Colloids and Surfaces A: Physicochem. Eng. Aspects 481 (2015) 229–236

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Confocal Raman microscopy for mapping phase segregation in individually dried particles composed of lactose and macromolecules

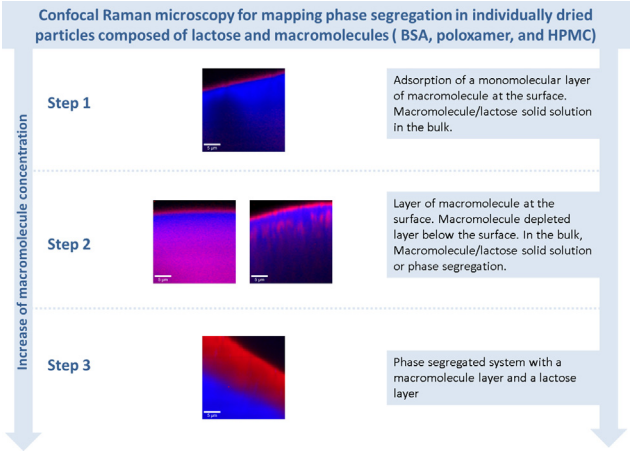
Marine Nuzzo[a,b,*], Jakob Sloth[c], Birgit Brandner[a], Björn Bergenstahl[b], Anna Millqvist-Fureby[a]

[a] SP Technical Research Institute of Sweden, Chemistry, Materials and Surfaces, Stockholm, Sweden
[b] Lund University, Food Technology, Engineering and Nutrition, Lund, Sweden
[c] GEA Process Engineering A/S, Process Engineering, Soeborg, Denmark

HIGHLIGHTS

- Confocal Raman microscopy is a powerful tool for internal mapping of particles.
- Phase segregation occurs even during the short drying time of particles.
- Macromolecules are enriched at the surface of the dried particles.
- A macromolecule depleted layer is observed below the surface.
- The macromolecules are segregated from the lactose matrix as a thick surface layer.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 27 April 2015
Accepted 29 April 2015
Available online 7 May 2015

Keywords:
Confocal Raman microscopy
Phase segregation
Composition
Mapping
Individually dried particles

ABSTRACT

The quality of powder is determined by its functionality such as dissolution, encapsulation and flowability. The functionality of powder is in turn determined by their primary properties such as morphology and composition which need to be studied. Hence morphology and surface composition has been largely investigated in spray dried powders and individually dried particles. On the contrary, there is only scarce information regarding the internal structure. With the aim of acquiring a better understanding of the localization of different ingredients in spray dried powders we have used confocal Raman microscopy to investigate the internal microstructure of individually dried particles. In this study three different macromolecules have been investigated: bovine serum albumin, hydroxypropyl methyl cellulose, and triblock co-polymer poloxamer in a lactose matrix are compared at various macromolecule to lactose ratios. The surface and internal component distribution in response to the macromolecule concentration has been established. For the first time phase segregation in particles during a short drying time range is

* Corresponding author at: SP Technical Research Institute of Sweden, Chemistry, Materials and Surfaces, Stockholm, Sweden. Tel.: +46 709806023.
E-mail address: marine.nuzzo@sp.se (M. Nuzzo).

http://dx.doi.org/10.1016/j.colsurfa.2015.04.044

shown. Macromolecules were enriched at the surface of the dried particles and a macromolecule depleted layer was observed below the surface. Macromolecule enriched domains were found segregated from the amorphous lactose matrix in the internal part of the particles. Confocal Raman microscopy was found to be a powerful tool for internal mapping in individually dried particles.

1. Introduction

Many food and pharmaceutical formulations are created through a drying process. Spray drying is one of the most common methods to provide stable ingredients in the format of powders. Drying conditions and formulation determine primary properties such as morphology and composition that finally provide functionality such as dissolution, encapsulation and flowability. Substantial efforts have been spent to predict particle properties and structure after spray drying. As a large number of parameters can influence the properties it has been shown that the use of simplified systems as individually dried particles can aid prediction of particle properties, e.g. morphology and composition [1–3]. The morphology and surface composition correlation between spray dried powders and individually dried particles has already been studied [4–6]. These studies revealed great similarity between spray dried powders and individually dried particles, indicating that individually dried particles can be used to predict the properties of spray-dried powders, although time and length scales differ. Various techniques have been used to analyze the distribution of compounds in powders. In spray-dried emulsion the surface fat is often determined as the extractable fat, as established by Buma [7]. Surface composition analysis by XPS has been shown to be a powerful tool in analysis of the chemical surface composition of spray dried powders [6,8–11]. Auty [12] showed that confocal scanning laser microscopy is also a relevant method to map the interior microstructure of whole milk powders. Fluorescent probes were used to localize fat and protein, and an optical horizontal section was analyzed to provide an internal map of the powder particle, as well as of other dairy products. Similarly, confocal Raman microscopy was used by Piot et al. [13] to localize the ingredients in wheat grain by utilizing the inherent fluorescence and thus avoiding the addition of dyes. Protein was found preferentially in the subaleurone endosperm compared to the central endosperm. However, the number of studies using these techniques for powder investigations is very limited.

During the drying of a droplet numerous phenomena take place. Adsorption of surface active compounds at the particle surface was shown by Fäldt et al. [8] who studied a lactose-sodium caseinate system analyzed by XPS (analysing 2–10 nm of the surface). Other studies have confirmed the adsorption of surface active materials [6,8,14], effects of competitive adsorption [15,16] and drying conditions [17]. In a study by Landström [18] on spray dried micellar casein and lactose solution the presence of protein at the powder surface was shown using TEM of thin sections of particles. In further investigations by Landström et al. [19] the amount of the total protein located in the surface layer was quantified using a fluorescence quenching technique. In spray-dried particles formed from solutions of protein and lactose the authors found that 40%–5% of the protein was at the surface when the protein fraction of the total solid was less than 5%. Recent research by Whiteside et al. [4] has revealed that polymer enriched domains (PEG 6000) and drug enriched domains (griseofulvin) can occur in spray dried particles. Furthermore, a large number of studies have been focused on phase separation in liquid mixed systems [20–23]. In a study by Fransson et al. [24] the mixture of gelatin and maltodextrin was investigated. It was found, by recording in real time with the help of CLSM, that gelatin and maltodextrin started to phase separate after a short time (100 s) in droplets. In a mixture of whey protein isolate and gellan gum the phase separation was found to be starting after 200 s by Wassén et al. [25]. Additionally phase segregation induced by freeze drying has been reported [26,27]. Indeed, while freezing the formation of ice crystals induce a great increase of the solutes concentration which promoted molecular interactions hence leads to phase segregation of the compounds.

Phase segregation as a consequence of drying has been observed in thin dried films using AFM, for instance in thin films formed from protein and starch [28]. Further, SEM micrographs of the cross-sections of HPMC/corn starch dried films revealed a polymer phase segregation [29]. However, there is only scarce information regarding the internal microstructure in spray-dried and individually dried particles. The functionality of powders is expected to be influenced by phase segregation of its components that makes phase segregation an important phenomenon to study. It can be expected that phase segregation can affect the chemical reactivity, crystallization resistance, encapsulation power, dissolution properties, etc. With the aim of acquiring a better understanding of the localization of different ingredients in spray dried powders we have used confocal Raman microscopy to investigate the internal microstructure of individually dried particles. A specific advantage of this method is that no dyes are needed to identify the different components and optical sections can be obtained. Furthermore, Raman spectroscopy also provides semi-quantitative information. In this study three different macromolecules (BSA, poloxamer, and HPMC) in a lactose matrix are compared at different macromolecule concentrations. The localization of the macromolecules and the evolution of an internal microstructure are determined and linked to the physical properties of these macromolecules.

2. Material and methods

2.1. Materials

Bovine serum albumin (BSA) (lyophilized powder with a purity of 96%) and hydroxypropyl methylcellulose (HPMC) (molar mass 10 000 g/mol and 1.8–2 methoxy and 0.2–0.3 propylene oxide per glucose unit) were purchased from Sigma–Aldrich, St. Louis, USA. Poloxamer 188 (Lutrol F68) (PEO_{80}–PPO_{27}–PEO_{80} molar mass 8–10 000 g/mol) was purchased from BASF, Germany. α-Lactose monohydrate was obtained from Merck KGaA, Darmstadt, Germany. Ultra purified water (MilliQ, Millipore Systems, MA 01821, USA) was used as solvent.

2.2. Methods

2.2.1. Preparation of solutions

Three systems with 10% solids content have been used in this study. Stock solutions of α-lactose, BSA, HPMC and poloxamer 188 respectively, were prepared at 10% concentration w/w. The lactose solution was adjusted to pH 7 by adding NaOH. The α-lactose solution was mixed with poloxamer 188 (system 1), BSA (system 2), hydroxypropyl methylcellulose (system 3), respectively, in the following proportions of protein/polymer to lactose: 1/99; 5/95; 10/90; 20/80. The compositions are given as the weight percentage of each component in solid. The solutions were allowed to equilibrate for 24 h before conducting the drying experiments.