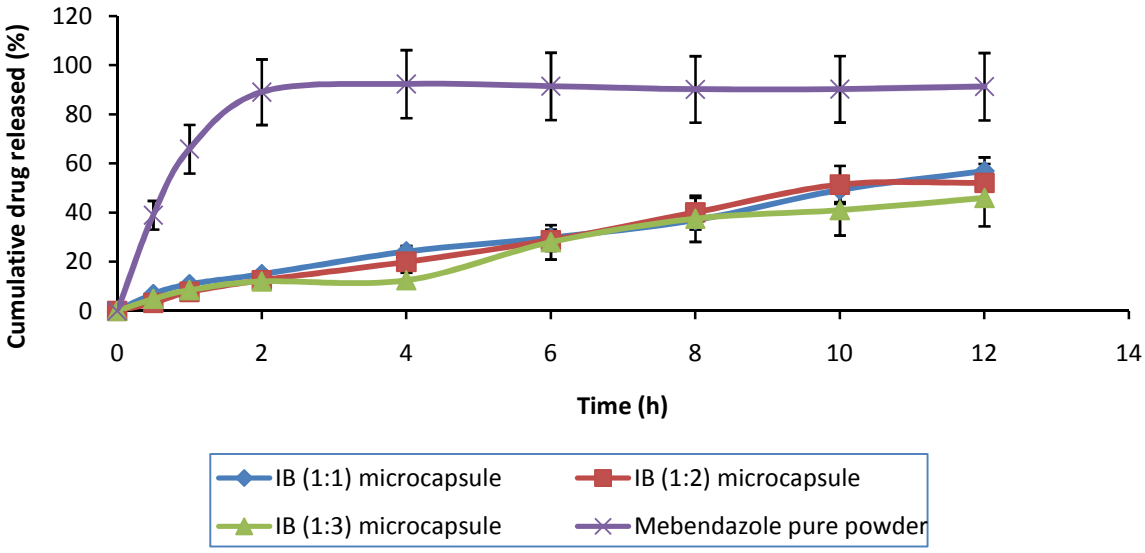


Figure 6A: Percentage Mebendazole release from microcapsules of PAPP inner coat in SIF.

Keys: IB (1:1) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:1, IB (1:2) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:2, IB (1:3) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:3.

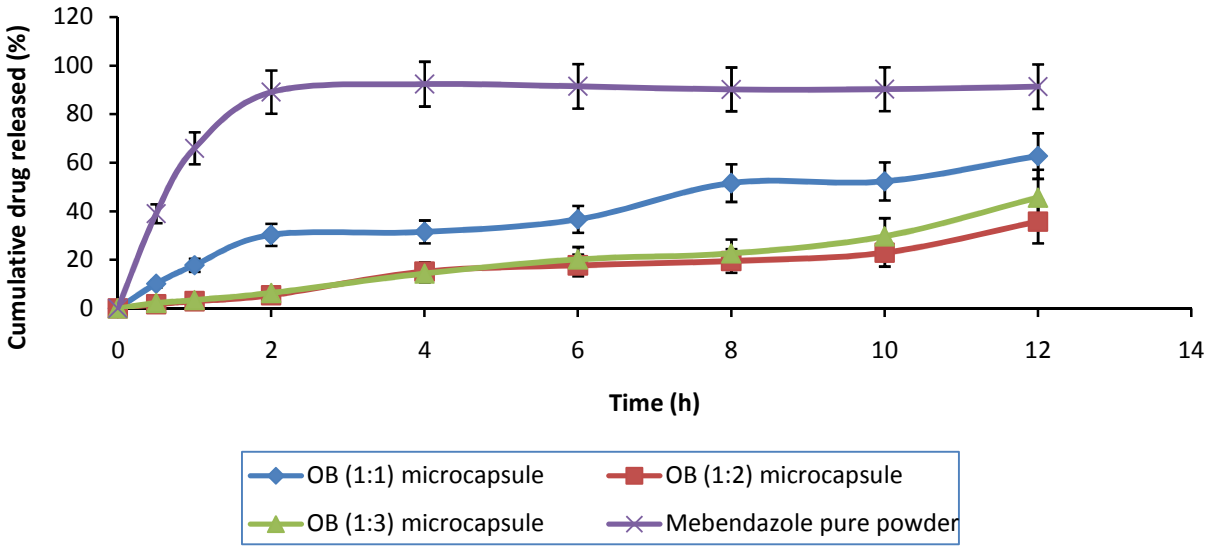


Figure 6B: Percentage mebendazole release from microcapsules of PAPP outer coat in SIF.

Keys: OB (1:1) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:1, OB (1:2) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:2 while OB (1:3) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:3.

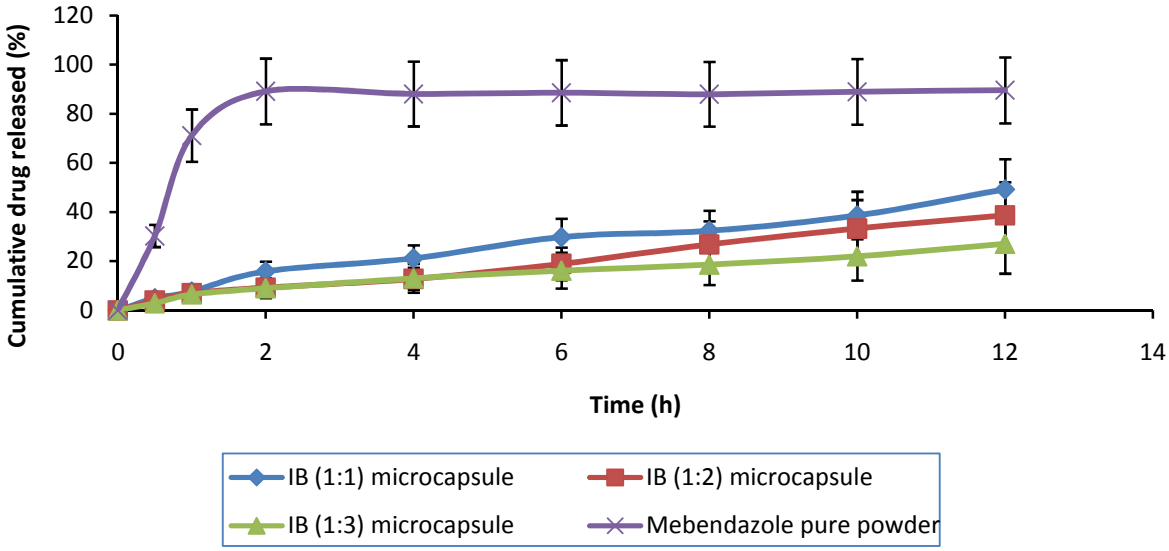


Figure 6C: Percentage mebendazole release from microcapsules of PAPP inner coat in SGF.

Keys: IB (1:1) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:1, IB (1:2) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:2, IB (1:3) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:3.

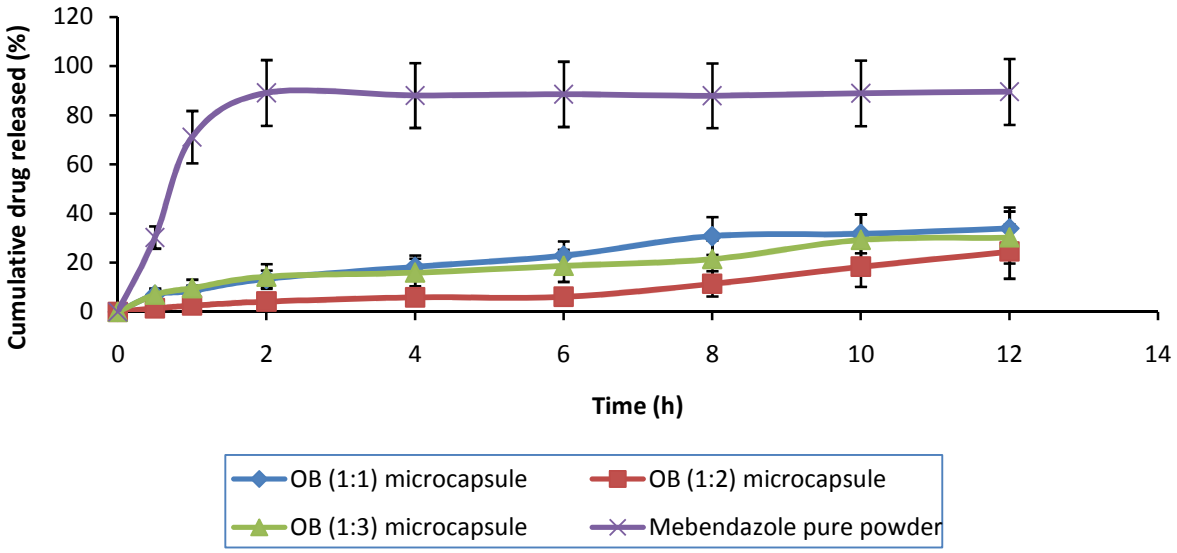


Figure 6D: Percentage mebendazole release from microcapsules of PAPP outer coat in SGF.

Keys: OB (1:1) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:1, OB (1:2) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:2 while OB (1:3) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:3

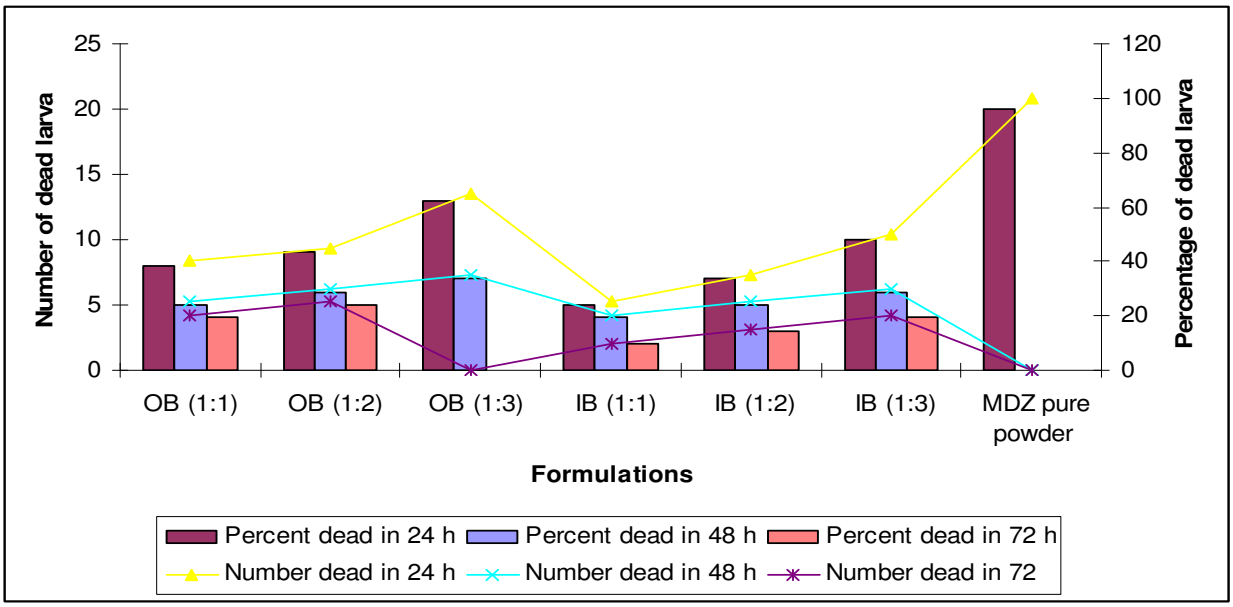


Figure 7: Larvicidal activity of the formulations.

Keys: IB (1:1) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:1, IB (1:2) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:2, IB (1:3) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:3, OB (1:1) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:1, OB (1:2) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:2 while OB (1:3) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:3.

Table 3: Kinetics of release of mebendazole from the microcapsules

Batch	Zero-order (r^2)	First-order (r^2)	Higuchi Square root (r^2)	Hixson-Crowell (r^2)
IB (1:1)	0.837	0.963	0.997	0.899
IB (1:2)	0.946	0.794	0.996	0.918
IB (1:3)	0.895	0.868	0.999	0.945
OB (1:1)	0.893	0.897	0.997	0.816
OB (1:2)	0.978	0.972	0.999	0.912
OB (1:3)	0.943	0.988	0.998	0.889

Keys: IB (1:1) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:1, IB (1:2) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:2, IB (1:3) means microcapsules prepared with drug and inner bark of PAPP at ratio 1:3, OB (1:1) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:1, OB (1:2) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:2 while OB (1:3) means microcapsules prepared with drug and outer bark of PAPP at ratio 1:3.

Table 4: Larvicidal activity of the formulations

Composition	Larva added	Parameter					
		No. dead with time (h)			% Dead with time (h)		
		24	48	72	24	48	72
1:1 Outer Coat microcap	20	8	5	4	40	25	20
1:2 Outer Coat microcap	20	9	6	5	45	30	25
1:3 Outer Coat microcap	20	13	7	-	65	35	-
1:1 Inner Coat microcap	20	5	4	2	25	20	10
1:2 Inner Coat microcap	20	7	5	3	35	25	15
1:3 Inner Coat microcap	20	10	6	4	50	30	20
Pure mebendazole (1%)	20	20	-	-	100	-	-

It may be inferred from these findings that mebendazole release is higher in alkaline than in acidic medium. This suggests that in the GIT, uptake of the drug will be higher in the intestine than in the stomach. Drug release from microcapsules should theoretically be slower as the concentration of polymer is increased because of an increase in the path length through which the drug has to diffuse [37]. Interestingly, results obtained indicate that increase in the concentration of PAPP inner bark caused a decrease in drug release rate from the microcapsule formulations. Furthermore, a characteristic feature of the release profile of unencapsulated mebendazole in both SIF and SGF is the biphasic pattern of release characterized by initial burst release of up to the maximum cumulative percent of 89 % within 2 hours. The initial rapid burst release would lead to dose dumping which is a demerit considering the adverse effects of the drug to patients. The microcapsules had the tendency to fully sustain the release of mebendazole, as had been demonstrated for mebendazole microscapsules in previous studies [18, 35, 36]. The high and rapid release of mebendazole from the microcapsules more in SIF than in SGF may also be a result of higher rate of hydration and swelling of the microcapsules hydrogels in SIF, which, in turn, could be attributable to the physicochemical properties of the excipients used in preparing the microcapsules especially PAPP. In addition, it could be due to the build-up of drug concentration in the dissolution medium in the course of time. By implication, the drug release was diffusion-controlled, consistent with earlier reports on mebendazole microcapsules [18, 35, 36]. Moreover, PAPP possesses bioadhesive properties [31]. This is an added advantage since the transit time of the dosage form would be prolonged in the small intestine for maximum absorption of the active ingredient.

Different mathematical models were used to describe the kinetics of mebendazole release from the microcapsules and the criterion for selecting the most appropriate model was on the basis of goodness-of-fit test. Table 3 depicts the result. A comparative evaluation of the (r^2) values for the microcapsules shows that all the formulae exhibited the highest regression coefficient when the percentage of the drug released was plotted against the square root of time. These data revealed that the Higuchi square root model was the predominant models of drug release from the polymeric matrix. These results conform to previous reports on microcapsules [16 – 18]. Higuchi's square root model describes drug release from a heterogeneous hydrated or solvated matrix where the drug is dispersed uniformly and it aims to describe the drug release by a combination of dissolution and diffusion out of the polymer matrix [19, 34]. The results (Table 3) indicate that all batches of the microcapsules

obeyed Higuchi square root model and thus exhibited diffusion-controlled release. The ability of the microcapsule hydrogel to hydrate in the medium enabled the drug to diffuse due to structural disorientation that led to the formation of pores through which the drug is eventually released in a controlled manner.

Figure 7 and Table 4 show the lavicidal activity of the formulations and the pure mebendazole sample which served as positive control. Microcapsules (1:3 outer coat) recorded 65 % death of mosquito larvae in 24 h while mebendazole recorded 100 % death in 24 h; microcapsules formulated with the 1:3 ratio of drug and inner coat recorded death of 50 % of the mosquito larva in 24 h. It could be deduced from Figure 7 and Table 4 that PAPP significantly influenced the larvicidal activity of the microcapsules since the higher the PAPP in the formulation the faster and greater the larvicidal activity. The 1:3 mebendazole microcapsules of the outer coat showed more larvicidal activity than the rest of the formulations while 1:1 of the inner coat had the least larvicidal activity. This could be attributed to the synergistic effect of the drug and PAPP since PAPP has been reported to possess activity against microbes [24, 27]. The larvicidal activity of mebendazole was sustained for up to 3 days in the formulations unlike the reference standard, mebendazole that killed all the larvae within 24 h. The high amount of pure mebendazole released within 24 h, which was responsible for 100 % death recorded, could pose serious toxicity concerns to humans unlike the more sustained release achieved with all the encapsulated drug. It could be deduced from the results that all the microcapsules sustained the lavicidal activity of mebendazole up to 3 days, except the microcapsules formulated with 1:3 ratio of drug and outer bark of PAPP which killed all the larvae within 48 hours. Earlier researchers reported that anthelmintic agents act by binding to the free proteins in the gastrointestinal tract of the host animal or glycoproteins on the cuticle thereby causing disruption of cell membrane integrity, culminating in the disruption of the metabolic pathways of the worms [46 – 48]. The predominant effect of mebendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Mebendazole by increasing chloride ion conductance of worm muscle membrane produces hyper-polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. In addition, mebendazole has been reported to cause paralysis in worm by disrupting the microfilaments, microtubules and β-tubulins component of their cytoskeletal structure [49]. Furthermore, the larvicidal activities of the formulations could be due to the fact that mebendazole passed through the egg shell such as ascaridole [50] and stopped segmentation of blastomers or paralysed larvae inside the embryonated egg. The high activity of the formulations on the larvae may be due to the fact that these free living stages were coming from their first moult in which they have lost energy and their cuticle is not yet hard. For this reason mebendazole may easily penetrate the cuticle and prevent the absorption of glucose, or block post synaptic receptors thus paralysing the larvae [51]. The drug may also induced the release of gamma aminobutyric acid (GABA) which blocked transmission of nerve impulses or decoupling the phosphorylation oxydative reaction which could lead to the exhaustion of the energy of the larvae [52, 53].

CONCLUSIONS

In this study, sustained-release mebendazole microcapsules were successfully prepared using PAPP by emulsification-coacervation technique. The results showed that particle sizes of the mostly irregularly shaped microcapsules were independent of polymer concentrations and suggest that PAPP at optimum amount could promote encapsulation of higher quantity of mebendazole. DSC thermograms indicate that mebendazole was molecularly dispersed in the polymeric microcapsules. The *in vitro* release study indicates that drug release in SIF followed a pattern: 1:1>1:2>1:3 (for the inner coat microcapsule) and 1:1>1:3>1:2 (for the outer coat) better than in SGF. Microcapsules prepared with PAPP produced more sustained larvicidal activity for up to 3 days compared with mebendazole powder that exhibited larvicidal activity in 24 hours. This indicates that delivery of mebendazole for effective control of worm infestation could be achieved by microencapsulation using the right microencapsulating material and formulation technique. This study suggests that PAPP could be employed in sustained delivery of mebendazole for improved patient compliance thus reducing the frequency of drug administration with associated side effects of mebendazole.

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