Nanobiotechnology: Applications in Agriculture, Food Science and Engineering

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Abstract

This paper reviews recent research focused on edible nanoparticles and sensor development for food and agricultural systems. Nanoparticles were produced from starch, BSA and gliadin by desolvation method. Spherical nanoparticles were produced in the range of 50 to 150 nm using different manufacturing methods for BSA. NPs of starch were manufactured with different amylose/amylopectin ratio which were achieved by mixing Hylon VII (70% amylose starch) and Amioca (98% amylopectin starch) to obtain various amylopectin/amylose ratios (30 to 100%). Spherical nanoparticles were produced in the range of 50 to 150 nm from different ratios. Gliadin nanoparticles varied in size from 180 to 900 nm depending on parameters used. Biocompatible nanotubes (NTs) were produced using Layer-by-Layer (LbL) deposition of Bovine Serum Albumin (BSA) and a positively charged biopolymer into a nano-porous template (400 nm). The robust NTs were produced by deposition of three bilayers. Quantum dots (QDs) were conjugated to gliadin antibody and used as a fluorescent probes to track gliadin protein in dough and baked bread samples. We also designed a novel and green platform for on-site detection of agriculture or food related target molecules from Zein, a prolamin protein of corn. In our project, a free-standing zein film having surface nano-patterns was formed. Nano-patterns combined with a thin layer of gold film enables the platform to be used as a SERS detection tool.

1. Introduction

In this overview, our objective is description of some applications of nanotechnology in biology especially focused on agriculture and on food science and engineering. First we'll introduce nanoparticulation of gliadin, a wheat protein, bovine serum albumin (BSA) and starch by using the desolvation method. Then layer-by-layer deposition method is introduced to prepare nanotubes from BSA and a poly-cation (Poly-D-Lysine, PDL). In the next step, we try to explain application of Quantum Dots (QDs) technique in evaluation of gliadin distribution in dough and bread sample. Finally, zein-based nanophotonic biosensors will be introduced.

2. Nanoparticles (NPs)

Starch NPs: Among other polymers, starch is inexpensive, renewable, biodegradable, and biocompatible material and it has been considered a promising polymer in various fields [1]. Two major molecules of starch, amylose and amylopectin, have a great impact on determining physicochemical characteristics of starch [2]. In this study, we have produced nanoparticles from different ratios of amylose/amylopectin content of starch and investigated the contribution of the change in amylose/amylopectin ratio to particle size and morphology of produced starch nanoparticles.

The different ratios of amylose/amylopectin content of starch was maintained by mixing desired amount of high amylose content starch (Hylon VII, 70% amylose) and high amylopectin content starch (AMIOCA, 99% amylopectin). Starch solutions were prepared by dissolution in NaOH/urea (0.8:1 % w/w) solution for 12 hours. Precipitation was achieved by drop wise addition of filtered starch solution into ethanol until the ratio was equal to 1:10 (starch solution/ethanol). The final solution became turbid due to precipitation in ethanol. The suspension was centrifuged at 8000 rpm for 5 min to separate particles from the supernatant. The sample was dried in a vacuum oven for 1 hour. For particle size measurement, particles were dispersed in water and analysis was conducted by Dynamic Light Scattering. The morphological analysis was performed by Scanning Electron Microscopy.

Depending on amylopectin content of starch the size of nanoparticles substantially changed. Higher amylopectin content led to bigger nanoparticles (Table 1). The Scanning Electron Microscopy (SEM) micrographs in Fig. 1 showed that more uniform and smaller nanoparticles with lower amylopectin content.

Table 1. Effect of amylose/amylopectin ratio of starch on particle size

	Amylopectin content of starch				
	100%	**90%**	**80%**	**70%**	**30%**
Particle size (nm)	**122.8± 5**	**119.2± 5**	**115.4± 5**	**106.3± 5**	**75.8± 5**

BSA NPs: Bovine Serum Albumin (BSA) is very strong carrier in blood and milk. BSA shows a wide range of physicological functions like binding, transport and distribution of bioactive compounds [3]. Our objective was to use different desolvating agents (ethanol, Acetone and mixtures of them) to produce BSA NPs. Then study the effects of different desolvating methods on particle morphology, zeta potential, size and distribution of the particle.

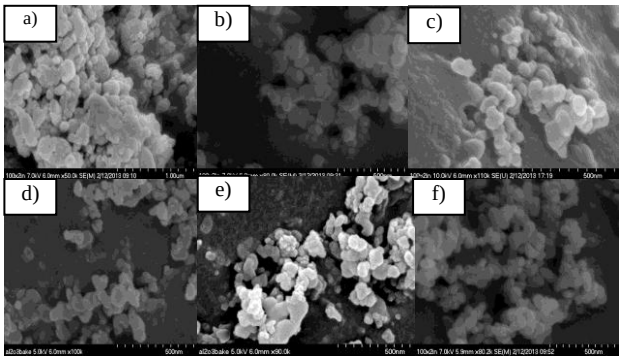

Fig. 1. SEM images of starch nanoparticles produced from varying amylopectin content of starch. a) 99%, b) 90%, c) 80%, d) 70%, e) 50%, f) 30%.

Fig. 2 shows the effect of this ratio on the particle size. For Ethanol, Et:Ac (70:30) and Et:Ac (50:50) by increasing the desolvation ratio. The particle size started to increase and after reaching the maximum became constant. The BSA molecules started to precipitate as big particles after adding more than 3 times acetone, and because of this phenomenon, higher ratios than 3 were not evaluated. The higher the desolvation ratio the bigger the particle size. The particulation was completed in 3X, 2X, 2X and 2X for ethanol, Et: Ac (70:30), Et: Ac (50:50) and acetone, respectively.

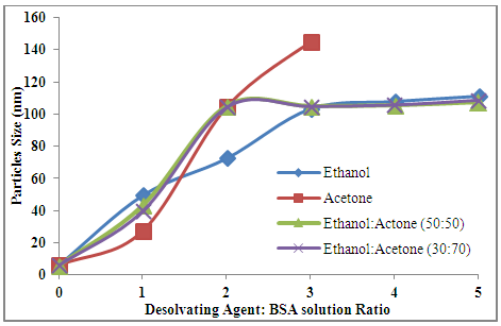

Fig. 2: The effect of different desolvating agents on nanoparticle size

To select the best ratio of desolvating agents, the nanoparticulation efficiency must be calculated. The nanoparticulation efficiency was 95.2±0.48, 96.8±0.42, 97.7±0.40 and 99.2±0.18 for ethanol (4X), Et: Ac 70:30 (4X), Et: Ac 50:50 (4X) and Acetone (3X), respectively. The nanoparticulation efficiency was measured only for the turbid samples where particulation was completed. Acetone was very effective to increase the nanoparticulation efficiency.

The SEM images and DLS results confirmed that ethanol, acetone and mixtures of them are capable to produce NPs from BSA. The particles size of the BSA NPs was between 100-150 nm. The SEM images and DLS results show that ethanol produced uniform and spherical with the particles size (PS) and polydispersity index (PI) of 110±3 nm and 0.045±0.007, respectively. Fig. 3 shows the SEM images of BSA NPs by using ethanol and acetone as desolvating agents.

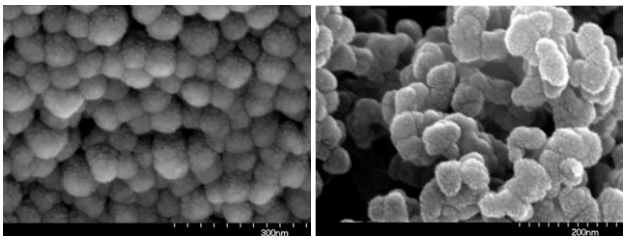

Fig. 3. The SEM images of BSA NPs, Ethanol (left) and Acetone as desolvating agent (right).

Gliadin NPs: Gliadins are a large and complex group of low molecular weight (~ 22,000 Daltons) proteins extracted from wheat flour. Gliadin were extracted and purified from crude gliadin (Sigma, St. Louis, MO, USA). . Gliadin was prepared by dissolving 7.5 mg of gliadin in 1.5 ml of different volume of ethanol solutions; this solution was poured into 3ml DI water containing 0.01% NaCl and 0,5 % pluronic F68 under stirring using magnetic stirrer (500 rpm) at room temperature (25 °C). Ethanol solution was poured into non solvent media for 3 min using syringe pump using the flow rate 0.5 ml/min.

The particle size was found to be around 210 nm between % 60 and % 62 ethanol solutions. The higher the ethanol concentration, the larger the particle size. Gliadin solutions were also heated before nanoprecipitation. It is clear that heat resulted in smaller particles because of increase in solubility of gliadin. Particle size of gliadin nanoparticles was around 300 nm between 200 and 700 rpm. A significant decrease in particle size was observed with treatment with 2-Mercaptoethanol and DDT. While the particle size of particles treated with 2-Mercaptoethanol was 190nm, the particle size of particles treated with DDT was measured around 240 nm. It was also observed that particle size of gliadin went down until non-solvent/solvent ratio reached to 6. After this point, there was not any significant change in particle size. In contrast to our work Bilati at al. [4] using PLA and PLGA as the polymer of interest with found that the solvent (MeCN) to non-solvent (ethanol) ratio had little impact on particle size. This is different than what we observed and also counterintuitive to the solubility difference forming the driving force for small particle formation. Fig. 4 shows the effect of different parameters on particle size of gliadin NPs.

3. Nanotubes

Nanotubes from synthetic materials are commonly produced [5-10]. The template assisted LbL method by the electrostatic interaction is more biocompatible because nanotubes can be produced by one or more biopolymers without using any chemical crosslinking agent. This method is conducted by deposition of negatively and positively

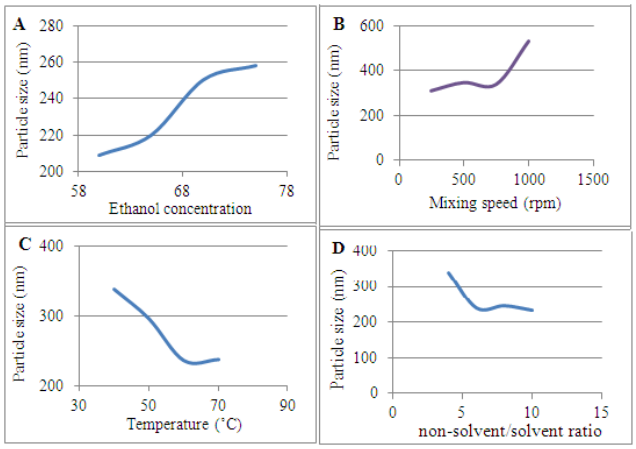

Fig. 4. The effect of Ethanol concentration (A), Mixing speed (B), temperature of good solvent (C), and good solvent/bad solvent ratio (D) on the particle size of gliadin.

charged polymers respectively on the surface of a template. If the template configuration is flat deposition results in a thin film on its surface but if the template has the nanoporous configuration, deposition causes the formation of the nanotubes into the nanopores of the template. Different templates have been used for nanotube preparation, two common types of which are anodic aluminum oxide (AAO) [11-14] and track-etched polycarbonate (PC) membrane [15-22]. The protein nanotubes were prepared according to the previously reported technique with some modifications [19].

The opposite electrostatic charges of PDL and BSA in wide range of pH are the main driving force for the interaction of the molecules. By deposition of PDL and BSA onto the template pores and cleaning both surface sides of the template, biopolymers formed very thin tubular film into the pores of the membrane. For three bilayers deposition $(PDL/BSA)_3$. The nanotubes were strong and robust, and they maintained their tubular structures after removing from template and drying (Fig. 5). These nanotubes have a great potential to be used as carrier for nutraceuticals.

4. QDs for imaging and probing

The type and quantity of gluten proteins, and their subfractions, gliadins and glutenins are critical for wheat flour and baked product functionality. Gliadin proteins are able to activate coeliac disease which causes severe damage

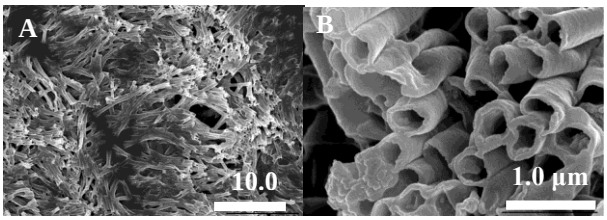

Fig. 5: The SEM images of NTs which produced by deposition of 3 BL (A and B).

to the digestive system. Therefore, it is important to develop a method which is able to accurately quantify the distribution of gliadin in food. There are some studies in regard to understand the structure and location of gluten protein using immunolabeling with gold label [23-25]. Also, Varriale et al. [26] labeled gliadin with organic dye fluorescein-5-isothiocyanate (FITC). However, quantum dots (QDs) have unique properties in fluorescent labeling technique. Therefore, gliadin antibody was conjugated and labeled with QDs to investigate the distribution of gliadin protein in dough and bread sample. Gliadin antibody was conjugated to QDs based on the protocol that Invitrogen Company provided. QDs conjugated with gliadin antibody were specifically bound to gliadin and monitored using confocal laser scanning microscopy (CLSM). The mean intensity value of QDs-anti-gliadin antibody conjugate was calculated with Axiovision software for each obtained images by CLSM from each sample. CLSM images and data which are obtained from ANOVA test showed that the distribution of gliadin protein was significantly difference between the samples. Fig. 6 shows gliadin distribution of dough sample after labeling with QDs.

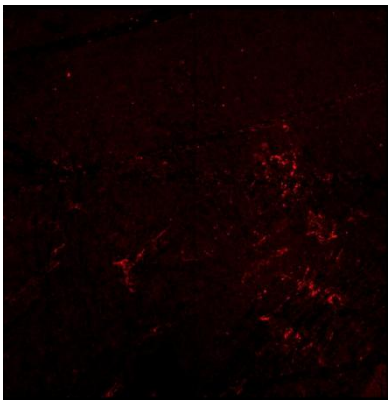

Fig 6: 3-D intensity profile of QDs-gliadin antibody labeled image of dough sample

5. Zein-based Biosensor

Zein films are successfully fabricated by solvent casting method and were shown successfully to sustain micro-features [27] and as a lab-on-a-chip platform to have microfluidic channels [28]. In this study, we demonstrated the ability to use thin zein films as a biosensor platform by using Surface Enhanced Raman Spectroscopy (SERS), which is known to be a highly-specific molecular detection tool.

Zein films were formed by soft-lithography method. Zein solution was prepared by solving zein powder in 75 % aqueous ethanol along with mixing oleic acid and monoglyceride at the ratio of 1 g of zein: 5 ml of ethanol: 1g of oleic acid: 0.05 g of monoglyceride. This solution was stirred at 70°C for 5 minutes and sonicated in order to remove air bubbles. Then it was casted on top the master molds covered with metal coating. The mixture was poured on top of the PDMS master with the desired pattern and

metal coating. Master mold was PDMS with a 200 nm thick gold film, the pattern on the master consisted of 2 um x 2 um base area pyramid shaped regular arrays. The cured films were peeled off the master and signals were measured with Raman Spectrometer with several test molecules.

As a result of this novel technique, nano-sized patterns as well as the gold film were able to be transferred onto zein surfaces with little to no distortion. In order to get Raman Signal, the roughness of the surface and a metal coating is crucial and by this method we were able to produce zein films with the desired two features. SERS measurements showed that these zein biosensors were able to enhance the signal 5 or 6 folds with one of the model molecules Rhodamine 6G. This study opens up the area of food protein based biosensors which can be used to detect analytes harming food and agricultural industry. Fig. 7 presents an example of zein sensor film with gold coating.

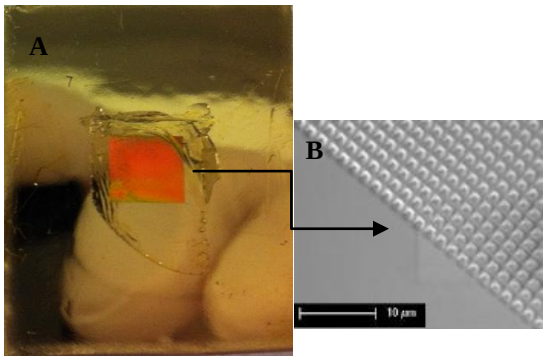

Fig. 7: An example of zein sensor film with gold coating, A) Macro scale B) Micro scale of pyramid structures [29]

5. References

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