# Some Physical Properties of *Vernonia amygdalina* and *Garcinia kola* Microspheres Prepared with High Molecular Weight Polyethylene Glycols

## *ERAGA, SO.; ARHEWOH, MI.; IVUONGBE, FE; EZE, HO

Department of Pharmaceutics and Pharmaceutical Technology,
Faculty of Pharmacy, University of Benin, Benin City 300001, Nigeria
*Corresponding author: Eraga, Sylvester O.
Department of Pharmaceutics and Pharmaceutical Technology
Faculty of Pharmacy, University of Benin,
Benin City, 300001, Nigeria, Tel.: +234-8030884928
E-mail: eragaso@uniben.edu

**ABSTRACT:** The effect of polymer concentrations on some of the physicochemical properties of *Vernonia amygdalina* (Linn) and *Garcinia kola* (Heckel) extracts loaded microspheres was evaluated. Microspheres of the aqueous extracts was prepared by emulsion solvent evaporation using polyethylene glycol (PEG) mixtures of molecular weight 4000 and 6000 at different ratios of 1:0, 0:1, 1:1, 1:2 and 2:1 while the amounts of the extracts incorporated was constant for all ratios. The microspheres were evaluated for their particles sizes, yield, flavonoid content, loading efficiency, moisture loss and flow properties. *In-vitro* release studies were carried out by monitoring flavonoid release rate from the microspheres. The microspheres were spherical and uniformly shaped and exhibited good flow characteristics. Their size range, yield, loading efficiency, moisture loss and flavonoid content were 76 - 83 μm, 49 - 76 %, 47 - 82 %, 2.18 - 4.60 % and 17.10 - 23.80 mg%,  respectively for *V. amygdalina* and 144 - 160 μm, 50 - 68 %, 51 - 68 %, 3.00 - 4.41 % and 20.00 - 28.70 mg%, respectively for *G. kola*. Flavonoids release from the microsphere was up to 90 % within 1 h and it followed a matrix release kinetic model with a super case-II transport mechanism. The concentrations of the polymers affected the yield, loading efficiency, moisture loss and the extent of flavonoid release of the microspheres but had no effect on their particle sizes and flavonoid content. These results may find useful application in the delivery of *V. amygdalina* and *G. kola* extracts since the combination of PEG of different molecular weights resulted in microspheres with good physicochemical and release properties. © JASEM

*Introduction*
*Vernonia amygdalina* is an edible plant of the Asteraceae family. It is commonly known as bitter leaf in Nigeria where the shrub grows up to three meters high. It grows in tropical African regions as well as in South Africa (Afolayan, *et al.*, 2006). *V. amygdalina* has been reported to be effective against amoebic dysentery; gastrointestinal disorders; hepatotoxicities and diabetes mellitus (Izevbigie, 2003). The reported bioactive compound of *V. amygdalina* are saponins, alkaloids, terpenes, steroids, coumarines, flavonoids, phenolic acids, lignans, xanthones and anthraquinones (Cimanga, *et al.*, 2004), edotides (Izevbigie, 2003), tannis (Harborne, 1973) and sesquiterpene lactone (Kupchan, *et al.*, 1969). These compounds have been shown to be responsible for the observed biological activities. For example, the antiplasmodial (antimalarial) activity of its extracts has been related to the presence of flavonoids, saponins and alkaloids. Some studies have associated coumarines and flavonoids in most plant with anti-tumor activities in human (Monroe, *et al.*, 1988). Sesquiterpene lactone

and edotides present in the plant extract are believed to be responsible for the ability of the extract to fight cancer (Izevbigie, 2003; Kupchan, *et al.*, 1969).

*Garcinia kola* Heckel (Guttifera) is a dicotyledonous plant found in moist forest and grows as a medium sized tree up to about 12 m high. The seeds have a bitter taste hence the plant is commonly called bitter kola in Nigeria. The seeds have been consumed as a stimulant (Atawodi, *et al.*, 1995). The seeds have been used in the treatment of liver disorders, and diarrhoea (Iwu, *et al.*, 1990). *G. kola* has been reported to possess some hepatoprotective and aphrodisiac properties (Akintonwa and Essien, 1990; Ajibola and Satake, 1992). The plant has been referred to as a "wonder plant" because every part of it has been found to be of medicinal importance (Dalziel, 1937).

Despite the many studies on the extracts of *V. amygdalina* and *G. kola*, only a few have attempted to formulate the extract into conventional dosage

*Corresponding author: E-mail: eragaso@uniben.edu*

forms (tablets, capsules, suspensions etc.), much less the more advanced delivery systems.

PEGylation can be described as the molecular attachment of polyethylene glycols (PEGs) of different molecular weights to active drug molecules or surface treatment of drug-bearing particles with PEGs. It is a promising strategy of improving the pharmacokinetic behaviour of therapeutic drugs. The main pharmacokinetic outcomes of PEGylation are summarized as changes occurring in overall circulation life-span, tissue distribution pattern, and elimination pathway of the parent drug/particle (Abuchowski, *et al.*, 1977; Fee, 2003).

In this study, an attempt is made into formulating the extracts of *V. amygdalina* and *G. kola* into microspheres using high molecular weight polyethylene glycols and to evaluate the effects of polymer concentration on the physicochemical properties and drug release profiles of the microspheres.

## MATERIALS AND METHODS

*Materials:* Polyethylene glycol (4000 and 6000), Tween 80 and quercetin were purchased from Sigma-Aldrich, Germany. Acetone and n-hexane (BDH Chemicals, England). Aluminium chloride (JHD Chemicals, China), Fehlings solution A and B and Dragendorffs reagent were obtained from our laboratory stock. All other chemicals used were of reagent grade and were used without further purification. *Vernonia amygdalina* leaves and seeds of *Garcinia kola* were bought from a local market in Benin City, Edo State, Nigeria and identified by Mr. Sunday Nweke of the Department of Pharmacognosy, University of Benin, Nigeria.

*Extraction processes:* Pesticide free fresh *V. amygdalina* leaves were air dried for 7 days and milled into powders. Six hundred grams of the powder was soaked in 4 litres of distilled water overnight at room temperature. The mixture was filtered through a clean white gauze to remove the particulate matter before filtration through a filter paper. The resulting extract was concentrated to dryness in vacuum oven at 40 ℃.

The seeds of *G. kola* were peeled to remove the outer covering, cut into pieces and air dried over a period of 10 days. The seeds were ground into fine powder and 1.5 kg of the powdered seeds was soaked in distilled water for 24 hours with continuous stirring every two hours to facilitate penetration of solvent into the powders. The mixture was filtered to obtain the extract and concentrated to dryness in vacuum oven at 40 ℃.

*Phytochemical analysis:* The following tests were performed on the aqueous extracts of the dried, seeds.

Test for starch was performed by means of iodine. Million's reagent was used to test for proteins while the test for alkaloids was performed using Wagner and Dragendorff's reagents. Other tests included Fehling's solution for glycosides, ferric chloride test for tannins and the presence of flavonoids was detected using ammonium hydroxide solution.

*Microsphere preparation:* The microspheres were prepared by emulsion solvent evaporation technique using the formula shown in Table 1. For batch A, five hundred milligram each of polyethylene glycol 4000 and 6000 were dissolved in 10 ml of water. Five hundred milligram of the extract was added to the PEG solution. The solution was poured into a 250 ml beaker containing 100 ml of liquid paraffin and 2 ml of Tween 80 as an emulsifying agent. The system was stirred (Silverson, UK) continuously for about 6 h at 500 rpm over a hot water bath maintained at 60 ℃ until the aqueous phase was completely removed by evaporation. The liquid paraffin was decanted and the collected microspheres were washed three times with 50 ml aliquots of n-hexane and acetone, filtered, air dried and kept in an air tight container at room temperature until evaluation.

*Percentage yield:* The percentage yield was calculated using the formula in Eqn 1, where $W_M$ is the weight of the microspheres recovered from each batch and $W_T$ is the total weight of extract and polymer(s) used in the preparation (Sengel, *et al.*, 2006).

$$Yield\ (\%) = \frac{W_M}{W_T} \times 100 \quad (1)$$

*Extract loading efficiency:* The loading efficiency was calculated using the formula in Eqn 2, where $W_M$ is the weight of the microspheres recovered from each batch and $W_E$ is the weight of extract used in the preparation (Sengel, *et al.*, 2006).

$$Extract\ Loading\ (\%) = \frac{W_M}{W_E} \times 100 \quad (2)$$

*Particle size analysis:* The microsphere size distribution was determined using an optical microscope (Labo Microsystems GmbH, Germany) with a calibrated stage micrometer and size was calculated by using this Equation 3.

$$D = 10 \times \left[ \frac{n \times \log x}{N} \right] \quad (3)$$

Where D is geometric mean diameter, n is number of particle in range, x is the midpoint of range and N is the total number of particles.

*Percentage moisture loss:* The microspheres (200 mg) were weighed and kept in a desiccator with

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activated silica gel at room temperature for 24 h. They were then re-weighed and the percentage loss in weight was computed as the moisture loss.

*Flavonoid estimation:* The colorimetric method of aluminium chloride was used (Akbay, *et al.*, 2003) to determine the quercetin-like flavonoids present in the extract-loaded microspheres. A Beer's calibration curve for quercetin was employed as the standard (Chang, *et al.*, 2002). This procedure was used as the index for content estimation.

*Content estimation:* Extract loaded microspheres (100 mg) were suspended in 100 ml mixture of methanol and water (2:98 %v/v). The resultant dispersion was agitated for 30 min for complete mixing and filtered. Five drops of freshly prepared aluminium chloride solution was added to 5 ml of the filtrate to obtain a yellow coloration and analysed spectrophotometrically at 415 nm to determine the flavonoid content.

*Micromeritic properties:* The following micromeritic properties of the microspheres were evaluated using standard methods; bulk and tapped densities, Hausner's ratio, Carr's index and angle of repose.

*In vitro release studies: In vitro* release studies were carried out using the BP basket (Type II) method for the various batches of the microspheres (Caleva ST7, UK). A dissolution medium of 900 ml containing 0.1 M HCl solution maintained at 37±0.5 °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 maintained at same temperature (37±0.5 °C) of the dissolution medium. The samples were filtered and diluted with an equal volume of 0.1 M HCl. This was continued for 60 min. The absorbances of the resulting solutions were measured at λmax of 415 nm after adding 5 drops of aluminium chloride solution. The amount of flavonoids released at each time interval was determined 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.

*Release kinetics:* Data obtained from *in vitro* release studies were fitted to various kinetic equations to determine the model and mechanism of flavonoid release from the microspheres. The kinetic models used were:

$Q = kt$ (zero-order equation)  (4)
$In (1-Q) = -kt$ (first-order equation)  (5)
$Q = kt^{1/2}$ (Higuchi equation)  (6)
$Log Q = log k + n log t$ (Korsemeyer-Peppas eqn.) (7)
Where, $Q$ is the fraction of flavonoid released at time $t$, $k$ is the release rate constant and $n$ is the diffusional exponent.

# RESULTS AND DISCUSSION

The percentage yield of the extraction process was 14.5 % for *V. amygdalina* and 9.93 % for *G. kola*. Lower and higher percentage yield values have been reported by different researchers in their aqueous extraction (Owolabi, *et al.*, 2013; Njume, *et al.*, 2011; Uko, *et al.*, 2001; Adiukwu, *et al.*, 2013). These differences are as a result of the methods used and the efficiency of the filtration process during the extraction.

The phytochemical analysis results in Table 2 showed that both extracts contain protein, carbohydrate, glycosides, tannins and flavonoids. The presence of alkaloids was only detected in the extract of *V. amygdalina*. Also the presence of flavonoids in the aqueous extract of the plants has been confirmed by previous studies (Imaga, *et al.*, 2013; Onunkwo, *et al.*, 2004).

The production yield and extract loading efficiency of the batches of microspheres ranged from 49 - 73 % and 47 - 82 % respectively for *V. amygdalina* extract and 50 - 68 % and 51 - 68 % for *G. kola* (Table 3). Batches C and H with equal PEG concentrations gave the highest production yield but not the highest loading efficiency which was achieved by batches D and I. This was as a result of the fractional concentration of the PEG co-polymers. Batches D and I had the highest concentration of the PEG 6000 and gave the highest percentage loading efficiency. This was due to increase in surface area provided by the polymers for more extract binding. Generally, the loading efficiency increases as the concentration of PEG 6000 increases. This further supports the theory of increased concentration equals increased surface area and hence more of the extract will be incorporated into the microspheres.

The particle sizes of the formed microspheres were found to be in the range of 76 - 83 μm for *V. amygdalina* extract and 144 - 160 μm for *G. kola* (Table 3). Although there are considerable size differences between the microspheres of the two extracts, nevertheless, there is a small size distribution within the batches of the individual extract. This is an indication of minute variations in the production of the batches as their sizes can be affected by factors such as variation in stirring time, stirring speed and rate of solvent evaporation during preparation. There were no appreciable effects of the concentration of the co-polymers on the particle sizes of the microspheres.

Moisture loss from the microspheres was at a minimum in all the formulation (Table 3). This was due to a stable and minute internal aqueous phase of the microspheres. Moisture loss increased with increase in the amounts of PEG 6000. This is expected since polyethylene glycol is a hydrophilic

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polymer and increase in the molecular weight and concentration will necessarily increase its water content.

All the batches of both extracts gave Hausner's ratio ≤ 1.18, Carr's index of 11.36 - 15.46 and angle of repose in the range of 25° - 33° (Table 4). These parameters are indication of good flow properties of the microspheres. The microspheres have been designed to stabilize the aqueous extracts within liquid paraffin, it would be expected to improve the flow characteristics and packing properties of the extracts since the extracts are reported to be effective in treating a vast array of disease conditions, the microspheres can further be packed into capsules shells and dispensed as capsules or compressed into tablets and the PEG matrix may also improve compaction properties.

There was no discernable pattern in the flavonoid content of the microspheres with regards to the concentrations of the polymers but it appears to decrease as the polymer concentrations were increased (Table 3). The flavonoid content depends on the production yield and loading efficiency of the microspheres preparation process. The application of flavonoid content as an index of activity is quiet interesting. However, the major limitation with this is that extracts are handled as a whole and not in parts. Their action is also wholistic, where different components of the extract work synergistically to exert their therapeutic effect.

The flavonoid release profile from the microsphere is shown in Figure 1 and 2. For *V. amygdalina*, batches D and E with the highest amount of PEG 6000 had the highest percentage release in 1 h (88 and 86 % respectively), followed by batch C (81 %) with equal concentration of the co-polymers and batch A released the lowest amount (60 %) while for *G. kola*,

batch H with equal concentration of the co-polymers had the highest percentage release in 1 h (90 %) but closely followed by batch I (89 %) and batch F released the lowest amount (70 %). These differences was due solely to the concentration of the polymers in the formulations as polyethylene glycol is water soluble can easily go into solution to release the extract incorporated in them. Higher concentrations of PEG 6000 in batches D, E, I and J may have facilitated the high release of the flavonoids because of their high extract loading efficiency. This reason could also account for the high release of batch H, whose loading efficiency almost equals those of batches I and J.

Highest $R^2$ values (Table 5) were obtained for Higuchi and Korsemeyer-Peppas model (n > 1) for both extracts, suggesting that the release of flavonoids from the extract loaded microspheres follows a matrix release kinetics model with a super case-II transport mechanism. PEG concentrations and type did not affect the $R^2$ values.

*Conclusion:* The polymer combination of polyethylene glycol 4000 and 6000 molecular weights resulted in microspheres with good yield and loading efficiency. The present study suggests that combination of both polymers shows sufficient release of the extract. High concentrations of polyethylene glycol might enhance the flavonoid release profile of *Vernonia amygdalina* and *Garcinia kola* microspheres. The study also presents another view in the possible options available for the formulation of aqueous extracts thus expanding the possible applications of PEG in the immobilization of plant extracts.

**Table 1**: Formula for preparation of microspheres

| Batch/ Ratio | PEG (4000) (mg) | PEG (6000) (mg) | Extract (mg) |
|---|---|---|---|
| *Venonia amygdalina* | | | |
| A (1:0) | 1500 | 0 | 500 |
| B (0:1) | 0 | 1500 | 500 |
| C (1:1) | 750 | 750 | 500 |
| D (1:2) | 500 | 1000 | 500 |
| E (2:1) | 1000 | 500 | 500 |
| *Garcinia kola* | | | |
| F (1:0) | 1500 | 0 | 500 |
| G (0:1) | 0 | 1500 | 500 |
| H (1:1) | 750 | 750 | 500 |
| I (1:2) | 500 | 1000 | 500 |
| J (2:1) | 1000 | 500 | 500 |

**Table 2**: Phytochemical analysis of *V. amygdalina* and *G. kola* extracts. Key: + = present; − = absent

| Parameter | *V. amygdalina* | *G.kola* |
|---|---|---|
| Starch | + | + |
| Proteins | + | + |
| Alkaloids | + | - |
| Glycosides | + | + |
| Tannins | + | + |
| Flavonoids | + | + |

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**Table 3:** Some properties of the microspheres evaluated (mean ± standard deviation)

| Batch/ Ratio | Production Yield (%) | Loading Efficiency (%) | Mean Particle Size (µm) | Flavonoid Content (mg %) | Moisture Loss (%) |
|---|---|---|---|---|---|
| *Vernonia amygdalina* | | | | | |
| A (1:0) | 49.00 | 47.88 | 76.23 ± 0.408 | 17.1 ± 0.646 | 3.06 ± 0.170 |
| B (0:1) | 61.00 | 63.56 | 82.31 ± 0.372 | 23.8 ± 0.566 | 2.18 ± 0.090 |
| C (1:1) | 76.25 | 67.08 | 80.67 ± 0.360 | 19.9 ± 0.487 | 3.27 ± 0.201 |
| D (1:2) | 73.80 | 73.00 | 83.45 ± 0.438 | 19.8 ± 0.450 | 4.60 ± 0.420 |
| E (2:1) | 67.90 | 70.00 | 81.65 ± 0.243 | 20.7 ± 0.450 | 4.10 ± 0.210 |
| *Garcinia kola* | | | | | |
| F (1:0) | 50.00 | 51.68 | 144.30 ± 0.404 | 23.0 ± 0.296 | 3.00 ± 0.296 |
| G (0:1) | 61.00 | 55.23 | 148.14 ± 0.115 | 20.0 ± 0.326 | 3.00 ± 0.326 |
| H (1:1) | 68.50 | 67.30 | 160.60 ± 1.133 | 27.2 ± 0.642 | 3.85 ± 0.260 |
| I (1:2) | 56.80 | 68.14 | 147.70 ± 0.874 | 22.1 ± 0.306 | 4.38 ± 0.131 |
| J (2:1) | 63.30 | 66.10 | 152.60 ± 1.250 | 28.7 ± 0.153 | 4.41 ± 0.778 |

**Table 4**: Micromeritic properties of the microspheres

| Batch/ Ratio | Bulk Density (g/L) | Tapped Density (g/L) | Hausner's Ratio | Carr's Index (%) | Angle of Repose (°) |
|---|---|---|---|---|---|
| *Vernonia amygdalina* | | | | | |
| A (1:0) | 0.514 | 0.608 | 1.18 | 15.46 | 33 |
| B (0:1) | 0.568 | 0.659 | 1.16 | 13.81 | 32 |
| C (1:1) | 0.512 | 0.584 | 1.14 | 12.33 | 29 |
| D (1:2) | 0.582 | 0.663 | 1.14 | 12.22 | 27 |
| E (2:1) | 0.441 | 0.505 | 1.15 | 12.74 | 26 |
| *Garcinia kola* | | | | | |
| F (1:0) | 0.416 | 0.490 | 1.18 | 15.28 | 31 |
| G (0:1) | 0.424 | 0.497 | 1.17 | 14.69 | 30 |
| H (1:1) | 0.625 | 0.720 | 1.15 | 13.19 | 29 |
| I (1:2) | 0.489 | 0.556 | 1.14 | 12.05 | 27 |
| J (2:1) | 0.458 | 0.516 | 1.13 | 11.36 | 25 |

**Table 5:** $R^2$ values for different release models

| Batch/ Ratio | Zero Order | First Order | Higuchi | Korsmeyer Peppas (n) |
|---|---|---|---|---|
| | | $R^2$ | | |
| *Vernonia amygdalina* | | | | |
| **A (1:0)** | 0.4302 | 0.9485 | 0.9896 | 0.9851 (1.3892) |
| **B (0:1)** | 0.5018 | 0.9251 | 0.9860 | 0.9706 (1.3841) |
| **C (1:1)** | 0.4181 | 0.9098 | 0.9376 | 0.9829 (1.4622) |
| **D (1:2)** | 0.3839 | 0.9171 | 0.9023 | 0.9735 (1.5191) |
| **E (2:1)** | 0.3716 | 0.9015 | 0.8670 | 0.9428 (1.5228) |
| *Garcinia kola* | | | | |
| **F (1:0)** | 0.5041 | 0.8956 | 0.9854 | 0.9942 (1.3138) |
| **G (0:1)** | 0.4179 | 0.8876 | 0.9579 | 0.9773 (1.3108) |
| **H (1:1)** | 0.5182 | 0.8695 | 0.8621 | 0.8325 (1.4516) |
| **I (1:2)** | 0.6469 | 0.8981 | 0.9198 | 0.9172 (1.3216) |
| **J (2:1)** | 0.5507 | 0.9211 | 0.9644 | 0.9660 (1.3211) |
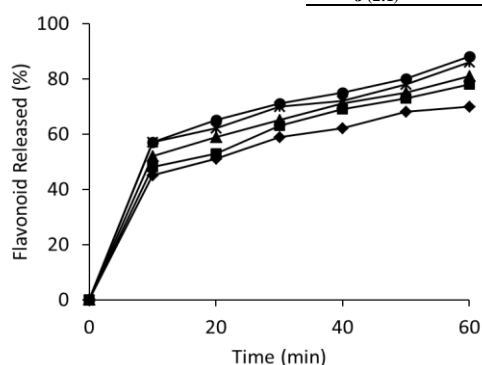


**Fig 1**: Release profile of flavonoid from *V. amygdalina* microspheres
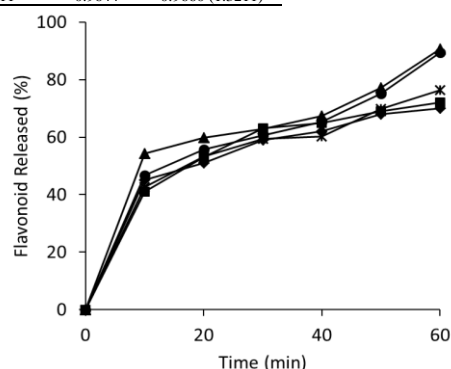


**Fig 2:** Release profile of flavonoid from *G. kola* microspheres

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