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Measurement of Microcapsule Physico-Chemical, Structural and Mechanical Properties

Andrew Gray^{1,2}, Stefan Egan², Serafim Bakalis¹ and Zhibing Zhang^{1*}

¹ School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

² Procter and Gamble, Newcastle-Upon-Tyne Innovation Centre, Whitley Road, Longbenton, Newcastle-upon-Tyne, NE12 9TS. UK

* Corresponding author (Email: z.zhang@bham.ac.uk)

Abstract

Research into the fundamental properties of microcapsules and the conversion of this work into development of a wide variety of products in industries such as printing, fast-moving consumer goods (FMCG), construction, pharmaceuticals and agrochemicals is a dynamic and ever-progressing field of study. For microcapsules to be effective in providing protection from harsh environments or delivering a large payload it is essential to have a good understanding of their properties to gain quality control during formulation, storage and application. This review aims to outline the commonly used techniques for analysing physico-chemical, structural and mechanical properties of microcapsules and highlights the interlinking nature of the three areas of interest with respect to the goal of the end-use industrial application, whatever that might be. Whilst providing information on techniques that are well-supported in the literature it also looks into techniques that are due to become more prevalent as microcapsule analytical techniques following new technological developments or extensions from other areas of study.

1 Introduction

Since the pioneering work of Barrett Green in the late 1930s through to the 1950s culminating the invention of carbonless copy paper, where microcapsules containing ink were embedded within the paper structure and broke to copy the information on the sheet above, the potential applications were soon realised and research into the use of microencapsulation of valuable material has been applied to a number of industry sectors (Green & Schleicher, 1956). Whether the need is to protect the core material during processing, the protection from external harsh conditions after preparation, the masking of odour and taste of undesirable ingredients, or the delivery of the core material at a high dosage, at a certain time, and in a certain place, microencapsulation is now widely considered to address these challenges.

Research into microencapsulation broadly falls into two categories: formulation of new microcapsules and characterisation of microcapsule properties/performance. On the formulation side, work has been focused on finding new methods of encapsulation particularly relevant to industrial applications (Andrade et al., 2015). In recent years there has been increased interest in areas such as supramolecular encapsulation (Calderón, Quadir, Strumia, & Haag, 2010; Nikolić et al., 2013; Walker, Oun, McInnes, & Wheate, 2011; Zimmerman, Quinn, Burakowska, & Haag, 2007), aqueous self-assembled systems (Jesorka & Orwar, 2008; Katz et al., 2010; Tsai, Stuhmann, & Koenderink, 2011) and emulsion based microcapsules (Bon & Chen, 2007; Ghosh, 2006; White et al., 2001). Sitting in the foundations of all of this new research is the need to characterise microcapsules to confirm their desirable properties and functions.

Building on the research that has been carried out in terms of advancing the understanding and breadth of formulation methods, microencapsulation has been translated into industrial practice in a wide range of industry sectors and the large number of patents attributed to the field is a sign that microencapsulation is not merely an academic endeavour (Arshady, 2003). An area of particular interest is the use of microcapsules in the pharmaceutical industry as drug delivery systems and examples such as Salbutamol (Pradnya, Raghavendra, & Doddayya, 2010), Captopril, verapamil (Mukherjee, Mahanti, Panda, & Mahapatra, 2005) and propranolol (Vishnu, Ravindrababu, Sudheer, Shireesh, & Naveen babu, 2011) have all been encapsulated in a polymer shell (wall) for clinical use. In a similar vein microcapsules have been used in agrochemicals (Trenkel, 2010), in the cosmetic products (Martins, Barreiro, Coelho, & Rodrigues, 2014; Ohlberg, Guder, Wiedmann, & Pommersheim, 2010;

Ravera & Ravera, 2013), and in the construction industry with the encapsulation of phase change materials for use in thermal insulation where a large number of patents have been filed (Boh & Šumiga, 2008).

An interesting novel application of microcapsules which highlights the widespread nature of possibilities of applications of microcapsules is in the work of Vericilla et al. (2015) in the capture of carbon dioxide from coal fired power plants. Highly permeable silicone shell microcapsules promote the diffusion of carbon dioxide into a liquid carbonate sorbent core. Following this the polymer microcapsules can be heated, releasing CO₂ at the desired moment when it can be collected. If deemed a successful progression the technique could allow for carbon capture at much lower capital, energy and chemical costs (Vericella et al., 2015).

In order to completely understand the capabilities of microcapsules in relation to their specific applications it is necessary to characterise them in terms of physico-chemical, structural and mechanical properties. Depending on the application the relative importance of the properties may change. For example, in the laundry industry it may be desirable for microcapsules containing fragrance to remain intact until the optimum moment when broken by mechanical force after the drying process, therefore the mechanical properties are pivotal. In the pharmaceutical industry the microcapsules may release the active ingredient via a diffusion mechanism and whilst the mechanical properties remain important, it is the release rate due to the porosity that becomes a dominating property in this case. It is also interesting to note the inter-relationship between these properties. The mechanical strength of a certain composition of microcapsule depends greatly on the shell composition, structure and thickness, and also particle size. In terms of the physico-chemical and structural characterisation of microcapsules there are often a variety of characterisation techniques on offer, each with its own capabilities and limitations and the choice of technique depends on type of information required and the accuracy required. The next sections will highlight the classical methods of characterising the physico-chemical properties including size and size distribution, chemical composition and surface morphology of microcapsules, structural properties of shell thickness and porosity, followed by mechanical properties on an individual level and on a population in the bulk of the system. In addition to the conventional methods, newer techniques that aim to build on limitations of classical techniques will be outlined along with literature examples of how they have been applied thus far giving an insight into the future prospects for their application.

Physico-chemical characteristics of microcapsules

Size and size distribution of microcapsules

Producing microcapsules from different methods can provide varying levels of sizes and ranges of size distributions. For example, microcapsules produced by microfluidics can have extremely narrow size distribution, but the rate of formation is extremely slow. Microcapsules produced in a stirred vessel or homogeniser on the other hand have a much wider size distribution but with a higher throughput. Taking into consideration the size of microcapsules is not only important in relation to their flow properties, their payload and adhesion to a surface, but the size is also inextricably linked to the mechanical properties in that a larger microcapsule with equal wall thickness can have a greater rupture force and lower nominal rupture stress than a smaller microcapsule (Xue & Zhang, 2009). It is therefore important to consider the requirements of the end-use application when choosing a method of synthesis for microcapsules.

The size and size distribution of microcapsules can be determined by various methods but it can be largely divided into two separate areas: laser diffraction methods and microscopy, with dynamic light scattering (DLS) becoming important for sub-micron particles. Each method has to be carefully considered in terms of the technical complexity and the accuracy requirements of the data before a decision is made on which technique is most suitable.

DLS examines the brownian motion of the particles in liquid and measures the rate at which the intensity of light scattered from the particles fluctuates as a function of time through a fast photon detector. Therefore, it is typically applied to particles including capsules in submicron region (1 nm -1 μm) (Cao, Landfester, & Ziener, 2012; Rahman & Elaissari, 2012). The smaller the particles, the faster the fluctuation of intensity, giving information on the size of particles. DLS is an ensemble method and through measuring the intensity of fluctuations caused by all capsules in a dispersion, this information is related to the size distribution over the whole sample (Myhra & Rivière, 2012). Laser diffraction methods are based upon measuring the variation in intensity of light as a laser beam of light passes through a suspension of microcapsules. The angular scattering intensity data is used to identify the average size and size distributions of the microcapsules of interest, typically in the range of 10 nm – 3 mm. A limitation of laser diffraction is that knowledge of the refractive index of the shell material is required for the measurement.

The other common technique for size analysis of microcapsules is microscopy. The benefit of microscopy over DLS and laser diffraction is that a true image of the particle can be obtained, which can also help when it comes to studying the morphology. A clear drawback however is the large amount of time it takes to image single samples and conduct subsequent image analysis. Due to this and the fact that it is extremely difficult to get the whole sample in the field of view, microscopy is largely used for individual size analysis but seldom used for size distribution analysis which is left in the remit of the reliable, repeatable laser diffraction technique (Chen & Zhou, 2015; Long, Song, York, Zhang, & Preece, 2013; Martins, Rodrigues, Barreiro, & Rodrigues, 2011; Mokarram, Mortazavi, Najafi, & Shahidi, 2009), and DLS if the particles of interest are sub-micron. Optical (light) microscopy though the simplest method in terms of sample preparation and operation is limited by the minimum wavelength of visible light (400 nm). It is still a useful technique in determining particle size, however structures outside the optical plane can distort the image through scattered or emitted light. If it is deemed necessary to achieve a higher resolution than optical microscopy, electron microscopy can be chosen as an observation method. Scanning Electron Microscopy (SEM) uses a high energy beam of electrons (20-30 keV) to generate an image with higher magnification (>100,000x) and resolution capabilities (down to 10 nm) than optical microscopy. The technique has seen widespread use in size analysis of microcapsules. Moreover, Environmental Scanning Electron Microscopy (ESEM) can be a useful alternative as the high vacuum required to prevent collisions in conventional SEM is not necessary, and samples can be imaged at their native states, meaning sample preparation and operation is much more simple (Bogdanowicz, Tylkowski, & Giamberini, 2013).

If the microcapsules are dry, an in-line image analysis sensor can be used as a means of studying individual microcapsule size and size distribution between a size range of 50 – 3000 μm (Treffer et al., 2014). The principle of the method is that light is directly illuminated onto the sample in pulses. A photometric stereo illumination arrangement means that 3D images can be produced alongside 2D. Image analysis methods and direct geometrical measurements are then used to estimate size of particles and volume-based size distributions can be derived (Merkus, 2009; Stieß, 2009; Young, Koleng, & McGinity, 2002). The direct illumination promotes ease of use as no material-based calibration is required as with DLS. There are drawbacks to the technique however, as the diffuse refracted light used for illumination prevents the study of black, reflective or transparent particles.

Surface roughness and morphology of microcapsules

A particularly rough or smooth surface on microcapsules can either promote adhesion to other substrate or aid transit. In this regard it is important to understand the surface roughness of the microcapsules before further development is undertaken.

In qualitative terms SEM has been used to study the surface roughness of microcapsules (Chowdhury et al., 2015), however it is not easy to quantify the surface roughness just by viewing the SEM image. If quantitative characterisation of surface roughness is required, atomic force microscopy (AFM) and interferometry can be used to aid researchers.

AFM will later be described as a method for characterising the mechanical properties of microcapsules from compression through a normal force load. A more conventional application is contact AFM whereby a sharp tip probe is attached to the end of a cantilever and the deflection of the cantilever as it scans the surface is monitored by a photodiode detector. As the name suggests, the probe tip is in contact with the surface at all times. AFM has the ability to resolve the surface down to fractions of nanometre level and there is no need to apply an ultra-high vacuum which makes the technique extremely attractive for investigating surface morphology of microcapsules and it is currently the most widespread technique used for such characterisation (Choi et al., 2013; Dorati et al., 2006; Lekka, Sainz-Serp, Kulik, & Wandrey, 2004; Wang et al., 2009; Xu, Hercules, Lacik, & Wang, 1998), see Figure 1 for example.

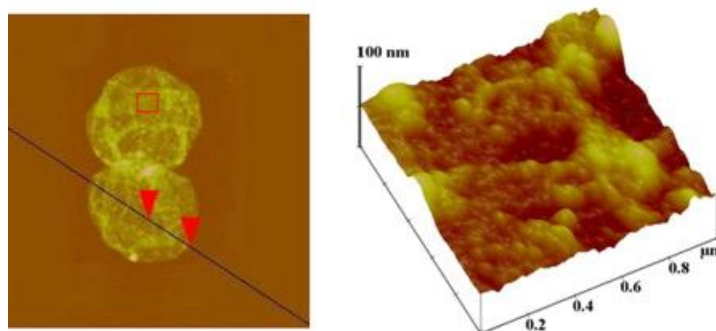


Figure 1: AFM images of microcapsules and part of the surface morphology $1 \times 1 \mu\text{m}^2$ (red rectangle in left image) (Wang et al., 2009).

White-light interferometry is a technique that has been used to study the surface roughness of microparticles (Adi et al., 2008) and pharmaceutical excipients (Bashaiwoldu, Podczec, & Newton, 2004; Narayan & Hancock, 2005), and it is starting to find use in microcapsule analysis (Zheng et al., 2014; Zheng et al., 2012). The technique is non-contact unlike AFM and is a form of profilometry whereby a source beam of light is split by a beam splitter such that one beam travels to the interferometer and the other hits the sample particle. The beams are then reflected back to the beam splitter and combined into interference fringes, captured by a charge coupled device (CCD) camera which creates an interference pattern from which the surface roughness pattern can be developed. In the vertical-axis there is a sub-nanometre resolution of 0.2 nm and in the horizontal plane a spatial resolution of 150 nm is possible (Haas, Birnie III, Zecchino, & Figueroa, 2001). An interesting facet to the technique is that in non-contact mode there are capabilities of scanning distances of 10,000 μm , making surface measurements of multiple particles (Adi et al., 2008).

Chemical composition of microcapsules

There are three relevant methods widely used to provide information on the chemical composition of microcapsule shells as a function of depth. These are Attenuated Total Reflectance Fourier Transform Infra-Red Spectroscopy (ATR-FTIR), X-ray Photoelectron Spectroscopy (XPS) and Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) (Rokstad, Lacik, de Vos, & Strand, 2014; Tam et al., 2005). Each of these techniques provides the capability to analyse the surface to different depths, meaning the information obtained from each is complementary to each other.

ATR-FTIR allows analysis of the sample to be conducted with little sample preparation to significantly increase the speed of analysis. When looking at microcapsule shell thickness ATR-FTIR can work to a depth of penetration in the micrometre range and is generally used to provide information on the functional groups characterising the bulk chemistry of the microcapsule shell (Levin & Bhargava, 2005).

XPS was the first technique identified for microcapsule wall composition analysis and the principle is that X-rays are fired at the surface to eject electrons (de Vos et al., 2003). The number of electrons and the kinetic energy are measured and the information allows identification of the elemental origin of the electrons, giving qualitative information on surface chemical groups up to a depth of 100 nm.

ToF-SIMS is a technique where a primary beam of ions sputters the surface of the target material, ejecting secondary ions of which the mass is recorded to characterise the compounds in the microcapsule wall. It is used for just the outermost region of the microcapsule wall with a depth of penetration of 1-2nm. Whilst ToF-SIMS is extremely useful for qualitative analysis, quantification of the individual elemental compositions is difficult, and other techniques such as energy dispersive x-ray spectroscopy must be used if this is required (Tam et al., 2005). An interesting comparison and collation of these methods was made by Tam et al. (2005) in modelling the wall structure of alginate-poly-L-lysine (APA) microcapsules which are used in the immuno-isolation of secretory cells. Poly-L-lysine (PLL) is used to form the semi-permeable cell membrane but if PLL is exposed at the surface of the microcapsule membrane it can generate an unwanted immune response. ATR-FTIR analysis confirmed that PLL was present in the outermost 3 μm of the microcapsule wall, though this is insufficient in attributing PLL to the surface where it will most likely generate an immune response. XPS was utilised to characterise the surface composition of the outermost 100 \AA and in terms of carbon content it was found that there were high levels of PLL found within this region. Finally ToF-SIMS was applied to study the outermost 1-2 monolayers of the structure and it was found that there was indeed PLL exposed in these layers. A change in peaks of the ToF-SIMS analysis also demonstrated that there are alginate complexes to the PLL in the outermost surface though this may not be consistent throughout the surface and any free PLL offers the complications of generating an immune response.

For elemental analysis of the surface or across the shell of microcapsules, Energy-dispersive X-ray spectroscopy (EDS) in conjunction with SEM can also be used. This relies on each element's unique atomic structure and forms a unique set of peaks on its X-ray emission spectrum that identify the presence of the element qualitatively and quantitatively (Han et al., 2011).

Surface charge and Zeta potential

Understanding the Zeta Potential, which is the charge that develops at an interface between a solid particle and surrounding medium, can help in predicting the stability of formulated microcapsule suspensions. If not adequately controlled aggregation of microcapsules can occur. The zeta potential is governed by the surface charge of the microcapsules, the pH of the suspension and the ionic strength of the suspending medium. If it is sought to prolong the

stability of an evenly dispersed suspension of microcapsules, zeta potential experiments should be carried out to identify the actions that should be taken to maximise the positive or negative zeta potential away from the iso-electric point (zeta potential of zero) to prevent aggregation. The zeta potential can be calculated following a number of different experimental methods and in relation to microcapsules it is the streaming potential and electrophoresis that are often chosen as the primary options for measurements. In the streaming potential technique the sample microcapsules are placed in a stationary state within a measuring cell and an electrolyte is forced through. There is a pressure drop associated with the resistance of flow in the measuring cell (de Vos, de Haan, Kamps, Faas, & Kitano, 2007). As the electrolyte is introduced to the cell the surface charge of the microcapsules is disrupted causing a potential difference i.e. the streaming potential (Xie et al., 2010). Electrophoresis uses an electric field to force charged particles to move in surrounding liquid. By measuring the electrophoretic mobility of the particles in the liquid, the zeta potential can be calculated (Chatterjee, Salaün, & Campagne, 2014; Mirabedini, Dutil, & Farnood, 2012). Both methods receive attention in literature associated with microcapsules but the choice is often based upon size limitations as electrophoresis has an upper size limit of around 30 μm due to sedimentation issues, whereas the streaming potential method can be used on microcapsules greater than 30 μm .

Structural properties of microcapsules

Shell thickness

SEM not only provides an excellent means of studying the size and size distribution of the microcapsules, but it also retains sufficient resolution for determining shell thickness, leading to it becoming a prevalent technique in such identification (O'Sullivan, Zhang, & Vincent, 2009; Polenz, Weitz, & Baret, 2015). The issue however relates to slicing of the microcapsules with microtomes to expose the cross section. To observe the true shell thickness it is important to splice the microcapsule at the equator as any deviation would result in an overestimation, which should be corrected (Mercadé-Prieto et al., 2011). Another issue relates to the complex preparation of samples as they are dehydrated and fixated for SEM which can cause shrinkage and the introduction of artefacts from sample preparation (Goldstein et al., 2003). A method to overcome this is cold stage scanning electron microscopy (Cryo-SEM). Usually reserved, but not restricted to biological samples (Allan-

Wojtas, Truelstrup Hansen, & Paulson, 2008; Lensen et al., 2011), Cryo-SEM is a technique which cryo-fixes a sample by plunging it into liquid nitrogen. It is then coated with a metal, usually gold or platinum to increase conductivity and then transferred to the conventional SEM chamber.

Focused Ion Beam (FIB) imaging is a technique in which accelerated heavy ions such as gallium, gold or iridium are rastered across a target material, causing a sputtering of surface ions. Momentum of the incident ions is transferred to the sample material and sputtering occurs if the binding energy of the atoms on the surface of the target material is overcome by the incoming kinetic energy (Wirth, 2009). In this regard the process is invasive and destructive to the specimens of interest, and in analysis of living cells this may be a cause of issue, but for microcapsules the sputtering can provide a means of carefully controlling the creation of a cross section in the sample through milling with FIB, which opens up possibilities for determining shell thickness. It is possible to combine the capabilities of SEM and FIB in a dual beam instrument configuration and this was introduced by Sudraud et al. (1988) where a Gallium FIB column was added to an scanning electron microscope and a number of reviews cover the developments over the years (Groeber, Haley, Uchic, Dimiduk, & Ghosh, 2006; Mobus & Inkson, 2007; Uchic, Holzer, Inkson, Principe, & Munroe, 2007). The scanning electron microscope is used to view the cross section as the focused ion beam mills through the sample perpendicular to the sample surface. This takes the advantage of the high resolution imaging from SEM and the accurate cross-sectioning of FIB to combine both into one system. This dual beam set up allows in-situ monitoring of the progress of the milling and has been used recently in microcapsule shell thickness characterisation of food-grade Zein-Lime shell-core microcapsules (Wang, Su, Schulmerich, & Padua, 2013) and poly(methyl methacrylate) (PMMA) microcapsules enclosing biocides (Andersson Trojer et al., 2013).

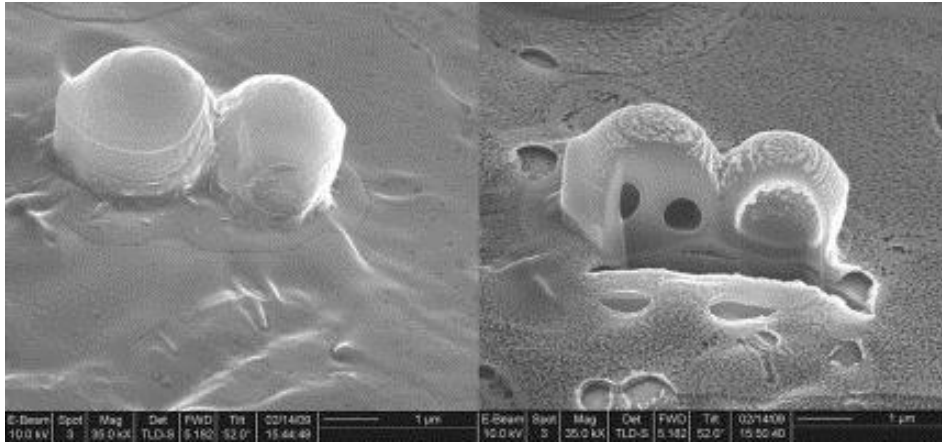


Figure 2: SEM-FIB images of the surface morphology and internal structure of Zein microcapsules (Wang et al., 2013)

One potential drawback of SEM-FIB is that the milling action of the FIB can cause ion beam damage to the shell to some extent, therefore creating uncertainty over the true shell thickness. This occurs because as the ion beam intensifies the individual disordered cascade region overlaps, creating a damaged surface layer (Mikmekova´ et al., 2011). As mentioned in the section for determining the chemical composition of microcapsules, SEM when combined with EDS can provide a method of chemical microanalysis of the surface of samples of interest (Lasagni, Lasagni, Holzapfel, Mücklich, & Degischer, 2006; Lasagni et al., 2007; Minor, 2005). Using the SEM-FIB setup in combination with EDS facilitates chemical analysis of the sample beyond the surface and deeper into the internal structure to enable the culmination of a 3D representation of elemental information. In this sense it is an extremely powerful technique but as the analysis is effectively conducted on several slices it is extremely time consuming and therefore there was a real need to automate the technique when EDS is necessary as proposed by Schaffer et al. (Schaffer, Wagner, Schaffer, Schmied, & Mulders, 2007).

Transmission Electron Microscopy (TEM) again utilizes a high energy beam of electrons, similar to SEM, but this time at 100-1000 KeV to transmit the image through a thin specimen, allowing for identification of internal structures including shell thickness of microcapsules (Yue Long, Vincent, York, Zhang, & Preece, 2010; Polenz et al., 2015). TEM has a resolution of around 0.2 nm and a maximum magnification >10,000,000x, both at least an order of magnitude greater than SEM, but it must be performed under high vacuum to

prevent collisions between electron and air molecules. Consequently it must be carefully considered whether TEM is absolutely necessary as not only is sample preparation laborious, but the method itself can be quite time consuming.

In order to fully utilise the resolution capabilities of TEM it is often necessary to embed the microcapsule samples in an acrylic resin, fixating them for imaging (Kumar, 2013). Despite being costly and time consuming this additional step in sample preparation is advantageous for many microcapsule formulations, however, for example, in microcapsules with a shell composition of PMMA, embedding in an acrylic resin causes the shells to dissolve (Pan, Mercadé-Prieto, York, Preece, & Zhang, 2013). In this situation an option is to turn to Confocal Laser Scanning Microscopy (CLSM). Usually applied to the study of biological samples, CLSM is a non-destructive technique that uses a fluorescent light source to excite fluorescent markers in structures of interest, such as microcapsule shells. The fluorescent light source is a narrow beam and as such it is scanned across the sample to obtain an image of the whole field. In comparison to widefield optical epi-fluorescence microscopes, CLSM only marginally improves resolution to 0.2 μm laterally, and 0.5 μm vertically but a distinct advantage of CLSM is that the confocal pinhole rejects out of focus fluorescent light, giving an extremely clear image (Schermelleh, Heintzmann, & Leonhardt, 2010). It is however worth remembering the deficit in resolution in comparison with TEM, emphasising that CLSM is a technique to consider if interaction with the embedding medium causes shell degradation. There are examples of the use of CLSM in various microcapsule studies where the shell thickness was determined by looking at intensity across mid-points of the resulting cross-section profiles (Lebedeva, Kim, & Vinogradova, 2004; Pan et al., 2013; Paramita, Iida, Yoshii, & Furuta, 2010; Tavera, Kadali, Bagaria, Liu, & Wong, 2009).

Moving away from analysing the microcapsule shell thickness from optical techniques and visualisation of images, small angle X-ray scattering (SAXS) is a non-destructive method of light scattering that can resolve structures from sizes <1 nm up to 200nm through investigating scattering patterns from X-rays that penetrate the material of interest. X-rays interact with electrons in the structure of the material and inhomogeneity in electron density causes changes to the diffraction pattern. Focusing on small angle scattering the effects can be translated into providing information on the structure and shape of an object of interest (Lifshin, 1999). SAXS can analyse microcapsules in liquid, which is not possible in SEM or TEM due to the need for a vacuum in the sample chamber. The same limitations apply to light scattering techniques as with microcapsule size analysis in that the data is related to the

average properties rather than individual measurements (Lifshin, 1999). The size restrictions of SAXS mean that nanocapsules have largely been the focus of analysis (Gutsche, Daikeler, Guo, Dingenouts, & Nirsche, 2014; Utama, Dulle, Forster, Stenzel, & Zetterlund, 2015), however ultra-small angle X-ray scattering can spatially resolve structures up to 10 μm , bringing it into the interests of microcapsule analysis, though there is not yet any published result.

Pore Size

As formulation techniques have advanced over the years the structure of microcapsules has developed from a simple core-shell morphology so that now it is possible to get multi-wall, multi-core and matrix morphologies to name a few. X-ray micro-computed tomography is a non-destructive technique that creates a cross section of an object. An X-ray source illuminates the object of interest and a planar X-ray detector collects magnified projection images. This is repeated hundreds of times as the object rotates which allows the computer to build up individual cross section slices of the object which can be summated at different depths using computer software to generate a 3D image of the whole microcapsule. The technique has been applied to look at the inner structure of calcium shellac beads of 1.35 mm average diameter (Law & Zhang, 2007). The advantages of the technique lie in the fact that it is non-destructive and capable of producing a complete image of the microcapsule structure, but the resolution at which this image can be produced is around 1-10 μm which limits its application in microspheres if an accurate representation of inner structure is desired.

In a similar vein to surface roughness, AFM can also be applied to investigate the porosity of microcapsules when in tapping mode (Wagdare, Marcelis, Boom, & van Rijn, 2011). Though porosity affects mechanical strength of the microcapsules to a certain degree, it has a much more profound impact on the efflux of core material to the surroundings. This may be desirable in the case of certain pharmaceuticals, but when the microcapsule is required to rupture under the influence of a certain trigger this diffusion of material from the capsule can lower the availability of core material and reduce the impact of the microcapsule.

A technique that has gained attention in investigating porous systems, largely polymers (Engbrecht, Green, Hillmyer, Olson, & Todd, 2013; Zaleski, Maciejewska, & Puzio, 2015) and films (Fukuzumi et al., 2011; He et al., 2013) is positron emission annihilation lifetime

spectroscopy (PALS). This technique has been used to investigate free volumes of diameters between angstroms to tens of nanometers in porous solids in order to determine pore size and pore size distribution up to 30 nm (Gidley, Peng, & Vallery, 2006). Positrons, the antimatter particle of the electron are emitted via a radioactive source (^{22}Na), releasing gamma rays. The positronium pseudo-atom (the bound state of a positron and electron) is formed randomly in the sample and can occur at any place. A positron is an unstable species and it can either annihilate intrinsically (125 ps), with its electron or via a pick off process with an electron in the surrounding material. The lifetime of the ortho-positronium in a solid with a free electron will be 1-4 ns due to the large number of electrons but in a void or pore within a sample the lifetime will be shorter than in a vacuum (142 ns) due to the annihilation with electrons in the wall (He et al., 2013). Upon annihilation more gamma ray radiation is emitted and the time between the initial radiation source and this annihilation radiation gives a time that is directly correlated to the pore size. Alongside the capability of identifying pores and defects on an atomic scale, PALS has advantages that the technique can identify closed pores and that measurements can be performed in-situ so that changes to pores over time can be examined (Sun, Gidley, Hu, Frieze, & Yang, 2002).

Crystallinity of microcapsules

The degree of crystallinity of the wall material can have important consequences on the stability of the microcapsule and can also affect flowability, dispersibility, permeability and mechanical properties. Crystallinity is intrinsically linked to the thermal properties of microcapsules and this can be vital information to understand when considering the end-use application. An example of this relates to the addition of microcapsules to detergents to encapsulate fragrance in order to increase the longevity of the fresh scent after the wash. Different countries wash under different typical temperatures and via different methods (washing machine, hand-wash) so that the requirements for the thermal stability of the microcapsule vary.

Wide angle X-Ray Diffraction (XRD) is a method used to determine the crystalline structure of microcapsules (Chai, Wang, & Wu, 2015; Jaya, Durance, & Wang, 2009). The technique can shed light on whether the shell is of a crystalline nature or whether it is of an amorphous composition. It can also provide information regarding the crystalline nature of any dopants in the shell, for example block copolymer microcapsules embedded with cobalt nanoparticles

(Qiao, Zhang, Qiu, Li, & Kang, 2007). It is often the case that Dynamic Scanning Calorimetry (DSC) and Thermo-Gravimetric Analysis (TGA) are used alongside XRD for analysis of the crystalline structure of microcapsules (Adeyeye et al., 2005; Emami et al., 2009). From analysing the results produced it is possible to identify thermal properties such as glass transition temperature, melting point and also point to any decomposition of shell material, which gives information on the purity and crystallinity of the sample.

Release of core material from the microcapsule

The most widely used technique to measure the release of core material from microcapsules is to gently agitate to create a well-mixed dispersion and then to measure the change in solute concentration over time. This can be done in discrete intervals or continuously if online analysis is available (Zhang, Law, & Lian, 2010). This then enables calculation of release kinetics and subsequently the diffusion coefficient, D_E . Long et al. (2010) measured the release kinetics of core oil from double shelled melamine formaldehyde- CaCO_3 microcapsules by placing the microcapsules in water to which an organic solvent hexane was added to extract the oil, and samples were taken from the hexane phase overtime for analysis using gas chromatograph. (Mercadé-Prieto, Allen, York, et al., 2012) studied the release of hexyl salicylate from melamine formaldehyde microcapsules using co-solvents of ethanol, propan-1-ol, propan-2-ol and 1,3-butanediol, which significantly increase the solubility of hexyl salicylate in them. The latter method is simpler and more advantageous, but requires a co-solvent which does not cause significant damage to the shell of microcapsules.

Mechanical properties of microcapsules

A key decision when encapsulating an active ingredient is the choice of shell material. Theoretically, it is the shell that imparts the mechanical properties on the microcapsule with a liquid core and they are subject to the chemical composition, the structure of the wall material, the size of the microcapsule and the shell thickness, interlinking the physicochemical and structural properties. Once the mechanical properties of microcapsule are known, giving information on its capabilities it is then possible to incorporate financial margins, environmental impact etc. to decide which shell material provides the most effective

solution to the encapsulation problem. One of the key benefits of microencapsulation is stabilisation of active ingredients and realisation of their controlled release, which requires the microcapsules to be mechanically stable in processing, storage and to have optimum mechanical strength if the release needs to be achieved using mechanical force as a trigger. The mechanical characteristics /properties of microcapsules can be characterised using bulk methods and individual measurements. Generally speaking, bulk methods offer quick acquisition of data and can be performed in an automated fashion, but as all microcapsules are simultaneously measured, any mechanical properties identified are average values across the batch.

Individual measurement of microcapsules can provide more detailed information on the variability between microcapsules, but can be time consuming to acquire a substantial collection of data.

Bulk methods

Measurements of mechanical characteristics on a bulk level have largely focused on resistance to shear forces generated by shaking (J. Chen, Jo, & Park, 1995; Uludag, De Vos, & Tresco, 2000; Y. J. Wang, 2000), bubble column (Lu, Gray, & Thompson, 1992; Martins dos Santos et al., 1997), and turbine reactor (Poncelet & Neufeld, 1989). This had an issue in that the breakage depends on the hydrodynamics of the processing equipment along with the mechanical properties of the microcapsules. A cone and plate viscometer technique was utilised to overcome the hydrodynamic issues, however in terms of studying microcapsule breakage this technique is extremely limited as the forces available are unable to break even weaker mammalian cells (Born, Zhang, Al-Rubeai, & Thomas, 1992). Moving away from looking at shear and focusing on applying a normal force was compression between parallel plates developed by Ohtsubo et al. in 1991. A sample of microcapsules was placed between two glass plates and through compressing the bulk sample between the plates it was possible to obtain mechanical properties over the whole sample (Ohtsubo, Tsuda, & Tsuji, 1991). However it is difficult to ensure the two plates to be parallel, which can lead to large experimental error when the gap between them is in the order of several to tens of microns.

For some microcapsules with a semi-permeable membrane/wall, their mechanical strength can be inferred from their damage when exposed to a suspending liquid with different

osmotic pressure (Van Raamsdonk & Chang, 2001). However, the change of the hydrodynamic pressure on the microcapsule membrane due to the change in osmotic pressure can be limited. Therefore, the technique can only be applied to relatively weak microcapsules.

Individual particle measurements

Experimental techniques to characterise single microcapsules (1 – 1000 μm in diameter) include optical tweezers, shear flow (spinning drop apparatus), micropipette aspiration, AFM and micromanipulation based on diametric compression between two surfaces and the magnitude of typical forces applied is shown in Figure 3.

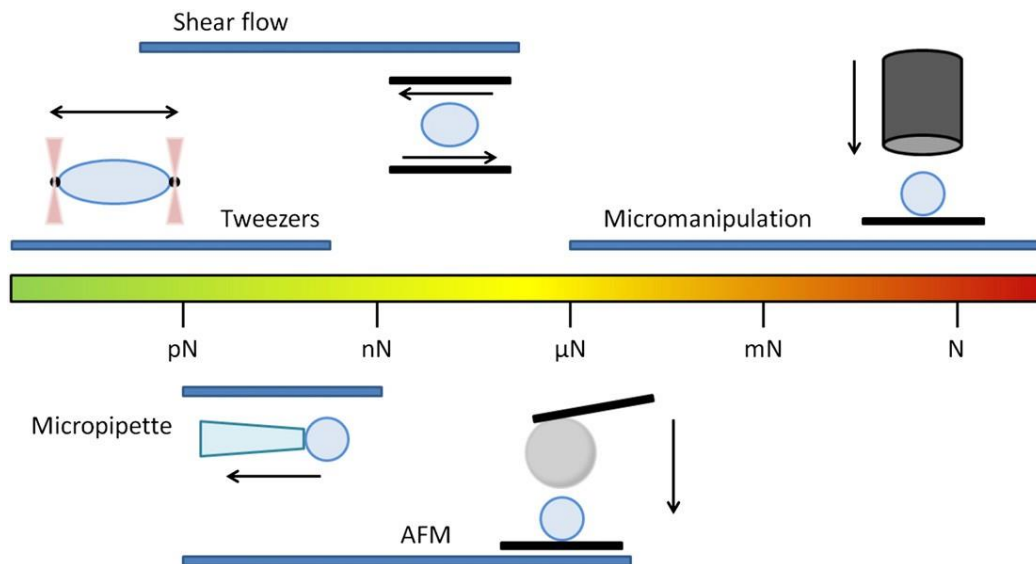


Figure 3: Schematic representation of single-capsule measurement techniques, each with typically available force range. Arrows indicate the directions in which forces are acting (Neubauer, Poehlmann and Fery, 2014).

The most sensitive technique available for characterisation of mechanical properties is optical or magnetic tweezers. This is generally used with biological samples but whilst the high sensitivity can be a benefit with regards to deformation of biological cells, the technique is limited up to an applied force of around 50 pN which prevents analysis of tougher artificial microcapsules. The principle of the technique is that photons from a laser beam are directed

at a dielectric object, at which point they undergo a change in momentum, which pushes the object towards the focal point of the laser beam. To investigate mechanical properties two beads are attached to a cell via specific or non-specific binding. One of the beads is fixed to the surface whilst the other trapped by the optical tweezers. The bead is then moved away from the bead attached to the surface leading to a force of extension in the cell of interest which can be measured to determine elastic parameters of the cell (Dao, Lim, & Suresh, 2003; Hénon, Lenormand, Richert, & Gallet, 1999; Sleep, Wilson, Simmons, & Gratzer, 1999).

Monitoring the flow of microcapsules in microfluidic channels is a method used to measure mechanical properties of microcapsule walls. In this method microcapsules are allowed to flow through a microchannel of comparable dimensions and the viscous stress from the suspending and internal liquids cause deformation of the microcapsules which is then measured as a function of flow rate. A theoretical model that incorporates the axisymmetric motion and deformation of the microcapsule in a cylindrical tube is used for inverse analysis of the results to assess the elastic properties. Experimentally this technique is fairly simple to execute, however the technique involves incorporation of a complicated model that needs substantial membrane deformation data and little experimental error. It is particularly useful for analysing microcapsules on the order of a few tens of microns and has been used for analysis of biological cells (Tomaiuolo, Simeone, Martinelli, Rotoli, & Guido, 2009) and artificial microcapsules (Chu et al., 2012; Leclerc, Kinoshita, Fujii, & Barthès-Biesel, 2011; Lefebvre, Leclerc, Barthès-Biesel, Walter, & Edwards-Lévy, 2008; She, Xu, Yin, Tong, & Gao, 2012).

A technique that can generate similar magnitudes of force to the shear flow technique is micropipette aspiration. This technique is mainly applicable to biological capsules (Chien, Sung, Skalak, Usami, & Tözeren, 1978; Evans & Yeung, 1989; Hochmuth, 2000; Jones et al., 1999; D. Kim, Wong, Park, Levchenko, & Sun, 2009; Rand & Burton, 1964; Sato, Theret, Wheeler, Ohshima, & Nerem, 1990; Shao & Hochmuth, 1996) with relatively weak mechanical strength and semi-permeable artificial microcapsules (Campillo, Pépin-Donat, & Viallat, 2007; Dieluweit et al., 2010; Jay & Edwards, 1968; Mabrouk et al., 2009; Olbrich, Rawicz, Needham, & Evans, 2000; Ratanabanangkoon, Gropper, Merkel, Sackmann, & Gast, 2003; Rawicz, Olbrich, McIntosh, Needham, & Evans, 2000). It comprises of a deformation opposite to that caused by micromanipulation and AFM whereby the microcapsule is

extended into the micropipette via suction rather than being compressed by a probe. A microcapsule is positioned at the open end of a capillary tube (0.2-0.8 times the size of the microcapsule) and a negative pressure differential is applied encouraging aspiration into the tube. The deformation is then captured by a high resolution microscope and the geometric changes can give information on the elastic and viscoelastic properties and flow resistance of the microcapsule. An issue with micropipette aspiration is that the mechanical characterisation is made by studying the interaction between the capillary wall and the shell of the microcapsule and doesn't take into consideration the whole microcapsule structure (Guo & Wyss, 2011). A technique that has been developed to overcome this limitation has been based on capillary micromechanics. A pressure gradient is applied to a tapered capillary with a tip diameter less than that of the microcapsule. This causes the microcapsule to be drawn into the capillary where it blocks further flow of fluid where pressure difference falls off across the length of the particle, applying external stresses to the capsule causing deformation. This is observed directly with an optical microscope to characterise compressive and shear modulus of microcapsules (Kaufman et al., 2014; Kong, Wang, Wyss, & Shum, 2014; Wyss, Franke, Mele, & Weitz, 2010). The method can be easily incorporated into existing microfluidic devices although it is primarily used on biological and soft cells where inhomogeneities make extracting elastic properties difficult in other techniques of comparable sensitivity. A drawback of the optical or magnetic tweezers, shear flow micropipette techniques is that they cannot operate at forces large enough to break the microcapsules so that whilst they can offer information about the elastic/viscoelastic properties, they cannot give information regarding rupture characteristics such as rupture force, wall tension and stress at rupture.

AFM is also a technique that applies a normal force of compression to individual microcapsules and along with micromanipulation it is the most widely used method for characterising the mechanical properties of microcapsules. The work done using AFM can be divided into two categories: indentation using a large colloidal probe or indentation using a sharp tip. The colloidal probe technique was first introduced by (Butt, 1991; Ducker, Senden, & Pashley, 1991) whereby a probe larger in size than the microcapsules is attached to a tipless cantilever. The main benefit of employing a colloidal probe rather than a sharp tip is that the geometry between the probe and microcapsules is well defined and the mechanical behaviour up to a significant deformation of the whole microcapsule can be investigated. In contrast, when a sharp tip is used for indentation there can be further compressive

stress/strain applied to the microcapsule which can damage the shell local structure, if this is of interest.

Micromanipulation is a technique that was first introduced in 1991 by Zhang et al. (Zhang, Ferenczi, Lush, & Thomas, 1991) to measure the bursting force of single mammalian cell, which was later modified to test a wide range of biological and non-biological microparticles including microcapsules (Zhang, Ferenczi, & Thomas, 1992; Zhang, Saunders, & Thomas, 1999; Zhao & Zhang, 2004; Stenkes et al, 2000; Kim et al., 2010). Single (dry or wet) microcapsules resting on a glass slide or in chamber with liquid of interest are compressed under a normal force by a cylindrical probe with a diameter larger than the microcapsules. The resulting force-displacement curves provide the information from which the mechanical properties can be determined, depending on the extent of displacement. Micromanipulation as a technique can access forces range greater than all other techniques listed in this review (μN - N) and therefore if it is necessary to compress to rupture it is certainly the technique of choice. From the force-displacement curves it is possible to extract information on the rupture force, deformation at rupture, nominal rupture stress and nominal wall tension (Liu, 2010). These properties are subject to the shell composition, the size of the microcapsule and the shell thickness. Aside from compressing to rupture, loading and unloading experiments at small deformations are performed to determine intrinsic material properties of the microcapsules, such as the Young's modulus, which were determined using different approaches. When single microcapsules were considered as a sphere, the Hertz model has been applied to estimate the Young's modulus of the whole sphere (K. Kim, Cheng, Liu, Wu, & Sun, 2010). The Young's modulus of the shell material was determined using analytical models (Feng & Yang, 1973; Liu, Williams, & Briscoe, 1996) or finite element analysis (FEA) (Mercadé-Prieto, Allen, Zhang, et al., 2012; Mercadé-Prieto, Nguyen, Allen et al., 2011; Mercadé-Prieto, Allen, York et al., 2011). The simulated relationship of force versus displacement in the elastic region for a given diameter and shell thickness of microcapsule was obtained using a commercial software package ABACUS, as shown in Figure 4.

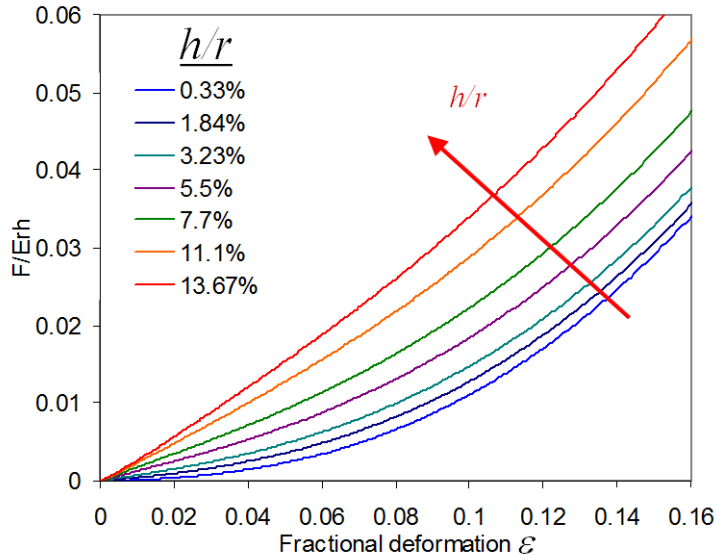


Figure 4: Dimensionless force F/Erh with the fractional deformation ϵ (ratio of the displacement to diameter) from FEA simulations at different wall thickness ratios. F -applied force (N), E -Young's modulus (Pa), r -microcapsule radius (m) and h – microcapsule shell thickness (h) (Modified from Mercadé-Prieto , Nguyen, Allen et al., 2011)

At moderate deformation, the elastic regime is assumed to be followed by perfectly plastic region. The elastic limit where the force profiles start to continuously deviate from the fully elastic scenario was determined using experimental data and simulation results from FEA. The yield stress was therefore determined. For melamine formaldehyde microcapsules, the yield stress and the corresponding strain were 130MPa and 0.0236 (corresponding to a fractional deformation in the range of 11% to 19%).

At large deformation, the shell material was assumed to behave perfectly plastic followed by strain hardening until rupture. The experimental data of force versus displacement from the beginning of compression to rupture were fitted with the simulation results of FEA using the pre-determined Young's modulus, yield stress with an adjustable strain hardening modulus. Therefore, the real stress and strain at rupture for the shell of melamine formaldehyde microcapsules have been determined, see Figure 5.

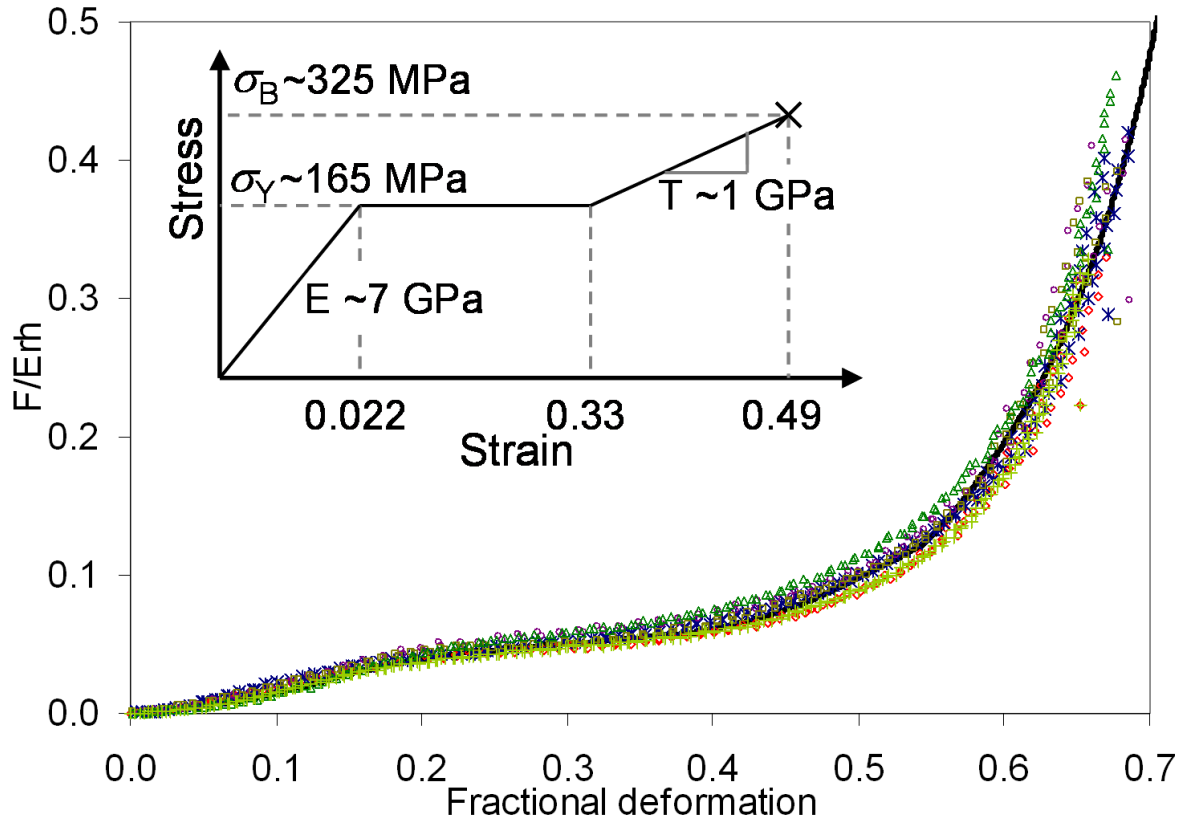


Figure 5: Experimental compression curves of 6 different melamine-formaldehyde microcapsules ($r = 2.5 - 6 \mu\text{m}$, $Eh \sim 1300 \text{ N m}^{-1}$ and $\sigma_Y h \sim 30 \text{ N m}^{-1}$). Continuous line is the FEA results for an elastic perfectly-plastic with strain hardening microcapsule, using $h/r = 4.8\%$, $\sigma_Y / E = 0.022$, $H_T = 0.33$ and $T / E = 0.15$. σ_Y - yield stress, H_T - strain corresponding to onset of strain hardening and T - strain hardening modulus. Inset shows the predicted stress-strain relationship of the MF shell using $h = 0.185 \mu\text{m}$ (Mercadé-Prieto, Allen, Zhang, et al., 2012).

The micromanipulation technique was extended to nanomanipulation in a chamber of an environmental scanning electron microscope (ESEM), and the particles tested by the technique were as small as 530nm in diameter (Liu, Donald and Zhang, 2005). Moreover, ESEM allows direct measurement of the rupture mode of single microcapsules (Figure 6) and the contact area between the microcapsules and the force probe/bottom surface and lateral extension of the deformed microcapsules, which can be used to validate the results from

finite element analysis. Currently, the smallest size of particles which can be mechanically characterized is limited by the resolution of ESEM under wet conditions (10-20nm). Improvement of the resolution can allow smaller sub-micron particles or even nanocapsules (< 100nm) to be measured directly.

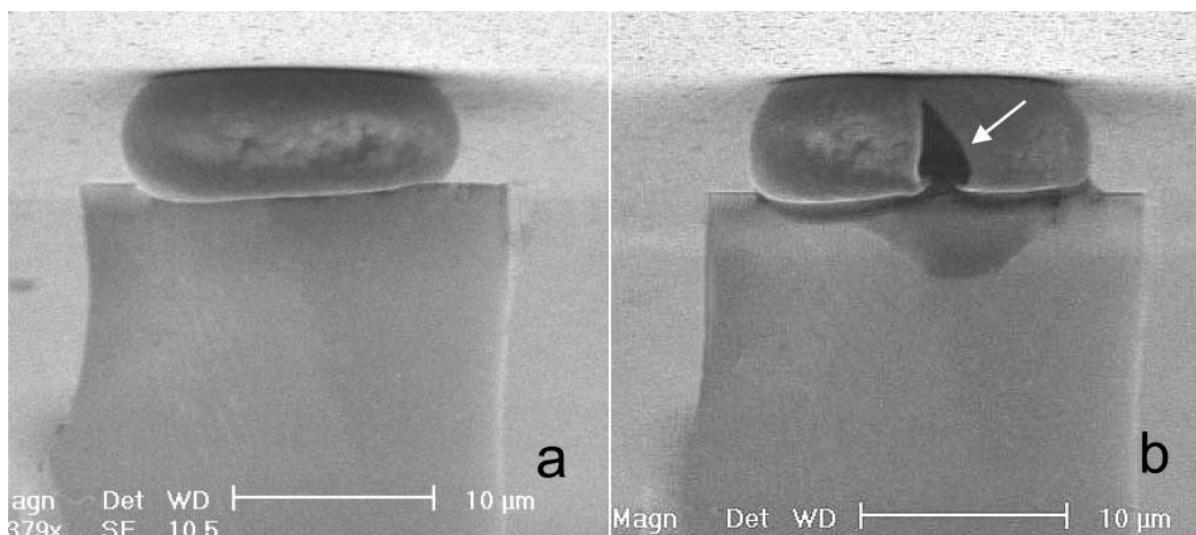


Figure 6: Images of a microcapsule (16.5 µm in diameter) during compression and after it was ruptured by nanomanipulation in the chamber of an environmental scanning electron microscope with high vacuum (5 kV, spot size 4). a - microcapsule being compressed; b: the microcapsule being ruptured (Ren, Donald and Zhang, 2007).

Conclusions

For all of the physico-chemical, structural and mechanical properties that have been outlined in this review it is important to note the interconnectivity of the properties and the necessity to take an interdisciplinary approach towards the analytical techniques in order to characterise microcapsules as fully as possible. Such is the interest in gaining quality control of microcapsules in all stages from the microencapsulation process to their end-use application. A key point to note when revisiting the analytical techniques for characterisation of each property is that more often than not there are multiple methods available for each, giving the researcher a range of choices depending on resolution required, the cost and the ease of experimental procedure. This highlights the desire for research to further develop analytical techniques for microcapsules in the pursuit of a more complete understanding of their properties in order to facilitate progression in industrial applications. New analytical techniques that can extend the limits of current ones, are more accurate, more user-friendly,

of lower cost and fully automated, which can be used in well-controlled environment such as temperature and humidity, should be highly desirable.

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