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Smart Mucoadhesive Film Former from Seeds of *Arachis Hypogea* and Its In-Built Properties for Pharmaceutical Applications

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ABSTRACT

Aim: Isolation of novel biopolymer from *Arachis hypogea* seeds to be used as bio-excipient in formulation of nanosized Topiramate loaded bio-flexy films for epilepsy treatment.

Method: Formulations containing nanosized Topiramate: *Arachis hypogea* biopolymer (in ratios of 1:0.5, 1:1; 1:3, 1:5, 1:6, 1:10) (FAO1-FAO6) were prepared by solvent casting method.

Results: *Arachis Hypogea* Biopolymer showed percentage yield of 20% ±0.01. The biopolymer was brown in color, odourless, partially soluble in water. Its colour changing point was found to be 210°C±2. It was tested positive for proteins and carbohydrates, amino acids were not present. Evaluation parameters of nanosized Topiramate loaded bio-flexy films containing *Arachis hypogea* biopolymer (FAO1-FAO6) revealed Thickness: 0.022 mm±0.004 to 0.044±0.003 mm, Folding Endurance: 140-169, Surface pH: 7.01±0.03 to 7.01±0.02, Weight Uniformity: 0.006±0.04 to 0.036±0.02, Drug Content Uniformity: 85.4%±0.68 to 93.4%±0.50, Swelling Percentage: 73%±0.6 to 89%±0.4, Percentage Moisture Uptake (PTU): 1.8%±0.10 to 2.4%±0.08, Mucoadhesion time: 45-150 minutes, Mucoretention time: 75-210 minutes. The drug release pattern for formulations FAO1-FAO6 containing *Arachis hypogea* biopolymer based on the T50% and T80% was found to be FAO2 (1:1) > FAO5 (1:6) > FAO6 (1:10) > FAO1 (1:0.5)> FAO4 (1:5) > FAO3 (1:3).

Conclusion: Based on all above-mentioned evaluation parameters, FAO2 (containing Topiramate: *Arachis hypogea* biopolymer (1:1)) Bio-flexy film having R^2=0.9215, Higuchi Matrix as best fit model, follows Fickian Diffusion (Higuchi Matrix) release mechanism, $T_{50\%}$: 44.52 hrs., $T_{80\%}$: 46.14 hrs. using BITS Software. Stability study revealed stable formulations. Isolated *Arachis hypogea* biopolymer showed in-built filmability, mucoadhesivity properties, was non-reactive and suitable for Soft Palatal Drug Delivery.

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Keywords: *Arachis Hypogea* Biopolymer, Bio-flexy Films, Nanosized Topiramate, Soft Palatal Delivery

Abbreviations

Abbreviations	Full forms (expanded meaning)
mm	Millimetre
hr	Hours
cm^2	Centimetres Square
mins.	Minutes
mL	Millilitre
gm.	Grams
mg	Milligram
nm	Nanometer
µg	Microgram
µM	Micrometer
d. nm	Diameter in Nanometer
mV	Millivolt
rpm	Revolutions per minute
ºC	Degree Centigrade
GABA	Gamma Amino Butyric Acid
KBr	Potassium Bromide
GIT	Gastro Intestinal Tract
API	Active Pharmaceutical Ingredient
U.V.	Ultraviolet Visible Spectroscopy
λ_{max}	Maximum Absorbance

pKa	Dissociation Constant
C_{max}	Maximum Concentration
T_{max}	Time to attain peak Concentration
t1/2	Half Life
SEM	Scanning Electron Microscopy
IR	Infra-Red Spectroscopy
DSC	Differential Scanning Calorimetry
NMR	Nuclear Magnetic Resonance Spectroscopy
MTT	3-(4, 5–Dimethyl Thiazol–2–yl)–5–Diphenyl Tetrazolium Bromide
GIT	Gastro Intestinal Tract
Sodium CMC	Sodium Carboxyl Methyl Cellulose Standard Polymer
Abbreviations	Full forms (expanded meaning)
FAO1-FAO6	6 Bio-Flexy Films Formulations of nanosized Topiramate with *Arachis hypogea* biopolymer in ratios of (1:0.5-1:10)
FEO1-FEO6	6 Flexy Films Formulations of nanosized Topiramate with Sodium Carboxyl Methyl Cellulose Standard polymer in ratios of (1:0.5-1:10)
RH	Relative Humidity
CDR	Cumulative Drug Release
$T_{50\%}$	Time during which 50% Drug is released
$T_{80\%}$	Time during which 80% Drug is released
ICH	International Conference on Harmonization

Introduction

In current research scenario, vast number of bio-excipients obtained from natural edible sources are used in pharmaceuticals. Biopolymers have numerous advantages over the synthetic polymer being biocompatible, biodegradable, non-toxic, having lesser side effects and lower cost etc. Bio polymers have been successfully investigated and employed in the different types of formulation like solid, liquid, and semisolid dosage forms and are specifically useful in the design of novel drug delivery systems. Groundnut is a legume obtained from plant *Arachis hypogea* belonging to family Fabaceae. It contains 30 Essential Nutrients and Phytonutrients, Niacin, Folate, Fiber, Vitamin E, Minerals like Potassium, Sodium, Magnesium, Manganese, Selenium and Phosphorus,Carbohydrates-9.5 to 19%, Protein-25%,Essential Amino Acids like Arginine and Histidine as primary metabolites, Coenzyme Q10, Resveratrol, p-Coumaric Acid, Polyphenols, Oleic Acid. Epilepsy is a neurological disorder occurring due to excess of excitatory neurotransmitter discharges in brain. It affects 65 million people globally as stated by World Health Organization [1-3]. Topiramate, antiepileptic drug (t1/2:19-30 hours; bioavailability: 80%; protein binding: 13-17%; water solubility: 9.8 mg/L). It is used for Partial Onset and Generalized Onset Seizures. It causes potentiation of activity of Gamma-Amino Butyrate (GABA) neurotransmitter. Soft palate or velum constitutes back of roof of mouth. Soft palatal drug delivery provides sustained, controlled and retentive drug delivery. It has non-keratinized histology, no bone, abundant blood and nerve supply, drug directly reaches systemic circulation, non-invasive, non-mobile, afford high bioavailability, lower doses, not interfering with patient's regular routine activities of talking, eating, drinking, non-interference of tongue, suitable for bitter drugs, protein molecules, offers a Novel mucoadhesive Drug Delivery Platform for Brain targeting. Trigeminal nerve directly connects soft palate to brain, thus nanosized drug can directly reach into brain by via inter and intra neural pathway. Bio-flexy films were prepared by solvent casting method. Formulations were screened for various evaluation parameters [4,5].

Materials and Methods

DRUG: Topiramate (procured from Cipla Ltd, Mumbai)
POLYMERS: Groundnut seeds 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 *Arachis Hypogea* [6]
Procured Groundnut seeds from local market. 100 gm. of Ground Nuts soaked in water overnight. Removed Groundnuts peels, added optimized quantity of water, crushed to obtain slurry. Filtered slurry, filtrate centrifuged at 17000 rpm. Supernatant separated, collected, naturally dried residue. Residue washed repeatedly with propan-2-one, removed excess oil. Air dried isolated polymer, powdered, sieve through Sieve No. 120. Optimized six time and calculated % yield. Stored in well closed container for further use.

Physicochemical Characterization of Isolated Biomaterial [7,8]
The Physiochemical characterization of isolated bio-material was performed like color, odor, solubility, melting point and various chemical tests were performed.
(a) Texture, (b) Color, (c) Odor were examined physically.

Color Changing Point
Determined by capillary method by Melting point apparatus. The Biopolymer was kept in a capillary tube and it was fitted in a Melting point apparatus. Temperature was determined by thermometer.

Solubility: Determined in chloroform, methanol, distilled water, acetone.

Test for Carbohydrates: Molisch Reagent Test: 2 mL of biopolymer solution (0.1gm Dissolved in 2 mL of distilled water) was taken in a test tube. 2 drops of Molisch reagent (Solution of α-naphthol in 95% Ethanol) was added. Solution was poured slowly into a test tube containing 2 mL of concentrated Sulphuric acid. Two layers were formed. Observed color change.

Test for Proteins: Biuret Test: Determines the presence of peptide bonds in protein content in isolated biomaterial. 2 mL of biomaterial solution (0.1 gm. dissolved in 2 mL of distilled water) was taken in a test tube. 1 mL of sodium hydroxide solution (1%) and then 1% of Copper (II) sulphate solution was added drop wise. Allowed the mixture to stand for 5 minutes and observed the color change.

Test for Starch: 2 mL of biomaterial solution (0.1 gm. dissolved in 2 mL of distilled water) was taken in a test tube.1-2 drops of iodine solution was added, observed the color change.

Test for Reducing Sugar: 2 mL of biopolymer solution (0.1 gm. dissolved in 2 mL of distilled water) was taken in a test tube. Added 1 mL each of Fehling's A (7 g $CuSO_4.5H_2O$ dissolved in distilled water containing 2 drops of dilute sulfuric acid) and Fehling's B (35 gm. potassium tartrate, 12 gm. of sodium hydroxide in 100 mL of distilled water). Test tube was placed in a water bath at 60°C. Observed color change.

Spectral studies of isolated biopolymer [7,8]
IR Spectroscopy: The IR spectroscopy of isolated biopolymer in solid form was performed by using Potassium Bromide Disc Method. 1mg of sample was finely admixed with 100 mg of KBr in mortar. Pressure of 10 tons was applied to mixture using hydraulic pump. Small pellet of 1-2mm in diameter was formed. Kept pellet in path of IR radiation and recorded the spectrum within the range of 4000-200cm-1. IR of pure Topiramate was also performed.

DSC (Differential Scanning Calorimetry)
Amount of the heat difference of Sample and Reference was measured against temperature for determination of Glass Transition temperature (GTT or TSg). 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. DSC of pure Topiramate was also performed.

ZETA Sizing and Particle Size Determination
A Zeta Potential value 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
Provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecule. Solvent used was DMSO (Dimethyl Sulfoxide). Spectrometer was connected to flow cell of 5 mm diameter. High flow rates were applied to sample. When the valve switches back, the flow cell in the instrument was rinsed again with the reaction mixture. Spectrum was sent to the automation computer where it was processed and analyzed.

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

Cell-Line Toxicity Study of Biopolymer (MTT Cytotoxicity Assay)
H9c2 cell line (cardiac cells) has been used for this Cell-Line toxicity study. 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. The ability of cells to reduce MTT provides an indication of the mitochondrial integrity and activity interpreted as a measure of viability and/or cell number. Dead cells or their products do not reduce Tetrazolium [9].

In-Vitro Mucoadhesivity of Isolated Biopolymer
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 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.

Standard Graph of Drug [10,11]
Preparation of Standard Curve of Topiramate In Distilled Water
Topiramate does not contain intrinsic chromophore, thus it cannot be analysed by ultraviolet, visible or fluorescence absorption without pre-treatment. Colour Development Method of Topiramate was developed by the reaction of functional group present in it with Ammonium Molybdate as chromogenic agent in presence of 2M Hydrochloric Acid. From the working standard drug solution of 1,2,3,4,5 mL (10-50 µg/mL drug solution) was placed in 5 different 10 mL volumetric flasks. Into this 2 ml of 5% of Ammonium Molybdate was added followed by 2 mL of 2M hydrochloric acid. Volume was made up to 10 mL with Distilled Water. Reaction mixture was kept in water bath for 35 minutes for the completion of reaction for full blue colour development and the absorbance was measured against a reagent blank.

Preparation of Standard Graph of Topiramate In Phosphate Buffer pH 7.4
10 mg of Topiramate 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) (λmax = 244 nm). Absorbance was measured against solvent blank.

Drug Excipient Interaction Studies
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 Topiramate [12].

Dry Method
Topiramate: *Arachis hypogea* biopolymer 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 λmax with that of pure Topiramate and reported.

Wet Method
Topiramate: *Arachis hypogea* biopolymer 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 λmax with that of pure Topiramate and reported.

Colorimetry Method
Topiramate: *Arachis hypogea* in ratio of 1:1 were mixed with Potassium Permanganate on glass plate. Observed color change, diluted suitably with distilled water, analyzed by UV. Repeated with Drug: Distilled Water and Drug: Potassium Permanganate.

Nanosizing of Drug
Solvent Evaporation Method
100 mg Topiramate 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. Residue was dried and stored [12].

Sonication Method

100 mg Topiramate 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. Diluted the mixture 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 [12].

Permeation Study of Topiramate Using M.S. Apparatus

Nanosized Topiramate (10 mg) was added in Donor compartment while Phosphate Buffer (pH 7.4) was filled in Receiver compartment. Egg Shell Membrane was tied over donor compartment. Study was conducted for up to 48 hours. Nanosized Drug would permeate through egg membrane into Phosphate Buffer. At specific time intervals ranging from 10 minutes up to 48 hours, samples of 5 mL were withdrawn and immediately restored with the same volume of fresh phosphate buffer. The amount of drug permeated was assessed by measuring the absorbance at 244 nm using U.V spectrophotometer and compared with that of control i.e., without nanosized drug and reported [13].

Formulation of Bio-Flexy Films (Solvent Casting Method)

100 mg of Nanosized Topiramate (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 (Solvent). To this dispersion, incorporated 10 mg of Dextrose (Flexicizer), 5 mg of Fructose (Flexicizer) and 10 µL of Glycerine (1% solution v/v) (Plasticizer) with continuous stirring. 0.6 gm. 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. Similarly, six different formulations of nanosized Topiramate with different isolated biopolymers and Standard Sodium Carboxyl Methyl Cellulose Polymer in different ratios of 1:1, 1:3, 1:5, 1:6 and 1:10 were prepared. (Tables 1, 2)

Table 1: Formulation of Nanosized Topiramate Loaded Bio-Flexy Films Using *Arachis Hypogea* Biopolymer

Formulation	FAO1 (1:0.5)	FAO2 (1:1)	FAO3 (1:3)	FAO4 (1:5)	FAO5 (1:6)	FAO6 (1:10)
Nanosized Topiramate (mg)	100	100	100	100	100	100
Arachis hypogea 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 Topiramate Loaded Flexy Films Using Sodium Carboxyl Methyl Cellulose Standard Polymer

Formulation	FEO1 (1:0.5)	FEO2 (1:1)	FEO3 (1:3)	FEO4 (1:5)	FEO5 (1:6)	FEO6 (1:10)
Nanosized Topiramate (mg)	100	100	100	100	100	100
Sodium Carboxyl Methyl Cellulose Standard Polymer (SCMC) (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

Evaluation of Formulated Bio-Flexy Films [14]

Thickness: The average thickness of formulations was determined using standard digital micrometer and reported with appropriate standard deviation.

Surface pH Study

The formulations were allowed to swell by keeping in contact with 1 ml of distilled water for 1 hour at room temperature. The pH was measured by bringing the electrode in contact with the surface of film and allowing it to equilibrate for 1 minute. The experiments were performed in triplicate and avg. values were reported.

Ex-Vivo Mucoadhesion Study of Formulations by Rotating Cylinder Method

Mucoadhesivity of formulated films was evaluated on soft palatal mucosa of Capra aegagrus (i.e., Goat). Bio-flexy films of area 1cm2 of each formulation were cut down using sharp blade. Tied the goat soft palatal 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 soft palatal mucosa. The dislodgement and detachment of films from mucosal surface was observed at regular intervals and reported.

Ex-Vivo Mucoretention Study of Formulations

Bio-flexy films of area 1cm2 of each formulation were cut down using sharp blade. Tied the Capra aegagrus (Goat) soft palatal mucosa over slanting condenser over which buffer was allowed to flow from a burette. It was applied over the inner surface of Goat soft palatal mucosa. The detachment and dislodgement of film from mucosal substrate was noted at regular intervals and reported.

Weight Uniformity of Formulated Nanosized Drugs Loaded Bio-Flexy Films

Weighed 10 formulations of 1 cm2 diameter. Determined average weight and reported.

Drug Content Uniformity of Formulated Nanosized Drugs Loaded Bio-Flexy Films

Dissolved the films in Phosphate Buffer (pH7.4) (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 λmax 750 nm.

Folding Endurance of Formulated Nanosized Drugs Loaded Bio-Flexy Films

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

1x1 cm2 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 for 24 hours. Calculated % Swelling Index and reported.

Percentage Moisture Uptake (PMU) of Formulated Nanosized Drugs Loaded Bio-Flexy Films

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.

$$\text{Percentage Moisture Uptake} = \frac{(\text{Final weight of Films} - \text{Initial weight of films}) \times 100}{\text{Initial weight of Films}}$$

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.

Stability Studies of Prepared Films as Per ICH Guidelines (Q1B)

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 at room temperature and after stability study (25 ± 2°C with 60 ± 5% RH).

Results and Discussion

Yield of Isolated Biopolymer

Biopolymer was isolated from natural edible source of *Arachis hypogea* by simplified economic process. The optimization of biopolymer isolation process was repeated six times and the % yield was calculated. The % yield of *Arachis hypogea* biopolymer was found to be 20% ±0.01.

Physicochemical Properties of Isolated Biomaterial

The biomaterial obtained from the seeds of *Arachis hypogea* and showed following characteristics: (a) Texture: Powder; (b) Color: Brown; (c) Odor: Odorless; (d) Solubility: partially soluble in water and insoluble in alcohol; (e) Color Changing Point: 210ºC±2.

Molisch Reagent Test for Carbohydrates

Appearance of purple color at interface of two layers due to formation of 5-hydroxy methyl furfural. This indicated presence of carbohydrates.

Biuret Test for Proteins

Color change was observed. Cu (II) ions form a violet-colored chelate complex that absorbs light at 540 nm. This indicated presence of proteins.

Test for Starch

Intense blue black color did not appeared confirmed the absence of Starch in isolated biomaterial.

Test for Reducing Sugar

brick red precipitate appeared indicating the presence of reducing sugar. It was due to formation of insoluble copper oxide.

Spectral Studies of Isolated Biopolymer

IR Spectroscopy:

IR Spectroscopy was performed for the isolated biomaterial to determine the presence of Functional Groups in biopolymer. IR Peaks of *Arachis hypogea* biopolymer were obtained at 3441 cm-1, 3223cm-1, 1647cm-1, 1612cm-1, 1082 cm-1, 1686 cm-1, 1409 cm-1 which indicated functional groups RCH2OH, RCOOH, R2C=CH2, RCONH2, RNH2, S=O. (Figure 1)

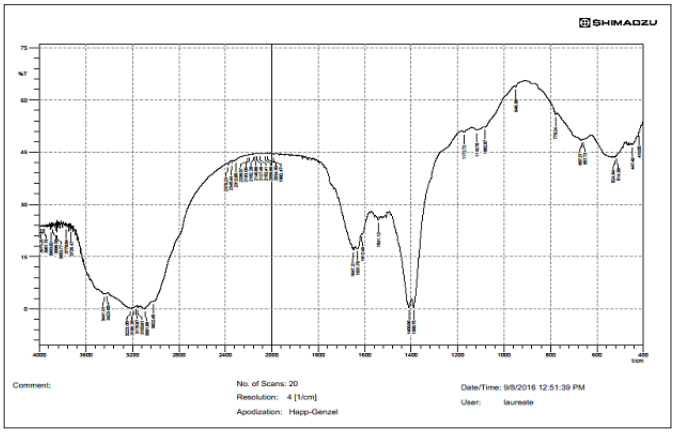

Figure 1: IR Spectra of *Arachis Hypogea* Biopolymer

Differential Scanning Calorimetery (DSC)

DSC Peak of *Arachis hypogea* biopolymer was obtained at 71.72ºC, Peak Height was 0.5482 mW, Delta H was 19.6931 J/g. (Figure 2)

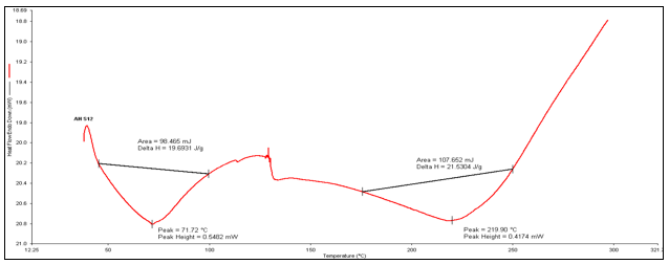

Figure 2: DSC Spectra of *Arachis Hypogea* Biopolymer

Zeta Sizing and Particle Size Determination

Particle Size Determination graph of *Arachis hypogea* biopolymer showed Peak at -11.9 mV, Standard Deviation of 4.82 mV, Conductivity 2.76 (mS/cm), Zeta Potential was -11.9 mV, Zeta Deviation was 4.82 mV, Area at 100%. It depicted monodisperse system. (Figure 3)

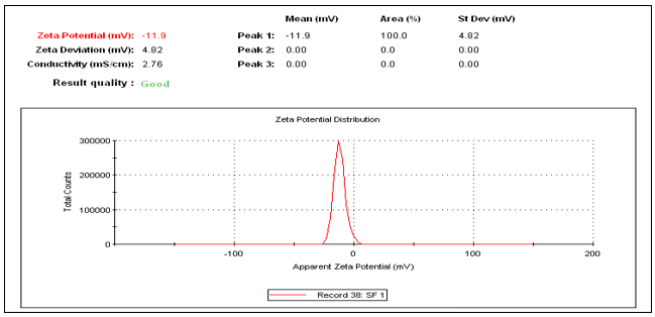

Figure 3: Particle Size Determination of *Arachis Hypogea* Biopolymer

Nuclear Magnetic Resonance Spectroscopy (NMR)

1HNMR Spectra of *Arachis hypogea* 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 3.4951 ppm. NMR studies revealed doublets and multiplets at 3.45δ and 3.34 δ respectively showing methyl groups. (Figure 4)

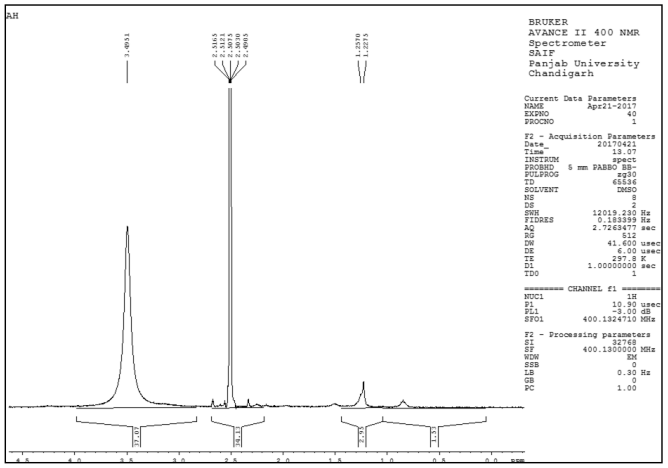

Figure 4: NMR Spectra of *Arachis Hypogea* Biopolymer

Scanning Electron Microscopy (SEM) of Isolated Biopolymer

SEM image of *Arachis hypogea* biopolymer showed size range of 100 µm, irregular structure with smooth texture. (Figure 5)

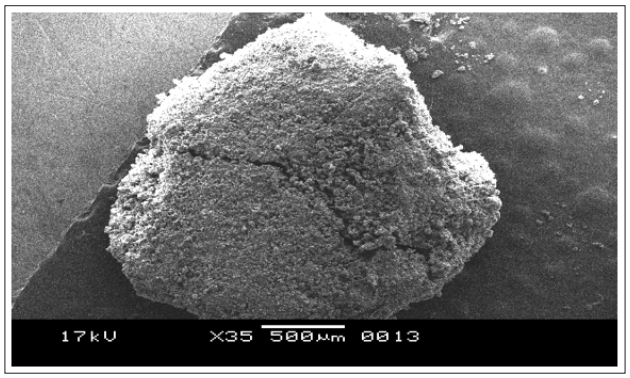

Figure 5: SEM of *Arachis Hypogea* Biopolymer

Cell-Line Toxicity Study Data of Isolated Polymer

The Cell-Line toxicity for isolated biopolymers was performed by MTT Assay Method using H9c2 Cell-Line. Cell-line toxicity data of *Arachis hypogea* biopolymer in concentrations ranging from 25-200 µM revealed IC50 (µM) of 6.86482 and mean % cell viability ranging from 75.9-94.1%. Hence isolated *Arachis hypogea* biopolymer was found to be safe and devoid of toxicity. (Figure 6)

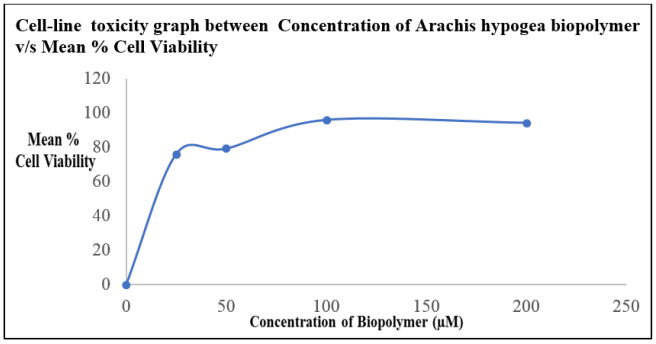

Figure 6: Cell-Line Toxicity Study Data Graph of *Arachis Hypogea* Biopolymer

In-Vitro Mucoadhesivity of Isolated Biopolymers by Shear Stress Method

Order of Mucoadhesivity of all concentrations of *Arachis hypogea* biopolymer was 10% *Arachis hypogea* biopolymer >8% *Arachis hypogea* biopolymer >6% *Arachis hypogea* biopolymer>4% *Arachis hypogea* biopolymer>2% *Arachis hypogea* biopolymer>1% *Arachis hypogea* biopolymer. (Table 3)

Table 3: In-Vitro Mucoadhesivity of *Arachis Hypogea* Biopolymer By Shear Stress Method

S. No.	Time (minutes)	Concentration of *Arachis hypogea* biopolymer solutions (%w/v)						Sodium CMC
		1%	2%	4%	6%	8%	10%	1%
1.	0 minute	50.68gm.***,a1	64.84gm.***,a1	85.5gm.***,a1	134.76gm.	143.25gm.	170.35gm.	186.85gm.
2.	10 minutes	71.9 gm.***,a1	85.41gm.***,a1	123.77gm.***,a1	167.42gm.	165.32gm.	200.48gm.	222.84gm.
3.	20 minutes	104.4gm.***,a1	145.94gm.***,a1	177.6gm.***,a1	204.85gm.	243.02gm.	260.26gm.	260.06gm.
4.	30 minutes	152 gm.***,a1	173.07gm.***,a1	216.58gm.***,a1	260.22gm.	284.6 gm.	313.64gm.	300.04gm.

***: 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

Spectral Studies of pure Topiramate

IR Spectra of Topiramate: IR Peaks of Topiramate were obtained at 240 cm^{-1}, 352 cm^{-1}, 928 cm^{-1}, 1022 cm^{-1}, 1102 cm^{-1}, which indicated functional groups at CH_3, SO_3, CH_2, C=O, NH2 respectively. (Figure 7)

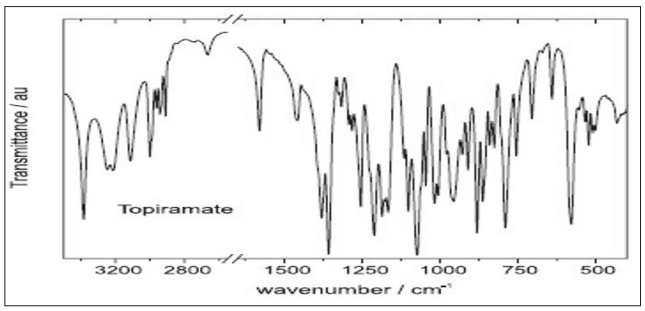

Figure 7: IR Spectra of Pure Topiramate

DSC Spectra of Topiramate

The DSC curves of Topiramate was recorded using Shimadzu-DSC 50, in dynamic Nitrogen atmosphere with a constant flow rate of 30 mL/min and heating rate of 2◦C/min, up to temperature 200◦C/ min using a mass of about 2 mg of sample packed in platinum pan. DSC equipment was preliminarily calibrated with standard reference of indium. Ten tablets were accurately weighed, amount equivalent to 2 mg of each drug substance was packed in the pan. Then the DSC curves were recorded. DSC Peak of Topiramate was obtained at 122.41ºC, Delta H at -95.56/g, The endothermic peak at 178 ºC is attributed to the first stage of decomposition, where Topiramate loses the sulfamate group, preceding mass loss. (Figure 8)

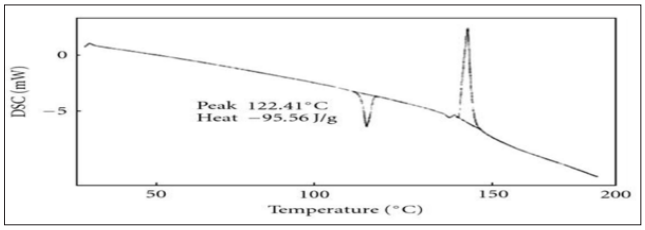

Figure 8: DSC Spectra of Pure Topiramate

Standard Graphs of Topiramate

Standard Graph of Topiramate in Distilled Water

The Standard Curve of Topiramate showed linearity range of 10 to 50 µg/ml at λ_{max} of 750 nm. R2 value was found to be 0.9994. (Figure 9 (a))

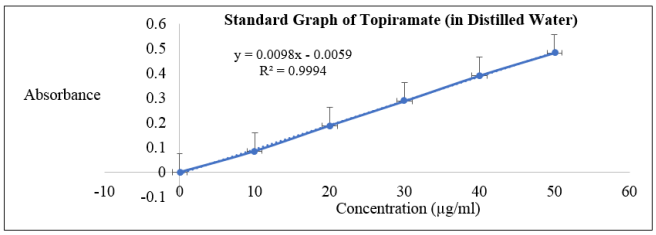

Figure 9 (a): Standard Graph of Topiramate In Distilled Water

Standard Graph of Topiramate in Phosphate Buffer pH 7.4

The Standard Curve of Topiramate showed linearityat λ_{max} of 244 nm. R^2 value was found to be 0.9945. (Figure 9(b))

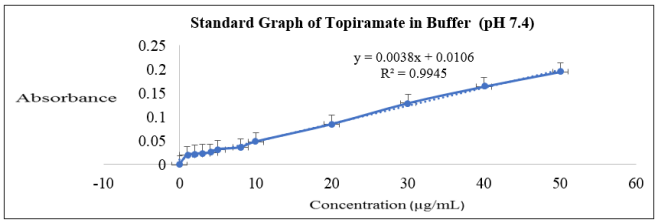

Figure 9 (b): Standard Graph of Topiramate In Phosphate Buffer pH 7.4

Drug–Polymer Interaction Study of The Isolated Biopolymer

Wet Method: λ_{max} at 752 nm showed no significant difference with that of pure Topiramate at 750 nm. Therefore, drug-excipient interaction did not occur as there was no shift in λ_{max}.

Dry Method

λ_{max} at 752 nm showed no significant difference with that of pure Topiramate at 750 nm. Therefore, drug-excipient interaction did not occur as there was no shift in λ_{max}.

Colorimetry: Drug showed color change from pink to brown with Potassium Permanganate while polymer showed no color change. No significant difference in shift of λ_{max} than that of pure drug observed.

Nanosizing of Topiramate

Compared %transmittance and λ max of nanosized Topiramate (by Novel Sonication and Standard Solvent Evaporation Method. (Figure 10)

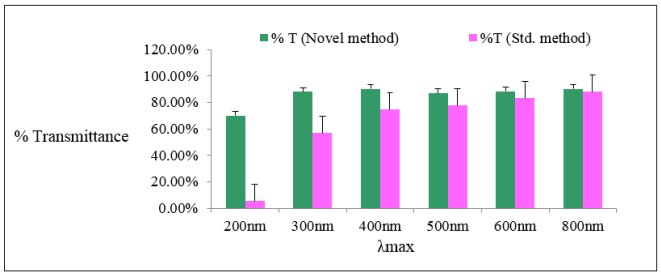

Figure 10: Comparative Graph between %transmittance and λ max of nanosized topiramate (By Novel Sonication and Standard Solvent Evaporation Methods

Permeation Study of Topiramate

Permeation study of pure and nanosized Topiramate using M.S. Apparatus revealed that nanosized Topiramate permeated more through egg membrane than pure Topiramate. (Figure 11)

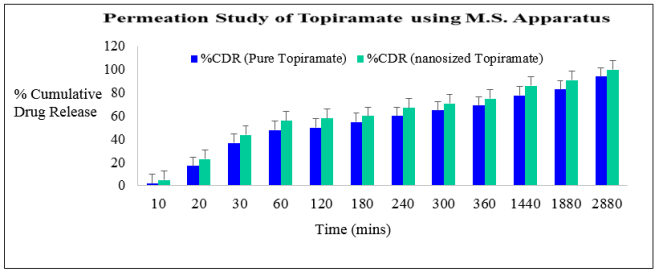

Figure 11: Permeation Study of Topiramate Using M.S. Apparatus

Evaluation Parameters of Formulation

Thickness of Formulated Bio-Flexy Films

As polymer concentration was increased, thickness of films increased proportionately. The thickness of nanosized Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6) was found to be in range of 0.022±0.004 mm to 0.044±0.003 mm.

Surface pH of Formulated Bio-flexy Films:

The Surface pH of nanosized Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6) was found to be in range of 7.01±0.03 to 7.01±0.02.

Ex-Vivo Mucoadhesion Study of Formulated Bio-Flexy Films Using Capra Aegagrus (Goat) Soft Palatal Mucosa

Ex-Vivo Mucoadhesion Study by Rotating Cylinder method revealed that nanosized

Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6)

showed mucoadhesivity on Capra aegagrus soft palatal mucosal surface for time period of 45-150 minutes.

Ex-Vivo Mucoretention Study of Formulated Bio-Flexy Films Using Capra Aegagrus (Goat) Soft Palatal Mucosa

Ex-Vivo Mucoretention Study revealed that nanosized Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6) were mucoretentive on Capra aegagrus soft palatal mucosal surface for time period of 75-210 minutes.

Weight Uniformity of Formulated Bio-Flexy Films

The Weight Uniformity of nanosized Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6) was found to be in range of 0.006±0.04 mg to 0.036±0.02 mg.

Drug Content Uniformity of Formulated Bio-Flexy Films

The Drug Content Uniformity of nanosized Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6) was found to be in range of 85.4%±0.68 to 93.4%±0.50.

Folding Endurance of Formulated Bio-Flexy Films

The Folding Endurance of nanosized Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6) was found to be in range of 140-169.

Swelling Percentage of Formulated Bio-Flexy Films

The Swelling Percentage of nanosized Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6) was found to be in range of 73%±0.6 to 89%±0.4.

Percentage Moisture Uptake of Formulated Bio-Flexy Films

The Percentage Moisture Uptake of nanosized Topiramate loaded Bio-Flexy films containing *Arachis hypogea* biopolymer (FAO1–FAO6) was found to be in range of 1.8%±0.10 to 2.4%±0.08.

In-Vitro Release Study of Formulated Bio-Flexy Films by Modified M.S. Diffusion Apparatus

The drug release pattern for formulations FAO1-FAO6 containing *Arachis hypogea* biopolymer based on the T50% and T80% was found to be FAO2 (1:1) > FAO5 (1:6) > FAO6 (1:10) > FAO1 (1:0.5)> FAO4 (1:5) > FAO3 (1:3). Based on all above mentioned evaluation parameters, FAO2 (containing Topiramate: *Arachis hypogea* biopolymer (1:1)) Bio-Flexy film was selected as the Best formulation as it showed significant values of $T_{50\%}$: 27 hours., $T_{80\%}$: 29 hours and having R2=0.9215, Higuchi Matrix as best fit model, follows Fickian Diffusion (Higuchi Matrix) release mechanism in comparison to other formulations of same biopolymer. (Figure 12) (Table 4)

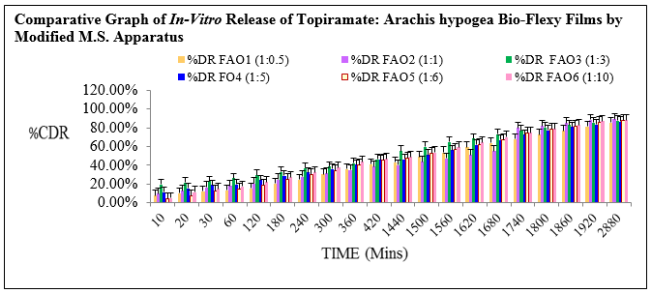

Figure 12: In-Vitro Drug Release Graph of nanosized Topiramate loaded Bio-Flexy Films using *Arachis hypogea* biopolymer by Modified M.S. Diffusion Apparatus

The drug release pattern for formulations FEO1-FEO6 containing Sodium Carboxyl Methyl Cellulose Standard polymer based on the $T_{50\%}$ and $T_{80\%}$ was found to be FEO4 (1:5) > FEO6 (1:10) > FEO5 (1:6)> FEO1 (1:0.5)> FEO2 (1:1) > FEO3 (1:3). Based on all above mentioned evaluation parameters, FEO4 (containing Topiramate: Sodium Carboxyl Methyl Cellulose standard polymer (1:5)) Flexy film was selected as the Best formulation as it showed significant values of $T_{50\%}$: 10.58 hours, $T_{80\%}$: 11.78 hours and having R2=0.9698, Peppas Korsmeyer as best fit model, follows Anomalous transport release mechanism in comparison to other formulations of same standard polymer. (Figure 13) (Table 5)

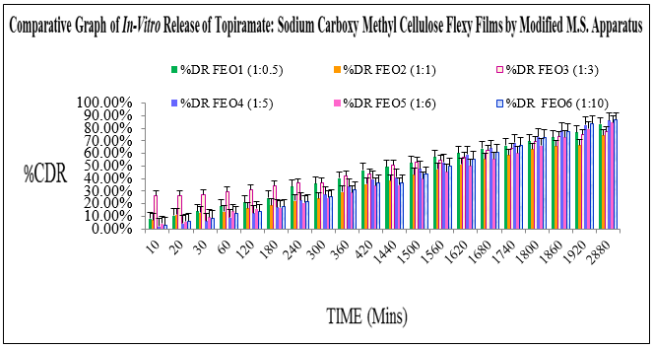

Figure 13: In-Vitro Drug Release Graph of nanosized Topiramate loaded Bio-Flexy Films using Sodium Carboxyl Methyl Cellulose standard polymer by Modified M.S. Diffusion Apparatus

Table 4: Kinetics Release of Topiramate-*Arachis hypogea* polymer Bio-flexy Films

Release Kinetics Analysis Dynamic Method Formulation of Topiramate: *Arachis hypogea* Bio-Flexy Films							
Formulations	**R2**					**Best Fit Model**	**Mechanism of Action**
	Zero order	**1st order**	**Higuchi Matrix**	**Peppas**	**Hixon Crowell**		
FAO1 (1:0.5)	0.9043	0.9045	0.9361	0.9606	0.9044	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)
FAO2 (1:1)	0.8362	0.8361	0.9215	0.9146	0.8361	Higuchi Matrix	Fickian Diffusion (Higuchi Matrix)
FAO3 (1:3)	0.8899	0.8904	0.9130	0.9537	0.8902	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)
FAO4 (1:5)	0.8733	0.8736	0.9221	0.9562	0.8735	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)
FAO5 (1:6)	0.8807	0.8810	0.9374	0.9677	0.8809	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)
FAO6 (1:10)	0.8696	0.8699	0.9330	0.9558	0.8698	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)

The drug release pattern for formulations FEO1-FEO6 containing Sodium Carboxyl Methyl Cellulose Standard polymer based on the $T_{50\%}$ and $T_{80\%}$ was found to be FEO4 (1:5) > FEO6 (1:10) > FEO5 (1:6)> FEO1 (1:0.5)> FEO2 (1:1) > FEO3 (1:3). Based on all above mentioned evaluation parameters, FEO4 (containing Topiramate: Sodium Carboxyl Methyl Cellulose standard polymer (1:5)) Flexy film was selected as the Best formulation as it showed significant values of $T_{50\%}$: 10.58 hours, $T_{80\%}$: 11.78 hours and having R^2=0.9698, Peppas Korsmeyer as best fit model, follows Anomalous transport release mechanism in comparison to other formulations of same standard polymer. (Figure 13) (Table 5)

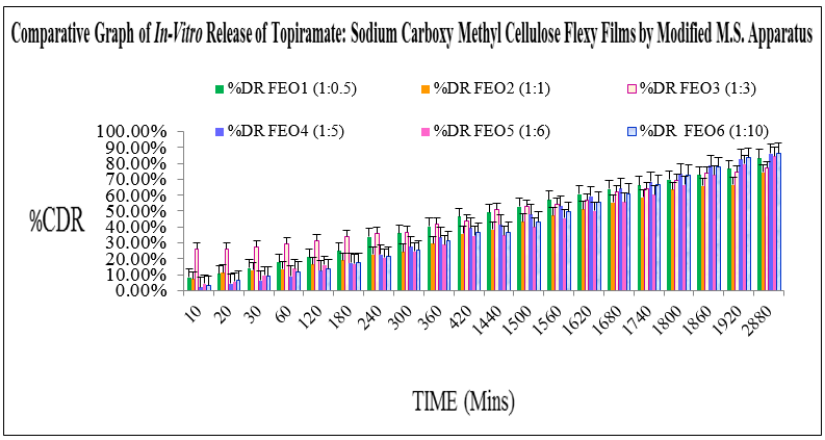

Figure 13: In-Vitro Drug Release Graph of nanosized Topiramate loaded Bio-Flexy Films using Sodium Carboxyl Methyl Cellulose standard polymer by Modified M.S. Diffusion Apparatus

Table 5: Kinetics Release of Topiramate-Sodium CMC Flexy Films

Release Kinetics Analysis Dynamic Method Formulation of Topiramate: Sodium CMC Flexy Films							
Formulations	**R2**					**Best Fit Model**	**Mechanism of Action**
	Zero order	**1st order**	**Higuchi Matrix**	**Peppas**	**Hixon Crowell**		
FEO1 (1:0.5)	0.8809	0.8813	0.9327	0.9761	0.8812	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)
FEO2 (1:1)	0.9170	0.9172	0.9311	0.9610	0.9171	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)
FEO3 (1:3)	0.8454	0.8460	0.8947	0.9009	0.8458	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)
FEO4 (1:5)	0.9049	0.9051	0.9425	0.9698	0.9051	Peppas Korsmeyer	Anomalous Transport
FEO5 (1:6)	0.8963	0.8963	0.9319	0.9566	0.8963	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matrix)
FEO6 (1:10)	0.8989	0.8989	0.9371	0.9614	0.8989	Peppas Korsmeyer	Anomalous Transport

Stability Studies of Formulated Nanosized Drugs Loaded Bio-Flexy Films As Per Ich Guidelines Q1B

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 was determined at room temperature and after stability study (25°C ± 2°C and 60 ± 5% RH). Bio-flexy films were found to be stable. (Figure 14)

Conclusion

In this study novel biopolymer was isolated from *Arachis hypogea* seeds by simplified economic process. It possessed in-built properties of filmability, mucoadhesivity, mucoretentivity and bioretardability. Hence, it was used as bio-excipient in formulations of nanosized Topiramate loaded bio-flexy films. Biopolymer was biodegradable, inert and non-reactive on soft palatal mucosa. Ratios were chosen at six levels for Drug: Biopolymer (1:0.5 to 1:10) and six levels for Drug: Sodium Carboxyl Methyl Cellulose (1:0.5 to 1:10) for formulating flexy-films. *Arachis hypogea* biopolymer showed percentage yield of 20% ±0.01. The biopolymer was brown in color, odourless, partially soluble in water. Its colour changing point was found to be 210ºC±2. It was tested positive for proteins and carbohydrates, amino acids were not present. Evaluation parameters of nanosized Topiramate loaded bio-flexy films containing *Arachis hypogea* biopolymer (FAO1-FAO6) revealed Thickness: 0.022 mm±0.004 to 0.044±0.003 mm, Folding Endurance: 140-169, Surface pH: 7.01±0.03 to 7.01±0.02, Weight Uniformity: 0.006±0.04 to 0.036±0.02, Drug Content Uniformity: 85.4%±0.68 to 93.4%±0.50, Swelling Percentage: 73%±0.6 to 89%±0.4, Percentage Moisture Uptake (PTU): 1.8%±0.10 to 2.4%±0.08, Mucoadhesion time: 45-150 minutes, Mucoretention time: 75-210 minutes. The drug release pattern for formulations FAO1-FAO6 containing *Arachis hypogea* biopolymer based on the $T_{50\%}$ and $T_{80\%}$ was found to be FAO2 (1:1) > FAO5 (1:6) > FAO6 (1:10) > FAO1 (1:0.5)> FAO4 (1:5) > FAO3 (1:3). Based on all above mentioned evaluation parameters, FAO2 (containing Topiramate: *Arachis hypogea* biopolymer (1:1)) Bio-flexy film having R2=0.9215, Higuchi Matrix as best fit model, follows Fickian Diffusion (Higuchi Matrix) release mechanism, T50%: 27 hrs., T80%: 29 hrs. using BITS Software. Stability study revealed stable formulations. Prepared nanosized Topiramate loaded bio-flexy films containing *Arachis hypogea* biopolymer were suitable for Soft Palatal Delivery.

Acknowledgement

We wish to acknowledge Mr. Anuj Aggarwal (Chairman, DIT University), Prof. K.K. Raina (Vice Chancellor, DIT University) for providing platform for conducting the research work.

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