AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Formulation and Evaluation of Phenytoin Loaded Biofilms using *Annona Squamosa* Biomaterial

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ABSTRACT

In the present paper an effort was made to isolate a biomaterial from the fruit pulp of *Annona squamosa* and evaluate its film forming capacity. *Annona squamosa* was isolated by an economic method and subjected to various physical evaluation parameters. The biomaterial was subjected to spectral studies like IR, ^{1}H NMR and DSC analyses. Phenytoin loaded biofilms were prepared using the isolated biomaterial and other co- processing agents. The prepared biofilms were evaluated for their film parameters and subjected to *in-vitro* drug release studies using static MS diffusion apparatus. The results were compared with the standard HPMC film. The experimental results revealed that the prepared biofilms possessed good folding endurance with appreciable release characteristics. The best film formulation was PS6 with percentage cumulative release of 91.85% over 36 hours. Hence it clearly indicated that the isolated biomaterial possessed inbuilt filmability.

Keywords: Biofilms, Biopolymers, *Annona squamosa*, Phenytoin.

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Received 22 March 2014, Accepted 09 April 2014

Please cite this article in press as: Ojha A *et al.* Formulation and Evaluation of Phenytoin Loaded Biofilms using *Annona Squamosa* Biomaterial . American Journal of PharmTech Research 2014.

INTRODUCTION

Annona squamosa is a small tree with edible fruits belonging to the family Annonaceae. The tree ranges from 10 to 20 feet in height with an open crown of irregular branches [1, 2]. The fruit is round and heart shaped with a diameter of 5-10 cm. On ripening the fruit becomes greenish-yellow with a white powdery bloom. The pulp is white, edible and sweetly aromatic having black, shiny and smooth seeds in each carpel. The fruit pulp is composed of chemical constituents like sugars, triglycerides, α-pinene, sabinene and limonene [3, 4]. It has nutritive and hypoglycemic properties. The seeds of *Annona squamosa* contain Annotemoyin, squamocin, quercetin and cholesteryl glucopyranoside, which have remarkable antimicrobial, antithyroidal and cytotoxic activities [2, 5].

Phenytoin is an antiepileptic drug, having molecular formula $C_{15}H_{12}N_2O_2$ and molecular weight 252.268 g/mol [6, 7]. Phenytoin acts by blocking voltage-sensitive sodium channels in the neurons. At higher concentrations, phenytoin prolongs the neuronal refractory period by delaying the activation of outward potassium currents in nerves. The anticonvulsant action of phenytoin may also be the result of its action on calcium channels or γ-amino butyric acid receptors (GABA) [6, 7]. The aim of our research work was to isolate a biomaterial from *Annona squamosa* fruit pulp and evaluate its film forming ability by making biofilms using phenytoin as the model drug.

MATERIALS AND METHODS

Phenytoin was obtained as a gift sample from Zaneka Healthcare Pvt. Ltd.; Haridwar. *Annona squamosa* fruit was procured from the local market; Dehradun. HPMC were purchased from Merck Specialties Pvt. Ltd.; Mumbai. IR spectral analysis performed in Laureate Institute of Pharmacy; Himachal Pradesh. ^{1}H NMR was obtained from SAIF; Panjab University; Chandigarh. DSC analysis was performed in Dibrugarh University; Assam.

Isolation of biomaterial

Initially fruit pulp of *Annona squamosa* was separated, minced with double distilled water and filtered. It was then treated with 90% alcohol and refrigerated for 10 hours. It was centrifuged at 3000 rpm for 30 min, dried in dessicator for 24 hours and finally passed through 120 mesh sieve to get uniform size particles. The percentage yield of isolated biomaterial was calculated [5, 8].

Characterization of isolated biomaterial

The isolated biomaterial was subjected for various physical tests like colour, odour, solubility, colour changing point, viscosity and surface tension [9, 10]. It was also subjected for IR spectral analysis in order to determine the functional groups present in the isolated biomaterial. Further

the biomaterial was subjected to ^{1}H NMR and differential scanning calorimetry (DSC).

Formulation of film formulations using the isolated biomaterial

The isolated biomaterial was used for formulating biofilms using phenytoin as the drug. Six different film formulations i.e. PS1, PS2, PS3, PS4, PS5 and PS6 were prepared using biopolymer & phenytoin in six different ratios by solvent casting method (Table 1). Initially, 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 biofilm. Phenytoin solution was prepared separately 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 films of 1 sq. cm [11, 12].

Table 1: Formulation of Phenytoin loaded film formulations using the biomaterial

S.No.	PS1 (1:0.5)	PS2 (1:1)	PS3 (1:2)	PS4 (1:3)	PS5 (1:4)	PS6 (1:5)
Phenytoin (mg)	100	100	100	100	100	100
Biopolymer (mg)	50	100	200	300	400	500
Dextrose(mg)	300	300	300	300	300	300
Mannitol (mg)	300	300	300	300	300	300
Water (ml)	20	20	20	20	20	20

Evaluation of film formulations

The prepared phenytoin loaded biofilms were evaluated for the parameters namely weight, thickness, folding endurance, surface pH, tensile strength, elongation, moisture uptake and content uniformity[12, 13]. The mean of three readings was determined for each parameter.

***In-vitro* drug release studies**

The *in- vitro* drug release studies of prepared film formulation were carried out by using a novel static M.S. diffusion apparatus which had two compartments; upper donor and lower receptor compartment. The formulated 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**.** 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 film of HPMC polymer[12, 14].

RESULTS AND DISCUSSION

Physical evaluation parameters of isolated biomaterial

Our experimental results revealed that the isolated biomaterial was yellowish brown powder with a colour changing point of 230^0C and percentage yield 15%. It was soluble in water, slightly soluble in chloroform and insoluble in alcohol. It 1% solution showed viscosity 2.5 cp and surface tension 79.66 dyne/cm.

Spectral analysis of isolated biomaterial

IR spectroscopy of the isolated biomaterial revealed peaks at 3387 cm-1 (OH stretching), 2924 cm-1 (C-H stretching), 1743 cm-1 (CHO stretching aldehyde), 1600 cm-1 (C=O stretching of carboxyl group) and 775 cm-1 (CH bending aromatic ring) **(**Figure 1). The presence of OH and COOH groups in IR spectrum confirmed the polymeric nature of the biomaterial.

^{1}H NMR of *Annona squamosa* biomaterial showed chemical shift at δ 2.1-3 ppm (-C≡CH, acetylenic proton), δ 3.1-4 ppm (-CH_3OR, ether proton), δ 4.5 ppm (-C=CH, vinylic proton) and δ 5.1 ppm (R-OH, hydroxyl proton) (Figure 2).

The DSC curve of *Annona squamosa* showed glass transition temperature 139.97^0C. Peak height was observed at 52.4591 mW, area was found to be 5860.800 mJ. The value of delta H was 586.0800 J/g (Figure 3).

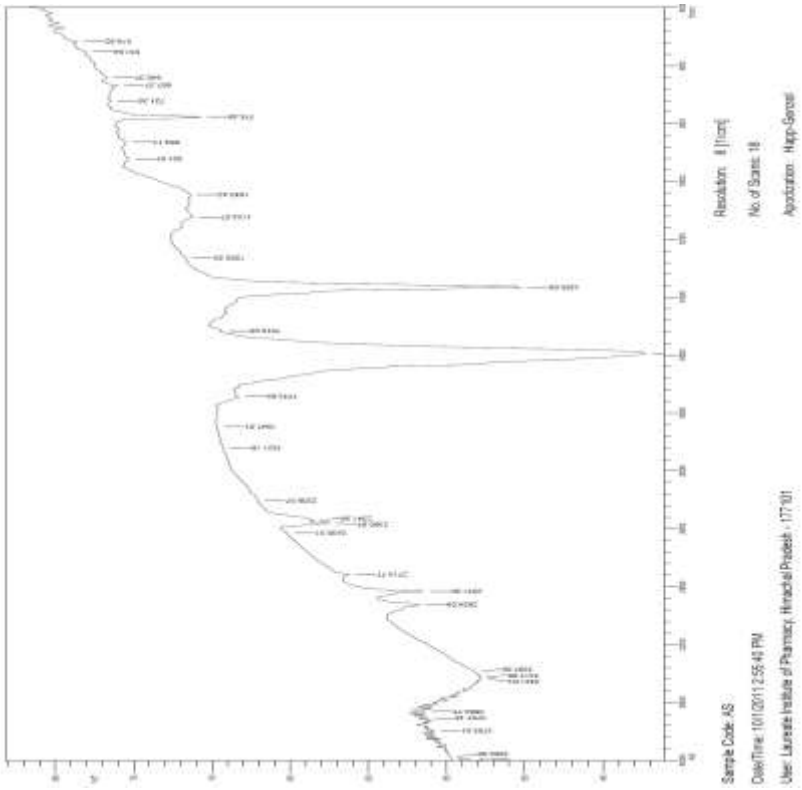

Figure 1: IR Spectra of *Annona squamosa* biomaterial

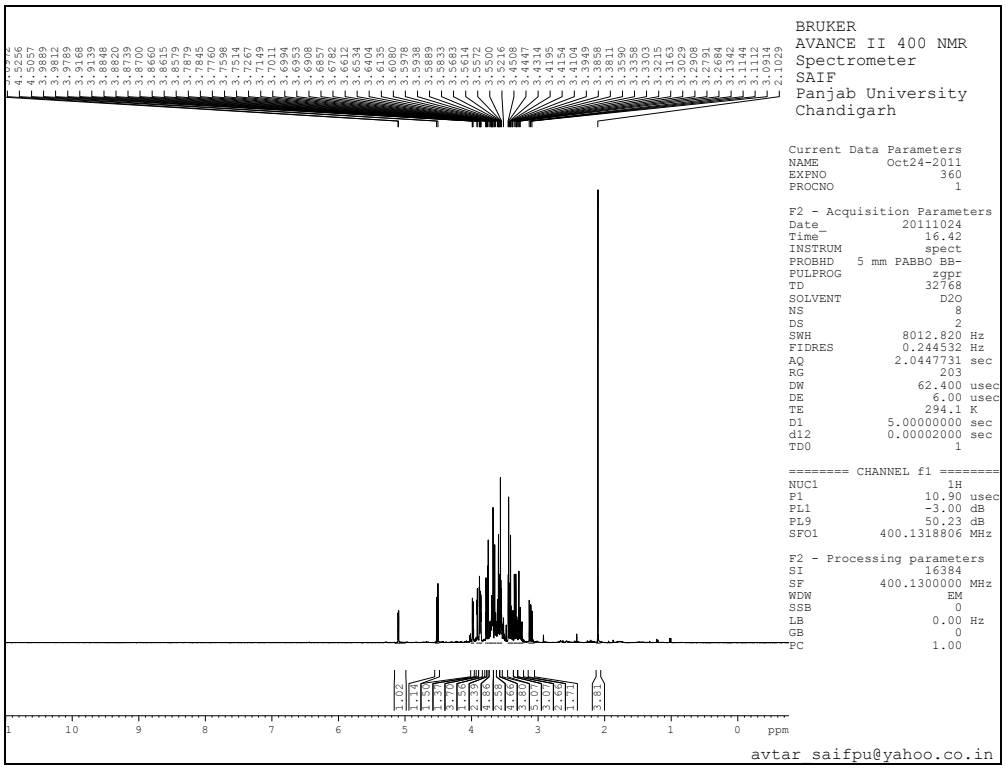

Figure 2: ^{1}H NMR Spectra of *Annona squamosa* biomaterial

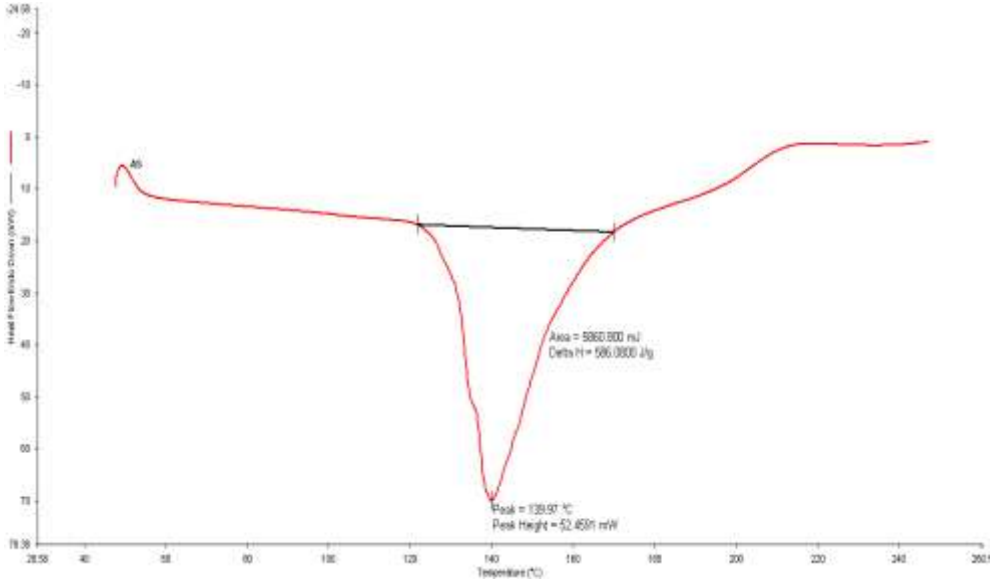

Figure 3: DSC curve of *Annona squamosa* biomaterial

Formulation and evaluation of film formulations using the biomaterial

Six biofilms were successfully prepared from *Annona squamosa* biomaterial in the ratio of drug: biomaterial 1:0.5, 1:1, 1:2, 1:3, 1:4 and 1:5 respectively. The weight of prepared phenytoin loaded biofilms of biomaterial varied from 47.62±0.49 mg to 65.26±0.42 mg for PS1 to PS6. The thickness of biofilms of biomaterial varied from 0.36±0.01 mm to 0.73±0.02 mm. The maximum folding endurance was obtained for film formulations PS6 (150.67±0.58). The measured surface

pH for all batches was found near to the neutral pH (Table 2).

The film formulations showed maximum tensile strength for PS6. The percent elongation ranged from 6.73±0.57 % to 8.29±0.24 %. All the biofilms showed % moisture uptake in the range 12.23±0.60 to10.29±0.33. The film formulation PS6 showed maximum content uniformity (Table 3).

Table 2: Evaluation parameters of phenytoin loaded biofilm of *Annona squamosa*

Formulation	Wt.Uniformity (mg)	Thickness (mm)	Folding endurance	Surface pH
PS1	47.62±0.49	0.36±0.01	113.67±1.52	6.8±0.15
PS2	49.07±0.43	0.37±0.01	114.00±1.73	7±0.17
PS3	52.28±0.59	0.47±0.01	122.67±1.53	7.1±0.1
PS4	57.63±0.32	0.59±0.02	122.00±1.00	7.2±0.1
PS5	62.11±0.30	0.63±0.02	149.33±0.58	7.0±0.1
PS6	65.26±0.42	0.73±0.02	150.67±0.58	7.2±0.15

Table 3: Additional evaluation parameters of phenytoin loaded biofilm of *Annona squamosa*

Formulation	Tensile strength	Elongation (%)	Moisture uptake (%)	Content uniformity (%)
PS1	90.97±0.46	6.73±0.57	12.23±0.60	78.01±0.49
PS2	97.88±0.57	6.71±0.63	12.44±0.30	86.10±0.45
PS3	130.68±0.53	7.23±0.51	11.56±0.66	88.75±0.57
PS4	131.74±0.46	7.7±0.35	11.46±0.68	88.88±0.84
PS5	140.96±0.46	7.75±0.40	10.38±0.45	90.36±0.38
PS6	142.51±0.33	8.29±0.24	10.29±0.33	93.50±0.36

***In-vitro* drug release of biofilms**

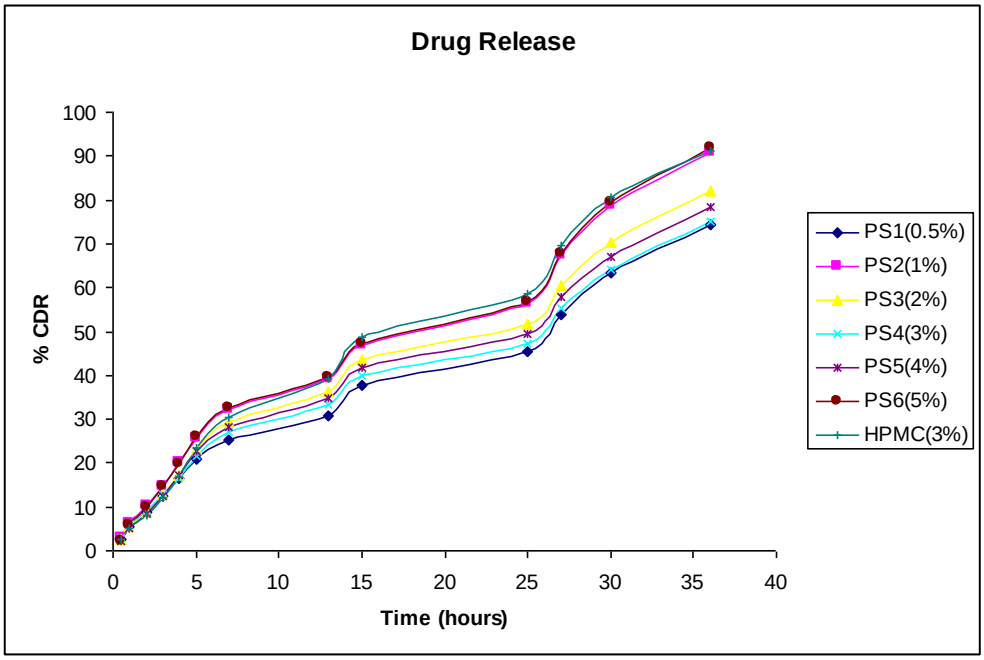

Figure 4: In-vitro drug release of phenytoin loaded biofilms of *Annona squamosa*

The percentage release of film formulations containing Phenytoin drug and Annona squamosa bio-polymer (0.5, 1, 2, 3, 4, 5, 6 %) was found to be in the order PS6 > PS2 > PS3 > PS5 > > PS4 > PS1 The maximum drug release was observed for PS6 formulation was 91.85 %, which was comparable to HPMC film (Figure 4).

CONCLUSION

In the present study the biofilms were prepared using *Annona squamosa* biomaterial that exhibited appreciable folding endurance. The *in vitro* studies confirmed that the biofilms prepared using isolated biomaterial showed a good release profile in a controlled manner. Hence this natural biomaterial can act as a promising excipient for formulating film formulations. Moreover the biomaterial has been isolated from an edible source; it can prove an effective alternative to conventional polymers.

ACKNOWLEDGEMENT

We are thankful to Zaneka Healthcare Pvt. Ltd.; Haridwar for providing the gift sample of phenytoin. We also acknowledge Laureate Institute of Pharmacy; Himachal Pradesh for providing IR spectral analysis, SAIF; Panjab University; Chandigarh for providing ^{1}H NMR as well as Dibrugarh University; Assam for providing DSC analysis of the isolated biomaterial.

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