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A Research Pepar-formulation and evaluation of in-situ gel for ophthalmic drug delivery system.

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Abstract
Acyclovir is an antiviral drug used against herpes simplex virus and varicella zoster virus. The aim of this research work was to formulated and characterize ophthalmic of in-situ gel of acyclovir .In-situ gel formulations were prepared by the ***precipitation -cold method****. The effect of important process parameters .e.; amplitude, different surfactant and its ratio, and sonication time were studies. The drug and interaction of excipient with drug was characterized by FTIR studies. The acyclovir ophthalmic in-situ gel formulations were prepared five formulations coded as F1, F2, F3, F4 & F5. All formulation has 0.3 g active drug and other excipients were taken in various ratio. All formulations were studied for pH, spread ability, drug content, viscosity, clarity, sterility test, In vitro drug release parameters and compared with marketed preparation TarinCare. Among all formulation, F3 was best formulation as per above consider parameters.*
Key word-*Formulation: FT-IR studies; In-vitro drug relase studies, acyclovir,gellan gum, carbopol,in-situ gel*

I. Introduction

Ophthalmic medication delivery is among the most fascinating and difficult tasks facing pharmaceutical scientists1,2. The eye is a highly resistant organ to foreign substances due to its structure, physiology, and biochemistry. substances. In clinical settings, numerous conventional topical medications can be used to treat the anterior portion of the eye (cornea, conjunctiva, and sclera). Poor bioavailability is displayed by delivery methods like solutions and suspensions. And healing reaction. Anytime an ophthalmic medication is applied topically Only a small portion (5%) of the total amount actually passes through the cornea and reaches the interior anterior tissue of the eyes. Rapid and effective noncorneal absorption, drainage by the nasolacrimal system, Because the cornea's relative permeability to both hydrophilic and hydrophobic molecules all contribute to such poor low ocular bioavailability 3,4. The objective the goal of pharmacotherapeutics is to treat an illness in a reliable and predictable manner fashion. It is presumpted that a drug's concentration at its target site of action and the subsequent pharmacological impact are correlated. Therefore, we must lengthen the drug's stay in the eye in order to maximise its ocular bioavailability. To increase ophthalmic bioavailability and extend residence periods of implanted doses, a variety of ophthalmic vehicles, including inserts, ointments, suspensions, and aqueous gels, have been developed4,5. However, due to several limitations, such as impaired vision from ointments or low patient compliance from inserts 6, these ocular medication delivery systems have not been widely adopted. Several in situ gelling systems have been created to increase patient compliance, extend a drug's precorneal residence duration, and increase ocular bioavailability as a result7. These systems experience sol-to-gel phase transitions when a particular physicochemical parameter (such as pH, temperature, or ions) changes in the cul-de-sac8. A polysaccharide made up of tetra saccharide repeating units, Gelrite (deacetylated gum) is one of the most intriguing environmentally responsive gelling polymers9. In the presence of, it solidifies the tear fluid contains cations. Once the formulations have gelled, they are resistant to the precorneal area's normal drainage process, which increases the amount of medicine absorbed and extends their resident time there9,10. The goal of the current work was to create an in-situ gelling system for the acyclovi derivative acyclovir which is used to treat external eye infections. The use of Gellan as a sole vehicle for the formulation of eye drops containing acyclovir (0.3 percent w/v), which gels when injected into the cul-de-sac of the eye and provides sustained drug release during the treatment of ocular infections, was investigated.

In-situ gelling system-

Ophthalmic *in-situ* gelling is comprising of environmentally sensitive polymers that will be altered structurally with the small changes in specific conditions like pH, temperature and ionic strength in the

environment. In-situ forming gels are liquids during instillation into the eye and then undergoes rapid gelation in the cul-de-sac of the eye to form viscoelastic gels in response to environmental changes lastly release the drug slowly under physiological conditions [11]. Consequently, the residence time of the gel formed in-situ will be extended and the drug is released in a sustained manner which leads to enhanced bioavailability, minimized systemic absorption and reduced frequent dosing regimen resulting to improved patient compliance [12]. Furthermore, some other potential advantages such as simple manufacturing process, ease of administration, and deliverance of accurate dose have been exhibited by in-situ gelling systems [13].

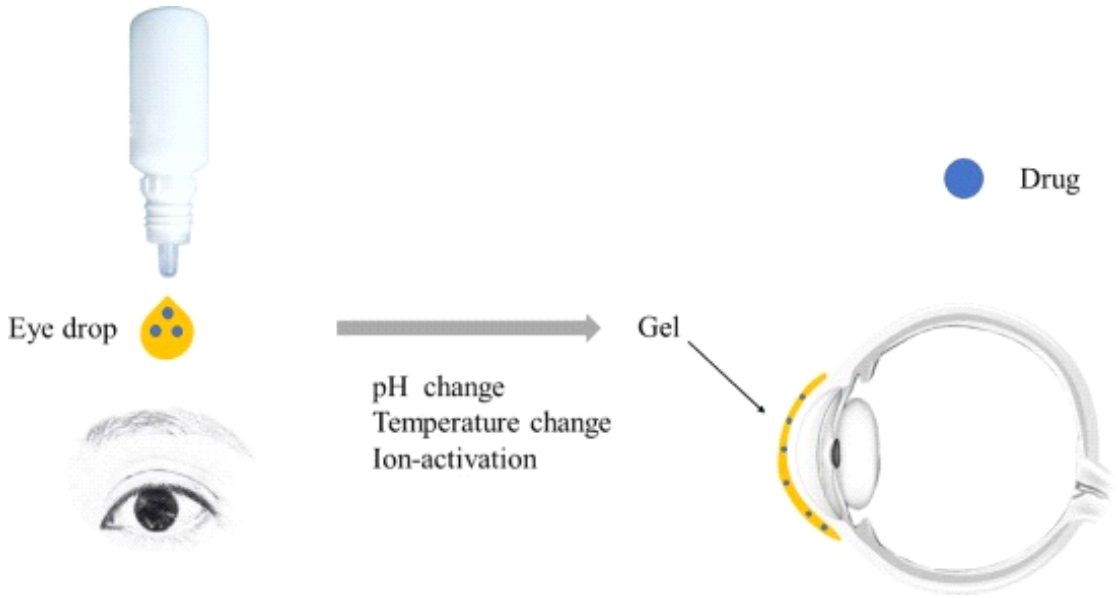


Fig no-1

II. MATERIALS AND METHODS

Material

Acyclovir was received as gift sample C.S.J.M. University Inst.of Pharmacy-Kanpur. Carbopol 934-P Was received as gift sample C.S.J.M. University Institute of Pharmacy -Kanpur.Gelrite Gellan gum applied to the Sisco Research laboratories Pvt.Ltd. used were purchased from local suppliers and of analytical grade unless mention.

Pre-formulation studies-

Pre-formulation is a level of development during which, the physiochemical properties of drug substance are characterize. Prior to the development of these major dosage forms, it is essential that certain fundamental physical and chemical properties of the drug molecules and other divided properties of the drug powder are determined. This information decides many of the subsequent events and approaches in formulation development. This first learning phase is known as pre-formulation.

Before beginning the formulation programs, the pre-formulation step must consider the following factors:

- The amount of drug available.
- The physicochemical properties of the drug already known.
- The nature of formulation should have or would like to have

Identification of drugs

The drug acyclovir was utilized during the whole work was firstly identified via different parameters.

Melting point of acyclovir-

Melting point equipment was used to determine the melting point of acyclovir. Firstly picked a little capillary tube and sealed one end of it. The material was filled into capillary tubes and allowed to rise to a height of 0.5 cm. The sample-containing capillary tube was then put into the equipment' sample holder. Finally, the melting point range was noticed by thermomete.

Fig no-2 Ambassador apparatus

Table no. 1 Melting point

S.no	Properties (drug)	Reported	Observed	Mean
1	Acyclovir	256.5 ℃	258-260℃ 257-258℃ 257-260℃	259

Solubility of Acyclovir-

Solubility studies ware done by equilibrium solubility method. According to this method, the pure acyclovir was added to different solvent medium and shaken for 24h.The saturation was confirmed by observation of presence of undissolved material. After centrifugation of the slurry .sample was analysed using UV visible spectrophotometer at lambda 252 nm.

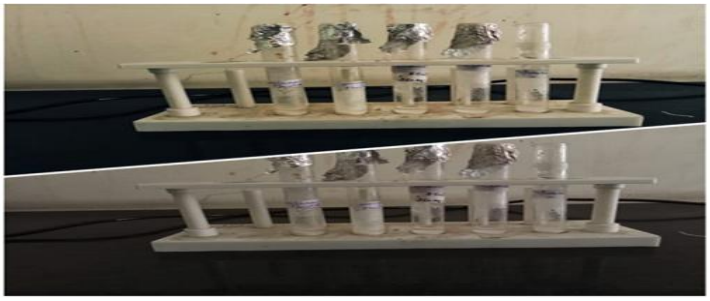


Fig no -3 Solubility of drug

Table no.2 -solubility of drug

S.no	Solvent	Volume required (ml)	Solubility
1	Water	6.5ml	Slightly soluble
2	Ethanol	30ml	Insoluble
3	Methanol	6.5ml	Insoluble
4	Phosphate buffer	7.5ml	Soluble

Partition coefficient -

Separating funnel method was used for determination of partition coefficient of acyclovir. This is a classical and most reliable method of log P determinations. Partition coefficient is a measurement of drug. Partition coefficient of acyclovir was taking 50 ml of benzene and 50 ml water. about 5mg of drug added to this solution and was shaken. after shaking the system remained undisbisburbed for 24 hrs. Two layer was separate through Whitman flter, and the amount of acyclovir solubilised, was determined by measuring the absorbance at 252nm against regent blank through double beam UV spectrometer in both the solution. partition coefficient was determined as ratio of concentration of drug in acyclovir to the concentration of drug in phosphate buffer (pH7.4) and the value were reported as log P.

Log P = Conc. Of drug in Aq. phase

Log P =Log 10 (partition coefficient)


Fig no -4 partition coefficient

Preparation of Calibration curves-

Preparation of calibration curve of acyclovir in Distilled water

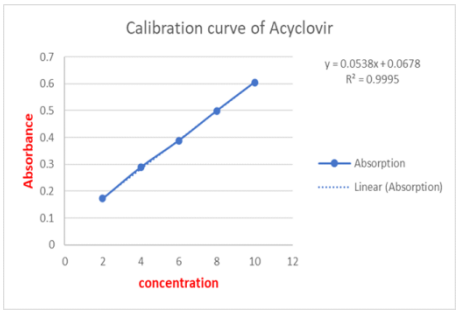
Accurately weighed quantity of acyclovir (10mg) was taken in 100 ml volumetric flask.It was dissolved in an adequate amount of distilled water and the volume was made upto 100 ml to obtain a stock solution of 100µg/ml .From the above stock solution appropriate dilutions were made in distilled water the concentration rang of 2,4,6,8 and 10µg/ml and absorbance was taken at λmax 252nm.

Table no 3 Acyclovir calibration curve distilled water

S. no	Concentration (µg/ml)	Absorbance(252nm)
1	0.2µg/ml	0.172
2	0.4µg/ml	0.269
3	0.6µg/ml	0.388
4	0.8µg/ml	0.509
5	10µg/ml	0.680

Calibration Curve of drug had to be performed by UV. Spectroscopy

Absorption maxima of Acyclovir were found to be at 252nm.



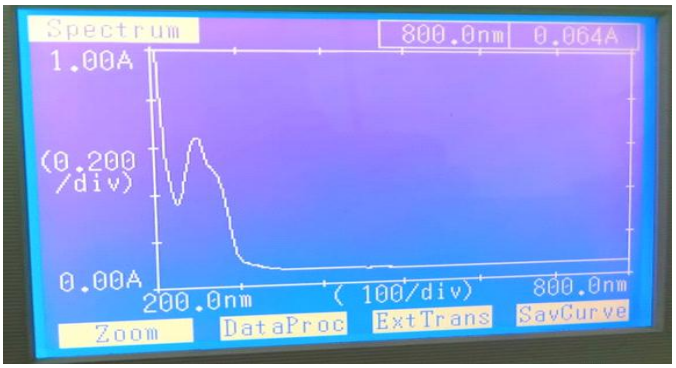
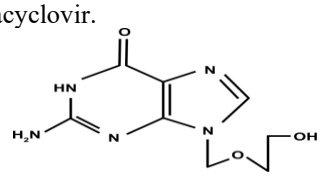


Fig no.5- Uv spectroscopy to acyclovir in water at 252nm

FTIR OF ACYCLOVIR-

This study was executed to check the compatibility of API and excipients in the final formulation. The analysis was executed in shimadzu-IR affinity spectrophotometer. The IR spectra of the sample were obtained using Kbr pellet, prepared with hydraulic press with small amount of each sample after careful grinding of each sample with Kbr. The spectral width was 400-4000cm-1

The FT-IR spectrum of the procured sample of the pure acyclovir obtained from shimadzu and was compared with the standard FT-IR spectra of pure acyclovir.



Spectral (FTIR) Analysis of acyclovir-

On comparing the IR spectrum of sample (Acyclovir) and reference spectrum, it was observed that all characteristic peak of drug was found as shown in fig 5.

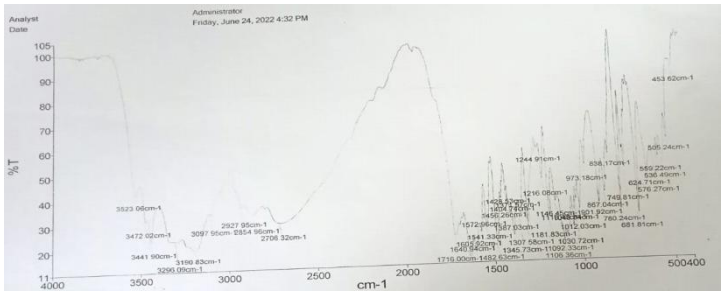


Fig no-6: FTIR of Acyclovir drug

Formulation-

Preparation of *in-situ* gel-

For the preparation of in-situ gel, gellan gum and carbopol934p was selected as co-polymer. A polymer solution was prepared according to the cold method. gellan gum and carbopol934p were accurately weighed and solubilization in required volume of distilled water by continuous stirring for 10 min. The concentration of gallan gum was optimized for *in-situ* gel formation among these concentration 0.2 mg, 0.25 gm and 0.3 gm. The optimized concentration of gellan gum was used for preparation of all formulation F1, F2, F3, F4 & F5. only the concentration of carbopol934p was varied from 0.1g to 0.25g. Based on the in-vitro gelation time and gelation capacity, optimum batch was selected for preparation and evaluation. Acyclovir loaded in-situ gel. During this study 0.1%w/v of methyl paraben was used as preservative along with 0.9%w/v NaCl for tonicity adjustment. The composition of different gelling system in shown in fig no 6 .

In-situ gel



Fig no-7 In-situ gel (After) **In-situ gel Before (S.T.F added)**

Table no.1 formulation table of *in-situ* gel

S.no	Gellan gum(mg)	Carbopol (gm)	Acyclovir(gm)	Distilled water(ml)
1	0.25mg	-	-	25ml
2	0.25mg	0.1gm	0.3gm	25ml
3	0.25mg	0.15gm	0.3gm	25ml
4	0.25gm	0.2gm	0.3gm	25ml
5	0.25gm	0.25gm	0.3gm	25ml

Tear liquid (TF)

The formulation was prepared in various concentration. When the artificial tear fluid was added to the formulation. formulation was gelled.

In a similar way, drug dilutions were prepared in TF (Table2) at a concentration of within the micrograms per ml range.

Preparation of TF (Table no.2)

Ingredients	quantity	Distilled water
Sodium chloride	0.136gm	20ml
Sodium bicarbonate	0.04gm	20ml
Calcium chloride dihydrate	0.0016gm	20ml
Potassium chloride	0.028gm	20ml

Evaluation of ophthalmic *in situ* gel -

Visual clarity and appearance- Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background**.**

Fig no-8 Clarity of *in-situ* gel

pH determination of various formulations-

pH is one of the most important parameters involved in ophthalmic formulations. The two areas of critical importance are the effect of pH on solubility and stability. The pH of an ophthalmic formulation should be such as to ensure formulation stability and at the same time to cause no irritation to the patient upon administration of

the formulation. Ophthalmic formulations should have a pH ranging between 5- 7.4. The developed formulations were evaluated for pH by using a digital pH meter.

Fig no-9 pH *in-situ* gel

Determination of drug content Uniformity-

The drug content was determined by diluting 1 ml of the formulation to 100 ml with freshly prepared simulated phosphates buffer having pH 7.4. An aliquot of 100 ml was withdrawn and further diluted to 1 ml with simulated phosphates buffer. acyclovir concentration was then determined at 252 nm wavelength using a UV-Visible spectrophotometer.

Viscosity and rheological studies-

Brookfield digital viscometer (model DV-II+Pro) was used for the determination of viscosity and rheological properties using spindle no 64. The viscosity of gel was measured at different angular velocities at a temperature of 29°C. A typical run comprised changing of the angular velocity from 8.2 to 50 rpm. The viscosity measurements were done before (at pH 7.2) and after gelling (at T. F pH7.4) .

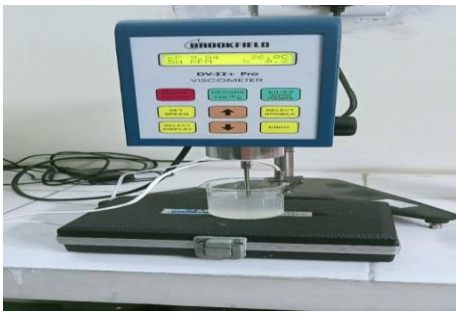
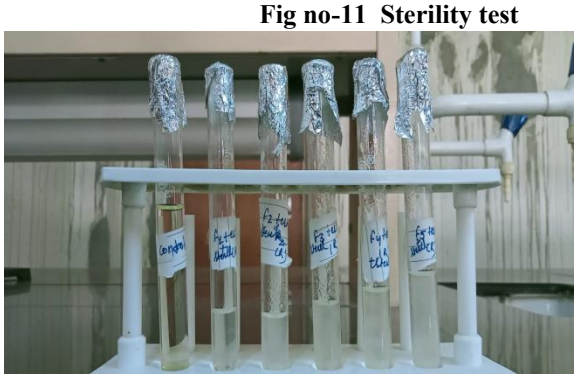


Fig no-10 viscosity

Test for sterility-

For an ophthalmic preparation, sterility is one of the most important prerequisites. In order to identify the presence of live microorganisms in produced ophthalmic formulations, sterility tests were performed.In this study, sterility tests for aerobic bacteria were conducted.

Fig no-11 Sterility test



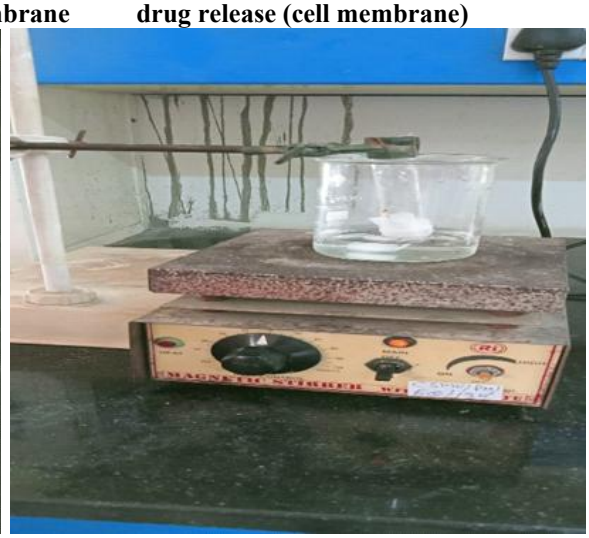
Autoclave

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

***In-vitro* drug release study of *in- situ* gel-**

In-vitro drug release study of *in-situ* gel was prepared by measuring the drug's diffusion across a diffusion cell membrane with a diffusion cell membrane; the release of acyclovir from the *in-situ* gel was evaluated. The supplier compartment and the receptor compartment made up each of the cell's two compartments. 100 ml of phosphate buffer served as the receptor medium in the receptor compartment**.** The magnetic stirrer was kept running at 50 rpm at 37 .0.5°C while the diffusioncell membrane was positioned over it aliquots of the drug release medium were taken out and diluted with the receptor media at predefined intervals. A similar volume of the receptor compartment was used as compensation. Contemporary receptor medium. At 252 nm, the drug concentrations in the release medium at different time points were Spectrophotometricallymeasured.

Fig no -12 Diffusion cell membrane drug release (cell membrane)



III. RESULTS AND DISCUSSION

Gellan gum and Carbopol 934p were used as polymers in the creation of the *in-situ* gelling system. The various concentration of polymer's used in formulations. All formulations were evaluated for clarity, pH, gelation duration, temperature, gelling capacity,*in-vitro* study.

Pre-formulation study of drug(Acyclovir)-

Appearance: Odourless, Light white crystalline powder as reported in literature.

Melting Point: Melting point of acyclovir was determined by open capillary method. The melting pointofacyclovirwas found to be 258°Cwhich complied with literature (258-270°C). The value indicating identity and purity of the drug sample**.**

Table no. 1melting point-

S. no	Reported(°C)	Observed (°C)	Mean (°C)
1	256.5°C	258-260	259
2		257-258	
3		257-260	

Solubility: Solubility of the sample was found to be 0.1mg/ml.solubility in different solvent such as water, phosphate buffer methanol and ethanol.

Table no. 2 Solubility of drug-

S.no	**Solvent**	**Volume required (ml)**	**Solubility**
1.	Water	6.5ml	Slightly Soluble
2.	Ethanol	1000-10000	Insoluble
3.	Methanol	1000	Insoluble
4.	Phosphate buffer	7.5ml	Soluble

Analytical method development for acyclovir by UV visible spectrophotometer.

UV Spectroscopy method was developed for the analysis of acyclovir using double beam Shimadzu 1700 UV Spectroscopy.

Identification of drug by UV spectroscopy-

The Acyclovir was identified by UV spectroscopy method. The acyclovir exhibited maximum absorption at 252 respectively. These wavelengths were considered as λmax for samples and all the observations by UV spectrophotometer to calculate the amount of drug were taken at this wavelength.

Standard curves of Acyclovir-

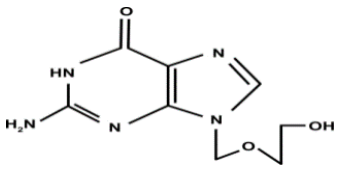
The standard curve of Acyclovir wasprepared in distilled water and results depicted in table no3.

The calibration curve was drawn for acyclovir in distilled water, and it shows straight line in range of concentration from (2, 4, 6, 8 to 10 µg/ml) with R^2 value of drugs like 0.9995respectively, indicating good linearity as shown in fig no. which follows Beer-Lambert law in the Concentration range 2-10µl.

Table no 3.Calibration curve of Acyclovir in Phosphate buffer at 7.4

S. no	Concentration	Absorption at 252nm
1	2µg/ml	0.172
2	4µg/ml	0.289
3	6µg/ml	0.388
4	8µg/ml	0.499
5	10µg/ml	0.605

Spectral (FTIR) Analysis of acyclovir-



On comparing the IR spectrum of sample (Acyclovir) and reference spectrum, it was observed that all characteristic peaks of drug were found as shown in fig .

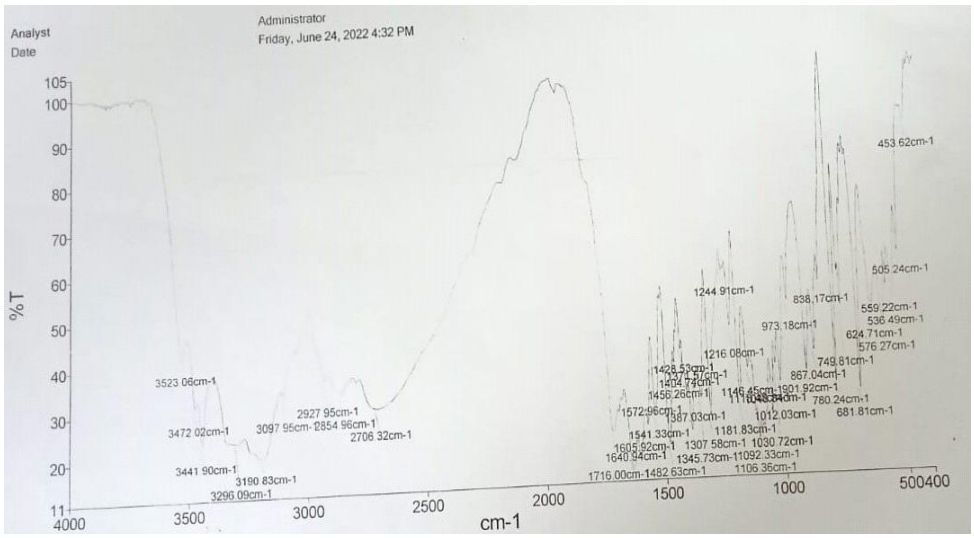


Fig no-13 FTIR Spectra of Acyclovir (API)

Table no .4 IR Characterization of Acyclovir-

S.no	Functional grp.	ReferenceWave no. (cm-1)	Observed wave no.
1.	OH hydroxyl	3500	3523
2.	C=O carbonyl	1695	1640.94
3.	NH2 amino group	3282	3296.09
4.	C=N	1487	1482.63
5.	C-N	1185	1181.83

Table no .5 Clarity test of various formulations -

Formulation code	Colour
F1	White\transparent
F2	Off white
F3	White
F4	White
F5	Light white

pH of *in-situ* gel- The pH of all formulations were determined by pH meter. All formulation were in pH range 6.5-7.4.

Table no 6. pH result of Formulated batches-

Formulation code	pH range (Average)
F1	6.5
F2	6.9
F3	7.4
F4	6.6
F5	7.0

Viscosity and rheological studies-

Table no 7. Viscosity of various formulated batches at 35^{o}C.

S.no	Spindle no.	Rpm 50	Viscosity
F1	64	50	6.36
F2	64	50	9.98
F3	64	50	13.7
F4	64	50	15.1
F5	64	50	27.0

Drug content Uniformity-

Fig no-14. Study of Drug content uniformity

Table no 8. Drug content uniformity of various formulations-

Formulation	Drug content (mean)
F1	89.01
F2	89.63
F3	97.067
F4	95.002
F5	93.143

***In-vitro* drug release study of various formulation**

The *in-vitro* release study for various formulation was performed by modified diffusion apparatus. Study revealed that all formulation had varied release percentage (51.71 to 70.26%). The F3 batch considered as best batch as this formulation showed maximum percentage release at time 60 min.

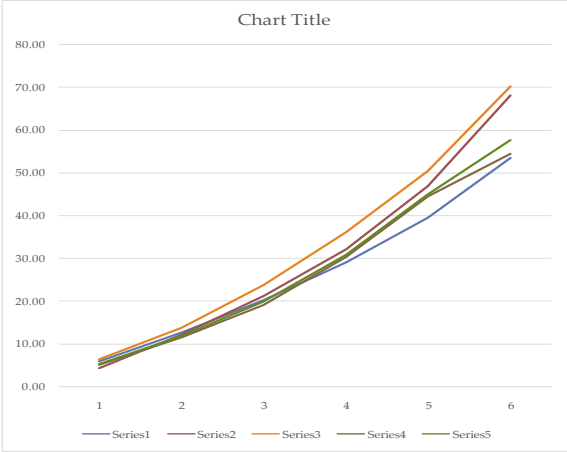


Fig no-15 Graph of *in-vitro*percentage drug release of various formulations.

Table no-9 Results of *in vitro* % release study of various formulations

Time in (min)	F1	F2	F3	F4	F5
5	5.85	4.32	6.41	5.01	5.15
15	12.55	12.13	13.80	11.43	11.84
25	20.21	21.19	23.84	19.09	19.79
35	29.00	32.06	36.11	30.25	30.80
45	39.45	46.84	50.46	44.47	45.02
60	53.53	68.03	70.26	54.50	51.71

IV. CONCLUSION

1.Pre-formlation studies of Acyclovir comply with the reported literature limits.
2.The normal eye drops have very poor bioavailability due to its rapid washout from the eye so, these problems can be overcome by modifying the drug *in-situ gel* forming ophthalmic solutions.
3.FTIR studies revealed no chemical interaction
4.The duration of action was performed by formulating *in-situ gel*
5.*In-situ gel* formulation of Acyclovir was prepared by cold method.
6.*In-situ gel* formulation eye drops formulation the duration of action was prolong by
7.In-situ gel sustained the drug released up to 10 hours which was comparatively longer periods than marketed eye drops.
8.In-vitro corneal permeation efficacy of in-situ gel might be a good alternative for conventional eye drops as it sustains the drug release for prolonged periods of time and may also reduced the number of applicationof the drug .