Journal of Advanced Multidisciplinary Research Studies and Development

ISSN : 2583-6404
May - June 2023

QUALITY BY DESIGN PERSPECTIVES FOR SILICA NANOPARTICLES

Astha Atul Karkhanis[1], Dr. Itishree Vaidya[2]

[1] *Research Scholar, Department of Quality Assurance, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Mumbai, Maharashtra, India.*

[2] *Research Guide, Department of Quality Assurance, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Mumbai, Maharashtra, India.*

Abstract

Silica nanoparticles have been widely studied in nanotechnology for their underlying benefits such as excellent biocompatibility, low degradation profiles, ease of synthesis and tunable morphology. We have used a green, sustainable and cost effective source for synthesizing Silica Nanoparticles on a lab scale by keeping Quality by design (QbD) viewpoint. A 2 factor 3 level factorial design was established to check the effect of critical process parameters on particle size of amorphous silica nanoparticles (ASiNP's). The QbD study has taken into account the risk assessment matrix and resulted in a statistically significant model for the experimental design space. ASiNP's resulting from optimized batch were characterized by FTIR, XRD, TEM and EDS analysis.

Keywords: Nanoparticles, Amorphous silica nanoparticles, Green synthesis, Quality by Design.

► *Corresponding Author: Astha Atul Karkhanis*

Introduction

In recent times silica nanomaterial is gaining more importance due to its excellent set of physical, chemical, electronic and optical properties. They show excellent biocompatibility, have low degradation profiles, ease of synthesis and have tunable morphology. The use of plant pre-materials for the synthesis of nanoparticles is regarded as eco-friendly due to its advantages namely easy availability, the low cost of plant materials, the elimination of chemical substances and the reduction of energy consumption over chemical methods.(1) Raw materials having an abundance of silica are rice husk, banana peel, coconut husk, corn cob etc. Sugarcane bagasse , a byproduct from the sugar industry, has about 97% of silica and hence suitable candidate for the green synthesis of Amorphous silica nanoparticles.(2) These plant materials can produce silica nanoparticles that can be used for various medical applications such as drug delivery of poorly soluble drugs or sensitive drugs as well.(3) Thus with the help of silica nanoparticles one can achieve even targeted drug delivery. Other applications include biosensing photocatalytic degradation etc.

The concept of quality by design (QbD) has gained increasing attention because of its expected benefits. Quality by design is increasingly becoming an essential and widely used term in the pharmaceutical industry quality system. The reason for this can be the fact that various challenges that come with synthesizing nanoparticles can be overcome by a solution of application of a process development tool. The aim is to improve the quality and avoid variability in the product. QbD begins with recognizing the quality target product profile, understanding critical processes, material attributes, and quality attributes. The underlying principles of QbD are explained in the quality guideline of the international conference on harmonization i.e. ICH Q8 for pharmaceutical development, ICH Q9 for quality risk management, and ICH Q10 for pharmaceutical quality system. (4) In this research work we have applied QbD principles for understanding critical quality attributes for silica nanoparticle synthesis from a cost effective source.

ISSN : 2583-6404
May - June 2023

Material and Methods:

Sugarcane bagasse which is a waste product of the sugar industry was procured from the local juice vendors of Thane district. Lab grade Hydrochloric Acid (MOLYCHEM), and Sodium Hydroxide pellets (MOLYCHEM) were used for the experimental work.

Methodology:

Preparation of Sugarcane Bagasse Ash:

Sugarcane Bagasse Ash (SBA) was used as the starting material for the extraction of amorphous silica. Washed Sugarcane bagasse was completely dried in a hot air oven at 70°C to 80°C and then incinerated in a muffle furnace at a temperature of 600°C - 700°C for 2 to 3 hours. This led to formation of a gray ash. Fig.1 (A) and Fig.1 (B) shows images of the sugarcane bagasse and sugarcane bagasse ash obtained after its incineration repectively.

Fig. 1 (A) Sugarcane Bagasse

Fig. 1 (B) Sugarcane Bagasse Ash

Preparation of Amorphous Silica Nanoparticles:

The processed gray ash was accurately weighed and treated with 1 M HCl under reflux for an hour to eliminate any metal impurities. This is also termed as a leaching procedure. Subsequently this leached ash was filtered and washed several times with distilled water and was refluxed with NaOH solution (0.1 M to 3M). The solution was kept under a constant stirring and temperature. The reacted ash was again filtered and the resulting sodium silicate solution was cooled down to room temperature and subsequently, the solution was titrated with HCl (1M) under slow and constant agitation. The solution pH was monitored and the titration was interrupted at pH 7. After 24 h of aging, the gel was smoothly fractured and washed with distilled water. The obtained xerogel was then centrifuged for 20 mins at 2000 to 4000 RPM with washings at regular intervals

ISSN : 2583-6404
May - June 2023

of distilled water. This was then dried in an oven and triturated in a glass mortar and pestle for further characterization.[5] Fig 2 (A) Shows amorphous silica nanoparticles (ASiNPs) in colloidal xerogel form and Fig 2 (B) shows their dried powder form.

Fig. 2 (A) ASiNPs as Colloidal Xerogel

Fig. 2 (B) ASiNPs as Dry Powder

Following is the chemical reaction that represents the synthesis procedure of amorphous silica nanoparticles.

$$SiO_2 + 2NaOH\ (aq) \rightarrow Na_2SiO_3\ (aq) + H_2O\ (l)$$
$$Na_2SiO_3\ (aq) + 2HCl\ (aq) \rightarrow SiO_2\ (s) + NaCl\ (aq) + H_2O\ (l)$$

Optimization Study Using Factorial Designs:

A 2 factor, 3 level factorial design was established to test the effect of Critical Quality Attribute and Critical Process Parameter such as concentration of Sodium Hydroxide and Stirring Temperature respectively. These factors were tested at 3 discrete levels. Design Expert ® ver. 12.0 was used to analyze the output parameters and how they are affected by the above mentioned factors. Following Table 1 shows the levels assigned to the factors.

ISSN : 2583-6404
May - June 2023

Table. 1 Factors and Levels for Study

Factors	Level 1	Level 2	Level 3
Conc. Of NaOH	0.5 M	1.5 M	3 M
Stirring Temperature	70 °C	100 °C	130 °C

A total of 9 runs were suggested by the Design Expert ® ver. 12.0 according to which optimization trials were carried out. The output parameter was chosen as Particle Size (nm), which is also an important Critical Quality Attribute of the silica nanoparticle. The study was carried out according to the following Table. 2 representing the batch numbers.

Table. 2 Batch Numbers of Experimental Trials

Batch No	Conc Of NaOH (M)	Stirring Temp (°C)
B1	3	100
B2	3	130
B3	0.5	130
B4	1.5	130
B5	1.5	100
B6	0.5	70
B7	1.5	70
B8	3	70
B9	0.5	100

Characterization:

The silica content and the other compounds present in the sol-gel , green synthesized amorphous silica nanoparticles and in the sugarcane bagasse ash powder were determined using various characterization techniques. The FTIR analysis was carried out to detect functional groups that may be present. The particle size analysis of the synthesized nanoparticles was also carried out using the Horiba Particle Size Analyser. The bagasse ash , as well as the amorphous character of the prepared powder samples was determined by X-ray diffractometry studies. The morphology of the prepared materials was investigated by scanning electron microscopy (SEM). The functional compositional analysis was undertaken using electron dispersive spectrometry and the amorphousness of the silica nanoparticles was found out using titrimetric analysis.

Result and Discussion

Optimization Study using Quality by Design

ICH Q8 guideline of product development lays down quality by design approach and its various concepts that have been used in the following study so as to carry out optimization of the synthesis procedure. In order to successfully understand the concept of quality by design one has to be aware of the elements of QbD. Quality By Design starts with having a complete understanding of the process and its various elements. Thus, the elements of quality by design were correlated to the entire synthesis procedure of the green synthesis of amorphous silica nanoparticles.

With reference to silica nanoparticles from the perspectives of QbD

Critical Quality Attributes (CQA): Particle Size, Zeta potential, Porosity, Polydispersity Index etc.

Critical Process Parameters (CPP): Stirring Temperature of the reaction, Stirring RPM of the reaction, The precipitation pH for conversion of solution to gel form, Gelling Time for xerogel

ISSN : 2583-6404
May - June 2023

formation, Temperature of the furnace for conversion of sugarcane bagasse into sugarcane bagasse ash.

Critical material Attributes (CMA): Concentration of acid, Volume of the acid used, Concentration of base, Volume of the base used, Quantity of the treated ash

In order to analyze the criticality of these input factors or variables on the output quality of the product, it was necessary to run certain test batches. These batches helped determine how critically any CPP or CMA had an effect on the CQA.

Hence, a series of test batches were taken with a set of parameters derived from the CPP and CMA and variables were introduced according to Table.3 in the following manner.

Table. 3 Testing Parameters and Variables

Parameters	**Variables**
Reaction Temperature	28˚C-130˚C
Stirring Time	1hr-2 hr
Conc Of NaOH	0.1 M to 3 M
Conc Of HCl	0.1 M to 2 .5 M
Precipitation pH	5 to 12
Gelling time	3 hours- 15 hours

This study helped establish a certain relationship between the CMA, CPP and CQA. The criticality of each quality attribute associated with the nanoparticles depends upon the factor that affects it the most. Following Risk Assessment Matrix (Fig.3) was tabulated with all the data produced from the test batches.

CQA	Critical Material Attributes			Critical Process Parameters			
	Furnace temp.	Conc. Of HCl	Conc of NaOH	Sitrring Temp.	Stiiring RPM	Precipitation pH	Gelling Time
Particle size	Medium	Low	High	High	Low	High	Medium
Zeta Potential	Low	Low	High	High	Low	High	Medium
PDI	Low	Low	Medium	Low	High	Medium	Medium
Porosity	Low	Low	Low	Medium	Medium	Medium	Low

Fig. 3 Risk Assessment Matrix

Particle Size of the Amorphous silica nanoparticle is chosen to be the Critical Quality Attribute and needed to be studied, as per the risk estimation matrix. The attribute was at a higher risk due to the variability in the factors selected of the alkali and the temperature as well as the effect of pH. The risk in terms of the attributes could not be neglected and hence was put under study. Hence the Critical Quality Attribute chosen was Particle size, The Critical Process Parameter chosen for study was Stirring temperature of the reaction and the Critical Material Attribute chosen was Concentration of the alkali used for alkali treatment, in this synthesis procedure the alkali being Sodium Hydroxide.

The runs suggested by the Design Expert ® software were carried out and the critical attribute chosen i.e. the particle size of the nanoparticle was analyzed. Following Table.3 represents the output measurement of the design that was carried out.

ISSN : 2583-6404
May - June 2023

Table. 3 Output Data of the Experimental Study

Batch No	Conc Of NaOH (M)	Stirring Temp (°C)	Particle Size (nm)
B1	3	100	917.8
B2	3	130	2802.5
B3	0.5	130	2765.1
B4	1.5	130	3682
B5	1.5	100	2957.3
B6	0.5	70	1106.9
B7	1.5	70	1958.5
B8	3	70	429.1
B9	0.5	100	1892

The results obtained in nm were then fitted into a quadratic model for each response factor. The relation between the selected parameters and the response was established using a polynomial equation.The statistical equation was as follows: *Particle Size = 2738.13 + 136.33*A + 941.82*B + 215.83*AB - 1625.77*A2 + 201.65*B2*. The developed model and its fit statistics are represented in the below Table.4

Table. 4 Fit Statistics of Developed Model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.122E+06	5	1.824E+06	20.03	0.0164	**Significant**
A-NaOH Conc	4.345E+05	1	4.345E+05	4.77	0.1169	
B-Stirring Temperature	5.627E+06	1	5.627E+06	61.76	0.0043	
AB	1.403E+05	1	1.403E+05	1.54	0.3028	
A^2	2.655E+06	1	2.655E+06	29.14	0.0125	
B^2	81325.45	1	81325.45	0.8926	0.4145	
Residual	2.733E+05	3	91107.95			
Cor Total	9.396E+06	8				
R^2	0.9709					
Adjusted R^2	0.9224					
Adequate Precision	14.5052					

Here the term ‘A’ represents the concentration of NaOH and term ‘B’ represents the Stirring Temperature. The coefficient of the model was calculated using the Analysis Of Variance (ANOVA) for the quadratic model as well. The *p value* was found to be lesser than 0.05 stating that the model is significant. The coefficient of correlation was also used to check the significance of the model. For this model the R2 value was 0.9709 which is at a reasonable agreement with the adjusted value which is 0.9224, also suggesting that the model is significant. Thus this model can be used to navigate the design space. The relationship between the input variables ie the critical process parameters and critical material attributes was represented by 3D response surface ad 2D contour plots generated by the equation obtained from the model. The plot represent the level of interaction between the input parameters on each other as well as that on the output parameter. The Fig. 4 (A) and Fig. 4 (B) show the representation of the response surface plot and counter plot respectively.

ISSN : 2583-6404
May - June 2023

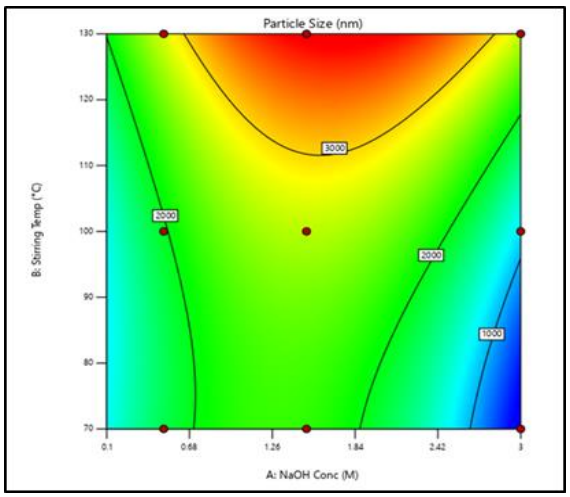

Fig. 4 (A) Response Surface Plot

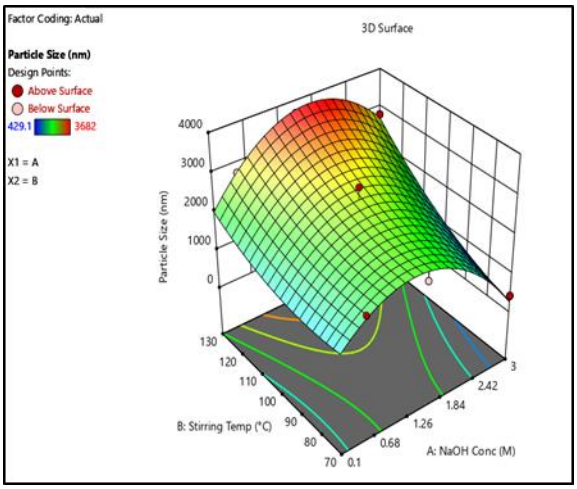

Fig. 4 (B) Counter Plot

As seen from the plots it is found that as the concentration of Sodium Hydroxide solution increases the particle size of the nano silica decreases, whereas when the stirring temperature increases the particle size also increases. The overlay plot for the given model also helped determine the optimized parameters for the set predefined criteria to obtain minimum particle size.

Fig.5 depicts the Overlay plot for the given experiment. The yellow zone represents the area where all the combinations of input parameters meet the predefined criteria on minimal particle size, while the gray area represents the combinations of input variables fail to meet the criteria. The yellow region with successful operating ranges also includes the Batch No.8 (B8) with concentration of NaOH as 3 M and Stirring Temperature set to 70 °C. This indicates the least particle size of 429.1nm which also coincides with the optimized batch suggested by the software which is X1 = 2.9 M and X2 = 74.4 °C (X1 representing conc of NaOH and X2 representing Stirring Temperature) having a suggested particle size of 427nm. Thus we can draw a conclusion from above plots that B8 can be denoted as the optimized batch that gives the least particle size for the amorphous silica nanoparticle.[6]

ISSN : 2583-6404
May - June 2023

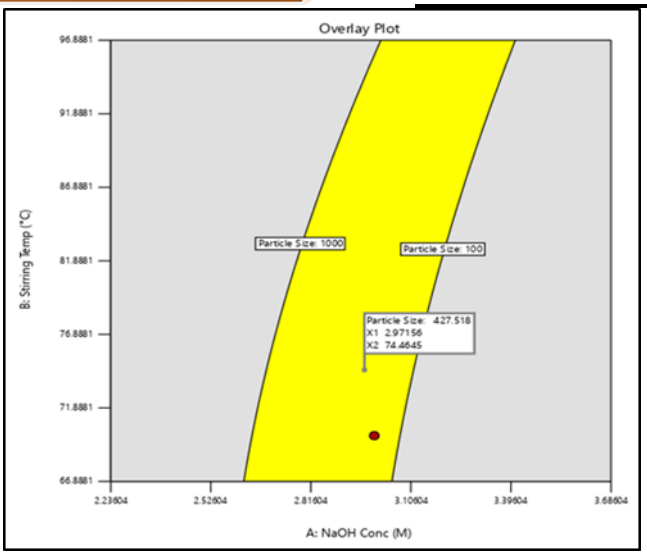

Fig. 5 Overlay Plot

Characterization Study

The FTIR spectra for the samples of the optimized batch of silica as well as that of the Sugarcane bagasse ash are represented in Fig. 6 (A) and Fig. 6 (B). Both the samples show a domination of the silanol (Si -O-H) and Siloxane (Si-O-Si) groups without the presence of any other impurity bands. The Bands at 3494.37 cm-1 and 3482.97 cm-1 for the Sample silica and Standard Silica respectively are attributed to the stretching vibration of O-H. The bands at 1077.38cm-1 and 1088.76 cm-1 are denoted to the asymmetric stretching vibration of Si-O-Si. The wavenumber at 796.64cm-1 and 799.49 cm-1 have arisen due to symmetric stretching vibration of Si-O bond while bands at 466.01 cm-1 are designated for Si-O bending of the siloxane group for sample and standard silica respectively.

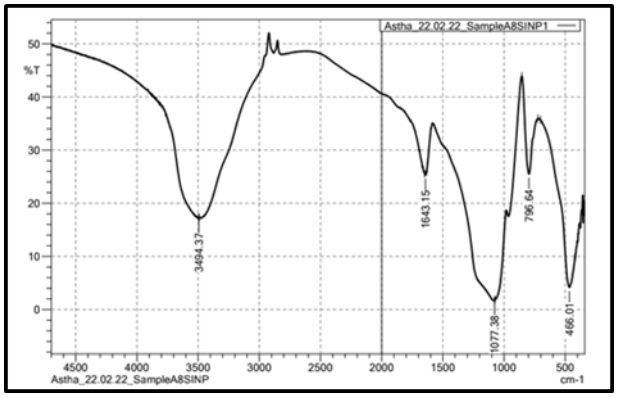

Fig. 6 (A) FTIR Spectra of the Optimized batch of (ASiNP)

ISSN : 2583-6404
May - June 2023

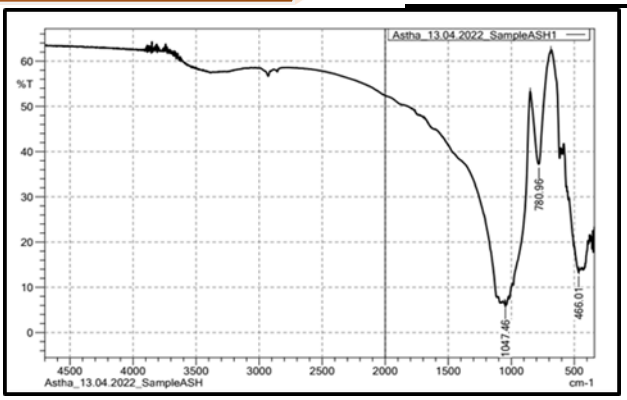

Fig. 6 (B) FTIR Spectra of Sugarcae Bagasse Ash

The X-Ray diffraction pattern In Fig. 7 (A) represents that of the Sugarcane bagasse ash and that in Fig 7 (B) represents that of the green method synthesized biogenic amorphous silica nanoparticles. A single broad peak at 2Θ angle of 22° was observed for both silica samples which illustrates the amorphous nature of the samples. Fairly a lower amount of crystalline silica was observed due to absence of any other strong and prominent peaks.[7]

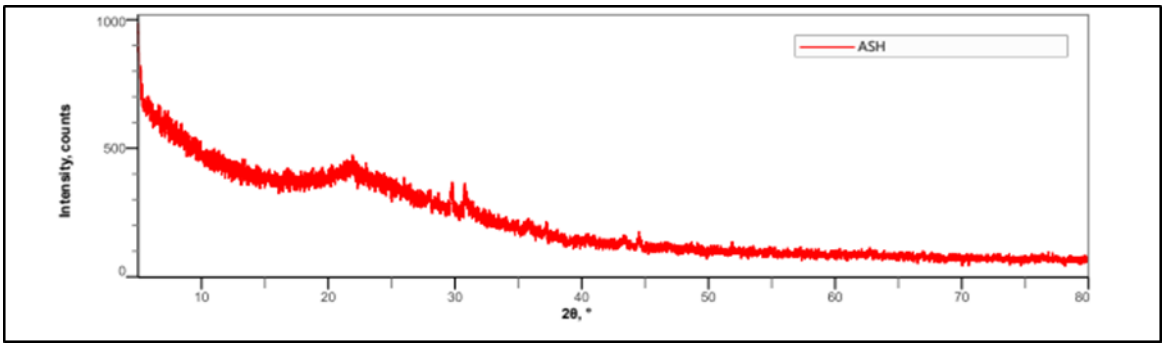

Fig. 7 (A) XRD Pattern of Sugarcane Bagasse Ash

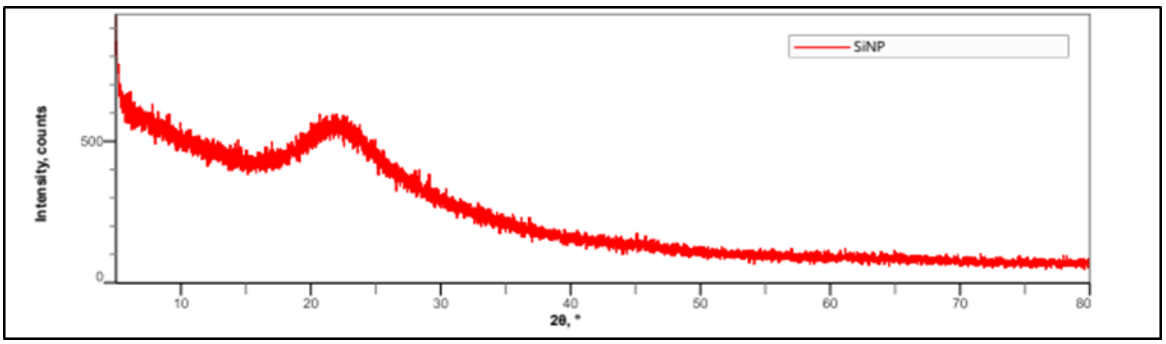

Fig. 7 (B) XRD Pattern of Optimized batch of (ASiNP)

The TEM images show silica nanoparticles have rough porous surfaces with irregular morphology. This can confirm that the silica nanoparticles are mesoporous in nature.(Fig. 8 (A) and Fig. 8 (B). The SEM Images in Fig. 9 (A) and Fig. 9 (B) show cluster-like irregular shaped arrangement with Porous and linked particles in nanoscale. The images are taken at magnifications of 50,000x and 1,00,000x respectively. The amorphous nature of the nano silica can be confirmed as no clear margins are visible and agglomerates can be clearly seen.

ISSN : 2583-6404
May - June 2023

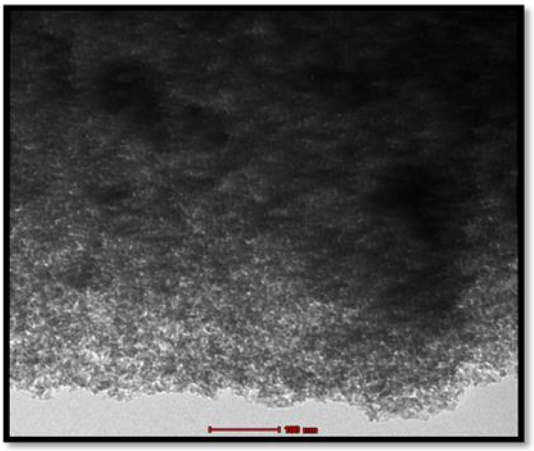

Fig. 8 (A)

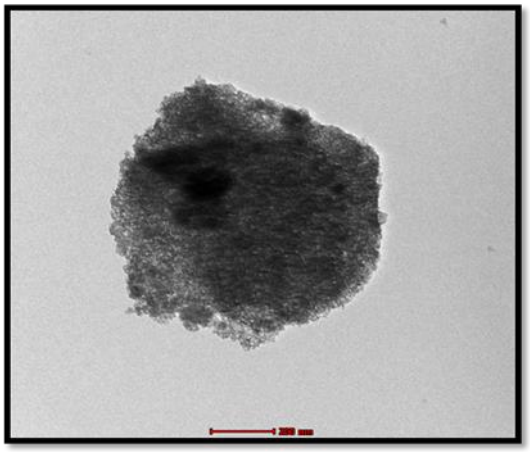

Fig. 8 (B)
Fig.8 (A) & (B) TEM Images of (ASiNPs)

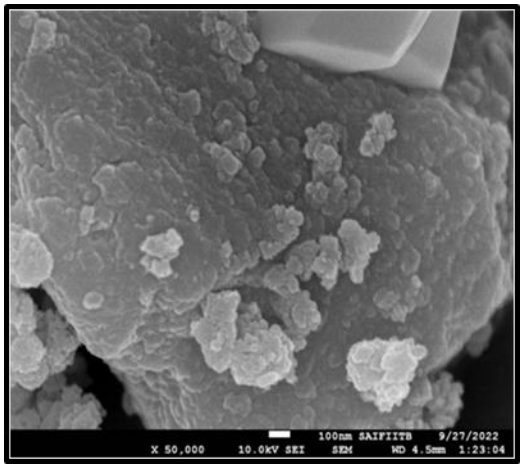

Fig. 9 (A)

ISSN : 2583-6404
May - June 2023

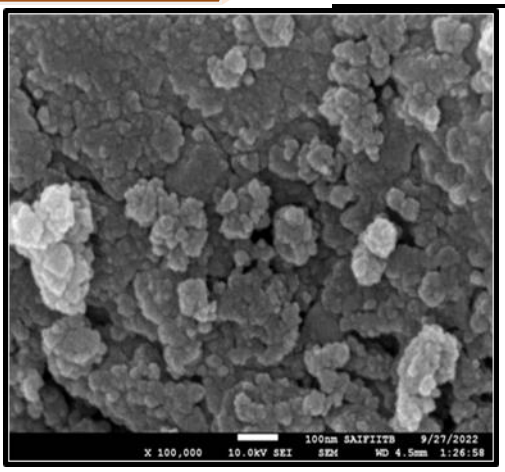

Fig. 9 (B)

Fig. 9 (A) & (B) SEM Images of (ASiNPs)

The elemental composition analysis was performed through EDS study and the results are presented in Table. 5. It was observed that only two strong peaks of O Kα and Si Kα were present in nano silica samples. Fig.10 represents the EDS plot with a small peak of Na Kα can also be seen, it is due to the presence of sodium ions from sodium silicate salt formed as an intermediate. No other peak in the EDS study denoted the sample is free from any other impurities.

Table. 5 Data for EDS Study

Element	**Weight%**	**Atomic%**
O Kα	53.14	66.04
Si Kα	41.87	29.64
Na Kα	4.99	4.32
Total	**100.00**	

Elemental maps of the optimized batch of amorphous silica nanoparticles are shown in Fig.11 (A) and Fig.11 (C) of Silicon and Oxygen as an element. Fig 11 (B) represents the microscopic image of the same cross section of the area under study. The images give an idea about the functional composition and imaging of the various elements present in the nano silica sample under study. Si and O maps characterize the silica particle's surface, revealing that the entire particle's surface consists of Si and O.[8]

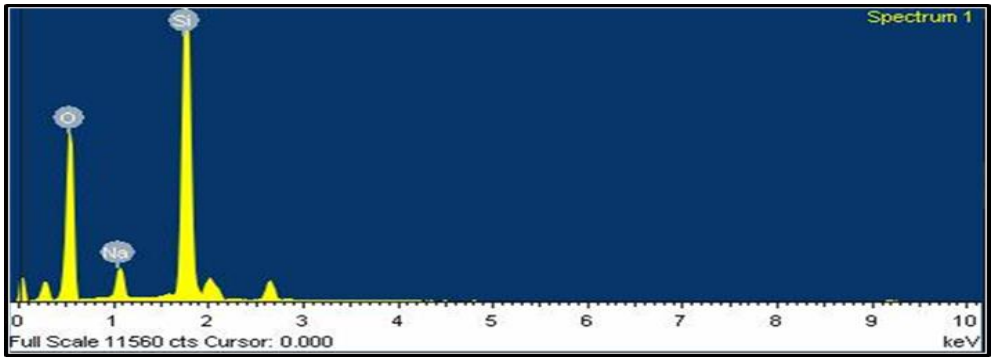

Fig. 10 Electron Dispersive Spectrophotometry of Optimized Batch of (ASiNPs)

ISSN : 2583-6404
May - June 2023

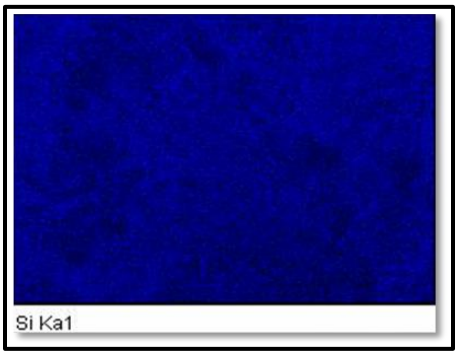

Fig. 11 (A) Silicon

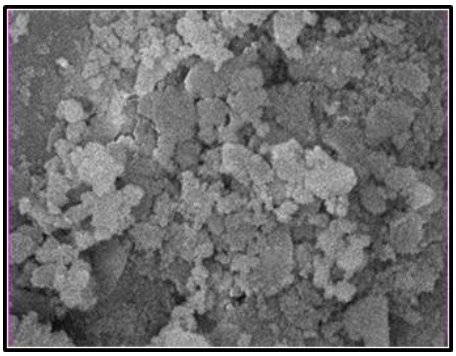
Fig. 11 (B) Crosssection Image

Fig. 11 (C) Oxygen

Fig. 11 Elemental maps of Silicon, & Oxygen

The Amorphousness of silica nanoparticles was found out using a rapid titrimetric analytical technique.[9] The process was mainly based upon reacting a test sample forming a glycerosilicate complex and titrating it with an aqueous Glycerolic Barium Hydroxide solution and the determination of free amorphous silica in glycerol was calculated using the below formula.

$$(FAS)_{gly} = \frac{m_2 (V_3 - V_2)}{m_1 (V_1 - V_2)} \times 100$$

Where m_1 and m_2 represents the quantity of standard silica and sample silica, and V_1, V_2, V_3 represent the volume of titrant for standard silica, for blank titration and for Sample of silica under study. Table 6. denotes the results of the rapid titrimetric analysis for determining free amorphous

ISSN : 2583-6404
May - June 2023

content of the silica nanoparticles. The % FAS for Batch C which was also the optimized batch of the silica nanoparticles was found to be 46.5 %

Table 6. Results of the Titrimetric Analysis for % FAS

Batch	**m_1 (Std)**	**m_2 (Sample)**	**V_1 (Std)**	**V_2 (Blank)**	**V_3 (Sample)**	**%FAS**
Batch A	200 mg	20 mg	13.3 ml	11 ml	19.2 ml	35 %
Batch B	200 mg	20 mg	13.5 ml	11 ml	16.8 ml	23.2 %
Batch C	200 mg	20 mg	13.0 ml	11 ml	20.3 ml	46.5 %

Conclusion

The product development tool of Quality by Design was successfully applied to green or biogenic synthesis of amorphous silica nanoparticles. The sol gel method of synthesis was applied and sugarcane bagasse, which is a by-product of the sugarcane industry, was used as a raw material for the precursor of nanoparticle synthesis.

The product development tool of Quality by Design and its various aspects such as CQA, CPP, CMA were applied for the optimization of the synthesis process. For the study the CQA was selected as particle size, the CPP was selected as Stirring Temperature, and the CMA was selected as concentration of NaOH. A risk assessment matrix was also established to determine the various interactions of the input variables and output variables. Factorial designs helped to choose a design space for the nanoparticle synthesis. The effect of various parameters and material attributes such as Concentration of sodium hydroxide and Stirring temperature were chosen as input variables and output parameter of particle size was analyzed. The model was found to be significant.

Various characterizations such as SEM, TEM, EDS, FTIR etc were carried out to gain insights on surface morphology and elemental composition and the amorphous nature of the nanoparticles was quantitatively determined using a rapid analytical technique. The software helped to establish a relationship between the input and output parameters with the help of Contour plots and response surface diagrams. Thereby aiding in the selection of the optimized batch that synthesized amorphous silica nanoparticles with the least particle size.

References

1. Abolghasemi, R.; Haghighi, M.; Solgi, M.; Mobinikhaledi, A. Rapid synthesis of ZnO nanoparticles by waste thyme (*Thymus vulgaris* L.). *Int. J. Environ. Sci. Technol.* 2019, *16*, 6985–6990.

2. Norsuraya, S., Fazlena, H., & Norhasyimi, R. 2016. Sugarcane bagasse as a renewable source of silica to synthesize Santa Barbara Amorphous-15 (SBA-15). *Procedia Engineering*, 2016, *148*, 839-846.

3. Sonal Rane and Dr. Itishree Vaidya, A Starring role of biogenic nano silica for drug delivery and photocatalytic degradation. *European Journal of Pharmaceutical and Medical Research, r,* 2021, 8(9), 443-450.

4. (Q8 (R2): Pharmaceutical Development, Revision 1, ICH Harmonized Tripartite Guidelines, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2007 (https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf)

ISSN : 2583-6404
May - June 2023

5. G. Falk, G.P. Shinhe, L.B. Teixeira, E.G. Moraes, A.P. Novaes de Oliveira, Synthesis of silica nanoparticles from sugarcane bagasse ash and nano-silicon via magnesiothermic reactions, *Ceramics International*, 45(17), 2019, 21618-21624
6. Temesgen Abeto Amibo, Surafel Mustefa Beyan, Tsegaye Markos Damite, Production and Optimization of Bio-Based Silica Nanoparticle from Teff Straw (*Eragrostis tef*) Using RSM-Based Modeling, Characterization Aspects, and Adsorption Efficacy of Methyl Orange Dye, *Journal of Chemistry*, 2022, 15
7. Nayak, P. P., & Datta, A. K, Synthesis of SiO2-nanoparticles from rice husk ash and its comparison with commercial amorphous silica through material characterization. *Silicon*, 2021 *13*(4), 1209-1214.
8. Rades, S., Hodoroaba, V. D., Salge, T., Wirth, T., Lobera, M. P., Labrador, R. H., & Unger, W. E. High-resolution imaging with SEM/T-SEM, EDX and SAM as a combined methodical approach for morphological and elemental analyses of single engineered nanoparticles. *RSC advances*, 2014, *4*(91), 49577-49587.
9. Payá, J., Monzó, J.M., Borrachero, M.V., Mellado, A., & Ordóñez, L.M. Determination of amorphous silica in rice husk ash by a rapid analytical method. *Cement and Concrete Research*, 2001, *31*, 227-231.