Microsphere formulations of ambroxol hydrochloride: influence of Okra (*Abelmoschus esculentus*) mucilage as a sustained release polymer

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Abstract

Ambroxol hydrochloride (AH), a secretion-releasing expectorant, is a good candidate for sustained delivery. Mucilages are biodegradable, inexpensive carriers in microsphere formulations. The study aimed to prepare microspheres of AH using Okra mucilage obtained from pods of *Abelmoschus esculentus* combined with sodium alginate at various polymer/drug ratios. Okra mucilage was characterized for morphology, swelling, viscosity and flow properties. AH microspheres were prepared by ionic emulsification method and characterized using size, entrapment efficiency, swelling index and dissolution time (t_{50}). A full 2 by 3 factorial experimental design using three factors (Okra mucilage/alginate ratio X_1 ; drug/polymer ratio X_2 ; and polymer concentration X_3), each at two levels, was used to determine the effects of formulation variables on the responses. Optimized formulations of AH microspheres had sizes ranging from 250.91 ± 16.22 to 462.10 ± 23.85 μm ; swelling index 1.35 ± 0.05 and 3.20 ± 0.03 and entrapment 55.70 ± 3.55 – $94.11 \pm 4.50\%$. The microspheres exhibited sustained release of AH over a prolonged period as revealed by the dissolution time (t_{50}) 2.85 ± 1.03 – 7.50 ± 0.96 h. Drug release kinetics generally followed zero order, implying that the process is constant and independent of the initial concentration of drug. Polymer concentration had the highest influence on microsphere size, entrapment efficiency and dissolution time while Okra/alginate ratio had the highest influence on swelling. Okra mucilage was a suitable polymer that could serve as an alternative to synthetic polymers in sustaining the release of ambroxol hydrochloride.

Keywords Ambroxol hydrochloride · Factorial design · Microspheres · Okra mucilage · Sodium alginate · Sustained release

Introduction

The use of microspheres as carriers as an approach for the controlled delivery of drugs to target sites has become increasingly popular (Odeku et al. 2014; Zheng et al. 2017). Microspheres are free-flowing, spherical particles of size range 1–1000 μm , consisting of natural or synthetic polymers (Jyothi 2010). Their small size and efficient carrying capacity make them valuable controlled delivery devices and their drug release behavior is often related to the nature of polymers used and their

concentrations. Natural polymers have the advantages of being non-toxic, degradable in biological fluids as a result of natural biological processes and can be injected, implanted or inserted into the body without requiring surgical removal of the polymer skeleton. Thus, they are preferred to synthetic polymers for use as coating materials in microsphere formulations. Mucilages are products of metabolism (physiological products) formed within cells. They are examples of natural polymers that are biocompatible, relatively cheap, and readily available. Okra mucilage obtained from the fresh fruits of *Abelmoschus esculentus* (family Malvaceae) has been evaluated for its safety as well as its suitability as excipients in several formulations for sustained release (Bakre and Jaiyeoba 2009; Ilango et al. 2010; Sharma et al. 2013). Furthermore, the mucilage has been investigated as a polymer for formulating gastric floating dosage systems in which better sustained release was imparted in comparison to those containing hydroxypropyl methyl cellulose and HPMC (Chodavarapu

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et al. 2011). Okra is an annual, high yielding crop with the global commercial production estimated at 4.8 million tons in 2011; India and Nigeria reported as the major producers (Gulsen et al. 2007). However, like many perishable vegetables, Okra pods have a high percentage of post-harvest loss annually (Dhalil et al. 2014). This wastage can be minimized by industrial application of the crops and the mucilage offers such an opportunity.

Ambroxol hydrochloride, a secretion-releasing expectorant, is a drug with short biological half-life (3–4 h) hence requiring frequent daily dosing; three to four times (Beeh et al. 2008). The drug is, therefore, a candidate for controlled delivery. The aim of this research is to extract mucilage from Okra (*Abelmoschus esculentus*) pods using solvent extraction, characterize the mucilage and utilize it as a polymer with sodium alginate in the formulation of microspheres of ambroxol hydrochloride using the ionic emulsification method. The effects of formulation variables on the properties of the ambroxol hydrochloride microspheres were determined using a 2³ factorial design.

Materials

The materials used included ethanol (Sigma-Aldrich GmbH, Germany); ambroxol hydrochloride (Xi'an Sgonek Biological Technology Co. Ltd, Xi'an City, China); sodium alginate (S.D. Fine Chem, Mumbai, India); petroleum ether, heavy liquid paraffin and calcium chloride (BDH Chemicals Ltd., Poole, England). Okra (*Abelmoschus esculentus*, Malvaceae) pods were purchased from traders in a local market in Ibadan, Nigeria.

Methods

Extraction of Okra mucilage

A 400 g sample of powdered Okra was dispersed in 2 L of distilled water and kept for 24 h at ambient temperature. The mucilage was then extracted with 1.5 L of ethanol 96% after clarification. The mucilage obtained was filtered and dried at 50 ± 2 °C for 24 h. The dried mucilage was blended into powder and screened through a 125-µm sieve.

Phytochemical constituents of Okra mucilage

Tests for tannins, flavonoids, saponins, alkaloids and anthraquinones were conducted to confirm the suitability of Okra mucilage as an excipient devoid of pharmacological activity.

Test for tannins

One gram of dried powder sample of the mucilage was boiled in 40 mL of water inside a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration.

Test for flavonoids

Exactly 0.5 g of the sample with 10 mL of ethyl acetate was heated over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia. A yellow coloration indicates a positive test for flavonoids.

Test for saponin

One gram of each powdered sample was boiled in 10 mL of distilled water in a water bath and filtered. A stable persistent froth was obtained from the filtrate by vigorously shaking a mixture of 5 mL filtrate with 2–3 mL of distilled water. The frothing was mixed with 1–2 drops of olive oil and shaken vigorously. It was then observed for the formation of emulsions.

Test for alkaloids

One gram of sample was mixed in 8 mL of 1% HCl, warmed and filtered. 2 mL of the filtrate was treated separately with Mayer's and Dragendorff's reagents. The presence of alkaloids was determined from the turbidity or precipitate developed.

Test for anthraquinone

One gram of the sample was boiled with 10 mL of sulfuric acid (H₂SO₄) and filtered while hot. Chloroform (5 mL) was mixed with the filtrate and shaken intimately. The chloroform layer was then transferred using a pipette into another test tube and dilute ammonia (1 mL) was added. The resulting solution was observed for color changes.

Preparation of ambroxol hydrochloride microspheres

Preformulation studies

Several microsphere trial formulations were prepared by varying the ratio of the Okra mucilage to sodium alginate, ratio of total polymer to drug, polymer concentration,

curing times, concentration of dispersion agent and stirring speeds. The batches of formulations selected were prepared as described below:

Preparation of microspheres through ionic emulsification method

The blend of Okra mucilage gel and sodium alginate gel in water was prepared at a polymer concentration of 2% (w/v) in which the ratio of Okra/alginate gum was varied as 1:1 and 2:1. An appropriate quantity of AH (1.0 g and 0.5 g, respectively) was added and mixed for 30 min to obtain homogeneous polymer–drug dispersions of polymer:drug ratio 2:1 and 4:1, respectively. The polymer dispersion was added in a thin stream to 50 mL of heavy liquid paraffin contained in a 250-mL beaker, while stirring at 500 rpm to emulsify the dispersion as fine droplets. A 20 mL of calcium chloride solution (10% w/v) was transferred into the emulsion while stirring at 400 rpm for 10–15 min to produce spherical microspheres. The microspheres were collected by decanting and washing repeatedly (four times) with petroleum ether to remove the liquid paraffin. The product was then washed again with distilled water and air-dried to obtain discrete microspheres. The total polymer concentration was increased to 3% (w/v) and the above procedure was repeated.

Characterization

pH determination

The pH determination of 1% (w/v) Okra mucilage was carried out using the pH/meter (Thermo Electron Corporation, MA, USA) at 25 °C.

Swelling index

An amount of 5 g of Okra mucilage powder was measured and transferred into a 100-mL measuring cylinder while noting the volume occupied as V_1 . Distilled water (90 mL) was added and after shaking the dispersion for 2 min, more distilled water was added to reach the 100-mL mark. After 24 h, the sedimentation volume (V_2) was recorded while swelling index was obtained from calculating V_2/V_1 .

Microspheres were poured into a 10-mL measuring cylinder up to the 1 mL mark. The microsphere bed was soaked in 5 mL phosphate buffer (pH 6.8) for 12 h and swelling index was calculated as the ratio of the volume after 12 h to that of the original volume.

Density measurements

To determine the bulk density of Okra mucilage powder, 10 g of the powder was poured, through a funnel at an angle of 45°, into a 50-mL measuring cylinder. The bulk density was determined as the ratio of mass to volume occupied by the Okra powder. The tapped density was determined by applying 100 taps to the 10 g of the Okra powder sample in a graduated cylinder. The particle density of the dried mucilage (powder) was determined by a pycnometer using xylene as non-solvent (Okunlola and Ghomorai 2017).

Determination of flow properties

The flowability of the mucilage powder was determined by calculating the Hausner's ratio (Eq. 1) and Carr's index (Eq. 2) (Carr 1965; Hausner 1967; Okunlola and Ghomorai 2017):

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk Density}}, \quad (1)$$

$$\text{Carr's index} = \frac{(\text{Tapped density} - \text{Bulk Density}) \times 100}{\text{Tapped density}}. \quad (2)$$

To determine the angle of repose, an open-ended glass cylinder was placed on a base of similar diameter. Okra mucilage powder (5 g) was allowed to flow freely through a funnel under gravity, to form a conical heap. The angle of repose was calculated from the following equation:

$$\tan \theta = h/r, \quad (3)$$

where h is the height of the cone and r stands for radius of the cylinder.

Determination of viscosity

The viscosity of 1 and 2% (w/v) aqueous slurry of Okra mucilage was determined at 50 and 100 rpm using Brookfield rheometer (Brookfield Engineering, USA) using CPE40. Spindle no 2.

Morphology

The morphology of the Okra mucilage powder and microspheres was observed using a scanning electron microscope (Hitachi, Tokyo, Japan) at 5.0 kV accelerating potential.

Fourier transform infra-red (FTIR) analysis

The Okra mucilage powder was analyzed by FTIR (Perkin Elmer, Waltham, MA, USA) in transmission mode. Transmission spectra were obtained using 64 scans with 8 cm^{-1} resolution in the spectral range $4000\text{--}400\text{ cm}^{-1}$.

X-ray diffractometry

The XRD pattern of pristine drug, alginate, Okra mucilage powder and drug-loaded microspheres was obtained using an X-ray diffractometer (Thermo Fisher Scientific, Landsmeer, The Netherlands) with copper–cobalt radiation. The XRD patterns were obtained over a scanning region from 5° to 70° of the diffraction angle (2^θ) and at a scan rate of $12^\circ/\text{min}$.

Differential scanning calorimetry (DSC)

The DSC of the pristine drug, alginate, Okra mucilage powder and drug-loaded microspheres was determined using a differential scanning calorimeter (Mettler Toledo, Columbus OH, USA). The sample (about 5 mg on a dry weight basis) was heated from 60 to 300°C in sealed aluminum pans at a scanning rate of $10^\circ\text{C}/\text{min}$.

Drug loading and entrapment efficiency

The ambroxol hydrochloride microspheres (100 mg) were crushed and suspended in 100 mL of phosphate buffer, pH 6.8, for 24 h. The resulting solution was filtered and the filtrate was analyzed using a UV/VIS spectrophotometer at wavelength of 244 nm.

Drug loading was computed by

$$\text{Drug loading (\%)} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}} \times 100. \quad (4)$$

Entrapment efficiency (E) was calculated by

$$E (\%) = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100, \quad (5)$$

where theoretical drug content refers to the amount of drug in 100 mg microspheres based on the total amount of drug added to each batch formulation.

Drug release study

To study drug release, dissolution test was carried out using the basket method (USP XXXVI), in 900 mL of phosphate buffer, pH 6.8 (pH of the small intestine),

rotated at 50 rpm and temperature of $37 \pm 0.5^\circ\text{C}$. Aliquots of 10 mL were withdrawn at predetermined time intervals for analysis of AH release. Sink condition was maintained by replacing samples with equal amounts of fresh medium. The amount of AH released was determined at λ_{max} of 244 nm, using a UV spectrophotometer (Spectrum lab 752s, Changsha Hunan, China).

Kinetic models of drug release

Drug release data obtained from the formulations were fitted to various kinetic equations including: zero order, first order, Higuchi, Korsmeyer–Peppas and Hopfenberg (Higuchi 1961; Hopfenberg 1976; Korsmeyer et al. 1983). The coefficient of determination (r^2) values obtained from different equations was computed and the model of best fit was identified by comparing the values.

Experimental design

Prior to the use of 2^3 factorial design, some preliminary trials of microsphere formulations were conducted to establish the important control factors and their levels. Three factors, the Okra/alginate ratio (X_1), polymer/drug ratio (X_2) and polymer concentration (X_3), were selected at two different levels each and experimental trials were performed at all possible eight combinations in a fully randomized order. The microbead entrapment efficiency, microsphere size and swelling as well as the time taken for 50% drug release (t_{50}) were selected as dependent variables. The main effects of these factors (X_1 , X_2 and X_3) represent the average result of changing one factor at a time from its lowest to highest values. The graphs of three-dimensional response surface plots were depicted to study the effect of the response variables. The data obtained were subjected to regression analysis using Minitab 16 statistic software (Minitab, USA).

Table 1 Phytochemical constituents of Okra mucilage

Parameter	Results
Tannins	–ve
Flavonoids	–ve
Saponin	+ve
Alkaloids	–ve
Anthraquinones	–ve

Results and discussion

Characterization of Okra mucilage powder

The yield of mucilage from Okra pods was 4.85% (w/w) on a dry weight basis. This was consistent with those reported earlier (Emeje et al. 2011; Gemedet et al. 2018). The extraction yield of Okra mucilage is a function of environmental factors and varies with factors such as climatic condition and crop age (Emeje et al. 2011). The results of phytochemical analysis of the extracted mucilage are presented in Table 1. The phytochemical constituents determine the therapeutic activities of plant species. The results showed the absence of tannins, flavonoids, alkaloids and anthraquinones. However, saponin was present in the mucilage in small amount. This may explain the folklore use of the mucilage as an anti-inflammatory agent (Lim 2012).

The material properties of Okra mucilage are presented in Table 2. These include values of size, pH, swelling index, density measurement, Carr's index, Hausner's ratio and angle of repose. The scanning electron micrograph showing the shape of the particles is presented in Fig. 1a. The particles of Okra mucilage powder were irregular with non-distinct shape and a mean size of 0.23 mm. Particle size and shape have been known to affect the surface properties of polymers used as carriers in pharmaceutical applications. The pH of the mucilage is near neutral and would minimize irritation to the gastro-intestinal tract (git), making it suitable as a polymer carrier (Malviya et al. 2011). Okra mucilage had a high swelling index in water suggesting its usefulness as a matrix agent since swelling is a major mechanism in diffusion-controlled release systems (Emeje et al. 2008). The swelling index of polymers is of significance in formulations such as microspheres because the degrading properties of polymer carrier can be influenced by their swelling and eroding action (Akin-Ajani et al. 2014).

Particle density is a major factor responsible for the difference in packing behaviour of materials during handling and processing of formulations (Riley and Adebayo 2010). The Hausner's ratio and Carr's index were derived from the bulk and tapped densities of the mucilage. A Hausner ratio of less than 1.25 is indicative of good flowability while values of 1.5 or higher is indicative of poor

flowability (Hausner 1967). The results showed that the Okra mucilage powder had good flowability and this was further confirmed by the value of Carr's index. Generally, Carr's index up to 16% indicates good flow behaviour, while its above 25% indicate cohesive or poor flow behavior (Carr 1965). The angle of repose of Okra powder; however, indicated fair flow being $> 25^\circ$ but $< 40^\circ$. This physical property is dependent on particle size and surface properties of individual particles. Due to the high dependence of angle of repose measurements on testing conditions, angle of repose is not a very robust means of quantifying powder flow (Amidon et al. 2017).

The Fourier transform infra-red (FTIR) spectrum of the mucilage powder is shown in Fig. 1b, while the X-ray diffraction (XRD) spectrum of Okra mucilage powder and the differential scanning calorimetry (DSC) endotherm of Okra mucilage powder are shown in Fig. 1c, d, respectively. The finger print region of the FTIR spectrum of Okra mucilage showed characteristic peaks between 894.30 and 1426.58 cm^{-1} which are attributed to the C–O bending. The band at 1645.66 cm^{-1} was assigned to the O–H bending of water. The carbonyl stretches in the 1732.12 cm^{-1} region indicates the presence of ester linkages (Mano et al. 2014). A sharp band is observed at 2937.4 cm^{-1} and this is due to the methyl C–H stretching. The broad band at 3458 cm^{-1} is due to the hydrogen-bonded hydroxyl. This spectrum confirms the polysaccharide nature of the mucilage. The XRD spectrum reveals the nature of Okra mucilage to be more amorphous than crystalline (Zaharuddin et al. 2014). The DSC endotherm of Okra mucilage also confirmed Okra mucilage to be a mixture of amorphous and crystalline structures.

The viscosity values of 1 and 2% (w/v) slurries of Okra mucilage are presented in Table 3. The viscosity values give a measure of material's resistance to flow. Okra mucilage gave significantly higher viscosity values at higher concentration, producing a thicker mass. This informed the choice of the concentration of the polymer used in the microsphere formulations. The gum with a higher viscosity would create a more dense material with heavier cross linkage of molecules, producing formulations with slower drug release (Kalu et al. 2007). It was observed that as the speed increased from 20 to 50 rpm, the viscosity of the mucilage reduced.

Table 2 Morphological and material properties of Okra mucilage powder

Particle size mm	Particle shape	pH	Swelling index	Particle density (gcm^{-3})	Bulk density (gcm^{-3})	Tapped density (gcm^{-3})	Carr's index (%)	Hausner's ratio	Angle of repose ($^\circ$)
0.23 ± 0.19	Irregular	6.48 ± 1.10	13.00 ± 1.10	1.415 ± 0.064	0.508 ± 0.07	0.594 ± 0.04	14.65 ± 4.99	1.17 ± 0.07	37.00 ± 0.06

Fig. 1 **a** Scanning electron micrographs (SEM) for Okra mucilage, **b** Fourier transform infra-red (FTIR) spectrum for Okra mucilage, **c** X-ray diffraction (XRD) spectrum for Okra mucilage, **d** differential scanning calorimetry (DSC) endotherm for Okra mucilage

Table 3 Viscosity of 1 and 2% (w/v) aqueous slurries of Okra mucilage

Sample conc % (w/v)	Viscosity cps		
	20 rpm	30 rpm	50 rpm
1.0	240	142	80
2.0	3395	2593	1884

Characterization of ambroxol hydrochloride microspheres

The ionic emulsification method was used to prepare ambroxol hydrochloride microspheres utilizing Okra mucilage–alginate blend as polymers. Okra mucilage is a polysaccharide that is an anionic natural macromolecule. The interaction between drug and polymer may be hydrophilic or hydrophobic and may result in the drug release and

Fig. 2 Scanning electron micrographs (SEM) of ambroxol hydrochloride microsphere mg $\times 300$

entrapment from micro-spherical systems (Crotto and Park 1997; Park et al. 1998). The size of the microspheres, the entrapment and release characteristics of drugs are influenced by microsphere production method (Martins et al. 2014). In the ionic emulsification method, low shear was used on adding the chelating agent (the solution of calcium chloride) to protect the initially fragile microspheres until they hardened on curing. Another advantage of this method was that there is a potential for scale-up to large-scale production using available large-scale equipment (Poncelet et al. 1992).

The SEM of the AH microspheres are shown in Fig. 2 while the results of the size, swelling and entrapment efficiency of AH microbeads are presented in Table 4. The scanning electron micrographs of ambroxol hydrochloride microspheres revealed they were discrete and approximately spherical particles. The mean diameter of the microspheres was within the range of 250.91 ± 16.22 – 462.10 ± 3.85 μm . Furthermore, increase in the concentration of Okra mucilage in the formulations led to a corresponding increase in the size of the microspheres produced. Higher concentrations of the Okra mucilage produced more viscous polymer solutions which required more energy to break into smaller droplets, thus resulting in the production of larger sized microspheres. The drug content increased with increase in the Okra/alginate ratio, suggesting that the Okra mucilage enhanced drug entrapment. The increased amount of Okra mucilage had higher

Table 4 Coded and real values of variables, predicted and obtained responses for ambroxol hydrochloride microsphere formulations

Batch code	Coded levels			Real values		Predicted particle size (μm)	Obtained particle size (μm)	Predicted swelling (v/v)	Obtained swelling (v/v)	Predicted entrapment (%)	Obtained entrapment (%)	Predicted t_{50} (h)	Obtained t_{50} (h)
	X_1	X_2	X_3	X_1 Okra/alginate	X_2 Polymer/drug	X_3 Polymer conc (%) w/v							
B ₁	–1	–1	–1	1:1	2:1	2	239.41	250.91 $\pm$ 16.22	1.41	55.70	55.70 $\pm$ 3.55	2.94	2.85 $\pm$ 1.03
B ₂	–1	+1	–1	1:1	4:1	2	309.48	297.98 $\pm$ 20.15	2.38	75.50	75.50 $\pm$ 1.36	4.01	4.10 $\pm$ 1.30
B ₃	–1	–1	+1	1:1	2:1	3	366.09	354.59 $\pm$ 19.67	2.36	79.80	79.75 $\pm$ 2.45	4.63	4.55 $\pm$ 1.18
B ₄	–1	+1	+1	1:1	4:1	3	362.74	374.24 $\pm$ 24.73	2.26	88.30	88.28 $\pm$ 4.05	4.89	4.80 $\pm$ 0.36
B ₅	+1	+1	–1	2:1	4:1	2	409.55	421.05 $\pm$ 14.25	3.21	83.71	83.70 $\pm$ 6.70	5.09	5.00 $\pm$ 0.50
B ₆	+1	–1	–1	2:1	2:1	2	291.60	280.10 $\pm$ 15.33	2.44	67.80	67.80 $\pm$ 2.58	3.31	3.40 $\pm$ 1.40
B ₇	+1	–1	+1	2:1	2:1	3	429.05	440.55 $\pm$ 13.85	3.28	89.45	89.45 $\pm$ 1.50	6.29	6.20 $\pm$ 0.45
B ₈	+1	+1	+1	2:1	4:1	3	473.60	462.10 $\pm$ 23.85	3.14	94.10	94.11 $\pm$ 4.50	8.49	7.41 $\pm$ 0.96

Fig. 3 X-ray diffraction (XRD) spectra of a Okra mucilage, b sodium alginate, c ambroxol hydrochloride and d ambroxol hydrochloride microspheres

degree of viscosity which created a more dense material with heavier cross linkage of molecules that enabled the system to entrap the drug more efficiently and produce microspheres with better release-retarding effects (Zaharuddin et al. 2014).

The XRD and DSC spectra of Okra mucilage, sodium alginate, pristine drug and ambroxol hydrochloride microspheres containing the Okra mucilage are shown in Figs. 3 and 4, respectively. X-ray powder diffractometry was carried out to investigate the effect of microencapsulation process on crystallinity of drug. Characteristic crystalline peaks of ambroxol hydrochloride were absent in the XRD of ambroxol hydrochloride-loaded microspheres, suggesting that the drug became amorphous when formulated in the microspheres. The absence of detectable crystalline domains in the XRD spectrum of the drug-loaded microspheres also suggested that ambroxol hydrochloride was dispersed completely in the formulation as the microspheres must have

been modified to an amorphous, disordered form (Gavini et al. 2006).

The dissolution profiles of the microbeads are presented in Fig. 5. From the plots, the values of dissolution time t_{50} (i.e., time required for 50% drug release) were determined and are also presented in Table 4. In vitro dissolution test of ambroxol hydrochloride from the Okra mucilage-based microspheres were carried out in phosphate buffer media, pH 6.8, to simulate the physiological conditions in the small intestine, being the site that was targeted for drug release. According to Stetinová et al. (2009), the high solubility and high permeability of ambroxol hydrochloride rank it among well-absorbed compounds and class I of BCS, Therefore, the oral dose fraction of ambroxol absorbed in human intestine is expected to be high. Figure 5 shows cumulative percentages of ambroxol released against time. The narrow error bars indicate good reproducibility. Drug release from all formulations showed a biphasic release pattern. Phase I

Fig. 4 Differential scanning calorimetry (DSC) endotherms of a Okra mucilage, b sodium alginate, c ambroxol hydrochloride and d ambroxol hydrochloride microspheres

Fig. 5 Dissolution profile of the batches of the ambroxol hydrochloride microspheres (batches B₁–B₈), $n = 3$

represents the initial burst release due to drugs that are freely and weakly bound on the particle surface. Phase I is followed by Phase II, a second slower drug release phase for all the formulations. Phase II is predominately governed by drug diffusion, either through water-filled pores or polymer. Drug release ranged from 2.85 to 7.5 h. The drug release from the microspheres was simulated with different kinetic models (zero order, Higuchi, Hopfenberg, Korsmeyer-Peppas (Higuchi 1961; Hopfenberg 1976; Korsmeyer et al. 1983)). The values of the kinetic release parameters and regression coefficients are presented in Table 5 while the plots of selected models are presented in Fig. 6. The kinetics of drug release is important due to their influence on drug bioavailability. The physicochemical properties of the drug and the polymer have been shown to govern the release of drug from formulations which could affect their release kinetics. Drug release from formulations B₃, B₄ and B₇ generally fitted the zero-order model implying that the process is constant and independent of the initial concentration of the drug in the delivery system. On the other hand, Formulations B₁ and B₅ fitted the Hopfenberg kinetic model, which describes the release of drug from spherical formulations and is used

Table 5 Kinetic release parameters and regression coefficients obtained by different kinetic models ($n=3$)

Batch	Zero order k/R^2	Higuchi k/R^2	Hopfenberg k/R^2	Korsmeyer–Peppas	
				R^2	n
B ₁	11.056/0.9396	18.697/0.9285	0.266/*0.9579	0.8232	0.8556
B ₂	9.577/0.9740	26.170/0.9237	0.234/0.9650	*0.9822	1.1638
B ₃	9.237/*0.9819	16.971/0.9369	0.229/0.9715	0.9429	0.6663
B ₄	9.446/*0.9852	23.216/0.9801	0.239/0.9705	0.9540	0.5103
B ₅	9.145/0.9874	15.484/0.9430	0.236/*0.9951	0.9438	0.6461
B ₆	9.903/0.9640	21.357/0.9812	0.240/0.9509	0.9501	0.5562
B ₇	7.662/*0.9736	25.655/0.9492	0.194/0.9220	0.9637	0.7991
B ₈	6.429/0.9586	29.657/0.9745	0.165/0.9014	*0.9966	0.7696

*Highest coefficient of determination for batch

to correlate the drug release from surface-eroding polymer so long as the surface remains constant during degradation process (Hopfenberg 1976). Only formulation B₆ fitted the Higuchi model. This model explains the diffusion-controlled release mechanism (Higuchi 1961). Formulations B₂ and B₈ fitted the Korsmeyer–Peppas model. The Korsmeyer–Peppas model relates drug release from a polymeric system with the mechanism of release in which the value of n characterizes the release mechanism of the drug. Since $n > 0.89$ for Batch B₂, the release corresponds to a super case II transport. Formulation B₈ had n value < 0.89 implying non-Fickian (anomalous) drug release. Of all the formulations prepared, B₈ with higher Okra mucilage content (2:1), higher polymer/drug (4:1) and higher concentration of polymer (3% w/v) had the lowest release rate constant value, with the most prolonged dissolution time ($t_{50} = 7.41 \pm 0.96$ h).

Experimental design

The 2³ full factorial experimental design was used to provide the quantitative effects of three selected parameters, viz., Okra mucilage/alginate ratio (X_1), polymer/drug ratio (X_2) and polymer concentration (X_3), chosen as independent variables, on the responses: particle size, swelling, entrapment efficiency and time for 50% drug release (t_{50}) (as dependent variables). The summary of the individual and interaction coefficients of the variables on responses is presented in Table 6. The main effects (X_1 , X_2 and X_3) represent the average result of changing one factor at a time from its low (denoted as -1) to high (denoted as $+1$) value. The interaction terms (X_1X_2 , X_1X_3 , X_2X_3) show the effects of simultaneously changing the three factors on the responses. The analysis determined the predicted responses of the formulations in comparison to the actual responses obtained. The analysis of variance (ANOVA) showing the level of significance of the influence of the independent variables on responses is presented in Table 7.

The influence of Okra mucilage/alginate ratio (X_1) on the size of microsphere was positive, indicating that a change

in ratio from 1:1 to 2:1 increased the sizes of microspheres. Similarly, the coefficients of polymer/drug ratio (X_2) and polymer concentration (X_3) on the size of microspheres were positive, showing that as the values of these factors are increased from low to high, there was a corresponding increase in the sizes of microspheres. The coefficients of the microsphere size were in the rank order: $X_3 > X_1 > X_2$ showing that polymer concentration (X_3) had the most influence on the size of the formulated microspheres. The significance of its effect on size was confirmed by the results of ANOVA analysis in Table 7, where $p = 0.018$. As the polymer concentration increased, there was increase in the viscosity of the polymer gel which made it difficult to break down into smaller droplets, thus resulting in microspheres with larger sizes. This increase in size caused a decrease in the surface area of the microspheres and reduced their exposure to water. Drug loss due to diffusion from the gel layer also decreased (Saravanan and Anupama 2011). Thus, for a given rate of drug diffusion through the microsphere, the rate of flux of drug out of the microsphere, per mass of formulation, increased with decreasing particle size (Okunlola and Owajori 2016). The magnitude of the interaction coefficients for microsphere size were in the rank order of $X_2X_3 > X_1X_2 > X_1X_3$. This indicated that interactions between polymer/drug ratio and polymer concentration (X_2X_3) had the most profound influence on microsphere size. The magnitude of the interaction coefficient (X_2X_3) was negative indicating that both variables interacted to produce microspheres of reduced size.

The positive coefficient values of X_1 , X_2 and X_3 on swelling showed that changing the ratio of Okra mucilage/alginate from 1:1 to 2:1, increasing the polymer/drug from 2:1 to 4:1 and increasing polymer concentration from 2 to 3% (w/v) increased swelling. The ranking of the coefficients on swelling was in the order: $X_1 > X_3 > X_2$ implying that Okra mucilage/alginate ratio had the most profound influence on the swelling. However, ANOVA analysis in Table 7 revealed that the effects of all variables were not significant ($p > 0.05$). The ranking of the values of the interaction

Fig. 6 Plots of kinetic models of the batches of ambroxol hydrochloride microspheres: **a** Zero order, **b** Higuchi, **c** Hopfenberg and **d** Korsmeyer-Peppas

coefficients on swelling was $X_2X_3 > X_1X_3 > X_1X_2$. The results indicated that interaction between polymer/drug and polymer concentration (X_2X_3) had the most influence on swelling

of microspheres. The results of ANOVA analysis showed the interactive effects of the factors on both size and swelling were not significant.

Table 6 Summary of individual and interaction coefficients of the variables on particle size, swelling, entrapment and dissolution time (t_{50}) of ambroxol hydrochloride microspheres

Factor	Coefficient	Particle size (μm)	Swelling	Entrapment (%)	t_{50} (h)
X_1	Effect	81.52	0.95	8.96	1.45
	p value	0.03	0.02	0.04	0.03
X_2	Effect	57.30	0.34	12.22	1.10
	p value	0.08	0.23	0.02	0.07
X_3	Effect	95.36	0.44	17.22	1.93
	p value	0.02	0.18	0.00	0.01
$X_1 X_2$	Effect	11.97	−0.01	−0.97	0.78
	p value	0.49	0.87	0.00	0.29
$X_1 X_3$	Effect	2.70	−0.03	−0.60	0.36
	p value	0.85	0.76	0.00	0.15
$X_2 X_3$	Effect	−18.36	−0.23	−2.81	−0.16
	p value	0.36	0.17	0.00	0.31

Entrapment efficiency of the microsphere formulations varied from 55.70 ± 3.55 to $94.11 \pm 4.50\%$. The coefficient values for X_1 , X_2 and X_3 were positive indicating that the three factors had a significant influence on entrapment efficiency. The ranking of the coefficients on entrapment was in the order: $X_3 > X_2 > X_1$ suggesting that entrapment of AH in the microspheres was mostly influenced by polymer concentration. The ANOVA analysis showed the level of significance to be significant at $p = 0.005$. The ranking of interaction coefficients on entrapment was $X_2 X_3 > X_1 X_2 > X_1 X_3$. The interaction between drug/polymer and polymer concentration had the most influence with a level of significance of $p = 0.001$.

In the in vitro dissolution studies of the release of ambroxol hydrochloride from the Okra mucilage-based microspheres, the time for 50% drug release (t_{50}) ranged from 2.85 to 7.5 h. The coefficient values of X_1 , X_2 and X_3 on dissolution time (t_{50}) were positive. This indicates that increasing the three factors resulted in the production of microspheres with longer dissolution time, i.e., sustained release. The ranking of the coefficients on t_{50} was in the order $X_3 > X_1 > X_2$ indicating that polymer concentration also had the most significant influence on the time for the release of ambroxol hydrochloride from the microspheres ($p = 0.031$). Formulations containing higher concentration of polymer showed smaller percentage of cumulative release due to slower ingress of dissolution medium into the microspheres. With increase in polymer concentration, there was an increase in viscosity of the polymer gel which became more resistant to dilution and erosion. This could have resulted in increase in diffusional path length, decrease in the diffusion coefficient of the drug and reduction in the release rate of the drug (Sinha and Rohera 2002; Akbari et al. 2011). The interaction coefficients for t_{50} were in the rank order of $X_1 X_2 > X_1 X_3 > X_2 X_3$ showing

that Okra mucilage/alginate ratio and drug: polymer ratio ($X_1 X_2$) interacted synergistically to extend the time of release of AH from microspheres.

The effect of X_1 was statistically significant ($p < 0.05$) for all the criteria. On the other hand, the influence of X_2 was statistically significant for entrapment only while influence of X_3 was significant for all the criteria except swelling. The largest particle size, highest swelling and entrapment and most prolonged dissolution were obtained from formulation B₈ in which all three factors had been used at the highest level. On the other hand, the least responses resulted from formulation B₁ in all factors were used at the lowest level. Therefore, formulation (B₈) was chosen as the best formulation, while the values of independent variables for this formulation were considered optimum for the preparation of ambroxol microspheres containing Okra mucilage.

The response surface plots in Fig. 7 represent the interaction patterns between the variables X_2 and X_3 and their influence on the responses. From the plots of Figs. 7a–d, as polymer/drug ratio and polymer concentration increased, the microsphere size, swelling, entrapment efficiency, dissolution time t_{80} , respectively, increased.

Conclusion

Okra mucilage obtained from *Abelmoschus esculentus* was successfully used in the formulation of ambroxol hydrochloride microspheres using ionic emulsification method at varying ratios with sodium alginate, at different drug/polymer ratios and polymer concentrations. Polymer concentration was the variable that had the most significant influence on size, swelling and entrapment of AH microspheres while the ratio of Okra/alginate had the strongest influence on dissolution time. Optimized formulations with Okra/

Table 7 Analysis of variance (ANOVA) to determine the significance of the variables

Source	DF	Seq	Adj SS	Adj MS	F	p
Particle size						
Regression	3	38,045.8	38,045.8	12,681.9	10.2338	0.023934
X1	1	13,291	13,291	13,291	10.7253	0.030646
X2	1	6567.7	6567.7	6567.7	5.2999	0.082743
X3	1	18,187.1	18,187.1	18,187.1	14.6762	0.018603
Interaction	3	3899.3	3899.3	1299.78	1.23	0.566
$X_1 \times X_2$	1	1146.7	1146.7	1146.73	1.08	0.487
$X_1 \times X_3$	1	58.1	58.1	58.1	0.05	0.853
$X_2 \times X_3$	1	2694.5	2694.5	2694.51	2.55	0.356
Residual	Error	1	1057.5	1057.5	1057.54	
Total	7	43,002.7				
Swelling						
Regression	6	2.8441	2.8441	0.474017	15.17	0.194
Linear	3	2.41465	0.50797	0.169325	5.42	0.304
X1	1	1.805	0.14533	0.145329	4.65	0.276
X2	1	0.2312	0.33916	0.339161	10.85	0.188
X3	1	0.37845	0.40644	0.406445	13.01	0.172
Interaction	3	0.42945	0.42945	0.14315	4.58	0.328
$X_1 \times X_2$	1	0.00125	0.00125	0.00125	0.04	0.874
$X_1 \times X_3$	1	0.005	0.005	0.005	0.16	0.758
$X_2 \times X_3$	1	0.4232	0.4232	0.4232	13.54	0.169
Residual	Error	1	0.03125	0.03125	0.03125	
Total	7	2.87535				
Entrapment						
Regression	6	1126.21	1126.21	187.702	1,668,459	0.001
Linear	3	1052.48	192.83	64.275	571,336.6	0.001
X1	1	160.47	160.47	160.47	8.71	0.042
X2	1	298.78	298.78	298.78	16.21	0.016
X3	1	593.23	593.23	593.23	32.18	0.005
Interaction	3	73.73	73.73	24.576	218,454	0.002
$X_1 \times X_2$	1	7.55	7.55	7.547	67,081	0.002
$X_1 \times X_3$	1	2.84	2.84	2.844	25,281	0.004
$X_2 \times X_3$	1	63.34	63.34	63.338	563,000.1	0.001
Residual	Error	1	0	0	0	
Total	7	1126.21				
t_{50}						
Regression	6	15.5438	15.5438	2.59063	42.3	0.117
Linear	3	14.0363	0.9453	0.31511	5.14	0.311
X1	1	4.205	4.205	4.205	10.72	0.031
X2	1	2.42	2.42	2.42	6.17	0.068
X3	1	7.4112	7.4112	7.4112	18.9	0.012
Interaction	3	1.5075	1.5075	0.5025	8.2	0.250
$X_1 \times X_2$	1	0.245	0.245	0.245	4	0.295
$X_1 \times X_3$	1	1.0512	1.0512	1.05125	17.16	0.151
$X_2 \times X_3$	1	0.2113	0.2113	0.21125	3.45	0.314
Residual	Error	1	0.0612	0.0612	0.06125	
Total	7	15.605

Fig. 7 Response surface plots for influence of: X_2 (polymer/drug ratio) and X_3 (polymer concentration) on **a** size, **b** swelling, **c** entrapment, and **d** influence of X_1 and X_2 on t_{50}

alginate 2:1, polymer/drug ratio 4:1 and concentration of 3% (w/v) gave the highest swelling, entrapment and sustained drug release. The study revealed the usefulness of

Okra mucilage as a suitable and more affordable substitute to synthetic polymers in drug delivery.

Data/code availability

Minitab 16 Statistical Software.

Author contributions The conception and design of the study was carried out by AO, MAO and MIA. The analysis and interpretation of results were carried out by AO. Drafting of the manuscript was done by AO. Thorough reading and approval of final version of manuscript was done by AO, MIA and MAO.

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Compliance with ethical standards

Conflict of interest The authors hereby declare that there is no conflict of interest.

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