Fixed dose combination of targeted release paediatric dry syrup containing ofloxacin and ornidazole using natural polymers: formulation and characterization

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Background
Tablets and capsules are difficult to swallow for children. Therefore, liquid formulations are preferred for administration to children. Most of the drugs have bitter taste and children do not support oral administration.

Aim
The aim of this study was to prepare a palatable paediatric dry syrup with ofloxacin and ornidazole as targeted released formulation using natural polymer and to mask the bitter taste of the drugs with suitable natural polymers.

Materials and methods
Extraction of the mucilaginous substances was carried out as per the method of extraction of pectin, followed by phytochemical examination, and physicochemical characterization was carried out. Lethal dose, 50% of the extracted natural polymer was determined as per OECD/OCDE guidelines 423. Compatibility study of natural polymer with formulation excipient was carried out. Preparation, characterization and evaluation of microspheres were carried out, including drug-release study in simulated gastric fluid and intestinal fluid, followed by preparation of dry syrup using suitable sweetening and flavouring agents.

Results
The release study states that the polymers released the drugs at the intestine (site of action) only and not (or little) at the stomach. Drug release from the optimum formulation F5 (Dillenia indica) was 99.97% for ofloxacin and 99.12% for ornidazole after 2.5?h in simulated intestinal fluid as compared with 8.3% for ofloxacin and 8.46% for ornidazole after 8?h in simulated gastric fluid. Microspheres of optimum formulation F5 D. indica were used for preparation of dry syrup. Statistical methods were utilized to analyse the results of different tests performed throughout the investigation.

Conclusion
Palatable dry syrup containing ofloxacin and ornidazole using natural polysaccharides was successfully prepared without any stability problem. It is expected to be effective/suitable for the paediatric patients with high compliance.

Keywords:
paediatric dry syrup, palatability; safe excipient, targeted release, taste masking

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Introduction

As ofloxacin and ornidazole are second-generation antibiotics and they are bitter in taste, their patient compliance is low as such. Therefore, we have tried to mask the bitter taste of these two drugs with microencapsulation using natural polymers, preparing a dry syrup with these microencapsulated drugs. Another objective of the formulation is to make the dry syrup palatable to increase paediatric compliance and to ensure safety of the paediatric population by making use of excipients that are safe for children.

Calculation of proper child dose is necessary before administration of drug in children. In addition to different dosing requirements, there is a need to use only selective dosage forms for the paediatric population. Dosage forms that permit fewer side effects of the drug and have a good paediatric compliance should be preferred – that is, a dosage form that is able to reduce the side effects of a drug by releasing the drug at the site of action only so that systemic side effects are minimized.

Paediatric patient compliance is a major problem, especially if the drugs have an unpleasant taste or odour. Antibiotics are among the most frequently prescribed medications in modern medicine. Most of

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them are bitter in taste and result in patient noncompliance while administering. Ofloxacin and ornidazole are second-generation antibiotics. Undesirable taste is one of the several important formulation problems that is encountered with certain drugs including these. Oral administration of bitter drugs with an acceptable degree of palatability is a key issue for healthcare providers, especially for paediatric patients. Thus, the problem of bitter and obnoxious taste of drug in paediatric and geriatric formulations is a challenge to the pharmacist in the present scenario [1–3]. Microencapsulation is a process of applying relatively thin coating to small particles of solid, droplets of liquid and dispersion. This is the method being widely used in pharmaceutical industries to mask the taste of bitter drugs as well as bioavailability. Coating agents used in microencapsulation are gelatin, povidone hydroxypropylmethylcellulose (HPMC), ethyl cellulose, carnauba wax, acrylics and shellac, or any other polymer [4]. Here, we used natural polymers such as sodium alginate (SA) and mucilaginous substances from Dillenia indica and Abelmoschus esculentus/lady's finger (LF) for the preparation of microspheres.

Another major consideration in case of paediatric formulation is the safety of this group of patients. The excipients used in the formulation must be safe enough for paediatric groups (http://www.americanpharmaceuticalreview.com/Featured-Articles/37186-Pediatric-Formulations) (http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm129477.pdf) [5–7] (http://apps.who.int/prequal/trainingresources/pq_pres/workshop_China2010/english/22/002-Excipients.pdf). Natural polymer extracted from DI/LF are regarded as safe as they are approved by the Food and Drug Administration (GRAS i.e. generally regarded as safe) as both are edible daily used vegetables. Considering all of these facts it can be concluded that an oral palatable liquid formulation containing excipients safe for children and capable of releasing drug only at the site of action will be the most suitable preparation for paediatric use. Although a large number of paediatric formulations are presently available in market, to the best of our knowledge, no one can satisfy all of these criteria together. In the present investigation, an attempt has been made to prepare a paediatric dry syrup formulation containing fixed dose combination of drugs ofloxacin and ornidazole using excipients safe for children, masking the bitter taste of both drugs and aiming for the release of drugs only at the site of action (intestine). Microspheres of both drugs were prepared for taste masking purpose. Natural polymers such as SA and mucilaginous substances from DI and A. esculentus/LF were used for preparation of microspheres.

Materials and methods

Materials

The fruits of DI and LF were procured from Namdang village of Sivasagar district of Assam (India). SA, calcium chloride and sodium carboxymethyl cellulose were purchased from Himedia Laboratories Pvt Ltd. (Vadhani Industrial Estate, L.B.S. Marg, Mumbai - 400 086, India) d-Sorbitol, sodium citrate, sodium benzoate, pure pectin (PP) and carbazole were purchased from Loba Chemie Ltd (Allahabad, Uttar Pradesh, India). Methanol, cyclohexane and dichloromethane were purchased from Rankem. Pancreatin and pepsin were purchased from Sigma Aldrich Corporation. Ofloxacin and ornidazole were obtained as gift samples from M/S Ozone Pharmaceutical Ltd (Guwahati, Assam, India). All chemicals were of analytical grade. Unless otherwise specified, all solutions were prepared in distilled water.

Methods

Extraction of mucilaginous substance from Dillenia indica and lady's finger

Extraction procedure followed was in accordance with that followed for extraction of pectin [8]. The fruits were collected, washed properly and cut into small pieces. It was then warmed at 55–60°C for 4 h with triple volume of distilled water on water bath. It was then cooled and passed through an ordinary tea mesh without pressing. The filtrate was diluted 1.5 times with water and kept undisturbed in a refrigerator for 12 h. The upper clear solution was decanted off and the sediments were rejected. The volume was reduced by heating on water bath at 55–60°C. The mass was then separated by precipitating with three times of acetone. This separated mass was then dried at 55–60°C in a hot air oven, powdered and stored in desiccators.

Phytochemical examination and physicochemical characterization of extracted natural polymer

The mucilage solutions were tested for the presence of carbohydrates by performing the preliminary standard tests, Molisch's test and Ruthenium red test. Dried powdered mucilage was studied for solubility, pH, weight loss on drying, swelling index, density and viscosity (http://www.pharmacopeia.cn/v29240/usp29nf24s0_m61250.html) [9–11]. Determination of pH was carried out using a pH metre (Eutech Instrument, pH Tutor; Riviera Glass Pvt Ltd, Mumbai, Maharashtra, India). The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. It was carried out using the hot air oven (NSW India, New Delhi, India)

dry method. Density and viscosity were measured with a Brookfield Viscometer (Brookfield Model LV).

Swelling index

The swelling of mucilaginous substances from DI and LF was observed in distilled water at 37°C. Initially, a measured quantity of dried mucilage was placed in each of three test tubes and distilled water was added. Thereafter, the test tubes were kept for 5–6 h maintaining the temperature at 37°C. The excess water was decanted and the weight of the swollen mucilages was measured. Subsequently, swelling ratio was determined using the following equation:

$$\text{Swelling ratio (Swt.\%)} = [(W_s - W_d)/W_d] \times 100\%,$$

where W_s is the weight of the swollen mucilage and W_d is the weight of the dried mucilage.

The average Swt.% was calculated and from this SD was calculated.

Determination of amount of pectin in mucilaginous substances using the carbazole test

0.1% (w/v) solution of mucilaginous substance was de-esterified by holding in 0.05 N NaOH for 30 min at 25–30°C. Afterwards, the solution was diluted to 0.002%. A volume of 12 ml of concentrated sulphuric acid was taken in a culture tube and cooled to about 3°C in ice bath, and 2 ml of diluted solution of mucilaginous substance was added to it. The contents were then mixed thoroughly and cooled again to 5°C in ice bath. The tube was then heated for 10 min in a boiling water bath and then cooled to 20°C. A volume of 1 ml of 0.15% carbazole reagent (prepared by taking weighed amount of carbazole in ethanol) was added, mixed thoroughly and allowed to stand at room temperature for 30 min Thereafter, the solution was analysed in a colorimetre at 520 nm and absorbance was recorded. Similarly, different solutions were prepared taking different amounts of mucilaginous substances and absorbances were recorded (http://pubs.acs.org/doi/abs/10.1021/ac60070a036). PP was taken as standard and different standard solutions of concentration varying from 0 to10 μg/ml were prepared and their absorbances were measured. A calibration curve was prepared by plotting absorbance versus concentration of PP.

Acute toxicity study of mucilaginous substances

The acute oral toxicity of the mucilaginous substances was determined by evaluating their lethal dose, 50% (LD_{50}) values (http://www.oecd.org/dataoecd/17/50/1948370.pdf) following the acute toxic class method described in the OECD guidelines (OECD/OCDE guidelines 423). Three healthy adult nulliparous and nonpregnant female rats were taken and subjected to overnight fasting. Each animal was then administered orally the solution of mucilaginous substance (2 ml/100 g body weight) at a dose of 300 mg/kg body weight and observed for 14 days. Depending upon the observations, the test was further proceeded according to guidelines, and LD_{50} was determined. The test was performed for SA, DI and LF separately.

Preparation of microspheres

Microspheres of ofloxacin and ornidazole were prepared using the ionic cross-linking technique [12] using varying proportions of SA and mucilaginous substances from DI and LF. Each formulation contained 57.14 mg of ofloxacin and 142.86 mg of ornidazole. The formulations are coded and mentioned in detail in Table 1; each contained different amounts of SA and mucilaginous substances from DI or LF.

The polymer solution was prepared by initially dissolving a definite amount of SA and mucilaginous

Table 1 Composition of different microsphere formulations

Formulation code	SA (mg)	DI (mg)	LF (mg)	Total polymer (mg)	Ofl (mg)	Orn (mg)	Total drug (mg)	Drug: polymer ratio
F1 (DI)	600	600	0	1200	57.14	142.86	200	1 : 6
F1 (LF)	600	0	600	1200	57.14	142.86	200	1 : 6
F2 (DI)	800	400	0	1200	57.14	142.86	200	1 : 6
F2 (LF)	800	0	400	1200	57.14	142.86	200	1 : 6
F3 (DI)	400	800	0	1200	57.14	142.86	200	1 : 6
F3 (LF)	400	0	800	1200	57.14	142.86	200	1 : 6
F4 (DI)	900	300	0	1200	57.14	142.86	200	1 : 6
F4 (LF)	900	0	300	1200	57.14	142.86	200	1 : 6
F5 (DI)	300	900	0	1200	57.14	142.86	200	1 : 6
F5 (LF)	300	0	900	1200	57.14	142.86	200	1 : 6
F6 (DI)	960	240	0	1200	57.14	142.86	200	1 : 6
F6 (LF)	960	0	240	1200	57.14	142.86	200	1 : 6

DI, *Dillenia indica*; LF, lady's finger; Olf, ofloxacin; Orn, ornidazole; SA, sodium alginate.

substance from DI/LF in distilled water (200 ml) using gentle heat. On complete dissolution, the weighed quantity of drugs was added and mixed thoroughly to make a homogeneous dispersion. The dispersion was added dropwise using a 20 G hypodermic needle fitted with a 10 ml syringe into 500 ml of 10% (w/v) of cross-linking agent (10 g of calcium chloride in 100 ml of distilled water) solution, being stirred at 100 rpm. The droplets from the dispersion instantaneously gelled into discrete drug–polymer–alginate matrices upon contact with the solution of cross-linking agent. The formed microspheres were further allowed to stir in the solution of cross-linking agent for a total of 3 h. On expiration, cross-linking agent was decanted and microspheres were washed with 2×500 ml volume of deionized water. The microspheres were dried at 50°C in a hot air oven.

Evaluation of microspheres

Drug entrapment efficiency (DEE): The amount of drug present in the microspheres was determined by extraction in methanol [13]. Fifty milligram of the crushed and powdered microsphere was taken and extracted in 50 ml of methanol and stirred overnight in a magnetic stirrer. The solution was filtered, and after suitable dilution the content of both drugs was determined spectrophotometrically at 287 and 319 nm, respectively, using simultaneous equations [14,15] obtained from the standard curves of the two drugs. The equations are as follows:

$$A_1 = 726C_1 + 150C_2 \text{ and} A_2 = 285C_1 + 382C_2,$$

where A_1 is the absorbance of ofloxacin, A_2 is the absorbance of ornidazole, C_1 is the per cent concentration of ofloxacin and C_2 is the per cent concentration of ornidazole. DEE was calculated as follows:

$$\text{DEE} = (\text{experimental drug content}/\text{heoretical drug content}) \times 100\%$$

The process was repeated for three different samples from each formulation and mean DEE±SD were calculated.

Particle size determination of microspheres

The particle size of the microspheres was determined using the microscopic method. The ocular micrometre was calibrated using a stage micrometre and each division of the ocular micrometre was measured in micrometre. One division of ocular micrometre is 100 divisions of stage micrometre – that is, 0.01 mm. On calibration, 1 division of ocular micrometre is 1.5 division of stage micrometre. Hence, it was calculated as follows:

$$1 \text{ division of ocular micrometer} = 1.5 \text{ division of stage} = 1.5 \times 0.01 \text{ mm} = 15\ \mu\text{m}.$$

The size of the microspheres was measured, and mean ±SD were calculated.

In-vitro drug-release study

The in-vitro drug-release study was carried out in a basket type dissolution test apparatus using intestinal fluid (IF) [simulated testing fluid (TS) of united states pharmacopoeia (USP)] and gastric fluid (GF) (simulated TS of USP) (http://www.pharmacopeia.cn/v29240/usp29nf24s0_m61250.html) as dissolution medium, adding 4 g of Tween 80 in dissolution medium to solubilize the drugs released (as the solubility of the drugs in the selected media was determined earlier). Volume of dissolution medium was 900 ml and bath temperature was maintained at 37±1°C throughout the study. Basket speed was adjusted to 100 rpm. At every interval of 0.5 h, 1 ml of sample was withdrawn and diluted 10 times with replacement of 1 ml of fresh medium and analysed for drug content using a UV visible spectrophotometre (UV-1800; Shimadzu; Shimadzu Corporation, Kyoto, Japan) at 287 and 319 nm. Cumulative percentage drug release was calculated using simultaneous equations obtained from the standard curves of the two drugs (simultaneous equations were described during determination of DEE). Thereafter, cumulative per cent release versus time curve was plotted [13] (http://www.pharmacopeia.cn/v29240/usp29nf24s0_ris1s126.html).

Selection of the optimum formulation based on observation of drug release and drug entrapment efficiency

One formulation was selected as optimum formulation, which showed the fastest release of the drugs from microspheres and showed good DEE

Release kinetics of the optimum formulation

Data obtained from the released study were fitted to various kinetic equations to find the pharmacokinetic model followed by the drug when released from the microspheres [16]. The kinetic models used were zero order, first order, Higuchi and Korsmeyer Peppas model.

Study of the shape and surface morphology of the optimum formulation using scanning electron microscopy

Scanning electron microscopy (SEM; Jeol JSM-6390, Tokyo, Japan) study was performed to study the shape and surface morphology of the optimum formulation. Coating of samples is required in the

field of electron microscopy to enable or improve the imaging of samples. Creating a conductive layer of metal on the sample inhibits charging, reduces thermal damage and improves the secondary electron signal required for topographic examination in the SEM. The coating technique used depends on the resolution and application. For the final sample preparation, one side was polished and etched (apply coating if required).

Polymer–drug compatibility study of the optimum formulation using Fourier transform infrared spectroscopy

Drug–polymer interactions were studied using Fourier transform infrared spectroscopy (FTIR; BRUCKER Alpha E, Ettlingen, Germany) spectroscopy. Two spectra were recorded, one for the physical mixture of ofloxacin, ornidazole, SA and mucilaginous substance from DI and another for their formulation. The scanning range was 450–4000/cm. The spectra were compared through the instrument to know if any interaction between the drugs and the polymers occurred.

Preparation of test formulations of dry syrup

Different test formulations were prepared taking F5 (DI) microspheres (optimized formulation) and excipients, as shown in Table 2.

The amount of microspheres was calculated considering DEE for F5 (DI) and Young's formula for child dose.

$$\text{Child dose} = \text{age (years)}/(\text{age} + 12) \times \text{adult dose}.$$

Age was taken to be 2 years for calculation.

Evaluation of dry syrup/reconstitutable suspension

The prepared dry syrup was evaluated in terms of pH and specific gravity, viscosity, redispersibility, sedimentation volume and leakage of the drugs from suspended microspheres [17–19]. Sedimentation volume (F) was measured at selected time intervals during storage without agitation for a period of 10 days and was recorded in terms of the ratio of the ultimate settled height (H_u) to the original height (H_o), as expressed by the following equation:

$$F = H_u/H_o.$$

Besides, an aliquot of 10 ml was drawn from each of the reconstituted suspensions at the end of 1st, 2nd, 4th, 6th and 10th day for determination of leakage of drug from suspended microspheres during storage. The aliquot was filtered and the microspheres were washed with water to remove the suspending vehicle and then dried in an oven at 37°C for 24 h. Dried

Table 2 Composition of various test formulations of reconstitutable suspension

Formulation code	Microsphere (%)	Sodium CMC (%)	D-Sorbitol (%)	Citric acid (%)	Sodium citrate (%)	Sodium benzoate (%)	Sunset yellow FCF (%)	Orange flavour
F1	26.54	1.6	25	1.44	0.72	0.2	0.015	q.s.
F2	26.54	2.0	25	1.44	0.72	0.2	0.015	q.s.
F3	26.54	2.4	25	1.44	0.72	0.2	0.015	q.s.
F4	26.54	2.6	25	1.44	0.72	0.2	0.015	q.s.
F5	26.54	2.8	25	1.44	0.72	0.2	0.015	q.s.

CMC, sodium carboxymethyl cellulose; FCF, for coloring food.

Table 3 Identification and characterization of mucilaginous substance from *Dillenia indica* and lady's finger

Tests	Results
Molisch's test (http://pubs.acs.org/doi/abs/10.1021/ac60070a036)	Purple ring appeared at the interface between the acid and test solutions of both DI and LF indicating the presence of carbohydrate.
Stiff gel test (http://pubs.acs.org/doi/abs/10.1021/ac60070a036)	A stiff gel was formed after cooling, indicating the presence of pectin
Test with alcohol (http://pubs.acs.org/doi/abs/10.1021/ac60070a036)	A translucent gelatinous precipitate was formed (characteristic of pectin)
Test with sodium hydroxide (http://pubs.acs.org/doi/abs/10.1021/ac60070a036)	A semigel was formed, indicating the presence of pectin
Solubility test (http://pubs.acs.org/doi/abs/10.1021/ac60070a036)	Insoluble in organic solvents like n hexane, chloroform, diethyl ether, ethanol 95 %, dichloromethane, ethyl acetate.Soluble (1 in 20 parts) in aqueous solvents such as water, 0.1 N NaOH and 0.1 N HCl.

DI, *Dillenia indica*; LF, lady's finger.

microspheres were crushed and dissolved in methanol. The dissolved drug amount was measured spectrophotometrically at 287 and 319 nm, respectively. Putting the absorbance values in the simultaneous equations (simultaneous equations were described during determination of DEE), the amount of drug present in the microspheres was determined.

Stability study of reconstitutable suspension

Reconstitutable suspensions (three samples of each formulation) were stored in an airtight amber-coloured container for 36 days at 45, 25 and 4°C and reconstituted by adding water, and then some tests were performed. Time for reconstitution was determined for each formulation. It was determined by adding water and observing the time required to make a proper suspension by shaking the container vigorously. Reconstituted suspensions were observed for probable change in colour, reduction in flavour, reduction in sweetness and for probable change in pH during first 10 days following reconstitution.

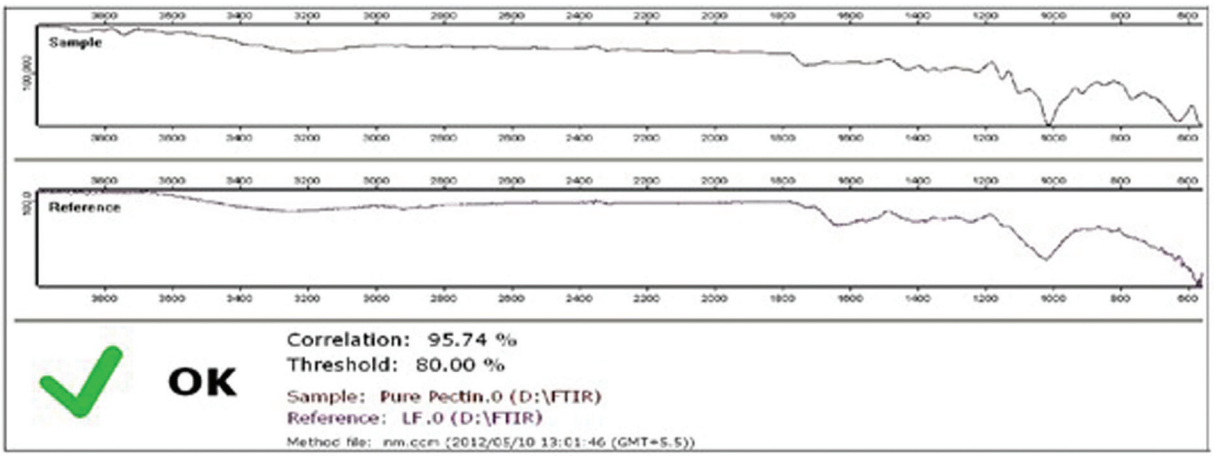
Selection of optimum formulation

One formulation was selected as optimum formulation based upon the results of various tests performed on the test formulations of reconstitutable suspension.

Statistical analyses

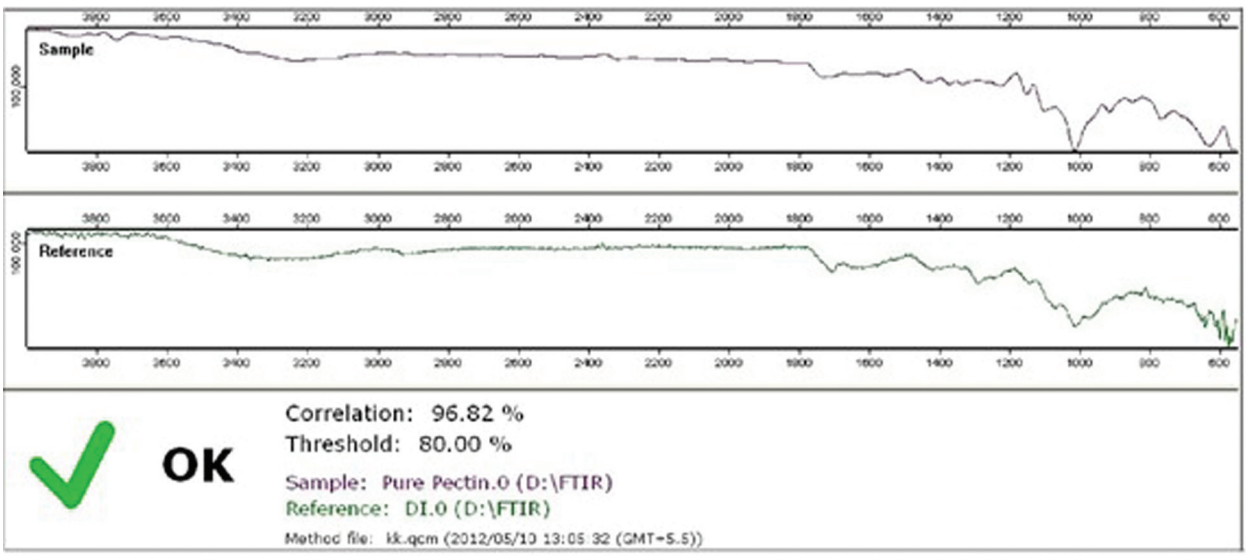
Per cent content of pectin in mucilaginous substance was determined, and their mean±SD were calculated. The process of DEE was repeated for three different samples from each formulation, and mean DEE±SD

Figure 1



Fourier transform infrared spectroscopy (FTIR) study of pure pectin and lady's finger.

Figure 2



Fourier transform infrared spectroscopy study of pure pectin and *Dillenia indica*.

were calculated. Data obtained from the release study of the microsphere was fitted to various kinetic equations to find the pharmacokinetic model followed by the drug when released from the microspheres. The kinetic models used were zero order, First order, Higuchi and Korsmeyer Peppas model. The size of the microspheres was measured, and mean±SD were calculated.

Results and discussion

Phytochemical examination and physicochemical characterization of extracted natural polymer

Identification and characterization of extracted natural polymer (LF, DI) are detailed in Table 3.

Fourier transform infrared spectroscopy analysis

The FTIR spectrum of PP and that of mucilaginous substance from LF showed 95.74% correlation, and the spectrum of PP and mucilaginous substance from DI showed a correlation of 96.82% (Figs 1 and 2). This result reveals that the mucilaginous substances from LF and DI contain pectin as one of their constituents.

Table 4 Drug entrapment efficiency and particle size of microspheres

Formulation code	DEE (%)	Particle size (μm)
F1 (DI)	82±1.05	239±2.15
F1 (LF)	86±0.58	252±2.76
F2 (DI)	88±1.44	245±3.28
F2 (LF)	80±2.45	234±2.89
F3 (DI)	89±0.34	241±4.33
F3 (LF)	87±1.18	228±2
F4 (DI)	79±2.14	249±4.12
F4 (LF)	84±3.12	239±1.88
F5 (DI)	89±1.98	231±2.34
F5 (LF)	88±0.98	229±1.5

DEE, drug entrapment efficiency; DI, *Dillenia indica*; LF, lady's finger. Values are represented as mean±SD (*n*=3).

Swelling ratio of mucilaginous substances

The study confirmed that mucilaginous substances from both DI and LF swell in distilled water at 37°C. Swelling ratio of DI was found to be 350 ±2.55% and that of LF was 1320.2±4.96%. Each value represents mean±SD (n=3).

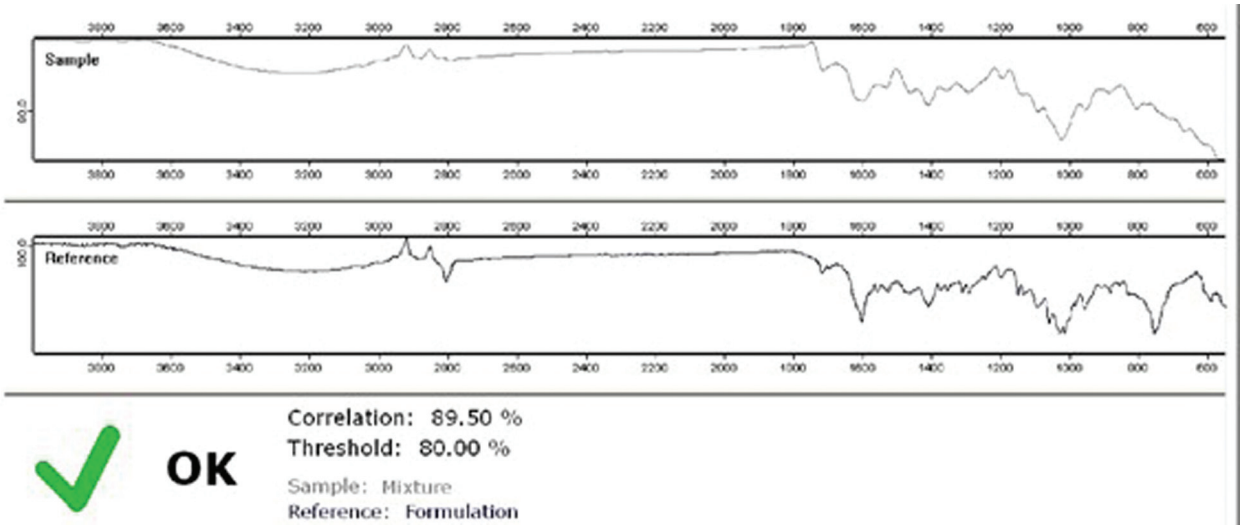
Determination of amount of pectin in mucilaginous substances using the carbazole test

The equation for calibration curve was y=0.046x +0.0016 and r^2=0.9982. From this standard curve, actual concentrations of pectin in different solutions of mucilaginous substance were calculated. Thereafter, per cent content of pectin, their mean±SD were calculated. From the quantitative carbazole test it was found that per cent content of pectin in mucilaginous substance from LF was 48.15±1.88% and that of DI was 52.95±2.1%. Each value represents mean±SD (n=3). From the study it can be concluded that both DI and LF are very rich in pectin content.

Acute toxicity study of mucilaginous substances

For all substances (SA, DI and LF), the LD_{50} value was found to be more than 5000 mg/kg body weight [globally harmonized system of classification and labelling of chemicals (GHS) category 5 or unclassified] (http://www.oecd.org/dataoecd/17/50/1948370.pdf). These values of LD_{50} indicate that the

Figure 3



Drug release from microsphere (lady's finger) at gastric fluid. Of, ofloxacin; Or, ornidazole.

materials may have toxicity only above regulatory limit doses, and hence they are quite safe for use in humans. Thus, these natural polymers could be safely used as excipients in paediatric formulations.

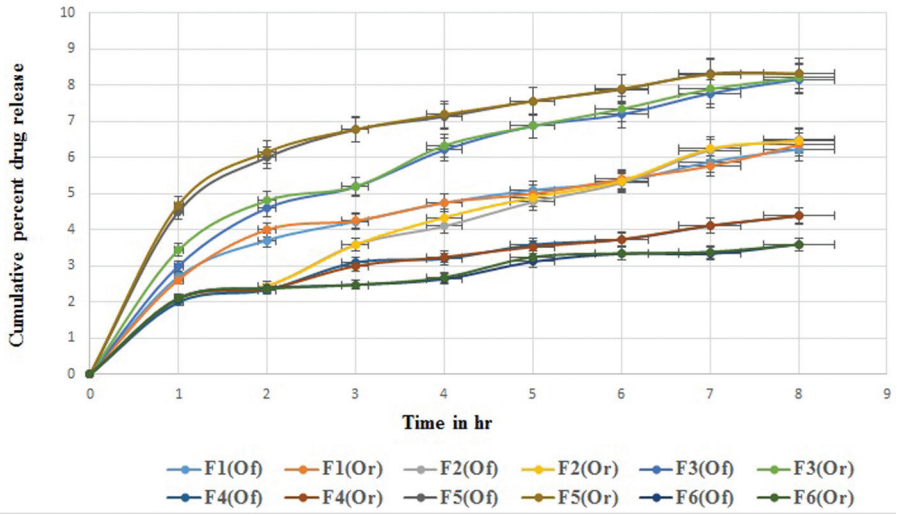
Drug entrapment efficiency and particle size of microspheres

All formulations showed almost uniform DEE. It ranged from a minimum of 75±1.77% to a maximum of 89±1.98%. However, maximum DEE was seen in case of F5 (DI) and F3 (DI). Particle size of the microspheres ranged from a minimum of 222±3.24 µm to a maximum of 252±2.76 µm. Details are reported in Table 4.

In-vitro drug-release study of microspheres

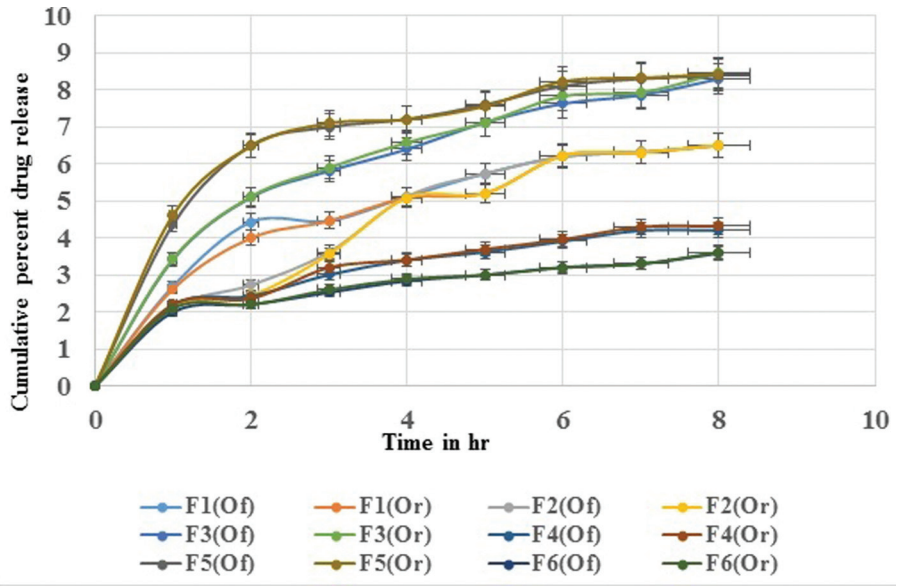
The drug-release study revealed that the release of drugs from all microspheres occurred at both IF and GF. However, complete and rapid release was seen only in IF. In contrast, release occurred much slowly and incompletely in GF. It might be due to the fact that pectin (which is the main constituent of mucilaginous substances from DI and LF) and SA release drugs at intestinal pH (pH 6.8) and not (or less) at gastric pH (pH 1.2). Hence, this type of formulation is suitable for targeted delivery of drugs in the intestine. Again, it was seen that formulations containing a higher amount of pectin than SA showed comparatively rapid release of drugs. However,

Figure 4



Drug release from microsphere (*Dillenia indica*) at gastric fluid. Of, ofloxacin; Or, ornidazole.

Figure 5



Drug release from microsphere (lady's finger) at intestinal fluid. Of, ofloxacin; Or, ornidazole.

formulations richer in SA content than in pectin content showed a delayed release pattern of drugs. Thus, the study led to the conclusion that a formulation having high pectin content and less SA content undergoes rapid and complete release of drugs at IF (pH 6.8), and hence this type of formulation may be suitable as immediate release dose form to deliver drugs at intestine (Figs 3–6).

Selection of optimum formulation based on observations of drug release and drug entrapment efficiency

The formulation F5 (DI) was selected as optimum formulation because it showed the fastest and complete release of the drugs from microspheres at intestinal condition and also showed excellent DEE. It released 100% of drugs within 2.5 h at pH 6.8 and showed highest DEE of 89±1.98%.

Release kinetics of the optimum formulation F5 (*Dillenia indica*)

Kinetic modelling of drug-release profile of the optimum formulation F5 (Dillenia indica) in intestinal fluid (pH 6.8)

The *R* values of the Higuchi model were found to be 0.9495 for release of ofloxacin and 0.9484 for that of ornidazole. The values were close to 1. The diffusion coefficient (*n*) values were found to be 0.2066 for release of ofloxacin and 0.2019 for that of ornidazole. As the R values of Higuchi matrix were close to 1, the drug release followed matrix diffusion kinetics. Again, the Higuchi plot showed linearity, indicating that diffusion was the main mechanism of drug release from the microspheres. Furthermore, the observed diffusion coefficient values are indicative of the fact that the drug release from the formulation follows Fickian type of diffusion.

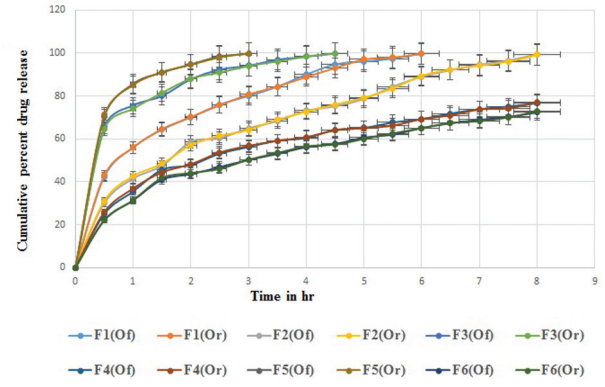
Study of the shape and surface morphology of the optimum formulation using scanning electron microscopy

From the SEM study it was observed that the microspheres were almost spherical in shape. Observation of the surface of the drug-loaded microsphere showed the presence of particulate matter on the surface. These might be drug particles attached on the surface of the microspheres. Drug particles might also be entrapped inside the microspheres (Fig. 7).

Polymer–drug compatibility study of F5 (Dillenia indica)

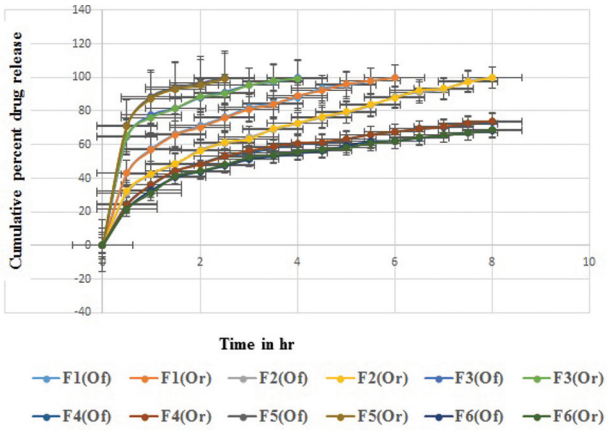
Comparison of FTIR spectrum of physical mixture of ofloxacin, ornidazole, SA and mucilaginous

Figure 6



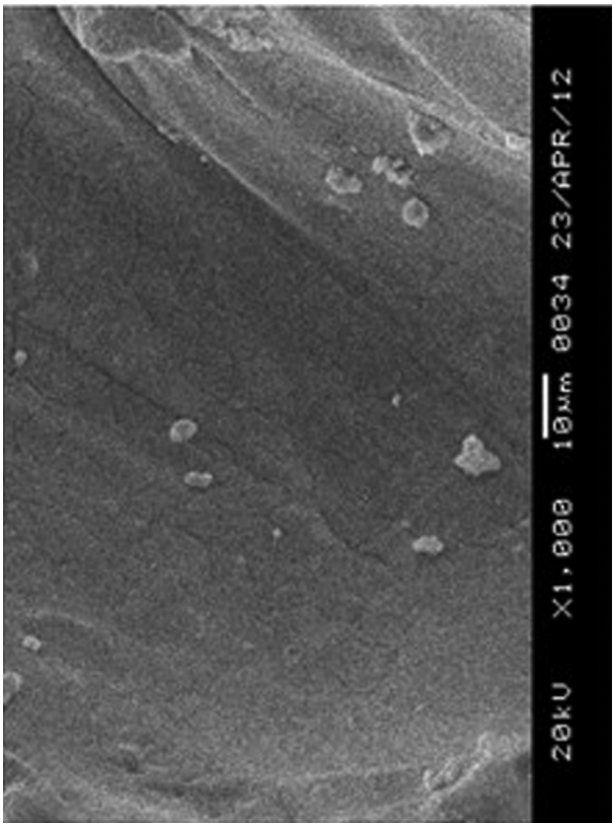
Drug release from microsphere (*Dillenia indica*) at intestinal fluid. Of, ofloxacin; Or, ornidazole.

Figure 7



Scanning electron microscopy image of the surface of a drug-loaded microsphere.

Figure 8



Fourier transform infrared spectroscopy study of physical mixture of microsphere and formulation (microsphere).

substance from DI with that of their formulation F5 (DI) showed a correlation of 89.50%, indicating that no prominent interaction occurred between the drugs and the polymers in formulation. Thus, both were supposed to be compatible. Figure 8 is detailed about it.

Measurement of pH, specific gravity, redispersibility, and viscosity of test formulations of dry syrup

Results are reported in Table 5. pH of all five formulations was almost same. Specific gravity of F5 was highest and that of F1 was lowest. F5 was found to be more viscous among the five. Redispersibility was least in F5 and more in F1. Thus, F5 more easily redispersible and stable.

Determination of the sedimentation volume

From the study it was found that the formulation F5 was the most stable suspension among all as its rate of sedimentation was the lowest.

Determination of the leakage of drugs from suspended microspheres

From the leakage study it was observed that a little release of drugs from the microspheres took place within the formulation. Leakage of drugs from the microspheres does not cause reduction in amount of drugs in each dose, as drug is released to the surrounding medium. However, it may affect the taste of the preparation to a little extent (Fig. 9).

Stability study of reconstitutable suspension

Reconstitutable suspensions were stored in airtight amber-coloured containers for 36 days at 45, 25 and 4°C and reconstituted by adding water, and different tests were performed. Time for reconstitution ranged from 2.1±0.516 to 2.5 ±0.236 min (mean±SD) for all reconstitutable suspensions stored at 4 and 25°C. The formulations stored at 45°C required 3.25±1.45–4.36±0.86 min for reconstitution.

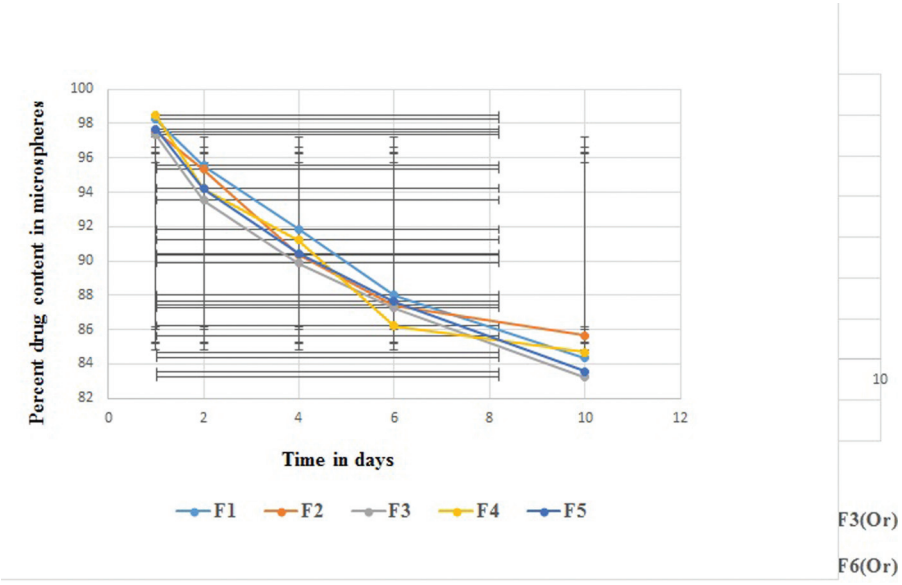
Reconstituted suspensions were observed for probable change in colour for 10 days, but no change in colour was noticed. All reconstituted suspensions maintained their flavour during the period of 10 days. Reconstituted suspensions showed very little reduction in sweet taste during the period of 10 days. Thus, the formulations showed sound stability.

Table 5 Measurement of pH, viscosity, specific gravity and redispersibility

Formulation code	pH	Viscosity at 25°C (Cp)	Specific gravity at 25°C (g/ml)	Redispersibility[a]
F1	3.55±0.045	340±3.22	1.323±0.034	6.33±0.577 to 7.00±0.00
F2	3.54±0.021	752±2.14	1.327±0.025	6.00±0.00 to 6.66±0.577
F3	3.54±0.016	1150±2.03	1.331±0.031	5.33±0.577 to 6.00±0.00
F4	3.55±0.022	1520±2.26	1.335±0.023	3.66±0.577 to 4.33±0.577
F5	3.56±0.034	1892±2.51	1.340±0.029	3.00±0.00 to 3.33±0.577

Cp, centipoises. Value are represented as mean±SD (n=3). [a]The maximum and the minimum mean±SD value observed during the period of 10 days are presented.

Figure 9



Leakage study of optimum formulation. Or, ornidazole.

Table 6 Change in pH after 10 days

Observed temperature (°C)	pH change
4	3.54±0.02 to 3.54±0.11
25	3.53±0.28 to 3.54±0.045
45	3.53±0.28 to 3.54±0.045

Change in pH

pH after 10 days (observed maximum and minimum values are expressed as mean±SD) is expressed in Table 6. No notable change in pH occurred, which is the sign of good stability.

Selection of an optimum formulation

The formulation F5 was selected as optimum formulation as it showed a lowest rate of sedimentation, was easily dispersible and showed good stability during storage.

Conclusion

Palatable dry syrup containing ofloxacin and ornidazole using natural polysaccharides was successfully prepared without any stability problem. It is expected to be effective/suitable for the paediatric patient with high compliance.

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Conflicts of interest

There are no conflicts of interest.

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