# Protein-based nanoparticles for hydrophilic coating

S. Kim, A. Biswas and K. Evans

National Center for Agricultural Utilization Research,
Agricultural Research Service, USDA
1815 N. University Street, Peoria, IL 61604, USA

## ABSTRACT

Cyanoacrylate nanoparticles have been studied in great detail over the past three decades. Conventionally, the mechanism of polymerization is anionic where the initiating species is the hydroxyl anion that was derived from dissociation of water. In the current research, amphiphilic copolymers were synthesized by utilizing amine groups on the surface of protein molecules as initiator for the polymerization. The produced amphiphilic copolymer forms a stable nanoparticle suspension in the reaction medium. The suspension containing these nanoparticles has an excellent coating capability on the surface of hydrophobic materials. A simple spray coating changes the wetting property of the material instantly and dramatically. Spray coating of window-glasses improves visibility on rainy days. Since both protein and poly(alkyl cyanoacrylate) are degradable polymers, the developed nanoparticles are degradable.

***Keywords***: nanoparticles, cyanoacrylate, hydrophilic coating, adsorption, protein

## 1 INTRODUCTION

Poly(alkyl cyanoacrylate) (PACA) nanoparticles have been studied in great detail with a view to their use as controlled release drug delivery materials during the last three decades [1, 2]. PACA's are biodegradable and can be used for the production of nanoparticles via emulsion polymerization. Currently, preparation methods of poly(alkyl cyanoacrylate) nanoparticles are well understood whereby nanoparticles with well-defined properties can be produced. The majority of PACA nanoparticles are obtained through anionic polymerization of the corresponding monomer [2-5]. Typical initiators are anions (i.e., $I^-$, $CH_3COO^-$, $CN^-$, $OH^-$, etc.), and amino acids embedded in proteins. In most cases, hydroxyl ions in water have been utilized as an initiator for the production of nanoparticles in previous research. In this report, a protein was employed for the same purpose. Since initiator itself is a polymer, amphiphilic copolymers could be prepared as a result of polymerization reaction. In other words, the reaction product is a copolymer consisting of two types of homopolymers, protein and PACA. Since proteins are highly hydrophilic, it works as the hydrophilic end in the resultant amphiphile molecule because of its charges on amino acids, while polymerized alkylcyanoacrylate works as the hydrophobic end because there is no charge on its surface. For our purpose, a wheat protein (gliadin) was chosen because of its ease of preparation and low production cost. As a monomer for PACA, ethyl cyanoacrylate (ECA) was chosen because of its low cost and availability.

The produced protein-based nanoparticle possesses peculiar properties. It readily adheres to hydrophobic surfaces and improves the wetting property instantly and dramatically. This report examines the properties of produced nanoparticles and investigate its optimum production condition.

## 2 EXPERIMENTAL

### 2.1 Reagents

Ethyl cyanoacrylate (ECA) monomer (E–Z Bond, viscosity; 5 cps) was purchased from K&R International (Laguna Niguel, CA). Wheat gliadin was a gift from MGP Ingredients, Inc (Atchison, KS). Ethanol and Hydrochloric acid were reagent grade.

### 2.2 Preparation of nanoparticle suspensions

Particles were prepared by emulsion polymerization of ethyl cyanoacrylate. 20 mg of gliadin was dissolved in 10g of 68 wt% aqueous ethanol solution that was premixed with 40 µL of 4N HCl. Then y µL ($40 < y < 200$) of ECA was slowly added during constant stirring with a magnetic stirrer at 500 rpm. Reaction time was set to 30 min to 3h depending on the added amount of ECA. As reaction proceeded, turbidity was developed, indicating nanoparticles were formed in the reaction medium. The resultant nanoparticle suspension was centrifuged at 1000x g for 20 min. The produced nanoparticle suspension (supernatant) was collected and stored at room temperature for the characterization of nanoparticles. To calculate the reaction efficiency, the weight of reaction byproduct (precipitate) was measured by obtaining its dry weight.

### 2.3 Reaction time

Reaction time was decided by monitoring the heat of reaction in a custom-built solution calorimeter. Polymerization reaction was performed in a dewar bottle,

and the temperature variation was monitored with a thermistor connected to a Wheatstone bridge. The output of the bridge circuit was measured with a digital voltmeter. For the conversion of the output voltage to the heat of reaction, calorimeter was calibrated by applying a calculated amount of heat with an electric heater.

## 2.4 Particle size measurement

Dynamic light scattering (DLS) experiments were carried out with the dispersions using a Particle Size Analyzer equipped with a 658 nm diode laser and an avalanche photodiode detector (Model 90 Plus, Brookhaven Instruments Corporation, Holtsville, NY, USA). All the samples (prepared by the procedure in the previous section) were diluted twenty times with the same solvent, and measurements were performed without filtration. All measurements were done at a 90° detection angle at 20.0°C. For each sample, ten DLS measurements were conducted and each run lasted 30 s. All measurements were processed using the software supplied by the manufacturer (9kpsdw, v.5.31), which provided the mean hydrodynamic diameter via a multimodal analysis. Data from ten measurements were averaged to obtain the size of nanoparticles.

## 2.5 Dynamic contact angle (DCA) measurement

DCA analysis was performed using a DCA 315 (Thermo Cahn Instruments, Madison, WI, USA) by the Wilhelmy plate method to determine the effect of nanoparticle coating on surface wettability [6]. Samples for DCA analysis were prepared by dipping plates made of the material to be tested into the nanoparticle suspension and rinsing with a stream of distilled water a few seconds. The prepared plate was consecutively immersed in and removed from distilled water at a speed of 60 mm/min. Curves relating the interfacial tension to the immersion depth were plotted and used to calculate the receding contact angle. A representative contact angle was calculated for each formulation using the mean and standard deviation of five independent measurements.

# 3 RESULTS AND DISCUSSION

## 3.1 Reaction condition

For the production of nanoparticles, anionic polymerization has been commonly used with hydroxyl ions as an initiator [2]. For this project, however, amine groups on the surface of gliadin molecules were used as an initiator. As a result of this polymerization, copolymers are produced. Since gliadin is hydrophilic and poly(ethyl cyanoacrylate) is hydrophobic, the resultant star copolymer behaves as an amphiphile. These copolymers form micelles in the solution. The reaction mechanism is shown in Figure 1.

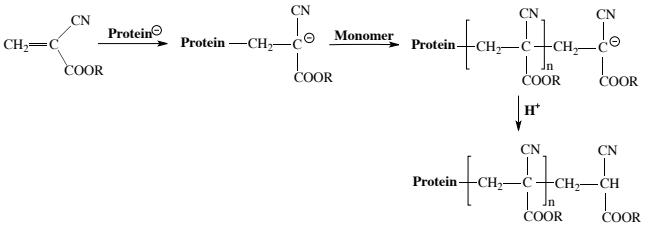


Figure 1: Reaction scheme.

This reaction is a living polymerization. Therefore, unless this reaction is terminated intentionally, this reaction proceeds until all monomers are consumed. The size of nanoparticles depends on the amount of ECA monomers reacted with each protein molecule. Since the reaction rate depends on the concentration of reactants, the reation time was investigated by varying the amount of ECA per 10 mg of gliadin. Data from a custom-built calorimeter shows that the reaction time varies depending on the amount of added ECA monomer (Figure 2). Termination of the reaction was indicated by arrows in the Figure. The reaction times obtained from this data were used for the preparation of the nanoparticle solutions used in the report.

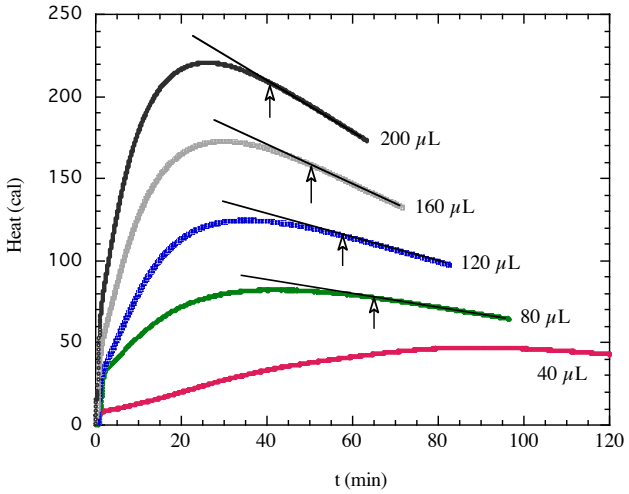


Figure 2: Reaction time measured by a solution calorimeter.

In the case of conventional synthesis of nanoparticles from cyanoacrylates, nanoparticles are fabricated in an aqueous medium that is acidified with hydrochloric acid. Since cyanoacrylates are hydrophobic monomers, they are not soluble in water. Therefore, reaction is performed via emulsion polymerization while the reactant mixture is vigorously stirred [7]. This type of two-phase reaction is slow and produces particles with broad size distribution. In our reaction scheme, amine groups on the surface of protein (gliadin) molecules react with ECA. To avoid two-phase reaction, ethanol/water mixture was chosen as a reaction medium. Aquesous ethanol is more hydrophobic than water, allowing both gliadin and ECA to dissolve in the same reaction medium. Acidic condition was necessary to control the size of nanoparticles. Neutral water supplies too much hydroxyl ions which make a large chunk of

poly(ethyl cyanoacrylate) (PECA) aggregates instead of nanoparticles. It is known that the size distribution of produced nanoparticles depends on the pH of the reaction medium [7].

## 3.2 Byproduct of the reaction

Reaction efficiency is another factor that has to be taken into account for the production of nanoparticles. As was stated in the Introduction section, ECA can also be polymerized with hydroxyl ion as an initiator. Therefore, there should be a competition of the two initiators, amine groups on gliadin molecules and hydroxyl ions that were dissociated from water. For hydroxyl ion-based products, particles are easily precipitated if a stabilizer (e.g., dextran) is not used [8]. On the other hand, protein-based nanoparticles form a stable suspension without a stabilizer. Therefore, these two types of reaction products can be separated by centrifugation. After the centrifugation, the precipitates, i.e., hydroxyl ion-based particles, are removed from the reaction product.

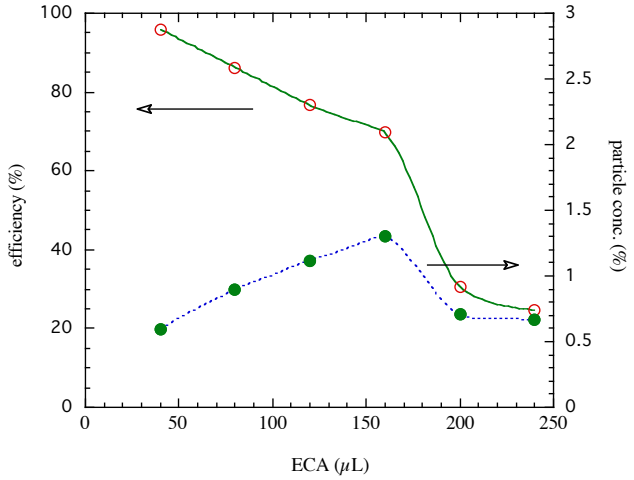


Figure 3: Reaction efficiency.

Figure 3 indicates that the reaction efficiency for the production of protein-based nanoparticle is higher with lower amount of ECA. The particle concentration data shows that nanoparticles with very large hydrophobic moiety are so unstable that significant amounts are removed from the suspension by precipitation. Despite this irregularity in the efficiency and particle concentration, the hydrodynamic diameter of the produced nanoparticles showed a monotonous increase as the ratio of monomer to protein molecule increased (Figure 4).

## 3.3 Structure of nanoparticles

Since the produced nanoparticles are assembled from amphiphilic copolymers and the reaction medium is aqueous ethanol, the hydrophobic moiety of the copolymers should be in contact with aqueous ethanol, which is more

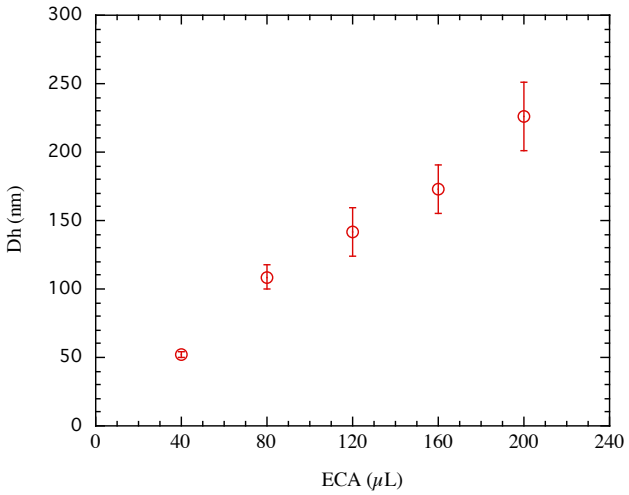


Figure 4: Hydrodynamic diameter of the produced nanoparticles measured by DLS.

hydrophobic than water. On the other hand, the hydrophilic moiety of copolymer (i.e., gliadin) will be more stable by staying in the core of the micellar structure. This means that gliadin molecules are in the core of the nanoparticles while PECA chains are in the corona.

Each gliadin molecule contains many amine groups that can be used as an initiator of the polymerization of ECA. Although it is not known how many of them are located on the surface of gliadin aggregates, it is reasonable to assume that more than one PECA chain are attached to each gliadin molecule. Therefore, produced nanoparticles can be described as an assembly of $(PECA)_n$-gliadin star copolymers where PECA chains are localized on the surface of each gliadin molecule [9].

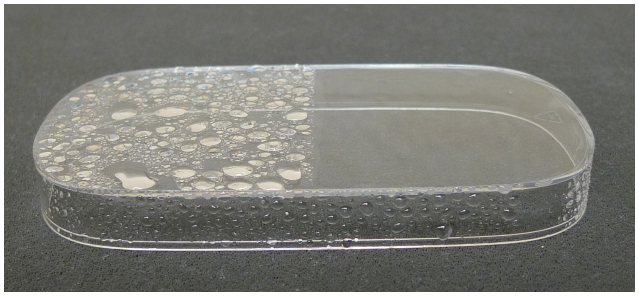

Figure 5: Demonstration of wetting behavior of nanoparticle-coated surface (right half).

## 3.4 Adhesion of nanoparticles

The produced nanoparticles readily adhere on hydrophobic surfaces. Simple spray on the target surface followed by washing with flowing water induces adherence of nanoparticles. The adhesion (coating) takes place instantly and the coated surface turns hydrophilic. Any hydrophobic surfaces such as glass panes, plexiglases, stainless steel, porcelain, and polymer films that were made of polyethylene, polypropylene, polystyrene, or PET

(poly(ethylene terephthalate)), etc. can be coated. A demonstration of this behavior is shown in Figure 5.

For quantitative evaluation of the functionality of produced nanoparticles, contact angle was measured before and after the coating. Data from a glass plate and a polystyrene (PS) sheet are shown in Fig. 6. As was expected, the contact angle of very hydrophobic PS was much larger than that of the glass plate. Coating with nanoparticles prominently decreased contact angle.

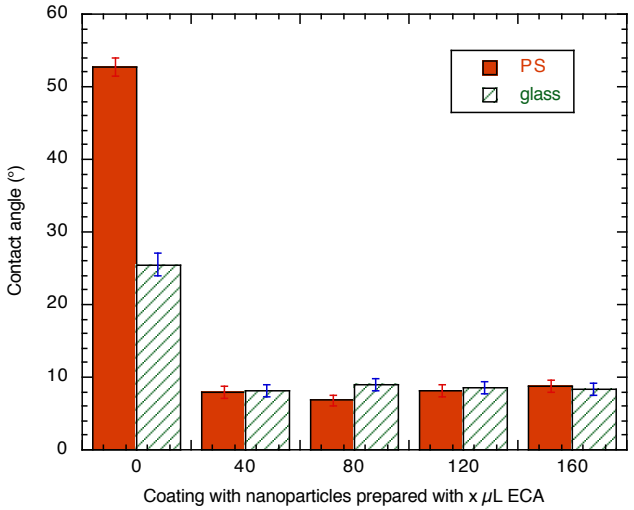


Figure 6: Contact angle of nanoparticle-coated surfaces.

In the examples shown in Figure 6, the effect of adhesion of nanopaticles was not dependent on the size of nanoparticles even though nanoparticles with longer hydrophobic polymer chains were expected to have stonger bonds on hydrophobic surfaces. Obviously, DCA is a good tool for the evaluation of the performance of the produced nanoparticles. According to the data shown in Figure 6, nanoparticle prepared with 40 µL ECA (smallest monomer to protein ratio that was examined) has a good enough functionality as a coating material.

## 4 CONCLUSIONS

In this article, it is shown that surface modifying nanoparticles can be produced by using protein (gliadin) as an initiator for the polymerization of alkyl cyanoacrylates. Unlike conventional procedures employed for the fabrication of cyanoacrylate nanoparticles, the presented procedure does not require stabilizers because the produced nanoparticles are composed of amphiphilic copolymers. The produced nanoparticle has a strong adsorption characteristic that changes the wetting property of hydrophobic materials. Since the average diameter of adsorbed nanoparticles is smaller than the wavelength of visible light, transparent materials such as glass or Plexiglas can be coated with the presented nanoparticles without deteriorating transparency. This characteristic is useful for improving visibility on rainy days because water-droplet formation on the surface of nanoparticle-coated windows will be suppressed.

In this report, ECA was employed, but other cyanoacrylates with different alkyl chains can also be used for the same type of reaction. For the same reason, many proteins other than gliadin can be used for the same purpose. In the case of other proteins, however, some reaction conditions (e.g., reaction medium) need to be modified.

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