ELSEVIER

PII: S0032-3861(96)00962-7

Polymer Vol. 38 No. 15, pp. 3937–3945, 1997

0032-3861/97/$17.00 + 0.00

New method for the characterization of domain morphology of polymer blends using ruthenium tetroxide staining and low voltage scanning electron microscopy (LVSEM)

G. M. Brown* and J. H. Butler
Exxon Chemical Company, Baytown Polymers Center, PO Box 5200, Baytown, TX 77520, USA
(Received 5 May 1996; revised October 1996)

A new method has been developed for the analysis of domain morphology of stained polyolefin blends by low voltage scanning electron microscopy (LVSEM). The component polymers of the blend are differentiated by heavy staining with RuO_4. LVSEM at low accelerating voltages provides high resolution imaging and minimal beam damage to the sample. The method is routinely applied to the analysis of domain morphology in moulded samples, fibres and films, to failure analysis and to the analysis of layer morphology of co-extruded films. © 1997 Elsevier Science Ltd.

(Keywords: low voltage scanning electron microscopy; RuO_4 staining; blend morphology)

INTRODUCTION

Ruthenium tetroxide staining for transmission electron microscopy (TEM) is a well proven technique for characterization of crystalline polyolefins and blends[1–4]. Staining has also been applied to the analysis of polymers by conventional scanning electron microscopy (SEM) and low voltage SEM. Goizueta *et al.*[5,6] stained binary blends of polybutadiene or ethylenepropylene/diene rubber in polypropylene with osmium and ruthenium tetroxides and analysed the domain morphology at ≥ 5 kV in the SEM. Berry[7] used topographical and compositional contrast to image the domain morphology of polymers in LVSEM and Himelfarb and Labat[8] successfully used LVSEM and RuO_4 staining to image domains of hydrogenated polystyrene/butadiene/styrene (H-SBS) blended with nylon. This report describes a new method in which RuO_4 staining and LVSEM imaging are used in the characterization of polyolefin blend morphology, especially blends of polypropylene modified by the addition of elastomers or plastics. The method is well suited to the analysis of domain morphology in moulded parts as well as spun fibres and extruded polymer films, to failure analysis and the analysis of layer morphology in certain co-extruded films.

The key parts of this method are the heavy staining of the sample with RuO_4 which creates compositional contrast between polymer phases, and the characterization of the stained sample in LVSEM. LVSEM provides high resolution imaging over a range of magnifications that spans optical microscopy and TEM. The use of low accelerating voltages improves resolution and minimizes beam damage. The examination of stained samples allows direct determination of the relative orientation of polymer phases and mineral fillers, and for this reason is preferred over solvent extraction or acid etching steps that physically remove components from the blend.

* To whom correspondence should be addressed

EXPERIMENTAL

Materials

The samples used in the development and application of this method were typically blends of isotactic polypropylene (PP) or random copolymers of polypropylene (RCP) modified with one or more elastomeric or plastic modifiers such as ethylene/propylene rubber (EPR), high density polyethylene, linear low density polyethylene and low density polyethylene (HDPE, LLDPE and LDPE) or plastomers, which are semi-crystalline polyolefins with densities below 0.915 g cm^{-3}. Comonomer content, density, crystallinity, molecular weight or other compositional information will not be provided for the wide range of the materials discussed here because detailed descriptions are unnecessary in defining the scope of the method. Fillers and pigments such as talc and carbon black were present in some samples. Analyses were performed on in-house test samples such as compression or injection moulded parts and real-world articles of commerce, including injection moulded parts, extruded films and fibres. The films described here were co-extruded cling films that are commonly used in pallet wrap and food preparation applications. Such films typically are 20–25 μm thick with skin layers of PP

and ethylene methyl acrylate that surround a LLDPE core layer.

Sample preparation

Three sample preparation steps are required prior to the LVSEM analysis and are to be performed in the order shown. (1) A plane or face of the desired orientation is cryogenically cut through the sample using glass knives in the cryomicrotome below the lowest glass transition temperature of the sample. (2) The cryosectioned face is then stained in the vapours of RuO_4 for 2.5 h, the optimal staining duration for blends containing EPR, any of the polyethylenes or plastomers. The staining solution is prepared *in situ* by a modification of the method of Montezinos *et al.*[2]. (3) To prepare a smooth flat surface for analysis by LVSEM, the stained sample is sectioned (or microtomed) to an optimal depth of roughly 0.5 μm. This is achieved by cutting 100 nm thick sections from the stained face at ambient temperature using a diamond knife. During sectioning on the diamond knife, the sections are floated on to the surface of the water in the boat and may be collected for analysis by TEM[9]. The stained face produced by ambient temperature sectioning is mounted and examined by LVSEM. Vacuum evaporation or sputter coating of the specimen with heavy metals or carbon is not recommended. Details pertaining to sample preparation and the preparation and use of the stain are described in the Appendix.

Instrumentation and operating conditions

All microtomy was performed using the Reichert Ultracut E ultramicrotome equipped with the FC-4D cryochamber for cryomicrotomy. Glass knives were used in cryomicrotomy whereas diamond knives equipped with boats were employed in ambient temperature ultramicrotomy. All samples were analysed using the Hitachi S-900 low voltage scanning electron microscope, typically at accelerating voltages of 0.8–1.2 kV; stained thin sections were analysed at 4 kV. Operational parameters were as follows: condenser lens settings = 10–12 (nominal), 300 μm condenser aperture, 30 μm objective aperture and 0° sample tilt. TEM analyses were performed on the Philips EM-300 TEM at 80–100 kV.

RESULTS

This method has been applied to a variety of blends prepared by different processing conditions. Typical examples are given to illustrate the strengths and limitations of the method. One should understand that the contrast that is commonly observed in TEM images of stained polymers is inverted in LVSEM images. In the SEM and LVSEM, RuO_4 stained polymers appear bright and poorly stained polymers appear dark[10].

Blend morphology

For years the solvent extraction method for SEM has been applied to the analysis of the domain morphology of EPR/PP blends. In that method, the blend sample is cryofaced and the soluble rubber phase is removed, leaving voids that represent the rubber domains in the PP matrix[11]. The method is generally restricted to blends containing a soluble rubber phase within an insoluble polymer matrix and generally fails to produce contrast between domains in blends where one component cannot be removed by solvent extraction at room temperature (i.e. LLDPE/PP blends). Limitations of the extraction method include the collapse of laminar morphologies during extraction and the inability to image sub-inclusions in the rubber domains. By comparison, the RuO_4 staining/LVSEM method directly images both the continuous and discrete phases, and sub-inclusions and

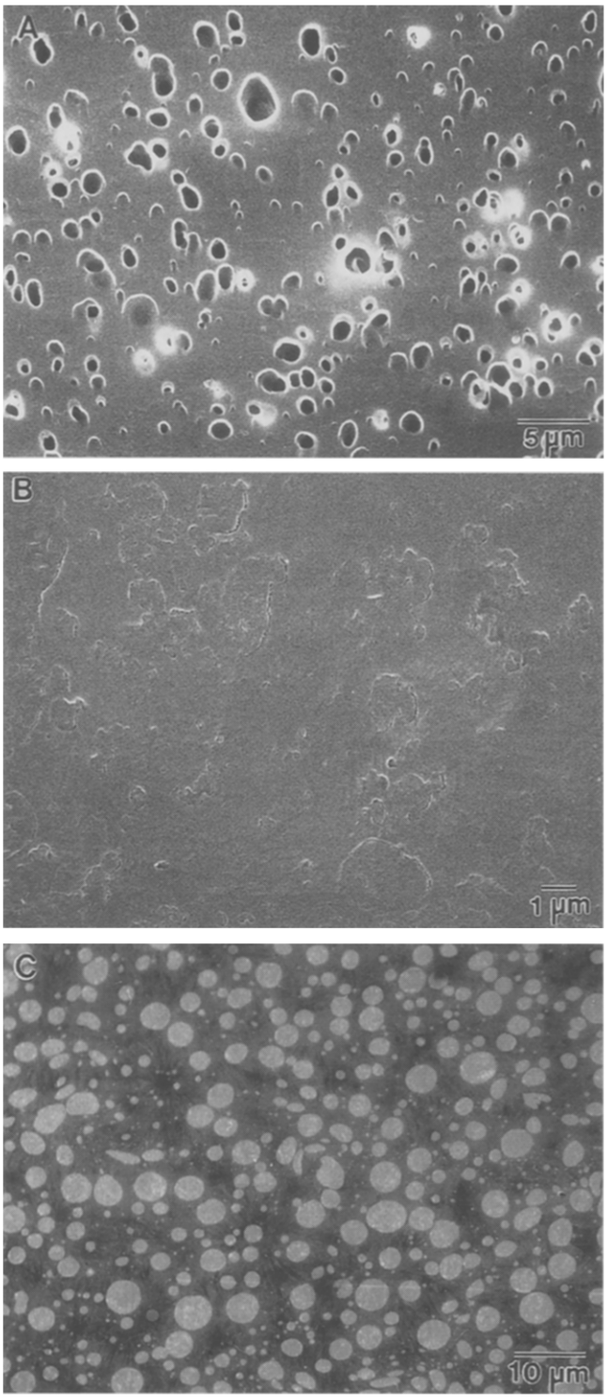

Figure 1 Comparison of solvent extraction/SEM and RuO_4 staining/LVSEM. (A) Xylene extraction removes EPR leaving voids in matrix of EPR/PP blend (SEM, 2kX, 20 kV). (B) Xylene extraction incompletely defines domain interfaces in LLDPE/PP blend (SEM, 5kX, 20 kV). (C) RuO_4 staining creates high contrast between phases in LLDPE/PP blend; LLDPE domains are bright and PP matrix is dark (LVSEM, 1kX, 1.0 kV)

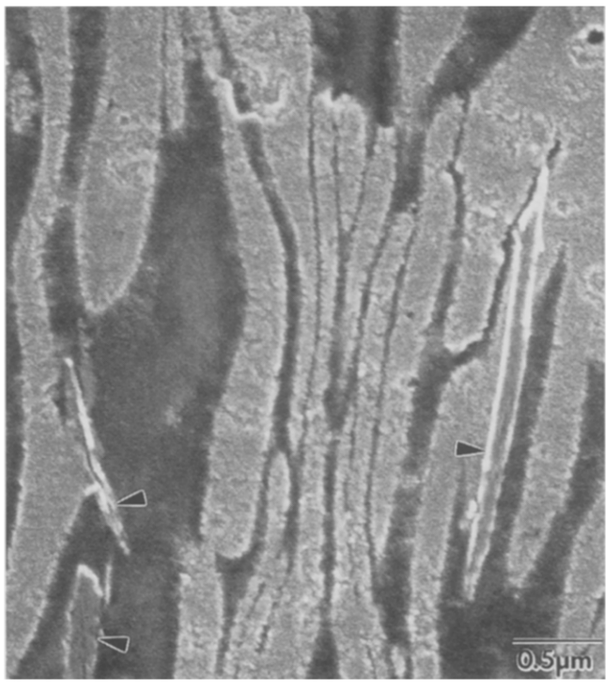

Figure 2 RuO_4 staining/LVSEM of a plastomer/RCP blend containing talc. Plastomer domains are contrasted against darker RCP matrix. Talc particles (at arrows) have bright borders (25kX, 1.2 kV)

mineral fillers as well. *Figure 1* compares the solvent extraction/SEM and RuO_4 staining/LVSEM methods for blends of EPR/PP and LLDPE/PP. Although EPR/PP blends may be addressed by either method, only the RuO_4/LVSEM method definitively images the domain morphologies in the LLDPE/PP blend.

This staining method is applicable to characterization of the blends containing a variety of polyolefin species. *Figure 2* shows the domain morphology of an injection moulded blend containing a plastomer, PP and talc. The high resolution of LVSEM and the high contrast produced by heavy staining allow differentiation of the well stained plastomer domains (bright) from the poorly stained PP matrix (dark) and the talc particles (at arrows). In spite of their relatively high secondary electron emission, talc particles are easily recognized by their texture following microtomy and by the bright halo that typically borders each particle. This halo represents negative charging of the talc particle relative to the surrounding stained polymer.

A strength of the LVSEM is the capability of high resolution analysis over relatively large areas of a sample. *Figure 3* shows the near-surface and the central region of an injection moulded ternary blend consisting of an RCP matrix and an elastomer phase containing the EPR, plastomer and HDPE (our unpublished observations indicate that these polymers typically reside within a single elastomer phase in such blends). The elastomer domains near the centre of the sample are well defined and only somewhat oriented. By comparison, the outer 15–20 μm of the surface consists of a laminar arrangement of highly oriented elastomer and RCP. Most elastomer domains are less than 100 nm thick and are often separated by even thinner layers of RCP.

Figure 4 compares LVSEM and TEM images from a blend of LLDPE/EPR/PP. In the LVSEM micrograph,

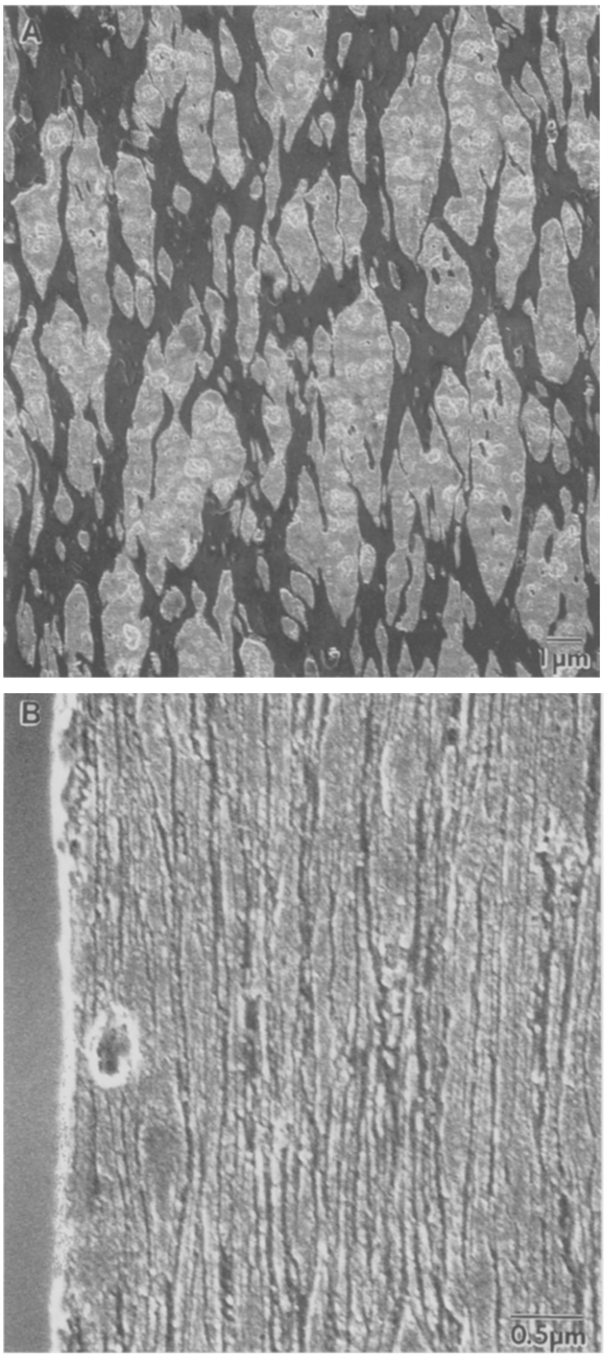

Figure 3 RuO_4 staining/LVSEM of a ternary blend with elastomer domains (containing EPR/plastomer/HDPE) in RCP matrix: (A) domain morphology near the centre of the moulded sample (5kX, 1.0 kV); (B) laminar morphology at the surface of sample (20kX, 1.0 kV)

elongated elastomeric domains are seen throughout the PP matrix. TEM shows thin LLDPE lamellae and amorphous polymer of the elastomer domains, and the PP lamellae of the matrix. PP sub-inclusions within elastomer domains are seen both by LVSEM and TEM. The lamellar morphologies of these ruthenium stained samples are consistent with those described by Sano and others[1–3]. Such side-by-side comparisons of TEM and LVSEM support interpretation of LVSEM data and illustrate the power of these complementary methods in

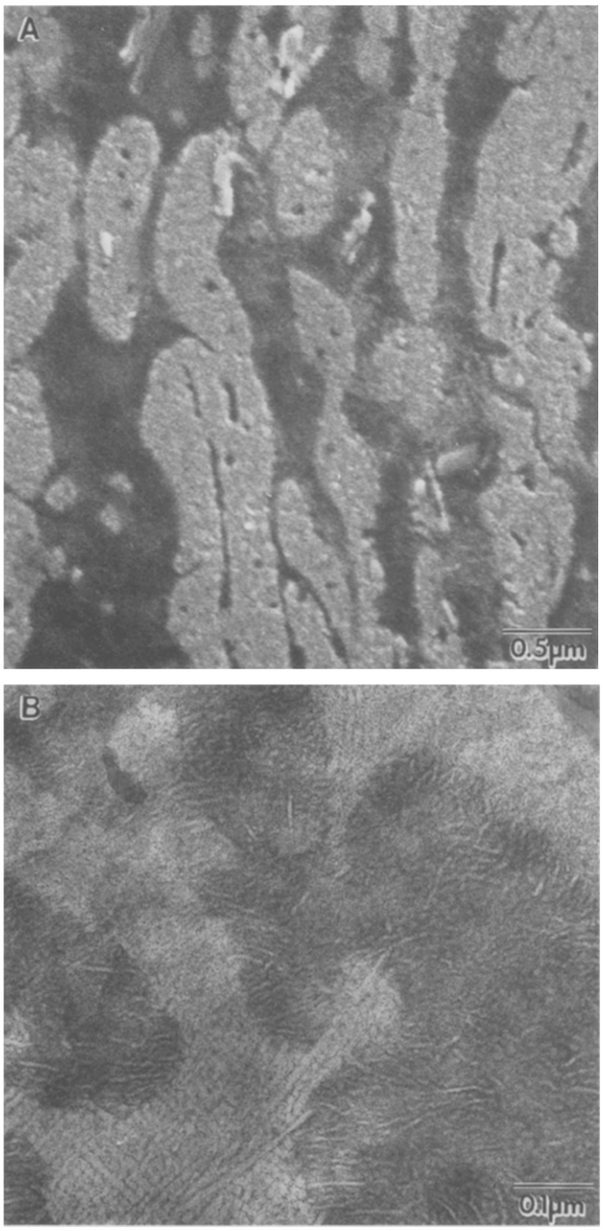

Figure 4 Comparison of LVSEM and TEM morphology of RuO_4 stained LLDPE/EPR/PP blend containing talc: (A) LVSEM shows elastomer domains (LLDPE/EPR) and PP sub-inclusions (25kX, 1.2 kV); (B) TEM confirms domain morphology and images crystalline lamellae in elastomer and PP matrix. Note cross-hatched lamellae in PP matrix and sub-inclusions (110kX, 80 kV)

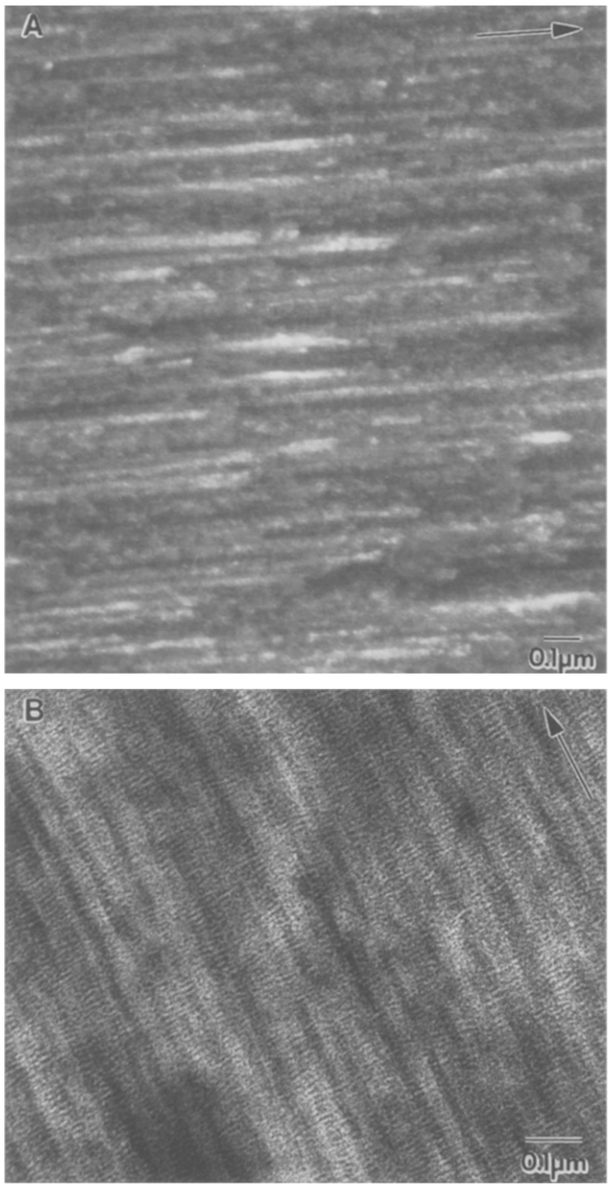

Figure 5 Blend morphology in a polybutene-1/PP blend containing a minor amount of LLDPE, stained with RuO_4 for LVSEM and TEM. (A) Polybutene-1 (and undifferentiated LLDPE) domains are well defined by LVSEM (50kX, 1.0 kV). (B) By TEM, polybutene-1 domains appear as dark streaks perpendicularly oriented to stacked PP lamellae of the matrix (80kX, 80 kV). Arrows indicate the fibre axis

the characterization of polymer blends. The LVSEM method accurately characterizes the domain morphology of the blends whereas TEM provides the lamellar morphology of the matrix and the elastomeric domains that facilitates more complete understanding of the blend.

Figure 5 shows the domain morphology of spun bond fibres made from a blend of PP, polybutene-1 and a small amount of an ethylene-rich compatibilizer. LVSEM of the RuO_4 stained blend provides sharp contrast between the PP matrix and the 15–40 nm thick polybutene-1 domains that are oriented parallel to the fibre axis. Further examination of these fibres by TEM confirms this morphology and complements it with the crystalline morphology of the fibre.

Failure analysis

The capability of LVSEM to differentiate domain morphology, especially at the surface of delaminated samples, is useful when the assignment of phase composition is essential to the determination of failure as cohesive or adhesive. TEM analyses of such failed samples may be compromised if the region where failure occurs is obscured by tearing, wrinkling or folding of the sections. Such problems are avoided in LVSEM by analysing the sectioned faces, which do not fold or wrinkle.

Ruthenium tetroxide staining and LVSEM are being

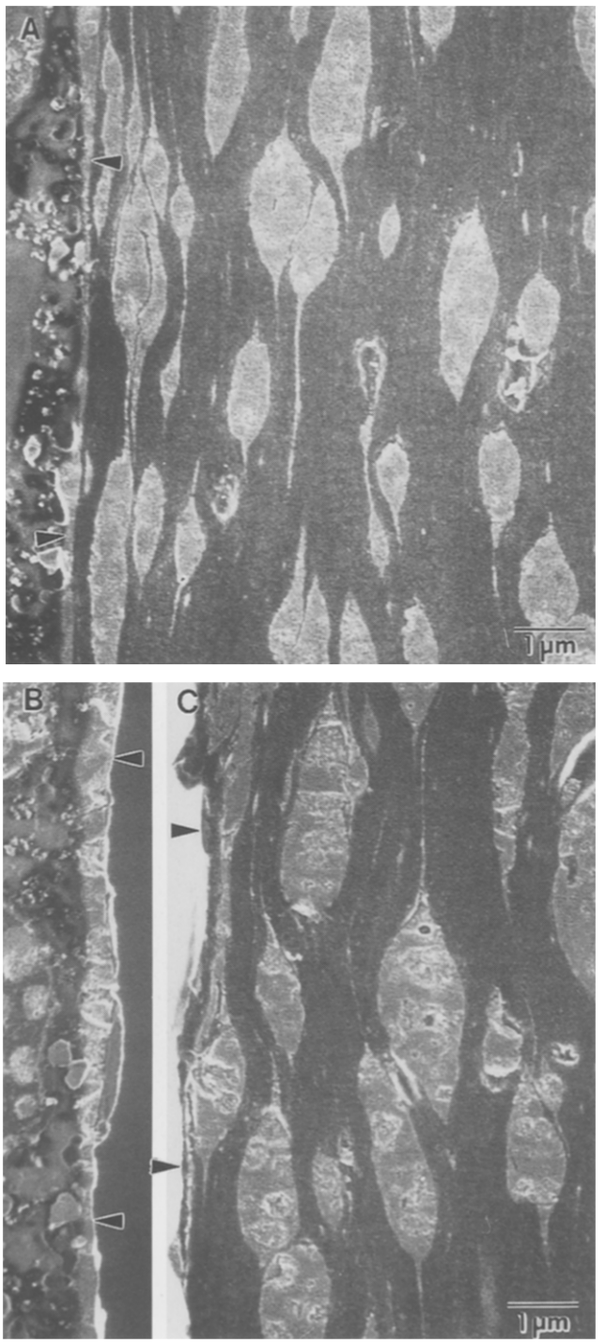

Figure 6 Analysis of failure in painted, injection moulded EPR/PP blend stained with RuO_4. (A) Near-surface region of intact sample. Note the thin layer of EPR (at arrows) at interface of blend and paint layer. (B) and (C) show complementary halves of the failed sample. (B) Paint and attached EPR layer (at arrows); (C) blend side with exposed PP surface (at arrows). Failure occurs within blend at interface of the EPR layer and adjacent PP matrix (LVSEM, 10kX, 1.1 kV)

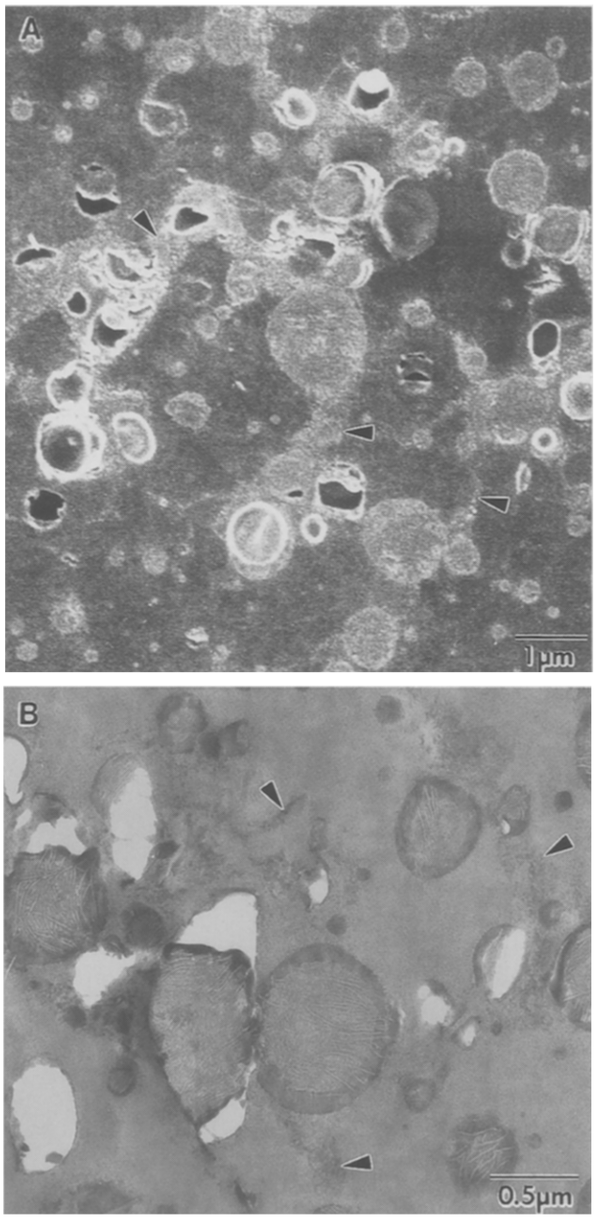

Figure 7 Failure occurring within a RuO_4 stained, compression moulded blend of EPR/HDPE/PP following impact testing: (A) LVSEM (10kX, 1.1 kV) and (B) TEM (25kX, 100 kV) show crazes (at arrows) and cavitated elastomer domains (voids)

used in the analysis of failure of painted blends used for automotive applications. *Figure 6* shows the morphology of a painted injection moulded sample comprised of PP and EPR. The painted control sample is characterized by a thin layer of EPR (at arrows) at the interface of the polymer blend and the paint. Analysis of opposing halves of a failed sample in which the paint appeared to delaminate from the moulded sample indicates that failure occurs at the interface between this thin EPR layer and the underlying PP matrix of the blend. Other types of failure analysis may be performed using this method. *Figure 7* shows both crazing and cavitation in a compression moulded blend containing EPR/HDPE domains in a PP matrix. Cavitation, or collapse, of the elastomer domains is obvious in both LVSEM and TEM micrographs. Crazes meander through the PP matrix, often intersecting with the elastomer domains. The increase in stain uptake by the crazes is explained by their high porosity.

Co-extruded film morphology

Designers of multilayer films combine layers of various polymers to achieve the specific physical properties needed for a given application. The polymers selected for skin layers of cling films determine the surface

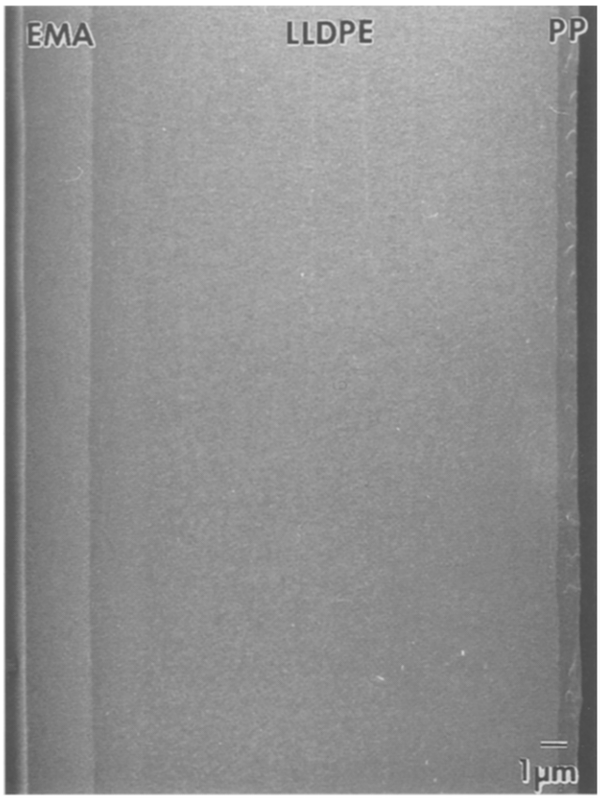

Figure 8 LVSEM of RuO_4 stained 3-layer co-extruded cling film. EMA and PP skin layers are well differentiated from the LLDPE core (3kX, 0.9 kV)

properties of the films whereas polymers used in the core layer are chosen for their bulk mechanical properties. *Figure 8* shows the construction of a co-extruded cling film consisting of PP and EMA skin layers surrounding an LLDPE core layer. The layers are differentiated from one another by their stain uptake. Note that the bright surface next to the EMA layer is the original surface of the film.

The determination of layer thickness and morphology of co-extruded films by this method offers a useful addition to the film characterization capabilities of commercial and industrial microscopy labs. Several types of commercially available co-extruded films (stretch and cling films are among the most common) often possess $\leq 1\,\mu$m thick skin layers that pose considerable analytical problems for the light microscopist. Such films are easily analysed and the layer thicknesses measured using staining and LVSEM. The method is limited by its inability to differentiate layers containing similarly staining polymers or provide information on the composition of individual layers. Such compositional analyses typically are obtained from hot stage optical microscopy or *FT*i.r. spectroscopy.

Morphology of crystalline lamellae

Since lamellae in most semi-crystalline polymers are sufficiently thick to be resolved by LVSEM, the challenge in imaging the crystalline morphology of ruthenium stained polymers lies only in resolving the ruthenium-rich zones that form at the basal surfaces of the lamellae. *Figure 9* compares LVSEM and TEM images of lamellae in a ~700 Å thick section of RuO_4 stained HDPE. In

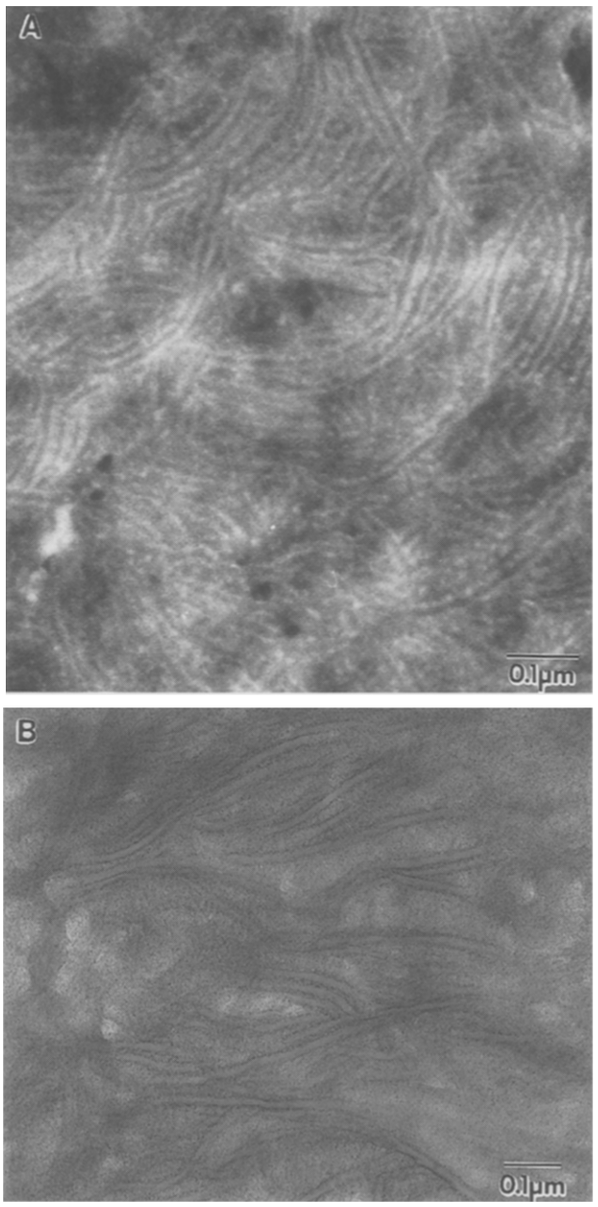

Figure 9 LVSEM and TEM images from a microtomed section of RuO_4 stained HDPE: (A) ruthenium-rich zones on basal surfaces of lamellae are imaged as bright lines in LVSEM (100kX, 4 kV); (B) the TEM image is characteristic of RuO_4 stained lamellae (80kX, 80 kV)

TEM, these ruthenium-rich zones on the basal surfaces of the lamellae appear dark[3] giving each lamella a 'railroad track' appearance. The LVSEM image of the same section (at 4 kV accelerating voltage) shows the same morphology, but with inverted contrast.

DISCUSSION

The keys to the success of this method are the heavy staining of the sample with RuO_4 tetroxide vapours and the superb imaging capabilities of LVSEM. Heavy staining embrittles the polymer, allowing ultramicrotomy at ambient temperature without ductile deformation, and produces strong contrast between differently stained phases of the blend when analysed at low voltage by LVSEM.

The differential staining of blend phases depends on the reactivity of the polymer functionalities to RuO_4 and the diffusion of RuO_4 within the sample. To achieve high contrast between phases, polyolefin blends must be heavily stained to compensate for their relatively poor reactivity to RuO_4[1]. Our experience indicates that diffusion of the stain to useful depths is dependent on the composition and crystallinity of the component polymers. Most elastomers, lower crystallinity polyolefins and copolymers that we have studied (i.e. ethylene/propylene rubbers and plastomers, LLDPE and LDPE and ethylene vinyl acetate copolymers) stain more heavily and to a greater depth than higher crystallinity polymers such as HDPE and PP.

When staining duration is optimized for a blend, the sample can be sectioned at ambient temperature without deformation, and effective differential staining of the polymer domains of the blend is achieved. In the example of an EPR/PP blend, EPR domains are highly embrittled and section without any deformation whereas the relatively poorly stained PP matrix may exhibit some ductile deformation during sectioning. The secondary electron signal across the smooth ambient sectioned face represents variations in the concentration of stain as a function of the chemical functionality and density of the component polymers of the blend. In the previous example, the high concentration of ruthenium atoms in the heavily stained EPR domains results in high secondary electron emission causing them to appear bright in LVSEM micrographs. The more poorly stained PP matrix appears darker because of its lower secondary electron emission. This contrast is reversed in amplitude bright field TEM images where stained polymers appear dark as the result of elastic scattering of electrons by the heavy atoms of the stain.

Low voltage–high resolution SEMs offer dramatic improvements in image quality and resolution relative to conventional SEMs. These improvements arise from the use of field emission guns (FEGs), immersion lenses (in which the sample is located inside the objective lens) and operation at low beam voltages. FEGs increase signal and resolution at low voltages by producing smaller probes with higher brightness and narrower energy spread than can be achieved using thermionic tungsten or LaB_6 sources. Immersion lenses improve the quality of the probe by decreasing chromatic and spherical aberrations as a function of the shortened focal length[12]. The positioning of the secondary electron detector above the objective lens of the LVSEM dramatically increases the detection efficiency for secondary electrons and improves resolution by minimizing the collection of SE III electrons[12,13]. The use of low accelerating voltages improves spatial and depth resolution by decreasing the dimensions of the interaction volume of the electron beam in the sample. In addition, beam induced sample damage is decreased at beam energies less than 2 kV[14,15].

Images of stained polymer blends with 3–4 nm resolution are routinely made at 1.2 kV over a range of magnification that encompasses both optical microscopy and TEM. Low voltage SEMs allow one to determine the accelerating voltages that provide best contrast without electrostatic charging or significant sample damage[14,15]. This imaging capability is achieved through the use of high brightness FEG, low voltage power supplies and electron optics that produce the small probe diameters and operational stability needed for high resolution imaging at accelerating voltages as low as 500 eV. Conventional SEMs, which are not optimized for work below 5 kV, are not suitably equipped for high resolution imaging at low accelerating voltages.

Our observations indicate that staining with RuO_4 increases the secondary electron yield, δ, of polymers, and that the secondary electron emission of the stained polymers is dependent on their ruthenium concentration. Empirical data compiled by Joy[16] shows that δ at 1 kV for several period 5 transition metals is only slightly higher than for polyethylene, suggesting that δ does not increase significantly with atomic number (although ruthenium metal was not included in this study the trend should still apply)[16]. However, the increase in δ of a polymer sample following staining might result from changes in the bonding environment of the ruthenium atoms, similar to the difference in δ observed for MgO relative to elemental magnesium[10,16]. Furthermore, ruthenium stained polymers are heterogeneous, consisting of ruthenium atoms embedded in the carbonaceous matrix of the unstained portions of polymer molecules. The lower inelastic scattering of secondary electrons by this matrix should enhance their escape from the surface of the stained sample[10].

Another parameter affecting image formation is $E2$, which is the voltage at which dynamic charge balance is achieved between the incident beam electrons and the secondary and back-scattered electrons emitted from the sample. $E2$, where $\delta = 1$, is the accelerating voltage at which the best contrast and image quality are seen in LVSEM[14,15]. Assuming that a sample is not charging[17], a region of the sample that is brighter than its surroundings at a given accelerating voltage, E_0, has $\delta > 1$ and $E_0 < E2$. Our experience shows that the optimal accelerating voltages for the examination of stained and unstained polymers are approximately equal, but the range of useful accelerating voltages for the analysis of heavily stained polymers is somewhat increased. This suggests that the $E2$ of the stained portions of the molecules has shifted upward as a result of its increased δ and that the $E2$ of the unstained portions of the molecules is unchanged. Staining, therefore, broadens the useful range of accelerating voltages and improves the ease and versatility of the analysis.

The primary limitations of this method are the difficulties of differentiating similarly stained polymers and imaging polymer lamellae. Compositional information on the samples is based on stain uptake. Therefore, polymers that differ in their lamellar morphology, but have similar stain uptake, such as EPR and LDPE, may be difficult to distinguish solely by their secondary electron signals. Problems that occasionally occur in the interpretation of stain contrast may be resolved in the light of data from TEM and other techniques. Once developed, this improved understanding of the contrast and morphology seen in an LVSEM analysis of a particular type of sample is often applicable to other problems.

CONCLUSIONS

A method for the LVSEM analysis of heavily stained polyolefin blends has been developed that provides more information on the domain morphology and the relationship of phases and fillers in blends than any other method except TEM. This method is preferred

over TEM for the analysis of the domain morphology of blends. The LVSEM method is faster than TEM and the excellent compositional contrast arising from differential staining with RuO_4, coupled with the high resolution and the ease of use of the LVSEM, make this method applicable to many problems encountered in commercial and industrial laboratories. In addition to the analysis of domain morphology in moulded samples, fibres and films, the method is useful in failure analysis and the characterization of layer morphology of co-extruded films.

ACKNOWLEDGEMENT

The authors thank Dr Hironari Sano of Mitsubishi Chemical Corporation for invaluable discussions and suggestions pertaining to the RuO_4 staining of polymers for TEM.

REFERENCES

1. Trent, J. S., Scheinbeim, J. I. and Couchman, P. R., *Macromolecules*, 1983, **16**, 589.
2. Montezinos, D., Wells, B. G. and Burns, J. L., *J. Polym. Sci.: Polym. Lett. Ed.*, 1985, **23**, 421.
3. Sano, H., Usami, T. and Nakagawa, H., *Polymer*, 1986, **27**, 1497.
4. Sawyer, L. C. and Grubb, D. T., *Polymer Microscopy*. Chapman and Hall, New York, 1987, p. 104.
5. Goizueta, G., Chiba, T. and Inoue, T., *Polymer*, 1992, **33**, 886.
6. Goizueta, G., Chiba, T. and Inoue, T., *Polymer*, 1993, **34**, 253.
7. Berry, V. K., *Scanning*, 1988, **10**, 19.
8. Himelfarb, P. B. and Labat, K. B., *Scanning*, 1990, **12**, 148.
9. Hayat, M. A., *Principles and Techniques of Electron Microscopy. Biological Applications*, Vol. 1. Van Nostrand Reinhold, New York, 1970, p. 183.
10. Goldstein, J. I., Newbury, D. E., Echlin, P., Joy, D. C., Fiori, C. and Lifshin, E., *Scanning Electron Microscopy and X-Ray Microanalysis*. Plenum Press, New York, 1981, p. 518.
11. Stehling, F. C., Huff, T., Speed, C. S. and Wissler, G., *J. Appl. Polym. Sci.*, 1981, **26**, 2693.
12. Joy, D. C., *Ultramicroscopy*, 1991, **37**, 216.
13. Autrata, R., *Electron Microsc. Soc. Am. Bull.*, 1992, **22**, 54.
14. Butler, J. H., Joy, D. C., Bradley, G. F., Krause, S. J. and Brown, G. M., *Microscopy: The Key Research Tool*. EMSA, 1992, p. 103.
15. Butler, J. H., Joy, D. C., Bradley, G. F. and Krause, S. J., *Polymer*, 1995, **36**, 1781.
16. Joy, D. C. (ed.), *A Data-Base of Electron–Solid Interactions*. University of Tennessee, 1995.
17. Joy, D. C. and Joy, C. S., *J. Microsc. Soc. Am.*, 1995, **1**, 109.

APPENDIX

Details not given in the text regarding the preparation and use of the RuO_4 staining solution, and other practical aspects of sample preparation, are described here.

Sample preparation

The sample preparation described in the Experimental section of the text applies to moulded samples. Polymer films and fibres must be handled somewhat differently. Most fibres and some polymer films must be embedded in an epoxy or other medium prior to sample preparation and analysis. The embedding resin should not anneal, melt or dissolve the sample. We use BIPAX Tra-Bond BB-2115 (Tra-Con, Inc.), an epoxy resin that cures at ambient temperature with low heat of curing. The embedded fibres or film are cryofaced in the appropriate orientation, then stained and sectioned as described above.

Some films may be cryofaced, stained and ambient sectioned without prior embedment in a mounting resin. These stained films are typically friable and difficult to section without breaking. In our laboratory, the stained film is carefully inserted into the ultramicrotome chuck between 1 mm thick polyethylene sheets with the stained face of the film protruding for sectioning.

Preparation and use of ruthenium tetroxide stain

The RuO_4 staining solution is prepared *in situ* using a modification of the Montezinos method[2]. Sodium hypochlorite solution (1 ml of 10 w/v%) is added to 0.02 g of $RuCl_3.3H_2O$ in a 5 ml glass vial, mixed with a Pasteur pipette, and immediately capped. This reddish-brown solution can be used immediately, but is stable for many hours when kept sealed. Samples are stained in the RuO_4 vapours above the staining solution by fastening the sample to the inside of the vial cap with adhesive tape. Following staining, the specimen should de-gas in the fume hood for several hours prior to ultramicrotomy. After staining is completed, the waste RuO_4 solution is reduced with aqueous $NaHSO_3$ (10% w/v) and properly disposed. Personal exposure to the hazardous vapours of RuO_4 should be prevented by keeping the stain in a fume hood and wearing safety glasses and vinyl gloves when working with the stain.

Reproducibility of staining is accomplished by keeping constant the size of the vial, the volume of stain and the duration of staining. Staining duration varies according to the type of analysis: 2.5 h for LVSEM of polyolefin blends; 2.5 h for TEM of the lamellar morphologies of polyethylenes, semi-crystalline elastomers and plastomers; and 7 h for TEM of the lamellar morphology of PPs.

Rigorous attention to the quality of the NaOCl solution ensures predictable staining and, in our laboratory, has eliminated concerns pertaining to the quality of the staining solution. The use of commercially available NaOCl from a reputable supplier (Mallinckrodt) is essential to the preparation of staining solutions of known potency; NaOCl solutions that have lost free chlorine by diffusion from solution cannot dependably produce potent RuO_4 staining solutions (unpublished observations). In our laboratory, we ensure the potency of the RuO_4 solution by refrigerating the solution and replacing it with a new bottle each month. Household bleaches (i.e. Clorox®) are not recommended as a source of NaOCl because the age and potency of these products are generally unknown. Our experience supports Montezinos' recommendation that pre-packaged ampoules of aqueous RuO_4 (0.5%) should not be used because the concentration of RuO_4 is inadequate for this application and because the potency of these solutions tends to vary[2].

Ultramicrotomy

Ultramicrotomy of the stained sample is the final major sample preparation step. The purpose of sectioning is to remove the overstained skin from the surface of the sample, thus exposing the underlying differentially stained polymer. This skin is typically less than 100 nm thick and so heavily stained that it possesses no differential contrast. Usually only a half-dozen sections need to be cut to expose a uniformly stained sample face; no advantage is gained by unnecessary sectioning. The

use of a water flotation bath on the diamond knife is essential to produce the extremely smooth stained surface needed for LVSEM analysis. Sectioning with a dry diamond knife at ambient or cryogenic temperatures typically causes extensive microfracture of the sample during sectioning. Although biological grade diamond knives are ideal for both LVSEM and TEM, the less expensive histoknives marketed by most diamond knife manufacturers perform very well in the LVSEM application. After ambient facing, the stained sample is mounted for examination in the LVSEM, using a structurally strong mounting material (we use Leit C, from Neubauer Chemikalien, which is available from most electron microscopy supply houses) to hold the specimen in place and prevent specimen shifting during analysis.