Formulation and *In vitro* Characterization of Phenytoin Loaded Mucoadhesive Biofilms of *Colocasia esculenta* for Translabial Drug Delivery System

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ABSTRACT: The aim of our research work was to isolate a biomaterial from *Colocasia. esculenta* and prepare phenytoin loaded mucoadhesive biofilms using this biomaterial. The biomaterial was isolated from *C. esculenta* tubers by an economical process. The isolated biomaterial was subjected to various physical evaluation, chemical tests as well as spectral analysis. The drug-biomaterial interaction study was performed to see if there was any interaction of biomaterial with phenytoin. Phenytoin loaded biofilms were prepared using biomaterial, flexicizer and other co- processing agents. The prepared biofilms were evaluated for physical appearance, weight, thickness, folding endurance, swelling index, surface pH, tensile strength, percent elongation, percent moisture uptake, percent moisture loss, vapor transmission rate and content uniformity. The mucoadhesivity of biofilms was investigated using rotating basket method. The *in-vitro* drug release study of biofilms was performed on static MS diffusion apparatus. The stability studies of biofilms were carried out at different conditions of temperature and relative humidity. The results were compared with the standard hydroxy propyl methyl cellulose (HPMC) and sodium carboxymethyl cellulose (Sodium CMC) films. The experimental results revealed that the phenytoin loaded biofilms of *C. esculenta* possessed excellent mucoadhesivity, sufficient stability as well as appreciable release characteristics. The best biofilm formulation was PK6 with a cumulative drug release of 95.35 % over 36 hours. Hence, *C. esculenta* biomaterial can serve as a potential film forming agent for transmucosal drug delivery systems.

Key words: Biofilm; Mucoadhesion; Biopolymer; *Colocasia esculenta*; Phenytoin.

INTRODUCTION

Colocasia esculenta is a tropical plant of the family Araceae. It is a tall tuberous herb with a stout short caudex. Leaves are simple, with a stout petiole. Seeds are oblong and stem is above the ground or slightly swollen at the base of the leaf-sheaths. The tubers contain globulins that account for 80% of the total tuber proteins. The total amino acids recorded in the tubers are in the range of 1,380-2,397 mg/100 g. The starch content of *C. esculenta* flour varies from 73-76% and the nitrogen varies from 0.33-1.35%. The uses include protection against asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders and skin disorders. The juice is widely used for treatment of body ache and baldness.[1,2]

Phenytoin is an antiepileptic drug, which is chemically 5, 5-diphenylimidazolidine-2, 4-dione. Its molecular formula is $C_{15}H_{12}N_2O_2$ and molecular weight 252.268 g/mol.[3] It exerts anticonvulsant effect by blocking voltage-sensitive and frequency-dependent sodium channels in the neurons. It stabilizes sodium channels in an inactive state, and this effect depends on the voltage and frequency of firing of the neuron.[3,4] At higher concentrations, phenytoin delays activation of outward potassium currents in nerves and prolongs the neuronal refractory period. It also exerts an anticonvulsant effect by influencing calcium channels or γ-amino

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butyric acid receptors (GABA). Phenytoin may cause neurological adverse effects like sedation, cerebellar ataxia, ophthalmoparesis as well as seizures. At high dose, it may cause a reduction in folic acid levels, causing megaloblastic anemia. Other side effects of phenytoin include agranulocytosis, aplastic anemia, leukopenia and thrombocytopenia. Phenytoin is also associated with drug-induced gingival enlargement. This effect involves bleeding upon probing, increased gingival exudate and pronounced gingival inflammatory response to plaque levels.[5,6]

In order to promote the direct systemic absorption of phenytoin by oral route, labial mucosa is one of the promising site. Hence use of labial mucoadhesive films can reduce the dose of orally administered phenytoin, thus minimizing the adverse effects of phenytoin. The aim of our research work was to isolate a biomaterial from the tubers of *C. esculenta* and prepare mucoadhesive phenytoin loaded biofilms using this biomaterial.

MATERIALS AND METHODS

Phenytoin was obtained as a gift sample from Zaneka Healthcare Pvt. Ltd.; Haridwar. *C. esculenta* tubers were procured from the local market, Dehradun. HPMC and sodium CMC were purchased from Merck Specialties Pvt. Ltd.; Mumbai. IR spectral analysis of isolated biomaterial was performed in BHU; Banaras, ^{1}H NMR and mass spectra was obtained from SAIF; Panjab University Chandigarh. SEM and elemental analysis were performed in Wadia Institute; Dehradun and DSC analysis was performed in Dibrugarh University; Assam.

Isolation of biomaterial. *C. esculenta* tubers (1000 gm) were accurately weighed. The outer covering was removed and fleshy portion was collected. It was mashed with double distilled water and filtered. It was then treated with 90% dimethyl ketone and refrigerated for 10 hours. It was centrifuged at 4000 rpm for 30 minutes. The supernatant was discarded and obtained biomaterial was dried in vacuum desiccator for 24 hours. The dried biomaterial was purified by hot dialysis method using ORCHID scientific dialysis apparatus. The purified biomaterial was dried in vacuum desiccator and then passed through sieve # 200 to get uniform particles. The procedure was optimized three times to calculate percent yield.[7,8]

Characterization of biomaterial. The isolated biomaterial was subjected to evaluation of various parameters like colour, colour changing point, viscosity, surface tension, solubility and chemical tests.[9,10] The isolated biomaterial was also studied by spectral analyses as IR, ^{1}H NMR, DSC, SEM and elemental analysis.

Acute toxicity study of isolated biomaterial. The single dose acute toxicity study of the biomaterial was performed on Wistar rats (either sex, 200-250 gm) for two weeks. The study protocol was approved by the Institutional Animal Ethical Committee (Registration No. 1156/AC/07/CPCSEA). The procedure was as per OECD 423 guidelines. Two groups of 6 albino rats, one for test and other for control, were used for the study. The study was performed by administering the biomaterial solution orally at 5g/kg body weight to the test group animals. The acute toxicity study was evaluated for a period of two weeks by observing body weight, changes in the skin, corneal reflex, respiratory rate, autonomic symptoms, salivation, diarrhoea, lethargy, sleep, behavioural patterns, and convulsions and compared with control group animals.[11,12]

Drug-biomaterial interaction studies. The interaction study of phenytoin with the isolated biomaterial was done with phenytoin to see whether the biomaterial is reactive or non reactive with phenytoin If interaction is found, it means that the isolated biomaterial is not suitable for the preparation of biofilms.

a) Dry method. Initially, λ_{max} of phenytoin was determined after scanning its solution by UV Spectroscopy. Then phenytoin and biomaterial were mixed three different ratios 1:1, 1:3, 3:1 and λ_{max} of mixtures was determined by UV Spectroscopy. The λ_{max} of mixtures was compared with that of phenytoin.

b) Wet method. Phenytoin was mixed with biomaterial in three different ratios 1:1, 1:3, 3:1 in petriplates using small amount of water and dried in an oven. Then dried mixtures were dissolved in 10 ml of methanol and scanned by UV Spectroscopy after proper dilution. The λ_{max} of mixtures was compared with that of pure drug.

Formulation of biofilms using the isolated biomaterial. The isolated biomaterial was used for formulating biofilms using phenytoin as the drug. Six different biofilms (PK1, PK2, PK3, PK4, PK5 and PK6) were prepared using biomaterial and phenytoin in six different ratios by solvent casting method (Table 1). The isolated biomaterial was dissolved in distilled water with constant stirring on a magnetic stirrer. Dextrose and mannitol were added as flexicizer for the formulations of biofilms. Phenytoin solution was separately prepared and added to the biomaterial solution containing dextrose and mannitol. This mixture was then transferred into petriplates uniformly and solvent was allowed to evaporate in a controlled manner. Dried biofilms were carefully scraped out and cut into biofilms of 1 sq. cm size.[13,14]

Table 1. Formulation of phenytoin loaded film formulations.

Ingredients	PK1	PK2	PK3	PK4	PK5	PK6	HPMC film	Sod CMC film
Phenytoin (mg)	100	100	100	100	100	100	100	100
Biomaterial (mg)	50	100	200	300	400	500	-	-
HPMC (mg)	-	-	-	-	-	-	300	-
Sodium CMC (mg)	-	-	-	-	-	-	-	300
Dextrose (mg)	25	25	25	25	25	25	25	25
Mannitol (mg)	25	25	25	25	25	25	25	25
Water (ml)	10	10	10	10	10	10	10	10

Evaluation of biofilms. The prepared phenytoin loaded biofilms were evaluated for the parameters namely physical appearance, weight uniformity, thickness, folding endurance, swelling index, surface pH, tensile strength, percent elongation, percent moisture uptake, percent moisture loss, vapour transmission rate and content uniformity. The average of three readings was determined for each parameter.

a) Weight uniformity. Weight uniformity was tested in order to ensure the uniformity in weight of biofilms. Three biofilms were weighed on a digital balance and mean was calculated.[14,15]

b) Thickness. Three biofilms were randomly selected and their thickness was determined using a micrometer screw gauge. The mean thickness of three biofilms was calculated.[14,15]

c) Folding endurance. Folding endurance of biofilm was determined by repeatedly folding the biofilm at the same place until it was broken. The number of times the biofilm could be folded at the same place without breaking was recorded. The measurement was repeated in triplicate.[15,16]

d) Swelling index. The swelling index was determined by placing the individual biofilm in the petriplate containing 10 ml of phosphate buffer pH 7.4.[13,15] Swelling index (S) was determined by using the following formula:

$$S = \left(\frac{X_t - X_0}{X_0} \right) \times 100$$

where, X_t = Weight of swollen biofilm after time t,

X_0 = Initial weight of biofilm

e) Surface pH. For determining the surface pH of biofilm, the individual biofilm was placed in a petriplate and moistened with 0.5 ml of water and kept for 30 sec. The biofilm surface was brought into contact with the electrode of pH meter and pH was determined.[14,15]

f) Tensile strength. Tensile strength of the biofilm was determined by a lab fabricated universal strength testing apparatus. It had a glass plate which

was fixed on lower base of apparatus, a pulley through which a string was attached, and a weight holder box which was connected with the strings.

g) Percent elongation. The percent elongation at break was calculated by lab fabricated universal strength testing apparatus.[14,16] The formula used was:

Percent elongation at break=

(Increase in length of biofilm / Initial length) x 100

h) Percent moisture uptake. For determining percent moisture uptake, the prepared biofilms were weighed individually. They were transferred to a watch glass and kept in a desiccator containing saturated solution of aluminium chloride at room temperature for 48 hours.[14,15] After 48 hours, the biofilms were reweighed and the percentage moisture uptake was determined by the formula:

Percent moisture uptake =

[(Final weight - initial weight / Initial weight)] × 100

i) Percent moisture loss. A percentage moisture loss study for all formulated biofilms was performed by taking a biofilm from each formulation. The biofilm was weighed and kept in desiccator containing fused anhydrous calcium chloride for 48 hours.[16,17] At the end, the weight loss from the biofilm was determined by the following formula:

Percent moisture loss =

[(Initial weight - Final weight / Initial weight)] × 100

j) Water vapour transmission. Water vapour transmission was determined using a glass bottle filled with anhydrous calcium chloride and an adhesive spread across its rim. The biofilm was fixed over the adhesive and the assembly was placed in a sealed desiccator containing saturated potassium chloride solution for 24 hours.[16,17] 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 biofilm exposed (cm^2),

T is exposure time.

k) Drug content uniformity. Three biofilms 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 using Shimadzu 1800 UV-Visible spectrophotometer.[17,18]

Determination of mucoadhesivity of biofilms on *Capra aegagrus* labial mucosa:

The mucoadhesivity of biofilms was evaluated by a lab fabricated rotating basket apparatus using *Capra aegagrus* labial mucosa. The *C. aegagrus* labial mucosa was placed around the rotating basket and biofilm was adhered onto it. It was allowed for rotation at 100 rpm. The detachment time of biofilm from mucosal substrate was noted at regular intervals and data was compared with standard film of HPMC and sodium CMC.[7,12]

***In vitro* drug release studies.** The *in- vitro* drug release study of biofilms was carried out by using a novel static M.S. diffusion apparatus. It had two compartments, upper donor and lower receptor compartment. The biofilm was adhered onto a biomembrane and fixed to a donor compartment at one end with the help of adhesive. This assembly was immersed in a double walled receptor compartment containing 10 ml of 7.4 pH phosphate buffer solution. Samples were withdrawn completely at regular intervals till 36 hours and replaced by fresh buffer after withdrawing the sample**.** The samples were analyzed by Shimadzu 1800 UV-Visible spectrophotometer at λ_{max} 216 nm. Concentration of drug in sample and % Cumulative drug release was calculated. It was compared with standard films of HPMC and sodium CMC polymer.

Stability studies. Stability studies of phenytoin loaded biofilms of *C. esculenta* were conducted as per ICH Guidelines at various conditions of temperature and relative humidity (RH). The biomaterial was subjected for stability studies under three conditions (5°C±3°C/60% RH, at room temperature i.e.25°C± 2°C /60 ±5% RH and at 40°C±

2°C/ 75% RH) for three months. The changes were observed in the characteristics of biomaterial and the results were reported.[15]

RESULTS AND DISCUSSION

Characterization of biomaterial. The *C. esculenta* biomaterial was brown in colour with a percentage yield of 4.2± 0.04 % w/w. Its colour changing point was 202^0C with darkening, viscosity 1.2 cp and surface tension 73.14 dyne/cm. It was soluble in water, insoluble in methanol & acetone. It passed Benedict test, Fehling test and iodine test.

Spectral analysis of biomaterial. The IR spectrum of *C. esculenta* biomaterial showed peaks at 3518 (OH stretching), 2924 cm^{-1}(CH stretching alkane), 2337 cm^{-1} (C=C alkene), 1600 cm^{-1} (C=O stretching of carboxylic acid), 1357, 1153, 1026 (C-N stretching amine) and 763 (CH bending Aromatic ring) (Figure 1). The presence of COOH and OH groups in biomaterial proved that biomaterial was polymeric in nature. ^{1}H NMR spectra of *C. esculenta* biomaterial showed chemical shift values at δ 1.6 (-CH saturated proton), δ 2.1 (R-$COOCH_3$, ester proton), δ 3.5-3.9 (-CH_3OR, ether proton), δ 6.9, 7.4-7.7 (Ar-H, aromatic proton), δ 8.2 (Ar-OH, aromatic hydroxyl proton) (Figure 2).

The DSC curve of *Colocasia esculenta* showed primary glass transition temperature is 92.27^0C and secondary glass transition temperature of 160.52^0C. Primary peak height was observed at 5.7280 mW, area was found to be 1072.661 mJ. The value of delta H for primary peak was 107.2661 J/g. Secondary peak height was observed at 0.9188 mW, area was found to be 86.844 mJ. The value of delta H for secondary peak was 8.6844 J/g (Figure 3). SEM image of the biomaterial revealed that biomaterial possesses smooth and irregular topography, thus confirming the polymeric nature of biomaterial (Figure 4). Elemental analysis showed that the biomaterial contains carbon as the major element and devoid of arsenic, lead, iron or other toxic element (Figure 5).

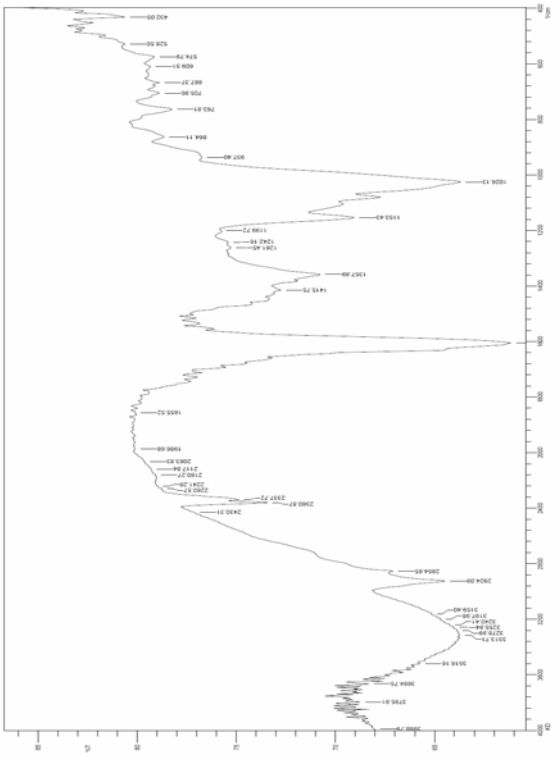


Figure. 1. IR spetrum of *Colocasia esculenta* biomaterial.

Acute toxicity studies of isolated biomaterial. In acute toxicity studies, the test animals did not reveal any significant change in their body weight, skin reaction, corneal reflex, respiratory rate, autonomic symptoms, salivation, diarrhea, lethargic conditions, sleeping conditions and convulsion. So, it was concluded that biomaterial was non toxic.

Drug-biomaterial interaction studies. The λ_{max} of phenytoin was found to be 216 nm. There was no significant change in λ_{max} of mixtures as compared to pure phenytoin, which revealed that the biomaterial was devoid of any interaction with the functional groups of the phenytoin. Hence, the biomaterial from *C. esculenta* can be safely used for preparation of biofilms of phenytoin.

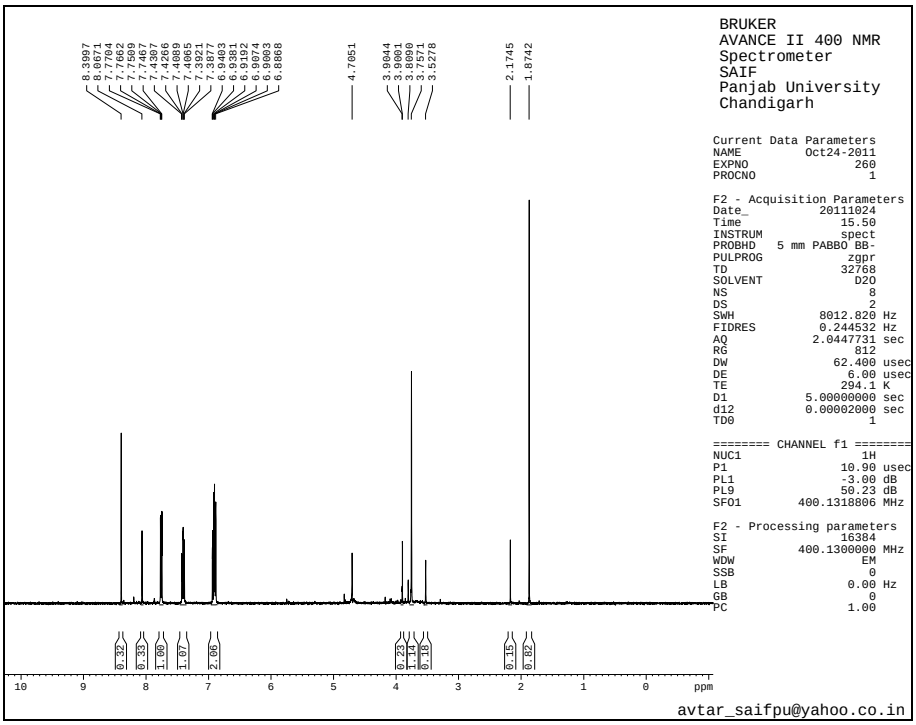


Figure. 2. [1]H NMR spetrum of *Colocasia esculenta* biomaterial.

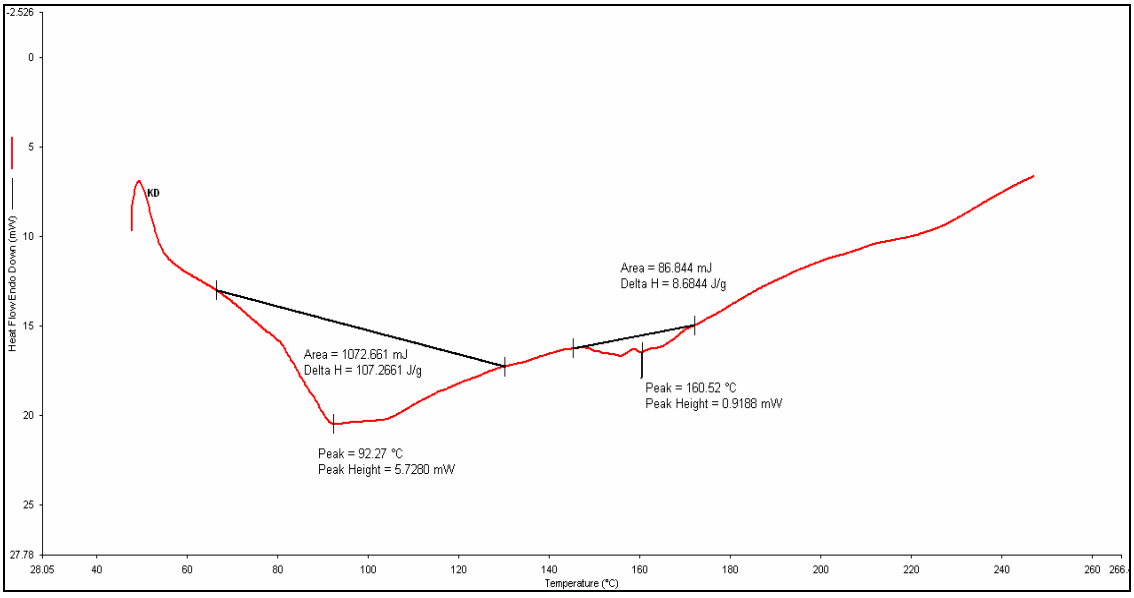


Figure. 3. DSC of *Colocasia esculenta* biomaterial.

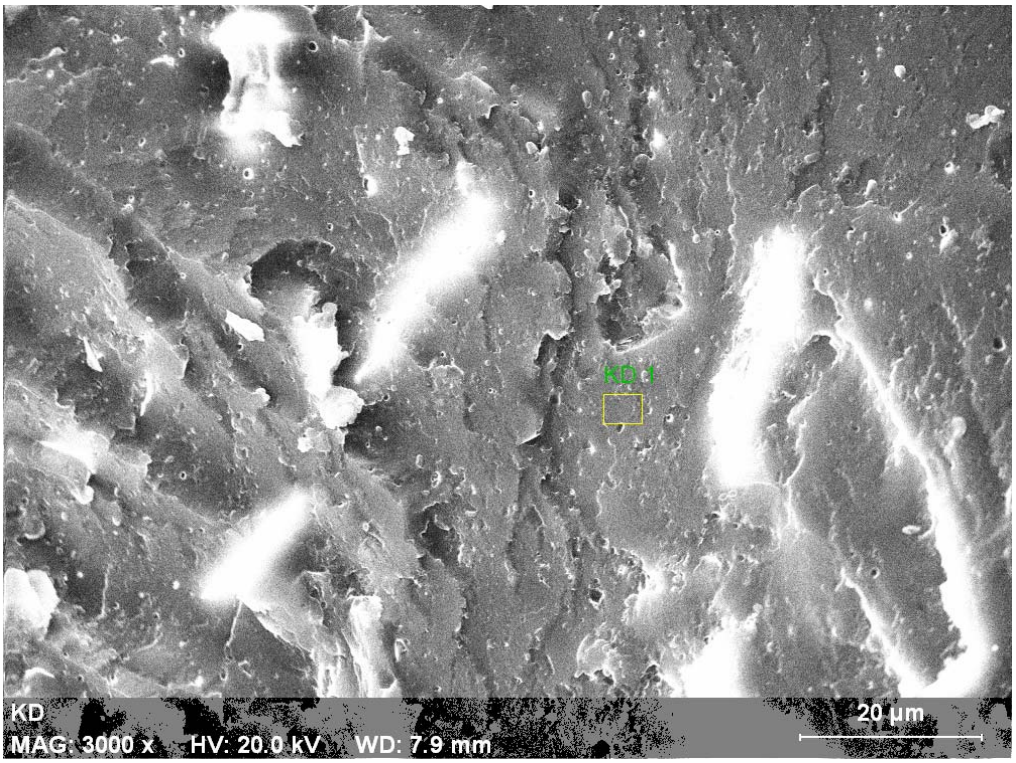


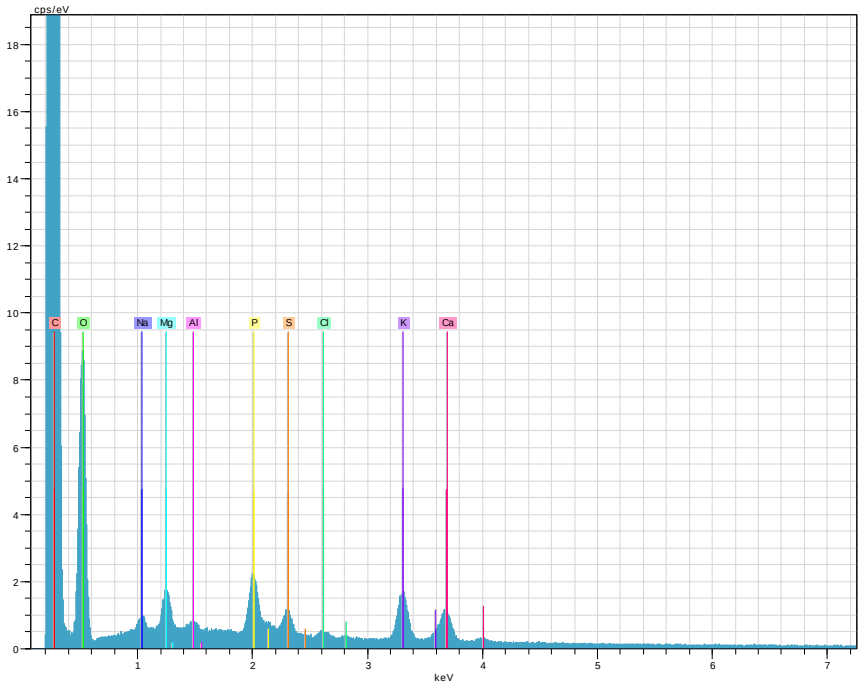
Figure 4. SEM image of *Colocasia esculenta* biomaterial.

Table 2. Comparative evaluation parameters of various film formulations.

Formulation	Wt.Uniformity (mg)	Thickness (mm)	Folding endurance	Swelling index	Surface pH
PK1	44.77 ± 0.66	0.49 ± 0.05	121.33 ± 0.57	55.10 ± 1.14	6.83 ± 0.05
PK2	46.17 ± 0.59	0.5 ± 0.07	120.66 ± 0.57	64.47 ± 0.17	6.60 ± 0.10
PK3	52.28 ± 0.56	0.56 ± 0.01	122 ± 1.00	61.70 ± 0.22	7.1 ± 0.10
PK4	52.87 ± 0.47	0.59 ± 0.02	110.66 ± 0.57	66.64 ± 0.36	6.93 ± 0.05
PK5	70.17 ± 0.08	0.69 ± 0.07	140.66 ± 1.15	67.39 ± 0.59	7.03 ± 0.15
PK6	82.79 ± 0.59	0.70 ± 0.06	141.33 ± 0.57	66.73 ± 0.44	7.03 ± 0.15

Table 3. Comparative evaluation parameters of various film formulations.

Formulation	Tensile strength	% elongation	% Moisture uptake	% Moisture loss	VTR ($gm/cm^2/hr$)	Content uniformity (%)
PK1	57.99 ± 0.75	5.67 ± 0.24	9.59 ± 0.54	10.15 ± 0.54	6.15 ± 0.05	81.23 ± 0.21
PK2	67.88 ± 0.57	6.05 ± 0.71	9.52 ± 0.50	9.73 ± 0.65	7.16 ± 0.16	86.83 ± 0.60
PK3	89.20 ± 1.05	6.07 ± 0.33	10.93 ± 0.54	11.62 ± 0.37	6.78 ± 0.65	81.62 ± 0.70
PK4	90.74 ± 0.82	6.79 ± 0.54	13.32 ± 0.87	13.85 ± 0.71	8.64 ± 0.25	90.30 ± 0.17
PK5	110.93 ± 0.51	7.28 ± 0.50	13.76 ± 0.69	14.46 ± 0.46	10.27 ± 0.80	89.8 ± 0.50
PK6	112.44 ± 0.21	7.06 ± 0.74	14.25 ± 0.99	14.27 ± 0.23	11.26 ± 0.26	93.28 ± 0.08



Spectrum: KD

Element	Series	unn. C [wt.-%]	norm. C [wt.-%]	Atom. C [at.-%]	Oxide	Oxid. C [wt.-%]
Carbon	K-series	54.66	54.66	63.38	CO2	94.43
Sodium	K-series	1.14	1.14	0.69	Na2O	0.73
Magnesium	K-series	1.24	1.24	0.71	MgO	0.97
Aluminium	K-series	0.35	0.35	0.18	Al2O3	0.31
Phosphorus	K-series	1.40	1.40	0.63	P2O5	1.52
Sulfur	K-series	0.50	0.50	0.22	SO3	0.59
Chlorine	K-series	0.10	0.10	0.04	Cl	0.05
Potassium	K-series	1.40	1.40	0.50	K2O	0.79
Calcium	K-series	0.93	0.93	0.32	CaO	0.62
Oxygen	K-series	38.28	38.28	33.32	O	-52.85
	Total:	100.00	100.00	100.00		

Figure 5. Elemental analysis of *Colocasia esculenta* biomaterial.

Formulation and evaluation of biofilms using the isolated biomaterial. Six biofilms (PK1, PK2, PK3, PK4, PK5 and PK6) were successfully prepared from *C. esculenta* biomaterial in the ratio of drug: biopolymer. The biofilms from all the batches were smooth, translucent and flexible without any sign of cracking. The weight of biofilms ranged from 44.77±0.66 mg to 82.79±0.59 mg and thickness ranged from 0.49±0.05 mm to 0.70±0.06 mm. The biofilms showed folding endurance 121.33±0.57 to 141.33±0.57. The swelling index of biofilms ranged from 55.10±1.14 to 66.73±0.44. All biofilms showed nearly neutral pH (Table 2).

The tensile strength of PK1 to PK6 biofilms was 57.99±0.75 to 112.44±0.21 and percent elongation was 5.67±0.24 to 7.06±0.74 %. Percent moisture uptake of biofilms was 9.59±0.54 to 14.25±0.99 %.

Percent moisture loss ranged from 10.15±0.54 to 14.27±0.23 % and vapour transmission rate was 6.15±0.05 to 11.26±0.26 gm/cm^2/hr. Finally content uniformity for all biofilms was determined which varied from 81.23±0.21 to 93.28±0.08 % (Table 3).

Mucoadhesivity of drug loaded biofilms. All the drug loaded biofilms (PK1 to PK6) showed potential inbuilt mucoadhesivity. The biofilm formulation PK6 showed maximum mucoadhesivity value that was comparable to standard polymeric film of HPMC and more than sodium CMC film (Figure 6).

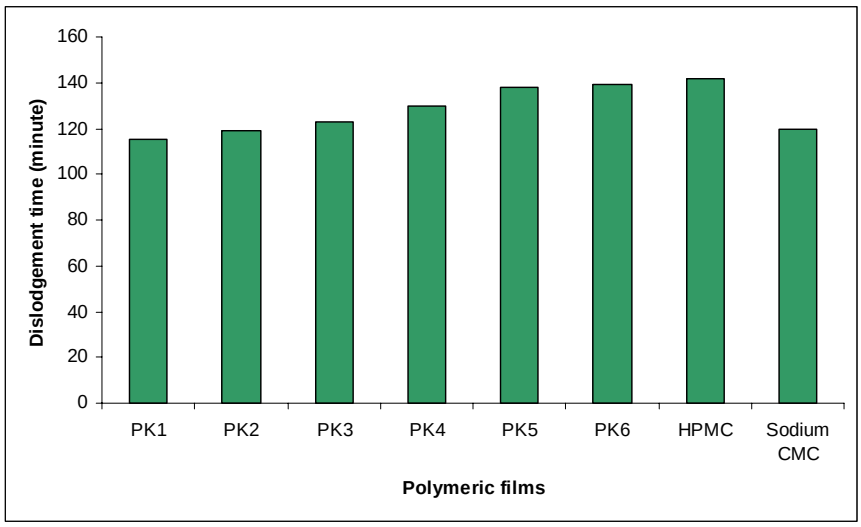


Figure 6. Determination of mucoadhesivity of various film formulations by rotating basket method.

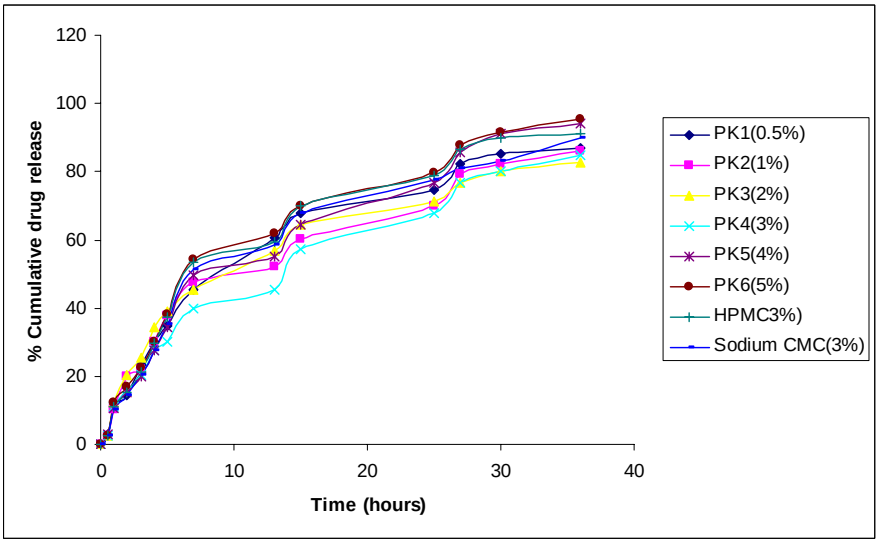


Figure 7. *In vitro* drug release of phenytoin loaded polymeric films.

***In vitro* drug release of biofilms.** The *in-vitro* percentage release data of biofilms was found to be in the order PK6 > PK5 > PK1 > PK2 > PK4 > PK3 (Figure 7). The drug release of PK6 formulation was 95.35% over 36 hours, which was more than HPMC film (91.23%) as well as sodium CMC film

(89.88%). The drug release of PK6 formulation followed first order kinetics with T_{50} 15.36 hours and T_{80} 24.24 hours. The mechanism of drug release was anomalous transport.

Stability studies. There was no significant change in the colour or physico-chemical properties of prepared phenytoin loaded biofilms of *C. esculenta* biomaterial during stability testing till three months. It revealed that biofilms were sufficiently stable.

CONCLUSION

Finally conclusion was drawn that *C. esculenta* biomaterial can serve as a potential film forming biopolymer 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.

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