RESEARCH ARTICLE

Design, Development and Evaluation Studies of Acyclovir Topical Gels for Anti-Viral Activity

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ABSTRACT

The aim of this study is formulation and development of Acyclovir loaded topical gel, to evaluate pre formulation and post formulation parameters of gel with effect of different gelling agent and their comparative study. In the first phase, pre-formulation studies were carried out then the Acyclovir formulation was prepared, using solvent dispersion method and then post formulation studies were carried out. Acyclovir as the API has effective anti-viral activity, which is helpful for treatment of Herpes Simplex Virus like infections. The gel will ensure longer retention time, both at site of application and also at the site of drug absorption, thus, giving the drug enough time for absorption. Different parameters are studied in formulation of gel like: Organoleptic properties, viscosity study, in-vitro drug release study, FT-IR of API and gelling agent evaluation of gel as well as in-vitro and in-vivo activities.

Keywords: Gel, Acyclovir, Herpes Simplex Virus infections, Evaluation of gel.

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INTRODUCTION

Intravenous infusion is recognized as an advanced method of drug administration, primarily to bypass hepatic "first pass" metabolism and maintain stable drug levels in the body.[1] This approach allows for direct delivery of the medication into the systemic circulation, although it does carry certain risks. On the other hand, topical administration is utilized to apply a drug directly at the site of need, ensuring that an adequate amount is absorbed into the systemic circulation to achieve therapeutic effects.[2] To facilitate continuous drug delivery through intact skin, various topical formulations are employed, one of which is gel. Gels are specifically designed for topical dosage forms, aimed at delivering medication to a localized area of the skin.[3]

Skin

The skin is classified as an organ because it is made up of tissues that are structurally connected to perform specific functions. It is one of the largest organs in the body in terms of surface area.[4]

For an average individual, the skin covers approximately 2 square meters (3000 square inches). The epidermis is made up of stratified squamous epithelium and contains four distinct types of cells:

1) Keratinocytes,
2) Melanocytes,
3) Langerhans cells (previously referred to as non-pigmented granular dendrocytes), and
4) Granstein cells. The keratinocytes in the epidermis are organized into the following layers:

Mechanism of Diffusion via the Stratum Corneum

The stratum corneum is a multilayered membrane, with the spaces between cells filled with lipid-rich amorphous substances. In a dry state, these intercellular spaces can constitute up to 5% of the total volume.

While molecules can diffuse through these intercellular areas, current evidence indicates that diffusion for water-soluble, non-electrolytes typically does not occur primarily through these spaces.

Instead, transcellular permeation is characterized by a relatively lower diffusion coefficient.[5] Consequently, molecules can diffuse through intercellular pathways while also penetrating via transcellular routes. The stratum corneum has a defined thickness, resulting in a brief lag time for diffusion after a drug is applied to the skin, during which the rate of transfer through the skin gradually increases until it stabilizes at a steady state.[6]

METHODOLOGY

Formulation of Gel

Gels were prepared by cold mechanical method. Accurately weighed (1% w/v, 2% w/v, 3% w/v, 4% w/v and 5% w/v) specified mass of Xanthan Gum and Carbopol 940. A beaker has been taken into which specified volume of distilled water mixed slowly in order to dilute polymer, Xanthan Gum as well as Carbopol 940 were added thoroughly (so as to stop particle agglomeration) in water present in beaker. Further completely done the mixing with the help of homogenize performed at 250 rpm. To reduce bubbles formation in the preparation, care should be done. Mixing was performed to perceive homogeneous mixture. Further mixed well, volume make up with purified water and Further over end of homogenising phase, entire ingredients are also mixed

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Table 1: Composition of different batches this polymer-based Acyclovir gel
Every 10 ml of formulation contains

S.N.	Formulation code	F-1C (gm)	F-2C (gm)	F-3C (gm)	F-4C (gm)	F-5C (gm)	F-6X (gm)	F-7X (gm)	F-8X (gm)	F-9X (gm)	F-10X (gm)	F-11X (gm)	F-12X (gm)
1	Acyclovir	0.10 gm	0.15 gm	0.2 gm	0.25 gm	0.30 gm	0.10 gm	015 gm	0.20 gm	0.25 gm	0.30 gm	0.30 gm	0.30 gm
2	Carbopol 940	0.10	0.20	0.3	0.40	0.50	-	-	-	-	-	-	-
3	Xanthan Gum	-	-	-	-	-	0.10	0.20	0.30	0.40	0.50	0.50	0.50
4	Methyl Paraben	0.8 ml	0.8 ml	0.8 ml	0.8 ml	0.8 ml	0.8 ml	0.8 ml	0.8 ml	0.8 ml	0.8 ml	0.8 ml	0.8 ml
5	Liquid Paraffin	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml
6	Agar	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm
7	Propylene glycol	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	2 ml	3 ml
8	Mint	0.2ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
9	Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

after which, Acyclovir was added.[7]

Evaluation of Formulation

Physical Examination

The Acyclovir gels were visually examined for color, homogeneity, consistency, spreadability and phase separation. pH of each gel (using pH meter calibrated at each use with standard buffer solutions pH 4, 7, 9) The electrode was inserted into the sample 10 minutes prior to reading at room temperature.[8]

Determination of Entrapment Efficiency of Preliminary Trial Batches of Acyclovir

In one glass tube containing 0.2gm of gel in that added 10ml of pH 6.8 phosphate buffer and sonicated by means of sonicator bath for 10 minutes then for 40 minutes centrifugation was carried out at 18000rpm at 5^0C. Collected supernatant was assayed by means of UV method subtracting the amount of free drug in the supernatant from total drug added, percent drug encapsulation efficiency was determined.[9]

$EE\% = (C_t - C_f / C_t) * 100$

In which, Ct means total concentration of a drug and C_f means concentration of free drug.

% Drug Content Determination

0.1gm of Acyclovir was weighed, diluted in 6.8 pH phosphate buffer. Further each prepared formulations were diluted in 6.8 pH buffer solution and examined under ultra violet spectrophotometer.[10]

The sample absorbance compared with that of standard to determine % drug content.

Drug content = (sample absorbance x 100) / standard absorbance

***In-vitro* Drug Release Study**

Preparation of Phosphate Buffer Solution pH 6.8

In 200 ml volumetric flask to 0.2 M Potassium dihydrogen phosphate (50 ml) and 0.2 M NaOH (22.4 ml) was added after that made up the volume upto 200ml with water.

Table 2: Visual Appearance of Formulations

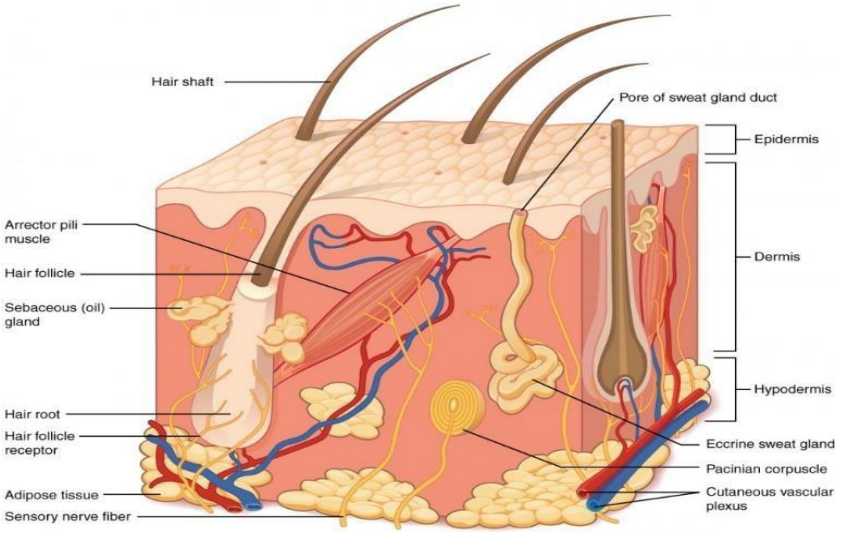


Figure 1: Layers of Skin

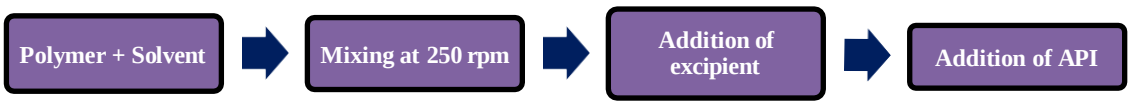


Figure 2: Steps involved in formulation of gel

Formulation code	Consistency	Homogeneity	Colour
F-1C	Excellent	Poor	White
F-2C	Excellent	Fair	White
F-3C	Very Good	Fair	White
F-4C	Excellent	Excellent	White
F-5C	Excellent	Excellent	White
F-6X	Excellent	Fair	White
F-7X	Fair	Excellent	Light Yellow
F-8X	Excellent	Excellent	White
F-9X	Excellent	Fair	Yellowish White
F-10X	Poor	Excellent	Yellowish White
F-11X	Excellent	Excellent	White
F-12X	Excellent	Excellent	White

Using modified Keshary-Chien diffusion cell, the release of Acyclovir from different gel formulations was investigated. To create a permeation cell, a conventional cellophane membrane that had been soaked in pH 6.8 for two hours before to usage was adhered to one end of the cylinder. The donor compartment of the cell contained 1 gram of gel, while the receptor compartment was submerged in a 100 ml beaker filled with drug-free phosphate buffer pH 6.8 (90 ml). A magnetic stirrer was used to stir the cell, which was submerged in the receptor compartment 1 cm below the phosphate buffer surface. A temperature of $32^0C \pm 1^0C$ was maintained. Over the course of 75min, a sample (5 ml) of the receptor compartment was obtained at different intervals (15, 30, 45, 60 and 75min) and tested for Acyclovir at 253 nm. Drug-free phosphate buffer was used to replenish the volume that was taken out each time. Acyclovir release amounts at different times were computed and displayed against time.[11,12]

Table 3: Table showing Entrapment Efficiency*(%) of Acyclovir with Carbopol and Xanthan Mixture.

Formulation code	Entrapment Efficiency*(%)
F-1C	12.07+1.5
F-2C	14.98+1.43
F-3C	17.15+0.72
F-4C	19.22+0.89
F-5C	20.18+0.70
F-6X	15.18+0.70
F-7X	17.85+0.69
F-8X	18.08+1.62
F-9X	16.08+0.49
F-10X	17.75+0.72
F-11X	19.27+0.46
F-12X	22.98+1.67

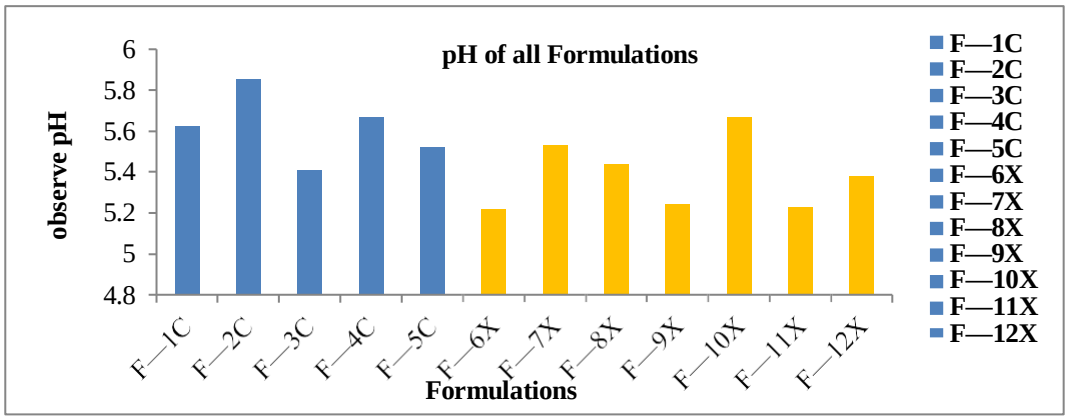


Figure 3: Showing pH analysis of all the formulation

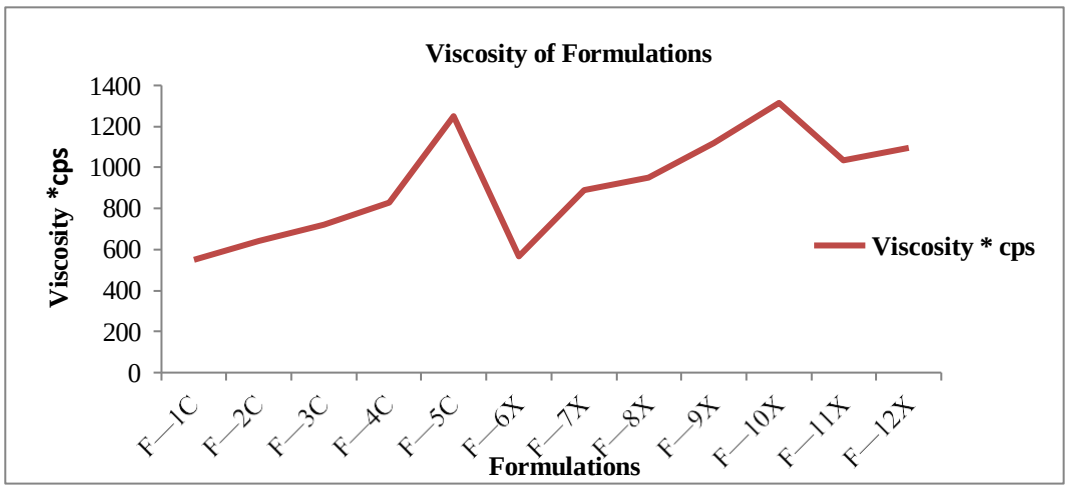


Figure 4: Showing Viscosity analysis of all the formulation

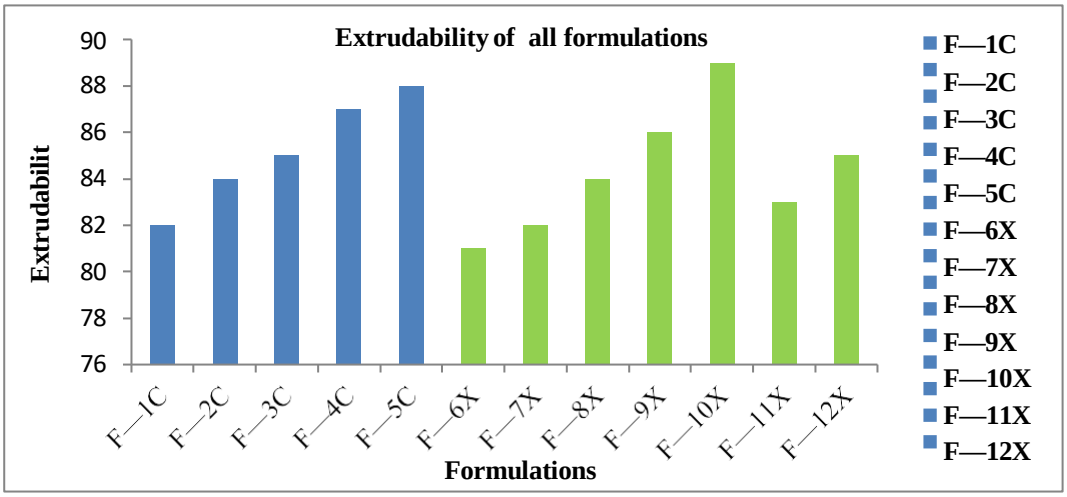


Figure 5: Showing Extrudability analysis of all the formulation

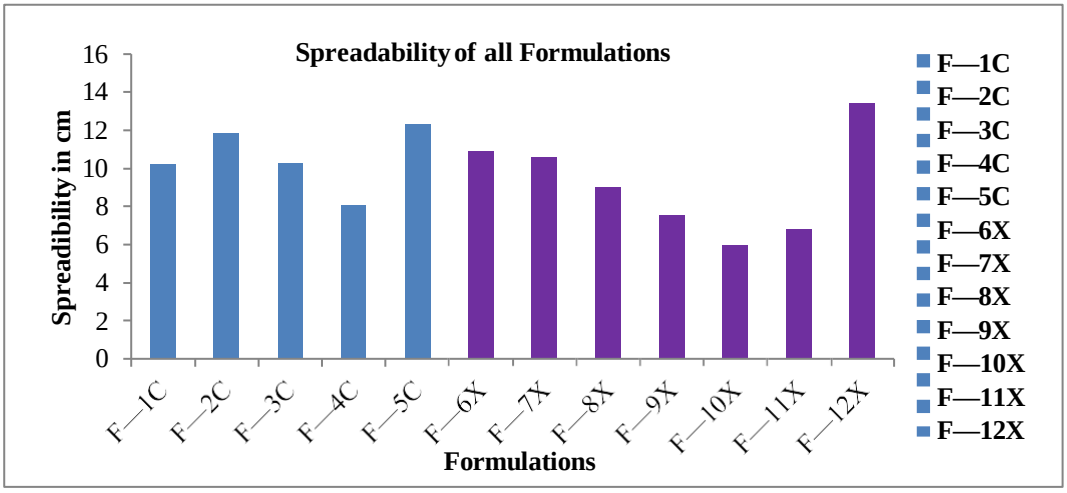


Figure 6: Showing Spreadibility analysis of all the formulation

Stability Studies

Keeping topical gel preparations at room temperature for three months is crucial for stability testing. During this period, checked several important factors to ensure the gel remains safe and effective. It was checked for how the gel appeared, making sure it doesn't change color or separate. The pH level was measured to confirm it stays balanced and won't irritate the skin. Also checked if the amount of drug in the gel stays the same over time.[13]

Table 4: pH of all the formulations is given in the table

Formulation Code	pH
F—1C	5.62
F—2C	5.85
F—3C	5.41
F—4C	5.67
F—5C	5.52
F—6X	5.22
F—7X	5.53
F—8X	5.44
F—9X	5.24
F—10X	5.67
F—11X	5.23
F—12X	5.38

Visual Appearance

The visual appearance of formulations from F-1C to F-12X was done on the basis of parameters which includes consistency of the formulations, homogenicity, and colour of the formulations.[14]

pH

Measured the pH of the samples using an electronic pH meter from Hanna Instruments. Three readings for each sample were taken and average was calculated. To find out when the liquid turned into a gel, special methods were used. Added small amounts of the liquid into vials and slowly heated them in a water bath. Starting at 4°C, and increased the temperature by 1°C at a time. Watched closely to see when the liquid stopped moving when the vial was tilted. This point, where the liquid became a gel, was called the critical gelation temperature.[15]

Viscosity

The viscosity of the different gel formulae was determined at 25°C using rotational Brookfield viscometer of cone and plate structure with spindle CPE-41 and CP-5221. The apparent viscosity was determined at shear rate 40 sec^{-1}. The flow index was determined by linear regression of the logarithmic form of the following equation.[16]

$\tau = k \gamma n$ ...Equation

Table 5: Showing Viscosity analysis of all the formulation

Formulation Code	Viscosity * cps
F—1C	550
F—2C	640
F—3C	720
F—4C	830
F—5C	1250
F—6X	567
F—7X	890
F—8X	950
F—9X	1120
F—10X	1315
F—11X	1034
F—12X	1098

Where "τ" is the shear stress, "γ" is the shear rate, k is the consistency index, and n is the flow index.
When the flow is Newtonian n=1, if n>1 or n

Extrudability

The study evaluated gel formulation extrudability by measuring the amount of gel squeezed out of a tube under a specific weight. A higher percentage of extruded gel indicated better extrudability. The gel was placed in a one-ounce aluminum tube with a 5 mm nasal tip opening. To test it, the tube was put between two glass slides and pressed with a 1 kg weight. Collected and weighed the gel that came out of the tip.[17] Then the percentage of gel extruded was calculated and grade was given. A good grade was marked as +++, fair as ++, and poor as +.[17]

Spreadibility

The device used to measure how easily the gel spreads was based on Mutimer's design, with some changes made in the lab. It has a wooden block with a pulley at one end and a glass plate on top. About 2 grams of gel were put on this plate. Another glass plate was placed on top of the gel, and a 1 kg weight was put on it for 5 minutes. This squeezed out air bubbles and made the gel spread evenly. Extra gel on the sides was removed. Then, a 50-gram weight was tied to the top plate with a rope. The time it took for the top plate to move 10 cm was measured. If this time was shorter, it meant the gel spread more easily. This simple test helps to understand how well different gels can be applied and spread on surfaces.[18]

Table 6: Showing Extrudability analysis of all the formulation

Formulation Code	Extrudability cm^2	Formulation Code	Extrudability cm^2
F—1C	82±0.55	F—7X	82±0.55
F—2C	84±0.65	F—8X	84±0.82
F—3C	85±0.82	F—9X	86±0.62
F—4C	87±0.12	F—10X	89±0.12
F—5C	88±0.62	F—11X	83±0.38
F—6X	81±0.45	F—12X	85±0.52

Table 7: Showing Spreadibility analysis of all the formulation

Formulation Code	Spreadibility (in cm)	Formulation Code	Spreadibility (in cm)
F—1C	10.23	F—7X	10.56
F—2C	11.85	F—8X	9.02
F—3C	10.27	F—9X	7.56
F—4C	8.06	F—10X	5.96
F—5C	12.28	F—11X	6.82
F—6X	10.89	F—12X	13.42

Phase Separation

All developed gel formulae were inspected for their homogeneity, Spreadability test, color; syneresis, phase separation and presence of lumps by visual inspection after the gels have been set in the container.[19]

Leakage

During the manufacturing process, it's crucial to check for leaks of gel formulations. These leaks can happen due to tiny holes, cuts, or faulty seals caused by problems like air bubbles in the release liner layers. To catch these issues, manufacturers need to test the products carefully. If they don't have automated technology to do this, they should perform specific tests during production. These tests help identify any defects that could lead to leaks. By doing these checks, manufacturers can ensure the quality and safety of their products.[20]

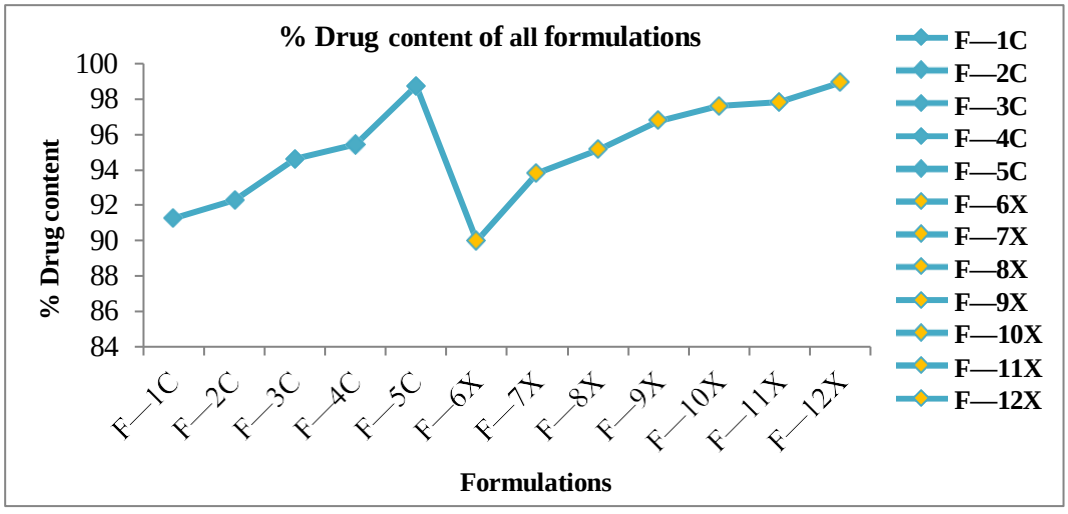


Figure 7: Showing percentage drug content of all the formulations

Table 8: Showing phase separation data of all the formulations

Formulation Code	Separated/ Clear/ Excellent
F—1C	Excellent
F—2C	Excellent
F—3C	Separated
F—4C	Smooth
F—5C	Excellent
F—6X	Excellent
F—7X	Separated
F—8X	Smooth
F—9X	Excellent
F—10X	Excellent
F—11X	Smooth
F—12X	Excellent

Table 9: Showing percentage drug content of all the formulations

Formulation Code	% drug content
F—1C	91.25
F—2C	92.30
F—3C	94.60
F—4C	95.43
F—5C	98.73
F—6X	92.55
F—7X	93.80
F—8X	95.12
F—9X	96.75
F—10X	98.60
F—11X	97.80
F—12X	98.92

Visual Inspection

A fixed number of tubes, defined based on batch size, need to be audited at random.

Leakage Check

Each sampled tubes should be checked visually for leakage. If any of the tubes tested leaks, the moment it is detected, the product ends.

Seal Integrity

Sealing of tubes should also be stress tested to avoid the effects of pressure causing seals to open and thus lead to leakage. A defined number (N1) of tubes, based on batch size, should be randomly checked. Visual inspection for leakage should be carried out on all sampled tubes. A weight is laid on top of the sampled tubes while the tubes is placed on a flat, hard surface so that it is subject to a pressure of 13.6 kg. Should keep the weight for 2 mins. The tube must be checked for leakage visually after removing the weight. However, if the number of tubes detected in the event of a leak exceeds the limit specified by the manufacturer, the product is considered faulty.[21]

Packaged Product Testing

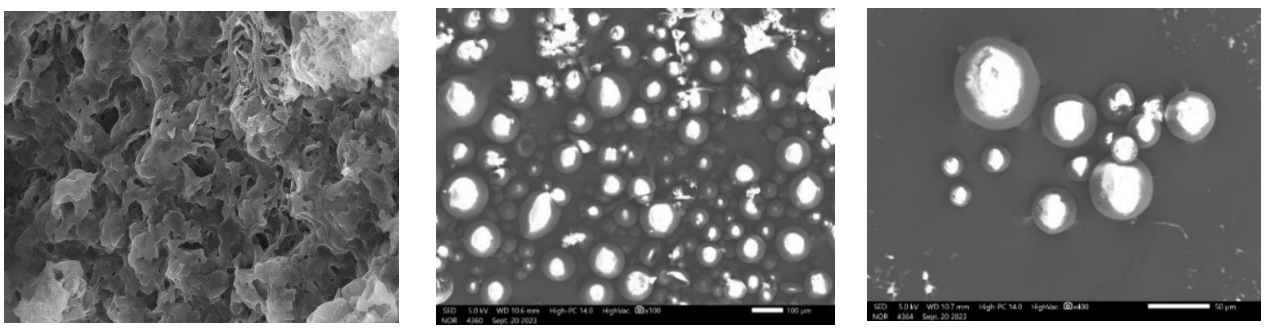
Semisolids are a popular way to deliver medicine through the skin. However, these systems can sometimes leak after being placed in their primary packaging. This leakage can happen during the packaging process itself or when a user opens the package. To ensure patient safety and drug effectiveness, it's crucial to check tubes for any leaks. This testing should be done both during manufacturing and after the tubes are put into their primary packaging. Regular leak checks help maintain the quality of products and protect users from potential issues caused by leaking drugs. After being placed in their primary packaging material, a specified number of tubes, which is defined based on batch size, should be randomly tested. The packages of sampled tubes must not be entered in the field and should be removed from the packaging and visually inspected for leakage. Registered tubes sample should then be evenly wiped with a solvent-moistened swab. Wipe the tubes backing side and the release liner side. Wipe the inside surface of the pouch too. The swab(s) is (are) then removed and the drug is assayed. If the amount of drug corresponding to the tubes and pouch exceeds a limit set by the manufacturer, then the product fails.[22,23]

Nature

The nature of the prepared formulations was studied on the basis of different parameters which includes appearance, colour and texture.

Drug Content Analysis of Active Ingredients

Percentage drug content is a key measure in medicine production. It tells us how much of the active ingredient is in a final product. This is important to make sure patients get the right amount of medicine. When making pills or other medicines, manufacturers aim for a specific percentage of the active drug. They check this carefully to ensure each dose has the correct amount. Too little of the drug might not work well, while too much could be harmful. Quality control teams use special tests to measure the drug content. They make sure each batch of medicine meets the required standards. This helps keep patients safe and ensures the medicine works as it should. Doctors and pharmacists



10X resolution 100X resolution 400X resolution

Figure 8: Figure showing SEM analysis of acyclovir topical gel

Table 10: Comparative percent cumulative drug release of F-1C to F-5C formulations of Acyclovir in phosphate buffer 6.8 pH

Time (minutes)	% drug release				
	F-1C	F-2C	F-3C	F-4C	F-5C
0	0	0	0	0	0
5	23.144	24.547	27.124	31.254	33.254
10	47.524	52.358	55.245	58.254	65.214
15	65.124	68.154	71.124	79.254	84.858
20	71.254	74.457	79.524	86.457	94.547
25	72.564	79.521	85.254	92.546	98.125

rely on accurate drug content information to give the right doses to patients.

The formula for calculating the percentage of a drug in a solution is:

Volume/volume (V/V) percent solution = volume of solute (mL) / volume of solution (mL) × 100

FTIR Spectroscopy of Formulation

The pure form of Acyclovir and polymers were performed to FT-IR studies individually as well as in combination, to evaluate the and drug polymers interferences.[25]

FTIR spectrum drug and drug–excipient blend was recorded. This mixture had been prepared immediately prior to mixing and/or heating on a FTIR spectrophotometer (SHIMADZU, IR PRESTIGE-21, JAPAN) over the range of 400–4000 cm^{-1}.

Potassium bromide disc method was used. The spectrum was the measure of ten consecutive scans at the same sample. FTIR data were processed using GRAMS/32 version 3.04.[26]

Scanning Electron Microscopy

SEM analysis of final formulation was done to study to surface morphology of the best finalised formulation. The study was done at different resolutions like 40X, 100X and 400X.[27]

Pharmacological Activity

The developed topical gelling system incorporated with ACV is suitable for variable routes of administration as indicated by the pH- independent in vitro drug release in different media. However, the Herpes Simplex Virus

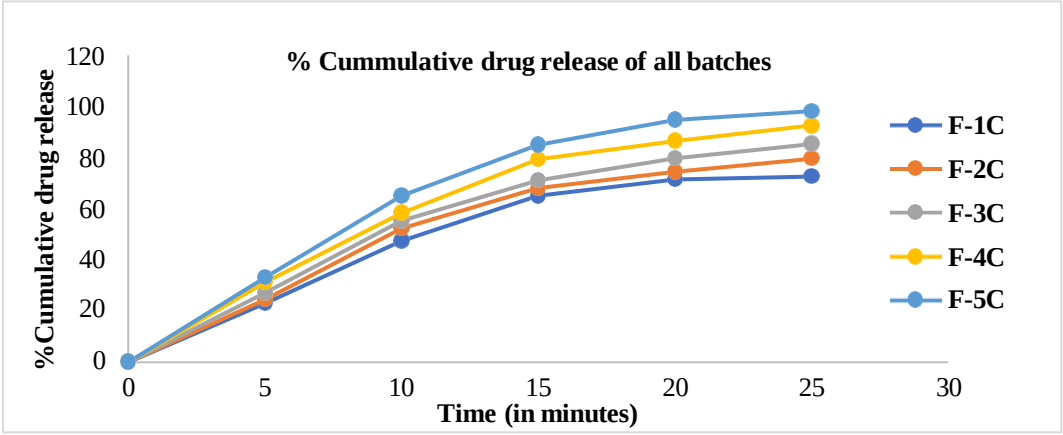


Figure 9: Comparative percent cumulative drug release of F-1C to F-5C formulations of Acyclovir in phosphate buffer 5.0 pH

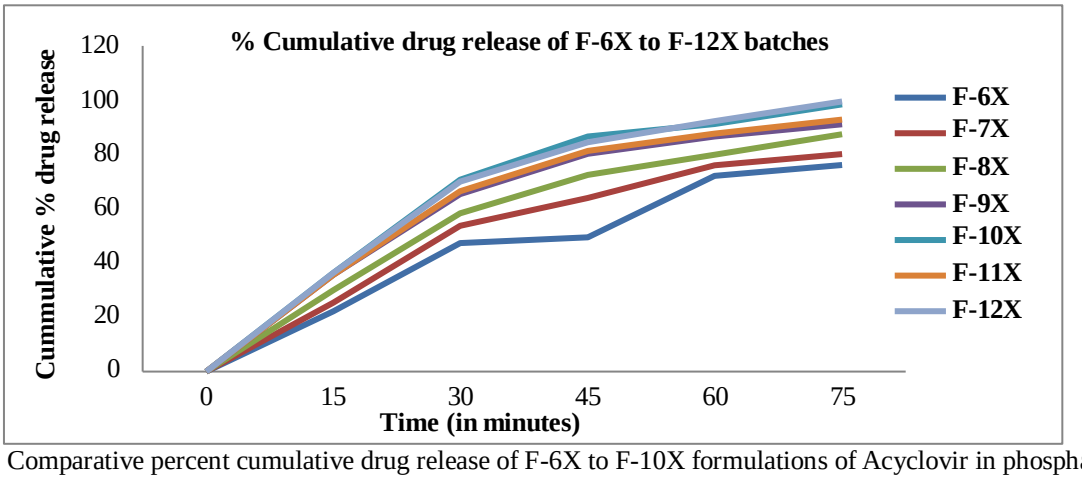


Figure 10: Comparative percent cumulative drug release of F-6X to F-10X formulations of Acyclovir in phosphate buffer 6.8 pH

infections are severe predominantly on the mucosal epithelial surface of ocular region and genitals. The gel can be administered in ophthalmic region or for vaginal application for treating the infections in eye or genital, respectively. Hence, the safety of the formulation was assessed through ex vivo cytotoxicity studies using the specific cell lines, where the formulation resides for 24 h in vivo.[28]

***In-vitro* Activity**

Screening of Compounds with MTT (3- (4, 5-Dimethylthiazol-2yl) 5-diphenyltetrazoii bromide) Assay

Cytotoxicity assay based on MTT was developed by Tolosa *et al.*, 2015. It is colorimetric assays, The Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. MTT is a yellow, water-soluble tetrazolium dye, which crosses both plasma and mitochondrial membranes. In the mitochondria of viable and glycolytically active cells, MTT is reduced by the action of NADH or NADPH dependent dehydrogenases to form an insoluble, purple formazan. The amount of the formazan product that results from this reaction is dependent on the number of viable cells.[29]

MTT is reduced to purple formazan in living cells. A solubilization solution (usually dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually 570 nm) by a spectrophotometer.[30]

The growth inhibitory effect of those compounds towards the PCS-480-010 (Vaginal Epithelial Cell line) cell line was measured by means of MTT assay. The cleavage and conversion of the soluble yellowish MTT to the insoluble purple formazan by active mitochondrial dehydrogenase of living cells has been used to develop an assay system for measurement of cell proliferation. PCS-480-010 cells 75000/100 µL media in 96-well culture plates were treated with various concentrations 1, 2.5, 5, 10, 20, 40 µM of F-1C to F-5C and F-6X to F-10X for 48 hrs. 20 µl of MTT solution (2.5 mg/ml) was added to each well and incubated at 37 0C for 4 hours before the termination of assay. The plates were discarded while the MTT-formazan crystals were dissolved in 150 µl of DMSO. The OD measured at 570 nm.

***Ex-vivo* Permeation Study**

The amount of drug diffusion across various excised membranes was measured using the Franz diffusion apparatus, which has an effective diffusion area of 2.54 cm^2. Goat organs from a nearby abattoir were used to isolate the biological membranes of the vagina, rectum, stomach, and cornea. The excised membrane was placed between the donor and the receptor after the receptor cell was filled (maximum filling capacity: 15 mL) with phosphate buffer pH 7.4 as the medium. The experiment involved a setup where a membrane separated two chambers. The bottom of the membrane touched a liquid with receptor cells, while a gel was placed on top. A small magnetic bead was put in the bottom chamber, and the whole setup was kept warm at 37°C. To keep things balanced, researchers took out 5 mL of liquid from the bottom chamber at set times and replaced it with fresh, warm liquid. They then measured how much of the drug (ACV) had moved through the membrane by looking at how the liquid absorbed light at 252 nm. This helped them compare how well the drug passed through different types of membranes over time.

RESULTS AND DISCUSSION

Evaluation of Acyclovir Gel

Visual Appearance

The formulations of Colloidal gel were yellowish white viscous cream like formulation having glossy appearance and even homogenous mixture.

Entrapment Efficiency

Determination of entrapment efficiency of preliminary trial batches of Acyclovir is mentioned in table 3.

pH Analysis

The pH values of all developed formulation were in range 5-6 which is considered acceptable to avoid the risk of irritation upon application to the skin. The observed data is shown in the table 4.

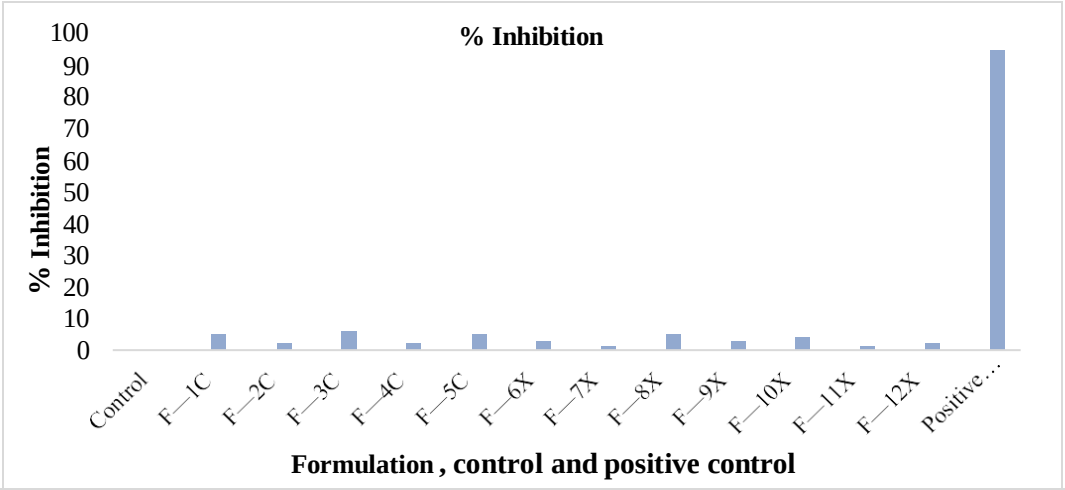


Figure 11: % Inhibition of F-1C to F-5c and F-6X to F-12X on PCS-480-010 cell line by MTT assay

Table 11: Comparative percent cumulative drug release of F-6X to F-12X formulations of Acyclovir in phosphate buffer 6.8 pH

Time	% drug release						
(minutes)	F-6X	F-7X	F-8X	F-9X	F-10X	F-11X	F-12X
0	0	0	0	0	0	0	0
15	22.253	25.254	29.854	35.654	36.458	35.759	36.248
30	47.241	53.846	58.458	65.478	70.654	66.523	69.982
45	49.458	64.145	72.451	80.254	86.458	81.321	84.598
60	72.145	75.845	79.889	86.457	91.257	87.795	92.346
75	76.124	80.147	87.458	91.125	98.458	92.854	99.524

Table 12: Cytotoxicity of formulation of F-1C to F-5c and F-6X to F-6X % growth inhibition and IC_{50} values against PCS-480-010 cell lines by MTT assay.

Cell line PCS-480-010 (Vaginal Epithelial Cell line)

S.N.	Sample Coad	CONC. (µM)	% Inhibition
1	Control	Only media100	0
2	F—1C	100	5
3	F—2C	100	2
4	F—3C	100	6
5	F—4C	100	2
6	F—5C	100	5
7	F—6X	100	3
8	F—7X	100	1
9	F—8X	100	5
10	F—9X	100	3
11	F—10X	100	4
12	F—11X	100	1
13	F—12X	100	2
14	Positive control (Camptothecin)	5	95

Viscosity

The viscosities (in cPs) of the gels were measured using a Brookfield digital viscometer DV II RVTDV-II USA. At 0.5 rpm, the spindle (TF 96) was revolved. Prior to measurements, gel samples were allowed to settle for 30 minutes at the assay temperature of 25 ±/1 °C.

Spreadibility

The spreadibility of all the formulations were determined. From F-1C to F-5C were found to be 8.06 to 12.28 and from F-6X to F-12X it was found to be 5.96 to 13.42. The decrease in the value spreadibility shows increase in gelling strength of the formulations.

Phase Separation

The phase separation study of all the formulation was done by visual inspection, and the data of all the formulations were expressed in terms of excellent / smooth and separated.

Drug Content Analysis of Active Ingredients

Results of drug content are shown in table 9. After formulation of various Acyclovir gel the drug content of the formulated gel was estimated and the results were in the official limits with range of 91.25 % to 98.92 %. The drug content determination also showed that the drug was uniformly distributed throughout the gel.

Surface Morphology of Acyclovir Topical Gel by SEM

The surface morphology of best formulation was determined by scanning electron microscopy, to study the morphological properties of the formulation. The study was done at 10X, 100X and 400X.

***In-vitro* Dissolution Studies**

The in-vitro drug release studies of all formulations with two different polymers were studied individually in the time interval of every 5 minutes from initial to 25 minutes in phosphate buffer of pH 5.0 using in-vitro dissolution apparatus (TDT-08L Electrolab, India).

In-vitro dissolution data of formulation showed maximum percentage drug release upto 72.564, 79.521, 85.254, 92.546, 98.125 for F-1C, F-2C, F-3C, F-4C, F-5C, respectively.

For In-vitro dissolution data of formulation showed maximum percentage drug release upto 76.124, 80.147, 87.458, 91.125, 98.458 for F-1X, F-2X, F-3X, F-4X, F-5X, respectively.

Pharmacological Activity

***In-vitro* Study**

Effect of Cytotoxicity of Formulation F-1C to F-5C and F-6X to F-10X on PCS-480-010 (Vaginal Epithelial Cell Line) Cell Line by MMT Assay

In-vitro screening of the compounds, formulation F-1C to F-5C and F-6X to F-10X was also carried out against PCS-480-010 (Vaginal Epithelial Cell line) cell line by MTT assay at the concentrations of 20 and 40 µM for 48 h. the results are shown in table 12 as % growth inhibition for corresponding concentrations used and the IC_{50} values were also determined for respective cell lines. Camptothecin (5 µM), was used as positive control in the assay. The formulation F-1C to F-5C and F-6X to F-10X was found to be in effective against PCS-480-010 (Vaginal Epithelial Cell line) and showed non-cytotoxic effect against PCS-480-010 cell line.

Comparison of *Ex-vivo* Permeation Profile

The optimised formulations F-5C and F-12X's ex vivo permeation profiles through different biological membranes are compared. Up until 12 hours, the total amount of drug that permeated through all of the membranes was found to be linear. After that, the cumulative amount of drug that permeated decreased, which could be explained by a reduction in the concentration gradient across the membrane. Compared to the other membranes, the corneal membrane had a higher rate of drug penetration. As a result, the corneal membrane's steady-state flux and associated permeability coefficient were greater for both formulations. It was discovered that the qualitative and quantitative permeation profiles through the vaginal and rectal membranes were

Table 13: Results of *ex vivo* permeability study of thermo sensitive gelling system incorporated with ACV

Formulaton code	Membrane	Q24 $\mu g/cm^2$	JSS $\mu g/cm^2/h$	K_p cm/s x 10^{-6}	T_L h
F-5C	Cornea	2452±178	139.80±17.24	3.88±1.72	0.18±0.15
	Vagina	1116±87	58.76±7.60	1.63±0.76	0.54±0.05
F-12X	Cornea	3020±123	177.70±11.10	4.93±1.11	0.22±0.05
	Vagina	1443±84	79.24±1.94	2.20±0.19	0.16±0.02

comparable.

All of the membranes in both topical gel formulations have significantly increased the permeability coefficient of ACV (1.19 x 10-6 cm/s). The permeability coefficient values, obtained for the ACV loaded gelling system were found to be higher than the previously reported ACV pro-drugs and different drug delivery systems like mucoadhesive liposomes and ball milled solid dispersions. Compared to the F-5C in situ gel, the F-12X gel showed noticeably ($P<0.05$) more drug penetration. As a result, the xanthan gum formulations system with increase in penetration enhancer like Propylene Glycol showed steady state flux and higher permeability coefficient across all membranes. This might be explained by the fact that the ACV-loaded gel using xanthan gum along with increased concentration of penetration enhancer (propylene glycol) allows greater penetration than normal gel formulation with minimal quantity of penetration enhancer and carbopol as gelling agent.

CONCLUSION

The pH analysis of all the formulations were done to detect the pH of all the formulation and it was found to be in the range of 5.24 to 5.85 which complies to the pH of the skin to prevent any kind of irritation effect over the skin.

The viscosity of all the formulation were determined to evaluate the flow property of the formulation. For the formulation from F-1C to F-5C, it was found to be in the range of 550 cps to 1250 cps and for F-6X to F-12X, it was found to be in the range of 567 cps to 1315 cps. With this study, we can predict that Xanthan Gum formulations show great ability in the formulation of Acyclovir topical gel.

The Spreadibility analysis was done to study the resistance in the spreading of the formulation. Upon increasing the concentration of polymers, the spreadibility reduces which shows the cohesive force development in the formulation.

The phase separation study in all the formulations were done and majority of the formulations shown excellent condition of the formulation.

SEM at different magnifications was used for investigating the morphological differences of samples of both polymer physical mixture that exists as irregularly shaped.

The release property of all the formulations were studied individually, the formulation F-5C shows % drug release of 98.125% in 75 minutes whereas the formulation F-12X shows % drug release of about 99.524% in 75 minutes.

All the formulations were evaluated for their *in-vitro* cytotoxicity is basically a colorimetric assay called MTT assay. In this experiment, the cytotoxicity potential of all formulation on 100 µM and no any morphological changes was observed in microscopical observation, positive control Camptothecin 5 µM is the 95 % of cytotoxicity was shown and cell lysis was observed in microscopic observation.

On the basis of overall evaluation of all the formulations, the formulation with code F-5C and F-12X were selected for in-vivo activities, which showed excellent penetration properties during the study and complies the in-vivo activity for the management of infections to vaginal epithelial cells.

This experiment proves that xanthan gum shows better gelling property in terms of physical properties of formulation, furthermore, increase in concentration of penetration enhancer like, propylene glycol helps the drug to penetrate through vaginal epithelial cells, increasing the infection management and decreasing the number of application.

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