Preparation and Evaluation of Tableted Microcapsules of Acyclovir for Oral Controlled Release

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Abstract

The most popular medication for infections like cutaneous herpes, genital herpes, chicken pox, and varicella zoster is acyclovir [9-(2-hydroxyethoxymethyl)guanine], a synthetic purine nucleoside analogue derived from guanine. It is thought to be the first agent to be licenced for the treatment of herpes simplex virus (HSV-1, HSV-2) infections. The bioavailability of acyclovir is only 20%. Its elimination half-life is 2-3 hours, and it proceeds through hepatic metabolism. Hence, creating a prolonged release mucoadhesive formulation is one method by which this might be overcome. By employing the polymers Sodium Alginate, Sod CMC, HPMC K4M, and Carbopol 940, the mucoadhesive microcapsules of Acyclovir (F1-F16) were successfully synthesized by the Emulsion Solvent Evaporation method. Studies using FTIR did not find any notable medication interactions. Scan-electron microscopy showed that the produced microcapsules had acceptable spherical geometry and a smooth surface. Acyclovir mucoadhesive microcapsules were discovered to have an average particle size between 289 and 399 micrometres. In terms of percentage yield (97.6%), entrapment efficiency (87.50.32%), mucoadhesion test (703.32%), and swelling index (74.62.24%), formulation F16 was chosen as the best formulation. After 12 hours, the in-vitro drug release (F16) was discovered to be (98.120.24%). The Higuchi mechanism and Zero order kinetics were used in the formulation F16. The improved formulation F16 was kept at high temperatures, such as 250°C and 400°C, respectively, for three months. Acyclovir mucoadhesive microspheres were made into tablets using the improved formulation F16. Acyclovir mucoadhesive microcapsules and optimized acyclovir mucoadhesive microcapsules were compared in dissolving trials, and after 12 hours, the percentage of drug release was found to be 98.120.24% and 91.70.54%, respectively. The formulation F1 acyclovir microcapsules tableted was determined to be the best formulation among all formulations and can be used in the treatment of herpes simplex virus (HSV-1, HSV-2) infections based on all the aforementioned evaluation criteria.

Keywords: Emulsion Solvent evaporation, Drug entrapment efficiency, Swelling index, *In-vitro* drug release

INTRODUCTION:

In order to localize medications to a specific target region of the gastrointestinal tract (GIT) over an extended period of time, mucoadhesive microcapsules that become adhesive upon hydration are utilized. Moreover, it is simple to administer, does not require patient compliance, and has flexible composition. Controlling the gastro retentive drug delivery system, which will offer significant treatment possibilities, is one of the most practical ways to achieve a prolonged and predictable drug administration in the gastrointestinal tract (GIT). The capacity of Mucoadhesive Microcapsules to stick to the mucosal surface and release the encapsulated medicine in a continuous release makes them appealing [1]. Mucoadhesion is connected to the mucin layer of a mucosal tissue, while the bioadhesion phenomena is connected to

biological surfaces. Mucoadhesive Microcapsules have benefits such as effective absorption, increased bioavailability of the pharmaceuticals, maximum usage of the drugs, and much more close interaction with intestinal cells. They also have higher patient compliance and are targeted to specific absorption sites[2].

The most popular medication for infections like cutaneous herpes, genital herpes, chicken pox, and varicella zoster is acyclovir [9-(2-hydroxyethoxymethyl) guanine] (ACV), a synthetic purine nucleoside analogue derived from guanine3,4. It is thought to be the first agent to be licensed for the treatment of herpes simplex virus (HSV-1, HSV-2) infections and is used to treat 5 ACV is classified as a BCS Class III medicine, meaning that it is soluble and has low intestinal permeability, and that it must be delivered orally or intravenously in large quantities to have the desired therapeutic effect. ACV is mostly absorbed from the upper gastrointestinal tract, with a maximum solubility of 2.5 mg/ml at pH 7.0, and is virtually entirely unionised (GIT). The bioavailability of acyclovir is only 20%. Its elimination half-life is 2-3 hours, and it proceeds through hepatic metabolism. In order to increase its oral bioavailability and sustained drug release, it is chosen as a suitable medication for the construction of mucoadhesive Microcapsules.

MATERIALS AND METHODS:

The Alpha drug laboratory in Indore provided the acyclovir sample as a gift. We bought sodium alginate from Finar Chemicals Limited in Ahmadabad. The supplier of carbopol 934P was S.D. Fine chem. Ltd. in Mumbai. From Mumbai's Yarrow Chemicals Ltd., HPMCK100M was bought. The rest of the reagents were all of analytical grade.

Table No: 01, Formulation of Mucoadhesive Microcapsules of Acyclovir

Ingredients	Formulation code															
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16
Acyclovir (mg)	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
Sodium Alginate (mg)	200	400	600	800												
Sodium Carboxy Methyl Cellulose (NACMC) (mg)	--	--	--	--	200	400	600	800	200	--	--	--	--	--	--	--
Hydroxy Propyl Methyl Cellulose (HPMC K4M) (mg)	--	--	--	--	--	--	--	--	200	400	600	800	--	--	--	--
Carbopol 940 (mg)	--	--	--	--	--	--	--	--	--	--	--	--	200	400	600	800
Ethanol (ml)	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Liquid paraffin (ml)	49	49	49	49	49	49	49	49	49	49	49	49	49	49	49	49
Span-80 (ml)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Formulation of Mucoadhesive Microcapsules of Acyclovir

Trial and Error Method:

(Preliminary experiments) Previously, numerous tests were conducted using various polymers and the emulsion solvent evaporation process to create mucoadhesive Microcapsules of Acyclovir. Experiments were conducted by adjusting the temperature, stirring rates, polymer concentration, and Span-80 concentration. Following so many tests, it was determined that temperature plays a crucial role in the continual stirring process that results in the creation of microcapsules. The procedure was optimised at every stage by conducting tests using the trial-and-error method.

Preparation of Mucoadhesive Microcapsules of Acyclovir:

Sodium alginate, Sod.CMC, HPMC K4M, and Carbopol 940 are the four polymers used in the Emulsion Solvent Evaporation process to create the acyclovir mucoadhesive microcapsules. The steps for creating microcapsules are listed below. In the first step of this procedure, 50ml of ethanol and the precisely weighed polymer are combined and continuously stirred at 500–600 rpm to homogenize the mixture. The drug was added to this precisely measured amount, and stirring was continued until an uniform dispersion was achieved. Separately, a mechanical stirrer was used to homogenize 50ml of liquid paraffin containing 1ml of Span80. Over the course of 2-3 minutes, a thin stream of the previously created polymer-drug dispersion (aqueous phase) was gradually added to the liquid paraffin. This emulsion was heated to 80 °C for 3–4 hours while being agitated at 2000 rpm. After that, the microspheres were separated by filtration, the extra paraffin oil was removed by repeatedly washing with n-hexane (three times), and finally the products were dried overnight at room temperature.

Evaluation and Characterization of Mucoadhesive Microcapsules of Acyclovir

Particle Size Determination:[6,7]

A stage micrometre was used in optical microscopy to measure the average particle size of the mucoadhesive Acyclovir microcapsules. A small quantity of Microcapsules were spread out on a glass slide and suspended in liquid paraffin for microscopic inspection. The glass slide was then set on the mechanical microscope stage. Each batch's size of 100 Microcapsules was evaluated in order to estimate the average particle size.

Percentage Yield:[7]

The total amount of mucoadhesive Microcapsules obtained were weighed and evaluated for percentage yield.

$$\text{Percentage yield} = \text{Actual yield} / \text{Theoretical yield} \times 100$$

Drug Entrapment Efficiency:[8]

A mortar and pestle was used to grind the mucoadhesive Microcapsules into powder. Microcapsules that were precisely weighed to be 20 mg of drug equivalent were suspended in 30 ml of pH 1.2 HCL buffer and sonicated for 30 minutes. The resultant mixture was filtered before being diluted with pH 1.2 HCL buffer to make 100 ml. After an appropriate dilution, the solution was filtered, and the amount of acyclovir in the filtrate was determined at 254 nm using a UV-Visible spectrophotometer. To determine the precise concentration of the medication entrapped, the measured absorbance was plotted on a standard curve. We may calculate the percentage of actual medicine encapsulated in Microcapsules by multiplying this concentration by the dilution factor and volume. The effectiveness of drug entrapment was calculated using the following relationship.

$$\%\ \text{Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Loose Surface Crystal Study (LSC):[8]

This research was done to determine how much medication was on the Microcapsules' surface. In 20ml of pH 1.2 HCL buffer, 20mg of mucoadhesive Microcapsules were suspended. In a mechanical shaker, the samples were agitated ferociously for 15 minutes. At 254 nm, the amount of medication that had leached out of the surface was measured spectrophotometrically. The percentage of drug release compared to drug that was entrapped in the sample was noted.

Swelling Index (SI):[9,10]

Mass spectrometry was used to analyze the dynamic swelling behaviors of Acyclovir mucoadhesive Microcapsules. The Microcapsules were incubated in petri plates with 25 ml of pH 1.2 HCL buffer at 37 °C. At various times, the Microcapsules were removed and the surplus surface liquid was gently blotted off without applying pressure. The electronic microbalance was used to weigh the swollen Microcapsules (Model BL-220H, Shimadzu, Japan). The studies were performed in triplicate and average values were taken in data analysis.

$$SI = \frac{\text{Weight of wet Microcapsules} - \text{Weight of dry Microcapsules}}{\text{Weight of dry Microcapsules}} \times 100$$

Mucoadhesive Testing by *In vitro* Wash-Off Test:[11,12]

The Acyclovir mucoadhesive Microcapsules' mucoadhesive ability was assessed using an in vitro adhesion testing technique called the wash-off method. Using poly cyanoacrylate glue, freshly removed sheep stomach mucosa samples (4 x 5 cm) were mounted onto glass slides (3 x 1 inch). Two glass slides were joined together with an appropriate sample of each wet, rinsed tissue, and then the supports were instantly attached onto the arm of a USP pill dissolving test device. When the disintegrating test machine was running, the tissue sample was slowly and consistently moved up and down in the test fluid (900 ml) at 37 °C enclosed in the machine's 1000 ml tank. The machine was stopped after 1 hour and at hourly intervals up to 12 hours, and the number of Microcapsules remained stuck to the tissue was counted.

The test was performed in stomach (pH 1.2). Mucoadhesion was calculated using formula:

% Mucoadhesion = Number of Microcapsules applied/ Number of Microcapsules adhered x100

Scanning Electron Microscopy (SEM):[13]

Scanning electron microscopy was used to observe the mucoadhesive Microcapsules. The Microcapsules were fixed directly onto the SEM sample stub using double-sided adhesive tape and covered with gold film with an attached vacuum system, ion splitter, and gold target with resolutions of 3nm, 10nm, and 40nm.

***In vitro* Drug Release Studies:**[14,15]

In vitro drug release research was done using a USP dissolving test device. 900ml of phosphate buffer, pH 1.2, was placed in a 900ml dissolution flask along with an amount of mucoadhesive microcapsules equivalent to 200mg of acyclovir microcapsules. The temperature was held constant at 370.50C throughout the trial. Using a syringe with a prefilter, 5 ml of samples were taken out at regular intervals for 12 hours and then put back into a dissolution flask containing buffer. After the necessary dilution with fresh medium, the sample's absorbance was measured at 254 nm (pH 1.2). There were three duplicates of each study.

Kinetics of *In vitro* Drug Release:[16]

Drug dissolution from solid dosage forms was represented by a kinetic model, where the amount of drug dissolved is a function of test time. Kinetic models were used to examine the precise mechanism of Acyclovir release from the microsphere in more detail. Zero order, first order, Higuchi, Korsmeyer

Peppa's, and Hixon Crowell models were used to assess the drug release data. The goodness of fit test was used to determine the criteria for choosing the best model.

Accelerated Stability Studies:[17]

The goal of a stability study is to provide proof of how a drug substance's or product's quality changes over time under the effect of various environmental conditions, including temperature, humidity, and light. The improved formulation, F16, was put through accelerated stability tests in accordance with ICH norms at 250°C and 400°C and 75% relative humidity for three months in airtight high density ethylene bottles. Samples were removed from the subjects at 0, 30, and 90 days. The various physicochemical parameters, including drug entrapment effectiveness, swelling index, and invitro drug release, were assessed for mucoadhesive microcapsules.

Preparation of Acyclovir Tableted microcapsules

Direct compression method:

The materials, including the microcrystalline cellulose (MCC) and acyclovir microcapsules, were all precisely weighed.

The materials were then combined in increasing weight order, with the exception of the lubricants, and blended for 10 minutes.

The lubricant was added after the ingredients had been thoroughly combined, and the mixture was then stirred once more for two minutes.

The resulting mixture of each formulation was then crushed using an 8 stage rotating tablet compress machine and an 8mm flat faced punch (Rimeck Mini Press KannavatiEng.L

Table 2: Composition of formulation. (All values are given in mg/tablet**)**

Ingredients (mg)	**FORMULATION CODE**
	F1
Acyclovir Microcapsules	200

Micro crystalline cellulose	290
Talc	5
Magnesium state	5
Total weight (mg)	500

Post compression parameters of Acyclovir Mucoadhesive Microcapsules tablets[18] :

Hardness and thickness:

The hardness of the pill was assessed using the Mansanto hardness tester. Between a fixed and movable jaw, the tablet was being held. The load was steadily increased until the tablet shattered when the scale was set to zero. The amount of force there provides a measurement of the tablet's hardness.

Hardness was expressed in Kg/cm^2.

Friability test: After dusting 10 tablets, their weight was measured after they were placed in the friabilator and rotated vertically at 25 RPM for 4 minutes. The remaining weight of the tablets after dusting was measured, and the percentage of friability was computed (% loss in weight).

$$F = \frac{(W_{initial}) - (W_{final})}{(W_{intial})} \times 100$$

W - initial weight of the tablet

Wt – weight of tablet dedusting

Weight variation:

To make sure a tablet has the right amount of medication in it, the weight of the tablet being created is frequently measured. For the USP weight variation test, 20 tablets were individually weighed, the average weight was determined, and the individual weights were then compared to the average. If no more than two tablets fall outside the acceptable percentage limits and no tablets differ by more than twice the acceptable percentage limit, the tablet passes the USP test. The following table shows the USP's official tablet percentage deviation limitations.

Table 03: Weight Variation Limits:

Weight variation	**Maximum % Deviation allowed**
130 or less	±10
130-324	±7.5
More than 324	±5

$$PD = \frac{(W_{avg}) - (W_{initial})}{(W_{avg})} X\,100$$

Where,
PD= Percentage deviation,
W_{avg}= Average weight of tablet,
$W_{initial}$ = individual weight of tablet.

Swelling Index [19]:

Each Acyclovir Mucoadhesive tablet was weighed (w1) individually before being set aside in petri dishes with 5ml of pH 1.2 phosphate buffer. When the internal time of 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, and 10 hours came, the tablet was taken out of the petri dish, and any extra surface water was carefully wiped away with filter paper. The tablets were reweighed (w2) after swelling, and the swelling index (SI) was calculated.

The swelling index is calculated by the formula:

$$\text{Swelling Index} = \frac{W2 - W1}{W1} \times 100$$

***In vitro* drug release study:**

Buccal tablets that had been manufactured were put through in vitro disintegration. The USP type paddle method was used for the dissolution test [apparatus 2]. Phosphate buffer with a pH of 1.2 was utilised as the dissolution media, which was kept at 37°C with 50RPM of stirring. At regular intervals of 12 hours, 5 ml samples were taken, filtered, and replaced with 5 ml of fresh dissolving medium. Dilutions were made as needed, and the samples were then tested for the presence of acyclovir at 254 nm using a UV-visible spectrophotometer.

RESULTS AND DISCUSSION

Percentage Yield:

When the yields of the various formulations, F1 through F16, were computed, they ranged from 87.5% to 97.6%, respectively. This increased yield % shows how effective the Emulsion Solvent Evaporation process was in creating Acyclovir mucoadhesive Microcapsules. The percentage yield for the formulation F16 was greater at 97.6%. The results were tabulated in the Table No: 02

Particle Size Analysis:

By using an optical microscope equipped with an ocular micrometre and stage micrometre, the particle size distribution of mucoadhesive Acyclovir microcapsules was measured. The size distribution is uniform across all mucoadhesive Microcapsules F1–F16 formulations. Acyclovir mucoadhesive microcapsules were discovered to have an average particle size between 289 and 399 micrometres.

Drug Entrapment Efficiency:

The range of the entrapment efficiency was discovered to be between 68.4 and 87.5 and 0.32 percent. The percentage of entrapment was found to be higher for the F4 formulation, 83.72.17%, 85.72.22%,

863.12%, and 87.50.32%. Using drug and carbopol 940 in a 1:4 ratio, the formulation F16 was determined to have the highest percentage of drug entrapment efficiency, or 87.5%. This improved entrapment efficiency is due to the greater proportion of polymers with respect to the amount of drug. The results are shown in Table No: 02.

Loose Surface Crystallography: (LSC)

For all of the drug-loaded formulations F1–F16, loose surface crystallography experiments were carried out. With a rise in polymer concentration, the mucoadhesive Microcapsules' surface-associated medication content dropped. From F1 to F4, F5 to F8, F9 to F12, and F13 to F16, the concentration of polymer demonstrated an increase in entrapment efficiency and, consequently, a decrease in surface drug contents. However, due to less effective entrapment, the surface associated drug content was higher in formulations F1 (31.60.22%), F50.17%, F90.15%, and F1323.40.32% with low polymer concentrations. Hence, the results were shown in Table No: 02

Table no: 2. Evaluation parameters of Mucoadhesive Microcapsules of Acyclovir Formulations F1-F16

Formulation Code	Percentage Yield (%)	Drug Entrapment Efficiency (%)	LSC (%)
F1	89.0	68.4±1.33	31.6±0.22
F2	93.3	75.8±1.91	24.2±0.36
F3	95.2	76.8±2.4	23.2±0.18
F4	96.2	83.7±2.17	16.3±0.10
F5	87.5	71.3±0.21	28.7±0.17
F6	94.3	72.6±0.17	27.4±0.21
F7	96.2	82±3.32	18±0.45
F8	97.1	85.7±2.22	14.3±0.32
F9	90.0	70.7±1.03	29.3±0.15
F10	95.0	76.2±2.5	23.8±0.16
F11	96.1	79±1.24	21±0.26
F12	97.3	86±3.12	13.5±0.18
F13	94.5	76.6±0.28	23.4±0.32
F14	95.6	80.3±1.38	19.7±0.16
F15	96.7	85.3±0.17	14.7±0.34
F16	97.6	87.5±0.32	12±0.24

All values are represented as mean ± standard deviation (n=3)

Swelling Index: (SI)

The concentration of polymers mostly affected swelling property. The ability for swelling increased as polymer concentration rose. In a pH 1.2 HCL buffer, the swelling index for each of the formulations, F1 through F16, was calculated. After 12 hours in a pH 1.2 HCL buffer, the swelling index increased from F1 (40.21.30%) to F4 (60.21.46%), F5 (44.81.04%) to F8 (643.98%), F9 (50.28.54%) to F12

(68.24.60%), F13 (436.1%) to F16 (74.62.24%). The rise in polymer concentration is what causes the swelling index to increase. By employing the medication and carbopol 940 in a 1:4 ratio, the improved formulation, F16, was discovered to have a high swelling index, or 74.672.24%, at the conclusion of 24 hours. The results are shown in Table No: 03

***In-vitro* Mucoadhesion Test:**

The outcome of the in vitro Mucoadhesion test after 12 hours is displayed in Table No. 03. With an increase in mucoadhesive polymer concentration, the percent mucoadhesion rose. Mucoadhesion percentages increased in formulations F4 (67.92%), F8, F12 (63.07%), and F16 (70.32%). By utilising the medication with carbopol 940 in a 1:4 ratio, the improved formulation F16 was found to have higher mucoadhesion, or 703.32% at the end of 12 hours.

Fig 1: *In vitro* Mucoadhesion Test

Table 3: Swelling Index Percentage and Percentage of Mucoadhesion of Formulations F1 to F16

Formulation Code	**Swelling Index (%) (In 12hrs)**	**Mucoadhesion (%) (In 12 hrs)**
F1	40.2±1.30	50±2.08
F2	44.6±0.52	59±1.0
F3	56.4±0.62	60±1.23
F4	60.2±1.46	67±0.92
F5	44.8±1.04	57±1.02
F6	52.2±1.20	62±0.98
F7	60.4±2.48	64±1.11
F8	64±3.98	68±1.13
F9	50.2±8.54	55±1.27
F10	62±7.64	57±1.13
F11	64.6±2.84	60±1.33
F12	68.2±4.60	63±2.07
F13	43.0±6.1	57±1.07

F14	56.2±0.86	62±0.56
F15	68.2±5.18	67±0.89
F16	74.6±2.24	70±3.32

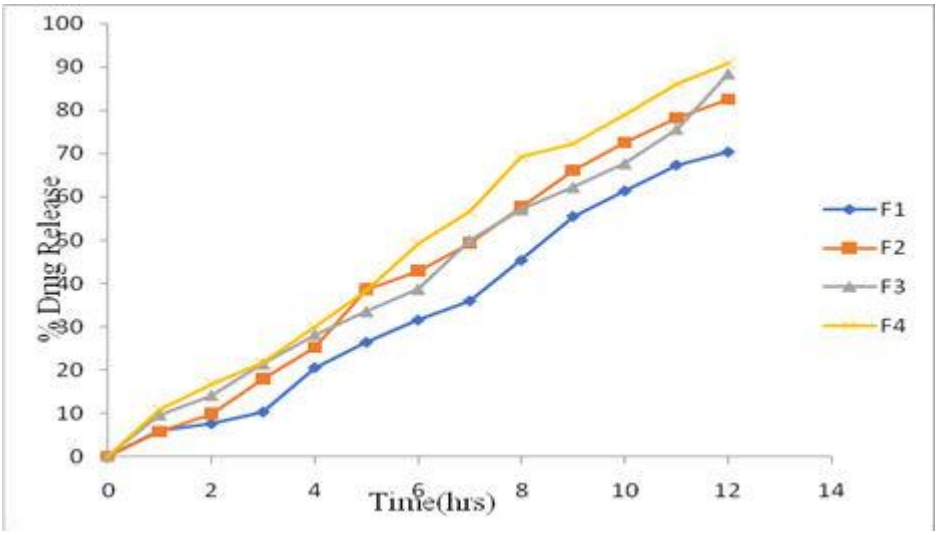
All values are represented as mean ± standard deviation (n=3)

***In-Vitro* Drug Release Studies:**

The Acyclovir mucoadhesive Microcapsules in-vitro drug release tests were completed using a pH 1.2 HCL buffer as the dissolution media. The results are shown in Table No. 04 to 07 and Fig. No. 02 to 05. The medication release increased proportionally with the mucoadhesive polymer concentration. At the end of 12 hours, it was discovered that the percentage of drug release for formulations F1–F4 was in the range of 70.37–0.11% to 90.89–0.28%, F5–F8 was discovered to be in the range of 70.7–0.82% to 89.8–0.56%, F9–F12 was discovered to be in the range of 72.5–0.62% to 90.2–0.50%, and F13–F16 was discovered to be in the range of 75–0.71%. At the conclusion of 12 hours, it was discovered that the optimised formulation F16 had a greater and more regulated percentage of drug release, or 91.70.54%. This is accomplished by mixing a medication in a 1:4 ratio with carbopol polymer. This information reveals that the formulations' use of polymers greatly restricted medication release, which would be beneficial in lowering the number of doses and enhancing patient compliance.

Table no. 4: *In vitro* drug release for formulations F1-F4

Time (hours)	**F1**	**F2**	**F3**	**F4**
1	5.81±0.1	5.6±0.32	9.5±0.11	10.82±0.4
2	7.5±0.16	9.86±0.24	14.11±0.19	16.79±0.13
3	10.22±0.10	17.9±0.11	21.35±0.44	21.62±0.19
4	20.45±0.17	25.1±2.1	28.1±0.16	29.9±0.11
5	26.33±0.22	38.6±0.19	33.48±0.21	38.49±0.7
6	31.49±0.13	42.94±0.31	38.64±0.35	49.27±0.19
7	35.92±0.32	49.38±0.10	49.91±0.41	56.6±2.5
8	45.27±0.18	57.64±0.44	57.06±0.10	69.3±0.52
9	55.43±0.22	66.1±0.25	62.2±0.45	72.4±0.27
10	61.31±0.43	72.5±0.33	67.6±0.34	79±0.19
11	67.29±0.19	78.3±0.28	75.6±0.22	86.18±0.43
12	70.37±0.11	82.5±0.30	88.41±0.51	90.89±0.28



All values are represented as mean ± standard deviation (n=3)

Fig 2: Comparison of *In-vitro* drug release for formulations F1-F4

Table 5: In vitro drug release for formulations F5-F8

Time(hours)	**F5**	**F6**	**F7**	**F8**
1	4.97±0.14	7.39±0.08	9.96±0.21	11.41±0.18
2	9.14±0.07	10.22±0.13	12.67±0.32	22.22±0.37
3	17.95±0.08	19.39±0.04	20.45±0.24	38.63±0.44
4	21.82±0.12	24.64±0.18	26.6±0.64	46.51±0.53
5	27.53±0.50	29.72±0.31	31.95±0.55	53.75±0.25
6	32.6±0.43	35.6±0.67	38.84±0.25	58.34±0.35
7	38.48±0.23	40.02±0.50	47.91±0.48	64.59±0.22
8	49.82±0.49	49.09±0.08	55.72±0.72	69.22±0.53
9	55.88±0.46	54.79±0.30	61.96±0.61	73.84±0.28
10	61.31±0.89	60.94±0.43	68.08±0.45	76.47±0.12
11	66.11±0.39	67.64±0.26	74.05±0.52	82.71±0.38
12	70.7±0.82	75.52±0.56	80.95±0.74	89.8±0.56

All values are represented as mean ± standard deviation (n=3)

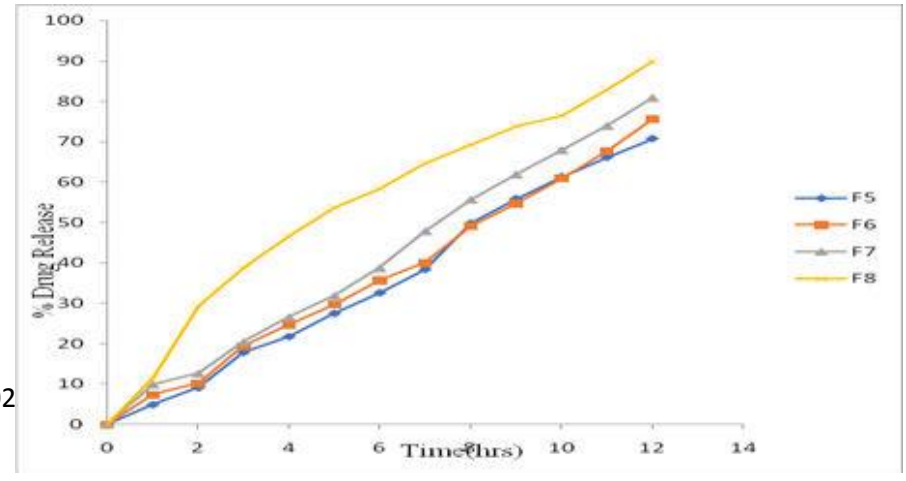


Fig 3: Comparison of *In vitro* drug release for formulations F5-F8

Table 6: *In vitro* drug release for formulations F9-F12

Time (hours)	**F9**	**F10**	**F11**	**F12**
1	4.16±0.5	8.5±0.11	8.32±0.4	9.3±0.15
2	8.68±0.10	10.95±0.19	17.94±0.17	18.76±0.28
3	17.95±0.15	19.27±0.12	27.17±0.22	25.17±0.33
4	23.99±0.23	25.79±0.32	33.6±0.10	30.15±0.11
5	29.43±0.18	32.31±0.16	38.66±0.17	39.86±0.26
6	35.76±0.33	38.57±0.19	45.55±0.22	49.28±0.19
7	40.84±0.48	43.09±0.53	59.43±0.55	58.88±0.35
8	45.82±0.28	50.07±0.65	63.67±0.37	65.39±0.50
9	51.25±0.19	58.39±0.27	69.38±0.25	71.18±0.44
10	59.41±0.56	65.46±0.16	73.9±0.65	79.03±0.32
11	66.57±0.32	71.97±0.42	80.88±0.29	85.45±0.74
12	72.55±0.62	78.23±0.39	86.22±0.43	90.2±0.50

All values are represented as mean ± standard deviation (n=3)

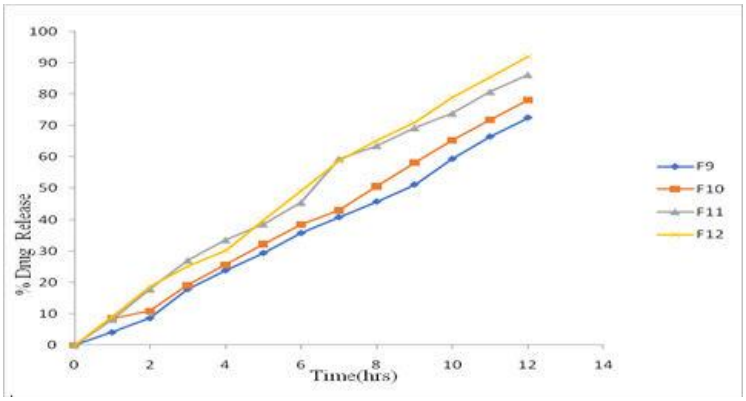


Fig 4: Comparison of *In vitro* drug release for formulations F9-F12

Table 7: In vitro drug release for formulations F13-F16

Time (hours)	**F13**	**F14**	**F15**	**F16**
1	5.33±0.9	7.71±0.21	8.24±0.12	9.04±0.18
2	9.5±0.15	18.31±0.32	16.58±0.33	19.59±0.74
3	13.39±0.20	26.45±0.53	22.65±0.45	28.17±0.32

4	21.26±0.18	32.51±0.68	25.17±0.67	35.15±0.53
5	28.96±0.33	37.59±0.55	30.77±0.51	43.12±0.54
6	36.75±0.29	42.39±0.33	38.67±0.62	49.54±0.22
7	41.46±0.19	49.1±0.61	48.16±0.58	55.15±0.45
8	48.72±0.44	56.72±0.53	55.25±0.87	68.23±0.55
9	55.97±0.31	61.94±0.72	68.96±0.66	77.92±0.38
10	60.47±0.54	67.92±0.84	75.74±0.71	81.5±0.26
11	66.57±0.32	73.25±0.77	81.97±0.55	87.7±0.54
12	75.05±0.71	80.32±0.65	89.50±0.36	91.7±0.54

All values are represented as mean ± standard deviation (n=3)

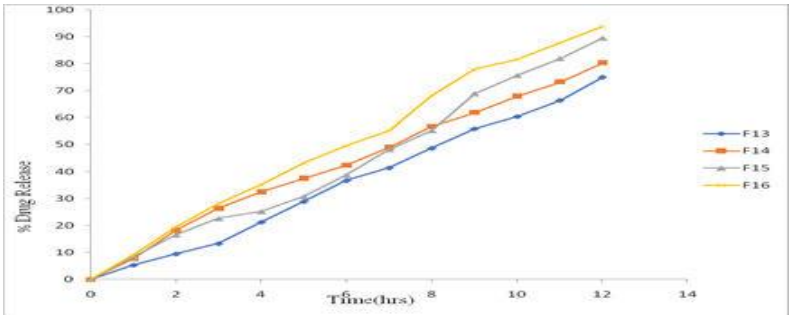


Fig 5: Comparison of *In vitro* drug release for formulations F13-F16

***In-vitro* Drug Release Kinetics of Formulations F1-F16**

By applying the drug released data to different kinetic models, including zero order, first order, Higuchi, Korsmeyer Peppas, and Hixon Crowell, it was possible to calculate the kinetics of in vitro drug release. Table No. 08 provided information on the drug release kinetics of Acyclovir mucoadhesive Microcapsules. The Higuchi mechanism and Zero order kinetics were used in the formulation F16.

Table 8: *In-vitro* Drug Release Kinetics data of Formulations F1-F16

Formulation Code	**Zero order (R^2)**	**First order (R^2)**	**Higuchi (R^2)**	**Korsmeyer Peppas (R^2)**	**n**	**Hixon Crowell (R^2)**
F1	0.993	0.981	0.994	0.991	0.835	0.991
F2	0.985	0.980	0.993	0.993	0.952	0.982
F3	0.994	0.969	0.983	0.985	0.924	0.985
F4	0.992	0.985	0.978	0.982	0.388	0.990
F5	0.995	0.964	0.985	0.965	0.569	0.979
F6	0.983	0.958	0.976	0.973	0.668	0.973
F7	0.990	0.945	0.981	0.994	0.870	0.985

F8	0.989	0.973	0.968	0.990	0.468	0.980
F9	0.994	0.979	0.973	0.992	0.358	0.989
F10	0.995	0.968	0.980	0.975	1.066	0.972
F11	0.992	0.973	0.958	0.960	1.239	0.965
F12	0.990	0.961	0.963	0.955	0.456	0.977
F13	0.997	0.971	0.978	0.994	1.017	0.980
F14	0.993	0.954	0.962	0.993	0.727	0.968
F15	0.989	0.975	0.986	0.978	0.796	0.988
F16	0.998	0.987	0.997	0.996	0.467	0.993

Scanning Electron Microscopy (SEM):

SEM analysis was used to examine the mucoadhesive Microcapsules' internal cross-sectional structure and surface shape. In Fig. No. 12, SEM photomicrographs of the optimised formulation were displayed. The Microcapsules have a smooth, sphere-like surface. Very little drug particle debris was seen on the surface of the microcapsules, indicating that the medication was distributed uniformly within the polymer network.

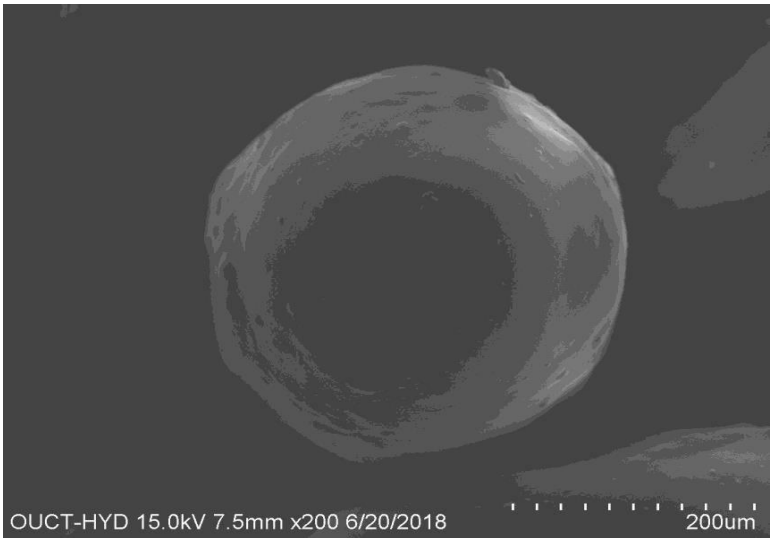


Fig 6: Scanning Electron Micrograph of F16 Formulation

Accelerated Stability Studies:

The improved formulation F16 was kept at high temperatures, such as 250°C and 400°C, respectively, for three months. According to the stability studies' findings, there were no appreciable changes in the drug entrapment effectiveness, swelling index, or in vitro drug release tests. The results were shown in Table no: 9

Table 9: Stability studies

	Initials	**30 days**	**60 days**	**90 days**

Characteristics	**25˚±2˚C 60±5 % RH**	**25˚±2˚C 60±5 %RH**	**25˚±2˚C 60±5% RH**	**40˚±2˚C 75±5 % RH**
Drug Entrapment Efficiency (%)	87.5±0.32	86.5±0.2	85.2±0.12	85.1±0.32
Swelling Index (%)	74.6±2.24	73.6±2.20	72.8±1.14	72.6±0.22
In vitro drug release (%)	98.12±0.24	97.20±0.21	96.75±0.19	96.2±0.55

Post compression parameters of Acyclovir Mucoadhesive tablets:

Weight variation test:

Each batch of formulations F1 underwent the weight variation test in accordance with I.P. The findings are displayed in table 10. The tablet passed the test for weight variation. The Acyclovir tablet's average weight was determined to be 497.40.557 mg, which is within the authorised legal limits (IP).

Hardness test:

Consumer acceptance and management of the tablet depend on its appropriate hardness. The results are reported in table 10 and the measured hardness of the tablet formulations, F1, was 6.1 0.15 kg/cm2. This guarantees careful treatment.

Friability test:

The formulation's friability test was conducted according to protocol. I.P. Table 10 is a summary of the friability test results. According to the statistics, the formulation's friability was less than 1%, assuring that the tablets were mechanically stable. The formulation F1shown 0.100.453% demonstrates the tablet's excellent friability.

Thickness:

The thickness was found to be 4.6±0.153 mm. Hence, it is concluded that the formulation compiled the thickness test and the results are shown in Table 10.

Table 10 : Weight variation, Hardness, Friability of Acyclovir Mucoadhesive tablet

SL.NO	Weight Variation (mg)	Hardness (kg/cm^2)	Friability (%)	Thickness (mm)
F1	497.4±0.557	6.1 ±0.15	0.10±0.453	4.6±0.153

All values are represented as mean ±standard deviation (n=3)

Swelling index:

The formulation F 1was the subject of a swelling investigation. Table 11 presents the outcome. Generally, all of the formulation was hydrated by dipping the tablet into the buffer for anywhere between one and ten hours. Swelling Index has increased as a result of an increase in polymer content.

Table 11 : percentage of hydration of Acyclovir Mucoadhesive tablets

All values are represented as mean ±standard deviation (n=3)

Formulation code	**1hr**	**2hr**	**4hr**	**6hr**	**8hr**	**10hr**
F1	81.8±0.65%	104.5±0.61%	125±0.30%	134±0.45%	145±0.32%	154±0.20 %

***In-vitro* Dissolution Study:**

The ratio of the medication to the carbopol 940 polymer in formulation F1 is 1:4. At the conclusion of 12 hours, formulation F1's in-vitro cumulative drug release profile indicated 98.120.24% drug release. The pill was originally swollen and non-erodible throughout the course of 12 hours, according to the study. It

was determined that the drug release rate from the tablets was raised by increasing the concentration of carbopol 940 in the formulation.

Table 12: *In-vitro* Cumulative drug release of F1

Time (hr)	Formulation code and %CDR
	F1
01	25.49±0.20
02	32.51±0.30
03	39.11±0.22
04	49.54±0.46
05	54.54±0.51
06	59.31±0.66
07	69.19±0.33
08	81.45±0.14
09	84.43±0.20
10	92.22±0.34
12	98.12±0.24

All values are represented as mean ±standard deviation (n=3)

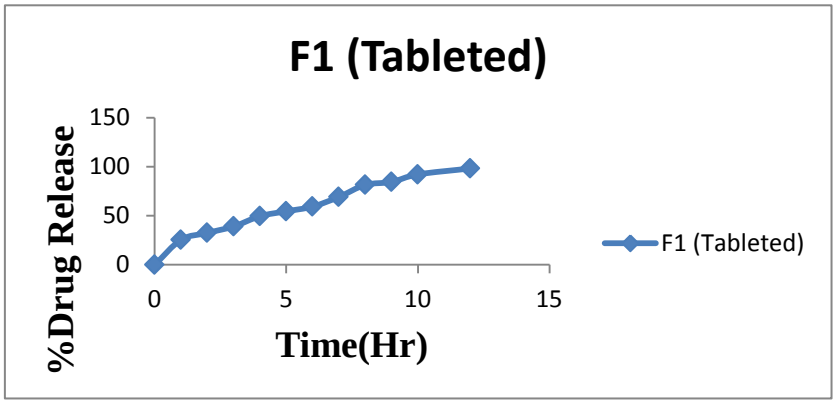


Fig 7: *In vitro* drug release for formulation acyclovir tableted F1

Comparative dissolution studies between acyclovir microcapsule and tableted:

Table 13: comparative dissolution studies between acyclovir microcapsule and tableted:

Time (hr)	Formulation code and %CDR	
	F1	F16
01	25.49±0.20	9.04±0.18
02	32.51±0.30	19.59±0.74
03	39.11±0.22	28.17±0.32
04	49.54±0.46	35.15±0.53

05	54.54±0.51	43.12±0.54
06	59.31±0.66	49.54±0.22
07	69.19±0.33	55.15±0.45
08	81.45±0.14	68.23±0.55
09	84.43±0.20	77.92±0.38
10	92.22±0.34	81.5±0.26
12	98.12±0.24	91.7±0.54

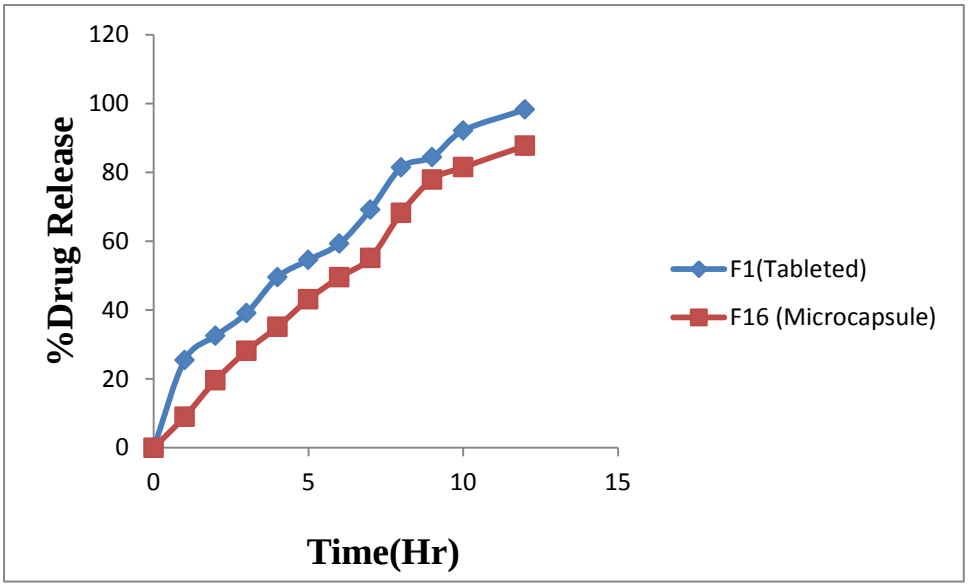


Figure 8: Comparison of *In vitro* drug release for formulations F1(Tableted) and F16 (Microcapsules)

Drug release kinetics:

Table 14: *In vitro* Drug Release Kinetics data of Formulations F1

Formulations	**Zero order(R^2)**	**First order (R^2)**	**Higuchi (R^2)**	**Korsemeyer-peppas (R^2)**	**N-values**
F1	0.912	0.910	0.983	0.955	0.7813

To determine the drug release mechanism, in-vitro drug release data for the formulation F1 was subjected to a release kinetic investigation using the zero order, first order, higuchi, and korsemeyer-peppas equations. The R^2 values were found to be greater in zero-order compared to first-order. Thus, zero-order kinetics was used in the formulation. However, in the instance of the drug release mechanism, the R^2 value was found to be larger in the korsemeyer-peppas equation and the release exponent "n" value less than 1, i.e. (n .0.5 and 0.89). This suggests that non-fickian diffusion was used in the formulation. Hence, it was determined that the formulation used non-fickian diffusion in conjunction with zero-order drug release.

Accelerated Stability Studies:

All values are represented as mean ±standard deviation (n=3)

Acyclovir tablet formulations chosen from the current investigation's development were kept at 40°C and 75% RH for three months. The product was assessed for a number of tablet qualities, including dissolution rate. After three months of storage, the tablets showed no discernible modifications. The

Characteristics	**Room Temperature**		**Accelerated Studies**	
	Initials	**30 days**	**60 days**	**90 days**
	$25^0\pm2^0$C 60±5% RH	**$25^0\pm2^0$C 60±5% RH**	**$40^0\pm2^0$C 75±5% RH**	**$40^0\pm2^0$C 75±5% RH**
Swelling Index(%)	154±0.20	152.8±0.26	150.5±0.12	149.4±0.32
In vitro drug release (%)	98.12±0.24	97.8±0.14	97.5±0.07	97.4±0.6

studied tablet formulation's dissolving properties and swelling index did not change over the course of storage. Based on the aforementioned stability experiments, it was determined that formulation F1 was

Table 15: Stability studies of Formulation F1 as per ICH Guideline

highly stable because there were no values that underwent any major changes. The results are shown in Table No: 15

CONCLUSION

The polymers Sodium Alginate, Sod.CMC, HPMC K4M, and Carbopol 940 were used to successfully create the mucoadhesive Microcapsules of Acyclovir utilizing the Emulsion Solvent Evaporation method approach. Based on the method's greater % yield, it has been determined to be the best method for producing these microcapsules. Studies using FTIR did not find any notable medication interactions. Scan-electron microscopy showed that the produced Microcapsules had good spherical geometry and a smooth surface. Comparing the main evaluation parameters, such as the percentage yield, entrapment effectiveness, swelling index, and in-vitro drug release. The optimum formulation was determined to be F16 due to its high percentage yield (97.6%), high entrapment efficiency (87.50.32%), low swelling index (74.62.24%), and high in vitro drug release (98.120.24%). Acyclovir mucoadhesive microspheres and optimised acyclovir mucoadhesive microcapsules were compared in dissolving trials, and after 12 hours, the percentage of drug release was reported to be 98.120.24% and 91.70.54%, respectively. Hence, it was determined that acyclovir mucoadhesive microspheres tablet was superior to acyclovir mucoadhesive microcapsule. The formulation F1 acyclovir microspheres presented was determined to be the best formulation among all formulations and can be utilised in the treatment of herpes simplex virus (HSV-1, HSV-2) infections based on all the above evaluation criteria.

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