Research Article

Influence of preparation methods of folic acid-β cyclodextrin complexes on its photostability

Vikas Yadav[1], Vikas Budhwar[1*], Manjusha Choudhary[2]

[1]*Department of Pharmaceutical Sciences, Maharishi Dayanand University Rohtak-124001 India*

[2]*University Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136118 India*

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Abstract

Objective: The present research aims toward studying the influence of method of preparation of folic acid-β cyclodextrin complexes in increasing its photostability, which is susceptible to degrade in the presence of light. **Materials and methods:** Folic acid-β cyclodextrin complexes were prepared by solvent evaporation, co-precipitation and kneading method and characterized by FT-IR, DSC and XRD. These complexes and the pure drug were simultaneously subjected to accelerated photostability studies according to Q1B ICH 1996 guidelines. The samples were withdrawn in duplicate everyday for seven days and were analyzed for the quantitative estimation of folic acid by UV visible spectrophotometer method and the results were compiled. **Result and conclusion:** After the seventh day the percentage drug degraded in the complexes was (48.05%, 58.05%, and 61.96%) by solvent evaporation, co-precipitation and kneading method respectively. In the end of seventh day pure drug degraded to 78.05%. The photo degradation follows first order kinetics.

***Keywords*:** Folic acid, photo degradation, kinetics, photostability, cyclodextrin complexes, derivatization

Introduction

In 1941, folic acid derived its name from the Latin word *Folium* Leaf. Folic acid (folacin, pteroyl glutamic acid, vitamin B_9) is one of the vitamins of B complex group. It is composed of the aromatic pteridine ring linked to para-aminobenzoic acid and one or more glutamate residues. Folic acid itself is inert. It is reduced to tetrahydrofolate by dihydrofoliate in the liver which is biologically active. Tetrahydrofolate is than metabolized into other derivatives in the liver (Bailey & Ayling 2009; Weinstein et al., 2003). Folic acid is required to conduct many bodily activities like DNA synthesis, DNA repair and DNA methylation. Apart from this it plays role as a cofactor in many biological reactions. Rapid cell division and cell growth is aided by folic acid especially for proper fetal growth in pregnancy and infancy. It helps in the synthesis of healthy red blood cells and prevent anemia in children & adults. Humans are not capable to synthesize folic acid in their body and depend upon external sources of folic acid to meet their daily requirement. Beans and legumes, dark leafy vegetables, citrus fruit, egg yolks and animal's liver are some of the rich sources of folic acid (Kaferle and Strzoda, 2009). Folic acid is also present in many microorganisms: *Bacillus subtilis, B. vilgalys, Serratia marcescens* and a gram –ve *Bacillus* from chick intestine. Poor folate status is associated with one of the causes of macrocytic anaemia and other negative health outcomes, for example inadequate maternal foliate status has been linked to abruption placenta, preeclampsia, spontaneous abortion, stillbirth, preterm delivery, low birth weight (Hibbard et al., 1965; Molly et al., 2008) and serious congenital anomalies of the brain and spine, such as neural tube defects (Mohammad, 1947).

Folic acid is one of the most useful constituents of food without which life is not possible. Millions of people could suffer from foliate deficiency throughout the world. According to a survey report by WHO varying degree of foliate deficiency is seen among different populations throughout the globe. The outcome of foliate deficiency has been witnessed in the form of child birth defects, complications in development of fetus and children, mental

Address for Corresponding Author:
Dr. Vikaas Budhwar
Assistant Professor,
Department of Pharmaceutical Sciences,
Maharishi Dayanand University, Rohtak-124001, Haryana, India
E-mail: vikaasbudhwar@yahoo.com

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disabilities and cardiovascular disorders (Food and Nutrition Bulletin 2008). After realizing the importance of foliate in the diet of people and its deficiency in public even in the developed country like USFDA layed stress on supplementation of the enriched breads, cereals, flours, corn meals, pastas, rice and other grain products with folic acid as a legal mandate which was acted in January 1, 1998 (Daly et al., 1997; Malinow et al., 1998). This aimed especially to decrease neural tube birth defects infants (Crandall et al., 1998).

Commercially available folic acid for dietary supplementation and cure of foliate deficiency diseases suffer from number of drawbacks like poor solubility and photosensitivity. Folic acid is slightly soluble in cold water (0.0016 mg/ml at 25°C). Aqueous solubility of folic acid is decomposed readily on exposure to sunlight or ultraviolet light and oxygen. It is unstable in acid solutions, practically below a pH 6.0. Alkaline solutions are sensitive to oxidation. It interacts with several B complexes vitamins causing destruction of the molecule (Allwood, 1941). Folic acid is incompatible with oxidizing agents, reducing agents and heavy metal ions. Owing to these reasons, this molecule even though is of immense importance for the general health of human, especially of pregnant woman and neonates, yet it is very difficult to formulate it in a dosage form which would promise to be stable and readily bioavailable at the same time.

Mylius, first time in 1886, observed the unusual complexations between several volatile compounds and hydroquinone. He also observed the entrapment of a molecule into another molecule without the presence of any chemical bond. These complexes were earlier known by some other names such as "occlusion compounds, adducts and clathrates" then Schlenk, a scientist, named the complexes as "inclusion compounds". The size of the guest molecule is an important parameter in this technique i.e. to form a stable complex with desired physiochemical properties, the size of guest molecule should be equivalent to the host molecule so that it can easily entrap into the cavity of the host molecule (Uekama et al., 1998; Kurokov & Loftsson, 2013; Crini, 2013).

Many researchers have proposed various classifications for the inclusion complexes like, on the basis of organization and structures in complexes, Frank classified the inclusion complexes into polymolecular, monomolecular and macromolecular complexes (Frank, 1975). Steed and Atwood proposed the compounds, on the basis of topological relationship between host and guest, named as cavitands and clathrands (Steed & Atwood, 2000). Dyadin and Terekhova proposed various inclusion complexes like tubulatoclathrates, intercalatoclathrates and cryptoclathrates, on the basis of shape and structure of the host's cavity (Dyadin & Terekhova, 2004). In 1891, a French scientist named Antoine Villiers isolated potato starch by bacterial digestion and described this material as dextrin which was further termed as "cellulosine" (Villiers, 1891). In 1903, Franz Schardinger discovered two crystalline compounds, α-dextrin and β-dextrin. He further identified cellulosine as β-dextrin which is presently known as α-cyclodextrin (α-CD) or β-CD (Schardinger, 1891; Schardinger, 1911). In 1935, Freudenberg et al. (1935) identified another compound known as γ-cyclodextrin (Freudenberg et al., 1935). In 1976, a formulation composed of cyclodextrin and prostaglandin (Prostarmon-E™sublingual tablets) was marketed by Japan for the first time. In 1977, Piroxicam or β-CD (Brexin® tablets) was the first formulation which was marketed in Europe and itraconazole/2-hydroxypropyl-β-CD oral solution (Sporanox®) was the first US-approved product (Loftsson & Duchene, 2007). Since then cyclodextrins have been used for the improvement of pharmaceutical characteristics of the drugs like aqueous solubility and stability including environmental protection of drugs and prevention of its optical rotation, cyclization etc. Improving photostability is yet another sphere explored by many researchers who were successfully able to formulate cyclodextrin complexes with α-tocopherol (Aytac and Uyar, 2016), Rhein (Petralito et al., 2009), flavonoids and geraldol (Sali et al., 2018)), Nootkatone (Kfoury et al., 2017), 13-cis-Retinoic (Yap et al., 2017), isradipine (Park et al., 2013) and could increase their shelf life by protecting them from deleterious effects of sunlight. Presently Aceclofenac-β Cyclodextrin, Ulgut/ Lonmiel, Betahist, Propulsid , Pansporin-T, Zyrtec, Australian dream, Fluner, Ryndthisol, Stada-Travel, Clear eyes, Pain relief gel, Mobitil, Transillium, Glymesason, Mena-Gargle, Cycladol/ Brexin/ Flamexin, Opalmon, Prostandin 500, Vitaseptol, Rofizgel, Flogene etc drug cyclodextrin complexes are approved for being marketed throughout the globe.

Materials and Methods

Folic acid and β-Cyclodextrin was procured from Fluka Goldie, Linco Scientific Instruments & Chemical (P) Ltd. Ambala, Haryana. Sodium nitrite and Potassium Dihydrogen Orthophosphate were obtained from C.D.H Pvt. Ltd. New Delhi and Molychem Mumbai respectively. All the chemicals used were of analytical grades.

Drug excipients compatibility studies

The drug excipients compatibility study was performed by using FT-IR Spectrophotometric method (FT-IR Bruker 1206 0280, Germany) by KBr disc technique. FT-IR analysis was performed over a range of 4000-400 cm^{-1} by taking above

mixture. The powdered drug (folic acid) and polymer (β-cyclodextrin) were taken in the molar ratio quantity 1:1 and dissolved in common solvent ethanol, dried in oven for 24 hour at 35°C and kept in dessicator for 24 hours before conducting the FTIR analysis (Patil et al., 2010; Kumar et al., 2013).

Preparation of inclusion complexes

Inclusion complexes of folic acid were prepared by three methods**:** drug and polymer are incorporated in the molar ratio.

Solvent Evaporation: A solvent evaporation method is the most simple when compared to other methods. Firstly the Folic acid (882 mg) and β- cyclodextrin (2724 mg) was weighed to make molar ratio of 1:1. Both the drug and the polymer were mixed separately in common miscible solvent ethanol. After both the solution were mixed together. Finally the solvent was evaporating in hot air oven at 35°C for 24 hour (Patil et al., 2010; Kumar et al., 2013).

Co-precipitation: β-cyclodextrin (2724 mg) was mixed in ethanol. Folic acid (882 mg) was added slowly in the above solution. The mixture was stirred at room temperature for one hour in the dark. Then the solvent of mixture was evaporated in the hot air oven at 35°C for 24 hour, stored in desiccators over fused calcium chloride (Shirse et al., 2012).

Kneading Method: 882 mg folic acid and 2724 mg β-CD was weighed to make molar ratio of 1:1. First β-cyclodextrin was added to the mortar, small quantity of ethanol was also added while triturating to get slurry like consistency. Folic acid was incorporated slowly into the slurry and triturated for 1 hour. The obtained paste was dried in hot air oven at 35°C for 24 hours, stored in desiccators over fused calcium chloride (Ammara et al., 2007).

Characterization of Folic Acid-β-Cyclodextrin inclusion complexes

Folic acid, β-cyclodextrin and the powder obtained after adapting the above mentioned process of complexes preparation was subjected to following studies.

FT-IR Analysis

The FT-IR of the inclusion complexes was performed on (FT-IR Bruker 1206 0280, Germany) instrument by KBr disc technique. The spectra were recorded (Figure 3) over the range of 4000- 400 cm^{-1} and the spectrum was obtained.

DSC Analysis

DSC measurements were carried out on DSC Q10 V9.9 Build 303. Sample (2mg) were placed in sealed aluminium pan and heated from 35°C to 300°C at a rate of 10°C/minute in an atmosphere of nitrogen gas by passing it at a flow rate of 60 ml/min and an empty pan used as a reference.

XRD Analysis

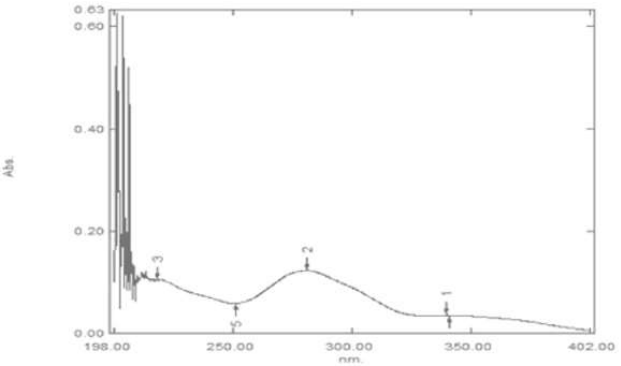
The X-ray powder diffraction studies were performed at PW1710 X-Ray Diffractometer with Cu as anode material and graphite monochromator, operated at a voltage of 35kV, current 40mA. The samples were analyzed in the 2θ angle range of 10-70° and scan time of 0.5 second.

Photostability studies

Photostability of folic acid and inclusion complexes was performed in photostability chamber (Thermolab ES2000 UV), equipped with a cool white fluorescent lamp and near UV fluorescent lamp, option 2 according to the Q1B ICH Guidelines for photostability testing (ICH 1996). Irradiance power was set to overall illumination of 1.2 million lux h^{-1} and near UV energy of (1.3W h m^{-2}). Temperature and relative humidity inside the chamber were maintained at 25°C and 60%, respectively, throughout the study. A weighed quantity of finely powdered folic acid (882 mg) and its complexes (3 gm) were spread as a thin layer in glass Petri dishes (diameter 6 cm). The Petri dishes were placed in the photostability chamber sufficiently apart to avoid shadowing and irradiated with visible lamps (1.2 million lux h^{-1}). The samples were withdrawn in duplicate from the chamber after every 24 hour for up to 7 days. The samples were analyzed by UV Spectrophotometer (UV- 1800, Shimadzu Corp., Japan).

The standard curve of folic acid was prepared by following two methods:

1. The drug 50 mg was dissolved in 50 ml phosphate buffer (pH 6.8) to make a solution of 1 mg/ml. 10 ml of this solution was diluted to 100 ml by phosphate buffer (pH 6.8) to make a final concentration of 2,4,6,8….18 mcg/ml. This solution was scanned in UV Spectrophotometer (UV- 1800, Shimadzu Corp., Japan) between 200-400 nm wavelengths against phosphate buffer pH 6.8 as blank. The λ_{max} obtained was 281 nm (Chinese Pharmacopoeia, 1995).



Peak Pick

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	339.80	0.04	
2	↑	281.20	0.12	
3	↑	218.40	0.11	
4	↓	341.00	0.03	
5	↓	251.20	0.06	

Figure 1. Peak pick for λ_{max} (Folic acid)

2. Derivatization Method: This method was adopted from (Hutchings et al., 1947) and (Nagaraja et al., 2002). Folic acid is reductively cleaved in hydrochloric acid by zinc. The product, p-ABGA, is diazotized and then coupled with 3AP. The coupled substance is yellow-orange coloured with maximum absorbance at 460 nm. Figure 2 show the reactions that take place in the process. A Shimadzu (UV- 1800, Shimadzu Corp., Japan) was used to measure the absorbance which was correlated to folic acid concentration. The detailed procedure is as under:

a) 2 ml of the 500 ppm folic acid (500 mg anhydrous folic acid to make 1000 ml suspension in purified water) was mixed with 1 g of zinc granules and 10 ml of 5 N HCl in a 250 ml glass jar. It was shaken every 5 minutes for 30 minutes to allow for the reductive cleavage reaction to go to completion.

b) 28 ml of purified water was added to the jar to get the final volume to 40 ml. The jar was shaken and zinc granules were allowed to settle to the bottom.

c) 2, 4, 6 ….18 ml (corresponding to 2-18 µg/ml of folic acid) were dispensed into labeled test tubes.

d) 2 ml of 5 N HCl and 1 ml of 2% sodium nitrite ($NaNO_2$) were added to each of the test tubes. The test tubes were mixed with a vortex and left to sit for 5 minutes to allow the diazotization step to go into completion.

e) 4% Sulphamic acid was added to each of the tubes. The test tubes were mixed with a vortex and left to sit for 5 minutes. This is to remove the excess nitrite.

f) 5 mL of 1% 3-AP (3-aminophenol) was added to each test tube and mixed with a vortex. The test tubes were heated in a boiling water bath for 10 minutes to allow the coupling reaction to go to completion. At this stage, the originally colorless solutions turned orange-yellow.

g) The test tubes were removed from the water bath, cooled to room temperature and 3 mL of 5 N HCl was added to each tube.

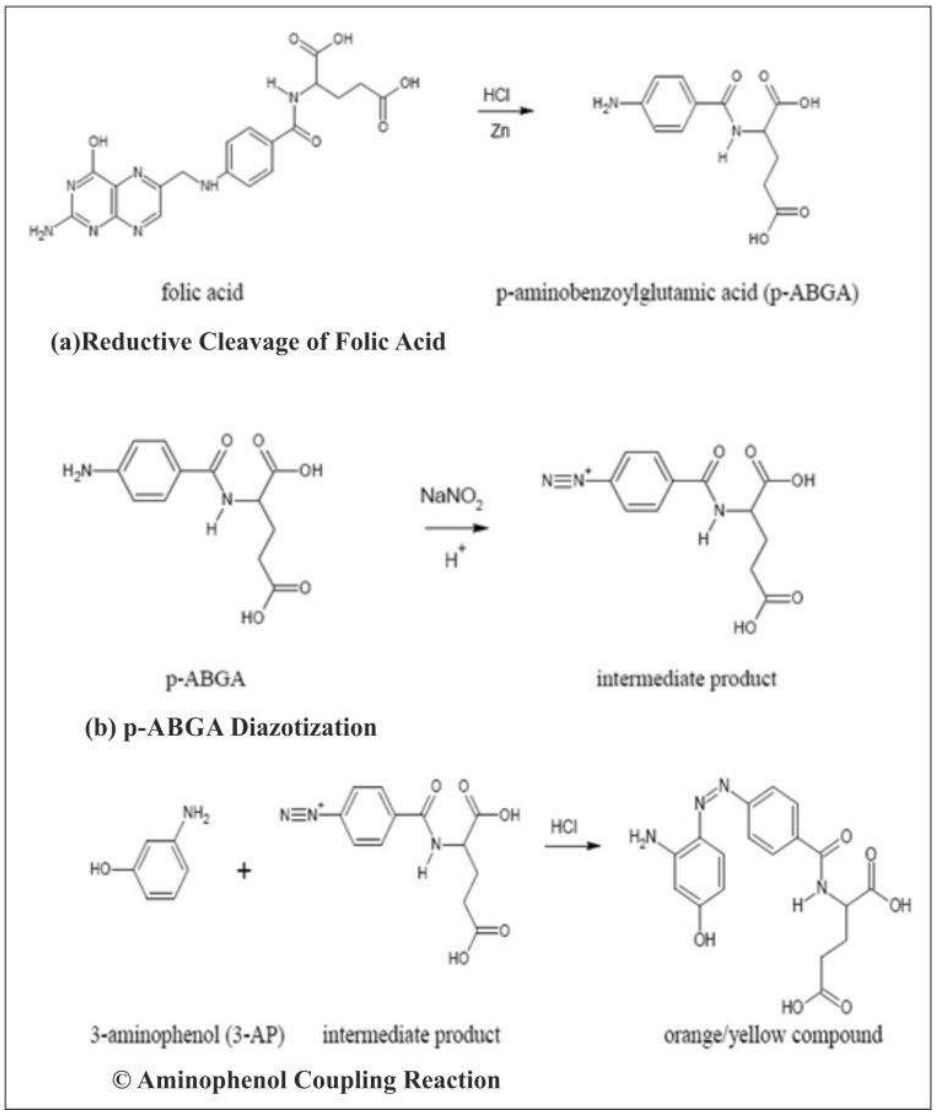


Figure 2. Chemical reaction of folic acid

h) Each solution was diluted to 25 ml with distilled water and mixed with the vortex.

i) The absorbance of each solution was measured at 460 nm.

Analysis of samples

The 80 mg folic acid-β-cyclodextrin inclusion complexes containing folic acid (25 mg) were placed in a 250 ml glass jar and 8 ml of (0.1 N sodium hydroxide) was added. The mixture was shook for 10 minutes to extract the folic acid from the salt. 4 mg of zinc granules and 40 ml of 5 N HCl were added to the above solution. The solution was shaken every 5 minutes for 30 minutes to allow for the reductive cleavage reaction to go to completion. After that 52 ml of distilled water was added to the jar (final volume = 100 mL). The jar was shaken and zinc granules were allowed to settle to the bottom. 2 ml of the jar's contents was distributed into labeled test tubes. Steps 3-9 from the Standardization procedure were repeated. The final concentration in each test tube was 10 µg/ ml.

The sequence in a brief overview of the chemical changes that occur during this process are given in figure 2.

Results and Discussion

Drug excipients compatibility studies

FTIR analysis of Folic acid, β-Cyclodextrin and their physical mixture was performed (Figure 3). The characteristic peaks of Folic acid C=C aromatic (stretch) 1485, C=N 1718, C=O 1485, C=O carboxylic group 1718, OH carboxylic of glutamic acid moiety 2927, NH group of pterin ring stretching 2927 were evident in folic acid spectra, β-Cyclodextrin OH stretching 3144, CH stretching 2924 were evident in β cyclodextrin spectra (Bratu et al., 2004; Patil et al., 2012).

The characteristic peaks of Folic acid and β-Cyclodextrin were also present in the FTIR spectrum of their physical mixture (Figure 3) with minor shifting in some of the peaks presents in the FTIR spectrum of folic acid and β-cyclodextrin is evident in the FTIR spectrum of the physical mixture. For example: 3543-3411, 2927-2925, 1718-1694 etc. This indicates that there might be some physical interaction between the folic acid and β-cyclodextrin like hydrogen bonding or dipole-dipole attraction (Bratu et al., 2004; Patil et al., 2012).

Characterization of Folic Acid-β-Cyclodextrin inclusion complexes

The product obtained after following the procedure for preparation of inclusion complexes by solvent evaporation, co-precipitation and kneading method was subjected to FTIR, DSC & XRD analysis and the results obtained can be summarized here.

FTIR Studies

Characteristics peaks of folic acid 3543, 3413, 3322, 2927, 2569, 1718 and β-cyclodextrin 3144, 2924, 1643 etc are seen in the FTIR spectrum of product formation (Figure 3). The

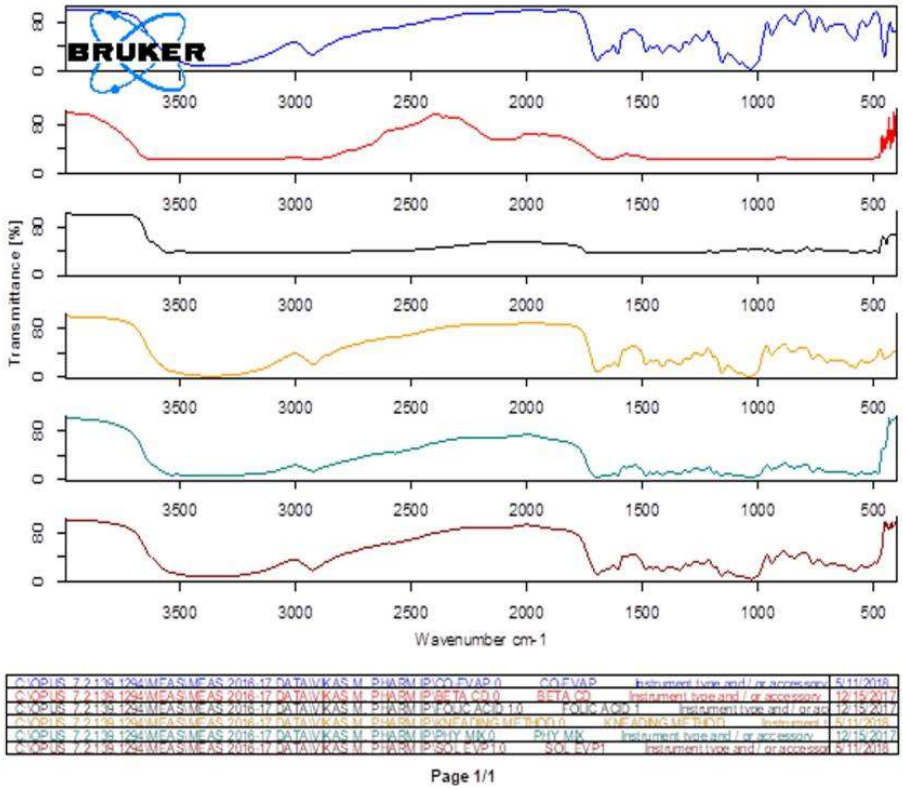


Figure 3. FT-IR spectrum of folic acid

spectrum of solvent evaporation method revealed characteristics peaks *viz.* 3409 for (N-H) Secondary amine group, 2925 for (–C-H) Alkane stretch, 1693 for C=C (Alkene) stretch, 1606-1483 for C=C (Aromatic) stretch, 1028-1190 for C-O stretch and 941 for =C-H (Alkene) bending respectively. The spectrum co-precipitation method revealed characteristics peaks *viz.* 3387 for N-H (Secondary amine) group, 2926 for –C-H (Alkane) stretch, 1694 for C=C (Alkene) stretch, 1606-1483 for C=C (Aromatic) stretch, 1028-1191 for C-O stretch and 941 for =C-H (Alkene) bending respectively. The spectrum kneading method revealed characteristics peaks *viz.* 3364 for N-H (Secondary amine) group, 2923 for –C-H (Alkane) stretch, 1693 for C=C (Alkene) stretch, 1607-1483 for C=C (Aromatic) stretch, 1030-1192 for C-O stretch and 941 for =C-H (Alkene) bending respectively (Bratu et al., 2004; Patil et al., 2012; El-Leithy et al., 2017).

DSC Studies

The DSC thermogram of folic acid shows a sharp endothermic peak at 196°C which is also evident in the DSC thermogram of folic acid-β cyclodextrin inclusion complexes at 195°C, 179°C and 175°C respectively by solvent evaporation, co-precipitation and kneading method (Figure 4). The DSC thermogram of β-cyclodextrin shows a broader peak at 123°C which is absent in the thermogram of all inclusion complexes of all the three methods solvent evaporation, co-precipitation and kneading. However broad endothermic peak at 145°C, 126°C, 118°C by solvent evaporation, co-precipitation and kneading method respectively. This shows that the complexes formed by kneading method broke down at lowest temperature. However solvent evaporation and co-precipitation method break down at high temperature (Vora et al., 2002; El-Leithy et al., 2017).

X-Ray diffraction (XRD) studies

The XRD study, which is a versatile nondestructive technique, was conducted to detect the crystalline behavior of different samples. Powder XRD patterns of folic acid, β-cyclodextrin and folic acid-β cyclodextrin inclusion complexes prepared by three different methods were displayed in figure 5. The comparative analysis of XRD diffraction patterns was used to observe the difference at some peaks in the diffraction patterns of the folic acid, β-cyclodextrin and folic acid-β-cyclodextrin complexes prepared by three different methods solvent evaporation, co-precipitation and kneading method.

By observing the XRD of Folic acid, sharp peaks at 2θ value of 11.88, 14.42, 16.66, 19.18, 22.82, 24.64° due to its crystalline properties (Figure 5). The diffraction pattern of β- cyclodextrin reveals obvious intense peaks 2θ value of 10.69, 12.68, 15.52, 17.18, 19.74, 20.76, 22.2, 31.18 and

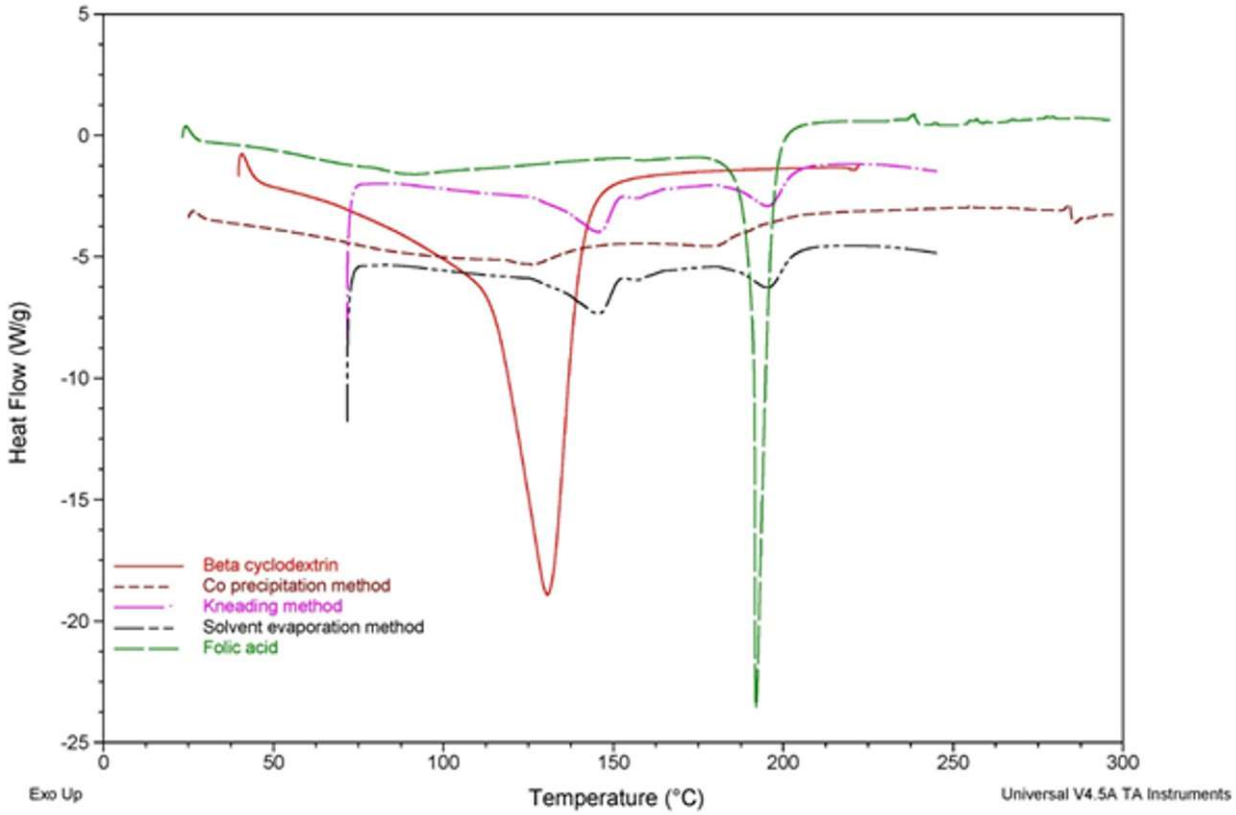


Figure 4. DSC Thermogram of: Folic Acid, β-Cyclodextrin and Folic Acid- β- Cyclodextrin Inclusion Complexes

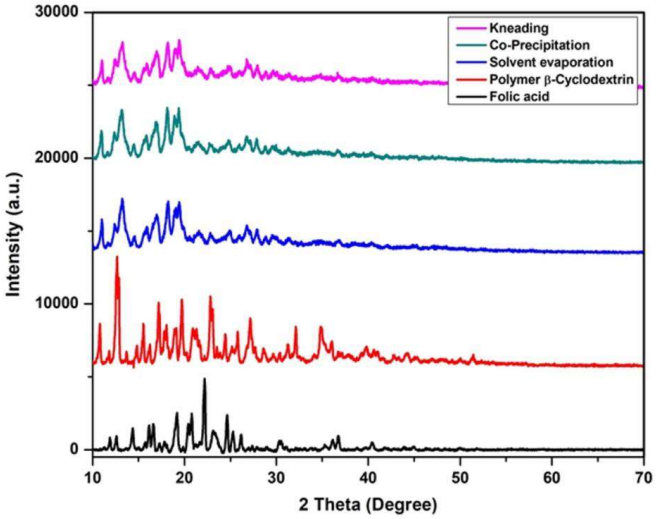


Figure 5. XRD pattern of Folic acid, β-Cyclodextrin and Folic acid-β Cyclodextrin Inclusion Complexes

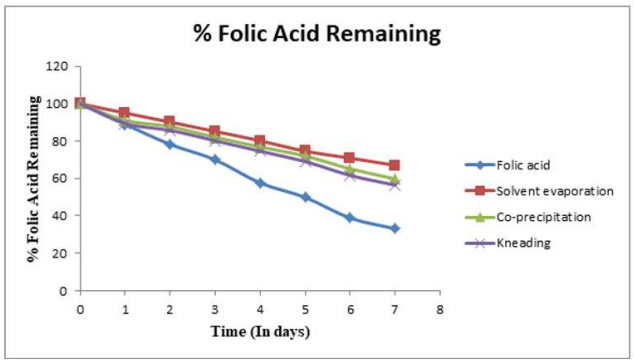


Figure 6. Degradation of Folic Acid in Pure Form and Its Inclusion Complexes Formulation after Exposure to Light during 7 Days Photostability Studies

34.62° indicating the high degree of crystallinity of β-cyclodextrin (Figure 5) (Ilic-Stojanovic et al., 2015; El-Leithy et al., 2017). While in folic acid-inclusion complexes the peaks were fewer and less intense. Lack of characteristic peaks of folic acid confirms the presence of amorphous form of drug. The following characteristics peaks of different methods are: solvent evaporation method based folic acid-β cyclodextrin inclusion complex shows peaks at 11.02, 13.24, 16.84, 18.18, 19.44, 21.42, 26.8 and 27.94, co-precipitation method peaks was observed at 11.02, 13.26, 15.98, 16.92, 18.2, 19.4, 21.6, 24.8, 26.9 and 27.86 and kneading method observed peak was found at 10.88, 13.18, 16.86, 18.12, 21.62, 24.76, 26.66 and 27.78. The loss of some peaks and with reduced intensity indicated that the drug molecules were protected from X-rays by the inclusion of the guest molecules in the hydrophobic cavities of the host molecules (Ilic-Stojanovic et al., 2015; El-Leithy et al., 2017).

Standard Curve of Folic Acid

Different concentrations of folic acid 2, 4, 6, 8, 10…..18 mcg/ml was prepared in phosphate buffer solution of pH 6.8 and absorbance was measured at 281 nm (Figure 1). Standard curve of folic acid with regression coefficient value ($R^2 = 0.998$) was obtained.

Different concentrations of folic acid 2, 4, 6, 8, 10…..18 mcg/ml were prepared by derivatization method and absorbance was measured at 460 nm. Standard curve of folic acid with regression coefficient value $R^2 = 0.999$ was obtained.

Photostability studies

In these investigations the photostability of folic acid in the pure state and the folic acid-β-cyclodextrin complexes by above mentioned three methods of preparation was observed at 25±2°C in Photostability chamber for 7 days and the amount of drug decomposed was calculated everyday using UV Spectrophotometer (UV- 1800, Shimadzu Corp., Japan).

Table 1. Photostability Data of Folic Acid and Folic Acid-β-Cyclodextrin Inclusion Complexes Prepared by Different Methods Measured at 281 nm

Days	Absorbance Mean ±SD (n=3)				% Folic Acid Remaining			
	D	C1	C2	C3	D	C1	C2	C3
1	0.481 ±0.001	0.511 ±0.002	0.490 ±0.001	0.481 ±0.001	88.95	95.00	91.11	89.44
2	0.423 ±0.001	0.485 ±0.001	0.472 ±0.001	0.461 ±0.001	78.26	90.18	87.77	85.74
3	0.378 ±0.001	0.458 ±0.001	0.441 ±0.001	0.431 ±0.001	69.98	85.18	82.03	80.18
4	0.311 ±0.000	0.431 ±0.001	0.413 ±0.002	0.401 ±0.001	57.64	80.18	76.85	74.62
5	0.269 ±0.001	0.401 ±0.000	0.387 ±0.001	0.371 ±0.001	49.90	74.62	72.03	69.07
6	0.209 ±0.000	0.381 ±0.001	0.350 ±0.001	0.331 ±0.001	38.85	70.92	65.18	61.66
7	0.178 ±0.001	0.360 ±0.001	0.321 ±0.001	0.310 ±0.001	33.14	67.03	59.81	56.48

D-Folic acid, C1-Cyclodextrin 1 (Solvent Evaporation) 1, C2- Cyclodextrin 2 (Co-precipitation), C3- Cyclodextrin 3 (Kneading)

Table 2. Photostability Data of Folic Acid and Folic Acid-β-Cyclodextrin Inclusion Complexes Prepared by Different Methods Measured at 460 nm

Days	Absorbance Mean ±SD (n=3)				% Folic Acid Remaining			
	D	C1	C2	C3	D	C1	C2	C3
1	0.476 ±0.001	0.511 ±0.001	0.490 ±0.001	0.481 ±0.001	73.91	81.52	76.95	75
2	0.420 ±0.001	0.490 ±0.001	0.470 ±0.001	0.461 ±0.001	61.73	76.95	72.60	70.65
3	0.372 ±0.001	0.461 ±0.000	0.437 ±0.001	0.431 ±0.001	51.30	70.65	65.43	64.13
4	0.310 ±0.001	0.435 ±0.001	0.410 ±0.001	0.403 ±0.001	37.82	65	59.56	58.04
5	0.273 ±0.001	0.407 ±0.001	0.381 ±0.001	0.371 ±0.001	29.78	58.91	53.26	51.08
6	0.252 ±0.001	0.387 ±0.001	0.347 ±0.001	0.331 ±0.001	25.21	54.56	45.86	42.39
7	0.237 ±0.001	0.375 ±0.002	0.329 ±0.001	0.311 ±0.001	21.95	51.95	41.95	38.04

D-Folic acid, C1-Cyclodextrin 1 (Solvent Evaporation) 1, C2- Cyclodextrin 2 (Co-precipitation), C3- Cyclodextrin 3 (Kneading)

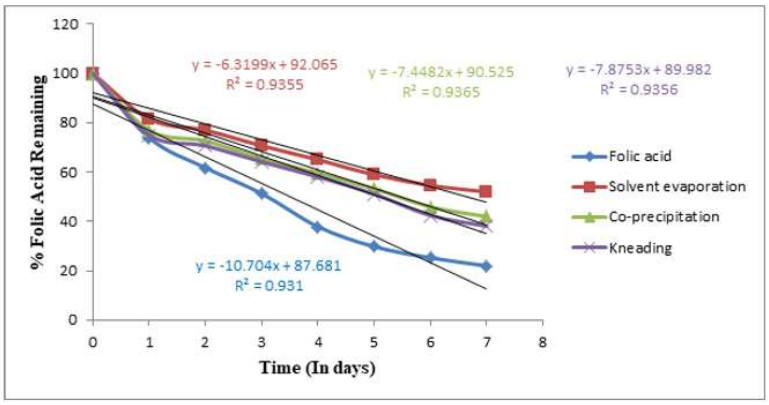


Figure 7. Degradation of folic acid in pure form and its inclusion complexes formulation after exposure to Light during 7 Days photostability studies

Table 3. First order for derivatization method

Time	Log % Folic acid Remaining	Log % Folic acid Remaining (Solvent evaporation)	Log % Folic acid Remaining (Co-precipitation)	Log % Folic acid Remaining (Kneading method)
0	2	2	2	2
1	1.86	1.91	1.88	1.87
2	1.79	1.88	1.86	1.84
3	1.71	1.84	1.81	1.80
4	1.57	1.81	1.77	1.76
5	1.47	1.77	1.72	1.70
6	1.40	1.73	1.66	1.62
7	1.34	1.72	1.62	1.58

Total drug percentage remaining in the pure form was 21.95 % while in the complexes state was 51.95 %, 41.95 %, 38.04 % for the complexes formed by solvent evaporation, co-precipitation and kneading method respectively after 7 days of the study (Table 2). The decrease in the total amount of drug after seven days of the photostability studies is greater when analyzed by the derivatization method probably due to the fact that the value of absorbance of folic acid at 281 nm in phosphate buffer (6.8 pH) is the total absorbance by folic acid and its impurities formed due to its photo-degradation. However in the later case the absorbance is only by folic acid which has formed its derivatives. Photo-degradation of folic acid analysis by derivatization figure 7 shows the relationship between time (in days) and percentage drug remaining which represents zero order kinetics while figure 8 shows the relationship between time (in days) & log % drug remaining which

Table 4. Correlation Coefficient (R^2) value for derivatization method

Chemical Kinetics	Folic Acid	Solvent Evaporation Method	Co-precipitation Method	Kneading Method
Zero Order (R^2)	0.931	0.935	0.936	0.935
First Order (R^2)	0.992	0.966	0.978	0.976

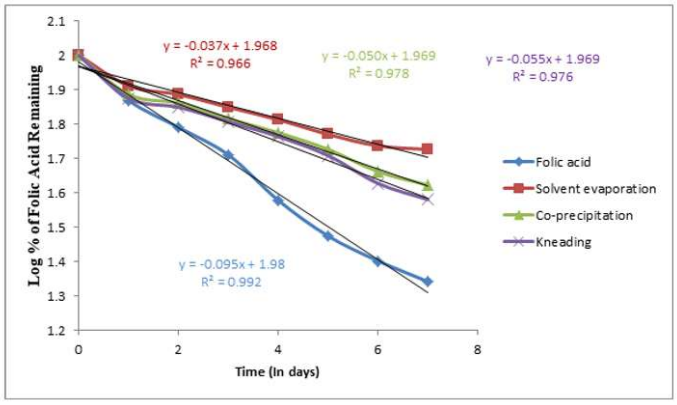


Figure 8. First Order Chemical Kinetic for λ_{max} at 460 nm

represents 1st order kinetics. The R^2 values for 1st order kinetics are 0.992, 0.966, 0.978 and 0.976 for folic acid, solvent evaporation, co-precipitation and kneading method. But in the case of zero order kinetics the value for R^2 are 0.931, 0.935, 0.936, and 0.935 for folic acid, solvent evaporation, co-precipitation and kneading method respectively (Table 3). This shows that the drug degradation follows 1st order release kinetics both in the pure drug & in β-cyclodextrin inclusion complexes. The slope of degradation of pure drug changed from 0.094 to 0.078 after 3rd day which shows a steep increase in drug degradation after 3rd day. This can be attributed to the accumulation of FPT (6-formylpterin) and PCA (6-carboxypterin) which play their role in accelerating the photo-degradation of folic acid (Gazzali et al., 2016). However this trend is not seen in the degradation pattern of inclusion complexes with all three methods of preparation. This could be because the total drug degradation in all the three cases is comparatively very less leading to the formation of FPT & PCA in smaller quantities that could not do a noticeable acceleration in the photo-degradation of folic acid. This shows that complexation of drug by β-cyclodextrin protected it significantly from deteriorating effects of light. Solvent evaporation is most effective method for enhanced the photostability of folic acid as compared to co-precipitation and kneading method.

Conclusion

β-cyclodextrin complexation technique promise to enhance the photostability of folic acid. The complexes obtained by solvent evaporation method were the most stable followed by Co-precipitation & kneading method which was evident through FTIR, XRD & DSC studies. Solvent evaporation method could exert the greatest photo-protective effect on the drug followed by co-precipitation & kneading method. The method of preparation influences the ability of photo-protection. *In vivo* studies, toxicity studies and clinical studies are required to further exploit this advantage of folic acid-β-cyclodextrin complexes.

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Conflict of interest

The authors have no conflict of interest.

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