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Review Article

Exploring application & recent advances of response surface methodology driven approach in drug design and nanotechnology

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Abstract

The response surface methodology (RSM) continues to gain better importance in the current scenario in pharmaceutical analytical/formulation R & D, and healthcare industries including nanotechnology for its statistical implementation using diverse designs. The present review signifies the different types of RSM driven techniques, counting its combinations with analytical procedures and related guidelines on the methods for obtaining the ICH Q2(R1), ICH Q8, Q9, Q10 criteria's by implementing a quality-by-design systematic holistic approach for product development. Apart from these, the comprehensive glance of the broadest applications of RSM approach in the nanotechnology and nano biotechnology are the major focus in this review. For utmost patient safety the GMP, quality assurance approved regulations provided ICH, WHO, and USFDA becomes more concern about current challenges, future prospectives of RSM driven novel evolving techniques in nanotechnology and nano biotechnology. Current review forecasts the broadest applications of RSM enabled liquid chromatographic hyphenated methodologies, screening risk assessment methodologies, regulatory significances, diversified formulations, analytical and casing bioanalytical method development, validations, stability studies to ascertain sustainable and economic product for better efficacy and patient compliance.

Keywords: Response surface methodology, Pharmaceutical, Nanotechnology, Screening.

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1. Introduction

For methods related to developing new analytical procedures, the definite subject of approaches for developing procedures for evaluating pharmaceutical products is necessary. This becomes essential when the other approaches fail and also assists in containing the costs likely to be incurred to enhance preciseness and robustness. Consequently, this leads to fine-tuning and validation of the developed methods and the first trials to confirm the correctness and repeatability of the methods.[1] Hence, method validation under the perspective of the mentioned viewpoint confirms whether the applying technique's specifics correspond to the demands prescribed in each science area. The recognition of conclusions derived from analytical procedures should always be gained and maintained. Validation is likely to ensure a certain level of confidence that the given processing system shall perform to the level of acceptance.[1,2] In the pharmaceutical industry, the statistically proved response surface methodology (RSM) tool is used since the precision of the established and verified analytical techniques is essential. Because of the availability of effective analysis and the optimality of the pertinent components and their interactions, the current approaches are guaranteed to be robust and reliable.[3] RSM conserves time and reduces the quantity of tests necessary in comparison to competing methodologies. Compared with RSM, it can

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establish several constituents and examine the interactions between them, while the other techniques need to assess the individual components step by step. This strategy eliminates two significant difficulties encountered during an analysis: time constraints and the inability to analyse the interrelations of the different components. Because of its high effectiveness, RSM has applications in the pharmaceutical industry that offer benefits like lower costs and quicker development times.[4]

The Response Surface Methodology, also known as RSM, is a systematic approach for optimising processes or products. **Figure 1** depicts various steps involved in response surface methodology and its significance in drug development.[5,6] The process consists of many stages: identification of the independent variables and the range of values that will be used for each variable, selection of experimental design, testing the samples or specimens and obtaining the data, modelling the fitting of data, assessment of the model's adequacy, and conclusion (**Figure 1**).[5-16]

RSM uses a variety of trial strategies, including the Central composite design commonly known as CCD and the Box-Behnken Design, which are very important and efficient and enable accurate results with a little number of experiments.[17] Additionally, RSM uses statistical measures like Analysis of Variance abbreviated as ANOVA to observe the significance of independent variables and how they interact with the response variables.

Moreover, RSM makes it possible to include extensions and techniques that optimise multiple responses at once. It is being adopted in several fields, such as the pharmaceutical business, since it provides optimised solutions for compounding and producing robust products **(Table 1)**. Complex regression analysis and testing can also be performed with the help of software such as Design Expert and Minitab to initiate the best experiment design.[18]

As RSM is a part of the Quality by Design (QbD) studies, **Table 1** demonstrates how RSM benefits pharmaceutical quality through design studies.[19,20]

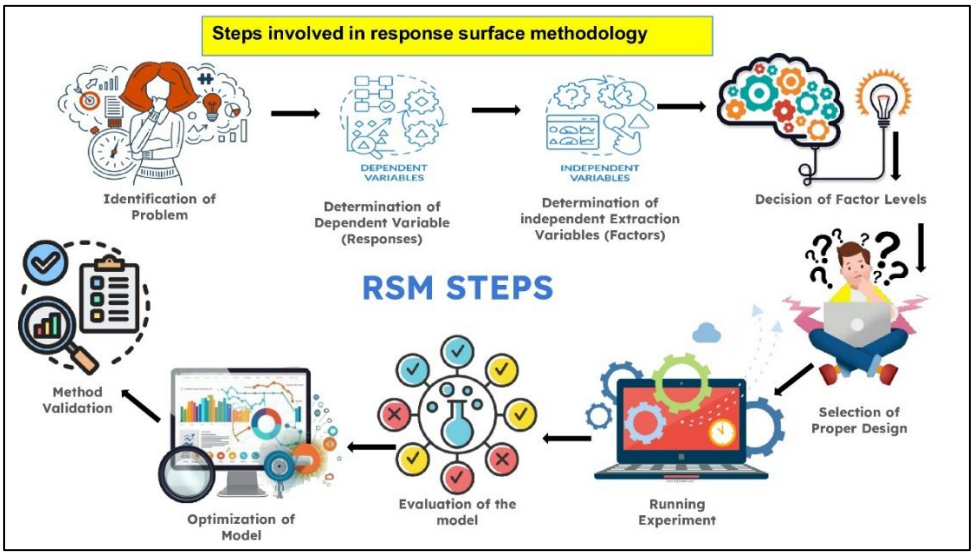

Figure 1: Various steps involved in RSM

Table 1: Quality by Design (QbD) parameters and its applications according RSM principles

Elements of QbD Paradigm	Applications of QbD parameters	Significance	References
Quality Target Product Profile	Characterise critical quality attributes (CQAs) and desired product performance	Guides of RSM experiments to optimise CQAs.	19,20
Product Design	Promote a robust formulation and understand its impact on product quality	Efficient identification of critical formulation factors.	19,20
Process Design	Optimise manufacturing processes using RSM	Enhanced process robustness with faster process development and validation.	19,20
Control Strategy and Continual Improvement	Develop effective control solutions.	Consistent product quality and Effective control strategies.	19,20

2. Numerous Designs used under RSM

Response surface methodology generally uses designs based on factorial designs: One approach is full factorial design, while the other method is fractional factorial design. Three types of response designs are mainly based on this factorial design: Central Composite design, Box-Bhenken design, and Doehlert design.[21]

2.1. Central composite design

The central composite design abbreviated as CCD, one of the key components of the response surface approach, is used to optimise numerous variables at once. To accurately ascertain if linear and curvature effects are present, this method makes use of axial and centre points as well as a 2k factorial design.[22]

The axial (star) and centre points comprise the factorial design. It is able to estimate both first- and second-order terms more quickly. The popularity of this approach is due to its ability to achieve acceptable accuracy for second-order models with a minimal number of trial runs. It allows for obtaining the required design characteristics, such as rotation capacity, with the help of varying axial points. Quadratic terms are then assessed by measuring some crucial aspects that are at a certain basic distance from the centre. Centre points assist in measuring the effectiveness of a model in performing the intended work and determining intrinsic errors.[22-26]

2.2. Box- behnken design

The Box-Behnken design abbreviated as BBD, is a commonly utilised experimental design in response surface technique. It is highly useful for determining links between inputs and responses. This design belongs to a category of second-order designs that may be rotated or almost rotated. It is derived from three-level fractional factorial designs.[23-26]

One benefit of BBD is the exclusion of combinations when all components are simultaneously at their maximum or lowest values, eliminating the need for measurements in extreme scenarios. According to some reviews, several investigations focus on membrane fouling, the primary disadvantage of membrane technology. This issue leads to a decline in system performance, mostly shown as reduced permeate flow or altered membrane selectivity. In addition, it is a precious tool for optimising chromatographic analysis.[21,27]

2.3. Doehlert design

Doehlert design shows a considerable number of advantages in terms of efficiency compared to the other designs due to the work of the quadratic response surface. It studies quadratic interactions in a spherical experimental region, giving accurate estimates of quadratic terms with fewer experiments.[19] This design is based on a spherical domain, although it lacks the typical characteristics of other designs. Even though their matrices lack orthogonality and rotational properties, the anticipated values do not exhibit uniform variance. Another significant attribute of this design is its capacity for traversing the experimental zone.[28]

Current studies reveal that the Doehlert design has been applied in many fields, such as food processing, synthesis of silver nanoparticles, and biodiesel production optimisation. This is evident in the case of using this technology to produce biohythane from organic waste, in which the objectives of increasing complex biochemical functions are well demonstrated.[28,29]

3. Types of Analytical Techniques used for Nanofomulations Development

Analytical techniques include various techniques used in the preparation, detection, identification, determination, and characterisation of individual components of a sample. Mostly DSC, FTIR are commonly used for Characterisation studies for Nano formulations preparations. HPLC, UPLC, UPLC are the powerful analytical tools used now-a-days for Evaluation of Nanofomulations and its quantifications for Quality Control and to find out the percentage of purity. In addition to this, diverse bioanalytical techniques (eg. Hyphenated techniques LC-MS, LC-NMR etc.) are used for estimation and evaluation during preparation and evaluation of Nano formulations such as polymeric nano-particles, preparation of mucoadhesive multiarticulate drug delivery systems (MMDDS), Self-nanoemulsifying drug delivery systems (SNEDDS), Liqui-Solid Techniques etc. for bioavailability enhancement of poorly soluble drugs.[38] These analytical techniques are mainly classified as classical and instrumental methods of analysis and Quality by design (QbD) and RSM approach can be broadly incorporated for generation of reliable, rapid, accurate, precise, economic product development for patient compliance as per USFDA, ICH recommended guidelines.[28,29] Schematic diagram elucidating various Systematic Process used in Analytical Method Development has been illustrated in **Figure 2**.[28,29]

The classical method is divided into several categories: separation of analytes, which encompasses the extraction, distilling, precipitating, and filtering; qualitative analysis, which covers the boiling and freezing point, colour, odour, density; and quantitative analysis, including volumetric and gravimetric analysis.[28,29]

The instrumental method is further classified as spectroscopic, electrochemical, chromatographic, and other methods like x-ray, radioactivity, and thermal methods. The spectroscopic and chromatographic methods are mostly used for development and validation purposes. Apart from that, electrophoresis is also used in some cases when trying to separate complex samples.[28,29]

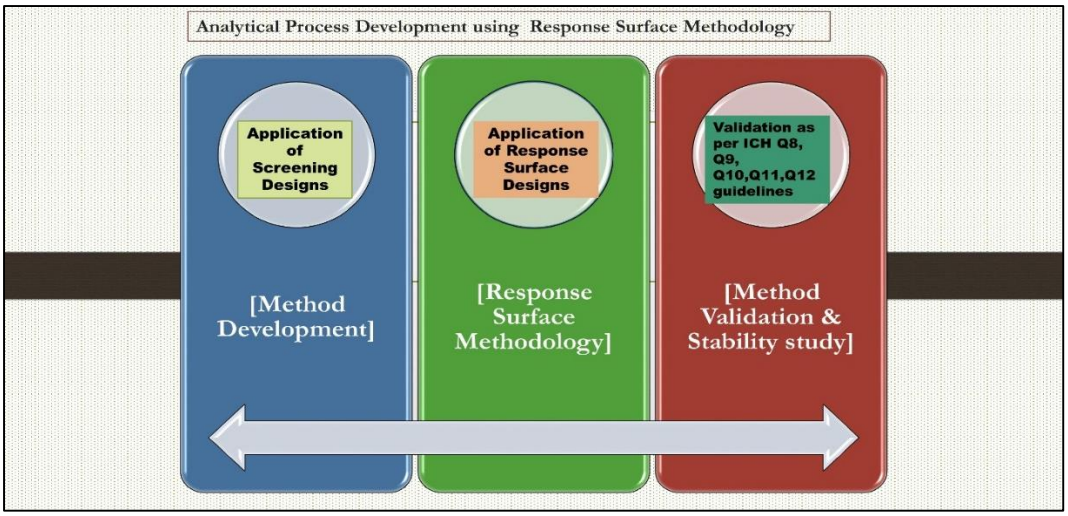

Figure 2: Analytical process development using RSM

3.1. Spectroscopic techniques

3.1.1. UV- visible spectroscopy

In the visible and ultraviolet regions of the electromagnetic spectrum, UV-visible spectrophotometry is still one of the most popular analytical methods for figuring out how much light is absorbed by molecules. Due to the provided data, it is possible to analyse the motion of electrons inside molecules and perform quantitative and qualitative assessments of various compounds. This technology has become useful in numerous areas of chemistry, biochemical, and pharmaceutical sciences since it increases concentration accuracy and helps study structures.[30]

3.1.2. Infrared spectroscopy

Infrared spectroscopy is an absolute technique determining the amount of infrared radiation molecules absorbed. It provides intricate information about the molecular structure and the functional groups. The application of this spectroscopy mainly covers organic chemistry, material chemistry and biology, and biochemistry areas, which are used to identify chemical components, quality analysis, and structural chemistry. It is an invasiveness method and does not require any sample preparatory steps.[31]

3.1.3. Nuclear magnetic resonance spectroscopy

One method of chemical analysis that investigates the magnetic properties of particular nuclei is magnetic resonance spectroscopy. Numerous molecule composition, movement, and chemical environment data are included in this. Nuclear magnetic resonance spectroscopy is applied in chemistry and biochemistry, and disease diagnosis is made via magnetic resonance imaging (MRI). This economical technique does not damage the sample under analysis, which can be in a liquid or solid phase.[32]

3.1.4. Mass spectroscopy

Mass spectroscopy is a spectrometric method used for the identification and analysis of molecules. The process involves disintegrating compounds into charged particles and determining their mass-to-charge ratio. This procedure generates a spectrum that displays the various particles present, aiding scientists in discerning the content and structure of materials. It is extensively used in the fields of chemistry, biology, and forensics for a multitude of purposes.[33,34]

3.1.5. Atomic absorption spectroscopy

A spectroanalytical method for quantitatively analysing chemical elements is atomic absorption spectroscopy (AAS), which observes how unbound atoms in the gaseous phase absorb optical energy, or light.[35] The approach employs a specimen's atomic absorbance spectra to calculate the amount present of individual analyses. To assess the link among observed absorbance and analyte quantity, standards with established analyte contents are required, which relies on the principle of Beer-Lambert.[36] Atomic absorption spectroscopy (AAS) comes in two varieties: Flame AAS and Graphite furnace AAS. The Flame Atomic Absorption Spectroscopy (FAAS) is often used to observe the percentage of metals in solutions at parts per million abbreviated as ppm, as well as parts per billion (ppb) ranges. Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) is a very sensitive technique primarily employed for detecting trace levels of metals (more than 1 ppb) in small volume samples.[35]

3.2. Advanced chromatographic techniques

These approaches are employed to distinguish the variety of a mixture through the differences in their ability to partition themselves between a stationary and a mobile phase. There are several classifications of chromatographic methods, and the most famous of them include Gas chromatography (G.C.), liquid chromatography (L.C.), and ion chromatography (I.C.).[28,29,37]

3.2.1. Gas chromatography

G.C. is a precise technique for analysing and identifying volatile substances in a sample due to the ability of chromatography to separate them. It entails converting the sample into a gaseous phase and directing this gaseous phase through a column with the help of a carrier gas. The elution is recognised at certain time points to determine the components quantitatively.[38]

3.2.2. TLC

TLC, or thin-layer chromatography, is a cost-effective method for drug analysis. The stationary phase and the mobile phase are the two separate stages of the process. A plate is covered with a thin layer of material, like silica gel, which serves as the stationary phase. TLC separates compounds as they migrate over this layer. Its flexibility, compatibility with different solvents, and ability to accommodate large samples have contributed to its popularity in drug analysis.[39]

3.2.3. HPTLC

High-performance thin layer chromatography (HPTLC) is an advanced technique for analysing drugs that are used on a global scale. It rapidly segregates and discerns the constituent elements of drugs in medicines. The benefits of this include rapidity, adaptability, and user-friendliness. It can quickly analyse samples of raw drugs and accurately determine various parameters without any time constraints.[40]

3.2.4. HPLC

High-performance liquid chromatography (HPLC) effectively separates intricate combinations of chemical and biological substances. Prior to use, it needs meticulous preparation and confirmation. It often employs a U.V. detector for sample analysis. This detector utilises repeated scans to identify the appropriate wavelength for each sample, enabling precise detection of various substances in the combination.[28,29,41]

3.2.5. RP-HPLC

Reverse phase liquid chromatography is an HPLC technique that separates molecules by their hydrophobic properties. The method employs a fixed phase that lacks polarity and a movable phase that has polarity. Compounds are partitioned as they traverse the column, with more hydrophobic molecules exhibiting greater affinity for the stationary phase and eluting more slowly. It is extensively used for the analysis of several sorts of compounds.[42]

3.2.6. UPLC

Ultra-performance liquid chromatography (UPLC) is a more advanced variation of HPLC. By using smaller particles and applying more significant pressures, this method enables quicker analysis and more effective separation of chemicals. It offers more distinct peaks, improved resolution, and enhanced sensitivity. This technique is very advantageous in pharmaceutical analysis since it provides faster results and requires less solvent as compared to standard HPLC.[43]

3.2.7. Ion chromatography

Ion chromatography, or I.C. is a modified version of high-performance liquid chromatography. This one distinguishes and measures anions, cations, and other chemicals that may be converted to ionic forms. However, this method is frequently applied in water quality diagnostics.[44]

3.2.8. Electrophoresis

Electrophoresis is a very efficient idea of work that sorts something because of the migration of an object in an electric field depending on the charge. It is usually applied when trying to separate complex samples of complex biomolecules like proteins, nucleic acids, or even small molecules relying on the size-to-charge ratios to carry out their separation.[29]

4. Hyphenated Analytical Techniques

The ability to name the two analytical procedures joined with a hyphen makes a great revolution to this field of chemical analysis because it offers scientists an unparalleled understanding of the systems of compounds or complex mixtures.[29] Hyphenated analytical techniques are recognised standard techniques, sometimes called GC-MS, LC-MS, LC-NMR, etc.[29,45-48]

4.1. GC-MS

The complex method known as gas chromatography-mass spectrometry, or GC-MS, combines the identifying powers of mass spectrometry with the separation characteristics of gas chromatography. In the context of gas chromatography (G.C.), a sample or a mixture to be analysed is changed into vapor, which is then carried through a column by an inert gas. Its constituents distribute themselves based on how they engage with the column's stationary phase and the boiling point.[45]

The individual chemicals get into a mass spectrometer after elution out of gas chromatography (G.C.). This is the procedure where molecules are ionised, most often with the help of electrons, and then fragmented. These are then sorted based on the mass-to-charge ratio; each chemical produces a different marked spectrum. GC-MS is critical in environmental analysis and forensic and metabolomic studies because it helps identify and characterise complex mixtures.[46]

4.2. LC-MS

Liquid chromatography-mass spectrometry (LC-MS) is one of the most useful analytical technologies at present, which combines the features of liquid chromatography used for the separation of components with the element of mass analysis and the possibility of selectively distinguishing compounds based on their mass to charge ratio.[47]

The liquid chromatography segment separates the individual components in a sample based on their particular interactions with a specialised column. Subsequently, these individual components are put into the mass spectrometer, where they are broken and their mass is measured.[44] This technique is extensively used in several domains, such as pharmaceutical research, environmental analysis, and food quality control, due to its ability to rapidly and precisely identify and quantify diverse substances.[49]

4.3. LC-NMR

LC-NMR integrates the separation capabilities of liquid chromatography with the analytical features of nuclear magnetic resonance spectroscopy. The hyphenated methodology employs chromatographic procedures to segregate complicated materials, then analysed by NMR. This facilitates the prompt characterisation and identification of compounds upon elution.[48,49] Some advantages of LC-NMR include the fact that it might present complex structural information of still unidentified compounds in very complex mixtures without preparing the samples for a long time and isolation of the compounds. A significantly high value of this research tool is seen in natural product research, metabolomics, and pharmacological analysis disciplines. However, there are challenges that LC-NMR faces, including limited sensitivity and the requirement of deuterated solvents, which sometimes make it difficult to establish methods and incur high costs.[49,51]

5. Modern applications of RSM in Method and Formulation Development in Drug Discovery

Response surface methodology (RSM) is a potentiated statistic tool frequently employed in analytical chemistry in the enhancement of systems. Helps a researcher to establish the type of connection between different parts of the study and how they impact the results of the experiment. RSM is favoured over the one-factor-at-a-time methodology since it enables the researcher to evaluate the effects of several factors simultaneously, hence expediting the procedure.[51]

As RSM is employed in optimising methods in the pharmaceutical field chromatography being one of the appealing applications of this method is hereby seen. It can assist to maximise/minimize certain crucial parameters like the identity of the fluid that separates compounds, what extent of acidity this fluym has, mobility of the fluym through the system and the temperature of the system. These factors can be adjusted in order to improve the compounded rate and identification.[52-54] Another related activity is method validation where efficiency of a given method is established for accuracy by RSM. In helping such scientists, it helps scientists determine how efficient a particular procedure is in delivering outcomes and how shifts in such parameters as the concentrations of solutions influence the results. This is particularly important when it is required to demonstrate that the applied method in the given study is correct, unerring, and stable.[47] Utilising RSM allows for the construction of a 'map' delineating the parameters deemed best for achieving a high effectiveness rate of a certain procedure, even amongst minor variations in experimental variables. **Figure 3** demonstrates the Ishikawa fishbone diagram as a part of Screening Tool to identify factors affecting the optimisation of a drug formulation or manufacturing process.[55,56]

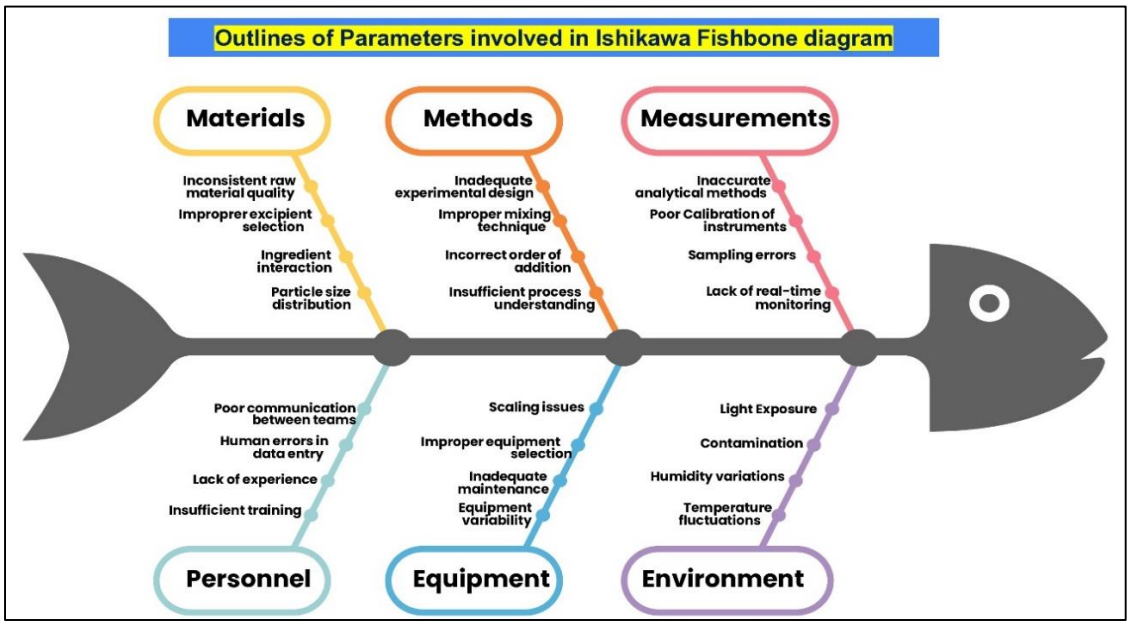

Figure 3: Outline parameters involved in ishikawa fishbone diagram

6. Different Screening and Optimisation Tools used During Formulation & Method Development in Drug Design

Statistical design is based on capturing various interrelationships of factors with quantitative response variables; the specific technique utilised can be univariate or multivariate, in order to predict results from these relations. The screening designs that are commonly investigated with respect to process improvements encompass full and fractionated factorial designs, response surface designs such as Box Behnken design, orthogonal central composite design, Taguchi rough designs, Plackett Burman designs and combination of these designs for screening. Many of these statistical designs are then enhanced through response surface techniques, and polynomial equations.[57]

6.1. Full factorial design

The types of full design that are being analysed include every potential combination of variables and levels that could be studied. It is the second most frequent design and gives a perfect understandable snapshot view of the process behaviour and is a useful statistical tool. While FFD covers all aspects, the management of elements and their interrelation is also included. A general type of FFDs is Feature Fusion and Decision that includes such sorts as those with two layers, three layers as well as four layers that offer different views. Two levels Full Factorial Design (2k, where k is the number of components) is predominantly used and three levels.[57]

6.2. Fractional factorial design

A full factorial design will take a longer time to complete but it is closely related to a fractional factorial design. The test works in a systematic manner, focusing on a specific subset of the factors for a reduction in number of trials and yet provide you with necessary results. This technique is fast and consumes less resources hence a method that is suitable when dealing with several elements. However, it may factor out complex interactions between variables or within and across domains. Fractional factorial designs are advantageous in preliminary investigations since they minimise the number of factors examined, allowing improved outcomes in subsequent applications of more robust experimental designs.[57,58]

6.3. Box-behnken design

Box-Behnken design known as BBD, a kind of rotational design, use the midpoints of the space and the internal midpoints inside the cubic design region. This technique minimises excessive experimental variables and minimises the chance of acquiring incorrect results. It is commonly applied when making an analysis involving at least three variables. It facilitates the monitoring of individual impacts of several variables and their potential interactions with the response variable, while simultaneously minimising the number of trials, time, and costs. Furthermore, the assertion that BBD is intricately linked to 'response surface methodology,' a statistical technique used to enhance processes.[57]

6.4. Central composite design

Central composite design abbreviated as CCD is the utmost prevalent statistical methods cast-off in optimisation. Optimisation of the design is possible and it can be constructed using either an orthogonal 2k complete factorial design or an orthogonal 2k−p fractional design that can contain axial and centre points. This particular design of experiment (DOE) also has the ability to include a full quadratic model. The most common form of the CCD is one that has three quite different variables with each having five levels.[25]

6.5. Taguchi design

The Taguchi design (T.D.) is a comprehensive and characteristic screening technique to search the active factors pertaining to the extraction of the metallic quantity and their qualities. Optimisation through more efficient procedures is achieved by methods of rage that cut trials, time, and costs. According to the Taguchi technique, the trials of experimentation are conducted with the help of the orthogonal array index. It depends on the total quantity of the parameters and their configurations where the dimensions are headed. This design enables one to decide on the optimum number of trials that will help in arriving at the most beneficial data given a certain number of parameters.[59]

6.6. Plackett- burman design

Plackett Burman design (PBD) is categorised as a kind of fractional factorial design, implemented using two stages. The design focusses on analysing $K = N - 1$ variables via N experiments, with N being a multiple of 4. This design lacks geometric or regular characteristics that preclude data representation in cube form, making it a valuable tool for optimising the process while evaluating multiple factors that may affect the response variable Y with a minimal number of experimental runs. One of the key advantages of such design is the possibility of rejecting unnecessary ones from the models and defining important variables for the subsequent improvement using some statistical designs as Box-Behnken or central composite design.[50,51]

7. Application of RSM in Nanotechnology

Nanotechnology is employed in various fields, including energy, healthcare and pharmaceuticals, nanobiotechnology, nanodevices, optical engineering, personal care products, bioengineering, nano fabrics, and the defence industry, because to its significant surface-area-to-volume ratio. Nanotechnology significantly contributes to contemporary biological research. Nanotechnology plays an enormous part in contemporary biological research.[60] This technology has

extensive uses in pharmaceuticals, delivery of drugs, gene therapy, safety in food, and environmental health. It has also been employed in the treatment of infections, cancer, allergies, diabetes, and inflammation.[61] The green-mediated synthesis of metal nanoparticles has attracted heightened interest in recent decades owing to its cost-efficiency, non-toxicity, and environmental sustainability for medicinal and cleanup purposes. Green-synthesised metal nanoparticles, including silver, copper, and zinc, are predominantly utilised due to their efficacy and safety.[61]

Considering the daily expansion of nanotechnology applications, the scientific community is focused on developing a cost-effective, straightforward, and environmentally friendly method for nanoparticle manufacturing, particularly the biosynthesis of stable nanoparticles with well-defined shapes and sizes.[62] There are numerous drawbacks to experimental optimisation using classical methods that change one factor at a time while fixing the others. These methods show the impact of each variable separately through a large number of experiments, but they ignore the effect of interactions between the various factors under study. Conversely, the application of statistical approaches addresses the challenges of selecting effective variables among numerous influencing factors. Moreover, it aids in comprehending the interplay of several significant elements.[63]

There are several methods to optimise the process of synthesizing these nanoparticles like completely randomised design (CRD), two-level factorial and fractional factorial design, response surface methodology (RSM) and Taguchi's method.[64] Among them, RSM demonstrated the most efficient use as a design. This methodology facilitates the examination of interactions among many factors and minimises the number of experiments by accounting for input variables. Most of the nanoparticle's synthesis optimisation has been done using the Box- Behnken design.[65]

7.1. Application of RSM in Nano biotechnology

The combination of nanotechnology with biotechnology is known as nanobiotechnology. It integrates molecular biology techniques with traditional microtechnology. Innovations and a significant role in several biomedical applications, including gene therapy, drug delivery systems, biomarkers, molecular imaging, and biosensors, are expected to be produced by nanobiotechnology.[66] Understanding, modifying, and applying matter at the nanoscale is the foundation of nano-science and nano-technology, which may find application in biochemistry, biomaterials, and bioengineering, among other scientific fields.[67,68] Nanoparticles possess distinctive characteristics and a unique structure.[69-73] Various nanoparticles, particularly metal-oxide nanoparticles, may be utilised in biological applications.[74] Zinc oxide nanoparticles (ZnO-NPs) are significant in numerous research domains. ZnO is a wide band-gap semiconductor that has been utilised in several applications.[75] Microorganisms such as yeast, bacteria, algae, and fungi are crucial in the reduction of metals, either intracellularly or extracellularly, forming the foundation for utilising these organisms in the eco-friendly manufacture of nanoparticles, so serving as intriguing nano-factories. These procedures are used for the synthesis of diverse metal nanoparticles, including silver, gold, and zinc. Bio-modelling and optimisation increase the understanding and implementation of biological experiments.[76]

In recent years, response surface methodology (RSM) has been used for optimisation in several biotechnological processes. RSM functions by evaluating the relationships between parameters and outputs to identify optimal conditions via the conduct of several experiments.[77] This technique has been extensively utilised to determine the optimal settings for various nanotechnological and biotechnological processes by assessing the interaction effects among process factors.[78] The recent reported studies related to nanotechnology and nanobiotechnology have been demonstrated in **Table 2**.[65,79-93]

Table 2: Reported research studies in Nanotechnology incorporating RSM approach

Reported Research Title	Type of dosage form developed	Objective of the study & Applied RSM approach	Design RSM Approach	Year of Publication	References
Response Surface Methodology (RSM) approach to formulate and optimise the bilayer combination tablet of Tamsulosin and Finasteride	Tamsulosin and Finasteride bilayer tablet	A bilayer tablet designed for oral administration was developed, incorporating Tamsulosin (TAM) for sustained release (SR) and Finasteride (FIN) for immediate release (IR). A response surface methodology was employed to formulate bilayer tablets featuring separate release layers, specifically sustained release (SR) and instant release (IR). In both cases, the independent variables selected were talc and triacetin for the outer layer, hydroxypropyl methylcellulose (HPMC) as the sustained release polymer, and avicel PH102 for the interior layer. A central composite design was employed in the response surface methodology to formulate and optimise the design.	Central Composite Rotatable Design (CCRD)	2024	79

Table 2 continued…

Formulation Optimisation of Erythromycin Solid Lipid Nanocarrier Using Response Surface Methodology	Erythromycin solid lipid nanocarriers	This research utilised response surface methodology (RSM) with a varied design model to optimise the preparation of erythromycin solid lipid nanocarriers (ERY-SLN). A two-factor, three-level factorial design was utilised for optimisation. The study of the optimal formulation of ERY-SLN examined three parameters: drug entrapment efficiency (EE), percentage drug loading (DL), and mean particle size, in relation to two independent variables: lipid content (X1) and surfactant to cosurfactant ratio (X2).	Two-factor three level factorial design	2014	80
Application of RSM and ANN for the prediction and optimisation of thermal conductivity ratio of water based Fe2O3 coated SiC hybrid nanofluid	Nanoparticle	A novel water-based hybrid nanofluid has been synthesided utilizing Iron oxide (Fe2O3) coated Silicon carbide (SiC) at different mixing ratios (Fe2O3: SiC) to investigate the impact of nanoparticle combinations on effective thermophysical properties. This study utilises artificial neural network (ANN) and response surface methodology (RSM) modeling techniques to estimate the thermal conductivity ratio of the nanofluid at an optimised nanoparticle mixing ratio.	Central Composite Design (CCD)	2021	81
The Utilisation of Response Surface Methodology (RSM) In the Optimisation of Diclofenac Sodium (DS) Liposomes Formulate through the Thin Film Hydration (TFH) Technique with Involving Computational Method	Diclofenac Sodium loaded liposomes	The research focused on enhancing the formulation of Diclofenac Sodium-loaded liposomes through Response Surface Methodology (RSM) and a computational technique for validation. The optimisation of DS-loaded liposomes was conducted using Response Surface Methodology (RSM), focusing on two key parameters: encapsulation efficiency (% EE) and in vitro drug release (% DR) over a 12-hour period, incorporating Quality by Design (QbD) principles. The investigational outcome demonstrates that the perceived responses correspond with the anticipated values, illustrating the relationship of Response Surface Methodology (RSM) in optimizing % Drug Release (DR) and % Encapsulation Efficiency (EE) in drug-loaded liposomal formulations.	Two-factor three level factorial design	2023	82
Response Surface Methodology (RSM) Powered Formulation Development, Optimisation and Evaluation of Thiolated Based Mucoadhesive Nanocrystals for Local Delivery of Simvastatin	Simvastatin nanocrystal	A novel water-based hybrid nanofluid has been synthesised utilising Iron oxide (Fe2O3) coated Silicon carbide (SiC) at different mixing ratios (Fe2O3: SiC) to investigate the effect of nanoparticle combinations on effective thermophysical properties. This study utilises artificial neural network (ANN) and response surface methodology (RSM) modeling techniques to estimate the thermal conductivity ratio of the nanofluid at an optimised nanoparticle mixing ratio.	Central Composite Design (CCD)	2022	83
AQbD Driven Development of an RP-HPLC Method for the Quantitation of Abiraterone Acetate for its Pharmaceutical Formulations in the Presence of Degradants	Abiraterone Acetate	An analytical quality by design (AQbD) technique was utilised in this work for the purpose of developing the medication abiraterone acetate. Drug separation was conducted with a Princeton Merck-Hibar Purospher STAR (C18, 250 mm × 4.6 mm i.d., 5 µm particle size) with UV detection at 235 nm. This was accomplished through the utilisation of chemometrics-assisted reverse phase high performance liquid chromatography (RP-HPLC).	Box-Behnken design	2021	84

Table 2 continued…

Synthesis of CuO nanoparticles through green route using Citrus limon juice and its application as nanosorbent for Cr(VI) remediation: Process optimisation with RSM and ANN-GA based model	Cupric Oxide Nanoparticles	This study seeks to synthesise cupric oxide nanoparticles (CuONPs) by an eco-friendly technique utilizing lemon juice extract as a bioreductant. The synthesised CuONPs were examined for their physicochemical and molecular properties. The integration of response surface methodology (RSM) with an artificial neural network hybridized with a genetic algorithm (ANN-GA) has attained a peak Cr(VI) adsorption of 98.8% under optimised conditions of an initial metal concentration of 22.5 mg/L, a pH of 3.81, a Cu ONPs dosage of 1.28 g/L, and a temperature of 37.1 °C.	Central Composite Design (CCD)	2015	85
Application of response surface methodology to optimise the extracellular fungal mediated nanosilver green synthesis	Nanosilver particles mediated by Trichoderma viride	This study aims to improve the production of nanosilver particles using Trichoderma viride ATCC36838, employing response surface methodology (RSM). Silver nanoparticles (AgNPs) were successfully biosynthesised by investigating the parameters that affect the reduction of silver ions (Ag+) to metallic nanosilver (Ag0) using culture filtrate under agitation.	Central Composite Design (CCD)	2017	86
Statistical optimisation of *Bacillus alcalophilus* α-amylase immobilization on iron-oxide magnetic nanoparticles	Iron oxide nanoparticles	This study examines the statistical optimisation of the immobilization of alkaline α-amylase [E.C. 3.2.1.1] derived from Bacillus alcalophilus onto nanoscale supermagnetic iron oxide nanoparticles (MNPs) to improve the cost-effectiveness of industrial applications involving MNP-bound α-amylase. Plackett-Burman factorial design and response surface methodology (RSM) were employed to assess the influence of different parameters and the central response on the binding process of α-amylase to iron oxide magnetic nanoparticles (MNPs).	Plackett-Burman factorial design	2010	87
Optimisation of angelica sinensis polysaccharide-loaded Poly (lactic-co-glycolicacid) nanoparticles by RSM and its immunological activity in vitro	Angelica sinensis polysaccharide-loaded Poly (lactic-co-glycolicacid) nanoparticles	The study's goals were to find the best conditions for making ASP-PLGA capsules, which would increase their packaging efficiency, and to look into how well they worked at boosting the immune system. Response surface methodology (RSM) was implemented to look at the amounts of Pluronic F68 (F68) (w/v), the ratios of the organic phase (o) to the internal water phase (w1), and the ratios of the external water phase (w2) to the organic phase (o). This helped find the best processing settings.	Box-Behnken design	2018	88
Optimisation of myco-synthesised silver nanoparticles by response surface methodology employing Box-Behnken design	Silver nanoparticles (AgNPs) induced by Penicillium citrinum.	The primary objective of the study was to improve the mean diameter of biosynthesised Silver Nanoparticles (AgNPs) produced by Penicillium citrinum. The response surface methodology (RSM) was utilised to optimise the average diameter of silver nanoparticles (AgNPs) through the Box-Behnken design (BBD). The critical factors in this method included the concentration of AgNO3 (mM) (X1), solution pH (X2), shaker incubator temperature (°C) (X3), incubator shaking rate (rpm) (X4), and incubation duration (hours) (X5). The R^2 value lies at 0.8894.	Box-Behnken design	2019	65

Table 2 continued…

QbD-based Formulation Optimisation and Characterization of Polymeric Nanoparticles of Cinacalcet Hydrochloride with Improved Biopharmaceutical Attributes	Polymeric Nanoparticles of Cinacalcet Hydrochloride	The objective of this study was to develop Quality by Design (QbD) optimisation and enhance the oral bioavailability of freeze-dried polymeric nanoparticles of cinacalcet hydrochloride, produced via nanoprecipitation and ultrasonication techniques utilising PLGA and poloxamer-188.	Taguchi and Box- Behnken design	2020	89
Optimisation of Cobalt Nanoparticles for Biogas Enhancement from Green Algae Using Response Surface Methodology	Cobalt Nanoparticles	This study optimised the concentration of Cobalt nanoparticles with response surface methodology (RSM). The mesophilic temperature range, pH (5–9), and Co NPs dosage were designated as the independent variables for response surface methodology (RSM). The performance indicators indicated that the mathematical model matched effectively with the data obtained from experiments.	Central Composite design	2023	90
Response surface methodology and reaction optimisation to product zero-valent iron nanoparticles for organic pollutant remediation	Zero-valent iron nanoparticles	This study employed design of experiments (DoE) and response surface methodology (RSM) to identify effective reaction parameters and optimal conditions for maximising the generation of ZVINPs via green tea extract. Initially, a fractional factorial design was employed to evaluate the reaction parameters (reaction time, temperature, leaf extract quantity, and metal precursor concentration) in the synthesis process. The central composite face (CCF) design was employed for optimising the reaction. The concentrations of green tea extract and iron precursor were identified as the most beneficial parameters.	Central Composite Face design	2019	91
Response Surface Methodology for Statistical Optimisation of Chitosan/Alginate Nanoparticles as a Vehicle for Recombinant Human Bone Morphogenetic Protein-2 Delivery	Chitosan/Algi nate Nanoparticles	The present study aimed to optimise the influential elements for encapsulating rhBMP-2 in Cs/Alg nanoparticles by response surface methodology (RSM) and the Box–Behnken design (BBD). The factors included the Cs/Alg molecular weight (Mw) ratios, pH, and stirring rates, while the responses encompassed particle size, zeta-potential, polydispersity index, drug loading efficacy cumulative drug release, and morphological degradation time (MDE).	Box- Behnken design	2020	92
Response surface methodology: Optimisation of myco-synthesised gold and silver nanoparticles by Trichoderma saturnisporum	Myco-synthesised gold and silver nanoparticles	In the present work, the parameters that are effective for the green synthesis reaction and central composite face design were employed to achieve the optimal reaction state. The model's execution employed a commendable foot. Normal probability plots, interaction plots, effect plots, analysis of variance (ANOVA), surface plots, contour plots, and Pareto charts were employed to optimise the components.	Face centred central composite design	2023	93

8. Regulatory Aspects of RSM for Product Development

Response surface methodology (RSM) investigates the relation between multiple response variables. It has gained acknowledgment from regulatory agencies globally, counting the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the U.S. Food and Drug Administration (FDA).[89-91]

The ICH Q2(R1) guideline provides instructions on validating different analytical procedures. The ICH Q8(R1) recognises the use of Response Surface Methodology (RSM) in validating the approach. This recommendation highlights that analytical processes are appropriate for their intended purpose.[90-94]

The World Health Organisation and the Food and Drug Administration (FDA) have both recognised the importance of RSM in the field of pharmaceutical research and quality control. The WHO'S guidelines Annex 2 describes the guidelines for quality risk management. Similarly, The FDA's process analytical technology (PAT) initiative describes using different analytical techniques and statistical tools, such as RSM, to enhance the understanding and oversight of processes.[89,91,92,95-97]

9. Conclusion

While implementing the response surface methodology (RSM) in the analytical process development and regulatory science there are enormous opportunities in the coming future for drug discovery in Nanoscience. One of the current issues is the complexity of the multiple factors influencing the biological and pharmacological systems, which might require assistance in refining them for modelling. Further, to equip the growing levels of data and specialised experimental designs, there is a great need for enhanced software tools. Conversely, there is a need for more efficient specialists who can efficiently identify and apply findings based on RSM in a framework of regulations. Also, integrating RSMs with modern technologies, including artificial intelligence and machine learning, is still in the initial stages. However, such type of research holds great potential for RSM enabled method development, validation and also even crucial to Formulation Developments in Pharmaceutical and Biotech Research Organizations.

10. Current Challenges and Future Prospective

According to different literatures it can be seen that the regulatory bodies are now focusing on risk assessment techniques and quality with a design approach. Thus, there is the expectation that RSM (Response Surface Methodology) will make a significant contribution to the enhancement and confirmation of processes. In future, it is possible to predict the further enhancement of the application of advanced RSM algorithms for a more accurate and efficient account of nonlinear dependencies and randomness. RSM may also be incorporated with real-time monitoring in order to offer continuous validation of the process. In the therapeutic pharmaceutical products and development of biosimilars, regulatory science is of critical importance, especially when there is a need for an extensive understanding of the process. As these challenges are being solved and more applications are developed, RSM use is expected to drastically rise and improve constructional, regulatory science, and analytical approaches.

11. List of Abbreviations

RSM: Response Surface Methodology
ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
FDA: Food and Drug Administration
WHO: World Health Organisation
CCD: Central Composite Design
ANOVA: Analysis of Variance
QbD: Quality by Design
CQA: Critical Quality Attributes
BBD: Box-Behnken Design
GC: Gas Chromatography
LC: Liquid Chromatography
IC: Ion Chromatography
TLC: Thin Layer Chromatography
HPTLC: High-Performance Thin Layer Chromatography
HPLC: High-Performance Liquid Chromatography
UV: Ultra Violet
RP-HPLC: Reverse Phase-High Performance Liquid Chromatography
UPLC: Ultra Performance Liquid Chromatography
GC-MS: Gas Chromatography Tandem Mass Spectroscopy
LC-MS: Liquid Chromatography Tandem Mass Spectroscopy
LC-NMR: Liquid Chromatography Tandem Nuclear Magnetic Resonance Spectroscopy
LC-MS-MS: Liquid Chromatography Tandem Mass Spectroscopy
LC-NMR-MS: Liquid Chromatography Tandem Nuclear Magnetic Resonance Spectroscopy Tandem Mass Spectroscopy
FFD: Full Factorial Designs
DoE: Design of Experiment
TD: Taguchi Design
PBD: Plackett-Burman Design
PAT: Process Analytical Technology

12. Source of Funding

Not applicable.

13. Conflict of Interest

The authors declare no conflict of interest.

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