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Microencapsulated *Garcinia kola a*nd *Hunteria umbellata* Seeds Aqueous Extracts - Part 2: Influence of Some Selected Pharmaceutical Excipients

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Abstract
Objective: This study investigates the effect of selected excipients on microcapsules properties of seeds extracts of *Garcinia kola* (GK) and *Hunteria umbellata* (HU).
Method: Extracts from macerated dried powdered seeds were microencapsulated with chitosan-alginate including either talc, HPMCAS, Eudragit®RLPO or Eudragit®L100 up to 25% in the core. Microcapsules were characterized using differential scanning calorimertry (DSC), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR) and *In vitro* release.
Results: DSC, XRD and FTIR showed no change in crystalline properties and functional groups of microcapsules and excipients in the formulations. *In vitro* extract release showed retardation of extract release in acid medium after 2 h and between 80 to 100% at pH 6.8. Generally, the microcapsules fitted more closely to first order model than the other models.
Conclusion: Inclusion of these excipients did not impart negatively in interaction and release profile on microcapsules containing GK and HU aqueous extracts and can therefore be added to facilitate microcapsule production and enhance dosage form performance.
Keywords: Excipients, Garcinia kola, Hunteria umbellata, talc, HPMCAS, polymethacrylic acid derivatives, polymers

1. Introduction

The International Pharmaceutical Excipients Council (IPEC) define an excipient as any substance other than the active drug or prodrug that is included in the manufacturing process or is contained in a finished pharmaceutical dosage form [1]. Drug dosage forms are rather complex systems containing many components in addition to active pharmaceutical ingredients (APIs). Formulators apply practical understanding of pharmaceutical excipients to develop optimal, robust formulations and the appropriate manufacturing processes. Excipients facilitate formulation design and perform a wide range of functions to obtain desired properties for the finished drug product[2]. Herbal medicines may contain excipients in addition to the active ingredients. Excipients can help ease manufacturing procedure hence formula including the amount of excipients should be described in details. Other goals such as controlled release have been reported to be achievable with the inclusion of suitable polymers [3]. The

granulated herb is first mixed or coated with an adjuvant or mixture of adjuvants selected from the group consisting of polymers such as polyvinyl, polyethylenes, cellulose, polyacrylate, fats, waxes and sugars, and then processed into a form selected from the group of microcapsules and pellets. The microcapsules or pellets are filled into hard gelatin capsules.

Some polymers and pigments used as excipients in drug formulation include chitosan, polymetacrylic acid derivatives, the cellulosics and talc. Chitosan is deacetylated chitin obtained from the shell of crustaceans. Its film forming abilities lend itself well as a coating agent for conventional solid dosage forms such as tablets [4]. Furthermore, its gel and matrix-forming abilities make it useful for solid dosage forms, such as granules and microparticles.

There are several ranges of polymers derived from metacrylates and metacrylic acid co-polymers depending on the method of production and their intended use. These polymers have wide range of applications including their use as filler for embedding active substances in the construction of matrix structures in depot or retard formulations. Eudragit RL has a low content of quaternary ammonium groups that are present as salts and give rise to the permeability of the lacquer films. They afford water – insoluble, but permeable film coatings [5].

Hydroxylpropyl methylcellulose acetate succinate (HPMCAS) is a white powder with an average particle size of less than 5 µm and can be dispersed readily in water. The characteristics of HPMCAS are related to its degree and type of substitution. The various types of HPMCAS dissolve more readily as the pH increases. HPMCAS has been reported to produce significant changes in the release profiles of microcapsules when it was incorporated in the microcapsule by coating the microcapsule membrane as well as blending it with the capsule core polymer in varying ratios. In fact, at pH 1.2, the modified microcapsules retained up to 60 % of the encapsulated drug after 24 hours [6].

Talc is a water insoluble mineral composed of hydrated magnesium silicate. It is the widely used substance known as talcum powder hence, the US Food and Drug Administration (FDA) considers talc (magnesium silicate) to be generally recognized as safe (GRAS) for use as an anti-caking agent in table salt in concentrations smaller than 2 % [7]. Talc is used in many industries such as paper making, plastic, paint and coatings, rubber, food, electric cable, cosmetics, ceramics, etc. and in pharmaceuticals, it finds use as a lubricant or glidant in tablet production, in baby powder. The objective of this study was to investigate the modulation of aqueous seed extract release of *Garcinia kola* (GK) and *Hunteria umbellata* (HU) from chitosan-alginate microcapsules prepared with either talc, HPMCAS, the acrylate metacrylic acids derivatives.

2. Materials and Methods

2.1 Materials

Fresh seeds of *Hunteria umbellata* (HU) and *Garcinia kola* (GK) were collected in Benin City, Nigeria between April and June. Chitosan was obtained from Sigma-Aldrich, Germany and Sodium alginate (Saltiagin®) was obtained from Sanofi Bio Industries, Canada. Hydroxylpropyl methylcellulose acetate succinate (HPMCAS), an enteric polymer is a product of Shin Etsu Chemical Co. Ltd, Japan, while Eudragit® RLPO and L100 were gifts from Rhom Pharma Chemical Co, Germany. All other chemicals used were of reagent grade and were used without further purification.

2.2 Methods

The method of extraction and preparation of plant material as well as preparation and characterization of microcapsules have been described in the part 1 of this study [8].

2.3 Preparation of excipient - sodium alginate - plant extract solutions

The alginate/plant extract mixture was mixed with another solution of plant extract containing 2% of either talc, HPMCAS, Eudragit L100 or Eudragit RLPO dispersed in it, in the ratio 3:1. (i.e., 75 ml of alginate-plant extract to 25 ml of excipient-plant extract). These were the highest proportions of excipients beyond which stable microcapsules could not form. Table 1 shows the different batches of microcapsules and their respective codes.

Table 1: Batch codes of microcapsules produced

Codes	Microcapsules types
GK	*Garcinia kola*
BM	Blank or microcapsule not containing any plant extract
PAGKA	Physical admixtures of dried GK extract and alginate powder
GKA	Microcapsules containing only alginate and GK
GKA3T1	GK microcapsules containing alginate and talc; ratio 3:1
GKA3H1	GK microcapsules containing alginate and HPMCAS; ratio 3:1
GKA3R1	GK microcapsules containing alginate and Eudragit® RLPO; ratio 3:1
GKA3L1	GK microcapsules containing alginate and Eudragit® L100; ratio 3:1
HU	*Hunteria umbellata*
PAHUA	Physical admixtures of dried HU extract and alginate powder
HUA	Microcapsules containing only alginate and HU
HUA3T1	HU microcapsules containing alginate and talc; ratio 3:1
HUA3H1	HU microcapsules containing alginate and HPMCAS; ratio 3:1
HUA3R1	HU microcapsules containing alginate and Eudragit® RLPO; ratio 3:1
HUA3L1	HU microcapsules containing alginate and Eudragit® L100; ratio 3:1

2.4 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, microcapsules (250 mg) were placed in each dissolution basket and dipped in 500 ml of the dissolution medium (0.1 M HCl) maintained at 37± 0.5^{o}C and then 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 ^{o}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 [9]. The dissolution values of the various formulations at different time points were compared using similarity factor (f2) and difference factor (f1) which were calculated using Equations 1 and 2.

$$F1 = \{[\Sigma_{t=1}^{n} |R_t - T_t|] / [\Sigma_{t=1}^{n} R_t]\} \times 100 \quad \ldots. (1)$$

$$f2 = 50 \times \log \{[1 + (1/n) \Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\} \quad \ldots. (2)$$

where R_t and T_t are the cumulative percentage dissolved at each of the selected 'n' time points of the reference and test product, respectively.

2.5 Data analysis

All experiments were conducted in triplicate and the results expressed as mean ± standard deviation (SD). Statistical analysis was carried out using Microsoft Excel, version 2007. 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 Effect of inclusion of excipients on the thermal properties of microcapsules

Figures 1 a and b show the effect of inclusion of excipients in the microcapsule core on the DSC thermograms of the microcapsules. There was an endothermic peak followed by a transition at 175^{o}C which likely represents thermal decomposition. The thermogram of microcapsules containing excipients was essentially similar to those of the microcapsules containing only the extract or the blank microcapsules. It is apparent that the chitosan-alginate microcapsules formed were physically stable and their thermal properties were not affected by the presence of the excipients added.

(a) (b)

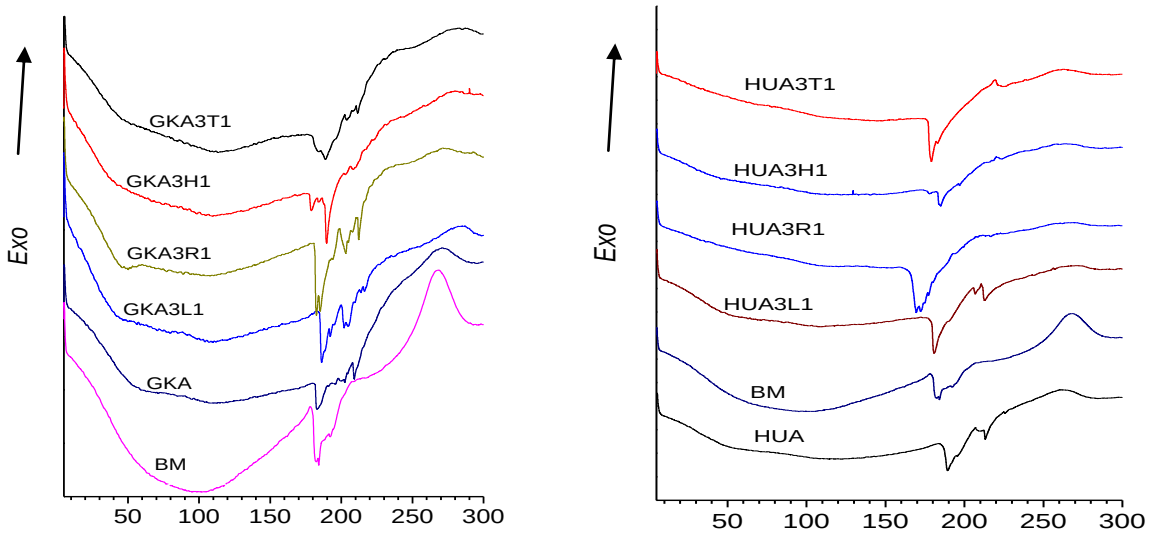
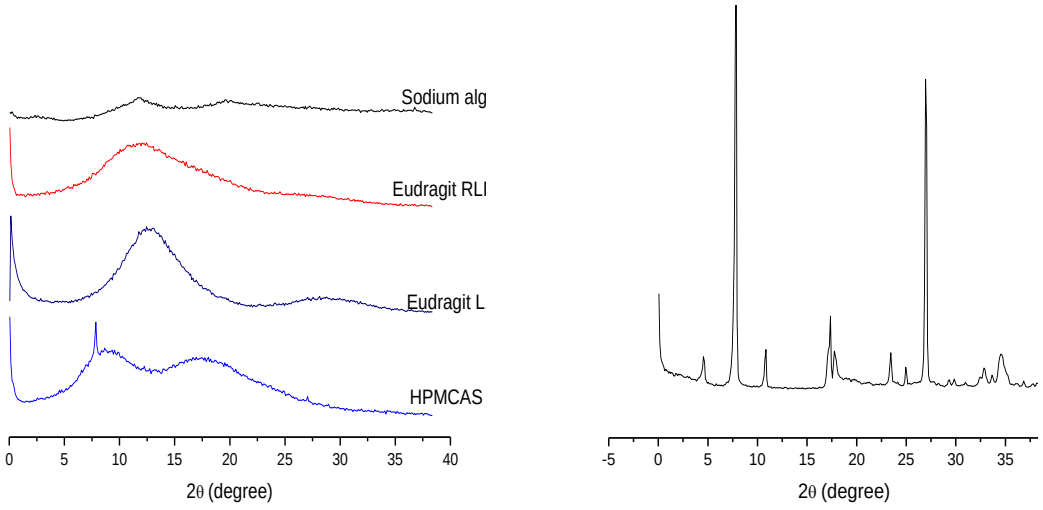


Figure 1: DSC Thermogram of GK (a) and HU (b) microcapsules modified with different excipients. Key: BM: Blank microcapsules; GkA or HuA:- microcapsules containing only alginate and GK or HU. A3T1 or A3H1 or A3R1 or A3L1:- microcapsules containing 3 parts of Alginate plus 1part of either talc (T) or HPMCAS (H) or Eugragit®RLPO or Eugragit®L100 (L)

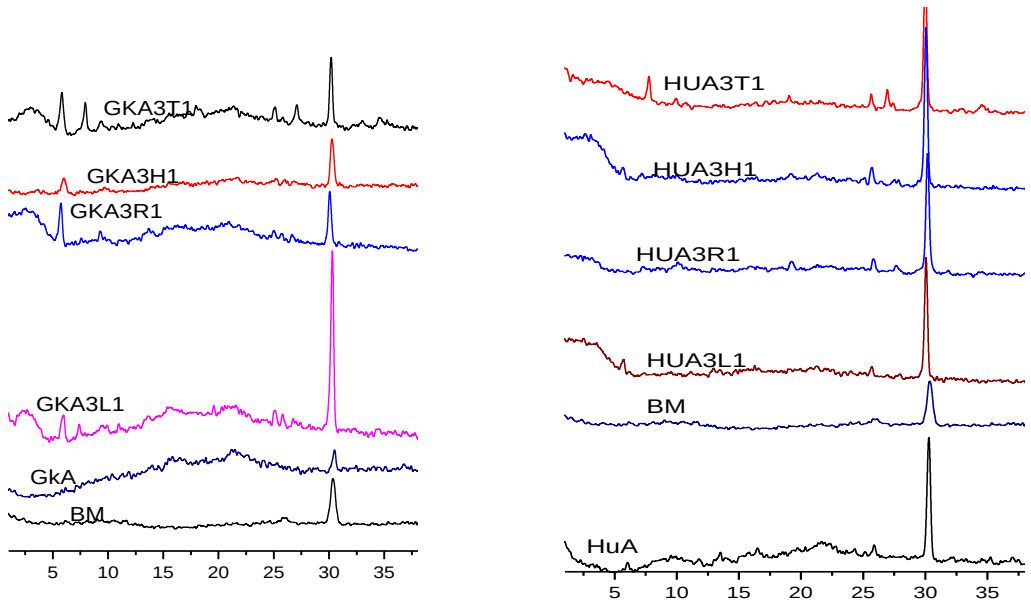
3.2 Effect of excipients on X-ray diffraction properties of microcapsules

The effect of inclusion of excipients on XRD spectra is displayed in Figures 2 a, and b. Talc is crystalline with peaks at 6^o and 27^o. Thus, crystalline properties of the blank chitosan alginate microcapsules (BM) were not affected by the presence of the aqueous plant extracts or the excipients. All the microcapsule formulations of the two plant extracts containing talc showed distinct crystalline peaks at 6^o and 27^o corresponding to that of talc. Furthermore, the crystalline peaks of chitosan-calcium-alginate at 31 ^{o} did not shift following microcapsule production when the extracts and excipients were incorporated. Thus the crystalline properties were not affected by the presence of the plant extracts and excipients.

(a) (b) Talc



(c) (d)

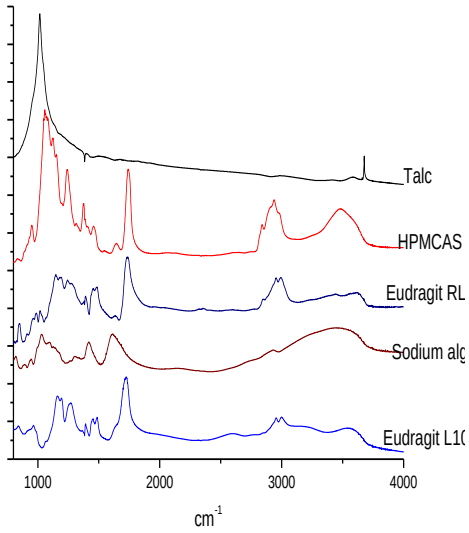


Figures 2 (a and b): X-ray powder diffractograms of excipients; **(c and d)** X ray powder diffractograms of microcapsules containing GK and HU, respectively. **Key**: BM: Blank microcapsules; GkA or HuA:- microcapsules containing only alginate and GK or HU; A3T1 or A3H1 or A3R1 or A3L1:- microcapsules containing 3 parts of Alginate plus 1 part of either talc (T) or HPMCAS (H) or Eugragit®RLPO or Eugragit®L100 (L)

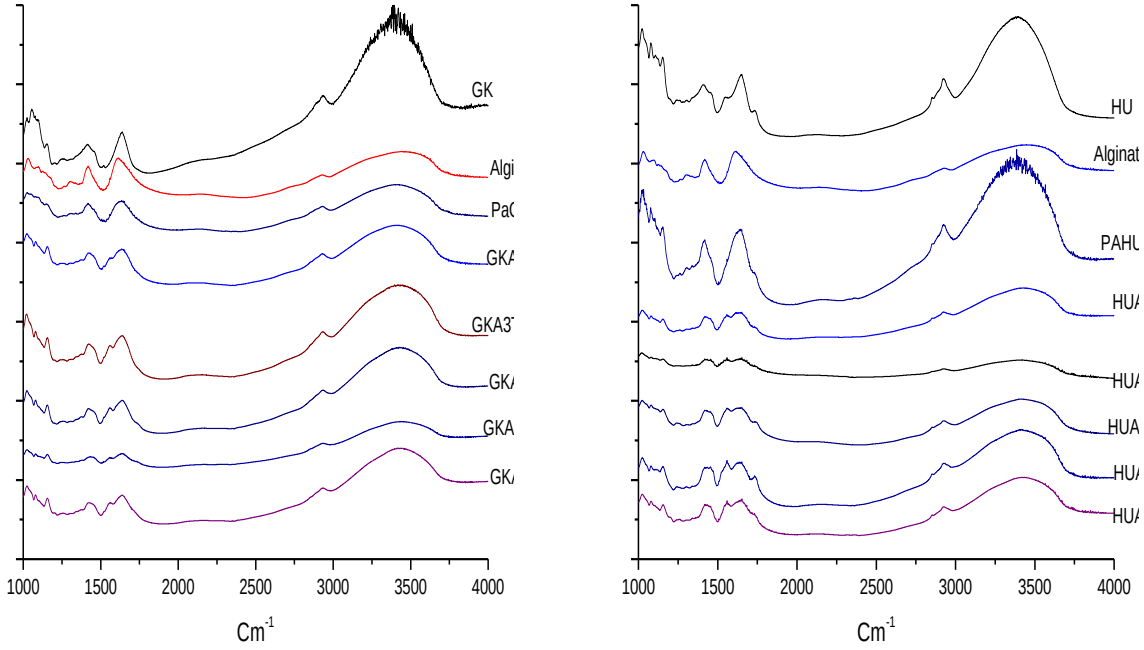
3.3 FTIR spectroscopy

The FTIR spectra of excipients and extracts are shown in Figure 3 a, while those of microcapsules containing excipients are in Figures 3 b and c. With the exception of talc, the spectra revealed obvious similarities in the functional groups of the primary constituents of the excipients, extracts and microcapsules, with characteristic absorption spectra for OH stretching at wavenumber of 3400-3550 cm^{-1}, and CH stretching of CH_2 and CH_3 at wavenumber of 1922 cm^{-1}. There were absorption bands between 1430 and 1730 cm^{-1} as well. There was no change in the functional groups of the different compounds in the microcapsules before and after extract encapsulation or as a result of inclusion of excipients. This indicates that there was no chemical reaction between the extracts and other materials in the formulation.

(a)



(b) (c)

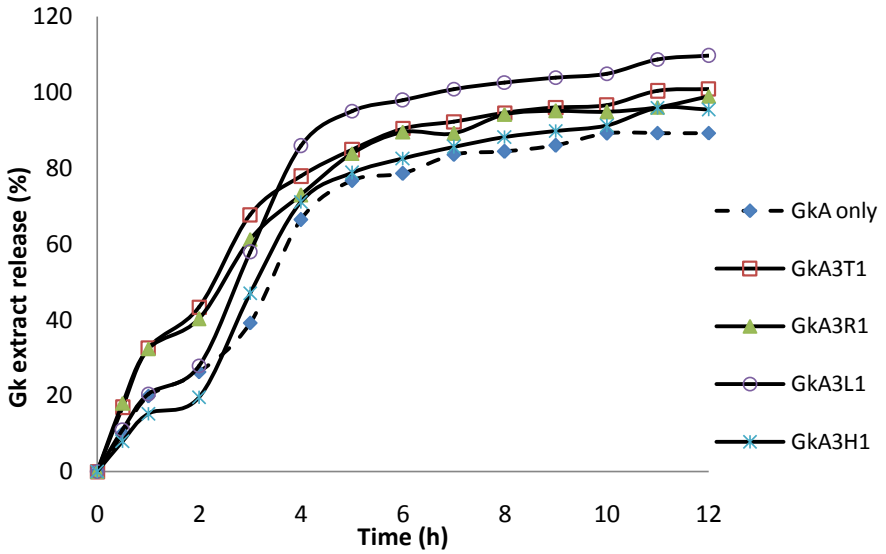


Figures 3 (a, b and c): FTIR spectra of microcapsules containing *Garcinia kola* and *Hunteria umbellata* extracts alone and with excipients ***Key:*** GK: *Garcinia kola*; BM: Blank microcapsules; PA GKA: Physical admixtures of GK and alginate powder; GKA: microcapsules containing only alginate and GK. T: Talc; H: HPMCAS; R: Eugragit® RLPO; L: Eudragit® L100; HU: *Hunteria umbellata* A3T1 or A3H1 or A3R1 or A3L1: microcapsules containing alginate and either T or H or R or L in 3:1 ratio

3.4 Release of Garcinia kola and HU extract from modified microcapsules

The GKA and GKA3L1 microcapsules released only 23 % of their contents while the least release was observed from the microcapsules containing HPMCAS (20 %) after 2 h. Though chitosan has been reported not to prevent drug diffusion in acid medium because of its acid solubility [6], there seem 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. While it was expected that the enteric polymers (Eudragit and HPMCAS) may have been responsible for retarding drug release in the case of the microcapsules containing them, it is not clear why the unmodified microcapsules also hindered drug release. *G. kola* is reported to contain natural gums [10] which may have played a role in compaction of the microcapsules and hence retarding extract diffusion out of the capsules [8]. Talc and Eudragit®RLPO containing microcapsules released over 40 % content within 2 h. it is probable that some extract diffused out of the microcapsules and dried around the coat during drying and this may have been responsible for the high amount observed in the first 2 h. Furthermore, talc and Eudragit®RLPO have mean larger particle sizes > 200 µm when compared with HPMCAS and Eudragit®L100 (< 100 µm) [11-14]. The particles are therefore more likely to interfere with the chitosan/calcium-alginate matrix thus creating cracks or flaws through which drug diffusion were facilitated.

(a)



(b)

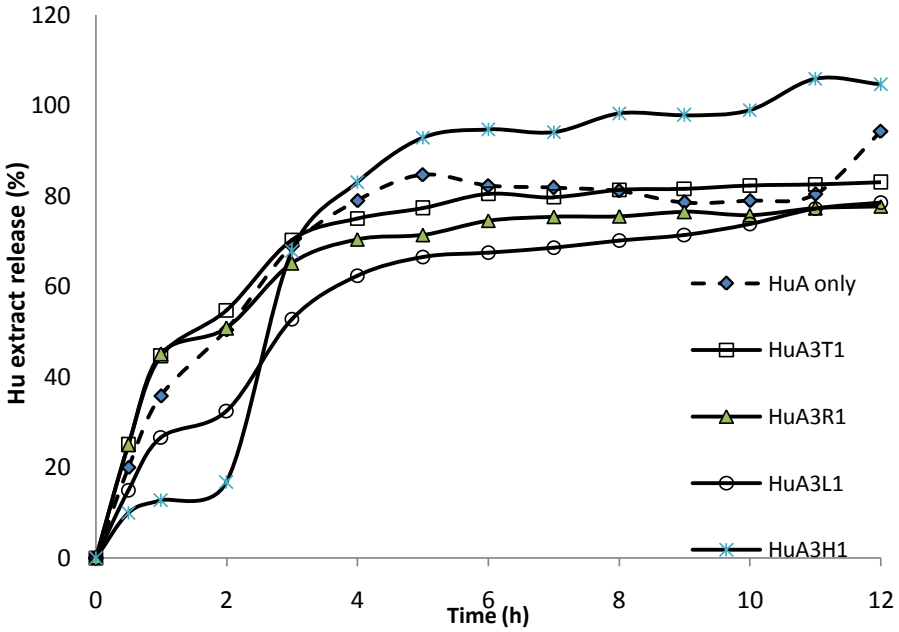


Figure 4 a and b: Release of extract from Gk (a) and Hu (b) microcapsules modified with either talc (T) or HPMCAS (H) or Eugragit ® RLPO or Eugragit ® L100 (L)

When the microcapsules were transferred into phosphate buffer pH 6.8 after 2 h and the dissolution continued for another 10 h, they all demonstrated steady release of their contents. Maximum release was obtained from microcapsules containing talc and Eudragit®L100 (Figures 4 a, and b). The excipients interfered with the gel structure of the microcapsules and hence reduced the compactness. The unmodified microcapsules did not release all their content after 12 h probably because it was more compact due to the alginate and natural gums present in the core. When the release profiles of all the microcapsules containing excipients were compared with that of the unmodified microcapsules (Table 2), the similarity and difference factor data show that the two extracts behaved differently. In the case of GK, extract release from the unmodified microcapsules (GKA) were significantly different from those containing the excipients (f1 values ≥ 15, while f2 < 50) except for GKA3H1. GK extract release from microcapsules containing HPMCAS showed a high level of similarity with the unmodified (f2 > 50). HPMCAS is an enteric polymer, which did not appear to interfere with the alginate/plant extract matrix significantly [15-16].

The results of the release studies on the microencapsulated extract of *Hunteria* are shown in Figure 4b. In 2 h, less than 20 % of the extract was released by HUA3H1 microcapsules compared with HUA3L1 which exhibited 33 % release within the same period. There was no significant difference ($p < 0.05$) between HUA, HUA3T1 and HUA3R1 in terms of extract release in acid medium as over 50 % was released from the microcapsules. The similarity and difference data between the release profiles show that the release from the HUA3L1 and HUA3H1 microcapsules were significantly different from HUA microcapsules (f1 values > 15; f2 < 50) while HUA3T1 and HUA3R1 microcapsules showed a high level of similarity in release with the unmodified (f2 > 50) (Table 2). After 10 h at pH 6.8, they all demonstrated steady extract release. Maximum release was obtained from HUA3H1 microcapsules.

Table 2: f1 and f2 values for dissolution profiles of *GK and HU* microcapsules

Microcapsule type	f1	f2
GKA/GKA3T1	17.98	43.35
GKA/GKA3R1	14.48	47.94
GKA/GKA3L1	22.54	38.56
GKA/GKA3H1	6.56	65.03
HUA/HUA3T1	5.54	63.72
HUA/HUA3R1	8.93	54.49
HUA/HUA3L1	16.55	43.75
HUA/HUA3H1	20.88	37.23

Note: f1 values ≤ 15 indicate minor difference in dissolution rate, while f2 values > 50 indicate similarity in dissolution rate

3.5 Kinetic Data Analysis

Table 3 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. The unmodified microcapsules had r^2 values above 0.9 for First order, and Millar and Peppas. Modulating the microcapsule core with the different excipients did not significantly affect r^2 values. In this study, the movement of dosage form from the acid pH of the stomach to intestinal fluid was simulated. Extract diffusion from microcapsules fitted more closely to first order release model than the other models, thus, extract diffusion rate decreased in proportion to the amount of extract remaning in the microcapsule. All the formulations fitted poorly with zero order and Higuchi models. The extract release from most of the microscapsules displayed a diffusion controlled mechanism ($n < 0.45$) in Korsmeyer's equation. The few exceptions were GKA, GKA3L1 and HUA3H1 in which n was greater than 0.45. The exact reason for this cannot be immediately explained since each batch of microcapsules varied significantly in terms of constituents.

Table 3: Regression coefficient (r^2) values for the different release models

	Zero order	First order	Higuchi	Millar and Peppas (n values in brackets)
HUA	0.5977	0.9679	0.7529	0.9120 (0.258)
HUA3T1	0.5819	0.9912	0.7080	0.9676 (0.213)
HUA3R1	0.5737	0.9825	0.6852	0.9736 (0.202)
HUA3L1	0.7626	0.9833	0.9176	0.9540 (0.368)
HUA3H1	0.7466	0.9227	0.8600	0.8601 (0.500)
GKA	0.8185	0.9665	0.9222	0.9225 (0.513)
GKA3T1	0.7714	0.9930	0.9288	0.9577 (0.381)
GKA3R1	0.7851	0.9903	0.9300	0.9560 (0.398)
GKA3L1	0.7871	0.9593	0.9056	0.9046 (0.496)
GKA3H1	0.8200	0.9584	0.9095	0.9123 (0.283)

4. Conclusion

Microcapsules containing *Garcinia kola* and *Hunteria umbellata* aqueous extracts were produced successfully and these can serve as suitable alternative to liquids and dried powdered leaf phytoformulations. Inclusion of excipients had no significantly negative effect on interaction and release profile of the extracts from microcapsules. Hence, these excipients can be included to facilitate microcapsule production process as well as enhance the formulation performance.

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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