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Applying the Taguchi Method to Optimise the Size of Silica Nanoparticles Entrapped with Rifampicin for a Drug Delivery System

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Abstract: *The aim of this study was to optimise the experimental conditions for the synthesis of silica nanoparticles. In achieving this, the amount of butanol, the amount of surfactant, the amount of silica precursor, the synthesis temperature and the agitation speed were optimised by applying the Taguchi orthogonal arrays method. The optimal synthesis conditions for silica nanoparticle production were a temperature of 50°C, 6 ml butanol, 7 ml Tween 80, 3 ml trimethoxyvinylsilane (TMVS), and an agitation speed of 320 rpm. The nanoparticle size was characterised to optimise the synthesis conditions and determined to be smaller than 100 nm using a Malvern Zetasizer Nano ZS and a transmission electron microscope (TEM).*

Keywords: Silica nanoparticles, rifampicin, drug delivery system, synthesis of silica, Taguchi method

1. INTRODUCTION

Over the past decades, the drug delivery system (DDS) concept has made great progress in the treatment of a variety of diseases and in cancer therapy. Some of the most important aspects of DDS include high stability in biological media, high carrier capacity, potential use for both hydrophilic and hydrophobic molecules, tuneable size for targeted delivery and controllable release of molecules. Nanoparticles for DDS are typically made of biocompatible and biodegradable materials such as polymers and ceramics. Ceramic nanoparticles such as those composed of silica have attracted much attention in recent years due to their unique properties, which include biocompatibility, chemical and mechanical stability, large specific surface area, and porous structure.[1–4]

The properties of silica nanoparticles are affected by various parameters such as the reaction temperature, butanol concentration, surfactant type, silica precursor and agitation speed used during synthesis.[5,6] The interaction between these variables is complex; therefore, the analysis of this system to optimise the

processing conditions is time and labour intensive. The use of Taguchi method makes it possible to develop an acceptable formulation using minimum raw materials and save time. The primary goal of Taguchi method is to analyse all variables simultaneously using a few experiments. Software that implements this method can be successfully utilised to optimise various formulations within a given set of independent variables.[7–10]

In this study, the micelle entrapment approach was used to synthesise silica nanoparticles as nano-carriers for DDS, and the essential parameters were considered. The objectives of this work were to optimise the experimental conditions for the preparation of silica nanoparticles and to select the significant parameters that most strongly affect the nanoparticle size by the Taguchi design method. In addition, the nanoparticle size was evaluated by dynamic light scattering (DLS), and transmission electron microscopy (TEM) was used to characterise the size and shape of the silica nanoparticles for DDS.

2. EXPERIMENTAL

2.1 Materials

The materials required for the synthesis of silica nanoparticles were as follows. Tween 80-viscous liquid, trimethoxyvinylsilane (TMVS 98% pure) and rifampicin were obtained from Sigma-Aldrich Co. (Missouri, U.S.). 2-Butanol (99% pure) and a 10 M ammonium hydroxide solution (31.5% NH_3 pure) were obtained from Fischer Scientific (Fairlawn, New Jersey, U.S.). All chemicals were of analytical grade and used without any purification. De-ionised water from a Millipore filtration system (with a conductivity of 18.2 MΩ cm) was used in this study.

2.2 Sample Preparation

Silica nanoparticles were prepared via the micelle formation approach. First, 5.5 ml Tween 80 was dissolved in 200 ml of de-ionised water. The mixture was stirred for 15 min before 200 µl of prepared NH_3 (1 ml NH_3 was dissolved in 1 ml de-ionised water) was added to ensure the pH was maintained within the range of 9–11. Then, butanol was poured into the mixture and continuously stirred for 5 min. The mixture was then transferred into a preheated reactor at a set temperature and continuously stirred at 320 rpm for 1 h. A prepared rifampicin drug solution (839 mg of rifampicin dissolved in 1.5 ml methanol) was then added to the abovementioned mixture and continuously stirred under the same conditions. After an hour, TMVS was added as a silica precursor. The mixture was continuously stirred overnight at 320 rpm and maintained at a set

temperature, which yielded a total volume of 250 ml. The produced silica was then subjected to dialysis for 5 days to remove the surfactant. Finally, the sample was collected in a bottle and stored in a refrigerator until further testing.

2.3 Characterisation

The synthesised silica nanoparticles were characterised using different techniques. The particle size was characterised by dynamic light scattering (Zetasizer Nano ZS, Malvern Instrument, U.K.), whereas images of the samples were captured using transmission electron microscopy (TEM; Philips, model CM12, Eindhoven, Netherlands). To prepare samples for TEM observation, a drop of silica nanoparticles was placed on a copper grid coated with carbon and air-dried for 3 min at room temperature. Then, the grid was examined by TEM without being stained.

2.4 Experimental Design

A specialised experimental design was used to develop an optimisation process for silica nanoparticle preparation. In this study, the Taguchi orthogonal arrays (OA) method was used to identify the optimal conditions and to select the synthesis parameters that have the most significant effect on the size of silica nanoparticles. To minimise the number of experiments, Taguchi OA was implemented using Design Expert statistical software version 8.0.6.1 (Stat-Ease, Inc., Minneapolis, U.S.). Taguchi OA was applied by choosing 5 parameters that could affect the size of silica nanoparticles. The levels of each parameter are listed in Table 1. The orthogonal arrays of the L_{16} type were used, indicating that 16 experiments were required to study all of the parameters involved, with the target output parameter being the particle size (particle diameter in nanometres), as shown in Table 2.

Table 1: Factors and levels employed in Taguchi method.

Code	Factor	Level			
		1	2	3	4
A	Temperature (°C)	40	50	60	70
B	Amount of 2-butanol (ml)	4	6	8	10
C	Amount of Tween 80 (ml)	3	5	7	9
D	Amount of TMVS (ml)	1	2	3	4
E	Agitation speed (rpm)	220	320	420	520

Table 2: Combination of variables of the Taguchi orthogonal experimental design (16 runs [4 levels and 5 factors]).

Run	A Temperature	B 2-Butanol	C Tween 80	D TMVS	E Agitation speed	Average particle size (nm)	S/N ratio (dB)
1	40	4	3	1	220	18.24	–25.22
2	40	6	5	2	320	31.22	–29.89
3	40	8	7	3	420	60.45	–35.63
4	40	10	9	4	520	101.53	–40.13
5	50	4	7	2	520	45.81	–33.22
6	50	6	9	1	420	63.66	–36.08
7	50	8	3	4	320	54.37	–34.71
8	50	10	5	3	220	68.98	–36.77
9	60	4	9	3	320	95.68	–39.17
10	60	6	7	4	220	111.47	–40.94
11	60	8	5	1	520	46.47	–33.34
12	60	10	3	2	420	87.74	–38.86
13	70	4	5	4	420	126.87	–42.07
14	70	6	3	3	520	69.69	–36.86
15	70	8	9	2	220	115.30	–41.24
16	70	10	7	1	320	89.54	–39.04

3. RESULTS AND DISCUSSION

3.1 Analysis of Experimental Design

An OA design was used to identify the optimal conditions and to select the parameters that have the greatest effect on the size of silica nanoparticles. 16 experiments were performed to estimate the best conditions for the synthesis of the nanoparticles. The structure of the OA design and the results of particle size measurements are shown in Table 2, and the smallest nanoparticle size (18.24 nm) is presented in run number 1. The particle size for each sample of silica nanoparticles was determined by DLS. Experiments were performed for each run number in Table 2.

In the Taguchi method, the signal/noise (S/N) ratio is a measure of signal quality and deviation from the desired value. The term "signal" represents the desired value (mean), whereas "noise" represents an undesired value (standard deviation from mean) for output characteristics.[11] The S/N ratio varies with the

type of characteristic considered. A "smaller-the-better" type of S/N ratio is used in analyses aimed at achieving high accuracy. This type of S/N ratio is defined as follows:

$$\text{S/N ratio [dB]} = -10 \log [y_1^2 + y_2^2 + y_3^2 + \ldots. /n]$$

Table 2 shows the S/N ratio for each particle size calculated by the above-described equation, and the results of particle size measurements performed by DLS. The mean S/N ratio for each level of the parameters and the response in terms of the S/N ratio is shown in Table 3. The highest value of A indicates that temperature has the most significant effect on the size of silica nanoparticles. The amount of TMVS has the second most significant effect on nanoparticle size, and the agitation speed has the weakest effect.

Table 3: S/N response table for silica nanoparticle size.

Factors	Mean S/N ratio (dB) for particle size				Contribution (%)
	Level 1	Level 2	Level 3	Level 4	
A	–32.72	–35.19	–38.19	–39.80	28.92
B	–35.03	–35.94	–36.23	–38.70	14.99
C	–33.91	–35.52	–37.21	–39.27	21.85
D	–33.42	–35.80	–37.22	–39.46	24.66
E	–36.04	–35.81	–38.16	–35.89	9.58

Table 4 shows the main effects of the various factors on the size of silica nanoparticles. The Model *F*-value of 4.64 implies the model is significant. There is only a 2.02% chance that such a large Model *F*-value could occur due to noise. Values of "Prob > *F*" less than 0.0500 indicate that the model terms are significant. Therefore, based on the results presented in Table 3 and Table 4, A and C, which represent temperature and the amount of TMVS, are significant model terms, and the R-squared value is equal to 0.7558.

Table 4: ANOVA table of silica nanoparticle size.

Sources	Degrees of freedom	Sum of squares	*F*-value	*p*-value prob > F
Model	6	0.72	4.64	0.0202
A – Temperature	3	0.35	4.52	0.0339
D – TMVS	3	0.37	4.76	0.0296
Residual	9	0.23	–	–
Cor total	15	0.95	–	–

The response graphs corresponding to the aforementioned factors are presented in Figure 1. As shown in the figure, the optimal conditions for the synthesis of silica nanoparticles are a temperature at level 2, an amount of 2-butanol at level 2, an amount of Tween 80 at level 3, an amount of TMVS at level 3, and an agitation speed at level 2.

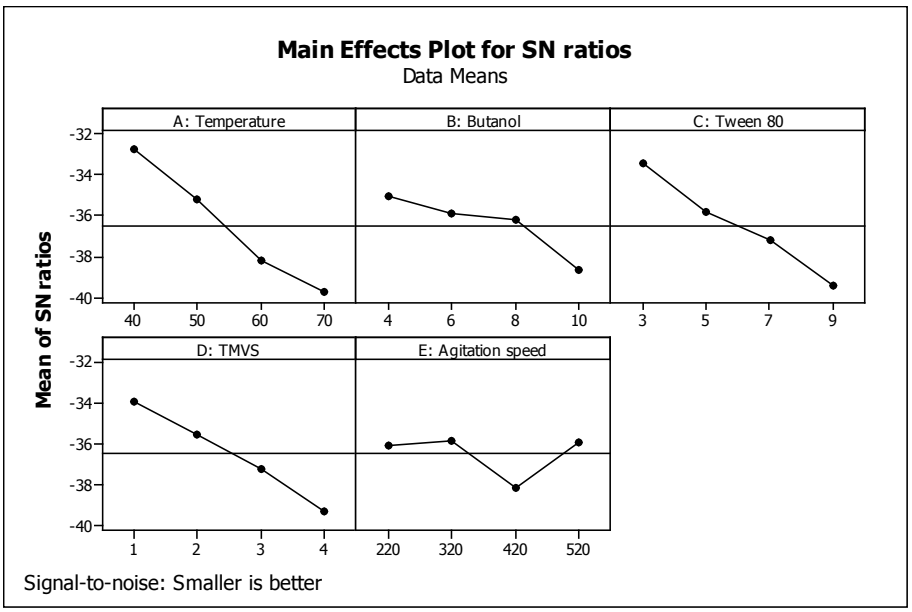


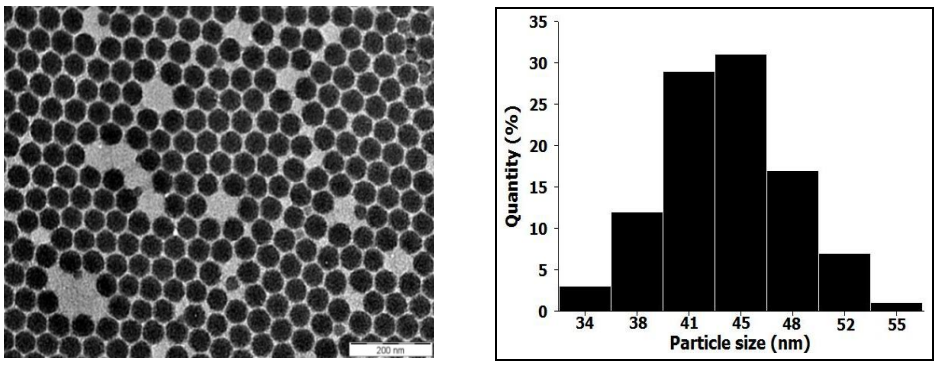
Figure 1: Response graph of S/N ratio for smaller-the-better analysis of nanoparticle size.

The following were determined to be the best parameters for the production of silica nanoparticles: temperature = 50°C; amount of 2-butanol = 6 ml; amount of Tween 80 = 7 ml; amount of TMVS = 3 ml; and agitation speed = 320 rpm. Under these conditions, the software program estimated the nanoparticle size to be 32.07 nm, whereas the nanoparticle size achieved by experiment was 28.91 nm, as shown in Figure 2. Experiments were conducted to validate the optimal parameters obtained by the Taguchi method. Good agreement was observed between the predicted particle size and the experimental particle size. Consequently, the size of synthesised silica nanoparticles can be improved through the Taguchi method.

3.2 Characteristics and Determination of Silica Nanoparticle Size Distribution

TEM micrographs and the particle size distribution of the silica nanoparticles obtained under optimal conditions are shown in Figure 2. Silica nanoparticles with a mean size of 28.91 nm were prepared, as illustrated in Figure

2 (a). It was observed that the size distribution of the prepared silica nanoparticles was unimodal and uniform. The average diameter of the silica particles, which were nearly spherical, was determined based on the diameter of approximately 100 particles using TEM micrographs of a sample, as illustrated in Figure 2 (b).



(a) (b)

Figure 2: TEM image (a) and histogram of particle size distribution (b) of silica nanoparticles obtained under optimal conditions.

4. CONCLUSION

This study demonstrated that the size of silica nanoparticles entrapped with rifampicin synthesised by the micelle entrapment approach can be manipulated by varying the process conditions. Using the Taguchi method, parameter A (temperature) was determined to have the most significant effect in tuning the size of silica nanoparticles. Based on the S/N ratio and ANOVA, the optimum conditions for preparing silica nanoparticles are a temperature of 50°C, 6 ml 2-butanol, 7 ml Tween 80, 3 ml trimethoxyvinylsilane (TMVS), and an agitation speed of 320 rpm. Under these conditions, silica nanoparticles measuring 28.91 nm were produced. This study shows that the Taguchi method is one of the most suitable methods for optimising experimental conditions to achieve the minimum size of silica nanoparticles for drug delivery systems.

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