Development and evaluation of Bio-Flexy films using a novel biopolymer from *Ananas cosmosus* loaded with nanosized tiagabine

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Background
Tiagabine, an anticonvulsant drug, has $t_{1/2}$: 7–9 h (low); protein binding: 96%; water solubility: 22 mg/l; it also acts as a selective GABA reuptake inhibitor. Side effects include abdominal pain, pharyngitis, suicidal thoughts, and sudden unexpected death.
Aim
The aim of this work was to formulate nanosized Bio-Flexy films using a novel biopolymer isolated from *Ananas cosmosus* fruit pulp containing tiagabine as a model drug. The soft palate drug delivery helps bypass first-pass metabolism in the liver and presystemic elimination in the gastrointestinal tract is avoided.
The biopolymer isolated from *A. cosmosus* was used to prepare Bio-Flexy films because of its biodegradability and biocompatibility, and because it is nontoxic and nonirritant, and nonreactive on soft palatal surfaces. Physicochemical characterization of the biopolymer showed its inbuilt property as film forming ability and mucoadhesivity which was confirmed, screened and authenticated.
Materials and methods
Bio-Flexy films were prepared using the solvent casting technique. The drug to polymer ratio was chosen at five levels for *A. cosmosus* FPA1–FPA5 with varying ratios of biopolymer from 1 to 10 and 1% of nanosized tiagabine and compared with sodium carboxyl methyl cellulose standard films. Bio-Flexy films were evaluated for thickness, surface pH, weight uniformity, folding endurance, in-vitro release, and stability studies.
Results
The percentage yield of the *A. cosmosus* biopolymer was found to be 0.972± 0.008%. The thickness of the formulated Bio-Flexy films ranged from 0.041 to 0.091 mm, the folding endurance was 65–95, the surface pH was 7.01±0.02 to 7.01±0.01, and weight uniformity was 0.001±0.02 to 0.032±0.01.
Conclusion
On the basis of all the above-mentioned evaluation parameters, formulation FPA3 [containing Tiagabine: *Ananas cosmosus* biopolymer (1:5)] was selected as the best film as results of an in-vitro release study showed prolonged durationof 48 h in prolonged manner. The best film showed R^2=0.969 release pattern by Peppas–Korsmeyer, as the Best Fit model, followed by anomalous transport release mechanism, which was confirmed by using BITS Software 1.12. Stability study showed stable Bio-Flexy films with no significant change in physical appearance and stable pH. Prepared formulations of tiagabine-loaded Bio-Flexy films are suitable for soft palatal delivery.

Keywords:
Ananas cosmosus, Bio-Flexy films, biopolymer, nanosized tiagabine, soft palatal delivery

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Introduction

Soft palate (velum) is the soft tissue that is suspended from the posterior border of the hard palate. It is part of the oral mucosa, protects the nasal passage, does not contain bone, and facilitates improved absorption into the blood stream compared with oral administration to the gastrointestinal tract. It is a more convenient means of drug administration [1]. It has promising nonkeratinized histology with unique thickness. The surface area of the oral mucosa (200 cm^2) is relatively small compared with the gastrointestinal tract (350 000 cm^2) and skin (20 000 cm^2).

Blood–brain barrier restricts the entry of most drugs; thus, brain targeting of drugs to a specific site but also to retain it for the desired duration to elicit a

pharmacological action is a challenging task. The therapeutic potential of many drugs can be improved. The trans-soft palatal route offers a novel drug-delivery platform for the systemic delivery of drugs for brain targeting (http://en.wikipedia.org/wiki/soft palate.html). It is well suited for retentive drug delivery and appears to be acceptable to the patient. With the right dosage form design andformulation, the permeability and the local environment of the mucosa can be controlled and manipulated to accommodatedrug permeation. Nanosized drug loaded mucoadhesive bio-flexy films formulations were designed that can offer significant permeability and sustainability in drug release to produce sustained drug action. Palatal drug delivery is a promising area for the systemic delivery of orally inefficient drugs as well as a feasible and attractive alternative for noninvasive delivery of potent peptide and protein drug molecules. Blood supply to the soft palate is through the middle meningeal artery; the accessory meningeal artery; the greater palatine branch of the maxillary artery; the ascending palatine branch of the facial artery; and the ascending pharyngeal artery (http://en.wikipedia.org/wiki/soft palate.html). The soft palate is innervated by the mandibular branch of the trigeminal nerve (cranial nerve V); the lesser palatine nerve; the greater palatine nerve; the nasopalatine nerve; the glossopharyngeal nerve; and motor nerves. When drug is administered in a nanosized form by this route, then, through the interneural and intraneural pathway, it can directly reach the brain through the trigeminal nerve that connects the soft palate to the brain (http://en.wikipedia.org/wiki/nerve supply.html).

Epilepsy ranks 7th position causing 3.3% total deaths worldwide. This disease can take 6th position causing 3.7% total death by year 2030 as per survey report. The antiepileptic drug tiagabine is available only as tablets and capsules, which result in delayed action because of first-pass metabolism in the gastrointestinal tract. In this research work, an inert, biodegradable cost-effective biopolymer obtained from *Ananas cosmosus* pulp was used to avoid toxicity caused by synthetic polymers. *A. cosmosus* contains water, carbohydrates, sugars, vitamins A, C, and carotene, protein, fat, flavonoids, and fiber, bromelain [2]. Drugs enter the systemic circulation directly [3]. This route is noninvasive and nonmobile, with a high mucoretention ability, and leads to high bio-availability; drugs enter the systemic circulation directly, and at lower doses, first-pass metabolism by the liver and metabolism by the gastrointestinal tract can be avoided. Thus, to decrease dose frequency and to minimize adverse drug reactions, nanosized tiagabine-loaded Bio-Flexy films were suitably formulated that could exert sustained drug action up to 3–4 days.

The importance and advantages of using biopolymer instead of synthetic polymers such as carboxyl methyl cellulose and hydroxyl propyl methyl cellulose [3] are as follows:

(1) Isolated biopolymers show significant muco-adhesivity, film ability, retardability, and bio-degradability comparable to synthetic polymers.
(2) They are cheap and environment friendly.
(3) They are suitable as a drug carrier for sustained-release dosage forms with suitable modification.
(4) They can be used in pharmaceutical industries and commercialized effectively.
(5) *A. cosmosus* biopolymer has the uniqueness of being pure and of natural origin, isolated from pineapple pulp.
(6) *A. cosmosus* biopolymer is isolated using acetone as a solvent, which belongs to class 3, and is thus less toxic and poses a lower risk to human health. No class 1, 2 carcinogenic solvents are used.
(7) Hydroxyl propyl methyl cellulose and carboxyl methyl cellulose are synthesized using various harmful chemicals. The biopolymer devoids of toxicity due to its inbuilt biodegradability and bio-safe nature as it is isolated from natural edible source.
(8) Biopolymer serves as a suitable carrier of drugs for the formulation of Bio-Flexy films.

Materials and methods

Drug

Tiagabine was procured from Sun Pharmaceuticals Industries Ltd (Dahej, Gujarat, India).

Polymers

A. cosmosus biopolymer (pineapple procured from local market).

Sodium carboxyl methyl cellulose (sodium CMC) was obtained from Central drug House (P) Ltd (New Delhi).

All other reagents used were of the highest purity and analytical grade.

Double-distilled water was used throughout the experimental work.

Isolation of biomaterial from *A. cosmosus*

500 g of the center part of *A. cosmosus* fruits was weighed and the skin was removed. Slurry was prepared using 200 ml of distilled water; the slurry was filtered using muslin cloth. To the filtrate, optimized quantity of propan-2-one was added in a proportion of 1 : 2. To isolate the biomaterial, the mixture was refrigerated at 2–8°C for 24 h. The mixture was centrifuged at 3500 rpm for 15 min. The supernatant was separated and the residue was collected. The biomaterial obtained was dried naturally, powdered, passed through sieve no. 120, packed, and stored for further use. The same procedure was repeated six times for optimization and percentage yield was calculated and reported.

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To the filtrate, an optimized quantity of propan-2-one was added in a ratio of 1 : 2

↓

The mixture was refrigerated at 2–8°C for 24 h

↓

The mixture was centrifuged at 3500 rpm for 15 min

↓

The supernatant was discarded and the residue was collected

↓

Biomaterial was dried naturally for 24 h

↓

Biomaterial was powdered, passed through sieve no. 120, packed, and stored

↓s

The same procedure was repeated six times for optimization. Percentage yield was reported

Physicochemical characterization

The physiochemical characterization of isolated biomaterial was performed, such as color, odor, solubility, and melting point, and various chemical tests were performed [3].

(1) Test for carbohydrates: Molisch reagent test: 2 ml of biopolymer solution (0.1 g dissolved in 2 ml of distilled water) was placed in a test tube. Two drops of Molisch reagent (solution of α-napthol in 95% ethanol) were added. The solution was then poured slowly into a test tube containing 2 ml of concentrated sulfuric acid. Two layers were formed. Purple color appeared at the interface of the two layers because of the formation of 5-hydroxy methyl furfural.
(2) Test for proteins: the Biuret test determines the presence of peptide bonds in the protein content in the isolated biomaterial. 2 ml of biomaterial solution (0.1 g dissolved in 2 ml of distilled water) was placed in a test tube. 1 ml of sodium hydroxide solution (1%), followed by 1% copper (II) sulfate solution were added drop wise. The test tube was then shaken vigorously. The mixture was allowed to stand for 5 min and the color change was observed. The Biuret test is based on the ability of Cu (II) ions to form a violet-colored chelate complex with the peptide bonds (CONH groups) under alkaline conditions. The chelate complex absorbs light at 540 nm; thus, it appeared violet. Color change indicated the presence of proteins.
(3) Test for starch: 2 ml of biomaterial solution (0.1 g dissolved in 2 ml of distilled water) was placed in a test tube. 1–2 drops of iodine solution were added. Then, the color change was observed. The appearance of an intense blue black color confirmed the presence of starch in the isolated biomaterial (transfer of charge between starch and iodide ion alters the spacing between the energy orbitals; thus, the starch–iodide complex absorbs light at a higher wavelength).
(4) Test for reducing sugar: 2 ml of biopolymer solution (0.1 g dissolved in 2 ml of distilled water) was placed in a test tube. Then, 1 ml each of Fehling's A (7 g $CuSO_4.5H_2O$ dissolved in distilled water containing two drops of dilute sulfuric acid) and Fehling's B (35 g potassium tartrate, 12 g of sodium hydroxide in 100 ml of distilled water) were added. The test tube was placed in a water bath at 60°C. The appearance of a brick red precipitate of insoluble copper oxide indicated the presence of reducing sugar in the isolated biomaterial.

Drug–excipient interaction study [3].

In Drug-Excipient Interaction study by wet method, Tiagabine and isolated biopolymer in ratios of 1:1, 1:3 and 3:1 were triturated, mixed in geometric progression manner followed by wetting with 1 ml of distilled water.

(1) The wet mixture was subjected to drying in oven for 30 min at 50°C, followed by dilution with 2 ml of methanol. An UV spectroscopy study was carried out. The shift in λ_{max} was reported in comparison with that of pure Tiagabine.
(2) Dry method: Drug–excipients at ratios of 1 : 1, 1 : 3, and 3 : 1 were obtained in their physical forms (dry) in three different petridishes. Mixtures were kept at room temperature for 2 h, followed by dilution with 2 ml of methanol. An UV spectroscopy study was then carried out. The shift in λ_{max} was reported in comparison with that of pure tiagabine.

Importance of determination of drug–biopolymer interactions using dry and wet methods

The two methods showed that no drug–biopolymer interactions occurred either in dry form or in the presence of a solvent. As the biopolymer was isolated from a natural source and used in the formulation, it had to be ascertained whether the biopolymer was inert under both dry (during storage) as well as wet (if used in oral drug delivery) conditions. Thus, to confirm inertness and nonreactiveness of the biopolymer with the drug, these two methods were used. The drug was found to be intact with the he biopolymer.

Spectral studies of isolated biopolymer [3,4]

Ultraviolet spectroscopy

Determination of λ_{max} and detection of functional groups were performed for qualitative and quantitative analyses. Schimadzu model 1800 was used for UV analysis of the biomaterial. In spectral study of isolated biopolymer by ultraviolet spectroscopy method, distilled water was used as blank initially. The baseline correction was done by using distilled water as blank. When baseline correction was completed, one of the cuvettes was replaced by pouring biomaterial solution; distilled water was used as a blank sample here. During scanning of the sample, a peak was observed, which yielded the maximum absorbance of the biomaterial. Thus, the absorbance of the sample was recorded as a function of wavelength.

Scanning electron microscope analysis

Morphological examination of the surface was performed and the internal structure of the biomaterial was determined using a scanning electron microscope (SEM). A small amount of biomaterial was placed on aluminum studs and coated with gold using a sputter coater under vacuum (pressure 1 mmHg). The biomaterial was then analyzed by SEM.

IR spectra

The physical form of the isolated biomaterial was solid; thus, the potassium bromide (KBr) disc method was used for IR spectroscopy, and in this technique, about 1 mg of the solid sample was mixed with about 100 mg of predried and desiccated solid KBr. The mixture was finely ground in a mortar, preferably under an IR lamp, to exclude any water vapors. The finely ground mixture was pressed under a pressure of about 10 tons using a hydraulic pump to create a small pellet about 1–2 mm in diameter. The resulting KBr disc was removed from the KBr die and positioned in a special holder in the path of the IR radiation and its spectrum was recorded within the range of 4000–200 cm^{-1}.

Conversion of tiagabine hydrochloride into tiagabine by the precipitation method

To 100 mg of tiagabine hydrochloride, 20 ml of distilled water was added in a test tube and shaken vigorously. The mixture was subjected to sonication for one cycle (each cycle of 3 min) in an ultrasonic bath sonicator. 10 ml of 1 N sodium hydroxide solution was added drop wise to the above Tiagabine solution. A precipitate was formed at the bottom of the test tube. The mixture was centrifuged for 15 min at 3500 rpm. Tiagabine was separated, washed with 10 ml distilled water, and air dried.

Preparation of the standard curve of tiagabine [4,5]

10 mg of tiagabine was dissolved in 30 ml of buffer (pH 7.4) in a 100 ml volumetric flask and diluted up to the mark with distilled water (100 μg/ml). Dilutions of concentrations (0.5,1, 2, 3, 4, and 5 μg/ml) were prepared in 10 ml volumetric flasks. The volume was made up to 10 ml with distilled water (λ_{max}=396 nm). Absorbance was measured against a solvent blank.

Nanosizing of tiagabine by a novel method

100 mg tiagabine was mixed with 10 mg dextrose, 5 mg fructose, and 10 ml distilled water in a mortar pestle and triturated. The mixture was transferred to a beaker and sonicated for five cycles (3 min/cycle in an ultrasonic bath sonicator). The mixture was diluted with 50 ml distilled water and again sonicated for five cycles. Absorbance, %transmittance, and %blockage (100% transmittance) were determined after every five cycles up to 15 cycles. After the 15th cycle, the residue was collected, dried, packed, and stored.

Nanosizing of tiagabine by a standard method

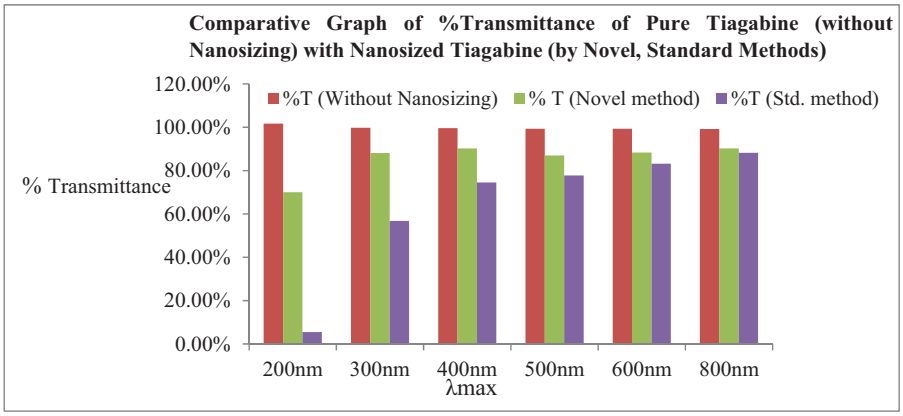
100 mg of tiagabine was mixed with 10 mg dextrose, 5 mg fructose, and 10 ml methanol in a mortar pestle and triturated. The mixture was transferred to a beaker and sonicated for five cycles (3 min/cycle in an ultrasonic bath sonicator). The mixture was diluted with 50 ml distilled water and again sonicated for five cycles. Absorbance, %transmittance, and %blockage (100% transmittance) were determined after every five cycles up to 15 cycles. After the 15th cycle, the residue was collected, dried, packed, and stored.

The main purpose of nanosizing tiagabine by two different methods was to compare the novel method with the published standard solvent evaporation method (Fig. 1).

Formulation of Bio-Flexy films (solvent casting method)

Nanosized tiagabine (0.1 g/100 ml) and *A. cosmosus* biopolymer solution (10% w/v) (in ratios 1 : 1, 1 : 3, 1 :

Figure 1



Comparative graph between %transmittance and λ_{max} of pure tiagabine (without nanosizing) with nanosized tiagabine (by novel and standard methods).

5, 1 : 6, and 1 : 10) were placed in a mortar. To this mixture, dextrose (film initiator) (10 mg/ml) and fructose (5 mg/ml) were added and triturated. Glycerine (10 µl) (plasticizer) and pectin (3%) (film former) were added. Distilled water (20 ml) was added and triturated uniformly for 2 min. Mixtures were subjected to magnetic stirring for 15 min, followed by sonication for up to five cycles (each cycle 3 min). Mixtures were poured into petridishes and dried. The prepared films were removed from petridishes using a 1% borax solution. The film ability of the prepared films was checked. The same procedure was followed for formulations of standard polymer films (Tables 1 and 2).

Table 1 Formulation of Bio-Flexy films using the *Ananas cosmosus* biopolymer

Formulation	FPA1 (1 : 1)	FPA2 (1 : 3)	FPA3 (1 : 5)	FPA4 (1 : 6)	FPA5 (1 : 10)
Tiagabine (mg)	100	100	100	100	100
Ananas cosmosus biopolymer (mg)	100	300	500	600	1000
Dextrose (mg)	100	100	100	100	100
Fructose (mg)	50	50	50	50	50
Glycerine (µl)	10	10	10	10	10
Distilled Water (ml)	10	10	10	10	10

Table 2 Formulation of a Bio-Flexy film using sodium carboxyl methyl cellulose

Standard polymer: formulation	FS1 (1 : 1)	FS2 (1 : 3)	FS3 (1 : 5)	FS4 (1 : 6)	FS5 (1 : 10)
Tiagabine (mg)	100	100	100	100	100
Sodium carboxyl methyl cellulose (mg)	100	300	500	600	1000
Dextrose (mg)	100	100	100	100	100
Fructose (mg)	50	50	50	50	50
Glycerine (µl)	10	10	10	10	10
Distilled water (ml)	10	10	10	10	10

Evaluation of formulated Bio-Flexy films [6]

Thickness

The thickness of randomly selected Bio-Flexy films from every batch was determined using a standard digital micrometer. The average thickness was determined and reported with the appropriate SD.

Folding endurance

Folding endurance of Bio-Flexy films was determined by repeatedly folding one of the films at the same place till it broke or folded up to 300 times manually, which was considered to indicate good properties. The number of times a film could be folded at the same place without breaking yielded the value of the folding endurance. This test was performed on randomly selected three flexi films from each batch.

Surface pH study

The surface pH of Bio-Flexy films was determined to investigate the possibility of any side effects *in vivo*. As an acidic or an alkaline pH may cause irritation to the soft palatal mucosa, it was decided to maintain the surface pH as close to neutral as possible. The Bio-Flexy films were allowed to swell by placing them in 1 ml of distilled water for 1 h at room temperature. The pH was measured by bringing the electrode in contact with the surface of the film and allowing it to equilibrate for 1 min. The experiments were conducted in triplicate and the average values were reported.

The surface pH of films was determined because the prepared Bio-Flexy films will be placed directly in the soft palatal region; thus, it is essential for the formulation (rather than the polymeric solution) to be neutral to the mucosal surface and compatible with soft palatal pH.

Weight uniformity study

The weights of ten Bio-Flexy films 1 cm^2 in diameter from every batch were determined and weighed individually on an electronic balance. The average weight was calculated.

Drug content uniformity

This was determined by dissolving the Bio-Flexy films (1 cm^2 in diameter) in 100 ml of phosphate buffer (pH 7.4) for 24 h under occasional shaking. A 5 ml solution was obtained and diluted with phosphate buffer, pH 7.4, up to 20 ml, and the resulting solution was filtered through a 0.45 mm Whattman filter paper. The drug content was then determined after proper dilution using a UV spectrophotometer at λ_{max} 257 nm.

Swelling percentage study

This was calculated as a function of weight and area increase because of swelling. Bio-Flexy films 1×1 cm^2 in size were weighed, placed in a petridish, and 10 ml distilled water was added. After 1 h, the films were weighed. The difference in the weights yields the weight increase because of absorption of water and swelling of patch. The study was carried out for 24 h.

$$\%S = (X_t X_o / X_o) \times 100,$$

where X_t is the weight or area of the swollen Bio-Flexy film after time t; X_o is the original Bio-Flexy film weight or area at 0 time.

Tensile strength

The tensile strength of a Bio-Flexy film is the total weight that is necessary to break or rupture the dosage form, and this was determined using a device with a rectangular frame with two plates. One plate is in the front and movable, and can be pulled by loading weights on the string, which is connected to the movable part. A 1×1 cm^2 Bio-Flexy film equivalent to 50 mg drug from each formulation was fixed between the stationary and the movable plate. The force needed to fracture the film was determined by measuring the total weight loaded in the string. The weight that caused breakage of the patches was the tensile strength.

$$\text{Tensile strength } (g/cm^2) = \frac{\text{Force at break } (g)}{\text{Initial cross} - \text{sectional area of bio} - \text{flexy film}}.$$

Percentage moisture uptake

PMU was calculated to determine the physical stability of the Bio-Flexy films under highly humid conditions. Three 1 cm diameter Bio-Flexy films were cut out and weighed accurately; then, the films were placed in a desiccator containing a saturated solution of aluminum chloride, with the humidity in the desiccator maintained at 79.5%. After 3 days, the Bio-Flexy films were removed, weighed, and the percentage moisture absorption was calculated. The average percentage moisture absorption of three films was determined.

$$\text{Percentage moisture uptake} = \frac{\text{Final weightinitial weight}}{\text{Initial weight}} \times 100.$$

Mucoadhesivity studies

The mucoadhesive property of the *A. cosmosus* polymer was assessed using the Modified Shear Stress Method at different concentrations, 1, 2, 4, 6, 8, and 10%, of biopolymer. The weight required to slide a glass plate using various concentrations of the biomaterial solutions ranging from 0 to 30 min was determined and compared with sodium CMC (1%).

Mucoretention time

Dynamic method (*rotating cylinder method*): In the rotating cylinder method, the mucoadhesive property of Bio-Flexy films was evaluated by *Capra aegagrus* (goat) intestinal mucosa. Bio-Flexy films of 1 cm^2 of each formulation were cut using a sharp blade. The goat intestinal mucosa was tied over the rotating basket of the i-disso apparatus (Electrolab India PVT. LTD, Mumbai, India). The dissolution medium was composed of 900 ml buffer, pH 7.4, maintained at 37°C, and subjected to rotation at 50 rpm. It was applied over the inner surface of goat intestinal mucosa until it became dislodged. The detachment and dislodgement of the film from the mucosal substrate were noted at regular intervals.

Static method: (*slanting condenser method*): Bio-Flexy films of 1 cm^2 of each formulation were cut using a sharp blade. The *C. aegagrus* (goat) intestinal mucosa was tied over the slanting condenser over which the buffer was allowed to flow from a burette. It was applied over the inner surface of goat intestinal mucosa until it became dislodged. The detachment and dislodgement of the film from the mucosal substrate were noted at regular intervals.

In-vitro drug release (by a modified MS apparatus) [7,8]

A thermostatically controlled compartment with vials containing buffer pH 7.4 was prepared. Egg membranes tied to the donor compartment (which contained formulations) were inserted into receiver compartment. The temperature was maintained at 37°C using an orbital shaker incubator. Samples were withdrawn at regular intervals ranging from 10 min to 30 h. The buffer was replaced completely each time. An ultraviolet spectral analysis was carried out.

Stability studies

Stability studies were carried out as per ICH Guidelines. Stability testing of pharmaceutical

product was performed to ensure the efficacy, safety, and quality of active drug substance and dosage forms and the shelf-life or the expiration period. The stability studies of the formulations were carried out at 40±2°C and 45±5% RH, 25±2°C and 60±5% RH, and 2±5°C temperature and RH values, respectively. After every 15 days, the aggregation, nature, color change, and in-vitro drug release of the formulations were determined [9,10].

Results

Isolation of the biomaterial

The percentage yield for the biomaterial from *A. cosmosus* was found to be 0.972±0.008%.

Physicochemical properties of the isolated biomaterial

The biomaterial obtained from the pulp of *A. cosmosus* was obtained in a powdered texture that was brown in color, had a characteristic odor, and was soluble in acetone. The change in color occurred at 200±4°C.

Drug–excipient interaction studies

A drug–polymer interaction study of the biomaterial isolated was carried out using UV techniques. The drug interaction study was carried out using a wet and dry method.

(1) Wet method λ_{max} was observed at 260 nm, and there was no significant difference from that of the pure drug.
(2) Dry method λ_{max} was observed at 260 nm, and there was no significant difference from that of the pure drug.

Thus, no drug–excipient interaction occurred.

Colorimetry

To 0.05 g of tiagabine, 0.05 ml each of potassium permanganate, crystal violet, iodine, copper sulfate, potassium dichromate, methyl red, methyl orange, and ferrous sulfate were added on different sections of the glass plate. The drug showed color change with potassium permanganate from pink to brown, indicating the reaction of potassium permanganate because of saturation of double bonds. Drug (0.05 g)+polymer (0.05 g) also showed a similar color change with potassium permanganate. This indicated that the drug was not entrapped. With the use of the UV method, λ_{max} of the drug–excipient mixture was found to be close to that of the pure drug. Thus, the drug–excipient interaction study showed that there was no interaction between the drug and the biomaterial and the biomaterial was compatible with the drug. As no interaction was found, it can be concluded that the biomaterial may be useful in the formulation of Bio-Flexy films. Drug +polymer showed no color change with the use of other reagents (Table 3).

Spectral studies of the isolated biomaterials

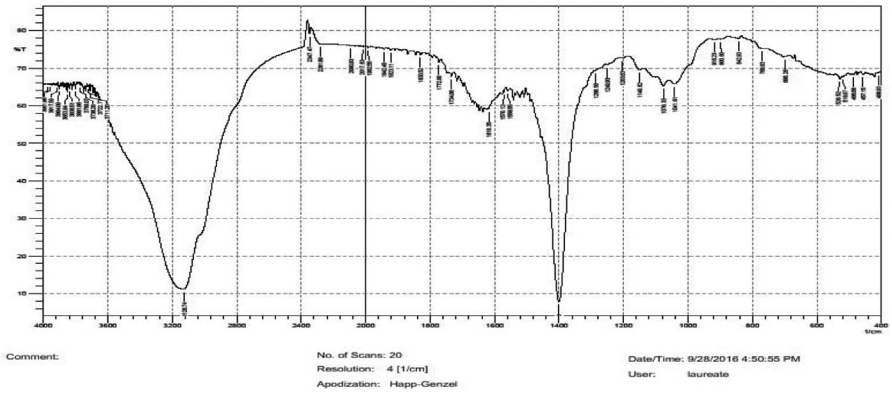
IR spectroscopy (using IRPal2.0 software: by Wolf van Heeswijk Bio-Flexy films were prepared in DIT University, Research Lab, Dehradun)

The results of IR spectra of biomaterial isolated from *A. cosmosus* showed peaks at 3904, 3126, 1287, and 1076 cm^{-1}, which clearly indicated an inbuilt mucoadhesive property with functional groups RCH_2OH, C=C-COOH, RCOOH, and RNH_2 (Fig. 2).

Table 3 Drug–polymer interaction with different reagents

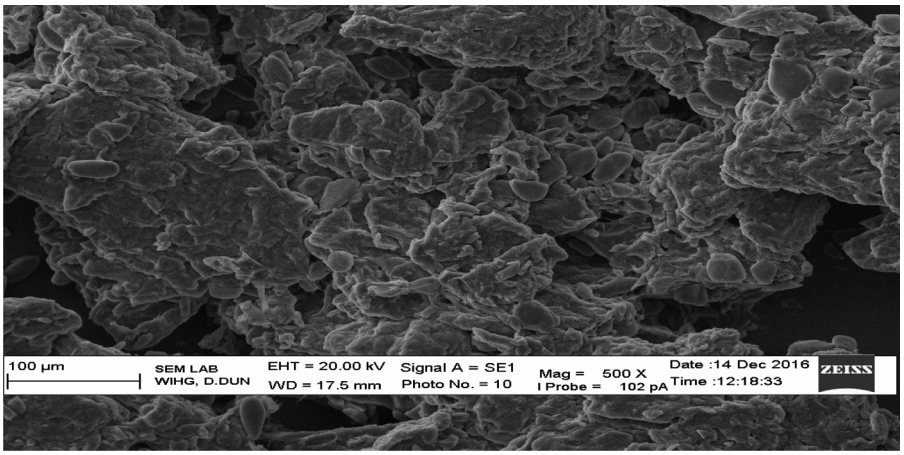
Potassium permanganate	Crystal violet	Iodine	Copper sulfate	Potassium dichromate	Methyl red	Methyl orange	Ferrous sulfate
Pink to brown color change	Purple	Light brown	Blue	Light color change	Red	Brown	Light brown

Figure 2



IR spectra of *Ananas cosmosus* biopolymer.

Figure 3



Scanning electron microscope of *Ananas cosmosus* biopolymer.

Scanning electron microscope analysis

The size range of the biopolymer was found to be 100 µm (Fig. 3).

Preparation of the calibration curve of the drug

The calibration curve of tiagabine was prepared in buffer (pH 7.4) and showed linearity. The R^2 value was found to be 0.9945 (Fig. 4).

Thickness of formulations

The thickness of nanosized tiagabine-loaded Bio-Flexy films containing the *A. cosmosus* biopolymer (FPA1–FPA5) was in range of 0.041–0.091 mm. The thickness of sodium CMC containing Bio-Flexy films (FS1–FS5) was found to be in range of 0.020–0.038 mm.

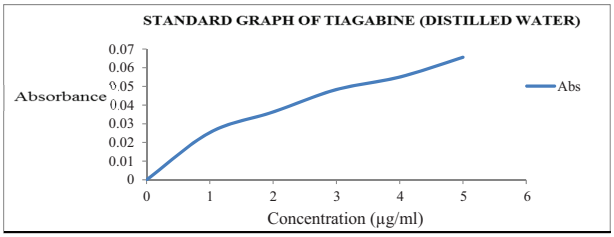
Folding endurance of formulations

The folding endurance was found to be in the range of 65–95 for nanosized tiagabine-loaded Bio-Flexy films containing the *A. cosmosus* biopolymer (FPA1–FPA5). The folding endurance was found to be in the range of 122–135 for formulations containing the sodium CMC (FS1–FS5) biopolymer.

Surface pH of formulations

The surface pH of nanosized tiagabine-loaded Bio-Flexy films containing the *A. cosmosus* biopolymer (FPA1–FPA5) was found to be in range of 7.01± 0.02 to 7.01±0.01. The pH was obtained to be in the range of 7.2±0.20 to 7.5±0.05 for Bio-Flexy films using the sodium CMC (FS1–FS5) synthetic polymer. The prepared formulations were suitable as a soft palatal delivery platform as they were within the range of physiological pH.

Figure 4



Standard curve of tiagabine in buffer pH 7.4.

Weight uniformity of formulations

The weight of nanosized tiagabine-loaded Bio-Flexy films containing the *A. cosmosus* biopolymer (FPA1–FPA5) was found to be in range of 0.001± 0.02 to 0.032±0.01 and that of sodium CMC (FS1–FS5) was found to be in range of 0.011±0.03 to 0.032±0.05.

Drug content uniformity

Drug content uniformity of nanosized tiagabine-loaded Bio-Flexy films containing the *A. cosmosus* biopolymer (FPA1–FPA5) was found to be in range of 82.2±0.65 to 92.8±0.22%. This indicates that the drug was dispersed uniformly in Bio-Flexy films.

Swelling percentage study

Swelling percentage of Bio-Flexy films containing the *A. cosmosus* biopolymer (FPA1–FPA5) ranged from 65±0.8 to 88±0.1%. Swelling increased with increasing biopolymer content. This indicates that the drug was released through Bio-Flexy films by swelling, followed by erosion.

Tensile strength of Bio-Flexy films

Swelling percentage of Bio-Flexy films containing *A. cosmosus* biopolymer (FPA1–FPA5) ranged from 75.02± 4.2 to 102.05±1.7 g. This shows that the prepared Bio-Flexy films can withstand any pressure or strain.

Table 4 Mucoadhesivity of *Ananas cosmosus* biopolymer

Solutions	Time (min) (g)						
	0	5	10	15	20	25	30
Water	6	8.4	10.5	11.1	13.2	16.6	22.2
Biopolymer 1%	8.8	9.4	10.8	13.4	17.8	22.4	28.6
Biopolymer 2%	14.4	15.6	19.2	26.3	29.6	34.5	38.5
Biopolymer 4%	15.2	20.0	26.4	34.6	38.2	42.8	49.4
Biopolymer 6%	22.2	26.2	38.6	45.8	56.5	58.2	67.5
Biopolymer 8%	35.6	40.4	48.5	58.7	66.8	72.4	88.6
Biopolymer 10%	52.4	55.5	58.0	65.5	68.8	73.7	78.9
Sodium carboxyl methyl cellulose 1%	48.6	52.2	59.4	74.2	98.4	112	128.2

Percentage moisture uptake of Bio-Flexy films

Percentage moisture uptake of Bio-Flexy films containing the *A. cosmosus* biopolymer (FPA1–FPA5) was found to range from 3.5±0.20 to 4.8±0.07%.

Mucoadhesivity study

Shear stress study of the *A. cosmosus* biopolymer indicated that 10% concentration showed promising mucoadhesivity comparable to the standard sodium CMC polymer (Table 4).

Mucoretention time

Mucoretentive study by dynamic and static methods showed that nanosized tiagabine-loaded Bio-Flexy films containing *A. cosmosus* are mucoretentive on *C. aegagrus* intestinal mucosa for a considerable time period (Tables 5 and 6).

Table 5 Dynamic method

Formulation	Dislodgement time (min)	Formulation	Dislodgement time (min)
FPA1 (1 : 1)	45	FS1 (1 : 1)	10
FPA2 (1 : 3)	60	FS2 (1 : 3)	15
FPA3 (1 : 5)	75	FS3 (1 : 5)	25
FPA4 (1 : 6)	120	FS4 (1 : 6)	30
FPA5 (1 : 10)	150	FS5 (1 : 10)	45

Table 6 Static method

Formulation	Dislodgement Time (min)	Formulation	Dislodgement Time (min)
FPA1 (1 : 1)	120	FS1 (1 : 1)	15
FPA2 (1 : 3)	150	FS2 (1 : 3)	25
FPA3 (1 : 5)	180	FS3 (1 : 5)	30
FPA4 (1 : 6)	210	FS4 (1 : 6)	35
FPA5 (1 : 10)	240	FS5 (1 : 10)	55

In-vitro release study by a modified MS apparatus

The best formulation was found to be FPA3 (Bio-Flexy film containing tiagabine: *A. cosmosus* biopolymer at a ratio of 1 : 5) as shown in Fig. 5 (comparative graph of in-vitro drug release of formulations FPA1–FPA5 by a modified MS apparatus) and Fig. 6 (comparative graph of in-vitro drug release of formulations FS1–FS5 by a modified MS apparatus).

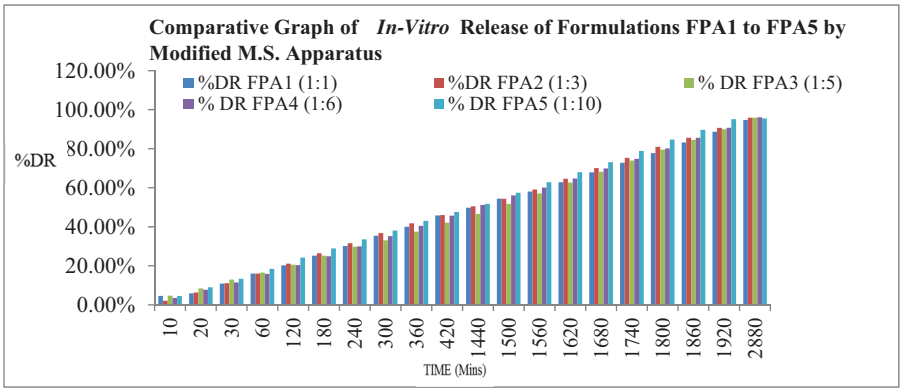
Stability studies

The formulations examined showed no physical changes, related to the color, odor, taste, etc. The drug content and in-vitro release were found to be the same; no significant change was observed. Therefore, it was concluded that the formulation of Bio-Flexy films of tiagabine was found to be stable (Tables 7–10).

Discussion

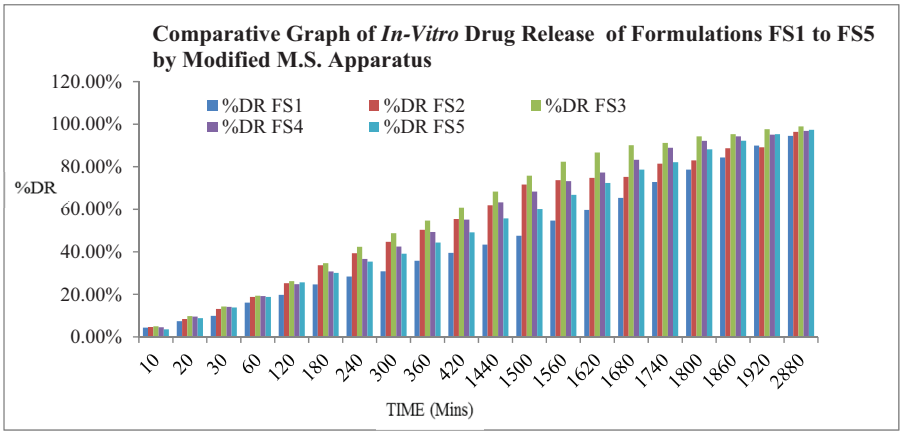
In this study, nanosized tiagabine-loaded Bio-Flexy films were formulated and evaluated for targeting to the brain through oro-soft palatal mucosa, a novelistic platform for systemic drug delivery. The aim of this study was to explore the potentialities of soft palatal mucosa as a drug-delivery platform for brain targeting. Biopolymer is used to prepare a flexy film because of its biodegradability, biocompatibility, its nontoxic and nonirritant nature, and the absence of reaction on the soft palatal surface. Physicochemical characterization of biopolymers such as color, odor, taste, and texture was performed, and chemical tests were carried out. The isolated biomaterial was rich in protein, starch, and carbohydrates. The biopolymer was found to be nontoxic in nature. Thus, these biopolymers are suitable for preparing Bio-Flexy films for trans-soft palatal delivery. Drug–polymer interactions were not observed because there was no change in the wavelength of the pure drug and the drug to polymer ratio. These biopolymers did not irritate the soft palate because of their inertness; thus, these biopolymers were selected for the formulation of tiagabine Bio-Flexy films. The biopolymers showed excellent film-forming properties along with mucoadhesive and mucoretentive properties. The functional groups present in the biopolymer were comparable to the groups present in

Figure 5



In-vitro drug release of tiagabine Bio-Flexy films using *Ananas cosmosus* biopolymer by a modified MS apparatus (dynamic method) (FPA1–FPA5).

Figure 6



In-vitro drug release of tiagabine Bio-Flexy films using sodium carboxyl methyl cellulose by a modified MS apparatus (dynamic method) (FS1–FS5).

Table 7 T50% and T80% values of tiagabine–*Ananas cosmosus* polymer Bio-Flexy films

Ratio	T50% (h)	T80% (h)
FPA1 (1 : 1)	1.84	5.25
FPA2 (1 : 3)	2.24	5.45
FPA3 (1 : 5)	**2.94**	**6.23**
FPA4 (1 : 6)	3.73	6.45
FPA5 (1 : 10)	1.81	5.41

Table 8 T50% and T80% values of tiagabine–sodium carboxyl methyl cellulose flexy films

Ratio	T50% (h)	T80% (h)
FS1 (1 : 1)	**6.24**	**6.82**
FS2 (1 : 3)	3.34	7.13
FS3 (1 : 5)	3.53	7.22
FS4 (1 : 6)	3.41	7.10
FS5 (1 : 10)	3.67	7.29

The bold values are the values of Best Formulation which is selected based on comparison of other Formulations and Standard Formulations.

the mucoadhesive polymers. Bio-Flexy films were prepared using the solvent casting technique, which is the easiest and most reproducible method to prepare flexy films without the need for any sophisticated instruments. A total of five drug to polymer ratios were chosen for *A. cosmosus*, FPA1 (1 : 1), FPA2 (1 : 3), FPA3 (1 : 5), FPA4 (1 : 6), and FPA5 (1 : 10), and five levels for sodium CMC: FS1 (1 : 1), FS2 (1 : 3), FS3 (1 : 5), FS4 (1 : 6), and FS5 (1 : 10). The prepared Bio-Flexy films of 1 cm^2 were cut using a round punch to determine the evaluation parameters and for stability studies. The practical yield of isolated Ananas cosmosus biopolymer was found to be 0.972±0.008%. The surface thickness of films ranged from 0.041 to 0.091 mm. Folding endurance was 65–95, indicating the flexibility of the Bio-Flexy films. The surface pH was found to be in the range of 7.01±0.02 to 7.01 ±0.01, which is in the range of physiological pH; thus, the prepared formulations were suitable for soft palatal formulation. Weight uniformity ranged from 0.001±0.02 to 0.032±0.01. Formulations FPA3

Table 9 Kinetic release of tiagabine–*Ananas cosmosus* polymer Bio-Flexy films

Release kinetics analysis dynamic method formulation of tiagabine: *Ananas cosmosus* Bio-Flexy films							
Formulations	R^2					Best-fit model	Mechanism of action
	Zero order	First order	Higuchi matrix	Peppas	Hixson–Crowell		
FPA1 (1 : 1)	0.7398	0.7402	0.9360	0.9575	0.7400	Peppas–Korsmeyer	Anomalous transport
FPA2 (1 : 3)	0.7194	0.7197	0.9341	0.9448	0.7196	Peppas–Korsmeyer	Anomalous transport
FPA3 (1 : 5)	**0.7499**	**0.7503**	**0.9322**	**0.9693**	**0.7501**	Peppas–Korsmeyer	Anomalous transport
FPA4 (1 : 6)	0.7530	0.7534	0.9359	0.9636	0.7533	Peppas–Korsmeyer	Anomalous transport
FPA5 (1 : 10)	0.7351	0.7356	0.9336	0.9639	0.7354	Peppas–Korsmeyer	Anomalous transport

The bold values are the values of Best Formulation which is selected based on comparison of other Formulations and Standard Formulations

Table 10 Kinetic release of tiagabine–sodium carboxyl methyl cellulose flexy films

Release kinetics analysis dynamic method formulations of tiagabine: sodium carboxyl methyl cellulose flexy films							
Formulations	R^2					Best-fit model	Mechanism of action
	Zero order	First order	Higuchi matrix	Peppas	Hixson–Crowell		
FS1 (1 : 1)	**0.8928**	**0.8929**	**0.9320**	**0.9641**	**0.8929**	Peppas–Korsmeyer	Fickian diffusion
FS2 (1 : 3)	0.8667	0.8673	0.9421	0.9602	0.8671	Peppas–Korsmeyer	Fickian diffusion
FS3 (1 : 5)	0.8548	0.8554	0.9442	0.9638	0.8552	Peppas–Korsmeyer	Fickian diffusion
FS4 (1 : 6)	0.8758	0.8763	0.9404	0.9650	0.8761	Peppas–Korsmeyer	Fickian diffusion
FS5 (1 : 10)	0.8841	0.8845	0.9351	0.9488	0.8844	Peppas–Korsmeyer	Fickian diffusion

The bold values are the values of Best Formulation which is selected based on comparison of other Formulations and Standard Formulations.

[containing riagabine: *A. cosmosus* biopolymer (1 : 5)], with R^2=0.9693, Peppas–Korsmeyer (best-fit model), followed an anomalous transport release mechanism, T50%: 1.73 h, T80%: 4.68 h. FS1 [containing tiagabine: sodium CMC (1 : 1)], with R^2=0.9641, Peppas–Korsmeyer (best-fit model), followed a fickian diffusion release mechanism, T50%: 6.24 h, T80%: 6.82 h. These were found to be the best formulations according to the release study. The stability study indicated stable Bio-Flexy films with no significant change in physical appearance and stable pH. Biomaterial was isolated and characterized for formulation to achieve controlled release for prolonged periods of time.

Conclusion

In this research work, nanosized tiagabine-loaded Bio-Flexy films were formulated using a novel biopolymer isolated from *A. cosmosus* fruit pulp and other coprocessing agents. The performance of the prepared formulations was assessed in comparison with tiagabine-standard polymer (sodium CMC) films. The study aimed to check and determine the feasibility of an oro-trans-soft palatal drug-delivery platform and also the suitability of the isolated biopolymer than the standard polymer. The results of all evaluation parameters showed that controlled drug release can be achieved by this drug-delivery route up to 48 h. Formulation FPA3 [containing tiagabine: *A. cosmosus* biopolymer (1 : 5)] was found to be the best film.

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Conflicts of interest

There are no conflicts of interest.

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