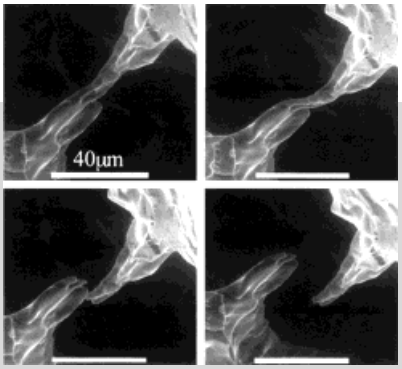
Characterisation of Soft Condensed Matter and Delicate Materials Using Environmental Scanning Electron Microscopy (ESEM)

By *Debbie J. Stokes**



Soft condensed matter and delicate specimens represent a wide class of materials including foods, pharmaceuticals, personal care products, polymeric coatings, gels, colloidal dispersions, emulsions and other complex fluids, biological specimens, and biomaterials. However, observational studies of soft condensed matter and delicate specimens in general have traditionally been restricted by the available experimental techniques. The recent advent of the environmental scanning electron microscope (ESEM) enables insulating and/or moist specimens, even liquid mixtures, to be observed directly, without the need for conventional EM preparation techniques such as freezing/drying and metallic coating. This represents a significant technological advance, extending electron microscopic capabilities to the study of soft condensed matter and delicate specimens in their native states. In addition, in situ experiments may be carried out including mechanical deformation and the observation of dynamic processes such as wetting and swelling behaviour of materials, thermal responses, the effects of hydration, dehydration and rehydration, and film-formation. A brief description of ESEM is presented, along with selected examples to highlight the potential usefulness of this unique instrument.

1. Introduction

Development of environmental scanning electron microscopy (ESEM) was originally driven by a growing need to overcome the limitations of conventional SEM[1] in order to facilitate the research and characterisation of a greater range of specimen types and expand the available experimental methodologies.

A fundamental limitation of conventional SEM is the need for high vacuum conditions (10^{-5}–10^{-7} torr) throughout the system, in order to prevent unacceptable scattering of the primary electron beam. However, many biological specimens, foams, gels, emulsions, food systems and so on, contain water, oil or other volatile substances that evaporate under high vacuum. Preparation of such specimens may therefore involve dehydration, chemical fixing, and freeze-drying, which can be very sophisticated or time consuming and may change the very structural features to be examined, particularly if the specimen contains delicate membranes, leading to unwanted artefacts. Furthermore, unless the specimen is electrically conductive, a build up of negative charge can quickly result. Hence insulators must be subjected to further treatment in the form of a metallic coating. Again, the introduction of artefacts is a possibility, along with the risk of obscuring fine structural details under the coating. Dynamic experiments become difficult or impossible under these circumstances. Coated specimens give only topographic contrast, due to the short escape depths of secondary electrons from metals, and therefore valuable compositional contrast from the underlying specimen is lost.

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ESEM does not require high vacuum conditions or conventional EM sample preparation, and this versatile microscope can be operated at three principal levels:

- High vacuum conditions as in conventional SEM, for dry, electrically conductive specimens,
- low vacuum conditions (nominally up to around 2 torr gas pressure) for dry, electrically insulating specimens,
- "wet" mode (around 4–10 torr pressure of water vapor) for moist or liquid specimens.

The work presented here will be concerned with the latter mode only, since this is often the most appropriate means of stabilising and imaging soft condensed matter and delicate specimens in their natural state. ESEM utilises two major innovations in order to operate in wet mode (also used for low vacuum conditions): differential pumping of the column and a gaseous secondary electron detector (GSED).

1.1. Differential Pumping

The column of the ESEM is divided into zones of varying pressure using an ion pump, diffusion pumps, and rotary pumps. Pressure limiting apertures then allow the electron beam to pass through, but are sufficiently small to maintain the pressure difference between each zone. The filament and upper parts of the column are thus maintained at high vacuum, whilst the sample chamber can be held at a much lower vacuum (typically up to 10 torr). The mean free paths of high-energy primary electrons are of the order of several millimeters under these low vacuum conditions. This allows the presence of a gas without destroying the criteria for imaging, provided that the working distance is appropriately short, typically around 8 mm from the objective lens. Inevitably, some scattering of the primary beam by the gas adds a broad "skirt" to the beam profile, but does not alter the distribution of the remaining focused beam. Hence the signal-to-background ratio remains sufficiently high and good quality images can be produced without too much interference from the probe skirt. Under optimum conditions, a resolution of 2 nm is possible. However, it is worth mentioning that, due to the large escape depths of secondary electrons (SE) from insulators in general,[2] images of soft, liquid or delicate materials may appear to lack the crisp definition associated with metal-coated specimens. Furthermore, if specimens have a covering of water or mucus, for example, then this will further serve to reduce the sharpness of specimen features. It is therefore important to appreciate that resolution may be limited, not by the microscopes specifications, but by the nature of the specimens themselves.

1.2. Gaseous Secondary Electron Detection

The microscope's SE detection system uses the principle of gaseous amplification to achieve a measurable signal. SE escaping the sample surface are accelerated towards a positively biased electrode (typically 300–500 V). Ionising collisions with gas molecules as SE traverse the specimen-to-detector gap give rise to environmental SE, which in turn can undergo ionising collisions, creating a cascade that amplifies the total signal. The efficiency of signal amplification varies as functions of gas pressure, specimen-to-detector gap and gas type.[3] Imaging gases may include nitrous oxide, carbon dioxide, helium, nitrogen, and water vapor, among others, each exhibiting different amplification properties. Indeed, water vapor has been found to be the most efficient imaging gas so far tested, amplifying the SE signal by a factor of up to 10^3.[4] By-products of gaseous amplification are positive ions that are swept towards the sample surface, helping to compensate for the build-up of negative charge deposited within the specimen by the primary electron beam, thus obviating the need for a conductive coating on the specimen. A schematic representation of gaseous amplification is shown in Figure 1. The absence of a metallic coating also means that specimen composition-dependent SE contrast can be detected, yielding further information about the material.

1.3. Environmental Control—"Wet" Mode

Maintaining specimens in a moist/liquid state is achieved by choosing appropriate environmental conditions in the sample chamber. Specifically, the specimen must be held in

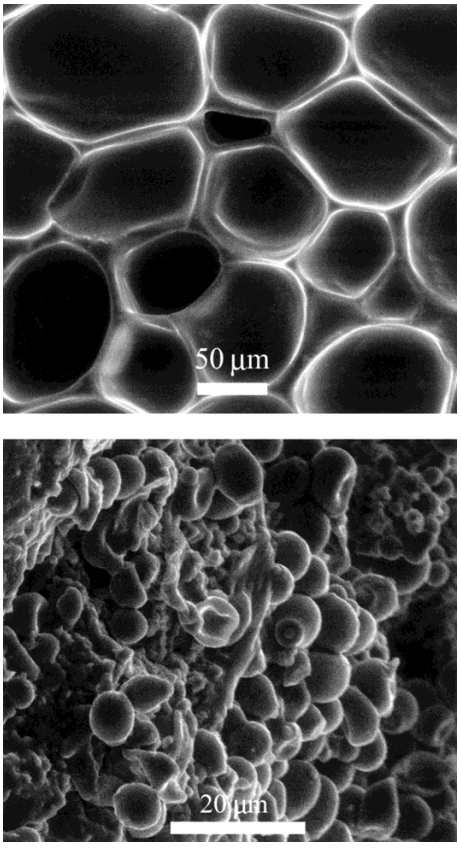

Fig. 1. Schematic illustration of gas cascade signal amplification utilised in ESEM. Emitted SE collide with gas molecules, creating further SE, leaving positive ions to compensate for any negative specimen charge.

an atmosphere close to 100 % relative humidity in order to be stabilised against moisture loss or gain over time. Figure 2 depicts the thermodynamic saturated vapor pressure curve for water. It can be seen that, quite conveniently, the pressure range up to around 10 torr meets the above criteria if the specimen temperature is lowered to a few degrees Celsius. Typically, a water-cooled Peltier stage is employed to keep the specimen at around 3 °C, within a chamber environment of water vapor at a pressure in the region of 4.5 torr, conferring specimen stability and yielding good quality images. It is also essential to ensure that the specimens natural moisture is preserved during the initial pumpdown of the chamber. It is therefore usual to perform a sequential pumpdown such that the air in the chamber is successively replaced with water vapor.[5] Figure 3 shows examples of two delicate microstructures, celery (upper image) and Stilton cheese (lower image), that have been stabilised and imaged following the regime outlined above. In particular, note how the celery cells and microbial spores in the cheese retain their structural integrity.

On a cautionary note, the absence of a coating makes delicate specimens particularly susceptible to radiation damage, and the effects appear to be enhanced when the specimen is surrounded by water vapor. Moreover, a study involving polypropylene[6] showed that the deposition of condensed water layers on the polymer surface significantly increased the likelihood of damage. It is thought that the number density and highly mobile nature of radicals in liquid water increases the rate of polymer hydrolysis, compared to the dry state. Care is therefore needed in order to avoid beam-induced artefacts, and a thorough assessment of a specimens stability under the electron beam should always be carried out prior to engaging in experiments or interpreting results.

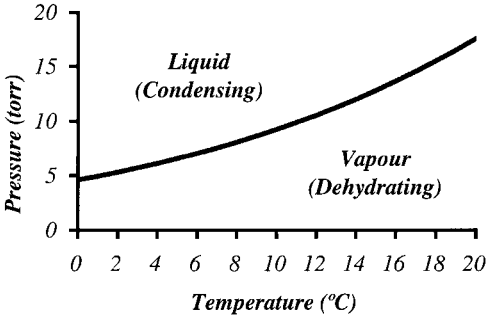

Fig. 2. Plot of the thermodynamic saturated vapor pressure curve for water.

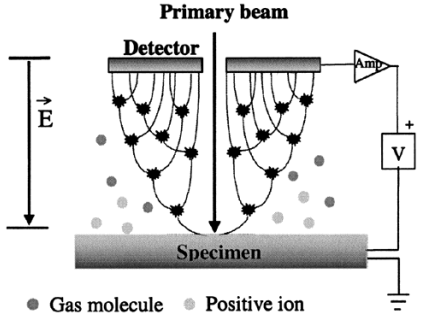

Fig. 3. ESEM micrographs of delicate microstructures stabilised against moisture loss or collapse under controlled environmental conditions. See text for discussion.

2. Liquid Specimens

One of the most unique features of ESEM is that it is has become possible to use electron microscopy for the observation of entirely liquid specimens with no prior treatment. A primary consideration is the volatility of the liquids concerned. Oily substances generally have sufficiently low vapor pressures to withstand vacuum conditions of a few torr. However, more care is needed with water-containing specimens, where environmental conditions must be controlled in order to preserve stability. Provided that the water phase is maintained at or near equilibrium with the water vapor above the specimen, by control of the chamber gas pressure and specimen temperature in accordance with Figure 2, an aqueous mixture can be studied for extended periods of time without water loss or gain. It is therefore possible to study the microstructures of a variety of liquid specimens, such as water–oil emulsions, gels and colloidal dispersions, at the high resolution and depth of field of an electron microscope. Figure 4 shows a protein-stabilised corn oil-in-water emulsion containing populations of both large and small oil droplets (approximately in the range 100 nm–50 μm). An extension to this type of microstructural characterisation of complex fluids is the potential for real-time observations of dynamic processes such as aggregation, coalescence and phase separation.[7]

Figure 4 also demonstrates that specimen-dependent compositional contrast can be obtained, as it can be seen that the water phase exhibits a more intense SE signal than the oil phase. In essence, the origins of this contrast can be traced back to differences in the electronic structures of the two liquids. The presence of double bonds in an unsaturated hydrocarbon liquid (corn oil in this instance) serve to attenuate excited electrons produced in that part of the specimen, there-

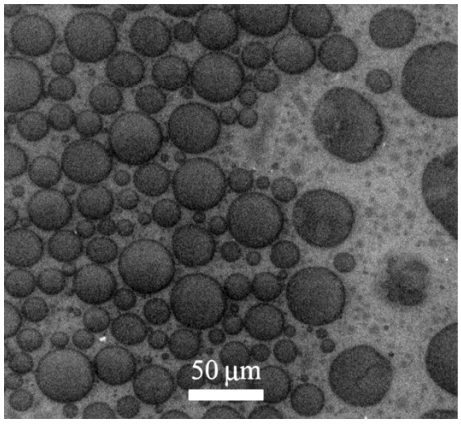

Fig. 4. An oil-in-water emulsion, demonstrating that liquid-state specimens can be stabilised and imaged in ESEM. Furthermore, with no conductive coating, specimen-dependent SE contrast provides compositional information (the water phase gives a more intense SE signal than the unsaturated hydrocarbon liquid).

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by reducing the yield of SE. Detailed discussions of the mechanisms of SE contrast from liquids can be found elsewhere,[8,9] however it is sufficient to note that differences in SE emission are very useful from the perspectives of distinguishing between phases, assigning relative compositions and thereby aiding image interpretation.

3. In Situ Evaporation or Condensation of Water

The ability to manipulate the water vapor environment within the specimen chamber makes possible a range of new dynamic experiments and observations involving the in situ removal or addition of water from specimens.

3.1. Polymer Latex Film Formation

Water acts as a solvent in many polymer latex systems, a commercially important example being water-based paint. A crucial property of paint is its ability to rapidly aggregate and coalesce upon application to a surface, forming a homogeneous, continuous film with good mechanical properties upon drying. ESEM can be used to explore the quality of such films during the drying process, providing evidence as to any inherent tendency for the development of flaws and cracks that would ultimately lead to an inferior coating.[10,11] Factors such as the surface chemistry of the latex particles promote or inhibit the overall coalescence and interdiffusion between particles as a function of time and these effects can be observed. The controlled removal of water from a polymer latex dispersion is illustrated in Figure 5, where it can be seen that particles begin to pack together and coalesce as the solvent is removed. Once dried, some regions have formed into a continuous film, while other areas possess good packing order but incomplete particle coalescence, leaving voids that could compromise the properties of the coating.

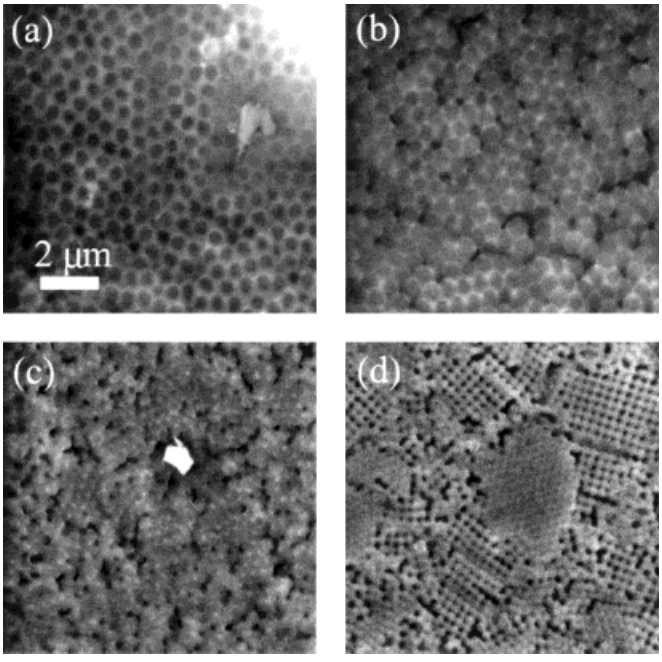

Fig. 5. Controlled removal of water from a polymer latex during film-formation. a) Wet specimen, 3 torr, 3 °C. b), c), and d), after 10, 15, and 20 min at 2.7 torr, 20 °C, respectively. Image courtesy of Dr. Nadia Stelmashenko.

3.2. Contact Angle Measurements

Condensation of water droplets onto a substrate can provide valuable information regarding the wetting behaviour and characteristics of materials, for example polymer substrates[12] and textile fibres.[13] Optical methods for the measurement of contact angles can be difficult due to ambiguities in judging substrate–water contact points and, for porous media, rapid diffusion of water droplets once formed. The use of an electron image makes the substrate–water contact points considerably easier to visualize and hence enables more accurate measurement of contact angles. Additionally, the in situ condensation of water onto a specimen in ESEM is a continuous, controllable process once initiated and hence a quasi-equilibrium between water diffusion and deposition can be established for porous media, such as textile materials, enabling stable droplets to form.[13] Figure 6 shows a textile fibre that has undergone in situ wetting, providing a clear demonstration of the usefulness of ESEM for this type of characterisation.

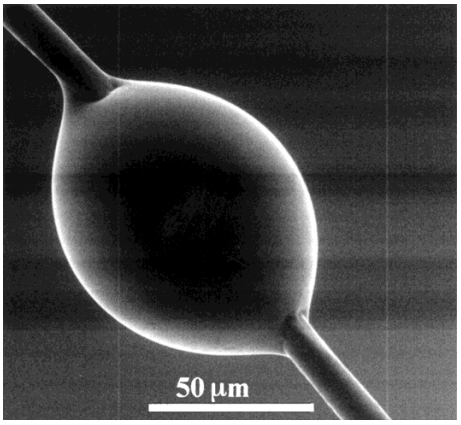

Fig. 6. Condensation of water onto a textile fibre, illustrating the use of ESEM for making contact angle measurements. Image courtesy of Dr. Lisa Jenkins.

4. In Situ Mechanical Deformation

ESEM can be used to probe the mechanical responses of a wide range of materials, including porous or cellular insulators, without the traditional restrictions placed by having rigid coatings on specimen surfaces as in conventional SEM. This allows real-time observations of the behaviour of specimens under tension, compression or during a "wedge" or "slice" test, in order to correlate stress–strain data with deformation and failure mechanisms.

A straining rig that incorporates a Peltier chip and heat-sink can be used to control specimen temperature, in conjunction with water vapor pressure in the chamber. This further extends the possibilities for experiments involving specimens that naturally have water confined within their structures, e.g., closed-cell fluid-filled structures such as fruit and vegetable tissues,[14] or whose mechanical properties vary as a function of relative humidity, e.g., open-celled biopolymer foams such as bread.[15] Figure 7 highlights the use of ESEM to follow the deformation and failure of a specimen that has been partially plasticised by water, in situ. The real advantage of this technique is that the modes of deformation and failure are observed in a continuous experiment, revealing far more information than conventional static observations of fracture surfaces.

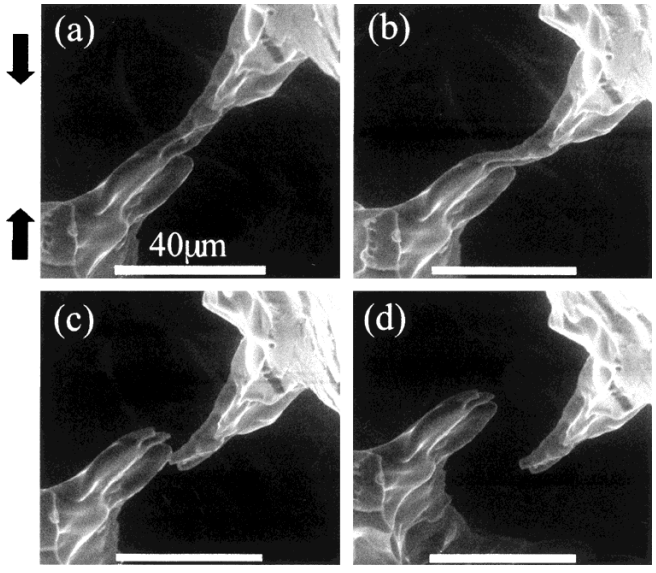

Fig. 7. ESEM micrographs obtained during an in situ compression test. The feature shown is a structural member within a partially hydrated breadcrumb and can be seen to distort before failure. Black arrows indicate direction of applied stress.

5. Summary

It has been shown that a number of new experimental methodologies have been made possible using ESEM. Particular strengths of this instrument are that specimens do not require any form of conductive coating and that specimens may be in a moist or even liquid state. This leads to the ability to observe, and perform dynamic experiments on, a much wider range of materials than was previously possible using conventional SEM. However, the examples provided here are not exhaustive and the interested reader is encouraged to consider the application of ESEM to their own area of expertise in order to give new insights or add complementary information. With many of the constraints of conventional SEM lifted, the way is open for researchers in many fields, such as materials science, engineering, physics, food technology, and biotechnology, to take advantage of a whole new way of exploring and characterising materials and systems. It is hoped that the impact of ESEM as a research and characterisation tool will be as significant for soft condensed matter and delicate materials as conventional SEM was for solid state materials.

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