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SMART AND NOVELISTIC BIOFLEXIFILM FORMER LOADED WITH LAMOTRIGINE FROM THE SEEDS OF *PHASEOLUS VULGARIS*

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ABSTRACT

Objective: The aim of our research work was to isolate a biomaterial from *Phaseolus vulgaris* and evaluate its filmability. *Phaseolus vulgaris* was subjected for isolating the biomaterial in a simplified and economic process. The biomaterial was investigated for various physico-chemical tests as well as spectral analyses like IR, ^{1}H NMR, DSC, XRD, SEM and elemental analysis. The mucoadhesivity of biomaterial was screened by rotating cylinder method. **Methods**: Lamotrigine loaded bioflexifilm were prepared using biopolymer, flexicizer and other co-processing agents for delivery of lamotrigine through oro-translabial mucosa. **Result:** The prepared films were evaluated for physical appearance, weight uniformity, thickness, folding endurance, surface pH, tensile strength, percent moisture uptake, vapour transmission rate, content uniformity and *in-vitro* drug diffusion using novel static M.S. diffusion apparatus. The results were compared with the standard HPMC film. The experimental results revealed that the biomaterial shows appreciable mucoadhesivity. The bioflexifilm LP6 possessed excellent folding endurance and 90.54 % drug diffusion through the biological membrane for a period of 36 hours. **Conclusion**: Finally conclusion was drawn that biopolymer exhibits inbuilt filmability, so it can be used as a bioflexifilm former for designing drug loaded bioflexifilms for translabial mucosal delivery.

KEYWORDS: Labial drug delivery, Bioflexifilms, Labial mucosa, *Phaseolus vulgaris*.

INTRODUCTION

Phaseolus vulgaris is an annual herbaceous plant of family *Leguminosae*. *Phaseolus vulgaris* seeds are associated with a decreased risk for wide variety of chronic diseases such as cancer, obesity, cardiovascular diseases and diabetes.[1] Phytochemical screening shows that *Phaseolus vulgaris* seeds contain alkaloids, anthocyanin, carbohydrate, fibers, flavonoids, quercetin, saponins, steroids, tannins, terpenoids and trypsin. It is high in starch, protein and dietary fiber and is an excellent source of iron, potassium, selenium, molybdenum, thiamine, vitamin B6, and folic acid.[2]

Lamotrigine is an antiepileptic drug, which is chemically 6-(2, 3-dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine. Its molecular formula is $C_9H_7Cl_2N_5$ and molecular weight 256.091 g/mol.[3] Lamotrigine inhibits voltage-sensitive sodium channels thereby stabilizing neuronal membranes and consequently modulating presynaptic release of excitatory amino acids.[4] The pharmacokinetics of lamotrigine follows first-order kinetics, with a half-life of 13.5 hours and volume of distribution of 1.36 L/kg. Lamotrigine is rapidly and completely absorbed after oral administration. Its absolute bioavailability is 98% and Cmax is 1.4 to 4.8 hours. It is used for partial seizures & primary generalized tonic-clonic seizures in patients more than two years of age. It is also indicated for the maintenance treatment of Bipolar Disorder.[5] The aim of our research work was to isolate a biomaterial from pulp of *Phaseolus vulgaris* and evaluate its film forming ability by making bioflexifilms using lamotrigine as the model drug.

MATERIALS AND METHODS

Lamotrigine was obtained as a gift sample from Zaneka Healthcare Pvt. Ltd.; Haridwar. *Phaseolus vulgaris* beans were procured from the local market, Dehradun. Sodium CMC & HPMC were purchased from Merck Specialties Pvt. Ltd.; Mumbai. IR spectral analysis was done in BHU; Banaras. ^{1}H NMR was obtained from SAIF; Panjab University Chandigarh. SEM, elemental and XRD analyses were performed in Wadia Institute; Dehradun. DSC analysis was performed in Dibrugarh University; Assam.

ISOLATION OF BIOMATERIAL

The biomaterial was isolated from the *Phaseolus vulgaris* seeds using simple and economical process [6].

Phaseolus vulgaris seeds were taken & soaked in water. The outer covering of seeds was removed & inner portion was collected. It was mashed with distilled water & filtered. Filtrate was subjected for centrifugation for 5 minutes at 3000 rpm. To the supernatant liquid, optimized concentration (1:2) of dimethyl ketone was added and kept in a refrigerator for a period of 10 hours. Then biomaterial was separated by centrifugation at 4000 rpm by discarding the supernatant and the collected biomaterial was subjected for drying in a desiccator for a period of 8 hours. It was screened through 120 mesh sieve.

CHARACTERIZATION OF BIOMATERIAL

a) Determination of Physico-chemical properties

The isolated biomaterial was tested for colour, solubility, colour changing point, viscosity, surface tension, pH and chemical tests.[7,8]

b) Spectral Analysis

The isolated biomaterial was subjected to various spectral analyses as IR, ^{1}H NMR, DSC, XRD, SEM and elemental analysis.

Infrared spectroscopy (IR spectroscopy) is a common analytical method that deals with the infrared region of the electromagnetic spectrum. The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near, mid and far infrared regions. The near-IR approximately ranges from 14000–4000 cm^{-1} (0.8–2.5 µm wavelength). The mid-infrared ranges approximately between 4000–400 cm^{-1} (2.5–25 µm). The far-infrared region ranges approximately between 400–10 cm^{-1} (25–1000 µm), lying adjacent to the microwave region, has low energy.

Nuclear magnetic resonance spectroscopy is a research technique that exploits the magnetic properties of certain atomic nuclei. It determines the physical and chemical properties of atoms or the molecules in which they are contained.

Differential scanning calorimeter (DSC) is a thermal analysis apparatus that measures the change in physical properties of a sample along with temperature against time. During the change in temperature, DSC measures the heat quantity, which is radiated or absorbed excessively by the sample on the basis of a temperature difference between the sample and the reference material. X-ray diffraction (XRD) is a tool used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of X-rays to diffract into many specific directions. By measuring the angles and intensities of these diffracted beams, a three-dimensional picture of the density of electrons within the crystal can be produce. From this electron density, the atomic positions, chemical bonds and their disorder can be determined. Scanning Electron Microscopy (SEM) is a technique that images a sample by scanning it with a beam of electrons in a raster scan pattern. The electrons interact with the atoms of the sample producing signals that contain information about the sample's surface topography and composition. Elemental analysis gives an idea of the elemental composition of the sample.

c) Determination of mucoadhesivity of isolated biomaterial

The mucoadhesivity of isolated biomaterial was determined by Rotating cylinder method using *Capra aegagrus* labial mucosa. The biomaterial film was prepared by casting method. The film was placed on labial and subjected for rotation at 100 rpm. The detachment and dislodgement of film from mucosal substrate was noted at regular intervals and data was compared with standard film of HPMC polymer.[9]

FORMULATION OF BIOFLEXIFILMS

The isolated biomaterial was used for formulating bioflexifilms using lamotrigine as model drug. Six different film formulations i.e. LP1, LP2, LP3, LP4, LP5 and LP6 were prepared using biopolymer & lamotrigine in six different ratios by solvent casting method **(Table 1)**. The biomaterial was dissolved in distilled water with constant stirring. Dextrose and mannitol were added as flexicizer for the formulations of film. Lamotrigine solution was separately prepared and added to the biomaterial solution containing dextrose and mannitol. This mixture was then transferred into petriplates and allowed for controlled evaporation of solvent at room temperature. Dried films were carefully removed, and cut into films of 1sq.cm.[10,11]

EVALUATION OF BIOFLEXIFILMS[10-14]

a) Physical appearance

The bioflexifilms were visually inspected for various factors like color, clarity, flexibility and smoothness in order to ensure the uniformity in physical appearance of the films.

b) Weight uniformity test

Weight uniformity test was done in order to ensure the uniformity in weight of the bioflexifilms. Three bioflexifilms were weighed on a digital balance and mean was calculated. The thickness of bioflexifilm was determined using a screw gauge.

c) Thickness

Three bioflexifilms were randomly selected and their thickness was determined using a micrometer screw gauge. The mean thickness of three bioflexifilms was calculated.

d) Folding endurance

Folding endurance of bioflexifilms was determined by repeatedly folding the film at the same place until it was broken. The number of times the bioflexifilms could be folded at the same place without breaking was recorded. The measurement was repeated in triplicate.

e) Surface pH

For determining the surface pH of bioflexifilm, the individual film was placed in a petridish and moistened with 0.5 ml of water and kept for 30 sec. The film surface was brought into contact with the electrode of pH meter and pH was determined.

f) Tensile strength

Tensile strength of the bioflexifilms was determined by a lab fabricated universal strength testing apparatus consisting of the glass plate which is fixed on lower base of apparatus, a pulley through which a string is attached, and a weight holder box which is connected with the strings.

g) Percent moisture uptake

For determining % moisture uptake, the prepared bioflexifilms were weighed individually. They were transferred to a watch glass and kept in desiccator containing saturated solution of aluminium chloride at room temperature for 48 hours. After 48 hours, the films were reweighed and the percentage moisture uptake was determined by the formula:

% moisture uptake = [(Final weight - initial weight / Initial weight)] × 100

h) Water vapor transmission

Water vapor transmission (WVT) is defined as the quantity of moisture transmitted through a unit area of film in unit time. It was determined using a glass bottle filled with anhydrous calcium chloride and an adhesive spread across its rim. The bioflexifilm was fixed over the adhesive and the assembly was placed in a sealed desiccator containing saturated potassium chloride solution for 24 hours. The bottle was reweighed and water vapour transmission was determined using the formula

Water Vapour Transmision= W/ST

Where, W is the increase in weight in 24 hours;
S is area of bioflexifilm exposed (cm^2);
T is exposure time.

i) Drug content uniformity

Three bioflexifilms from each formulation were randomly selected and transferred individually into a 100 ml volumetric flask containing phosphate buffer (pH 7.4) and methanol. The flask was stirred on a magnetic stirrer. The obtained solutions were filtered and drug content was then determined after proper dilution by Shimadzu 1800 UV-Visible spectrophotometer.

2.5. *IN-VITRO* DRUG DIFFUSION STUDIES

The *in- vitro* drug diffusion study was carried out by using a novel static M.S. diffusion apparatus having two compartments; upper donor and lower receptor compartment. The formulated film was adhered onto biomembrane and fixed to a donor compartment at one end with the help of adhesive. This assembly was immersed in the receptor compartment which is double walled containing 10 ml of buffer solution (pH 7.4). Samples were withdrawn completely at regular intervals till 36 hours and replaced by fresh buffer**.** The samples were analyzed by Shimadzu 1800 UV-Visible spectrophotometer at λ_{max} 308 nm. Concentration of drug in sample and % Cumulative Drug Release was calculated. It was compared with standard film of HPMC polymer. The drug release kinetics of film formulations was determined by BIT software.

RESULTS AND DISCUSSION

Physico-chemical characterization of biomaterial

Phaseolus vulgaris biomaterial was yellowish white in color with colour changing point 220^0C, pH 6.4, viscosity 0.9 cp, percentage yield 12 % and surface tension 71.12 dyne/cm. It was slightly soluble in water, insoluble in methanol & acetone. It passed Fehling test and Molisch test.

Spectral analysis of biomaterial

The bioflexifilm showed promising mucoadhesivity as the biomaterial contains functional group like C=O of carboxyl group, which is confirmed by IR spectra and presence of OH group, which was confirmed by IR and NMR spectra. The IR spectrum of *Phaseolus vulgaris* biomaterial showed peaks at 3377 cm^{-1} (OH stretching), 1660 cm^{-1} (C=O stretching of carboxylic acid), 1531 cm^{-1} (C=C aromatic ring), 1246 cm^{-1}, 1111 cm^{-1} (C-N stretching) and 615 cm^{-1} (CH bending aromatic ring) **(Figure 1).**

^{1}H NMR of *Phaseolus vulgaris* biomaterial showed chemical shift values at δ 0.9-1.5 ppm (-CH saturated proton), δ 2-3 ppm (-C≡CH, acetylenic proton), δ 3.4-4 ppm (-CH_3OR, ether proton), δ 4.5 ppm (-C=CH, vinylic proton), δ 5.2 ppm (R-OH, hydroxyl proton), δ 6.4 ppm (Ar-H, aromatic proton) **(Figure 2).** The DSC curve of *Phaseolus vulgaris* showed glass transition temperature 122.05^0C. Peak height was observed at 1.0015 mW, area was found to be 1613.720 mJ. The value of delta H was 161.7230 J/g **(Figure 3).** X-ray diffraction curve of *Phaseolus vulgaris* indicated that it was amorphous in nature **(Figure 4).** SEM image of the biomaterial revealed that biopolymer possesses smooth and irregular topography **(Figure 5).** Elemental analysis showed that it contains carbon as the major element and devoid of arsenic, lead, iron or other toxic element **(Figure 6).**

Determination of mucoadhesivity of isolated biomaterial

The detachment time for *Phaseolus vulgaris* biomaterial was 147 minutes, which was more than HPMC **(Figure 7)**. So the biomaterial contains notable mucoadhesivity.

Formulation and evaluation of bioflexifilms using the isolated biomaterial

Six bioflexifilms LP1, LP2, LP3, LP4, LP5 and LP6 were successfully prepared from *Phaseolus vulgaris*

biomaterial in the ratio of drug: biopolymer 1:1, 1:2, 1:3, 1:4 1:5 and 1:6 respectively. The bioflexifilms from all the batches were smooth, translucent and flexible without any sign of cracking. The bioflexifilms LP1 to LP6 had 47.61 ± 0.55 mg to 82.48 ± 0.13 mg weights and 0.43 ± 0.03 mm to 0.65 ± 0.02 mm thickness. The bioflexifilms showed folding endurance 133.3 ± 1.15 to 154.7 ± 0.58. All bioflexifilms showed nearly neutral pH **(Table 2).**

The tensile strength of LP1 to LP6 bioflexifilms was 70.78 ± 0.49 to 113.27 ± 0.46. Percent moisture uptake of bioflexifilms was 11.47 ± 0.28 to 14.88 ± 0.41 %. Vapour Transmission Rate was found to be 6.23 ± 0.27 to 11.29 ± 0.67 gm/cm^2/hr. Finally content uniformity for all bioflexifilms was determined which varied from 87.26 ± 0.65 to 92.98 ± 0.67 % **(Table 3).**

***In-vitro* drug diffusion of bioflexifilms**

The *in-vitro* percentage drug diffusion of bioflexifilms containing lamotrigine drug and *Phaseolus vulgaris* biomaterial was found to be in the order LP5 > LP6 > LP4 > LP3 > LP2 > LP1 **(Figure 8).** The drug release of LP5 formulation was 90.54 %, which was comparable to HPMC films (91.23 %). LP5 release followed Zero order kinetics with T_{50} 22.00 hours and T_{80} 35.20 hours. It gave good fit to the Korsmeyer–Peppas model and mechanism of drug release was anomalous transport.

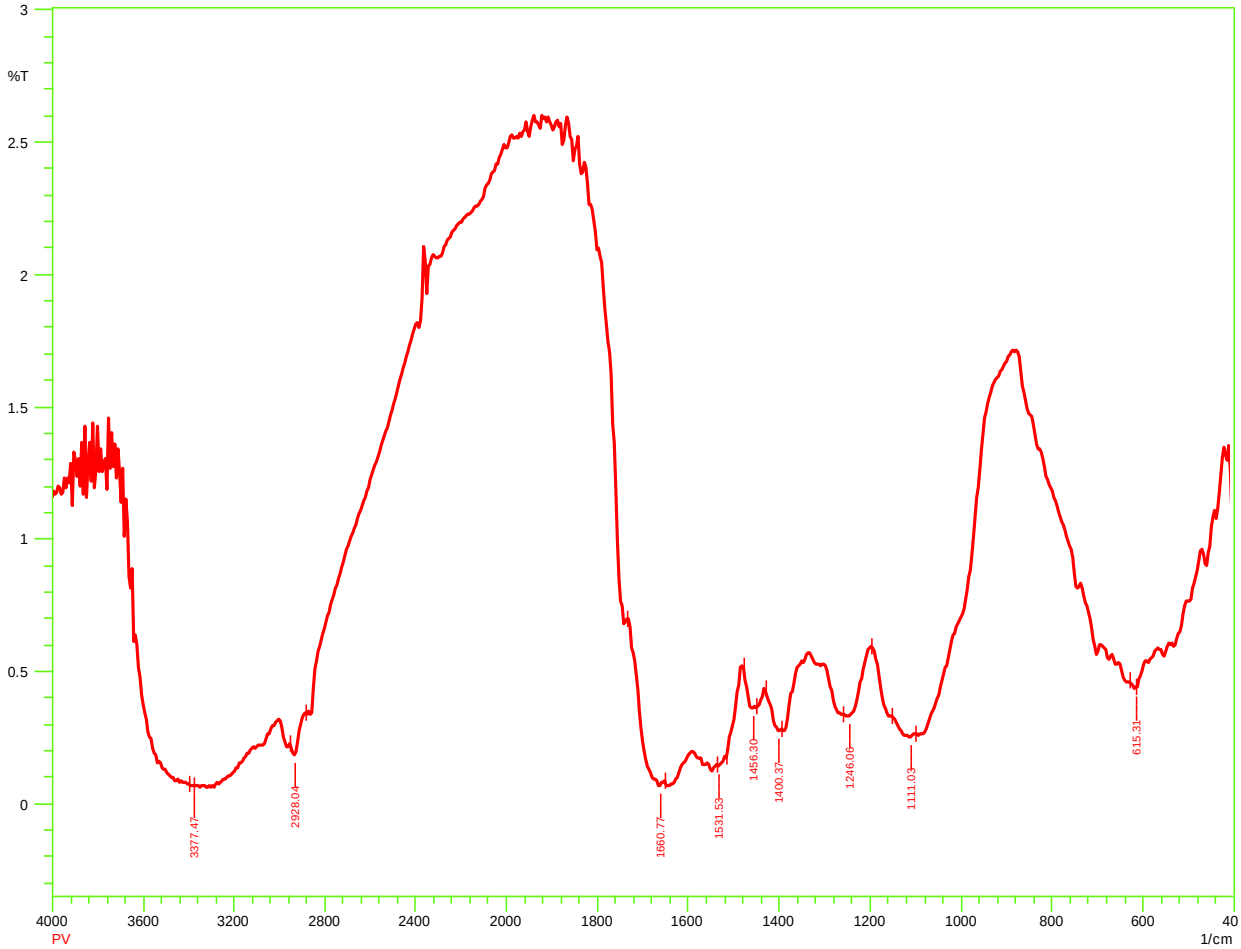

Figure 1: IR of *Phaseolus vulgaris* biomaterial.

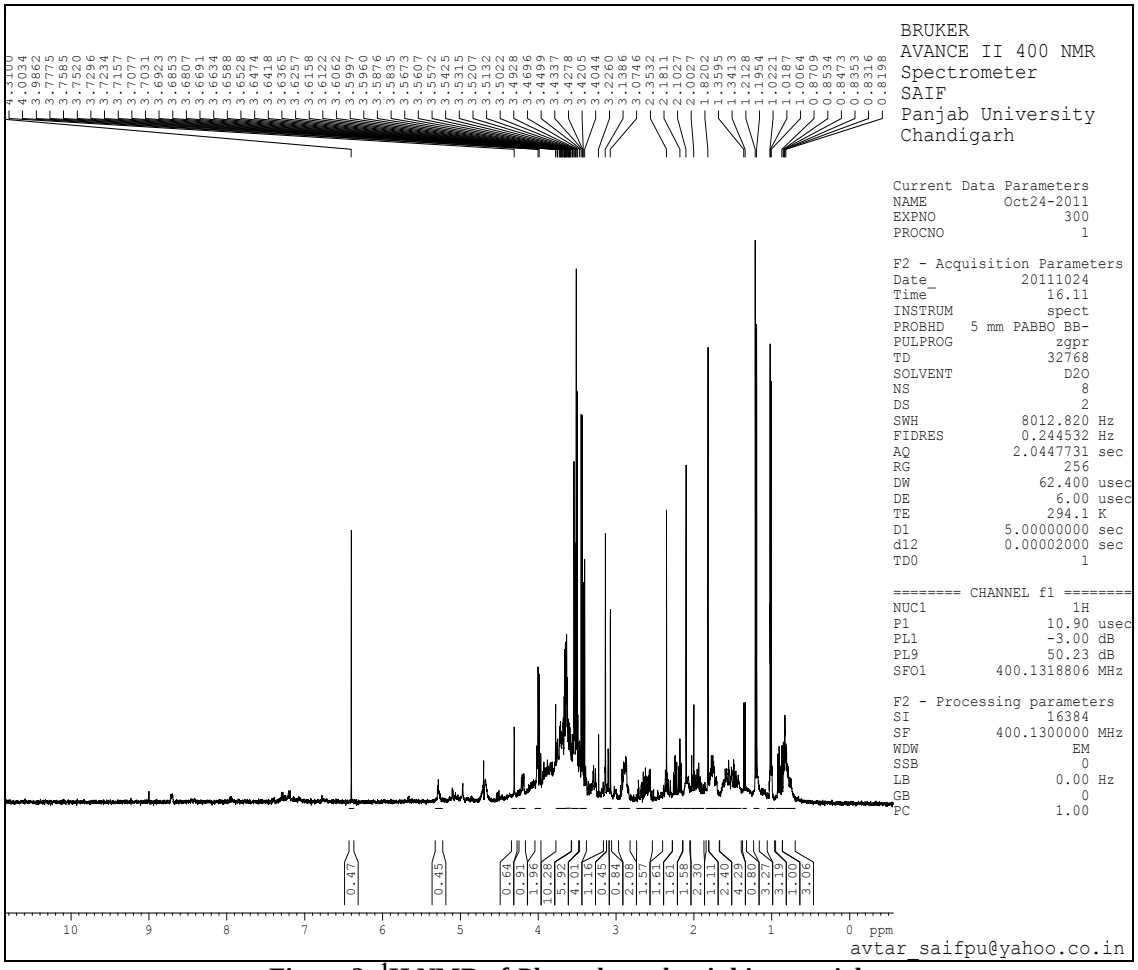

Figure 2: ^{1}H NMR of *Phaseolus vulgaris* biomaterial.

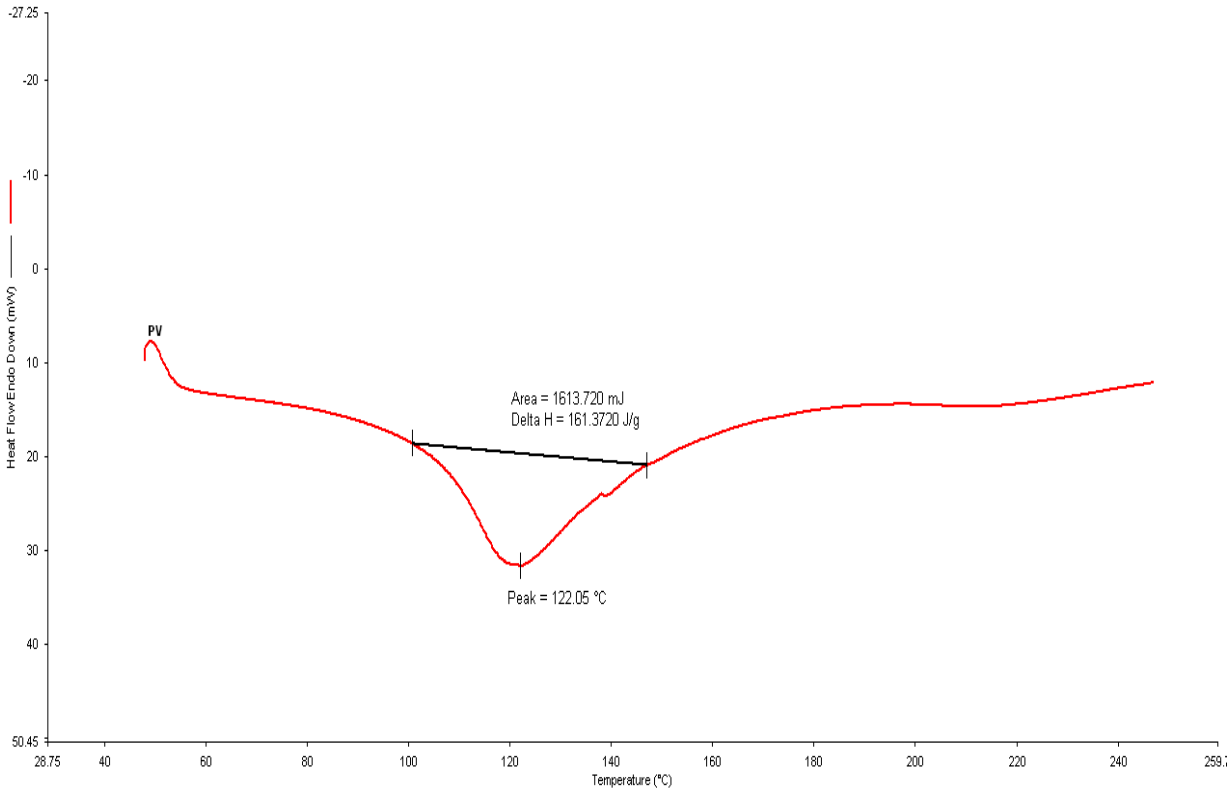

Figure 3: DSC of *Phaseolus vulgaris* biomaterial.

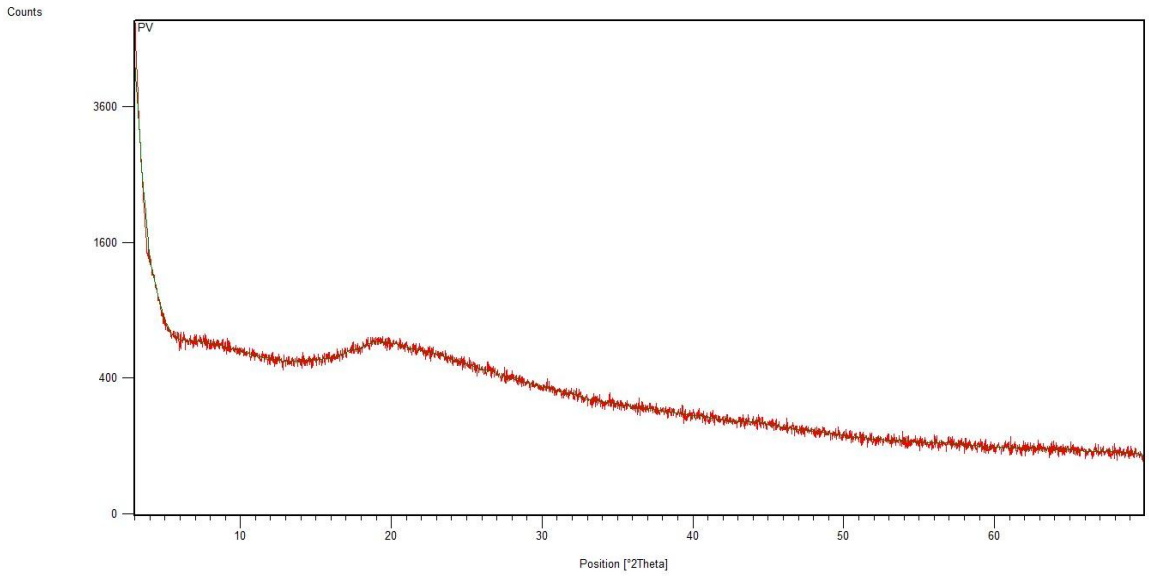

Figure 4: XRD of *Phaseolus vulgaris* biomaterial.

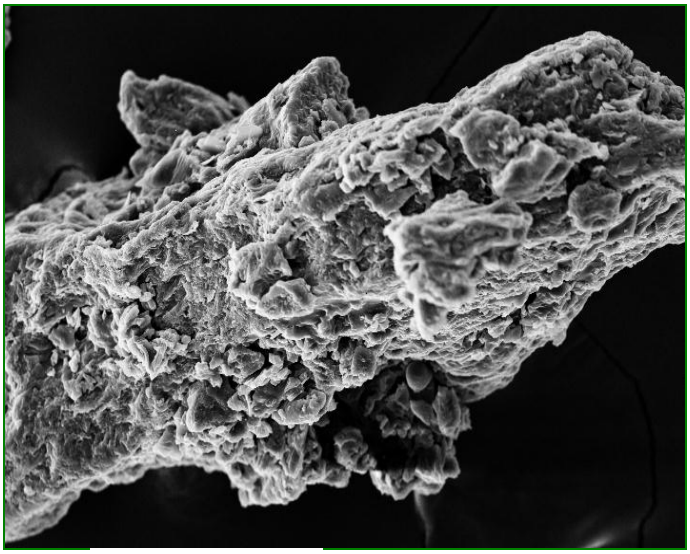

Figure 5: SEM Analysis of *Phaseolus vulgaris* biomaterial.

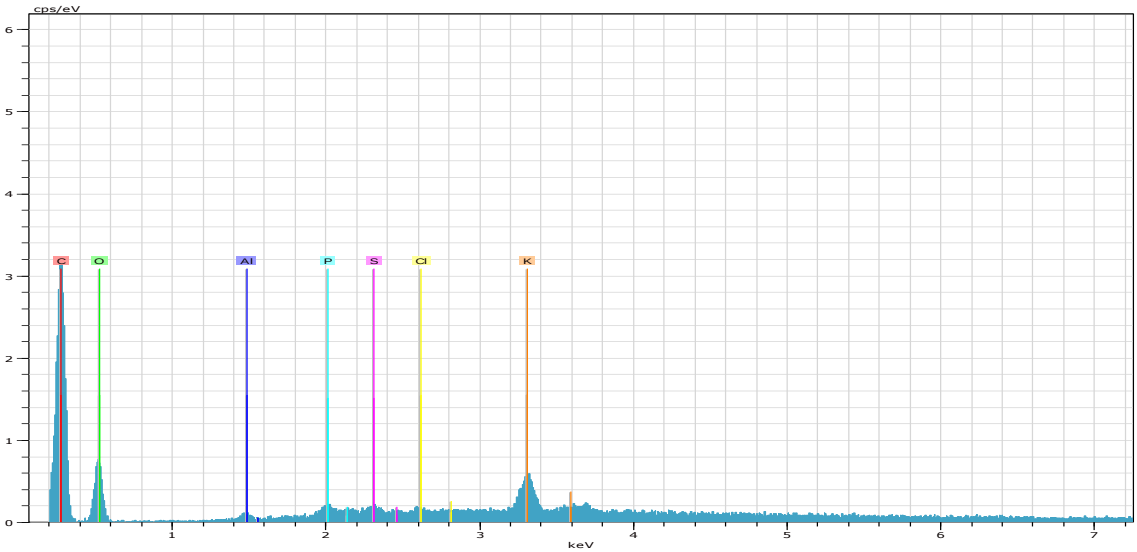

Figure 6: Elemental Analysis of *Phaseolus vulgaris* biomaterial.

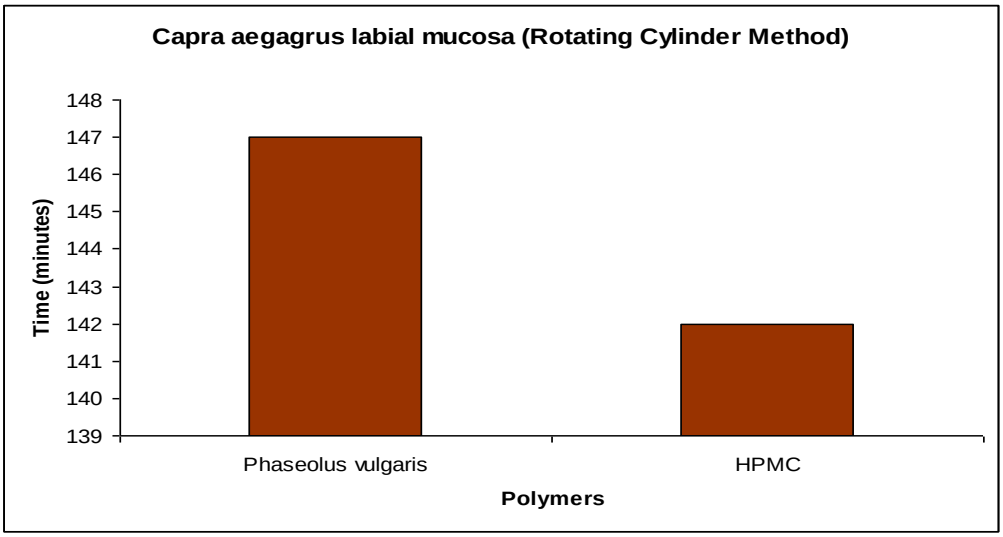

Figure 7: Mucoadhesivity of *Phaseolus vulgaris* biomaterial.

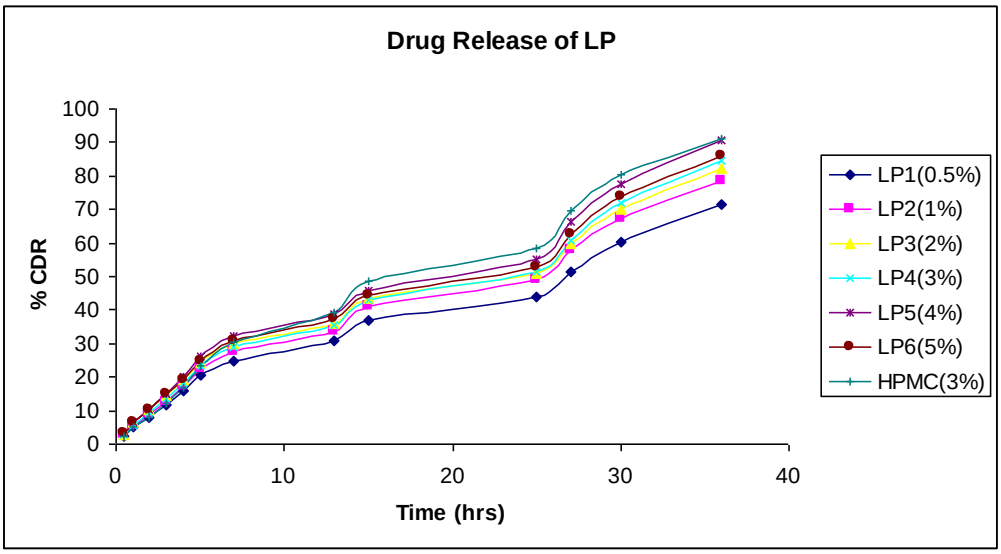

Figure 8: *In-vitro* drug release of various bioflexifilm formulations.

Table 1: Lamotrigine loaded bioflexifilm of *Phaseolus vulgaris.*

S.No.	LP1 (1:1)	LP2 (1:2)	LP3 (1:3)	LP4 (1:4)	LP5 (1:5)	LP6 (1:6)
Lamotrigine (mg)	50	50	50	50	50	50
Biopolymer (mg)	50	100	150	200	250	300
Dextrose(mg)	50	50	50	50	50	50
Mannitol(mg)	50	50	50	50	50	50
Water(ml)	20	20	20	20	20	20

Table 2: Comparative evaluation parameters of various film formulation.

Formulation	Wt.Uniformity (mg)	Thickness (mm)	Folding endurance	Surface pH
LP1	47.61 ± 0.55	0.43 ± 0.03	133.3 ± 1.15	6.63 ± 0.15
LP2	47.85 ± 0.85	0.46 ± 0.03	139.3 ± 1.53	6.73 ± 0.06
LP3	51.54 ± 0.67	0.47 ± 0.047	142.3 ± 2.08	7.17 ± 0.15
LP4	57.04 ± 0.49	0.49 ± 0.03	140.3 ± 2.52	6.7 ± 0.15
LP5	70.47 ± 0.45	0.62 ± 0.03	151.3 ± 0.58	7.0 ± 0.05
LP6	82.48 ± 0.13	0.65 ± 0.02	154.7 ± 0.58	7.0 ± 0.15

Table 3: Comparative evaluation parameters of various films formulations.

Formulation	Tensile strength	% Moisture Uptake	VTR ($g/cm^2/hr$)	Content Uniformity (%)
LP1	70.78 ± 0.49	11.47 ± 0.28	6.23 ± 0.27	87.26 ± 0.65
LP2	77.99 ± 0.48	10.52 ± 0.30	7.26 ± 0.18	82.56 ± 0.87
LP3	80.77 ± 0.49	11.51 ± 0.51	7.28 ± 0.08	89.52 ± 0.74
LP4	87.04 ± 0.16	12.70 ± 0.47	8.12 ± 0.19	91.60 ± 0.49
LP5	110.43 ± 0.60	14.64 ± 0.32	10.89 ± 0.46	94.39 ± 0.39
LP6	113.27 ± 0.46	14.88 ± 0.41	11.29 ± 0.67	92.98 ± 0.67

CONCLUSION

Finally conclusion was drawn that *Phaseolus vulgaris* biopolymer can serve as a potential film former in pharmaceutical preparations. Since this natural film forming agent is edible, it is easily biodegradable and may provide an alternative to conventional synthetic/semisynthetic film forming agents.

Authors' contributions:
All authors have equally contributed for making this project to be successful.

Competing interests:
The authors declare no conflicts of interest.

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