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Formation of nanostructured colloidosomes using electrostatic deposition of solid lipid nanoparticles onto an oil droplet interface

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ABSTRACT

This study describes the assembly of colloidosomes by adsorbing solid lipid nanoparticles (SLN) onto the interfaces of oil-in-water emulsion droplets via electrostatic deposition technique. Oil-in-water emulsions (10% *w*/*w* corn oil, 1% *w*/*w* whey protein isolate in water) and SLN (10% octadecane, 1% sodium dodecyl sulfate in water) were prepared using a microfluidizer. The surface saturation concentration (C_{sat}) of negatively charged SLN adsorbed onto the positively charged oil-in-water emulsions (at oil droplet concentration of 0.5%) at pH 3 was investigated by measuring ζ-potential, and particle size, as well as assessing the microstructure by optical microscopy. Each of these methods depicted C_{sat} between 1.1–1.5% (*w*/*w*). The results were explained by calculating theoretical C_{sat} using molecular forces acting between the adsorbed droplets as described by the DLVO theory. We demonstrated that the surface saturation of SLN on emulsion droplets depends on the particle size population.

1. Introduction

Solid lipid nanoparticles (SLN) are colloidal encapsulation systems that have been developed to incorporate, protect and deliver hydrophobic bioactive ingredients, such as functional lipids and drugs (Das & Chaudhury, 2011; Helgason et al., 2009; Jenning, Thunemann, & Gohla, 2000; Salminen, Gömmel, Leuenberger, & Weiss, 2016; Salminen, Helgason, Kristinsson, Kristbergsson, & Weiss, 2013; Weiss et al., 2008; Westesen, Bunjes, & Koch, 1997). The next step in formation of functionalized particles is to assemble nanosized structures (such as SLN), which are then used to form more complex structures. By assembling nanosized building blocks in a specific way, the assembled particles can have functionality incorporated into the structure. Such an example is colloidosomes, which have been gaining interest in recent years.

Colloidosomes have been defined as capsules whose interface is composed of closely packed solid particles (Dinsmore et al., 2002; Gu, Decker, & McClements, 2007; Hsu et al., 2005; Kim et al., 2007; Rossier-Miranda, Schroën, & Boom, 2009; Sagis, 2008; Simovic & Prestidge, 2008; Subramaniam, Gregory, Petkov, & Stone, 2007; Thompson, Williams, & Armes, 2015; Velev, Furusawa, & Nagayama, 1996; Zhao, Dan, Pan, Nitin, & Tikekar, 2013). The inner phase of the colloidosome shell can contain oil or water, while the outside phase (continuous phase) is usually water. The colloidosomes are first assembled by absorbing solid particles (e.g., silica or latex nanoparticles) directly onto an oil and water droplet, using reduction in interfacial tension (Dinsmore et al., 2002). Then the adsorbed solid particles are fused together, thus forming an interconnected network of solid particles on an oil droplet interface. In some cases, the inner oil phase is removed resulting in colloidosomes with water as both inner and outer phases (Dinsmore et al., 2002; Kim et al., 2007; Sagis, 2008; Simovic & Prestidge, 2008; Velev et al., 1996). Conversely, other researchers leave the oil phase intact, thus forming colloidosomes with oil as an inner phase and water as continuous phase (Gu et al., 2007; Hsu et al., 2005; Subramaniam et al., 2007). This indicates that colloidosomes can act as delivery systems for both hydrophobic and hydrophilic compounds. The advantages of these types of structures are that the

Abbreviations: *A*, area; A_{12}, Hamaker constant; A_{coll}, surface area of colloidosome; A_{hex}, surface area of a hexagon; c_i, molar concentration of ion *i*; C_{sat}, minimum concentration of polyelectrolyte required to fully saturate the particle surfaces; d_i, diameter; d_{32}, surface area mean diameter; d_{43}, volume mean diameter; δ, layer thickness; *e*, charge of an electron; ε_0, permittivity of vacuum; ε_r, relative dielectric constant of the solution; ε_1, ε_2, ε_3, dielectric constants of phase 1, 2, and medium; Γ, absorbed weight; *h*, Planck constant; *I*, ionic strength; *k*, Boltzmann constant; κ^{-1}, Debye screening length; *MVol*, molar volume of solvent; MWCO, molecular weight cut-off; N_A, Avogadro's number; n_i, number of particles; N_{SLN}, number of SLN; n_1, n_2, n_3, refractive indices of phases 1, 2, and medium; PDI, polydispersity index; ϕ, volume fraction of the particles; φ, surface potential; pI, isoelectric point; *r*, radius; r_{eff}, effective radius; R_{hex}, radius of hexagon; ρ, density; *s*, separation distance; SDS, sodium dodecyl sulfate; SLN, solid lipid nanoparticles; T, absolute temperature; WPI, whey protein isolate; Γ_{sat}, surface load of the polyelectrolyte at saturation; *V*, volume; v_e, absorption frequency; $V_E(s)$, electrostatic (Coulombic) interaction potential; $w(s)$, interaction potential; $V_S(s)$, steric interaction potential; $V_{total}(s)$, interaction pair potential; $V_{VDW}(s)$, van der Waals (dispersion) interaction potential; χ, Flory–Huggins parameter; z_i, charge number of an ion.

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colloidal shell can be fused together resulting in controlled pore size and extensively increased physical stability of the structure. The high physical stability is underlined by the fact that the inner oil phase can be removed without destabilizing the colloidal shell.

Unfortunately, the size of colloidosomes formed is limited by the formation procedure. Because the solid particles are adsorbed directly onto an oil droplet interface (a procedure called Pickering stabilization), the diameter of the inner droplet has to be much larger than the diameter of the solid particle (Aveyard, Binks, & Clint, 2003). Furthermore, the solid particle has to be partly hydrophobic and partly hydrophilic in order to adsorb to an oil droplet interface (Aveyard et al., 2003). An interesting solution to both these problems is to form colloidosomes using electrostatic deposition, which makes it possible to adsorb, for example, negatively charged solid particles onto the surface of a positively charged oil droplet interface (Gu et al., 2007; Salminen & Weiss, 2014b). Therefore, there will be almost no limits to the size range of the inner particle compared to the outer solid particle.

The purpose of this study was to investigate the formation of nanostructured colloidosomes by assembly of nanosized oil-in-water emulsions and solid lipid nanoparticles. We hypothesized that SLN can be adsorbed onto the interface of oil-in-water emulsion droplets through electrostatic deposition, thus creating a colloidosome structure. We investigated the surface coverage (i.e. saturation concentration) of SLN on the interface of oil-in-water emulsion, and compared the surface coverage with theoretically calculated values.

2. Materials and methods

2.1. Materials

Octadecane (purity 99%) was purchased from Fluka (Buchs, Switzerland). Corn oil was obtained from Mega eG (Stuttgart, Germany). Food-grade whey protein isolate (WPI) (DSE 9273, dry matter 99.0%, protein 93.9%, lactose 0.5%, fat 0.2%, moisture 5.2%, ash 1.5%) was donated by Fonterra GmbH (Hamburg, Germany). Sodium acetate anhydrous was obtained from Merck (Darmstadt, Germany) and glacial acetic acid was from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Sodium azide, and sodium dodecyl sulfate (SDS) (purity 98.5%) were purchased from the Sigma-Aldrich (Steinheim, Germany). Double distilled, deionized water was used throughout the study.

2.2. Oil-in-water emulsion preparation

Aqueous surfactant solution was prepared by dispersing 1% (*w*/*w*) WPI in water and stirred overnight to fully solubilize the protein. Then corn oil (10% *w*/*w*) was added to the aqueous WPI solution (90% *w*/*w*) and mixed with a standard unit homogenizer (Labworld, Staufen, Germany) at high speed (24,000 min^{-1}) for 2 min followed by five passes through a microfluidizer (Microfluidizer Processor M-110EH-30, Microfluidics, Newton MA, USA) at 10,000 psi.

2.3. SLN preparation

Aqueous surfactant solution was prepared by dispersing 1% (*w*/*w*) SDS in water. SLN were prepared using the hot high-pressure homogenization method as described earlier (Helgason, Salminen, Kristbergsson, McClements, & Weiss, 2015). A lipid phase (10% *w*/*w* octadecane) was fully melted by heating to 40–45 °C, and mixed with the aqueous SDS surfactant solution (90% *w*/*w*) held at 40–45 °C with a standard unit homogenizer (Labworld, Staufen, Germany) at high speed (24,000 min^{-1}) for 2 min to form a coarse emulsion premix. The hot emulsion premix was homogenized with 5 cycles at 10,000 psi using a microfluidizer (Microfluidizer Processor M-110EH-30, Microfluidics, Newton, MA, USA). The microfluidizer was heated up prior homogenization by cycling hot water (40–45 °C) through the machine to prevent solidification during the homogenization procedure. The finely dispersed emulsion was then placed at 4 °C to ensure octadecane crystallization.

2.4. Dialysis of emulsion and SLN

Both the emulsion and SLN were dialyzed using a cellulose ester dialysis membrane (MWCO 100,000; Spectrum Laboratories, Inc., Rancho Dominquez, CA, USA) prior electrostatic deposition to remove excess proteins and SDS from the aqueous phases that could interfere with the adsorption process. Water was used as dialysate for the emulsions. Dialysates were changed three times after an equilibrium time of at least 3 h. Sodium azide (0.02% *w*/*w*) was added to the dialyzed samples to prevent microbial growth.

2.5. Formation of colloidosomes

Colloidosomes were formed using the electrostatic deposition technique (Gu et al., 2007; Guzey & McClements, 2006; Salminen & Weiss, 2014b). This was carried out by first mixing corn oil-in-water emulsions (10% oil, 1% WPI, pH 6) with SLN solution (10% octadecane, 1% SDS, pH 6), and then diluting with 10 mM sodium acetate buffer (pH 3 or 5) (Fig. 1). After mixing, the final emulsion oil droplet concentration in the solutions was 0.5% (*w*/*w*), while the SLN concentration varied (0–2.0% *w*/*w*).

2.6. Particle size determination

The particle size of the samples was measured using a dynamic light scattering instrument (Nano ZS, Malvern Instruments, Worcestershire, UK) that reports the mean particle diameter (z-average) and the polydispersity index (PDI) ranging from 0 (monodisperse) to 1.0 (very broad distribution), and a laser diffraction instrument (LS230 Small Volume Module Plus, Beckmann Coulter Inc., Miami, FL) that reports the surface area mean diameter: $d_{32} = \Sigma n_i^3 / \Sigma n_i^2$, and volume mean diameter: $d_{43} = \Sigma n_i^4 / \Sigma n_i^3$, where n_i is the number of particles of diameter d_i in the population. Samples were diluted 1:100 using a 10 mM acetate buffer solution (pH 3 or pH 5) to prevent multiple scattering effects. Refractive indices of 1.50, 1.43, and 1.33 were used for solid octadecane (Christenson, 2006), corn oil, and water, respectively. Refractive indexes of materials and (colloidal) particles define how they interact with electromagnetic radiation (Hunter, 1986), which in turn impacts the scattering and absorption of light by these particles (McClements, 2015). Consequently, the accuracy of particle size devices based on light scattering depends on the correct knowledge of refractive indexes.

2.7. ζ-potential measurement

The ζ-potential was measured at 25 °C using a laser-Doppler microelectrophoresis device (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). Samples were diluted as described in **2.6**.

2.8. Optical microscopy

Microscopic images were taken using an AXIO Scope.A1 Light Microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany) equipped with a Canon Powershot G10 Digital camera (Canon, Tokyo, Japan). All images were recorded at magnification of 10×. Representative images for microscopic imaging were chosen from among at least four similar images.

2.9. Statistical analysis

All measurements were repeated 3 times using triplicate samples. Means and standard deviations were calculated using Excel (Microsoft, Redmond, WA, USA).