Applying the Taguchi Method for Optimized Fabrication of "-Lactalbumin Nanoparticles as Carrier in Drug Delivery and Food Science

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Abstract: Protein nanoparticles fabrication as well as characterization have been extensively studied in our previous works as suitable carrier for drug delivery and food science, since they are biodegradable, non-toxic and non antigenic. The objective of the present study was to optimize the fabrication of "-lactalbumin nanoparticle by applying the Taguchi robust method which is a statistical approach to overcome the limitation of the factorial and fractional factorial experiments. The process variables were pH, temperature and agitation speed. The optimal levels of the different factors for the nanoparticle production based on coacervation method were pH 2.5, temperature 50°C and 750 rpm for agitation speed. The nanoparticle size at the determined condition was less than 220 nm. The mechanistic of the optimum conditions for preparing "-lactalbumin nanoparticles and their characterization as a drug delivery vehicles are strongly discussed.

Key words: "-lactalbumin %Nanoparticles %Drug carrier %Optimization %Taguchi method

INTRODUCTION

Although, the drug delivery system (DDS) concept is not new, great progress has recently been made in the treatment of a variety of diseases. Targeting delivery of drugs to the diseased lesions is one of the most important aspects of DDS and controlled drug delivery technology represent one of the frontier areas of science, which involves multidisciplinary scientific approach, contributing to human health care. These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficiency, reduced toxicity and improved patient compliance and convenience. To convey a sufficient dose of drug to the lesion, suitable carrier of drug is needed [1]. Nanoparticles (NPs) have been developed as an important strategy to deliver conventional drugs, recombinant proteins, vaccines and more recently nucleotides. Nanoparticles and other colloidal drug delivery systems modify the kinetics, body distribution and drug release of an associated drug. Other effects are tissue or cell specific targeting of drugs and the reduction of unwanted side effects by a controlled release [2].

Polymeric nanoparticles have attractive physicochemical properties such as size, surface potential, hydrophilic-hydrophobic balance etc. and for this reason they have been recognized as potential drug carrier for bioactive ingredient such as anticancer drugs, vaccines, oligonucleotides, peptides, etc [3,4].

Among colloidal systems those based on proteins may be very capable. Proteins are a class of natural molecules that have unique functionalities and potential applications in both biological as well as material fields [5].

In spite of successful elaboration of many synthetic polymers as delivery systems, these cannot be used in food applications that require compounds which are generally recognized as safe (GRAS). Food biopolymers, specifically food proteins are widely used in formulate food because they have high nutritional value and are generally recognized as safe.

Clear advantages of food protein matrices include their high nutritional value, abundant renewable sources and acceptability as naturally occurring food components degradable by digestive enzymes.
"-Lactalbumin is the major whey protein found in milk. "-Lactalbumin is a protein present in the milk of all mammals. The molecular weight is 14176 Da and the isoelectric point is between 4.2 and 4.5. In addition, "-lactalbumin has significant nutritional properties and is associated with some positive health effects upon consumption [6].

It has been shown that particle size is an important factor for the tissue and organ distribution of nanoparticles. For example, larger particles are more rapidly removed by the liver and spleen than smaller particles. Reducing the particle size of colloidal carriers below a threshold of 100 to 200 nm introduces the possibility of escaping the vascular system via fenestration or cavities in the lining of blood vessels [7]. Different in vitro studies indicate that the particle size also influences the cellular uptake of nanoparticles [8-10]. Therefore size optimization of the nanoparticles is one of the important purposes in the fabrication of "-lactalbumin nanoparticles.

The properties of "-lactalbumin nanoparticles fabricated by coacervation method are affected by various parameters such as pH value, temperature, agitation speed. The interrelationships between the mentioned parameters are complex and therefore the analysis of this system for optimizing the factors is a time and labor consuming work. The Taguchi method is a powerful problem solving technique for improving process performance and reducing rework and manufacturing costs due to excessive variability in processes [11].

In this work simple coacervation method is used for manufacturing of "-lactalbumin nanoparticle as a drug delivery system and the essential parameters are considered. The objective of the present study is the optimization for the preparation of "-lactalbumin nanoparticles by the Taguchi design method which shows a controllable particles diameter and a narrow size distribution. In addition, SEM characterizes the shape and morphology of the products. This study is intended to establish a rational basis for the controlled production and application of protein-based nanoparticles as drug carrier system.

**Experimental**

**Materials:** "-Lactalbumin (purity > 85%) and glutaraldehyde (25% solution) were commercially supplied by Sigma Aldrich. Acetone and all other chemicals were supplied from Merck (Germany).

**Preparation of "-lactalbumin Nanoparticles:** Two-step desolvation technique was implemented for preparation of "-lactalbumin nanoparticles. 25 mg.ml⁻¹ aqueous solution of "-lactalbumin was stirred on 500 rpm magnetic stirrer at 50°C for 10 min and then 4 ml acetone (desolvating agent), all at once was added to cause sedimentation of high molecular weight (HMW) component of "-lactalbumin. The first step is performed to discard low molecular weight (LMW) component of "-lactalbumin which would make the production of stable nanoparticles with a uni-model size distribution impossible. After 24 h incubation at room temperature and resolvation of sediment in 1 ml purified water and pH-adjustment (pH 2.5), acetone was added drop-wise until the solution become just turbid. After desolvation process, 30 µl of 25% glutaraldehyde was added for cross linking and stirred continuously at room temperature for 12 h. The formed nanoparticles were purified by 3 cycles of centrifugation. For each centrifugation step, supernatant was centrifuged at 15000 g for 20 min. Shape and morphology of "-lactalbumin nanoparticles were determined by scanning electron microscopy (SEM).

**Taguchi Method**

**Taguchi Approach to Parameter Design:** Taguchi methods are statistical methods developed by Genichi Taguchi to improve the quality of manufactured goods and more recently also applied to, engineering, biotechnology, marketing and advertising. This method provides a systematic approach for conducting experimentation to determine optimum settings of design parameters. Taguchi method pushes quality back to the design stage, seeking to design a product/process, which is insensitive to quality problems [12, 13]. It can reduce research and development cost by simultaneously studying a large number of parameters. The method can significantly reduce the number of experimental configurations by using orthogonal arrays. In order to analyze the results, Taguchi method uses a statistical measure of performance called ‘signal-to-noise’ ratio, (S/N), where S is the standard deviation of the performance parameters for each array experiment and N is the total number of experiment in the orthogonal array [14]. After performing the statistical analysis of S/N ratio, an analysis of variance (ANOVA) needs to be employed for estimating error variance and determining the relative importance of various factors. From their relative importance and from the S/N ratio, the optimum condition of factors is chosen [3, 15, 16]. Figure 1 provides a brief overview of the process followed by Taguchi’s approach to parameter design [14, 17].
Determine the Quality Characteristic to be Optimized

Identify the Control Factors and their Alternative Levels

Design the Matrix Experiment and Define the Data Analysis Product

Conduct the Matrix Experiment

Analyze the Data and Determine Optimum Levels for Control Factors

Predict the Performance at These Levels

Fig. 1: Flowchart of the Taguchi Method

Table 1: Variable (factors) and their levels employed in Taguchi method

<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>4</td>
<td>25</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>2.5</td>
<td>7.5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Agitation speed (rpm)</td>
<td>250</td>
<td>500</td>
<td>750</td>
<td></td>
</tr>
</tbody>
</table>

Details of Experiments: As it was found, three important factor that influence the ","-lactalbumin nanoparticle size were pH value, temperature and agitation speed. Therefore in order to minimize the number of experiments, Automatic Design and Analysis of Taguchi Experiments were employed through Qualitek-4 software (version IV). Taguchi's orthogonal array table was used by choosing these three parameters that could affect the particle size. Table 1 shows the parameters and levels used in this experiment. The orthogonal array of L 9 type was used. L and subscript 9 means Latin square and the number of experiment, respectively.

RESULTS AND DISCUSSION

Fabrication of "-lactalbumin Nanoparticles: The effects of parameters and an alternative explanation about protein nanoparticle fabrication have been introduced in our previous publications [5, 6, 13, 18].

Table 2: Experimental measured values for particle size of "-lactalbumin nanoparticles (Taguchi orthogonal array table of L9).

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Agitation (rpm)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>420</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>450</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>348</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>380</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>270</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>210</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>380</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>300</td>
</tr>
</tbody>
</table>

Table 3: The ANOVA table of particle size.

<table>
<thead>
<tr>
<th>SFactors</th>
<th>Degree of freedom</th>
<th>Sums of squares</th>
<th>Variance</th>
<th>F - Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature(°C)</td>
<td>2</td>
<td>17934.204</td>
<td>8967.102</td>
<td>7.639</td>
</tr>
<tr>
<td>pH</td>
<td>2</td>
<td>1360.867</td>
<td>680.433</td>
<td>0.579</td>
</tr>
<tr>
<td>Agitation speed(rpm)</td>
<td>2</td>
<td>26054.199</td>
<td>13027.099</td>
<td>11.098</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>2347.613</td>
<td>1173.808</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>47696.888</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Taguchi Array Design and Analysis of Variance: A Taguchi orthogonal array design was used to identify the optimal conditions and to select the parameters having the most principal influence on the particle size of \(-\)-lactalbumin nanoparticles. Table 2 shows the structure of Taguchi’s orthogonal array design and the results of measurement by PCS. The variance of the particle size in Table 2 (analysis of variance) was calculated, the optimum conditions can be determined through the response table of ANOVA-TM software. The level average graph of the raw data is illustrated in figure 2, which shows that data analyzed by the Taguchi method is in good agreement with experimental finding and the results are shown in Table 3. The purpose of the analysis of variance (ANOVA) is to investigate which factors significantly affect the quality characteristic.

The horizontal axis shows the different levels of the each significant factor. The lines represent the trend of each factor with respect to different levels. Based on the S/N ratio and ANOVA analysis, the optimal parameters for nanoparticle size are the pH at level 1, temperature at level 3 and agitation speed at level 3. Under these conditions the program estimated the \(-\)-lactalbumin nanoparticles diameter as 207.5 nm, while in the experiment 220 nm was achieved for the nanoparticle diameter.

Physical Characteristics of \(-\)-lactalbumin Nanoparticles: Simple coacervation process of \(-\)-lactalbumin nanoparticles was evaluated based on the particle size. The particle sizes as well as the light intensity counts of the samples were determined by photon correlation spectroscopy (PCS) for all the experiments. The SEM image of \(-\)-lactalbumin nanoparticles is shown in figure 3. It was clear that the most of the resulting protein nanoparticles were smooth and semispherical. Based on these characteristics; \(-\)-lactalbumin nanoparticles were good enough to be candidate for loading drugs on/in them.
CONCLUSION

"-lactalbumin nanoparticles were fabricated successively by exploiting robust process of coacervation. According to the literature, the results demonstrated that produced nanoparticles have sufficient properties as a carrier for drug delivery systems. Here, Taguchi design method was used to optimize the parameter values for obtaining desired characteristics. The nanoparticle size fabricated here was influenced by several process variables including pH value, temperature and agitation speed. By optimal conditions (at pH 2.5, temperature 50 °C and agitation speed 750 rpm) of this method, the "-lactalbumin nanoparticle diameter of 220 nm was achieved and these results are in good agreement with data analyzed by Taguchi method.

Application of these nanoparticles in food technology and drug delivery will be the next step of the work and subject of our further publication.

REFERENCES