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Original Research Article

# Bio-flexy film formulation for delivery of tiagabine via oro trans-soft palatal route and its in-vitro stability study approach

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Soft palatal delivery

**ABSTRACT**

The aim of research work was to formulate bio-flexy films using a novel biopolymer isolated from *Rosa polyantha* petals containing tiagabine anticonvulsant drug. The soft palate drug delivery helps bypass first-pass metabolism in the liver and pre-systemic elimination in the gastrointestinal tract gets avoided. *Rosa polyantha* biopolymer used as bio-excipient due to its biodegradability, biocompatibility, inbuilt filmability, mucoadhesivity, non-toxicity, non-reactiveness on soft palatal surface. Bio-flexy films were prepared by solvent casting technique. Formulations containing different ratios of nanosized tiagabine: *Rosa polyantha* biopolymer (1:0.5, 1:1, 1:3, 1:5, 1:6, 1:10) (FRT1-FRT6) were prepared and compared with nanosized tiagabine loaded sodium CMC standard flexy films (FET1-FET6). The percentage yield of *Rosa polyantha* biopolymer was found to be 2.24±0.01%. Evaluation parameters for formulations revealed thickness of nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer (FRT1-FRT6):0.027 mm±0.005 to 0.039±0.004 mm, folding endurance: 83-130, surface pH: 7.00±0.04 to 7.00±0.01, weight uniformity: 0.008±0.05 to 0.044±0.03, drug content uniformity: 85.6%±0.48 to 94.8%±0.37, swelling percentage: 66%±0.2 to 75%±0.1, percentage moisture uptake (PTU): 2.5%±0.14 to 3.8%±0.10. Mucoadhesivity: 90-1440 mins, Mucoretentivity: 110-240 mins. Based on all above mentioned evaluation parameters, FRT5 (containing tiagabine: *Rosa polyantha* biopolymer (1:6)) bio-flexy film having $R^2$= 0.9295, higuchi matrix as best fit model, follows fickian diffusion (higuchi atrix) release mechanism, T50%:7 hrs., T80%: 30 hrs. was found to be best formulation. Stability studies conducted as per ICH guidelines revealed stable formulations at 40 °C ± 2 °C and ± 45± 5% RH, 25 °C ± 2 °C and 60 ± 5% RH and 2 °C ± 5 °C temperature and RH values in terms of In-vitro drug release, folding endurance, surface pH. Significance: The purpose of this research work is to provide effective treatment of epilepsy for sustained action up to 48 hours in reduced drug dose, frequency and minimized side effects.

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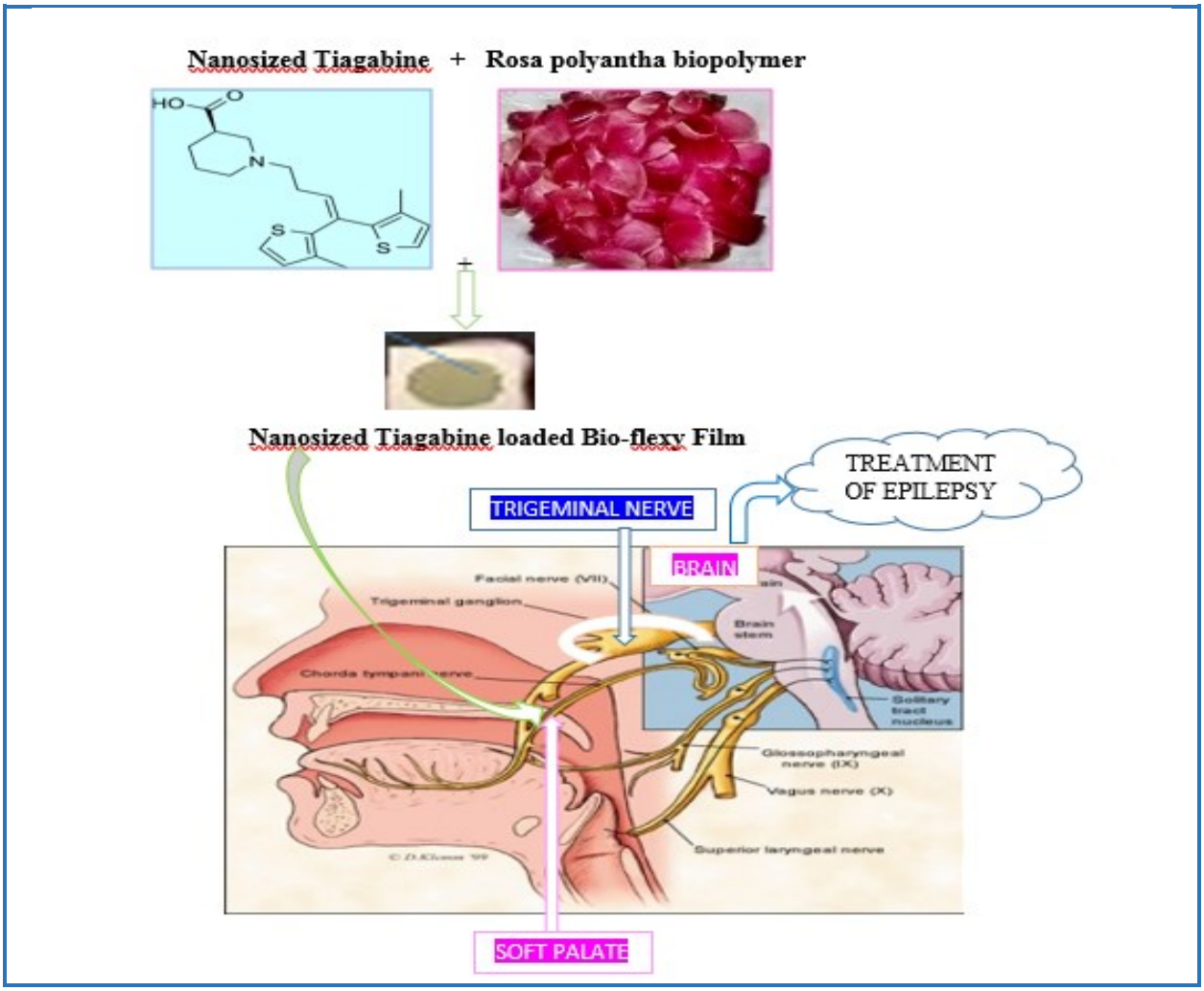
**Graphical Abstract**




## Introduction

Epilepsy is a central nervous system disorder (neurological disorder) in which nerve cell activity in the brain becomes disrupted, causing unprovoked, recurrent seizures or unusual behavior, sensations or even unconsciousness. Epilepsy ranks 7$^{th}$ position causing 3.3% total deaths worldwide, that is expected to rise up to 6$^{th}$ position causing 3.7% of total deaths till year 2030.

Anticonvulsants drugs are pharmacological agents used in the treatment of epileptic seizures. They suppress the rapid and excessive firing of neurons during the seizures also prevents the spread of the seizure within the brain. Tiagabine is used for treatment for Epilepsy. The aim of current research work was to explore a novelistic route for targeting to the brain through Soft Palate by formulating bio-flexy films using Tiagabine as a model drug permitting better control over epilepsy. Soft Palate (velum) is the part of oral mucosa, suspended from the posterior border of hard palate. It protects nasal passage, does not contain bone and provides improved absorption into blood stream as compared with oral administration to GIT. It is more suitable

route of drug administration [1]. It has promising Non-keratinized histology with unique thickness. Surface area of the oral mucosa (200 $cm^2$) relatively small compared with the GIT (350,000 $cm^2$ and skin (20,000 $cm^2$). Trans-Soft Palatal route offers a Novel Drug Delivery Platform for systemic delivery of drugs for Brain targeting [2]. Therapeutic potential of many drugs can be improved by this route. It is well suited for a retentive drug delivery and appears to be acceptable to the patient. With the right dosage form design and formulation, the permeability and the local environment of the mucosa can be controlled and manipulated in order to accommodate drug permeation. Palatal drug delivery is a promising area for systemic delivery of orally inefficient drugs as well as a feasible and attractive alternative for non-invasive delivery of potent peptide and protein drug molecules.

Middle meningeal artery; accessory meningeal artery; greater palatine branch of maxillary artery; ascending palatine branch of facial artery; ascending pharyngeal artery supplies blood to soft palate [3].

Nerve supply to soft palate is by mandibular branch of trigeminal nerve (Cranial nerve V); Lesser palatine nerve; greater palatine nerve; nasopalatine nerve; glossopharyngeal nerve; motor nerves. When nanosized drug is administered by this route, then, it can directly reach into brain by via inter and intra neural pathway. Trigeminal nerve directly connects soft palate to brain [4].

In this research work, an inert, biodegradable cost effective biopolymer obtained from *Rosa polyantha* petals was incorporated. *Rosa polyantha* contains geraniol and l-citronellol, rose camphor; an odorless solid composed of alkanes, which separates from rose oil, β-damascenone [5].

*Importance and advantages of using biopolymer instead of using synthetic polymers like CMC and HPMC* [6]

*i*) Isolated biopolymer exhibited significant mucoadhesivity, filmability, retardability an biodegradability comparable to synthetic polymers.
*ii*) Economically cheap and environment friendly.
*iii*) Suitable as a drug carrier for sustained release dosage forms with suitable modification.
*iiii*) It can applicable in Pharmaceutical Industries and commercialized effectively.
*v*) Rose biopolymer is having uniqueness of being pure, natural origin isolated from rose petals.
*vi*) Rose biopolymer is isolated using acetone as solvent that belongs to Class 3 so is less toxic, possess lower risk to human health than. No class 1, 2 carcinogenic solvents used.
*vii*) HPMC, CMC are synthesized using various harmful chemicals. Thus, biopolymer avoids toxicity that is by synthetic polymers.
*viii*) Biopolymer serves as a suitable carrier of drug for the formulation of bio-flexy films.

By this route, direct entry of drugs the systemic circulation can take place [7]. It is non-invasive, non-mobile with highly mucoretention ability, afford high bioavailability, lower doses, first-pass metabolism by the liver and metabolism by 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 can provide sustained drug action up to 2 days determined by *in-vitro* release studies using modified M.S. apparatus method which showed almost 100% drug release till 48 hours.

*Similar study about using this biopolymer for preparation of mucoadhesive drug delivery systems*

No similar study is available for this biopolymer as we are pioneer to isolate biopolymer from petals of rose. Earlier our research group has isolated bio penetrant from Rosa centifolia used for trans-ungal drug delivery. It's out comings were filed for patent.

## Experimental

*Materials and methods*

Tiagabine (procured from Sun Pharmaceuticals Industries Ltd., Gujarat) POLYMERS: *Rosa polyantha* biopolymer (Rose petals (pink) procured from local market) Sodium Carboxyl Methyl Cellulose (Central drug House (P) Ltd. New Delhi) All other reagents used were of highest purity and analytical grade. Double distilled water was used throughout the experimental work.

*Isolation of biomaterial from Rosa polyantha*

Rose petals were procured from local market. 500 gm. of Rose petals were weighed accurately, transferred into 1000 mL beaker. To this 1000 mL of methanol was added, covered with aluminum foil and refrigerated at 2-8 °C for over a period of 24 hours. The mixture was subjected for heating on a water bath at 70 °C in order to decolorize the petals for a period of 4 hours in continuous stirring mode using a mechanical stirrer at 500 rpm. The mixture was subjected for filtration using a muslin cloth. Decolorized petals were collected by discarding the filtrate. The decolorized petals were air dried for 2 hours, powdered using mortar and pestle. 300 gm. of powdered decolorized petals were treated with 500 mL purified water. Petals were soaked in 500 mL of purified water, kept for refrigeration for 2 hours. Then the mixture was removed from refrigeration, subjected to filtration. Optimized quantity of propan-2-one was added and uniformly mixed for period of 5 minutes. The mixture was subjected for refrigeration for over a period of 24 hours at 2-8°C in order to separate the biopolymer from the petals. The mixture was subjected for Centrifugation at 4000 rpm for 30 minutes. Biomaterial was collected by decanting the supernatant. Biomaterial was dried naturally for period of 24 hours, powdered, passed through Sieve No. 120. Stored in well closed container for further use. Similar procedure was repeated 6 times for optimization. Calculated % yield and reported.

*Physicochemical characterization*

*Solubility of isolated biopolymers*

10 mg biopolymer was dispersed in 2 mL distilled water on watch glass. This dispersion was then added to various solvents (10 mL each) like distilled water, methanol, acetone, ethanol, methanol, chloroform, carbon tetrachloride and isopropanol taken in different test tubes so as to determine solubility of isolated biopolymer.

*Physical and chemical tests of isolated biopolymers* [7]

The isolated bio-material was characterized for its physicochemical properties.

*Physical parameters*

Color, odor, texture and color changing point. Color, odor, texture of all biopolymers were examined physically. Color changing point was determined by capillary method by melting point apparatus.

Firstly the bio-polymer was kept in a capillary tube and it was fitted in a melting point apparatus. Temperature at which biopolymer

melts and changes its color was determined by means of a thermometer and reported.

*Chemical parameters*

Tests for presence of carbohydrates, proteins and starch. Prepared 1% biopolymer aqueous solution.

*Molisch reagent test for presence of carbohydrates*

To 2 mL of aqueous solution of biopolymer in test tube, added 2 drops of molisch reagent. Poured the solution slowly into another test tube that is containing 2 mL of concentrated sulphuric acid. Observed for formation of 2 layers and appearance of purple color at interface and reported.

*Biuret test for presence of proteins*

To 2 mL of aqueous solution of biopolymer in test tube, 1 mL of 1% sodium hydroxide solution was added. 1% copper (II) sulphate solution was then incorporated drop wise followed by vigorous shaking. The mixture was allowed to stand for 5 minutes. Color change was observed color change and reported.

*Test for presence of starch*

To 2 mL of aqueous solution of biopolymer in test tube,added 1-2 drops of Iodine solution and the color change was observed and reported.

*Test for presence of reducing sugar*

To 2 mL of aqueous solution of biopolymer in test tube, 1 mL each of Fehling's A and B solutions were added. Heated the test tube at 60 °C. Appearance of precipitate was observed and reported.

*Spectral analysis of isolated biopolymers* [7, 8]

*IR spectroscopy*

IR spectroscopy was performed using Shimadzu IR Affinity-1S Equipment (S/N ratio: 30,000:1, resolution: 0.5 $cm^{-1}$, autodryer, Dimensions: 514 (W) × 606 (D) × 273 (H) mm). The IR spectroscopy of isolated biopolymer in solid form was performed by using potassium bromide disc method. 1 mg of sample was finely admixed with about 100 mg of potassium bromide (KBr) in mortar. Pressure of 10 tons was applied to mixture using hydraulic pump. Small pellet of 1-2 mm in diameter was formed. The prepared pellet was kept in path of IR radiation and recorded the spectrum within the range of 4000-200 $cm^{-1}$.

*DSC (differential scanning calorimetry)*

Amount of the heat difference of sample and reference was measured against temperature. It was performed for determination of glass transition temperature (GTT or Tg). For DSC the perkin elmer instrument, Model-JADE DSC was used, with the heat flow of 50-250 °C at the rate of 10 °C/minute and nitrogen rate of flow of 20 mL/minute was used.

*ZETA sizing and particle size determination*

Zeta sizing was performed using malvern zeta sizer (Size range of sample: 0.3 nm-10 µM). It incorporates a zeta sizing analyzer that uses electrophoretic light scattering for particles, molecules and surfaces, and a molecular weight analyzer using static light scattering. Autodilution modules reduce the concentration to the optimum level so as to eliminate coincidence errors while still counting enough particles to completely define the particle size analyzers distribution. Zeta Potential values provides an indirect measurement of net charge on the particles. When charged nanosized particles are dispersed in a liquid, a layer of ions

of opposite charge strongly bound to their surface forming a charged thin layer called stern layer. This induces the formation of second diffuse out layer, composed of loosely associated ions called diffusive ion layer. These two layers are collectively called electrical double layer. When the nanosized particles moved in liquid phase due to applied electric field, there exists a boundary between the ions in diffuse layer that move with the particle and the ions that remain with the bulk dispersant. The electrostatic potential at this slipping plane boundary is zeta potential.

*NMR (Nuclear magnetic resonance) spectral analysis*

Exploits the magnetic properties of atomic nuclei, determines the physical and chemical properties of atoms or the molecules in which they are contained. It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecule. Solvent used was DMSO (Dimethyl sulfoxide). The spectrometer was connected to flow cell of 5 mm diameter. High flow rates were applied to the sample, a valve switch was activated to stop the flow for quick measurement. When the valve switches back, the flow cell in the instrument was rinsed again with the reaction mixture. The spectrum was sent to the automation computer where it can be processed and analyzed.

*SEM analysis*

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

*Cell-line toxicity study of biopolymer (MTT cytotoxicity assay)*

MTT [3-(4, 5 Dimethyl Thiazol–2–yl)–5–diphenyl tetrazolium bromide] is taken up by the viable cells and reduced to formazan by the "succinate-tetrazolium reductase" system that belongs to the mitochondrial respiratory chain functioning in metabolically active cells. Formazan formed, is a purple colored water-insoluble product that is largely impermeable to cell membranes, thus resulting in its accumulation within the healthy cells which is solubilized by adding dimethyl sulphoxide (DMSO). The optical density (OD) of purple colored solution developed was read using a conventional ELISA plate reader at 590 nm (maximum absorbance). The ability of cells to reduce MTT provides an indication of the mitochondrial integrity and activity, which, in turn, may be interpreted as a measure of viability and/or cell number. The ability of the cells to survive a toxic insult is the basis of this cytotoxicity assay. Dead cells or their products do not reduce tetrazolium.

*In-vitro mucoadhesivity of isolated biopolymers*

*In-vitro* Mucoadhesivity of isolated biopolymers was determined by modified shear stress apparatus. Different concentrations 1%, 2%, 4%, 6%, 8% and 10% of biopolymer solutions were placed between two glass plates of modified shear stress apparatus. Subjected to shear stress for assessment for *in-vitro* adhesive strength in terms of weight required for breaking adhesive bonds between the biomaterial and the glass plate after specified contact time from 0-30 minutes. The results were reported and graphs were plotted (Figure 1).

**Figure 1.** Shear stress apparatus for determination of mucoadhesivity of isolated biopolymer

*Standard graphs of tiagabine*

*Preparation of standard curve of tiagabine in distilled water*

10 mg of Tiagabine was dissolved in 30 mL of distilled water 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. Volume made up to 10 mL with distilled water ($\lambda_{max}$ =257 nm). Absorbance was measured against solvent blank [9].

*Preparation of standard curve of tiagabine in phosphate buffer pH 7.4*

10 mg of tiagabine was dissolved in 30 mL of phosphate buffer (pH 7.4) in a 100 mL volumetric flask and diluted up to the mark with phosphate buffer (100 µg/mL). Dilutions of concentrations (1, 2, 3, 4, 5, 8, 10, 20, 30, 40, 50 µg/mL) were prepared in 10 mL volumetric flasks. Volume was made up to 100 mL with phosphate buffer (pH 7.4) ($\lambda_{max}$=396 nm). Absorbance was measured against solvent blank.

*Drug-excipient interaction study* [6]

In this study 3 different ratios of drug: isolated biopolymer i.e., 1:1, 1:3 and 3:1 were taken. Absorbance was measured and compared with that of pure tiagabine.

*Dry method*

Drug-biopolymer were taken in ratios of 1:1, 1:3 and 3:1 were taken in three petridishes in dry form, kept at room temperature for about two hours. The mixtures were then diluted by using 2 mL methanol. Measured absorbance, observed shift in $\lambda_{max}$ with that of pure tiagabine and reported.

*Wet method*

Drug-biopolymer were taken in ratios of 1:1, 1:3 and 3:1 were taken in three petridishes. The mixtures were wetted with 1 mL of distilled water followed by drying at 50 °C for 30 min in oven. The mixtures were then diluted by using 2 mL methanol. Measured absorbance, observed shift in $\lambda_{max}$ with that of pure tiagabine and reported.

*Colorimetry method*

Drug:polymer 1:1 were mixed with potassium permanganate on glass plate. Observed color change, scrapped, diluted suitably with distilled water, analyzed by UV. Similarly repeated with drug:distilled water and drug:potassium permanganate.

*Importance of determination of drug-biopolymer interaction by dry and wet methods*

The two methods revealed that no interaction of drugs-biopolymers occurred either in dry form on in presence of solvent. Since biopolymers were isolated from natural source and used in formulation, it has to be ascertained whether the biopolymers were inert in both dry (during storage) as well as wet (if used in oral drug delivery) conditions. Thus in order to confirm inertness and non-

reactiveness of biopolymers with drugs, these two methods had been performed. The drugs were found to be intact with biopolymer.

*Preparation of tiagabine from tiagabine hydrochloride by precipitation method*

Tiagabine is available as tiagabine hydrochloride salt form. Thus, in order to enhance absorption and bioavailability of drug, to avoid ill-effects it is converted to its pure form by precipitation method. To 100 mg of tiagabine hydrochloride, 20 mL of distilled water was added in a test tube and shaken vigorously. Mixture was subjected to sonication for 1 cycle (each cycle of 3 minutes) in ultrasonic bath sonicator. 10 mL of 1N sodium hydroxide solution was incorporated drop wise in above tiagabine solution. Precipitate was formed at bottom of test tube. Mixture was centrifuged for 15 minutes at 3500 rpm. Tiagabine was separated, washed with 10 mL distilled water and air dried. Isolated tiagabine was analyzed using U.V. spectrophotometer.

*Nanosizing of tiagabine*

*Solvent evaporation method*

100 mg tiagabine was admixed with 5 mg of fructose, 10 mg of dextrose and 10 mL of methanol in mortar pestle. Sonication of mixture was performed for 5 cycles (180 sec/cycle) in ultrasonic bath sonicator. The mixture was then diluted with 50 mL distilled water and sonicated up to 15 cycles. Measured % transmittance, absorbance, % blockage (100–% Transmittance) after every 5 cycles. The residue was then dried and stored for further use [10].

*Sonication method*

100 mg tiagabine was admixed with 5 mg of fructose, 10 mg of dextrose and 10 mL of distilled water in mortar pestle. Sonication of mixture was performed for 5 cycles (180 seconds/cycle) in ultrasonic bath sonicator. The mixture was then diluted with 50 mL distilled water and sonicated up to 15 cycles. Measured % transmittance, absorbance, % blockage (100% transmittance) after every 5 cycles. The residue was then dried and stored for further use [10].

The main purpose of nanosizing tiagabine by two different methods was to compare novel sonication method with published standard solvent evaporation method (Figure 13).

*Nano size range determination by preliminary U.V. spectroscopic method*

It is a novel primarily screening method for nano-size range particles by U.V. spectroscopy. Transmittance is based on the concept of tyndall effect. When light of specified wavelength passes through the media containing particles less than or greater than the specified particle range, the % blockage represents particles beyond the size range whereas the % transmittance is considered that the particles lies above the size range at particular range [10].

*Formulation of bio-flexy films (solvent casting method)*

100 mg of nanosized tiagabine (Anticonvulsant) was triturated with 50 mg of biopolymer (Mucoadhesive, film forming cum retarding agent) (in ratio of 1:0.5) for 2 minutes using pestle mortar. Added 10 m of distilled water. To this dispersion, incorporated 10 mg of dextrose (Flexicizer), 5 mg of fructose (Flexicizer) and 10 µL of glycerin (1% solution v/v) (Plasticizer) with continuous stirring. 0.6 gr of Pectin (Film initiator) was added. Mixture was further uniformly triturated for 5 minutes. Volume was made up to 20 mL using distilled

water. Mixture was subjected to magnetic stirring for 15 minutes, followed by sonication for up to 5 cycles (each cycle 3 minutes). Clear dispersion obtained was poured into petridish. Kept for drying at room temperature for 24 hours. Removed prepared nanosized drug loaded bio-flexy film from petridish. Bio-flexy film formulation obtained was cut in 1 sq. cm dimension, packed in well closed air tight container for further use. Similarly, six different formulations of nanosized tiagabine with standard sodium carboxyl methyl cellulose polymer in different ratios of 1:1, 1:3, 1:5, 1:6 and 1:10 were prepared and evaluated (Tables 1 and 2).

**Table 1.** Formulation of nanosized tiagabine loaded bio-flexy films using rosa polyantha biopolymer

| Formulation | FRT1 (1:0.5) | FRT2 (1:1) | FRT3 (1:3) | FRT4 (1:5) | FRT5 (1:6) | FRT6 (1:10) |
|---|---|---|---|---|---|---|
| Nanosized tiagabine (mg) | 100 | 100 | 100 | 100 | 100 | 100 |
| *Rosa polyantha* biopolymer (mg) | 50 | 100 | 300 | 500 | 600 | 1000 |
| Dextrose (mg) | 10 | 10 | 10 | 10 | 10 | 10 |
| Fructose (mg) | 5 | 5 | 5 | 5 | 5 | 5 |
| Glycerine (µl) | 10 | 10 | 10 | 10 | 10 | 10 |
| Pectin (gm.) | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Distilled water (mL) | 20 | 20 | 20 | 20 | 20 | 20 |

**Table 2.** Formulation of nanosized tiagabine loaded bio-flexy films using sodium carboxyl methyl cellulose standard polymer

| Formulation | FET1 (1:0.5) | FET2 (1:1) | FET3 (1:3) | FET4 (1:5) | FET5 (1:6) | FET6 (1:10) |
|---|---|---|---|---|---|---|
| nanosized tiagabine (mg) | 100 | 100 | 100 | 100 | 100 | 100 |
| Sodium carboxyl methyl cellulose Standard polymer (mg) | 50 | 100 | 300 | 500 | 600 | 1000 |
| Dextrose (mg) | 10 | 10 | 10 | 10 | 10 | 10 |
| Fructose (mg) | 5 | 5 | 5 | 5 | 5 | 5 |
| Glycerine (µl) | 10 | 10 | 10 | 10 | 10 | 10 |
| Pectin (g) | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Distilled water (mL) | 20 | 20 | 20 | 20 | 20 | 20 |

*Evaluation of formulated bio-flexy films* [12]

*Thickness of formulated bio-flexy films*

Standard digital micrometer was used to determine thickness of formulated films and reported [13].

*Surface pH*

Surface pH of formulated films was determined by using digital pH meter. It should be neutral or close to soft palatal pH otherwise formulation might cause irritation to the soft palatal mucosa. The formulated bio-flexy films were kept in contact with 1 mL of distilled water at room temperature for 1 hour. pH was then measured in triplicate and reported. Compatibility of formulations with soft palatal pH is essential [13].

*Ex-vivo mucoadhesion study of formulations by rotating cylinder method*

In this method, the mucoadhesivity of formulated films was evaluated on intestinal mucosa of *Capra aegagrus* (i.e., Goat). Bio-flexy films of area 1 cm 2 of each formulation were cut down using sharp blade. Tied the goat intestinal mucosa over the rotating basket of I dissolution apparatus. The dissolution media was 900 mL of buffer (pH 7.4), maintained at 37 °C, subjected for rotation at 50 rpm. Films were applied over the inner surface of goat intestinal mucosa until they got dislodged. The dislodgement and detachment of films from mucosal surface was observed at regular intervals and reported (Figure 2) [13].

**Figure 2.** I-dissolution apparatus

*Ex-vivo mucoretention study of formulations*

Bio-flexy films of area 1 $cm^2$ of each formulation were cut down using sharp blade. Tied the *Capra aegagrus* (Goat) intestinal mucosa over slanting condenser over which buffer was allowed to flow from a burette. It was applied over the inner surface of Goat intestinal mucosa until it got dislodged. The detachment and dislodgement of film from mucosal substrate was noted at regular intervals and reported [13].

*Weight uniformity of formulated nanosized drugs loaded bio-flexy films*

Weight uniformity of formulated films was determined by weighing 10 formulations of 1 $cm^2$ diameter. Determined average weight and reported [13].

*Drug content uniformity of formulated nanosized drugs loaded bio-flexy films*

Drug content uniformity of formulated films was calculated by dissolving the films in Phosphate Buffer (pH 7.4) (100 mL) for 24 hours with occasional shaking. Diluted 5 mL of solution with phosphate buffer pH 7.4 up to 20 mL. Filtered through whattman filter paper of 0.45 mm. The drug content determined by UV analysis at $\lambda_{max}$ 750 nm for nanosized topiramate loaded formulations and at 257 nm for nanosized tiagabine loaded formulations [13].

*Folding endurance of formulated nanosized drugs loaded bio-flexy films*

Folding endurance of flexy film was determined by repeatedly folding one of the film at the same place till it broke or folded up to 300 times manually, which was considered satisfactory to reveal good properties. The number of times of film could be folded at the same place without breaking will give the value of the folding endurance. This test was done on randomly selected three bio-flexy films from each drug:biopolymer ratio.

*Swelling percentage study of formulated nanosized drugs loaded bio-flexy films*

It was determined as increase in weight and area because of Swelling. 1×1 $cm^2$ sized films were weighed, transferred in petridish and added 10 mL of distilled water. After one hour, reweighed the films. Absorption of water and swelling of films caused increased in weights of films. The study was performed for 24 hours. calculated % swelling index and reported.

*Percentage moisture uptake (PMU) of formulated nanosized drugs loaded bio-flexy films*

Percentage moisture uptake of formulations was determined so as to check the physical stability of the prepared bio-flexy films in high moist conditions. Bio-flexy films of 1cm diameter were kept in saturated solution of aluminum chloride in desiccator. The humidity inside the desiccator was maintained at 79.5%. Removed the films after 3 days, weighed, calculated percentage moisture absorption and reported.

$$\% \text{ MU } \frac{\text{Final weight of Films} - \text{Initial weight of films}}{\text{Initial weight of Films}} \times 100$$

*In-vitro drug release study of formulated nanosized drugs loaded bio- flexy films*

*In-vitro* drug release study of formulations was performed by using modified M.S. *In-vitro* diffusion apparatus. Buffer pH 7.4 was filled in 36 vials (receiver compartment). These were kept in thermostatically controlled compartment. Tied egg membranes to donor compartment (containing formulations). Donor compartments were inserted into receiver compartments. Temperature was kept constant at 37 °C with orbital shaker incubator. Sampling was done at regular intervals from 10 min to 48 hours. Buffer was completely replaced after every sampling. Performed ultra violet spectral analysis of every sample (Figure 3).


**Figure 3.** Modified M.S. *in-vitro* diffusion apparatus

*Stability studies of prepared films as per ICH guidelines*

Stability studies of prepared films were conducted as per ICH guidelines. Stability testing of pharmaceutical product is done to ensure the efficacy, safety and quality of active drug substance and dosage forms and shelf life or expiration period. The stability studies of the formulations were performed at 40 °C ± 2 °C with ± 45 ± 5% RH, at 25 ± 2 °C with 60 ± 5% RH and at 2 ± 5 °C conditions of temperature and relative humidity for 3 months. Observed for change in pH, folding endurance, *in-vitro* drug release of formulations (Figure 4).

**Figure 4.** Stability chamber

## Results and Discussion

*Isolation of biomaterial*

Biopolymer was isolated from petals of *Rosa polyantha* by simplified economic process. The optimization of biopolymer isolation process was repeated six times and the % yield was calculated. During optimization the results obtained were reproducible with insignificant variation and can be adopted for scaling up in bulk manner. The % yield of *Rosa polyantha* biopolymer was found to be 22.4%±0.01.

*Physicochemical properties of isolated biomaterial*

Solubility of isolated biopolymer was determined in different solvents and reported. *Rosa polyantha* biopolymer was sparingly

soluble in water, insoluble in acetone *Rosa polyantha* biopolymer was obtained in powder state in pinkish brown color with faint odor. The color changing point was found to be 150 °C±4. The biopolymer confirmed the presence of carbohydrates, reducing sugar and protein.

*Spectral analysis of isolated biopolymer*

*IR spectroscopy*

IR spectroscopy was performed for the isolated biomaterial to determine the presence of functional groups in all biopolymers. The data showed that the biopolymer comprises of carboxyl (-COOH), hydroxyl (-OH) groups which clearly indicated biopolymer possessed in-built mucoadhesive property.

IR peaks of *Rosa polyantha* biopolymer were obtained at 3121 $cm^{-1}$, 38881 $cm^{-1}$, 1653 $cm^{-1}$, 1418 $cm^{-1}$, 922 $cm^{-1}$, 1686 $cm^{-1}$ which indicated functional groups at C=C COOH, $RNH_2$, $R_2C=CH_2$, $RCH_2OH$, RCOOH, $RCONH_2$ (Figure 5).

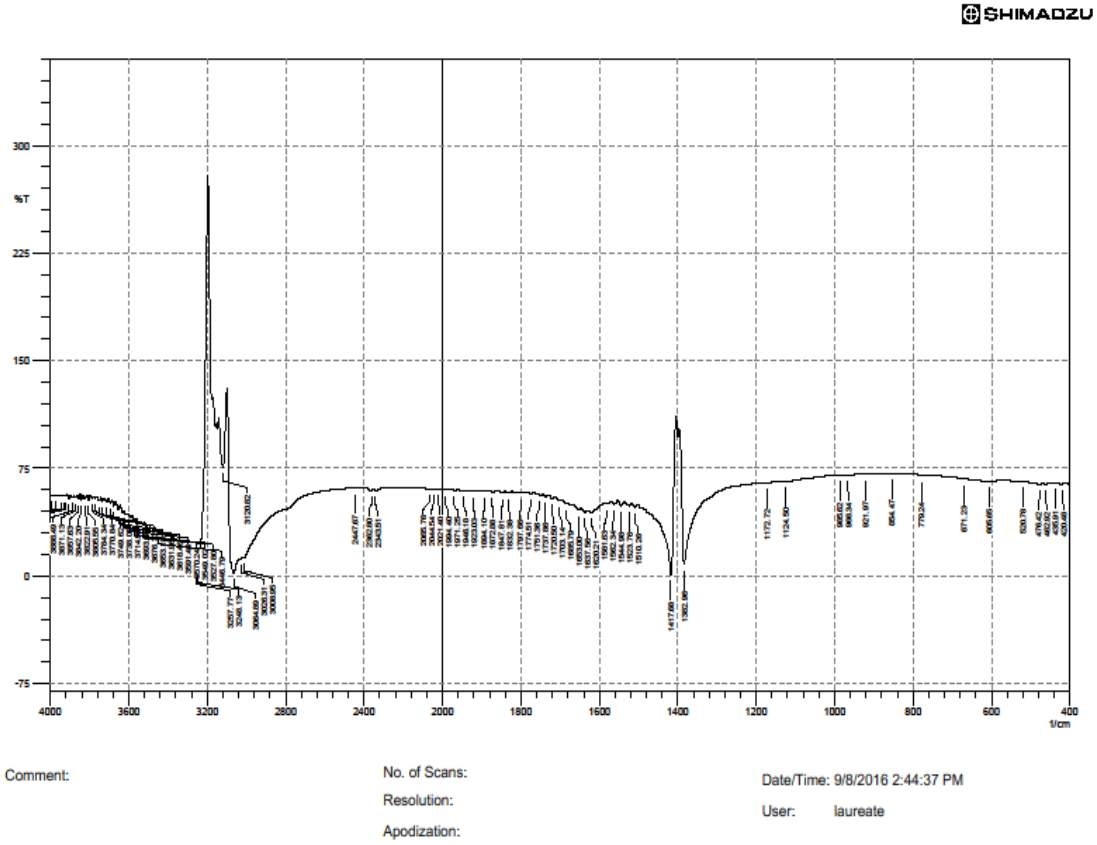



**Figure 5.** IR spectra of *Rosa polyantha* biopolymer

*Differential scanning calorimetery (DSC)*

DSC Peak of *Rosa polyantha* biopolymer was obtained at 130.59 °C, delta H at 79.0352 J/g, peak height at 7.9855 mW, peak area was 790.352 mJ (Figure 6).

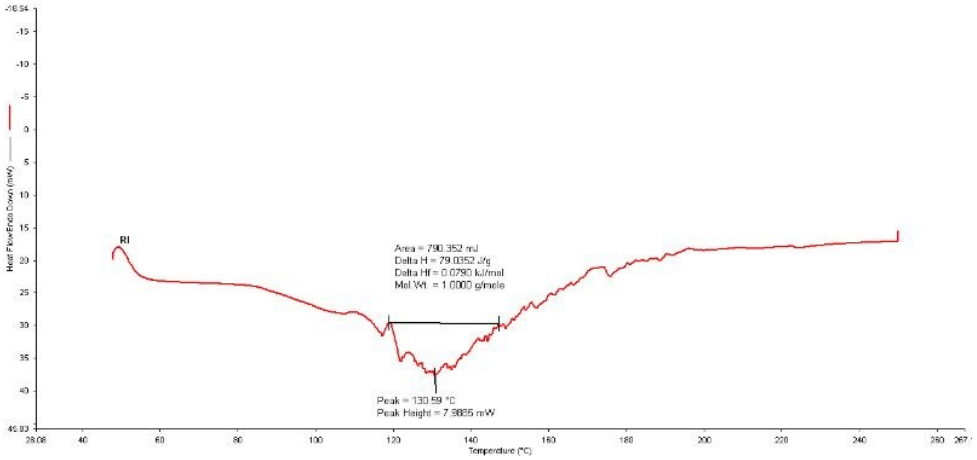



**Figure 6.** DSC of *Rosa polyantha* biopolymer

*Zeta sizing and particle size determination*

Particle size determination graph of *Rosa polyantha* biopolymer showed peak at 240.9 d.nm, standard deviation of 86.58 d.nm, polydispersity index (PDI) at 0.358, Z average (d. nm) was at 252.3, intensity at 100%, intercept at 0.935. It depicted monodisperse system (Figure 7).

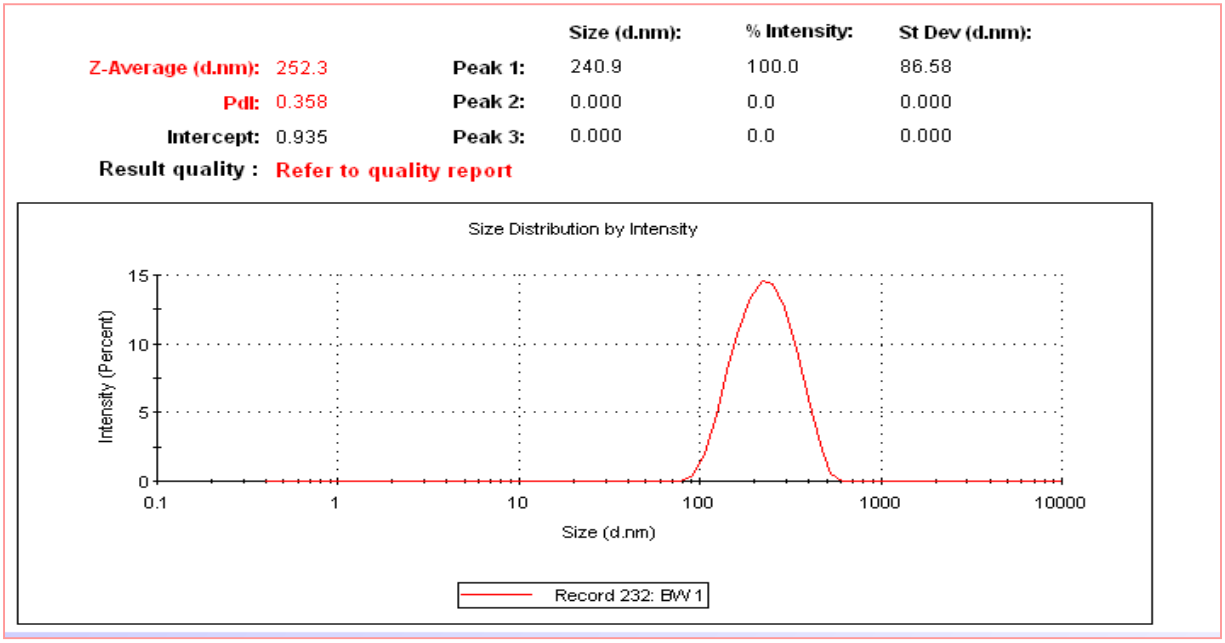



**Figure 7.** Particle size determination of *rosa polyantha* biopolymer

*Nuclea magnetic resonance spectroscopy (NMR)*

$^{1}$HNMR spectra of *Rosa polyantha* biopolymer confirmed the presence of carbohydrates residue within the biopolymer extracted as shift of carbohydrate protons were 3-6 ppm and the spectra when compared reflected the peak at 3.1996 (Figure 8).

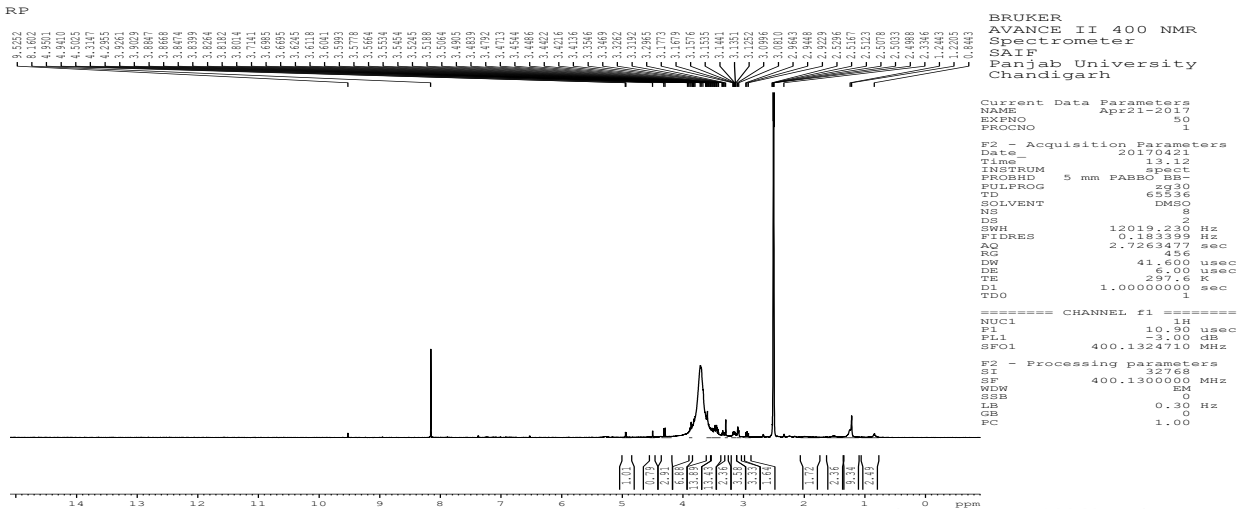



**Figure 8.** NMR spectral data of *Rosa polyantha* biopolymer

*Scanning electron microscopy (SEM) of isolated biopolymer*

Scanning electron microscopy image of *Rosa polyantha* biopolymer showed size range of 100 µm, irregular surface and smooth texture (Figure 9).

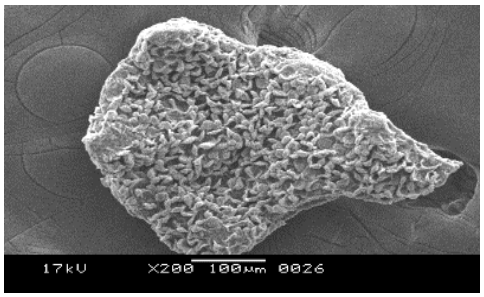



**Figure 9.** SEM of *Rosa polyantha* biopolymer

*Cell-line toxicity study data of isolated polymers*

The cell-line toxicity for isolated biopolymers was performed by MTT assay method using cell-line. Cell-line toxicity data of *Rosa polyantha* biopolymer in concentrations ranging from 25-200 µM, revealed IC50 (µM) of 250.676 and mean % cell viability ranging from 76.1-98.2%. Hence isolated *Rosa polyantha* biopolymer was found to be safe and devoid of toxicity (Table 3) (Figure 10).

*Safety of biopolymer*

**Table 3.** Cell-line toxicity data of *Rosa polyantha* biopolyme

| $H_9C_2$ Compound ID | 48 Hr Incubation treatment Concentration(µM) | Absorbance at 540 nm | | | Mean% cell death | Mean % cell viability | SEM |
|---|---|---|---|---|---|---|---|
| RP | 25 | 0.213 | 0.191 | 0.176 | 23.9 | 76.1 | 4.2 |
| (Rosa | 50 | 0.224 | 0.207 | 0.199 | 17.3 | 82.7 | 2.9 |
| polyantha) | 100 | 0.262 | 0.224 | 0.207 | 9.1 | 90.9 | 6.4 |
| biopolymer | 200 | 0.258 | 0.252 | 0.238 | 1.8 | 98.2 | 2.3 |

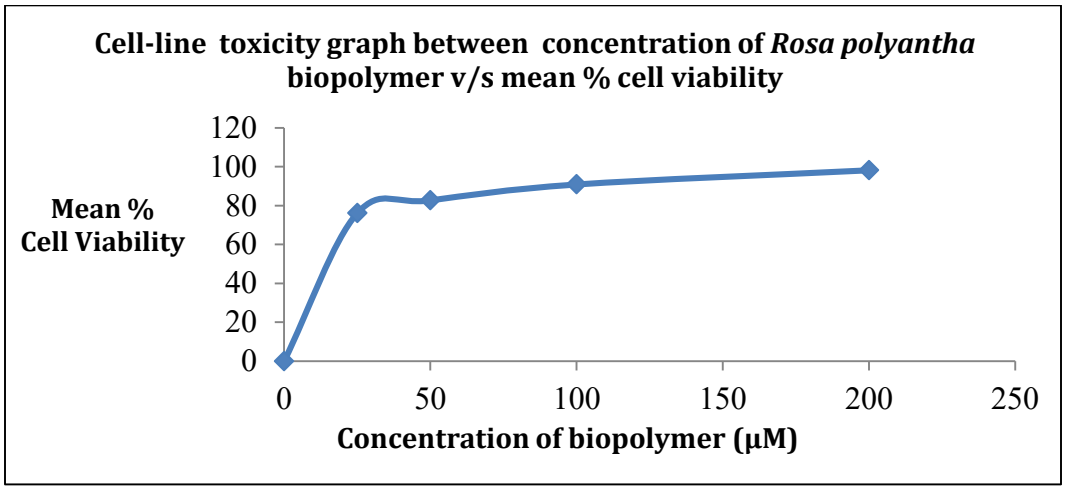



**Figure 10.** Cell-line toxicity graph between concentration of *Rosa polyantha* biopolymer (µM) v/s mean % cell viability

*In-vitro mucoadhesivity of isolated biopolymers by shear stress method*

Isolated biomaterial in concentrations 1%, 2%, 4%, 6%, 8% and 10% were subjected to shear stress for assessment for *in-vitro* adhesive strength in terms of weight required for breaking adhesive bonds between the biomaterial and the glass plate of modified shear stress apparatus after specified contact time from 0-30 minutes. The results were compared with standard polymer sodium carboxyl methyl cellulose (Sod CMC) 1% solution. The results showed significant Mucoadhesion strength of biomaterials as their concentrated was increased from 1%-10% with increased contact time from 0-30 minutes proportionately.

*In-vitro* mucoadhesion study data of *Rosa polyantha* biopolymer revealed that 1% biopolymer concentration showed significant results with p value<0.05 when compared to 10% biopolymer concentration and also with 1% sodium carboxyl methyl cellulose standard polymer. Order of mucoadhesivity of all concentrations of *Rosa polyantha* biopolymer was 10% *Rosa polyantha* biopolymer>8% *Rosa polyantha* biopolymer>4% *Rosa polyantha* biopolymer>2% *Rosa polyantha* biopolymer>6% *Rosa polyantha* biopolymer >1% *Rosa polyantha* biopolymer (Table 4) (Figure 11).

**Table 4**. *In-vitro* mucoadhesivity of *Rosa polyantha* biopolymer by shear stress method

| S. No. | Time (minutes) | Concentration of *Rosa polyantha* biopolymer solutions (%w/v) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1% | 2% | 4% | 6% | 8% | 10% |
| 1 | 0 minute | 50.58 g$^{***,a1}$ | 91.14 g | 92.9 g | 59.36 g | 92.15 g | 167.25 g |
| 2 | 10 minutes | 76.4 g$^{***,a1}$ | 180.61 g | 168.77 g | 102.12 g | 201.12 g | 199.08 g |
| 3 | 20 minutes | 112.6 g$^{***,a1}$ | 219.54 g | 251.3 g | 215.65 g | 258.62 g | 330.44 g |
| 4 | 30 minutes | 231 g$^{***,a1}$ | 258.87 g | 361.18 g | 326.39 g | 404.1 g | 450.42 g |

$^{***}$: p<0.05 as compared to 10% w/v biopolymer

$^{***, a1}$: p<0.05 as compared to 1%w/v sodium carboxyl methyl cellulose standard polymer significance level at 0.05, One way ANOVA using T test calculator

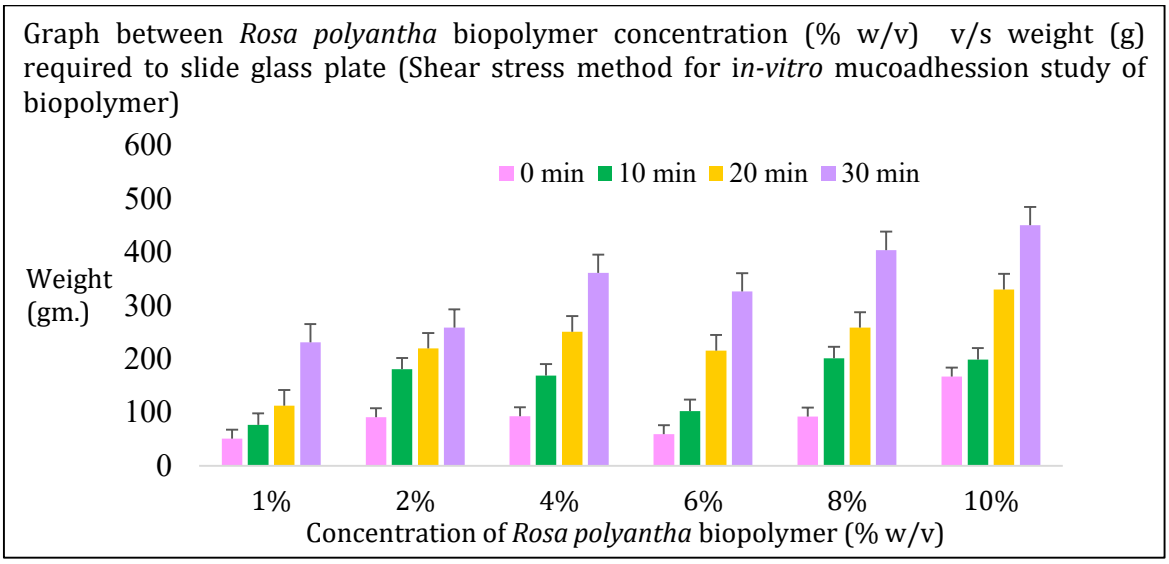



**Figure 11.** *In-vitro* mucoadhesion study graph of *Rosa polyantha* biopolymer

*Standard graph of tiagabine in distilled water*

Calibration curve of tiagabine was prepared in distilled water. The standard graph of tiagabine was obtained by plotting concentration versus absorbance. The standard curve of tiagabine showed linearityat a $\lambda_{max}$ of 257 nm. $R^2$ value was found to be 0.9993 (Figure 12**a**).

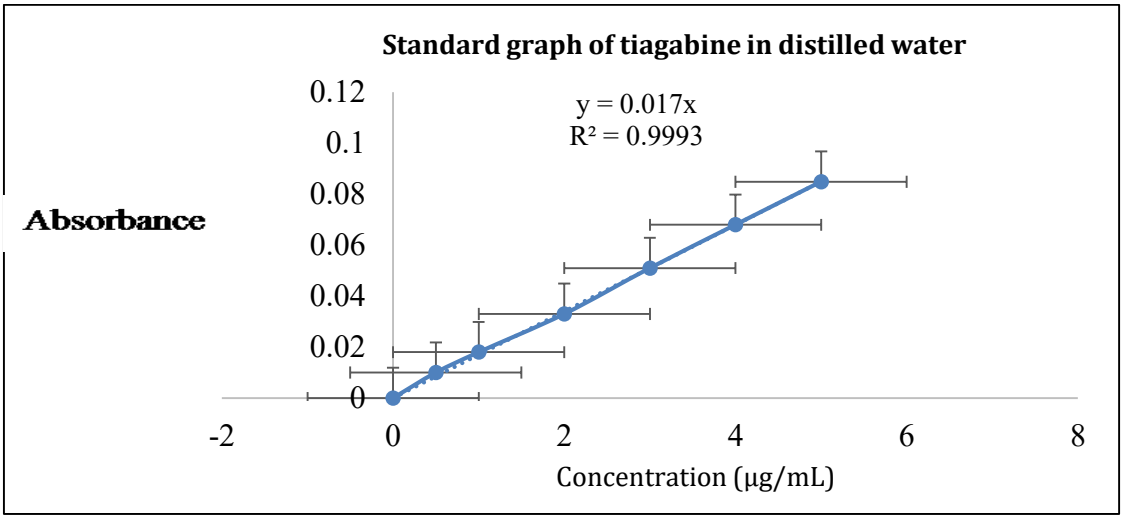



**Figure 12.** a) Standard graph of tiagabine in distilled water

*Standard graph of tiagabine in phosphate buffer pH 7.4*

Calibration curve of tiagabine was prepared in phosphate buffer pH 7.4. The standard graph of tiagabine was obtained by plotting concentration versus absorbance. The standard curve of tiagabine showed linearity at λmax of 396 nm. R2 value was found to be 0.9967 (Figure 12**b**).

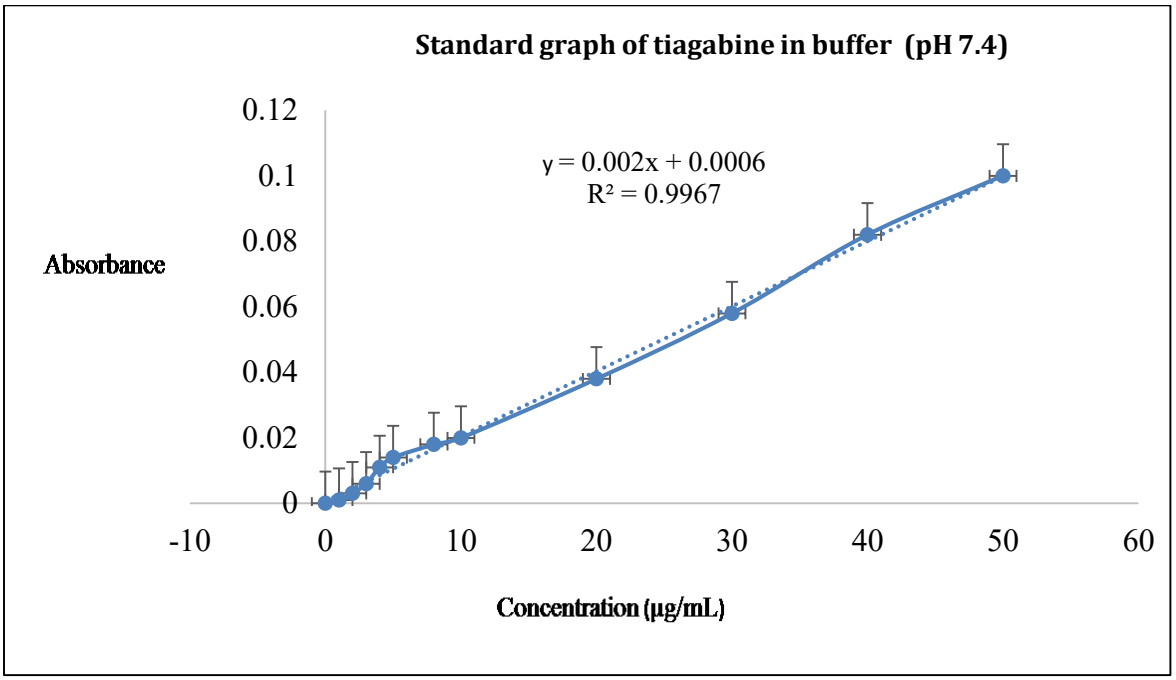



**Figure 12.** b) Standard graph of tiagabine in buffer (pH 7.4)

*Drug excipient interaction studies*

Drug-polymer interaction study of biomaterial isolated was done by UV techniques. Drug interaction study was performed by using wet and dry method.

*Wet method*

$\lambda_{max}$ was observed at 260 nm, no significant difference than that of pure drug tiagabine at 257 nm. Therefore, drug-excipient interaction did not occur as there was no shift in $\lambda_{max}$.

*Dry method*

$\lambda_{max}$ was observed at 260 nm, no significant difference than that of pure drug Tiagabine at 257 nm. Therefore, drug-excipient interaction did not occur as there was no shift in $\lambda_{max}$. Drug polymer interaction was not observed because no change in wavelength of pure drug and drug to biopolymer ratio.

*Colorimetry*

0.05 g of tiagabine 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)+biopolymer (0.05 g) also showed a similar color change with potassium permanganate. This indicated that the drug was not entrapped. With the use of U.V. method $\lambda_{max}$ 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 1:1 were mixed with potassium permanganate on glass plate. Observed color change, scrapped, diluted suitably with distilled water, analyzed by UV. Similarly repeated with drug:distilled water and drug:Potassium permanganate. Drug showed color change from pink to brown with potassium permanganate while polymer showed no color change. No

significant difference in shift of $\lambda_{max}$ than that of pure drug observed.

*Nanosizing of tiagabine:*

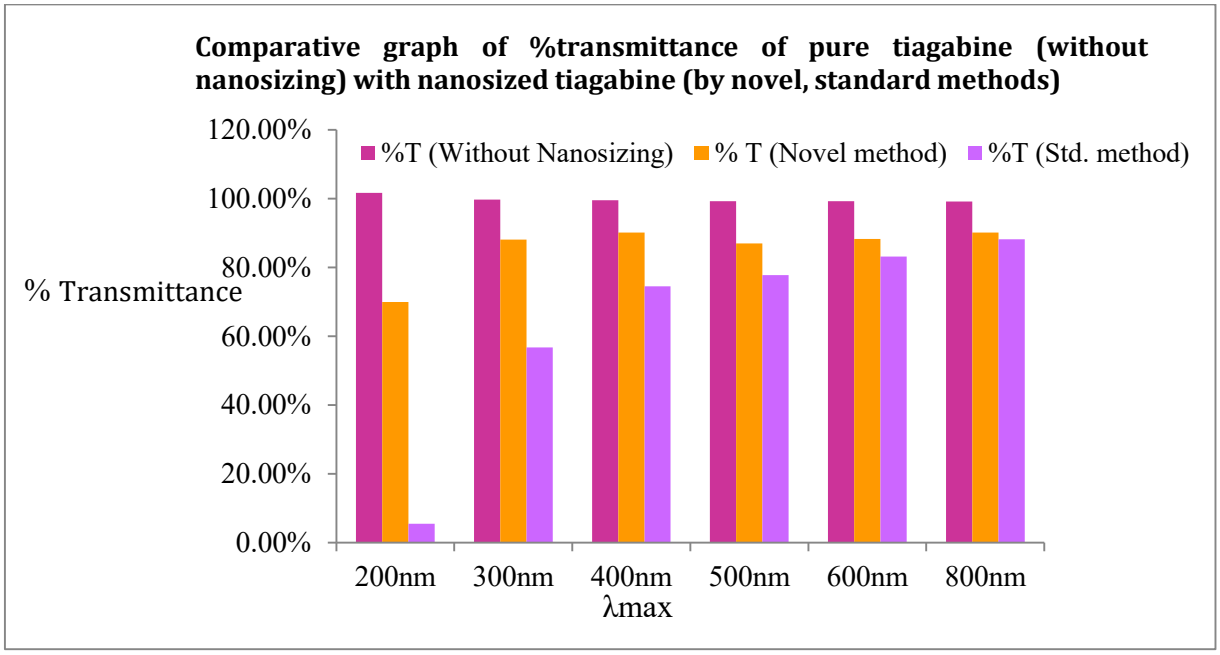



**Figure 13.** Comparative graph between % transmittance and λ max of pure tiagabine (without nanosizing) with nanosized tiagabine (by novel and Standard methods)

*Evaluation parameters of prepared bio-flexy films formulations*

*Thickness of formulations*

Thickness of formulations was measured using digital micrometer. As polymer concentration was increased, thickness of films increased proportionately. The thickness of nanosized tiagabine loaded bio-flexy films containing Rosa polyantha biopolymer (FRT1–FRT6) was found to be in range of 0.027±0.005 mm to 0.039±0.004 mm.

*Surface pH*

Prepared formulations suitable for soft palatal delivery platform as they are in the range of physiological pH. The surface pH of nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer (FRT1–FRT6) was found to be in range of 7.00±0.04 to 7.00±0.01.

*Ex-vivo mucoadhesion study of formulated bio-flexy films*

Ex-vivo mucoadhesion study by rotating cylinder method revealed that nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer (FRT1–FRT6) showed mucoadhesivity on Capra aegagrus mucosal surface for time period of 90-1440 minutes.

*Ex-vivo mucoretention study of formulated bio-flexy films*

Ex-vivo mucoretention study revealed that nanosized tiagabine loaded bio-flexy films containing Rosa polyantha biopolymer (FRT1–FRT6) were mucoretentive on capra aegagrus mucosal surface for time period of 110-240 minutes.

*Weight uniformity of formulated bio-flexy films*

The weight uniformity of all the formulations was proportionally increased as polymer concentration was increased. The weight uniformity of nanosized tiagabine loaded bio-flexy films containing rosa polyantha biopolymer (FRT1–FRT6) was found to be in range of 0.008±0.05 mg to 0.044±0.03 mg.

*Drug content uniformity of formulated bio-flexy films*

The drug content uniformity of all the formulations was proportionally increased as polymer concentration was increased. The drug content uniformity of nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer (FRT1–FRT6) was found to be in range of 85.6%±0.48 to 94.8%±0.37.

*Folding endurance of formulated bio-flexy films*

Folding endurance of all the formulations was measured and it showed that flexibility was proportionately increased significantly as concentration of polymer in formulation was increased. The bio-flexy films were devoid of brittleness showing significant folding endurance due to presence of dextrose and fructose as excipients in optimized ratio. The folding endurance of nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer (FRT1–FRT6) was found to be in range of 83-130.

*Swelling percentage of formulated bio-flexy films*

The swelling percentage of nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer (FRT1–FRT6) was found to be in range of 66%±0.2 to 75%±0.1.

*Percentage moisture uptake of formulated bio-flexy films*

The percentage moisture uptake of nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer (FRT1–FRT6) was found to be in range of 2.5%±0.14 to 3.8%±0.10 due to ability to imbibe maximum amount of water.

*In-vitro release study of formulated bio-flexy films by modified m.s. diffusion apparatus*

*In-vitro* release study of formulated bio-flexy films was performed by modified M.S. diffusion apparatus for up to 48 hours. The in-vitro drug release pattern for all the formulations were calculated and compared. T50% and T80% values along with the kinetics data were calculated using BITS software. The release kinetics mechanism was analyzed by comparing R2 values. Formulations were arranged based upon the above parameters is descending manner. The drug release pattern for formulations FRT1-FRT6 containing Rosa polyantha biopolymer based on the T50% and T80% was found to be FRT5 (1:6) > FRT4 (1:5) > FRT6 (1:10)>FRT1 (1:0.5)>FRT3 (1:3)>FRT2 (1:1). Based on all above mentioned evaluation parameters, FRT5 (containing Tiagabine: Rosa polyantha biopolymer (1:6)) bio-flexy film was selected as the Best formulation as it showed significant values of T50%: 7 hours., T80%: 30 hours and having R2=0.9295, higuchi matrix as best fit model, follows fickian diffusion (Higuchi Matrix) release mechanism in comparison to other formulations of same biopolymer (Figure 14 and Table 5).

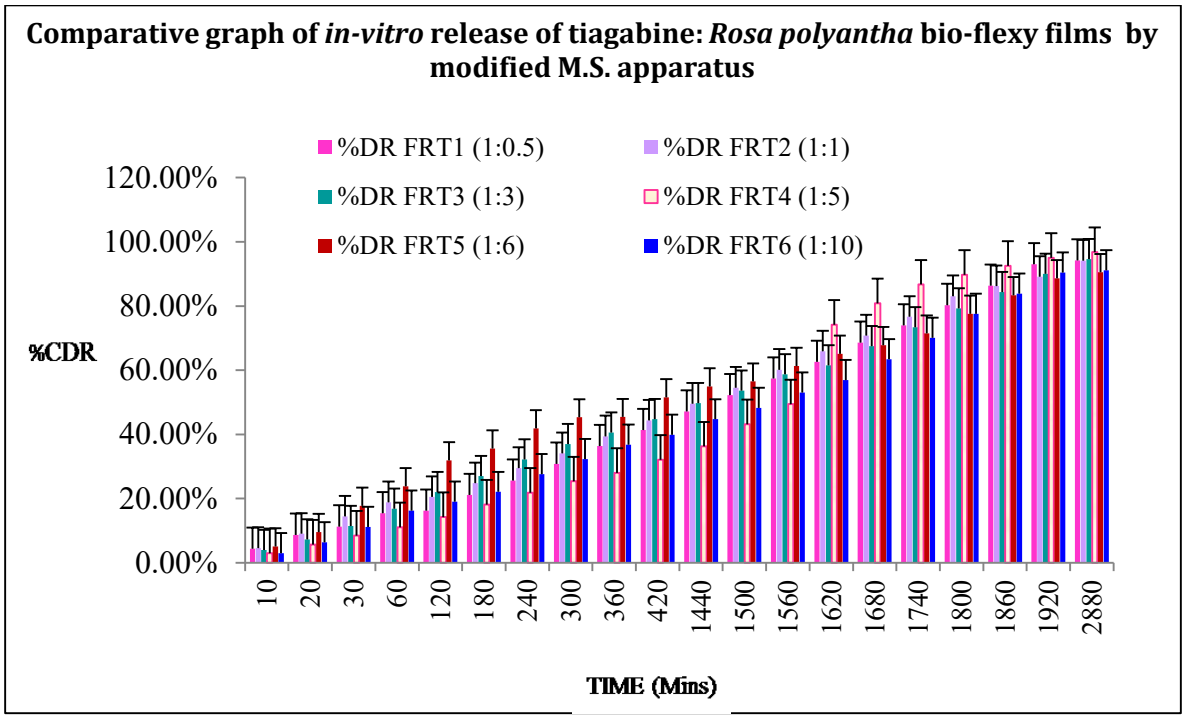



**Figure 14.** *In-vitro* drug release graph of nanosized tiagabine loaded bio-flexy films using *Rosa polyantha* biopolymer by modified M.S. diffusion apparatus (FRT1-FRT6)

**Table 5.** Kinetics release of tiagabine-rosa polyantha polymer bio-flexy films

| Release kinetics analysis dynamic method formulation of tiagabine: *Rosa polyantha* bio-flexy Films | | | | | | | |
|---|---|---|---|---|---|---|---|
| Formulations | $R^2$ | | | | | Best Fit Model | Mechanism of Action |
| | Zero order | 1st order | Higuchi Matrix | Peppas | Hixon Crowell | | |
| FRT1 (1:0.5) | 0.9064 | 0.9065 | 0.9366 | 0.9678 | 0.9065 | Peppas Korsmeyer | Fickian Diffusion (Higuchi Matrix) |
| FRT2 (1:1) | 0.9004 | 0.9006 | 0.9337 | 0.9608 | 0.9005 | Peppas Korsmeyer | Fickian Diffusion (Higuchi Matrix) |
| FRT3 (1:3) | 0.8863 | 0.8866 | 0.9333 | 0.9580 | 0.8865 | Peppas Korsmeyer | Fickian Diffusion (Higuchi Matrix) |
| FRT4 (1:5) | 0.8764 | 0.8762 | 0.9427 | 0.9553 | 0.8763 | Peppas Korsmeyer | Anomalous Transport |
| FRT5 (1:6) | 0.8275 | 0.8281 | 0.9295 | 0.9160 | 0.8279 | Higuchi Matrix | Fickian Diffusion (Higuchi Matrix) |
| FRT6 (1:10) | 0.8836 | 0.8836 | 0.9310 | 0.9477 | 0.8836 | Peppas Korsmeyer | Anomalous Transport |

The drug release pattern for formulations FET1-FET6 containing sodium carboxyl methyl cellulose standard polymer based on the T50% and T80% was found to be FET5 (1:6) > FET1 (1:0.5) > FET2(1:1)> FET3 (1:3)> FET4 (1:5) > FET6 (1:10). Based on all above mentioned

evaluation parameters, FET5 (containing tiagabine: sodium carboxyl methyl cellulose standard polymer (1:6)) flexy film was selected as the best formulation as it showed significant values of $T_{50\%}$: 40.66 hours, $T_{80\%}$: 43.79 hours and having $R^2$=0.9301, higuchi matrix as best fit model, follows fickian diffusion (Higuchi matrix) release mechanism in comparison to other formulations of same standard polymer. The best formulations of each biopolymer were further compared and again arranged in descending manner based on their $T_{50\%}$ and $T_{80\%}$ values. (Figure 15 and Table 6).

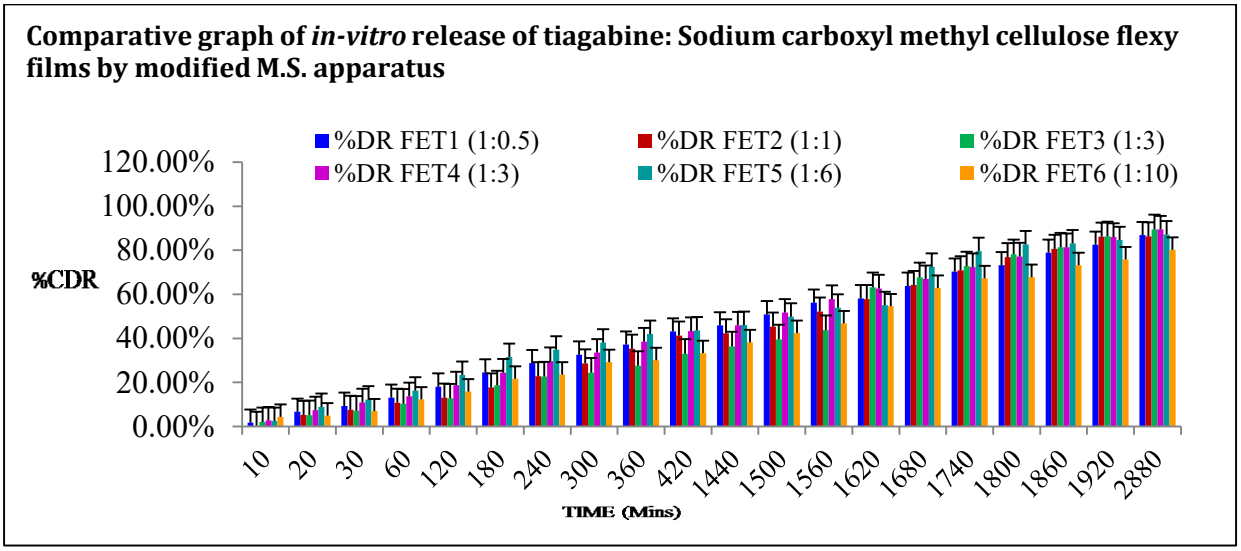



**Figure 15.** *In-vitro* drug release graph of nanosized tiagabine loaded bio-flexy films using sodium carboxyl methyl cellulose standard polymer by modified M.S. diffusion apparatus (FET1-FET6)

**Table 6.** Kinetics release of tiagabine-sodium CMC flexy films

| Release kinetics analysis dynamic method formulations of tiagabine: sodium CMC flexy films | | | | | | | |
|---|---|---|---|---|---|---|---|
| Formulations | $R^2$ | | | | | Best Fit Model | Mechanism of Action |
| | Zero order | 1st order | Higuchi Matrix | Peppas | Hixon Crowell | | |
| FET1 (1:0.5) | 0.8894 | 0.8897 | 0.9356 | 0.9300 | 0.8896 | Higuchi-Matrix | Anomalous Transport |
| FET2 (1:1) | 0.8852 | 0.8853 | 0.9324 | 0.8424 | 0.8853 | Higuchi-Matrix | Anomalous Transport |
| FET3 (1:3) | 0.8868 | 0.8868 | 0.9377 | 0.9550 | 0.8868 | Peppas Korsmeyer | Anomalous Transport |
| FET4 (1:5) | 0.8906 | 0.8908 | 0.9361 | 0.9514 | 0.8908 | Peppas Korsmeyer | Anomalous Transport |
| FET5 (1:6) | 0.8360 | 0.8363 | 0.9301 | 0.9084 | 0.8362 | Higuchi-Matrix | Fickian Diffusion (Higuchi Matrix) |
| FET6 (1:10) | 0.8960 | 0.8962 | 0.9372 | 0.9692 | 0.8961 | Peppas Korsmeyer | Anomalous Transport |

*Stability studies*

Stability studies were conducted as per ICH guidelines. Stability testing of pharmaceutical product is done to ensure the efficacy, safety and quality of active drug substance and dosage forms and shelf life or expiration period. The stability studies of the formulations were conducted at 40 °C ± 2°C and ± 45± 5% RH, 25 °C ± 2 °C and 60 ± 5% RH and 2 °C ± 5 °C temperature and RH values respectively period of three months. In-vitro drug release, folding endurance, surface pH of formulations were determined. Bio-flexy films were found to be stable (Figure 16).

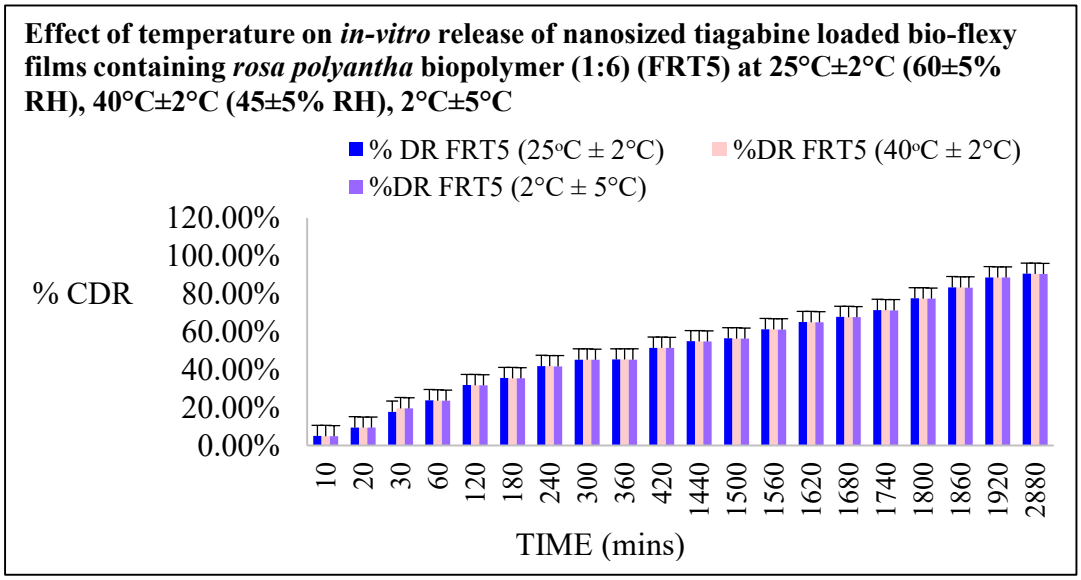



**Figure 16.** Stability study graph of best formulations of nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer

## Conclusion

In this research work, nanosized tiagabine loaded bio-flexy films were formulated using a novel biopolymer isolated from *Rosa polyantha* petals. Biopolymer possessed biodegradability, biocompatibility, non-toxicity, filmability, mucoadhesivity properties and was suitable for preparing bio-flexy films for trans-soft palatal delivery. Bio-flexy films were prepared by solvent casting technique. Drug to polymer ratio was chosen at five levels for Rosa polyantha; FRT1 (1:0.5), FRT2 (1:1), FRT3 (1:3), FRT4 (1:5), FRT5 (1:6), FRT6 (1:10), and five levels for Sodium CMC FET1 (1:0.5), FET2 (1:1), FET3 (1:3), FET4 (1:5), FET5 (1:6), FET6 (1:10). Prepared bio-flexy films of 1cm$^2$ were cut using a round punch for evaluation parameters and stability studies. *Rosa polyantha* biopolymer was found to be 22.4±0.01%. Thickness of nanosized Tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer (FRT1-FRT6) was ranging from 0.027 mm±0.005 to 0.039±0.004 mm, folding endurance: 83-130, surface pH: 7.00±0.04 to 7.00±0.01, weight uniformity: 0.008±0.05 to 0.044±0.03, Drug content uniformity: 85.6%±0.48 to 94.8%±0.37, swelling percentage: 66%±0.2 to 75%±0.1, percentage moisture uptake (PTU): 2.5%±0.14 to 3.8%±0.10. mucoadhesion study by dynamic method revealed that nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer were mucoadhesive for time period of 90-1440 minutes. Mucoretentive

Study revealed that nanosized tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer were mucoretentive for time period of 110-240 minutes. The drug release pattern for formulations FRT1-FRT6 containing Rosa polyantha biopolymer based on the $T_{50\%}$ and $T_{80\%}$ was found to be FRT5 (1:6) > FRT4 (1:5) > FRT6 (1:10) > FRT1 (1:0.5)> FRT3 (1:3) > FRT2 (1:1). *In-vitro* drug release was performed for all the formulations and the data indicate that drug loaded formulations show the sustained release behavior. Graph was plotted between %CDR and time, the $R^2$ value, $T_{50\%}$ and $T_{80\%}$ were calculated from graph. Based on all above mentioned evaluation parameters, FRT5 (containing tiagabine: *Rosa polyantha* biopolymer (1:6)) Bio-flexy film having $R^2$= 0.9295, higuchi matrix as best fit model, follows fickian diffusion (Higuchi matrix) release mechanism, $T_{50\%}$: 7 hrs., $T_{80\%}$: 30 hrs. using BITS software 1.12. Stability study revealed stable bio-flexy films with no significant change in physical appearance and stable pH. Prepared formulations of tiagabine loaded bio-flexy films containing *Rosa polyantha* biopolymer were suitable for soft palatal delivery. This research enlightens the potentiality of Oro-Soft palatal mucosa as a novelistic drug delivery platform for delivery to desired site of brain region in order to elicit anticonvulsant properly. Formulation FRT5 (containing Tiagabine: *Rosa polyantha* biopolymer (1:6)) was selected as Best Film.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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