Taguchi Orthogonal Array Design for the Optimization of Hydrogel Nanoparticles for the Intravenous Delivery of Small-Molecule Drugs

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ABSTRACT: In this study, a 16 runs Taguchi method was applied as an experimental design to establish the optimum conditions for hydrogel nanoparticle preparation. Five relevant factors, chitosan (CS) concentration, pentasodium tripolyphosphate (TPP) concentration, CS-to-TPP volume ratio, addition time of the TPP solution to the CS solution, and temperature, were selected as the main determinants, and the effects of each factor on the size of the hydrogel nanoparticles were studied at four levels. The statistical analysis revealed that the most important factors contributing to the achievement of minimum particle size were the CS-to-TPP volume ratio and the CS concentration. By solving a set of equations derived from the differentiation of the final model, we established the optimum conditions for hydrogel nanoparticle preparation as follows: CS concentration = 0.28% w/v, TPP concentration = 3.17% w/v, TPP/CS = 1 : 8, temperature = 25.66°C, and addition time of the TPP solution to the CS solution = 0.4 min. Also, an analysis of response at the different levels of the factors indicated that there was no remarkable interaction between them. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

Key words: hydrogels; modeling; nanoparticles

INTRODUCTION

Hydrogel nanoparticles (popularly referred to as *nanogels*), a new family of nanoparticulate systems, have been a point of considerable attention as a promising carrier for drug-delivery purposes. These nanogels have considerable potential and capability because they contain the characteristic features of the beneficial properties of hydrogels (because of their high degree of hydrophilicity) and nanoparticles (because of their small size) in drug delivery with the possibility of being actively or passively targeted to a desired biophase (e.g., tumor sites).[1] Besides the commonly used synthetic polymers, active research has focused on the preparation of nanoparticles with naturally occurring hydrophilic polymers, such as chitosan (CS). CS is a natural polysaccharide obtained via the partial deacetylation of chitin from crustacean shells. Chemically, CS is a copolymer of glucosamine and *N*-acetyl glucosamine. CS nanoparticles offer many advantages because of their proper stability, low toxicity, simple and mild preparation methods, and provide versatile routes of administration; they have gained more attention as a drug-delivery carrier. They prevent the use of hazardous organic solvents during particle fabrication because they are soluble in aqueous acidic solution.[2] CS has been widely studied in the preparation of nanoparticles for drug delivery and controlled drug release.[3–10] Different methods have been developed for the preparation of CS-based hydrogel nanoparticles.[11] Preparation methods based on the polyionic crosslinking of cationic CS molecules with polyanions have been particularly attractive because of the ease of the method, availability and low cost of the materials, and the possibility of loading different drugs into nanoparticles during the preparation procedure.[1] In these methods, a crosslinking network is formed via the formation of electrostatic intramolecular and intermolecular bonds throughout the CS chains mediated by a polyanion (e.g., a polyphosphate). This kind of spontaneous gel formation, generally referred to as *ionophoretic gelation*, is a very simple and available procedure, needs mild conditions, and results in highly homogeneous small-particle populations with controllable sizes of nanoparticles; therefore, it has been used extensively in recent years for the preparation of hydrogel nanoparticles loaded with different drugs and other

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bioactive agents.[11] Many researchers have explored the capacity of the CS–pentasodium tripolyphosphate (TPP) nanosystem for loading peptides, proteins, oligonucleotides, and plasmid DNA for potential pharmaceutical uses.[12–14]

The theoretical concepts and importance of optimization methodology and experimental design in research and development efforts has been illustrated in many studies.[15–17] As a general rule, experimental design techniques primarily allow the investigator to select which factors and which level has a significant effect on the final response defined in the model assumptions. This, in turn, helps one to reach the optimum conditions. One of these experimental design methods is the Taguchi orthogonal array (OA), which was first introduced by Genichi Taguchi.[18,19] The basic concept behind the Taguchi method is simple in theory: products are designed to be robust enough to achieve high quality despite environmental fluctuations, and the manufacturing process is improved through improved process design rather than through expensive process-control technologies. In this robust parameter design, the primary goal is for one to find factor settings that minimize response variations while adjusting the process on target. A process designed with this goal will produce more consistent output. Robust parameter design using a Taguchi matrix (OAs) allows us to analyze many factors with few runs. Taguchi designs are balanced; that is, no factor is weighted more or less in an experiment; this allows factors to be analyzed independently of each other and in the presence of each other. In this study, an experimental design based on the Taguchi model was used for optimization of the hydrogel nanoparticles produced by the ionotropic gelation method, intended for the delivery of small-molecule drugs. The simple, efficient approach, which was used throughout this study, allowed us to optimize a preparation method on the basis of a minimum number of experimental runs while not losing the valuable data dispersion and central indices within the array of findings. Finally, it is shown that this model had a good robustness for the loading of various small-molecule drugs.

EXPERIMENTAL

Materials

CS, with a deacetylation degree of 85% (lot no. 212F498-89), was from Sigma-Aldrich (St. louis, MO, USA). TPP (lot no. K36643499-742) was purchased from Merck Co. (Darmstadt, Germany). Sodium valproate was kindly donated by Rouz Darou Pharmaceuticals (Tehran, Iran). Desferrioxamine mesylate (Desferal 500-mg vials, Novartis Pharma AG, Basle, Switzerland) was purchased locally and was used as the drug active material throughout the study. All other chemicals and reagents were from the highest available purity grades and were purchased locally.

Methods

Preparation of the hydrogel nanoparticles

The hydrogel nanoparticles were prepared with the ionotropic gelation method, that is, via the ionic cross-linking of CS with TPP. As the typical finally optimized setting, the TPP solution (3.2% w/v) was added to CS solution (0.3% w/v) in acetate buffer (0.67*M*, pH 4) in a dropwise manner at a volume ratio of 1 : 8 (TPP/CS) over a 25-s time period with constant magnetic stirring (1500 rpm) at 25°C, and the stirring was continued for an additional 20-min period after all of the TPP solution was added. Finally, the resulting nanodispersion was centrifuged at 3000 g for 5 min (to separate it from out-of-range undesired associations), and the supernatant containing our target nanoparticles was divided for size analysis.

Preparation and characterization of the drug-loaded nanoparticles

To evaluate the drug-loading capability of the prepared nanoparticles, two widely used small-molecule drugs, sodium valproate (an antiepileptic agent) and desferrioxamine (an iron chelating agent), were loaded into the nanoparticles by the dissolution of both drugs separately in TPP solution before the addition of this solution to CS for gelation according to the optimal method variables settings described in the previous section. On the basis of a series of concentration–titration experiments, the selected optimal initial concentrations of valproate and desferrioxamine in the TPP solution were 2.5 and 6.25 mg/mL, respectively. The following drug-loading parameters were determined in each case for the evaluation of the nanocarrier capacity of the prepared nanoparticles:

$$\text{Loading amount (LA)} = (\text{Total drug concentration} - \text{Unloaded drug concentration}) \times \text{Total sample volume}$$

$$\text{Loading efficiency (LE)} = (\text{LA}/\text{Total drug added during the loading procedure}) \times 100$$

$$\text{Loading ratio (LR)} = [(\text{Total drug concentration} - \text{Unloaded durg concentration})/\text{Total drug concentration}] \times 100$$

The total drug concentrations in the nanodispersions were determined after the destruction of the

TABLE I
Variables and Their Corresponding Values in the Taguchi Orthogonal Experimental Design for Hydrogel Nanoparticle Preparation with Ionotropic Gelation

Code	Variable	Level 1	2	3	4
A	Concentration of CS (w/v %)	0.15	0.2	0.25	0.3
B	Concentration of TPP (w/v %)	0.5	1	2.5	5
C	TPP/CS solutions volume ratio	1 : 8	1 : 4	1 : 2	1 : 1
D	Time of addition of the TPP solution (min)	0	1	2	5
E	Temperature (°C)	25	35	45	55

nanoparticles via the addition of 0.015 mL/mL $HClO_4$ (70%) to the dispersions and, then, measurement of the drug concentration in the resulting solution with the developed high performance liquid chromatography (HPLC) method. The unloaded drug concentrations were determined after the separation of nanoparticles from the aqueous medium containing unloaded drug via filtration through a 50-nm membrane filter (Millipore, Bedford, CT) and, then, measurement of the amount of free (unloaded) drug in the filtrates with the developed HPLC method.

The particle morphology and possible aggregation was examined by transmission electron microscopy (TEM; Philips, model CM10, Eindhoven, Netherlands). The samples were immobilized on copper grids. They were dried at room temperature and then examined with TEM without being stained.

The *in vitro* release profiles of sodium valproate and desferrioxamine from the prepared hydrogel nanoparticles were determined as follows: the drug-loaded nanoparticles were prepared with the optimal methodological setup, and the final nanodispersion was then divided into fifteen 1-mL portions in 1.5-mL polypropylene microtubes. The samples were shaken gently (15 rpm) while being incubated at 37°C with a vertically shaking incubator designed and assembled in-house. At the beginning of the test and at intervals of 1, 2, 4, 8, 12, 24, 48, and 72 h and 1 and 2 weeks, one of the aliquots was harvested, and after a 1 : 10 dilution with distilled/filtered water, the total drug concentration in the nanodispersion was determined by destruction of the nanoparticles by the addition of 0.015 mL of $HCLO_4$ (70%) to the 1-mL dispersion followed by HPLC analysis for the drug content. At the same time, the free drug content in each sample was determined after the nanodispersion was passed through a 50-nm membrane filter (Millipore) and HPLC analysis of the drug content in the filtrate. The free drug concentration in each sample was the ordinate of the release profiles. The release experiment was repeated three times.

Particle size analysis

The statistical central and dispersion indices of the particle sizes (represented by the particle diameters) of the freshly prepared hydrogel nanoparticles were determined throughout the study with a

TABLE II
Combination of Variables of the Taguchi Orthogonal Experimental Design [16 Runs (4 Levels and 5 Factors)]

Run number	Concentration of CS	Concentration of TPP	TPP/CS (v/v)	Time of addition	Temperature	Particle size (nm)
1	0.15	0.5	1/8	0	25	100.333
2	0.15	1	1/4	1	35	520
3	0.15	2.5	1/2	2	45	339
4	0.15	5	1/1	5	55	2280
5	0.2	0.5	1/4	2	55	244.667
6	0.2	1	1/8	5	45	158
7	0.2	2.5	1/1	0	35	1310.67
8	0.2	5	1/2	1	25	306.667
9	0.25	0.5	1/2	5	35	537.333
10	0.25	1	1/1	2	25	514.667
11	0.25	2.5	1/8	1	55	110.667
12	0.25	5	1/4	0	45	81
13	0.3	0.5	1/1	1	45	522
14	0.3	1	1/2	0	55	459
15	0.3	2.5	1/4	5	25	107.333
16	0.3	5	1/8	2	35	63

TABLE III
Sodium Valproate and Desferrioxamine Loaded Hydrogel Nanoparticles

Parameter	Hydrogel nanoparticles	
	Valproate-loaded	Desferrioxamine-loaded
Particle size (number-based) before drug loading (nm)	62 ± 2.01	62 ± 0.57
Particle size (volume-based) before drug loading (nm)	78 ± 2.09	77 ± 1
Particle size (number-based) after drug loading (nm)	63 ± 1	62 ± 0.57
Particle size (volume-based) after drug loading (nm)	79 ± 3.21	77 ± 0.57
Mean ζ potential (mV)	−6.80	+4.14
LA (mg)[a]	3.68 ± 0.66	42.91 ± 1.05
LE (%)[b]	21.8 ± 3.90	26.1 ± 0.63
LR[c]	23.7 ± 4.54	30.26 ± 0.59

[a] LA = (Total drug concentration − Unloaded valproate concentration) × Sample volume.
[b] LE = (LA/Total drug added during the loading procedure) × 100.
[c] LR = [(Total drug concentration − Unloaded drug concentration)/Total drug concentration] × 100.

laser-diffraction-based particle size analyzer (Shimadzu, model SALD-2101, Kyoto, Japan). The particle size measurements were performed with a quartz cell in the manual mode. Samples were diluted to appropriate concentrations with deionized/filtered water. Analysis was done in triplicate for each sample.

In addition, to gain insight into the finally optimized nanoparticle surface charges, a parameter with remarkable impact on the *in vivo* behavior of the nanoparticles, the ζ potential of the finally optimized nanoparticles, was measured both without the drugs and as loaded by both drugs with a ζ sizer (Malvern, Worcestershire, United Kingdom).

Experimental design

An experimental design was used to develop a model for optimal hydrogel nanoparticle preparation. The effective variables were coded according to the following equation:

$$X_a = (X_i - X_c)/\Delta X_i$$

where X_a is the coded value of an independent variable, X_i is the independent variable's real value, X_c is the independent variable's real value at the center point, and ΔX_i is the step change value. The number-based particle diameter was taken as the dependent variable or response.

In this study, a Taguchi OA was designed to identify factors having a significant effect on the size of the hydrogel nanoparticles. A Taguchi OA is usually categorized with fractional factorial designs. The major benefit of the application of a factorial design is the reduced number of experiments needed to be carried out to obtain maximal information. Because of the nature of the ionotropic gelation procedure intended for nanoparticle fabrication in this study, a five-variable, four-level values matrix was constructed (Table I), with the target output parameter being the particle size (the diameter in nanometers).

The Taguchi array, which led to an optimized combination through 16 experiments, is listed in Table II. All of the variable levels were determined and used as input functions in our statistical model on the basis of our extensive preoptimization experiments, which resulted in preliminary point estimates for the variables. All of the trials were replicated three times to obtain the precision needed for the final statistical inference. Finally, the optimized run, selected by the statistical analysis, was repeated three times with and without drug to validate the estimated optimal settings of the variable values.

Data analysis

The statistical analysis of the results was performed with Design Expert statistical software version 6.0.10 (Stat-Ease, Inc., Minneapolis, MN). The qualities of the fitted models were examined by the coefficient of determination (R^2).The data were analyzed by analysis of variance (ANOVA) combined with the F test to evaluate whether a given term had a significant effect on the target value ($p < 0.05$). The location of the optimum was determined by the solution

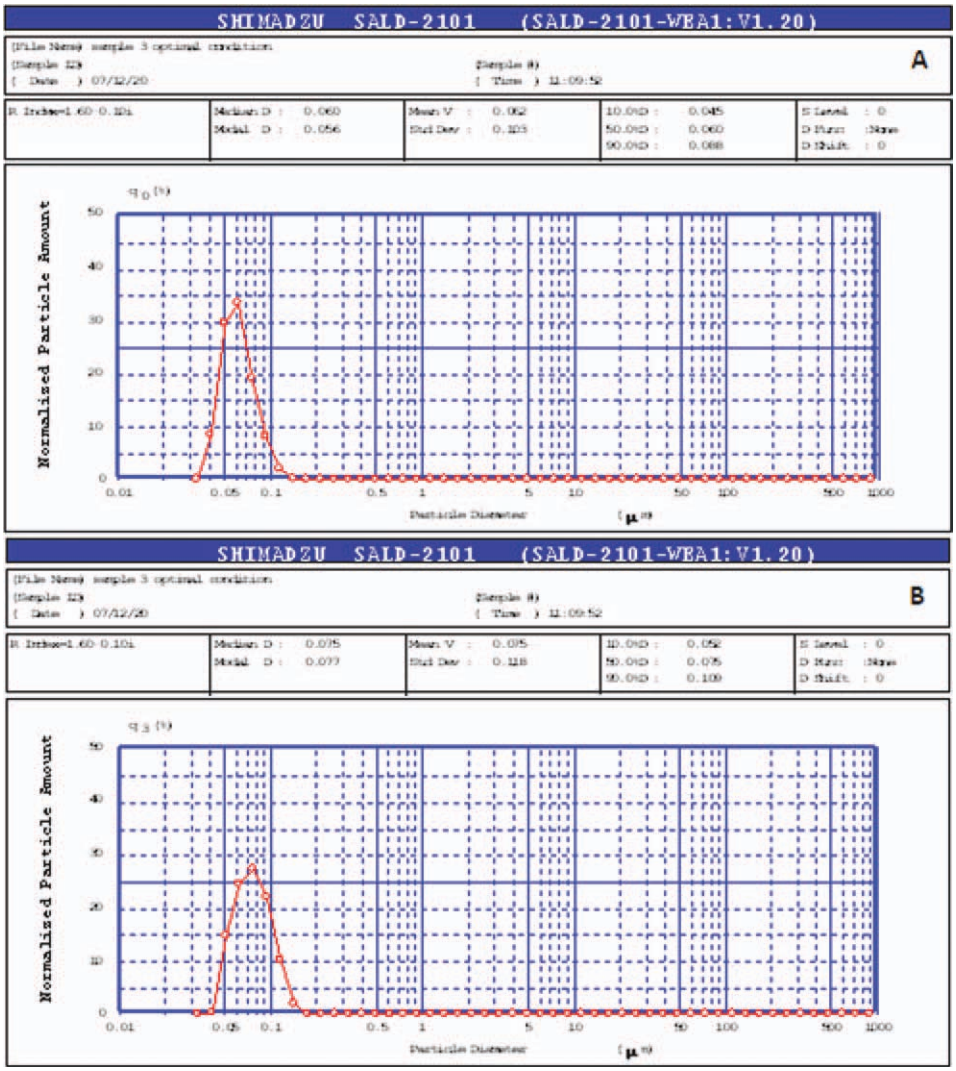

Figure 1 (A) Number-based and (B) volume-based diameter distributions of the hydrogel nanoparticles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of the set of equations derived by the differentiation of the final model.

RESULTS AND DISCUSSION

Preparation and characterization of the hydrogel nanoparticles

Small-molecule-drug-loaded hydrogel nanoparticles were prepared successfully and highly reproducibly with the set of optimal method variables determined by the statistical approach of this study (Table III).

The particles obtained in this study were unidispersed (unimodal curves) with appropriate sizes (<100 nm) in terms of the ultimate goal of the study, a time-controlled, intravenous drug-delivery system (Fig. 1). Most importantly, all of the mean, median, and modal diameter values remained without any significant changes after both of the drugs, desferrioxamine and sodium valproate, were loaded ($p > 0.05$). Furthermore, the size dispersity of the nanoparticles population was about the same in the drug-loaded and unloaded nanoparticles. The polydispersity indices for the optimized nanogels were 0.34 and 0.36 for the unloaded and drug-loaded ones, respectively. This means that under optimum conditions, the drug-loading procedure had no significant effect on the particle size or size distribution of the nanoparticles. Other similar studies have reported polydispersity index values larger than ours.[20,21] The particle diameters of about 60 nm obtained in this study for the drug-loaded hydrogel nanoparticles were remarkably lower compared to those of 300–400 nm for insulin-loaded hydrogel nanoparticles[22,23] and were greater than that of 120 nm for ammonium glycyrrhizinate-loaded hydrogel nanoparticles,[24] both prepared by ionotropic gelation processes. In these and most other similar studies,

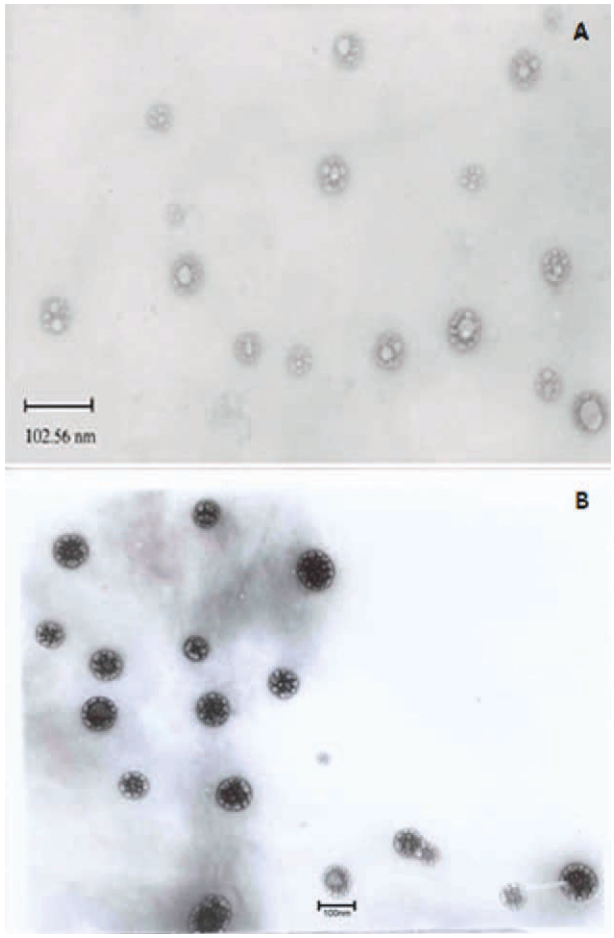

Figure 2 TEM images of the hydrogel nanoparticles loaded by (A) sodium valproate and (B) desferrioxamine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the size of the nanoparticles increased with therapeutic agent loading, whereas the size of our nanoparticles was not changed significantly after drug loading. Interestingly, the small size of the drug-loaded nanoparticles obtained in this study is a highly favorable prerequisite for a long-circulating drug-delivery system.

The drug-loading parameters obtained in this study, although deserving further improvement, is promising given the purpose of the study, that is, providing a circulating drug reservoir capable of the timed control release of the drug for a given period of time. It is obvious that dose calculation should be carried out in practice on the basis of these data via consideration of an appropriate volume of the nanodispersion to be administered to the organism to reach a target plasma concentration.

The ζ potentials of both drug-loaded nanoparticles, although having opposite charges, were in the near-zero range, which is appropriate for a long-circulating carrier. Clearly, the positive net surface charge of the desferrioxamine-loaded nanoparticles was a result of contribution of the positively charged drug molecules and the CS itself as a polycationic polymer, and the negative net charge of the valproate-loaded nanoparticles reflected the presence and dominance of valproate with ionized carboxylic acid groups in the particle surfaces.

Figure 2 shows the morphological characteristics of the nanoparticles obtained by TEM. Size analysis of a spread of sample particles under TEM, although confirming the size profiles obtained from our particle size analysis, indicated that the size ranges of the valproate-loaded and desferrioxamine-loaded CS–TPP nanoparticles were apparently identical, and both had a spherical shape. The shapes of the particles were approximately spherical and smooth in texture, with almost a homogeneous structure; this was attributed to the relatively gentle preparation conditions of the nanoparticles. In addition, the presence of some satellite particles around the main

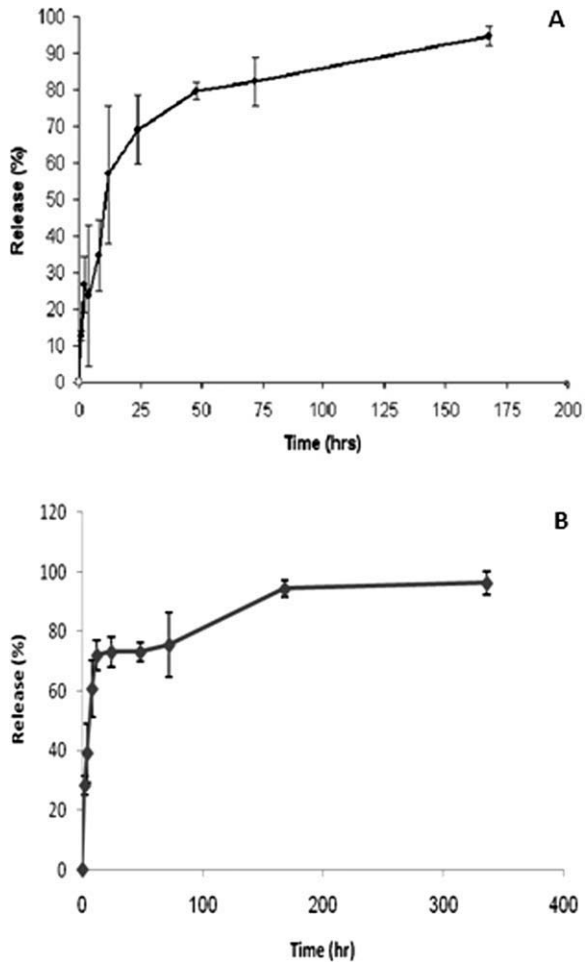

Figure 3 Release profiles of (A) sodium valproate and (B) desferrioxamine from the hydrogel nanoparticles.

TABLE IV
ANOVA for the First-Order Model

Source	Sum of squares	Degrees of freedom	Mean square	*F* value	$p > F$	
Model	2.51	5	0.50	11.70	0.0006	Significant
A	0.31	1	0.31	7.25	0.0226	Significant
B	0.039	1	0.039	0.90	0.3644	
C	2.02	1	2.02	47.22	<0.0001	Significant
D	0.046	1	0.046	1.08	0.3231	
E	0.088	1	0.088	2.06	0.1816	
Residual	0.43	10	0.043			

particles as typical behavior of the samples was noteworthy.

The release profiles of sodium valproate and desferrioxamine from the hydrogel nanoparticles while they were shaken at 37°C are shown in Figure 3. In the case of matrix devices such as the one we prepared in this study, the drug is uniformly distributed/dissolved in the matrix, and the release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than the matrix degradation, the mechanism of drug release becomes mainly governed by Fickian diffusion; otherwise, it depends on the degradation rate. Because we performed the drug-release experiments directly on the nanosuspension as prepared, without the replacement of the medium, to avoid any shock due to the external conditions on the nanoparticles and also because of the drug LR in the nanodispersion, we concluded from the release data that there was an initial burst release in the first 24 h of sampling, including about 60 and 70% of the loaded drug for sodium valproate and desferrioxamine, respectively. This release rate became gradually lower and was followed by a linear phase between the 2nd and 7th days. The burst release was hypothetically caused by a portion of the drug associated weakly with the nanoparticles, for example, those adsorbed on the surface of the microsphere.[25] The terminal apparently zero-order release, however, seemed to be related to a portion of the drug either bound to the nanoparticles more strongly, for example, via electrostatic associations, or entrapped deeply inside the nanogel structures. Drug association with hydrogel nanoparticles was evaluated by Fourier transform infrared spectroscopy (the data are not shown). This investigation revealed that there were some interactions between the drug molecules and polymer chains, especially in the sodium valproate-loaded carriers. As mentioned before, the slow release of the terminal phase might have been due to these interactions.

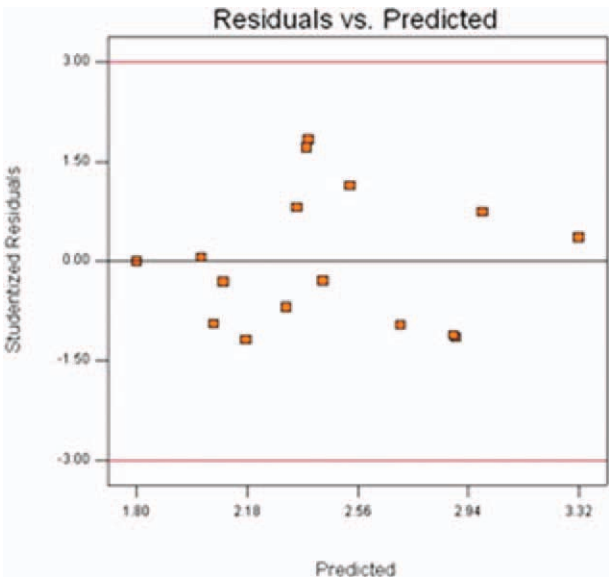

Figure 4 Studentized residuals versus predicted response by the final model. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

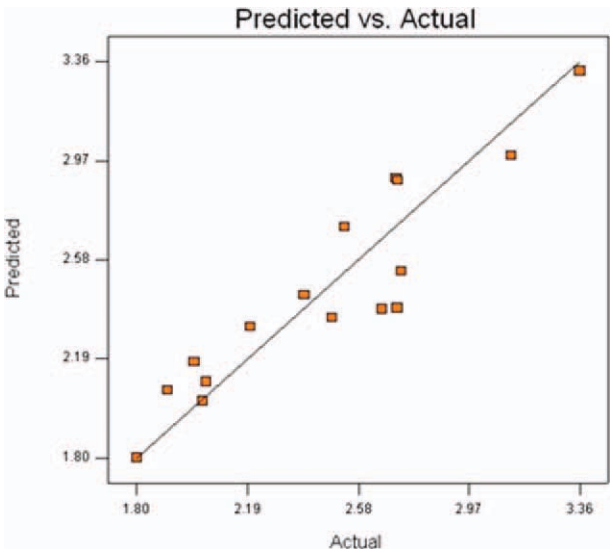

Figure 5 Predicted versus actual values by the final model. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

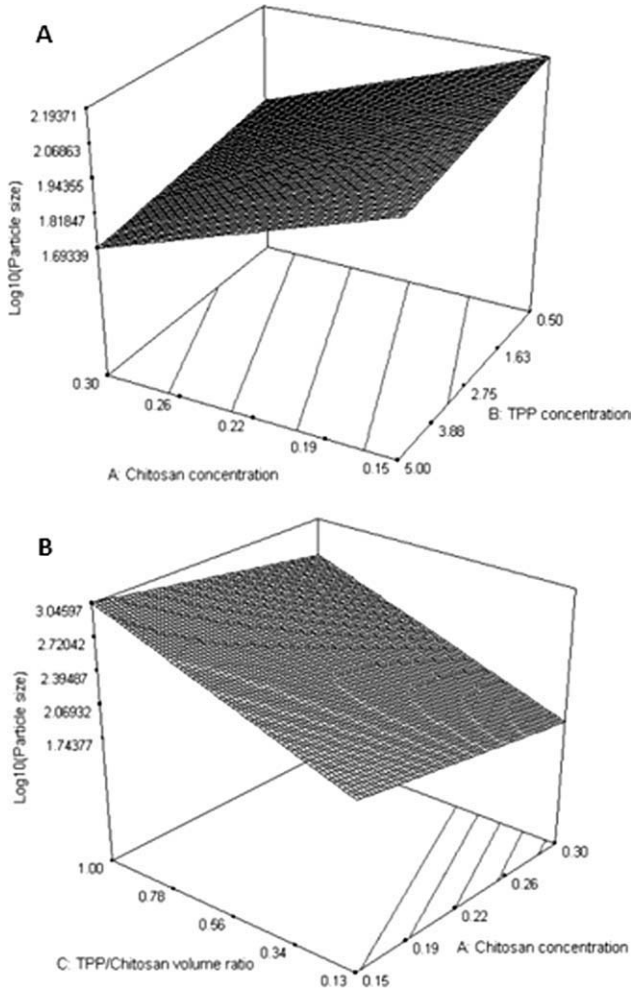

Figure 6 Response surface of the hydrogel nanoparticles size for different levels of (A) CS concentration and TPP concentration and (B) CS concentration and TPP/CS volume ratio.

Experimental design

The purpose of the first optimization step was to identify the factors that had a significant effect on the hydrogel nanoparticle size. As shown in Table II, 16 Taguchi OA experiments were considered. On the basis of the size data obtained, statistical treatment was then carried out with Fisher's statistical test. The overall F value of 11.70 implied that the model was significant. There is only a 0.06% chance that a model F value of 11.70 can occur because of the noise. Also, a p value of less than 0.05 indicates that the model terms are significant at the probability level of 95%. The p value of 0.0001 for the TPP/CS volume ratio showed that it had a very significant effect on the nanoparticle size. Also, the changing levels of CS concentration affected the particle size significantly, whereas other factors, including the TPP concentration, the addition time of the TPP solution, and the temperature, did not significantly influence the particle size within the level ranges tested. The ANOVA for the selected model is summarized in Table IV.

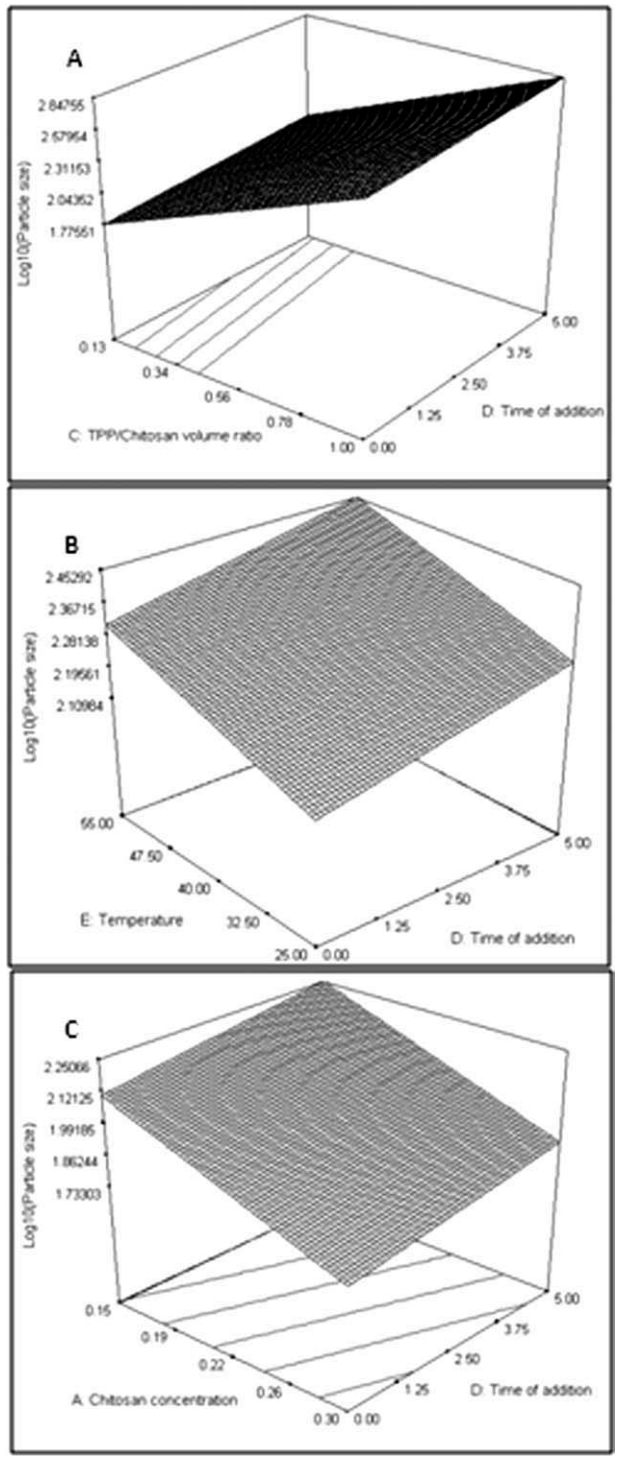

Figure 7 Response surface of the hydrogel nanoparticles size for different levels of (A) time of addition and TPP/CS volume ratio, (B) time of addition and temperature, and (C) time of addition and CS concentration.

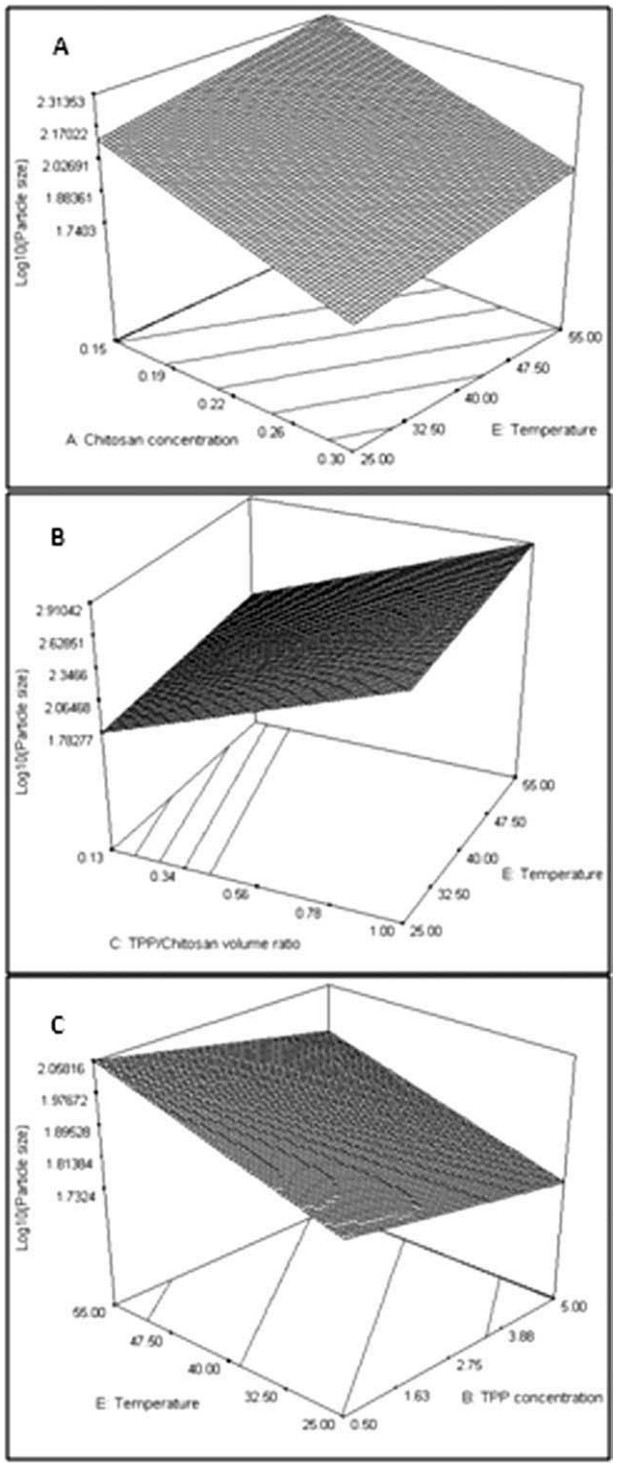

Figure 8 Response surface of the hydrogel nanoparticle size for different levels of (A) temperature and CS concentration, (B) temperature and TPP/CS volume ratio, and (C) temperature and TPP concentration.

To approach the vicinity of the optimum, a first-order model was fitted to the data obtained from the Taguchi OA design experiments. With the first-order model, the response surface was represented by a sloping plane. The values of the regression coefficients were calculated, and the following equation was derived with the coefficients of the coded variables:

$$\mathrm{Log}_{10}(\text{Particle size}) = 2.56 - 0.19A - 0.063B + 0.46C + 0.072D + 0.100E$$

Also, the final equation in terms of actual factors was

$$\begin{aligned}\mathrm{Log}_{10}(\text{Particle size}) &= 2.26601 - (2.49260 \times \text{CS concentration}) \\ &- (0.028094 \times \text{TPP concentration}) \\ &+ (1.06092 \times \text{TPP/CS volume ratio}) \\ &+ (0.028748 \times \text{Addition time of the TPP solution to the CS solution}) \\ &+ (6.64458 \times 10^3 \times \text{Temperature})\end{aligned}$$

The goodness of fit was expressed by R^2, which was calculated to be 0.85; this indicated that 85% of

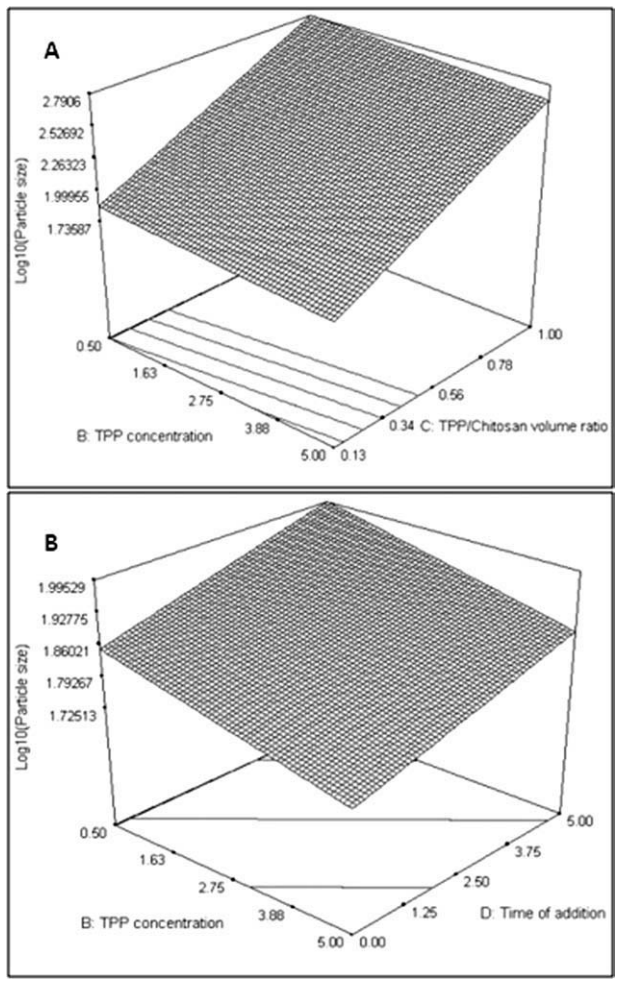

Figure 9 Response surface of the hydrogel nanoparticles size for different levels of (A) TPP concentration and TPP/CS volume ratio and (B) TPP concentration and time of addition.

the variability in the response could be explained by the model. Furthermore, the final model had an adjusted coefficient of determination (R^2_{Adj}) equal to 0.78. This supported the hypothesis that the model equation was sufficient to describe the response of the experimental observations pertaining to the hydrogel nanoparticle size. The ANOVA showed that the model F value of 11.70 was significant and that there was only a 0.06% likelihood that this large model F value could occur by chance. Also, the predicted coefficient of determination (R^2_{Pred}) of 0.6487 was in a reasonable agreement with the R^2_{Adj} of 0.7811. This means that the model equation had a sufficient ability to predict the particle size. In addition, adequate precision measured the signal-to-noise ratio. A ratio greater than 4 is desirable. Our ratio of 12.004 indicated an adequate signal.

Figure 4 displays the residual versus predicted response as determined from the final model. It shows a nearly constant variance throughout the response range. Figure 5 presents the predicted versus actual responses. It indicates that there was excellent agreement between the model and experimental data.

The location of the optimum was determined to be $A = 0.28$, $B = 3.17$, $C = 0.13$, $D = 0.4$, and $E = 25.66$, as obtained by the differentiation of the model given by the model equation. The predicted optimal hydrogel nanoparticle size corresponding to these values was 61.38 nm [Log_{10}(Particle size) = 1.788]. To confirm the model adequacy for predicting the minimum particle size, three additional experiments with this optimum condition were performed. The three replicated experiments yielded an average minimum size of 62 ± 2.01 nm. The good agreement between the predicted and experimental results verified the validity of the model and the existence of an optimal point. The response surfaces shown in Figures 6–9 were based on the final model, holding three variables constant at their optimum level, with the other two varied within their experimental range. As shown in Figure 6(A), the minimum response [Log_{10}(Particle size) = 1.69] occurred when both the CS concentration and TPP concentration were at their highest level. Figure 6(B) indicates that when the CS concentration was at its highest level and the TPP/CS volume ratio was at its lowest, the particle size was at a minimum [Log_{10}(Particle size) = 1.74]. According to Figures 7(A–C), we reached minimum particle sizes [Log_{10}(Particle size) = 1.77, 2.10, and 1.73] at the lowest level of addition time of the TPP solution to the CS solution versus the highest level of CS concentration and the lowest levels of temperature and TPP/CS volume ratio. Also, for the lowest level of temperature versus the highest levels of CS concentration and TPP concentration and the lowest level of TPP/CS volume ratio, the minimum particle size resulted [Figs. 8(A–C)]. Finally, the minimum response was seen with a high level of TPP concentration, along with low levels of TPP/CS volume ratio and addition time of the TPP solution to the CS solution on the basis of Figure 9(A,B). The analysis of response at the different levels of the factors revealed that there was no remarkable interaction between the factors.

CONCLUSIONS

In this study, a Taguchi OA design proved to be a valuable tool for optimizing hydrogel nanoparticle preparation. The Taguchi OA was efficient for screening which factors among the selected parameters were significant. More specifically, the TPP/CS volume ratio and the CS concentration were very significant, whereas other factors, including the TPP concentration, addition time of the TPP solution, and temperature were proven to be not very important parameters with regard to the hydrogel nanoparticle diameter. However, the attendance of all parameters in the final equation led to higher R^2, R^2_{Adj}, and R^2_{Pred} values. As the second step, response surface analysis was useful in determining the optimum levels of the factors for production of hydrogel nanoparticles with minimum possible size. The optimum condition for hydrogel nanoparticle preparation was established as follows: CS concentration = 0.28% w/v, TPP concentration = 3.17% w/v, TPP/CS = 1 : 8, temperature = 25.66°C, and addition time of the TPP solution to the CS solution = 0.4 min.

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