IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Bioavailability Enhancement Of Folic Acid By Improving Solubility

Ms. Parbhane* Monali B, Dr.Amjadkhan A. Pathan
PhD Scholar, PhD Guide
Pharmaceutical Science
Shri Jagdishprasad Jhabarmal Tibrewala University,
Vidyanagari, Jhunjhunu, Rajasthan – 333010

ABSTRACT

Folic acid, an BCS class IV essential nutrient, having low solubility and permeability, limiting its bioavailability and therapeutic efficiency. This study aims to enhance folic acid solubility and permeability through solid dispersion and inclusion complex formation utilizing Gelucire 50/13 and hydroxypropyl-beta-cyclodextrin. Solid dispersions of folic acid with Gelucire 50/13 (1:4 ratios) increased solubility by 8.5 fold, respectively. Using HP-β-CD to create inclusion complexes increased permeability by 20-30 fold. These data illustrate the potential of this combination method to considerably increase folic acid solubility and permeability.These results show that this combined method has the potential to greatly improve folic acid solubility and permeability, hence increasing its bioavailability and therapeutic potential.

Keywords: folic acid, solubility, permeability, solid dispersion, inclusion complex, Gelucire 50/13, and hydroxypropyl -beta-cyclodextrin, BCS class IV.

INTRODUCTION

Folic acid is a form of folate, which is a vitamin B_9 .It aids in the production of new cells in your body, including skin, hair, nails, brain, and blood cells. Folate is necessary for all new cells in the body. [1]

The hard effort of Lucy Wills, a medical researcher who earned a degree as a botanist and geologist from Cambridge University, led to the discovery of folic acid. She worked in India in 1930 and was particularly concerned in the issue of severe anemia in impoverished pregnant textile workers. [2]

Fortified foods and supplements contain folic acid, a synthetically produced water-insoluble vitamin. With divalent metals (Cu2+, Fe2+, Co2+, etc.), folic acid forms complex compounds that are insoluble in water. Folic acid with these cations produce stable adducts in biological fluids, such as blood. [3] Folic acid may play a role in the body's removal of divalent cations, according to research .Plants that naturally contain folate include dark green leafy greens. Humans cannot produce folate on their own, thus dietary intake of folic acid pills or foods high in this vitamin provides the daily requirements. Numerous health issues and symptoms, such as neural tube abnormalities, diarrhea, macrocytic anemia, pregnancy troubles, and mental confusion, can be brought on by a folic acid deficiency. [4]

For women who are pregnant or intend to get pregnant, folic acid is very crucial. The risk of neural tube abnormalities (NTDs), which impact the developing fetus's brain and spine, is decreased by adequate intake prior to conception and during the first trimester of pregnancy. Compared to the general population, pregnant women should consume a substantially larger amount. Supplements are frequently advised because of the significance of folic acid, particularly for expectant mothers. Supplementation should often be started prior to conception and continued during the first trimester, according to medical professionals. [5]

- Slow growth
- Forgetfulness
- Gingivitis
- Appetite loss
- Breathlessness
- Diarrhea

Inflammation Of the tongue:

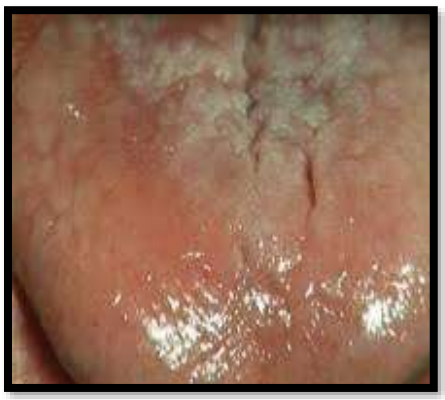


Fig. 1.1 Inflammation of the Tongue

Birth defect:

Fig. 1.2 Birth Defect (Neural Tube Defect)

Heart disease:

Age related hearing loss:

According to one study, folic acid supplementation may aid older adults with high homocysteine levels and inadequate dietary folate postpone the onset of age-related hearing loss. Whether healthy elderly would benefit is unknown. [10]

Depression:

One study suggests that older persons with high homocysteine levels and little dietary folate may benefit from folic acid supplementation to delay the development of age-related hearing loss. It's unclear if healthy seniors would gain from this. [11]

Cancer:

Certain types of cancer appear to be prevented by dietary folic acid, including: Cancer of the colon Cancer of the breast Cancer of the cervical region Pancreatic cancer Cancer of the stomach. [12] This information, however, is supported by population studies that indicate a decreased incidence of these cancers in those who consume adequate folate in their diet. The precise way that folate may help prevent cancer is unknown to researchers. Folic acid, according to some, protects DNA from mutating and causes cancer. [13] Supplementing with folic acid does not appear to prevent cancer. A balanced diet that includes adequate folate is the best course of action because it will help shield you from a variety of illnesses. [14]

Lack of folate in the diet may raise the risk of breast cancer, especially in women who consume alcohol. Frequent alcohol consumption—more than one and a half to two glasses per day—is linked to an increased

risk of breast cancer. According to a big study that monitored over 50,000 women over time, getting enough folate may lower the incidence of alcohol-related breast cancer. [15]

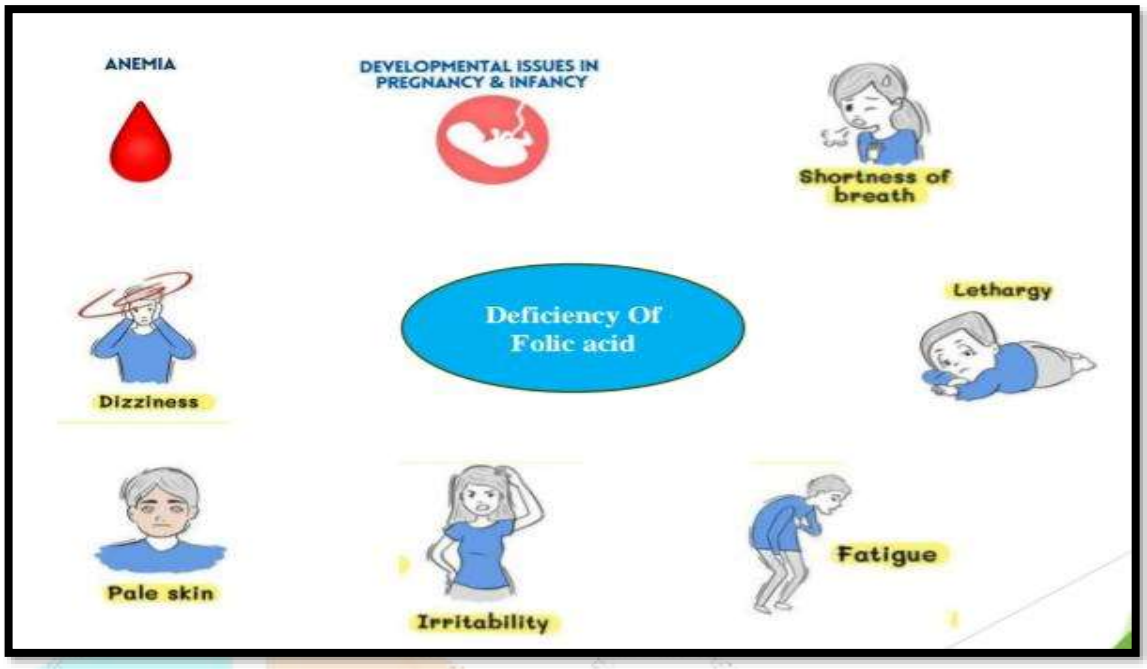


Fig. 1.3 Deficiency of Folic acid

BCS Classification

A sophisticated method for grouping pharmacological compounds according to their water solubility, intestinal permeability, and dissolution is the biopharmaceutical categorization system (BCS).

Three rate-limiting stages of oral retention are covered by the BCS conceptual framework. [17]

- Drug release from dosage form;
- Disintegrated form arrangement in the gastrointestinal (GI) tract
- Saturation into the hepatic circulation via the GI membrane

The BCS method divides medications into four categories depending on their solubility and intestinal permeability. The classification of BCS is based on important factors that regulate absorption, such as permeability, solubility, and rate of dissolution.

Solubility:

Solubility is the quantity of a material that can dissolve in a specific volume of solvent. A medication is considered a good dissolved pharmaceutical if it dissolves in 250 milliliters or less of water with a pH range of 1 to 8. [22]

Permeability:

The attribute or state of being permeable is called permeability. An exception occurs when a medication remains stable in the stomach and absorbs more than 90% of the recommended dosage pharmacological penetration. [23]

Dissolution Rate:

Dissolution is the process by which a solute turns into a solution by dissolving into a solvent. The drug product is considered to have quick dissolving when 85% of the indicated quantity of drug ingredient dissolved in 30 minutes using USP equipment 1 at 100 rpm or apparatus 2 at 50 rpm in a volume of 900 mL buffer solutions (0.1 N HCl/pH 4.5 buffer/pH 6.8 buffer without enzymes). [24]

Class IV drug:

Many BCS class IV medications (e.g., amphotericin B, furosemide, acetazolamide, ritonavir, folic acid, paclitaxel) have properties that make them difficult to give orally. [27] Some of the issues include low water solubility, poor permeability, unpredictable and poor absorption, inter and intra subject variability, and a large positive feeding impact, all of which contribute to low and variable bioavailability. [28] Furthermore, the majority of class IV medicines are substrates for P-glycoprotein (low permeability) and CYP3A4 (extensive pre-systemic metabolism), exacerbating the problem of their low therapeutic potential. [29] A decade ago, severe examples of class IV compounds were the exception rather than the rule, but today, many therapeutic

candidates in the development pipeline fell into this group. Formulating and developing an effective delivery system for BCS class IV medications is a tremendous challenge for any formulator. [30]

Table NO. 1.2 Solubility Enhancer

Example	Example
Ethanol	**Sodium Glycerate**
Propylene Glycol	**Sodium Salicylate**
Sorbitol	**Cyclodextrine**
Polyethylene Glycol	**Polyoxy Ethylene Sorbitol**
Tween series	**Glycerine**
Sodium Benzoate	

Table No. 1.3 Permeation Enhancer

Classification	Example
Surfactant	**Anionic: Sodium lauryl sulfate Cationic: Cetyl pyridinium Chloride Nonionic: Span, Tween**
Bile Salts	**Sodium glycodeoxycholate, Sodium glycocholate, Sodium taurodeoxycholate**
Cyclodextrins	**A,β,yCyclodextrin, Methylated ß- Cyclodextrins**
Fatty acids	**Oleic acid, Methyloleate, Lauric acid, Caprylic acid, Phosphotidylcholine**
Cationic compounds	**Poly-L-arginine, L-lysine**
Chelators	**Sodium salicylate, EDTA, Sodium citrate, citric acid**
Positive charged polymer	**Chitosan, Trimethylchitosan**

APPROCHES TO SOLUBILITY AND PERMEABILITY:

Folic acid's solubility is therefore essential for its uses; yet, it is essentially insoluble in water, with a solubility of 1.6 mg/L at 25 °C. Therefore, we are interested in folic acid's solubility improvement, which is rarely discussed in the literature. Drug delivery and development depend heavily on the water solubility of medications, which is a crucial aspect of their bioavailability that controls their dissolution and transfer process. [32] Due to their poor cell membrane permeability and water solubility, some recently developed medications have failed to reach the market. Many solubility-enhancing methods have been developed to address this problem, and numerous enhancing auxiliary methods are being researched to increase the dissolution of medications with low water solubility. [33] Following are some solubility enhancement technique as follow:

SOLUBILITY ENHANCEMENT TECHNIQUES:

A variety of techniques can be used to increase a drug's solubility and bioavailability if it is poorly soluble in water. Micronization, chemical modification, solid dispersion, complexation, co-solvency, micellar solubilization, hydrotropy, and others are the methods typically used for medication solubilization. [34] A common problem in screening tests of novel chemical entities and formulation design and development is the solubilization of poorly soluble medications. Any medication that is to be absorbed needs to be present at the absorption site as an aqueous solution.[35] Enhancement methods can change or modify solubility and permeability, which are the key factors that determine the drug's in-vivo absorption. The maximum amount of solute that may dissolve in a specific volume of solvent is known as "solubility."[36] It can also be described both qualitatively and statistically. It is defined quantitatively as the solute concentration in a saturated solution at a specific temperature. The spontaneous interaction of two or more substances to create a homogeneous molecular dispersion is a qualitative definition of solubility. When the solute and solvent are in balance, the solution is said to be saturated. [37] Different concentration expressions, including parts, %,

molarity, molality, volume fraction, and mole fraction, are used to represent a drug's solubility. When the solute and solvent are in balance, the solution is said to be saturated. Different concentration expressions, including parts, %, molarity, molality, volume fraction, and mole fraction, are used to represent a drug's solubility.[38]

Solid Dispersion:

There are various techniques for solubility enhancement. Solid dispersion is one of the best approaches for solubility enhancement.[39] The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or amorphous; basically amorphous is having good solubility than crystalline substance because no energy is required to break up the crystal lattice of a drug during dissolution process.Drug solubility andwettability may be increased by surrounding hydrophilic carriers. [40]

First Generation Solid Dispersion:[41]

Solid dispersions were first described by Sekiguchi and Obi in 1961 in which they used concept of eutectic Mixtures. They mentioned that the formulation of Eutectic mixtures improve the rate of drug release and Thus increase bioavailability of poorly soluble drug.

Thus first generation solid dispersions were prepared using crystalline carriers.

↓

Eutectic mixtures are binary Systems comprising of poorly water soluble drug and Highly water soluble carrier and at eutectic point drug Crystallizing out simultaneously only in the specific Composition.

↓

When eutectic mixture is dissolved in Aqueous medium, the carrier part will dissolve quickly and drug will be released in the form of fine Crystals.[42]

The main disadvantage of first-generation Solid dispersion is crystalline nature which leads to Less solubility as compare to amorphous form, However, they possess good thermodynamic Stability. [43]

Second Generation Solid Dispersion:

In second generation instead of crystalline carriers, amorphous carriers were used to disperse drugs which are generally polymers. [44] Polymeric carriers can be of fully synthetic origin like povidone, polyethylene Glycols and polymethacrylates whereas natural Product based polymers comprise of cellulose derivatives like hydroxypropyl methylcellulose, ethyl Cellulose or starch derivatives, like cyclodextrins. Amorphous solid dispersions are further classified as Solid solutions, solid suspension or mixture of both as Per molecular interaction of drug and carrier. Amorphous carriers: Polyethylene glycol, Povidone, Polyvinyl acetate, Polymethacrylate, cellulose derivatives.[45]

Third Generation Solid Dispersion:

In the third generation solid dispersion surfactants carrier or mixture of polymer are used as carrier. [46] If carrier has surface active or self-emulsifying properties, the dissolution profile of poorly soluble drug can be improved and hence result in increased bioavailability. Typically used surfactants as solid dispersion carriers are poloxamer 407, gelucire 44/14, compritol 888ATO27, inulin.[47]

Advantages:

There are various reasons for the improvement of solubility of poorly water-soluble drug using solid dispersion technology. The reasons for solid dispersion or advantages of solid dispersions are as follows:

1) **Particle with reduced particle size:**

Solid dispersion, represent the last state on particle size reduction and after inert carrier or matrix dissolution the drug is molecularly dispersed in the dissolution medium. A high surface area is formed which results an increased dissolution rate and further improved the bioavailability of the poorly water soluble drug.[48]

2) **Particles with improved wettability:**

The solubility enhancement of the drug is related to the drug wettability which can increase bioavailability.[49]

3) **Particles with higher porosity:**

Particles in solid dispersions have been found to have a higher degree of porosity and the increase in porosity also depends on the properties of the carrier.[50] When polymers having linear structure are utilized it

produces larger and more porous particle as compared with solid dispersion that prepared with polymers. More, the porous nature of the
particle more increase in dissolution rate.[51]

4) Drug in amorphous state:

Poorly water-soluble crystalline drugs, when in the amorphous state tend to have higher degree of solubility. Drug in its amorphous state shows higher drug release because no energy is required to break up the crystal lattice during the dissolution process.[52]

Disadvantage:

The major disadvantages of Solid dispersion are related to their instability. Several systems have shown changes in crystallinity and a decrease in dissolution rate on ageing. By absorbing moisture, phase separation, crystal growth or a change from metastable crystalline form to stable Form can take place which leads to the reduction of the solubilty. [53] Moisture and temperature have more of deteriorating effect on solid dispersions that on physical mixture. Sometimes it's difficult to handle because of tackiness.[54]

TYPES OF SOLID DISPERSION:

Binary Solid Dispersion: It consists of drug and a polymeric carrier. [55]

Ternary Solid Dispersion: It consists of drug, a polymeric carrier and a surfactant.[56]

Surface Solid Dispersion:

Surface Solid dispersion is formulated with polymers such as polyvinyl pyrrolidone, polyethylene glycol and polyvinyl pyrrolidone-vinyl acetate copolymer by fusion technique to improve its solubility. [57] It is appropriate to classify various systems of solid dispersion on the basis of their major fast release mechanisms. Solid dispersions in to the following six representative types:[58]

Based on their molecular arrangement:

1. Type 1- Simple eutectic mixture
2. Type 2-Amorphous precipitation of crystalline matrix
3. Type 3-Glass suspension
4. Type 4-Glass solution
5. Type 5-Solid solutions

MECHANISM OF SOLID DISPERSION:

There are two sets of observations with regard to the mechanism of drug release from solid dispersions.

1) Carrier Controlled Release

Corrigan (1986) provided a very valuable contribution by not only measuring the dissolution rate of the incorporated drug but also assessing that of the polymer itself, in this case PEG.[59] He found that the dissolution rate of the drug in the polymer and the polymer alone were in fact equivalent, leading to the suggestion of carrier-controlled dissolution whereby the dissolution rate of the drug is controlled by that of the inert carrier. This finding was supported by the work of Dubois and Ford (1985) who noted that the dissolution rates of a range of drugs in a single carrier, under comparable conditions, were identical in most cases. In this instance the particles dissolve into the polymer-rich diffusion layer at a sufficiently rapid rate that there is insufficient time for the particles to be released intact into the medium. Consequently, the drug is molecularly dispersed this concentrated layer.[60]

2) Drug Controlled Release:

Sjokvist and Nystrom (1991) measured the particle size of griseofulvin particles released from the dispersions and produced the dissolution rate enhancement was a direct function of the size of the released particles.[61] In an attempt to reconcile these contradictions Sjokvist-Saers and Craig (1992 used a homologous series of drug (Para aminobenzoates) in PEG 6000 in an attempt to interrelate the solid-state structure, drug solubility and dissolution rate. These noted that there was a linear relationship between the intrinsic dissolution rate of the model drugs in the dispersions and the drug solubility, clearly linking the properties of the drug (and not the polymer) to the dissolution rate; it may be helpful this stage to refer to such behaviour as drug-controlled dissolution as opposed to carrier-controlled dissolution. Here the dissolution into the polymer diffusion layer is comparatively slow and the drug is released as solid particles.[62] Consequently, the dissolution will not be associated with the polymer but will instead be dominated by the properties (size, physical form, etc.) of the drug itself. This may still lead to considerable

improvements in dissolution compared to conventional dosage forms due to the higher surface area associated the particles and the possibility of improved wetting and decreased agglomeration.[63]

Common method used for preparation solid dispersion:[64]

Various methods used for preparation of solid dispersion system. These methods are given bellow.

1) Melting Method
2) Solvent Method
3) Melting Solvent Method (melt evaporation)
4) Melt Extrusion Method
5) Lyophilization Techniques
6) Melt Agglomeration Process
7) Use of Surfactant
8) Electro Spinning
9) Super Critical Fluid technologies

MATERIAL & METHOD –The following reagents were acquired and used without any further treatment. Folic Acid, Magnesium stearate, Starch Cyclodextrin (β, Hydroxypropyl), Sodium hydroxide, Potassium bromide, Gelucire 50/13**.**

METHODOLOGY

SOLUBILITY ENHANCMENT (Solid Dispersion)

Drug Excipient Compatibility study

For the identification of any probable chemical reaction between the APIs and the carrier, an infrared spectra matching technique was applied. A 1:1 physical mixture of selected APIs and carrier was made and combined with the appropriate amount of potassium bromide. A hydraulic press was used to compress the mixture into a clear pellet. In a Shimadzu 8400 DRS FTIR spectrophotometer, it was scanned IR range. The physical mixture's IR spectra was compared to that of pure drug and polymers, and matching was performed.

Saturation Solubility Study of Drug

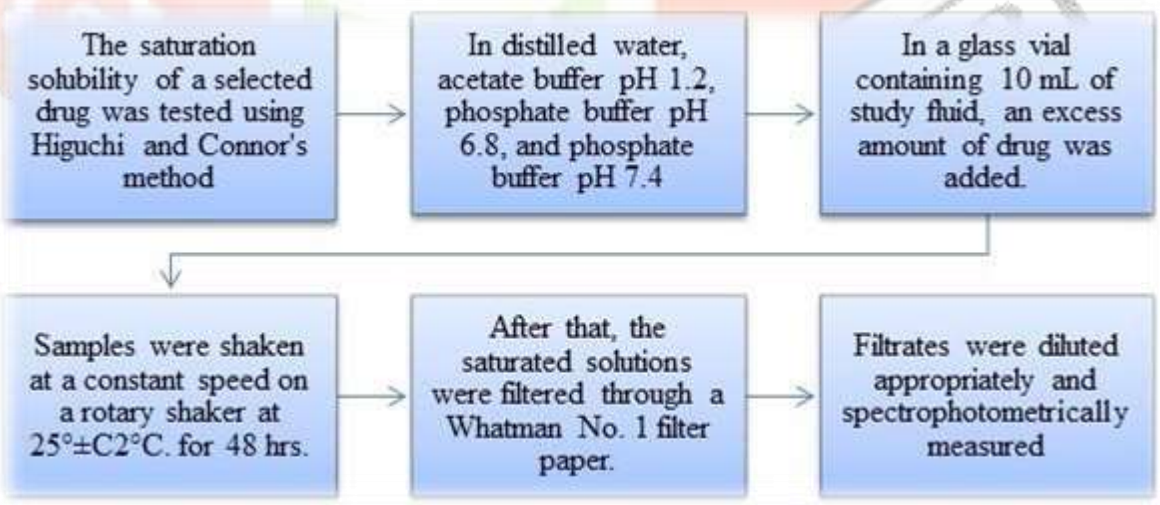


Figure 1.4 Saturation Solubility Study of Drug [68]

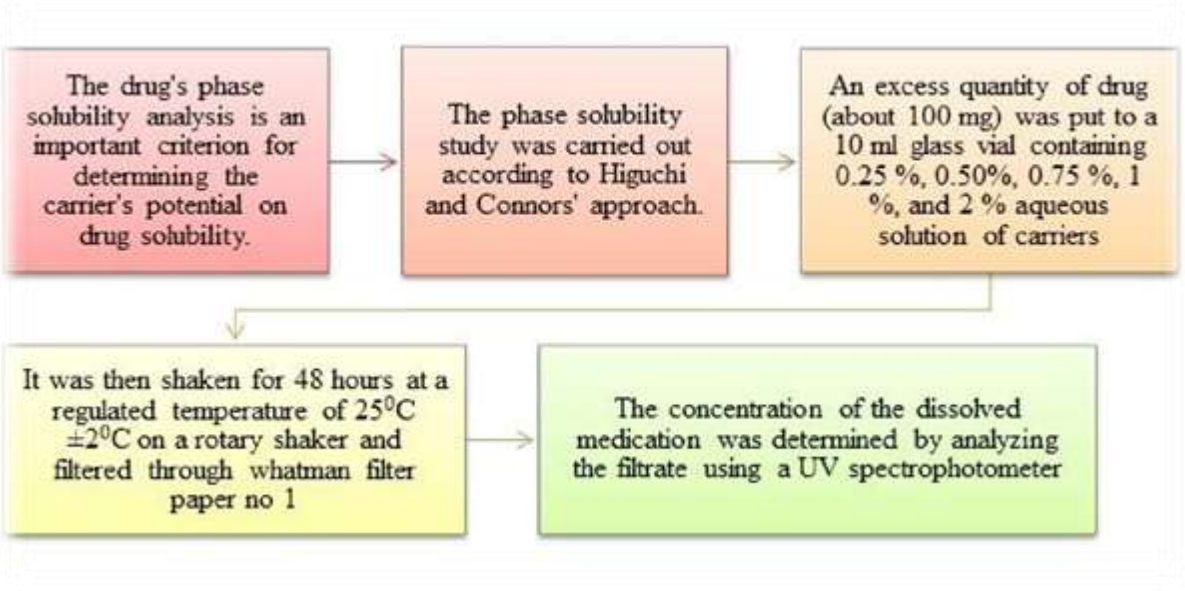


Figure 1.5 Phase Solubility Study of Drug [69]

Gibbs-Free Energy Calculation

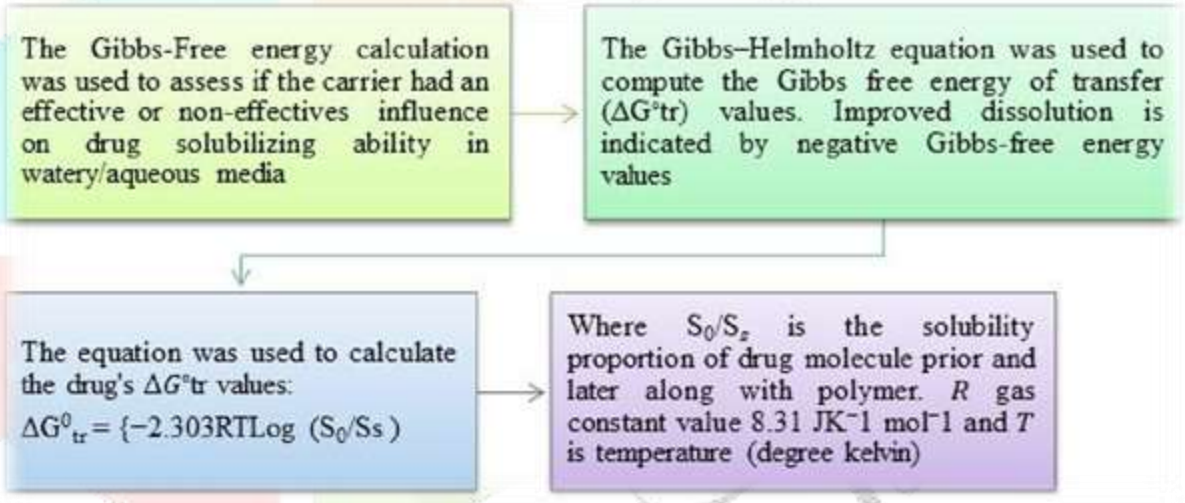


Figure 1.6 Gibbs-Free Energy Calculation [70]

PREPARATION OF SOLID DISPERSION OF FOLIC ACID

Formulation of Physical Mixture of Folic Acid [71]

By conventionally blending the API and polymer, a physical mixture of Folic acid was created using carriers, Gelucire 50/13 in different medication to transporter (carrier) proportion as shown in table.

In a mortar and pestle, the required quantities of two materials were blended to make a physical combination.

To achieve a homogeneous size distribution, the mixture was sieved at number 60 and held in a desiccator until needed.

Table NO. 1.4 Formulation of Folic acid Physical mixture

Formulation	Carrier	Batch Code	Proportion
Physical Mixture	Gelucire 50/13	FA1	1:1
		FA2	1:2
		FA3	1:3
		FA4	1:4
		FA5	1:5

Formulation of Solid Dispersion of Folic acid: [72]

Kneading method is utilized for the preparation of solid dispersion. Folic acid solid dispersion was made using Gelucire 50/13 in various drug and carrier ratios.

In this procedure the drug and carrier were combined in a mortar and vigorously kneaded for 20 mins with water and methanol (1:1).

↓

The kneaded composition were then dried in an oven until they attend a consistent weight.

↓

After drying the resultant dried mass was then crushed and screen through an 60- mesh screen before being kept in a desicator for further research.

Table NO. 1.5 Formulation of Folic Acid Solid Dispersion

Formulation	Carrier	Batch Code	Proportion
Solid Dispersion	Gelucire 50/13	FA1	1:1
		FA2	1:2
		FA3	1:3
		FA4	1:4
		FA5	1:5

Here are some specific results for solubility enhancement of folic acid by solid dispersion method using Gelucire by varying their concentration:

Gelucire-Based Solid Dispersion:

1. **Gelucire 50/13:** Solubility enhancement (SE) of folic acid increased with increasing Gelucire 50/13 concentration**:**
   - 1:1- (folic acid: Gelucire 50/13): 2.5-fold SE
   - 1:2- 4.2-fold SE
   - 1:3- 6.1-fold SE
   - 1:4- 8.5-fold SE (optimal)

Determination of Class of Solubility: (Solubility Test)

By adding a known amount of the Folic acid solid dispersion to a fixed volume of solvent (e.g. Water) and stirring until equilibrium is reached. Then measure the concentration of the dissolved substance using a suitable analytical method.

For the modified folic acid powder, the solubility in water was found to be 1.6 mg/ml, which would classify it as 'Soluble'.

Result: Modified folic acid powder is soluble in water.

PERMEABILITY ENHANCEMENT: (Inclusion Complex Formation) [73]

It Worked by:

1. Improving Solubilty and Permeability: Cyclodextrin increases folic acid aqueous solubility and permeability.
2. Stabilization: Cyclodextrin shields folic acid from degradation.

Mechanisms:

- By forming inclusion complexation folic acid enters into the cyclodextrin cavity.
- There is a occurance of hydrophobic interaction leads to cyclodextrin enhance folic acid membrane interaction.
- Cyclodextrin opens the tight junction and folic acid penetrate into the small paracellular space.

Types of Cyclodextrin:

1. α – Cyclodextrin
2. β - Cyclodextrin
3. γ – Cyclodextrin
4. Hydroxypropyl-β- Cyclodextrin

Factors Influencing Permeability Enhancement:

1. Cyclodextrins Concentrations: Optimal ratio of folic acid and cyclodextrin can increase the permeability. (1:4)
2. Cyclodextrin type
3. pH
4. Temperature

Procedure:

Weigh the needed amount of folic acid and HP-β-CD

↓

Dissolve the folic acid in a small quantity of water or buffer solution

↓

Dissolve HP-β-CD in separate water or buffer solution

↓

Slowly add the HP-β-CD solution to the folic acid solution while stirring continuously

↓

Continue stirring for 30 mins to 2 hrs to allow the complex to develop

↓

Adjust the pH of the solution to the appropriate range (pH- 4-8)

↓

Let the mixture equilibrate for 24 hrs at room temperature or in the refrigerator

↓

Filter the solution to eliminate any undissolved materials

Table No.1.6 Formulation of folic acid Inclusion complex

Formulation	Permeation Enhancer	Batch Code	Proportion
Inclusion Complex	β Cyclodextrin	FA1	1:1
		FA2	1:2
		FA3	1:3
		FA4	1:4
		FA5	1:5
	Hydroxypropyl-β-Cyclodextrin	FA1	1:1
		FA2	1:2
		FA3	1:3
		FA4	1:4
		FA5	1:5

Here are some specific result for permeability enhancement of folic acid by inclusion complex formation method using β Cyclodextrin and Hydroxypropyl-β-Cyclodextrin by varying their concentration:

- HP-β-CD: 20-30-fold increase. (1:4 Optimal)
- β-Cyclodextrin: 10-20 fold increase.
- γ-Cyclodextrin: 5-15-fold increase.

Preformulation Studies of Modified Folic Acid Powder:
Identification:
A. Organoleptic properties:
1) **Colour**: A small quantity of Folic Acid powder was taken in butter paper and viewed in well illuminated place.
Orange-Yellow crystalline powder.
2) Taste and Odor: very less quantity of folic acid as use to get taste with the help of tongue as well as smelled to get odor.
Odorless and Bitter tatse.

B. Flow properties:
1) Angle of repose:

$$\tan\theta = \frac{h}{r}$$

where,
θ= angle of repose, h= height of heap, r-radius of base of heap circle.
2) Bulk Density: [74]
Bulk Density = $\frac{Weight\ of\ sample\ in\ gm}{Bulk\ volume}$ gm/ml

3) Tapped Density:[75]
Tapped density = $\frac{Weight\ of\ sample\ in\ gm}{Volume\ after\ tapping}$ gm/ml

4) Compressibility index and Hausner ratio:[76]
Compressibility index = $\frac{Tap\ density \times Bulk\ density}{Tapped\ density} \times 100$

Hausner ratio = $\frac{Tapped\ density}{Bulk\ density}$

Loss On Drying (LOD): Determined 1 gm by drying in an oven at 100°c to 105° for 3 hours. [77]
Mixed & accurately weighed the substances to be tasted Tarred a glass stoppered, shallow weighing bottle that had been dried for 30 min under the same condition to be employed in the determination Weighed the empty bottle (W1) Put the sample in the bottle,replaced the cover, & accurately weighed the bottle & the content (W2) By gentle, sidewise shaking, distributed the sample as evenly as practicable to a depth of about 5 mm. placed the loaded bottle in the drying chamber Dried the sample at the specified temperature for constant weight. Upon opening the chamber, closed the bottle promptly, & allowed it to come to room temperature in a desiccators before weighing Weighed the bottle (W3) The difference between successive weights should not be more than 0.5 mg. The loss on drying is calculated by the formula-

%LOD = (W2-W3)/(W2-W1) X 100

C. Solubility- [78]
A semi-quantitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity of solute or vice versa.After each addition the system was vigoursly shaken and examined visually for any undissolved solid particles.The solubility is expressed in terms of ratio of solute.

D. FT-IR Spectroscopy [79]
The IR spectrums of the sample & of the Folic acid working/reference standard in the range of 4000cm o 400 cm were taken by preparing dispersion in dry potassium bromide under the same operational conditions. Superimposed these spectra. The transmission minima (absorption maxima) in the spectrum obtained with the sample corresponded in position & relative size to those in the spectrum obtained with the Lisinopril working/reference standard.

Compatibility studies:
FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. The FT-IR spectra of drug with polymers were compared with the standard FT-IR spectrum of the pure drug.

UV Spectrophotometric Study – [80**]**

Standard calibration curve of Folic Acid

Preparation of calibration curves:
The standard curves in at pH 6.8.

Determination of λ max in pH 6.8:

- Preparation of Potassium dihydrogen phosphate (0.2 M) solution.
  Potassium dihydrogen phosphate (27.218 g) was dissolved in water and made the volume with water to 1000 ml.
- Preparation of Sodium hydroxide (0.2 M) solution
  Sodium hydroxide (8 gm) was dissolved in 1000 ml water.
- Preparation of Phosphate buffer
  In 200 ml volumetric flask, Potassium dihydrogen phosphate (50 ml, 0.2 M) was taken and to this solution Sodium hydroxide (22.4 ml, 0.2 M) was added and made the volume with water.
- **Preparation of stock solutions:**

Weighed accurately 10 mg of Folic acid and dissolved in a few ml of phosphate buffer in a 100 ml volumetric flask.

↓

Then the volume was made up to 100 ml with Phosphate Buffer which gives 1 mg/ml concentration (Stock A).

↓

From that 10 ml solution was taken and dissolved in a few ml of phosphate buffer in a 100 ml volumetric flask.

↓

Then the volume was made up to 100 ml with the phosphate buffer, which gives 100 µg/ml concentrations (Stock B).

↓

From the second stock solution 2, 4, 6, 8, 28, 30 µg/ml dilution was prepared.

↓

The absorbance of each sample was measured at 282 nm against blank mixed phosphate buffer. Standard curve of concentration (µg/ml) Vs absorbance was plotted.

4) Drug content & content uniformity[81]

Ten tablets from each formulation were taken, crushed and mixed. From the mixture, 10 mg of Folic acid equivalent of mixture was extracted thoroughly with 100 ml of water. The amount of drug present in extract was determined using UV Spectrometer at 282 nm.

5) Invitro drug release [82]

The United States of Pharmacopoeia (USP) XXIV rotating paddle method was used to study the drug release from the tablet. The dissolution medium consisted of 900 ml of phosphate buffer (pH 6.8). The release was performed at 37oC ± 0.50C, with rotation speed of 50 rpm. Samples (5ml) were withdrawn at predetermined time intervals (2,4 and 6... 10 min) and volume was replaced with the fresh medium. The samples were filtered through Whatman filter paper and analyzed after appropriate dilution by UV spectrophotometry at 282 nm. The experiments for different formulations were conducted in triplicate and average values were recorded and found the release kinetics such as zero order, first order, Higuche and Hixconcrowell were determined.

6) Drug diffusion study [83**]**

Diffusion cell apparatus was used to study the diffusion of the drug from the goat intestinal mucosa of goat. The goat intestinal mucosa was cut in appropriate size to fit into the cell.freshly cut buccal mucosa was used. The dissolution medium consisted of 6 ml of phosphate buffer (pH 6.8). The diffusion was performed at 37oC ± 0.5oC, with rotation speed of 50 rpm. Samples (1 ml) were withdrawn at predetermined time intervals (2,4 and 6... 20 min) and volume was replaced with the fresh medium. The samples were filtered through Whatman filter paper and analyzed after appropriate dilution by UV spectrophotometry at 282 nm .

Appartus	Diffusion Cell
Dissolution Medium	Phosphate Buffer Ph(6.8)
Temperature	37+0.5 °C
Volume	6 ml
Speed	50 rpm
Sample Withdrawn	1 ml
Running Time	120 min

FORMULATION

METHOD OF PREPARATION OF TABLET (DIRECT COMPRESSION)

Tablets containing 10 mg of modified Folic acid were prepared by Direct compression and the various formulae used in the study are shown in the Table No.

↓

Weigh accurately drug, binder and all the diluents.

↓

The active ingredient folic acid, all polymers and diluents except lubricant were get sifted through sieve no. 44#.

↓

Then all the ingredients are mixed in mortar.

↓

Then the mixture is pass through sieve no. 44#

↓

The prepared mixture was compressed into tablets by using 8 mm punch using 12-station tablet punching machine.

Table NO. 1.10 Formulation Batches for Tablet

Batch	Modified Folic Acid (mg)	Magnesium Stearate (mg)	Hydroxypropyl-β-Cyclodextrin (mg)	Starch (mg)
FA1	10	15	10	165
FA2	10	15	20	155
FA3	10	15	30	145
FA4	10	15	40	135
FA5	10	15	50	125

EVALUATION OF PREPARED TABLETS

a) Thickness of tablets: [84]

The tablet thickness should be controlled within a +5% variation of a standard value.

b) Hardness:- [85**]**

Hardness tester (Monsanto type) was used to measure hardness of tablets. The whole experiment was performed in triplicate. It is expressed in Kg/c-.

c) Friability: [86]

An adequate resistance for powdering and friability are the necessary requisites for consumer acceptance. This test was carried out by using tablet friability test apparatus (Roche). Twenty pre-weighed tablets were rotated at 25 rpm for 4 min. The tablets were then de-dusted and reweighed. The percentage friability was measured using following formula.

$$\%F = \frac{Wo - W}{W} \text{ X } 100$$

W_0--- Initial weight

W-----Final weight

d) Weight variation:[87**]**

The weight variation test is done by taking 20 tablets randomly and weighed accurately. The composite weight divided by 20 provides an average weight of tablet. Not more than two of the individual weight deviates from the average weight by 10% and none should deviate by more than twice that percentage. The average weight and standard deviation of the tablets were calculated.

e) Uniformity of content: [88**]**

The uniformity of content of Lisinopril tablet was determined by dissolving one tables in 100 ml Phosphate buffer 6.8. An aliquot of 2 ml sample was withdrawn and diluted to 10 ml and analyzed by UV spectrophotometer at 282.5 nm.

In-Vitro drug release studies: [89**]**

The influence of technologically defined condition and difficulty in simulating in-vitro conditions has led to the development of a number of in- vitro release methods for buccal formulations, however, no standard method has yet been developed in-vitro release rate study of tablets of folic acid was carried out using USP TDT-08L rotating paddle apparatus (Type –II). The dissolution medium consisted of 900 ml of phosphate buffer (pH 6.8). The release study was performed at 37 °C ± 0.5 °C with a rotation speed of 50 rpm. The sample (5 ml) was withdrawn at time interval of 30, 60 and 120 minutes upto 8 h and replaced with 5 ml of dissolution media. The amount of Lisinopril released was determined spectrophotometrically at 282 nm.

Table No. 1.11 Parameters were used for the dissolution study

Apparatus	USP Dissolution apparatus (Type II)
Dissolution Medium	Phosphate buffer (pH 6.8)
Temperature	37±0.5 ^{O}C
Volume	900 ml
Speed	50 rpm
Sample withdrawn	5 ml
Running Time	10 hrs

Drug diffusion study:[90**]** -Diffusion cell study the diffusion of the apparatus was used to drug from the buccal mucosa of goat The goat buccal miscone was cut in appropriate size to fit into the cell. Freshly cut buccal mucosa was used. The dissolution medium opra ml of phosphate buffer (pH 6. 8). The diffusion was performed at 37oC.SoC, with rotation speed of 50 rpm. Samples (1 ml) were withdrawn at predetermined time intervals 1 15, 30, 1. 2. hrs) and volume was replaced with the fresh medium. The samples were filtered through Whatman filter paper and analyzed after appropriate dilution by UV spectrophotometry at 282 nm.

Table No. 1.12 Parameters were used to drug diffusion study

Apparatus	Diffusion cell
Dissolution medium	Phosphate Buffer (pH 6.8)
Temperature	37+0,5 c
Volume	6 ml
Speed	50 rpm
Sample withdrawn	1 ml
Running time	8 hrs

Drug Release Kinetics [91] -Drug transport inside pharmaceutical systems involves multiple steps provoked by different physical or chemical phenomenon, making it difficult, or even impossible, to get a mathematical model describing it in the correct way The release models with major application and best describing drug release phenomena are, in general, the Higuchi model, zero order model, first order model and Korsemeyer-Peppas model. Further, it can be added that the physicochemical properties of the drug as well as polymer and the drug to polymer ratio govern the release of drug from the formulation and thus, modify the release kinetics accordingly-

1. Zero order-The plot made-cumulative drug release vs time
2. First Order-The Plot made-log cumulative of % drug remaining vs time
3. Hixson Crowell cube root law-The plot made cube root of drug remaining in matrix vs. time
4. Higuchi Model - The plot made - cumulative % drug release vs. square root of time
5. Korsmeyer - Peppas model - The plot made - log cumulative drug release vs. log time

RESULT AND DISCSSION

COMPATABILITY STUDY

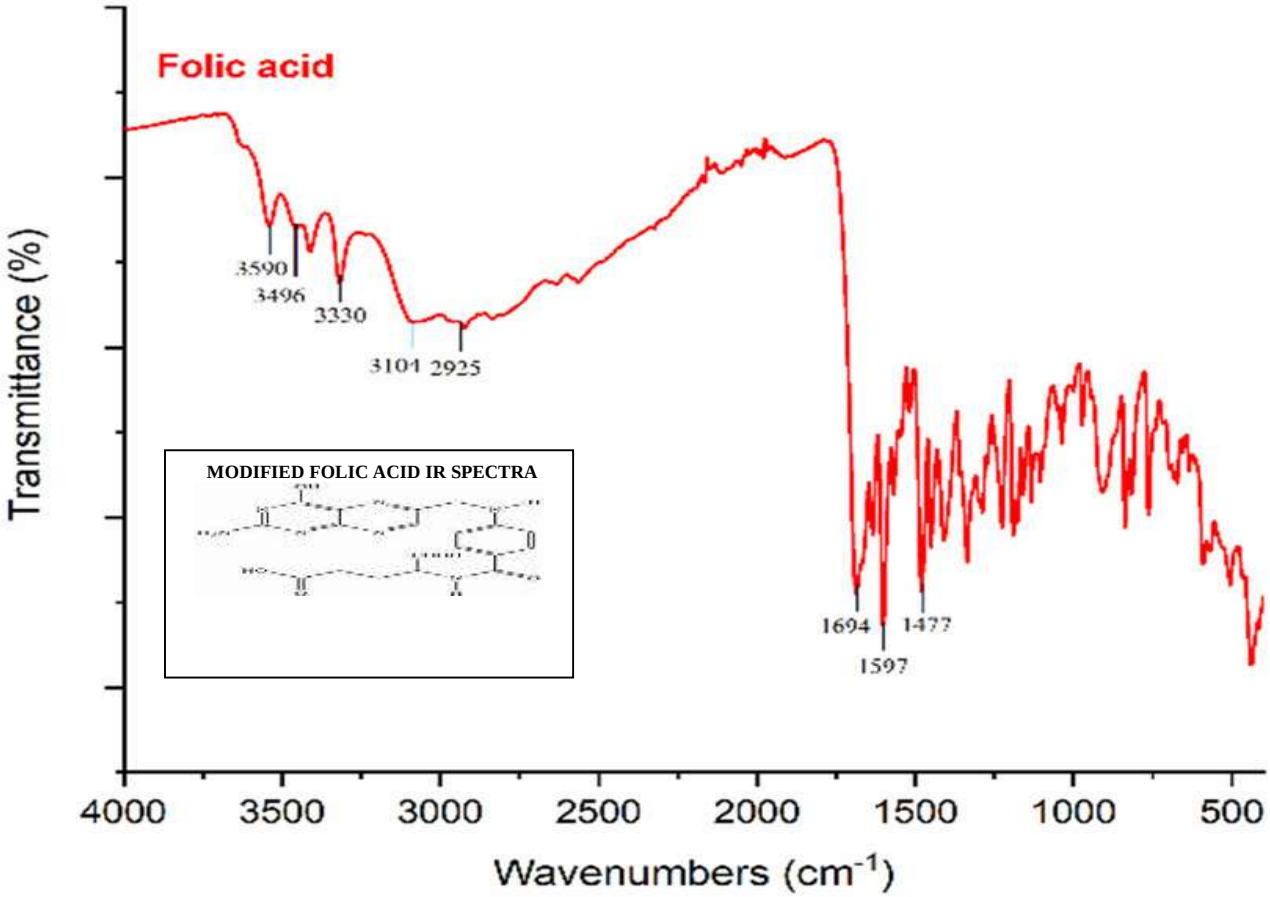
INFRA RED SPECTROSCOPY

By using FT-IR technique, modified folic acid drug was identified in pure form and in combination of excipients used in formulation of tablet.

IDENTIFICATION OF DRUG:

IR SPECTRA DATA (POTTASIUM BROMIDE PELLETS) FOR PURE MODIFIED FOLIC ACID

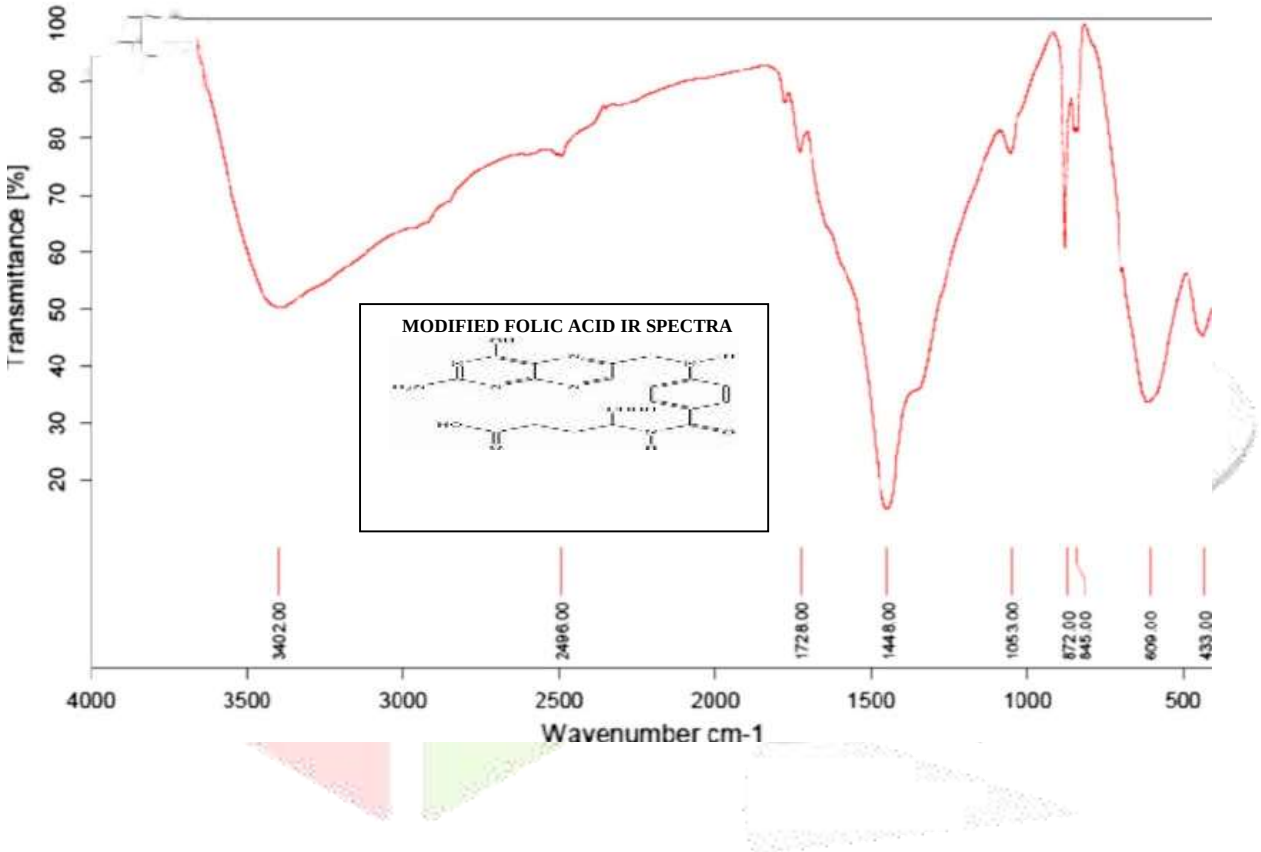
Frequency cm^{-1}	Group Assigned
3690-3496	Hydroxyl Group
1694-1605	Amide Group
1485-1477	Phenyl Ring
1731-1700	Carboxylic Group
1694-1670	N-H bending
3091-2927	C-H Aromatic ring



IR SPECTRA DATA (POTASSIUM BROMIDE PELLETS)

FOR MODIFIED FOLIC ACID TABLET OPTIMUM FORMULATION

Frequency cm^{-1}	Group Assigned
3690-3496	Hydroxyl Group
1694-1605	Amide Group
1485-1477	Phenyl Ring
1731-1700	Carboxylic Group
1694-1670	N-H bending
3091-2927	C-H Aromatic ring



3) Preparation of standard of modified Folic Acid

Determination of λmax in pH 6.8 Solution

The Solution of modified Folic Acid in above medium was scanned in order to determine the λmax for its estimation in the respective media.

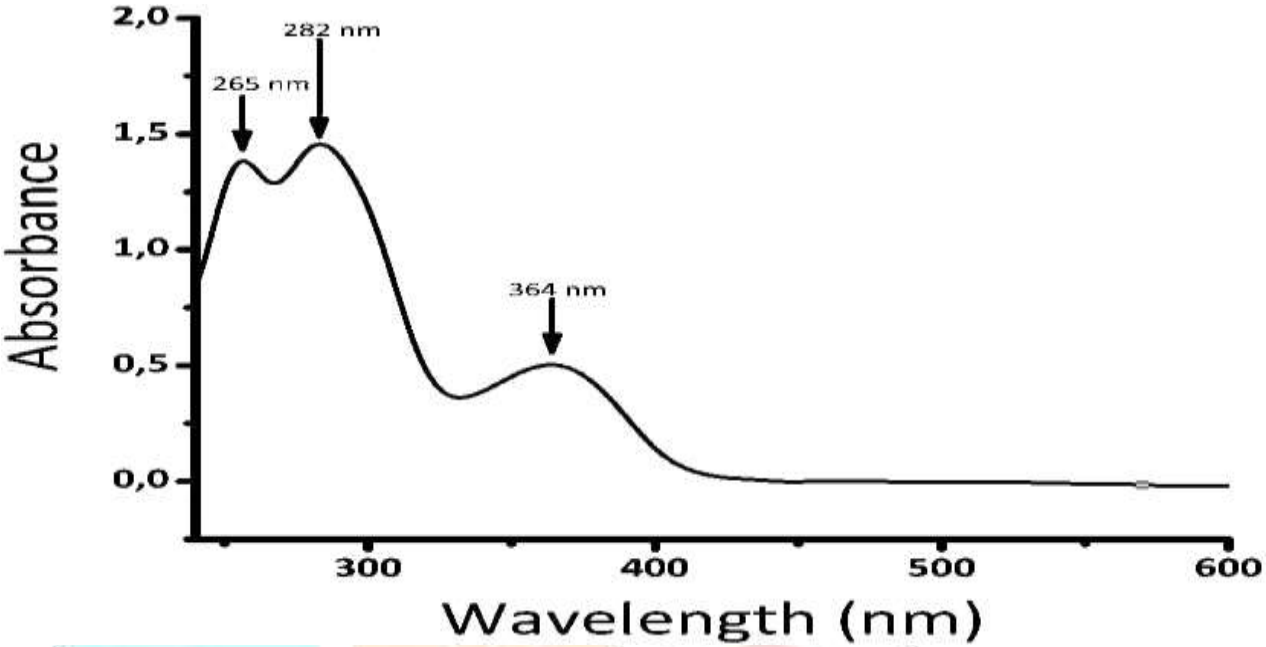


Fig No. 1.7 –Spectrum of Modified Folic acid in pH 6.8 Solution

Table No.1.13 – λmax of Modified Folic acid in pH 6.8

Sr. No.	Medium	λmax (nm)
1.	Phosphate buffer (pH 6.8)	282 nm

Table No.1.14–Absorbance of Modified Folic acid in different concentration at λmax 282 nm

Sr. No.	Concentration ($\mu g/ml$)	Absorbance
1	2	0.07
2	4	0.121
3	6	0.181
4	8	0.245
5	10	0.297
6	12	0.37
7	14	0.421
8	16	0.483
9	18	0.544
10	20	0.61
11	22	0.56
12	24	0.722
13	26	0.789
14	28	0.838
15	30	0.645

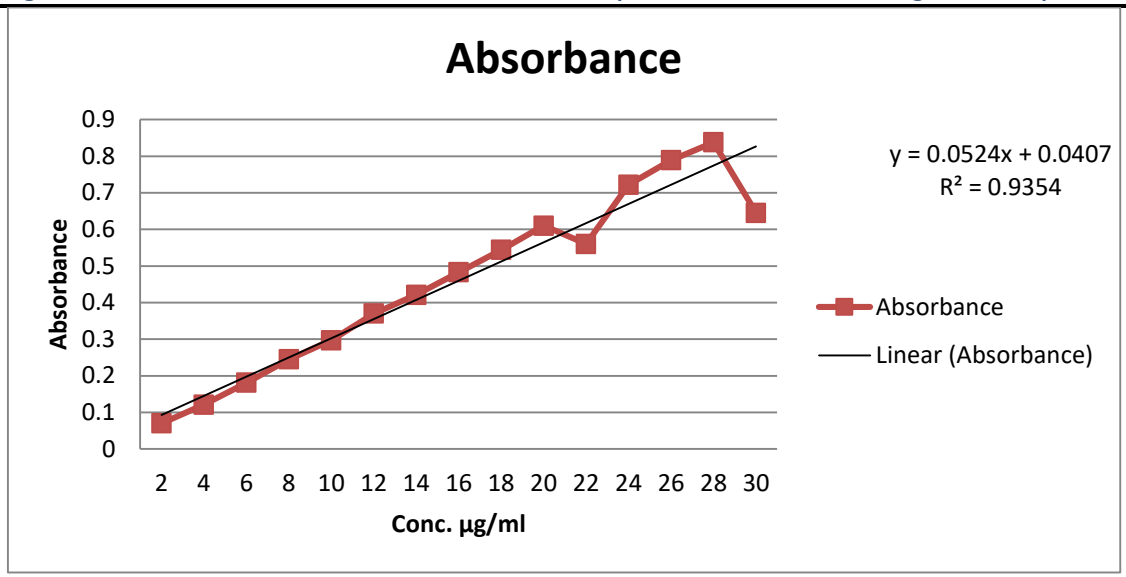


Fig. No. 1.8 –Calibration curve of Modified folic acid in pH 6.8 at 282 nm

Preformulation Study:

Table No.1.15 – Preformulation study of modified Folic acid powder

Sr. No.	TEST	USP STANDARD	RESULT
1.	Color	Orange-yellow Crystalline Powder	Compiles
2.	Odor	Odorless	Compiles
3.	Taste	Bitter	Compiles
4.	Solubility test	Soluble in water	Compiles
5.	Melting Point	482°F (250°C)	253°C
6.	Sulphate ash	Max 0.2%	1.2%
7.	Loss on Drying	8-9.5 in 0.1 gm	Compiles

Table No.1.16–Density study on pure form of Modified Folic acid powder

Bulk Density ±SD (n=3) gm/ml	Tapped Density ±SD (n=3) gm/ml	Angle of Repose ±SD (n=3)	Compressibility Index ±(n=3)	Hausner Ratio ± (n=3)
0.65±0.01893	0.714± 0.03	22.16° ± 1.54	13.86 ± 2.97	0,064±0.04

Precompression Study-

Table No.1.17– Precompression Study on mixture

Bulk Density± SD (n=3) gm/ml	Tapped Density gm/ml± SD(n=3)	Angle of Repose± SD (n=3)	Cpmpressibility index% ±SD (n=3)	Hausner Ratio ±SD (n=3)
0.851±0.03	0.914±0.05	25.74°±1.50	6.8±1.50	1.07±0.46

Determination of Melting Point-

Table No.1.18 Determination Melting Point

Standard Melting Point	Observed Melting Point
250°C	253°C

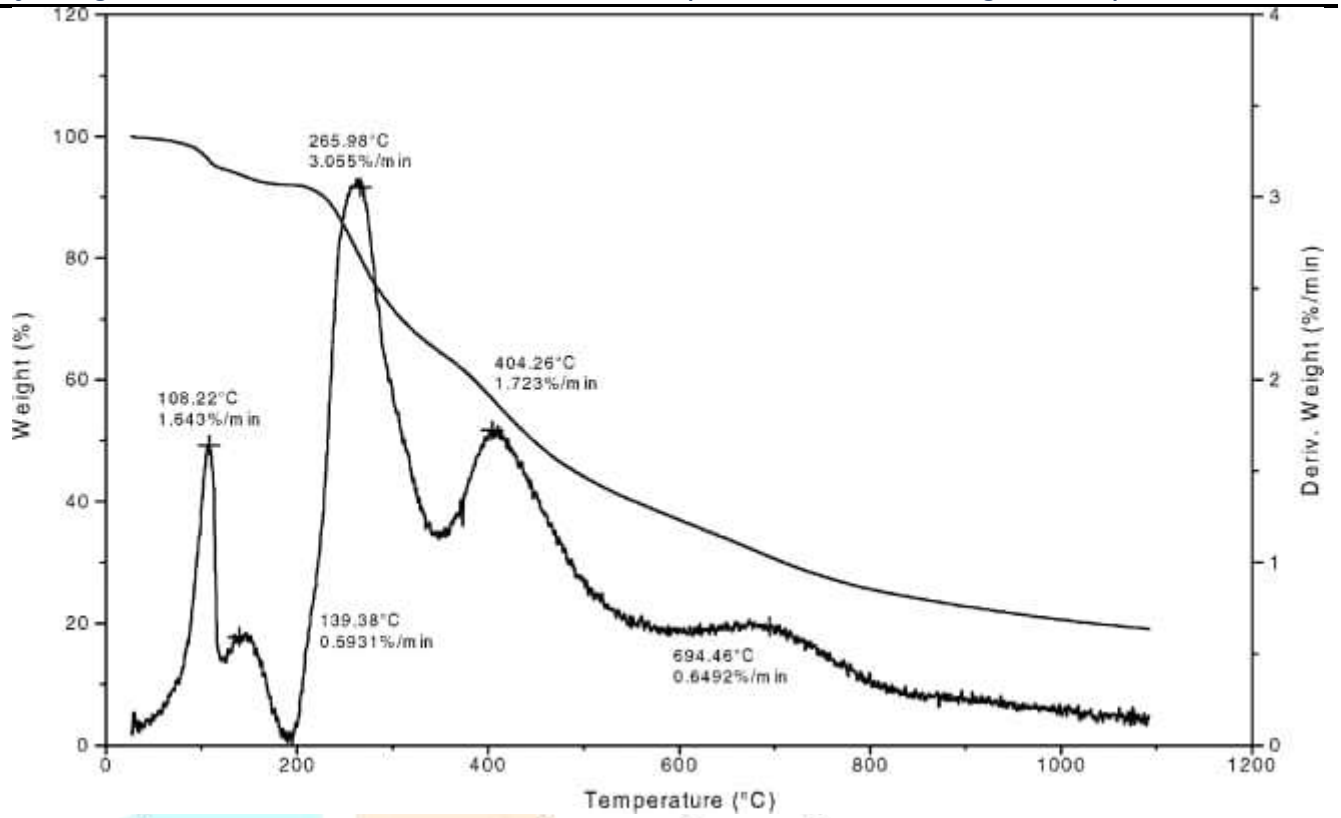


Fig. No.1.15 –DSC 0f pure modified Folic acid powder-Heating rate-20°C/min

Table No.1.19– Tablet Evaluation

Batch No.	Hardness ±SD (n=3) kg/cm^2	Weight uniformity ±SD (n=5) mg	Friability ±SD (n=3) %	Thickness ±SD (n=3) mm	Drug Content ±SD (n=3) %
FA1	5.14±0.26	199.9±4.04	0.45	3.47±0,04	92.64±4.69
FA2	5.03±0.22	199.05±5.01	0.77	3.45±0.04	104.03±3.34
FA3	4.98±0.19	201.55±4.12	0.74	3.49±0.03	96.96±3.98
FA4	6.21±0.31	199.95±4.97	0.70	3.52±0.02	104.65±4.22
FA5	4.82±0.19	199.95±3.96	0.62	3.47±0.03	97.49±4.11

All the tablet preparations were evaluated for various physical parameters like weight variation, hardness, thickness and friability. Tablet standard average wt was 200mg and its weight variation was 7.5% .The result shows weight variation lying within the limit. Tablet thickness in all batches varied between 3.40 mm to 3.50 mm and tablet hardness between 4.5 to 55kg/cm^2. Thus, all the physical parameters of the compressed tablets were within control. The shows the content Uniformity are presented in the table The result shows Drug content lying within limit.

In-Vitro Dissolution Study

Table No.1.20-% Drug Release of Tablet

Time (hrs)	% Drug Release				
0 min	FA1	FA2	FA3	FA4	FA5
10	39.37	23.21	11.45	35.81	14.76
20	52.17	30.14	37.54	44.56	37.09
30	52.23	58.15	46.46	53.64	41.08
40	54.12	62.43	60.46	63.07	49.55
50	56.34	80.12	83.14	71.99	75.87
60	69.12	86.24	86.46	60.36	84.46
70	78.78	71.52	91.13	85.78	75.46
80	80.67	80.87	92.78	93.67	80.66

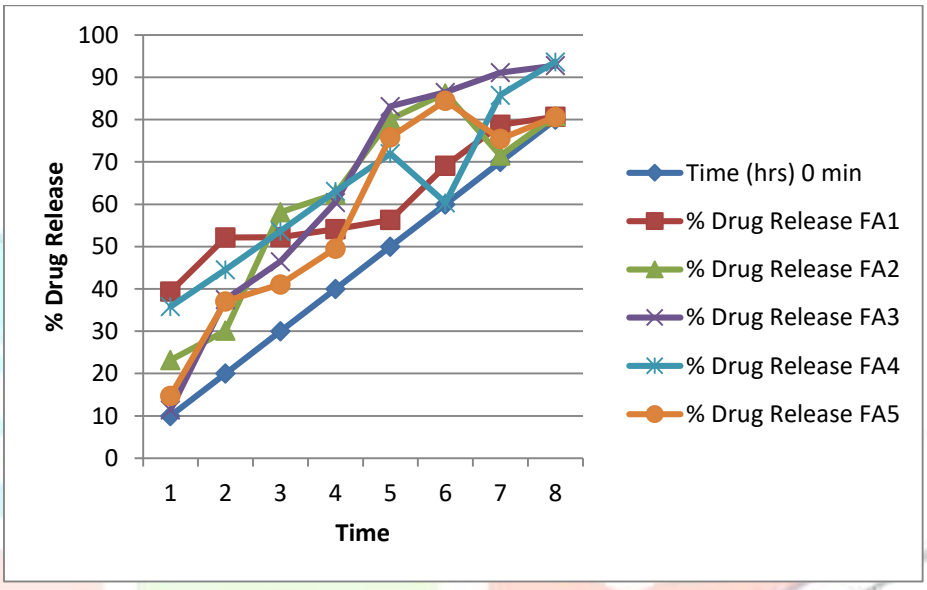


Fig. No.1.12 – Comparison of % drug release through tablet from goat intestinal mucosa

% Drug diffuse through goat intestinal mucosa

Table No.1.21- % Drug diffusion of tablet via intestinal mucosa

Time (hrs)	% Drug Diffused				
0 min	FA1	FA2	FA3	FA4	FA5
10	32.82	28.11	26.1	22.13	24.11
20	29.22	32.96	34.21	41.7	30.4
30	45.12	38.58	35.23	45.78	31.99
40	50.01	48.79	47.21	52.17	40.81
50	55.26	56.27	54.35	61.74	46.03
60	61.23	80.17	71.57	80.20	51.04
100	65.24	88.04	82.1	91.58	57.75

Drug diffusion from the tablet was increased with increase in hydroxypropyl-β-cyclodextrin concentration, formulation FA4 give maximum drug release.FA2 and FA3 also give a desired drug release than other formulation.

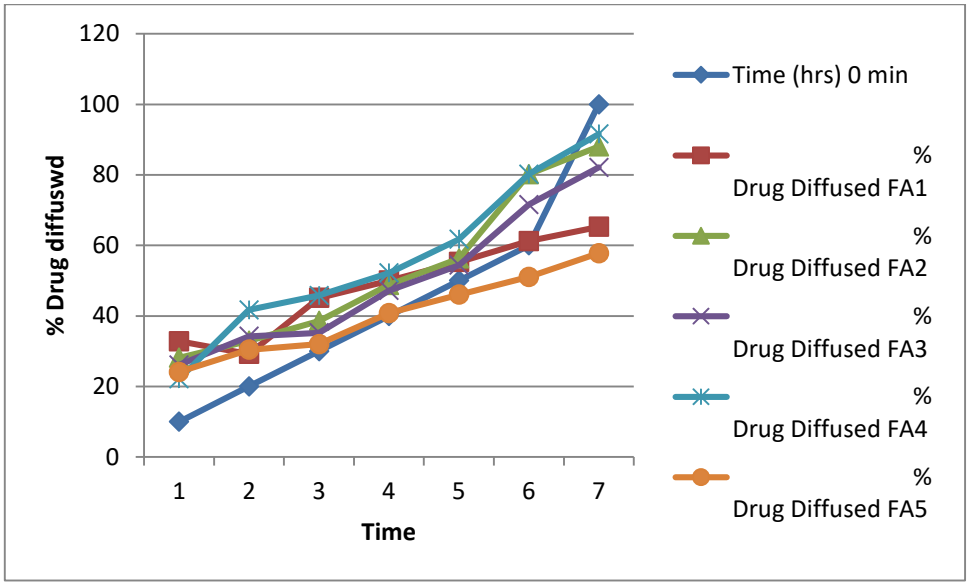
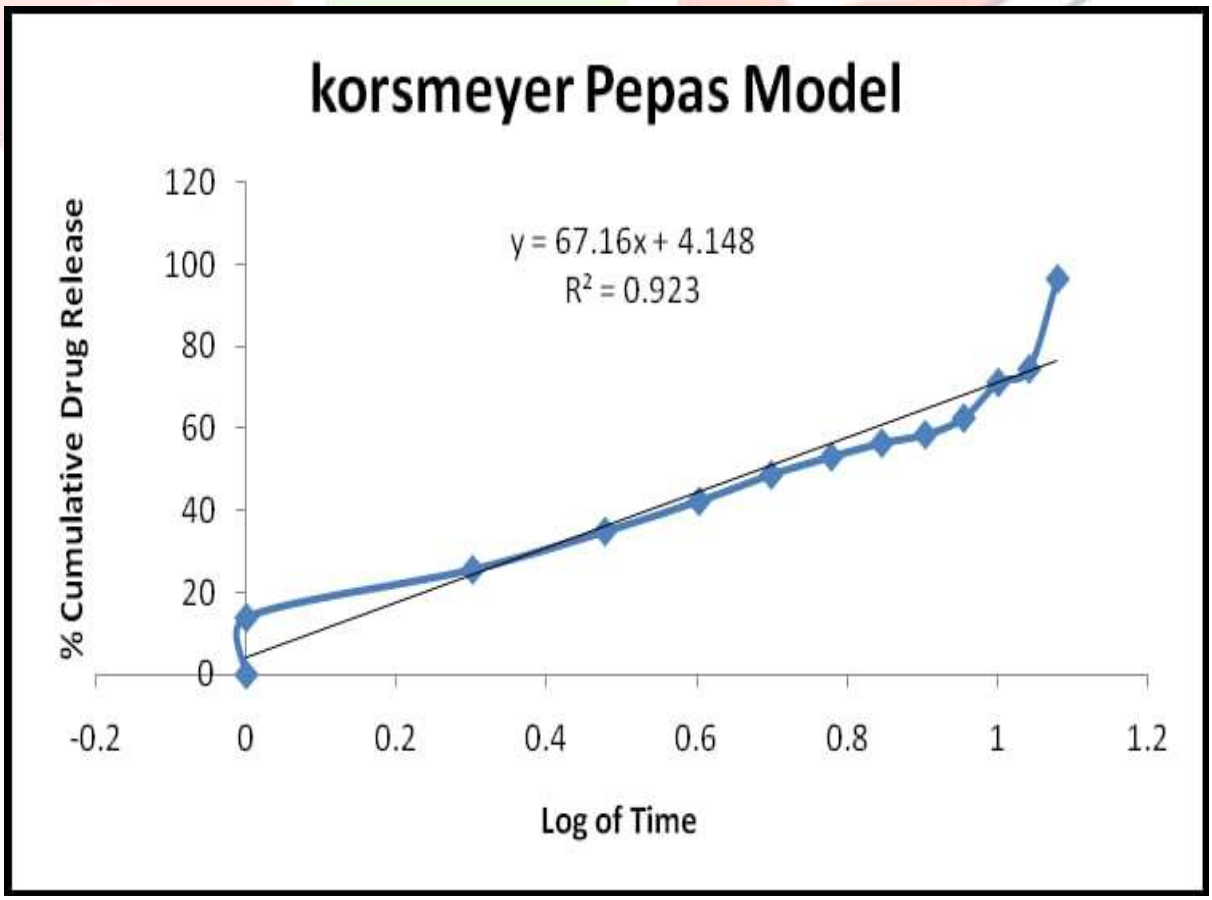


Fig. No. 1.10 - % Drug diffusion profile of Tablet across goat mucosa

Table No.1.22–Kinetic study on Tablet Formulation FA4

ORDER OF REACTION	FA4 Formulation
	R^2
ZERO ORDER	0.931
FIRST ORDER	0.977
HIXON CROWELL	0.971
KORSMEYER-PEPPAS	0.923
HIGUCHI PLOT	0.984
Best Model	KORSMEYER-PEPPAS



The graph was plotted between log % drug release(DR) vs log time .The regression values for drug release profiles of formulation FA4 was found to be 0.923. This indicates that, erosion is the mechanism of drug release from the system.

SUMMARY AND CONCLUSION

SUMMARY

- This research was aimed of utilizing solid dispersion techniques to rises the drug solubility, dissolution rate and bioavailability of less aqueous dissolvable, BCS class IV API's Folic Acid.
- Folic acid are mainly utilized in the cure and management of folic acid deficiency anaemia, Neural tube defect in pregnancy, cardiovascular disease, Depression. Folic acid drugs are falls in class IV as per BCS classification and possesses limited water solubility and permeability, resulting in poor bio-availability.
- SD formulation of drug folic acid was developed by using one grades of polymer, Gelucire 50/13 1:1, 1:2, 1:3, 1:4 and 1:5 drug to polymer proportion respectively by kneading technique.
- Solubility study performed on solid dispersion of an folic acid showed multifold increased in solubility as compare to pure form of drug. SD of modified folic acid prepared with Gelucire 50/13 and showed 8.5 fold rise in water solubility of drug respectively.
- Drug dissolution test displayed surge in speed of dissolution of drug contain in SD an drugs.
- By considering the drug solubility and dissolution rate, Modified folic acid SD formulation (FA4) prepared using Gelucire 50/13 (1:4 proportion) and Hydroxypropyle-β-cyclodextrin was pick out as better formulation and was chosen further for in vitro testing.
- Stability study conducted on optimized SD formulation (FA4) suggest no major changes in solubility of drug and % content during and after stability study period and hence formulation is found to stable.

CONCLUSION

From the present study following conclusion were observed

- The solid dispersion of Modified folic acid prepared successfully by kneading technique by using Gelucire 50/13 as a polymer has shows showed multifold improvement in solubility. All the prepared SD formulations of drugs showed rapid drug release in 90 min time, when tested for in vitro dissolution. Polymer used for SD preparation, Gelucire 50/13 gives better solubility and drug release. Among all methods of solubility enhancement of drugs, Solid dispersion approach was seen more promising in enhancing multifold solubility of drug. The order of solubility of drug enhanced by different approaches was found as SD > > pure drug. As it increase the solubility by 8.5 folds as it compare with the parent drug. According to findings of this research investigation, the conclusion was made that solid dispersion technique is effective in boosting the solubility, dissolution and ultimately bioavailability of less aqueous soluble API's like folic acid.
- As for the class IV drug, drug permeability is a most important and rate limiting step so this modified release FA are formulated in a tablet dosage form By forming inclusion complex using Hydroxypropyle-β-cyclodextrin the (FA4) formulation shows the better enhancement of permeability by 20-30 fold.

REFERENCE:

1) Sarwar MF, Sarwar MH, Sarwar M. Understanding Some of the Best Practices for Discipline of Health Education to the Public on the Sphere. International Journal of Innovation and Research in Educational Sciences, 2015.
2) Molloy, A.M. Folate bioavailability and health. Int. J. Vitam Nutr. Res. 2002, 72, 46–52.
3) Braga CB, Vannucchi H, Freire CM, Marchini JS, Jordão AA, da Cunha SF. Serum vitamins in adult patients with short bowel syndrome receiving intermittent parenteral nutrition. JPEN J Parenter Enteral Nutr. 2011 Jul;35(4):493-8. [PubMed]
4) Bailey LB, Caudill MA. Folate. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell; 2012:321-42.

5) Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group, authors. Lancet. 1991; 338:131–137.
6) Scaglione F, Panzavolta G. Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica. 2014 May; 44(5):480-8. [PubMed]
7) Rieder MJ. Prevention of neural tube defects with periconceptional folic acid. ClinPerinatol. 1994; 21:483–503.
8) Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press; 1998
9) Clarke R, Halsey J, Lewington S, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: Meta-analysis of 8 randomized trials involving 37 485 individuals. Arch Intern Med 2010; 170:1622-31.
10) Pusceddu, I.; Herrmann, W.; Kleber, M.E.; Scharnagl, H.; Hoffmann, M.M.; Winklhofer-Roob, B.M.; März, W.; Herrmann, M. Subclinical inflammation, telomere shortening, homocysteine, vitamin B6, and mortality: The Ludwigshafen Risk and Cardiovascular Health Study. Eur. J. Nutr. 2020, 59, 1399–1411
11) Boushey CJ, Beresford SA, Omenn GS, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA. 1995;274(13):1049–1057. doi:10.1001/jama.1995.03530130055028.
12) Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J. 2015; 14(1):1–10. doi: 10.1186/1475-2891-14-6.
13) WCRF/AICR: Food, nutrition and the prevention of cancer: a global perspective:. World Cancer Research Fund / American Institute for Cancer Research. 1997
14) Bailey LB, Caudill MA. Folate. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell; 2012:321-42.
15) Lee JE, Willett WC, Fuchs CS, et al. Folate intake and risk of colorectal cancer and adenoma: modification by time. Am J Clin Nutr 2011; 93:817-25.
16) Dahan A., Miller J.M., Amidon G.L. Prediction of solubility and permeability class membership: Provisional BCS classification of the world's top oral drugs. AAPS J. 2009;11:740–746. doi: 10.1208/s12248-009-9144-x.
17) 17)Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 1995;12:413–420. doi: 10.1023/a:1016212804288.
18) 18) Gregory K Webster, Robert G Bell, J Derek Jackson CRC Press, 2017.
19) 19) A Dahan, JM Miller, GL Amidon - The AAPS journal, 2009 – Springer
20) 20)MG Issa, HG Ferraz - Dissolution Technol, 2011 - researchgate.net
21) 21) Mahmood - Journal of Health Research and Reviews (In …, 2014 - journals.lww.com
22) 22) Martinez MN, Papich MG, Riviere JE. Veterinary application of in vitro dissolution data and the biopharmaceutics classification system. Stimuli to the revision process. Pharmacopeial Forum (United States Pharmacopeial Convention) 30(6): 2–10, 2004.
23) 23) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. J. Pharmacol. Toxicol. Methods 44, 235-249 (2000).
24) 24) A. Dahan, A. Beig, D. Lindley, J.M. Miller The solubility–permeability interplay and oral drug formulation design: Two heads are better than one Adv. Drug Deliv. Rev., 101 (2016), pp. 99-107, 10.1016/j.addr.2016.04.018
25) 25) Kaushansky, K. · Kipps, T.J.Hematopoietic agents: growth factors, minerals, and vitamins Brunton, L.L. · Goodman, L.S. · Gilman, A. ...Goodman & Gilman's the pharmacological basis of therapeutics McGraw-Hill Medical, New York, NY, 2011; 1067-1099.
26) 26)Brahmankar DM, Jaiswal SB. Biopharmaceutics and Pharmacokinetics, Vallabh Prakashan, 1st Edn. 1995:347-352.

27) 27)Amann LC, Gandal MJ, Lin R, Liang YL, Siegel SJ. In Vitro-In Vivo Correlations of Scalable PLGA-Risperidone Implants for the Treatment of Schizophrenia. Pharm. Res. 2010;27:1730–1737. doi: 10.1007/s11095-010-0152-4.

28) 28)C.A. Lipinski et al.Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings Adv. Drug Deliv. Rev.(2012).

29) 29)Abrahamsson et al., 1996 B. Abrahamsson, M. Alpsten, U.E. Jonsson, P.J. Lundberg, A. Sandberg, M. Sundgren, A. Svenheden, J. Tölli Gastro-intestinal transit of a multiple-unit formulation (metoprolol CR/ZOK) and a non-disintegrating tablet with the emphasis on colon Int. J. Pharm., 140 (1996), pp. 229-235.

30) 30)Lieu CH, William WN, Lippman SM. Cancer chemoprevention. In: Garrett-Mayer E, ed. Principles of Anticancer Drug Development. New York, NY: Springer; 2011. p. 463-81. 10.1007/978-1-4419-7358-0_16

31) 31) Anil K. Nair, Om Anand, Nam Chun, Dale P. Conner, Mehul U. Mehta, Duong T. Nhu, James E. Polli, Lawrence X. Yu & Barbara M. Davit volume 14 page 664-666.

32) 32) C.A. Bergström, R. Holm, S.A. Jørgensen, S.B. Andersson, P. Artursson, S. Beato, et al. Early pharmaceutical profiling to predict oral drug absorption: current status and unmet needs Eur J Pharm Sci, 57 (2014), pp. 173-199.

33) 33)Naninck E.F.G., Stijger P.C., Brouwer-Brolsma E.M. The Importance of Maternal Folate Status for Brain Development and Function of Offspring. Adv. Nutr. 2019;10:502–519. doi: 10.1093/ADVANCES/NMY120. - DOI - PMC – PubMed

34) 34) Krishnaiah Y.S. Pharmaceutical technologies for enhancing oral bioavailability of poorly soluble drugs. J. Bioequiv. Availab. 2010;2:28–36. doi: 10.4172/jbb.1000027.

35) 35)Martin A., Bustamanate P., Chun A.H.C. Physical Pharmacy. Volume 4. BI Wavely Pvt. Ltd.; New Delhi, India: 1994. p. 223.

36) 36)Bruce J Aungst Journal of pharmaceutical sciences 106 (4), 921-929, 2017.

37) 37)Yogesh S Thorat, Indrajeet D Gonjari, Avinash H Hosmani International journal of pharmaceutical sciences and research 2 (10), 2501, 2011.

38) 38)AT Florence, D Attwood, AT Florence, D Attwood Physicochemical principles of pharmacy, 152-198, 1998.

39) 39)Ladan Akbarpour Nikghalb, Gurinder Singh, Gaurav Singh, Kimia Fazaeli Kahkeshan Journal of Applied Pharmaceutical Science 2 (10), 170-175, 2012.

40) 40)Gupta Sachin, Srivastav Shruti, Vajpai Meenakshi Journal of Pharmacy Research 2010, 3(4).

41) 41)Gupta Sachin, Srivastav Shruti, Vajpai Meenakshi Journal of Pharmacy Research 2010, 3(4).

42) 42)Giovana Carolina Bazzo, Bianca Ramos Pezzini, Hellen Karine Stulzer International Journal of Pharmaceutics 588, 119741, 2020.

43) 43)Deng-Guang Yu, Jiao-Jiao Li, Gareth R Williams, Min Zhao Journal of controlled release 292, 91-110, 2018.

44) 44)Tu Van Duong, Guy Van den Mooter Expert opinion on drug delivery 13 (12), 1681-1694, 2016. Sonal V Bhujbal, Biplob Mitra, Uday Jain, Yuchuan Gong, Anjali Agrawal, Shyam Karki, Lynne S Taylor, Sumit Kumar, Qi Tony Zhou Acta Pharmaceutica Sinica B 11 (8), 2505-2536, 2021.

45) 45)Athira R Nair, Yarlagadda Dani Lakshman, Vullendula Sai Krishna Anand, KS Navya Sree, Krishnamurthy Bhat, Swapnil J Dengale AAPS PharmSciTech 21, 1-20, 2020.

46) 46)Rowe RC, Sheskey P, Quinn M. Handbook of pharmaceutical excipients. Libros Digitales-Pharmaceutical Press 2009.

47) 47)Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement techniques. ISRN Pharm. 2012;2012:195727. doi: 10.5402/2012/195727.

48) 48)Poonam Kesarwani, Sameer Rastogi, Vijay Bhalla, Vandana Arora International Journal of Pharmaceutical Sciences and Research 5 (8), 3123, 2014.

49) 49)Hirofumi Takeuchi, Shinsuke Nagira, Hiromitsu Yamamoto, Yoshiaki Kawashima International Journal of Pharmaceutics 293 (1-2), 155-164, 2005.

50) 49)Deng-Guang Yu, Jiao-Jiao Li, Gareth R Williams, Min Zhao Journal of controlled release 292, 91-110, 2018.
51) 50)Sharma KS, Sahoo J, Agrawal S, et al. Solid dispersions: a technology for improving bioavailability. J Anal Pharm Res. 2019;8(4):127-133.
52) 51)P. Pandi, R. Bulusu, N. Kommineni, W. Khan, M. Singh Amorphous solid dispersions: an update for preparation, characterization, mechanism on bioavailability, stability, regulatory considerations and marketed products Int J Pharm, 586 (2020), Article 119560.
53) 52)Abhishek Singh, Guy Van den Mooter Advanced drug delivery reviews 100, 27-50, 2016.
54) 53)Athira R Nair, Yarlagadda Dani Lakshman, Vullendula Sai Krishna Anand, KS Navya Sree, Krishnamurthy Bhat, Swapnil J Dengale AAPS PharmSciTech 21, 1-20, 2020.
55) 54)Shambhavi Borde, Sagar Kumar Paul, Harsh Chauhan Drug Development and Industrial Pharmacy 47 (7), 1011-1028, 2021.
56) 55)Agus Rusdin, Amirah Mohd Gazzali, Nur Ain Thomas, Sandra Megantara, Diah Lia Aulifa, Arif Budiman, Muchtaridi Muchtaridi Polymers 16 (2), 286, 2024.
57) 56)Harpreet Sandhu, Navnit Shah, Hitesh Chokshi, A Waseem Malick Amorphous solid dispersions: Theory and practice, 91-122, 2014.
58) 57)DQM Craig Drug Development and Industrial Pharmacy 16 (17), 2501-2526, 1990.
59) 58)Ruochen Yang, Geoff GZ Zhang, Dmitry Y Zemlyanov, Hitesh S Purohit, Lynne S Taylor Pharmaceutical Research 40 (12), 2817-2845, 2023.
60) 59)Duncan QM Craig International journal of pharmaceutics 231 (2), 131-144, 2002.
61) 60Zhang X., Xing H., Zhao Y., Ma Z. Pharmaceutical dispersion techniques for dissolution and bioavailability enhancement of poorly water-soluble drugs. Pharmaceutics. 2018;10:74. doi: 10.3390/pharmaceutics10030074.
62) 61)Berben et al., 2018a P. Berben, A. Bauer-Brandl, M. Brandl, B. Faller, G.E. Flaten, A.-C. Jacobsen, J. Brouwers, P. Augustijns Drug permeability profiling using cell-free permeation tools: overview and applications Eur. J. Pharm. Sci., 119 (2018), pp. 219-233.
63) 62)Win Loung Chiou, Sidney Riegelman Journal of pharmaceutical sciences 60 (9), 1281-1302, 1971
64) 63)Brahmankar D.M. et al, Bio pharmaceutics and Pharmacokinetics 2009; 349-357.
65) 64)Mason JB, Levesque T. Folate: effects on carcinogenesis and the potential for cancer chemoprevention. Oncology.1996; 10:1727-1736, 1742-1743.
66) 65)Ball, G. F. M. 2004. Nutritional aspects of vitamins. In Vitamins: Their Role in the Human Body, pp. 8-11. Oxford, UK: Blackwell Publishing Ltd.
67) 66)M. Lucock Folic acid: nutritional biochemistry, molecular biology, and role in disease processes Mol Genet Metab (2000).
68) 67)SB Sapkal, SA Shinde, RA Darakhe, VN Shikhande MOJ Bioequiv Availab 5 (6), 313-9, 2018.
69) 68)SR Kumar, T Naga Satya Yagnesh Indo American Journal of Pharmaceutical Research 6 (11), 7036-7056, 2016.
70) 69)Ming Xu, Chungang Zhang, Yanfei Luo, Lishuang Xu, Xiaoguang Tao, Yanjiao Wang, Haibing He, Xing Tang Drug development and industrial pharmacy 40 (1), 126-135, 2014.
71) 70)Subhra Sahoo, TU Patel, MJ Baldaniya, Ashwini Chavan, Saladi Murmu International Journal of Chemical Studies 6 (3), 3248-3250, 2018.
72) 71)Patil JS, D Kadam, S Marapur, M Kamalapur Int. J. Pharm. Sci. Rev. Res 2, 29-34, 2010.
73) 72)M. B. Parbhane FORMULATION & EVALUATION OF MODIFIED RELEASE DRUG DELIVERY SYSTEM OF LISINOPRIL thesis pharmaceutics 69-83.
74) 73)Herbert A Lieberman, Lean Lachman & Joseph B Schwartz, Pharmaceutical dosage form : Tablet Volume I , published by Marcel dekker, page no. 75,195-245,419-580.
75) 74)Brahamanker D.M. and Jaiswal S.B. Biopharmaceutical and Pharmaceutics- A Treatise, 1st Edition, pp 347-371, 1995.
76) 75)Ezzat Chan Abdullah, Derek Geldart Powder technology 102 (2), 151-165, 1999.

77) 76)Muhammad Ali Kaleem, Muhammad Zubair Alam, Mushtaq Khan, Syed Husain Imran Jaffery, Badar Rashid Metal Powder Report 76, S50-S54, 2021.
78) 77)Cezar A Bizzi, Juliano S Barin, Aline L Hermes, Sergio R Mortari, Érico MM Flores Journal of the Brazilian Chemical Society 22, 376-381, 2011.
79) SR Kumar, T Naga Satya Yagnesh Indo American Journal of Pharmaceutical Research 6 (11), 7036-7056, 2016.
80) Ming Xu, Chungang Zhang, Yanfei Luo, Lishuang Xu, Xiaoguang Tao, Yanjiao Wang, Haibing He, Xing Tang Drug development and industrial pharmacy 40 (1), 126-135, 2014.
81) Subhra Sahoo, TU Patel, MJ Baldaniya, Ashwini Chavan, Saladi Murmu International Journal of Chemical Studies 6 (3), 3248-3250, 2018.
82) Patil JS, D Kadam, S Marapur, M Kamalapur Int. J. Pharm. Sci. Rev. Res 2, 29-34, 2010.
83) M. B. Parbhane FORMULATION & EVALUATION OF MODIFIED RELEASE DRUG DELIVERY SYSTEM OF LISINOPRIL thesis pharmaceutics 69-83.
84) Herbert A Lieberman, Lean Lachman & Joseph B Schwartz, Pharmaceutical dosage form : Tablet Volume I , published by Marcel dekker, page no. 75,195-245,419-580.
85) Brahamanker D.M. and Jaiswal S.B. Biopharmaceutical and Pharmaceutics- A Treatise, 1st Edition, pp 347-371, 1995.
86) Ezzat Chan Abdullah, Derek Geldart Powder technology 102 (2), 151-165, 1999.
87) Muhammad Ali Kaleem, Muhammad Zubair Alam, Mushtaq Khan, Syed Husain Imran Jaffery, Badar Rashid Metal Powder Report 76, S50-S54, 2021.
88) Cezar A Bizzi, Juliano S Barin, Aline L Hermes, Sergio R Mortari, Érico MM Flores Journal of the Brazilian Chemical Society 22, 376-381, 2011.
89) R Gopinath, RAS Naidu International Journal of Pharmaceutical and Biological Archives 2 (5), 1391-1400, 2011
90) N. K. Jain –Advance in controlled & Novel drug delivery –CBS publication, Page No.70-86.
91) Amira H Kamal, Samah F El-Malla, Sherin F Hammad Eur J Pharm Med Res 3 (2), 348-60, 2016.
92) Shaynan Hill, Andrew S Varker, Kelly Karlage, Paul B Myrdal Journal of Managed Care Pharmacy 15 (3), 253-261, 2009.
93) Gautam Singhvi, Mahaveer Singh Int J Pharm Stud Res 2 (1), 77-84, 2011.
94) Edward R Garrett, Pramod B Chemburkar Journal of Pharmaceutical Sciences 57 (6), 944-948, 1968.
95) Priyanka Nagar, Kusum Singh, Iti Chauhan, Madhu Verma, Mohd Yasir, Azad Khan, Rajat Sharma, Nandini Gupta Journal of Applied Pharmaceutical Science, 35-45, 2011.
96) Hitesh Chaturvedi, Ayush Garg, Udiabhan Singh Rathore Eur J Pharm Med Res [Internet] 4 (11), 526-30, 2017.
97) Muniyandy Saravanan, Kalakonda Sri Nataraj, Kettavarampalayam Swaminath Ganesh Biological and Pharmaceutical Bulletin 25 (4), 541-545, 2002.
98) James A Seitz, Gerald M Flessland Journal of pharmaceutical sciences 54 (9), 1353-1357, 1965.
99) Shaynan Hill, Andrew S Varker, Kelly Karlage, Paul B Myrdal Journal of Managed Care Pharmacy 15 (3), 253-261, 2009.

100)Gautam Singhvi, Mahaveer Singh Int J Pharm Stud Res 2 (1), 77-84, 2011.
101)M Padmaa Paarakh, Preethy Ani Jose, CM Setty, GV Peterchristoper International Journal of Pharmacy Research & Technology (IJPRT) 8 (1), 12-20, 2018.
102)Prasad Deshmukh, Sangam Nimkar, Amruta Phuse, Dipti Ruikar, Dipti Bonde,A Systemic Review on Techniques of Solubility Enhancement for Poor Water-Soluble Drugs, Asian Journal of Pharmaceutical Research and Development ;2024,12 (3), 247-256.
100) S Wadhwa, Mukesh Gupta,A Review on Absorption Enhancer and Bioenhancer,International journal of health sciences;2022,6 (S6), 1854-1866.
101) Muhammad Farhan Sarwar, Muhammad Haroon Sarwar, Muhammad Sarwar,Deficiency of vitamin B-Complex and its relation with body disorders,B-complex vitamins-sources, intakes and novel applications,2021, Page No.:-79-100.

102) BCS class IV drugs: Highly notorious candidates for formulation development Rohan Ghadi, Neha Dand Journal of Controlled Release ,2017;248,Page No.:-71-95.
103) Ketan T Savjani, Anuradha K Gajjar, Jignasa K Savjani,Drug solubility: importance and enhancement techniques, International Scholarly Research Notices 2012 (1), 195727.
104) Donnelly, J. G. ,Folic Acid. Critical Reviews in Clinical Laboratory Sciences, 38(3), 183–223.
105) Theresa O Scholl, William G Johnson,Folic acid: influence on the outcome of pregnancy,The American journal of clinical nutrition,2000,71 (5), 1295S-1303S.
106) Yue Wang, Xinyi Tan, Xinyu Fan, Linxuan Zhao, Shuhang Wang, Haibing He, Tian Yin, Yu Zhang, Xing Tang, Lingyan Jian, Jian Jin, Jingxin Gou,Current strategies for oral delivery of BCS IV drug nanocrystals: challenges, solutions and future trends,Expert Opinion on Drug Delivery,2021,18 (9), 1211-1228.
107) DM Shinkar, AN Patil, RB Saudagar,Solubility Enhancement by Solid Dispersion Asian Journal of Pharmacy and Technology 2017,7 (2), 72-76.
108) Prasad Deshmukh, Sangam Nimkar, Amruta Phuse, Dipti Ruikar, Dipti Bonde,A Systemic Review on Techniques of Solubility Enhancement for Poor Water-Soluble Drugs, Asian Journal of Pharmaceutical Research and Development ;2024,12 (3), 247-256.
109) S Wadhwa, Mukesh Gupta,A Review on Absorption Enhancer and Bioenhancer,International journal of health sciences;2022,6 (S6), 1854-1866.
110) Muhammad Farhan Sarwar, Muhammad Haroon Sarwar, Muhammad Sarwar,Deficiency of vitamin B-Complex and its relation with body l disorders,B-complex vitamins-sources, intakes and novel applications,2021, Page No.:-79-100.
111) BCS class IV drugs: Highly notorious candidates for formulation development Rohan Ghadi, Neha Dand Journal of Controlled Release ,2017;248,Page No.:-71-95.
112) Ketan T Savjani, Anuradha K Gajjar, Jignasa K Savjani,Drug solubility: importance and enhancement techniques, International Scholarly Research Notices 2012 (1), 195727.
113) Donnelly, J. G. ,Folic Acid. Critical Reviews in Clinical Laboratory Sciences, 38(3), 183–223.
114) Theresa O Scholl, William G Johnson,Folic acid: influence on the outcome of pregnancy, The American journal of clinical nutrition,2000,71 (5), 1295S-1303S.
115) DM Shinkar, AN Patil, RB Saudagar,Solubility Enhancement by Solid Dispersion Asian Journal of Pharmacy and Technology 2017,7 (2), 72-76