The Plackett-Burman model-An Improved Alternative to Identify the Significant Factors Implied in the Preparation of Tramadol Hydrochloride Microsphere

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ABSTRACT

Background and Purpose: According to Process Analytical Technology perspective, drug product quality should be ensured by manufacturing process design. Initial step of the process analysis is investigation of critical process parameters (CPPs). Quality is considered to be the major tool in pharmaceutical industry and failures in the quality of upcoming new chemical entities have been seen in the recent years due to complications in the formulation process. This paper describes the use of Design of Experiments tool for selection of the CPPs. **Methods:** Albumin microspheres were prepared by heat denaturation method. Different factors like Drug Conc., amount of Tween, Glutaraldehyde conc., Stirring speed were selected as independent variables at two levels in the Plackett and Burman (PB) design. By using software the effect of different factors were studied. The microspheres were characterized for percentage burst effect, percentage encapsulation efficiency. **Results:** The CPPs affecting encapsulation efficiency were Drug Conc., amount of Tween, Glutaraldehyde conc., Stirring speed. FTIR study showed compatibility of the drug with excipients without having any significant interactions. **Conclusion:** The study concludes that the statistical PB design could be useful to identify influencing variables such as Drug Conc., amount of Tween, Glutaraldehyde conc., Stirring speed.

Key words: Microspheres, Plackett Burman design, Tramadol Hydrochloride (TH).

INTRODUCTION

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm.[1] Microspheres can also offer advantages like limiting fluctuation of drug concentration within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance. The use of natural biodegradable polymer to deliver drugs continues to be an area of active research, despite the advent of synthetic biodegradable polymers. Some of the materials taken from nature for microsphere preparation include lipids and waxes, proteins like albumin[2] and gelatin, polysaccharides like alginate[3,4] and chitosan.[5] Albumin is a widely distributed natural protein. It is considered as a potential carrier of drug or proteins (for either their site specific localization or their local application into anatomical discrete sites). Preparation of uniformly sized Albumin Microspheres.[6] (AMS) was

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first reported in the late 60's and early 70's.[7,8] AMS have received wide attention during the recent decades due to their specificity[9] biodegradability[10] and other desirable characteristics such as non-toxicity and biocompatibility[11] as an ideal drug carrier.

TH is a centrally acting oral analgesic that blocks pain through opoid receptor binding and inhibition of nor epinephrine and serotonin reuptake. Short half life (about 2 h) requires frequent administration of dosage form to maintain therapeutic level of drug in body.[12] Hence, TH was selected as a model drug to design modified release microspheres.

Among many development strategies, statistical Design of Experiments (DoE) is considered as most beneficial tool for the scientific knowledge acquisition, since it is relevant for multi-factorial relationships investigation. Generally, for test of *k* factors each at 2 levels, the factorial design requires 2^k runs of experimentation. As the number of factors or levels increases, the number of runs increases rapidly: 4 factors at two levels need to be tested within 16 runs but 6 factors at two levels require 64 runs. The classical method of medium optimization involves changing one variable at time by keeping others at fixed levels. Being single dimensional it is laborious and time consuming method does not guarantee the determination of optimal conditions. On the other hand carrying out experiments with possible factorial combination of the test is impractical because of large number of experiments. Experimental design technique offers a considerable advantage over-one-factor at time for microsphere formation. Statically approach effectively tackles problems which involves specific design of experiments which minimizes the error in determine the variables. PB design allows short listings of medium components. As in this experiments, the Drug Conc., amount of Tween, Gluteraldehyde conc., Stirring speed were selected as independent variables at two levels in the Plackett and Burman (PB) design. The microspheres were characterized for percentage burst effect, percentage encapsulation efficiency. Plackett and Burman design is the best reported method for screening.[13] The basic objective of quality by design (QbD) is to develop safe, effective and customer friendly dosage form.

The variables were correlated using the following polynomial equation with PB design.

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_4 + \ldots\ldots\ldots\ldots + A_nX_n$$

Where, Y is the response, A_0 is the constant, and A_1 is the coefficients of the response.[14]

MATERIALS AND METHODS

Materials

TH was procured from Matrix Laboratories, Bangalore. Other ingredients like Tween, Gluteraldehyde, Stirring speed were procured from S.D. Fine chemicals, Mumbai.

Preparation of Microsphere

Albumin microspheres were prepared by modifying heat denaturation method.[15] A solution of albumin (1 g in 25 ml) was prepared and the drug of weight 0.2 gm was added to the albumin solution. The contents were slowly added to a beaker containing 100 ml of preheated 60ºC liquid paraffin containing Tween 80 as emulsifying agent and stirred for 1 hr. The temperature was reduced to 40ºC for hardening process and was maintained for 25 min. The resulting microspheres were stabilized using gluteraldehyde solution (25%v/v) for a period of 15 min. The microspheres were collected by decantation and washed with n-hexane and dried at room temperature. Number of microsphere formulations was prepared and after formulation suitable formulations are only shown in Table 1.

EVALUATION OF MICROSPHERES

Drug Polymer Interaction Studies

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for TH and physical mixture of TH: albumin (0.2:3). Samples were prepared in KBr pellets with a hydrostatic press at a force of 5.2 τ cm^{-2} for 3 minutes. The scanning range was selected 400-4000 cm^{-1}. Results is shown in Figure 2 and 3.

Size analysis and morphology

The formations of microspheres were monitored during preparation steps by an optical microscope with transmitted light at 40 X magnification. The mean particle size of microspheres was calculated with the help of a calibrated ocular micrometer and particle size distribution is as shown in Figure 1. But for the shape and surface morphologies of the microspheres scanning electron microscope (SEM) was used.

Drug entrapment

Accurately weighed microspheres equivalent to 20 mg of drug was suspended in 25 ml of methanol and sonicated for 3 mins. The solution was then filtered, diluted suitably and analyzed for drug content spectro photometrically at 272 nm. The percentage drug entrapment was calculated as below and results shown in Table 2.

Table 1: Composition of Formulations Albumin Microsphere				
Formulation Code	**Drug (X1 gm):Albumin (gm)**	**Tween (X2)**	**Gluteraldehyde (X3)**	**Stirring speed (X4 rpm)**
F1	0.2:1	50 m g	50 ml	1000
F2	0.2:1.5	50 mg	60 ml	1000
F3	0.2:2	100 mg	100 ml	500
F4	0.2:2.5	100 mg	50 ml	1200
F5	0.2:3	50 m g	60 ml	1200
F6	0.25:4	50 mg	60 ml	1200
F7	0.2:5	50 m g	70 ml	700

Table 2: Data for %Yield and Drug Encapsulation Efficiency		
Formula Code	**Percentage yield (%)**	**Drug encapsulation efficiency (%)**
F1	43.0	38.35
F2	46.0	41.89
F3	63.1	53.60
F4	80.0	60.75
F5	85.0	77.50
F6	88.83	64. 71
F7	88.5	69.85

Table 3: Data for *In-Vitro* Drug Release Profile							
Time in (Min)	**Cumulative % Drug Release**						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
30	12.32	27.41	39.91	37.86	39.68	19.18	48.62
60	13.73	32.36	45.59	44.00	45.36	21.96	66.62
90	24.77	37.32	48.59	47.64	48.32	31.68	87.45
120	27.36	39.82	51.55	54.05	56.09	40.05	99.51
150	32.26	41.41	56.68	64.16	65.27	44.32	91.88
180	38.59	45.73	59.05	68.27	73.81	50.73	81.42
240	41.55	47.23	63.00	75.50	81.03	59.82	72.36
300	50.00	55.73	69.23	80.00	82.84	69.73	56.39
6 hr	53.05	58.82	75.14	84.53	88.73	75.55	48.62
7 hr	61.55	68.59	85.18	89.07	93.31	80.05	66.62
9 hr	74.68	88.14	89.73	91.59	97.87	85.90	87.45

Table 4: Data for Stability Studies of Batch F5				
Microspheres Formulation	**Days (0)**	**Days (60)**	**Days (120)**	**Days (180)**
F5	97.87	96.73	97.85	96.97

Out of the different batches, the suitable batch F_5 was selected for to study design.

Table 5: The Independent Variables and Levels of PB Design of Low-Medium-High Units			
Independent Variables	**Low(-)**	**Medium**	**High(+)**
Drug Conc.(X1)	0.2 gm	1.35 gm	0.25 gm
Tween Conc.(X2)	50 mg	75 mg	100 mg
Gluteraldehyde conc.(X3)	50 ml	75 ml	100 ml
Stirring speed(X4)	500 rpm	850 rpm	1200 rpm

Table 6: Observed Responses of PBF (Plackett-Burman Design Formulation)

PBF NO	X1	X2	X4	X5	Drug encapsulation efficiency (%)	Burst effect (%)
PBF1	1	-1	1	-1	77.21	87.89
PBF2	1	1	-1	1	69.67	42.78
PBF3	-1	1	1	-1	60.69	86.07
PBF4	1	-1	1	1	62.16	79.2
PBF5	1	1	-1	1	71.41	41.9
PBF6	1	1	1	-1	77.28	87.3
PBF7	-1	1	1	1	46.56	84
PBF8	-1	-1	1	1	56.55	82.7
PBF9	-1	-1	-1	1	79.6	45.8
PBF10	1	-1	-1	-1	70.78	74.38
PBF11	-1	1	-1	-1	81.98	62
PBF12	-1	-1	-1	-1	77.59	88.4

Table 7: Statistical Analysis of Burst effect (Y_1) & Encapsulation efficiency (Y_2)

Independent Variables	Burst effect (Y_1)		Encapsulation efficiency (Y_2)	
	Coefficient	p-value	Coefficient	p-value
Drug (X1)	-2.960	0.281	2.1283	0.406
Tween (X2)	-4.5267	0.117	-1.3583	0.590
Glutaraldehyde conc.(X3)	12.658	0.002	-5.8817	0.044
Stirring speed (X4)	-9.1383	0.009	-4.965	0.078

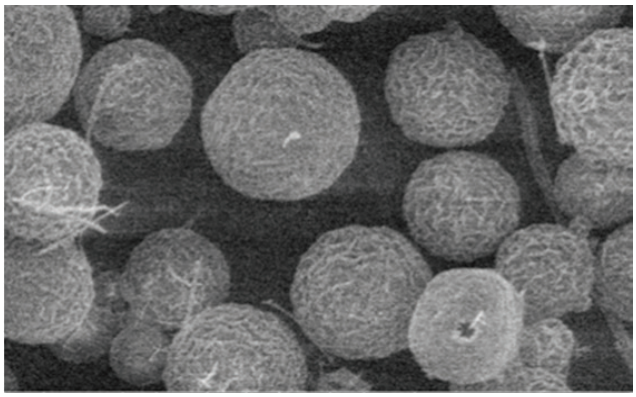

Figure 1: Particle size distribution

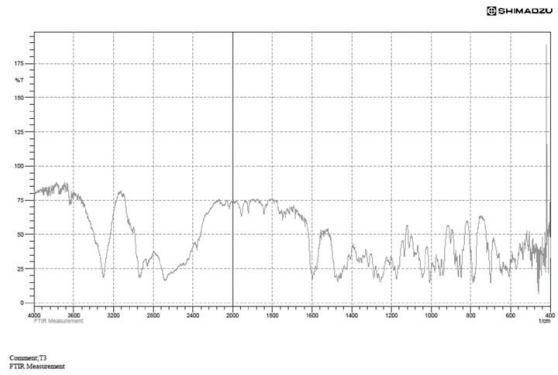

Figure 2: FTIR spectra of drug Tramadol Hydrochloride

% Drug Entrapment = (Practical drug loading / Theoretical drug loading) X 100

Dissolution studies

Dissolution test was performed in USP XXIII dissolution test apparatus by paddle method. The dissolution media used was 900 ml of phosphate buffer pH 7.4 maintained at 37 ± 0.5 C and rotated at 100 rpm. The *in vitro* release of microspheres showed biphasic release pattern, i.e. initial burst release followed by slow release. The burst effect was probably due to quick release of drug present on the surface of microspheres. Aliquots samples were withdrawn at specified time intervals and replaced with same volume of fresh media, filtered and analyzed spectro photometrically (Shimadzu 1600) at 272 nm for cumulative drug release. Results are given in Table 3.

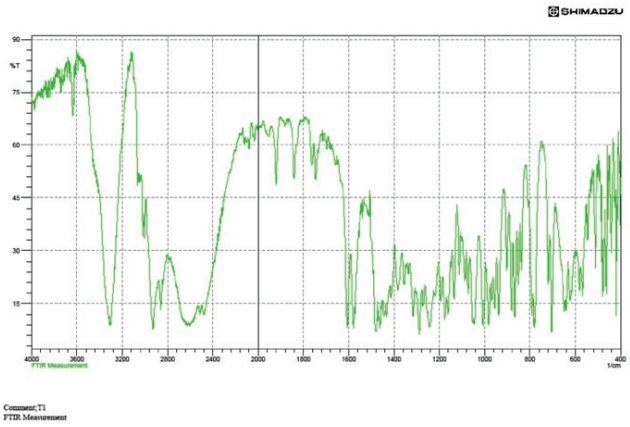

Figure 3: FTIR spectra of physical mixture of Tramadol Hydrochloride and albumin

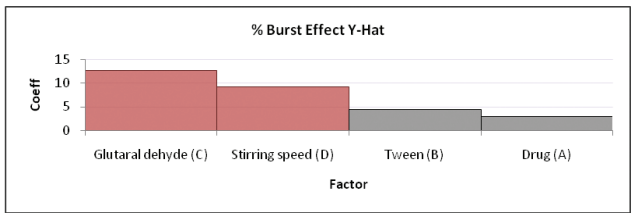

Figure 4: Pareto-Plot for Plackett-Burman parameter estimates for % Burst Effect (Y_1)

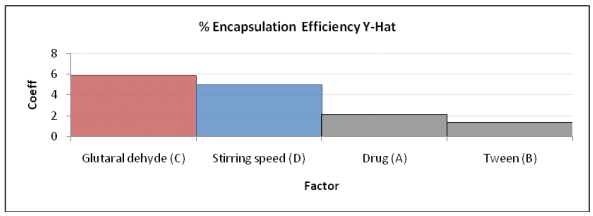

Figure 5: Pareto-Plot for Plackett-Burman parameter estimates for % Encapsulation Efficiency (Y_2)

Stability Studies

The stability protocol was designed based on the ICH 'Q1AR2' guidelines. The microspheres formulations F5 chosen was stored at 40 ± 20ºC and 75 ± 5% RH for a period of 6 months. The stored samples were tested for their drug content and for any physical change. The testing was carried out at 0, 2, 4 & 6 months for accelerated storage condition and results are shown in Table 4.

RSELUTS AND DISCUSSION

Drug excipients compatibility studies

Stability of formulation based on selection and quality of raw material hence it is advisable to check drug excipients compatibility. From the result of FTIR we can conclude that there is no interaction between drug and polymers.

Percentage yield

Any process can be economic to be used for industrial scale production only if yield is high. The p value>0.05 shows that the % yield was not significantly affected by investigated factors.

Influence of investigated parameters on burst release (Y_1)

It is worth while to note that design of experiment is an important element of QbD. In order to identify critical material attributes (CMA) and critical process parameters (CPP), multiple regression analysis was carried out. A linear model describing the relationship between the independent variables and % encapsulation efficiency was evaluated.

$$Y_1 = 71.868-2.960X_1-4.5267X_2+12.658X_3 -9.1383X_4$$

The effect for burst release shows the impact of evaluating factors on encapsulation efficiency. Positive value for a coefficient indicates that the independent variable favors the response and a negative value indicates an inverse relationship between response and factor. The higher amount of glutaraldehyde appears to favor the cross-linking reaction, and hence spherical free-flowing microspheres were obtained with an increase in loading efficiency.

Influence of investigated parameters on Encapsulation efficiency (Y_2)

A linear model describing the relationship between the independent variable and burst drug release at 9 hr was evolved.

$$Y_2= 69.29+2.3783X_1-1.3583X_2-5.8817X_3-4.965X_4$$

The Pareto chart indicates that the factors Gluteraldehyde conc. X_3 and Stirring speed X_4 possess significant influence as confirmed by least p value of in Table 6.

CONCLUSION

Statistical experimental designs are strongly recommended in identifying critical variables in the development of pharmaceutical products, particularly extended release dosage forms. The Plackett Burman Design allows the screening of critical parameters. In summary, this study presents the screening of critical processing parameters using Plackett Burman design are Drug Conc., Tween conc., Glutaraldehyde conc. and stirring speed. The stirrer rotation speed and Glutaraldehyde conc. seems to be significant parameter affecting formulation of TH Microsphere. The use of design of experiment is dem-

onstrated to meet the primary requirements of quality by design (QbD). The study indicates the use of Placket and Burman Design to identify the important variables for microsphere formulation.

ACKNOWLEDGEMENT

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CONFLICTS OF INTREST

Authors declare that there is no any conflict of interest.

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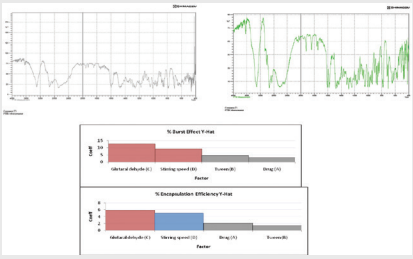
PICTORIAL ABSTRACT



ABBREVIATIONS USED

TH: Tramadol Hydrochloride; **SEM:** Scanning electron microscope; **QbD:** Quality by design; **DoE:** Design of Experiments.

SUMMARY

- Statistical experimental designs are strongly recommended in identifying critical variables in the development of pharmaceutical products, particularly extended release dosage forms.
- The Plackett Burman Design allows the screening of critical parameters. In summary, this study presents the screening of critical processing parameters using Plackett Burman design are Drug Conc., Tween conc., Glutaraldehyde conc. and stirring speed.
- The stirrer rotation speed and Glutaraldehyde conc. seems to be significant parameter affecting formulation of TH Microsphere.
- The use of design of experiment is demonstrated to meet the primary requirements of quality by design (QbD). The study indicates the use of Placket and Burman Design to identify the important variables for microsphere formulation.

About Author

Amaresh Prusty, working as Asst.Professor at College of Pharmaceutical Sciences, Puri. Odisha. Author also pursuing Ph.D under Biju Patnaik University of Technology, Odisha. His research project mainly focused on extended release tablets.