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Solubility Enhancement for some BCS Class IV Drug

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Abstract In the present study, inclusion complexes of Gamma oryganol were developed with β-cyclodextrin (β-CD) and hydroxy propyl β-cyclodextrin (HPβ-CD). The complexes were prepared by kneading and solvent evaporation. Inclusion complexes of Gamma oryganol with both β-CD and HPβ-CD in aqueous solution were evaluated by phase solubility study. The phase solubility diagram with both the complexing agent was classified as A_L-type, indicating the formation of 1:1 steriochiometric inclusion complex. The apparent complexation constant obtained were 164.41 $M^{-1}$and 130.92 M^{-1}, respectively. No endothermic and characteristic diffraction peaks of Gamma oryganol was observed in both the inclusion complexes in Differential scanning colorimetry (DSC) and X-ray diffractometry (XRD). The drug was compatible with the polymers used. It was confirmed by fourier-tranform infrared spectra. The study indicated the presence of intermolecular hydrogen bonds between Gamma oryganol and both complexing agents, resulting in the formation of amorphous form. Aqueous solubility and dissolution profiles were markedly increased in inclusion complexes, compared with the drug alone and physical mixture. Moreover, Gamma orygano-HPβ-CD Complex performed better than Gamma oryganol-β-CD complex. The inclusion complex prepared by Solvent evaporation was shown better aqueous solubility and dissolution profile than complex prepared by kneading method as well as complex prepared by 1:2 M ratio shows higher dissolution profile as compared to 1:1 M ratio, which indicate as the ratio of complex increase there is increase in dissolution behavior of drug.

Keywords BCS Class IV Drug, β-cyclodextrin, Gamma oryganol, Solubility

Introduction

Solubility is the property of a solid, liquid, or gaseous chemical substance called *solute* to dissolve in a solid, liquid, or gaseous solvent to form a homogeneous solution of the solute in the solvent. The solubility of a substance fundamentally depends on the solvent used as well as on temperature and pressure. The extent of solubility of a substance in a specific solvent is measured as the saturation concentration where adding more solute does not increase its concentration in the solution [1]. The solvent is generally a liquid, which can be a pure substance or a mixture of two liquids. One may also speak of solid solution, but rarely of solution in a gas. The extent of solubility ranges widely, from infinitely soluble (fully miscible) such as ethanol in water, to poorly soluble, such as silver chloride in water. The term insoluble soften applied to poorly or very poorly soluble compounds [2]. Solubility occurs under dynamic equilibrium, which means that solubility results from the simultaneous and opposing processes of dissolution and phase joining (e.g., precipitation of solids). Solubility equilibrium occurs when the two processes proceed at a constant rate. Under certain conditions equilibrium solubility may be exceeded to give also-called supersaturated solution, which is metastable [3].

Material and Methods

The following materials that were either AR/LR grader the best possible pharma grade available were used as supplied by the manufacturer.

Calibration curve for Gamma oryganol in distilled water 30

100 of Gamma oryganol was accurately weighed into 100ml volumetric flask and dissolved in small quantity of methanol.The volume was made upto 100ml with the methanol to get a concentration of (1000 µg/ml.) SS-I. From this, 2 ml was withdrawn and diluted to 100ml to get a concentration of (20 µg/ml) SS-II. From the standard stock solution (SS-II), 1,2,4,6 and 8ml were withdrawn and volume was made up to 10ml with Distilled water to give a concentration of 2,4,8,12 and 16 µg/ml. Absorbance of these solutions was measured against a blank of Distilled water at 264 nm for Gamma oryganol and the absorbance values are summarized in Table 1 Calibration curve was plotted, drug concentrations versus absorbance was given in the Figure 1.

Calibration curve for Gamma oryganol in Methanol

100 mg of Gamma oryganol was accurately weighed into 100ml volumetric flask and dissolved in small quantity of methanol. The volume was made up to 100ml with the methanol to get a concentration of (1000 µg/ml.) SS-I. From this, 2 ml was withdrawn and diluted to 100ml to get a concentration of (20µg/ml) SS-II. From the standard stock solution (SS-II), 2,4,8,12 and 16ml were withdrawn and volume was made up to 10 ml with methanol to give a concentration of 2,4,8,12 and 16 µg/ml. Absorbance of these solutions was measured against a blank of methanol at 264 nm for Gamma oryganoland the absorbance values are summarized in Table 2. Calibration curve was plotted, drug concentrations versus absorbance was given in the Figure 2.

Calibration curve for Gamma oryganol in 7.2 pH phosphate Buffer

100 mg of Gamma oryganol was accurately weighed into 100ml volumetric flask and dissolved in small quantity of methanol. The volume was made up to 100ml with the methanol to get a concentration of (1000 µg/ml.) SS-I. From this, 2 ml was withdrawn and diluted to 100ml to get a concentration of (20 µg/ml) SS-II. From the standard stock solution (SS-II), 1,2,4,6 and 8 ml were withdrawn and volume was made up to 10 ml with 7.2 pH Phosphate buffer to give a concentration of 2,4,8,12 and 16 µg/ml. Absorbance of these solutions was measured against a blank 7.2 pH Phosphate buffer of at 264 nm for Gamma oryganol and the absorbance values are summarized.

Phase solubility studies

The most widely used approach to study inclusion complexation is the phase solubility method described by Higuchi and Connors, which examines the effect of a solubilizer, i.e., CD or ligand on the drug being solubilized, i.e., the substrate.

50mg gamma oryzanol was added to 15ml distilled water containing 0- 10mM beta cyclodextrin and transferred to 25ml stoppered conical flask. The mixture was shaken for 72hrs. Aliquots of 2ml were withdrawn and filtered immediately using 0.45µ nylon disc filter. The filtered samples were diluted suitably and assayed for gamma oryzanol by measuring absorbance at 264 nm against blank. The experiments were conducted in triplicate. The same procedure was followed to HP beta-CD.

The apparent solubility constant (Kc) according the hypothesis of 1:1 stoichiometric ratio of complexes was calculated from the phase-solubility diagram using following equation.

$$K_{a:b} = \frac{\text{slope}}{S_0\ (1\text{-slope})}$$

The slope is obtained from the initial straight line portion of the plot of gamma oryzanol against cyclodextrin concentration, and So is the equilibrium solubility of gamma oryzanol in water.

Compatibility Study with IR

Fourier Transform Infrared Spectroscopy

Infrared spectroscopy is one of the most powerful analytical techniques that offer the possibility of chemical identification. The IR spectra of Gamma oryganol and their complexes were obtained by KBr pellet method by JASCO FT/IR- 5300 spectrometer. A resolution of 4 cm^{-1} was used and 4 scans were co-added for each spectrum over a frequency range of 4000-400 cm^{-1}. All sample was analyzed in duplicate. Preparation and charecterization of Gamma oryganol-β-cyclodextrin and HPβ-cyclodextrins inclusion complexes 26,43,45. Complexes of Gamma oryganol with β-cyclodextrin (β-CD) and hydroxypropyl β cyclodextrin (HPβCD) were prepared by different methods using different molar concentrations of β-CD and (HP β-CD).

Methods used in present work

Physical mixture: Gamma oryganol with β-CD in different molar ratios (i.e.1:1M, 1:2M) and with HPβ-CD in ratio (i.e.,1:1M,1:2M) were mixed in a mortar for about one hour with constant trituration, passed through sieve No.100 and stored in adesic cators over fused calcium chloride.

Kneading method: Gamma oryganol with β-C Dindifferent molar ratios (i.e., 1:1M, 1:2M) and with HPβ-CD in ratios (i.e.1:1M) were taken. First cyclodertrin is added to the mortar, small quantity of 50% Ethanol is added while triturating to get slurry like consistency. Then slowly drug is incorporated into the slurry and trituration is further continued for one hour. Slurry is then air dried at 25°C for 24 hours, pulverized and passed through sieve no. 100 and stored in desiccators over fused calcium chloride.

Solvent evaporation method: Weighed amount of drug and β-CD were dissolved in required amount of methanol–water 50%v/vmixture. Mixed the both solution than the clear solution was kept for stirring on a magnetic stirrer till all the solvent got evaporated. The mass obtained was dried at 50°C and further sieved through no. 100 sieve. Same was repeated for drug and HP-β-CD.

Drug Evaluation Studies

Drug Content Estimation: Inclusion complexes prepared by physical mixture, kneading, and solvent evaporation methods were assayed for Gamma oryganol content by dissolving a specific amount of the complexes in methanol and analyzing for the Gamma oryganol content spectrophotometrically at 264 nm on a spectrophotometer.

Saturation solution study: pure drug, physical mixture and inclusion complex were weighed accurately (approx. 100mg) and transferred in three screw capped bottles containing 10ml distilled water. Tubes were kept for shaking at maximum speed in mechanical shaker. After 48 hrs, tubes were removed and kept aside 1 hour to attain equilibrium. Sample solution were filtered through Whatman filter paper No. 42 and absorbance taken at 264nm.

In vitro dissolution rate studies Gamma oryzanol-β-CD and HPβ-CD complexes:26.Dissolution studies of gamma oryzanol, its complex with β-CD and HPβ-CD and its physical mixture was performed using USP dissolution apparatus type (USPII)with 900-ml dissolution medium at 37˚C± 0.5˚C and 75 rpm for 45 min and 7.2 pH Buffer were used as dissolution media. At fixed time intervals,5-mlaliquots were withdrawn, filtered, suitably diluted, and assayed for Gamma oryganol content by measuring the absorbance at 264nm using a spectrophotometer. Equal volume of fresh medium at the same temperature was replaced into the dissolution medium after each sampling to maintain its constant volume throughout the study. Dissolution studies were perform in triplicate (n=3) and calculated mean values of cumulative drug release were used while plotting the release curves. The percent drug released at various time intervals was calculated and plotted against time.

Characterization of complexes

Differential scanning calorimetry (DSC): The DSC measurements were performed using a Perkin Elmer Pyris (Shelton, CT) and mettle equipped with an intercooler 2P cooling accessory. Samples of 4mg were placed in standard aluminum pans and sealed with a lid. Heating scans by10˚C/min were applied with a nitrogen purge of 20ml/min, over a temperature range of 30˚C to 285°C. An empty aluminum pan was used as reference.

Fourier transformation infrared spectroscopy (FTIR)

Infrared spectra were recorded using a jasco FTIR spectrometer 5300 (japan) using KBr disks. The scanning range was kept from 4000 to 400cm^{-1}.

Stability study

In any rational drug design or evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining the iracceptance or rejection.

Stability of a drug can be defined as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity is not less than determined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously.

b) Objective of the Study: 36, 37 The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. The International Conference on Harmonization (ICH) Guide linestitled "Stability Testing of New Drug substance and Products" (QIA) describes the stability test requirements for drug registration applications in the European Union, Japan and the United States of America.

ICH specifies the length of study and storage conditions.

Long-term Testing: 25˚C ± 2˚C / 60 % RH ± 5 % for 12 Months.

Accelerated Testing: 40˚C ± 2˚C / 75 % RH ± 5 % for 6 Months.

Stability studies were carried out at 25˚C / 60 % RH and 40˚C / 75 % RH
for the selected formulation for the period of 6 weeks.

Method: The selected formulations were packed in amber-colored bottles, which were tightly plugged with cotton and capped. They were then stored at 25˚C / 60% RH and 40˚C / 75 % RH for 6 weeks and evaluated for their physical appearance, drug content and drug excipients compatibility at specified intervals of time.

Result and Discussion

Calibration Curve

Standard Calibration Curve of Gamma Oryganol in Distilled Water

Standard calibration curve of Gamma Oryganol was drawn by plotting absorbance v/s concentration. The λ_{max} of Gamma Oryganol in distilled water was determined to be 264 nm. The absorbance values are tabulated in Table 1. Standard calibration curve of Gamma Oryganol in the Beer's concentration range between 0-20 µg/ml is shown in Figure 1.

Table 1: Standard Calibration Curve of Gamma Oryganol in Distilled Water λ_{max} 264

S. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.081
3	4	0.156
4	6	0.274
5	8	0.389
6	10	0.426
7	12	0.562
8	14	0.648
9	16	0.794
10	18	0.836
11	20	0.927

*Average of three determination

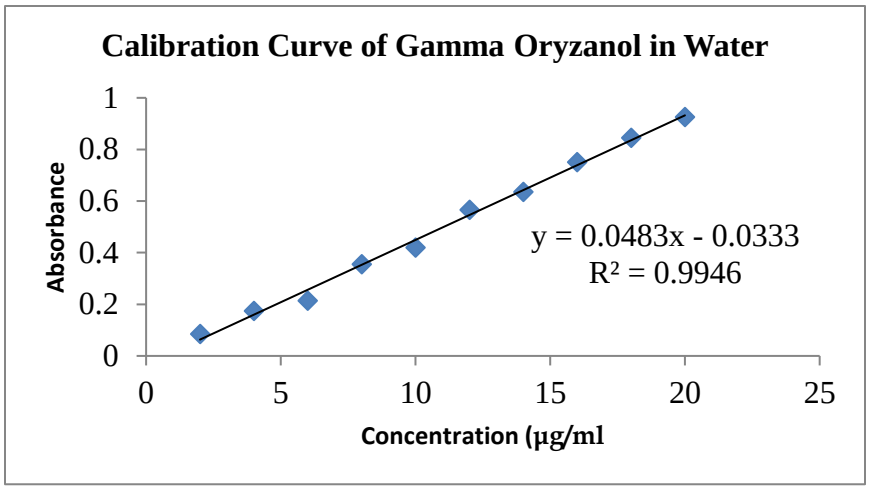

Figure 1: Calibration Curve of Gamma Oryganol in Water

The linear regression analysis standard curve in distilled water:

The linear regression analysis was done on absorbance data points.

The results are as follows:

The Slope = 0.0481

The intercept =0.047

The correlation coefficient =0.995

A straight-line equation (y = mx + c) was generated to facilitate the calculation for amount of drug. The equation is as follows.

Absorbance = 0.0481 X Concentration

Standard Calibration Curve of Gamma Oryganol in Methanol λ_{max} 264

Standard calibration curve of Gamma Oryganol was drawn by plotting absorbance v/s concentration. The λ_{max} of Gamma Oryganol in methanol was determined to be 264 nm. The absorbance values are tabulated in Table 2. Standard calibration curve of Gamma Oryganol in the Beer's concentration range between 0-20 µg/ml is shown in Figure 2.

Table 2: Standard Calibration Curve of Gamma Oryganol in Methanol

S. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.078
3	4	0.164
4	6	0.242
5	8	0.387
6	10	0.494
7	12	0.543
8	14	0.664
9	16	0.751
10	18	0.891
11	20	0.967

*Average of three determination

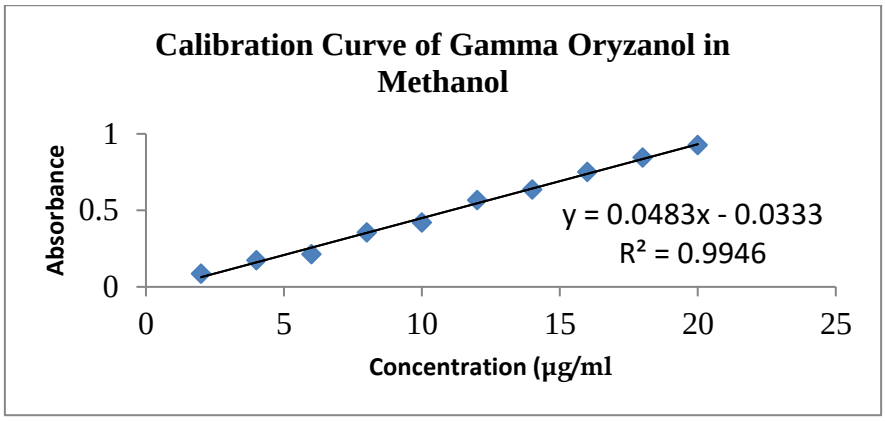

Figure 2: Calibration Curve of Gamma Oryganol in Methanol

The linear regression analysis standard curve in methanol

The linear regression analysis was done on absorbance data points.

The results are as follows:

The Slope = 0.0500

The intercept =0.034

The correlation coefficient =0.996

A straight-line equation (y = mx + c) was generated to facilitate the calculation for amount of drug. The equation is as follows.

Absorbance = 0.048 X Concentration

Table 3: Standard Calibration Curve of Gamma Oryganol in pH 7.2 Buffer

S. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.086
3	4	0.175
4	6	0.214
5	8	0.356
6	10	0.420
7	12	0.567
8	14	0.636
9	16	0.751
10	18	0.846
11	20	0.926

*Average of three determination

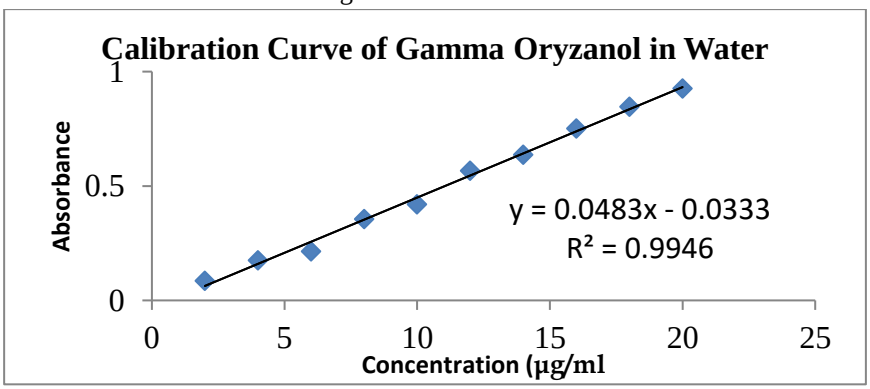

Figure 3: Calibration Curve of Gamma Oryganol in pH 7.2 Buffer

Phase Solubility Study

Table 4: Phase Solubility Study for Gamma Oryganol/β–Cyclodextrin

S. No.	Concentration of β–Cyclodextrin	Concentration of Gamma Oryganol
1	0	0.0124
2	2	0.0138
3	4	0.0194
4	6	0.0203
5	8	0.0268
6	10	0.0291

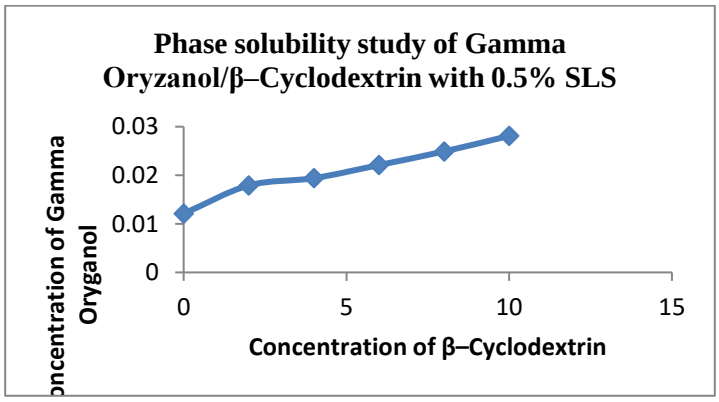

Figure 4: Phase solubility study of Gamma Oryzanol/β–Cyclodextrin with 0.5% SL

Table 5: Phase Solubility Study for Gamma Oryganol/β–Cyclodextrin with 0.5% SLS

S. No.	Concentration of β–Cyclodextrin	Concentration of Gamma Oryganol
1	0	0.0121
2	2	0.0179
3	4	0.0194
4	6	0.0221
5	8	0.0249
6	10	0.0281

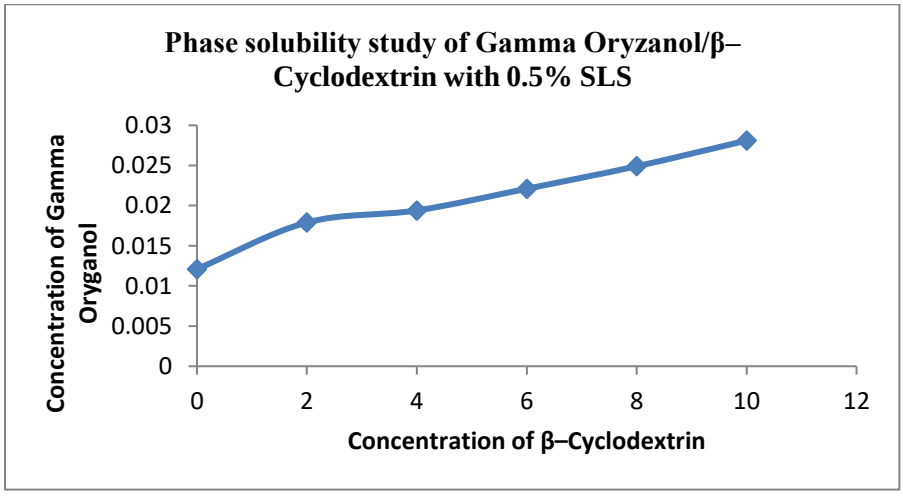

Figure 5: Phase solubility of Gamma Oryganol/β-Cyclodextrin with 0.5% SLS

Drug Content Uniformity

The percentage of drug content was found to be in the range of 73.41 to 97.90 showed in table 6.

Table 6: Drug content uniformity of Gamma Oryganol/β-cyclodextrin and Gamma Oryganol/β-cyclodextrin with 0.5% SLS

S. No.	Formulation	Quantity of complex taken (mg)	Theoretical drug content (mg)	Practical drug content (mg)	% drug content
1	F1	50	12.6	10.8	85.71
2	F2	50	16.8	16.1	95.83
3	F3	50	12.5	10.1	80.80
4	F4	50	13.6	11.8	86.76
5	F5	50	11.1	9.1	81.98
6	F6	50	15.2	14.1	92.76

*Each reading is an average of 3 readings

Solubility Study

The pure drug, physical mixture and inclusion complex was analysed by adding phosphate buffer pH 7.2. The concentration was found to be in the range of 0.028-0.499. (Table 7)

Table 7: Solubility Study

S. No.	Sample	Absorbance	Conc. X	Concentration (µg/ml)	Concentration (mg/ml)
1	Pure drug	0.024	0.2841	28..41	0.028
2	Physical mixture	0.219	2.5647	256.47	0.256
3	Inclusion complex	0.426	4.8654	486.54	0.486

***In vitro* Drug Release**

The *in vitro* drug release study of pure Gamma Oryganol in pH 7.2 Buffer is given in Table 8.

Table 8: *In vitro* drug dissolution of pure Gamma Oryganol in pH 7.2 Buffer

Time (min)	√T	Log T	Absorbance	Concentration (µg/ml)	Concentration (mg/900ml)	% Drug release	Log% Drug Release
2	1.41421	0.30103	0.0161	0.191017	0.171915	1.7191	0.23531
5	2.23606	0.69897	0.0442	0.524407	0.471966	4.7196	0.67391
10	3.16227	1	0.0886	1.051186	0.946068	9.4606	0.97592
15	3.87298	1.17609	0.1267	1.50322	1.352898	13.528	1.13126
20	4.47213	1.30103	0.1634	1.938644	1.74478	17.447	1.24174
30	5.47722	1.47712	0.2223	2.637458	2.373712	23.737	1.37542
45	6.70820	1.65321	0.301	3.571186	3.214068	32.140	1.50705

***Each reading is an average of 3 readings**

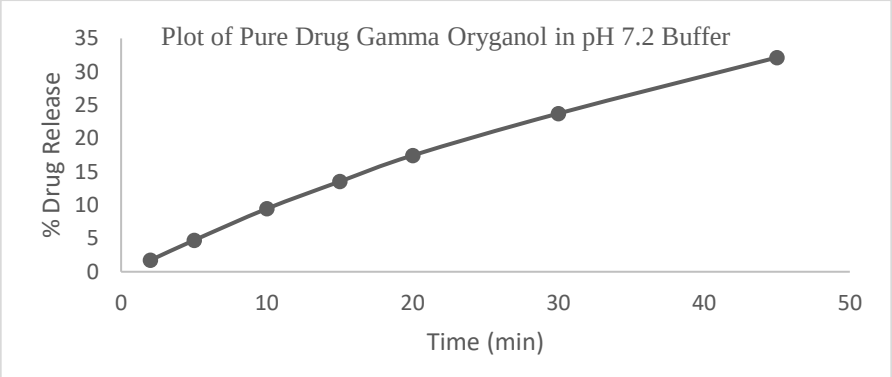

Figure 6: In vitro drug dissolution profile of pure Gamma Oryganol in pH 7.2 Buffer

Table 9: *In vitro* drug dissolution of F1 complexes in pH 7.2 Buffer

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Concentration (mg/900ml)	% Drug release	Log% Drug Release
1	2	0.0161	0.3744	0.3370	3.12	1.1379
2	5	0.0246	0.5721	0.5149	4.77	1.5618
3	10	0.0364	0.8465	0.7619	7.05	1.9536
4	15	0.0546	1.2698	1.1428	10.58	2.3591
5	20	0.0789	1.8349	1.6514	15.29	2.7272
6	30	0.0954	2.2186	1.9967	18.49	2.9171
7	45	0.1231	2.8628	2.5765	23.86	3.1721

***Each reading is an average of 3 readings**

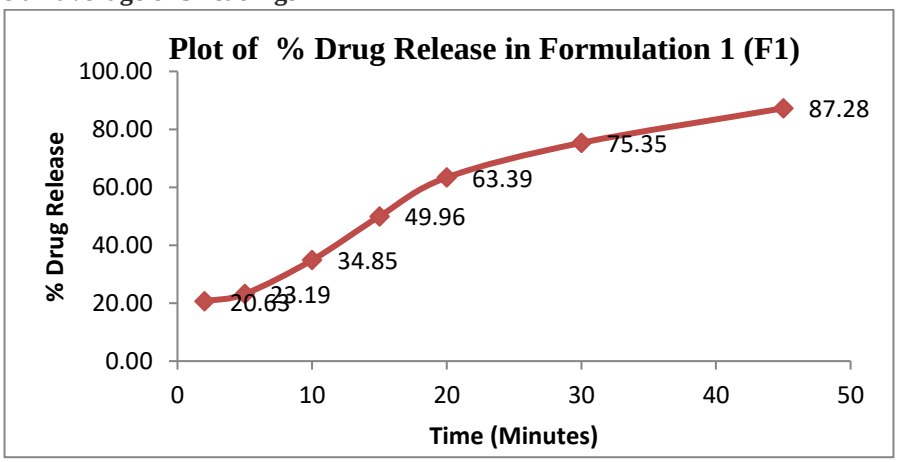

Figure 7: In vitro drug dissolution of F1 complexes in pH 7.2 Buffer

Table 10: *In vitro* drug dissolution of F2 complexes in pH 7.2 Buffer

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Concentration (mg/900ml)	% Drug release	Log% Drug Release
1	2	0.0459	1.0674	0.9607	5.97	1.7863
2	5	0.1246	2.8977	2.6079	16.20	2.7849
3	10	0.1879	4.3698	3.9328	24.43	3.1957
4	15	0.2786	6.4791	5.8312	36.22	3.5896
5	20	0.3786	8.8047	7.9242	49.22	3.8963
6	30	0.4298	9.9953	8.9958	55.87	4.0231
7	45	0.5312	12.3535	11.1181	69.06	4.2349

*Each reading is an average of 3 readings

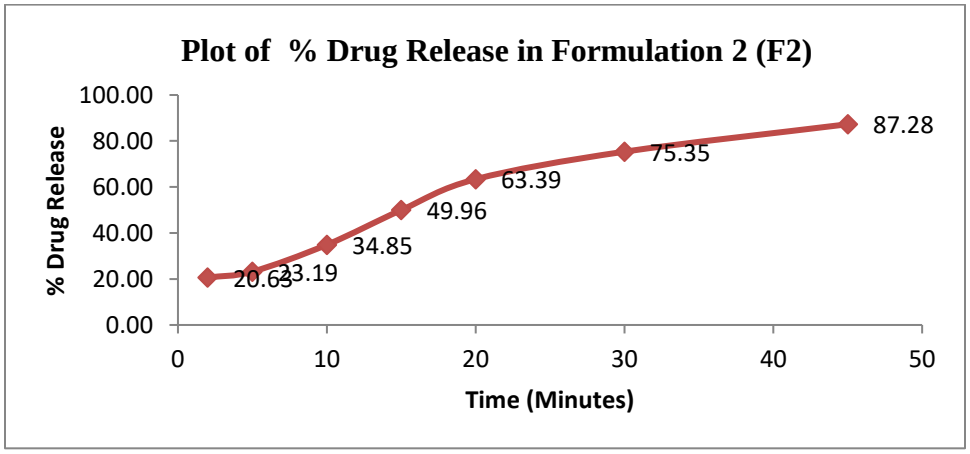

Figure 8: In vitro drug dissolution of F2 complexes in pH 7.2 Buffer

Table 11: *In vitro* drug dissolution of F3 complexes in pH 7.2 Buffer

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Concentration (mg/900ml)	% Drug release	Log% Drug Release
1	2	0.0869	2.0209	1.8188	11.30	2.4245
2	5	0.1697	3.9465	3.5519	22.06	3.0938
3	10	0.2149	4.9977	4.4979	27.94	3.3300
4	15	0.3897	9.0628	8.1565	50.66	3.9252
5	20	0.4687	10.9000	9.8100	60.93	4.1098
6	30	0.5314	12.3581	11.1223	69.08	4.2353
7	45	0.5987	13.9233	12.5309	77.83	4.3546

*Each reading is an average of 3 readings

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