View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Archivio della ricerca - Università degli studi di Napoli Federico II

Asian Journal of Pharmaceutical and Health Sciences

www.ajphs.com

Chitosan-alginate microparticles of *Andrographis paniculata* and *Annona muricata* extracts for Controlled Release

Matthew I. Arhewoh[1], Augustine O. Okhamafe[1]*, Finizia Auriemma[2], Claudio De Rosa[2], Rocco Di Girolamo[2]

1 Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City

2 Dipartimento di Scienze Chimiche , Università di Napoli Federico II, Complessso Monte Sant Angelo, via Cintia, 80126 Napoli, Italy.

ARTICLE HISTORY

Received: 02.01.2016

Accepted: 12.03.2016

Available online: 30.03.2016

Keywords:

Andrographis paniculata, Annona muricata, microencapsulation, aqueous extract.

***Corresponding author:**

Email : arhewoh@uniben.edu

Tel.: +234 8055306846

ABSTRACT

This study investigates the properties of microparticles prepared from *Andrographis paniculata* (AP) and *Annona muricata* (AM) aqueous extracts for controlled release. Extracts obtained by maceration of the dried powdered plant leaves were microencapsulated by counterion coacervation method. Microcapsules were characterized using Fourier-transform infrared-spectroscopy (*FTIR), x-ray difractometry (XRD) and differential scanning calorimetry (DSC)*.*In vitro* release studies were carried out at pH 1.2 for 2 h and 6.8 for a further 10 h. Release was monitored at274 and 230 nm for AM and AP, respectively. Encapsulation efficacy was less than 52% for AP and 70% for AM. *In vitro* drug release at pH 1.2 showed less than 40% release from the microcapsules after 2h while over 90% of extract was released after 6h at pH 6.8. Conventional capsules released the content within 1 h in simulated gastric fluid. *FTIR, XRD and DSC results indicate the stable character of the extract within the microcapsules.* Microencapsulation with chitosan-alginate controlled the release of *Andrographis paniculata* (AP) and *Annona muricata* (AM) aqueous extracts.

INTRODUCTION

Andrographis paniculata (Acanthaceae) also known commonly as "King of Bitters," has been used for centuries in Asia to treat disorders of the gastrointestinal tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases[1]. It is found in the Indian Pharmacopoeia and is a prominent component in at least 26 Ayurvedic formulas as well as in Traditional Chinese Medicine (TCM) [2]. Research conducted in the 1980s and 1990s confirmed that when *Andrographis* is properly administered, it has a range of pharmacological effects, some of which are extremely beneficial. Andrographolide is the principal alkaloid in the plant and it appears to have a relatively short half-life of approximately six hours and is excreted by one of several routes (urine, faeces, exhaled air, sweat, or other body excretions). In some studies, 80 % of the administered dose of andrographolide is removed from the body within 8 - 10 h [3,4].

Annona muricata Linn, (Annonaceae) is a popular plant of the tropical states of India, America and Africa. The leaves are described as evergreen. Phytochemically, it is rich in miscellaneous lactones and isoquinoline alkaloids. The leaf, stem, bark and seeds contain varying amounts of a novel group of chemicals believed to be biologically active, called *Annonaceous acetogenins* [5].The plant extracts have been used traditionally for their antimicrobial [6], antidiabetic [7] and antineoplastic [8] properties. Its antioxidant activity which was studied as a function of its radical DPPH scavenging activity has been reported [9]. It is reported to be selectively potent against cancer cells while leaving the human cells unharmed [5,8]. *Annona muricata* leaves are mostly used (in capsule form) in the management of cancer due to its content of acetogenins. These capsules generally contain the powdered leaves administered as 6 to 8 g daily in 3 to 4 divided doses. However, the issue of high multiple daily dosing to achieve therapy makes them inconvenient for patients. Furthermore, treatment for cancer is usually over a long period of time hence most patients are usually faced with the challenge of adherence. Compounds with short half-life need to be administered often since they do not stay in the body for long.

Microencapsulation which involves the reversible coating of solids, liquids etc with thin polymeric films will have tremendous benefits in taste masking, stability and controlled release. This research effort was aimed at formulating Chitosan-alginate microcapsules for controlled release of the aqueous extract of *Andrographis paniculata* and *Annona muricata*.

MATERIALS AND METHODS

Materials

Fresh leaves of *Andrographis paniculata* (AP) and *Annona muricata* (AM) were collected in Southern Nigeria and air-dried. 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.

Collection and Identification of Plant material

AP and AM leaves were harvested in farms 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. Voucher numbers 107852 (AM) and 108458 (AP) were assigned and samples deposited in the herbarium of the same Institute. The leaves were plucked off from the stem, rinsed and air-dried in a dust-free environment for two weeks following which they were pulverized and passed through a sieve (1 mm aperture size). The powders collected were separately packed in air tight containers prior to utilization.

Extraction

The powdered sample (100 g) was macerated with 1000 ml of distilled water at room temperature for 24 h. The solution was vigorously shaken at 30 min interval for the first two hours. The extracting solution was filtered and the filtrate obtained was concentrated to dryness using a vacuum oven at 35°C and the dried mass was stored in a refrigerator before drug production. Preliminary investigations of the extracts have been reported in a previous study [9].

Preparation of microcapsules

Preparation of 0.1 % chitosan solution

The solution was prepared by dissolving 1 g of chitosan 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) and polysorbate 80 (0.2 ml) were added. 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 [10].

Preparation of sodium alginate and aqueous plant extract solutions

The dried extract of each plant (2 g) was re-dispersed 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 give a 2% solution of sodium alginate.

Microencapsulation process

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 21G, 90° blunt tip needle (Chromatographic Specialties, Canada) through which the mixtures were extruded. 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. This had two cables of which the 'live' was connected to the needle while the 'ground' cable was placed in the collecting solution. The gelation process occurs spontaneously due to the formation of calcium-alginate gel thereby trapping the extract in the gel matrix. The particles were allowed to remain for 2 min after extrusion of alginate/extract solution to allow the formation of chitosan coat. The microcapsules were then filtered, rinsed with water and air dried in an air conditioned room (reduced temperature and humidity) for 48 h.

Conventional capsules were also prepared by loading 600 mg of dried pulverized leaves (< 0.710 mm) into suitable capsule shells.

Determination of microcapsule size

A light microscope (model 745917, 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.

Entrapment efficiency (EE)

Microcapsules equivalent to 50 mg of the extract was "citrated" by dispersing it in 20 ml of 0.5 M sodium citrate and kept overnight (citration was used to breakdown the gel structure containing the entrapped dug). The microcapsules, together with the citrate solution were then transferred to a glass mortar and crushed with a pestle to effect maximum extract release into solution [10]. The absorbance of each solution was monitored at the respective absorbance of each extract and the EE was calculated using Equation 1

EE (%) = Aa/At x 100/1 .... (1)

where "Aa" is the absorbance of the actual amount of drug present and "At" is the expected absorbance of theoretical drug load

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. 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 the instrument blank.

Thermal Analysis

Thermograms of all the samples were obtained by differential scanning calorimetry (DSC 822, Mettler Toledo) equipped with a thermokinetic analysis software. Between 5 to 10 mg of the microcapsules and dried plant extracts were weighed into sealed aluminium 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 nitrogen atmosphere with a flow rate of 75 ml/min. Isothermal condition was maintained at 25°C for 5 min. Thermogravimetric analysis (TGA) of all the samples were obtained by TA instrument Inc. USA, equipped with simultaneous DSC-TGA software. The microcapsules and dried plant extracts (10 to 20 mg) were weighed into an aluminium pan. The pan was placed in the combustion chamber. The equipment

was set to heat from 0 to 300°C at a ramp (heating rate) of 10°C per min under compressed air with a flow rate of 75 ml/min.

X-ray diffraction Studies

The X-ray diffraction patterns of the dried plant powders 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 levelled to form a plane surface. The disc with the powder was placed in the sample holder and the scan performed over a wide angle (2θ). The patterns were recorded using Cu-Kα radiation at 35 kV and 30 mA for 30 min, continuous scan, scanning rate of 0.1deg (Δ2θ)/ 5s (Δ*t*).

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 (rotating basket method) [11]. For each batch, 250 mg 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 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 500 ml 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 in order to maintain sink conditions. Release of the extracts from the microcapsules was monitored spectrophotometrically at 274 and 230 nm for AM and AP, respectively [12,13].

Drug release kinetic

The data obtained from release studies were fitted to various release models, namely, zero order, first order, Higuchi, and Millar and Peppas using SigmaPlot 8.02 software (Sigma Systat Software Inc. CA, USA). These models were first built into the regression library of the software (Equations 2 5). The model that best represent extract release was determined.

$$F = Kt \quad \text{.... (2)}$$

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

$$F = Kt^{½} \quad \text{.... (4)}$$

$$F = kt^{n} \quad \text{.... (5)}$$

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 [14].

Data analysis

All experiments were conducted in triplicate and the results expressed as mean ± standard deviation (SD, n = 3). 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 spectra data from FTIR, DSC, TGA and XRD.

RESULTS

Physical characteristics of the microcapsules

The wet microcapsules formed were spherical in shape and appeared uniform in size distribution. Microcapsules containing

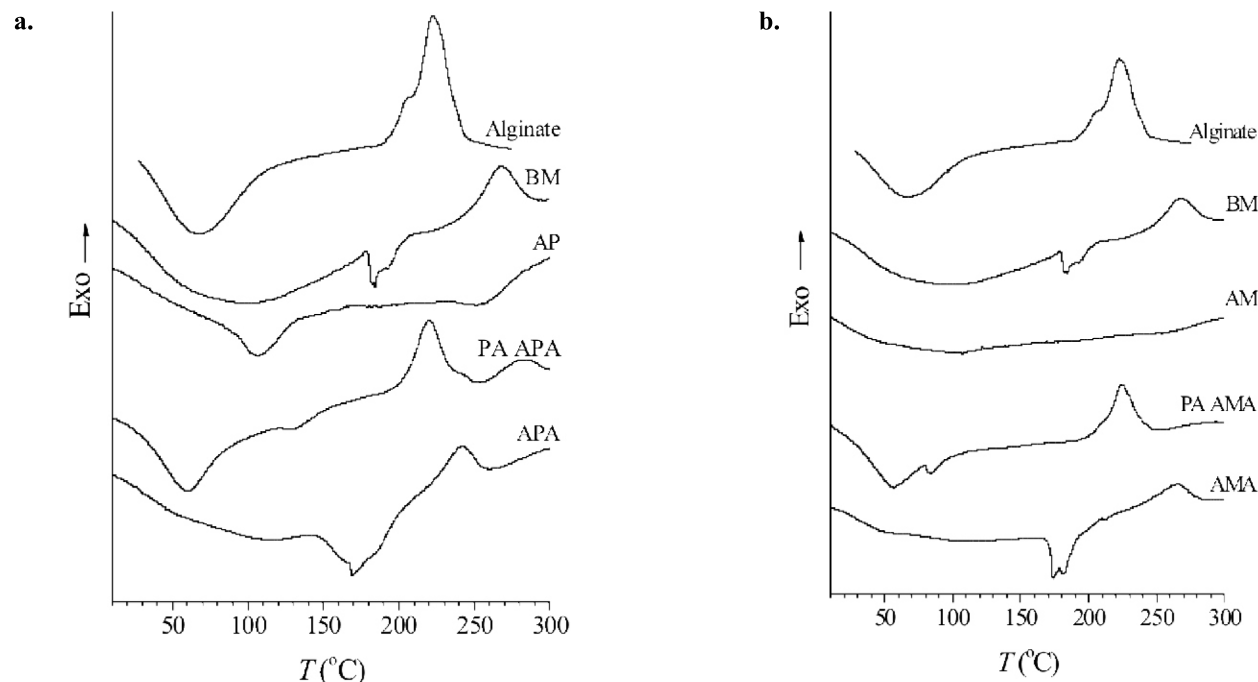

Figure 1 a and b: DSC thermograms of microcapsules containing *Andrographis paniculata andAnnona muricata*
Key: AP: *Andrographis paniculata*; BM: Blank microcapsules; PA APA: Physical admixtures of AP and alginate; APA: microcapsules containing only alginate and AP; AM: *Annona muricata*; PA AMA: Physical admixtures of AM and alginate powder; AMA: microcapsules containing alginate and AM in the core

the leaf extract were green in colour. The microcapsules shrank to approximately 10 % of its original wet size and became hard and irregular in shape after drying. The mean particle size (µm) ± SD produced at 0 kV were 855.5 ± 12.9 and 887.5 ± 12.9 for AP and AM, respectively. This size reduced significantly when the applied voltage was increased to 10 kV to 273.9 ± 23.6 and 325.9 ± 23.6, respectively. This finding is similar to earlier reports that increasing voltage above 6 kV results in decreased microcapsule size [15].

There was over 70 % entrapment for microencapsulated *Annona muricata* while microcapsules containing *Andrographis paniculata* extract had over 52 % entrapment. The reason for this high entrapment values could be attributed to the spontaneous gelation during the encapsulation process. The drying process was slow (2 days) though the gelation process was spontaneous. The microcapsules were air-dried [16]. This resulted in the drainage of some of the extract out of the microcapsules during drying. This was more prominent for the *Andrographis paniculata* microcapsules which showed the lowest entrapment.

Thermograms

The DSC thermograms of the microcapsules shown in

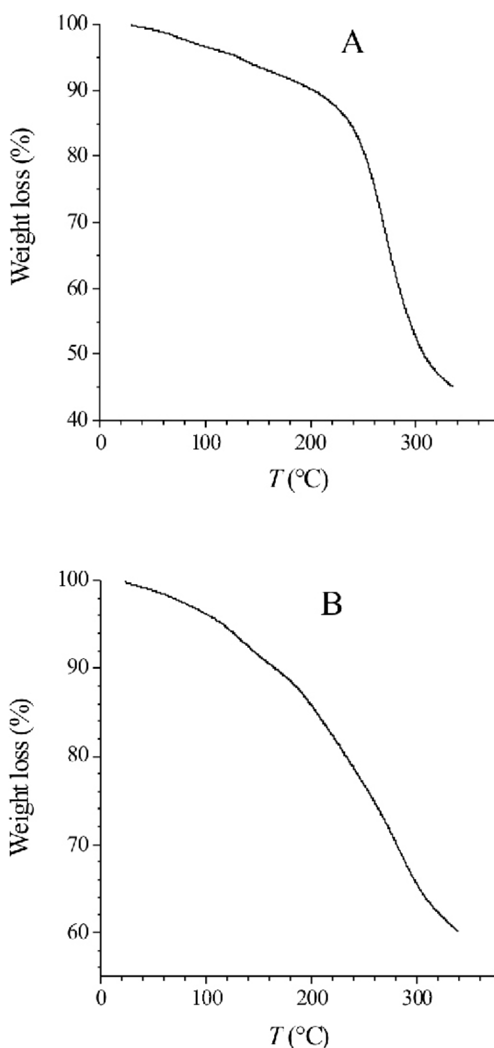

Figure 2 : Representative TGA thermograph of (A) extracts and (B) microcapsules

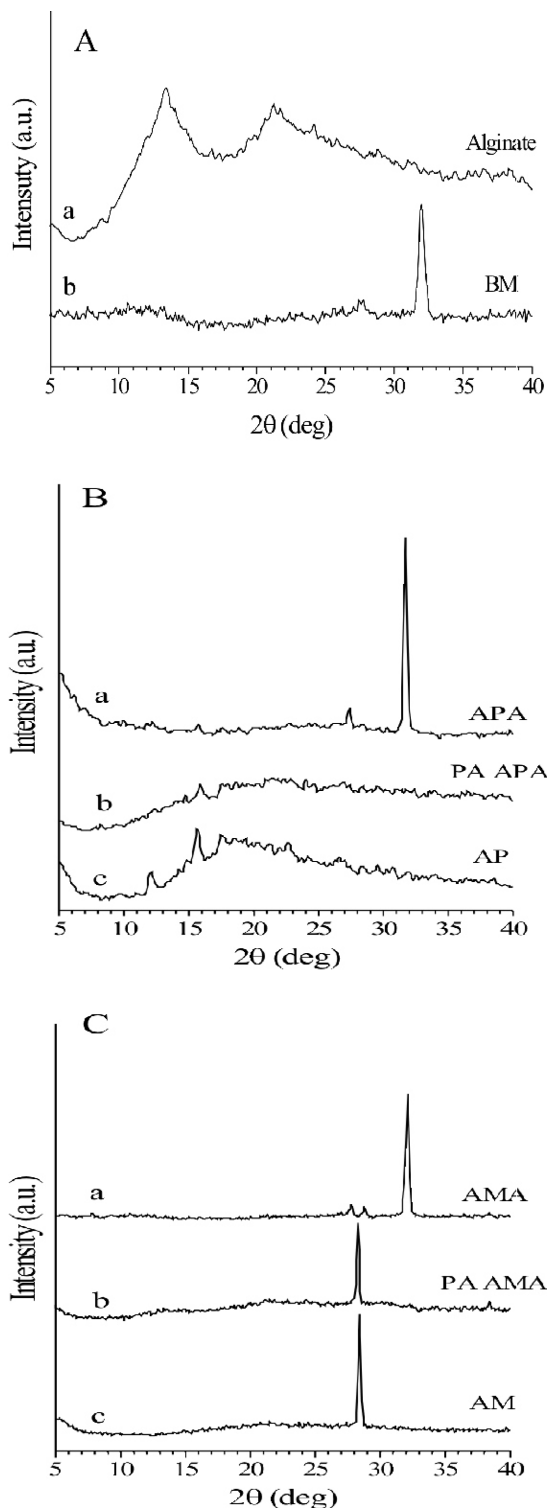

Figure 3: X-ray powder diffraction profiles of A: Alginate (a) and Blank microcapsules (b); B: *Andrographis paniculata (c), corresponding physical admixtures with alginate (b) and microcapsules (a); C: Annona muricata (c), corresponding physical admixtures with alginate (b) and microcapsules (a).* **Key:** BM: Blank microcapsules; AP: *Andrographis paniculata*; PA ApA: Physical admixtures of AP and alginate; APA: microcapsules containing only alginate and AP. AM: *Annona muricata*; PA AMA: Physical admixtures of AM and alginate powder; AMA: microcapsules containing only alginate and AM in the core.

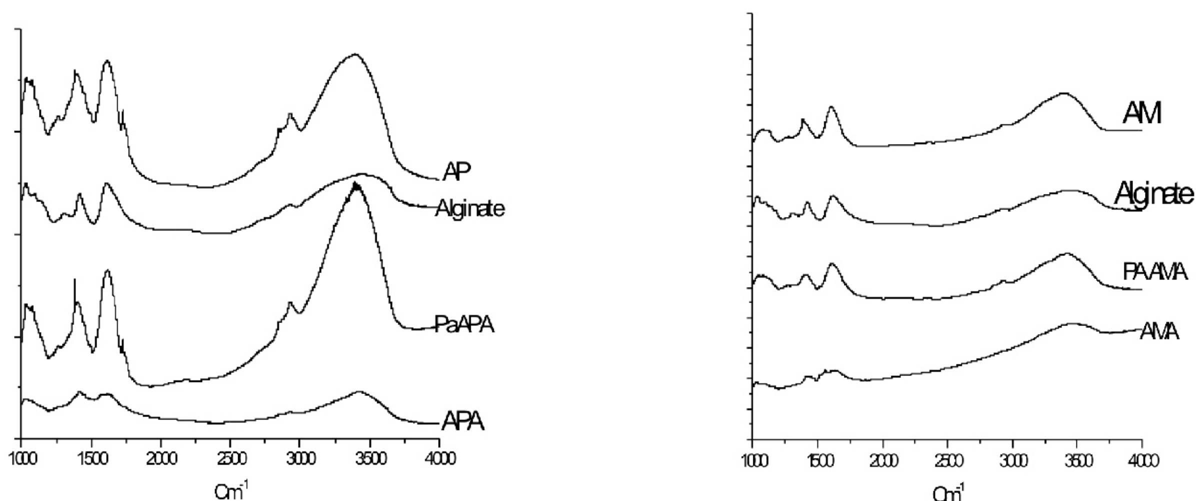

Figure 4 (a and b): FTIR spectra of (a) AP extract and microcapsules and (b) AM extract and microcapsules

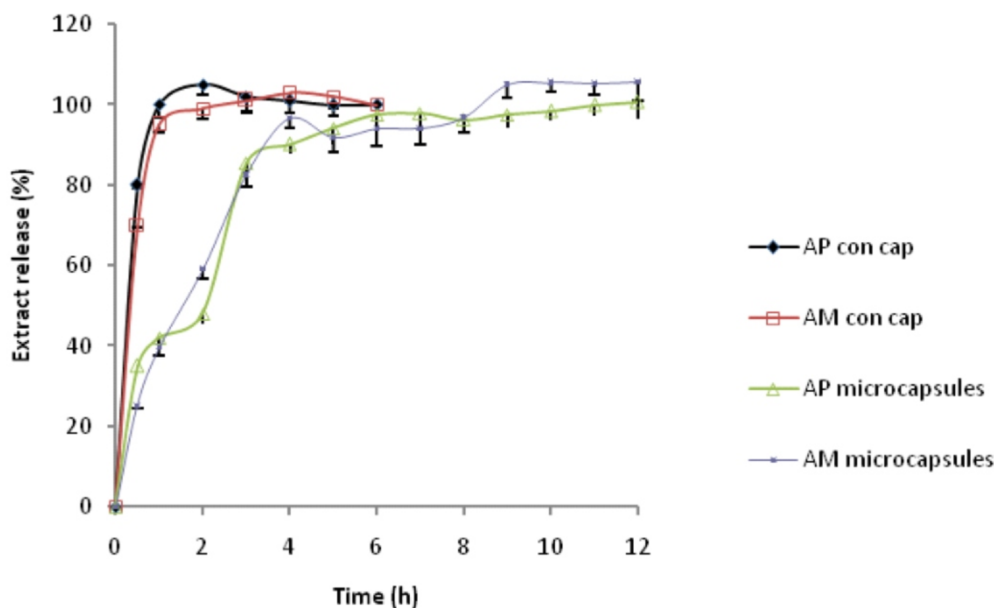

Figure 5: Release of *Andrographis paniculata* and *Anona muricata* extract from microcapsules
Key: AP conventional capsules (♦); AM conventional capsules (□); AP microcapsules (Δ); AM microcapsules (x)

Table 1. : Regression coefficient (R2) values for the different release models

	Zero order	First order	Higuchi	Millar and Peppas (n values in parenthesis)
APA	0.6255	0.9716	0.7934	0.9131 (0.284)
AMA	0.6763	0.9829	0.8404	0.9447 (0.297)

Figures 1a and b, revealed the presence of endothermic peaks in the range 60 - 120°C, 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. Figure 2 (a and b) are representative TGA thermograms of extracts and microcapsules. There was a progressive weight loss in all the samples with increase in temperature. The initial loss might have been due to moisture loss. This weight loss partly confirms that the changes observed in the DSC thermograms particularly for the microcapsules were primarily as a result of thermal degradation or decomposition.

X-ray diffractograms

The X-ray diffractograms of the microcapsules are shown in Figures 3A-C. They indicate that the amorphous sodium alginate (curve 'a' of Figure 3A) and chitosan reacted with the crystalline calcium chloride to form a complex (chitosan-alginate) with crystalline peak at 32°(curve 'b' of Figure 3A).

FTIR spectra

The FTIR spectra of microcapsules are shown in Figures 4a and b, the spectra revealed obvious similarities in the functional groups of the primary constituents of the extracts and microcapsules, with 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 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 other materials in the formulation.

Drug release

The effect of microencapsulation on the release of the extract from microcapsules is shown in Figure 5. In simulated gastric fluid (pH 1.2), the conventional capsule formulations released completely the extract within 1 h while the microcapsule formulations demonstrated reduced extract release by up to 50% in the first 2 h.

DISCUSSION

A number of formulations containing *Andrographis paniculata* have been reported [16]. It is also an ingredient in some herbal formulations and bitters. These commercial formulations are however very bitter and the andrographolides have a relatively short half-life (6.6 h) after oral administration [4] hence warranting frequent administration. This renders it difficult to formulate stable preparations with prolonged release. The DSC thermograms of APand AM extracts showed a pronounced endothermic peak as the temperature was increased from 25°C. *Andrographis paniculata* dried extract showed a transition endotherm at 100 to 110°C followed by decomposition between 90 and 125°C. Plant extracts comprise a wide variety of materials from components like calcium oxalate to macromolecules like sugars, proteins and a vast array of secondary metabolites [17]. The presence of all these components in the extracts was responsible for the broad endothermic peak observed in the DSC study since they undergo decomposition at different times and under different thermal conditions. These endotherms are 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 [18]. The endotherm for sodium alginate powder disappeared when used in microencapsulation, due to the formation of a new compound (calcium-alginate). 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 thermograms observed. The thermograms of the new complex formed appeared not to have been affected by the inclusion of the extract while the physical admixtures of sodium alginate and dried plant extract did not affect the endotherm of the pure sodium alginate.

The XRD of blank microcapsules (BM) indicate their crystalline nature with a peak at 32° (curve b of Figure 3A). The crystalline peaks of calcium-alginate did not shift when the extracts were incorporated in the microcapsules (curve a, of Figure 3B,C). Thus the crystalline properties of the chitosan-alginate microcapsules were not affected by the presence of the extracts. *Annona muricata* extract on the other hand showed a crystalline peak at 28° (curve c, of Figure 3C). BM and AM peaks were distinct and there was no shift and no creation of new peak was observed following the incorporation of *Annona muricata* extract into the microcapsules (curve c, of Figure 3C). These data strongly suggest that the crystal structure of extracts was not affected by encapsulation process.

The AM microcapsules released up to 60 % of the extract while those of AP released 40 % of the extract. *A. muricata* extract was more soluble in the release medium and therefore diffused out of the microcapsules more rapidly. Nevertheless, extract release from the microcapsules was considerably slower than from the conventional capsule preparation of finely powdered leaves of AM or AP). The initial high amount released may be due to the fact that the extract diffused from the microcapsule core to its surface during drying. This in itself may be beneficial where continuous drug release is desired, as the remaining 55 - 65 % of the extract in the microcapsules was released over the next 5 h when the microcapsules were transferred to simulated intestinal fluid (SIF).When the microcapsules were transferred into phosphate buffer (pH 6.8) (simulated intestinal fluid) and dissolution continued for another 10 h, maximum release was achieved from AMA microcapsules after 8 h. *Annona muricata* leaves are mostly used (in capsule form) in the management of cancer due to its content of annonaceous acetogenins. These capsules generally contain the powdered leaves administered as 4 to 6 g daily in 3 to 4 divided doses. However, the issue of high multiple daily dosing to achieve therapy makes them inconvenient for patients hence the potential benefit of these microcapsules.

Table 1 presents the regression coefficient (R^2) values for the release models to which the extract release data were fitted. The R^2 values were above 0.9 for first order, and Millar and Peppas. While a reasonable amount of the extract diffused out at acid pH, often the bulk of the extract diffused out at pH 6.8. The microcapsules fitted more closely to first order model followed by Millar and Peppas model. Thus, extract diffusion decreased in proportion to the amount of extract remaining in the microcapsule. All the formulations fitted poorly to zero order and Higuchi models. Extract release from microcapsules was by matrix-diffusion mechanism

CONCLUSION

Formulations of the aqueous extracts of these plants in chitosan-alginate microcapsules slowed down *in vitro* release of the extracts. This work indicates that it is feasible to formulate

herbal extracts into microcapsules with the aim of reducing dosing frequency. The successful application of this technology to two different plants with very different constituents suggests that the technique can also be applied to a wide variety of plant extracts from different sources.

REFERENCES

1. Gupta S, Choudhry MA, Yadava JNS, Srivastava V, Tandon JS. Anti-diarrhoeal activity of diterpenes of *Andrographis paniculata* (Kalmegh) against *Escherichia coli* enterotoxin in in vivo models. Int. J. Crude Drug Res. 1990: 28: 273-283.

2. Tang W, Eisenbrand G. Chinese drugs of plant origin. Springer, Berlin, 1992.p.97.

3. Matsuda T, Kuroyanagi M, Sugiyama S, Umehara K, Ueno A, Nishi K. Cell differentiation inducing diterpenes from *Andrographis paniculata*, Chem. Pharm. Bul. 1994: 42: 1216-1225.

4. Niranjan A, Tewari SK, Lehri A. Biological activities of *Kalmegh (Andrographis paniculata Nee)* and its active principles A review. Ind. J. Nat. Prod. Res. 2010: 1(2): 125-135

5. Chang FR. Novel cytotoxic *Annonaceous acetogenius* from Annona muricata. J. Nat. Prod. 2001: 64(7): 925-931

6. Takahashi JA. Antibacterial activity of eight Brazilian annonaceae plants. Nat. Prod. Res. 2006: 20(1): 21-26.

7. Adewole SO, Caxton-Martins EA. Morphological changes, hypoglycemic effects of *Annona muricata Linn. (Annonaceae)*.leaf aqueous extract on pancreatic B-cells of Streptozotocin-treated diabetic rats. Afr. J. Biomed. Res. 2006:9:173-187

8. Kojima N. Systematic synthesis of antitumor annoceous acetogenins. Yakugaku Zashishi. 2004: 124(10): 673-681

9. Arhewoh MI, Falodun A, Okhamafe AO, Bao Y, Sheng Q. Ultrasonic Assisted Extraction and radical scavenging activity of some selected medicinal plants, *J. Pharm. Res. 2011: 4(2): 408-410.*

10. Okhamafe AO, Goosen MFA and Arhewoh IM. Formulation and *in vitro* release studies on chitosan-alginate microcapsules modified for fish vaccine delivery. Bull. Anim. Hlth. Prod. Afr. 2008: 56: 98-108

11. USP-NF. Product Information: General Information-Nutritional Supplements. United States Pharmacopoeial Convention, Rockville, USA, 2005.p 2663.

12. Arhewoh MI and Okhamafe AO. Spectrophotometric Validation of Assay Method for Selected Medicinal Plant Extracts. Int. J. Phytopharmacy. 2014: 4(4): 109-112, DOI:10.7439/ijpp

13. Nair VDP, Kanfer I. Development of dissolution tests for the quality control of complementary/alternate and traditional medicines: Application to African potato products. J. Pharm. Pharm. Sci. 2008: 11(3): 35-44.

14. Lu DR, Abu-Izza K and Mao F. Non-linear data fitting for controlled release devices: an integrated computer program. Internat. J. Pharm. 1996: 129: 243-251

15. Goosen MFA. Experimental and modelling studies of mass transfer in micro encapsulated cell systems, Trop. J. Pharm. Res. 2002: 1(1): 3-14.

16. Ahonkhai EI, Arhewoh IM, Okhamafe AO. Effect of solvent type and drying method on protein retention in chitosan-alginate microcapsules. Trop. J. Pharm. Res. 2006: 3(2):583-588.

17. Adesanya SA, Sofowora A. Phytochemical investigation of plants used in traditional medicine. Hostettman K, (Edn). Oxford UK; OUP. 1994.p.1-98

18. Pillay V, Fassihi R. *In vitro* release modulation from cross-linked pellets for site-specific drug delivery to the gastrointestinal tract. II. Physicochemical characterization of calcium-alginate, calcium-pectinate, calcium alginate-pectinate pellets, J. Control. Rel. 1999: 59: 234-256.