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A REVIEW OF METHODS AND TECHNIQUES FOR CHARACTERIZATION OF STRUCTURE, MORPHOLOGY AND DISPERSION STABILITY OF MICROCAPSULES

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Abstract: Many different active materials such as flavours, enzymes, drugs, vitamins, etc., are subject to microencapsulation inside microspheres made from different polymeric and non-polymeric materials. These microcapsules release their content at certain time and depending on the end use of encapsulated moieties, they could be implemented in agriculture, food, cosmetic, pharmaceutical, textile or other products. The paper reviews the existing methods and techniques for characterization of microcapsules with various morphologies, structures and properties. These characterization techniques include methods for determination of composition, visualizing tools, size measurements, examination of structure, crystallinity, and dispersion stability of microcapsules. The paper summarizes the common physicochemical techniques for microcapsule characterization and thus, provides a useful tool for researchers working in this area.

Keywords: Microcapsules, Morphology, Structure, Composition, Characterization methods.

INTRODUCTION

Microencapsulation is an effective method that wraps gas, solid and/or liquid substances by using polymer or non-polymeric capsules with size from 1 to 999 μm . The ingredient coated is referred as core, fill, payload, or internal phase whereas the polymer coat is termed as shell, wall, coating, capsule, membrane, or external phase (Fig. 1). The encapsulated ingredients can be released at a controlled manner under the influence of specific conditions. Typical mechanisms for releasing the core material are dissolution of the wall, diffusion through the wall, dry up, enzymatic degradation, rupture (mechanical, light, etc.) or melting of the wall whereas the release effects could be immediate, pulsed or delayed. The microcapsule stored substance is isolated and protected from the surrounding degrading factors like oxidation, dehydration, heat, acidity, moisture, enzymes, evaporation, etc. Besides the protection, microencapsulation also separates the reactants, reduces the toxicity, volatility, etc. All this made microencapsulation suitable for diverse applications such as pharmaceuticals, cosmetics, biotechnology, food, electronics, catalysis, pesticides, textile, adhesives, waste treatment, etc. (Park, J.H. et al., 2007; Specos, M.M.M et al., 2010). Various techniques of microencapsulation have been reported including chemical (coacervation, interfacial and in situ polymerization, phase separation, etc.), physico-chemical and physico-mechanical such as sol-gel encapsulation, emulsion solvent evaporation, spray drying, spray congealing, solvent diffusion, pan coating, fluidized bed coating, centrifugal extrusion process, etc.

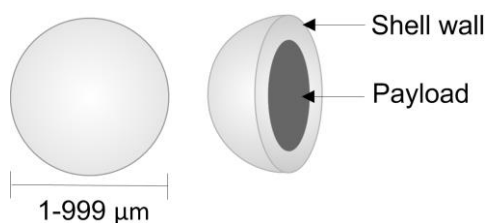


Fig. 1. Schematic representation of the principle structure of microcapsules.

The paper aims at summarizing the common physicochemical techniques for microcapsule (MC) characterization and thus, to provide a useful tool for researchers working in this area.

EXPOSITION

Composition determination and visualizing tools

The visualizing tools allow the shape and morphology of the microcapsules to be visualized in a solution or when impregnated in textiles. In optical microscopy, by attaching a thin layer of microcapsule paste on microscope slide glass, direct measurements of particle size in solution can be done by using digital cameras to generate micrographs and interactive software. The microscopic image analysis techniques are able to measure agglomeration, phase separation, particle size distribution, and volume-surface mean diameter together with the standard deviation. Both transmitted and reflected light modes are suitable for MC observations. For generating increased contrast dark field illumination where the contrast comes from light scattering from the sample could be used. Another option is applying phase contrast that allows advance illumination setups for monitoring the structural changes in dispersions. Using phase contrast, the sample contrast comes from interference of different path lengths of light through the sample. Additionally, the swelling behavior of MCs stirred slowly with dH₂O for 10 min can be observed at different time intervals (Alvim, I.D. & Grosso, C.R.F., 2010). Statistical representative distribution of size can be constructed by measuring tens to thousands of particles. This static imaging method allows cheap in situ observations although suffering from low resolution limit restricted by the wavelength of light and lens used. For increasing optical resolution and contrast, confocal laser scanning microscope (CLSM) can be used. By scanning over a regular raster, it captures multiple two dimensional co-planar section images at different depths through the object allowing 3D reconstructions, image analysis, and surface profiling of microcapsules (Tromp, R.H., et al., 2001). Using multiple mirrors, CLSM scans the laser across the sample and “de-scan” the image across a pinhole and detector. This method allows direct, non-invasive observation of MCs with a minimum sample preparation. Scanning with a fine tip over the surface, atomic force microscopy (AFM), topographic characterization on encapsulated particles (immobilized or in a solution) can be also made. Demonstrating resolution of fractions of a nanometer, AFM plots a color mapping through changing the x-y position of the tip while scanning and recording the measured variables corresponding to each coordinate. The method could be used to determine surface 3D surface profiling, roughness, size of MC particles, aggregates, etc. at ambient conditions without requiring any special treatment of the samples. Nonetheless, limitations of AFM are the slow scanning rate and tiny scanning area of about 150 × 150 μm and a maximum height of 10-20 μm.

Alternatives to optical microscopy which do not use visible light are scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM uses signals produced from the interaction of the electron beam with atoms at various depths within the sample. For conventional SEM observations in high vacuum, the MC samples should be initially dried. Epoxy resin may also be used for loading the microcapsules. After that the consolidated sample

is fractured, washed with acetone and dried. To make the samples conductive, a thin layer of sputtered conductive materials (such as Au, Au/Pd, Pt/Ag, carbon, etc.) is applied to prevent charging under the electron beam. For environmental SEM (ESEM) non-conductive specimens in wet conditions could be observed without coating. SEM allows analysis of shape and morphology of MCs, mean particle diameters (more than 250 particles are recommended (Yuan, L., et al., 2006)), shell thickness (for larger MCs, the shell could be ruptured by razor blade to facilitate thickness measurements), effectiveness of adhesion of microcapsules impregnated on a certain textile, etc. Although SEM provides essential information on size, shape, size distribution, TEM analysis can be used to determine the granularity, size, crystal structure, and morphology of the capsules at atomic scale. It gives variety of nanometer and atomic-resolution information if the sample is thin enough to be electron transparent which makes TEM analysis relatively time consuming and the field of view is relatively small. By coupling TEM or SEM with energy dispersive X-ray spectrometer (EDS), elemental composition of samples can also be measured. The method allows high count rate collection and reduced sample damage. However, the accuracy of the measured composition is affected by the nature of the samples. Usually, the light atoms such as H, C, O or N are determined by high uncertainty. To measure the elemental concentration of C, H, S, and N in a given sample with accuracy and precision, Carbon-Hydrogen-Nitrogen (CHN) analyzer can be used to calculate their percentage based on “Dumas” method (Fig. 2). After combustion of the sample at 700-1000°C, the oxidized compounds are detected by using thermal conductivity detection (TCD) or infrared spectroscopy. This method uses no hazardous chemicals and performs fast automated analysis of up to 150 samples. However, it could not discriminate between different forms of C, H, and N species. Brown et al. used CHN analyzer to determine the fill content of the microcapsules. The capsules were combusted at 980 °C in an oxygen atmosphere to form H₂O, CO₂ and N_xO_y. Knowing the chemical composition of the shell and filling substance and assuming that water was the only impurity in the combusted sample, the weight fractions of all compounds were calculated (Brown, E.N., et al., 2003).

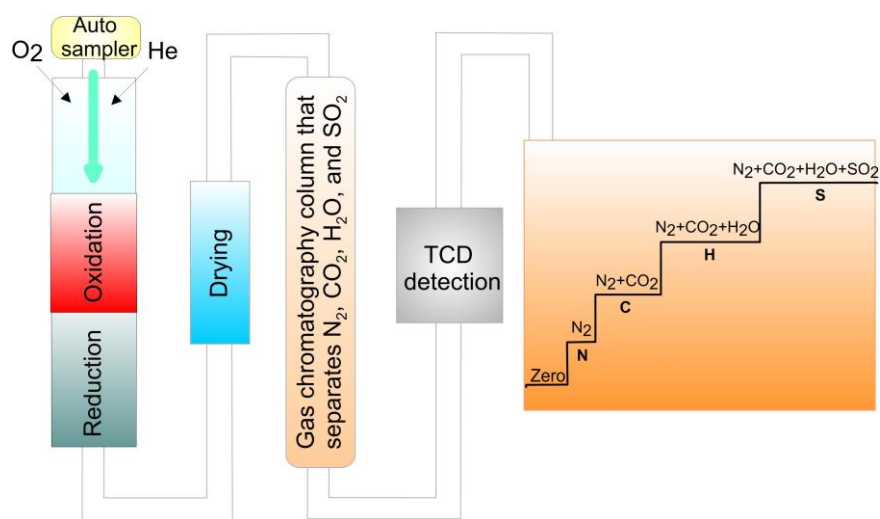


Fig. 2. Principle scheme of elemental analysis based on “Dumas” method.

Microcapsule size measurements

By far, one of the most valuable physical property of particulate samples is the particle size. This property influences the reactivity, dissolution rate, the stability in suspension, viscosity, texture, etc. To characterize the particle size different measurement techniques have been used. For example, dynamic light scattering (DLS), also called photon correlation spectroscopy (PCS), measures the particle size distribution by using laser dynamic light scattering of a certain wavelength and analyzing the Brownian motions of particles in suspension or emulsion. The monochromatic light causes a Doppler effect when the light hits the moving

particles which change the wavelength of the incoming light. This change corresponds to the size of the particles. Thus, the technique gives the average hydrodynamic radius of the particles obtained from the peak position in the distribution curve. The hydrodynamic radius depends on the surface structure and the concentration and type of ions in the medium which means that the size can be larger than measured by electron microscopy. The size measurements are usually performed at fixed, for example 90° scattering angle of the capsules (given as size (μm)) and intensity autocorrelation functions in (%). The method is suitable for sub-micron down to 1 nm sized particles suspended in a liquid. During the measurement it is critical the sample to consist of individual particles and not of agglomerates. In a wet dispersion with water, adding small amount of surfactant can improve the particle dispersion. Evaporating of the dispersant allows analysis of the particles in a dry state. Static light scattering such as laser diffraction may give a volume weighted distribution related to the volume of the particles (equivalent to mass if the density is uniform). It is suitable for particle sizing of materials ranging from hundreds of nm up to several mm. The laser diffraction measures the angular variation in intensity of the scattered light as the beam passes through the samples (both wet and dry) according to ISO 13320:2009. The large particles scatter the light at small angles whereas the small particles are responsible for large angle scattering (Fig. 3). In dynamic automated imaging system the sample flows through the image capture optics. This technique allows measuring the shape differences, detection of agglomerates, non-spherical size measurements, etc. The image is captured by using digital ccd camera when the sample is illuminated from behind. The images captured provide qualitative and quantitative results. Additionally, electrophoretic light scattering (ELS) measures the electrophoretic mobility of particles in dispersion. The system containing two electrodes between which electric field is applied and the charged particles migrate towards the oppositely charged electrode with velocity known as electrophoretic mobility. The velocity is measured by a laser Doppler technique determining both frequency and phase shift (Fig. 4b). The mobility could be converted to zeta potential that will be discussed later. Thus, combining ELS with DLS both particle size and zeta potential can be measured.

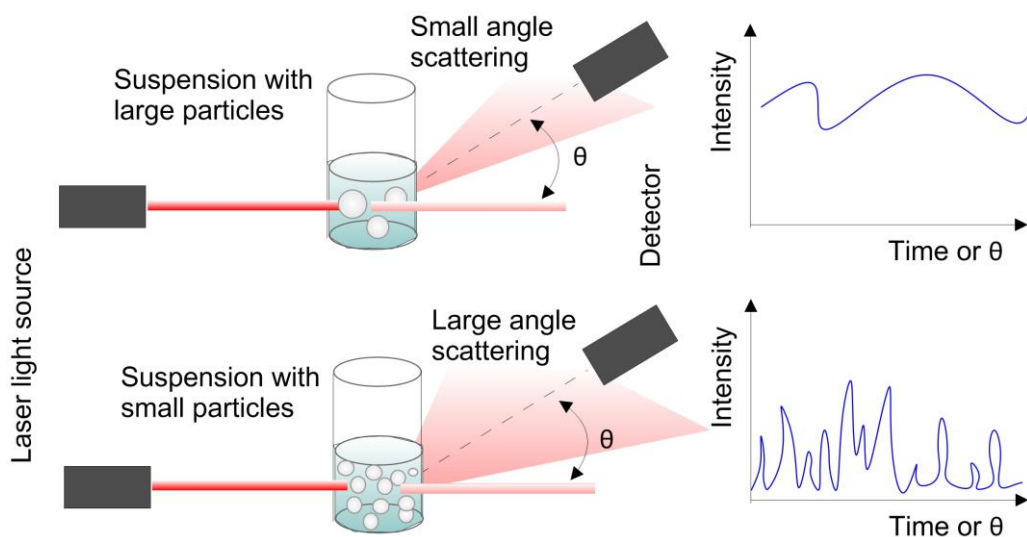


Fig. 3. Principles of dynamic (static) light scattering and fluctuation in intensity of the scattered light from suspensions of different size particles

Structure, crystallinity, and dispersion stability of MCs

After freeze drying, MCs can be subjected to non-destructive X-ray diffraction (XRD) analysis. It allows identification of crystalline phases of the obtained structures, determination of the degree of crystallinity, recognition of crystallite size using variants of Scherrer equation, and determination of crystalline orientation through Hermans orientation function (Bandeira, B., et al., 2016). However, polymers are not highly absorbing to X-rays. Because of the large number of defects, the diffraction peaks are usually broadened. Due to the nano-sized polymer

crystallites (with dimensions between 1 and 100 nm), small angle X-ray scattering (SAXS) at diffraction angles below 6 degree is appropriate to be used. The method allows diffraction of large lattice spacing (even thousands of interatomic distances) scattering by perturbed and non-periodic structures of amorphous and mesomorphic materials (Chu, B., & Hsiao, B.S., 2001). SAXS analyzes the elastic scattering behavior of X-rays and is capable of determining the size distribution of nanoparticles, pore size, resolve the size and shape of monodisperse macromolecules, etc. This technique can also analyze the structure of coacervate phases determined by a collection of various pH values in the structures (Weinbreck, F., Tromp, R.H., & de Kruif, C.G., 2004). Similarly, microstructure investigations could be done by small angle neutron scattering (SANS). The possibility to substitute hydrogen to deuterium makes this method useful for understanding the polymers conformation, rheology, morphology, thermodynamics, etc. It could also determine the characteristics size, shape, internal organization, inner composition, and water content of complex polymer structures (Schmidt, I., et al., 2009).

Spectroscopic analysis on a microscopic level that provides important information about chemical composition and morphology at the same time is essential for the characterization of MCs. Any adsorption spectroscopy measures the light absorption of the sample at each wavelength. The measured intensity indicates how much light is emitted at that wavelength. A routine Raman spectroscopy irradiates the samples with visible wavelength laser to study the molecular vibrations. However, to reduce the energy of the photons that strike the samples for a long time causing fluorescence and photodecomposition, photons with energy lower than the threshold of excitation are used (Bel jebbar, A., Angiboust, J.-F. & Manfait, M., 1993). To avoid the absorption process that gives rise to fluorescence, long wavelength near-infrared laser can be easily applied. Due to the distributive property of Fourier transform (FT), the fluctuation noise on each strong line is redistributed throughout the frequency domain degrading the noise. The use of Fourier transform Raman spectroscopy enables rapid and efficient collection of structurally relevant information for the vibration bands and secondary structures of polymers with superior spectra resolution and frequency accuracy compared with dispersion-based spectrometer (Xue, G., 1997). Fourier transform infrared (FT-IR) spectrometer analysis makes it possible to reveal the composition of the encapsulated substance by analyzing its functional groups. After encapsulation, FT-IR could give the composition of the shell material and the way the encapsulated substance and polymer interacts. By using radio waves, solid state nuclear magnetic resonance (NMR) can observe the local magnetic field around the atomic nuclei. Similarly to FT-IR, its spectrum reveals the molecular structure of the compounds formed and their individual functional groups. The technique is based on the mobility determination of water molecules through relaxation time measurements. A very important factor for the water molecules' motion is the electrostatic interaction of complexes (Ducel, V., et al., 2008).

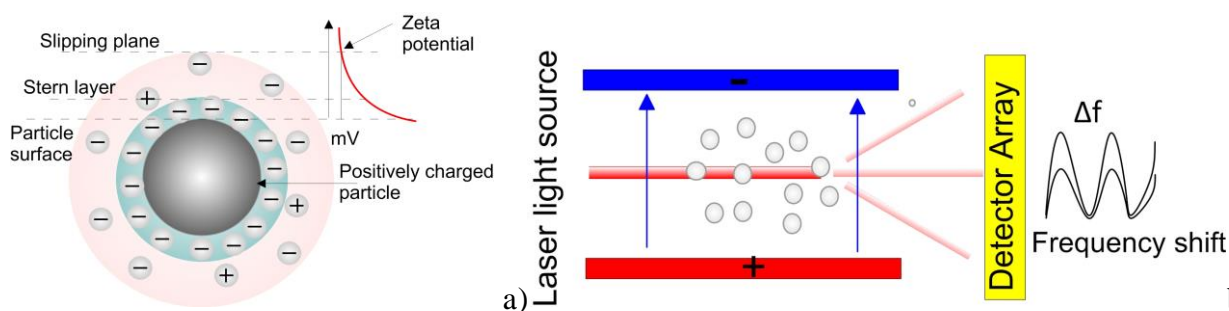


Fig. 4. Electrical double layer and zeta potential (a) and schematic diagram for measuring electrophoretic light scattering (b).

When suspended in fluids, MCs that are large and heavy enough settle rapidly to the bottom in contrast to small particles. The small particles cause the liquid to appear turbid. Turbidity is usually studied by UV-Vis spectrophotometer at a particular wavelength. Maximum

turbidity is developed when the interaction between opposite charged polymers tend to maximum. At this point the turbidity of the supernatant (after centrifugation) is minimum due to precipitation (Devi, N., et al. 2016). A solution turbidity could be measured and quantified by using turbidimeter. A fundamental parameter affecting the dispersion stability is zeta potential. It measures the magnitude of electrostatic attraction of charge repulsion among particles in a suspension. Zeta potential constitutes the electric potential difference between the layer of fluid attached to the dispersed particles and the dispersion medium (Fig. 4a). This potential is indirectly determined by the electrophoretic mobility of particulates (Dukhin, A.S. & Goetz, P.J., 2002) by using different experimental techniques such as microelectrophoresis or electrophoretic light scattering (ELS), Fig. 4b.

The dispersion stability of MC emulsion could be determined by using graduated cylinders (at room temperature for 60 days). During the storage, the emulsion separates into creamy top layer and a transparent serum layer at the bottom of the cylinder. The total height of the emulsion (H_E) and serum (H_S) are constantly measured. Then, the extent of creaming (the creaming index (H)) could be calculated by equation 1 (Sovilj, V.J., et al., 2010):

$$H = 100 \cdot \frac{H_S}{H_E} \quad (1)$$

The higher the creaming index, the worse the emulsion stability.

Another approach used for studying the stability of protein polysaccharide coacervates applies diffusion-wave spectroscopy (DWS) (derived from dynamic light scattering) as a function of time. It considers the multiple scattering of light by the medium containing microcapsules where the transport of light is diffusive and follows multiple number of paths during the medium (Schmitt, C., et al., 2001). The rheological behavior of the complex fluids containing MCs can range from visco-elastic medium to soft solids. By means of conventional macroscale rheometers or microfluidic shear viscometers the shear rheology of fluids with MCs is characterized.

CONCLUSION

Microcapsules having different core and shell materials and variable properties are subject to rigorous testing to ensure their quality and production efficiency. The selection of characterization technique is much dependent on the specification of microcapsules such as materials of the shell and core, thickness and mechanical properties of the shell, capsule size, etc. The limitations of each method for examination should also be taken into account. To obtain satisfactory outcome, the long term stability of microcapsules is of prime importance. Moreover, standard testing procedures for microcapsule characterization are also needed.

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