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RESEARCH ARTICLE

FORMULATION DEVELOPMENT AND EVALUATION OF TABLETS CONTAINING *ANOECTOCHILUS FORMOSANUS* EXTRACT: PROCESS OPTIMISATION BY FULL FACTORIAL DESIGN

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ABSTRACT

*Anoectochilusformosanus*Hayata (Orchidaceae) is an indigenous and valuable Taiwanese medicinal plant and has been used popularly as a nutraceutical herbal tea in Taiwan and other Asian countries.The present research investigation is focused on development of compressed tablets of *Anoectochilusformosanus*Hayata (Orchidaceae) after its successive extraction with petroleum ether, chloroform, methanol and water by wet granulation using a 2^2 full factorial design with centre point, considering amount of polyvinylpyrrolidone K-30 and croscarmellose sodium as two independent variables. All the five batches of tablets prepared were portrayed satisfactory results in hardness, friability, weight variation and content uniformity tests. An immediate drug release pattern was evident from the dissolution profiles and mechanism of drug release was Non-Fickian transport.Slope of Weibull model had indicated that dissolution curves were sigmoid, S-shaped, with upward curvature followed by a turning point.Normal probability plots of response variables had supported the chosen interaction model. Furthermore, drug release pattern was found to be increasing with croscarmellose sodium and decreasing with polyvinylpyrrolidone K-30, which had been concluded from the regression equations, contour plots and corresponding response surface plots. Optimised values of polyvinylpyrrolidone K-30 and croscarmellose sodium were 60 mg and 52 mg respectively for the target values of 50% and 90% drug released after 20 and 40 minutes respectively.

Key words: *Anoectochilusformosanus,* Crosscarmellose sodium, Dissolution, Extracts, Full factorial design, Mathemaical models, Tablets

INTRODUCTION:

*Anoectochilusformosanus*Hayata (Orchidaceae) is an indigenous and valuable Taiwanese medicinal plant and has been used popularly as a nutraceutical herbal tea in Taiwan and other Asian countries. This herbal plant is also called “King Medicine” because of its diverse pharmacological effects such as liver protection, cancer prevention, and diabetes and for treatment of cardiovascular diseases. *A. formosanus*possesses hepatoprotective effect on carbon tetrachloride(CCl_4) and acetaminophen induced acute hepatitis.[1, 2]and its effect on arachidonate metabolism has also been reported.[3]In addition to these, aqueous extracts of *A. formosanus*inhibits cell damage induced by CCl_4in primary cultured rat hepatocytes.[4] Recently, it wasalso confirmed that an aqueous extract of *A. formosanus*attenuated hepatic fibrosis induced by both $CCl_4$and dimethylnitrosamine in rats.[5, 6] The plant contains kinsenone, and a number of known flavonoid glycosides also.[7]The formulation of solid oral dosage foms, and tablets in perticular, has undergone rapid change and development over the last several decades with the emergence of precompression, induced die feeding, high speed and now ultra high speed processes, automated weight controlled systems and many more. The conventional dosage form gained so much patience compliance and advantages that it has been further explored in to immediate release tablets, delayed release tablets, controlled rease tablets and other modified relese tablets in order toachive different release patterns

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of the active pharmaceutical ingredients.[8]Major advantages of the tablet includes, lightest compact tamperproof dosage form, its lowest cost among all oral dosage forms, easiest and cheapest to package and ship, suitability for large scale production wheather manufactured by wet granulation, direct compression or dry granulation methods.[9]In the present research investigation an attempt has been made towards development of compressed tablets of *Anoectochilusformosanus*Hayata (Orchidaceae)after its successive extraction with petroleum ether, chloroform, methanol and water. Aqueous extract of whole part of the plant *Anoectochilusformosanus*(dry powder) was subjected to wet granulation for tablet compressionusing a 2^2 full factorial design with centre point. Amount of binder and disintegrant were considered as two independent variables (factors) in the experimental design. The study also includes analysis of the effect of independent variables on the release profile of extract and optimisation of their amounts for a particular set of target values.

MATERIALS AND METHODS:

Materials:

Whole part of the plant *Anoectochilusformosanus*(AF)were collected in the month of September from Nilgir district, Tamilnadu and authenticated by Dr. N. K. Dhal, Taxonomist, Institute of Minerals and Materials Technology, Bhubaneswar, Odisha. Croscarmellose sodium (CCS) was obtained as a gift samples from Torrent Pharmaceuticals, Baddi, H.P., India. Polyvinylpyrrolidone K-30 (PVP), magnesium stearate (MS) and talc were purchased from Lobachemie. Pvt. Ltd, Mumbai. D-Mannitol (DM), isopropyl alcohol (IPA) and hydrochloric acid (HCl) were purchased from Merck Pvt. Ltd. Double distilled water (DDW) was prepared in the laboratory from demineralised water. All the reagents used were of analytical grade and were used as received.

Extraction of plant materials:

The collected plants (whole part of the plant of *Anoectochilusformosanus*) were washed thoroughly in water and chopped, dried for a week (35-40^{0}C) and pulverized in an electric grinder. The powder obtained was successively extracted in petroleum ether (60-80^{0}C), chloroform, methanol and DDW. The extracts were then made to powder by using rotary evaporator under reduced pressure. Accordingly, powdered aqueous extract of this plant was prepared in sufficient quantity and stored in a well closed tight container for further use.

Formulation of tablets:

Accurately weighed quantities (as specified in Table 1) of AF extract, D-Mannitol and CCS were passed through a 350µm sieve to get uniform size particles, then were dry blended for 20 minutes by a double cone blender (Laboratory scale, Excel Enterprises, Kolkata). Accurately weighed quantity of PVP was dissolved in isopropyl alcohol (IPA) to prepare a binder solution. The binder solution was addedto the dry blend gradually with constant kneading to form a homogeneous mass. Thedough mass formed was passed through a 500 µm sieve and the granular mass was allowed todry at room temperature. These dried granuleswere lubricated with magnesium stearate and talc followed by compression into tablets using an8-station rotary tablet compression machine (CadmatchRimek, India with oblong faced punches of 16 mmlength).

Table 1: Tablet formulation of AF extract

Formulation Code	Ingredients in mg/tablet						
	AF	D-Mannitol	PVP	CCS	MS	Talc	Total
T1	500	188	40	16	16	40	800
T2	500	140	40	64	16	40	800
T3	500	108	120	16	16	40	800
T4	500	60	120	64	16	40	800
T5	500	124	80	40	16	40	800

Physical evaluation and drug content study of tablets:

Different batches of compressed tablets (T1 to T5) were subjected to weight variation, hardness, friability and content uniformity test.The friability test of all the tablets was conductedin a triplicate manner using a Roche friabilator by taking 10 tablets in each replicate. Monsantohardness tester was used for the determination of hardness of tablets in a triplicate manner. Weight variation test was carried out by taking 20 tablets as per United States Pharmacopeia (USP).[10]For the determination of drug content,30 tablets were randomly selected from each batch and 10 of them were assayed individually by dissolving in 0.1 M HCl followed by dilution up to 1000 ml with 0.1 M HCl. Then 5 ml of the above

solution was diluted up to 50 ml with 0.1 M HCl.The resulting solution was subjected to absorbance measurement under UV-visible spectrophotometer at 287 nm against 0.1 M HClas reference standard or blank.

In-vitro dissolution study and mechanism of drug release:

In vitro dissolution study of compressed tablets of different batches was conducted in USP apparatus-1 (basket type, LABINDIA, DISSO.). Dissolution test was carried out in 900 ml of 0.1M HCl at 37±0.5^{0}C and the basket was rotated at 60 rpm. Samples withdrawn at an interval of 5 minutes from the starting of dissolution were diluted up to 25 ml with 0.1 M HCl and analysed for the amount of AF extract released under UV-visible spectrophotometer at 287 nm against 0.1 M HCl as reference standard or blank.Each time after sample withdrawn 5 ml of 0.1 M HCl was also replaced. Data of the in-vitro dissolution study were fitted into different mathematical models like Zero order (ZO),[10]First order (FO),[11]Higuchi (HG),[12] Hixson Crowell (HC),[13]KorsmeyerPeppas model (KP)[14] and Weibull model (WB)[15, 16]and their correlation coefficient (R^2) values were used as an indicator of the best fitting for each of the models. KorsmeyerPeppas model was fitted to identify the mechanism of drug release,[17] which was determined from the slope of the model or release exponent (n) values.[18] Dissolution rate constants of zero order(K_0 in % min^{-1}), first order (K_1 in min^{-1}), Higuchi (K_H in % $min^{-1/2}$) and Hixson Crowell (K_{HC} in $mg^{1/3}$ % min^{-1}) models were determined from their slope values whereas those of KorsmeyerPeppas (K_{KP} in min^{-n}) and Weibull (K_{WB} in min^{b}) models were determined from their respective y-intercept values.

Experimental design:

A 2^2 full factorial design with a centre point was employed considering amount of PVP (X_1) and amount of CCS(X_2) as two independentvariables (factors) and 5 different batches of tabletswere prepared (table 1). Percentage of drug released (% DR) at 20 minutes (Q_{20}) and 40 minutes (Q_{40}) were taken as two response variables (dependent variables) to study the effect of PVP and CCSon the release profile of AF extractfrom its tablet dosage forms in a triplicate manner. Regression coefficients of the independent variables were determined to emphasize the effect of PVP and CCS on the response variables. Residuals and percentage bias were calculated along with construction of normal probability plot to check the model accuracy. Analysis of variance (ANOVA) was performed to study the statistical significance of factors and the interaction term. Minitab 15 and SAS (for optimisation) were used for statistical and mathematical analyses of the full factorial design.[19] Full factorial design with centre point for all the five batches of tablet formulations were given in Table 2.

Table 2: Full factorial design for tablet formulations

Formulation code	Coded values		Actual values in mg	
	PVP	CCS	PVP	CCS
T1	-1	-1	40	16
T2	-1	+1	40	64
T3	+1	-1	120	16
T4	+1	+1	120	64
T5	0	0	80	40

RESULTS AND DISCUSSION:

Physical evaluation and drug content study of tablets:

The hardness test indicated good mechanical strength as far as all the five batches are concerned, which is sufficient to render them tamper proof. Hardness wasranged from 6.3 to 8.7 Kg/cm^2 in case of all the tablets, whereas it was lowest in case of T1 (6.5 ± 0.93 Kg/cm^2) and T2 (6.3 ± 0.80 Kg/cm^2). The reason may be attributed towards lowest value of binder (PVP). It is clearly indicated that as the amount of PVP increases, hardness value also increases, but effect of CCS was complex on the hardness value.Friability was ranged from 0.52 % to 0.74 % (less than 1%), which indicated that tablets had good mechanical resistance. Friability was found to be lowest (0.52 ± 0.15 %) in case of T3 and highest (0.68 ± 0.12 %) in case of T2. These results have depicted that as the amount of PVP increases, friability decreases, although friability is a function of many factors, for instance, amount of binder, amount of disintegrant, compression forces and sometimes method of granulation also. Weight variation test of all the five batches revealed that all the tablets fall within the ± 5 % limit, as allowed by USP weight variation test. Drug content was found to be high (more than 98 %) in case of all the batches. It was ranged from 98.75% to 99.44 % anduniform in all tablet formulations. All the batches have passed the content uniformity test, as percentage of drug content was no more than ± 15 % in case of all the tablets.An ultraviolet (UV) spectrophotometric method

was used for thedetermination of drug content as well as analysis of dissolution samples. Wave length of absorption maxima was determined by scanning differentconcentrations of AFsolution in 0.1 M HCl. Absorption maxima was 287 nm and method obeysBeer's law in concentration range of 10 to 100 µg/ml, with good correlation coefficient (Equation of standard curve was $Y = 0.0058\,X + 0.0009$ having R^2 – value = 0.9995 ± 0.0002, in a triplicate manner). Results of physical evaluation and drug content determination were summarised in Table 3.

Table 3: Physical evaluation and drug content study of tablets

Formulation code	Parameters in Average ± SD			
	Hardness in Kg/cm^2	Friability (% loss)	Weight variation in mg	Drug content (%)
T1	6.5 ± 0.93	0.66 ± 0.05	803.85 ± 21.96	98.92 ± 8.26
T2	6.3 ± 0.80	0.68 ± 0.12	809.62 ± 26.22	99.03 ± 9.06
T3	8.7 ± 0.46	0.52 ± 0.15	812.46 ± 31.08	99.44 ± 7.79
T4	8.4 ± 0.62	0.56 ± 0.10	797.87 ± 19.26	98.75 ± 9.13
T5	7.9 ± 0.95	0.63 ± 0.09	792.08 ± 24.13	99.39 ± 8.86

In-vitro dissolution study and mechanism of drug release:

All the five batches of tablets had shown more than 90 % of drug release after one hour, an immediate release pattern was observed; having highestpercentage drug released (% DR) in case of T2 (37.57 % after 5 minutes) and lowest in case of T3(10.77 % after 5 minutes). This may be due to the fact that T2 contains highest amount of CCS (64 mg) and lowest amount of PVP (40 mg), on the other hand, T3 contains highest amount of PVP (120 mg) and lowest amount of CCS (16 mg). Moreover, all the formulations had agreed with more than 60 % DR after half an hour. Dissolution profile of all the 5 batches had shown in Figure 1, which clearly revealed that as the amount of CCS increases consequently drug release increases from the tablets.R^2 values of different mathematical models depicted that dissolution profile of T1, T2 and T3 were best fitted to Hixson Crowell model, values were 0.9861 ± 0.0073, 0.9765 ± 0.0023 and 0.9892 ± 0.0042 respectively. However, dissolution profile of T4 and T5 were best fitted to KorsmeyerPeppas model as their R^2 values were 0.9808 ± 0.0076 for T4 and 0.9869 ± 0.0043for T5.R^2 values of different mathematical models were given in Table 4.The release exponent (n value or slope of the KorsmeyerPeppas model) ranges from 0.4419to 0.9249 in case of all the batches which indicated that the drug release occurred through Non-Fickiantransport mechanism (0.45 <n – value < 0.89).[15, 16]Dissolution rate constants (DRC) of Zero order model indicated that its value was highest in case of T1(1.6080 ± 0.0280 % min^{-1}) and lowest in case of T2 (1.1896± 0.0423 % min^{-1}). The shape parameter, b is used to characterize the curve as determined from the slope of Weibull model. Value of b was more than 1 in case of all the formulations, which indicated that dissolution curves were sigmoid, S-shaped, with upward curvature followed by a turning point.DRC values of all the models were summarised in Table 5 along with slope of Weibull and KorsmeyerPeppas model.

Figure 1: Dissolution profile of all the tablet batches

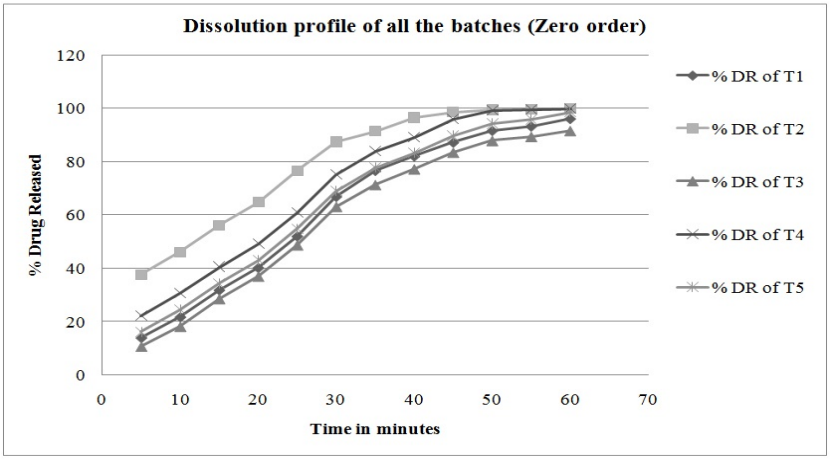


Table 4: R^2 - values of different mathematical models for all the batches

Mathematical models	R^2 – values of different tablet batches (Average ± SD), n = 3				
	T1	T2	T3	T4	T5
Zero Order	0.9524 ± 0.0156	0.8847 ± 0.0133	0.9560 ± 0.0067	0.9396 ± 0.0179	0.9578 ± 0.0098
First Order	0.9619 ± 0.0193	0.9214 ± 0.0409	0.9838 ± 0.0027	0.8588 ± 0.0327	0.8822 ± 0.1275
Higuchi	0.9757 ± 0.0099	0.9489 ± 0.0094	0.9790 ± 0.0056	0.9709 ± 0.0124	0.9802 ± 0.0070
Hixson Crowell	0.9861 ± 0.0073	0.9765 ± 0.0023	0.9892 ± 0.0042	0.9661 ± 0.0100	0.9765 ± 0.0215
KorsmeyerPeppas	0.9844 ± 0.0054	0.9701 ± 0.0054	0.9859 ± 0.0018	0.9808 ± 0.0076	0.9869 ± 0.0043
Weibull	0.9707 ± 0.0101	0.9237 ± 0.0144	0.9846 ± 0.0055	0.9132 ± 0.0144	0.9519 ± 0.0150

Table 5: Dissolution rate constants of different batches and slope of KorsmeyerPeppasand Weibull model

DRC (units)	Dissolution rate constants of different batches (Average ± SD), n = 3				
	T1	T2	T3	T4	T5
K_0(% min^{-1})	1.6080 ± 0.0280	1.1896± 0.0423	1.5870 ± 0.0288	1.5441 ±0.0237	1.6005 ±0.0351
K_1(min^{-1})	0.0578 ± 0.0060	0.1158 ± 0.0197	0.0463 ± 0.0023	0.1143 ±0.0167	0.0735 ±0.0108
K_H(% $min^{-1/2}$)	16.8035 ± 0.2379	12.7187 ± 0.4184	16.5799 ± 0.2952	16.2056 ±0.2182	16.7155 ±0.3512
K_{HC}($mg^{1/3}min^{-1}$)	0.0940 ± 0.0054	0.1178 ± 0.0067	0.0826 ± 0.0028	0.1268 ±0.0062	0.1046 ±0.0061
K_{KP}(min^{-n})*	0.0344 ± 0.0060	0.1787 ± 0.0162	0.0241 ± 0.0042	0.0704 ±0.0094	0.0437 ±0.0056
K_{WB}(min^{b})#	0.0130 ± 0.0023	0.0469 ± 0.0084	0.0105 ± 0.0018	0.0156 ± 0.0004	0.0143 ± 0.0033
Slope of Weibull	1.3196 ± 0.0440	1.1534 ± 0.0622	1.3350 ± 0.0450	1.3857 ± 0.0246	1.3279 ± 0.0681
Slope of Korsmeyer Peppas	0.8433 ± 0.0465	0.4419 ± 0.0230	0.9249 ± 0.0470	0.6737 ± 0.0326	0.7853 ± 0.0334

* "n" is the release exponent or slope of the KorsmeyerPeppas Model
# "b" is the shape parameter or slope of the Weibull model

Mathematical and statistical analyses:

A linear model with interaction terms was utilised in order to analyse the effect of PVP and CCS on the response variables (Q_{20} and Q_{40}). Linear terms were having statistical significant effects on the Q_{20} (p-value < 0.001) and Q_{40} (p-value < 0.01) as revealed from the results of ANOVA. However, effect of two-way interaction terms were having little and no significant effect on the Q_{20} (p-value = 0.066) and Q_{40} (p-value = 0.404) respectively. Accuracy of this interaction model was evident from the normal probability plot for the residuals for Q_{20} and Q_{40} (Figure 2 and 3 respectively) as the points on these plots are reasonably close to a straight line (R^2 value = 0.9564 for Q_{20} and 0.8940 for Q_{40}).[19]Normal probability plots had also depicted that residual values were minimal and vary within -4 to +2 in case of Q_{20}, whereas the value lies within -4 to +4 in case of Q_{40}.Regression equations including linear and interaction terms were utilised to evaluate the effect of PVP and CCS on the response variables (Q_{20} and Q_{40}). The regression equations describing percentage of drug released (% DR) at 20 minutes (Q_{20}) and 40 minutes (Q_{40}) were as follows.

$$Q_{20} = 46.778 - 4.694\,X_1 + 9.136\,X_2 - 3.115\,X_1X_2$$
$$Q_{40} = 85.555 - 3.051\,X_1 + 6.549\,X_2 - 0.654\,X_1X_2$$

Figure 2: Normal probability plot of Q_{20}

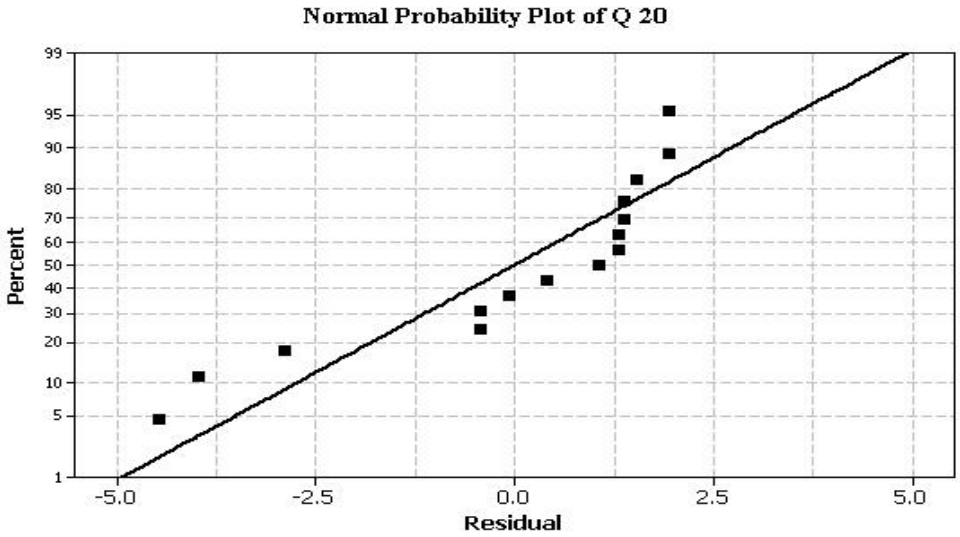
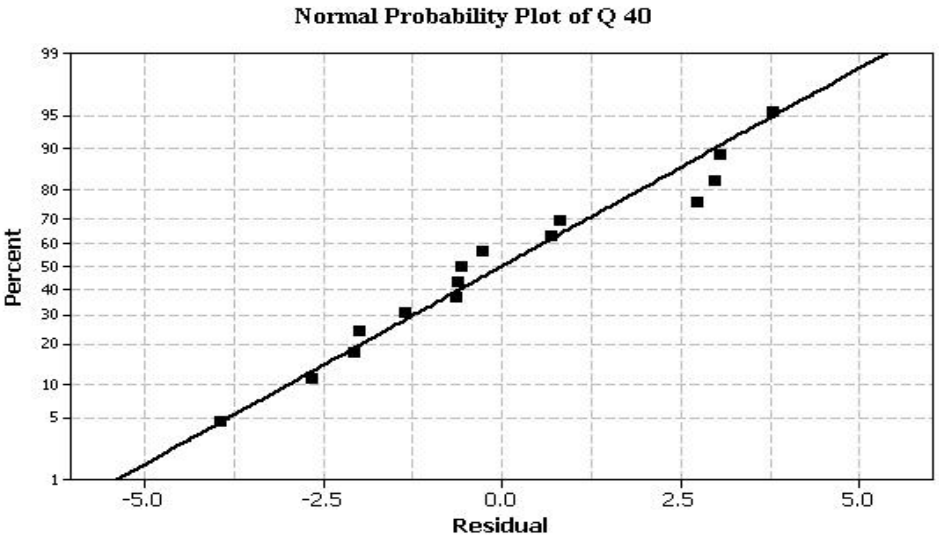


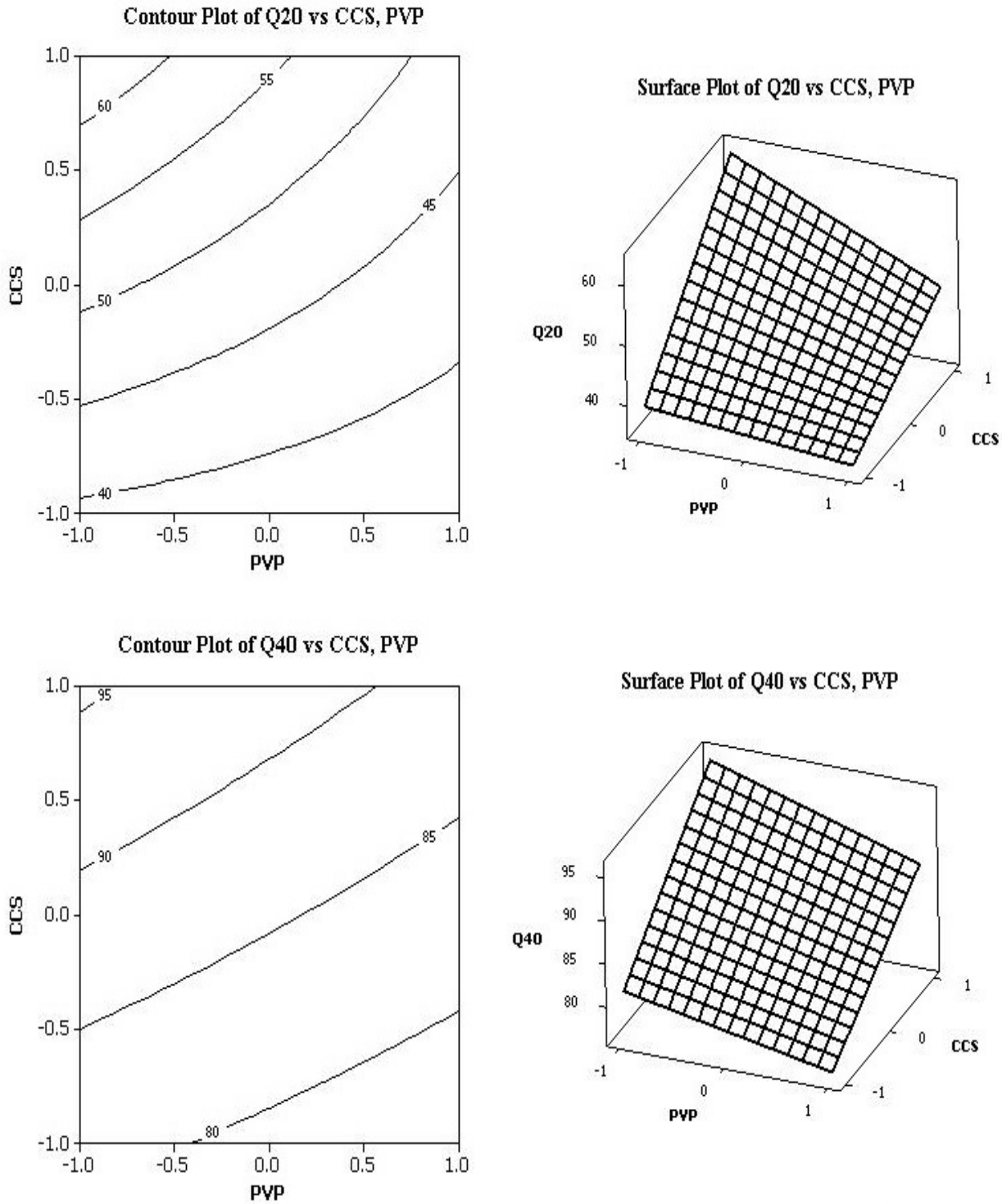
Figure 3: Normal probability plot of Q_{40}



It was clearly evident from the regression equations that highest amount of CCS produces higher values of both Q_{20} and Q_{40} and has more contribution towards immediate release, this is due to the fact that coefficient of amount of CCS (X_2) is positive in both the regression equations, which is used as a super disintegrant in order to achieve immediate drug release.At the same time, coefficient of PVP (X_1) is negative in the equations, thus, response variables decreases with increased values of PVP, which acts as a binder in the tablet formulations. Furthermore, interaction effects produced decreased drug release, though these effects were having less or no statistical significance as far as results of ANOVA (p-value > 0.05) were concerned. Contour plot of Q_{20} (Figure 4) revealed that as the amount of CCS increases from low (-1) to high (+1) level, keeping the value of PVP as constant at medium (0) level, percentage drug released was also increases, however, % DR was going down with increased values of PVP. Similar results were also found while contour plot of Q_{40} was analysed in the context of percentage drug released. Effect of CCS was proved to be more pronounced as compared to that of PVP on the response variables, because the slope of contour lines were positive in both the plots, which is also supported by the higher magnitude of the coefficients of CCS than that of PVP in their respective regression equations. Contour lines of Q_{40}(Figure 4) were almost linear, whereas that of Q_{20} were slightly curvilinear, which indicated that the interaction effect is more pronounced in case of Q_{20}, but were without any statistical significance (p-value > 0.05). Similar observations regarding main effects of CCS and PVP as well as that of interaction terms were also concluded from the corresponding response surface plots. Contour plots of Q_{20} and Q_{40} along with their response surface plots were given in Figure 4.Optimised values of PVP and CCS, as

determined by SAS were 60 mg and 52 mg respectively (target values of Q_{20} and Q_{40} were set at 50% and 90% respectively). The predicted response values (50.857 % for Q_{20} and 90.3544 % for Q_{40}) and observed responses (51.838 ± 2.8059 % for Q_{20} and 91.7622 ± 3.2778 % for Q_{40}, number of observations, n = 3) for the optimisedAF tablets shown no statistical significant difference (paired t-test, p – value = 0.6064 and 0.5344 for Q_{20} and Q_{40} respectively).

Figure 4: Contour plots and corresponding response surface plots of Q_{20} and Q_{40}



Contour plots and corresponding response surface plots of Q20 and Q40

CONCLUISIONS:

Aqueous extract of whole part of the plant *Anoectochilusformosanus* (dry powder) was successfully compressed to tablets by wet granulation. Total 5 successful batches were produced with hardness more than 6 Kg/cm^{2}which is sufficient to make the tablets tamper proof. Results of friability test and weight variation tests were satisfactory, to illustrate, friability weight loss were less than 0.7 % in all the batches and weight of all the batches fall within the USP weight variation limit (± 5% of the average weight). In addition to this, all the batches had passed the content uniformity test, as percentage of drug content was no more than ± 15 %. All the five batches of tablets had shown an immediate drug release pattern with more than 90 % of drug released after one hour by employing croscarmellose sodium as a super disintegrant. Dissolution profile of T1, T2 and T3 were best fitted to Hixson Crowell model, whereas that of T4 and T5 was best fitted to KorsmeyerPeppas model. Mechanism of drug release was found to be Non-Fickian transport as slope of the KorsmeyerPeppas model varies within 0.45 and 0.89. A 2^2 full factorial design with centre point was successfully explored for studying the effect of PVP and CCS on the drug release pattern after 20 and 40 minutes with sufficient model accuracy as revealed from the normal probability plots. Regression equations had indicated that drug release increases with CCS and decreases with PVP, moreover, less significant effect of the two way interaction termswere concluded. Optimised values of PVP and CCS were 60 mg and 52 mg respectively. Results of paired t-test confirmed that predicted and observed responses for the optimised tablet formulations had shown no statistical significant difference.

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