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Fundamentals and Applications of Scanning and Transmission Electron Microscopes

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Abstract

Electron microscopes are equipment that use an accelerated electron beams as probes to generated images with magnifications and resolution not possible to obtain with optical microscopes (due to fact that electron wavelength can be 100,000 times shorter than visible light photons). Electron microscopes operating in the conventional high vacuum mode require conductive imaging specimens; therefore, non-conductive materials need the deposition of a conductive layer (Au-Pd alloys, carbon and osmium, among others). Low voltage mode of modern microscopes makes possible to observe non-conductive uncoated specimens. Transmission electron microscopes require thin samples (below 100 nm), placed onto appropriate sample holders. Electron microscopes are state of the art equipment that requires high operation and maintenance standards, therefore having a clear understanding of the operation fundamentals, equipment capabilities, suitable sample preparations and appropriate results interpretation is of critical importance to use the technique in the most suitable fashion.

Keywords:* *Electron microscopy; Morphological characterization; Chemical characterization; Crystallographic characterization

Introduction

When a beam of charged particles hits the constituent atoms of a sample, they interact with the sample atoms and yield several effects that are generally a function of the nature of charged particle, its energy and the type of atoms of which the sample is constituted. Most electron microscopy characterization equipment uses an electron beam to probe the sample, although there are equipment's that can use positively charged particles.

Secondary electrons occur when an electron from the beam passes very close to the sample atom nucleus, providing enough energy to one or more of the inner electrons to overcome the energy that holds them attached to the nucleus and to migrate out of the sample. These electrons are very low in energy (generally less than 5eV), so they must be very close to the surface to escape; they provide valuable topographic information of the sample and are used mainly in scanning

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electron microscopy. Backscattered electrons are produced when an beam electron directly interacts with the nucleus of an atom of the sample, being repelled in the opposite direction outside the sample, the signal intensity of the backscattered electrons is strongly related to the atomic number (Z) of the atoms that constitute the sample, the signal generated in this type of interactions allows to generate spatial distribution images of composition of the sample, especially in scanning electron microscopy. When a secondary electron is ejected from the atom, another electron at an outside energy level may jump inward to fill the energy vacuum generated by the ejected secondary electron.

The excess energy caused by this displacement is balanced by the emission of X-rays, which have energy values that are characteristic of each chemical element of the sample, so they are used to obtain information about the chemical composition in techniques such as spectroscopy (EDS, Energy Dispersive X-ray Spectroscopy). Transmitted or non-dispersed electrons are those that pass through the sample without interacting with the sample atoms; the number of electrons transmitted is generally inversely proportional to the thickness and density of the sample; such differentiation in intensity by thickness and density produce lighter or brighter areas in the image formed from the transmitted electrons [1-3]. Elastically dispersed electrons are those that are deviated from their original path by the atoms of the sample without loss of energy and which are then transmitted (not dispersed) through the sample. In crystalline materials, these electrons are deflected at a given angle that is dependent on the wavelength of the incident beam and the distance between the atomic planes of the sample, providing electron diffraction images that give important information of the spatial distribution of the atoms in the observed sample (Bragg's Law). The interference of these electrons with the transmitted ones dramatically increases the contrast and is essential to obtain high resolution images (HRTEM). Inelastic dispersed electrons are those that are diverted from their original trajectory by the atoms of the sample with a loss of energy and are then transmitted or dispersed through the sample. Electrons that are elastically dispersed a second time form the so-called Kikuchi lines, of great importance in the study of crystalline structures [1-4].

Methodology

Electron microscope techniques

Scanning electron microscope: The Scanning Electron Microscope (SEM) is a characterization instrument that forms images of the surface of the sample observed by the interaction of a beam of high energy electrons in a sweeping sequence on the surface of the material. The electron beam penetration in the sample depends on many variables, the most important are the electron beam acceleration and the nature of the material to be characterized [5-9].

Beam electrons interact in different ways with the sample atoms, each of these interactions generates characteristic signals and contain information of the topography of the surface, composition, and other properties of the sample [10-13]. Another of the interaction signals of the electron beam with the sample corresponds to the secondary electrons, which are collected in a type of detector called Backscattered Secondary Electrons (BSE), which converts them into an amplified voltage signal, this amplified signal is transmitted to an image generating device Which in many cases is a charge-couple camera (CCD) on which a variable light intensity point is generated, depending on the intensity of the amplified signal that produces it. The image of the sample surface is formed from the combination of multiple points of varying intensity on the screen of the CCD [14].

SEM can produce detailed images of the surface of the sample with a resolution generally ranging from 10 to 50 nm. Due to the way the beam of electrons is generated and directed on the sample, SEM images are characterized by having a reduced depth of field producing images with three-dimensional information useful for understanding the structure of the surface of the sample [14-16]. FIG. 1 presents scanning electron microscopy photographs of ZnO hexagonal crystals synthesized from aqueous solutions of zinc nitrate hexahydrate and hexamethylenetetramine [17].

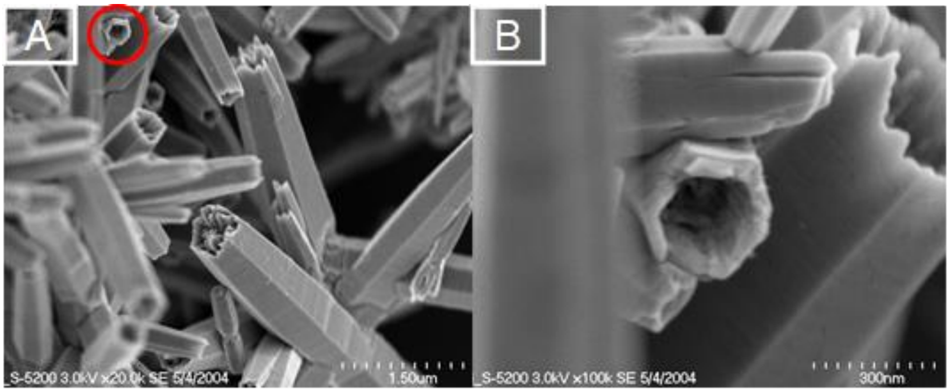

FIG. 1. **SEM photograph of hexagonal ZnO crystals synthesized from aqueous solutions of zinc hexahydrate and hexamethylenetetramine.**

FIG. 1 is an excellent example of SEM's ability to generate high resolution photographs in which topographic details of the surface of the sample can be observed; in this case crystals of ZnO, that under natural conditions, grow in random directions are forced to growth in only a specific direction forming hexagonal structures by means of a precise steric suppression caused by molecules of the amino group that block the growth of the crystals in directions other than those of the plane (101). In FIG. 1A, ZnO crystals can be observed as structures with elongated hexagonal shapes having lengths ranging from 2 to 10 microns and cross sections also varying in the order of 200 to 800 nanometers; In addition, this same figure shows that the longitudinal walls of the hexagonal crystals tend to be smooth while the cross sections are seen with many topographical accidents which appear to consist of small crystallites of pointed shapes. FIG. 1B is a magnification of the section enclosed between the red circle of FIG. 1A, in which the cross section of one of these hexagonal crystals is clearly seen, allowing details to be observed in high definition; FIG. 1B shows that the terminal section of the hexagonal crystals is formed by external walls with an empty section in the center. In particular, for the hexagonal crystal of 1B, those walls have a thickness of approximately 30 nm. This morphology seems to indicate that the growth of the hexagonal crystals starts in the walls and diffuses inwards.

Sample preparation for SEM: One of the most important versatilities of SEM equipment is its ability to characterize materials whose samples present a wide variety of external dimensions, given that there are SEM equipment’s with different chamber volumes to accommodate samples of varying sizes and also multiple sample holder designs. This versatility allows the characterization of samples whose macro dimensions are measured in tens of centimeters up to samples that have been prepared for use in transmission electron microscopes (generally in the order of 10 to 150 nm) [18]. A requirement that the sample must meet in order to obtain images with great detail is to have a good electrical conductive (at least in its surface), in addition to reduce the accumulation of charges on the surface of the sample it is necessary to establish a suitable ground pole between the sample and the sample holder; metallic samples require little preparation to be observed in a SEM. Non-conducting samples tend to be charged electrically when they are swept by the electron beam causing sweeping defects, distortions and mirages in the generated image, especially when the secondary electrons are used to form the image. It is advisable to deposit an ultrafine layer of a conductive material on the surface of the non-conducting samples, this ultra-thin layer generally formed of metals such as gold, gold-palladium alloys, platinum, osmium, iridium, tungsten, chromium and graphite, among others [19,20].

In the case of biological samples, in which the deposition of an ultrafine layer of conductive material may alter or ruin the sample, the technique of selective chemical spotting has been explored [13,21,22]. There is a possibility of scanning electron microscopy images of non-conducting samples under certain special operating conditions; the most common operating modes for these conditions are referred as Environmental Scanning Electron Microscopy (ESEM) and Field Scanning Electron Microscopy (FEG-SEM).

ESEM irradiates the sample with a beam of accelerated electrons at a low voltage, keeping the irradiation chamber and the microscope column at a relatively high vacuum operating pressure and maintaining a differential vacuum in the area of the irradiation electrode. As a result of this operation the accumulation of charges on the surface is reduced due to the low acceleration of the electron beam, the charges that are generated are neutralized thanks to the effect of the high pressure around the sample which increases the signal-to-noise ratio of the secondary electrons; on the other hand, the operating conditions and the type of emission electrode used in FEG-SEM allow the generation of high-bright primary electrons. The operating conditions must be adjusted in such a way that the local volumetric charge in the region near the surface of the sample is almost neutralized and with a secondary electron density sufficient to neutralize any positively charged sites generated on the surface [23-27]. It is always important to establish the appropriate acceleration of the electron beam with which the sample is irradiated, since delicate samples may suffer permanent damage if the beam is focused over it for a long period of time on the same area in the sample as illustrated in FIG. 2 [28].

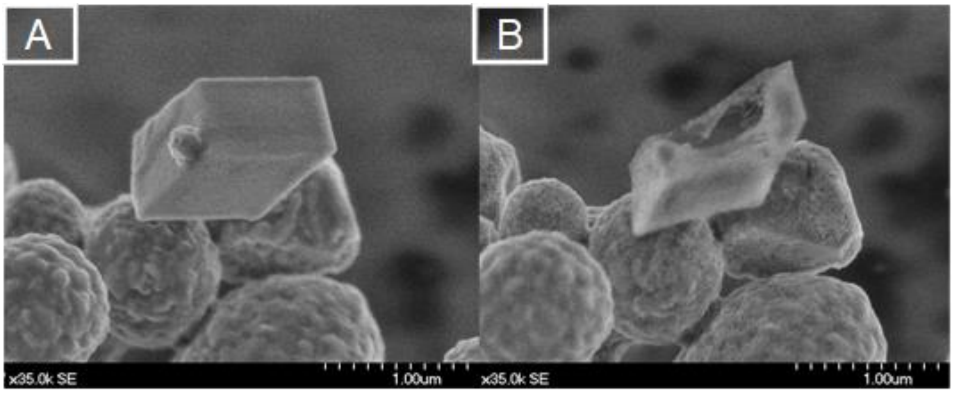

FIG. 2. **Permanent damage caused by the electron beam in an SEM equipment on crystals of aluminum compounds.**

X-ray energy dispersion spectroscopy

X-ray energy dispersion spectroscopy (EDS) is an analytical technique used for elemental analysis or chemical characterization of a sample. The quantitative accuracy of the EDS spectrum can be affected by several factors, given the multiple energy levels that the constituents of the sample may have, in some cases the characteristic peaks of the spectrum for different energy levels can be overlapped, for example the characteristic peaks of the emissions Ti Kβ K alpha and V, Mn and K alpha Kβ Fe. The precision in determining the elemental chemical composition of the sample can be affected by the nature of the sample and the energy of the irradiating electron beam.

The penetration of the electron beam in the sample is directly proportional to the energy of acceleration and inversely proportional to the density of the sample. Samples consisting of atoms of heavier materials allow less penetration of the beam of irradiating electrons; according to these conditions, it is possible that for a same acceleration energy in a sample whose density is low the X-ray emission is generated from the bulk of the specimen, the elemental chemical composition resulting from the analysis of the ESD spectrum will correspond to a global composition of sample; an elemental analysis under the same electron beam acceleration conditions in a sample with a high density value, the electron penetration capacity of the radiating beam will be smaller and therefore the X-ray emission will only come from the atoms located on the surface or near the surface, which means that the determined elemental chemical composition will not be global on the contrary it would be a local composition; an example of the determination of surface composition by means of the combined use of SEM and EDS is shown in FIG. 3 [29].

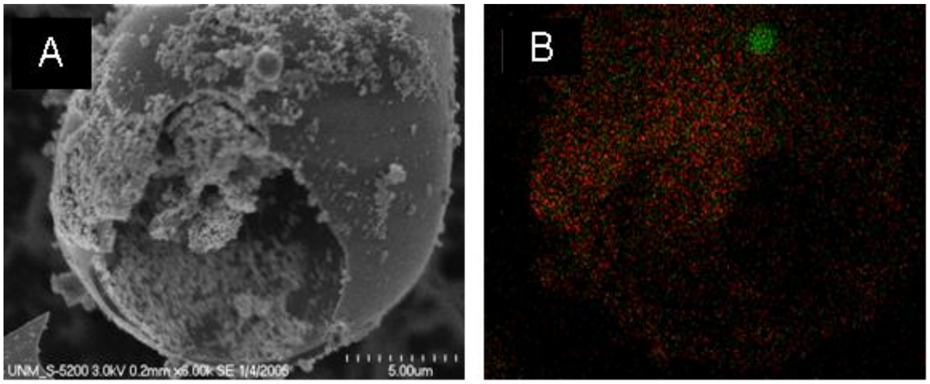

FIG. 3. **Photograph of secondary electrons taken in a SEM (FIG. 3A) and composition mapping obtained by means of EDS (FIG. 3B), of Pd and Zn alloy samples.**

FIG. 3 shows SEM photographs of particles of a plasma torch prepared alloy of Pd and Zn; in FIG. 3A it is possible to observe the photograph obtained by means of the secondary electron detector, in which the morphology of a spherical particle of size close to 10 or 15 microns is evident, with a fracture in its surface in the lower section of the sphere, this fracture reveals the sphere is a hollow structure. Additionally, agglomerated particles, probably in the nanometer size range, decorated or are around the micrometric spherical particle. FIG. 3B is a mapping photograph of elemental chemical composition obtained by means of EDS for the same particles photographed in FIG. 3A, the red dots and the green dots represent the presence of Pd and Zn respectively, it could be concluded that the chemical distribution of the elements Pd and Zn is homogeneous along most of the surface of the sample with the exception of an agglomerated size particle which are mostly composed of Zn, which is observed as a high density cluster of green dots in FIG. 3B, this same particle observed in the photograph of secondary electrons (FIG. 3A) is seen as a sphere of size close to 1 micron. Since the vast majority of SEM and TEM equipment have coupled X-ray detectors, obtaining morphological and elemental chemical composition analysis simultaneously is one of the major advantages of these techniques.

Detection of backscattered electrons

An additional source of information from the electron beam interaction with the sample is backscattered electrons (BSE). These are characterized by being high energy electrons that are reflected or backscattered due to an interaction with the nuclei of the atoms of the sample. The energy of the backscattered electrons is increased with the atomic number of the element with which they collided; in this way backscattered electrons of heavy elements appear brighter in the image than those generated by lighter elements, thus allowing to detect zones with different chemical compositions [30-33]. An example of backscattered electron photography is presented in FIG. 4.

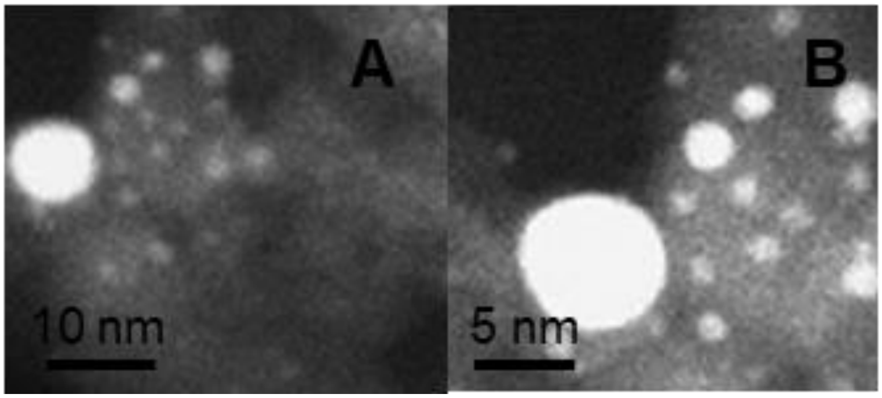

FIG. 4. **Back scattering electron images of metallic Pd supported on carbon.**

FIG. 4A and FIG. 4B show the photographs at different magnifications obtained from backscattered electrons of Pd on carbon catalysts prepared in a plasma torch; the Pd nanoparticles are clearly seen as bright spots on the dark surface of coal. Although a photograph of electron microscopy formed by the non-dispersed electrons would give reliable information on the size of these particles of Pd, the photograph formed from backscattered electrons allows to observe nanoparticles of Pd that are located in the hidden side of the carbon support, feature that would be probably not be clearly observed using only secondary electrons.

Transmission Electron Microscopy

In general, a TEM equipment consists of an electron emission source, which may be a tungsten or lanthanum hexaboride (LaB6) filament, connected to a high voltage source (typically 100-300 kV). The upper electromagnetic lenses of the TEM column allow the manipulation of the electron beam to the desired size and direction to interact with a specific section of the sample. Generally, a TEM uses several systems of electromagnetic lenses with very different purposes: the condenser lenses, objective aperture lenses, intermediate lenses and projector lenses. Condenser lenses are responsible for the formation of the primary beam of radiant electrons while objective aperture lenses, intermediate lenses are responsible for focusing the beam down on the sample. Projector lenses are used to expand the beam of undispersed electrons onto the screen of a CCD where the image is formed. The magnification increase of the image in a TEM is due to the ratio of the distances between the sample and the plane of the lens of the image [14]. The operating principle of the Electronic Transmission Microscope (TEM) is similar in its operation to SEM. The source of accelerated electrons irradiates the sample and a fraction of them does not disperse through the volume of the sample. These non-dispersed electrons are refocused by means of electromagnetic lenses and in this way an image of the sample is constructed. The characteristics of the image can be controlled by modifying the exposure aperture of the non-dispersed electron beam from the sample. If the exposure aperture allows the passage of most of the non-dispersed electrons, a so-called "clear field" or "bright field" image is obtained. In contrast, if only specific cross-sections of non-dispersed electrons are allowed to pass through the modulated aperture, an image is generated that is denominated “dark field”. Non-dispersed electrons from the sample and that passed through the modulated aperture are detected in the CCD to form the image. The resolution that can be obtained in a TEM is in the order of nanometers with magnifications ranging from 10X to 1000000 X [34-36]. The most common mode of operation of TEM equipment is bright field image mode. In this mode the contrast between the different areas of the image is formed directly by the occlusion and the absorption of electrons in the sample. Sample regions that are thicker or regions with higher atomic numbers will appear darker in the image, while regions where the sample is thinner or with lower atomic density will appear brighter. Images generated in TEM equipment can be considered as a two-dimensional projection of the sample in the path of the optical axis [36].

Results and Discussion

TEM has a substantially higher image resolution than an SEM and is therefore widely used for structure analysis, crystallographic characterization and the observation of defects in crystalline and non-crystalline materials. It is also used for the characterization of nanoparticles, in particle agglomeration analysis, the effects of aggregation, dispersion in a matrix, etc. The electron beam that radiates the sample not only experiences dispersion, but on the contrary, they experiment also the other interactions previously described between charged particles and the constituent atoms of the sample, these interactions can be used to obtain information of the elemental chemical composition being this reason why this equipment (TEM) generally include X-ray and secondary electrons detection systems. Among the most common detectors are the X-ray energy dispersion spectroscopy (EDS) and the electron loss energy spectroscopy (EELS), the latter technique has the advantage that allows the identification of samples containing light elements such as carbon, nitrogen, oxygen, etc, with high spatial resolution of the order of 1 nm, which cannot be determined using EDS [33,37,38]. Bright image photographs obtained by means of TEM, accompanied by an elemental chemical analysis of particles obtained by EDS are presented in FIG. 5.

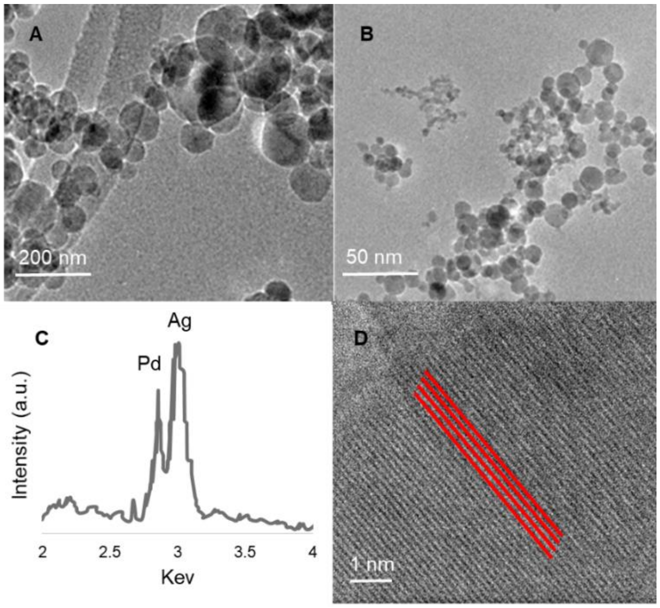

FIG. 5**. TEM images of particles of Pd and Ag alloy and analysis of selected particulate chemical composition.**

FIG. 5 shows TEM photographs of alumina-supported Pd and Ag alloy particles prepared from aqueous solutions of inorganic salts of the respective metals and heated in chemically controlled atmosphere. In FIG. 5A and 5B shows conglomerates of particles with size that varies between 15 to 80 nm. FIG. 5D is a higher magnification photograph of one of the particles of the Pd and Ag alloy in which the lines of the crystallographic planes are evident. The high resolution of this photograph allows to calculate the interplanar distance between the crystallographic planes (2.6 A^{o}), a value corresponding to the interplanar distance of an alloy with an atomic ratio 1/1 Pd and Ag; The EDS spectra presented in FIG. 5C was determined for the particle in picture 5D, revealing also a close to equal composition of Pd and Ag in the observed particle.

Given that the electron beam can experience wave diffraction phenomena, if these diffractions generate coherent interference (Law of Bragg), a sample with crystalline characteristics will produce diffraction points in the back focal plane of the sample [3]. By manipulating the aperture allowed for the passage of the diffracted electron beam from the sample in its back plane, i.e., by properly manoeuvring the aperture target, specific reflections of the Bragg Law points can be observed as the diffracted electrons are projected onto the image. If the reflections are selected in such a way that they do not include the dispersed electron beam the image appears dark where there is no dispersion, this is known as a "dark field" diffraction, which can be seen in FIG. 6 [39]. Some sample holders allow the sample to be tilted at different angular positions with respect to the plane of the radiant electron beam in order to obtain specific diffraction conditions [40-45].

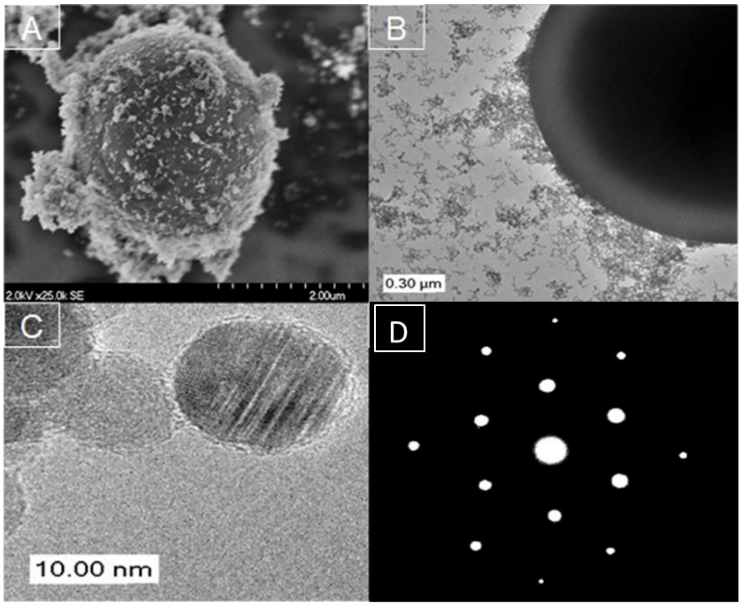

FIG. 6. **SEM, TEM and Electron Diffraction of aluminium and titanium oxides.**

FIG. 6 is an excellent example of the combination of characterization capabilities provided by complementary equipment such as SEM and TEM. The photographs correspond to the characterization of solid solution particles of aluminium and titanium oxides produced using the plasma torch technique. FIG. 6A corresponds to a SEM photograph of a spherical particle approximately 2.5 micrometers in size whose surface is not smooth and which appears to be surrounded by aggregations of nanoparticulate material, however the magnification and resolution of SEM photography does not allow determining with accuracy if those aggregations are composed of nanoparticles; FIG. 6B corresponds to a bright field TEM photograph of the same micrometric particle from which SEM photograph of FIG. 6A was obtained, in which it can be clearly seen the spherical particle of micrometric size surrounded by clusters of particles, which can now be assured to correspond to nanoparticles; In this photograph the edge of the micrometric particle becomes clearer than its center, this is because the path that the non-dispersed electrons from which the photograph was obtained must pass through a sample cross-section which is thinner at the edges than in the centre of the sphere. FIG. 6D is a high magnification TEM photograph of one of the nanoparticles that make up the aggregations, on this particle it is possible to observe some lines of crystalline planes. FIG. 6C is an electron diffraction image taken on TEM, which gives information on the crystalline structure of the nanoparticles of the solid solution of aluminium and titanium oxide.

TEM sample preparation techniques: Since the image of the sample in TEM is formed from the non-dispersed electrons passing through the sample, the sharpness, definition and resolution of the image depends to a large extent on the amount of undispersed electrons arriving at the detector; To increase the amount of undispersed electrons arriving at the detector it is recommended the thickness of the sample to be below 100 nm. There are different sample preparation techniques that allow obtaining samples with these specifications, those samples whose thickness is thin enough to be transparent to the passage of electrons (ultrafine powders, nanoparticles, among others) require minimal preparation since they can easily be supported on a grid holder.

In the cases the sample thickness is too high, the microtome is a device that allows to obtain very thin sections of samples and which is of wide application in the preparation of biological samples for TEM [46]. This method is used to obtain thin samples with minimum deformations that allow the observation of tissue samples [46-48]. To avoid the accumulation of charge on the surface of the sample, the tissue samples should be coated with a thin layer of conductive material, such as carbon, this coating generally does not exceed a thickness of a few nanometres. This can be achieved through an arc deposition process, using a sputtering coating device, chemical deposition, etc. In the case where the sample is sensitive to the vacuum pressure in which the TEM operates, the sample may be selectively spotted generally with heavy metal compounds such as osmium, lead or uranium [49]; These compounds interact with the electron beam by dispersing them differently and generating distinct areas of brightness in the TEM image, depending on the place in the sample where they have been selectively placed. This procedure requires a detailed understanding of the mechanisms by which heavy metals bind to biological tissues [35,50]. Mechanical polishing is a TEM sample preparation technique generally used when the sample has a surface of medium or high hardness; the surface to be polish is placed in contact with a surface coated with materials of high hardness and with very fine grain sizes, which remove surface layers from the sample.

Once the desired thickness has been achieved and depending on the level of homogeneity required of the polished surface, a final polishing can be made using techniques such as ion-polishing, which can eliminate imperfections the mechanical polishing may create on the surface. The most used polishing ions are argon or gallium, which are ionized by an electric field and directed on the surface. The polishing rate depends on the type of ion used and the energy applied to it, however, it rarely exceeds micrometers per hour, which limits the application of the method to polish only extremely fine samples. A procedure analogous is the chemical polishing; in this technique, a chemical compound is used as removal agent, which selectively attacks the surface of the material, especially metal surfaces. This process of corrosive attack on the material can be controlled by means of a detailed manipulation of currents and voltages in a corrosion cell [49].

Conclusion

Scanning Electron Microscope has the capacity to generate high resolution photographs of electrically conductive samples; non-conducting samples require the deposition of an ultrafine layer of a conductive material. If the deposition of an electrically conductive layer is not suitable with the sample is advisable to use Environmental Scanning Electron Microscopy (ESEM) and Field Scanning Electron Microscopy (FEG-SEM), this technique requires special operating conditions that may hinder the resolution of the photograph.

SEM also can bring compositional chemical information via Backscattered electrons (BSE) and X-ray energy dispersion spectroscopy (EDS). In BSE heavy elements appear brighter in the image than those generated by lighter elements, but not further information is obtained. On the contrary, EDS bring elemental chemical information, coming from the specific X-ray emission of the individual atoms forming the sample, even spatial composition is obtained when the mapping option is use. Electronic Transmission Microscope (TEM) operating principles are similar to SEM. In the case of the TEM, non-dispersed electrons are refocused by electromagnetic lenses creating, either bi-dimensional bright field or dark field images. TEM has a substantially higher image resolution than an SEM and is therefore widely used for structure analysis, crystallographic characterization and the observation of defects in crystalline and non-crystalline materials. Since the image of the sample in TEM is formed from the non-dispersed electrons passing through the sample, the sharpness, definition and resolution of the image depends to a large extent on the amount of undispersed electrons arriving at the detector, to increase the amount of undispersed electrons arriving at the detector it is recommended the thickness of the sample to be below 100 nm.

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