PREFORMULATION STUDY ON IDENTIFICATION AND CHARACTERIZATION OF ACYCLOVIR

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ABSTRACT

In compendial analytical methods, the identification of Acyclovir is confirmed through its distinct infrared absorption characteristics. This identification is carried out using a liquid chromatography technique. Other compendial sources also employ the infrared absorption method for identification. The guidelines categorize assay methods into two groups: one for Acyclovir as a bulk drug substance and another for Acyclovir in pharmaceutical formulations. An official assay method for Acyclovir utilizes potentiometric titration and outlines various spectrophotometric techniques for assessing Acyclovir in pharmaceutical preparations, including cream formulations, eye ointments, intravenous infusions, and tablets.

INTRODUCTION

For several decades, a chronic disease or acute disease is primarily regulated by drug delivery through kinds of pharmaceutical formulations, such as tablet, fluid dosage form, cream, capsules, aerosol, suppositories, and parenteral as a drug carrier. In the various direction for medication, oral way is viewed as the most normal course of of medication system. GIT drains the drug taken, to the liver that means first pass metabolism with poor bioavailability [1, 2].

Topical drug administration refers to the delivery of drugs across the body through topical pathways such as the skin, vagina, and ophthalmia. With skin being the most accessible organ in the human body for topical treatment, it acts as the primary transport route for drug delivery. Examples of topical formulation include semi-solid dosage forms, and liquid dosage formulations, such as transparent gels, ointments, patches, sprays; there are many more, and each of these has numerous applications for therapeutic considerations. They are most commonly used in cosmetic or medical fields[3, 4].

The virus particle enters the host's cytoplasm after attaching itself and passing through the plasma membrane. Penetration is the term for this process of overcoming the membrane barrier, and it calls for metabolic energy. Three primary processes are employed by viruses to penetrate: i) receptor-mediated endocytosis, ii) fusion, and iii) translocation [5].

The method of viral penetration that is most frequently employed is receptor-mediated endocytosis. Well-known techniques include bulk-phase endocytosis, phagocytosis, non-clathrin, non-caveolin, endocytosis, caveolin-mediated endocytosis, and clathrin-mediated endocytosis. A number of enveloped viruses enter a host cell by fusing. Viral fusion proteins cause the viral envelope to fuse with the host's plasma membrane. Viral nucleocapsid enters the host cell as a result of the fusion [6].

PREFORMULATION STUDIES

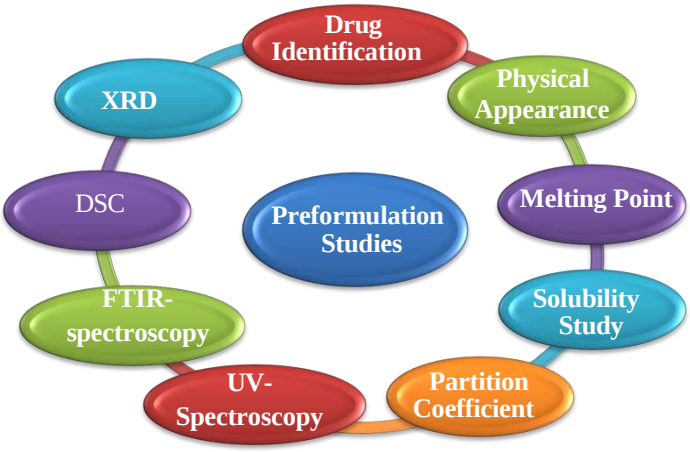
Preformulation study is essential for determining the drug's physical and chemical properties before incorporating it in formulation development. The nature of the drug highly affects the processing parameters like the method of preparation, entrapment efficiency, compatibility, and pharmacokinetics response of the formulation. Preformulation studies are an essential method of developing a dosage form that is safe, effective, and stable way. Preformulation tests were carried out in order to provide the best conditions for a therapeutically useful delivery system.

Authentication of drug

Melting point determination, UV spectroscopy, infrared spectroscopic analysis (FTIR), and differential scanning calorimetry (DSC) were used to confirm the medication [7].

UV spectroscopy

The absorption maximum was determined by scanning 10 µg/ml solution of Acyclovir in phosphate buffer pH 6.8 between 230-360nm [8].



Procedure

The standard stock solution of Acyclovir was prepared using 6.8 pH phosphate buffer. Accurately weighed 100 mg of drug was dissolved in 100 ml of phosphate buffer pH 6.8 in 100 ml volumetric flasks with aid of sonication in bath sonicator for 20 min. The concentration of Acyclovir was 100µg/ml and for the analytical purpose concentration of

Acyclovir was taken 10µg/ml. This sample was scanned under ultra-violet spectrophotometer range from 230-360nm and Path length: 1 cm. From this spectrum of Acyclovir drug, the wavelength with maximum absorbance was chosen for further analysis [9].

Infrared spectroscopic study

Fourier Transform Infra- red spectroscopy used in this research work for the chemical identification of Acyclovir. This is the most powerful analysis technique that provides useful information about the chemical structure of molecule quickly, without any lengthy and tiresome evaluation methods.

The main application of Infra- red spectroscopy is the determination of the identity of a compound by means of spectral comparison with that of an authentic sample and verification of the presence of functional groups in an unknown molecule. So, the identity of sample was established by taking the Infra- red of the drug sample and comparing with the pharmacopoeial spectra [10].

Method of placing sample in FT-IR

For placing the sample in FT-IR, disc of potassium bromide was employed. The powdered sample of Acyclovir was intimately mixed with dry potassium bromide. The mixture of drug and potassium bromide was compressed into transparent disc under the high pressure. This disc was placed in Fourier Transform Infra- red spectrophotometer using sample holder and the spectrum of Acyclovir was recorded at FTIR was scanned from 4000-400 cm^{-1}. The spectrum of Acyclovir obtained between the % transmittance and the wave number (cm^{-1}), that is inverse of λ.

Melting point

The fused capillary method used to determine the melting point of the drug.

Procedure

In this method a small amount of pure Acyclovir was taken in a capillary tube. This tube is open at one and closed at another end. This capillary was placed in Thieles melting point apparatus. The apparatus was filled with liquid paraffin. This apparatus is tied in stand and put over a Bunsen burner. The temperature at which the drug melted was noted and average of triplicate readings was taken [11].

Solubility determination

Solubility is defined in quantitative termed as the concentration of solute in a saturated solution at a certain temperature, in qualitative way, it can be defined as the spontaneous

interactions of two or more substances to form a homogeneous molecular dispersion. The solubility of Acyclovir was determined in different solvents. For solubility studies, a known amount of drug was dissolved in various solvents and the solubility was determined.

The drug Acyclovir belongs to BCS type III, which means it is high soluble, low permeable drug molecule. In spite, of having high water solubility, Acyclovir is slightly soluble in water making difficulties while manufacturing formulations. The reason for its low solubility is a nucleoside analogue with a purine base structure. Though it has hydrophilic groups such as hydroxyl and amide groups, the entire structure includes hydrophobic components (the purine base) that does not dissolve well in water.

Another reason for low water solubility is due to its crystalizing nature which are difficult to solubilize in water. One more reason behind its low solubility is that it is a weakly ionisable chemical, which does not form charged species easily in water, which tends to make it low soluble in water. Solubility study of the drug plays an important role to know about the characteristics of a drug in aqueous systems. Bioavailability of the drug completely depends on the aqueous solubility. Solubility of Acyclovir was determined by shaking flask method. The absorbance is measured by UV spectroscopy and solubility is calculated [12].

Table 1: Limits of Solubility as Per Indian Pharmacopoeia

S. No.	Descriptive term	Part of solvent required per part of solute
1	Very soluble	Less than 1
2	Freely soluble	1 to 10 ml
3	Soluble	10 to 30ml
4	Sparingly soluble	30 to 100ml
5	Slightly soluble	100 to 1000ml
6	Very slightly soluble	1000ml to 10,000ml
7	Practically insoluble or insoluble	10000 or above 10,000

Acyclovir apparent solubility was assessed at 37°C in distilled water, methanol and buffers with pH values of 6.8. Each of the eighteen vials contained 10 mg of Acyclovir. Each drug-containing vial received a 10 mL aliquot of each type of solvent. The vials were then stored for 24 hours at 37 ± 0.5 ºC in a shaker incubator. The vials were shaken and then incubated for 12 hours at 37 ± 0.5 ºC to achieve equilibrium. The filtrate was then subjected to spectrophotometric analysis at 270 nm after the solution was passed through a 0.45 µm millipore filter [13].

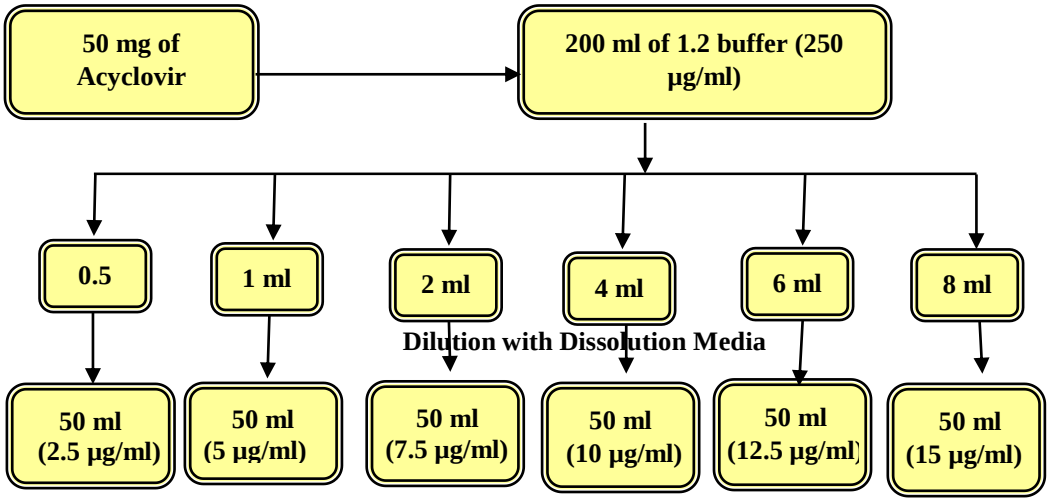
Standard Calibration Curve

Standard Calibration Curve of Acyclovir in 1.2 pH Buffer

Stock solution of Acyclovir was prepared by dissolving 50 mg of drug in 200 ml of 1.2

phosphate buffer. Aliquots of 0.5, 1, 2, 4, 6, 8 ml (2.5 to 15 µg/ml) were transferred separately in to 50 ml volumetric flasks from the stock solution. Volume was adjusted up to the mark Acyclovir the same solvent. Absorbance of the above solutions was taken at 253 nm against the blank. Graph of absorbance Vs concentration was plotted.

Standard (Stock) solution



Procedure for Reagents

Preparation of 0.2 M Sodium Hydroxide Solution

An accurately weighed 8g of sodium hydroxide pellets were dissolved in 1000ml of distilled water and mixed.

Preparation of 0.2 M Potassium Dihydrogen Phosphate

An accurately weighed 27.21 g of monobasic potassium dihydrogen phosphate was dissolved in 1000ml of distilled water and mixed.

Procedure for buffer

An accurately measured 50ml of 0.2 M potassium dihydrogen orthophosphate was transferred to a 200 ml volumetric flask and 39.14 ml of 0.2 M sodium hydroxide was added to it. The volume was made up with distilled water, mixed and pH was adjusted to 1.2 with 0.2 M sodium hydroxide [14].

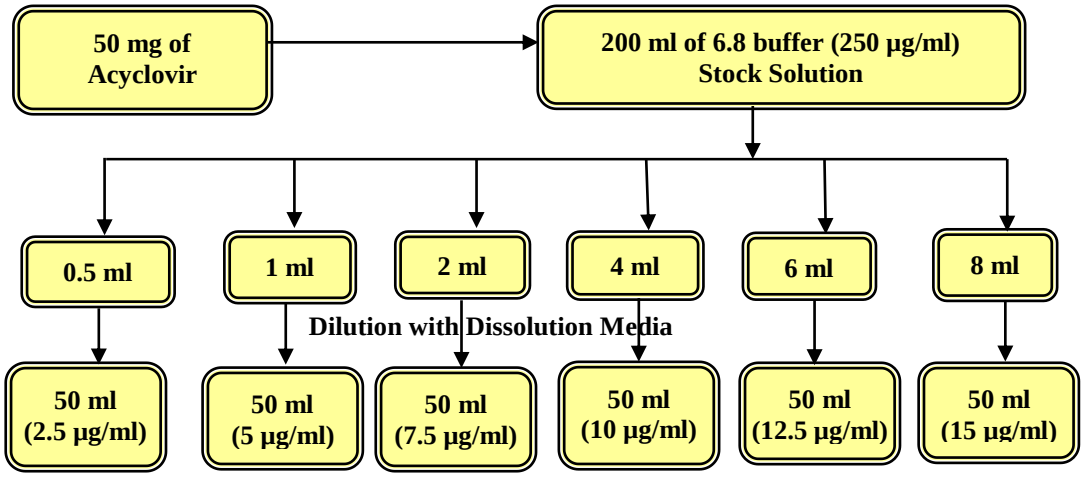
Preparation of standard curve of Acyclovir in 6.8 pH Buffer

1st Stock solution

50 mg of Acyclovir dissolved in up to 250 ml with 6.8 Phosphate buffer. This stock solution having concentration of 250µg/ml of Acyclovir.

Aliquots of 0.5, 1, 2, 4, 6, 8 ml (2.5 to 15 µg/ml) were transferred separately in to 50 ml volumetric flasks from the stock solution. Volume was adjusted up to the mark Acyclovir the same solvent.

The absorbance of each concentration was measured using UV-Visible spectrophotometer at 253 nm as λ max and the graph plotted against the concentration and absorbance [15].



Drug – Excipient Compatibility Study

Drug excipient compatibility studies were done by FTIR to check the compatibility of drug with excipients. The FTIR spectrum obtained of pure drug with excipients was compared with the standard FTIR spectrum of pure drug [16].

Partition Coefficient

A major criterion in evaluation of the ability of a drug to penetrate the lipid membranes is its apparent oil/water partition coefficient. So, the lipid solubility of a drug is determined from its oil/water partition coefficient ($K_{o/w}$). This value is a measure of the degree of distribution of drug between lipophilic solvent and an aqueous phase.

The partition coefficient measured from the following formula

$K = C_O/C_W$

Where C_O is the equilibrium concentration of all forms of the drug in an organic phase, C_W is the equilibrium concentration of all forms of the drug in aqueous phase [17].

Procedure for determining partition coefficient

The partition coefficient of both drugs was performed using n-octanol as the oil phase and phosphate buffer pH 6.8 as the aqueous phase. The both phases were mixed in equal quantities and were saturated with each other on a mechanical shaker at 37^{o}C for 24 hours. The saturated phases were separated by centrifugation at 1000 rpm. An accurately weighed amount (10mg) of both drugs was added into 10 ml each of n- octanol and aqueous phase in a screw capped tube. The aqueous phase was filtered, diluted and the amount of Acyclovir solubilized in aqueous phase was determined by measuring the absorbance at 253 using UV spectrophotometer [18].

The partition coefficient was calculated from the ratio between the concentration of drugs in organic and aqueous phase using the following equation:

$$P_{o/w} = (C_{oil} / C_{pH\ 7.4})$$

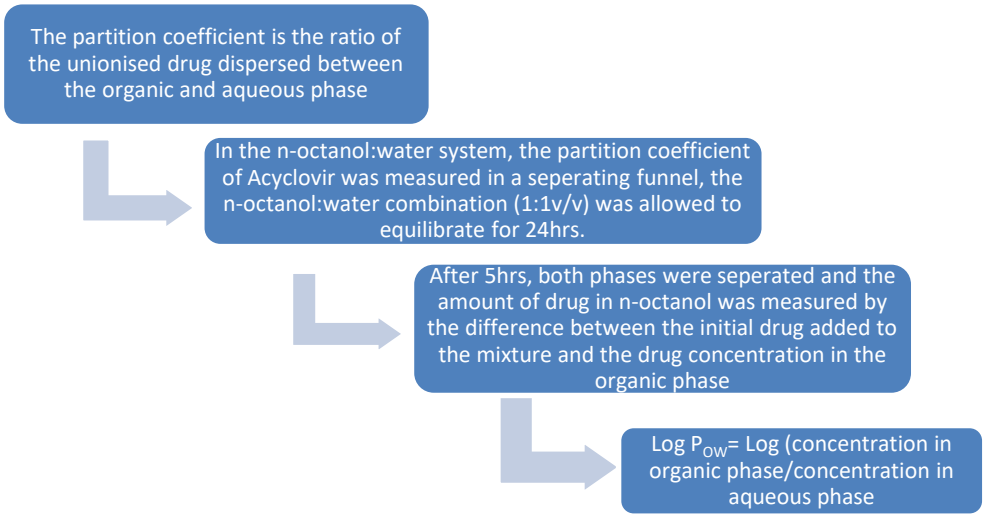


Figure 1: Flowchart for the partition coefficient evaluation.

Differential scanning calorimetry (DSC)

DSC verifies the drug nature and thermal transitions, polymorphic transitions engaged in energy variation through the formulation process. The DSC curve of Acyclovir, and formulation were assessed by DSC (Mettler Toledo stare DS822, Germany) in perforated aluminum-sealed pans at a heating rate of 5°C/min as of 10 to 340°C with Nitrogen gas (50ml/s) [19].

Particle Size Measurement

Particle size analysis was carried out by Malvonizer apparatus and sieve analysis method [20].

Stability Study

The stability study was carried out for drug and polymer mixture according to ICH guidelines. The mixture of drug and polymer were placed in screw capped glass container and stored at ICH storage ($40^0C \pm 2^0C/75\%RH \pm 5\%RH$) condition for a period of 90 days. The samples were analyzed for physical appearance and for the drug content [21].

Table 2: ICH guidelines for stability study

Study	Storage condition	Time period
Long term	$25^0C \pm 2^0C/60\%RH \pm 5RH$	12 month
Intermediate	$30^0C \pm 2^0C/65\%RH \pm 5\%RH$	6 month
Accelerated	$40^0C \pm 2^0C/75\%RH \pm 5\%RH$	6 month

Results & Discussion

Preformulation Studies

Organoleptic properties:

Identification of the procured drug sample and ensuring its purity is a prerequisite before proceeding with the formulation development. The identification tests and the inferences for the drug sample based on its appearance, solubility and odour are summarized in Table 3.

Table 3: Organoleptic properties of acyclovir

Properties	Results
Description	White or almost white powder
Taste	slightly unpleasant
Odour	Odourless
Colour	White

Melting point

The melting point determined by the capillary method is the temperature at which the last solid particle of a compact column of a substance in a tube passes into the liquid phase. Melting point of Acyclovir was observed in the following range enlisted below in table no 6.1 and get complies with the standard literature value.

Table 4: Melting Point Study of Acyclovir

Sr. No.	Drug	Observed Temperature	Literature Temperature
1.	Acyclovir	256°C	256-257°C

Solubility

The solubility of a drug is one of the most important physiochemical properties. as it is essential components of pharmaceutical development programs. The solubility depends on the physical form of the solid, the nature and composition of solvent medium as well as temperature and pressure of system. The observed solubility of plain drug in various solvent, solution is enlisted below in table no 5 for further studies.

Table 5: Observed Solubility of Acyclovir

Sr. No	Category		
	Solvent	Drug	ved Solubility (mg/ml)
1.	Methanol	Acyclovir	19.56 ± 0.056
2.	Distilled water	Acyclovir	0.2165 ± 0.0015
3.	Phosphate buffer pH6.8	Acyclovir	0.2376 ± 0.0016

Differential Scanning Calorimetry

The difference in the amount of heat needed to raise a sample's temperature is measured as a function of temperature using the DSC thermos-analytic approach. The sample holder temperature rises linearly with time thanks to the design of the temperature program for a DSC analysis. At a particular temperature, the drug and polymer's DSC thermogram displays an endothermic peak, maintaining the drug and polymer peak. The DSC thermograms of the pure drug show that there have been no phase changes indicating that the drug is chemically compatible.

Figures 2 show the thermal behavior of the pure drug. The crystalline nature of the pure medication Acyclovir is indicated by the DSC curve, which shows a prominent endothermic peak (T peak = 251.75° C) correlating to its melting. However, as seen in Figure 2, the distinctive endothermic peak that corresponds to drug melting was widened and moved towards lower temperatures with less intensity in the physical combination of Acyclovir.

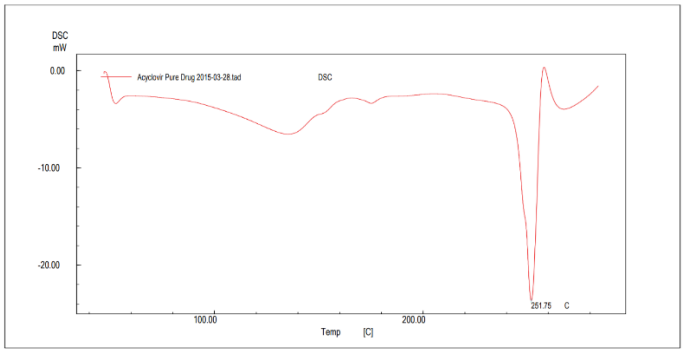


Figure 2: DSC thermogram of Acyclovir pure drug

Table 6: Observed DSC Thermograme of Acyclovir

Drug	Peak Temp (^{0}C)	Onset (^{0}C)	End set (^{0}C)	Heat (mJ)
Acyclovir	251.75	250.14	255.44	-154.57 mJ

Partition coefficient

The average melting point of pure drug is 256 ^{0}C which is complies with stander melting point of drug.

The partition coefficient is the concentration ratio of a compound in a mixture of two immiscible phases at equilibrium. These coefficients measure the compound's solubility difference in these two phases (Table 7). The concentration of Acyclovir in both phases was estimated and partition coefficient was calculated using the formula partition coefficient = concentration in the organic phase (noctanol)/ concentration in the aqueous phase (water).

Table 7: Partition coefficient of Acyclovir

Reported Log P	Observed Log P
-1.59 to -1.76	-1.64

FTIR Spectra of Acyclovir

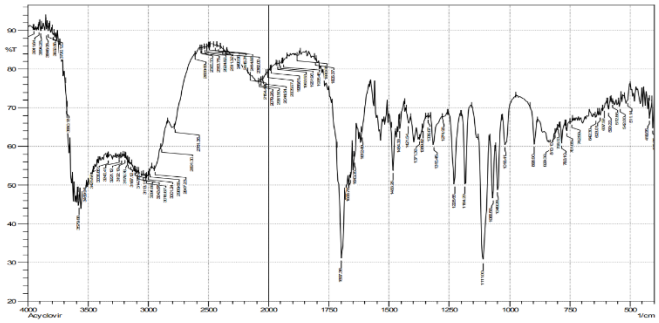


Figure 3: FTIR Spectra of Acyclovir

The analysis of the spectra for Acyclovir indicates that the experimental vibrational spectra reliably reflect the existence of specific intermolecular interactions associated with characteristic groups. The pure Acyclovir compound exhibited a broad band featuring peaks at 3167, 3336, and 3425 cm-1. This broad peak encompasses several low-intensity peaks. The observed values suggest the presence of hydrogen-bonded $–NH_2$ and OH groups. Consequently, the peaks in this region provide insights into the O-H and N-H stretching of the OH, NH_2, and NH groups. The broad band is likely attributed to the hydrogen bonding among these functional groups.

UV Spectrometric Studies

Assay and Determination of wavelength: An assay refers for qualitatively assessing or quantitatively measuring the presence or amount or the functional activity of a target entity (the analyte) which can be a drug or biochemical substance. The assay of Acyclovir was carried out by UV spectrometric method in different medium of methanol, 0.1N NaOH and phosphate buffer at PH 6.8 in strength of 2.5µg/ml concentration individually as specified in I.P. in a scanning range of 230 to 360 nm. The λ_{max} obtained are recorded in table no 8 and comparison with the literature value authenticated the study.

Table 8: λ_{max} Studies of Acyclovir

Sr. No.	Solvents (2.5µg/ml)	Experimental λmax	Literature λ_{max}	Observed absorbance
1.	Phosphate Buffer	252	251/252/253	0.714

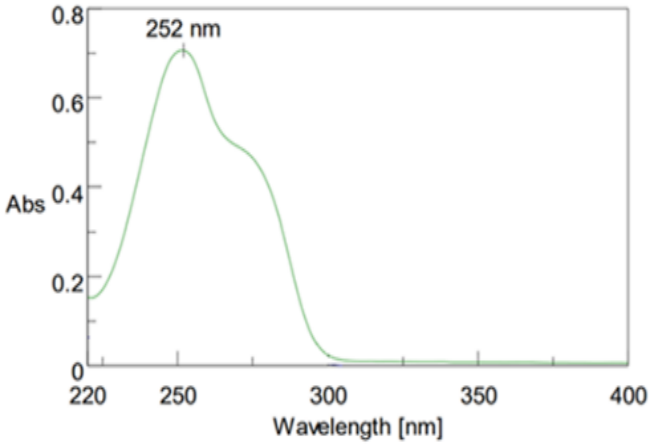


Figure 4: UV Spectrum Studies of Acyclovir

Analytical Method Development

Calibration curve for Acyclovir in pH 6.8 Phosphate buffer

Phosphate buffer pH 6.8 was used for the preparation of Acyclovir concentration and absorption was measure by Shimadzu UV-1700 UV/Vis double beam spectrophotometer. The λ max of Acyclovir was found to be 252 nm. The result was showed in the table no. 9 and figure no. 5.

Table 9: Standard Calibration Curve of Acyclovir in Phosphate buffer pH 6.8

Sr. No.	Concentration (µg/ml)	Absorbance			Average Absorbance
		1	2	3	
1	2.5	0.125	0.121	0.130	0.125
2	5.0	0.250	0.255	0.252	0.257
3	7.5	0.377	0.378	0.380	0.374
4	10.0	0.502	0.499	0.498	0.507
5	12.5	0.633	0.630	0.631	0.627
6	15.0	0.762	0.763	0.760	0.767
Correlation Co-efficient (R^2) = 0.9995 **Absorbance (y)** = 0.0509_x conc - 0.0025					

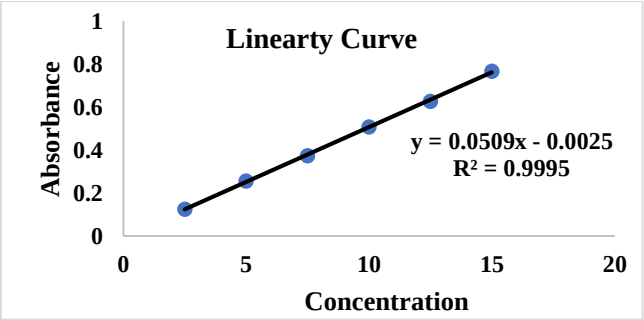


Figure 5: Drug calibration curve in Phosphate buffer pH 6.8

Calibration curve of Acyclovir in 0.1 N HCl

Acyclovir concentration was prepared in 0.1 N HCl and absorption were measure by Shimadzu-1700 UV/Vis double beam spectrophotometer. The λ max of Acyclovir was found to be 252 nm.

Table 10: Standard Calibration Curve of Acyclovir in 0.1 N HCl

Sr. No.	Concentration (µg/ml)	Absorbance			Average Absorbance
		1	2	3	
1	2.5	0.125	0.121	0.130	0.127
2	5.0	0.250	0.255	0.252	0.252
3	7.5	0.377	0.378	0.380	0.378
4	10.0	0.502	0.499	0.498	0.500
5	12.5	0.633	0.630	0.631	0.631
6	15.0	0.762	0.763	0.760	0.762
Correlation Co-efficient (R^2) = 0.9998 **Absorbance (y)** = 0.0507_x conc - 0.0017					

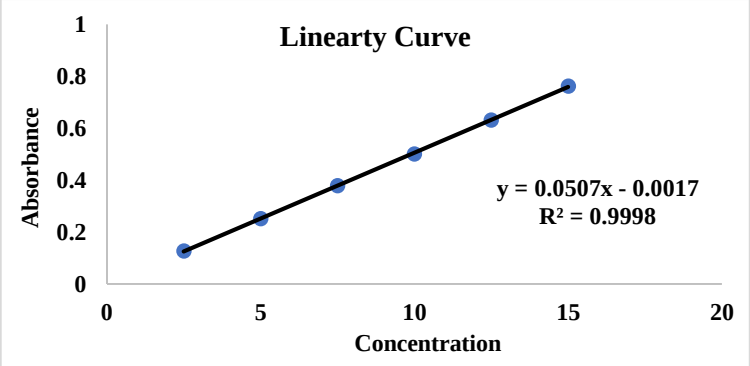


Figure 6: Drug calibration curve in 0.1 N HCl

Drug excipients compatibility study by FTIR spectroscopy

Drug excipients compatibility study of Acyclovir by FTIR spectroscopy

The FTIR spectrums of acyclovir alone and with excipients were shown in Fig.7. The detected peaks of Acyclovir were interpreted below.

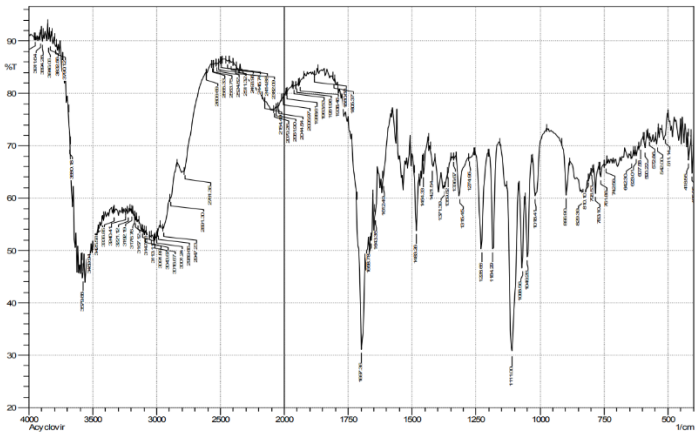


Figure 7. Interpretation of FTIR spectrum of Acyclovir

Different spectral peaks of FTIR spectra of acyclovir includes- 3949.5, 3847.29, 3791.37, 3669.87, 3563.81, 3444.24, 3347.82, 3251.4, 2856.06, 2763.49, 2350.8, 1714.41, 1594.84, 1482.99, 1421.28, 1307.5, 1143.58, 1083.8, 869.73, 779.1, 682.67, 680.469, 636.11

FTIR, or Fourier Transform Infrared Spectroscopy, is a helpful tool for identifying the chemical makeup of substances. When looking at acyclovir, a common antiviral drug, FTIR shows several important peaks. These peaks represent different parts of the acyclovir molecule. For example, there's usually a strong peak around 3200 cm^{-1}, which comes from the O-H and N-H stretching in the molecule. Another noticeable peak appears near 1700 cm^{-1}, showing the C=O bond stretching. The region between 1600-1500 cm^{-1} often has peaks related to the ring structures in acyclovir. Peaks in the 1300-1000 cm^{-1} range typically come from C-N and C-O stretching. These FTIR peaks helps to confirm that a sample is indeed acyclovir and check its purity.

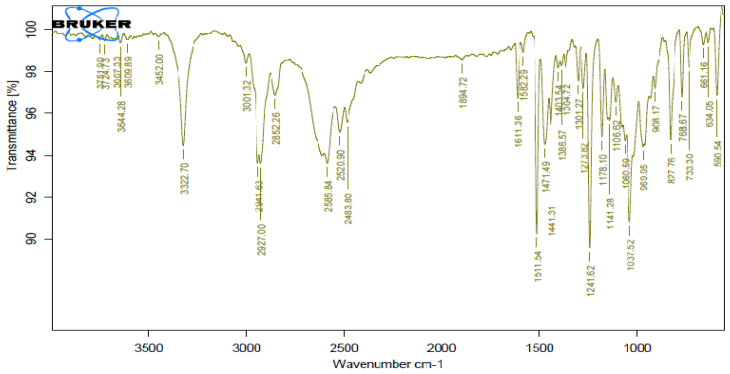


Figure 8: FTIR of Carbapol

Carbopol, a popular polymer used in various industries, shows distinct peaks in FTIR (Fourier-transform infrared spectroscopy) analysis. These peaks help identify the chemical structure of Carbopol. The most noticeable peak appeared around 1700 cm^{-1}, which represents the stretching of the carbonyl group (C=O). Another important peak is seen near 2950 cm^{-1}, indicating the presence of C-H bonds. A broad peak around 3300 cm^{-1} suggests the presence of hydroxyl groups (O-H). The fingerprint region, between 1500 and 500 cm^{-1}, contains several smaller peaks that are unique to Carbopol's structure. These peaks help scientists and researchers confirm the identity and purity of Carbopol samples. By looking at these FTIR peaks, experts can quickly determine if a sample is indeed Carbopol and if it has the expected chemical makeup.

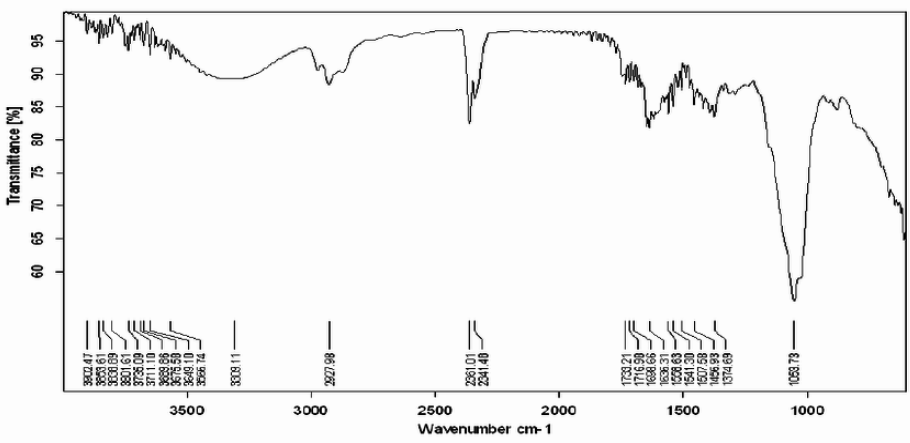


Figure 9: FTIR spectra of Xanthan gum

Spectral Analysis of Xanthan Gum

Xanthan gum is a commonly used food additive that can be studied using FTIR spectroscopy. When looking at the FTIR peaks of xanthan gum, we can see several important features. The spectrum typically shows a broad band around 3400 cm^{-1}, which is due to the stretching of O-H bonds in the molecule. Another significant peak appears near 2900 cm^{-1}, representing C-H stretching. The region between 1000 and 1200 cm^{-1} is particularly interesting, as it contains peaks related to C-O-C and C-O stretching in the sugar rings of xanthan gum. Additionally, a peak around 1600 cm^{-1} can be observed, which is associated with the carboxylate groups in

the molecule. These characteristic peaks help scientists identify and analyze xanthan gum in various products and studies.

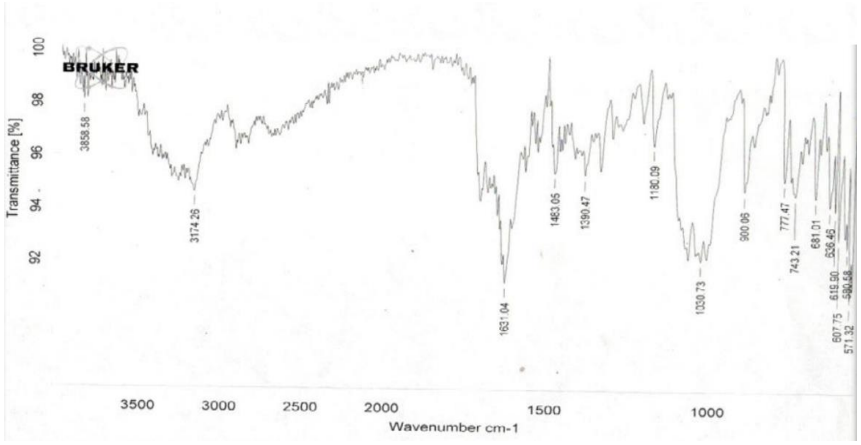


Figure 10: FTIR spectra of Acyclovir - Xanthan gum physical mixture

FTIR analysis of acyclovir mixed with xanthan gum shows distinct peaks that help identify the drug and its interaction with the gum. The main peaks of acyclovir appear around 3400 cm^{-1} for O-H and N-H stretching, 1700 cm^{-1} for C=O stretching, and 1630 cm^{-1} for C=N stretching. When combined with xanthan gum, some of these peaks may shift slightly or change in intensity. Xanthan gum itself shows peaks around 3400 cm^{-1} for O-H stretching and 1000-1200 cm^{-1} for C-O stretching. The mixture of acyclovir and xanthan gum might show a broader peak in the 3400 cm^{-1} region due to the overlap of O-H groups from both compounds. This FTIR analysis helps to know how acyclovir interacts with xanthan gum, it indicates no physico-chemical interaction between them.

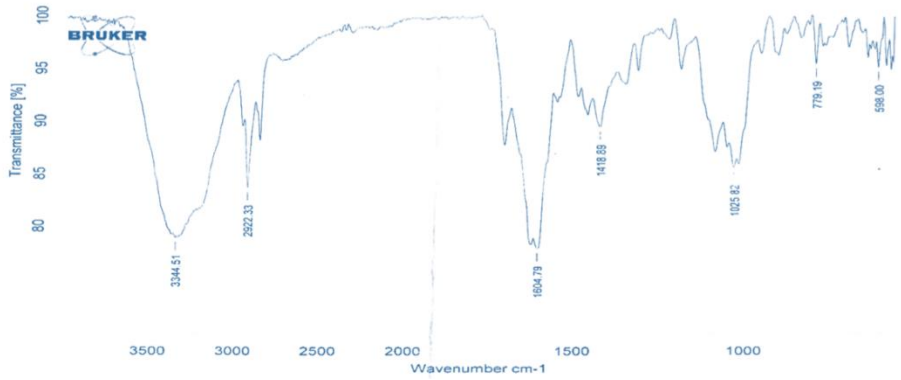


Figure 11: FTIR spectra of Acyclovir - Carbopol physical mixture

Standards peaks (cm^{-1})	Standard peaks (cm^{-1})	Peak Assigned
3050-3000	3040	O-H
1650- 1600	1625	C=C
3000-2840	2810	CH_3
1050-1000	1040	C-F
1750-1700	1730	C=O

1392-1360	1360	C-H

FTIR analysis of acyclovir mixed with Carbopol 940 shows distinct peaks that help identify the drug and polymer. The spectrum reveals characteristic bands for both components. Acyclovir typically displays peaks around 3440 cm^{-1} for O-H and N-H stretching, 1695 cm^{-1} for C=O stretching, and 1630 cm^{-1} for C=N stretching. Carbopol 940 exhibits a broad peak near 3000-3500 cm^{-1} for O-H stretching and a strong peak around 1700 cm^{-1} for C=O stretching of the carboxylic acid groups. When combined, these peaks may shift slightly or change in intensity due to interactions between acyclovir and Carbopol 940. The FTIR spectrum of the mixture helps confirm the presence of both substances and indicates no physico-chemical interaction between them.

6.1.1 Stability Study

The physical mixture was stability study. It is evident from the results that each physical mixture was found to be stable based on physical description (color) and % drug release up to 90 days at ACC storage condition, which is hopefully the most promising formulation of present invention.

Table 11: Stability of physical mixture

Formulation Coad	Temp (ºC)/ RH (%)	Time (months)	Appearance	Particle size* (nm)	PDI*	Zeta Potential* (mV)	Drug content (mg/ml)
PM of Xanthan Gum	$40^0C±2^0$ C/ 75% RH±5% RH	1	White	90.1 ± 2.1	0.172±0.003	-21.2±1.9	250
		2	White	91.2 ± 1.9	0.175±0.007	-21.1±2.0	250
		3	White	93.9 ± 2.0	0.181±0.009	-20.9±1.5	250
PM of Carbopol 940		1	White	386.1±1.9	0.183±0.008	-21.2±1.6	250
		2	White	388.5±1.6	0.184±0.006	-21.1±1.7	250
		3	White	387.9±1.6	0.185±0.002	-21.2±1.5	250

The developed formulations were found to be stable for 3 months at 30±2ºC/65 ± 5% RH. No change in the physical appearance of the formulation was observed during the stability studies. No significant change in the Z-average size, PDI and zeta potential were observed during the stability studies when analyzed using student's t-test.

Conclusion

The initial phase of the study involves the preformulation studies which mainly includes organoleptic properties of the drug i.e., Acyclovir (ACV). The melting point of the drug was evaluated and it was found to be 256^0C. The UV spectral shows peak absorbance at 252nm, that complies the purity of Acyclovir.

Pre-formulation experiments were conducted in the first phase to ascertain the drug's organoleptic characteristics, solubility and its melting point for Acyclovir. The medication satisfies all physico-chemical and organoleptic requirements. The drug's solubility was assessed, and it was discovered to be soluble in distilled water 0.2165 mg/ml, in Phosphate buffer pH6.8 0.2376 mg/ml and the drug shows excellent solubility in Methanol 19.56 mg/ml.

Acyclovir melting point was determined to be 256°C, falling between 256-257°C.

When the pure medication was subjected to UV spectroscopy, the absorbance was found to be to be 252 nm. The drug was determined to be compatible with all of the hydrotropic agents employed in the formulations based on FT-IR analysis of the pure drug and in combination with hydrotropic agents to determine the drug's vibrational frequencies.

Acyclovir was calibrated in 0.1 N HCL and Phosphate buffer pH6.8 to measure the drug's absorbance at 252 nm at various concentrations. The absorbance of the sample rises as the drug concentration does, indicating that the drug satisfies Beer's Lambert's Law, which states that the absorbance of the drug is directly proportional to the drug concentration. In order to comprehend the medication's solubility and release in the presence of several hydrotropic agents, the calibration of the drug in conjunction with these agents was also established.

The FTIR study of drug alone and in combination with excipients were done to justify the purity of the drug and the excipients and also to study any physical and chemical interaction between them. According to compatibility study, the drug shows no interaction with the polymers used in the formulations.

Infrared spectra of Acyclovir showed sharp characteristic peaks 3167, 3336, and 3425 cm^{-1} assigned to of hydrogen-bonded $–NH_2$ and OH groups stretching vibrations respectively, display a downward due to intermolecular hydrogen bond type interactions. Also, presence of OH, NH_2, and NH groups at 1737.70 cm^{-1}.

Also to study any physical and chemical interaction between them. According to compatibility study, the drug shows no interaction with the polymers used in the formulations. The DSC thermogram of Acyclovir exhibited characteristic sharp endothermic peak at 251.75 ºC respectively. The partition coefficient Log P of Acyclovir was observed -1.64 respectively.

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