

Microencapsulated *Garcinia kola* and *Hunteria umbellata* Seeds Aqueous Extracts – Part 1: Effect of microencapsulation process

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Abstract

Objective: This study investigates microencapsulated aqueous extracts of *Garcinia kola* (GK) and *Hunteria umbellata* (HU) seeds.

Method: Extracts obtained after maceration of dried powdered seeds were prepared as microcapsules with chitosan-alginate by counterion coacervation method. Microcapsules were characterized using differential scanning calorimetry (DSC), x-ray diffractometry (XRD) and fourier transform infrared (FTIR) spectroscopy. *In vitro* release studies were carried out at pH 1.2 for 2 h and 6.8 for a further 10 h.

Results: Between 20 and 50% extract release occurred from microcapsules after 2 h while conventional tablets released 100% after 1 h at simulated gastric pH. At pH 6.8, >80% of extract was released from microcapsules after 6 h. DSC revealed the presence of complex materials. XRD and FTIR showed stable character of the plant extracts within the microcapsules.

Conclusion: Controlled release of aqueous extracts derived from these plants was achieved by microencapsulation and therefore can be developed as suitable delivery devices.

Keywords: Microencapsulation, chitosan-alginate, aqueous extraction, medicinal plants, drug release

1. Introduction

Garcinia kola Heckel (Guttiferaceae) also called bitter kola, kola bitter, false kola or male kola is found in moist forest and grows as a medium sized tree, up to 12 m high. It is cultivated and distributed throughout West and Central Africa [1]. Traditional African medicinal uses of *Garcinia kola* include treatment of cough, as an antiparasitic and antimicrobial [2]. It is used in the treatment of bronchitis and throat infections [3] and liver disorder [4]. Aphrodisiac [5] and antioxidant activities [6] has been reported.

Hunteria umbellata K. Schum (Apocynaceae) is found in West and Central Africa. The plant is locally known as *Abeere* among the Yorubas (South-West Nigeria)[7]. Pulverized dry seeds of *Hunteria umbellata* are highly valued for the treatment of fever, pain, abdominal colic, diabetes mellitus and obesity in its suspected sufferers [7]. Various parts of the plant have been used in herbal medicine for the treatment of peptic ulcers, piles, yaws, dysmenorrhea, fevers, infertility, and helminthic infections [8].

Despite the fact that these herbal drugs have become popular both in developing and developed Countries, there is still a big challenge in formulating them into dosage forms. The instability of most plant products outside

their natural environment, bulkiness and bitter taste of plant extracts are some of the challenges of formulating these herbal drugs into suitable dosage forms. In some cases their active principles have short half-life warranting frequent dosing. Microencapsulation of herbal medicines will have tremendous benefits in taste masking, stability and controlled release. This study investigates the modulation of release of aqueous extracts of *Hunteria umbellata* and *Garcinia kola* seeds aqueous extracts from chitosan-alginate microcapsules.

2. Materials and Methods

2.1 Materials

Dried seeds of *Hunteria umbellata* (HU) and *Garcinia kola* (GK) were purchased from a local market in Benin City, Nigeria. Botanical identification was done by Dr Abere Tavs of the Department of Pharmacognosy, University of Benin and authentication was done by the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Herbarium samples with voucher numbers FHI107678 and FHI39154, respectively, were assigned and samples of the plants were deposited in the institute. Chitosan was obtained from Sigma-Aldrich, Germany and sodium alginate (Saltiagin[®]) was a product from Sanofi Bio Industries, France. All other chemicals used were of reagent grade.

2.2 Preparation of plant material

The HU and GK seeds were peeled and chopped into small pieces with a knife to facilitate drying. They were separately dried in sunlight and milled. The particles obtained were passed through a 1.0 mm aperture sieve, separately packed in air-tight containers and stored in a refrigerator prior to analysis.

2.3 Extraction and yield

Extraction was carried out by maceration. Each powdered sample (50 g) of HU, and GK were macerated with 1000 mL distilled water at room temperature for 24 h. The filtrate was concentrated to dryness by a vacuum oven at reduced pressure and 35°C. The dried extracts were stored at 4°C pending further analysis. The extraction yield was calculated as a percentage of the weight of the dried plant powder.

2.4 Microencapsulation process

The coating solution (0.1 %w/v chitosan solution) was prepared according to the method of Okhamafe and co workers⁹. Chitosan (1 g) was dissolved in 500 mL of distilled water, containing 10 mL of glacial acetic acid, with the aid of a magnetic stirrer. Calcium chloride dihydrate (20 g) was added and polysorbate 80 (0.2 mL) was also added to lower the interfacial tension/barrier during microencapsulation. The pH of the solution was then adjusted to 5.5 using sodium hydroxide solution. The solution was filtered and the volume made up to 1000 mL.

The core material was prepared by redispersing 2 g of the extract of each plant in 80 mL of distilled water with the aid of a magnetic stirrer followed by the addition of sodium alginate (2 g), and then stirred until they completely dissolved. The volume was then made up to 100 mL with more distilled water to make a 2% solution.

The electrostatic droplet generator was used for microcapsule preparation. Spherical droplets were formed with a syringe pump (Cole-Parmer Instrument Company, USA) to which was fitted a 21 G, 90° blunt tip needle (Chromatographic Specialties, Canada) through which the plant extract/alginate mixtures were extruded into the chitosan/calcium chloride solution. The potential difference was controlled with a voltage power supply (Model 30R, Bertan Associates, USA) with a maximum current of 0.4 mA and variable voltage of 0 to 30 kV. The particles formed were allowed a further 2 min post extrusion in the chitosan/calcium chloride solution to enable the chitosan in solution to form a coat of chitosan-alginate around the microcapsules. The microcapsules were filtered, rinsed with distilled water and air-dried.

Conventional tablets were also prepared by granulating 500 mg each of dried seed extracts using 10% maize starch mucilage and then blending with 5 % each of magnesium stearate and talc before compressing.

2.5 Characterization of microcapsules

2.5.1 Determination of microcapsule size

A light microscope (Olympus, Tokyo) with a calibrated eyepiece was used to study the size of the particles. All particles appearing within each field of view was counted and sized with the aid of the calibrated eyepiece graticle. For each sample, the diameter of each particle in four representative fields of view was used in the size analysis and the mean result calculated.

2.5.2 Entrapment efficiency (EE)

Assay of the microcapsules for initial drug content was carried out spectrophotometrically at 292 and 280 nm for GK and HU, respectively [10]. Microcapsules equivalent to 50 mg of the extract was “citrated” by dispersing it in 20 mL of 0.5 M sodium citrate in a test tube and kept overnight (citrating was used to breakdown the gel structure of the microcapsules). The microcapsules, together with the citrate solution were then transferred to a glass mortar and crushed with a pestle to effect maximum drug release into solution [9]. The absorbance of each solution was monitored at the respective wavelength of maximum absorbances of each extract and the EE was calculated using Equation 1:

$$EE = A_a/A_t \times 100/1 \dots (1)$$

Where A_a is the absorbance of the actual amount of drug present and A_t is the expected absorbance of theoretical drug load

2.5.3 Thermal Analysis

Thermograms of the samples were obtained by differential scanning calorimetry (DSC 830, Mettler Toledo, Switzerland) equipped with a thermokinetic analysis software. Between 5 to 10 mg of the microcapsules and dried plant extracts were weighed into sealed aluminum pans and the seal was perforated. The pan was placed in the DSC combustion chamber while an empty pan was used as the reference. The equipment was set to heat from 0 to 300°C at a heating rate of 3°C per min under liquid nitrogen at a flow rate of 75 ml/min. Isothermal condition was maintained at 25°C for 5 min.

2.5.4 X-ray diffraction Studies

The x-ray diffraction patterns of the dried plant extract and microcapsules were obtained using a Phillips X-ray diffractometer equipped with an X'pert Data collector version 3.0 (Sybase Inc, USA). The microcapsules and dried extracts were crushed to powder with a mortar and pestle. The powder was spread over a flat disc and leveled to give a plane surface. The disc with the powder was placed in the sample holder and the scan performed in the range of diffraction angle 2θ between 1.60 and 40° using Cu-K α radiation ($\lambda = 1.5418 \text{ \AA}$, continuous scan, scanning rate of 0.1 deg ($\Delta 2\theta$)/ 5s (Δt)).

2.5.5 Fourier-transform infrared (FTIR) spectroscopy

Spectra of the microcapsules and dried aqueous extract were obtained with a Fourier transform infrared (FTIR) spectrophotometer (Jas Co, Italy). The microcapsules and dried extracts were crushed to powder with a mortar and pestle. Dried potassium bromate powder (200 mg) was blended with 1 to 2 mg of the crushed sample. The mixture was then compressed into tablet shaped discs using a Potassium bromide press. The tablets produced were placed in the sample holder of the FTIR spectrometer and their spectra taken from 4000 – 1000 nm at a resolution of 4 cm⁻¹ and 128 scans per sample. Plane potassium bromide disc was used as blank for the instrument.

2.6 Dissolution studies

In vitro release studies were carried out at pH 1.2 for 2 h and 6.8 for a further period of 10 h using the USP type 1 method. For each batch, 250 mg of microcapsules were placed in each dissolution basket and dipped in 500 mL of the dissolution medium (0.1 M HCl) maintained at 37± 0.5°C and rotated at 100 revolutions per min (rpm) for 2 h. Following this 2 h period the basket holder was lifted and immediately transferred into another medium containing phosphate buffer pH 6.8 at 37± 0.5°C and the test continued for another 10 h. Samples (5 ml) were collected every hour and filtered using a Whatmann No 1 filter paper. After each withdrawal, 5 ml of fresh buffer maintained at the same temperature was used to replenish the dissolution medium. Release of the extracts from the microcapsules was monitored spectrophotometrically at 292 and 280 nm for GK and HU, respectively.[10]

2.7 Kinetic data analysis

The data obtained from release studies were fitted to various release models, namely, zero order (Equation 2), first order (Equation 3), Higuchi (Equation 4) and Millar and Peppas (Equation 5). These models were first built into the regression library of SigmaPlot 8.02 (Sigma Systat Software Inc. CA, USA). The software contains several built-in equations and graphs and has the capability to fit input data into the built-in curves automatically. Since some of the parameters in the equations are similar such as solubility of plant extract and sample weights, the equations were simplified as follows:

$$F = Kt \quad \dots (2)$$

$$F = 100(1 - e^{-Kt}) \quad \dots (3)$$

$$F = Kt^{1/2} \quad \dots (4)$$

$$F = kt^n \quad \dots (5)$$

where F = amount of drug released at time t and k = release constant and n is the characteristic exponent of the diffusion behaviour of swelling controlled release systems. The model that best represent extract release was determined.

2.8 Data Analysis

Statistical analysis was carried out using Microsoft Excel, (version 2007) and the results are expressed as mean \pm standard deviation (SD). Differences between means were determined with one-way analysis of variance (ANOVA) at level of significance of $p < 0.05$. Origin lab[®] software version 7.0, was used for elaboration of spectral data from FTIR, DSC and XRD.

3. Results and Discussion

3.1 Extraction yield and microcapsule properties

Extraction yield was 18.7 and 25.5 %w/w, respectively, for GK and HU. There was a significantly high level of aqueous soluble constituents in the powdered seeds of these plants. This result also agrees with earlier published findings [11][12]. The relatively higher yield earlier reported for HU¹⁰ was due to the differences in the method of filtration of the extract from the macerate. The result of size of the microcapsules is shown in Table 1.

Table 1: Effect of applied voltage on GKA and HUA Microcapsule size

Applied voltage (kV)	0	10
Microcapsules	Mean particle size (μm) \pm SD	
GKA	987 \pm 32.9	325.8 \pm 23.6
HUA	1,017 \pm 27.7	405.1 \pm 26.5

The wet microcapsules formed were spherical in shape and appeared uniform in size distribution. Microcapsules containing the seed extract were cream-colored. The microcapsules shrank to approximately 10% of the wet size and became hard and irregular in shape after drying. There was over 80% entrapment of the bioactive in the microcapsules containing GK followed by 70% entrapment for microencapsulated HU. When the applied voltage was increased from 0 to 10 kV, the microcapsules size reduced significantly from $>987 \mu\text{m}$ to less than $500 \mu\text{m}$ for both microcapsules. The mechanism of droplet production by the electrostatic droplet generation method has been described in previous study [13]. The findings on the applied voltage in this study are similar to earlier reports that increasing voltage above 6 kV results in decreased microcapsule [13]. There was, however, no significant difference ($p > 0.05$) between different microcapsules obtained from the two plant extracts though they were generally larger than have been reported in previous studies for other drugs like proteins when a similar voltage was applied.[9] This is because plant extracts have more components than pure chemicals in solution. Furthermore, microcapsules of HU extract were larger than that of GK though the difference in size was also not significant ($p > 0.05$).

The natural gums present in GK extract contributed in minimizing diffusion of the extract out of the microcapsules during drying hence the relatively higher entrapment efficiency obtained from microcapsules containing GK when compared with HU microcapsules. The biflavonoids present in GK are water-soluble hence the loss of approximately 20 % of the extract during drying. Though the gelation process was spontaneous, the drying process was slow (2 days). The microcapsules were air-dried because air-drying was cheaper [14]. This resulted in the drainage of some of the extract out of the microcapsules during drying. Therefore, it may be necessary to apply faster drying methods such as fluidized bed, vacuum or freeze-drying to minimize loss during drying.

3.2 Characterization studies

Figure 1 shows the DSC thermogram of the microcapsules, extract and their mixtures. There was a pronounced endothermic trough as the temperature was increased from 25°C , probably due to water loss and the presence of complex materials 15 and then decomposition which occurred between 90 and 125°C . Plant extracts comprise a wide variety of materials like sugars, proteins and large varieties of secondary metabolites. The presence of all these components in the extracts was responsible for the broad endothermic trough observed in the DSC study since they undergo decomposition at different times and under different thermal conditions.

More precisely the DSC thermograms of the microcapsules shown in Figure 1 revealed the presence of endothermic trough in the 60–120°C range, typical of sodium alginate, followed by an endothermic transition at 175°C in the blank and extract-loaded microcapsules. For pure sodium alginate powder, a transition occurred at 50–70°C while the other thermograms obtained from the microcapsules exhibited broad endotherms over 50 to 150°C range.

The endotherms obtained by DSC have been attributed to the presence of large varieties of secondary metabolites and sugars in the extracts including the fact that sodium alginate is a complex macromolecule polymer.[15] The endotherm for sodium alginate powder disappeared when used in microencapsulation, due to the formation of calcium alginate by the interaction between sodium alginate and calcium chloride. The blank microcapsules (BM) had a similar thermogram as those containing the extracts, thus suggesting that the reaction of sodium alginate and calcium chloride resulted in the formation of the new thermogram observed.

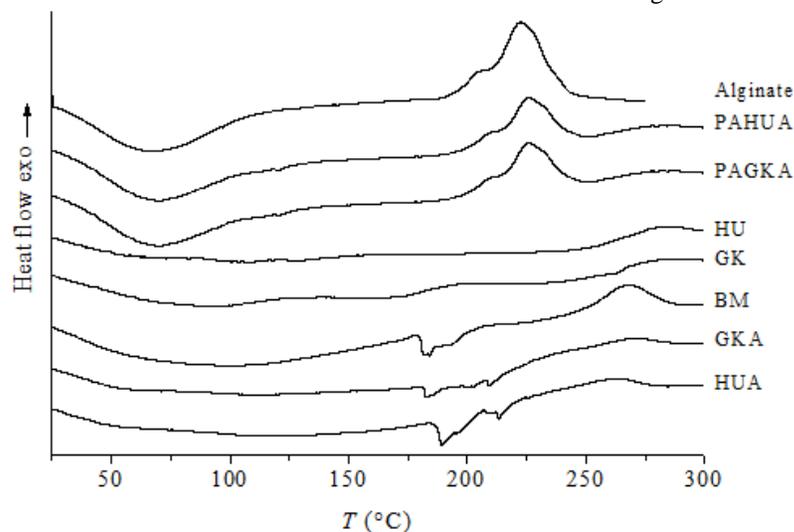


Figure 1: DSC thermograms. **Key:** GK: *Garcinia kola*; BM: Blank microcapsules; PAGKA: Physical admixtures of GK and alginate powder; GKA: microcapsules containing only alginate and GK; HU: *Hunteria umbellata*; PAHUA: Physical admixtures of HU and alginate powder; HUA: Microcapsules containing only alginate and HU.

Figure 2 show the X-ray diffractogram of microcapsules. They indicate that the amorphous sodium alginate and chitosan reacted with the crystalline calcium chloride to form a complex (chitosan-calcium alginate) with a crystalline peak at $2\theta \approx 32^\circ$. The reaction between calcium chloride and sodium alginate occurred first leading to the formation of calcium alginate gel, which then entrapped the bioactive material. Subsequent reaction of calcium alginate with chitosan resulted in the formation of a coat around the microcapsule core. The X-ray diffraction profile of blank microcapsules (BM) indicates their crystalline nature with a peak at $2\theta \approx 32^\circ$. The crystalline peaks of calcium-alginate ($2\theta \approx 32^\circ$) did not shift with the inclusion of extracts in the microcapsules. In GKA and HUA, the amorphous region produced by the extract and the crystalline peaks of the microcapsules similar to that of BM are distinct. Thus, the presence of the extracts had no influence on the crystalline properties of chitosan-alginate microcapsules.

The FTIR spectra of extracts and microcapsules are shown in Figures 3. There were the characteristic absorption spectra for OH stretching at wave number of 3400-3550 cm^{-1} , and CH stretching of CH_2 and CH_3 at wave number of 1922 cm^{-1} . There were absorption bands between 1430 and 1730 cm^{-1} as follows: C=O stretching of acetyl or carboxylic acid (1730 cm^{-1}), H—O—H bending of absorbed water (1648 cm^{-1}), Carbonyl stretching with aromatic ring (1634 cm^{-1}) and CH_2 bending (1430 cm^{-1}). Finally absorption bands appearing between 1000 and 1200 cm^{-1} indicate CO stretching of ether linkage (1250 cm^{-1}) COC antisymmetric bridge stretching (1166 cm^{-1}), and CO symmetric stretching of primary alcohol (1062 cm^{-1}). There was no change in the functional groups of the different compounds in the microcapsules before and after extract encapsulation. This indicates that there was no chemical reaction between the extracts and the other materials in the formulation.

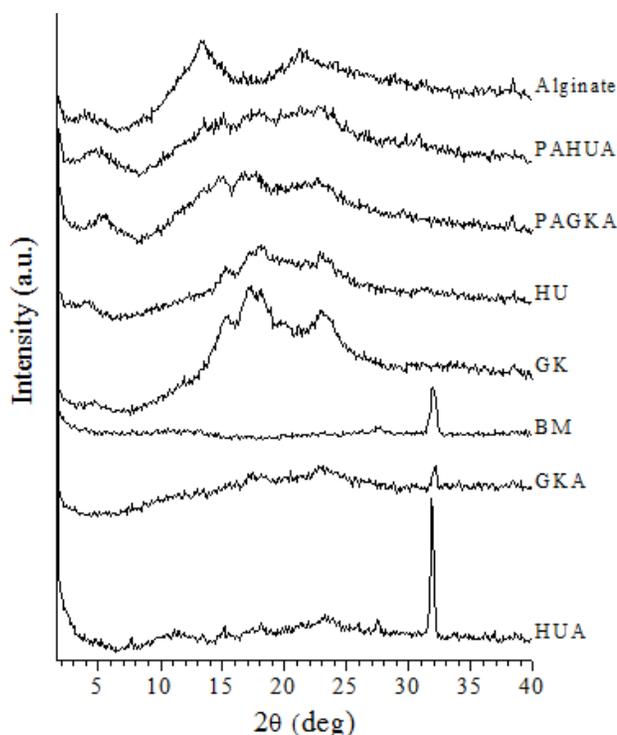


Figure 2: X-ray diffractogram of microcapsules containing *G. kola* and *H. umbellata*. **Key:** BM: Blank microcapsules; GKA: microcapsules containing only alginate and GK; PAGKA: Physical admixtures of sodium alginate and GK extract; HU: *Hunteria umbellata*; PAHUA: Physical admixtures of HU and alginate powder; HUA: microcapsules containing only alginate and HU.

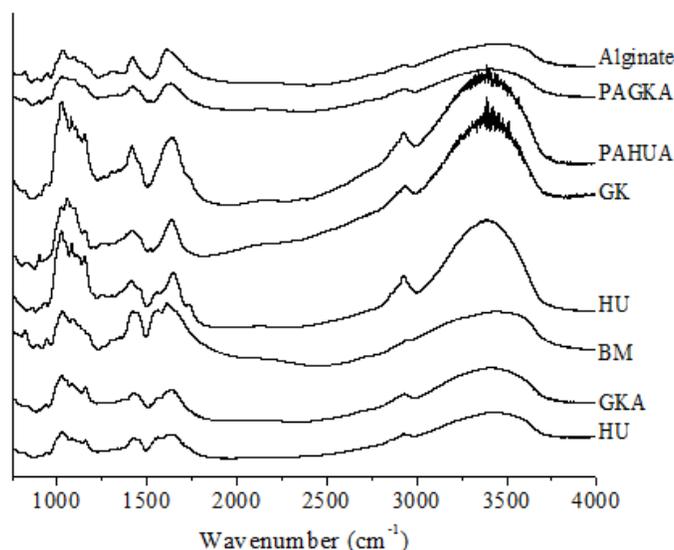


Figure 3: FTIR spectra of microcapsules containing *G. kola* and *H. umbellata*. **Key:** GK: *Garcinia kola*; BM: Blank microcapsules; PAGKA: Physical admixtures of GK and alginate powder; GKA: microcapsules containing only alginate and GK; HU: *Hunteria umbellata*; PA HUA: Physical admixtures of HU and alginate powder; HUA: microcapsules containing only alginate and HU.

3.3 Release of *G. kola* and *H. umbellata* extracts from microcapsules

In simulated gastric fluid, the conventional dosage form released all their contents within 1 h while all the microcapsules showed delay in the release of their contents within the first two hours. The GKA microcapsules released only 27% while HUA released 50% of their contents after 2 h. Transfer of the microcapsules into phosphate buffer pH 6.8 after 2 h resulted in over 80% of their content released after 6 h with no further drug release even after

10 h of study. The extract release study from microcapsule showed an initial lag in the acid medium. Though chitosan has been reported not to prevent drug diffusion in acid medium because of its acid solubility [9], there seems to be an impediment to the release of the extract from the microcapsules especially as they remained intact over the 2 h period in simulated gastric fluid. It is not clear why the unmodified microcapsules hindered drug release. *G. kola* is reported to contain natural gums [4] and probably HU contain components that interacted physically with alginate leading to compaction of the microcapsules and hence retarding extract diffusion out of the tablets in acid medium. *Garcinia kola* seed extract formulations in the form of syrups and tablets have been reported earlier [16][17]. The formulations exhibited good pharmaceutical and antimicrobial properties. These studies however, did not address the problem of the short half-life of biflavonoids (less than 2 h), which meant that the extract stability was low and frequent dosing would be required. On the other hand, *Hunteria umbellata* seeds are not currently available in any known standardized dosage form though it is widely used in ethnomedicine in the management of several disorders. Extract from this plant is very bitter and this will likely lead to poor patient acceptance/compliance since it is primarily administered orally. In traditional medicine, usually the seeds are boiled in water and the extract swallowed at regular intervals daily.

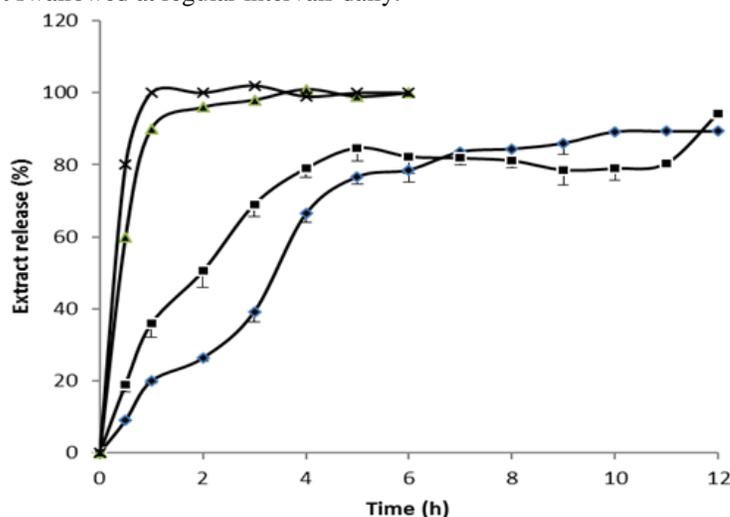


Figure 4: Release of HU and GK extracts from conventional tablets and microcapsules (Key: GK microcapsules (-♦-), GK tablets (-▲-), HU tablets (-x-), HU microcapsules (-■-))

In this study, the movement of dosage form from the acid pH of the stomach to the neutral/alkaline pH in the intestine was simulated. While a reasonable amount of the extract diffused out at acid pH, the bulk of the extract diffused out at pH 6.8. Hence, when the release in both pH are combined, they all demonstrated steady release of their contents. The microcapsules did not release all their content after 12 h probably because it was compact due to the alginate and natural gums present in the core.

3.4 Kinetic Data Analysis

Table 2 presents the regression coefficient (R^2) values for the release models to which the extract release data were fitted. The R^2 values were generally low with very few exceptions.

Table 2: Regression coefficient (R^2) values for the different release models for GKA and HUA microcapsules

	Zero order	First order	Higuchi	Millar and Peppas (n values in brackets)
HUA	0.5977	0.9679	0.7529	0.9120 (0.258)
GKA	0.8185	0.9665	0.9222	0.9225 (0.513)

The unmodified microcapsules had R^2 values above 0.9 for First order, and Millar & Peppas models. Extract diffusion from the microcapsules fitted more closely to first order model than the others. This was followed by the Millar & Peppas model. Thus, extract diffusion rate decreased in proportion to the amount of extract remaining in the microcapsule. The drug release from the HUA microcapsules displayed a diffusion controlled mechanism. This was confirmed from the value of the release exponent (n) in Korsmeyer’s equation having $n < 0.5$. The formulations fitted poorly with zero order and Higuchi models.

4. Conclusion

Chitosan – alginate microcapsules retarded the release of aqueous plant extracts at pH 1.2 when compared to conventional tablets of similar extracts but enhanced release at pH 6.8 in a controlled manner. Thus, chitosan-alginate microcapsules have the potential to serve as carrier for the controlled delivery of biologically active compounds obtained from these plants.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MIA: Carried out the preparation and characterization of the microcapsule formulations and drafted the manuscript. AOO: Supervised and participated in drafting the manuscript. FA: Supervised and participated in analysis of the drug. CDR: Also supervised and participated in analysis and characterization of the microcapsules. RDG: Participated in characterization of the microcapsules. All authors read and approved the final manuscript.

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