

Changes in Particle Area Measurements Due to SEM Accelerating Voltage and Magnification

MARÍA JULIA YAÑEZ¹ AND SILVIA ELENA BARBOSA^{2*}

¹Centro Regional de Investigaciones Básicas y Aplicadas Bahía Blanca (CRIBABB), Argentina

²Planta Piloto de Ingeniería Química (PLAPIQUI), Bahía Blanca, Argentina

KEY WORDS area calculations affected by accelerating voltage and magnifications; artifacts; kV effects; scanning electron microscopy

ABSTRACT Scanning electron microscopy (SEM) images of polymer blends followed by digital image analysis is a rapid and easy method for the measurement of particle size and dispersion. The particle size determination is done with appropriate off-line image analysis software. However, it is necessary to understand how machine parameters involved in the formation of the SEM image influence area measurements of morphological features. In this work, the influence of the accelerating voltage used during image acquisition was examined with standard samples and with polymer blend samples. A systematic study centered on two mutually exclusive assumptions of area variation or no area variation with accelerating voltage was carried out. The off-line image analysis software was then calibrated according to the assumptions. The main conclusion of this study was that kV has an important influence on area measurement in SEM images. This effect was observed for different standard materials (metallic and polymeric) and for the range of magnifications used. The higher the accelerating voltages, the greater the error at high magnification for polymer samples. As the beam energy increases, the primary electrons penetrate more deeply into the solid specimen, producing low-resolution signals. These signals degrade the image and surface details, which became less well defined. Therefore, images of polymer samples must be taken at lower accelerating voltages so the desired surface details can be imaged clearly. To avoid area measurement errors, particle measurement must be done with the calibration of the off-line image analysis software corresponding to the accelerating voltage and magnification used for the acquired images. *Microsc. Res. Tech.* 61:463–468, 2003. © 2003 Wiley-Liss, Inc.

INTRODUCTION

Polymer blends are important technological materials. These blends make possible the creation of new materials. A majority of the most important blends available on the plastic market are incompatible, and when mixed segregate into two or more phases. The principal advantages of hetero-phase blends can be a reinforced structural effect or an improvement in toughness. Several authors (Utracki, 1990; Sperling 1997; Wu, 1982) report that the final blend properties depend on factors such as blend composition (relative content of polymer A/polymer B) and phase morphology. The morphological properties can be evaluated by the adhesion between phases, the size and distribution of the dispersed phase in the predominant phase, the surface-to-surface dispersed phase interparticle distance, etc. For a blend composition, evaluation of the morphology and the measurement of the principal morphological parameters (particle form and dimensions and interparticle distance) are very important factors in determining blend characteristics. Microstructure measurements correlate the microstructure with the blend's final macroscopic properties. For this reason, morphological characterization techniques need to be easy and quick for rapid morphological evaluation and for eventual modification of blend preparations to achieve the desired properties.

Direct observation of the fracture surface of thermo-plastic blends, using scanning electron microscopy (SEM) followed by digital image analysis, is the most widely used method for the measurement of particle sizes in the dispersed phase. This SEM technique is useful because the images provide an excellent depth of field, giving good three-dimensional morphology. It should be remembered that the size of a feature in 2D images is related to the corresponding object in 3D (Geladi et al., 1986, Russ, 1999). Numerous authors use this method to measure dispersed-phase particles. Hobbs and Watkins (2000) have done an analysis of the morphology characterization. Dumolín et al. (1991) used this technique for the measurement of the dispersed phase in polypropylene/polyethylene blends and used these results to analyze the morphology and the rheology of this blend. Dirlikov et al. (1996) measured the different phase domains and analyzed the phase

*Correspondence to: S.E. Barbosa, Planta Piloto de Ingeniería Química (PLAPIQUI), Cno. La Carrindanga Km. 7 (8000) Bahía Blanca, Argentina.

This article was presented at the Multinational Congress on Electron Microscopy, Lecce, Italy, Sept., 2001.

Received 17 June 2002; Accepted in revised form 4 August 2002

Contract grant sponsors: CONICET (National Research Council of Argentina), and UNS (National University of the South).

DOI 10.1002/jemt.10309

Published online in Wiley InterScience (www.interscience.wiley.com).

separation of two-phase epoxy thermosets that contain epoxydized tridlyceride oils using techniques suggested by Hobbs and Watkins (2000).

The domains of the rubber-toughening polycarbonate-nylon blends were also evaluated by SEM measurements by Chang et al. (1996). Martuscelli (1995) analyzed the elastomer domains by this technique and related the structure and mechanical properties of rubber-toughened polypropylene. Sawyer (1996) made an extensive compendium of the morphology of a two-phase polymer system and particle measurements using SEM images.

The SEM irradiates an area with a finely focused electron beam that penetrates the specimen. The penetration of the primary electrons in the solid (interaction volume) depends on its average atomic number, the beam energy, and specimen tilt. A lot of signals are produced when the electron beam impinges on a specimen, such as secondary, backscattered, and Auger electrons, characteristic X-rays, etc. The secondary electron signal is the most frequently used in SEM imaging due to the fact that it gives topographical images of the surface. With the secondary signal, the size, distribution, and morphology of some specimen's characteristics can be directly observed. This kind of signal is defined (Goldstein, 1992) on the basis of its kinetic energy; that is, all electrons emitted from the specimen with an energy less than 50 eV. The secondary electrons produced by the primary beam contain the high-resolution signal that preserves both the lateral spatial resolution of the focused beam when the secondary electrons (SE1) are gathered from a shallow sampling depth. Also, backscattered electrons generate other kinds of secondary electrons (SE2), responsible for a low-resolution signal as they leave the specimen. Backscattered electrons striking the chamber walls produce a third source of secondary electrons (SE3).

Errors can arise in measurements depending on topographical contrast, defocus, aberrations, and other artifacts recorded in the image. Goldstein et al. (1992) attributes these errors in the image to instrument faults such as astigmatism, vibration drift, poor focus, uneven illumination, projection distortion, etc. These faults are only visible in the image while the sample remains undamaged by the electron beam. Other imaging faults occur due to the specimen-beam interaction. These faults include charging, specimen heating degradation, and contamination of the sample surface. Many of these problems can be overcome by modifying some of the SEM operating parameters.

Sawyer (1996) reveals that polymer blends have benefited from the use of low-voltage field emission SEM, as have other polymer imaging applications, because the contrast is improved, surface details can be observed, and the beam damage is reduced. Only in particular cases is it necessary to use high-beam voltage because details at greater depth in the specimen can be imaged and analyzed.

Particle measurement in an SEM image is usually done with appropriate off-line software for image processing and analysis (OLSIPA). The human eye is capable of recognizing the foreground as particles, but a computer needs to detect and filter the particle images to recognize them as individual objects. OLSIPA uses a binary image or thresholding process to perform these

steps and to digitize the particle shape and position in an optimized list of coordinates. It is important to point out the difference between image processing and image analysis. Russ (1999) pointed out that image processing implies the rearrangement of pixels according to neighboring pixel brightness or shifted to another place in the array by image warping without changing their sheer quantity. Image analysis is used to find the descriptive numeric parameters (areas, distances, radius, etc) which represent the relevant information in the image.

The domain's area is the most basic measurement in OLSIPA because it is directly determined by counting the number of pixels within the domain (pixel-based representation). However, OLSIPA requires a conversion factor that translates a pixel into real dimensions so that pixel counting gives real area values. This conversion factor is determined by OLSIPA's calibration and is obtained from images of standards. Pixel counting made on a standard is correlated with its known dimensions.

Most commercial OLSIPAs ask for conversion factors for several magnifications. However, they do not have requirements or specifications about how the image of the standards was made. They assume that the imaging geometry does not vary with changes in the microscope beam voltage used during imaging. As a consequence, a single unique conversion factor is used for evaluating measurements on images taken at different electron beam voltages.

Chang (1989) suggested that "the ease of use of modern image analysis systems does not lead to the equipment being used as a 'black box' but rather with constant interrogation as to what the quantities really do not mean." According to this statement, we performed a systematic study of the influence of beam voltage on the actual area measured. The study was carried out for a wide range of electron beam voltages and two magnifications were used for SEM image acquisition. The strong influence of electron beam voltage on area measurements was observed and analyzed.

EXPERIMENTAL Materials

Standards. Two kinds of standards were used: 1) Polystyrene microspheres of $\phi = 5 \mu\text{m}$, supplied and certified by Duke Scientific Corp., were dispersed in a vial with bidistilled water and immersed in a low-power ultrasonic bath (~30 seconds) to separate and spread the particles. A drop of this suspension was placed on a glass slide and dried at room temperature for this standard sample. 2) Copper aperture standard ($\phi = 200 \mu\text{m}$), Eastern Analytical PTY.

Blend Sample. Polypropylene/ethylen-propylen-rubber blends (PP/EPR 70/30) were obtained by blending 70 weight percent of PP (injection grade) with 30 weight percent of EPR in a twin screw extruder at 190°C. The blend was pelletized and rectangular plates were obtained by injection molding. The specimens for microscopy analysis were the cross-sections of the injection plates. To avoid polymer and elastomer deformations, the fracture was performed under liquid nitrogen. Taking into account that the particle sections are not perfectly distinguishable from the polymer, for an accurate measurement it is necessary to contrast it.

In this way, a toluene etching was performed. The superficial elastomeric phase was extracted and the area of the voids gave a direct measure of the EPR domains because there is no form variation in the etching process.

Procedure

Both samples and calibration standards were coated with a 300 Å gold layer in a sputter coater (PELCO 91000) and observed in scanning electron microscope (JEOL JSM-35 CF) with a secondary electron detector.

SEM images were acquired at 512×400 pixels resolution and at different magnifications for each sample. The Cu aperture standard was acquired at $300\times$ and the PS microspheres standard or blend sample were taken at $4,000\times$. The accelerating voltage was changed from 5 kV to 30 kV in steps of 5 kV, while other operating conditions such as load current, condenser lens aperture, work distance, and magnification were constant (same value for all voltages).

The processing of images and area calculation on the SEM acquired images were carried out with Analysis PRO™ off-line software for image processing and analysis. To avoid possible errors in the particle measurement due to image warping, different spatial OLSIPA calibrations (conversion factors) were established for the horizontal and vertical directions. The two following methods were used, depending on the calibration applied.

Method I. OLSIPA was calibrated only with 5 kV image and this conversion factor was used for all the area measurement in the higher voltage images. This procedure assumes that an area does not vary with the kV applied during imaging.

Method II. OLSIPA was calibrated for each imaging kV. This means that a standard was measured at each kV and OLSIPA used voltage-specific conversion factors. All image area measurements were performed using the corresponding kV conversion factor. In this case, we assumed that an area measurement changes with the accelerating voltage and it is necessary to calibrate the analysis software for each imaging kV. This procedure is schematized in Figure 1 to clarify the methodology of analysis applied.

The mean overall kV (\bar{X}), the ratio between the standard deviation and the mean (σ/\bar{X}) and the standard error of the mean or dispersion ($\sigma_{\bar{X}}$), were calculated either for standard samples or for four particles from a polymer blend sample. For standard samples the absolute percent errors ($e\%$) committed in the calculation was estimated as:

$$e\% = \left| \frac{\bar{X} - S}{S} \right| \cdot 100 \quad (1)$$

where S is the known actual area of the standards. These values are summarized in Table 1.

RESULTS AND DISCUSSION

The measured area results obtained for the copper aperture standard as a function of kV using Method I (filled squares) and Method II (open circles) and compared with the actual area (solid line) are shown in Figure 2. The principal statistical parameters for the

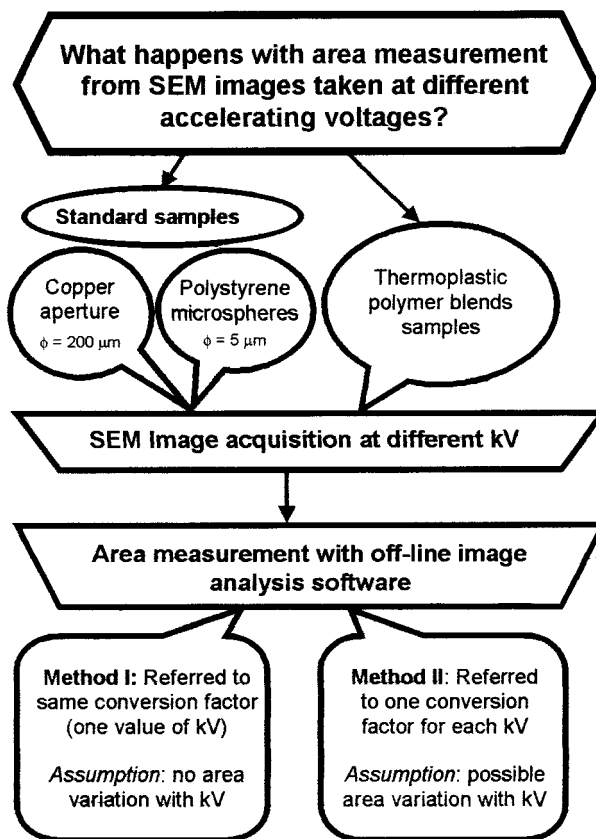


Fig. 1. Flow sheet of the methodology used for this study.

TABLE 1. Principal statistical parameters of all samples measured by Methods I and II

Standards sample	Method	\bar{X} [μm^2]	σ/\bar{X} (%)	$\sigma_{\bar{X}}$ [μm^2]	$e\%$
Copper aperture	I	30134	4.75	585	4.08
	II	31249	0.08	10.6	0.53
Polystyrene spheres	I	20.21	8.21	0.68	2.93
	II	19.55	0.10	$8.1 \cdot 10^{-3}$	0.42
Particles from blend sample	Method	\bar{X} [μm^2]	σ/\bar{X} (%)	$\sigma_{\bar{X}}$ [μm^2]	
A	I	5.17	7.35	0.15	
	II	5.01	4.09	0.10	
B	I	7.42	7.85	0.24	
	II	7.18	2.67	0.08	
C	I	9.87	7.22	0.29	
	II	9.56	2.19	0.08	
D	I	8.42	7.11	0.24	
	II	8.16	1.98	0.07	

distributions using Methods I and II and the absolute errors are summarized in Table 1. When the area variation is not considered (Method I), an absolute error of about 4% in the mean area results. Also, the ratio (σ/\bar{X}) has a similar error and the measurements done with this method have a greater dispersion ($\sigma_{\bar{X}}$). The Method II area measurements are more accurate ($e\% \approx 0.53\%$) and have a narrower spread or dispersion. This last

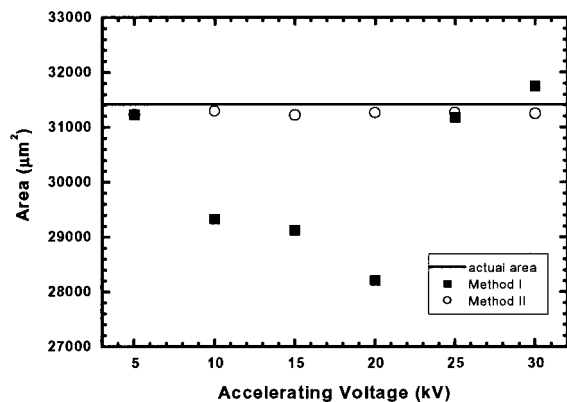


Fig. 2. Area measurements using Method I (■) and using Method II (○) compared with the actual area value (solid line).

observation is evident when comparing the filled square with the open circles in Figure 2. This figure also shows that the error, employing Method I, for mean area evaluation is not so big. However, an error of almost 15% results if a 5 kV-conversion factor is used to measure an image area taken at 20 kV. The results presented above for low-magnification images analysis (300×) indicate that the area measurement is a function of the kV applied.

To analyze kV influence on the area measure at high magnifications, polystyrene microsphere standards were observed at 4,000×. The results in Figure 3 show the polystyrene microspheres area measure vs. kV for both methods compared with the actual area. They show that kV strongly influences the area measurement. The error (standard and absolute), the ratio σ/\bar{X} , and the standard error of the mean for Method I are greater than for Method II. Note that when Method I is used the absolute error of the mean for the measurements of polystyrene microspheres is lower than for the copper aperture. However, the ratio σ/\bar{X} is greater, indicating that the measurements are less accurate. This conclusion is also evident in Figure 3, where an error of about 20% results when a 5 kV-conversion factor is applied to an area measurement at 30 kV. Figures 2 and 3 show that the measurements performed for each kV are randomly distributed around the mean for Methods I and II.

Experiments performed on two different materials, inorganic (Cu aperture) and organic (PS), make it possible to conclude that the area measurement, with OLSIPA, depends on a specific kV conversion factor for each image area. This result does not appear to depend on the material studied. Also, the area measurement kV-influence appears to be independent of magnification.

Using the conclusions from this study, the question to be answered is: What error occurs when the particle area of a polymer blend sample is measured without taking into account the area variation with kV?

The procedure we used is schematized in Figure 1 and was carried out on a polymer blend sample. The images of the blend, taken at 5 and 30 kV, are shown in Figure 4a,b, respectively. The particles measured are identified in the images with letters (A–D). In Figure

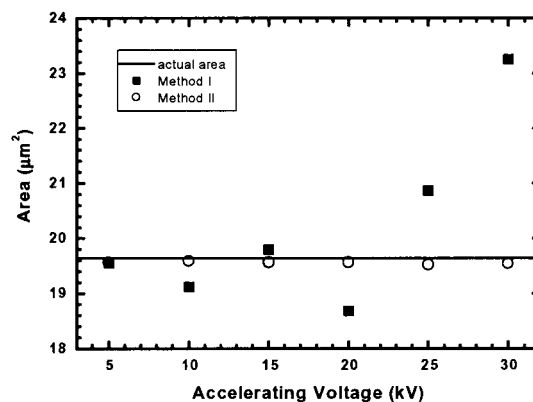


Fig. 3. Area measurements using Method I (■) and using Method II (○) compared with the actual area value (solid line).

4b (30 kV applied), the particles appear to be greater than in Figure 4a (5 kV). Also in Figure 4b, details deeper in the sample can be observed because the topographical contrast is improved. As the accelerating voltage increases, the primary beam penetrates into the specimen more deeply, producing high-resolution secondary electrons, SE1, and a significant number of a low-resolution SE2. The SE2 degrade the surface details of the image.

This contrast effect can also create errors in the particle size measurement using OLSIPAs because edge definition is affected. To avoid the negative influence of edge effect, only well-contrasted particles were analyzed.

Method I was used to measure the area of four particles recorded for all kV in Figure 4 and the results are presented in Figure 5a and compared with the actual particle area (solid line). The particle area measured shows different values for each kV. In Table 1, the dispersion of area measurement for all the particles, is high and the value of the ratio σ/\bar{X} has a large spread. In Figure 5a the particle area measured at different kV shows an irregular variation with respect to the actual area (solid line). At 30 kV this effect for all particles is most evident. The difference between the maximum and minimum area measured can be as high as 20% of the mean for all kV's for a single particle.

Figure 5b shows the area measured for the same particles using Method II. It is evident that the spread or dispersion of area measurements decreases. All area measurements with Method II are close to the actual mean area value (see solid lines in Fig. 5b). The difference between the maximum and minimum area measurement is no more than 4% of the mean.

CONCLUSIONS

Direct observation of liquid nitrogen fractured thermoplastic polymer blends with SEM is a straightforward and rapid method for characterizing polymer blend morphology. The morphological parameters such as size, form, and dispersed phase dimensions and interparticle distance can be evaluated in images using OLSIPA. Polymer scientists routinely use this method with SEM polymer blend images. There are no previ-

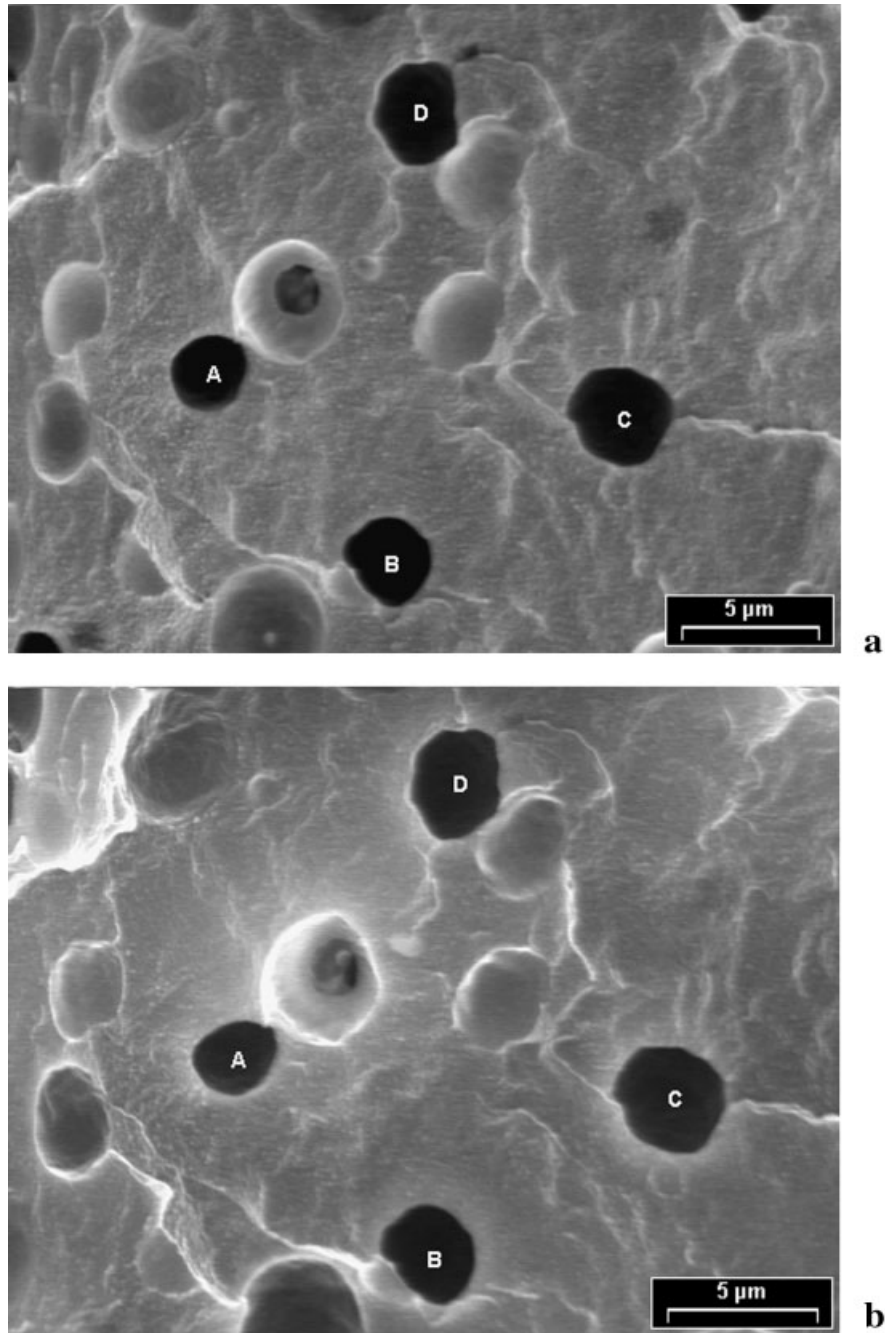


Fig. 4. SEM images of PP/EPR blend (4,000 \times) made at (a) 5 kV and (b) 30 kV.

ous studies in the literature on how the SEM accelerating voltage used in the acquisition of SEM images influence area measurements of polymer blend morphological features using OLSIPA.

The principal conclusions from the systematic study performed on standards and polymer samples are:

- The SEM accelerating voltage influences area measurements. This effect was observed for metallic and polymeric materials and at magnifications of 300 \times and 4,000 \times .
- The magnitude of the error in the area measurement at low magnification is lower ($\sim 15\%$) than at high magnifications (20%) using OLSIPA.
- For the same magnification, the error resulting using Method I is higher than 20% of the mean value, while using Method II it is no more than 4% of the mean.
- The higher the accelerating voltage, the greater the deviation from the actual particle area at high magnification (4,000 \times) for polymer blend samples. As the electron beam energy increases, the primary elec-

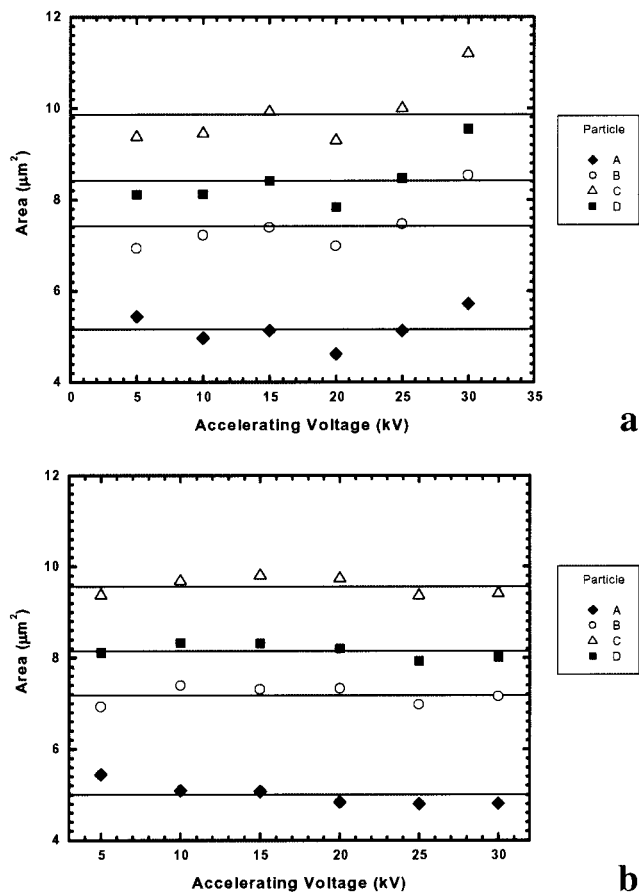


Fig. 5. Area measurement of the particles from Figure 4 by: (a) Method I (b) Method II.

trons penetrate more deeply into the solid, resulting in the production of secondary electrons SE1, as well as a significant number of SE2. The SE2 signal degrades the image and makes particle borders less well defined.

In order to avoid errors in area determinations, the following recommendations are important:

- An accurate calibration of the conversion factors for OLSIPA in x-y directions for each accelerating voltage and magnification used.

- The area measurement must be done with the conversion factor corresponding to the accelerating voltage and magnification used for image acquisition (Method II).
- After an SEM's annual maintenance, the conversion factors of the off-line image analysis software should be reentered using standards at different kVs at least once a year.
- Images of polymer samples should be taken at low accelerating voltages (~ 5 kV) to visualize the desired surface detail.

ACKNOWLEDGMENTS

The authors thank Lic. Viviana Sorrivias of SECEGRIN (CRIBABB - Argentine) for suggestions and useful discussion, and Ing. Francisco Spinella for electronic technical assistance.

REFERENCES

- Chang SL. 1989. Image analysis. In: Roulin-Moloney A, editor. Fractography and failure mechanism of polymers and composites. London: Elsevier. p 145-192
- Chang F, Chou D. 1996. Rubber-toughening of polycarbonate-nylon blends. In: Keith Riew C, Kinloch AJ, editors. Advances in chemistry series 252: toughened plastics II. Washington, DC: American Chemical Society. p 279-290
- Dirlikov S, Frischinger I, Chen Z. 1996. Phase separation of two-phase epoxy thermosets that contain epoxidized triglyceride oils. In: Keith Riew C, Kinloch AJ, editors. Advances in chemistry series 252: toughened plastics II. Washington, DC: American Chemical Society. p 95-109.
- Dumoulin M, Utracki L, Carreau P. 1991. Melt rheology and morphology of linear low density polyethylene/polypropylene blends. In: Utracki L, editor. Two-phase polymer system. Munich: Hanser. p 185-212.
- Geladi P, Esbensen K, Wold S. 1986. Image analysis, chemical information and chemometrics. Chemometrics meeting III CAC, Lerici.
- Goldstein J, Newbury D, Echlin P, Joy D, Romig A, Lyman CH, Fiori CH, Lifshin E. 1992. Scanning electron microscopy and X-ray microanalysis. New York: Plenum Press.
- Hobbs SY, Watkins VH. 2000. Morphology characterization by microscopy techniques. In: Paul DR, Bucknall CB, editors. Polymer blends, vol. 1. New York: John Wiley & Sons. p 239-290
- Martuscelli E. 1995. Structure and properties of polypropylene-elastomer blends. In: Karger-Kocsis J, editor. Polypropylene: structure, blends and composites, vol. 2. London: Chapman & Hall. p 95-140.
- Russ J. 1986. Practical stereology. New York: Plenum Press.
- Russ JC. 1999. The image processing handbook. Boca Raton, FL: CRC Press.
- Sawyer LC, Grubb DT. 1996. Polymer microscopy. 2nd ed. London: Chapman & Hall.
- Sperling LH. 1997. Polymeric multicomponent materials — an introduction. New York: John Wiley & Sons.
- Utracki L. 1990. Polymer alloys and blends — thermodynamics and rheology. Munich: Hanser.
- Wu S. 1982. Polymer interface and adhesion. New York: Marcel Dekker.