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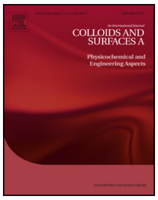

Morphology-controlled functional colloids by heterocoagulation of zein and nanoparticles

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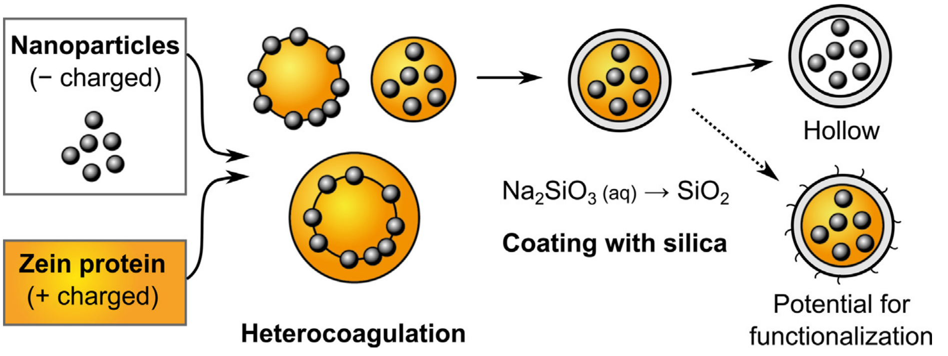
HIGHLIGHTS

- We present a general synthesis method for composite colloids of varying morphologies.
- Positively charged zein is combined with different negatively charged nanoparticles.
- A layer of silica is deposited on the zein particles to increase applicability.
- Hollow silica shells containing nanoparticles can be formed.

GRAPHICAL ABSTRACT



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ABSTRACT

A general and reproducible heterocoagulation method is presented to prepare sub-micron sized zein protein particles, loaded with negatively charged nanoparticles. These composite carrier particles can be obtained in three different morphologies, and each morphology can be prepared using nanoparticles of various size, shape and composition. An important feature of the zein composites is their long-term stability in water even in conditions where free nanoparticles often aggregate within days. Additionally, we modify the composite particles by coating them with a thin layer of silica via condensation of sodium silicate, opening possibilities for highly specific, functionalized carrier particles. Finally, the formation of hollow silica shells containing negatively charged nanoparticles is demonstrated, using the zein composites as a template.

1. Introduction

Nanoparticles provide many interesting new properties over their bulk material counterparts, because of their small size and high surface-to-volume ratio. The preparation of composite nanoparticles out of multiple materials can present further, important modifications of the nanoparticle properties, such as decreased reactivity [1], increased stability [2], combined properties within

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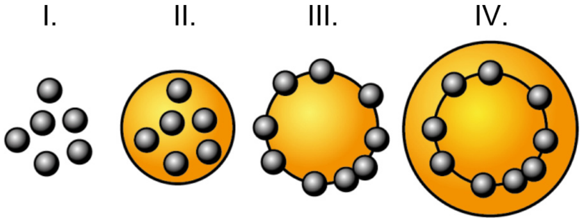

Fig. 1. Schematic representation of the four particle morphologies: (I) bare nanoparticles (NP), (II) zein with embedded nanoparticles (NP-Z), (III) zein with surface coverage of nanoparticles (Z-NP), and (IV) zein with an internal shell of nanoparticles (Z-NP-Z).

one system (such as optic and magnetic) [3] or highly specific and targeted interactions, useful in medical imaging [4] or drug delivery [5]. However, synthesis methods for such particles are usually laborious and require expensive starting materials or equipment. In addition, synthesis methods often lack general applicability, as they are designed for one specific material or morphology [6–8].

To alleviate some of these problems, we report in this paper a general and reproducible method to prepare sub-micron sized, core–shell particles that can contain a broad variety of nanoparticles, using the protein zein as a template material. Our method involves the heterocoagulation of positively charged zein together with, in principle, any negatively charged nanoparticle, into one composite colloid, without the use of any other additives.

Zein is a major storage protein from corn (*Zea mays*). It is used in a variety of foodstuffs, pharmaceuticals and other applications such as binders, adhesives and fabrics [9,10]. Because of its hydrophobic nature and biocompatibility, its use as a potential drug or nutrient delivery mechanism has received increased attention recently [11]. Our method of incorporating nanoparticles expands the number of possible applications of zein-based particles. For instance, incorporation of magnetic or optically active materials may result in particles relevant for medical imaging or (targeted) drug delivery.

The application of zein particles is hindered in several ways, related to its poor colloidal stability in aqueous media [12], with an iso-electric point around pH 6. Patel et al. [12] show that the particle stability could be increased by coating the zein particles with sodium caseinate. We provide an alternative method to increase the stability of zein composite particles by coating them with a thin layer of silica via the condensation of sodium silicate. This layer additionally expands the versatility of zein composites, as it provides a starting point for a variety of surface functionalization through the use of silane coupling agents [13]. In addition, we also show that the protein can be removed after the growth of a silica layer, leaving hollow silica shells containing nanoparticles.

In this work we investigate the incorporation of six different types of nanoparticles into the zein protein matrix: iron (III) pyrophosphate (FePP$_i$), magnetite (mag), cobalt ferrite (CoFe), gold (Au), silver (Ag) and hematite spindles (hem). Three different composite morphologies are prepared, schematically shown in Fig. 1: zein with embedded nanoparticles (NP-Z), zein with nanoparticles on its surface (Z-NP) and Z-NP particles with an added layer of zein (Z-NP-Z). Silica coating is applied to bare zein particles and composite systems of NP-Z containing magnetite and silver.

2. Methods

2.1. Materials

The following chemicals were obtained from their respective suppliers: zein protein, ammonia solution (28–30% in water), $FeCl_2{\cdot}4H_2O$ (98%), $FeCl_3{\cdot}6H_2O$ (ACS Reagent Grade, 97%), $NaBH_4$ (reagent grade, ≥ 98.5%), $Fe(ClO_4)_3{\cdot}6H_2O$ (crystalline), $HAuCl_4$ (puriss. p.a.) and sodium silicate solution (reagent grade) from Sigma–Aldrich Chemie B.V., ethanol (100%) from Interchema, tetramethyl ammonium hydroxide solution (TMAH, 25% in water), NaH_2PO_4 (purum p.a., ≥99.0% (T)), $CoCl_2{\cdot}6H_2O$ (purum, ≥98.0 (KT)) and Dowex 50Wx8-200 ion exchange resin from Fluka Analytical (Sigma–Aldrich Chemie B.V.), $Na_4P_2O_7{\cdot}10H_2O$ (ACS reagent grade), trisodium citrate dehydrate (crystalline), NaOH (pellets, pure), HCl (fuming, 37%) and HNO_3 (GR p.a., 65%) from Merck, urea (ACS, 99%) and $AgNO_3$ (ACS, 99.9%) from Brunschwig Chemie B.V and $Fe(NO_3)_3{\cdot}9H_2O$ (99+%, p.a.) from Acros Organics.

Chemicals were used as received. In all experiments, deionized water was used from a Millipore Synergy purification system.

2.2. Nanoparticle preparation

2.2.1. Iron pyrophosphate (FePP$_i$)

Iron pyrophosphate nanoparticles were prepared by coprecipitation following the method described by Rossi et al. [15]. In a typical preparation, 50 mL of an aqueous solution of 0.86 mol $FeCl_3$ is added drop-wise to 100 mL of an aqueous solution of $Na_4P_2O_7$, over a period of 15 min. A turbid white suspension forms in the final minutes of the addition (at pH 4). Nanoparticles were prepared shortly before further processing with zein, as colloidal iron pyrophosphate aggregates over time in water [16].

2.2.2. Magnetite (Mag)

Superparamagnetic magnetite (Fe_3O_4) nanoparticles stabilized by tetramethylammonium hydroxide (TMAH) were prepared by the Massart coprecipitation method [17]. A typical preparation involves mixing 0.02 mol $FeCl_2{\cdot}4H_2O$ dissolved in 10 mL 2 M hydrochloric acid with 0.04 mol $FeCl_3{\cdot}6H_2O$ dissolved in 40 mL water. After brief ultrasonication, the mixture is added to 500 mL 0.7 M ammonia while stirring vigorously, where a black precipitate forms immediately. The mixture is stirred for another 10 min, after which the magnetite is collected by a hand magnet and the supernatant is discarded. The particles are redispersed in 50 mL 1 M TMAH solution and stirred overnight. To remove excess TMAH, the particles are collected again and redispersed in water, yielding a typical stock solution of 2.7 g/L.

2.2.3. Cobalt ferrite (CoFe)

Nanoparticles of cobalt ferrite ($CoFe_2O_4$) were prepared following the coprecipitation method described by Claesson [18], following earlier work by Tourinho [19]. In a typical preparation 0.01 mol $CoCl_2$ is dissolved in 5 mL 2.4 M hydrochloric acid and 0.02 mol $FeCl_3$ is dissolved in 40 mL water. The solutions are heated to 50 °C, mixed and then immediately added to 200 mL of boiling 1 M NaOH solution while stirring vigorously. After 30 min of stirring at 100 °C, the black mixture is cooled to room temperature. The particles are washed four times with 100 mL water and then redispersed in 30 mL 2 M HNO_3. To this dispersion 30 mL 0.35 M $Fe(NO_3)_3$ is added and it is heated to 100 °C, at which it remains for 45 min. After cooling to room temperature, the particles are sedimented on a magnet, redispersed in 50 mL 1 M TMAH and stirred overnight. To remove excess TMAH, the particles are collected again and redispersed in 50 mL water.

2.2.4. Hematite (Hem)

Spindles of hematite (α-Fe_2O_3) were prepared by the method described by Ocaña [20,21]. The preparation consists of dissolving 0.1 mol $Fe(ClO_4)_3$, 0.1 mol urea and 0.004 mol NaH_2PO_4 in 200 mL and ageing the mixture for 24 h at 100 °C. The mixture is purified by centrifugation and the particles are redispersed first in water, then in 1 M TMAH and finally in again in water.