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Optimization and fabrication of gumghatti nanoparticles containing cyclophosphamide by plackett burman factorial design

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ABSTRACT

In this research work, Gumghatti loaded Cyclophosphamide nanoparticles has been developed to evaluate the antitumor activity. The fabrication of Gumghatti loaded Cyclophosphamide nanoparticles by suitable method were optimized by Plackett Burman method. Optimization of the formulation requires proper designing of the research. Consequently, the placket burman method has been utilized for the formulation of nanoparticles encompassing Cyclophosphamide in natural Gumghatti for antitumor activity. Ten critical parameters influencing the formulation has been selected and designed in Plackett Burman method of experimentation for 12 trials to assess critical variables influencing the experimental outcomes. The results shows that the 6th trial with optimum particle size of 143.4 nm with zeta potential of 28.5 mV. However, it has been found conducive, to prepare the nanoparticles containing the anticancer agents like Cyclophosphamide in natural Gumghatti as a polymer.

Keywords: Design of experiment, critical parameters, optimization, characterization of nanoparticles.

1. INTRODUCTION

A clear trial outline is essential in numerous investigations of diagnostic and other formulation processes. Complete factorial designs, which study every components (test variables) influencing the response, utilizing not less than two levels (values) for every variable, can offer ascent to an unsuitably extensive number of trial investigations. In addition these elements may influence the framework reaction intuitively, i.e. the impact of one variable may rely on upon the levels of others. So it is more basic to utilize incomplete factorial plans in which some data, particularly about interactions, may be yielded in light of a legitimate concern for a manageable number of investigations [1-5].

A popular and economical approach that gives information only on the effects of single factors, but not on interactions, is the Plackett–Burman (PB) method, introduced in 1946 when the authors were working for the British Ministry of Supply. This method is well suited to identifying formulation critical parameters, i.e. establishing whether the outcome of formulation is affected by changes in each relevant factor [6]. The most important feature of PB designs is that they all involve 4n experiments, where n ¼ 1, 2, 3.In each case the maximum number of factors that can be studied is 4n -1, so an 8-experiment PB design can study no more than 7 factors, a 12-experiment design will handle up to 11 factors, and so on. This may seem to be inconvenient, but it turns out to be a valuable feature of the method. Suppose we wish to study four factors. Four experiments will be then insufficient, so we shall have to use eight experiments in a PB design, and have seven factors. This means that three of the latter will be dummy factors; they will have no chemical meaning at all [7].

Nevertheless it turns out that the apparent effects of these dummy factors can be used to estimate the random measurement errors. The more dummy factors there are, the better the estimate of such errors, so it is not uncommon for experimenters to use a larger PB design than is strictly necessary, thus getting higher quality information on the significance of each “real” factor. PB designs utilise two levels for each factor, the higher level being denoted (+) and the lower (-

) as usual [8]. A further feature of the PB method is that the (+) and (-) signs for the individual trial experiments are assigned in a cyclical manner.

2. EXPERIMENTAL

2.1. Materials

Cyclophosphamide and Gumghatti was purchased from Sigma Aldrich, All the reagents and solvents used were analytical grade and standard.

2.2. Methods

2.2.1. Plackett Burman Factorial Design for fabrication of nanoparticles

Utilization of factorial design for the optimization of a process allows testing of a large number of factors concomitantly and prevents the use of an unwanted number of runs or trials, thus it prevents material wastage and time consumption. Statistical design of experiment is a perfect methodology to conduct and execute plan of experiments in order to extract the maximum amount of information with limited number of inputs. The most critical factors selected for the formulation along with the proper selection of design of experiment input a tool proves to be superior. DOE identifies optimal formulation conditions for these NPs provide understanding of the underlying relationship. Most commonly applied screening designs is the Plackett-Burman design that evaluates large number of factors and identify critical one in a minimal number of trials. The utilization of Plackett-Burman experimental design paves the way for the study of numerous factors in a systematic and logical way to select optimized runs. The important significance of Plackett-Burman design method is quicker screening obtained with minimum possible experimental runs. [9-11]

However, the process parameters which includes. Consequently, PLB design was exploited to optimize the procedure parameter at lesser (-) and upper (+) level. Twelve investigational runs exploiting 8 self-regulating progression variables at superior and inferior niche were generated exploiting Expert Design ® Version 9.

2.2.2. Characterization of the fabricated nanoparticles containing Cyclophosphamide

2.2.2.1. Encapsulation efficiency of Cyclophosphamide Gumghatti Nanoparticles

Encapsulation efficiency, which is the percentage of the actual amount of drug encapsulated in the polymeric carrier relative to the total amount of drug taken for Nanoparticles preparation, is calculate by using the following equation:

% Encapsulation Efficiency = (Actual drug loading/ Theoretical drug loading) × 100

To calculate actual drug loading an accurately weighed quantity of Cyclophosphamide was sonicate in 10 ml of methanol for 5 minutes and filter through 0.45 µl syringe filter. Cyclophosphamide concentration is analyzed by measuring the absorbance at 287 nm using UV–Vis spectrophotometer [12].

Table - 1: Optimization process parameters at lower and higher levels

Code	Variables	Levels	
		Lower (-)	Higher (+)
A	Cyclophosphamide (Drug)	100	105
B	Polymer quantity	150	200
C	Surfactant quantity	50	100
D	Aqueous solvent	10	20
E	Organic solvent	10	20
F	Stirring time	30	60
G	Stirring rate	1000	2000
H	Adding the component	Org to Aqueous	Aqueous to org
I	Addition mode	All at once	incremental
J	Stirring mode	Blade	Homogenizer

* Twelve experimental runs (Table 7.2) involving 10 process parameters at higher and lower levels were generated by Design-Expert®

* Prepared dual loaded flavono polymeric nanoparticles were characterized for average particle size, polydispersity index and zeta potential

Table - 2: Scheme of fabrication of Cyclophosphamide loaded nanoparticles by Plackett-Burman method

Trials	Drug (mg)	Polymer (mg)	Surfactant (mg)	Aqueous (ml)	Organic (ml)	time (min)	Stirring (rpm)	Additing component	Addition Mode	Stirring Mode
1	100	200	50	20	20	60	1000	O to A	All at once	H
2	100	200	50	20	20	30	2000	A to O	incremental	H
3	105	150	100	20	20	30	1000	O to A	incremental	H
4	105	150	50	20	10	60	2000	O to A	incremental	B
5	100	150	100	10	20	60	1000	A to O	incremental	B
6	100	150	100	20	10	60	2000	O to A	All at once	B
7	100	250	100	10	10	30	2000	O to A	incremental	H
8	105	200	50	10	10	60	1000	A to O	incremental	B
9	105	200	100	10	20	60	2000	O to A	All at once	H
10	105	150	50	10	20	30	2000	A to O	All at once	H
11	105	200	100	20	10	30	1000	A to O	All at once	B
12	100	150	50	10	10	30	1000	O to A	All at once	B

O -→ A = Organic to aqueous; A-→ O = Aqueous to organic; H→ Homogenizer; B→ Blade

Table - 3: Scheme of fabrication of Cyclophosphamide loaded nanoparticles by Plackett-Burman method higher (+) & lower (-) limits

Trials	Drug (mg)	Polymer (mg)	Surfactant (mg)	Aqueous solvent (ml)	Organic solvent (ml)	Stirring time (min)	Stirring rate (rpm)	Additing component	Addition Mode	Stirring Mode
1	-	+	-	+	+	-	-	-	-	H
2	-	+	-	+	+	-	+	+	+	H
3	+	-	+	+	+	-	-	-	+	H
4	+	-	-	+	-	+	+	-	+	B
5	-	-	+	-	+	+	-	+	+	B
6	-	-	+	+	-	+	+	-	-	B
7	-	+	+	-	-	-	+	-	+	H
8	+	+	-	-	-	+	-	+	+	B
9	+	+	+	-	+	+	+	-	-	H
10	+	-	-	-	+	-	+	+	-	H
11	+	+	+	+	-	-	-	+	-	B
12	-	-	-	-	-	-	-	-	-	B

Table - 4: Optimized formula for the fabrication of Cyclophosphamide loaded Gumghatti nanoparticles										
Trials	**Drug (mg)**	**Polymer (mg)**	**Surfactant (mg)**	**Aqueous solvent (ml)**	**Organic solvent (ml)**	**Stirring time (min)**	**Stirring rate (rpm)**	**Adding component**	**Addition Mode**	**Stirring Mode**
6	100	150	100	20	10	60	2000	O to A	All at once	B

Table 5: Optimized formula for the fabrication of Cyclophosphamide nanoparticles higher and lower limits										
Trials	**Drug (mg)**	**Polymer (mg)**	**Surfactant (mg)**	**Aqueous solvent (ml)**	**Organic solvent (ml)**	**Stirring time (min)**	**Stirring rate (rpm)**	**Adding component**	**Addition Mode**	**Stirring Mode**
6	-	-	+	+	-	+	+	-	-	B

Table - 5: Characterization of prepared nanoparticles			
Options	**Average Particle Size (nm)**	**Polydispersity Index**	**Zeta Potential (mV)**
1	625.3	0.657	0.230
2	200.7	0.072	11.4
3	270.0	0.370	14.9
4	993.0	0.845	2.70
5	1300.0	0.971	3.10
6	143.4	0.160	28.5
7	1005.8	0.602	1.16
8	199.1	0.240	9.5
9	1207.4	0.163	3.6
10	679.5	0.632	0.239
11	357.5	0.259	-0.078
12	238.9	0.790	8.65

3. RESULT AND DISCUSSION

3.1. Characterization of Nanoparticles of Twelve Formulations

For all the 12 trials the average particle size, polydispersity index and zeta potential of the formulation were obtained and the results are mentioned in the table 5. The 6th trial has shown a particle size of 143.4 nm, polydispersity index 0.16 and zeta potential of 28.5 respectively. The encapsulation efficiency for the 6th trial was observed to be higher 93.56%, Drug loading capacity has been 83.55% and reconciliation was observed to be 76.54% as mentioned in the table 6.

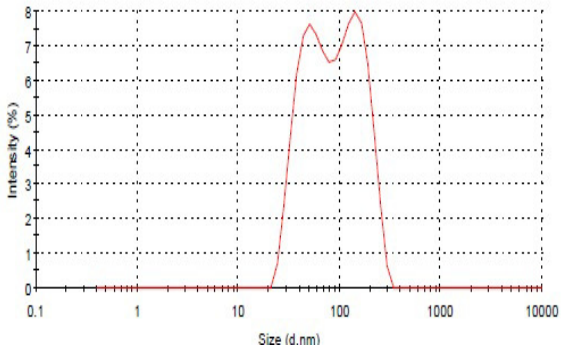

Figure - 1: Distribution of Nanoparticles of trail 6.

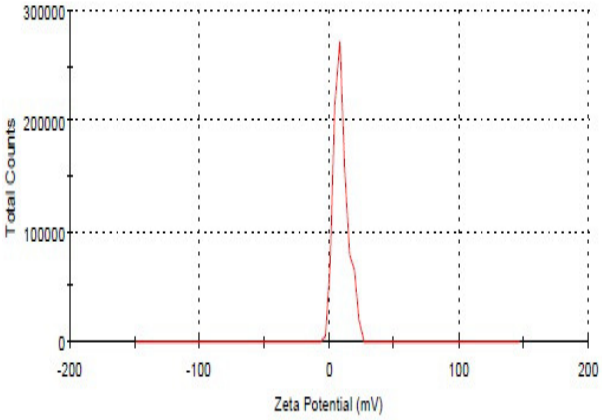

Figure - 2: Distribution of Zeta potential Trial 6.

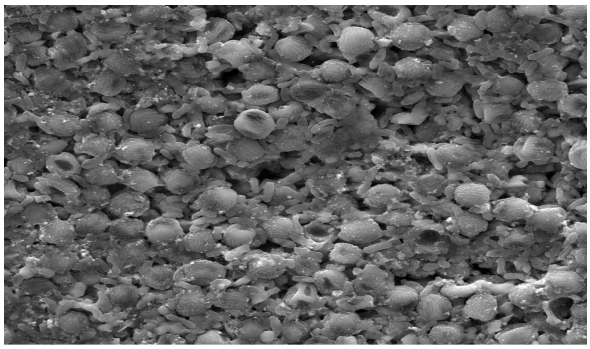

Figure - 3: SEM image of the prepared Nanoparticles.

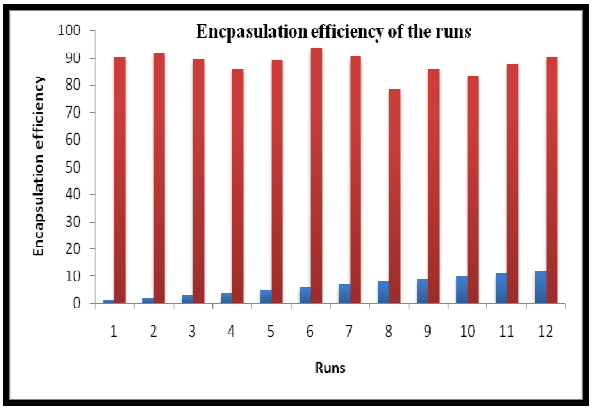

Figure - 4: Encapsulation efficiency of the Cyclophosphamide Nanoparticles.

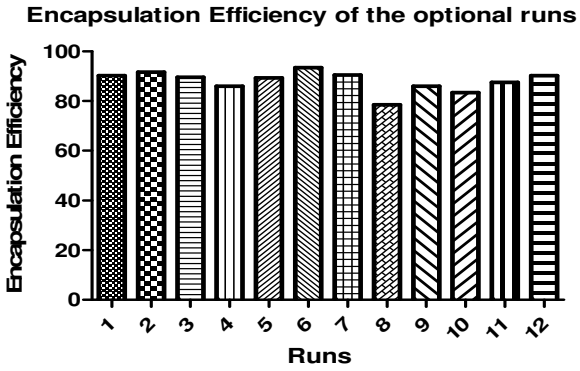

Figure - 5: Scheme of Encapsulation efficiency of the Cyclophosphamide Nanoparticles.

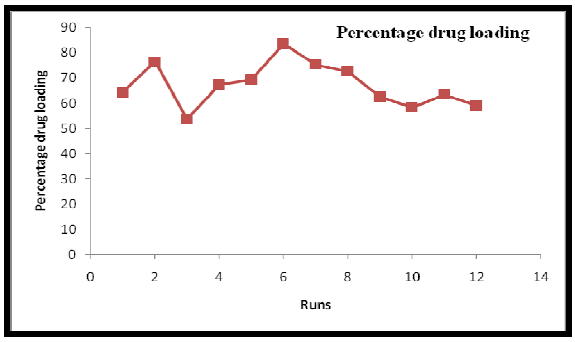

Figure - 6: Percentage drug loading of the Cyclophosphamide Nanoparticles.

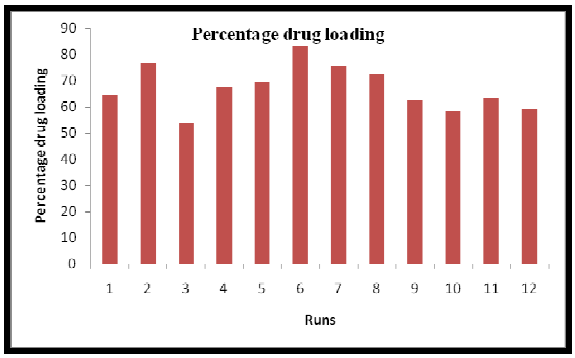

Figure - 7: Scheme of drug loading of the Cyclophosphamide Nanoparticles.

Table - 6: Parameters of the prepared nanoparticles			
Options	**Encapsulation Efficiency (EE) Percentage**	**Drug loading (DL)**	**Percentage Yield (PY)**
1	90.33±0.781	64.33±1.030	52.63±0.063
2	91.66±0.057	76.43±0.052	43.76±0.941
3	89.64±0.064	53.66±0.067	59.71±0.045
4	86.03±0.045	67.42±0.850	49.37±0.786
5	89.34±0.890	69.33±0.054	55.92±0.871
6	93.56±0.032	83.55±0.053	76.54±0.980
7	90.44±1.030	75.33±0.650	65.66±0.094
8	78.56±0.070	72.62±0.072	48.33±0.856
9	85.99±0.750	62.54±0.082	70.38±0.83
10	83.45±0.540	58.36±0.069	60.87±0.942
11	87.56±0.067	63.47±0.065	70.33±0.673
12	90.22±0.009	59.03±0.032	50.33±0.057

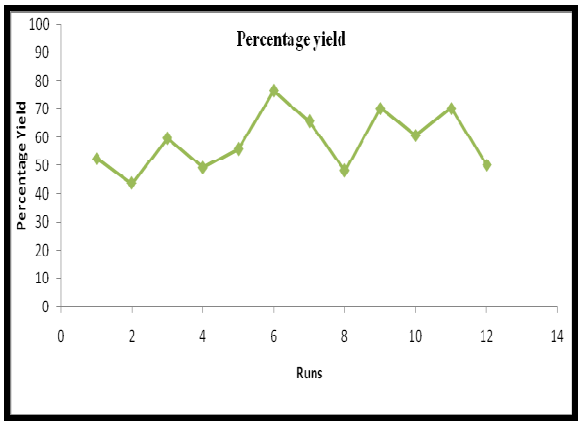

Figure - 8: Percentage yield of the Cyclophosphamide Nanoparticles (12 runs).

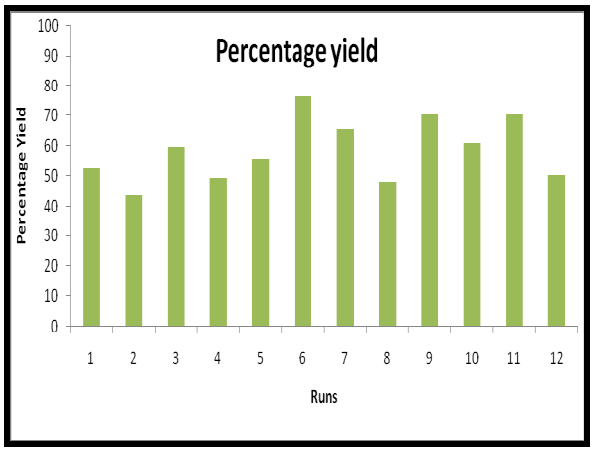

Figure - 9: Scheme of percentage yield of the Cyclophosphamide Nanoparticles.

4. CONCLUSION

The fabrication of Gumghatti nanoparticles containing the Cyclophosphamide for the anticancer activity, by Plackett Burman method was successfully executed and the method was also conducive and feasible. The critical factors governing the successful experimental results were identified by using the Plackett Burman factorial design. It has been observed the proposed Plackett Burman factorial design of 10 independent variables obtaining 12 trials was helpful in optimizing the formulation successively. The scale up of optimized formulation has been taken to perform other research work of the formulation.

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