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# Formulation And Evaluation Of Herbal Tablets Containing Ethanolic Extract Of *Acalypha Indica* –A Quality By Design (QBD) Approach

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**ABSTRACT**
The present paper deals with formulation and evaluation of herbal tablets prepared from ethanolic extract of the selected plant. A solid unit pharmaceutical dosage formulation using a novel dry plant extract (stem) using various excipients Polyvinylpyrrolidone (PVP) K 30, Croscarmellos sodium (CCS), Lactose, Magnesium stearate, Talk Finally, the Round and flat shaped tablets, with average weight of 600 mg were compressed. Response surface methodology was used to formulation and evaluations of the herbal tablets made from Ethanolic extract of stem of ***Acalypha indica*** belonging family of Euphorbiaceae, was used for the anti-hyperlipidemia. A design of expert (DOE) was used in the optimization of formulation, which was formed by three factors, namely, the Polyvinylpyrrolidone (PVP) K 30, the Croscarmellose sodium (CCS), and the Lactose, at three levels. Stability study of optimize formulation was done, both physically and chemically, for a period of 3 months at accelerated stability conditions ($25^0C \pm 2^0C$ and 60 ± 5% RH) and ($40^0C \pm 2^0C$ and 70 ±5% RH).

**KEY WORDS-** Optimization, Excipients, Polyvinylpyrrolidone (PVP), the Croscarmellose sodium (CCS), Lactose, Magnesium stearate, Talk, etc.

## INTRODUCTION-

Plants are always an exemplary source of drug. In fact, many of the currently available drugs were derived either directly or indirectly from the plants. The plant kingdom represents a rich source of organic compounds, many of which have been used for medicinal and other purposes[1].

Herbal products may contain a single herb or combination of several different herbs believed to have complementary and/ synergistic effect. Some herbal products, including many traditional medicines formulations, also derived from animal or mineral origins[2].

Herbal medicinal products are defined as any medicinal product, exclusively containing one or more active substances. WHO report 80% of the world population relies on the drug from natural origin[2]. *Acalypha indica* belonging to the family Euphorbiaceae commonly known as haritha manjari is an erect annual herb with stem dark green, quadrangular and longitudinal furrows and wings . It is used as expectorant, purgative, emetic, gastrointestinal irritant, diuretic, cathartic and anthelmintic, hyperlipidemia and high cholesterol. It is also used in constipation, skin diseases, ulcers bronchitis. The plant contain various phyto-constitutents such as alkaloids, flavonoids, glycosides, lactones, terpenoids, cyanogenetic glucosides and glucosinolates, phenantherenes, quinines, phenolic acids etc[3].

## MATERIALS AND METHODS

**Plant material-** The plants were collected from tropical and desert areas of Jaipur, Rajasthan Plant was identified and authenticated at Botany Department of the Apex University, Jaipur.

### Preparation of Extract-

In successive solvent extraction, a dried material was extracted with different solvents, starting from solvent of low polarity. The extraction was continued until the solvent in the thimble. After extraction by one solvent, material is removed from thimble, dried and again recharged, extracted with solvent of successively high polarity .Successive solvent extraction was done by using petroleum ether (60-80), Ethyl Acetate, and ethanol and purified water. The extract was filtered and concentrated. The extract was dried in vacuum drier and stored below 10°C.

### Phytochemical tests of extracts

The various phytochemicals tests performed for selected plants extracts for further study are following

**Test for carbohydrates:-** Benedict's test: About 0.5 ml of the filtrate was taken to which 0.5 ml of Benedict's reagent is added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

Molisch's test: To about 2ml of the sample, 2 drops of alcoholic solution of α-napthol was added and to the mixture after being shaken well. Few drops of conc.$H_2SO4$ were added along the sides of the test tube. A violet ring indicates the presence of sugars[4].

**Test for alkaloids**
Mayer‟s test - To a few ml of the filtrates, a drop of Mayer‟s reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive[5].

**Tests for glycosides**
Liebermann's test:- 2 ml of the organic extract was dissolved in 2 ml of chloroform and 2 ml of acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside)[6].

**Tests for steroids**
A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid added indicates the presence of steroids[6].

**Test for Phenolic compounds and Tannins**
**Ferric Chloride test**
The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric Chloride solution is added. A dark green color indicates the presence of phenolic compound[7].

**Test for Flavonoids:-**
**Shinoda test:** To 1ml of the extract, add 8 – 10 drops of concentrate HCl and a pinch of magnesium powder or filing. Boil for 10 to 15 minutes and cool. A red colorations indicates the presence of flavonoids[8].

**Test for Triterpinoides:-**
**Salkowski's test: -** Filtrate + few drops of conc $H_2SO_4$ (Shaken well and allowed to stand) Golden yellow layer (at the bottom) [8].

**Gums and mucilage Test:-**To 1ml of extract, distilled water, 2ml of absolute ethanol was added with constant stirring white or cloudy precipitate indicates the presence of gums or mucilage[5].

**Table 1. – Table-1 Show Results of Phytochemical investigations of the extracts of Stem of *Acalypha Indica* plant.**

| S.No. | Phyto-constitutents | Petroleum Ether (6080 $^0$C) (Extract) | Ethyl Acetate (Extract) | Ethanol (Extract) | Purified Water (Extract) |
|---|---|---|---|---|---|
| 1. | Flavonoids | - | - | +++ | + |
| 2. | Alkaloids | - | + | ++ | + |
| 3. | Glycosides | - | - | +++ | + |
| 4. | Steroids | - | + | ++ | - |
| 5. | Phenolic compounds and Tannins | - | + | ++ | + |
| 6. | Saponin | - | | +++ | ++ |
| 7. | Carbohydrate | - | + | - | + |
| 8. | Triterpenoids | - | - | +++ | + |
| 9. | Fixed oils and Fats | + | ++ | - | - |
| 10. | Gum and Mucilage | + | + | - | - |

'+'and '-'indicate the presence and absence of the compounds respectively. '+ +'indicates moderate degree of presence and '+ + +' indicates the high degree of the presence.

**Formulation of Tablets**
The software Design Expert version 12.0.1.0 (StatEase) was used for the design of the formulation. The total of 13 runs(formulations) were designed and the relationship of the dependent and independent variables was studied by gaining the surface responses, and finally, the significant model was achieved.

**Steps in Tablet formulations[15,16]**
This procedure use for the optimize formula of tablets.
1) The herbal ingredients were accurately weighed and passed through sieve separately. Due to hygroscopicity of extract was triturated with accurately weighed quantity of anhydrous lactose (dry mixture)

2) Starch was weighed and made into translucent granulating paste with required quantity of water.
3) The remaining herbal ingredients i.e., *Acalypha indica* Stem extract along with Croscarmellose sodium were mixed with this granulating paste until a wet coherent mass was formed.
4) The resulting wet coherent mass was then passed through sieve 10 to form granules. The granules were kept in oven at 40-45° until the granules were properly dried.
5) After proper drying the granules were passed through sieve no. 22 mesh superimposed on sieve no. 44 mesh to separate granules from fines.
6) The granules were then mixed thoroughly with the accurately weighed quantity of talc and magnesium stearate
7) Finally, the Round and flat shaped tablets, with average weight of 600 mg were compressed by using single tablet Punch machine, press with a die size of 6 mm.

**Table-2 Composition of Formulation**

| Formulations | *Acalypha Indica* (Stem) Ethanolic Extract(mg) | Polyvinylpyrrolidone (PVP) K 30 (mg) | Croscarmellose (CCS) sodium (mg) | Lactose (mg) | Magnesium Stearate (%) | Talk |
|---|---|---|---|---|---|---|
| **F 1** | 400 | 50 | 5 | 50 | 0.7 | q.s |
| **F 2** | 400 | 30 | 5 | 70 | 0.7 | q.s |
| **F 3** | 400 | 30 | 17.5 | 50 | 0.7 | q.s |
| **F 4** | 400 | 30 | 5 | 30 | 0.7 | q.s |
| **F 5** | 400 | 30 | 30 | 70 | 0.7 | q.s |
| **F 6** | 400 | 10 | 5 | 50 | 0.7 | q.s |
| **F 7** | 400 | 10 | 17.5 | 70 | 0.7 | q.s |
| **F 8** | 400 | 50 | 17.5 | 70 | 0.7 | q.s |
| **F 9** | 400 | 10 | 30 | 50 | 0.7 | q.s |
| **F 10** | 400 | 30 | 30 | 30 | 0.7 | q.s |
| **F 11** | 400 | 50 | 30 | 50 | 0.7 | q.s |
| **F 12** | 400 | 50 | 17.5 | 30 | 0.5 | q.s |
| **F 13** | 400 | 10 | 17.5 | 30 | 0.5 | q.s |

**Pre-compression Evaluation Parameters Bulk density**
Bulk density was carried out in 100 ml dried measuring cylinder. Then after pouring the powder into the graduated cylinder the powder bed was made uniform without disturbing. Then the volume was measured directly from the graduation marks on the cylinder as ml. calculated by using the following formula. Bulk density = Mass of the granules/Bulk volume of the granules[9]

**Tapped density**
Tapped density was carried out by pouring of dried granules in 100 ml measuring cylinder.100 tapping was done, note down the volume and calculate by using the following formula.

**Dt = M/Vt**
Where, M is the mass of powder and Vt is tapped volume of powder[9]
**Angle of repose:** Flow properties of the physical mixtures of all the formulations were determined by calculating angle of repose by fixed height method. A funnel with 10 mm inner diameter of stem was fixed at a height of 2 cm. over the platform. About 10 gm of sample was slowly passed along the wall of the funnel till the tip of the pile formed and touches the steam of the funnel. A rough circle was drawn around the pile base and the radius of the powder cone was measured.
Angle of repose was calculated from the average radius using the following formula. = tan1 (h/r)
Where,
= Angle of repose h = Height of the pile r = Average radius of the powder cone[10]

**Table 3: Relation between Angle of repose and Type of Flow[11]**

| S. No | Angle of repose (ɵ) | Flow property |
|---|---|---|
| **1.** | 20-25 | Excellent |
| **2.** | 25-30 | Good |
| **3.** | 30-40 | Fair-aid not needed |
| **4.** | 41-45 | Passable-may hang up |
| **5.** | 46-55 | Poor-must agitate vibrate |
| **6.** | 56-65 | Very poor |
| **7.** | >65 | Very, very poor |

**Hausner's ratio:** It provides an indication of the degree of densification which could result from vibration of the feed hopper.
Hausner ratio= Tapped density/ Bulk density
Lower Hausner ratio Better flow ability
Higher Hausner ratio Poor flow ability[4]

**Percentage Compressibility (Carr's Index)**
Percentage Carr's Index (% compressibility) was calculated as 100 times the ratio of the difference between the tapped density and bulk density to the tapped density. Carr's index (CI) is calculated as follows[12]: CI (%) = [(Tapped density – Bulk density) / Tapped density] x100

**Post-compression Evaluation**
**General appearance**
The formulated tablets are evaluated for general appearance such as colour, Shape and appearance[12].

**Thickness**
The thickness and diameter of the tablets were determined using a vernier caliper. Totally, 5 tablets from each type of formulation were used and average values were calculated. It is expressed in mm[12].

**Hardness**
Hardness also term as crushing of the tablets Tablet hardness was measured with Monsanto hardness tester.. The reading at the marked scale was recorded for the pressure at which breakdown of the tablet occurs[13]. Hardness was expressed in kg/cm$^2$.

**Weight variation**
The test was performed as per IP by weighing 20 tablets randomly on electric balance, calculating the average weight, and comparing the individual tablet weight to the average weight[13].

**Friability test**
This test is carried out by using Friability apparatus. The weighted tablets are placed in the apparatus and it is rotated at 25 rpm for 4 minutes. After sometimes tablets are removed out from apparatus and again they are weight. The friability is calculated by using following formula:
It should be preferably below 1.0%[14].
% Friability = $W_1$- $W_2$/ $W_1$ x100
Where,
W1= weight of tablets before test,
W2 = weight of tablets after test

**Disintegration Time**
In-vitro Disintegration time was performed by using Electrolab Disintegration tester. One tablet was placed in each of the six tubes of the basket. The apparatus was operated using deionized water as the immersion fluid maintained at 37±0.5°C. The time taken for complete disintegration of the tablet with no passable mass remaining on the mesh in the apparatus was measured in minutes[14].

**Stability study**
The herbal tablet formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing ($25^0$C±$2^0$C and 60 ± 5% RH) and ($40^0$C±$2^0$C and 70 ±5% RH) for 3 months. The formulation was checked for Physical appearance, Hardness, friability test and disintegration test at the interval of 30, 45, 60, 90 days (3

month) months. The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Formulation was analysed for the change in Physical appearance, Hardness, friability test and disintegration test. All Results were compared against final formulation of 0 days as the reference[17,20].

## RESULT AND DISCUSSION

The full factorial design was applied to the formulation design of the herbal tablets. The three different factors are evaluated in this design at two different levels. The three independent variables selected are PVP K30, CCS and lactose. There are two different levels for the selected variables as low and high, and they are coded as − 1, and + 1, respectively. The responses are considered as dependent variables, and they are friability and disintegration time of the designed and formulated herbal tablets[17]. The software Design Expert version 12.0.1.0 (StatEase) was used for the design of the formulation. The total of 13 runs (formulations) were designed and the relationship of the dependent and independent variables was studied by gaining the surface responses, and finally, the significant model was achieved[18]. Tablets are formulate for used in the treatment of Hyperlipidemia[19].

**Table 4: Evaluation parameter of granule formulation**

| Formulation | Angle of repose | Bulk Density | Tapped density | Carr's index ratio | Hausner's ratio | Loss on drying |
|---|---|---|---|---|---|---|
| **F 1** | 27.38±0.21 | 0.486±0.06 | 0.515±0.01 | 5.63±0.13 | 1.05±0.001 | 1.14 |
| **F 2** | 31.23±0.13 | 0.455±0.03 | 0.496±0.03 | 8.26±0.25 | 1.09±0.011 | 1.0 |
| **F 3** | 26.48±0.15 | 0.395±0.04 | 0.476±0.06 | 17.01±0.22 | 1.20±0.009 | 0.95 |
| **F 4** | 25.14±0.19 | 0.380±0.09 | 0.466±0.04 | 18.45±0.16 | 1.22±0.006 | 0.87 |
| **F 5** | 29.76±0.20 | 0.420±0.08 | 0.451±0.04 | 6.87±0.14 | 1.07±0.008 | 1.06 |
| **F 6** | 28.56±0.26 | 0.328±0.07 | 0.391±0.02 | 16.11±0.11 | 1.19±0.016 | 0.88 |
| **F 7** | 25.86±0.22 | 0.318±0.05 | 0.341±0.02 | 6.74±0.10 | 1.07±0.013 | 0.97 |
| **F 8** | 27.86±0.22 | 0.350±0.03 | 0.388±0.03 | 9.79±0.21 | 1.10±0.008 | 1.16 |
| **F 9** | 25.33±0.17 | 0.336±0.2 | 0.375±0.02 | 10.4±0.51 | 1.11±0.002 | 1.06 |
| **F 10** | 26.65±0.18 | 0.366±0.07 | 0.401±0.05 | 8.72±0.34 | 1.09±0.003 | 1.11 |
| **F 11** | 29.21±0.20 | 0.396±0.04 | 0.440±0.01 | 10.0±0.23 | 1.11±0.012 | 0.98 |
| **F 12** | 28.50±0.18 | 0.366±0.03 | 0.398±0.02 | 8.04±0.26 | 1.08±0.007 | 0.99 |
| **F 13** | 24.11±0.24 | 0.377±0.06 | 0.399±0.03 | 5.51±0.29 | 1.05±0.006 | 1.26 |

**Table 5: Evaluation parameter of tablet formulation**

| S. No | Formulation | Thickness (mm) | Hardness (kg/cm$^2$) | Friability (%) | Weight variation (%) | Disintegration time (min. sec) |
|---|---|---|---|---|---|---|
| **1** | F 1 | 6.0±0.01 | 5.2±0.12 | 0.17±0.11 | 600±1.23 | 12.33 |
| **2** | F 2 | 6.1±0.03 | 5.1±0.05 | 0.15±0.13 | 601±1.03 | 16.4 |
| **3** | F 3 | 6.0±0.03 | 5.4±0.06 | 0.21±0.17 | 601±0.84 | 13.17 |
| **4** | F 4 | 5.9±0.04 | 5.5±0.06 | 0.30±0.15 | 600±1.03 | 14.51 |
| **5** | F 5 | 6.0±0.08 | 5.6±0.09 | 0.55±0.12 | 602±0.73 | 14.2 |
| **6** | F 6 | 6.2±0.07 | 5.2±0.03 | 0.48±0.18 | 601±1.01 | 14.28 |
| **7** | F 7 | 6.1±0.06 | 5.5±0.04 | 0.46±0.17 | 604±1.11 | 13.55 |
| **8** | F 8 | 6.0±0.06 | 4.5±0.07 | 0.27±0.09 | 602±1.23 | 18.22 |
| **9** | F 9 | 6.0±0.07 | 8.1±0.04 | 0.19±0.11 | 601±1.56 | 16.53 |
| **10** | F 10 | 6.0±0.01 | 5.2±0.09 | 0.18±0.12 | 600±0.86 | 15.42 |
| **11** | F 11 | 6.1±0.03 | 4.2±0.12 | 0.42±0.08 | 600±1.44 | 13.11 |
| **12** | F 12 | 6.0±0.03 | 3.2±0.08 | 0.54±0.13 | 603±0.99 | 9.36 |
| **13** | F 13 | 6.1±0.05 | 8.4±0.11 | 0.16±0.09 | 602±1.15 | 16.45 |

### Optimization of formulation By Design of expert (DOE) Approach

The responses observed were fit to 13 runs, and it has been noted that the best fit model was the linear model for the two dependent variables. The significance of the model with that of comparing with the other model for the analysis by

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analysis of variance (ANOVA). In polynomial equations, positive sign before the factor shows the linear correlation between response and factor, while the negative sign shows the inverse relation between the same. All the responses recorded for 13 runs and the relation of independent and dependent variables are presented in table

**Build Information**

**Table 6: Build information of DOE software**

| | | | |
|---|---|---|---|
| **File Version** | **12.0.1.0** | | |
| **Study Type** | Response Surface | **Subtype** | Randomized |
| **Design Type** | Box-Behnken | **Runs** | 13 |
| **Design Model** | Quadratic | **Blocks** | No Blocks |
| **Build Time (ms)** | 1.0000 | | |

**Table 7: Coaded and actual Factors of formulation**

| Factor | Name | Units | Type | Minimum | Maximum | Coded Low | Coded High | Mean | Std. Dev. |
|---|---|---|---|---|---|---|---|---|---|
| **A** | PVP K30 | mg | Numeric | 10.00 | 50.00 | -1 ↔ 10.00 | +1 ↔ 50.00 | 30.00 | 16.33 |
| **B** | CCS | mg | Numeric | 5.00 | 30.00 | -1 ↔ 5.00 | +1 ↔ 30.00 | 17.50 | 10.21 |
| **C** | Lactose | mg | Numeric | 30.00 | 70.00 | -1 ↔ 30.00 | +1 ↔ 70.00 | 50.00 | 16.33 |

**Formulation trials as per Box–Behnken design**

**Table 8: Formulation trials**

| Formu la tions | Factor 1 (PVP) K 30 (mg) | Factor 2 CCS (mg) | Factor 3 Lactose (mg) | *Acalypha Indica* (Stem) Ethanolic Extract(mg) | Magnesi um Stearate (%) | Talk (mg) | Response 1 Disintegra tion time | Response 2 Friability test |
|---|---|---|---|---|---|---|---|---|
| **F 1** | 50 | 5 | 50 | 400 | 0.7 | q.s | 12.33 | 0.17 |
| **F 2** | 30 | 5 | 70 | 400 | 0.7 | q.s | 16.4 | 0.15 |
| **F 3** | 30 | 17.5 | 50 | 400 | 0.7 | q.s | 13.17 | 0.21 |
| **F 4** | 30 | 5 | 30 | 400 | 0.7 | q.s | 14.51 | 0.3 |
| **F 5** | 30 | 30 | 70 | 400 | 0.7 | q.s | 14.2 | 0.55 |
| **F 6** | 10 | 5 | 50 | 400 | 0.7 | q.s | 14.28 | 0.48 |
| **F 7** | 10 | 17.5 | 70 | 400 | 0.7 | q.s | 13.55 | 0.46 |
| **F 8** | 50 | 17.5 | 70 | 400 | 0.7 | q.s | 18.22 | 0.27 |
| **F 9** | 10 | 30 | 50 | 400 | 0.7 | q.s | 16.53 | 0.19 |
| **F 10** | 30 | 30 | 30 | 400 | 0.7 | q.s | 15.42 | 0.18 |
| **F 11** | 50 | 30 | 50 | 400 | 0.7 | q.s | 13.11 | 0.42 |
| **F 12** | 50 | 17.5 | 30 | 400 | 0.5 | q.s | 9.36 | 0.54 |
| **F 13** | 10 | 17.5 | 30 | 400 | 0.5 | q.s | 16.45 | 0.16 |

**Limits of Variables (Constraints)**

**Table 9: Variables operating range for herbal tablet formulation**

| Name | Goal | Lower Limit | Upper Limit | Importance |
|---|---|---|---|---|
| **A:PVP K30** | is in range | 10 | 50 | 3 |
| **B:CCS** | is in range | 5 | 30 | 3 |
| **C:Lactose** | is in range | 30 | 70 | 3 |
| **Disintegration time** | none | 9.36 | 18.22 | 3 |
| **Friability test** | none | 0.15 | 0.55 | 3 |

Selection of working method was done on the basis of Disintegration time and Friability test of formulation. A factorial design was used to study the effect of independent variables on the dependent variables (In below table).

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**Table 10: Independent variables**

| S. No. | Coding | Variables |
|---|---|---|
| **1.** | X1 | A: Polyvinylpyrrolidone (PVP) K 30 (mg) |
| **2.** | X2 | B: Croscarmellose sodium (CCS) (mg) |
| **3.** | X3 | C: Lactose (mg) |

**Table 11: Dependent variables**

| S. No | Coding | Variables |
|---|---|---|
| **1.** | Y1 | Disintegration time |
| **2.** | Y2 | Friability test |

**Fit Summary**

**Table 12: Response 1: Disintegration time**

| Source | Sequential p-value | Adjusted $R^2$ | Predicted $R^2$ | |
|---|---|---|---|---|
| **Linear** | 0.5094 | -0.0440 | -0.7020 | |
| **2FI** | **0.0233** | **0.6444** | **0.1189** | **Suggested** |
| **Quadratic** | 0.6606 | 0.5538 | | |
| **Cubic** | | | | Aliased |

**ANOVA for 2FI model**

**Table 13: Response 1: Disintegration time**

| Source | Sum of Squares | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|
| **Model** | 50.99 | 8.50 | 4.63 | 0.0423 | **significant** |
| **A-PVP K30** | 7.59 | 7.59 | 4.13 | 0.0884 | |
| **B-CCS** | 0.3784 | 0.3784 | 0.2060 | 0.6659 | |
| **C-Lactose** | 5.49 | 5.49 | 2.99 | 0.1345 | |
| **AB** | 0.5402 | 0.5402 | 0.2940 | 0.6072 | |
| **AC** | 34.57 | 34.57 | 18.82 | 0.0049 | |
| **BC** | 2.42 | 2.42 | 1.32 | 0.2950 | |
| **Residual** | 11.03 | 1.84 | | | |
| **Cor Total** | 62.02 | | | | |

Factor coding is Coded.
Sum of squares is Type III - Partial
The Model F-value of 4.63 implies the model is significant. There is only a 4.23% chance that an F-value this large could occur due to noise.
P-values less than 0.0500 indicate model terms are significant. In this case AC is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve suggested model.

**Table 14: Coefficients in Terms of Coded Factors**

| Factor | Coefficient stimate | Standard Error | 95% CI Low | 95% CI High | VIF |
|---|---|---|---|---|---|
| **Intercept** | 14.43 | 0.3760 | 13.51 | 15.35 | |
| **A-PVP K30** | -0.9738 | 0.4793 | -2.15 | 0.1990 | 1.0000 |
| **B-CCS** | 0.2175 | 0.4793 | -0.9552 | 1.39 | 1.0000 |
| **C-Lactose** | 0.8287 | 0.4793 | -0.3440 | 2.00 | 1.0000 |
| **AB** | -0.3675 | 0.6778 | -2.03 | 1.29 | 1.0000 |
| **AC** | 2.94 | 0.6778 | 1.28 | 4.60 | 1.0000 |
| **BC** | -0.7775 | 0.6778 | -2.44 | 0.8810 | 1.0000 |

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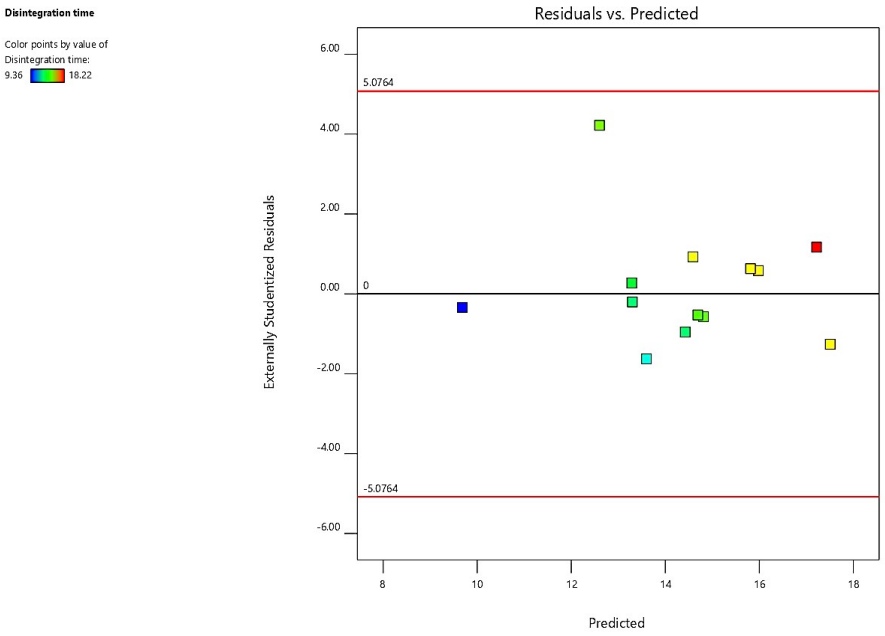
The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

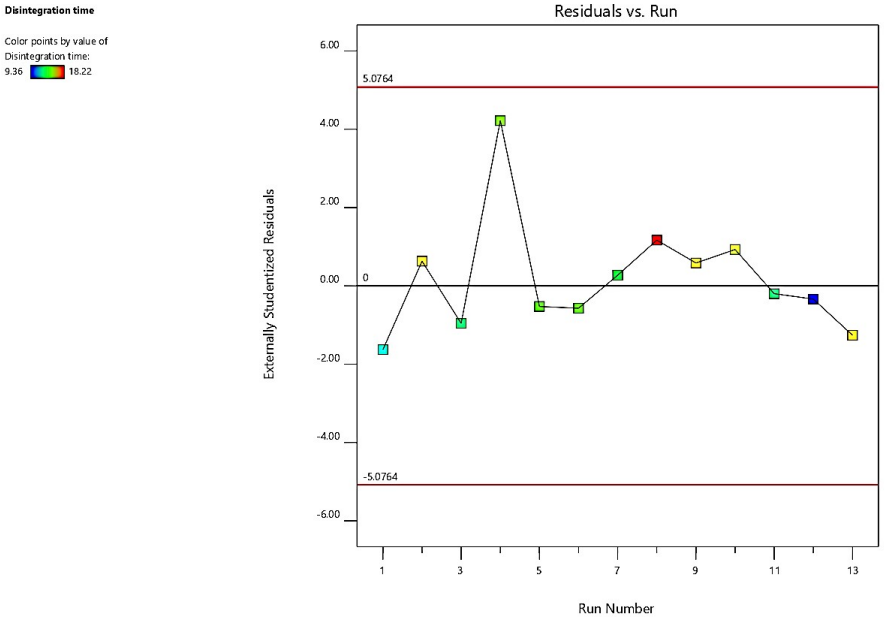
**Final Equation in Terms of Coded Factors**

**Disintegration time =** +14.43 -0.9738 A +0.2175 B +0.8287 C -0.3675 AB +2.94 AC -0.7775 BC

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.




**Graph 1: Residuals vs actual value of Disintegration time dependent variable**



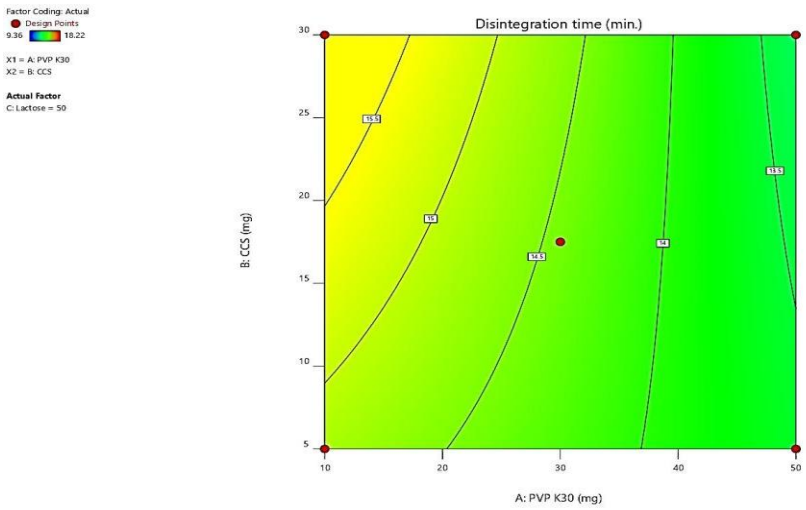

**Graph 2: residual vs run of Disintegration time dependent variable**

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25 No.1 (2024) http://www.veterinaria.org
Article Received-1 June 2024 Revised -09 June 2024 Accepted- 14 June 2024

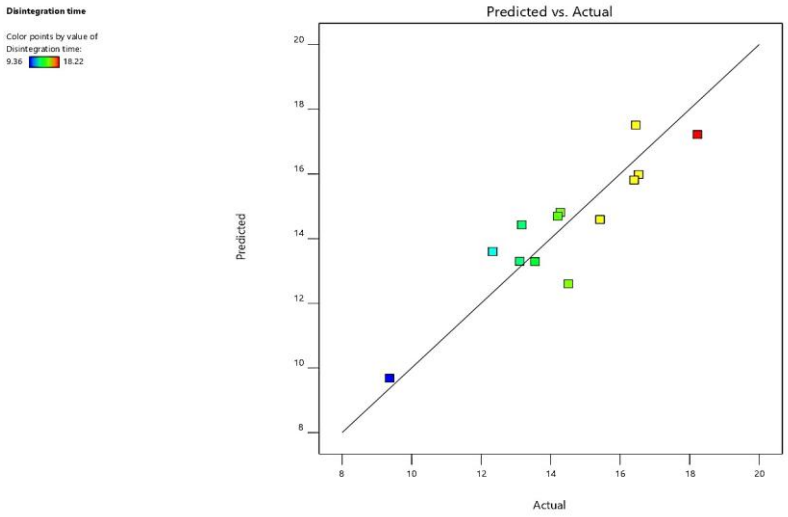
**Predicted and actual value of Disintegration time (All formulation)**

**Table 15**: **Predicted and actual value of Disintegration time**

| Formulation code | Actual Value | Predicted Value |
|---|---|---|
| **F1** | 12.33 | 13.60 |
| **F2** | 16.40 | 15.81 |
| **F3** | 13.17 | 14.43 |
| **F4** | 14.51 | 12.60 |
| **F5** | 14.20 | 14.69 |
| **F6** | 14.28 | 14.81 |
| **F7** | 13.55 | 13.29 |
| **F8** | 18.22 | 17.22 |
| **F9** | 16.53 | 15.98 |
| **F10** | 15.42 | 14.59 |
| **F11** | 13.11 | 13.30 |
| **F12** | 9.36 | 9.68 |
| **F13** | 16.45 | 17.51 |




**Graph 3: Response surface plot of Disintegration time dependent variable**




**Graph 4: predicted vs actual value of Disintegration time dependent variable**

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25 No.1 (2024) http://www.veterinaria.org
Article Received-1 June 2024 Revised -09 June 2024 Accepted- 14 June 2024

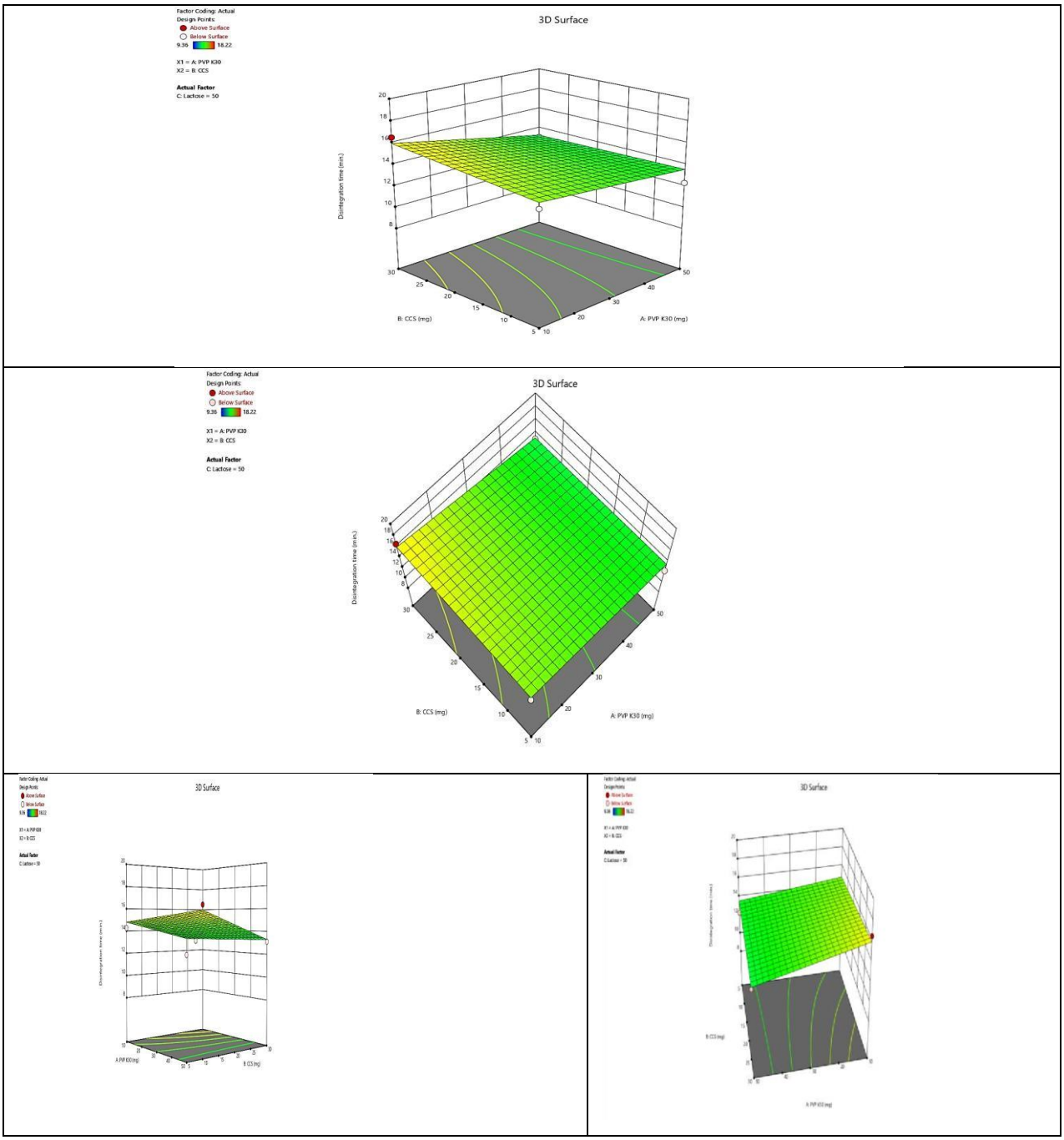



**Figure 1: Response surface plot showing combined effect of PVP K 30 and CCS on Disintegration time of tablet formulation**

The results of formulations as per the design when fitted into various models, a linear model was found to be significant for Disintegration time with F value of 4.63 and P value 0.0423. The model equation is as follows: Disintegration time = +14.43 -0.9738 A +0.2175 B +0.8287 C -0.3675 AB +2.94 AC -0.7775 BC. The effect of both the factors A, B and C can be explained with the help of the 3D response surface plot as shown in above Figure.

**Effect of formulation variables on Friability**

**Table 16: Response 2: Friability test (Fit Summary)**

| Source | Sequential p-value | Adjusted $R^2$ | Predicted $R^2$ | |
|---|---|---|---|---|
| **Linear** | 0.9039 | -0.2559 | -1.0400 | |
| **2FI** | **0.0105** | **0.6748** | **0.2589** | **Suggested** |
| **Quadratic** | 0.5904 | 0.6285 | | |
| **Cubic** | | | | Aliased |

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25 No.1 (2024) http://www.veterinaria.org
Article Received-1 June 2024 Revised -09 June 2024 Accepted- 14 June 2024

**ANOVA for 2FI model**

**Table 17: Response 2: 2FI model (ANOVA 2FI model )**

| Source | Sum of Squares | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|
| **Model** | 0.2382 | 0.0397 | 5.15 | 0.0332 | significant |
| **A-PVP K30** | 0.0015 | 0.0015 | 0.1962 | 0.6733 | |
| **B-CCS** | 0.0072 | 0.0072 | 0.9339 | 0.3712 | |
| **C-Lactose** | 0.0078 | 0.0078 | 1.01 | 0.3530 | |
| **AB** | 0.0729 | 0.0729 | 9.46 | 0.0218 | |
| **AC** | 0.0812 | 0.0812 | 10.54 | 0.0176 | |
| **BC** | 0.0676 | 0.0676 | 8.77 | 0.0252 | |
| **Residual** | 0.0463 | 0.0077 | | | |
| **Cor Total** | 0.2845 | | | | |

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**
The **Model F-value** of 5.15 implies the model is significant. There is only a 3.32% chance that an F-value this large could occur due to noise.
**P-values** less than 0.0500 indicate model terms are significant. In this case AB, AC, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve suggested model.

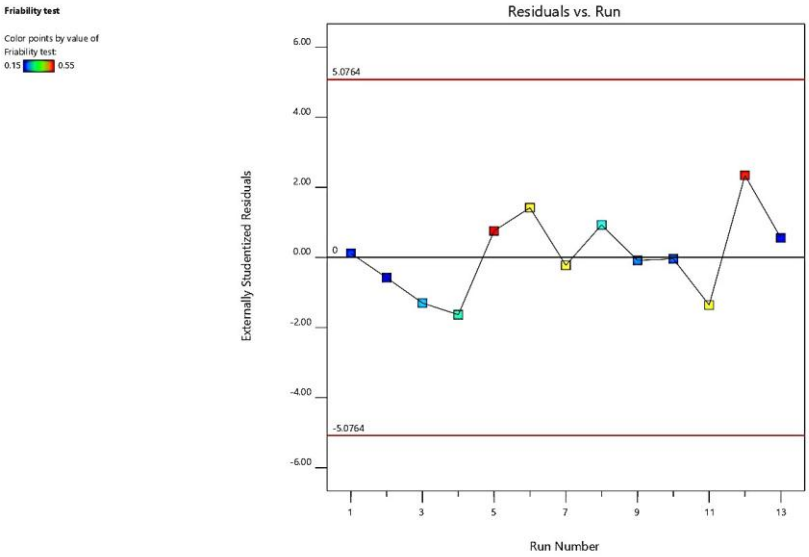
**Table 18: Coefficients in Terms of Coded Factors**

| Factor | Coefficient Estimate | Standard Error | 95% CI Low | 95% CI High | VIF |
|---|---|---|---|---|---|
| **Intercept** | 0.3138 | 0.0244 | 0.2543 | 0.3734 | |
| **A-PVP K30** | 0.0137 | 0.0310 | -0.0622 | 0.0897 | 1.0000 |
| **B-CCS** | 0.0300 | 0.0310 | -0.0460 | 0.1060 | 1.0000 |
| **C-Lactose** | 0.0313 | 0.0310 | -0.0447 | 0.1072 | 1.0000 |
| **AB** | 0.1350 | 0.0439 | 0.0276 | 0.2424 | 1.0000 |
| **AC** | -0.1425 | 0.0439 | -0.2499 | -0.0351 | 1.0000 |
| **BC** | 0.1300 | 0.0439 | 0.0226 | 0.2374 | 1.0000 |

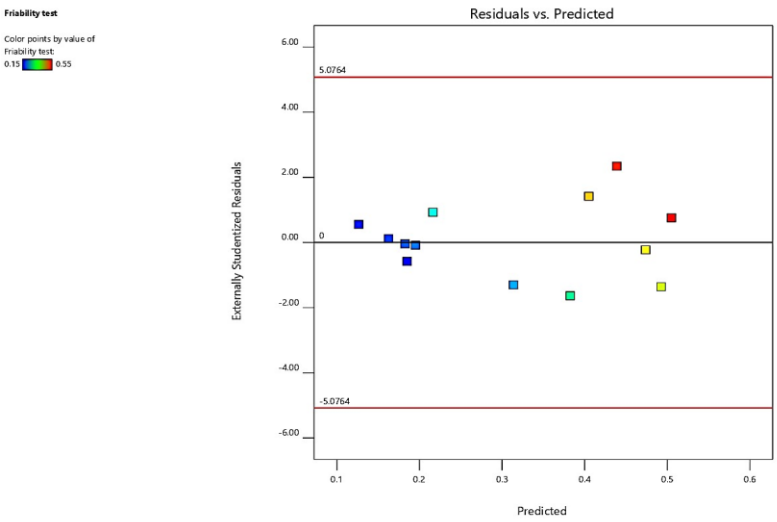
The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

**Final Equation in Terms of Coded Factors**
Friability test = +0.3138 + 0.0137 A +0.0300 B +0.0313 C +0.1350 AB -0.1425 AC +0.1300 BC
The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25 No.1 (2024) http://www.veterinaria.org
Article Received-1 June 2024 Revised -09 June 2024 Accepted- 14 June 2024




**Graph 5: Graph 6: normal plot of residual**



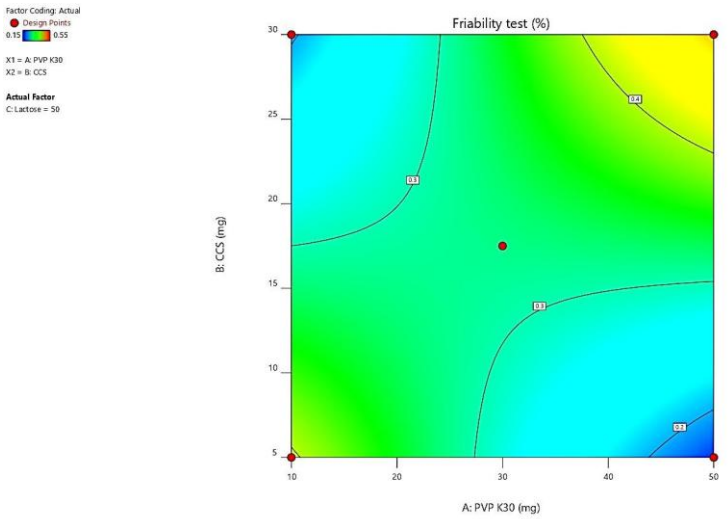

**Graph 7: residual vs run of Friability test dependent variable**

**Predicted and actual value of Friability test (All formulation)**

**Table 19: Predicted and actual value of Friability test**

| Formulation code | Actual Value | Predicted Value |
|---|---|---|
| **F1** | 0.1700 | 0.1626 |
| **F2** | 0.1500 | 0.1851 |
| **F3** | 0.2100 | 0.3138 |
| **F4** | 0.3000 | 0.3826 |
| **F5** | 0.5500 | 0.5051 |
| **F6** | 0.4800 | 0.4051 |
| **F7** | 0.4600 | 0.4738 |
| **F8** | 0.2700 | 0.2163 |
| **F9** | 0.1900 | 0.1951 |
| **F10** | 0.1800 | 0.1826 |
| **F11** | 0.4200 | 0.4926 |
| **F12** | 0.5400 | 0.4388 |
| **F13** | 0.1600 | 0.1263 |

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25 No.1 (2024) http://www.veterinaria.org
Article Received-1 June 2024 Revised -09 June 2024 Accepted- 14 June 2024




**Graph 8: counter plots of Friability test dependent variable**

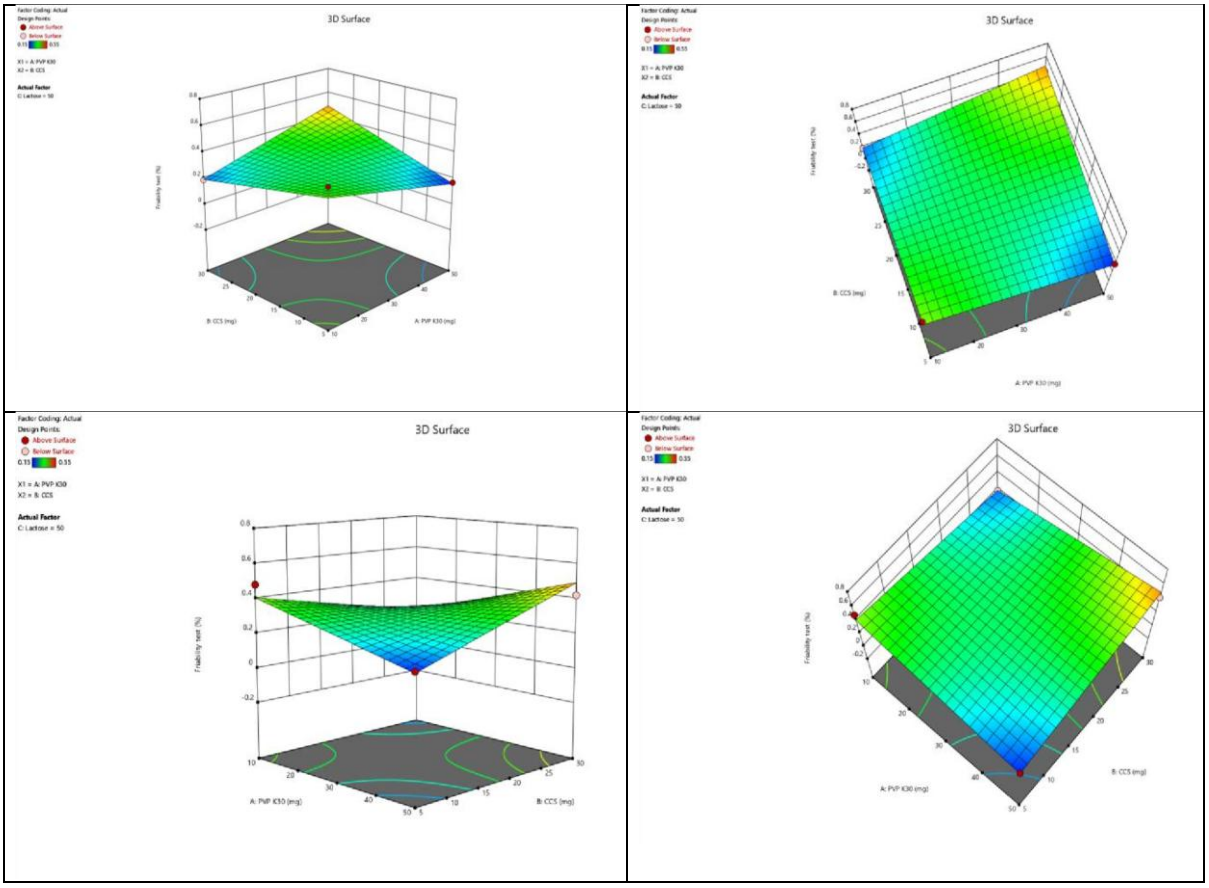
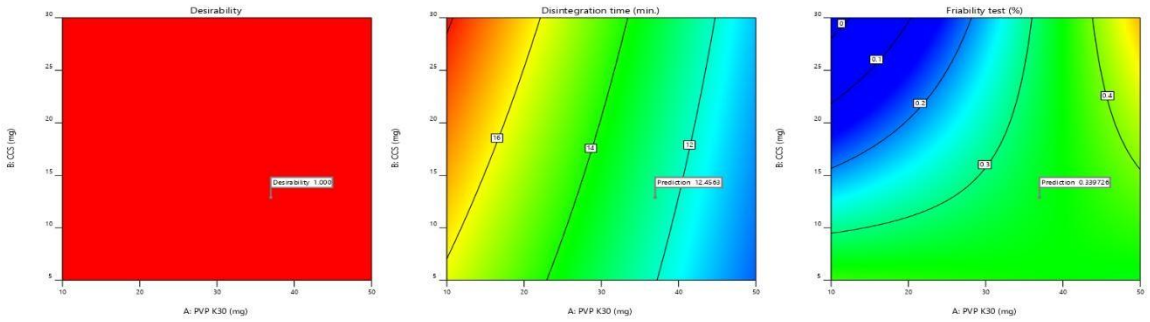
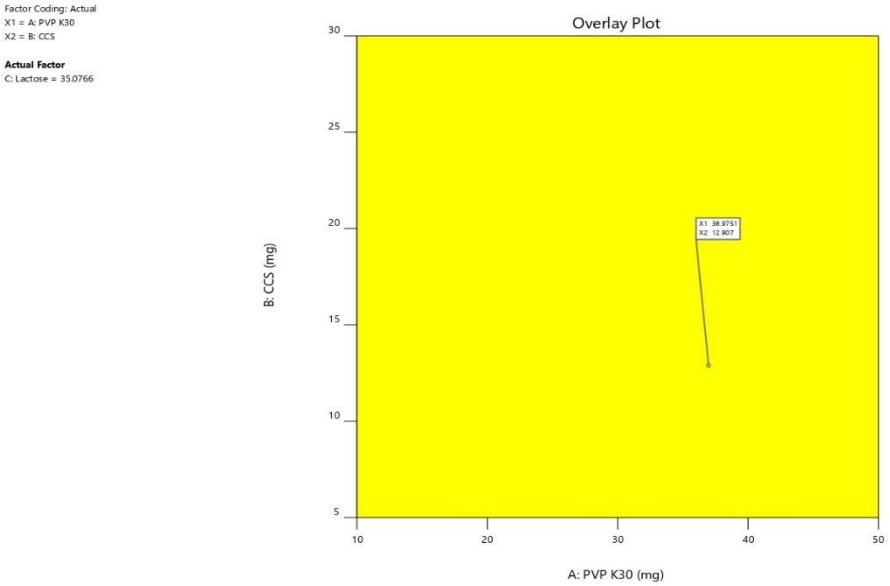



**Figure 2: Response surface plot showing combined effect of (PVP) K 30and CCS on Friability test of tablet formulation**

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25 No.1 (2024) http://www.veterinaria.org
Article Received-1 June 2024 Revised -09 June 2024 Accepted- 14 June 2024




**Graph 9: overlay plot of Friability test and disintegration time for optimization dependent variable Optimized formula of herbal tablet formulation**

**Table 20: Optimized formula of tablet formulation**

| S. No | PVP K30(mg) | CCS(mg) | Lactose (mg) | *Acalypha Indica* (Stem) Ethanolic Extract(mg) | Magnesium Stearate (%) | Talk (q.s) | Disintegration time | Friability | Desirability | |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 18.875 | 7.133 | 46.007 | 400.00 | 0.7 | q.s | 14.649 | 0.343 | 1.000 | |
| **2** | **36.975** | **12.907** | **35.007** | **400.00** | **0.7** | **q.s** | **12.456** | **0.340** | **1.000** | **Selected** |
| **3** | 15.999 | 24.359 | 47.189 | 400.00 | 0.7 | q.s | 15.256 | 0.240 | 1.000 | |

The results of formulations as per the design when fitted into various models, Linear model was found to be significant for Friability test with F value 5.15 and P value 0.0332. The model equation is as follows: Friability test = +0.3138 + 0.0137 A +0.0300 B +0.0313 C +0.1350 AB -0.1425 AC +0.1300 BC. The effect of factor A and B can be explained with the help of the 3D response surface plot as shown in above Figure. The results when analysed and optimized had generated numerical optimized solutions based on this experimental design. From the numerical optimization results, a

solution was selected randomly, coded as optimized formulation and considered as optimized tablet formulation. Box-Behnken design using response surface methods was used to finish the optimization process. Excipients were considered as independent variables, along with their effects on Friability and disintegration time of formulation in that order. In order to examine correlations across several variables with fewer experimental runs, the Box-Behnken design was utilized. Disintegration time and friability test for formulation were taken into consideration when choosing the operating method. The impact of both independent and dependent factors was examined using a factorial approach.

**Characterization of optimized formulation**

**Table 21: Evaluation parameter of granule**

| Formulation | Angle of repose Ɵ | Bulk density (g/cm3) | Tapped Density (g/cm3) | Carr's index ratio | Hausner's ratio | % of LOD |
|---|---|---|---|---|---|---|
| **Granules** | 25.34 | 0.369 | 0.398 | 7.28 | 1.07 | 0.97 |

**Table 22: Evaluation parameter of Optimized tablet formulation**

| Formulation | Hardness (kg/cm2) | Thickness (mm) | Weight variation (%) | Disintegration time (min. sec) (Actual value) | Disintegration time (min. sec) (Predicted value) | Friability ( %) (Actual value) | Friability (%) (Predicted value) |
|---|---|---|---|---|---|---|---|
| **Tablet** | 5.7 | 6.1 | 602 | 12.34 | 12.45 | 0.327 | 0.340 |

**STABILITY STUDY**

**Table-23 Stability study of optimized formulation**

| S.No | Time (Days) | $25^0$C±2 $^0$C and 60 ± 5% RH | | | $40^0$C±2 $^0$C and 70 ±5% RH | | |
|---|---|---|---|---|---|---|---|
| | | Hardness | Disintegration time | Friability | Hardness | Disintegration time | Friability |
| **1.** | 0 | 5.7 | 12.34 | 0.340 | 5.7 | 12.34 | 0.340 |
| **2.** | 30 | 5.9 | 12.29 | 0.346 | 5.9 | 12.38 | 0.349 |
| **3.** | 45 | 5.7 | 12.31 | 0.342 | 5.3 | 12.30 | 0.337 |
| **3.** | 60 | 5.8 | 12.31 | 0.337 | 5.4 | 12.32 | 0.343 |
| **4.** | 90 | 5.8 | 12.33 | 0.338 | 5.5 | 12.35 | 0.339 |

Formulation was found to be stable, both physically and chemically, for a period of 3 months at accelerated stability conditions ($25^0$C±$2^0$C and 60 ± 5% RH) and ($40^0$C±$2^0$C and 70 ±5% RH)[21]. Physicochemical parameters, including Physical appearance, Hardness, friability test and disintegration test studies were not altered significantly. Results of evaluation criteria at periodic time points of stability studies are summarized in Table. All Results were compared against final formulation of 0 days as the reference.

**CONCLUSION-**

The software Design Expert version 12.0.1.0 (StatEase) was used for the design of the formulation. The total of 13 runs (formulations) was designed and the relationship of the dependent and independent variables was studied by gaining the surface responses, and finally, the significant model was achieved. Tablets were prepared successfully from the ethanolic extract of stem of *Acalypha indica* plant with appropriate ingredients by using optimized formula of tablet formulation, Show in Table- 20 **.**Granules and Tablets were evaluated for different parameters showed results in table21,22. Stability study of optimize formulation was done, both physically and chemically, for a period of 3 months at accelerated stability conditions ($25^0$C±$2^0$C and 60 ± 5% RH) and ($40^0$C±$2^0$C and 70 ±5% RH). Results stability studies are summarized in Table-23. All Results were compared against final formulation of 0 days as the reference.

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