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COLOCASIA ESCULENTA CORMS MUCILAGE-ALGINATE MICROSPHERES OF OXCARBAZEPINE: DESIGN, OPTIMIZATION AND EVALUATION

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Abstract: The present investigation was undertaken with an objective of formulating sustained release microspheres of oxcarbazepine (OXC), an anti-epileptic drug, to overcome poor patient compliance and exposure to high doses associated with currently marketed OXC dosage forms. Ionic gelation technique was used to prepare OXC microspheres by using sodium alginate along with rate controlling polymer *Colocasia esculenta* mucilage (CEM) matrix as well coated form. The microspheres have been characterized by differential scanning calorimetry (DSC) for understanding thermal stability and Fourier transform infrared (FT-IR) spectroscopy to investigate the chemical interaction as well as to assess the structure of drug-loaded formulation. Surface morphology of the microspheres was investigated by scanning electron microscope (SEM). The size distribution of OXC microspheres as studied by optical microscopy was in the range of 394-575 µm. The microspheres exhibited encapsulating efficiency from 75 to 92%. The release of drug from the microspheres at pH 1.2 is negligible. Under neutral conditions, the microspheres were swell and release was attributed mainly to polymer relaxation. The release pattern from microspheres followed Korsmeyer-Peppas model and the value of $n > 1$ showed that drug released by anomalous (non-Fickian) diffusion. The data obtained thus suggest that a microparticulate system can be successfully designed by using CEM with alginate for sustained delivery of OXC.

Keywords: oxcarbazepine, microspheres, sodium alginate, *Colocasia esculenta* mucilage, Korsmeyer- Peppas model

Over the past few decades, a great deal of attention has been paid to the development of polysaccharide based microspheres through ionotropic gelation technique, which are useful as potential carriers in controlled drug delivery (1). The well known natural polymers: aloe mucilage, guar gum, sodium alginate, gelatin, linseed mucilage are applicable in different pharmaceutical dosage forms like matrix controlled systems, microspheres, nanoparticles, buccal films and viscous liquid formulations (2). In previous literature, a few investigations have been carried out on the development of microspheres using sodium alginate and other polymer blend (3). However, not a single report is available in the literature regarding the use of *Colocasia esculenta* corms mucilage as a material for the development of alginate based microspheres for controlled drug delivery.

Colocasia esculenta is an annual herbaceous succulent plant belonging to family Araceae, commonly known as arvi , widely cultivated in the tropical areas of the world (4). *Colocasia esculenta* corms contain a high percentage of mucilage and is already established as mucoadhesive polymer, emulsifying agent, binding agent for tablet dosage form as well as a suspending agent for suspension due to its flowability, weakly acidic pH, swelling potential and viscous nature (5, 6). Swelling property was confirming the suitability of mucilage as an excipient in novel drug delivery systems for controlled drug delivery (7).

Oxcarbazepine (OXC) is a newer anti seizure drug, released in the United States in 1999. Because of the short half-life of OXC (1 to 2 h), only trace amounts of the drug are detectable in human peripheral blood (8). OXC has been approved for partial and generalized tonic-clonic seizures in adults and children but may have a broader spectrum of use. It is currently under study for management of mood

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disorders, post-traumatic stress disorders, neuropathic pain, bipolar disorder and schizophrenia. Several significant adverse effects have been seen with OXC therapy. These include hyponatremia, secretion of antidiuretic hormone and hypersensitivity (9).

Hence, the objective of the present study is to develop sustain release OXC microspheres based on sodium alginate and *Colocasia esculenta* as drug release retardants which reduces the need for multiple dosing thereby increasing patient compliance and to treat epilepsy without any adverse effects.

EXPERIMENTAL

Chemicals

OXC was obtained as a gift from Novartis Pharmaceuticals Pvt. Ltd., Pakistan, sodium alginate was purchased from Sigma-Aldrich France, calcium chloride was from Merck, Germany. The fresh *Colocasia esculenta* corms were collected from main market in local government area of Punjab in Pakistan**.** All other chemicals and reagents used were of analytical grade.

Table 1. Physicochemical properties of *Colocasia esculenta* corms mucilage.

Parameter	Observation
Bulk density	0.714
Tapped density	0.833
Carr's index	14.28
Hausner's ratio	1.16
Angle of repose	18 ± 0.14°
pH of mucilage	6.5 ± 0.02
Loss on drying	6%
Viscosity	3.66 mPa
Mean particle size	78.66 ± 0.33 µm
Solubility	Swells in cold water, dissolves in hot water, insoluble in organic solvents

Extraction of mucilage from *Colocasia esculenta* corms

Fresh corms of C*olocasia esculenta* were collected from local market and washed with water. We removed the outer surface of the corms and cut them into small slices. These small slices were washed with water to clear the impurities. Small slices of same size were incorporated in the water for 4 h, boiled for 30 min and left to stand for 1 h to allow complete release of the mucilage into the water. The mucilage was extracted using a muslin cloth bag to remove the marc from the solution. Ethanol (in the volumes of three times to the volume of filtrate) was added to precipitate the mucilage. The mucilage was separated, dried in an oven at 40^{o}C, collected, ground, passed through a # 80 sieve and stored in an air tight container. Isolated mucilage was evaluated for organoleptic and physicochemical properties (5).

Drug-excipient compatibility studies

Fourier transform infrared (FT-IR) spectral analysis

The interactions between drug and rate controlling polymers were studied by FT-IR spectroscopy. Disks for FT-IR analysis were prepared by mixing drug/polymer/microspheres with KBr (2 mg sample in 200 mg KBr). The prepared disks were then examined in FT-IR spectroscope at the resolution of 2 cm^{-1} with scanning range of 4,000-400 cm^{-1} (10). Pure drug, polymers and drug loaded microspheres were subjected to this process and their spectra were recorded.

Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) was performed using a DSC-60 (Shimadzu-Kyoto, Japan) calorimeter to study the thermal behavior of

Table 2. Formulation composition for the preparation of OXC microspheres.

Formulations	Polymer Type	Drug (g)	ALG (g)	*CEM* (g)	$CaCl_2$ (g)	Distilled water (mL)
OXC-1	Alginate (Plain)	1	2	-	7	100
OXC-2		1	4	-	7	100
OXC-3	Alginate + *CEM* (Matrix)	1	2	2	7	100
OXC-4		1	4	2	7	100
OXC-5	Alginate + *CEM*	1	4	1	7	100
(OXC-6	Coated)	1	4	2	7	100

drug alone and mixtures of drug and polymer. The instrument was comprised of calorimeter (DSC-60), flow controller (FCL-60) thermal analyzer (TA-60) and operating software (TA-60). The samples were heated in sealed aluminium pans under nitrogen flow (10 mL/min) at a scanning rate of 5°C /min from 20 to 500°C. An empty aluminum pan was used as a reference. The heat flow as a function of temperature was measured for the drug and drug-polymer mixture (11).

Preparation of microspheres

Preparation of sodium alginate OXC microspheres

Microspheres of OXC were prepared by ionotropic gelation method. Weighed quantity of sodium alginate was added in the distilled water with stirring at about 300 rpm (12). OXC was added to this aqueous mucilage very slowly and stirred for about 5 min, to disperse the drug uniformly. The resultant dispersion was then added dropwise into 100 mL of 7% calcium chloride solution (13). After incubating for a predetermined time, the microspheres were filtered, washed with purified water and then dried for 6 h at 40°C.

Preparation of CEM-matrix alginate OXC microspheres

Briefly, weighed quantity of sodium alginate was dispersed uniformly in distilled water with stirring at about 300 rpm and then calculated amount of CEM was added to alginate dispersion. OXC was added to CEM-alginate dispersion**.** The resultant dispersion was then added dropwise *via* a needle fitted with a 24-G needle into 100 mL of 7% calcium chloride solution (13).

Preparation of CEM coated alginate OXC microspheres

Dipping method was used for coating alginate microspheres in this experiment. The coating solution was prepared by dissolving CEM in propylene glycol. The alginate microspheres were added to the polymer-coating solution and stirred mildly with the magnetic bar for 30 min to evenly coat the surface of alginate microspheres. After coating, the microspheres were rinsed with distilled water, filtered and then dried for 8 h using a speed-vacuum drier at room temperature (14).

Six formulations of microspheres (OXC-1, OXC-2, OXC-3, OXC-4, OXC-5 and OXC-6) were prepared by varying the process variables like change in the polymer concentration in blended form as well as coating material. Composition of OXC loaded microspheres is presented in Table.2.

Evaluation of microspheres

The bulk density (ρb) of the OXC microspheres was determined as the quotient of the weight to the bulk volume of each batch (15). The tapped density (ρt) was determined as the quotient of weights to the volumes of microspheres after tapping 100 times the measuring cylinder containing the samples and recording the tapped volume. Hausner's quotient was derived as the ratio of the tapped density to the bulk density, while % compressibility was calculated as the ratio of the difference between the tapped density and bulk density to the tapped density (16).

The microspheres were passed from the funnel which was initially fixed in a stand. Microspheres falling from a height of 6 cm (distance between top of funnel and surface) form a heap at the surface. The radius and height of the heap was calculated in order to measure angle of repose θ (15).

$$\text{Tan } \theta = h/r$$

where 'θ' denotes angle of repose while 'h' and 'r' denote height and radius of heap, respectively. Angle of repose < 30° shows excellent flow properties. All values were taken in triplicate and then compared with standards to assess the micrometric properties.

Determination of drug entrapment efficiency

A hundred mg of microspheres were crushed using pestle and mortar. The crushed powders of drug containing microspheres were placed in 250 mL of phosphate buffer of respective pH and kept for 24 h with occasionally shaking at 37 ± 0.5°C. The polymer debris formed after disintegration of microspheres was removed by filtering through Whatman filter paper (No. 40). The drug content in the filtrate was determined using a UV spectrophotometer (Shimadzu, Japan) at 256 nm against appropriate blank (3). The DEE (%) of these prepared microspheres (n = 3) was calculated by the following formula:

$$\text{DEE (\%)} = \frac{\text{Actual drug content in microprospheres}}{\text{Theoretical drug content in microprospheres}} \times 100$$

Percentage yield

In this case total amount of drug and polymer used was noted, then microspheres were dried and weighed again, %age yield (w/w) was determined by using the given formula (17). The formula for calculation of percentage yield (n = 3) is as follows:

$$\% \text{ yield} = \frac{\text{Weight of microspheres}}{\text{Weight of polymer + weight of drug}} \times 100$$

Microsphere size analysis

Particle size of 100 dried microspheres from each batch was measured using an optical micro-

scope fitted with an ocular and stage micrometer (SZ-6045, Olympus, Tokyo, Japan). The calibrated eye-piece was fitted with the micrometer which was used to determine the particle size (18).

Swelling characteristics of microspheres

Swelling behavior evaluation of microspheres containing OXC was carried out in their respective phosphate buffers. A hundred mg beads were placed in vessels of dissolution apparatus containing 500 mL of respective media. The experiment was carried out at 37 ± 1°C under 50 rpm paddle speed. The swelled microspheres were removed at predetermined time interval and weighed after drying the surface by using tissue paper (3). Swelling index (%) was determined using the following formula:

$$\text{Swelling index (\%)} = \frac{\text{Weight of microspheres after swelling} - \text{Dry weight of microspheres}}{\text{Dry weight of microspheres}} \times 100$$

Surface morphology analysis by scanning electron microscope (SEM)

Morphology and surface characteristics were studied by scanning electron microscopy. The samples for the SEM analysis were prepared by sprinkling the microspheres on an aluminium stub with the help of double adhesive tape. The stub was then coated with fine gold-palladium alloy to the thickness of 150-200 Å) using coat sputter JFC 1100 (JOEL, JSM-6100 SEM, Japan). The microspheres were then observed with the scanning electron microscope and photograph was taken (19).

***In vitro* drug release studies**

The release profiles of OXC from microspheres were examined in three different buffer solutions to mimic the various physiological GI-tracts. The media of pH 1.2 represented the gastric condition; pH 6.8 was a compromise condition between the gastric pH and the small intestine and pH 7.4 which is phosphate buffer solution. The dissolution process was carried out using a USP 6 rotating basket apparatus (Model Electrolab, TDT- 06T, Mumbai). The drug-loaded microspheres (equivalent to 100 mg of OXC) filled in empty capsule shells were put in the basket, rotated at a constant speed of 50 rpm and at temperature of 37°C. The 900 mL of the dissolution medium, pH 1.2, were added and test was conducted for 2 h , at the end of 2 h the test was continued changing the dissolution medium with pH 6.8 buffer solution up to 24 h for matrix microspheres. For coated microspheres the test was conducted for 2 h in pH 1.2 and then was continued for phosphate buffer of pH 7.4 up to 24 h. At scheduled time intervals, a 5 mL sample was withdrawn and replaced with the same volume of fresh medium. The samples were filtered through 0.45 µm membrane filter and after appropriate dilution, OXC concentration was estimated spectrophotometrically at 256 nm (Shimadzu 1201 spectrophotometer, Japan). Finally, the corresponding drug content in the samples was calculated from calibration curve of OXC to determine the drug release pattern (20).

Analysis of *in vitro* drug release kinetics and mechanism

In order to predict and correlate the *in vitro* release behavior of OXC from formulated microspheres, it is necessary to fit into a suitable mathematical model. The *in vitro* drug release data were evaluated kinetically in important mathematical models (21):

Zero-order model: $Q = kt + Q_0$

where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

First-order model: $Q = Q_0 e^{kt}$

where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

Hixson-Crowell model: $Q^{1/3} = kt + Q_0^{1/3}$

where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

Higuchi model: $Q = kt^{0.5}$

where Q represents the drug released amount in time t, and k is the rate constant.

Korsmeyer-Peppas model: $Q = kt^n$

where Q represents the drug released amount in time t, k is the rate constant and n is the diffusional exponent, indicative of drug release mechanism.

The accuracy and prediction ability of these models was compared by calculation of squared correlation coefficient (R^2) and model selection criteria (MSC) using DDSolver software. Again, the Korsmeyer-Peppas model was employed to distinguish between competing release mechanisms: Fickian release (diffusion-controlled release), non-Fickian release (anomalous transport), and super Case-II transport (relaxation-controlled release). When n is = 0.43, it is Fickian release, the n value between 0.43 and 0.85 is defined as non-Fickian release. When, n = 0.85, it is case-II transport (22).

Stability studies of microspheres

After determining the drug content, the optimized drug-loaded microspheres were charged the accelerated stability studies according to ICH guidelines. To assess long-term stability, accurately weighed drug loaded microspheres equivalent to 100 mg of OXC were filled into hard gelatin capsules manually and sealed in aluminum packaging

coated inside with polyethylene. The studies were performed at 25 ± 2°C , 40 ± 2°C with 60 ± 5% and 75 ± 5% relative humidity (RH) in desiccators with saturated salt solution for up to 6 months (23). A visual inspection for drug content was conducted every month for the entire period of the stability study.

Statistical analysis

All experiments were repeated thrice, the average values were taken and standard deviation was calculated. The R^2 and MSC for evaluation of accuracy and prediction ability of various kinetic models were calculated using DDSolver software.

RESULTS

Extraction of mucilage from *Colocasia esculenta* corms

The yield of extracted mucilage from *Colocasia esculenta* corms was found to be 22.39% w/w. The physical evaluation study of isolated mucilage shows that it is off white, odorless powder with smooth regular texture and having characteristics taste. It swells in cold water to form a gel, soluble in hot water, insoluble in ethanol, acetone and benzene. The isolated mucilage was studied for physicochemical parameters (Table 1), and it was found that isolated mucilage had excellent flow properties (5).

Drug/polymer compatibility studies

Fourier transform infrared (FT-IR) spectroscopic analysis

The spectra for all forms: (a) OXC, (b) CEM, (c) sodium alginate, (d) overlay of CEM-alginate blank microsphere and CEM-alginate microsphere contained OXC (Fig. 1). The spectrum of OXC is characterized by the presence of a strong absorption bands at 3466 cm^{-1}, 3344 cm^{-1} which are indicative of amines (-NH- group). The carbonyl-stretching mode appears as a very strong doublet at 1664 cm^{-1}

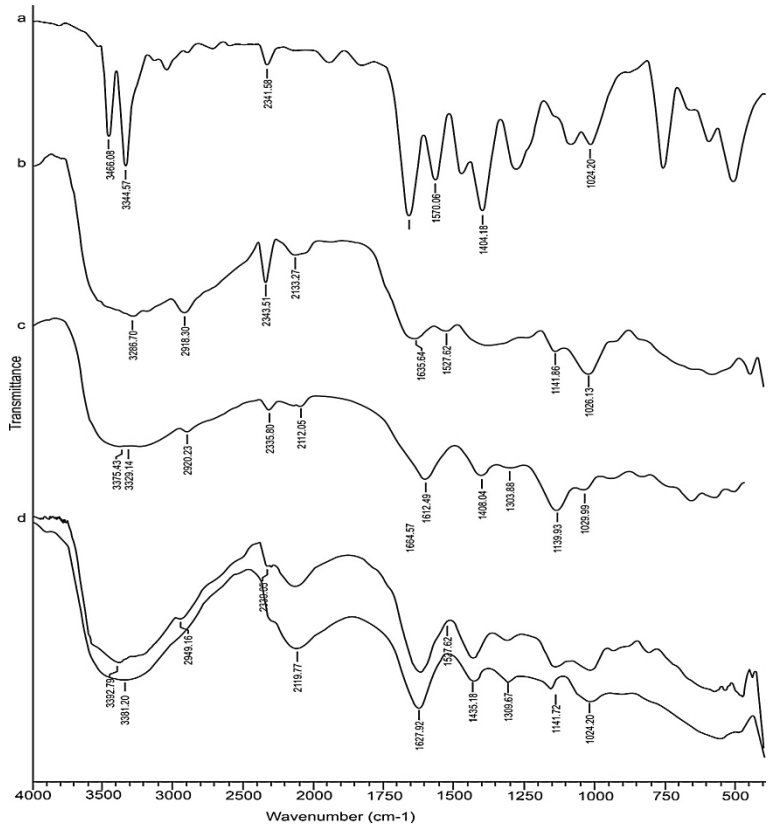

Figure 1. FTIR spectra of (a) OXC ,(b) CEM , (c) sodium alginate, (d) Overlay of CEM-alginate unloaded and CEM-alginate loaded microspheres

(C=O stretching) and absorption bands at 1570 cm^{-1} were denoted for stretching vibrations of C=C in aromatic ring and they are clearly seen in the spectra of loaded microspheres of OXC (24). The -OH groups present in CEM and sodium alginate are clearly seen at 3300 cm^{-1} and they are also present in the case of overlay forms. The peaks attributed to the $–CH_2$ groups present at 2918 cm^{-1} and 2920 cm^{-1} in CEM and sodium alginate, respectively, are also shown in overlay spectra. CEM showed 1527 cm^{-1} N-O asymmetric stretching, 1026 cm^{-1} for C-O stretching are also present in the spectra of formulated microspheres (25). In sodium alginate the -OH group is clearly seen at 3400 cm^{-1} and they can be clearly seen in the case of formulated microspheres. The peaks attributed to the $–CH_2$ groups are present at 2931 cm^{-1} and some distinct peaks such as carboxyl group showed strong absorption bands at 1612 cm^{-1}, 1408 cm^{-1} and 1303 cm^{-1}, due to carboxyl anions asymmetric and symmetric stretching vibrations (26), clearly seen in spectra of formulated microspheres.

Differential scanning calorimetry

The DSC curves of pure drug, CEM and OXC microspheres are in Figure 2. The DSC curve of OXC show a sharp endothermic peak at its melting point with an onset temperature at 217^{o}C. No significant degradation was seen to occur below 240^{o}C. The melting temperature determined from the DSC curve was in accordance with that described in the literature, 219-221^{o}C (27).

Measurement of micrometric properties

The rheological parameters like angle of repose, bulk density and tapped density of all microspheres confirm good flow and packaging properties (Table 3).

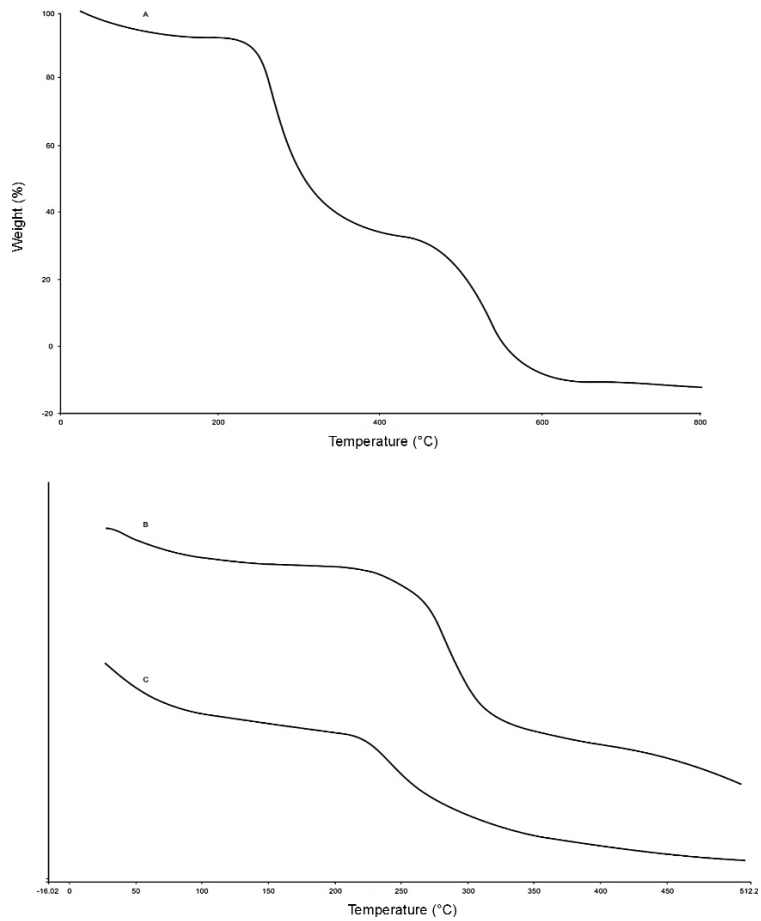

Figure 2. DSC thermograms of A: oxcarbazepine, B: CEM, C: OXC microspheres

Table 3. Micromeritic properties of OXC microspheres.

Formulation	Angle of repose (°)	ρb (g/cm³)	ρt (g/cm³)	Carr's index(%)	Hausner's ratio
OXC-1	25.39 ± 0.33	1.039 ± 0.007	1.278 ± 0.006	18.70 ± 0.1	1.230 ± 0.016
OXC-2	26.22 ± 0.42	1.138 ± 0.005	1.313 ± 0.005	13.32 ± 0.13	1.153 ± 0.021
OXC-3	24.15 ± 0.51	0.854 ± 0.004	0.934 ± 0.003	8.56 ± 0.06	1.093 ± 0.001
OXC-4	23.94 ± 0.47	0.833 ± 0.003	0.909 ± 0.004	8.36 ± 0.01	1.091 ± 0.012
OXC-5	24.53 ± 0.37	0.775 ± 0.001	0.840 ± 0.002	7.73 ± 0.03	1.083 ± 0.002
OXC-6	24.31 ± 0.34	0.769 ± 0.003	0.830 ± 0.003	7.34 ± 0.01	1.079 ± 0.001

Data are expressed as the mean ± S.D. of at least triplicate values.

Table 4. Percentage yield and drug encapsulation efficiency of OXC microspheres.

Formulation	%Yield	% Entrapment efficiency
OXC-1	88 ± 0.18	75.73 ± 0.02
OXC-2	89 ± 0.34	80.29 ± 0.02
OXC-3	90 ± 0.27	86.81 ± 0.04
OXC-4	92 ± 0.44	92.25 ± 0.01
OXC-5	90 ± 0.29	84.45 ± 0.05
OXC-6	91 ± 0.20	85.96 ± 0.03

l values are expressed as the mean ± S.D, n = 3.

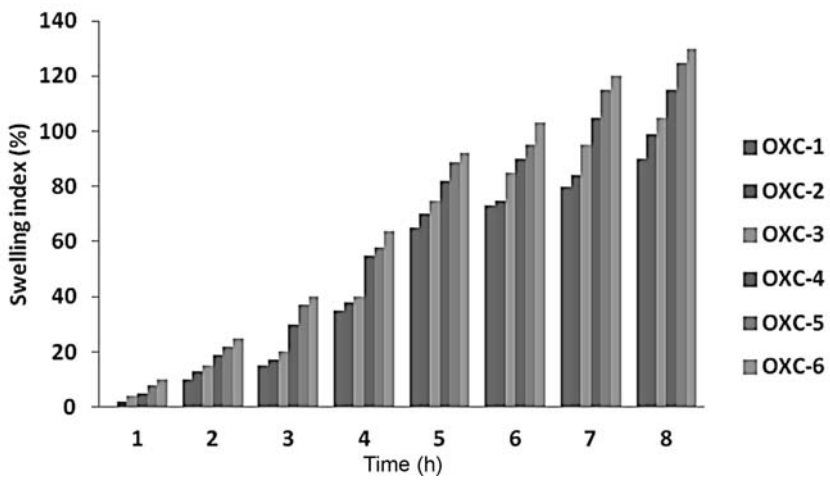

Figure 3. Swelling behavior of OXC microspheres in 0.1 M HCl [mean ± SD, n = 3]

Drug entrapment efficiency and yield

Percentage entrapment efficiency of microspheres were in the range between 75.73 ± 0.02 to 92.25 ± 0.01% (Table 4).

Microsphere size

The mean particle size of various formulations of OXC were obtained in the range between 394 ± 0.42 and 575 ± 0.28 µm (Table 5).

Swelling behavior of microspheres

The swelling behavior of various formulations of OXC was evaluated in 0.1 M HCl, pH 2.0, and phosphate buffer pH 6.8 for matrix and phosphate buffer pH 7.4 for coated microspheres (Figs. 3 and 4).

Microsphere surface morphology

The SEM photograph of *Colocasia esculenta* mucilage revealed that the surface of particles found

to be smooth and regular (Fig. 5). The SEM photomicrographs of alginate plain, polymer matrix and coated microspheres are in Figure 6.

In vitro drug release studies

The drug release profiles of all formulations are in Figure 7. All formulations showed a negligible drug release at pH 1.2 (< 7% w/w) which may be due to stability of the polymers at lower pH. On the other hand, the polymers eroded at alkaline pH and the contents are released in a sustained manner by both diffusion and slow erosion of polymer matrix (28). Drug release studies revealed that for microspheres made with sodium alginate (OXC-1, OXC-2) the drug release rate was found to be fast and of 99.07 and 98.67% till 10 and 12 h, respectively, which may be attributed to absence of other release retarding polymers, respectively.

Table 5. Mean diameter of OXC microspheres.

Formulation	Mean diameter (μm) Mean ± S.D
OXC-1	504 ± 0.32
OXC-2	476 ± 0.20
OXC-3	575 ± 0.28
OXC-4	512 ± 0.22
OXC-5	394 ± 0.42
OXC-6	423 ± 0.36

Kinetics of drug release

The *in vitro* dissolution data was analyzed using different kinetic models which describes the drug release mechanism. The kinetic model showing the highest correlation coefficient was considered as the most appropriate model for the dissolution data.

Table 6. Results of curve fitting of the *in vitro* drug release data from OXC microspheres with CEM.

Model	Parameters	OXC-1	OXC-2	OXC-3	OXC-4	OXC-5	OXC-6
Zero order	R^2 MSC	0.9446 2.13	0.9699 2.75	0.9805 3.27	0.9858 3.83	0.9326 2.12	0.8943 1.53
First order	R^2 MSC	0.8383 1.06	0.8814 1.38	0.9112 1.76	0.9467 2.51	0.8292 1.19	0.8450 1.53
Higuchi model	R^2 MSC	0.7186 0.51	0.7689 0.71	0.8139 1.02	0.7631 1.03	0.6734 0.54	0.6770 0.79
Korsmeyer-Peppas model	R^2 MSC n	0.9797 2.93 1.35	0.9783 2.90 1.14	0.9805 3.12 1.008	0.9928 4.38 1.127	0.9692 2.76 1.334	0.9746 3.20 1.30
Hixson-Crowell	R^2 MSC	0.8735 1.31	0.9147 1.71	0.9439 2.22	0.9628 2.82	0.8644 1.42	0.8789 1.77

R^2: squared correlation coefficient; MSC: Model selection criteria; n: diffusional exponent.

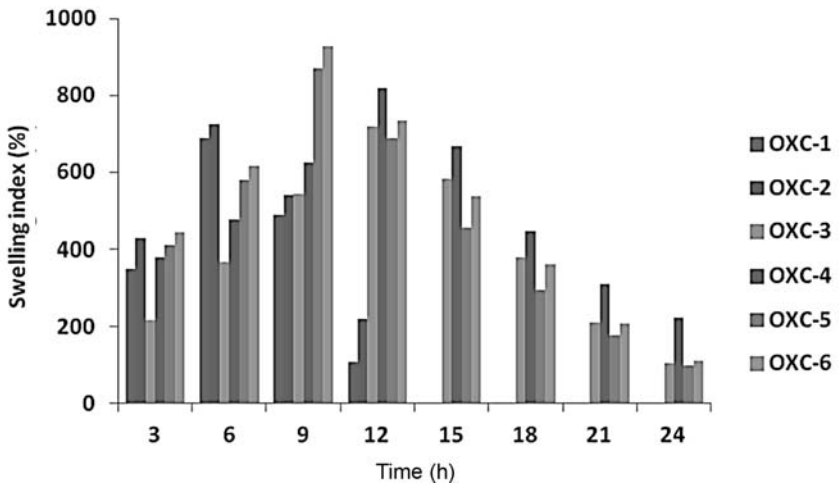

Figure 4. Swelling behavior of OXC microspheres in phosphate buffer [mean ± SD, n = 3]

Table 7. Stability data for OXC drug loaded microspheres.

Sampling interval (months)	Drug-contents of microspheres			Physical characteristics		
	5°C %w/w	25°C/60 %RH %w/w	40°C/75 %RH %w/w	5°C	25°C/60%RH	40°C/75%RH
0	100	100	100	*	*	*
1	100	100	99.89	*	*	*
2	100	100	99.10	*	*	*
3	100	99.81	98.34	*	*	*
4	100	98.96	97.32	*	*	*
5	100	97.64	96.66	*	*	*
6	100	96.86	95.45	*	*	*

*No change

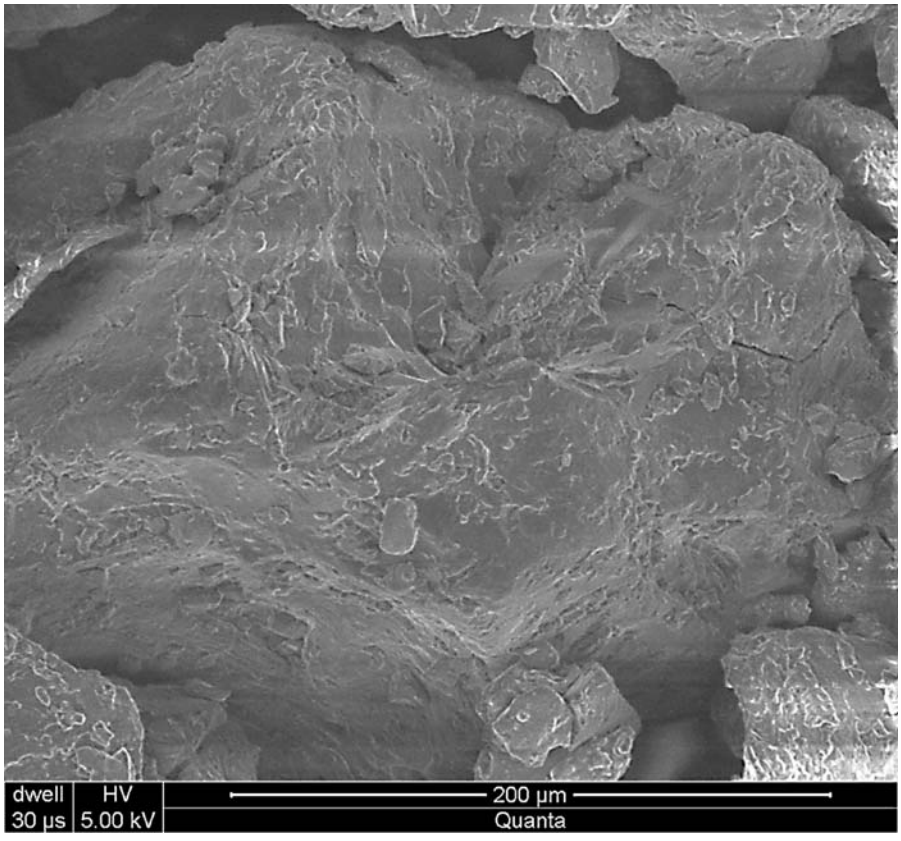

Figure 5. Scanning electron micrograph of *Colocasia esculenta* mucilage

For the values of n higher than 1 (n > 1), the mechanism of drug release is regarded as super case-II transport and this is used to analyze the release of pharmaceutical polymeric dosage forms when more than one type of release phenomena was involved. The values of correlation coefficient were found for equations and compared as mentioned in Table 6.

Stability studies

The developed optimum formulations were subjected to stability studies at 25°C/60% RH and 40°C/75% RH for up to 6 months. The dissolution profiles and capsule potency results for all the stability conditions were within 90 to 110% of the label claim (Table 7).

DISCUSSION

The careful observation of the overlay spectra of blank and microspheres containing OXC reveals that all the major peaks of the pure drug and polymers appear with negligible variation, indicating that there is no chemical interaction between the drug and polymers. In short, the CEM-alginate microspheres containing OXC had significant characters of OXC in the FT-IR spectrum, suggesting, that there was no interaction between the drug OXC and the polymers used. In case of CEM, endothermic peak corresponded to its melting point of 245-251^{o}C. The DSC curve of formulated OXC microspheres also proved the existence of particular thermal peaks at the melting point of OXC, which definitely suggest the compatibility of drug with polymers used to control drug release.

All the formulations showed excellent flowability represented in terms of angle of repose (< 30^{o}) (28). Bulk and tapped density of microspheres showed an acceptably good range, which indicates that the microspheres have good packability. Carr's index values and Hausner's ratio explain that the formulated microspheres had excellent compressibility and good flow properties (29).

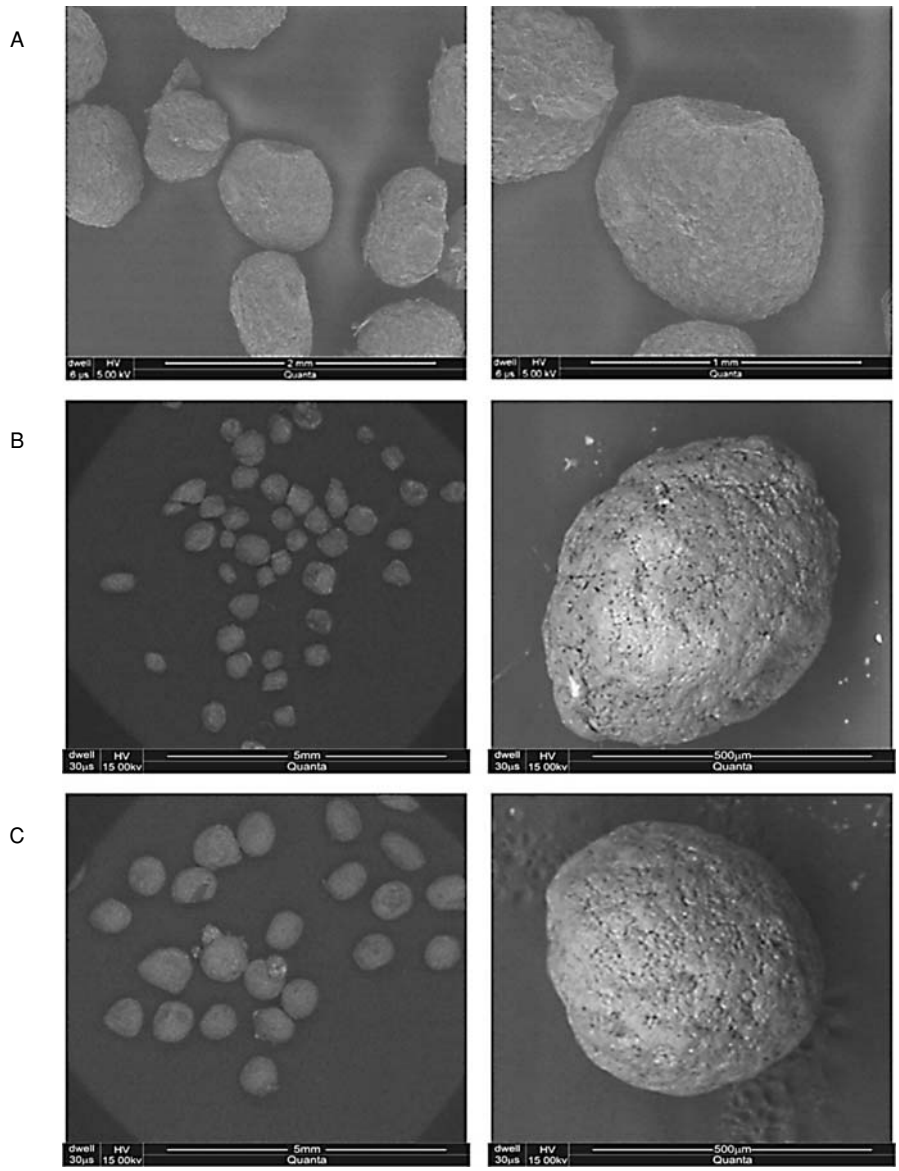

Figure 6. Scanning electron micrograph of (A) plain microspheres at 2 and 1 mm, (B) matrix microspheres at 5 mm and 500 µm, (C) coated microspheres at 5 mm and 500 µm

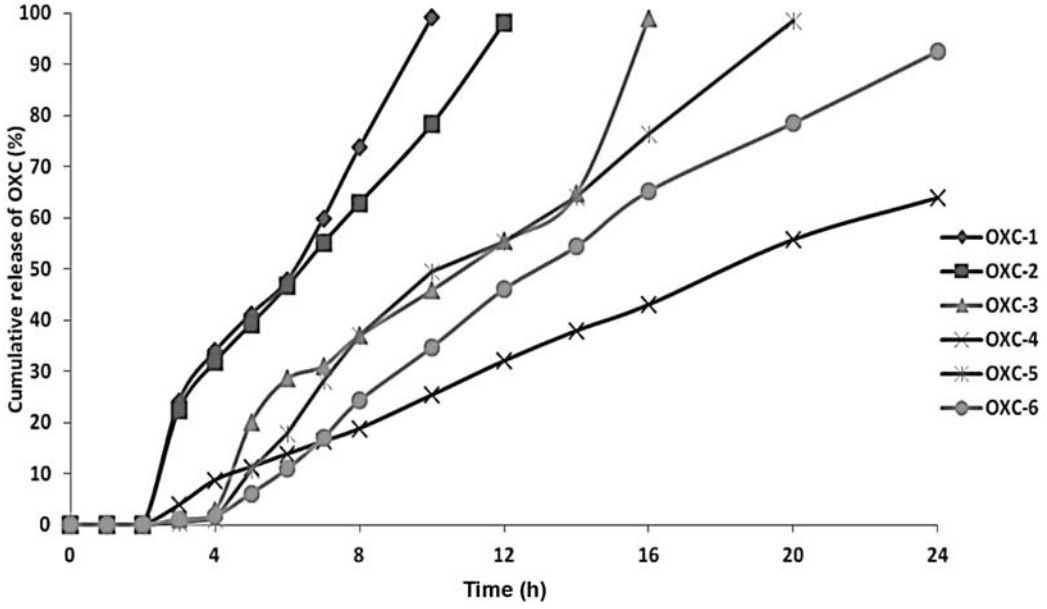

Figure 7. *In vitro* release profiles of various OXC microsphere formulations

The highest entrapment efficiency was observed in CEM-alginate matrix microspheres, this phenomenon might be due to the increase in viscosity of the polymeric solution by the addition of CEM with alginate, and it might have been prevented drug leaching to the cross-linking solution and this behavior was also proved previously while using FSM-alginate beads (3). Drug leaching during hydrogel preparation and rapid dissolution of alginate at higher pH are major limitations, as it results in low entrapment efficiency in alginate microspheres. To overcome these limitations, guar gum was included in the alginate matrix (30), chitosan was used in combination with alginate (31) as well as fenugreek seed mucilage-alginate (3) has been used in matrix form to increase the entrapment efficiency.

Matrix microspheres were found to be increased in size as compared to alginate and coated microspheres, this could be explained due to the increase in viscosity of the polymer-matrix solution with the incorporation of CEM that, in turn, increased the droplet size during addition of the polymer-blend solution to the cross-linking solution (3). As the amount of polymer in the coating solution increased, the average diameter of coated microspheres increased (14).

The swelling ratio of the microspheres was dependent on pH of the solution. Under acidic conditions, swelling of microspheres occurs scarcely. Under neutral conditions, the microspheres will swell and drug release depends on the swelling and erosion process. The low swelling in acidic media at pH 1.2 was probably due to proton-calcium ion exchange forming insoluble alginic acid regions followed by solvent penetration into the gel network.

It has been also reported that the swelling of calcium alginate-based beads can be enhanced by the presence of phosphate ions (in phosphate buffer), which act as calcium sequestrant (31). The swelling of microspheres was ultimately increased in basic media at pH 6.8 and pH 7.4, this was due to increased solubility of the polymer in basic pH leading to the relaxation of the cross-linked polymeric network. It has been reported that the swelling can be enhanced by the presence of phosphate ions at higher pH, which displaces the Ca^{2+} ions within the microspheres (3). In systems based on sodium alginate cross-linked with calcium chloride alone or in combination with *Colocasia esculenta*, the osmotic pressure gradient that exists between the alginate gel and environment comprises an important factor in the swelling process.

When we compared the overall results of the swelling ratio of all formulations in phosphate buffer, OXC-1 and OXC-2 alginate microspheres showed maximum swelling at 6 h and microspheres completely eroded at the end of 12 h and as the quantity of sodium alginate increased it showed more swelling. Matrix microspheres OXC-3 and OXC-4 have showed maximum swelling at 12 h and then slowly eroded and dissolute up to 24 h, but still these

were not eroded completely. The ratios of polymer to alginate OXC-3 of 2 : 2 showed less swelling as compared to OXC-4. OXC-4 of 4 : 2 ratio showed maximum swelling and not completely eroded until 24 h. While coated microspheres OXC-5 and OXC-6 showed maximum swelling at 9 h and most of them eroded at 24 h, swelling of the microspheres increased with the coating of the polymer. The swelling studies showed that with an increase in polymer concentration, swelling of microspheres was significantly increased (14). Swelling of OXC microspheres followed the pattern: matrix > coated > plain, while erosion, dissolution of OXC microspheres followed the pattern: matrix < coated < plain.

Alginate plain microspheres showed spherical shape but having cracks while CEM-alginate matrix microspheres showed approximate to spherical in shape with little bit irregularity in shape but without cracks. Due to the cross-linking of the polymer, the surface of the microspheres were found to be rough with slight ridges, as such behavior has been reported (32) while using natural source in formulating microspheres. CEM-coated alginate microspheres showed more spherical shape without cracks while the existence of clustered alginate microspheres surrounded by CEM coacervates was observed during coating alginate microspheres with CEM what may be caused by partly collapsing the polymeric gel network during drying (33).

CEM-alginate matrix microspheres showed an ideal linear release profile. The alginate microspheres swell and then disintegrate due to release of calcium ions by sodium or phosphate (14). CEM-alginate matrix microspheres swelled but withstood the disintegration longer than other alginate microspheres. As we increased the ratio of CEM with sodium alginate as in matrix microspheres (OXC-3, OXC-4), the drug release at a constant rate was found to be of 98.52% and 63.87% till 20 and 24 h, respectively; there was a decrease in the drug release because of higher polymer content that lowered the release of drug more effectively (34). CEM was found to be an effective additive polymer for controlling the release rates (7).

The swelling and disintegration of alginate microspheres is an important factor in the release of the drug. To prevent these factors , alginate microspheres were coated with CEM, which could strengthen the alginate microspheres and reduce membrane permeability. The CEM-alginate interaction may be proper to control the release rate and enhance the bioavailability of the drug in the intestine as reported with chitosan-alginate coating microspheres (14). Coated microspheres (OXC-5 and OXC-6) showed drug release with 98.52 and 92.41% for 20 and 24 h, respectively. Our results showed that as the concentration of coating solution of mucilage increased there was a decrease in drug release. Similar findings were observed previously that the higher polymer content of coating solution lowered the release of drug more effectively (35).

The R^2 value of Korsmeyer-Peppas and zero-order model is very near to 1 than the R^2 values of other kinetic models. Thus it can be said that the release follows Korsmeyer-Peppas model and zero-order model mechanism. The n value of Korsmeyer-Peppas model is n > 1 indicating that the release patterns from these microspheres followed the super Case-II transport mechanism controlled by swelling, relaxation and distanglement of polymeric chains (36).

Overall, results from the stability studies indicated that capsules were physically stable but the drug content at 40°C /75%RH was slightly reduced to 93.15% (w/w) after 6 months. Good stability was observed at lower temperature for more than 6 months (23).

CONCLUSION

This study reveals that oral sustained release of OXC microspheres can be successfully achieved by ionotropic gelation technique using sodium alginate and CEM as a release retardant polymer. Sustained release of OXC from microspheres not only depends upon the nature of polymer but also on its percentage used. The studies showed that the presence of CEM and calcium chloride crosslinking increases entrapment efficiency and prevent the rapid dissolution of alginate in alkaline pH. The assessment of the release kinetics revealed that drug release followed Korsmeyer Peppas model and was found to be non-Fickian type. Thus, the prepared microspheres could be a promising delivery system for OXC with sustained drug release profiles, reducing the dosing frequency and eliminating the dose related adverse effects.

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