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Formulation of oxybutynin chloride microparticle-loaded suppositories: in vitro characterization and in vivo pharmacokinetic study

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Abstract

Background: Oxybutynin chloride (OXC) is used to treat overactive urinary bladder. OXC is metabolized in the liver to N-desethyloxybutynin, which is mainly responsible for the anticholinergic side effects of OXC. Conventional oxybutynin suppositories formulated earlier have shown most common side effects, such as dry mouth, constipation and serious anticholinergic reaction. Hence the present research work deals with the formulation and characterization of OXC microparticle-loaded mucoadhesive suppositories which may remain adhered in the lower rectum and avoid first pass metabolism. The emulsification–ionic gelation method is employed to prepare OXC microparticles. Two formulation factors at three levels, i.e. polymer concentration and stirring speed, were selected. Sodium alginate (concentration 1–2%) and 1% w/v Carbopol 971P were used to prepare OXC microparticles. OXC microparticles were evaluated for various parameters such as production yield, entrapment efficiency, mucoadhesive strength, shape, size, zeta potential, Fourier Transform Infrared spectroscopy, differential scanning calorimetry, X-ray diffraction, in vitro dissolution studies and stability studies. Suppositories loaded with OXC microparticles were prepared by the fusion method using Poloxamer 188 and propylene glycol and evaluated for various parameters like weight variation, disintegration time, in vitro dissolution study, stability study and pharmacokinetic study.

Results: Results of in vitro characterization revealed that optimized batch of OXC loaded microparticles exhibited production yield 94.024% entrapment efficiency 95.378% and mucoadhesion strength 95.544%, particle size range 764.04–894.13 µm, zeta potential − 14.5 mV, with 0.946 desirability. Consequences of DSC and XRPD evaluation shown that drug was effectively entrapped inside the microparticles. In vitro release studies revealed improvement in drug dissolution as a consequence of its entrapment into microparticles. SEM results showed that micelles were sphere-shaped. On rectal administration of OXC microparticles loaded suppository in male Sprague–Dawley Rats, the relative bioavailability was found 173.72%.

Conclusion: In vivo study elicits rapid increase in absorption of drug from microparticles loaded suppository when compared with the oral formulation and drug loaded suppository in rats. OXC microparticles loaded suppository is novel and promising drug delivery system for rectal administration and may avoid anticholinergic side effects of hepatic metabolite, N-desethyloxybutynin. These rectal drug delivery systems will be advantageous for efficient absorption of drugs and to avoid first pass metabolism.

Keywords: Oxybutynin chloride, Microparticles, Sodium alginate, Carbopol 971 P, Poloxamer, Suppository

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Background

One in six people over the age of 40 suffers from an "overactive bladder" and the incidence increases with age. Many studies of published research do not report people at lower-socio-economic levels and may be more prone to an overactive bladder [1]. Overactive bladder (OAB) is a disorder with urinary indications, increased occurrence of urination, waking up at night-time to urinate, or urinating involuntarily. The main indication of OAB is urinary urgency. Co-morbid patients with OAB reported more health problems and saw an average of 84% more annual visits to physicians as compared to patients not suffering from OAB [2]. Postmenopausal women with urge incontinence have a considerably greater risk of falling and enduring a fracture than women without urge incontinence [3].

OAB is related to the lower urinary tract and occurs with clinical symptoms of nocturia, urge urinary incontinence, frequency, and urgency. Oxybutynin chloride (OXC), an anti-muscarinic agent is used effectively to treat OAB. When administered orally, OXC is metabolized in the liver to N-desethyloxybutynin, which mainly exhibits the anticholinergic side effects of oxybutynin. Side effects of N-desethyloxybutynin include dry mouth, constipation, dry or itchy eyes, blurred vision, indigestion, urinary tract infections, bladder retention, and drowsiness. OXC is well soluble in water but insoluble in alkaline media [4–6]. Moghimipour E. et al. were developed transdermal patches to minimize the hepatic metabolism and systemic harmful effects of oxybutynin metabolites, but long-term usage of transdermal patches produces skin irritation [7, 8]. In another research study, conventional oxybutynin rectal suppositories were developed and administered to patients, but the work was limited because patients reported the most common side effects, such as dry mouth and constipation, while one patient with polymyositis developed a serious anticholinergic reaction that necessitated hospital treatment [9]. These side effects were due to development of conventional suppositories as these reaches to the colon and results into partial hepatic metabolism. Considering above facts, this research work has been proposed to develop a novel suppository incorporating OXC microparticles that will adhere to the rectal mucosa and allow the drug to be absorbed from the lower rectum, will avoid hepatic metabolism, potentially improving bioavailability [10] as well as minimising or inhibiting anticholinergic effects of N-desethyloxybutynin.In earlier research studies, to increase gel strength and mucoadhesion, hydrogel forming mucoadhesive polymers such as carbopol and sodium alginate have been combined with thermosensitive polymers (Poloxamers). The carboxyl groups of hydrogels, can form a strong bond with the cross-linked poloxamer gel, allowing the molecules to be located between the gel to greatly increase strength. This strong mucosal retention of hydrogels facilitates modified drug release and absorption. To improve the therapeutic efficacy of drugs for local and systemic therapy, microparticulate systems have been included into rectal dosage forms. Microparticulate rectal drug delivery systems differ from traditional rectal drug delivery systems in that the drug is encapsulated or loaded into microparticles before being dispersed in a suppository base. Microencapsulation facilitates the modification of drug release kinetics and improves the solubility of hydrophobic substances [11–13].

Carbopol 971P was selected as a mucoadhesive polymer considering its wide application in microparticles and rectal administration [14, 15]. Alginate is a linear anionic polysaccharide. It consists of D-mannuronic acid and L-guluronic acid units (in different ratios) linked by glycosidic bonds. Various divalent or trivalent cations are used to crosslink the monomers of alginate. Cross-linked calcium alginate is clinically safe, hence most frequently used as crosslinking ion [16]. Alginates when interact with $CaCl_2$, the carboxyl groups of guluronate moieties of alginates form intermolecular crosslinks with Ca^{2+} to form "egg-box" structure, whereas Ba^{2+} do not form MG linkages [17, 18]. Poloxamers are synthetic, hydrophilic and non-ionic copolymers having surfactant properties. Poloxamer consists of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of Polyethylene glycol [19, 20]. Considering all these facts an objective was set to develop and evaluate a mucoadhesive suppository containing OXC loaded microparticles to deliver the drug in the rectal cavity.

Methods

Materials

OXC was kindly donated by Sun Pharma, Baroda (Gujarat, India). Carbopol 971 P was gifted by Noveon Pharmaceuticals Private Limited, Cleveland (USA). Poloxamer 188 was donated by Mylan Laboratories (Nasik, India). Sodium alginate was gifted by Thomas Baker Chemicals Private Limited, Mumbai, India. Liquid paraffin, potassium dihydrogen phosphate, calcium chloride, sodium hydroxide and propylene glycol were obtained from SD Fine Chemicals, Mumbai (Maharashtra, India). Methanol and acetonitrile were procured from Rankem Limited, India. Freshly excised rectal mucosa of goat was obtained from a local butcher (Nasik, India). All the other chemicals and reagents were of analytical grade and were used without further purification.

Formulation of OXC microparticles

The emulsification–ionic gelation method was used to prepare OXC microparticles. A dispersion (sodium alginate, (1–2%) and Carbopol 971P, (1% w/v) in water was prepared and kept overnight to form a homogeneous dispersion. The OXC was added to this polymer dispersion and dispersed thoroughly using a bath ultrasonicator (Trans-o-sonic, Mumbai). The drug-polymer dispersion was then poured drop-wise into 200 mL of liquid paraffin containing Span 80 (2%). The dispersion was stirred at 30 min using a magnetic stirrer (Remi, India). Thirty millilitres of a 20% w/v $CaCl_2$ solution was added drop-wise to the system. The resultant microparticles were filtered, dried in air at room temperature and stored in desiccator [21].

Design of experiment

The preparation of OXC microparticles was carried out using Design Expert 8.0.7.1 software. A face-centred ($\alpha = 1$) three-level, two-factor central composite design with a quadratic model was developed using Design Expert Software® (version 8.0.3.1 Stat-Ease Inc.). The minimum number of trials needed to optimize the microparticle formulation was determined using this model [22, 23]. Considering earlier study and the literature survey [24, 25] the concentration of sodium alginate (X_1) and the stirring speed (X_2) were considered as independent formulation (or process) parameters. These two parameters were taken at three levels (− 1, 0, + 1) during the formulation of microparticles. Thirteen microparticle batches were designed and formulated to study the effect of the independent variables on the properties of the formulation (Table 1). The effects of these two independent factors on three dependent factors (production yield, per cent entrapment efficiency (%EE) and mucoadhesion strength) were studied. The resultant data were then used to optimize the formulation and were subjected to statistical analysis. These dependent factors or responses (Y_1, Y_2 and Y_3) were subjected to analysis and were fitted to various models (linear, quadratic and two-factor interaction (2 FI) models). A model was selected on the basis of the highest correlation coefficient values (predicted r^2, adjusted r^2 and actual r^2) (Table 2).

Evaluation of microparticles

Production yield:

The production yield was calculated using the formula

$$\text{Production Yield} = \frac{\text{Practical yield of microparticles after drying}}{\text{Theoretical yield}\left(\text{Total amount of drug and polymers added initially}\right)} \times 100 \quad (1)$$

Entrapment efficiency (%EE) and drug loading (%DL):

The amount of OXC in the microparticles was determined by triturating the microparticles in a mortar and then sonicating them with water for 1 h. They were filtered using a membrane filter and diluted with distilled

Table 1 Formulation of OXC microparticles using face-centred central composite design

S. no	Formulation code	Drug	Level (Concentration of sodium alginate)	Carbopol 971P:sodium alginate ratio	Level stirring speed	Stirring speed (RPM)
1	B1	1	0	1:1.5	+ 1	1041
2	B2	1	+ 1	1:2.21	− 1	758
3	B3	1	0	1:1.5	0	900
4	B4	1	+ 1	1:2.21	0	900
5	B5	1	+ 1	1:2.21	+ 1	1041
6	B6	1	0	1:1.5	0	900
7	B7	1	0	1:1.5	− 1	758
8	B8	1	0	1:1.5	0	900
9	B9	1	− 1	1:0.793	0	900
10	B10	1	0	1:1.5	0	900
11	B11	1	0	1:1.5	0	900
12	B12	1	− 1	1:0.793	+ 1	1041
13	B13	1	− 1	1:0.793	− 1	758

Table 2 Results of oxybutynin chloride Microparticles formulated using face-centred central composite design

S. no	Formulation code	Production yield (%)	Entrapment efficiency (%)	Mucoadhesion strength (%)	Drug loading (%)
1	B1	93.70 ± 3.67	91.80 ± 3.92	88 ± 3.26	23.19 ± 0.54
2	**B2**	**92.90 ± 4.26**	**95.98 ± 4.44**	**96 ± 1.88**	**24.62 ± 0.45**
3	B3	89.93 ± 3.78	89.65 ± 6.68	89.33 ± 4.98	22.79 ± 0.37
4	B4	94.75 ± 4.21	96.36 ± 6.90	92 ± 3.26	23.00 ± 0.54
5	B5	87.82 ± 5.01	95. 28 ± 4.02	92 ± 1.88	23.23 ± 0.45
6	B6	90.02 ± 2.98	89.04 ± 3.94	90.67 ± 1.88	21.82 ± 0.40
7	B7	88.39 ± 3.98	92.15 ± 4.92	89.67 ± 3.26	23.65 ± 0.58
8	B8	90.25 ± 4.34	89.36 ± 6.24	92 ± 1.88	23.19 ± 0.54
9	B9	84. 54 ± 3.87	78.53 ± 4.92	85.33 ± 3.77	22.59 ± 0.34
10	B10	89.90 ± 4.22	90.92 ± 4.68	89.67 ± 1.88	21.79 ± 0.37
11	B11	90.41 ± 4.78	89.69 ± 4.36	89.33 ± 4.98	22.84 ± 0.51
12	B12	79.55 ± 3.66	84.68 ± 2.48	86.66 ± 1.88	22.43 ± 0.25
13	B13	86.30 ± 4.44	87.15 ± 3.36	85.33 ± 3.77	22.29 ± 0.35

B_2 is the optimized batch based on Numerical optimization that is further evaluated for different parameters as well as to develop microparticle loaded suppository

water. The absorbance at 221 nm was measured with UV visible spectrophotometer (Shimadzu UV-2501PC) to determine the drug concentration. The %EE and %DL were determined as

$$\%EE = \frac{\text{Practical amount of drug entrapped in microparticles}}{\text{Theoretical amount of drug added microparticles}} \times 100 \quad (2)$$

$$\%DL = \frac{\text{Practical amount of drug entrapped in microparticles}}{\text{Total weight of microparticles}} \times 100 \quad (3)$$

Determination of in vitro mucoadhesion strength of microparticles by falling liquid film method

Freshly excised rectal mucosa of goat was procured from a local slaughter house and was cleaned with normal saline solution. This rectal mucosal tissue was then pinned onto a polyethylene sheet inclined at an angle of 60°. A container was placed beneath the polyethylene sheet. Twenty-five (N_0) microparticles of each batch were placed on the mucosal surface. The surface was then hydrated. The water and the microparticles were allowed to interact for 15 min. Fifty millilitres of phosphate buffer (pH 7.4) was made to flow over the tissue at a rate of 40 drops/minute. The microparticles that did not adhere to the mucosa and were washed away by the buffer were collected in the container below the polythene sheet. The total number of microparticles that remained on the mucosa (*Ns*) was calculated [18, 24]. The per cent mucoadhesive strength was calculated as

$$\%\,\text{adhesive strength} = \frac{Ns}{No} \times 100 \quad (4)$$

Particle size analysis and zeta potential

OXC microparticles were dispersed in oil. Particle size analysis was performed using a Motic DMW2-223 digital microscope (Motic Instruments Inc., Canada) equipped with a 1/399 CCD camera imaging accessory and computer-controlled image analysis software (Motic images 2000, version 1.3). A zeta cell was filled with a dispersion of OXC microparticles in a pH 7.4 phosphate buffer and placed in a Zeta Sizer (Nano ZS90, Malvern Instruments, UK) to determine the zeta potential of the dispersion [24].

Scanning electron microscopy (SEM)

SEM analysis was performed to determine the surface properties and shape of the OXC microparticles. The surface morphology of the OXC microparticles was examined using a scanning electron microscope (JSM 6390, JEOL) with an accelerating voltage of 10 kV [25].

Differential scanning calorimetry (DSC)

DSC thermograms of the OXC, OXC microparticles and blank polymeric microparticles were obtained using a differential scanning calorimeter (Mettler-Toledo AG Analytical, Switzerland). Precisely weighed samples (2 mg) were placed in an aluminium pan (capacity 40 µL). The

pan was sealed with an aluminium lid, which was pierced with a pin from the top, and placed on the sample holder. Samples were heated at a heating speed of 10 °C/minute over a temperature range of 40–300 °C in an atmosphere of nitrogen gas, and a thermogram was recorded [24, 26, 27].

X-ray diffraction (XRD)

XRD analysis of the OXC, OXC microparticles and blank polymeric microparticles was carried out at a diffraction angle (2θ) range of 10°–80° using a Philips-PW-1050 scanner with a Ni filter. Cu-Ka radiation, a voltage of 40 kV and a current of 30 mA were used. All the samples were measured within a diffraction angle (2θ) range of 10°–80° and with a step size of 0.020 [24, 25].

Formulation of microparticle-loaded suppositories

Poloxamer 188 and propylene glycol were mixed in different ratios (80:20, 70:30, 50:50) and heated up to 55 °C. OXC microparticles were then dispersed slowly into the molten base with constant agitation to avoid precipitation of the microparticles. The molten base containing the microparticles was then poured with caution into a suppository mould. The mould was placed in an ice bath to cool it. Drug-loaded suppositories were prepared using the same method, dispersing OXC in a molten base. These pure-drug suppositories were considered as the standard for further analysis [28].

Evaluation of microparticle-loaded suppositories

Weight variations

Twenty suppositories were weighed, and the average weight was calculated. The weight of each suppository was then determined using an electronic balance. The weights of not more than two suppositories were to deviate by more than 5% from the average weight [29].

Disintegration test

Disintegration test was performed using disintegration test apparatus. Three suppositories of each type (with different Poloxamer bases/propylene glycol ratios) were placed on the lower perforated discs and then the devices were inserted into cylinder and to the sleeves. This assembly was placed 90 mm below the surface of phosphate buffer (pH 7.4) in a vessel fitted with a slow stirrer and a temperature-measuring device. The apparatus was inverted every 10 min to check the disintegration of the suppository. The disintegration of a suppository was considered to be complete when it had completely dissolved, disintegrated, sunk to the bottom or become soft with a considerable change in shape [30].

In vitro drug release study

OXC-loaded microparticles

A quantity of OXC microparticles equivalent to 5 mg of OXC was subjected to a release rate study in a 500 mL of pH 7.4 phosphate buffer USP dissolution test paddle apparatus (apparatus II—Model: Tablet Dissolution Test Apparatus, Labindia) with autosampler. The rotation speed of paddle apparatus was 100 rpm. The release rate of OXC was also studied. Five milligrams of OXC was used in the study. A 5 mL sample solution was pipetted out at set time intervals. The same volume of dissolution medium was added to maintain the sink condition. The drug concentration in the sample was determined by knowing an absorbance of sample UV visible spectrophotometer at 221.4 nm.

Drug and microparticle-loaded suppositories

Suppositories containing OXC microparticles were placed into semi-permeable membrane tubes. The tubes were sealed at both ends by tying with thread to avoid leakage of the contents and then subjected to a release rate study in a pH 7.4 phosphate buffer using a paddle apparatus (apparatus II—Model: Tablet Dissolution Test Apparatus, Labindia) with an auto sampler. Five-millilitre sample solutions were pipette out at set time intervals. The same volume of dissolution medium was added each time to maintain the sink condition. The drug concentration in the sample was determined by knowing an absorbance of sample using UV visible spectrophotometer at 221.4 nm [24].

Stability study

OXC microparticles of the optimized formulation were packed into sealed glass vials and subjected to a temperature of 40 °C ± 2 °C and a relative humidity of 75% ± 5% for 90 days in a stability study carried out as per ICH guidelines. The physical properties and %EE of the microparticles were determined at 30, 60 and 90 days. A short-term stability study was carried out wherein suppositories individually wrapped in aluminium foil and packed in cardboard boxes were subjected to a temperature of 4 °C) for 6 weeks. Samples were taken after 6 weeks, and the physical appearance was noted and the drug content estimated [24, 29, 30].

Pharmacokinetic study

Prior to this experimental study, male Sprague–Dawley rats (body weights 350–400 g) were fasted for 36 h but with free access to water. The animals were placed in three groups with five animals in each group. The protocol for this investigation was approved by the Institutional Animal Ethics Committee in accordance with the

disciplinary principles and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA/IAEC/SPTM/P-43/2015).

The animals received a suspension of OXC through an oral tube. OXC suppositories and OXC microparticle-loaded suppositories were inserted into the rectum (with a dose equivalent to 5 mg of OXC/kg). The formulations were administered through different routes, after which 0.5 mL blood samples were collected through retro-orbital plexus of the rat at predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h). The blood samples were allowed to clot and then centrifuged for 10 min at 3000 rpm. The serum samples that were obtained were subjected to deep-freezing (− 20 °C) and HPLC analysis. A mixture of water, acetonitrile and 1 M ammonium acetate in the ratio 13:85:2 v/v/v was selected for the mobile phase. This mobile phase was buffered at pH 7 with acetic acid and then pumped at 1 mL/minute. Kromasil C18, 150*4.6 mm was used as a column. The detection wavelength was 235 nm [31, 32].

Assessment of pharmacokinetic analysis parameters

The Kinetica 5.0® software package was used to assess non-compartment pharmacokinetic analysis parameters such as C_{max}, Tmax and AUC.

$$\%F_{rel} = \frac{\text{AUC test}}{\text{AUC std}} \times 100 \quad (5)$$

where $\%F_{rel}$ = relative bioavailability in per cent, AUC_{test} = AUC after administration of OXC microparticle-loaded suppository (test), AUC_{std} = AUC after administration of OXC suppository (std).

Results

Formulation of OXC microparticles

OXC microparticles were developed using sodium alginate and Carbopol 971P using the emulsification–ionic gelation technique. A three-level, two-factor face-centred ($\alpha = 1$) central composite experimental design was used. The concentration of sodium alginate (X_1) and stirring speed (X_2) was designated independent processing variables. The dependent or response parameters designated were production yield, EE and mucoadhesion strength.

Evaluation of microparticles

Production yield

The production yield OXC microparticle was in the range from 79.55 ± 3.66% to 94.75 ± 4.21% (Table 2).

Mucoadhesion strength

Mucoadhesion strength was verified by studying the adhesion of the formulation to excised goat rectal mucosa. The results indicate that microparticles effectively adhere to rectal mucosa and will prevent ascending migration and drug entry into the portal vein (Table 2) [24].

Particle size analysis and zeta potential

The size range of the OXC microparticles is 764.04–894.13 µm (Fig. 1A). Zeta potential of OXC microparticles is found to be − 14.5 (Fig. 1B).

Scanning electron microscopy (SEM)

The SEM analysis showed that the OXC microparticles had a smooth but ruptured surface and a nearly spherical shape (Fig. 1C).

Differential scanning calorimetry (DSC)

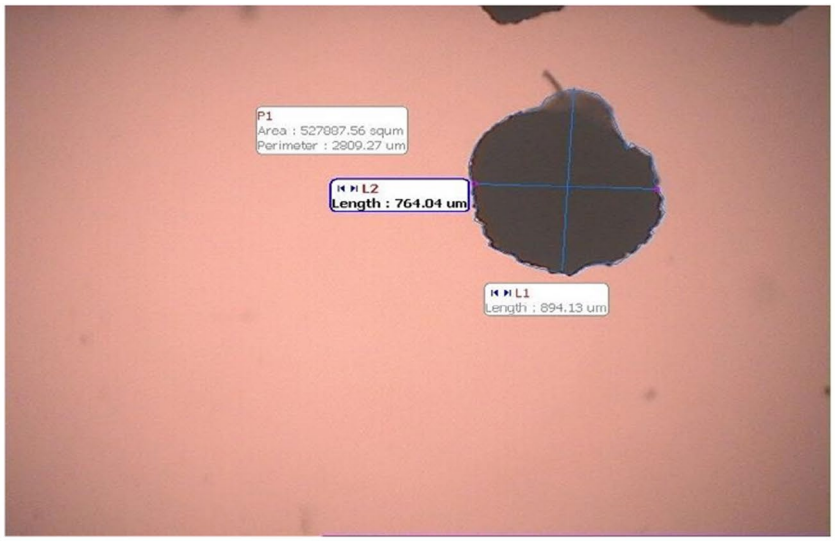
An endothermic peak was observed at 130 °C in the thermogram of OXC (Fig. 2).

FTIR

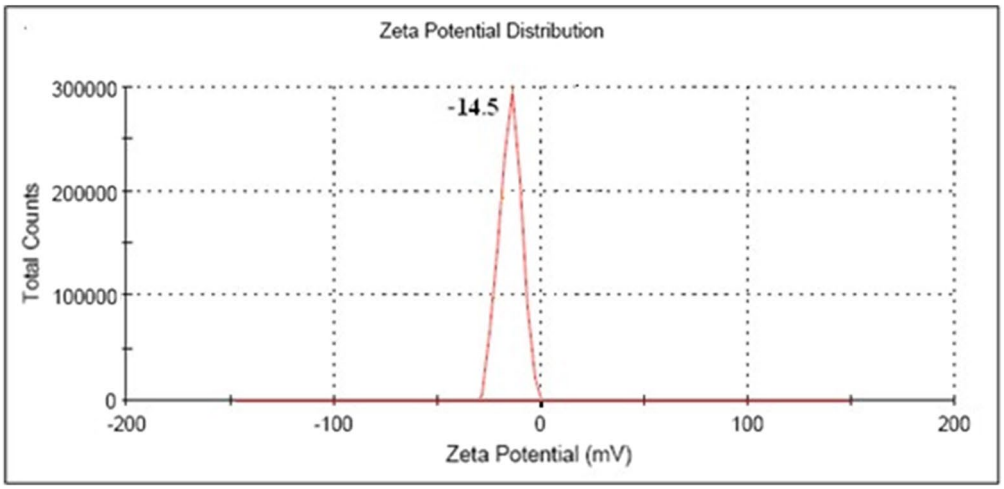
The IR spectrum of OXC exhibits absorption bands at 3315 cm^{-1}, 2928 cm^{-1}, 2845 cm^{-1}, 2460 cm^{-1}, 1754 cm^{-1}, 1622 cm^{-1}, 1350 cm^{-1}, 1246 cm^{-1}, 1209 cm^{-1}, 731 cm^{-1} in relation to O–H stretching, aromatic C–H stretching, aliphatic C–H stretching, alkyne stretching, C=O stretching, C=C stretching, C–N stretching, C–O–C stretching, O–H bending and C–Cl stretching, respectively. The spectrum of the blank microparticles had peaks at 2924 cm^{-1} due to the $-CH_2$ group, at 1629 cm^{-1} due to C=C stretching, at 1427 cm^{-1} due to the weak absorption band of the carboxyl group, at 1219 cm^{-1} due to O–H bending and at 1026 cm^{-1} due to the CH–OH group. The spectrum of the OXC microparticles had peaks at 1745 cm^{-1}, 1462 cm^{-1} and 1377 cm^{-1}, representing C=O stretching, aromatic C–H stretching and C–N stretching, respectively. The spectrum of Carbopol 971P IR had peaks at 1710 cm^{-1}, 1454 cm^{-1} and 1442 cm^{-1} which represent C=O stretching, aliphatic C–H bending and the C–C bond, respectively (Fig. 3) [33].

X-ray diffraction

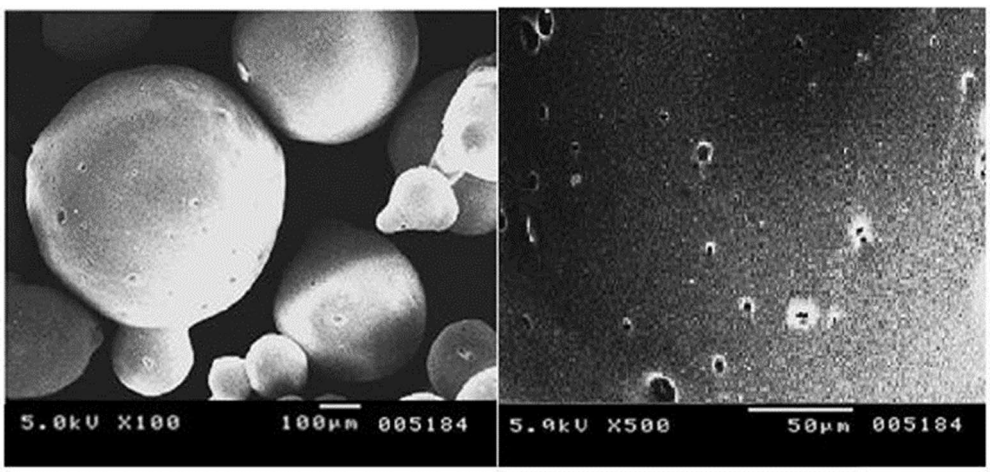
The X-ray diffraction pattern of OXC has sharp peaks at about 10.76°, 14.22°, 15.37°,15.90°, 17.31°, 19.09°, 25.15°, 26.17°, 26.61°, 26.88°, 28.23°, 30.68°, 32.90°, 34.12° 43.67°, 45.05° and 47.11°, which implies that it is crystalline in nature. Characteristic peaks between 2θ values of 10° and 50° were absent in the pattern of the blank microparticles. In the spectra of OXC microparticles there were some characteristic intense peaks at 2θ values of 33.07° and 45.75°, but their intensities were low (Fig. 4).



(A)



(B)



(C)

Fig. 1 Evaluation of OXC microparticles **A** optical microscopic image, **B** zeta potential distribution curve, **C** SEM photomicrograph

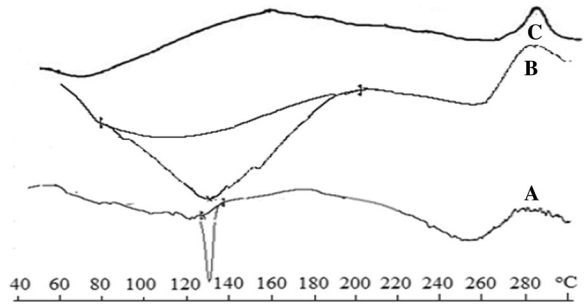


Fig. 2 DSC curve of **A** OXC, **B** OXC microparticles, **C** blank polymeric microparticles

Production of drug and microparticle loaded suppositories

Suppositories were produced using the fusion method. The dimensions of the micro-suppositories were 5 mm × 14 mm, and they weighed about 0.350–0.375 g.

Weight variation

The weight variation of the suppositories was in the acceptable range (< 5%).

Disintegration time

The disintegration time of the drug-loaded and microparticle-loaded suppositories was found to be in the range from 15.98 ± 0.56 min to 18.62 ± 0.41 min.

In vitro release study

The in vitro release study was performed in pH 7.4 phosphate buffer for 6 h. Pure drug (OXC) and OXC microparticles have shown % cumulative release 46.88 ± 0.63 and 90.41 ± 0.85, respectively, at the end of 6 h. Pure drug (OXC) loaded suppositories, optimized batch microparticles loaded suppositories 1 and 2 (prepared with different ratios of suppository bases) have shown the % cumulative release 67.60 ± 1.61, 94.04 ± 0.44 and 92.49 ± 1.52,

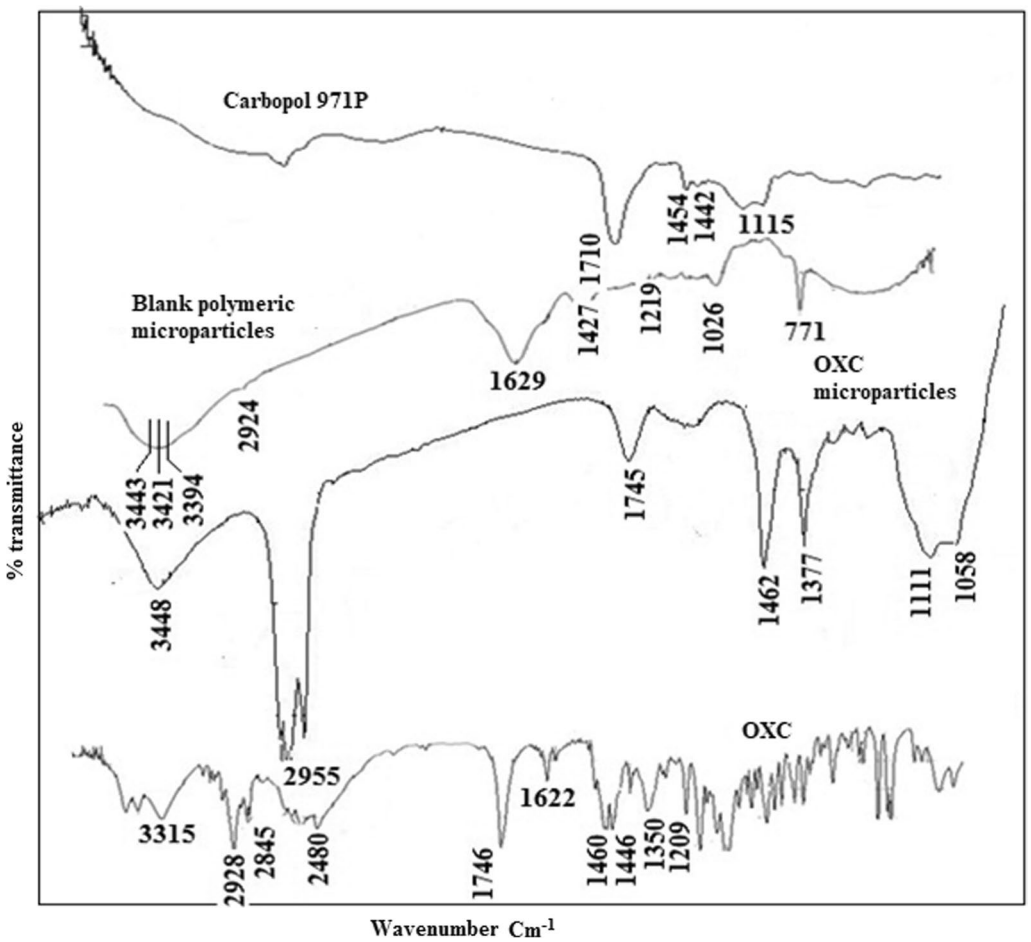


Fig. 3 IR Spectra of OXC, OXC microparticles, Blank polymeric microparticles, Carbopol 971P

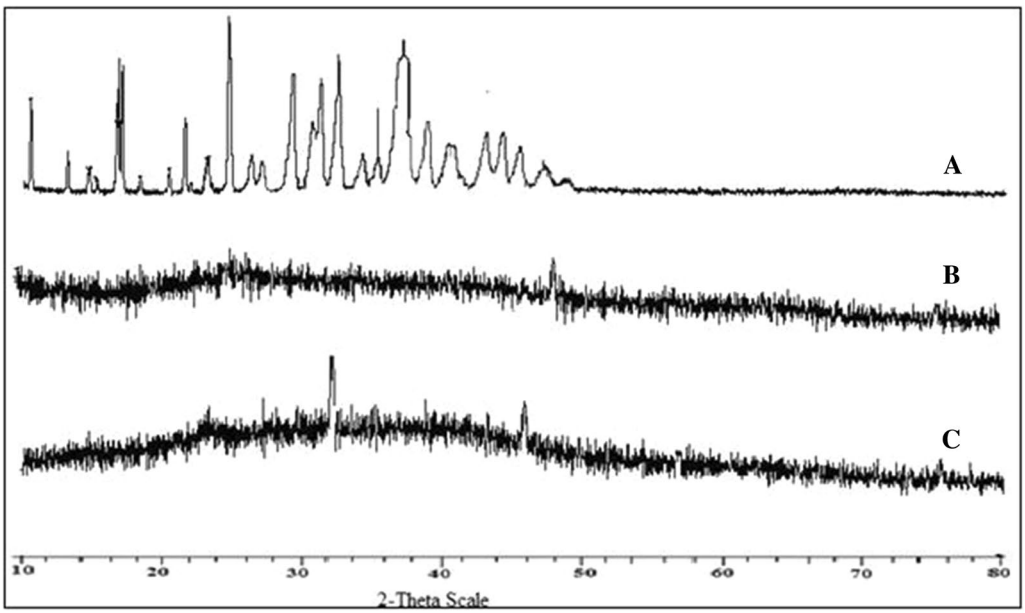


Fig. 4 X-ray diffractogram **A** oxybutynin chloride, **B** blank polymeric microparticles, **C** OXC microparticles

respectively. To ascertain the exact mechanism of drug release, the data from the in vitro release study were treated with different dissolution kinetic models (zero-order and first-order models, the Higuchi model and the Korsmeyer–Peppas model) [34].

Stability study

The stability of the optimized OXC microparticles was evaluated according to ICH guidelines over 3 months' storage at 40 °C and 75% RH. The drug content of the formulations was determined at intervals of 1 month. The entrapment efficiency of optimized batch of OXC microparticles after 3 months was found to be 91.97% ± 0.926.

Pharmacokinetic parameters of OXC formulations

The important pharmacokinetic parameters, such as the peak plasma concentration (C_{max}), time to reach peak plasma concentration (t_{max}) and AUC, are shown in Table 3.

Discussion

Different models were tested using the Design-Expert software application to assess the fitness of the responses generated during the formulation and evaluation of the OXC microparticles. The per cent production yield (Y1) was calculated using a linear model. The model's *F*-value of 21.67 indicates that it is statistically significant. Due to noise, there is only a 0.02 per cent chance that high *F*-value may occur. Model terms are significant if "Prob > *F*" is less than 0.0500. The "Lack of Fit *F*-value" of 3.45 indicates that the Lack of Fit is not significant in comparison to the pure errors.

%EE (Y_2) followed a linear model. The Model *F*-value of 13.86 implies the model is significant. There is only a 0.13% chance that an *F*-value this large could occur due to noise. Values of "Prob > *F*" less than 0.0500 indicate

Table 3 Pharmacokinetic parameters of oral and rectally administered OXC formulations ($n = 5$)

Parameters	OXC oral suspension	OXC loaded suppository	OXC microparticles loaded suppository
C_{max} (ng/mL)	1.911 ± 0.34	2.546 ± 0.89	3.472 ± 0.45
T_{max} (h)	2	3	3
AUC_{total} (ng h/mL)	21.34 ± 7.20	25.47 ± 5.674	38.58 ± 6.879
$AUC_{0-\infty}$ (ng h/mL)	22.62 ± 4.32	25.87 ± 5.16	44.94 ± 6.43
$F_{rel\ (\%)}$	–	–	173.72

Table 4 Summary regression analysis results

Models	R^2	Adjusted R^2	Predicted R^2	SD	% CV
Response Y_1 % production yield Linear model Eqh $Y_1 = 89.05 + 3.82X1 - 1.16X2$	0.8125	0.7750	0.6600	1.48	1.67
Response Y_2 %EE Linear model Eqh $Y_2 = 89.58 + 5.71 - 0.085X2$	0.7349	0.6819	0.4933	2.66	2.97
Response Y_3 % mucoadhesion strength 2FI $Y_3 = 89.69 + + 3.82\,X1 - 0.76\,X_2 - 1.28X1X2$	0.8942	0.8590	0.8207	1.13	1.26

Table 5 Results of ANOVA for measured response

Parameters	DF	SS	MS	*F*	Significance	Adeq. Prec	PRESS	*P*
Production yield								
Model	2	95.48	47.74	21.67	0.002	13.959	39.96	Significant
Residual	10	22.03	2.20	–	–	–	–	–
Total	12	117.51	49.94	–	–	–	–	–
Entrapment efficiency								
Model	2	195.67	97.83	13.86	0.0013	9.081	134.92	Significant
Residual	10	70.58	7.06	–	–	–	–	–
Total	12	266.25	104.89	–	–	–	–	–
Mucoadhesive strength								
Model	3	97.40	32.47	25.36	0.0001	16.235	19.53	Significant
Residual	9	11.52	1.28	–	–	–	–	–
Total	12	108.91	33.75	–	–	–	–	–

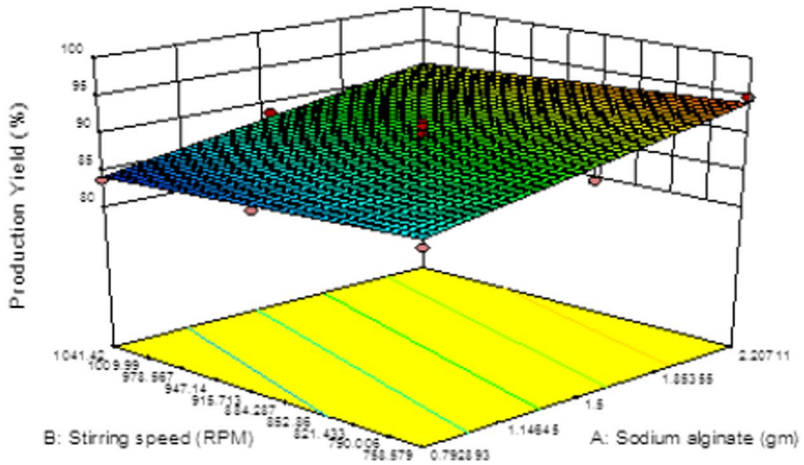
model terms are significant. The "Lack of Fit *F*-value" of 3.35 implies the Lack of Fit is not significant relative to the pure error.

% mucoadhesion strength (Y_3) followed a 2FI model. The Model *F*-value of 25.36 implies the model is significant. There is only a 0.01% chance that an *F*-value this large could occur due to noise. Values of "Prob > *F*" less than 0.0500 indicate model terms are significant. The "Lack of Fit *F*-value" of 0.88 implies the Lack of Fit is not significant relative to the pure error.

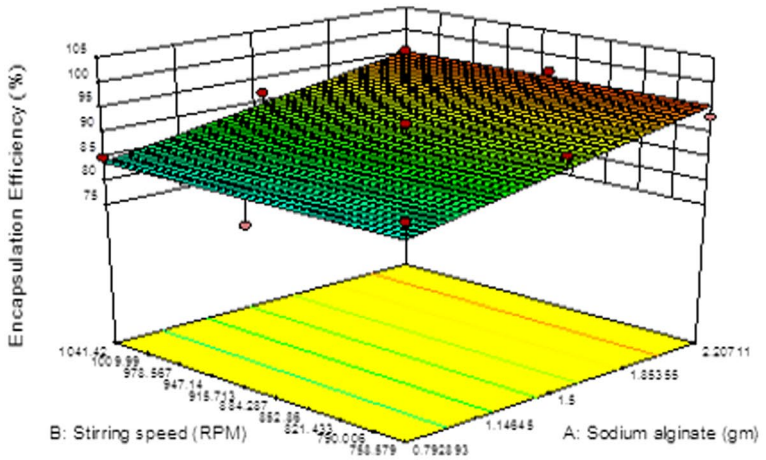
ANOVA was used to analyse polynomial models (Tables 4, 5) to assess the importance of the response surface models. The predicted R^2 was in reasonable agreement with the adjusted R^2 as the difference between them was less than 2 [35]. The reliability of the experiments was confirmed as the values of the coefficient of variance were relatively low.

The optimal independent variables, alginate concentrations and stirring speed were determined to be 2.21% and 758 rpm, respectively. Theoretically, the optimized OXC-loaded microparticles have a 94.024 ± 3.29% production yield, a 95.378 ± 2.44% EE and a 95.544 ± 4.32% mucoadhesion strength with 0.946 desirability. The OXC microparticles were formulated using design of experiments techniques. Formulation B2 was the optimized formulation. The experimental values of both the independent and dependent variables of formulation B2 are in accordance with the software-generated theoretical values [21, 22, 25]. Response surface plots were drawn to study the effects of the independent variables on the resultant responses, namely the % production yield, %EE and % mucoadhesion strength. Figure 5A shows a response surface plot that depicts the effects of alginate concentration and stirring speed on the % production yield. Figure 5B, C shows response surface plots depicting the effects of alginate concentration and stirring speed on % mucoadhesion and % entrapment efficiency strength, respectively.

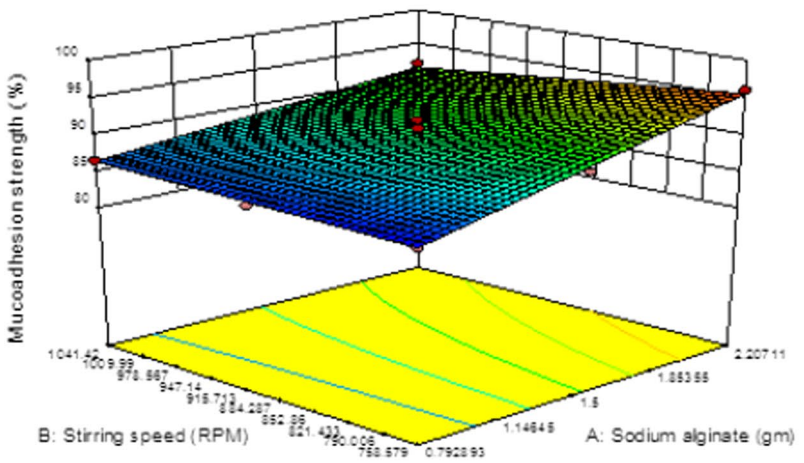
Sodium alginate and Carbopol 971P are widely used as mucoadhesive polymers. It was hypothesized that microparticles formulated using these polymers will exhibit a mucoadhesive ability. This was verified by studying the adhesion of the formulation to excised goat rectal



(A)



(B)



(C)

Fig. 5 Response surface plots for Independent variables to study the effect on **A** % production yield, **B** % entrapment efficiency, **C** mucoadhesion strength

mucosa. The results indicate that microparticles effectively adhere to rectal mucosa and will prevent ascending migration and drug entry into the portal vein (Table 2) [25].

The microparticles batch B2 was selected for further assessment on the basis of the primary evaluation. The dimensions of the extrusion device utilized the viscosity of the polymer have a big impact on the size of microparticles produced using the emulsification ionic gelation process. A greater polymer concentration will result in a more viscous polymer solution. As a result of the high viscosity, large droplets are formed during the addition of the drug–polymer mixture to the liquid paraffin, and larger microparticles are produced. The dimensions of the extrusion device also influence the microparticle size [24, 34]. The negative charge on the microparticles that an anionic polymer is present on the surface of the microparticles. The OXC-loaded microparticles had a higher zeta potential than the blank polymeric microparticles. This is due to interactions between anionic polymer groups and cationic drug moieties during microparticle formation. The charge on the microparticles may contribute mucoadhesive properties [24, 29].

The OXC microparticles exhibited a smooth but ruptured surface and a nearly spherical appearance, according to SEM examination (Fig. 1C). An endothermic peak was observed at 130 °C in the thermogram of OXC (Fig. 2). In the DSC thermogram of the OXC microparticles, the distinctive peak of OXC was seen to be broadening. The drug's dispersion in the polymer matrix was shown by the widening of the characteristic peak at the melting temperature. The polymer-drug interaction is confirmed by the FTIR investigation. The drug's molecular dispersion in the polymer matrix is indicated by the absence of OXC diffraction peaks as well as the reduction in peak intensity (Fig. 4).

Suppositories were produced using the fusion method. The drug or microparticles were dispersed in the molten suppository base. Different ratios of the base were used to produce suppositories. The micro-suppositories required for the in vivo pharmacokinetic study on rats were prepared using the fusion method and a specially designed mould. The weight variation of the suppositories was in the acceptable range (< 5%). During the disintegration test, it was observed that suppositories softened initially and then disintegrated completely into soft lumps. Suppositories with higher proportions of Poloxamer 407 disintegrated readily. Another base contained a combination of Poloxamer 188 and propylene glycol. Increasing the proportion of propylene glycol decreases the disintegration time of the suppository. Addition of propylene glycol promotes rapid disintegration of the suppository.

The poor dissolving profile of a drug might be due to its wettability. Drug dissolution is increased by better drug wettability, partial conversion of the crystalline form of the drug to an amorphous form, and improved solubilisation of the drug by molecular dispersion inside a water-loving polymer carrier matrix. The hydration of the polymer matrix happens when water molecules penetrate the hydrophilic polymer matrix, resulting in matrix swelling and drug release by diffusion through the dissolved polymer gel layer. The fast degradation of alginate is triggered by an exchange of Ca^{2+} ions attached to the carboxyl groups of the

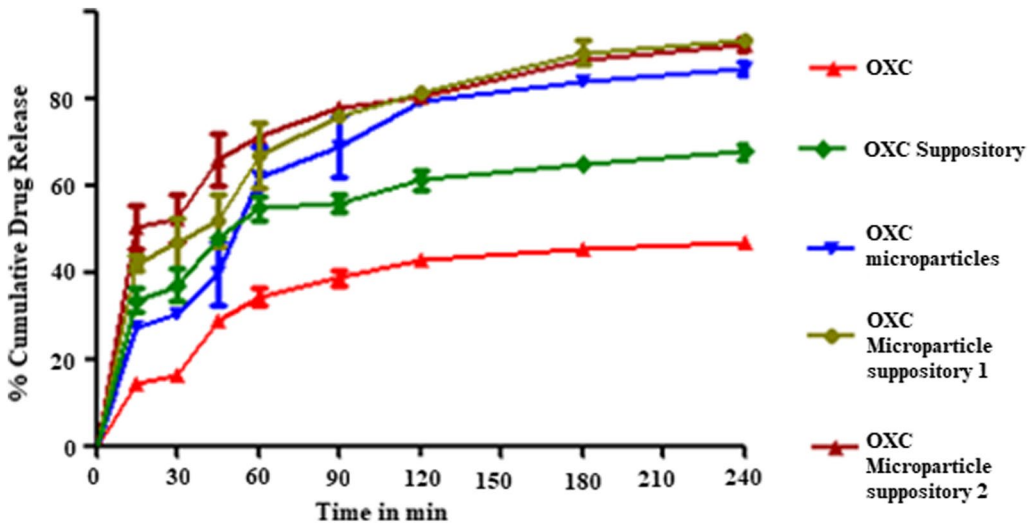


Fig. 6 Release profiles of OXC formulations

alginate with the phosphate ions of the medium during microparticle production. [36, 37]. The increase in the number of hydrophilic pores in the microparticles made it much easier for water to penetrate the microparticles, which facilitated the erosion of the swelling matrix, leading to drug's release from the microparticles. During the dissolving process, drug molecules diffuse through the gel layer that develops around the drug. [34, 38]. The microparticles when immersed in a dissolving media, such as a pH 7.4 phosphate buffer, the phosphate ions present in the phosphate buffer withdrew calcium from the microparticles. This caused the microparticles to expand, allowing the drug to escape. Calcium is extracted from microparticles in two ways. Ion exchange with sodium or phosphate ions in the dissolving liquid and electrostatic repulsions destroy the alginate network (Fig. 6).

To ascertain the exact mechanism of drug release, the data from the in vitro release study were treated with different dissolution kinetic models (zero-order and first-order models, the Higuchi model and the Korsmeyer–Peppas model). The values of the correlation coefficient (r^2) for the optimized OXC microparticle formulation B2 were 0.852 (zero-order model), 0.980 (first-order model), 0.9399 (Higuchi model) and 0.9175 (Korsmeyer–Peppas model). Considering correlation coefficient, the release profile of OXC microparticle formulation as well as OXC microparticle loaded suppositories could be best fitted to the first-order model. This relates to the sustained release of drug over a period of time. Subsequently, the OXC release rate remained dependent on the drug load remaining in the microparticle. The release exponent (n) and the kinetic constant (k) of the Korsmeyer–Peppas model were determined and found 0.4813 and 0.851, respectively, for formulation B_2. The n values of the OXC microparticle-loaded suppositories 1 and 2 were found to be 0.4633 and 0.5392, and the values of k were 1.224 and 1.4093, respectively. Higher values of the kinetic constant, k, represent higher dissolution rates, whereas the higher values of the release exponent ($0.45 < n < 0.89$) indicate a non-Fickian type drug release [39].

During the stability phase, the drug was retained within the microparticles. Because there was no noticeable change in the drug content, the optimized formulation of OXC microparticles was determined to be stable even under stress conditions of temperature and moisture. After 6 weeks of storage at a freezing temperature, the physical properties and pharmacological content of the suppositories remained intact.

Rectal administration of microparticle-loaded suppositories produced the maximum plasma concentration of OXC. The peak plasma concentration of the drug was produced by rectal formulations after more time compared with an oral formulation. This may be due to the low solubility of OXC at alkaline pH values.

Conclusion

OXC microparticles were successfully prepared using the emulsification–ionic gelation method, optimized and characterized. This is the first attempt to develop OXC microparticles loaded suppositories for rectal delivery. Poloxamer 188 and propylene glycol were used in combination as the suppository base. The suppositories were prepared using the fusion method. There was a substantial improvement in the bioavailability of OXC in the rat model when the drug was administered through a suppository compared to with an oral suspension. The microparticulate drug delivery system formulated in this research work will be advantageous for efficient absorption of OXC. This can be administered rectally, and apart from enhancing the bioavailability, its use can avoid hepatic metabolism or hepatic toxicity in relation to OXC. It can be concluded that the microparticle loaded suppository approach has a great deal of potential for developing a formulation that will provide an alternative route of administration to avoid hepatic metabolism or toxicity. This approach may enhance bioavailability of drugs with poor bioavailability due to hepatic metabolism or minimize hepatic metabolite induced toxicity.

Abbreviations

OXC: Oxybutynin chloride; DSC: Differential scanning calorimetry; XRPD: X-ray powder diffraction; SEM: Scanning electron microscopy; OAB: Overactive bladder; OROS: Osmotic-controlled release oral delivery system; USA: United States of America; $CaCl_2$: Calcium chloride; %EE: Per cent entrapment efficiency; nm: Nanometer; C_{max}: Peak plasma concentration; t_{max}: Time to reach peak plasma concentration; AUC: Area under the curve; FTIR: Fourier Transform Infrared Spectroscopy.

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Authors' contributions

APB performed the experiments and compile data for manuscript preparation. SPD helped in data analysis, and HSM conceived the idea design experiments, supervised the project and gave the final shape to the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data provided in the manuscript are available upon request.

Declarations

Ethics approval and consent to participate

The authors declare that this work involved animal study and the protocol for this investigation was approved by the Institutional Animal Ethics Committee in accordance with the disciplinary principles and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA/IAEC/SPTM/P-43/2018).

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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