FORMULATION DEVELOPMENT AND CHARACTERIZATION OF EPIGALLOCATECHIN GALLATE LOADED MUCOADHESIVE VAGINAL FILM FOR THE CERVICAL CANCER

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ABSTRACT

Cervical cancer remains a significant health concern worldwide, necessitating innovative approaches for effective treatment. This study explores the development and evaluation of mucoadhesive vaginal films loaded with *Epigallocatchin Gallate*, a promising phytoconstituent, for targeted therapy against cervical cancer. The solvent casting method was employed to formulate films using hydroxypropyl methylcellulose (HPMC) and sodium alginate as polymers, along with propylene glycol as a plasticizer. FTIR spectroscopic analysis confirmed the compatibility of *Epigallocatchin Gallate* with the selected excipients. Increasing polymer concentration led to controlled drug release, particularly at a ratio of 0.4:1 (plasticizer:polymer). The prepared films underwent comprehensive evaluation for various parameters including weight variation, thickness, folding endurance, surface pH, swelling index, drug content, moisture absorption, moisture loss, in-vitro disintegration, ex-vivo diffusion, and cell line studies. The formulations exhibited a pH range of 4.25 to 5.2, indicating compatibility with vaginal secretions. Standard graph analysis demonstrated linearity of *Epigallocatchin Gallate* concentrations from 10 to 60 µg/ml, adhering to Beer Lambert's law. Formulation F8 demonstrated optimal drug content (88.36%) and ex-vivo diffusion (86.74%), warranting further investigation. Cell line studies using the HeLa cell line revealed promising anticancer activity, with a 28% inhibition rate at 50 µM/ml compared to the vehicle control. Pharmacokinetic analysis suggested a first-order kinetics model ($R^2 = 0.9554$) with diffusion-controlled release ($n = 0.1935$), and Higuchi kinetics indicated diffusion as the predominant permeation mechanism ($R^2 = 0.976$). Overall, the study underscores the potential of *Epigallocatchin Gallate*-loaded mucoadhesive vaginal films as a promising therapeutic approach for cervical cancer. These findings suggest the viability of natural therapeutic agents like *Epigallocatchin Gallate* in mitigating cervical cancer complications, offering a more efficacious and acceptable alternative to conventional drug delivery systems with sustained release profiles for enhanced therapeutic efficacy.

Key Words: Cervical cancer, *Epigallocatchin Gallate,* HPMC, Higuchi kinetics

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INTRODUCTION

Cancer is a deadly disease which is caused due to rampant growth of the cells and forms from the extra mass tissue known as a tumor. Cancer is a disease of cells in the body. It is a disease in which some of the body‟s cells grow uncontrollably and spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Cancer is recognized as an abnormal growth of cells and is originated due to lack of proper regulation in cell cycle. Normally, human cells grow and multiply to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. Sometimes this orderly process breaks down, and abnormal or damaged cells grow and multiply when they shouldn‟t. These cells may form tumours, which are lump of tissues.

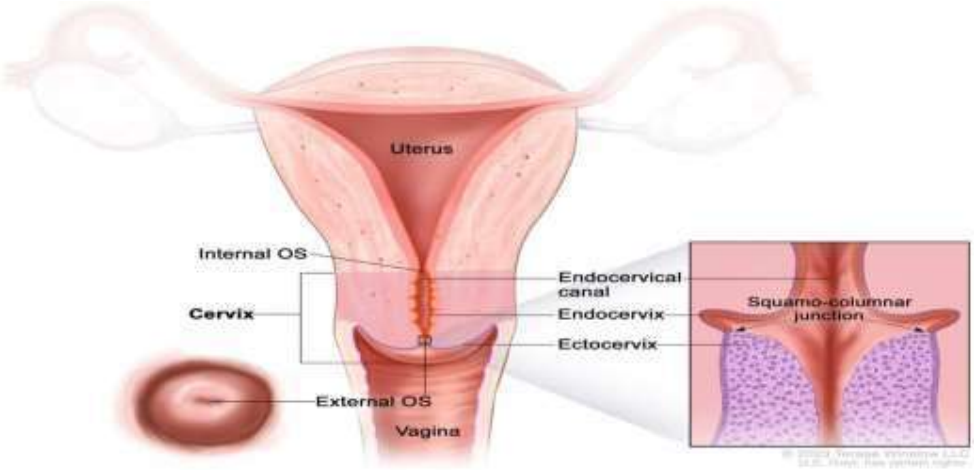


Figure 1. Diagrammatic Representation of Anatomy of Cervix

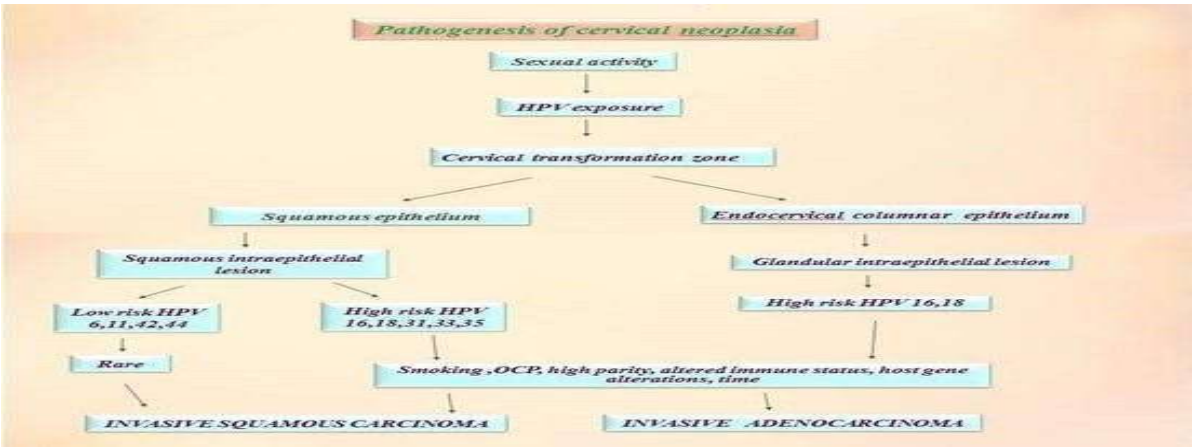


Figure 2. Schematic Representation of Pathogenesis of Cervical Cancer



Figure 3. Diagramattic Representation of *Camellia sinensis* plant

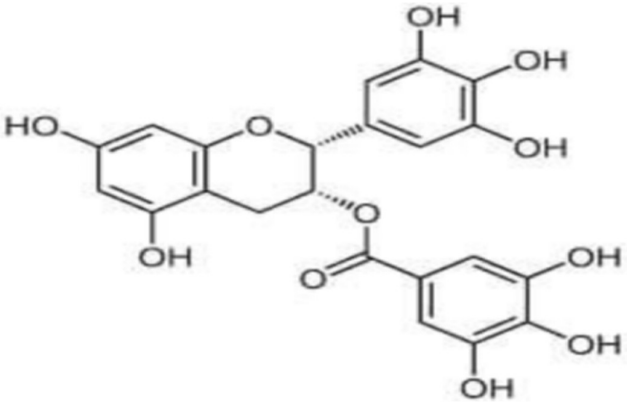
EPIGALLOCATCHIN GALLATE

Synonyms : Tea catechin

Source : *Camellia Sinesis*

IUPAC name : [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl] 3,4,5-trihydroxybenzoate

Chemical structure



Structural formula : C22H18O11

Molecular weight : 458.4 g/mol.

Appearance : It appears as a fine, off-white to pale yellow powder in its purified form..

Bioavailability ***: 45%***

METHODOLOGY

Preformulation Studies

Pre-formulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of pre-formulation testing is to generate information useful to developing the stable and bio-available dosage form obviously, the type of information needed will be depends on the dosage form to be developed. The use of pre-formulation parameter minimizes the chances in formulation and acceptable, safe, efficacious and stable product and at same time provides the basis for optimization of the drug product quality.

Solubility Studies of Epigallocatchin gallate

Preformulation solubility analysis was done, which include the selection of suitable solvent, to dissolve the respective drug. The solubility was done by adding the solute in small incremental amounts to the fixed volume of solvents, after each addition, the system was vigorously shaken and examined visually for the undissolved solute particles. When some amount of the solute remains undissolved, the total amount added up to the point served as a good and rapid estimate of solubility.

Melting Point by Capillary Tube Method

The melting point of Epigallocatchin gallatee was determined by the capillary tube method A sufficient quantity of Castine powder was filled into the capillary tube to give a compact column of 4-6 mm in height. The tube was introduced in an electrical melting point apparatus and the temperature was noted. The melting point was recorded, which is the temperature at which the last solid particle of Epigallocatchin gallatee in the tube passed into liquid phase.

Determination of Absorption Maxima (λmax) of Epigallocatchin gallate

A stock solution is prepared by 50 mg of Epigallocatchin gallate was accurately weighed and transferred to a 50 ml volumetric flask. The drug was dissolved in 5 ml ethanol and the volume was made up to 50 ml by using acetate buffer pH 4.6 to obtain a stock solution of 1000 µg/ml. 1 ml of this stock solution was again diluted with pH acetate buffer pH 4.6 up to 10 ml to obtain a solution of 100 µg/ml. The resulting solution was scanned at 200-400nm in UV-Visible spectrophotometer t to attain the absorption maximum max.

Preparation of Acetate buffer pH 4.6

The buffer was prepared by using distilled water following standard method. Dissolve 5.4 g of sodium acetate in 50ml of water and add 2.4ml of glacial acetic acid and dilute with water to 100ml. A stock solution is prepared by 50 mg of Epigallocatchin gallate was accurately weighed and transferred to a 50 ml volumetric

flask. The drug was dissolved in 5 ml ethanol and the volume was made up to 50 ml by using acetate buffer pH 4.6 to obtain a stock solution of 1000 µg/ml. 1 ml of this stock solution was again diluted with pH acetate buffer pH 4.6 up to 10 ml to obtain a solution of 100 µg/ml. The resulting solution was scanned at 200-400nm in UV-Visible spectrophotometer to attain the absorption maximum max.

Standard Curve for Epigallocatchin gallate

A stock solution of Epigallocatchin gallate was prepared by dissolving 100mg of Epigallocatchin gallate in 10ml of ethanol in a 100ml volumetric flask and shaking the flask few seconds. Then the final volume was made up to the mark with acetate buffer pH 4.6 to obtain the stock solution concentration of 1000 µg/ml. From the above standard stock solution, 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with acetate buffer pH 4.6 to get a working standard of 100µg/ml. From the working standard solution 1ml, 2ml, 3ml, 4ml, 5ml were pipetted out into 10ml volumetric flasks and volume was made up to the mark with acetate buffer pH 4.6. The absorbance of these solutions was measured by UV spectrophotometric method at 347nm against blank. Based on this information, the calibration curve was obtained by plotting concentration versus absorbance.

Drug-Excipient Compatibiloty Studies

Compatibility study of Epigallocatchin gallate with the excipients such as HPMC, Sodium alginate and PG was determined by FTIR Spectroscopy using shimadzu FT-IR. Spectral analysis of Epigallocatchin gallate, HPMC, Sodium alginate and PG and combination was carried out to investigate the changes in chemical composition of the drug after combining it with excipients. The compatibility study on FTIR was carried by shimadzu FT-IR specteophotometer in the frequency range 4000- 400 cm^{-1}

Formulation of Mucoadhesive Film

Mucoadhesive films were prepared by solvent evaporation technique. Specific amount of Epigallocatchin gallate 200mg was dissolved in 20 ml distilled water and then various ratios of the film forming polymers HPMC and sodium alginate were added and PG were added as plasticizers. The polymeric dispersion was stirred on magnetic stirrer for 30 minutes and sonicated for 15 minutes to remove the entrapped air bubbles. The polymeric dispersion obtained was uniformly spread on a petri-dish and allowed to dry for 24 hour. Dried films were peeled off from the plate with the help of a sharp knife. The film was sealed in a plastic pouch and stored at temperature of 30±2°C with relative humidity of 37±1% for further analysis.

Table 1. Composition of Mucoadhesive Film Formulation

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Epigallocatchin gallate (mg)	200	200	200	200	200	200	200	200
HPMC (mg)	200	100	200	200	200	100	200	200
Sodium alginate(mg)	-	200	100	200	-	200	100	200
PG (mg)	0.2:1	0.2:1	0.2:1	0.2:1	0.4:1	0.4:1	0.4:1	0.4:1
Distilled water(ml)	20	20	20	20	20	20	20	20

Calculated by using the following formula: $SI = [\frac{(W - Wo)}{Wo}] \times 100$

RESULTS AND DISCUSSION

Preformulation Studies

The following preformulation studies were performed.

Solubility Study of Epigallocatchin gallate

The solubility of Epigallocatchin gallate was checked in different solvents such as distilled water and ethanol and the results are given in the table 4. Solubility studies have been performed for pure extract. Result showed that pure extract of Epigallocatchin gallate was found to be soluble in distilled water, freely soluble in ethanol and acetone. So distilled water is used as common solvent in the preparation of mucoadhesive vaginal film.

Table 2. Solubiltiy Test of Epigallocatchin gallate

S.No	Media	Solubility
1	Distilled water	Soluble
2	Ethanol	soluble
3	Acetone	soluble

Melting point by capillary tube method

The melting point of Epigallocatchin gallate was observed for quality determination, it matches with standard value and are given in Table 5. Epigallocatchin gallate was found to be at 186°C in the capillary tube method.

Table 1. Melting Point Determination of Epigallocatchin gallate

Drug	Standard value	Experimental value
Epigallocatchin gallate	112 - 147°C	136°C

Determination of Absorption Maxima λmax for Epigallocatchin gallate

The absorption maximum of Epigallocatchin gallate was determined using uv visible spectrophotometer. The maximum absorbance of Epigallocatchin gallate was found to be 347 nm and it was selected for the quantitative drug content analysis.

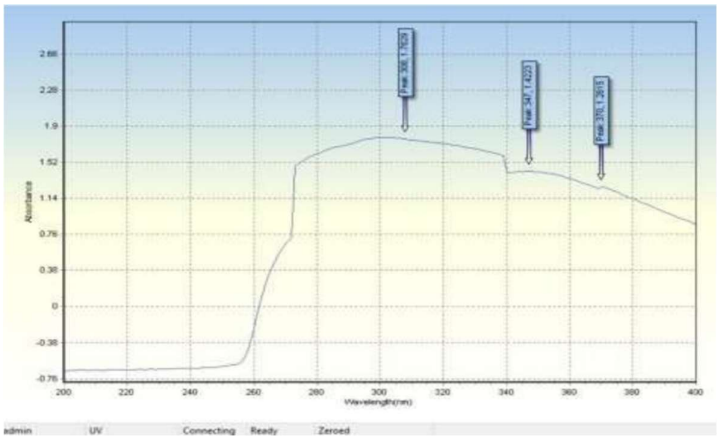


Figure 4. Representation of Absorption Maxima λmax for Epigallocatchin gallate

Standard Curve for Epigallocatchin gallate

The UV Visible spectrophotometric method was used to analyze Epigallocatchin gallate at wavelength of 347 nm. The solution of Epigallocatchin gallate in acetate buffer pH 4.6 was suitably diluted to give concentration ranging from 10-60µg/ml. The

absorbance was measured at 347 nm and the values were given in Table 5. It was found that the working standard solutions of Epigallocatchin gallate had shows standard linearity (R^2=0.9916) in the concentration of 10-60µg/ml and obeys Beer Lambert‟s law.Table 6. Calibration Curve of Epigallocatchin gallate in Acetate Buffer pH 4.6

S. No	Concentration(µg/ml)	Absorbance at 347nm
1	0	0
2	10	0.157
3	20	0.301
4	30	0.412
5	40	0.536
6	50	0.652
7	60	0.728

Drug excipients compatibility study

Fourier transform InfraRed (FTIR) is the tool for solid state characterization of active pharmaceutical ingredients and excipients. FTIR Spectroscopy of pure drug, excipients and physical mixture of them were carried out on Shimadzu FTIR 84005 model to investigate any possible interaction between the drug, Epigallocatchin gallate and the utilized polymers (HPMC, Sodium alginate). The samples were finely grounded with KBr to prepare the pellets under a hydraulic pressure of 600 psi and a spectrum was scanned in the wavelength range of 4000 and 400 cm-1 using FTIR spectrophotometer. The compatibility of drug in the formulation was confirmed by comparing FTIR spectra of pure drug with FTIR of its formulation.

The peak values obtained from spectra under stressed condition shows that the excipient does not affect the characteristic peaks of the drug. No significant change in the position of peak and relative intensities of the endothermic peak of the drug was observed. This indicates that there is no interaction between drug and polymer.

The peak observed in the FTIR spectrum for Epigallocatchin gallate, HPMC and Sodium alginate is shown in the figure 22. It shows no shift and no disappearance of characteristic peaks of drug and hence suggests that there is no interaction between the Epigallocatchin gallate, HPMC and sodium alginate.

The peak observed in the FTIR spectrum for Epigallocatchin gallate physical mixture is shown in figure 23. It shows no shift and no disappearance of characteristic peaks of drug and hence suggests that there is no interaction between the Epigallocatchin gallate physical mixture.

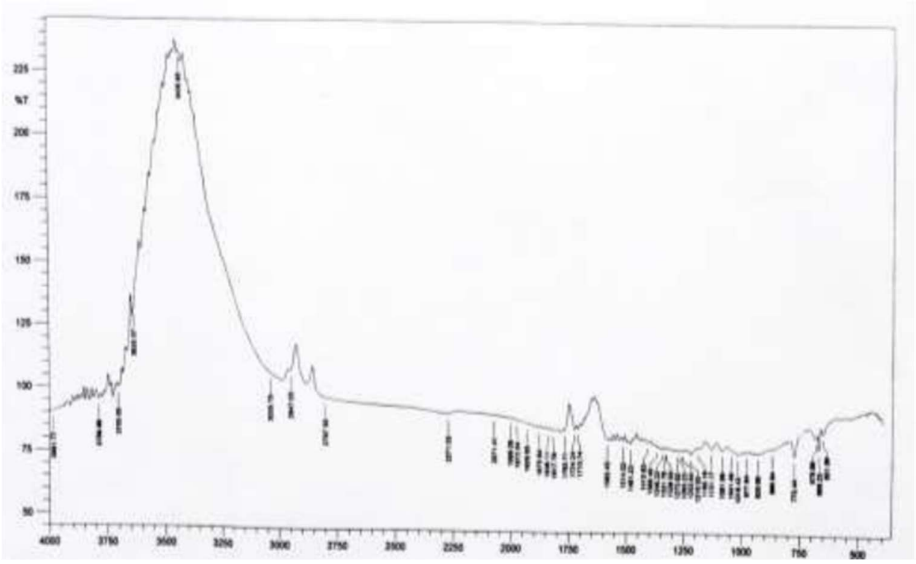


Figure 5. FTIR Spectrum of Epigallocatchin gallate

Table 3. FTIR spectral interpretation of Epigallocatchin gallate

S.No	Wave Number (cm^{-1})	Functional Group
1	3635.57	O-H Alcohol (Stretching)
2	3435.95	N-H Primary amine (Stretching)
3	2271.02	N=C=O Isocynate (Stretching)
4	1765.54	C=O Carboxilic acid (Stretching)
5	1424.12	C=O Aliphatic ketone (Stretching)
6	1218.93	C-O Vinyl ether (Stretching)
7	772.44	C-H 1,2,3-trisubstituted (Stretching)

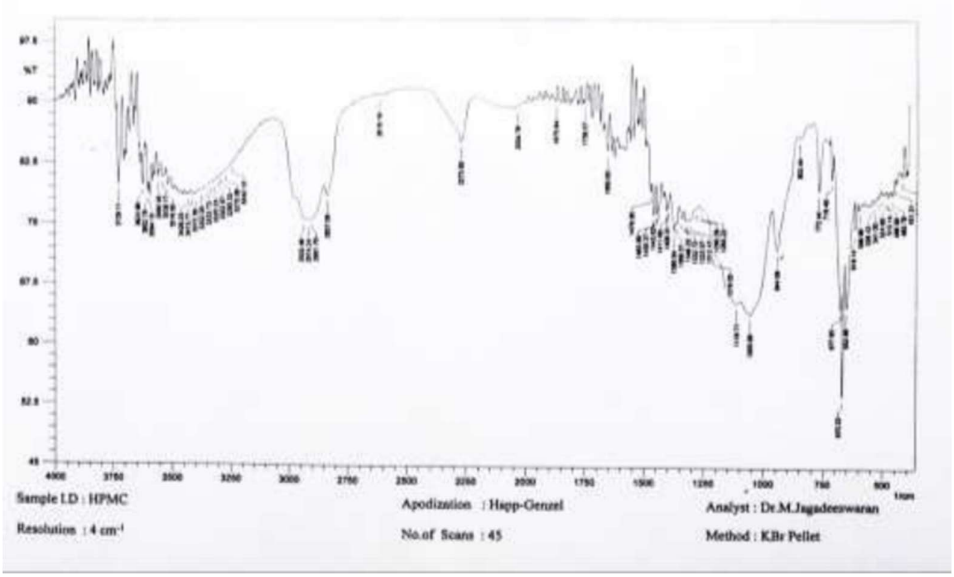


Figure 6. FTIR Spectrum of HPMC

Table 4. FTIR spectral interpretation of HPMC

S.No	Wave number (cm^{-1})	Functional group
1	3624.96	O-H Alcohol (Stretching)
2	3594.10	O-H Alcohol (Stretching)
3	3516.95	O-H Alcohol (Stretching)
4	1660.60	C=C Alkene (Stretching)
5	1443.62	C-H Alkane (Stretching)
6	1380.94	O-H Phenol (Stretching)

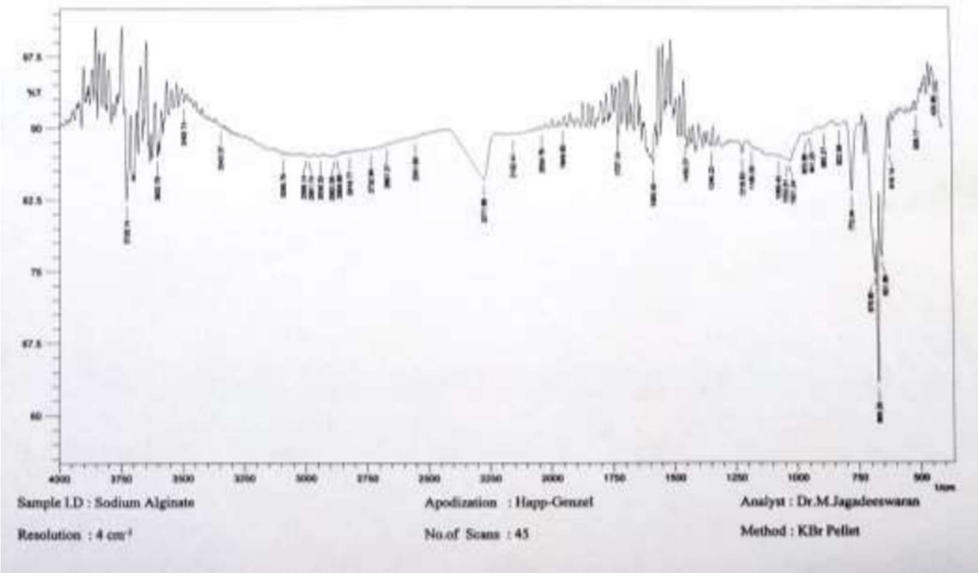


Figure 7. FTIR Spectra of Sodium alginate

Table 5. FTIR spectral interpretation of Sodium alginate

S.No	Wave Number (cm^{-1})	Functional Group
1	3602.78	O-H Alcohol (Stretching)
2	3600.50	O-H Alcohol (Stretching)
3	1727.24	C=O α,β unsaturated ester (Stretching)
4	1450.37	C-H Alkane (Stretching)
5	772.44	C-H 1,3 disubstituted (Stretching)

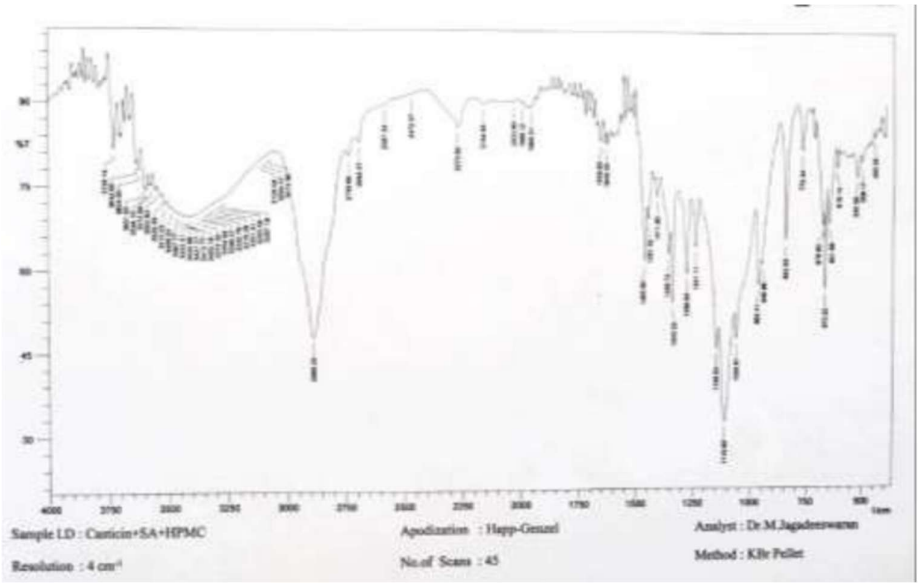


Figure 8. FTIR Spectrum of Epigallocatchin gallate and Polymer

Table 6. FTIR spectral interpretation of Epigallocatchin gallate and Polymer

S.No	Wave Number (cm^{-1})	Functional Group
1	3634.60	O-H Alcohol (Stretching)
2	3594.10	O-H Alcohol (Stretching)
3	2888.20	C-H Alkane (Stretching)
4	1465.80	C-H Alkane (Stretching)
5	1343.33	S=O Sulfonamide (Stretching)
6	1110.92	C-O Secondary alcohol (Stretching)
7	962.41	C=C Alkene (Stretching)
8	842.33	C-Cl Halo compound (Stretching)

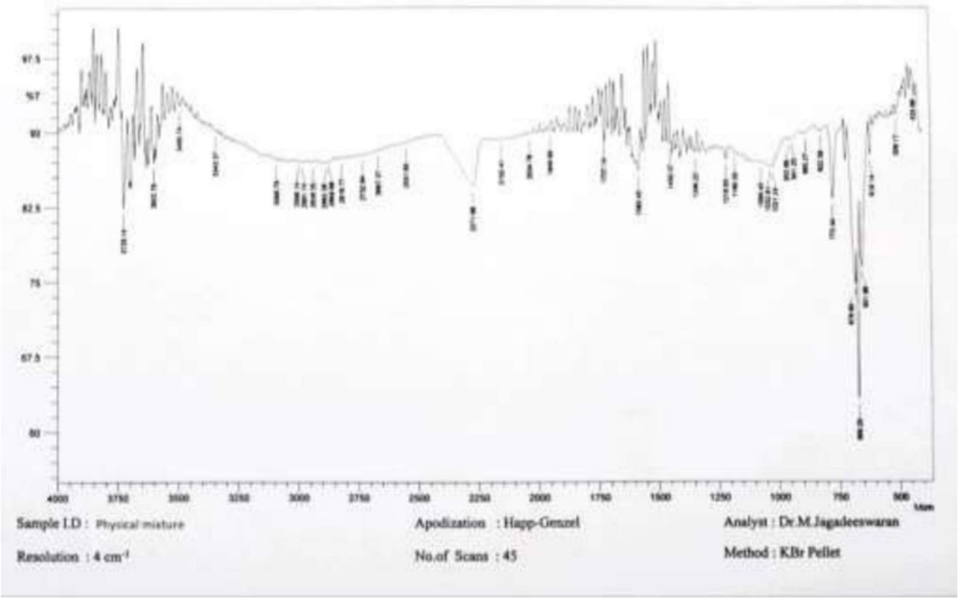


Figure 9. FTIR Spectrum of Physical mixture

Table 7. FTIR spectral interpretation of Epigallocatchin gallate physical mixture

S.No	Functional group	Wave number (cm^{-1})
1	O-H Alcohol (Stretching)	3634.60
2	O-H Alcohol (Stretching)	3594.10
3	C-H Alkane (Stretching)	2888.20
4	C=O α,β unsaturated ester (Stretching)	1727.24
5	C-H Alkane (Stretching)	1450.37
6	O-H Phenol (Stretching)	1380.94
7	C=C Alkene (Stretching)	962.41

Evaluation of Prepared Epigallocatchin gallate Loaded Mucoadhesive Film

Appearance of the film

The overall appearance was found to be brownish yellow and semi transparent which shown uniform distribution of drug. The prepared formulation of Epigallocatchin gallate loaded mucoadhesive vaginal film was shown in the figure 22.



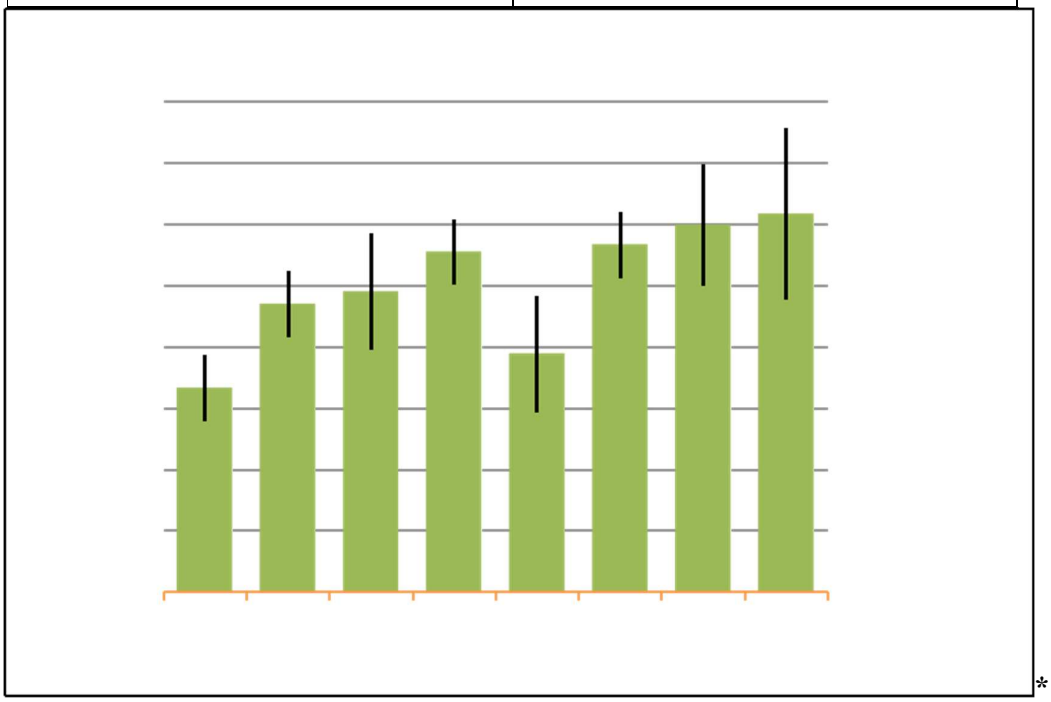
Figure 10. Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Weight Variation

Three films of 2×2 cm^2 size were cut randomly and they were individually weighed on electronic balance. The resultant weight was ranged from 25.66 ± 0.54 to 28.17 ± 1.4. Epigallocatchin gallate loaded mucoadhesive vaginal films was prepared with different concentrations of HPMC and sodium alginate. Weight of the films were F1(25.33 ± 0.54) mg, F2(26.7 ± 0.54) mg, F3 (26.90 ± 0.95), F4 (27.55
± 0.54) mg, F5 (25.89 ± 0.95), F6 (27.67 ± 0.54), F7 (27.99 ± 1.0) and F8(28.17 ± 1.4) mg when prepared with HPMC and sodium alginate in the ratio of (1:0), (0:1), (1:2) and (2:1) respectively. The results portray that increasing the ratio of polymer, increases the weight of the film.

Table 8. Weight Variation Test for Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	Weight Variation* (mg)
F1	25.33 ± 0.54
F2	26.7 ± 0.54
F3	26.90 ± 0.95
F4	27.55 ± 0.54
F5	25.89 ± 0.95
F6	27.67 ± 0.54
F7	27.99 ± 1.0
F8	28.17 ± 1.4



*

Mean ± S.D (n=3)

25.33 26.7 26.9 27.55 25.89 27.67 27.99 28.17

Figure 11. Representation of Weight variation of Epigallocatchin gallate loaded Mucoadhesive Vaginal Film

Thickness

The thickness of patch was directly related to drug content uniformity. So it was essential to find uniformity in the thickness of the film. It can be measured by calibrated

digital vernier calipers. The thickness was measured at 5 different spots of the patch and average was taken. The film thickness ranged from 0.136 ± 0.006mm to 0.212 ± 0.007mm. Epigallocatchin gallate loaded mucodhsive vaginal films were prepared with different concentrations of HPMC and sodium alginate. Thickness for the films (F1-F8) given in the table12 were prepared with HPMC and sodium alginate in the ratio of (1:0), (0:1), (1:2) and (2:1). Increasing the ratio of polymer was found to increase the thickness of the film.

Table 9. Thickness of the Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	Film Thickness (mm)*
F1	0.137 ± 0.018
F2	0.136 ± 0.006
F3	0.14 ± 0.006
F4	0.16 ± 0.006
F5	0.140 ± 0.006
F6	0.187 ± 0.009
F7	0.212 ± 0.007
F8	0.202 ± 0.007

***Mean ± S.D (n=3)**

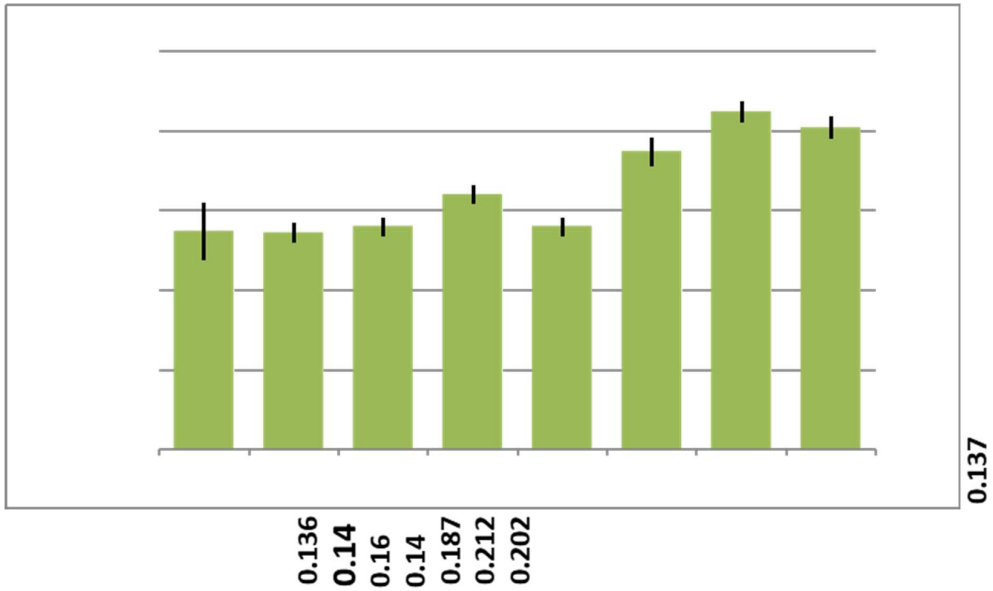


Figure 12. Representation of Thickness of Epigallocatchin gallate loaded Mucoadhesive Vaginal Film

8.2.1. Surface pH

Patch was slightly wet with help of water. The pH was measured by bringing the electrode in contact with the surface of the patch. The study was performed on three patch of each formulation and average was taken. The pH of the vagina ranges from
3.8 to 5.0. The prepared Epigallocatchin gallate loaded mucoadhesive vaginal film formulations F1 to F8 are ranges from 4.25 ± 0.20 to 5.2 ± 0.01. Formulation F1, F5, F6,F7 and F8 were within the vaginal pH.

Table 10. Surface pH of the Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	Surface pH*
F1	5.0 ± 0.05
F2	5.1 ± 0.05
F3	5.12 ± 0.05
F4	5.2 ± 0.01
F5	4.4 ± 0.19
F6	4.8 ± 0.04
F7	4.6 ± 0.05
F8	4.25 ± 0.20

***Mean ± S.D (n=3)**

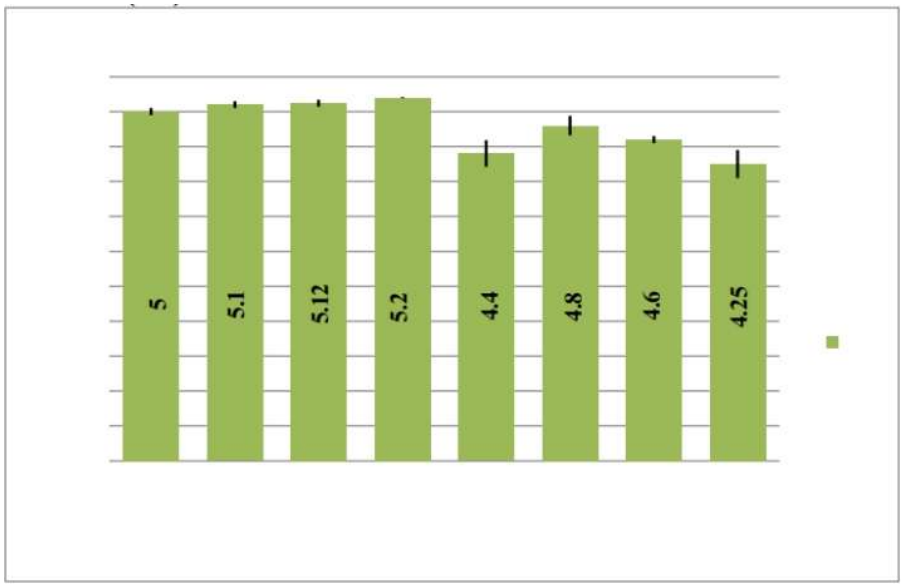


Figure 13. Representation of Surface pH of Epigallocatchin gallate loaded Mucoadhesive Vaginal FilmFolding Endurance

The folding endurance of the film was used to estimate the mechanical strength of the patch to with stand the folding or the ability to withstand the brittleness. It was measured by repeatedly folding a film at the same line before it breaks. The folding endurance was the number of times the film was folded without breaking. Higher the folding endurance value greater was the strength of the film.

Folding endurance of the Epigallocatchin gallate loaded mucoadhesive vaginal film depends on the polymer and plastisizer used This property of the film varies with the different ratio of the polymers HPMC and sodium alginate (1:0), (0:1), (1:2) and (2:1), and plastisizer Propylene glycol (0.2:1) and (0.4:1). Higher the rate of the polymers and plastisizer folding endurance of the film increases. Folding endurance of the formulation F8 is higher when compared to others.

Table 11. Folding Endurance of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	Folding Endurance*
F1	228 ± 4.74
F2	220 ± 8.22

F3	266 ± 2.74
F4	250 ± 3.59
F5	240 ± 1.64
F6	295 ± 1.451
F7	320 ± 1.09
F8	352 ± 2.39

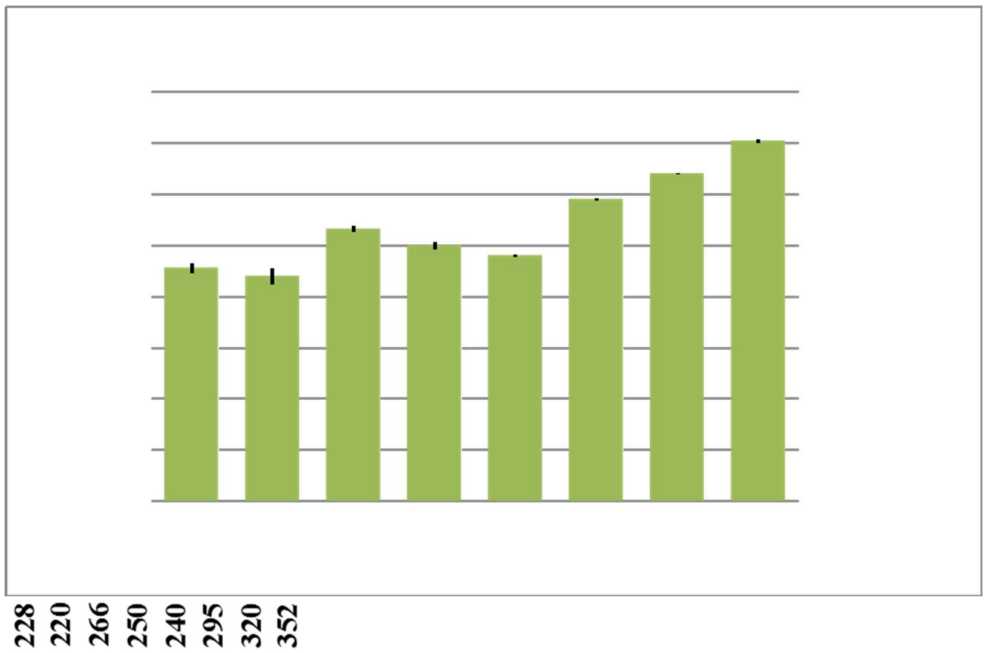


Figure 14. Representation of Folding Endurance of Epigallocatchin gallate loaded Mucoadhesive Vaginal Film

8.2.2. Swelling Index

The initial weight of the patch was determined and placed in the pre-weighed clean petri plate. The system was dipped in 15ml acetate buffer pH 4.6. The increase in the weight of the patch was noted by weighing the system at regular intervals.

Calculated by using the following formula

$$\%SI = \frac{[(W - Wo)]}{Wo} \times 100$$

Where,

%SI - Percentage swelling index

Wo - Initial weight of film

W - Final weight of film

Swelling property of the Epigallocatchin gallate loaded mucoadhesive vaginal film depends on the polymer used and quantity of polymer. The swelling index values of the film ranges from 18.50 ± 1.04 to 45.75 ± 0.79. The formulation F1 showed the lowest swelling index and formulation F8 showed the highest swelling index. Swelling increases with the increase in HPMC and sodium alginate concentration due to the presence of more hydroxyl group.

Table 12. Swelling index of the Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	Swelling Index (%)*
F1	18.50 ± 1.04
F2	25.74 ± 0.79
F3	32.11 ± 0.33
F4	42.20 ± 0.38
F5	25.36 ± 0.03
F6	27.69 ± 1.44
F7	34.21 ± 1.04
F8	45.75 ± 0.79

Mean ± S.D (n=3)

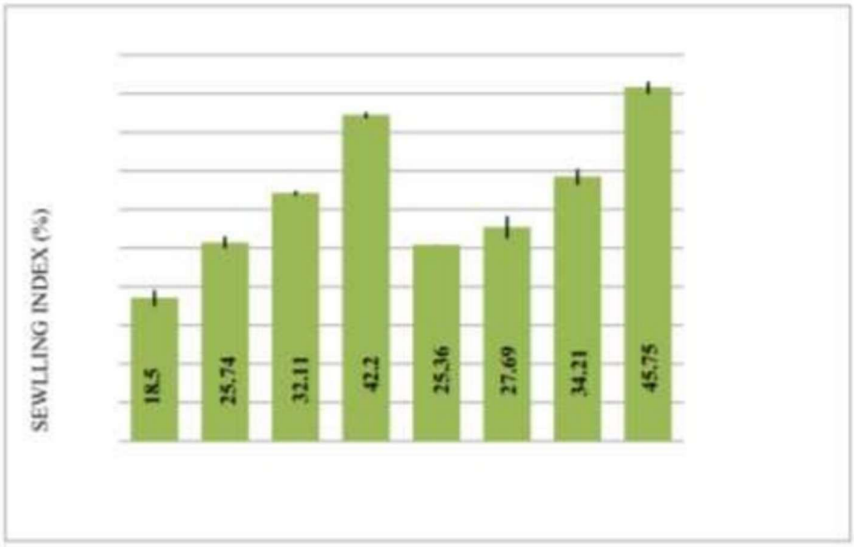


Figure 15. Representation of Swelling index of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Percentage Drug Content

The Epigallocatchin gallate loaded mucoadhesive vaginal film was added in 100ml of acetate buffer pH 4.6, stirred continuously in bench top orbital shaker and filtered through whatman filter paper. The samples were assayed at 347nm wavelength using a ultraviolet UV Visible spectrophotometer.

All the batches of the film contain 70.02±1.05 to 88.36±1.02 % of drug which indicate that there is no loss of drug during preparation of the Epigallocatchin gallate loaded mucoadhesive vaginal film. All the batches of the film exhibit drug content within limit, which is within the desirable range due to the equal distribution of drug in the solution. Formulation F8 had the higher percentage of drug content of 88.36±1.02 compared to others. The results indicate that the drug is distributed uniformly in all film formulations and will deliver the dose of drug accurately.

Table.13. Percentage Drug Content of the Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	**Drug Content (%)***
F1	71.02 ± 1.05
F2	72.68 ± 1.33
F3	73.63 ± 1.20

F4	79.09 ± 2.56
F5	71.49 ± 2.71
F6	73.39 ± 1.43
F7	81.23 ± 1.59
F8	88.36 ± 1.02

***Mean ± S.D (n=3)**

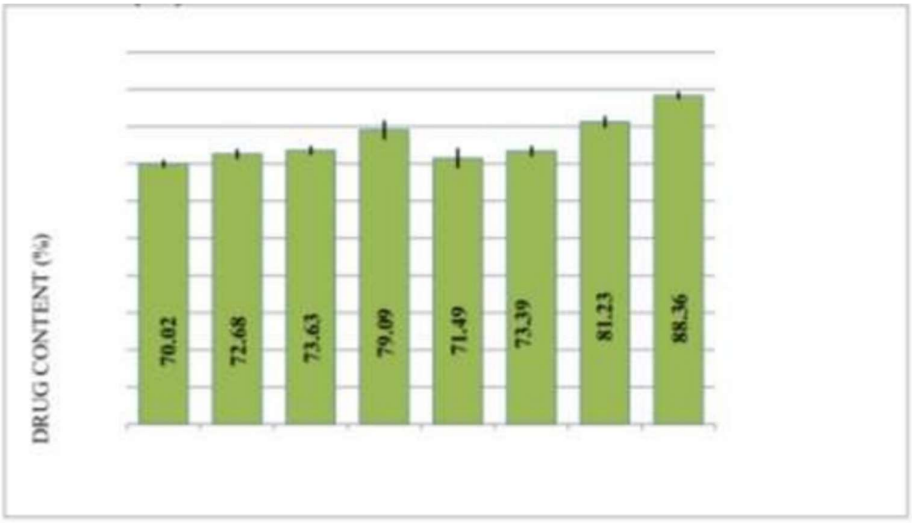


Figure 16. Representation of Percentage Drug Content of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Percentage Moisture Absorption

The percentage moisture absorption test was carried out to ensure physical stability or integrity of Epigallocatchin gallate loaded mucoadhesive vaginal film films. The film was weighed and kept in a desiccator containing saturated ammonium chloride solution at 40°C for 24 h. Films were removed from the desiccator and weighed until a constant weight was obtained.

The moisture absorption of Epigallocatchin gallate loaded mucoadhesive film was found to be in the range of 0.3933±0.24 to 3.0842±0.516. It was done to check the integrity of patch at hygroscopic condition. The moisture absorption for the formulation F7 and F8 is maximum compared to the other formulation.This ensure integrity of the film.

Table 14. Percentage Moisture Absorption of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	% Moisture absorption*
F1	0.3933 ± 0.24
F2	1.7477 ± 0.543
F3	1.8586 ± 0.353
F4	2.7878 ± 0.869
F5	0.7871 ± 0.38
F6	2.4067 ± 5244
F7	2.25988 ± 0.5
F8	3.0842 ± 0.516

***Mean ± S.D (n=3)**

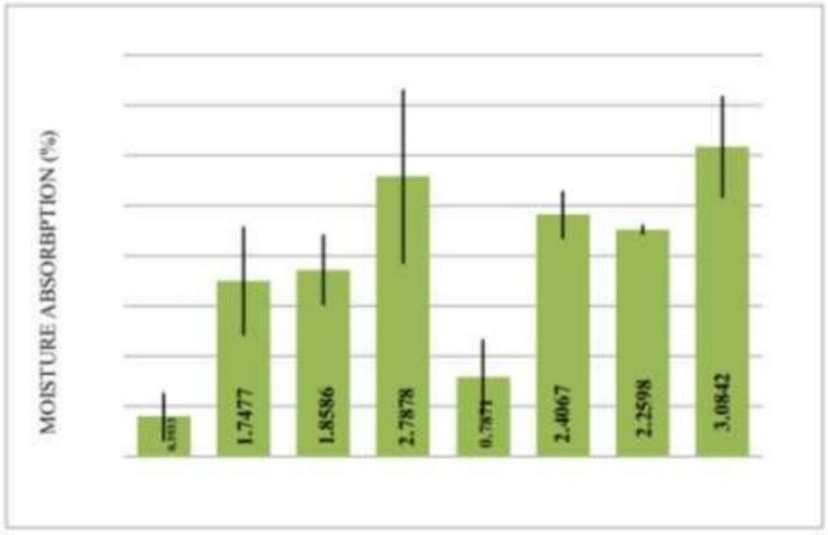


Figure 17. Representation of Percentage Moisture Absorption of Epigallocatchin gallate loaded Mucoadhesive Vaginal Film

8.2.3. Percent Moisture Loss

The percentage moisture loss test was carried out to ensure physical stability or integrity of films. It was done to check the integrity of patch at dry condition and hygroscopicity of patch. Three patch of 2 x 2 cm size were cut out and weighed accurately. Then the patch was rested in a desiccator containing anhydrous calcium chloride. After 24h the patches are removed, weighed and percentage weight loss were calculated.

The moisture loss of Epigallocatchin gallate loaded mucoadhesive film was found to be in the range of 0.6667±0.219 to 2.1509±0.339. It was done to check the integrity of patch in dry condition.The moisture loss for the formulation F7 and F8 is maximum compared to the other formulation.Table 19. Percentage Moisture Absorption of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	**% Moisture loss***
F1	0.6667 ± 0.219
F2	0.749 ± 0.356
F3	1.3749 ± 0.66
F4	2.3939 ± 0.491
F5	0.6587 ± 0.217
F6	1.3558 ± 0.023
F7	1.6969 ± 0.399
F8	2.1509 ± 0.339

Mean ± S.D (n=3)

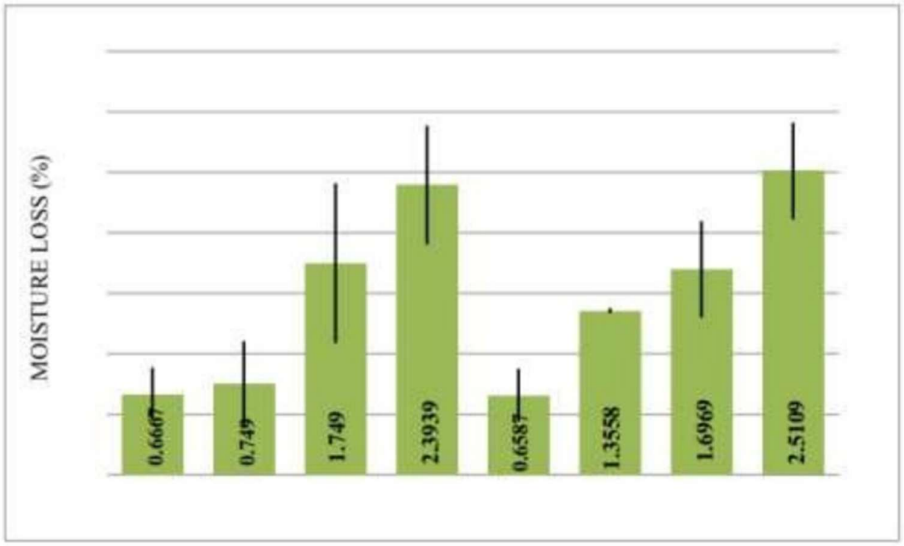


Figure 18. Representation of Percentage Moisture Loss of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Percentage Elongation

Percentage elongation of film was performed under the texture analyser. It indicates that the elastic nature of the film. This parameter indicates the physical and mechanical property of the Epigallocatchin gallate loaded mucoadhesive film.

Percentage elongation was calculated using the following equation:

The elongation of Epigallocatchin gallate loaded mucoadhesive film was found to be in the range of 23.3±2.45 to 81.54±1.02 revealing that the films had good mechanical strength and flexibility The percentage elongation for the formulatio F8 is maximum compared to the other formulation. It indicates that the formulation F8 has more mechanical strength due to presence of high amount of polymer HPMC and sodium alginate, and plastisizer PG.

Table 15. Percentage Elongation of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	Elongation (%)*
F1	23.3 ±2.45
F2	30.21 ± 2.21
F3	37.92± 1.981
F4	75.34 ± 1.65
F5	27.82 ± 2.02
F6	40.27 ± 2.53
F7	52.63± 1.30
F8	81.54 ± 1.02

***Mean ± S.D (n=3)**

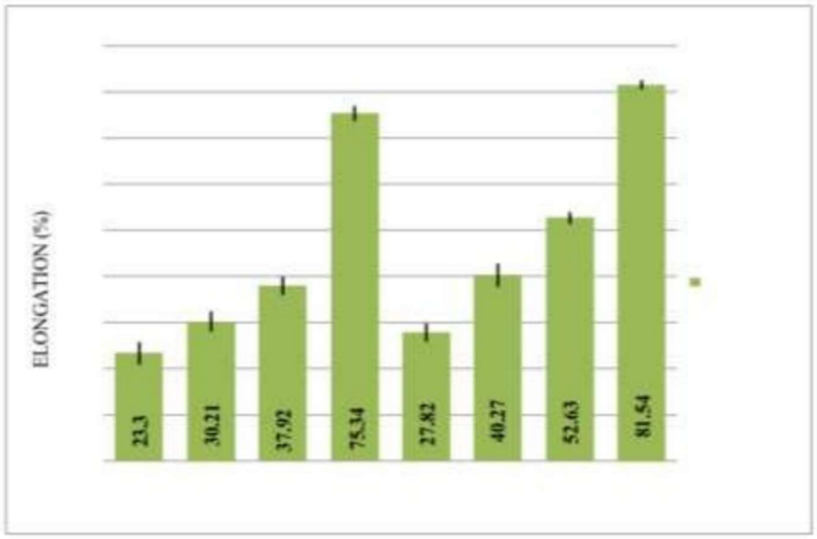


Figure 19. Representation of Percentage Elongation of Epigallocatchin gallate loaded Mucoadhesive Vaginal Film

8.2.4. Tensile Strength

The tensile strength parameter of film was performed under the texture analyser that indicates that the elastic nature of the film. This property denotes the mechanical property of the Epigallocatchin gallate loaded mucoadhesive film. The film was placed between the clamp of the stand & clip through which the weighing pan was attached above the ground level in the air. Measurement of tensile strength of the film the weights were added to the pan till the film breaks.

Calculated by the following equation

$$\text{Tensile strength} = \frac{\text{Force at breakage}}{\text{Film thickness (cm}^2\text{)} \times \text{Film width (cm}^2\text{)}}$$

Tensile strength of prepared Epigallocatchin gallate loaded mucoadhesive vaginal film varies from 1.3208± 2.45 to 7.8315±1.02 N/mm² revealing that the films had good mechanical strength and flexibility. Tensile strength of this film increases with the increase in the polymeric concentration. Formulation F1 showed the lowest tensile strength 1.3208±2.45 N/mm², whereas formulation F8 showed the highest tensile strength 7.8315±1.02 N/mm²

Table 16. Tensil strength of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	Tensile strength (N/cm²)*
F1	1.3208 ± 2.45
F2	1.8211 ± 2.98
F3	2.5917 ± 2.67
F4	4.3028 ± 2.56
F5	2.5918 ±2.71
F6	6.1620 ± 1.43
F7	7.1568 ± 1.59
F8	7.8315 ± 1.02

***Mean ± S.D (n=3)**

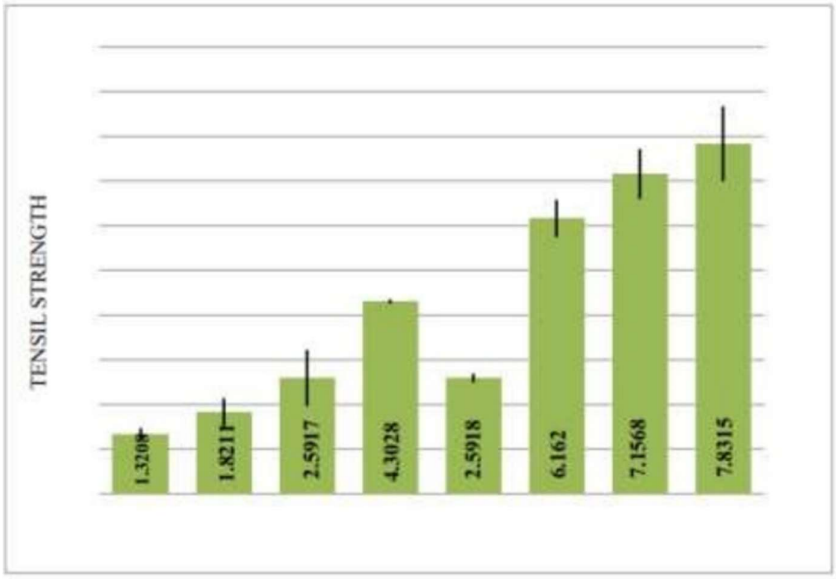


Figure 20. Represent Tensil strength of Epigallocatchin gallate loaded Mucoadhesive Vaginal Film

***Invitro* Disintegration**

The disintegration test was performed under the orbital shaker to read the disintegration of time of the film. A glass conical flask was filled with 15 mL acetate buffer of pH 4.6 and the film was carefully placed at the centre. The conical flask was left undisturbed in the orbital shaker and allowed to agitate the orbital shaker at 100rpm .The time taken for the film to completely disintegrate into fine particles is noted.

The disintegration of prepared Epigallocatchin gallate loaded mucoadhesive vaginal film varies from 12 ± 0.57 to 25±0.90 minutes revealing that the films had good physical property. Disintegration time of this film increases with the increase in the polymeric concentration. Formulation F1 showed lowest disintegration time 12±0.57 minutes, whereas formulation F8 showed the highest disintegration time 25±0.90 minutes.

Table 17. Disintegration time of Epigallocatchin gallate Loaded Mucoadhesive Vaginal Film

Formulation	Disintegration time*
F1	12 ± 0.57
F2	13 ± 0.54
F3	14 ± 0.18
F4	15 ± 0.74
F5	20 ± 0.64
F6	24 ± 0.33
F7	25 ± 0.75
F8	25 ± 0.90

Mean ± S.D (n=3)

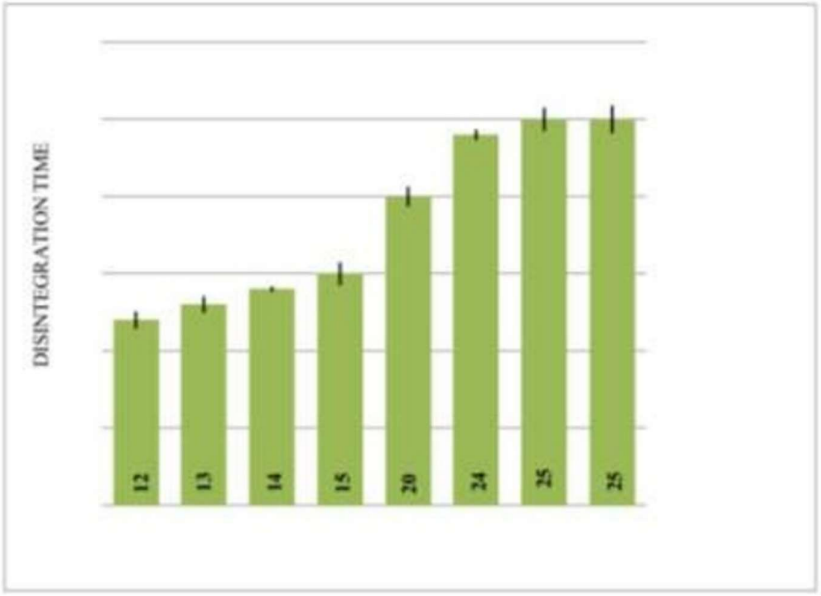


Figure 21. Represent Disintegration time of Epigallocatchin gallate loaded Mucoadhesive Vaginal Film

8.2.5. *Ex vivo* Diffusion study

The film of 2×2 cm^2 equivalent formulation of Epigallocatchin gallate loaded mucoadhesive vaginal film was kept in donor compartinent. The cylinder was attached to stand and suspended in 500ml of dissolution medium maintained at 37°C. The goat vaginal membrane just touching the receptor medium surfaces. The dissolution medium was stirred at 500 rpm speed using Teflon coated magnetic bead. Aliquots, each of 5ml volume were withdrawn periodically at predetermined time interval of 1, 2, 3, 4, 5 and 6 hours replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with the receptor medium and analysed by UV Visible spectrophotometer at 347nm using acetate buffer as blank and the percentage of drug permeation was calculated.

The cumulative percentage drug release (%CDR) of Epigallocatchin gallate loaded mucoadhesive vaginal film formulations (F1 to F8) for 6 hours were analysed by UV spectrometer at 347nm and results were calculated. The cumulative percentage of drug release of F1 to F8 formulation was quoted in above table. The drug release was found to be in the range of 68.5% to78.54% and gives good satisfactory results. A maximum percentage of 78.54% was obtained from the Epigallocatchin gallate loaded

mucoadhesive vaginal film during 6 hours study The formulated film was undergone with in-vivo studies.

Table 18. Cumulative Percentage of Drug Release (F1-F4)

S. No	Time (hrs)	F1	F2	F3	F4
1	0.5	11.17	12.15	12.8	14.1
2	1	20.29	22.24	23.22	24.52
3	1.5	28.43	34.95	35.92	37.88
4	2	34.62	45.05	46.02	47.33
5	3	38.53	55.14	56.12	57.42
6	4	50.91	62.31	63.61	64.59
7	5	56.77	69.31	70.13	72.41
8	6	68.5	71.43	75.99	80.88

Table 19. Representation of Cumulative Percentage of Drug Release (F5-F8)

S. No	Time (hrs)	F5	F6	F7	F8
1	0.5	11.82	12.47	14.75	16.38
2	1	20.94	22.57	25.75	29.08
3	1.5	32.51	33.64	38.86	36.58
4	2	39.83	41.14	41.46	42.11
5	3	48.3	48.95	48.95	52.86
6	4	55.79	57.42	57.1	66.52
7	5	63.61	69.48	74.36	75.01
8	6	70.78	74.04	81.85	86.74

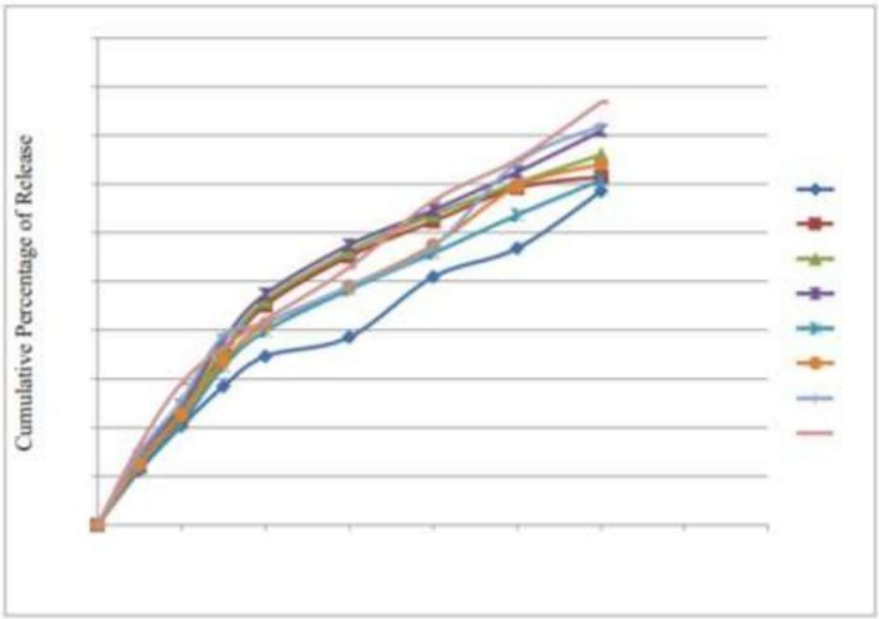


Figure 22. Cumulative Percentage of Drug Release (F1-F8)

8.2.6. Release kinetics

The diffusion data of formulation F8 were examined for zero order, first order, Higuchi and Korsemeyer-Peppas model.

The mucoadhesive film of formulation F8 profile exhibits an 86.74%

permeation at 6h on goat vaginal mucosa, the permeation profile was relatively steady and the amount consistently permeated with duration of time. The cumulative percentage of drug release of F8 shown in the table 24.

Table 20. Percentage drug diffusion of formulation (F8)

Time (hrs)	Cumulative Rercentage of Drug Release (F8) (%)
0.5	16.38
1	29.08
1.5	36.58
2	42.11
3	52.86
4	66.52
5	75.01
6	86.74

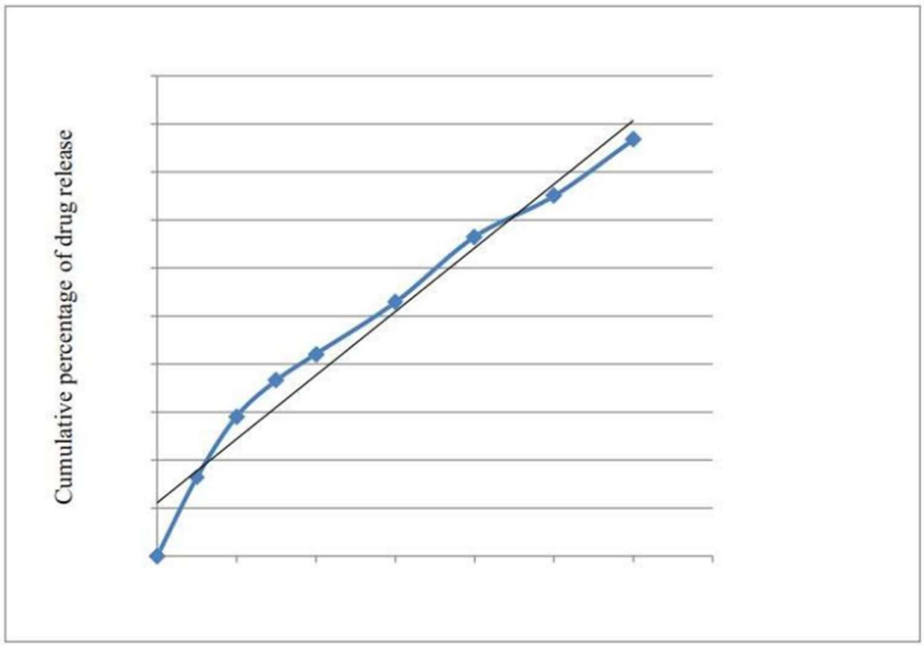
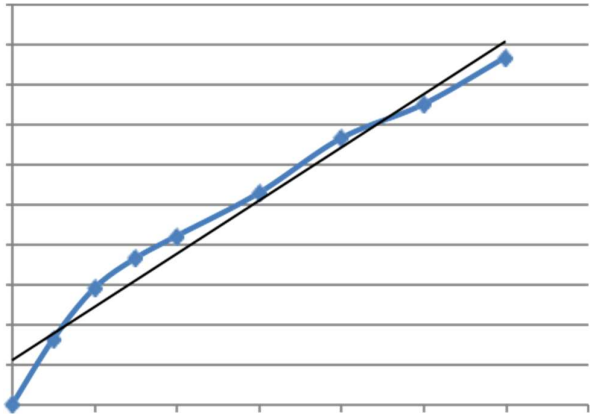


Figure 23. Representation of Cumulative Percentage of Drug Release of F8

Table 21. Release Kinetics of Formulation (F8) at 347nm

Time (hrs)	Square root of time	Log time	Absorbance	%CDR	Log %CDR	Log % CDR remaining
0.5	0.707	-0.30	0.083	16.38	1.21	1.93
1	1	0	0.122	29.08	1.46	1.87
1.5	1.224	0.17	0.145	36.58	1.56	1.78
2	1.414	0.3	0.162	42.11	1.62	1.76
3	1.732	0.477	0.195	52.86	1.72	1.7
4	2	0.6	0.237	66.52	1.82	1.63
5	2.23	0.69	0.263	75.01	1.87	1.4
6	2.44	0.77	0.299	86.74	1.93	1.25

Figure 24. Zero order plot of formulation F8 at 347nm



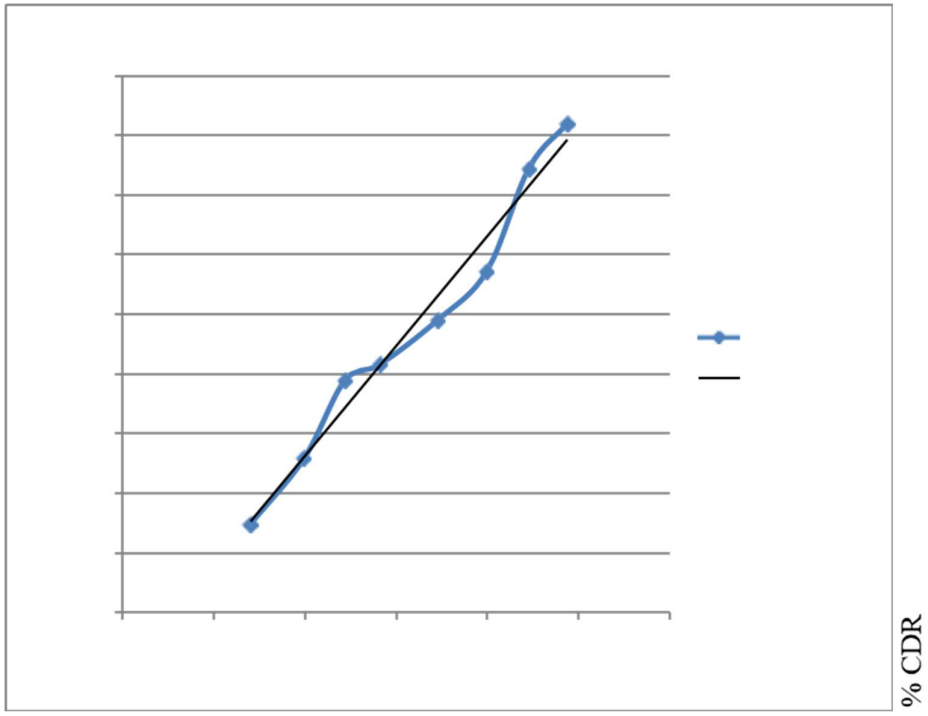


Figure 25. First order releasekinetics of formulation F8 at 347nm Figure 41. Korsmeyer-Peppas plot of formulation F8 at 347nm

The coefficient of determination (R^2) was taken as criteria for choosing the most appropriate model. The R^2 values of various models are given in Table 9.19.

Table 22. R² value of various kinetic models

Formulation	Zero order		First order		Higuchi		Korsmeyer Peppas	
	Slope	R²	Slope	R²	Slope	R²	Slope	R²
Formulation F8 Epigallocatchin gallate loaded mucoadhesive vaginal film	13.271	0.9638	0.1151	0.9554	36.929	0.976	0.1174	0.8492

The *Ex vivo* drug release showed highest regression value for the zero order kineties and release data was best fit with Higuchi model kinetics because the value of R^2 was greater in this model. The formulation follows the diffusion controlled mechanism for drug release. The value n = 0.1935 was found to be in range of $n \leq 0.45$ indicating that the drug release mechanism was diffusion and fickian release.

In-Vitro cell line study

The formulated Epigallocatchin gallate loaded mucoadhesive vaginal film was tested for percentage cell viability and in vitro cytotoxicity, using HeLa (Human Cervical Cancer Cells) cell lines by MTT assay. These cell lines are described as immortal, remarkably durable and prolific line of cells as they are derived from the cervical cancer cells.

Assay Description

The HeLa cells were treated with test sample (500 µM/ml of starting concentration then two- fold diluted up to eight times). After 24 hrs incubation, the cells were treated with MTT for 3 hours in CO2 incubator at 37° C. Later, the cell was then washed with PBS, and 100 µL. DMSO was added to determine the cytotoxicity at 570 nm.The results shown that treated group has 28% activity at 50µM/ml as compared to vehicle control (Acetate buffer).

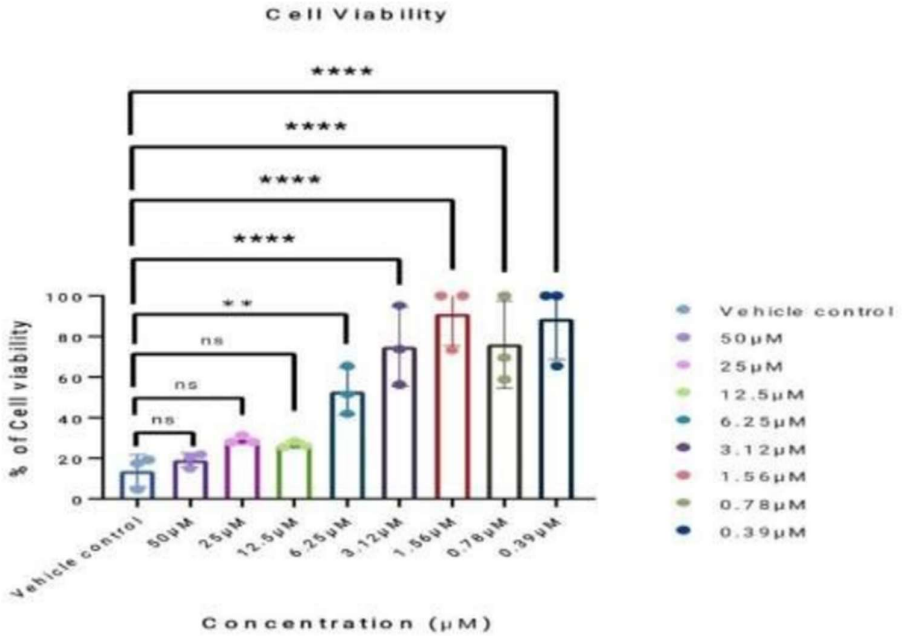


Figure 26. Diagrammatic Representation of Percentage of Cell Viability

Celll Cytotoxicity (%)

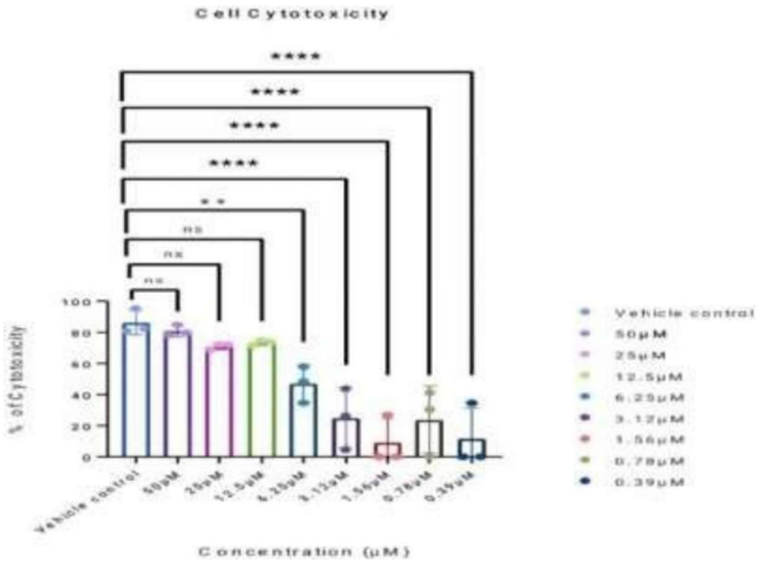


Figure 27. Diagrammatic Representation of Percentage of Cell Cytotoxicity

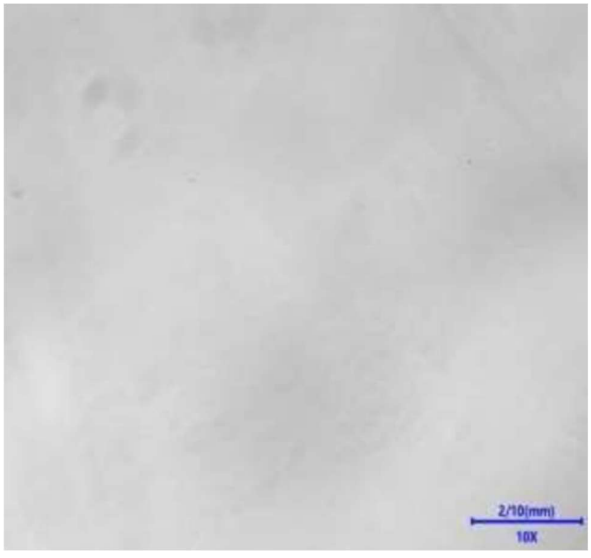


Figure 28. Microscopic Image of HeLa with Blank

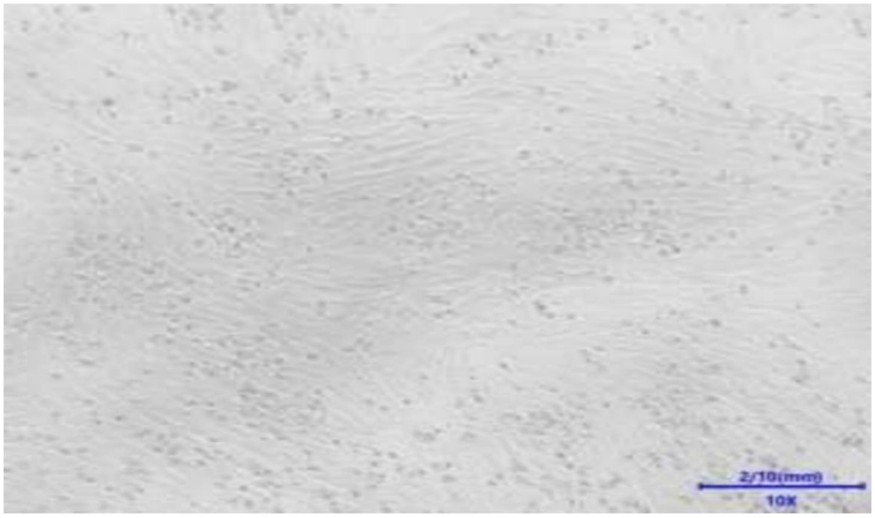


Figure 29. Microscopic Image of HeLa with Vehicle control



Figure 30. Microscopic Image of HeLa with Positive control

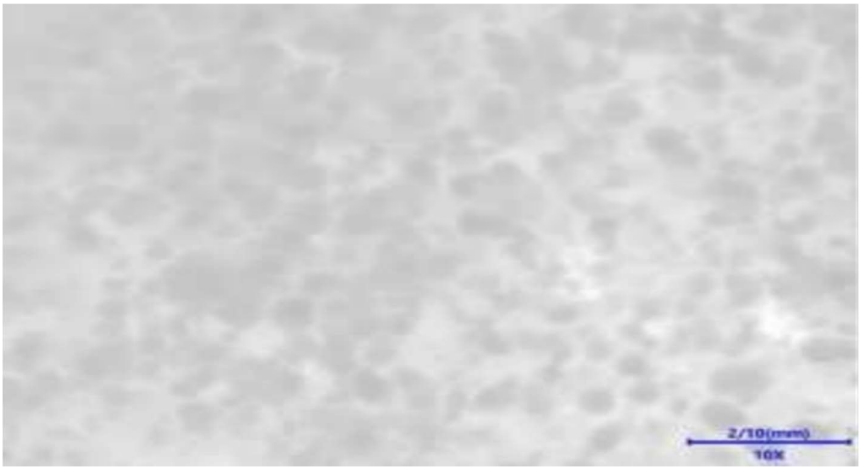


Figure 31. Microscopic Image of HeLa with Negative control

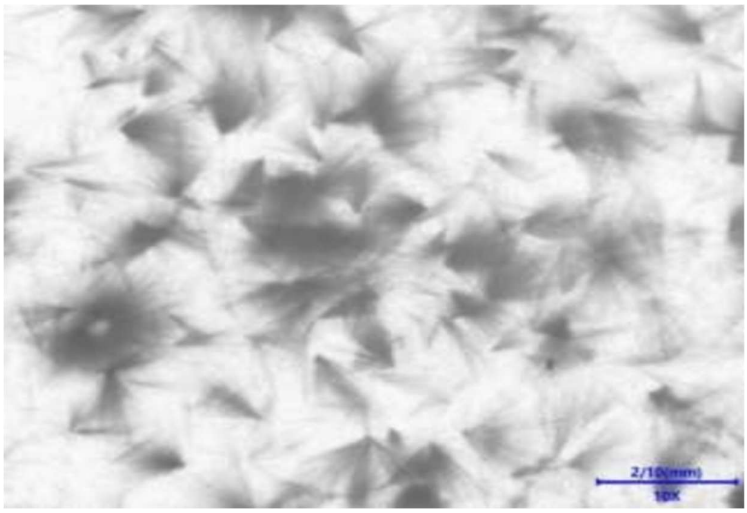


Figure 32. Microscopic Image of HeLa with 0.39μM/ml

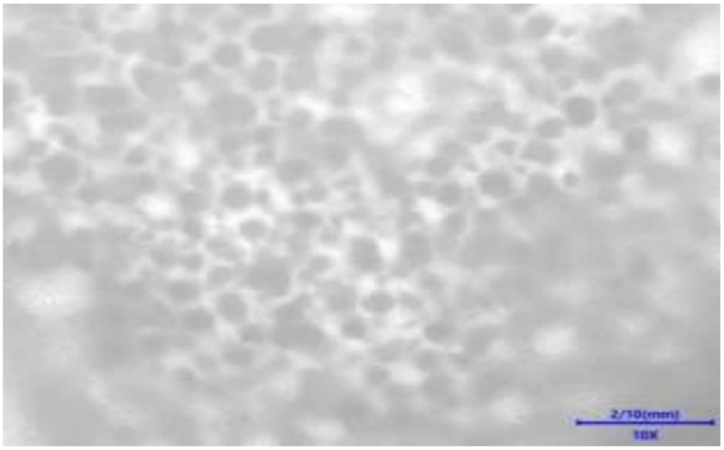


Figure 33. Microscopic Image of HeLa with 0.78μM/ml

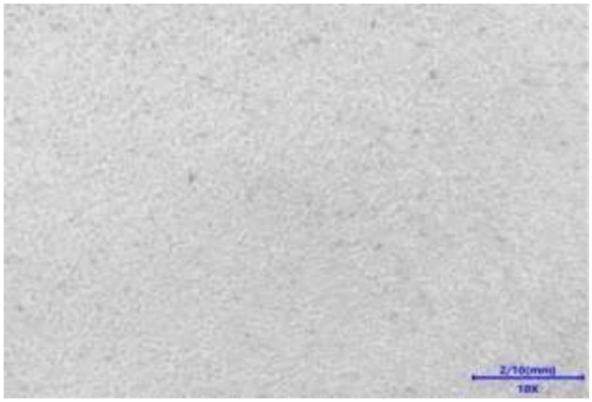


Figure 34. Microscopic Image of HeLa with 1.56μM/ml

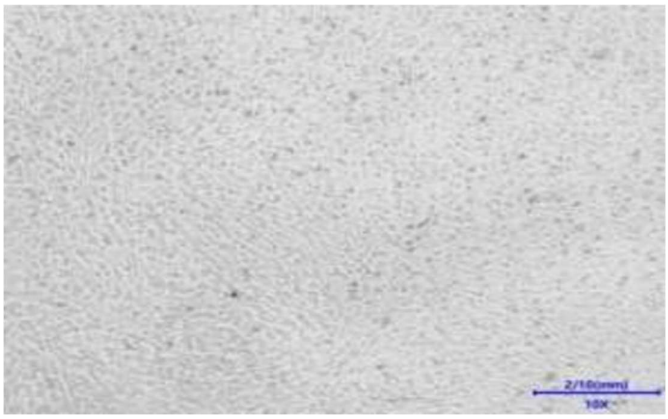


Figure 35. Microscopic Image of HeLa with 3.12µM/ml

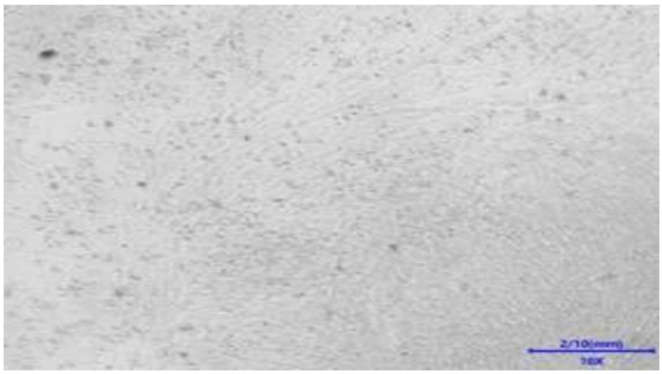


Figure 36. Microscopic Image of HeLa with 6.25µM/ml

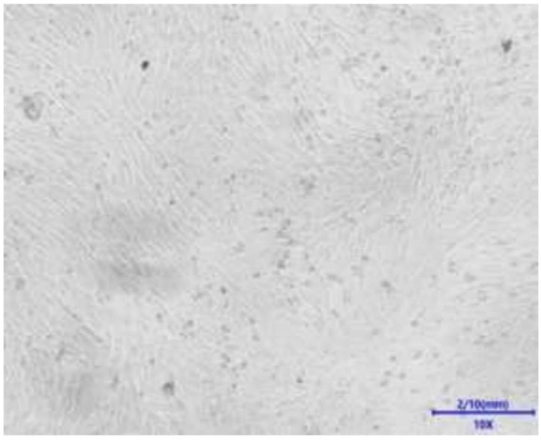


Figure 37. Microscopic Image of HeLa with 12.5µM/ml

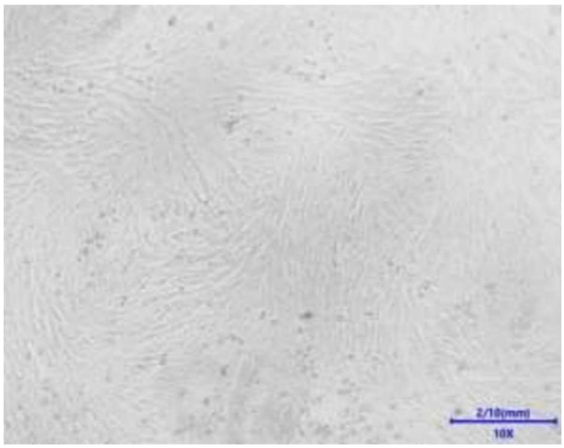


Figure 38. Microscopic Image of HeLa with 25µM/ml

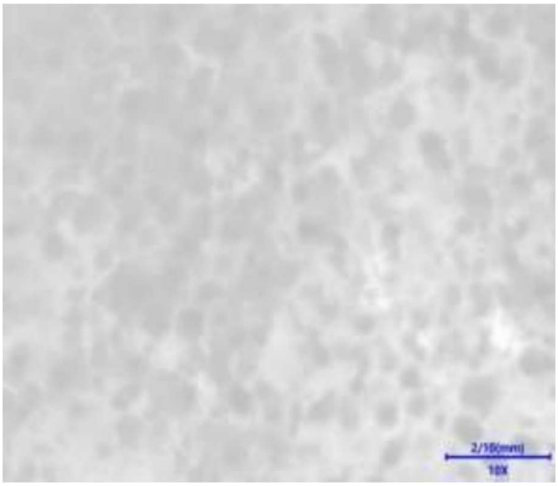


Figure 39. Microscopic of Image of HeLa with 50µM/ml

Stability studies

The studies revealed that there is slight reduction in the drug content and % transmittance after storage for 6months. Results were recorded in table 6.9. From the data it was indicated that the optimized Epigallocatchin gallate loaded can be stable up to 6months.

Table: 23 Effect of temperature on stability of the optimized Microemulsion formulation (n=3, mean± SD)

Temperature (°c)	Phase separation		% Transmittance		% of Assay	
	After 4months	After 6months	After 4months	After 6months	After 4months	After 6months
2-8 °c	No	No	94.08 ±0.8	92.87 ±1.3	95.23 ±0.2	92.30 ±0.1
Room temperature	No	No	94.72 ±1.2	93.48 ±1.5	92.23 ±0.4	94.60 ±1.1
Elevated temperature	No	No	93.22 ±1.2	92.80 ±0.6	93.00 ±0.7	92.28 ±0.3

SUMMARY AND CONCLUSION

Epigallocatchin gallate loaded mucoadhesive vaginal films represent an innovative approach to cervical cancer. The phytoconstitutent, Epigallocatchin gallate was successfully loaded within bioadhesive film for the effective treatment of cervical cancer.

In this work an attempt was made to formulate and evaluate bioadhesive vaginal film containing Epigallocatchin gallate for controlled release by Solvent casting method using HPMC and sodium alginate as polymers and propylene glycol as plastisizer.

The FTIR spectroscopic studies were carried out to study the compatibility between the drug and excipients. The results showed that there was no interaction between the drug and the selected excipients. An increase in polymer concentration causes controlled release drug delivery at the concentration of 0.4:1 (plastisizer:polymer).

The mucoadhesive vaginal film was tested for weight variation, thickness, folding endurance, surface ph , swelling index, percentage drug content, moisture absorbed, moisture loss, *in- vitro* disintegration *ex-vivo* diffusion and cell line studies.
The prepared formulations showed pH range of 4.25 to 5.2 which confirms the compatibility of the formulations with vaginal secretions.

Standard graph was drawn for pure Epigallocatchin gallate and it was found that they showed linearity at the concentration of 10 to 60µg/ml and obeyed Beer Lambert's law.

Among the prepared formulations (F1 to F8) F8 showed maximum drug content i.e. 88.36% and *ex-vivo* diffusion of F8 showes 86.74% of drug release which is used for further evaluation studies.

Cell line study was performed for the caticin loaded mucoadhesive vaginal film formulation F8 to analyse the anticancer activity against cervical cancer conducted in the HeLa cell line showed that the treated group has 28% activity at 50 µM/ml as compared to the vehicle control. From the *Ex-vivo* release kinetics, the pharmacokinetic model reveals that the mechanism of drug release from mucoadhesive film formulation followed first order kinetics with R^2 value of 0.9554 and release mechanism was diffusion controlled as per the calculated „n" value value 0.1935 from Korsmeyer peppas plot.

The results reveals that the mechanism of drug permeation follows diffusion from higher R^2 0.976 value for Higuchi kinetics.*Ex-vivo* release kineics showed that the pure phytoconstitutent of Epigallocatchin gallate exhibits a significant anticancer against cervical cancer. The study implies that the extracts could be used as natural therapeutic drugs to prevent complications related to cervical cancer.The above study concluded that the possibility of the making of mucoadhesive drug delivery system for Epigallocatchin gallate which will be more efficacious and acceptabale than the Conventional drug delivery of and also having satisfactory sustained release profile which provide an increased therapeutic efficacy.

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