Review Article

Design of Experiments: Optimization and Applications in Pharmaceutical Nanotechnology

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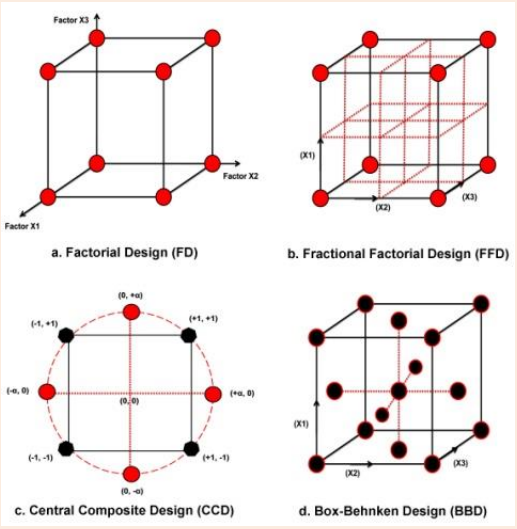
Abstract

The application of Design of Experiments (DoE) in the pharmaceutical industry is becoming a mandatory tool in recent times. It uses a simple experimental design to screen and optimize a number of experimental parameters in formulation development. DoE provides maximum information about the design with fewer initial experiments or trials. In the last couple of decades, nanotechnology based drug delivery systems have gained importance because of their enhanced oral bioavailability, controlled release, targeting, etc., and few of the products were also successfully launched in the market. However, preparation of most of the nanoparticles till today follows a trial and error method because of the involvement of many critical process parameters and difficulty in their optimization. Hence, this article would review the application of DoE in optimization of various types of nanoparticles and also discusses about some of the different types of nanoparticles optimized and prepared using DoE in the past 5 years.

Keywords: Nanoparticles, Experimental design, Optimization, Response surface methodology, Central composite design, Box–Behnken design, Factorial design, Fractional factorial design.



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Terminology used in DoE

Factors	:	Process inputs an investigator manipulates to cause a change in the output. Some factors cannot be controlled by the experimenter but may effect the responses.
Coding Factor Levels	:	Transforming the scale of measurement for a factor so that the high value becomes +1 and the low value becomes -1
Treatment	:	A treatment is a specific combination of factor levels whose effect is to be compared with other treatments
Responses	:	The output(s) of a process. Sometimes called dependent variable(s)
Effect	:	How changing the settings of a factor change the response. The effect of a single factor is

		also called a main effect
Interactions	:	Occurs when the effect of one factor on a response depends on the level of another factor(s)
Randomization	:	A schedule for allocating treatment material and for conducting treatment combinations in a DOE such that the conditions in one run neither depend on the conditions of the previous run nor predict the conditions in the subsequent runs
Analysis of Variance (ANOVA)	:	A mathematical process for separating the variability of a group of observations into assignable causes and setting up various significance tests
Center Points	:	Points at the center value of all factor ranges
Design	:	A set of experimental runs which allows you to fit a particular model and estimate your desired effects
Design Matrix	:	A matrix description of an experiment that is useful for constructing and analyzing experiments
Error	:	Unexplained variation in a collection of observations
Experimental Unit	:	The entity to which a specific treatment combination is applied
Model	:	Mathematical relationship which relates changes in a given response to changes in one or more factors.
Random Effect	:	An effect associated with input variables chosen at random from a population having a large or infinite number of possible values
Random error	:	Error that occurs due to natural variation in the process
Replication	:	Performing the same treatment combination more than once.
Resolution	:	A term which describes the degree to which estimated main effects are aliased (or confounded) with estimated 2-level interactions, 3-level interactions, etc.
Response Surface Designs	:	A DOE that fully explores the process window and models the responses.
Rotatability	:	A design is rotatable if the variance of the predicted response at any point x depends only on the distance of x from the design center point

Introduction

DoE is an efficient procedure for planning experiments from which the data obtained can be analyzed for a valid objective and conclusion. Doe based experimental designs have been proven as an important tool for the pharmaceutical industry, for the purpose of developing any formulation or product with predefined quality. These designs eliminate the dissipation due to trial and error method and save time as well as money. DoE gives the better understanding of the relationship between the independent and dependent variable in formulation development. Preliminary data achieved from the previous experiments play an important role in the DoE, as it imparts the important information about process variability, which can affect the quality of product [1, 2]. The Response surface design (RSD) and factorial designs (FD) are the most commonly employed designs in pharmaceutical industry. The response surface designs are a collection of statistical and mathematical techniques based upon the collection of experimental data from the experimental design. The Box-behnken design (BBD) is the most popular among all

response surface methodology (RSM) because it requires fewer runs in 3 factor experimental design than all other RSM designs [3]. Generally factorial designs allow the estimation of the effect and interactions between the independent variables [4]. A nanoparticle is microscopic particle with at least on dimension less than 100 nm. Nano-carrier system has been proposed as a promising alternative to conventional drug delivery system. In the last couple of decades, nanotechnology based drug delivery systems have gained vital importance and few of the products were also successfully launched in the market (Table 1). They provides large number of advantages include improved bioavailability of poorly water soluble drugs, reduces the numbers of doses and dose frequency, reduces side effects, provides protection to the entrapped drug from enzymatic degradation and improves the therapeutic efficacy [5] etc., However, preparation of most of the nanoparticles till today follows a trial and error method because of lack of optimized procedures. Hence, this article would review the application of DoE in optimization of various types of nanoparticles in pharmaceutical nanotechnology and also discusses about some of the different types of nanoparticles prepared by applying DoE in the past 5 years.

Table 1 Marketed nanoparticulate products.

Type of nanoparticle	Marketed Product
Liposomes	AmBisome® (Amphotericin B), DaunoXome® (Daunorubicin), DOXIL® (Doxorubicin), Myocet® (Doxorubicin), LipoDox® (Doxorubicin), Thermodox® (Doxorubicin), Marqibo® (Vincristine), Visudyne® (Verteporfin), DepoCyt (Cytarabine), DepoDur (Morphine sulfate), Lipoplatin™ (Cisplatin), and Arikace™ (Amikacin).
Nanoemulsions	Liple® (Palmitate alprostdil), Limethason® (Dexamethason), Diprivan® (Propofol), Ropion® (Flurbiprofenaxtil) and Vitalipid® (Vitamins A, D, E and K)
Nanocrystals	Semapimod® (guanylhydrazone), Paxceed® (Paclitaxel), Theralux® (Thymectacin), Nucryst® (Silver), Rapamune® (Sirolimus), Emend® (Aprepitant), Tricor® (Fenofibrate) and Triglide® (Fenofibrate).
Polymeric Nanoparticles	Copaxone® (L-Glutamic acid, L-alanine), Genexol-PM® (Methoxy-PEG-poly(D,L-lactide)taxol), Adagen® (PEG–adenosine deaminase), Macugen® (PEG-anti-VEGF aptamer), Pegasys® (PEG–a-interferon 2a), Neulasta® (PEG–GCSF), Somavert® (PEG–HGF), Oncaspar® (PEG–L-asparaginase) and Renagel® (Poly(allylamine hydrochloride).
Metal Nanoparticles	Resovist® (Iron), Feridex® (Iron) and Acticoat® (Silver)
Nanofibres	Pyrograf® (Carbon nanofiber)

Why Experimental Design?

Statistical experimental design based approach has brought a revolutionary change in pharmaceutical industry. Introducing a formulation which has been statistically optimized will reduce the burden on both the formulator as well as regulatory authorities. Using scientific knowledge instead of an empirical approach is a better idea for any formulator, but there still is a lot of confusion as to why preference is still given to experimental design. Enumerated are some points which will help clear this confusion [6].

- Its aids in the design and development of the pharmaceutical formulation and modifies the manufacturing process to ensure product quality.
- One can save time and financial resources by employing a statistics based approach.
- Optimizing and validating any formulation using these experimental designs gives a better understanding of the factors which can affect the final product performance.
- DoE provides experimental recipes, i.e., number of runs which do not depend on the system.
- DoE provides precise and accurate results on which one can rely easily.

- DoE is a quality based design which provides more efficient technology transfer to the manufacturing process i.e., commercialization.

How to Select an Experimental Design?

Before selecting an experimental design, there are a number of parameters which have to be taken into consideration failing which may lead to an error. Selection of any experimental design depends upon the objective and goal. Therefore, based on the experimental objective, the experimental designs are selected as follows [7].

Comparative objective: If there are one or more factors to be examined and the main aim is to screen one important factor among other existent factors and its influence on the responses, then it infers to a comparative problem which can be solved by employing comparative designs.

Screening objective: The objective of this design is to screen the more important factors among the lesser ones. Under this objective we can select full or fraction factorial designs or Plackett-Burman design (PBD).

Response surface method objective: When there is a need of investigating the interaction between the factors, quadratic effects or when the requirement involves the development of an idea in relation to the shape of response surface, in such situations, a response surface design is used. These designs are used to troubleshoot the process problems and to make a product more robust so as to not be affected by the non controllable influences. The BBD and CCD are the most popular designs under this category. Apart from all these criteria, the selection of experimental designs also depend on the number of factors to be entered, as each design has a limitation of entering the factors more or less of which will not be accepted. For instance, in BBD the minimum number of numeric factors to be entered is 3 and maximum number of numeric factors to be entered is 21.

Optimization Strategies of Experimental Designs [8]
There are various designs and plots (Figure 1) are available in DoE to obtain an optimized formulation. The most widely used designs in pharmaceutical applications are RSM and FD, both of which serve different purposes. The best criteria to select a design is that which can give an optimized formulation in fewer runs that in turn saves time as well as money.

Factorial designs (FD)
These designs help in screening the critical process parameters which can affect the process and product with the help of interactions between the factors.

Two level factorial design (2-21 factors): Full and fractional design will explore many factors by setting each on two levels i.e. higher and lower. This design is helpful in identifying the most significant factors among many others that are involved in design.

Min Run, Res V factorial designs (6-50 factors): These class of designs containing the minimum number of trials to estimate all main effects and all two-factor interactions (Resolution V) while maintaining treatment balance within all factors.

Min Run, Res IV factorial designs (5-50 factors): These class of designs which has a minimum run (or with 2 extra runs), and resolution IV. This design allows all main effects to be estimated, clear of two-factor interactions. The two-factor interactions will be aliased with each other.

Irregular fraction designs (4-11 factors): It allows the estimation of main effects and two factor interactions by involving lesser number of runs and more power of resolution than the normal fractional factorial design.

General factorial designs (1-12 factors): These designs are used to design an experiment where each factors can have different number of level (2-999). The layout of the design generated by this design will include all possible combination of the factors level.

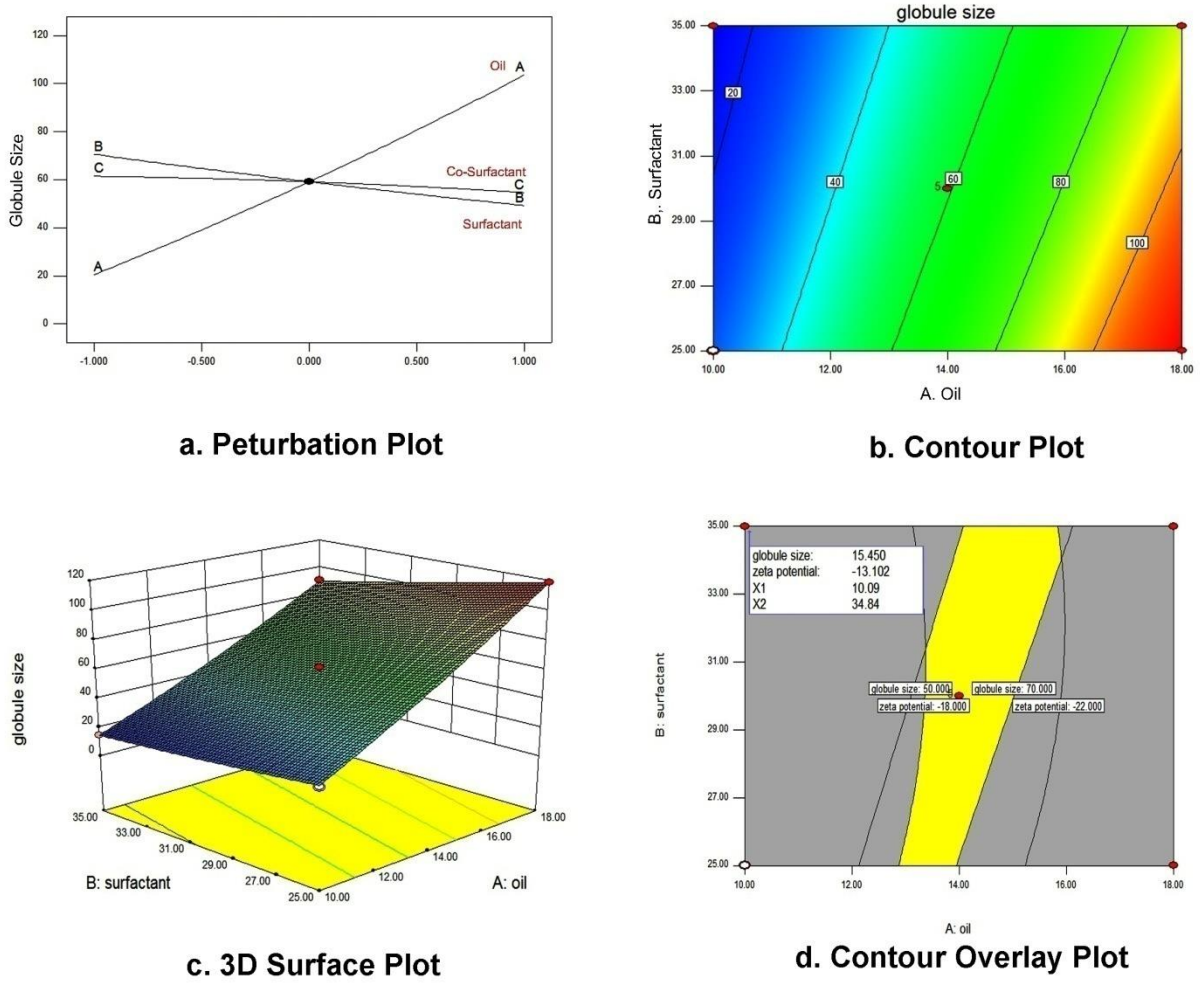

Figure 1 Typical plots obtained in nanoparticles optimization using DoE

Optimal design (2-30 factors): This design is similar to general factorial design which may produce a design with more number of runs. The number of runs generated depends on the model you want to estimate. These designs should be used carefully, taking into account subject matter knowledge to decide if the design is acceptable.

Plackett-Burman designs (up to 31 factors): These are highly confounding designs. The main useful application of this design is for validation where one can hope to find no or very little effect on the responses due to any factors.

Taguchi orthogonal array designs (up to 63 factors)
Response surface design (RSD)
RSM quantifies the relationship between several explanatory variable and one or more responses. It helps in finding the ideal process settings to achieve optimal performance.

Central composite design (CCD): The most popular design used in response surface methodology. Regular central composite designs have 5 levels for each factor, although this can be modified by choosing alpha value 1.0, a face-

centered CCD. The face-centered design has only three levels for each factor. This design is insensitive to missing data and has been created to estimate quadratic model.
Box behnken design (BBD): This is also a popular design among response surface designs; this design has 3 levels for each factor and generates a lesser number of trials in comparison to central composite design. This design is sensitive to the missing data and provides strong coefficient estimates near the center of the design space (where the presumed optimum is), but weaker at the corners of the cube (where there are no design points).

One factor at a time (OFAT): This design is used where only one continuous factor is meant to be estimated. Categoric factor can be added to this design for each categoric combination design is duplicated.

User defined: This design is user friendly and allows selecting all classes of candidate points as per requirement; vertices, centre of edges etc. One can select the number of factors and levels and can add constraints to limit the factor space to reasonable combination. One can even select the model desired to fit by using this design.

Mixture design
This design is applied when the factors are proportion of blend.
Simplex lattice: This design is used when all factors ranges are the same. It creates the design by imposing a grid over the design space with uniformly spaced points. This design should be augmented to allow for detection of the lack of it.

Simplex centriod: It is almost similar to simplex-lattice design but differs in creating a design from inside the centroid out.

Screening: These designs are created to estimate gradient effect for individual component and also useful for determining which ingredient to include in follow up experiments.

Combined designs
Combined designs are optimal and user defined. While working with categoric factor in addition to continuous factors or when there are constraints on experiment optimal design, this is used to minimize the number of trials.
Studies on Different Types of Nanoparticles Optimized Using DoE.

There are 30 reported studies from last 5 years have been accounted in this paper (Table 2), out of which one critical study from each category of nanoparticles have been taken and described in this section.

Table 2 Nanoparticles optimized using DoE.

Type of Nanoparticles	Design	Factors Optimized	Application	Year
Polymeric Nanoparticles				
Zidovudine PLGA nanoparticles	2^3FD	Polymer and surfactant concentration. pH of aqueous phase	Brain targeting	2014 [16]
Sildenafil citrate PLGA nanoparticles	BBD	Mass ratio of drug to polymer, volumetric proportion of the water to oil phase and the concentration of polyvinyl alcohol.	Treatment of erectile dysfunction	2013 [17]
Amoxicillin Chitosan–Alginate polyelectrolyte	BBD	Chitosan, drug and surfactant concentration	Mucopenetration and localization in gastric mucosa helps in	2011 [18]

complex nanoparticles			eradication of *H. pylori.*	
Rifampicin chitosan nanoparticles	2^3FD	Chitosan and tripolyphosphate concentration. Homogenization speed	Tuberculosis.	2013 [9]
Insulin chitosan nanoparticles	2^3FD	Chitosan and arabic gum concentration. Insulin dose	Protects insulin against enzymatic degradation and enhancing its transport across the intestinal mucosa into the systemic circulation	2009 [19]
Nimesulide- PLGA-nanoparticles	3^2FD	Surfactant percentage and the drug/polymer ratio	Rheumatoid Arthritis.	2013 [5]
Cyclosporine PLGA Nanoparticles	CCD	Concentration of Polymer PLGA and Eudragit RL100	Treatment of dry eye disease	2014 (20)
Lipid based nanoparticles				
Methazolamide solid lipid nanoparticle	BBD	Lipids and surfactant concentration.	Ophthalmic delivery	2014 [3]
Chloramphenicol solid lipid nanoparticle	BBD	Lipid, surfactant, and drug/lipid ratio	Ophthalmic delivery	2011 [21]
Simvastatin solid lipid nanoparticles	2^3FD	Lipid and surfactant concentration. Volume of solvent.	Hypolipidemic	2010 [4]
Haloperidol solid lipid nanoparticles	BBD	Drug to lipid ratio, surfactant concentration and stirring speed	Antipsychotic	2013 [22]
Terbinafine solid lipid nanoparticles	3^3FD	Drug: lipid ratio, surfactant concentration and volume of organic solvent	Anti-fungal	2013 [23]
Nanoemulsions				
Palm-Based Levodopa nanoemulsion	CCD	Palm oil:MCT oil, Lecithin and Cremophor EL concentration. Addition rate (ml/min)	Parkinson's disease (PD)	2012 [24]
Palm kernel oil esters-based Diclofenac sodium nanoemulsion	CCRD	Water content, oil and surfactant ratio. Mixing rate and mixing time	Alleviation of pain, fever, and inflammation.	2014 [25]
β-Casein nanoemulsions	D-optimal design	Water content (60%–80%, w/w) and oil and surfactant (O/S) ratio (0.17–1.33), as well as high-shear emulsification conditions, mixing rate (300–3,000 rpm) and mixing time (5–30 mins)	Food formulation	2011 [26]
Finasteride nanoemulsion	BBD	Sonication time and concentration of span-80 and	Competitive and specific steroidal inhibitor of	2013 [27]

		tween-80.	type II 5 α-reductase	
Curcumin nanoemulsion	BBD	Oil, surfactant and co-surfactant concentration	Alzheimer's disease	2013 [28]
Liposomes				
Peptide loaded liposomes	Plackett–Burman design	Peptide concentration, Lipid concentration, Number of freeze-thawing cycles, Mixing time.	Reduce the angiogenic development	2010 [29]
5-Fluorouracil liposomes	3^2 FD	Lipid: Drug ratio, egg choline : Cholesterol ratio	Colorectal Cancers	2013 [30]
Itraconazole PEGylated liposomes	Screening fractional factorial design, Full FD and CCD	Temperature (rotary) Rehydration time (min) Sonication type, lipid and drug concentration.	Antifungal	2013 [31]
Salvianolic acid B–Tanshinone II A–Glycyrrhetinic acid compound liposomes	BBD	Ratio of glycyrrhetinic acid to lipid, ratio of salvianolic acid B to lipid and pH of buffer	Against hepatic fibrosis	2014 [1]
liposomes	RSM	Total flow rate and the flow rate ratio	Biomedical Applications	2014 [2]
Inorganic/Metal Nanoparticles				
Gold nanoparticles	BBD	Stirring rate, sodium citrate concentration and ionic strength	Biomedical Applications	2013 [32]
Silver nano structures	2^5FD	Reaction time, injection speed, injection time and temperature	Antimicrobial activity	2012 [33]
Silver nano particles	OFAT	$AgNO_3$ concentration, incubation temperature and agitation speed	Antimicrobial activity	2013 [34]
Silver nano particles	CCD	$AgNO_3$ concentration, incubation period, pH level and inoculum size	Antimicrobial activity	2014 [35]
Nanofibers				
PLGA nanofiber scaffold	RSM	PLGA concentration , potential, feeding rate and the spinneret to collector distance	Artificial salivary gland tissue construct,	2010 [14]
Polyvinylidene fluoride electrospun Nanofiber	full FD	Voltage , collector distance and polymer flow rate	Biomedical engineering	2013 [36]
Nanocrystals				
NVS-102 nanosuspension	FD	Rpm, bead size and drug concentration.	Enhancing Solubility	2012 [37]
Naproxen nanosuspension	CCD	Bead volume, milling time, polymer and surfactant concentrations	Anti inflammatory	2014 [15]

Paper 1: "*Rifampicin Loaded Chitosan Nanoparticles*" by Patel B.K *et al*. [9]

In this paper ionic gelation method was used for the preparation of polymeric nanoparticles. Polymeric nanoparticles ranges from 1-100nm and are composed of polymers (chitosan, caprolactum, poly (lactic-co-glycolic) (PLGA) etc.) with or without copolymers which are dispersed in their respective matrices. Different concentrations of chitosan solution were prepared by dissolving chitosan in 1% acetic acid under continuous stirring at room temperature. After obtaining a homogenous mixture, the surfactant was added to this solution and the drug was dissolved in dichloromethane. For preparing an o/w emulsion, this oil phase was slowly added to the aqueous phase with stirring at different speeds by the use of a high speed homogenizer for 5 min. Cross-linking of the nanoparticles was achieved by adding tripolyphosphate solution in different concentrations to the emulsion under constant stirring at 500 rpm. The resultant emulsion was kept overnight at 40°C to ensure the complete removal of the organic solvent. Nanoparticles were centrifuged and isolated. This method was optimized using 2^3 FD which resulted in 8 sets of experiments. Three independent variables (chitosan concentration, tripolyphosphate concentration and homogenization speed) were checked for their effect on particle size, encapsulation efficiency and drug loading with the help of ANOVA, interaction studies etc. Particle size, encapsulation efficiency and drug loading for the optimized batch was found to be 221.9 nm, 44.17 ± 1.98% w/w and 42.96 ± 2.91% w/w respectively. It was observed ANOVA results in a polynomial equation which concludes that concentration of chitosan, concentration of TPP has positive effect on all responses where as homogenization speed has negative effect on all responses; by increasing concentration of chitosan, concentration of TPP, particle size and encapsulation efficiency increases and with increase in homogenization speed there is decrease in particle size and encapsulation efficiency. These effects were concluded with the help of perturbation plot as well as contour plots. For validation of the formulation the author has conducted 3 batches and found that there is no significant difference between actual and predicted values.

Paper 2: "*Clobetasol propionate solid lipid nanoparticles cream*" by Kalariya M *et al*. [10]

This paper has deliberated about the preparation of solid lipid nanoparticles by high pressure homogenization technique. Solid lipid nanoparticles are defined as the colloidal dispersion, whose matrix is comprises of biodegradable lipids. This lipid based nanoparticulate system includes several advantages like stability, tolerability, biocompatibility etc. In this method the lipid was melted and the drug was dissolved in it by sonication using a probe sonicator. Subsequent to this, the surfactant was added to the lipid phase under constant stirring. Aqueous phase was prepared by dissolving the surfactant in water and heated at the same temperature as that of lipid. Under continuous stirring, lipid phase was mixed with the aqueous phase and this mixture was homogenized for three cycles in a high pressure homogenizer by maintaining at a constant temperature. This paper studies four independent variables (type of lipid, drug: lipid molar ratio, concentration of surfactant, and homogenization pressure) to achieve maximum polydispersity index and particle size. For studying these factors, a 9 run taguchi orthogonal array design for four factors with three factor level $L_9(3^4)$ was performed. The optimized batch had a particle size of 177 nm and entrapment efficiency of 92.05%. Homogenization pressure was found to have significant impact on the particle size. The optimized batch was validated by preparing six batches using the same composition at six different days and mean was calculated. No significant differences (P <0.05) in particle size and PDI were observed within and among the batches.

Paper 3: *"Fullerene nanoemulsion"* by Ngan CL *et al*. [11]

This paper discuss about the high shear homogenization method for the preparation of nano emulsion. Nano emulsions are defined as oil in water dispersions in which one phase is being dispersed throughout the other in small droplet sizes. The average droplet diameter ranges from 1-500 nm. In this method the aqueous phase was slowly added to the oil phase while being homogenized using a polytron high shear homogenizer at room temperature. The resultant premix emulsion was ultrasonicated. The formulation was optimized using the BBB and central composite rotatory designs (CCRD). Three independent variables (homogenization rate, sonication amplitude and sonication time) were studied. BBD gave 17 runs and CCRD has given 20 runs. With the help of these designs, the effect of independent variables on particle size, zeta potential and viscosity was studied and the optimized formulation was obtained. This experimental design results the optimized batch with particle size, zeta potential and viscosity of 148.5 nm, -55.2mV, and 39.9 Pa seconds respectively. RSM designs (BBD, CCRD) used in this paper suggest linear /

quadratic model. Lack of fit for these models was insignificant which affirmed the fitness of model. Sonication amplitude and high shear homogenization rate were affecting the particle size, where as all independent variable have negative effect on viscosity. Comparing the two designs the author has suggested that CCRD is better design than BBD because it predict more accurate data and produces lower residual standard error for all independent variables. The model was validated and the actual and predicted values were found to be in close agreement with each other.

Paper 4: "*Propolis Flavonoids Liposomes"* by Yuan J *et al.* [12]

In this paper liposomes were prepared by using ethanol injection method. Liposomes are defined as "microscopic spherical-shaped vesicles consisting of an internal aqueous compartment entrapped by one or multiple concentric lipidic bilayers". In this method liposomes were prepared by dissolving lecithin, cholesterol, and propolis flavonoids in 10 mL of ethanol. This ethanol solution was then injected into the buffer as drop-by-drop and continued to thermostat mixing. After further evaporation of ethanol, liposomes were formed. To form small single chamber liposomes, the resulting mixture was homogenized with ultrasonication for 30 min. In order to optimize this method as well as the formulation, BBD was used with three factor three levels which resulted in 17 runs. Three independent factors (ratio of lipid to drug, ratio of soybean phospholipid to cholesterol and speed of injection) were examined for their influence on response (entrapment efficiency). The optimized formulations demonstrated an encapsulation efficiency of 91.67 ± 0.21%. The model was selected based upon the sequential model sum of square, lack of it and model summary. The R squared value (0.9898) of ANOVA for this model suggests that the particular model is significant. The predictive model was verified by selecting the optimum condition (which has been set to obtained predictive values) and the batch was analyzed, the actual value was found to be in close agreement with the predicted value.

Paper 5: "*Silica Sand Nanoparticles"* by Rizlan Z and Mamat O. [13]

The method used for the preparation of metal nanoparticles in this paper was ball milling. Metal nanoparticles have at least one dimension in nano-scale and are composed of metal. Gold and silver are the most often used metals for the preparation of metal nanoparticles. In this method sand silica was collected, washed to ensure the removal of impurity and kept in the oven at 120°C for 1 h to dry. Sand was then meshed and inserted into grinding jars together with grinding balls and milled for 2 h. After milling, the sand was again meshed to remove impurities and large agglomerates, and was dried in the oven at 120°C for 1 h. After every 2 h of milling, the sand was meshed and dried until the total milling time reached 10 h. This method was optimized using Taguchi orthogonal array design by involving 3 factors (ball-to-powder weight ratio, volume of milling jar and rotation speed) which resulted in 9 sets of experiments. In order to determine the effect of each independent variable on response (particle size), signal to noise ratio for each set of experiments was calculated. This design has suggested that in order to gain optimum particle size, the ball-to-powder weight ratio, volume of milling jar and rotation speed should be 20:1, 1.0 L, and 95 rpm respectively.

Paper 6: "*Polymeric Nanofiber Scaffolds"* by Jean-Gilles R et al. [14]

The widely used industrial method for the preparation of nanofibers is electrospinning. Nano fibers comprise of fibers with a diameter of 50-500 nm. They have wide applicability in the biomedical field as well as the textile industry. In this method in order to electrospin a nonwoven mat of nano and micro scale, PLGA was dissolved in hexafluoro-2-propanol or dimethylformamide and loaded into a 3 mL syringe. The syringe was loaded into an automatic syringe pump. For the purpose of shuttling the polymeric solution from the syringe to a metal needle tip, polytetrafluoroethylene tubing was used. Over a grounded aluminum collector plate, the needle was suspended vertically and voltage was supplied to the metal needle with an alligator clip. The author conducted a RSD in order to evaluate the effect of four factors on the response which are PLGA concentration, potential, feeding rate and spinneret-to-collector distance. The optimized batch of nano fibers had a mean average diameter of 247.2nm. Potential and spinneret to collector distance were found to have negative impact on diameter of nano fiber in comparison to feeding rate. The R squared value calculated by software was more than 95 %. The model was validated by comparing the actual values with the predicted values and setting the values of independent variable

given by the software (on basis of predicted value). No significant difference was observed between the actual and predicted values.

Paper 7: "*Febuxostat nanosuspension"* by Ahuja BK et al. [15]

In this particular paper the method used for preparation of nanocrystals was wet media milling method. Nano crystals are composed of atoms either in a single or poly-crystalline arrangement having particles size less than 100 nm. In this method the drug was dispersed in an aqueous solution containing primary and secondary stabilizers. The resulting suspension was poured into a glass vial which contained a zirconium bead and stirred on a magnetic stirrer for 1 h at room temperature. The authors have conducted CCD in order to optimize the formulation. For this purpose, they have selected four independent variables (bead volume, milling time, polymer concentration and surfactant concentration). The design yielded 30 runs under CCD. The particle size, PDI and zeta potential of the optimized batch was found to be 251.45±2.82 nm, 0.102±0.01 and 20.3±0.41 mV. Based on the sequential model sum of square, lack of it and model summary statistic, the design suggested two models i.e., quadratic and 2FI (two factor interaction) which can efficiently navigate to design space. Non-significant lack of fit, low PRESS value indicates best fit of model. The equation obtained from the statistical calculation has explained the positive and negative effect of independent variable on dependent variable. The model was validated and the actual values and predicted values were found to be in close agreement with each other.

Conclusion

The process of preparing nanoparticles is may be easy and not be costly but the time and skills involved in optimization and producing rock stable nanoparticles are tedious and costly. Statistical software and innovative tools are receiving greater recognition the world over and a direct consequence of this is related to DoE; however, inappropriate design selection and experimental domain can only prove detrimental to the whole concept of DoE. Hence, we can anticipate a greater product transfer to the market through the successful application of DoE by the identification of critical process parameters and nanoparticulate optimization. In the field of nano medicine many approaches for their development have been approved as a fruitful results, in future incorporation of DoE technology as a valuable tool will be occur very soon with best and positive results.

Conflict of interest and funding

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