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"Formulation and Evaluation of Nano-Gel Using Effective Combination of Acyclovir and Omeprazole for Enhanced Anti-Viral Activity"

Mayuresh Tambade [1], Shweta Shriwas[2], Dr. Rakesh Kumar Jatav[3]
[1]*PG Scholar, School of Pharmacy, Dr. A.P. J. Abdul Kalam University, Indore*
[2]*Associate, Professor, School of Pharmacy, Dr. A.P. J. Abdul Kalam University, Indore.*
[3]*Professor and Principal, School of Pharmacy. A.P. J. Abdul Kalam University, Indore.*

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ABSTRACT:
The aim of the research is to formulate nano transdermal using effective combination ofacyclovir and omeprazole to enhanced anti-viral activity to evaluate the Preformulating characters to formulate acyclovir and omeprazole nanogel by solvent diffusion method to Characterization and evaluation of formulated nanogel compare the *in-vitro* drug release with marketed formulation. It can be concluded that the experimental study carried out that the formulation of a Nanogel containing anti-viral drug and anti-ulcer drug yields a formulation with spherical and smooth surface, nano in size range. The prepared nanogel was opaque, without any lumps, particle and aggregates. So, all the formulations are homogenous. Based on all the factors the nanogel drug delivery system F9 shows good drug content compare to other. The particle size of the nanogel formulation is optimum and it is less than 1000 nm. So, it concluded that the particles are in tiny and nano in size range. All nanogel formulations shows pH in the range of 6.1 to 6.9. Formulation F9 shows highest pH of 6.9. Because the pH range of nanogel were 1 to 7 pH. Based on the Spreadability diameter study it shown the nanogel is having good Spreadability. Nanogel formulations shown viscosity range from 3268-3528 cps. It concluded that they are stable in nature. Formulation F9 shows highest percentage of drug release compare to other formulations. In-vitro diffusion studies show F9 formulation shows controlled release pattern of drug from the formulation. The formulation was found to be stable in short term stability studies. Here we have selected F9 has an optimized formulation which shown good morphological features, drug content efficiency and controlled drug release. Hence the F9 formulation is efficient than the marketed formulation of acyclovir ointment (ACIVIR).

KEYWORDS: Nano Transdermal, Acyclovir, Enhanced Anti-Viral Activity

I. INTRODUCTION

1.1 VIRUS

The word "virus" is derived from the Latin word for poison. Viruses are associated with all forms of life (bacteria, archaea, and eukaryotes). Viruses are infectious, obligate intracellular parasites whose genomes consist of either DNA or RNA.Virus genomes direct their own replication and the synthesis of other viral components, using cellular systems in appropriate host cells.

Virus particles (known as virions) are formed by assembly from newly synthesized components within the host cell. Virions are the vehicle for transmission of the genome to the next host cellor organism, where their disassembly initiates the beginning of the next infectious cycle. A minimal virus consists of a genome that has an origin of replication, plus a protein aceous coat,known as a capsid.

For enveloped viruses, the capsid is enclosed in a host cell-derived lipid bilayer studded with virus-specified glycoproteins. Viruses are dependent on host cells for biosynthesis of proteins and other critical macromolecules

1.2VIRUS TAXONOMY AND NOMENCLATURE

Taxonomy is a relational discipline that classifies organisms according to shared and distinguishing properties. The various virus lineages appear to have independent evolutionary origins, thus there is no overriding phylogeny for viruses.The two major classification schemes used for viruses are the comprehensive formal taxonomy developed over the past 40-plus years under the aegis of the International Committee for Taxonomy of Viruses (ICTV), and a scheme developed by David Baltimore (the Baltimore system) in which viruses are grouped on

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the basis of the path from their genome type to production of translatable mRNA. The ICTV system will be discussed in this section and the Baltimore system will be discussed in the section on virus replication cycles.Within the ICTV system [1], the two major taxonomic divisions are the viruses with RNA genomes and those with DNA genomes. Subsequent taxonomic levels are based on the size and structure of the capsid (icosahedreal, helical, or complex), whether the capsid is enveloped,and then the nature of the genome (single-stranded or double-stranded, linear or circular,segmented or non-segmented). This information is sufficient to define the major groups of genetically distinct viruses into families, with some families being grouped into orders Families are subdivided into genera, which are collections of related but distinct virus species. Some large families are divided into subfamilies that are then divided into genera. Subfamilies, genera, and species are defined by properties such as gene organization, replication mechanism,susceptibility to physical stresses and chemical agents, cell tropism, and immunologic andpathogenic propertie

II. MATERIALS AND METHODS

S.No	Ingradients	Vendor
1	Acyclovir	Mercury Medicare(gift sample)
2	Omeprazole	Enal drugs pvt .Ltd (gift sample)
3	Carbopol 940	Himedia laboratories pvt.ltd.
4	Tragacanth gum	Loba chemie pvt .ltd
5	Propylene glycol	Nice chemicals pvt. ltd
6	Ethanol	Changshu hongsheng fine chemical co.ltd
7	Triethanolamine	Thermo fisher scientific india pvt.ltd

Preformulation studies involve physical, chemical and biological characterization of new drug substances in order to develop stable, safe and effective dosage form. Preformulation testing encompasses all studies enacted on a drug compound in order to produce useful information for subsequent formulation of a stable and bio-pharmaceutically suitable drug dosage form.

III. Physical characteristics

By visual examination the drug was tested for its physical characters like colour, odour andtexture.

Melting Point:

The digital melting point apparatus was used to determine the melting point of drug. A capillary tube was taken and fused at one side with the help of a Bunsen burner. The drug acyclovirand omeprazole was introduced into the capillary tube through the unsealed end and then placed in a melting point viewer. Then the temperature at which drug starts melting was considered as themelting point of the drug[131].

Solubility test:

Acyclovir and Omeorazole powder (about 1mg) was taken in a test tube and solubility in ethanol, water, PH buffer 7.4 and methanol was tested [137].

Determination of λ max:

10 mg of accurately weighed acyclovir and omeprazole was dissolved in 10 ml of 7.4 pH buffer in a 100 ml volumetric flask. It was made up to 100 ml by using distilled water to get a concentration of 100 µg/ml (Stock A). From the above stock solution A, concentration of 2µg/mlwas prepared by pippeting 0.2 ml and made up to 10 ml using the medium. The solution was scanned by using double beam UV visible spectrophotometer between the wavelength ranges of 200 nm to 400 nm[137

10 mg of accurately weighed acyclovir and omeprazole was separately dissolved in 10 mlof 7.4 pH buffer and distilled water in a 100 ml using phosphate buffer pH 7.4 to get a concentrationof 100 µg/ml (Stock A). From the above stock solution A, concentration ranges from 2µg/ml to 10µg/ml was prepared by pippeting 0.2 ml to 1 ml. It was made up to 10 ml using medium. The absorbance of each concentration was analyzed in the UV visible double beam Spectrophotometer at 252 nm and 305 nm respectively. The correlation coefficient (r^2) was determined from the graph. A calibration curve was plotted with concentration on the x-axis and absorbance on the Y-axis. [146].

FT-IR Studies (Drug- Polymer Compatibility):

Drug polymer compatibility was determined by KBr pellet method using Fourier Transform Infrared

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Spectrophotometer. The samples were prepared by KBr pellet press method and it was scanned between 400-4000 cm^{-1}. [145].

IV. FORMULATION OF ACYCLOVIR AND OMEPRAZOLE NANOGEL

Preparation of acyclovir and omeprazole Nanogel: Acyclovir and omeprazole nanogel prepared by Nano solvent diffusion method Accuratelyweighed quantity of drug is dissolved in ethanol and propylene glycol with stirring (organic phase). In the second step aqueous phase is prepared by using Carbopol -940 dissolved in water with continuous stirring and heat for a 20 min in a magnetic stirring. And the drug phase is sonicated under ultrasonic bath Sonicator for 10 min. On next step drug phase is added drop by drop into aqueous phase during high speed homogenization for 30 min at 6000 rpm to from emulsion. The emulsion is converted into nanodroplet by homogenizer results in o/w emulsion formed. Then o/wemulsion is homogenized for 1 h at 8000 rpm and triethanolamine is added with continues stirring to from nanogel (using a combination of ultra-sonication and high speed homogenization). Carbopol and tracaganth were used as a gel forming polymer which were taken individually and in combination. The formulation code has been shown in table :4 .[137].

Table 4: Formulation Table of Nanogel.

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Acyclovir	5	5	5	5	5	5	5	5	5
Omeprazole	160	160	160	160	160	160	160	160	160
Tracaganth	200	400	600	800	-	-	-	-	400
Carbopol	-	-	-	-	200	400	600	800	400
PPG	4	4	4	4	4	4	4	4	4
Triethanolamine	4	4	4	4	4	4	4	4	4
water	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

Fig- 4: Nanogel formulation of acyclovir and omeprazole

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V. RESULTS AND DISCUSSION

PREFORMULATION STUDIES

Physical Characteristics

Acyclovir And Omeprazole was checked for its colour, odour and texture. Acyclovir AndOmeprazole is White colored powder in appearance, odourless and amorphous in nature.

Melting Point:

Melting point of acyclovir was determined by capillary tube method and it was found to be256^{0}C respectively, which confirms the purity of the drug.

Melting point of omeprazole was determined by capillary tube method and it was found to be156^{0}C respectively, which confirms the purity of the drug.

Solubility studies:

The solubility of Acyclovir and omeprazole in various solvents like water, methanol, ethanol andpH 7.4 was done. The results shows that the Acyclovir drug is sparingly soluble in water , methanol and its highly soluble in buffer PH 7.4 and ethanol . Omeprazole is sparingly soluble inethanol and its highly soluble in water, methanol and buffer pH 7.4.

Solubility Studies Data of Acyclovir and Omeprazole nanogel

S.No	Acyclovir with solvent	Inference	Omeprazole with solvent	Inference
1	Water	Sparingly soluble	Water	Soluble
2	Methanol	Sparingly soluble	Methanol	Soluble
3	buffer pH 7.4	Soluble	buffer pH 7.4	Soluble
4	Ethanol	Soluble	Ethanol	Sparingly soluble

Determination of λ max:

The highest concentration (µg/ml) from the dilution was chosen for determination of λ max. Theλ max of acyclovir was found to be 251 nm.

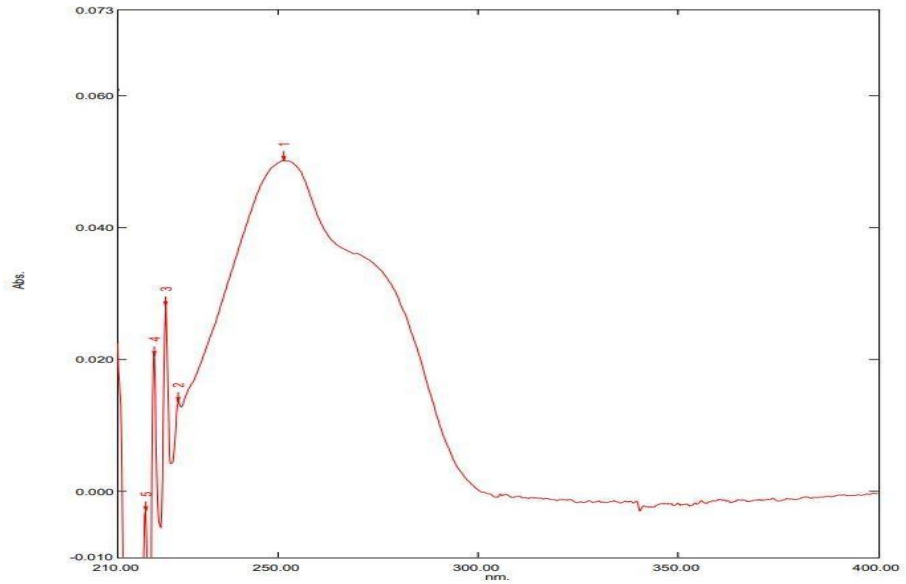

Fig- 5 : λ max of acyclovir

7.1.1 Determination of Omeprazole λ max:

❖ The highest concentration (µg/ml) from the dilution was chosen for determination of λmax. The λ max of omeprazole was found to be 302 nm.

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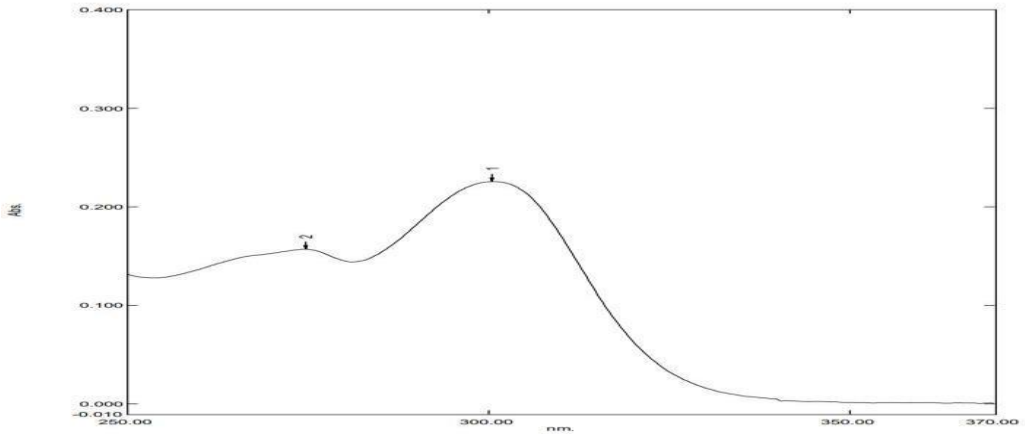

Fig-6: λ max of Omeprazole

Determination of Standard graph:

Standard graph was constructed with concentration of 2 to 10 µg/ml. The absorbance was determined corresponding to their concentration were shown in table 6 . Correlation coefficient was found to be r^2=0.9918 which shows standard graph was linear.

Table 6: Calibration data of Acyclovir

S.No	Calibration data	Absorbance at 251 nm
1	2	0.050
2	4	0.118
3	6	0.202
4	8	0.247
5	10	0.307

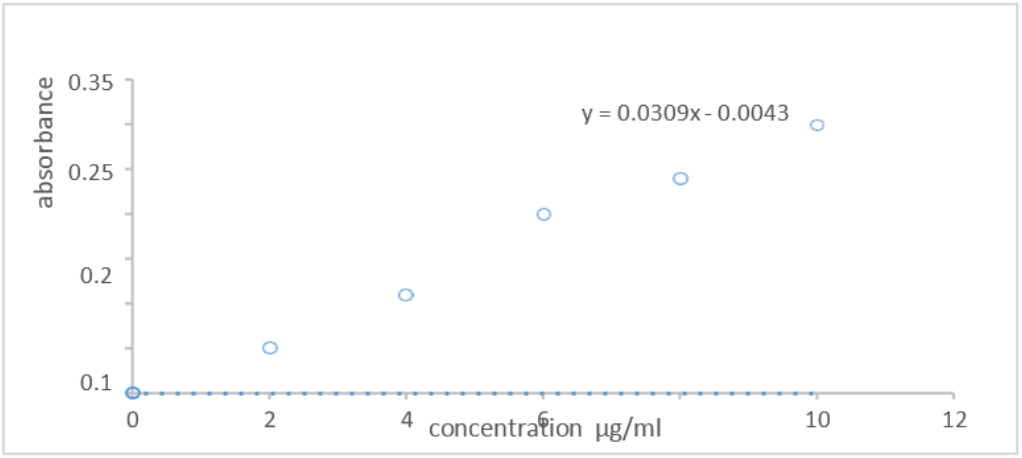

Fig 7: Standard graph curve of acyclovir

Determination of Standard graph:

Standard graph was constructed with concentration of 2 to 10 µg/ml. The absorbance was determined corresponding to their concentration were shown in table 7 . Correlation coefficient was found to be r^2=0.9872 which shows standard graph was linear.

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Table 7: Calibration data of Omeprazole

S.No	Calibration data (µg/ml)	Absorbance at 305 nm
1	2	0.116
2	4	0.312
3	6	0.452
4	8	0.647
5	10	0.870

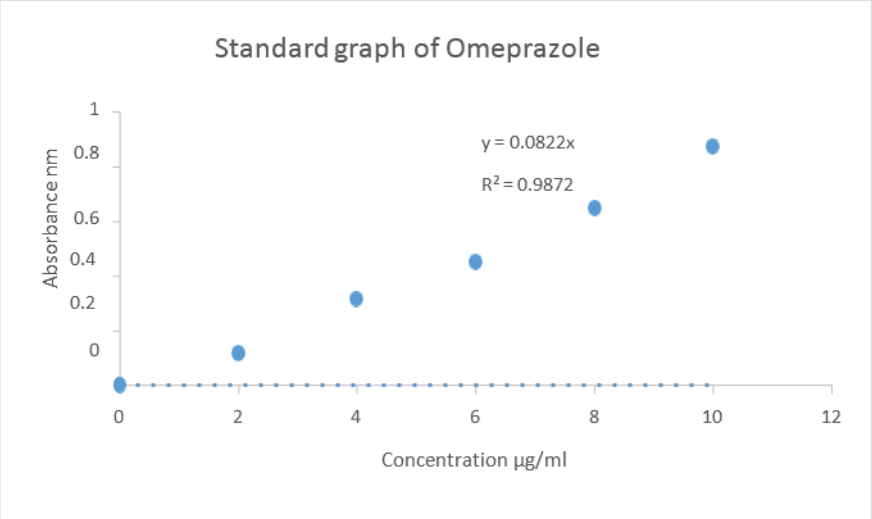
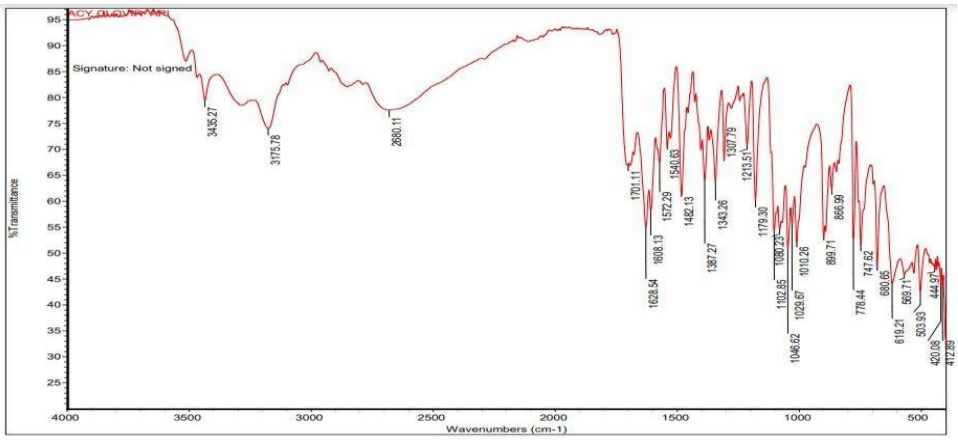


Fig-8 : Standard graph of Omeprazole

Excipient Compatibility Studies :

FT IR Study:
Drug excipients compatibility study was performed by FT IR.There is no incompatibilityobserved with the drug and excipients used in the formulation.

Acyclovir:



FT-IR of Acyclovir

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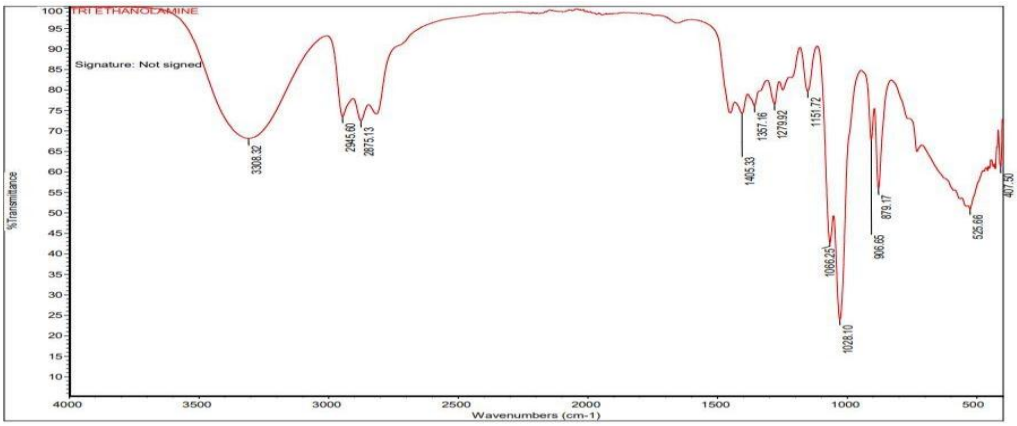
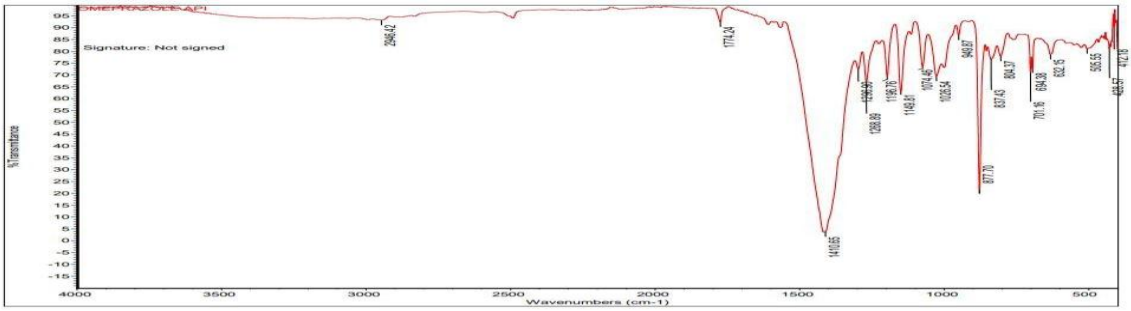
8:FT- IR data of Acyclovir

Sl. No.	Wave number cm^{-1}	Assignment
1	3435.27	NH stretching
2	3175.78	OH stretching
3	2680.11	CH stretching
4	1213.51	C – O stretching
5	1701.11	C=O stretching
6	1628.54	C=N stretching



7.5.1 Omeprazole:



7.5.1 Carbapol 940

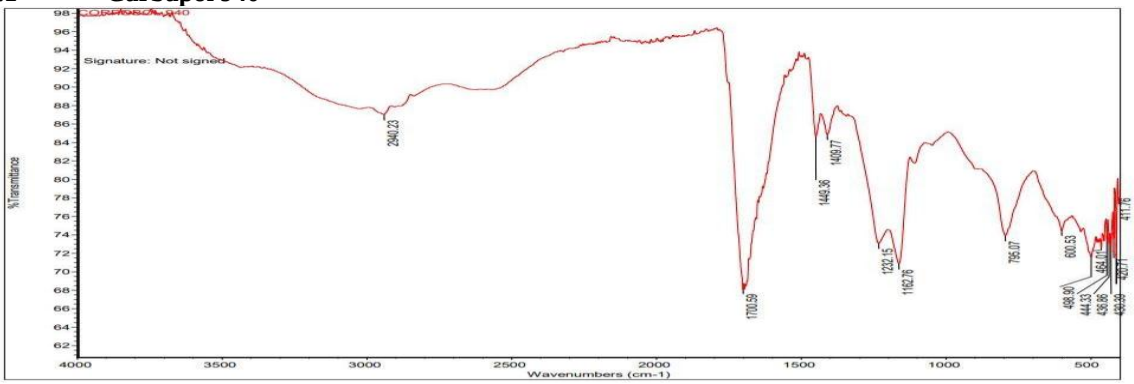


Fig- 11 : FT-IR of Carbapol-940

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7.5.2 Tri-ethanolamine:

7.5.3 Acyclovir Nanogel:

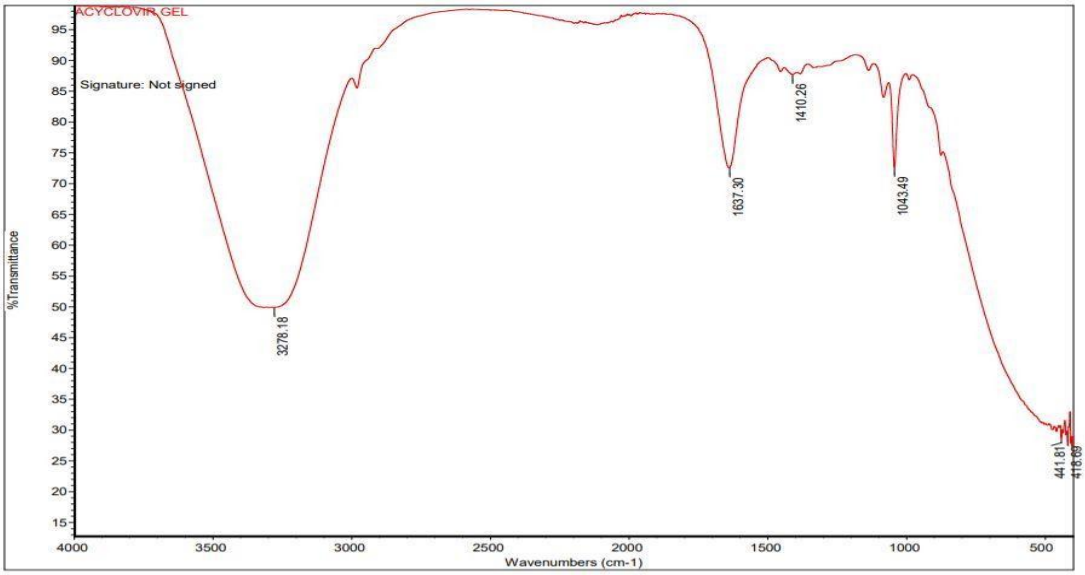

Fig- 12: FT-IR of Acyclovir Nanogel

7.5.4 Acyclovir + Omeprazole Nanogel :

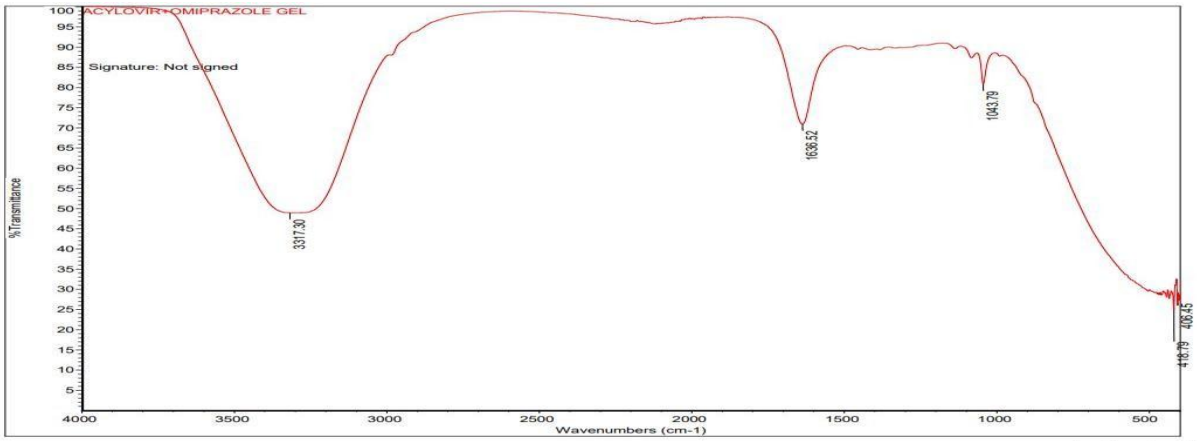

Fig-14 : FT-IR of Acyclovir and Omeprazole Nanogel

7.6 FORMULATION OF NANOGEL:

Selection of polymers for the formulation of Acyclovir and Omeprazole by emulsion solvent diffusion method was based on the trial batches carried out by using different polymers such as carbopol, triethanolamine, PPG in Table Drug: polymer ratio was selected based on the literature.The results indicated that F9 was found to be suitable for the formulation of Acyclovir and Omeprazole

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Table 14: Trial batches for formulation of Acyclovir and Omeprazole

Drug	Formulation code	Observed
Acyclovir and Omeprazolenanogel	F1	Light yellow
	F2	Solid in nature
	F3	Brown
	F4	Liquid in nature
	F5	Pale yellow
	F6	Pale orange
	F7	Semiliquid
	F8	Hard gel
	F9	transparent gel

7.5 CHARACTERISATION OF ACYCLOVIR AND OMEPRAZOLE NANOGEL:

7.5.1 Determination of pH

The pH of different formulation from F1 to F9 were showed in Table No. 15. The pH varies fromone formulation to another according to their polymer ratios with drug.

7.5.2 Homogeneity

All the gel formulations (F1-F9) showed good homogeneity with absence of lumps. Gels were found to be transparent and were free from presence of particles, uniformity of gel, aggregates, foreign matter and phase separation. Results are shown in Table No. 15.

7.5.3 Spreadability

Spreadability diameter for different formulations F1-F9 showed good spreadability i.e. gel is easily spreadable. The results are shown in Table No. 15.

7.5.4 Viscosity

All the formulations of Nanogel were subjected to Brookfield viscometer used to measure the viscosity (in cps) by dropping a cone attached to a holding rod from distance of 10 cm in such a way that, it should fall on center of the glass cup filled with Nanogel.

Table No 15: Evaluation of formulated batches of Nanogel.

Formulation code	**pH**	**Homogeneity**	**Spreadibility**	**Viscosity**
F1	6.1	Homogenous	2.5	3459
F2	6.3	Homogenous	3.5	3356
F3	6.2	Homogenous	2.9	3268
F4	6.5	Homogenous	3.4	3498
F5	6.2	Homogenous	2.6	3295
F6	6.3	Homogenous	2.9	3501
F7	5.9	Homogenous	3.2	3340
F8	6.4	Homogenous	2.8	3351
F9	6.9	Homogenous	3.5	3528

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7.5.5 Percentage yield analysis

Table 16: Percentage yield of Acyclovir and Omeprazole Nanogel

S. No.	Formulation code	Percentage yield (%)
1	F1	65.76
2	F2	32.55
3	F3	34.14
4	F4	44.56
5	F5	68.89
6	F6	71.18
7	F7	86.80
8	F8	78.25
9	F9	96.02

The percentage yield was minimum for formulation F2 (32%) and maximum for formulation F9 (96.02%).From the results we can conclude that as the F9 has the highest percentage yield . The percentage yield of all formulations is depicted in Figure 16 and table 16

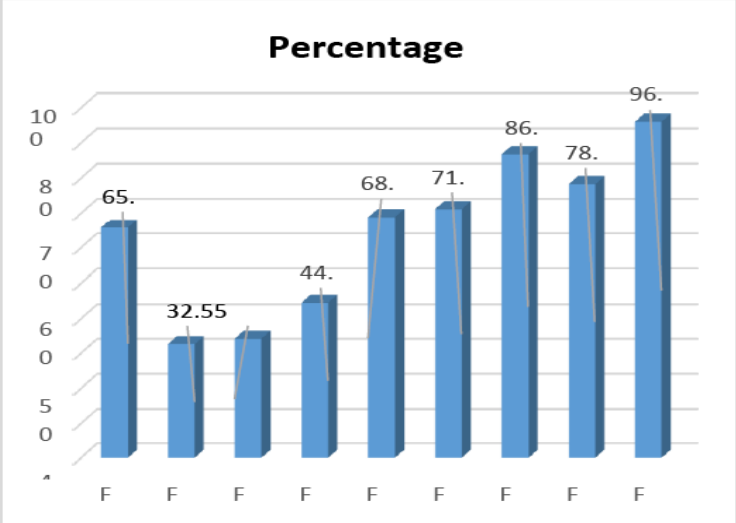

Fig 15 : Percentage yield of Acyclovir And Omeprazole Nanoge

7.6 Drug content

Table 17: Drug content of Acyclovir And Omeprazole Nanogel

S.No.	Formulation code	Drug content (%)
1	F1	60.54
2	F2	29.00
3	F3	30.84
4	F4	40.55

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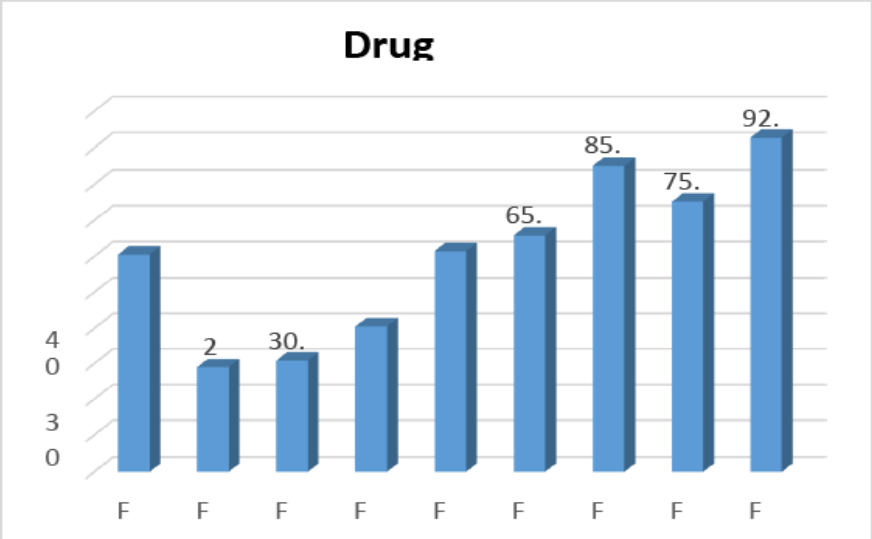
5	F5	61.45
6	F6	65.78
7	F7	85.11
8	F8	75.24
9	F9	92.65



7.7 *IN VITRO* DRUG RELEASE STUDIES

In vitro drug release study of the prepared Acyclovir and Omeprazole Nanogel was carried out using cellophane memberane by frantz diffusion cell. Amount of drug released in different time intervals were observed.

Table 18:*In vitro* drug release profile of Acyclovir and Omeprazole Nanogel (F1-F3)

S.NO	**TIME (h)**	**Cumulative percentage drug release (%)**					
		F1		**F2**		**F3**	
		A	**O**	**A**	**O**	**A**	**O**
1	**0**	0	0	0	0	0	0
2	**1**	3.4	0.5	1.2	1.1	1.5	1.5
3	**2**	12.5	9.5	8.8	7.2	10.6	8.8
4	**4**	25.2	20.8	24.5	15.4	18.7	17.3
5	**6**	43.8	35.1	40.5	27.9	29.3	23.9
6	**8**	53.6	50.5	50.9	43.5	35.6	39.6
7	**12**	60.4	55.7	55.3	51.4	42.1	46.4
8	**16**	65.5	60.8	60.4	69.6	56.6	52.5
9	**20**	79.9	70.0	62.9	72.3	65.2	65.7
10	**24**	80.1	75.9	70.4	75.7	72.7	69.1

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Fig 17:*In vitro* drug release profile of Acyclovir and Omeprazole Nanogel (F1-F3)

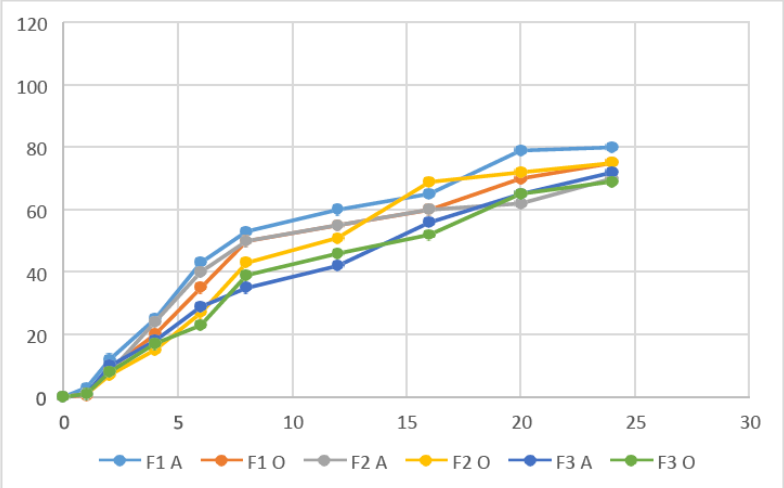

Table 19: *In vitro* drug release profile of Acyclovir and Omeprazole Nanogel (F4-F6)

S.NO	TIME (h)	Cumulative percentage drug release (%)					
		F4		F5		F6	
		A	O	A	O	A	O
1	0	0	0	0	0	0	0
2	1	2.2	1.5	1.5	3.8	8.1	5.2
3	2	8.6	12.4	11.1	15.5	17.6	12.2
4	4	20.8	24.1	23.3	28.9	30.2	23.8
5	6	33.5	33.8	32.9	45.2	38.8	31.3
6	8	46.5	48.9	41.5	51.1	41.5	43.8
7	12	55.2	56.1	52.7	65.8	48.2	51.1
8	16	61.9	66.6	61.2	69.7	51.8	63.8
9	20	68.8	73.4	73.8	71.3	68.3	68.2
10	24	71.5	75.2	75.4	78.8	75.4	72.8

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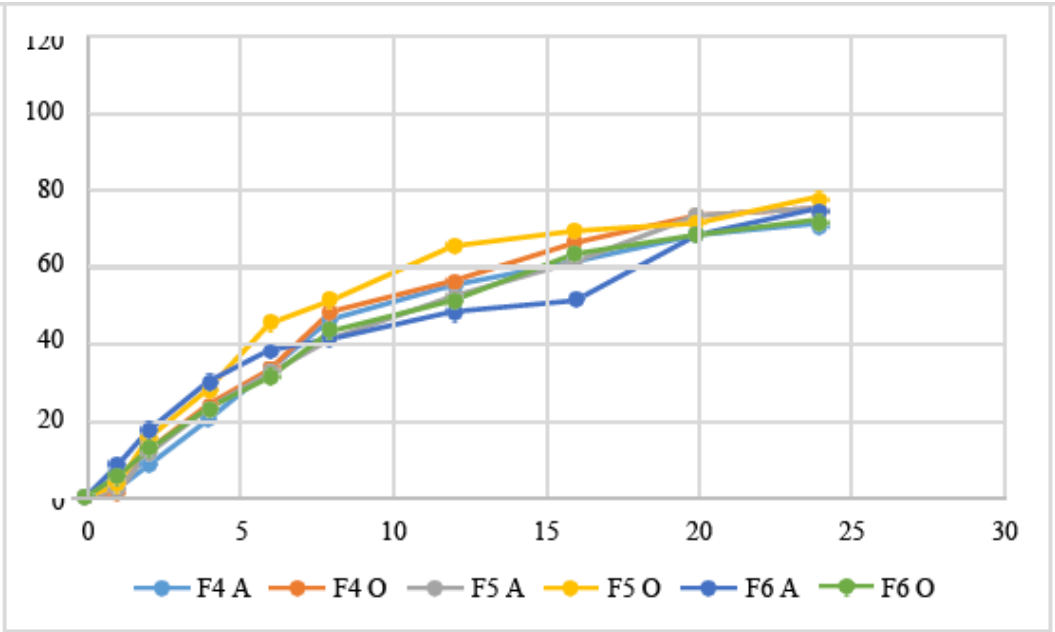

Fig 18: *In vitro* drug release profile of Acyclovir and Omeprazole Nanogel (F4-F6).

Table 20: *In vitro* drug release profile of Acyclovir and Omeprazole Nanogel (F7-F9)

S.NO	TIME (h)	Cumulative percentage drug release (%)					
		F7		F8		F9	
		A	O	A	O	A	O
1	0	0	0	0	0	0	0
2	1	2.4	4.7	3.7	1.4	15.2	8.8
3	2	10.2	12.5	11.5	9.5	23.5	17.6
4	4	26.5	28.1	27.6	25.4	39.2	35.2
5	6	45.3	47.8	46.4	44.6	48.8	45.5
6	8	55.8	57.4	56.1	54.7	61.5	58.2
7	12	60.5	62.7	61.8	59.3	73.1	72.6
8	16	66.5	68.2	67.1	65.5	81.6	83.2
9	20	79.9	81.9	80.4	78.8	92.4	93.5
10	24	80.2	82.2	81.9	79.5	98.1	97.9

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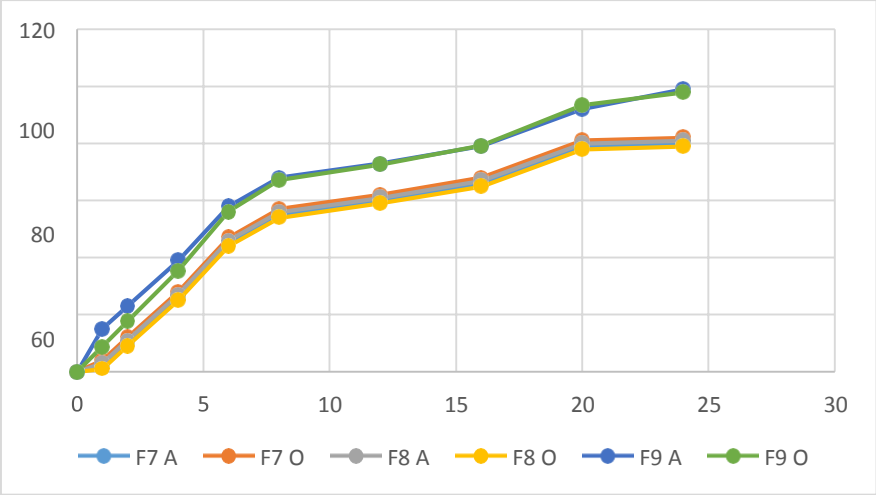

Fig 19:*In vitro* drug release profile of Acyclovir and Omeprazole Nanogel(F7-F9) From the in vitro release data it was found that formulations F9 showed the best release of 99%and 97.5% respectively at the end of 24 hrs among all the nine formulations of Acyclovir andOmeprazole Nanogel.

7.8 OPTIMIZATION OF ACYCLOVIR AND OMEPRAZOLE NANOGEL BYCHARACTERISATION:

Table 21: Optimization of Acyclovir And Omeprazole Nanogel By Characterization

S.NO	METHODS	OBSERVED	RESULT
1	pH	6.9	F9
2	Homogeneity	Homogenous	F9
3	Spreadability	3.5	F9
4	Viscosity	3528	F9
5	Percentage yield	96.02%	F9
6	Drug content	92.65%	F9
7	In-vitro dissolution study	99 (A)and 97.2(O)	F9

According to characterization of acyclovir and omeprazole nanogel have good drug release properties in F9 formulation.

7.9 EVALUATION OF OPTIMIZED FORMULATED F9:

7.9.1 Particle size and Zeta Potential:

The particle size is one of the most important parameter for the characterization of nanogel. The average particle sizes of the prepared F9 nanogel measured using Malvern zeta sizer.

Particle size analysis showed that the average particle size of Acyclovir and omeprazole nanogel formulated using (F9) was found to be 678.4 nm with polydispersity index (PDI) value 0.842 and with intercept 0.857. The zeta size distribution of - Acyclovir and omeprazole nanogel is depicted in Figure 20.

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Table 22: Particle size and Zeta potential of formulation F9

S.No	FORMULATION CODE	PARTICLE SIZE	PDI	ZETA POTENTIAL
1.	F9	678.4	0.842	-43.7

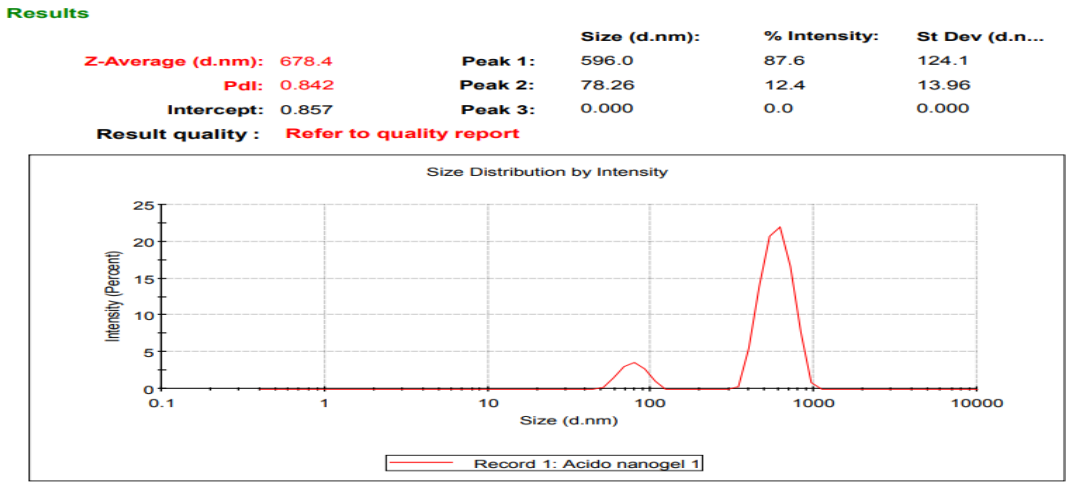

Figure 20: Particle size and PDI of the formulation F9

7.9.2 Determination of Zeta Potential

Zeta Potential was determined using Malvern zeta-sizer instrument. Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. The magnitude of zeta potential is predictive of the colloidal stability. Nanoparticles with zeta potential value greater than +25 mV or less than -25 mV typically have high degrees of stability. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repeleach other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating.

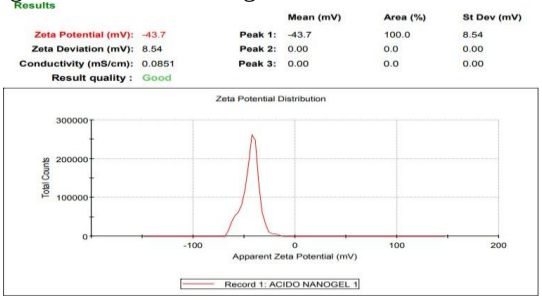

Figure 21: Zeta Potential of formulation

7.10 TEM (transmission electron microscope):

Transmission electron microscopy (TEM) is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. The specimen is most often an ultrathin section less than 100 nm thick or a suspension on a grid.

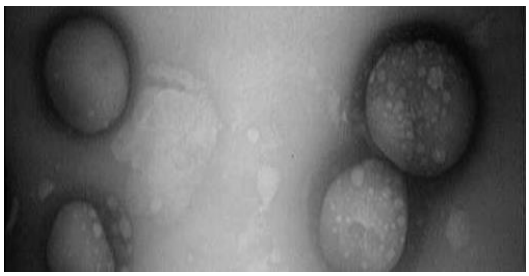

Fig 22: TEM image

7.11 *IN-VITRO* DRUG RELEASE KINETICS

The data obtained from the *in vitro* release study was used to fit into kinetic models. This was doneto find out the mechanism of drug release from Acyclovir and omeprazole nanogel F9. In order todetermine the release model, the *in vitro* release data were analyzed according to zero order kinetics. The preference of a certain mechanism was based on the coefficient of determination (r^2)

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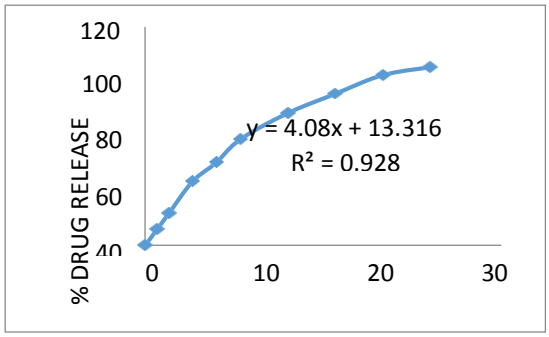
ACYCLOVIR



OMEORAZOLE

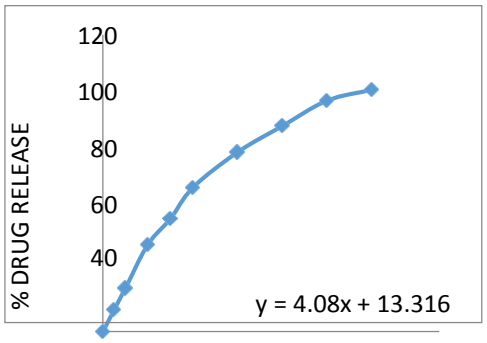


Fig : 23 Graph of zero order kinetics

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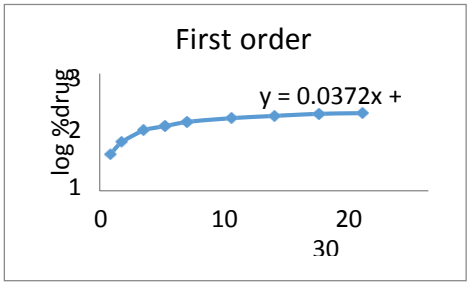

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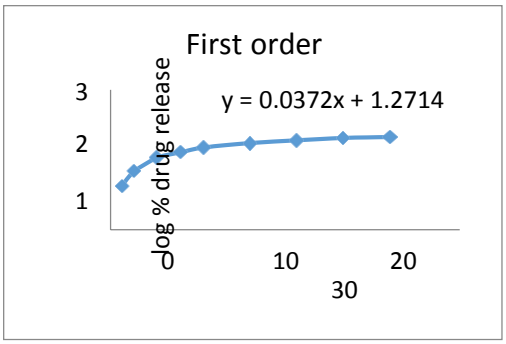

Fig 24 : Graph of first order kinetics

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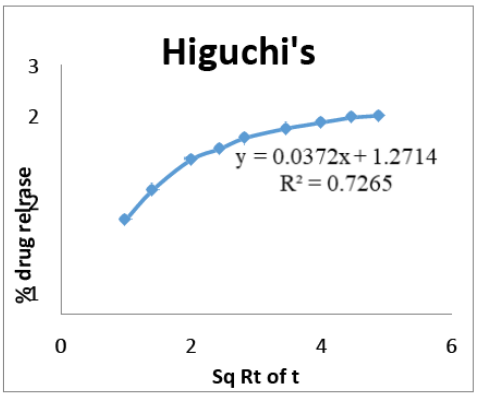

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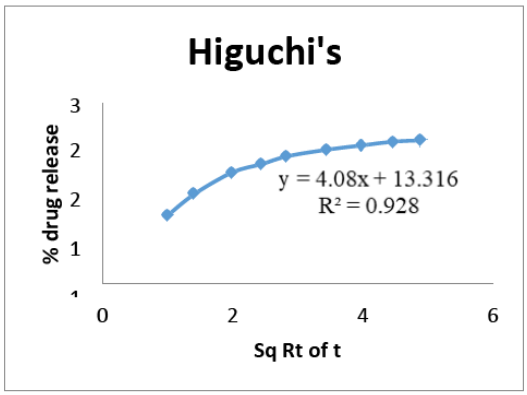

Fig 25: Graph of Higuchi's

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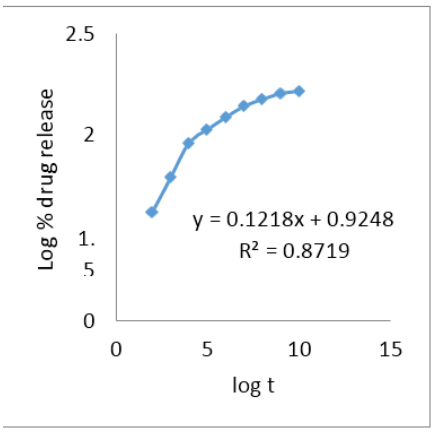
ACYCLOVIR



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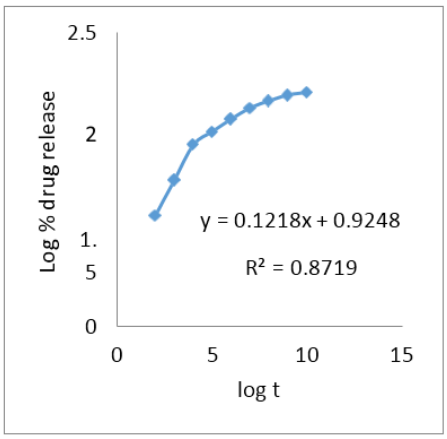

Fig 26:Graph of peppa's

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