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An optimized methodology to analyze biopolymer capsules by environmental scanning electron microscopy

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ABSTRACT

The aim of this paper is to describe an optimized methodology to study the surface characteristics and internal structure of biopolymer capsules using scanning electron microscopy (SEM) in environmental mode. The main advantage of this methodology is that no preparation is required and, significantly, no metallic coverage is deposited on the surface of the specimen, thus preserving the original capsule shape and its surface morphology. This avoids introducing preparation artefacts which could modify the capsule surface and mask information concerning important feature like porosities or roughness. Using this method gelatin and mainly fatty coatings, difficult to be analyzed by standard SEM technique, unambiguously show fine details of their surface morphology without damage. Furthermore, chemical contrast is preserved in backscattered electron images of unprepared samples, allowing visualizing the internal organization of the capsule, the quality of the envelope, etc.… This study provides pointers on how to obtain optimal conditions for the analysis of biological or sensitive material, as this is not always studied using appropriate techniques. A reliable evaluation of the parameters used in capsule elaboration for research and industrial applications, as well as that of capsule functionality is provided by this methodology, which is essential for the technological progress in this domain.

1. Introduction

Encapsulation is used in various domains, like food, feed, cosmetic, and pharmaceutics industry, to protect various bioactive molecules like vitamin, peptide, aromas or pigments. The main objective is to build a barrier between the component in the particle and the environment. This barrier may protect against oxygen, water, and light, and prevent contact with other ingredients, in e.g. a ready meal or control diffusion. The efficiency of protection mainly depends on the composition and structure of the wall. The barrier is generally made of bio compounds for food, feed and cosmetic industry products. The encapsulating agents are often gums (e.g. acacia, shellac…), carbohydrates (e.g. starch, modified starch, maltodextrin, sucrose…), polysaccharides of marine origin (e.g. alginate, carrageenan…) [1] and protein (e.g. milk protein, gelatin, pea protein, soy protein…) [2]. Lipids-like fats and waxes are commonly used as encapsulating agents [3].

Encapsulation process is the envelopment of small solid particles, liquid droplets or gases in a coating. The size of encapsulated particles may vary over a large range, going from the sub-micrometer scale to the millimeter scale. The development of a successful encapsulation system is based on a good knowledge of the component to be encapsulated the properties of the material used for encapsulation and the control management of the delivery system (capsule). Most encapsulation technologies use a liquid (complex coacervation, interfacial and in situ polymerization or solvent evaporation from emulsion), or a gas as suspending medium (spray-drying or spray-cooling, fluidized-bed coating or co-extrusion) [4].

In this study, the word "capsule" will be used for all the particles produced in the various processes used.

The shape and structure of the final capsule is an important factor in the design of a functional product. Purity, chemical homogeneity and internal structure are also very important parameters for the encapsulation of products of inorganic origin (e.g. calcium, silica), with for example, a fatty envelope [3]. The ability to distinguish the different parts of a capsule (core and shell) highlights the quality and the protective efficiency of this envelope. The thickness of a coating acting as a barrier is a very important parameter for a controlled release. As an example, for aroma encapsulation: porosity, size and distribution over the coating surface are instrumental as they determine the time and speed of release of the molecule.

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Finally, coating roughness and shape are also important because it can act as an interface with the environment, and is a valuable parameter from a purely esthetic role and comfort point of view with capsules used for cosmetic products.

Scanning electron microscopy is a powerful technique that provides high enough resolution and contrast in three-dimensional images to evaluate the above-mentioned parameters. To be successfully observed under high vacuum conditions, the sample surface has to be a good electrical and thermal conductor [5]. The analysis of non-conductor particles like capsules under high vacuum generally involves placing a metallic film, for instance of carbon, platinum, gold or gold alloys, on the surface [6]. The goal is to avoid the accumulation of electrical charges on the sample surface. Moreover, it is widely accepted that metallization helps to avoid damage to the sample due to the heating caused by the electron beam [7]. However, substances like fat with low fusion temperature are very sensitive to the energy transfer from the primary electron beam and thus particularly difficult to prepare and to observe in high-vacuum mode. Although a standard technique, metallization cannot be used in many cases because it modifies the original surface coating, covering porosities and masking very important surface information such as fine roughness. The deposited film is also detected in energy dispersive spectroscopy (EDS) spectra and its peaks can overlap with those of elements present in the coating resulting in the loss of important sample information.

Environmental scanning electron microscopy (ESEM) has been used in recent years as an alternative methodology for the observation of non-conductors and/or hydrated samples [8,9]. Thanks to the low partial pressure of an inert gas or water vapor introduced in the specimen chamber, these samples can be observed without any preparation. This is possible because the gas or water vapor molecules surrounding the sample surface are ionized by electrons emitted or re-emitted by the sample itself when it is bombarded by the primary electron beam. Electrons removed from gas atoms by ionization have an energy level that is proportional to that of the electron generating the ionization. The ionization proceeds in a cascade and the electrons it produces are identified by detectors adapted to the pressured medium (Large-Field or Gaseous, depending on the pressure range). The image formed using these electrons provide topographical information similar to that obtained with direct secondary electrons [10]. The positively charged ionized gas molecules are attracted by the negative charges accumulated on the surface of the sample, restoring the electrical equilibrium on it. In fact, it is essential that the surface has a neutral charge to avoid a repulsion of the primary beam, which would impede obtaining a useful image. This effect is equivalent to that obtained by covering the surface with a metallic film with the advantage that no artefact is introduced. These ionized molecules also contribute to the equilibrium of the surface temperature. For humid samples, the possibility to adjust the gas or water vapor pressure minimizes surface drying during the observation.

Backscattered electrons (BSE) result from elastic collisions between electrons from the primary beam and sample surface atoms and have energy of the same order of the incident beam [11]. Their path is mainly directed towards the bottom of the objective lens, where the BSE detector is usually placed. The solid-state detector (SSD) used in high-vacuum mode is usually divided into two sectors which are able to detect BSE signal independently. By adding signals detected simultaneously point-by-point by the two sectors, an image providing a compositional contrast is obtained. In ESEM mode, some loss in the detected BSE signal might be observed mainly for high pressures, i.e., several mbar in gas or water vapor. As BSE possess a relatively high energy, the fraction scattered far away from the initial direction of emission due to collisions with gas molecules is negligible, if the travel distance between the sample surface and the detector is not too large [12]. In order to minimize the BSE signal loss in ESEM mode a special detector equipped with a vertical cone directed towards the sample surface may be used. However, for pressures below 2 mbar, which can be considered as low, the signal loss is not significant. Consequently, the BSE detector used in high-vacuum mode can also be used in this pressure range to obtain a compositional contrast from non-conductor and/or humid sample surfaces.

In the literature SEM observations are often used to show general morphological aspects of capsules like shape and size. Very few studies go into the fine details of sample surface roughness. Moreover little information is provided about sample preparation and/or observation parameters (Table 1). No systematic study that investigated the influence of these parameters on the results was found, despite the fact that this information is crucial to correctly evaluate the capsule characteristics.

In this work the potential of ESEM methodology to provide information about the adequacy of capsule fabrication process and the functionality of its original surface was investigated.

The influence of artefacts generated in the capsule surface morphology after a metallic film deposition for conventional SEM observations in high-vacuum mode was also evaluated. Three different types of capsules were studied: 1) garlic extract entrapped by fat; 2) salt particles coated by gelatin or fat; and 3) a wet alginate bead. Surface details with and without metallization were compared. The thermal effects observed on covered surfaces are discussed. The goal is to propose a methodology for the analysis of capsules without covering them, thus preserving the original surface from both morphological and chemical points of view.

2. Experimental

2.1. Materials

2.1.1. "A" capsule: garlic extract in hydrogenated vegetable oil

"A" capsules containing garlic extract powder and coated by hydrogenated vegetable oil (SIO, France) were obtained by the method of spray chilling [4]. This process was performed with a WSG5 Glatt fluidized bed apparatus (Glatt, Switzerland).

The powder was divided into three portions, each prepared and observed in a different mode. For the first portion (labeled "A1"), the external surface of the garlic capsules was metallized with sputtered gold. Some capsules were cut with a scalpel and observed in environmental mode. The same sample was then observed in high vacuum at different voltages. The capsules of the second portion (labeled "A2") were first cut and observed in environmental mode. They were then metallized and observed in high vacuum mode. For the third portion (labeled "A3") no metallization was performed, and the capsules were observed in environmental mode by varying two parameters, water vapor pressure and the accelerating voltage of the primary beam

2.1.2. "B" capsule: sodium chloride coated by gelatin or by hydrogenated vegetable oil

"B" capsules are constituted of an inorganic salt (sodium chloride, Quaron, France), coated by: a) 10% of type A gelatin (Lapi Gelatine, Italy) (labeled "B1") (fluidized bed coating process); or b) 30% of hydrogenated vegetable oil (SIO, France) (labeled "B2") (hot melt process [24]). Productions were performed with a WSG5 Glatt fluidized bed apparatus (Glatt, Switzerland). SEM observations on coatless and coated salt using BSE compositional contrast in environmental and in high vacuum mode were then compared.

2.1.3. "C" capsule: alginate bead

"C" Capsule was an alginate bead, formulated with a solution of sodium alginate (2000 kDa, M:G ratio. 1.6, FMC Biopolymer, USA) to 2% w/w in distilled water, and obtained by gelation in a calcium chloride (Sigma Aldrich, USA) bath (10% w/w) during 10 min. Beads have a relative humidity of 24.8% w/w (infrared balance method). Beads were divided in two portions, each prepared and observed in a different mode. For the first portion (labeled "C1"), the external surface of the alginate beads was metalized with sputtered gold and observed in high vacuum mode. For the second portion (labeled "C2"), the original surface of the alginate beads was observed in environmental mode.