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Selection of Effectual Variables For Formulation Development of Prolonged Release Nanoparticles For Diltiazem Hydrochloride

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ABSTRACT

The purpose of this study was selection of most influential variable for the preparation of prolonged release nanoparticulate formulation by desolvation method for diltiazem hydrochloride with bioadhesive polymer gelatin. Formulation and processing variables which effect various response variables were studied by a Taguchi design. Independent variables studied were the amount of polymer, amount of glutaraldehyde, amount of Poloxamer 237, acetone addition rate, pH, stirring time and stirring speed. The dependent variables considered were the particle size, polydispersity index, amount of drug released in 6 h, time required to release 60 % of drug, mucoadhesiveness, entrapment efficiency and loading efficiency. Pareto charts showed that the two significant factors affecting the response variables were amount of glutaraldehyde and amount of polymer.

Keywords: Optimization; Nanoparticles; Gastoretentive; Mucoadhesive; Diltiazem.

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INTRODUCTION

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Diltiazem HCl (DTZ) is an antihypertensive agent that antagonizes the action of beta-1 receptor. DTZ when given orally is well absorbed from the gastrointestinal tract and is subject to an extensive first-pass effect. DTZ undergoes extensive metabolism in which only 2% to 4% of the unchanged drug appears in the urine. Drugs which induce or inhibit hepatic microsomal enzymes may alter DTZ disposition (Lee et al., 1991)[2]. It has been reported that the absolute bioavailability of DTZ when given orally is 30-40%. The biological half-life of DTZ is 4-6 hour and the main site of absorption is proximal small intestine (Brunton et al., 2008)[1].

The reduced bioavailability of DTZ may be because of transportation of dosage form from the region of absorption window to site where it is less absorbed. Therefore there was a need to increase gastroretention time of dosage form so that drug would be available at the site of absorption and results in improved bioavailability. Hence drug like diltiazem is considered as a suitable candidate for the design of nanoparticulate drug delivery system with a view to improve oral bioavailability and patient compliance

Gastroretentive dosage forms are retained in the stomach for several hours and would release the drug in a controlled and prolonged manner, so that the drug could be supplied continuously to the upper gastrointestinal tract at its absorption window (Singh and Kim, 2000, Streubel et al., 2006)[3,4]. Prolonged gastroretention is suitable for local drug delivery to the stomach and proximal small intestine. It is also improves solubility of drugs that are less soluble in a high pH environment, reduces drug waste, and improves bioavailability (Kharia et al., 2010)[5].

The conventional method to optimize a formulation or process entails studying the influence of one factor at time (OFAT), while keeping other factors constant. Using OFAT approach, solution of a specific problematic property can be achieved some way, but realization of the true optimum composition or process is by no means guaranteed. This may be ascribed to the presence of interactions, i.e., the influence of one or more factors on others. The final product may be satisfactory but generally suboptimal, as a better formulation might still exist for the studied conditions. Thus, the conventional OFAT approach of drug formulation development suffers from several pitfalls, like being strenuous, uneconomical, and inept to reveal interactions (Singh et al., 2004, 2006, 2009)[6-9].

Further, the OFAT methodology results only in “just satisfactory” solutions, as a detailed study of all variables is not possible. As one cannot establish “cause-and-effect” relationships using OFAT,

it becomes futile when all variables are changed simultaneously. Of late, the systematic optimization approaches are being widely practiced to alleviate such inconsistencies. This holistic approach encompassing the application of apt experimental designs coupled with the generation of mathematical equations and graphic outcomes, and depicting a complete picture of variation of the response(s) as a function of the factor(s) is termed as design of experiments (DoE). DoE techniques are thus far more beneficial, as they overcome most shortcomings inherent to the traditional OFAT approach. Prominent among all, DoE techniques yield the "best solution" in the presence of competing objectives and require fewer experiments to achieve an optimum formulation. By and large, low-resolution designs like FDs (full or fractional), Plackett Burman design, or Taguchi designs are enough for the purpose of simpler screening of a large number of experimental parameters (Singh et al., 2004)[7].

Taguchi methods or an orthogonal array have been widely used to optimize the reaction variables by formulating a minimum number of experiments. It is a method of designing experiments that usually requires only a fraction of the full factorial combinations. An orthogonal array means the design is balanced so that factor levels are weighted equally. Because of this, each factor can be evaluated independently of all the other factors, so the effect of one factor does not influence the estimation of another factor. This approach facilitates to categorize the influence of individual factors and ascertain the relationship between variables and operational conditions (Dasu et al., 2003)[9].

The aim of the present study was to screen most influential factors which effect formulation of prolonged release nanoparticulate formulation of DTZ by Taguchi approach. Gelatin was selected as a mucoadhesive polymer to prepare prolonged release nanoparticulate formulation as they strengthen the contact between dosage form and the site of absorption (Lueben et al., 1994; Park and Robinson, 1984)[10,11]. These mucoadhesive polymeric nanoparticles in the stomach will offer diverse advantages such as (a) Higher drug concentration at the site of adhesion absorption, which will create a driving force for the paracellular passive uptake. (b) Longer residence time of the dosage form on gastric mucosa which will improve absorption of the drug and increase the bioavailability. (c) Immediate absorption from the bioadhesive drug delivery system without previous dilution and probable degradation in the luminal fluids (Hejazi and Amiji, 2003)[12].

MATERIALS AND METHOD

Materials

Diltiazem hydrochloride was obtained as a gift sample from M/s Modern Laboratories, Indore, India. Gelatin and Poloxamer 237 were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Dialysis tubing (cut-off 12 kDa) was purchased from Sigma (USA). All other ingredients used throughout the study were of analytical grade and were used as received.

Preparation of Nanoparticles

Nanoparticles of DTZ were prepared by desolvation method as described by Coester et al. with slight modification. Gelatin was dissolved in distilled water (50 mL) under gentle heating and DTZ (120 mg) was then added to the polymeric solution. Poloxamer 237 was then added as stabilizer and the pH of solution was adjusted (by 1N hydrochloric acid or 1N sodium hydroxide). Then 50 mL of acetone was added at specified addition rate, after 10 min of acetone addition glutaraldehyde was added for cross-linking of nanoparticle. After stirring for specified time the nanoparticles were purified by three fold centrifugation (15000 g for 30 min at 4°C) and redispersion in 10 mL mixture of acetone: water (3:7). The supernatant was removed and the pellets were resuspended in distilled water and finally, the nanoparticles were freeze-dried and stored in vials (Coester et al., 2000)[13].

EVALUATION PARAMETERS

Particle Size and Particle Size Distribution

Particle size (average particle size) and particle size distribution (polydispersity index) were determined using the zeta sizer (Zetasizer- ZEN 2600 Malvern Instrument Ltd., Worchestershire, UK) equipped with the Malvern PCS software. For analysis nanoparticles were diluted five times with 0.45 µm membrane filtered bidistilled water (Elshafeey et al., 2010)[14].

Entrapment Efficiency

For determination of entrapment efficiency, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV spectrophotometer at 237 nm (UV-1700 Spectrophotometer, Shimadzu Scientific Instruments, Inc. Maryland, USA). The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (W) (Das et al., 2005)[15]. Percentage drug entrapment was obtained by using following equation

% Drug Entrapment = (W-w) × 100 / W

Drug Loading

The drug content in the nanoparticles was determined by crushing the drug loaded nanoparticles (10 mg) followed by immersing them in 100 mL simulated gastric fluid (SGF, pH 1.2, without enzymes) with agitating for 12 h at room temperature. The drug concentration was determined spectrophotometrically after filtration through a 0.45 µm membrane filter (Millipore) at the

wavelength of 237 nm. The filtered solution from the empty nanoparticles (without drug) was taken as blank. The drug loading (DL) was calculated according to the equation given below, all samples were analyzed in triplicate (Ma et al., 2008)[16]

DL (%) = WD/WT × 100

Where, DL: drug loading; *W*D: the weight of the drug loaded in the nanoparticles;

*W*T: the total weight of the nanoparticles.

Drug Release Study

The in vitro drug release studies were performed by dialysis membrane diffusion technique using glass tube of 10 cm length open at its both ends having 2.5 cm diameter. The dialysis membrane of 12,000 Mwco (Spectra por, Sigma, USA) was used for release study, because it retains nanoparticles and allows free drug to diffuse in the release media. The lower end of the glass tube was covered with the pretreated membrane to keep the nanoparticulate formulation on the donor side. The nanoparticles (equivalent to 10 mg of drug) were placed in donor compartment by dispersing in 3 mL of SGF (pH 1.2) where the drug was allowed to freely diffuse over the receptor compartment containing 100 mL of SGF (pH 1.2). The entire system was kept at 37±0.5°C with continuous magnetic stirring at 100 rpm. Samples of 5 mL were withdrawn at predetermined time intervals (0.5, 1, 2, 4, 6, 9, 12, 15, 18 and 24 h) and replaced with fresh SGF (Elshafeey et al., 2010; Kamel et al., 2009)[14,17]. The withdrawn samples were suitably diluted to carry out UV Spectrophotometric analysis at 237 nm.

Measurement of Mucoadhesive Strength

Mucoadhesive properties of nanoparticles were evaluated by Texture analyzer (M/s TA. XT. Plus, Stable Microsystem, UK) using porcine gastric mucosa. The method is based on the measurement of shear stress required to break the adhesive bond between a mucosal membrane and the formulation. The formulation is sandwiched between two mucosal membranes fixed on flexible supports in the assemblies for a sufficient period of time. After the adhesive bond has formed, the force (weight) required to separate the bond was recorded as mucoadhesive strength. This parameter was used to compare mucoadhesive property of various formulations.

Stomach of pig was washed with fresh water to remove non-digested food from stomach then placed in SGF at 4 ºC (used within 6 h). The porcine gastric mucosal membrane was then attached both to the stainless steel probe (using dual side adhesive tape) and on the base of texture analyzer. Probe is then fixed to the mobile arm of the texture analyzer. The 20 mg of nanoparticulate formulation was placed on the membrane placed on lower surface moistened with 2 mL of SGF. The mobile arm with attached membrane was lowered at a rate of 0.5 mm s^{-1} until contact with the

formulation was made. A contact force of 20 g was maintained for 300 s, after which the probe was withdrawn from the membrane. After the adhesive bond has formed, the force of detachment (g) required to separate the bond was recorded as mucoadhesive strength (Thirawong et al., 2007)[18].

Screening of Influential Variables

In current studies, Taguchi design was applied for seven factors at two levels each as given in table 1 for screening of various process and formulation variables, influencing development of nanoparticulate formulation. The number of experiments during screening was kept as small as possible, to limit the volume of work carried out during initial stages. This was conducted to identify the potential factors for further systematic DoE optimization studies, as the nature of influence of such variables is not widely known from prior literature reports.

Table 1 List of variables employed in Taguchi L8 orthogonal array experiment.

Factors	Levels	
	Low (-1)	High (+1)
Amount of Gelatin (mg)	200	1000
Amount of Glutaraldehyde (ml)	0.2	1.0
Amount of Poloxamer 237 (mg)	50	500
Acetone addition rate (ml/min)	0.5	5
pH	2	5
Stirring time (h)	1	4
Stirring speed (rpm)	500	2000

A standard orthogonal array L8 was used to examine this system where L and subscript 8 denote the latin square and the number of the experimental runs, respectively. Design-Expert® (Stat-Ease Inc., USA) was used as software for the DoE modeling. Table 2 illustrates the orthogonal array L8 for the seven factors at two levels by Taguchi design was adopted in the studies. Particle size, polydispersity index (PDI), amount of drug released in 6 h (Q6), time required to release 60 % of drug (T60%), entrapment efficiency, loading efficiency and mucoadhesiveness were the key response variables investigated thoroughly for selecting the significant formulation and response factors. A run involved the corresponding combination of levels to which the factors in the experiment were set are shown in table 3. The effects of the proposed experiments on the responses were then analyzed by the Design Expert software to obtain independently the main effects of these factors, followed by the analysis of variance (ANOVA) to determine which factors were statistically significant. Pareto charts were constructed to demonstrate the influence of each parameter on the responses. The most influential factor among the seven factors will be derived by

selecting factors which are showing maximum standardized effect for most of the responses in Pareto charts.

Table 2 L8 array layout as per 7 factors, 2 levels Taguchi screening design.

Formulation Code	Amount of gelatin (mg)	Amount of Glutaral-dehyde (ml)	Amount of Poloxamer 237 (mg)	Acetone addition rate (ml/min)	pH	Stirring time (h)	Stirring speed (rpm)
F1	-1	-1	-1	-1	-1	-1	-1
F2	-1	-1	-1	1	1	1	1
F3	-1	1	1	-1	-1	1	1
F4	-1	1	1	1	1	-1	-1
F5	1	-1	1	-1	1	-1	1
F6	1	-1	1	1	-1	1	-1
F7	1	1	-1	-1	1	1	-1
F8	1	1	-1	1	-1	-1	1

Table 3 Preparation of nanoparticles on the basis of Taguchi screening design.

Formulation	Amount of gelatin (mg)	Amount of Glutaralde-hyde (ml)	Amount of Poloxamer 237 (mg)	Acetone addition rate (ml/min)	pH	Stirring time (h)	Stirring speed (rpm)
F1	200	0.2	50	0.5	2	1	500
F2	200	0.2	50	5	5	4	2000
F3	200	1	500	0.5	2	4	2000
F4	200	1	500	5	5	1	500
F5	1000	0.2	500	0.5	5	1	2000
F6	1000	0.2	500	5	2	4	500
F7	1000	1	50	0.5	5	4	500
F8	1000	1	50	5	2	1	2000

Drug amount was kept constant at 120 mg

RESULTS AND DISCUSSION

The current study was undertaken to screen most influential factors in optimization of a nanoparticulate system suitable for mucoadhesion along with low particle size, low PDI, sustained release, high entrapment and loading efficiency. The results for evaluation of prolonged release nanoparticulate formulation are given in table 4.

Table 4:Evaluation of formulations of Taguchi screening design.

Formulation	Particle Size (nm)	PDI	Bioadhesion (g)	Q6 (%)	T60% (h)	Entrapment Efficiency%	Drug Loading%
F1	318.7	0.196	10.351	27.60	16.3	40.15	15.62
F2	220.8	0.302	8.655	42.25	12.0	37.55	26.57
F3	469.9	0.604	6.112	34.16	14.4	21.25	34.17
F4	1011.0	0.814	7.455	32.55	13.6	35.59	16.65
F5	585.2	0.312	10.121	41.26	11.5	52.21	28.65
F6	495.6	0.238	5.565	38.58	12.9	44.66	42.15
F7	386.5	0.292	6.589	19.05	17.4	34.55	25.57

F8	858.4	0.875	8.151	20.14	18.2	59.45	36.65
Minimum	220.8	0.196	5.565	19.05	11.5	21.25	15.62
Maximum	1011.0	0.875	10.351	42.25	18.2	59.45	42.15

*Mean (n = 3).

Where Q6 is amount of drug released in 6 h; T60% is time required for 60 % cumulative drug release.

Implementation of the L8 array design helps in identifying the most significant factors for further detailed investigation with minimum experimentation thus saving considerable time, efforts and resources. Data obtained after the evaluation of the prepared formulation was subjected to ANOVA by using Design Expert Software which provides the coefficient values for the selected responses. These coefficient values were used as standardized effect and plotted against various responses to construct Pareto charts. The "standardized effect" determines each factor's relative strength, the higher the absolute value the greater the effect of that factor on the response. A positive effect value indicates an effect that favors the response, and a negative value represents an inverse relationship between the response and the factor.

EVALUATION OF FORMULATIONS

Influence of various factors on particle size

It has been shown that particle size and size distribution are the most important characteristics of nanoparticles systems. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system. It was found that increasing the quantity of Poloxamer 237 results in reduction in size of the formulation because it decreases the surface tension between organic and aqueous phase and leads to the formation of smaller solvent droplets, which in turn causes decrease in particle size. It also stabilizes newly generated surfaces and prevents aggregation of the particles which is similar to the previous studies (Schubert and Muller, 2003)[19]. It was observed that increase in amount of cross linking agent results in increase in particle size which may be due to destabilization of the surface and thereby causing aggregation in later case. It was observed that increasing polymer concentration results in reduced particle size which may be due to reduced amount of cross linking agent available for interaction. Lower value of pH favors nanoparticles with small size because of maximum solubility of gelatin at pH values far from its isoelectric point (pH 4.7-5.2 for gelatin type B).

Influence of various factors on particle size distribution

It was found that on increasing the amount of glutaraldehyde and acetone addition rate PDI was increased. While increase in Poloxamer 237 and pH resulted in lowering of PDI. Considering that

the PDI is calculated from the square of the standard deviation/mean diameter, less value of polydispersity index indicates enhanced homogeneity of the nanoparticles.

Influence of various factors on loading and entrapment efficiency

Entrapment efficiency of the formulation increases with increasing the amount of polymer. Maximum loading and entrapment efficiency was obtained when drug is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption. Loading and entrapment efficiencies were more on increasing the acetone addition rate, while increasing Poloxamer 237 resulted in decreased loading and entrapment efficiency. On increasing stirring speed and stirring time reduces loading efficiency which may be because of leaching of drug from the formulation before cross linking step completes proficiently.

Influence of various factors on drug release

The results of drug release study for various formulations are shown in figure 1. Drug release was found to decrease with increase in the polymer ratio. This can be attributed to the release retarding effect of polymer which reduces the release of drug from the nanoparticulate formulation. Release rate was also reduced with increase in amount of cross linking agent which may be due to rigidization of surface of nanoparticles.

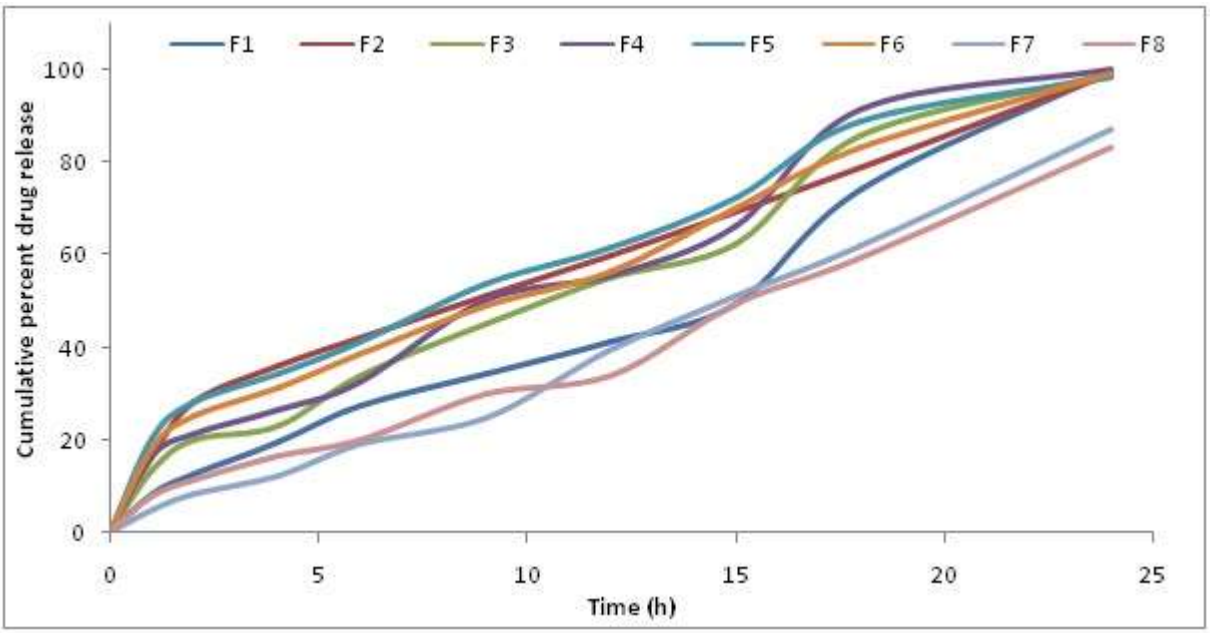


Figure 1 Drug release study of formulations of Taguchi screening design.

Influence of various factors on mucoadhesion

Mucoadhesion studies were performed by using texture analyzer and representative graph showing force of detachment is given in figure 2. Point F represents the force at which the breaking of the adhesive bond starts (i.e., the force of detachment). It was found that increase in polymer ratio

increases the mucoadhesiveness of the formulation due to bioadhesive nature of the polymer. While increase in concentration of glutaraldehyde results in lowering of mucoadhesiveness which may be due to greater cross linking of mucoadhesive polymer by glutaraldehyde.

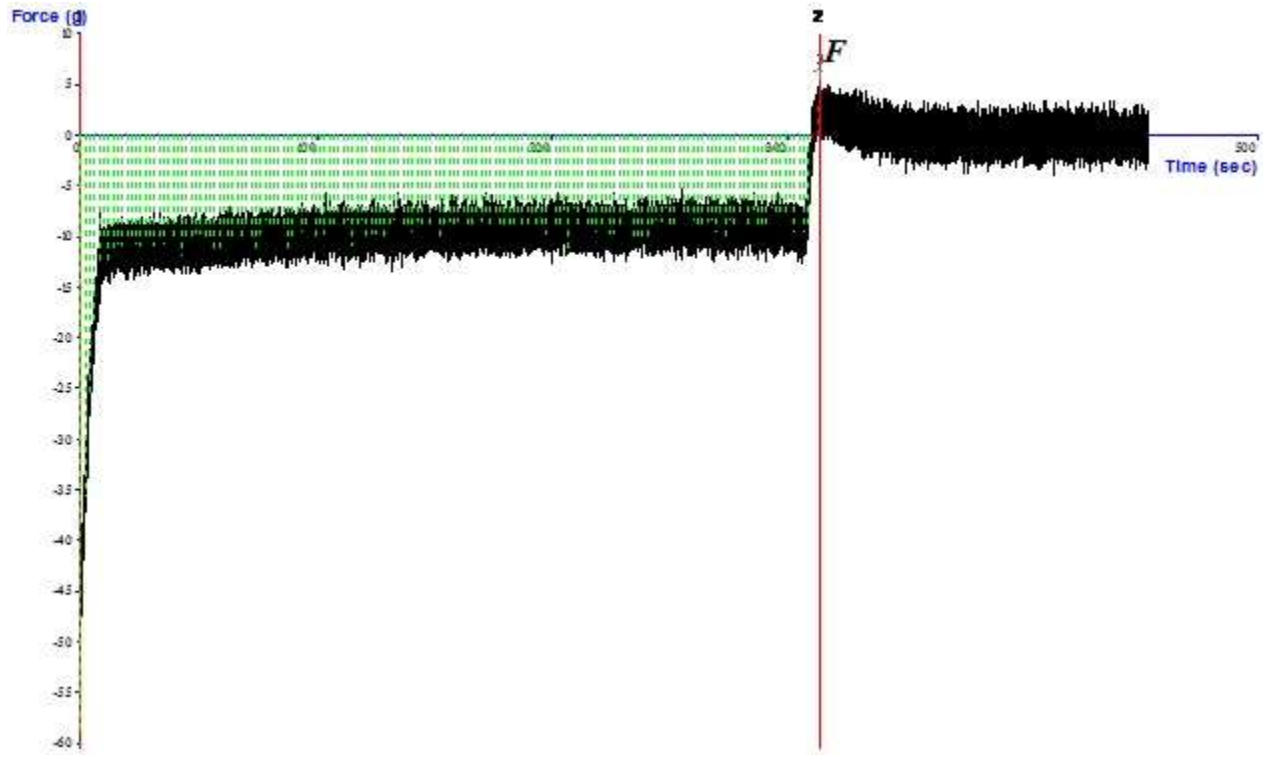


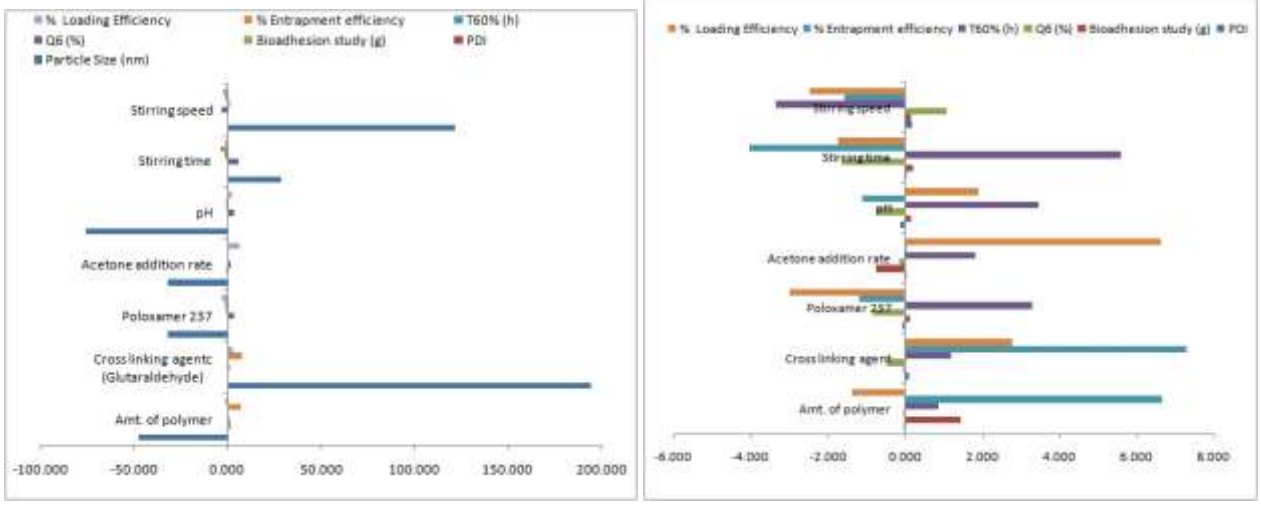
Figure 2 Graph showing force of detachment (point F represents the force of detachment).

Screening of influential formulation and process variables

Taguchi has developed a method based on "orthogonal array" experiments which gives much reduced "variance" for the experiment with "optimum settings" of control parameters. Seven formulation and process variables including amount of gelatin (mg), amount of glutaraldehyde (mL), amount of Poloxamer 237 (mg), acetone addition rate (mL/min), pH, stirring time (h) and stirring speed were studied each in two levels

Pareto ranking analyses showed that from the magnitude of coefficient for the studied response variables *viz.* Particle size, PDI, Q6, T60%, entrapment efficiency, loading efficiency and bioadhesiveness, variable X1 (Amount of gelatin) and X2 (Amount of gluataraldehyde) were the most influential factors. Based on the results of Taguchi screening further experimental design such as central composite design are suggested to generate polynomial equations for various responses, feasibility followed by intensive grid search and overlay plot method for selection of optimized formulation. The criteria for selection of suitable feasible region will be based upon the best possible values of particle size, PDI, Q6, T60%, entrapment efficiency, loading efficiency and

mucoadhesiveness. Validated formulations will be evaluated for their performance and the results will be critically compared with those predicted using RSM.



(a) **(b)**

Figure 3 a) Pareto charts of the standardized effects of various formulation and process factors on selected responses.

b) Pareto charts of the standardized effects of various formulation and process factors on selected responses except particle size.

CONCLUSION

A Taguchi design was presented to screen the effect of formulation and processing variables on the response variables by applying computer optimization technique. Screening studies showed that the most influential independent variables were amount of gelatin and cross linking agent (Glutaraldehyde). Based on the results of optimization studies it was concluded that further experimental design are required to study the main, interaction, quadratic effects of these variables on the responses and obtain the optimized formulation.

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