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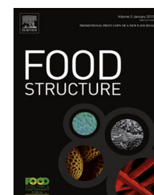

Morphological stability of microencapsulated vitamin formulations by AFM imaging

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The morphological stability of microencapsulated lipophilic vitamin formulations directly influences the long-term storage stability and performance in final applications. The internal particle morphology of three commercial dry Vitamin A acetate formulations differing in their matrix composition (hydrocolloid/polysaccharide and plasticizer) was systematically characterized by atomic force microscopy (AFM). The evolution of the particle internal morphology after *per-se* storage at 25 and 40 °C for ten months was investigated. Furthermore, an accelerated stress test by *in-situ* heating and AFM imaging was developed. Surface roughness parameters from the AFM images were used to semi-quantitatively estimate the evolution of the particle internal microstructure, and thus the aging and destabilization of the lipophilic vitamin formulations. The influence of humidity level on the thermal morphological restructuring of one of the commercial samples was also investigated. This study has shown that such AFM investigations of microparticles are a novel analytical tool to rapidly estimate the physical stability of the lipophilic vitamin dried emulsions.

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1. Introduction

Micronutrients and nutraceuticals such as vitamins, carotenoids and polyunsaturated fatty acids (PUFAs) are essential for human and animal health and well-being (Teleki, Hitzfeld, & Eggersdorfer, 2013). Today complementary to a healthy diet, these compounds are supplied either in fortified food and feed products or in dietary supplements such as multivitamin-multimineral tablets. Water-soluble vitamins like Vitamin C can easily be added to most food or feed matrices and might only require a dilution step (*e.g.* for Vitamin B12 or folic acid). On the other hand, fat-soluble vitamins, like Vitamin A, or PUFAs have to be delivered in microencapsulated product formulations (Gonnet, Lethuaut, & Boury, 2010). Typically, they are chemically instable (*e.g.* to oxidation, heat) and they cannot easily be added to a hydrophilic food environment like a beverage. Thus, such vitamins are mostly sold in microencapsulated product formulations to protect the active ingredient from the surrounding environment (Augustin & Hemar, 2009; Gouin, 2004; Kuang, Oliveira, & Crean, 2010; Madene, Jacquot, Joël, & Desobry, 2006). The main benefits of microencapsulation technologies are the extension of the vitamin's shelf-life, accurate and controlled release, easier handling process of a free-flowing powder compared to an oil, increased bioavailability, and usually improved sensory properties with respect to appearance and taste (*e.g.* fishy off-taste of PUFAs) (Desai & Park, 2005).

On an industrial scale, the formulation process typically consists of an emulsification step followed by drying to produce the dry product particles containing the microencapsulated active ingredient. During the first step, emulsifiers are homogenized with the oily active ingredient to form a fine oil-in-water emulsion (Jafari, Assadpoor, He, & Bhandari, 2008). The emulsifier typically also has the dual function as wall- or matrix-forming material during the subsequent drying step. The wall materials used for drying should fulfill a range of properties such as no reactivity with the active ingredient, ability to provide maximum protection during the process as well as in the final application, complete release of the core during desirable conditions, desired rheological properties and solubility in water (Jafari et al., 2008). Furthermore, the cost position, regulatory status as well as customer perception of the formulation ingredients are important. Proteins, especially gelatin, are commonly used for microencapsulation by drying (Jafari et al., 2008). An advantage of a protein like gelatin is its ability to assemble at interfaces thereby also stabilizing the

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vitamin emulsion that is dried (Augustin & Hemar, 2009). However, customer demands for animal-free formulations have driven the development of microencapsulated product formulations based on plant-based proteins, gums or carbohydrates as well as mixtures thereof. While most food carbohydrates like starches or maltodextrins are not surface active, gum acacia is often applied due to its emulsifying capacity (McNamee, O'Riorda, & O'Sullivan, 1998). Starch can be chemically modified with *n*-octenyl succinic anhydride (OSA) to improve its emulsifying properties (Augustin & Hemar, 2009; Drusch & Schwarz, 2006; Sweedman, Tizzotti, Schäfer, & Gilbert, 2013). Many studies have focused on the factors affecting encapsulation efficiency by spray-drying microencapsulation. Thus the influence of parameters such as emulsion droplet size and drying temperatures on final product properties like particle morphology and surface oil are well understood (Jafari et al., 2008).

However, the control of the thermal and aging stability of microencapsulated lipophilic compounds still remains challenging. Storage stability of microencapsulated product formulations often has to be warranted up to 36 months at ambient conditions. Furthermore, many applications require stability at harsher conditions such as increased temperature and humidity. However, such storage conditions can not only result in the degradation and loss of the encapsulated active ingredient, but also in a destabilization of the whole matrix, *e.g.* by a phase separation of the individual components. This is undesirable as it can not only accelerate loss of the encapsulated active but also alter the properties of the formulation such as the dissolution rate (Qi, Belton, Nollenberger, Gryczke, & Craig, 2011). During the research and development of novel product formulations, the stability of the encapsulated active ingredient is typically monitored in time-consuming studies, storing the product formulations at different environmental conditions and assessing the concentration of the active ingredient at several time points.

Therefore, it is critical to study and ultimately control the phase separation in a dry lipophilic vitamin formulation (Hughes et al., 2016; Tedeschi, Leuenberger, & Ubbink, 2016). The detection limit of conventional commercially available analytical tools such a differential scanning calorimetry (DSC) or powder X-ray diffraction (XRD) is not low enough to sense the early stage of this process, which represents the critical point of the thermodynamical destabilization. Hence, early detection would be highly desirable as these tools cannot deliver information on the distribution of the phase separation at molecular level. Up to now, few investigations have tried to identify low levels of phase separation at micro- and nanoscale. Lauer et al. (2011, 2013) used atomic force microscopy (AFM) in combination with Raman microscopy as a screening tool to assess the molecular homogeneity and physical stability of amorphous solid dispersions made by hot-melt extrusion. The methodology serves as a rapid assay to identify stable formulations promising for further development. AFM can directly and non-destructively examine the molecular surface landscapes and properties in an ambient environment and the molecular surface roughness can be quantified. Furthermore, during Tapping-Mode™ operation, the mechanical material heterogeneity can be qualitatively displayed by phase mapping (Lauer et al., 2013). Morphological evolution both in the bulk and on the surface can be observed by fracturing the samples (Lauer et al., 2011). Analysis can also be performed after aging the samples at certain humidity and temperature (Lauer et al., 2013; Qi et al., 2011). Quiroga and Bergenståhl (2007) investigated the phase separation in amylopectin and β-lactoglobulin dry films by AFM. The moisture-induced surface crystallization of spray-dried amorphous lactose particles was also studied by AFM and mechanistic and kinetic information about the process were extracted (Mahlin, Berggren, Alderborn, & Engström, 2004). Recently, Christensen, Rasmussen, and Simonsen (2015) introduced a methodology for *quantitative* roughness analysis of single nanoparticles by AFM. Surface parameters for topography maps of casein micelles that describe excess surface area and topography height variations were proposed. In combination, these parameters can capture surface height variations to monitor changes in surface morphology due to environmental or processing influences (Christensen et al., 2015).

In this study, the objective was to investigate the internal particle morphology of dry microencapsulated product formulations containing a lipophilic vitamin. Commercial Vitamin A acetate microparticles formulated with either gelatin, gum acacia or OSA-modified food starch were characterized by AFM. The AFM images were analyzed both qualitatively and semi-quantitatively by calculating several surface roughness parameters. Evolution of morphological properties after *per-se* storage at 25 and 40 °C for several months was investigated. Furthermore, an accelerated stress test by *in-situ* heating and AFM imaging was also developed to study and compare the morphological evolution of the internal structure of the commercial vitamin formulations at the nanoscale.

2. Material and methods

Commercial microencapsulated, dry Vitamin A acetate products (referred to as beadlets in the following) were all provided by DSM Nutritional Products Ltd. Three different Vitamin A acetate beadlet formulations were investigated that vary in their matrix composition. The main emulsifier and matrix material in these beadlets was gelatin (GEL), gum acacia (GA) or OSA-modified food starch (MFS), respectively. The GEL and MFS microparticles also contain sucrose as a plasticizer, while GA has been formulated with the addition of maltodextrin. All samples were stored in aluminium bags at 4 °C when not analyzed. Furthermore, the Vitamin A acetate microparticles were stored (*per-se* stability) in aluminium bags in 5 g aliquots in climatic chambers at 25 °C/60% RH as well as 40 °C/75% RH. The storage stability was thus only affected by temperature, as the aluminium packaging is impermeable to water vapor (Lange & Wyser, 2003). The samples were kept at 4 °C once they were extracted from the *per-se* stability after ~300 days of storage in either of the climatic chambers. Furthermore, the Vitamin A acetate retention after storage in the climatic chambers for 30, 60, 90 as well as 180 days was determined by HPLC analysis. For each sample, a double determination was carried out. A calibration curve was prepared by using Vitamin A acetate standard solutions of known purity (Dr. Ehrenstorfer GmbH, Augsburg, Germany). The Vitamin A acetate beadlet formulations were accurately weighed into volumetric flasks. They were then dissolved in an ultrasonic water bath at 45 °C for 20 min after addition of protease Multifect PR 6 L (Brenntag) as well as an ammonium hydroxide solution (0.02%, Fluka). The dissolved beadlet solution was then cooled down to room temperature and an extraction solution (1:1, v/v) consisting of THF (tetrahydrofurane, ≥99.5%, Fluka) and acetone (≥99.5%, Fluka) was added under sonication for 10 min at 45 °C. The final solution was then centrifuged until a clear supernatant was obtained and diluted to appropriate concentration with methanol (≥99.8%, Merck). The clear supernatant was injected into the HPLC, 2% water in methanol was used as mobile phase.

Microparticles as-received as well as from *per-se* stability studies were analyzed by *atomic force microscopy* (AFM). The particles were cut into two equal parts with a razor blade under an optical microscope. Particles that were visually severely damaged or deformed during the cutting procedure were discarded from further analysis. The hemispherical part was stuck and fixed on the magnetic disk using a double-sided tape. The plane area of the hemispherical microparticle was deposited upwards onto the substrate for AFM analysis. Several particles (~5–10) were

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