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Ruthenium Tetraoxide Staining of Polymers for Electron Microscopy

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ABSTRACT: The need for selective staining agents to enhance electron density contrast for transmission electron microscope (TEM) studies of heterogeneous polymer systems is widely acknowledged. In this study, we describe the use of ruthenium tetraoxide as a staining agent for thin films of various polymers for examination in the TEM. Ruthenium tetraoxide is shown to stain both saturated and unsaturated polymer systems that contain in their unit structure ether, alcohol, aromatic, or amide moieties. High-density polyethylene, linear polyethylene wax, poly(vinyl methyl ketone), and both isotactic and atactic polypropylene were also stained. Ruthenium tetraoxide did not stain poly(methyl methacrylate), poly(vinyl chloride), poly(vinylidene fluoride), and polyacrylonitrile. Optical and electron micrographs demonstrating the utility of this staining agent are presented and the action of RuO_4 on the stained polymers is discussed in terms of previously reported RuO_4–small molecule interactions.

1. Introduction

1.1. General. It is well-known that the micromorphology of polymeric materials is dependent on their heat treatment, composition, and processing and that in turn mechanical properties of these materials such as toughness, impact strength, resilience, fatigue, and fracture strength can be highly sensitive to morphology. Consequently, an explanation of these (and other) mechanical properties should include the nature of relations between heat treatment, composition, and consequent physical microstructure. The transmission electron microscope (TEM) is an established instrument for the characterization of the structure of heterogeneous polymer systems at a high level of resolution. However, it is often necessary to enhance image contrast for polymers by use of a staining agent. Although osmium tetraoxide is useful for unsaturated polymers, a suitable image-contrast enhancing stain for saturated polymers has been lacking. This situation has been improved somewhat by independent reports[1,2] of the discovery of ruthenium tetraoxide (RuO_4) as an effective staining agent for the TEM examination of morphology in both saturated and unsaturated polymeric systems. Vitali and Montani[1] observed improved image contrast for polybutadiene lattices, a terpolymer of acrylonitrile, butadiene, and styrene (ABS), and an acrylonitrile–styrene–acrylonitrile (ASA) polymer. Trent, Scheinbeim, and Couchman[2] demonstrated the usefulness of RuO_4 vapor staining in TEM studies of polystyrene/poly(methyl methacrylate) (PS/PMMA) blends and high-impact polystyrene (HIPS) films, due in both cases to a preferential action of the stain on the PS component.

The purpose of the present study is to determine which types of polymers are stained effectively by RuO_4 for examination in the TEM. Polymer films containing aromatic, ether, alcohol, amide, olefin, ester, nitrile, sulfone, halogen, carbonyl, or unsaturated moieties were exposed to RuO_4 vapor for various times; optical and electron micrographs of the results are presented and the interaction of the staining agent with each polymer is discussed.

1.2. Ruthenium Tetraoxide. Ruthenium, discovered in 1826,[3] is a rare metal[4] in the platinum group and is known to exist in ten different oxidation states (−2, 0, 1, 2, 3, 4, 5, 6, 7, 8),[5,6] of which ruthenium tetraoxide occurs in the 8th state. Although ruthenium tetraoxide was first prepared in 1860,[7] it was not used as an oxidant for organic compounds until 1953.[8] Typically, it is prepared by the oxidation of ruthenium compounds of lower oxidation states (usually ruthenium trichloride, ruthenate ion, or hydrated ruthenium dioxide).[9] Hydrated ruthenium dioxide ($RuO_2{\cdot}2H_2O$)[10,11] has been suggested as the most convenient starting material for the preparation of RuO_4 by a reaction with an excess of sodium periodate ($NaIO_4$) in water, followed by extraction of the tetraoxide with carbon tetrachloride.

Ruthenium tetraoxide is a far more vigorous oxidant than OsO_4;[3,10] many organic compounds that are inert to oxidation by OsO_4 react readily with RuO_4.[8-10,12,13] This highly reactive oxidant was first used as a fixative and stain for electron microscopy by Gaylarde and Sarkany.[14] When

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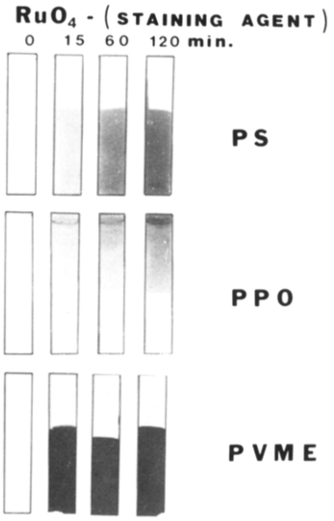


Figure 1. Optical micrographs of PS, PPO, and PVME films exposed to RuO_4 vapor for various times (min). All films in Figures 1–5 were stained in glass slides, which are outlined in black.

RuO_4 was used instead of OsO_4, excellent staining of the cytoplasmic and nuclear membranes of rat liver and kidney was observed. Some of the polar lipids that showed no reaction with OsO_4 reacted strongly with RuO_4 and, in addition, the tetraoxide reacted strongly with proteins, glycogen, and the common monosaccharides, slightly with mucopolysaccharides, and negligibly with the basement membranes of some tissues. Ruthenium tetraoxide also attacks a variety of other functional groups.[9,13]

1.3. Toxicity of Ruthenium Tetraoxide. Although ruthenium tetraoxide appears to be somewhat less toxic than osmium tetraoxide, the biological and physiological properties of the former have not been studied extensively. Inhalation should be avoided since RuO_4 oxidizes tissue readily, leaving a deposit of ruthenium dioxide (RuO_2).[15,16] It is also a powerful irritant to both the eyes and respiratory system[17] and should be used with this in mind.

2. Experimental Procedure

Clean glass slides (50 mm × 10 mm) were dipped in 2% or 0.75% solutions (by weight) of polymer, the former for Figures 1–12 and the latter for Figures 13–16, and the solvent was subsequently evaporated under vacuum at 50 °C for 24 h. The films in Figures 6–16 were removed from the glass substrate by immersing the slides in distilled water and then lifting the floating film from the water surface onto copper microscope grids. The high-density polyethylene films in Figures 13–15 were supported by carbon-film covered grids and annealed at 100 °C for an additional 2 h.

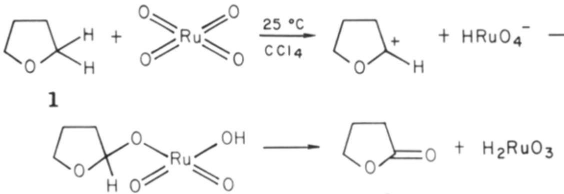
A 0.5% (by weight) solution of RuO_4 in distilled deionized water was used for staining. The aqueous solution, golden yellow when fresh, was found to be effective for a considerable time (up to 6 months) if kept between uses in a firmly sealed glass container in a freezer. Otherwise, the tetraoxide reduced to more stable (insoluble) forms within hours, the liquid becoming colorless. The ruthenium tetraoxide used was purchased from Alfa Products (Thiokol/Ventron Division) in crystal form.

Film-covered slides and grids were vapor stained in a glass-covered dish.[2] Optical micrographs were taken to examine the intensity of staining. These were complemented by transmission electron micrographs to illustrate detailed morphological features that can be brought out by our staining procedure. Electron micrographs were taken with a JEOL JEM-100CX electron microscope at an accelerating voltage of 80 kV.

3. Results and Discussion

Although the chemical interaction of RuO_4 with large molecules is not well understood, the observed staining for several polymers, taken together with reports of chemical reactions of some small-molecule liquids containing pertinent functional groups, provides the basis for our comments on the action of the stain. Table I gives a complete list of the polymers tested, together with their unit chemical structures and (for films cast from solution) the solvents used.

3.1. Oxidation of Arenes and Ether Moieties. The oxidation effect of ruthenium tetraoxide on aromatic rings and ethers was first demonstrated by Djerassi et al.,[8] who found that a small amount of RuO_4 added to benzene and anhydrous ether caused an explosive reaction and an instantaneous black precipitate (ruthenium dioxide). Similarly, RuO_4 oxidizes phenylcyclohexane and *p-tert*-butylphenol respectively to cyclohexanecarboxylic acid and pivalic acid.[18] Under controlled conditions, aliphatic ethers can be oxidized smoothly to esters or, in the case of cyclic ethers, to lactones. For example, the oxidation of tetrahydrofuran (**1**) to γ-butyrolactone (**2**) was reported in nearly quantitative yields under mild conditions.[12] A mechanism for this reaction, in which RuO_4 abstracts a hydride ion from the α-position of the ether, has been proposed and is outlined in reaction 3.1.1. The oxygen



1

2

(3.1.1)

of the ether linkage exerts an activating effect on the adjacent methylene, but this effect is absent in the esters. Attempts to oxidize γ-butyrolactone to succinic anhydride failed.[19] Although RuO_4 oxidizes aromatics and ethers very rapidly, the aromatics are oxidized more slowly than the ethers.[12]

Since ruthenium tetraoxide is capable of reaction with the aromatic nucleus of an alkylbenzene and also with the methylene groups activated by adjacent oxygens, it seemed likely that the polymers polystyrene (PS), poly(2,6-dimethyl-1,4-phenylene oxide) (PPO), and poly(vinyl methyl ether) (PVME) would be readily attacked. Figure 1 shows optical photographs of PS, PPO, and PVME films prepared and exposed to RuO_4 vapor as outlined in section 2. All three polymers were darkened by the action of the staining agent. The vigorous reaction of RuO_4 with many organic substances and tissue[17] and the consequent black coloration of the solvent suggests that as the RuO_4 vapor reacts with the functional groups in the polymers of Figure 1, the tetraoxide is reduced to RuO_2[8,9] and other lower oxide states to give inactive complexes.[20,21] Ruthenium dioxide is a dark blue-to-black compound that is insoluble in water and most other common solvents[3] and, in contrast to RuO_4, is very stable and not volatile. It is evident from Figure 1 that PVME was stained far more quickly and more intensely than both PPO and PS. While films of PVME became completely black within 15 min, the films of PPO and PS were seen to darken gradually.

The ether moiety in PVME (located in the pendant side chain) is probably responsible for the significant staining of this polymer and as there is an ether moiety located in the chain backbone of PPO, it might be thought surprising that PVME and PPO are not stained equally. The difference in the staining behavior of PPO compared to PVME may result from the fact that the carbon–oxygen bond in aryl ethers is considerably more resistant to thermal, acid, and oxidative cleavage than the alkyl ether bond, due to resonance with the ring.[22] This can be un-

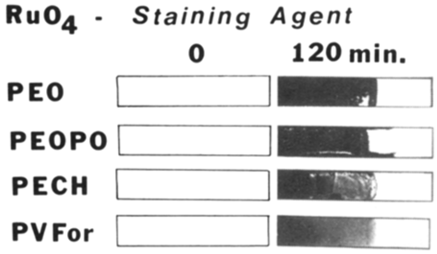
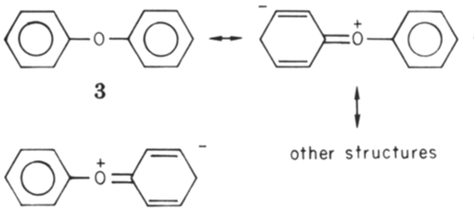


Figure 2. Optical micrographs of PEO, PEOPO, PECH, and PVFor films exposed to RuO_4 vapor for 120 min.

derstood by noting the stability of diphenyl ether[3] molecules (reaction 3.1.2). The presence of the phenyl groups



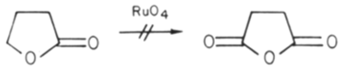
3

(3.1.2)

decreases the basicity of the oxygen and imparts some double-bond character to the ether link. In addition, it has been shown that RuO_4 extracts a hydrogen from the carbon adjacent to the oxygen atom in the alkyl ether molecule,[12] but oxidation at this point for aryl ethers would not be expected since there are no hydrogens available for extraction. Therefore, RuO_4 should react mainly at the aromatic ring in PPO, yielding a stain intensity approximately the same as that of PS (which contains no ether linkages).

To test whether the position of the ether moiety in the polymer repeat unit plays an important role in the staining behavior of RuO_4 vapor, films of ether-type polymers poly(ethylene oxide) (PEO), poly(ethylene oxide-*co*-propylene oxide) (PEOPO), poly(epichlorohydrin) (PECH), and poly(vinylformal) (PVFor) were cast from solution and stained. The results of the staining are shown in Figure 2. All the polymers darkened rapidly to a brownish black color, similar to the PVME film of Figure 1, but not as intensely.

3.2. Oxidation of Various Alcohol and Vinyl Groups. Since the oxidative effect of ruthenium tetraoxide is significantly greater than that of OsO_4, reactions of the former with small molecules are normally carried out in a suitable solvent. Of the many common solvents available, the alcohols cannot be used because RuO_4 converts primary alcohols into aldehydes or carboxylic acids and secondary alcohols into ketones.[9,12,23] The solvents reported suitable for reactions involving RuO_4 are carbon tetrachloride, alcohol-free chloroform, low molecular weight paraffins (see section 3.3), ketones, esters, and water.[9] The inertness of these solvents can be demonstrated by the following examples: A solution of the tetraoxide in carbon tetrachloride is reported to have remained unchanged for a year[8] and attempts to prepare succinic anhydride (4) from the ester γ-butyrolactone using RuO_4 as the oxidant were unsuccessful (reaction 3.2.1).[19] Ketones are often



4

(3.2.1)

a final product of organic oxidation reactions with RuO_4. Finally, we have kept this tetraoxide in cold water up to 6 months without change (reduction of RuO_4 is very slow).

In principle, polymers of saturated halogens, ketones, esters, and paraffins (discussed in section 3.3) should prove to be relatively inactive toward oxidation by RuO_4 vapor,

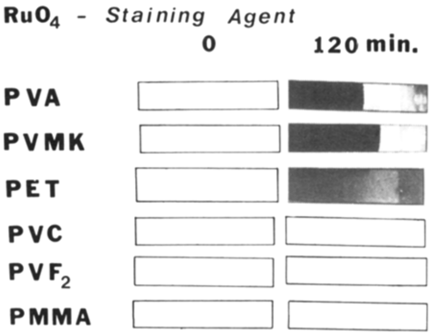


Figure 3. Optical micrographs of PVA, PVMK, PET, PVC, PVF_2, and PMMA films exposed to RuO_4 vapor for 120 min.

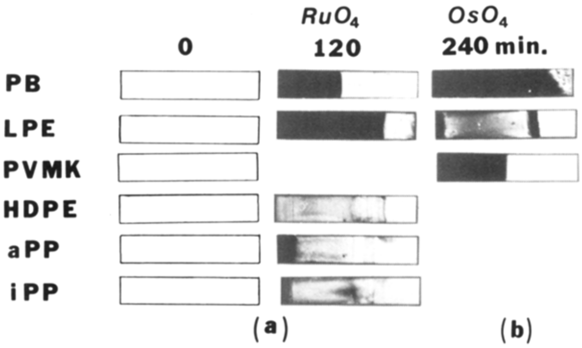
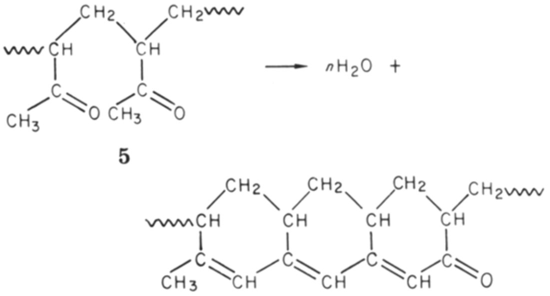


Figure 4. Optical micrographs of (a) PB, LPE, HDPE, aPP, and iPP films exposed to RuO_4 vapor for 120 min and (b) PB, LPE, and PVMK films exposed to OsO_4 vapor for 240 min.

while polymers with hydroxyl groups should be reactive to the tetraoxide. This idea was tested by selecting various vinyl and related polymers shown in Figure 3 and exposing them to RuO_4 vapor. Figure 3 shows that poly(vinyl alcohol) (PVA) and poly(vinyl methyl ketone) (PVMK) were deeply stained by RuO_4 vapor, poly(ethylene terephthalate) (PET) was lightly stained, and poly(vinyl chloride) (PVC), poly(vinylidene fluoride) (PVF_2), and PMMA were little affected by RuO_4.

It was surprising to observe that PVMK[5] was highly stained by RuO_4 vapor. It has been reported that this polymer is very unstable at high molding temperatures,[24] undergoing an aldol condensation reaction between pairs of adjacent repeating units (reaction 3.2.2) to form cyclic



5

(3.2.2)

ketone structures and conjugated sequences of various lengths with the elimination of H_2O.[25-27] Poly(vinyl methyl ketone) degrades thermally, becoming yellow, then orange, and finally red. Although this polymer can be made clear and colorless,[24] when stored for as short a time as 1 month it becomes yellow. The PVMK purchased and used in these experiments was a very light yellow, indicating the presence of some unsaturation. This unsaturation could be responsible for the observed staining behavior. To test this hypothesis, PVMK was exposed to osmium tetraoxide vapor. Figure 4b shows that OsO_4 vapor darkened the PVMK film.

Table I
Polymers Exposed to Ruthenium Tetraoxide Vapor

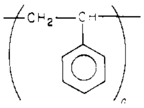
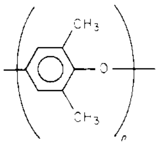
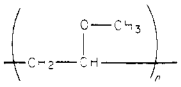
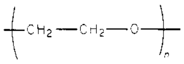
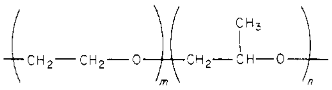
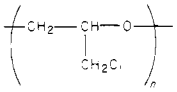
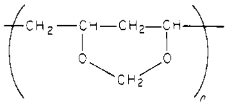
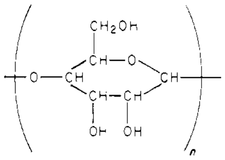
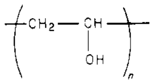
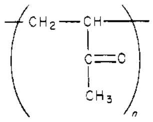
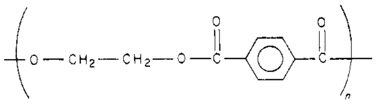
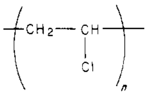
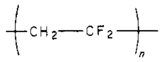
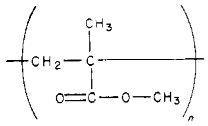
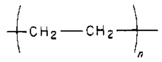
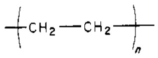
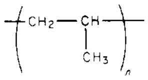
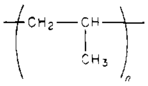
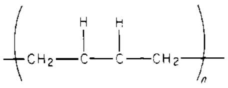
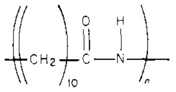
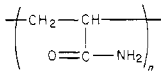
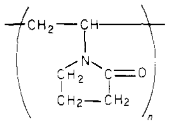
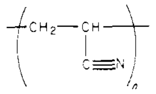
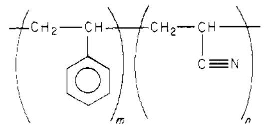
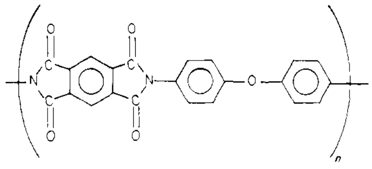
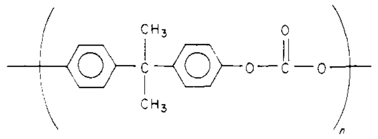
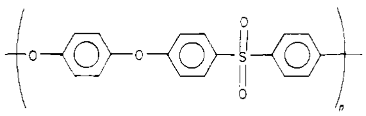
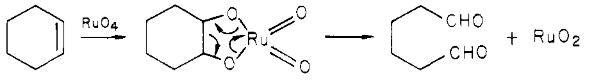
	polymer	abbreviated name	unit structure	solvent	stained
1.	polystyrene	PS		toluene	yes
2.	poly(2,6-dimethyl-1,4-phenylene oxide)	PPO		xylene	yes
3.	poly(vinyl methyl ether)	PVME		toluene	yes
4.	poly(ethylene oxide)	PEO		toluene	yes
5.	poly(ethylene oxide-*co*-propylene oxide)	PEOPO		toluene	yes
6.	poly(epichlorohydrin)	PECH		*N,N*-dimethylacetamide	yes
7.	poly(vinylformal)	PVFor		chloroform	yes
8.	wood and cotton (contains cellulose)	Q-tip			yes
9.	poly(vinyl alcohol)	PVA		distilled, deionized water	yes
10.	poly(vinyl methyl ketone)	PVMK		chloroform	yes
11.	poly(ethylene terephthalate)	PET			yes
12.	poly(vinyl chloride)	PVC		*N,N*-dimethylacetamide	no
13.	poly(vinylidene fluoride)	PVF_2		tetrahydrofuran	no
14.	poly(methyl methacrylate)	PMMA		toluene	no
15.	high-density polyethylene	HDPE		xylene	yes
16.	linear polyethylene wax	LPE			
17.	atactic polypropylene	aPP		toluene	yes

Table I (*Continued*)

	polymer	abbreviated name	unit structure	solvent	stained
18.	isotactic polypropylene	iPP	 $\left(CH_2-\underset{CH_3}{CH}\right)_n$	xylene	yes
19.	poly(*cis*-1,4-butadiene)	PB	 $\left(CH_2-\overset{H}{C}-\overset{H}{C}-CH_2\right)_n$	toluene	yes
20.	nylon 11	nylon 11	 $\left(\left(CH_2\right)_{10}-\overset{O}{\overset{\|}{C}}-\overset{H}{N}\right)_n$	trifluoroacetic acid	yes
21.	polyacrylamide	PAA	 $\left(CH_2-\underset{O=C-NH_2}{CH}\right)_n$	distilled, deionized water	yes
22.	poly(vinylpyrrolidone)	PVP		chloroform	yes
23.	polyacrylonitrile	PAN	 $\left(CH_2-\underset{C\equiv N}{CH}\right)_n$	*N,N*-dimethylacetamide	no
24.	styrene/acrylonitrile copolymer (75/25)	SAN		*N,N*-dimethylformamide	yes
25.	polyimide	H-film			yes
26.	polycarbonate	PC		chloroform	yes
27.	5 min Epoxy				yes
28.	poly(*p*-phenylene ether sulfone)	PPES		*N,N*-dimethylacetamide	yes

3.3. Oxidation of Olefins. Although there is no information on the oxidation of alkanes, it has been suggested that RuO_4 with a cooxidant might prove to be an effective reagent for the oxidation of alkanes.[9] Alkenes are very susceptible to electrophilic attack by RuO_4.[9,12] Osmium tetraoxide also reacts with alkenes, but in a manner considerably different from that of RuO_4. Oxidation of alkenes by OsO_4 results in the hydroxylation of the double bonds while RuO_4 cleaves the unsaturation to give ketones, aldehydes, or carboxylic acids.[9,12] A possible scheme for the oxidation of cyclohexane (**6**) to adipaldehyde (**7**) by RuO_4[12] is given in reaction 3.3.1.



6 7

(3.3.1)

Polymers containing some level of unsaturation, such as poly(*cis*-1,4-butadiene) (PB), should be attacked strongly by RuO_4. This fact was confirmed by the exposure of PB to RuO_4 vapor, resulting in an intensely stained film (Figure 4a). Poly(*cis*-1,4-butadiene) was also stained with OsO_4 vapor, but for a longer time due to the lower reactivity of this tetraoxide compared to RuO_4 (Figure 4b).

As low molecular weight paraffins can be used as solvents for reactions involving RuO_4, it seemed likely, at first glance, that linear polyethylene wax (LPE), high-density polyethylene (HDPE), and both isotactic and atactic polypropylene (respectively iPP and aPP) should be relatively inert to this tetraoxide. However, it is very difficult to eliminate unsaturation during the preparation of a saturated polyolefin. For example, several types of carbon–carbon double bonds may be present in polyethylene in very small concentrations (up to 0.25% in low-density polyethylene).[28] These are the terminal type ($RCH=CH_2$), the internal type ($RCH=CHR$), and the side-chain type ($R_2C=CH_2$).[28-30] Therefore, oxidation of olefins by RuO_4 may occur through these unsaturated moieties.

Linear polyethylene wax, HDPE, aPP, and iPP were exposed to RuO_4 vapor and the results are presented in

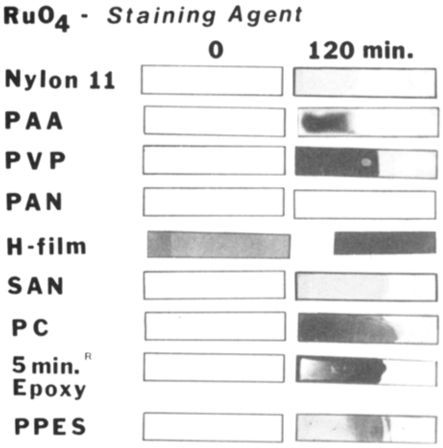
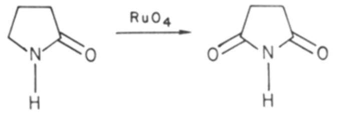


Figure 5. Optical micrographs of nylon 11, PAA, PVP, PAN, H-Film, SAN, PC, 5 min Epoxy, and PPES films exposed to RuO_4 vapor for 120 min.

Figure 4a. All samples were stained by the tetraoxide. In addition, a linear polyethylene wax film exposed to OsO_4 (Figure 4b) was stained lightly. This suggests that unsaturation may indeed be responsible for the staining of these polymers.

3.4. Oxidation of Nitrogen-Containing and Miscellaneous Polymers. Although esters are stable to RuO_4, amides (which are weaker reductants than amines) are oxidized smoothly by this tetraoxide. For example, γ-butyrolactam (**8**) was converted to succinimide (**9**) with a 49% yield,[12] reaction 3.4.1. Amines are not oxidized as smoothly as alcohols or ethers;[12] triamylamine, diethylamine, and piperidine treated with RuO_4 all gave intractable products. However, hexylamine gave a product whose infrared spectrum indicated the presence of aldehyde and nitrile moieties.[12] To date, we have not found any reports concerning the oxidation of nitrile groups by RuO_4 in the literature. The triple bond in the nitrile group is thought to be very stable toward oxidative cleavage due to resonance effects.



8 9

(3.4.1)

It appears that polymers which contain amine or amide moieties in their repeat unit should be active to RuO_4, while those having nitrile groups may be inert to this staining agent. Accordingly, nylon 11, poly(vinylpyrrolidone) (PVP), polyacrylamide (PAA), and polyacrylonitrile (PAN) were exposed to RuO_4 vapor, with the results illustrated in Figure 5. As expected, nylon 11, PAA, and PVP were stained by the tetraoxide, while PAN was unaffected by the stain.

In addition, some miscellaneous polymers were exposed to RuO_4 vapor: a polyimide (H-film), poly(styrene-*co*-acrylonitrile) (SAN), polycarbonate (PC), poly(*p*-phenylene ether sulfone) (PPES), and 5 min Epoxy (Devcon Corp.) (Figure 5).[46] Due to the presence of oxidizable moieties all were stained by the tetraoxide.

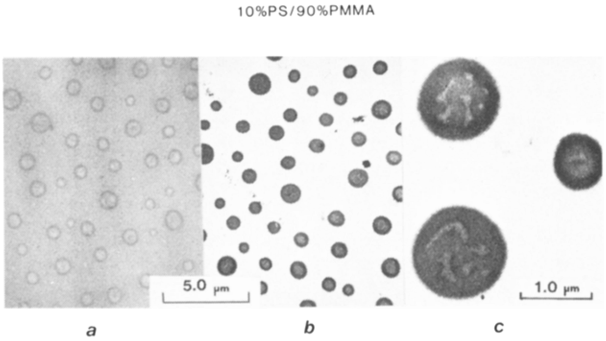


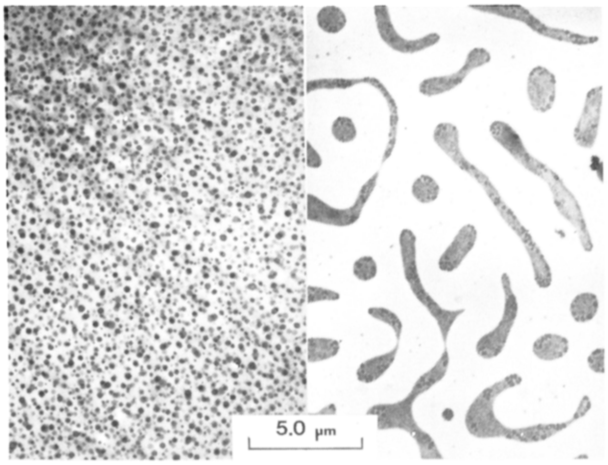
Figure 6. Electron micrographs of a 10% PS/90% PMMA polymer film cast from toluene (a) unstained and (b and c) stained with RuO_4 vapor. PS is the darker phase.

3.5. Electron Microscopy of Polymers Stained by Ruthenium Tetraoxide Vapor. The optical micrographs of Figures 1–5 demonstrate that RuO_4 vapor reacts with several polymers containing either an ether, alcohol, aromatic, amine, or unsaturated moiety in their unit structure and illustrate the variation of stain intensity with time and moiety. In addition, these data are of assistance in determining which polymers are unaffected by RuO_4. Several of the polymers listed in Table I were selected to demonstrate directly the usefulness of RuO_4 as a staining agent for electron microscopy. Solvent-cast thin films of these polymers and their blends were made and exposed to RuO_4 vapor. Crazes were formed in some of these films prior to staining by straining the film-covered copper grids with a pair of tweezers. The plastic strain in the copper grid held the polymer film under strain after the load was removed.

Since the amorphous single-component films such as PS, PPO, and SAN stained uniformly and appeared structureless when viewed in the TEM, only stained semicrystalline polymers and incompatible amorphous polymer blends are presented in our TEM studies. All of the polymer films examined below, except HDPE, were stained for 30 min. A longer staining time of 60 min was needed to bring out adequate contrast for HDPE.

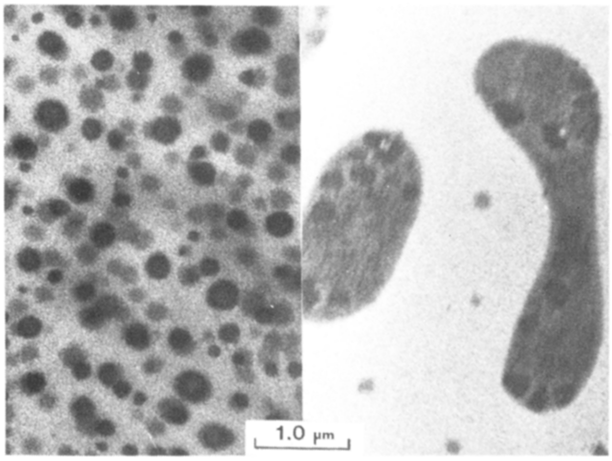
3.5.1. Morphology of Some Incompatible Polymer Blends. Figure 6 shows a comparison of a 10% PS/90% PMMA polymer film cast from toluene (a) unstained and (b and c) stained with RuO_4. The contrast mechanism in Figure 2a probably results from thickness and density fluctuations between the two phases. However, much structural detail is missing and the contrast is poor. Since PMMA was not stained by RuO_4 (see Figure 3), only the PS phase darkened, revealing PS droplets suspended in a PMMA matrix (Figure 6b,c). Figure 6c is a magnified view of the stained droplet shown in Figure 6b. Notice that the stain also brought out structural detail within the PS droplets not discernible in Figure 6a. The droplets appear to be a composite of irregular-shaped PMMA material in a regular-shaped PS matrix. As is the case with ABS (Figure 9) and HIPS,[2] these inclusions of PMMA tend to raise the apparent volume fraction of droplets present in the PMMA matrix.

The effect of solvent on microstructure in polymer blends can be studied effectively in the TEM by RuO_4 staining of the film. This was illustrated by preparing films 10% PS/90% PMMA (by weight) cast from both tetrahydrofuran (THF) and xylene and subsequently staining them with RuO_4 (Figures 7). Comparison of Figures 6a, 7a, and 7b shows that dramatic morphological changes occurred as the solvent was varied. Figure 8a is an enlarged view of Figure 7a, showing the finely dispersed PS-rich particles, with an average diameter of $\sim$0.2 μm formed within a PMMA-rich matrix as the THF evaporated from the film. When xylene was used as the solvent (Figure 7b), the PS phase formed both irregularly shaped and roughly spherical particles that contained some internal structure. Note than many of these large spherical particles are greater than 2 μm in diameter, considerably in excess of the film thickness, 0.3–0.5 μm. Figure 8b is an enlarged view of Figure 7b showing that this internal structure



a *b*

Figure 7. Electron micrographs of a 10% PS/90% PMMA film cast from (a) THF and (b) xylene and subsequently stained with RuO_4 vapor.



a *b*

Figure 8. Electron micrographs showing the magnified images of (a) Figure 7a and (b) Figure 7b.

Table II
Technical Data for Some Polymers and Solvents

polymer or solvent	δ, $(cal/s)^{1/2}$	bp, °C	vapor pressure at 25 °C, mmHg	ref
PS	8.5–9.7			31
PMMA	9.0–9.5			31
THF	9.5	65–67	162	32–34
toluene	8.9	111	~32	32, 34
xylene	8.8	~144	~8.3	31, 32, 34

appears as spherical PS-rich particles within a PS-rich phase.

Since the interaction (solubility) parameters for the solvents, PS, and PMMA are similar, a major factor in the formation of the different morphologies observed in Figures 6b, 7a, and 7b would seem to be the solvent evaporation rate. Table II gives the interaction parameters (δ) for both solvents and polymers tested, solvent boiling point (bp), and solvent vapor pressure. The film prepared from the most volatile solvent, THF, contained small well-dispersed PS particles, which may not have had time to coalesce, while the less volatile solvents, toluene and xylene (in order of decreasing volatility), gave films that contained far larger PS particles.

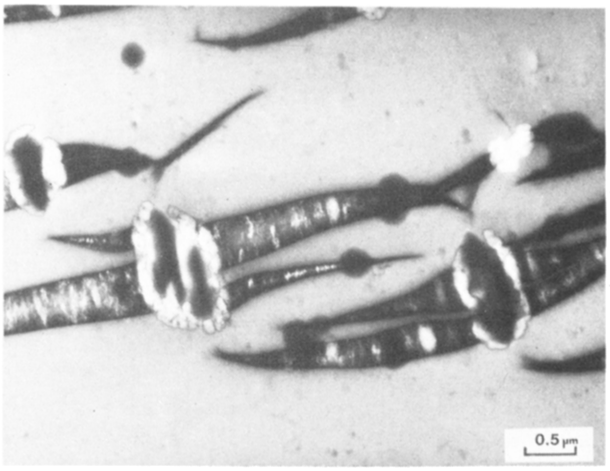


Figure 9. Electron micrograph of a stressed film of ABS cast from ethyl acetate and stained first with OsO_4 and then with RuO_4 vapor.

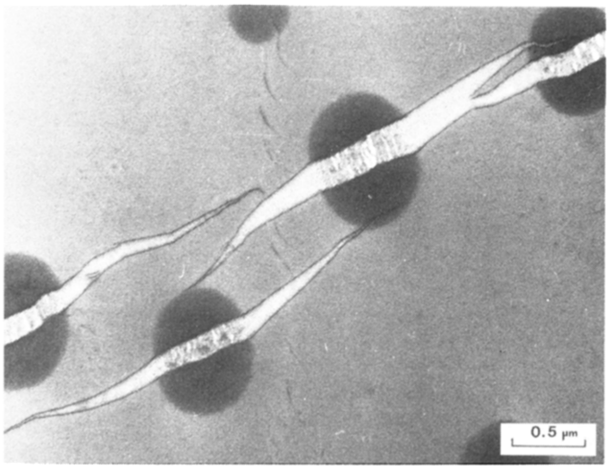


Figure 10. Electron micrograph of a stressed film of 4% PS/96% PMMA cast from toluene and stained with RuO_4. The dark phase is PS.

Figure 9 is an electron micrograph of a stressed film of ABS stained first with OsO_4 (to enhance the rubber phase) and then with RuO_4. As observed in Figure 6c, the rubber particles were a composite of SAN and butadiene. Not only was the film darkened by the stain, but the crazes were stained intensely by RuO_4, enhancing the fibrils. The darkening of the crazes by the stain illustrates clearly the blunted craze tips. Craze tip blunting has been attributed to shear deformation, leading to stress relaxation at the craze tip, thus preventing further tip advance.[35]

As observed in Figure 9, the presence of rubber particles in rubber-modified plastics serves to trigger yielding in the continuous phase and in particular the formation of crazes involving localized volume increases. The fact that the T_g of the rubber phase is well below room temperature is not a necessary criterion for the initiation of multiple crazes. The stressed film shown in Figure 10 is a blend of 4% PS/96% PMMA stained with RuO_4. The dark phase is polystyrene. Since PS yields at a lower strain level than PMMA and the maximum stress concentration for spherical particles occurs at the equator perpendicular to the direction of applied stress,[36,37] the crazes observed probably initiated at or within the surface of the PS phase. The craze tips propagated along the equatorial direction until the stress field associated with their tips interacted with the stress fields around the particles or other crazes. Fibril formation was only seen within the PS particles at

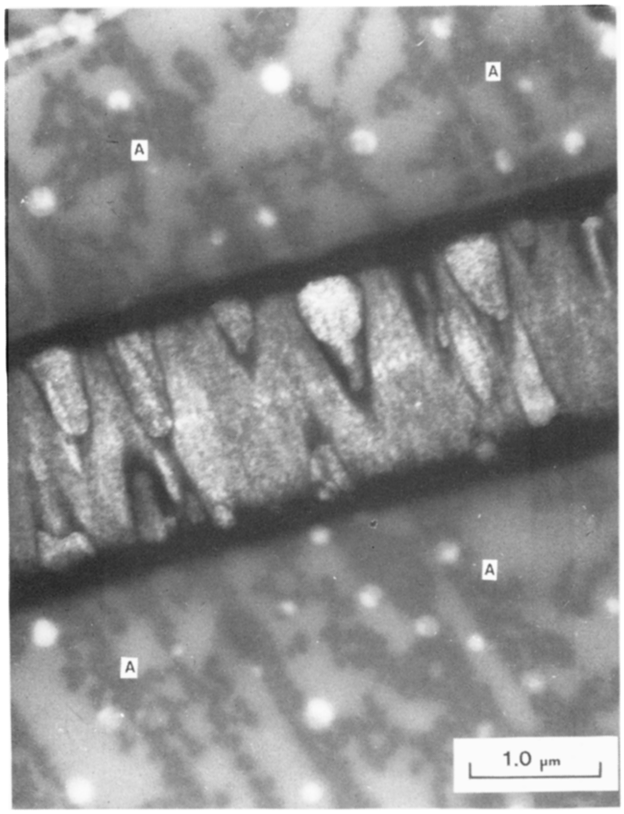


Figure 11. Electron micrograph of a 96% PS/4% PMMA film cast from toluene, stressed, and stained with RuO_4. The PMMA-rich regions are the light areas.

this level of deformation. Not only did the action of RuO_4 darken the craze boundaries within the PS particles but the boundaries in the PMMA matrix were stained as well. Figure 10 shows that PS particles, having a T_g well above room temperature but slightly below that of the PMMA matrix (T_g for PS and PMMA are 100 and 105 °C, respectively), can also serve to initiate crazes similar to those formed in rubber-reinforced polymers.

Figure 11 is an electron micrograph of a 96% PS/4% PMMA film cast from toluene (the reverse concentration to that of Figure 10), stressed and subsequently stained with RuO_4. The PMMA-rich regions are the light areas. The dark irregular phase observed on either side of the craze (indicated by the letter A) would seem to be a PS-rich precipitate. Notice again that the boundaries of the craze were darkened heavily by the stain. Also, RuO_4 enhanced the two-phase nature of the craze and exposes clearly the characteristic midrib along the center of the craze. Without staining, several of the morphological features of this sample would have been much more difficult to see or may have been overlooked.

We also examined a polymer blend in which one phase (PS) is stained more intensely than a second phase (SAN) for equal exposure times. Figure 12 is a stressed film of 87% SAN (25% AN)/13% PS stained with RuO_4, where the darker phase is PS. Complete separation of the PS particles from the SAN matrix occurred at the PS–SAN interface within the deformation zone. Partial mixing at the boundary would be responsible for some of the SAN material adhering to a few PS particles, which then deformed as shown in Figure 12. Ruthenium tetraoxide also darkened the edges of the holes more than the craze material, possibly a surface area effect. The boundaries of the crack formed within the deformation zone was not darkened by RuO_4 because it was initiated by the electron beam in the TEM after the staining process.

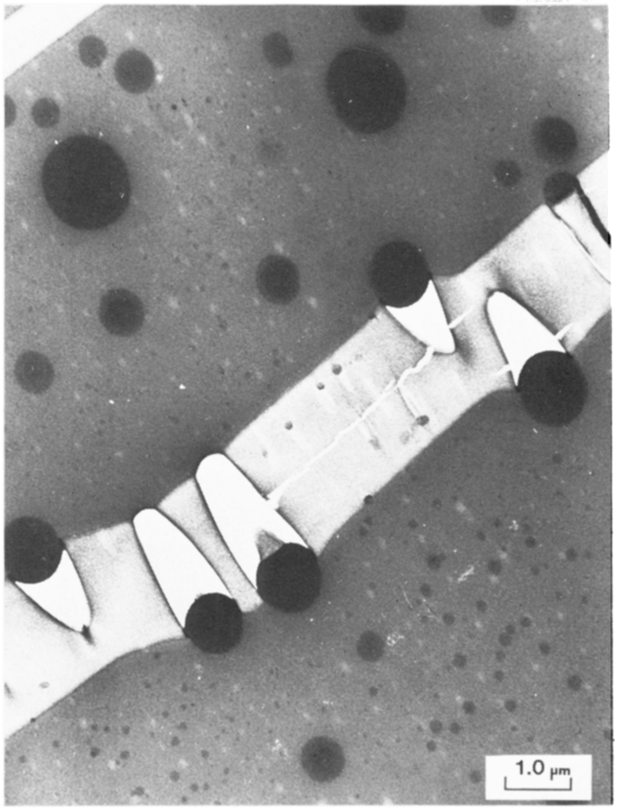


Figure 12. Electron micrograph of a stressed film of 87% SAN (25% AN)/13% PS cast from toluene and stained with RuO_4 vapor. The darker phase is PS.

3.5.2. Morphology of High-Density Polyethylene and Nylon 11. Both HDPE and nylon 11 exhibit the tendency to form spherulites when crystallized from either the melt or solution. Control of the spherulitic properties and morphology is of primary importance in the engineering design of polymers. For example, large spherulites are known to enhance brittleness in polymers[38,39] and may in some ways play a role similar to the grain structure in metals. For these reasons several methods have been developed to study and observe the morphology of spherulites. The optical microscope has been used to view spherulites, which have the familiar Maltese cross appearance when examined between crossed polaroids. Replication techniques have been used extensively to view indirectly the micromorphology of HDPE and nylon spherulites in the TEM.[40–42] More recently, bulk HDPE samples have been stained with chlorosulfonic acid and uranyl acetate,[43–45] followed by sectioning at −100 °C using a cryoultramicrotome,[45] to view spherulitic morphology in the TEM.

We have found that the spherulites of HDPE and nylon 11 film cast from xylene and 50% phenol/50% formic acid, respectively, were stained with RuO_4 vapor and gave excellent contrast in the TEM image (Figures 13–15). Not only was the preparation of these films and their subsequent staining relatively easy but the action of RuO_4 also stabilized the film to electron beam damage. High beam intensities can produce stresses, localized heating, cross-linking, and loss of carbon in polymer films, leading to drastic induced morphological changes. An example of this fact is shown in Figure 13, an electron micrograph of an unstained thin film of HDPE exposed directly to the electron beam. The film melted in various places and folded upon itself, completely destroying the original spherulitic morphology. However, when the film was exposed to RuO_4 vapor for 60 min before viewing in the

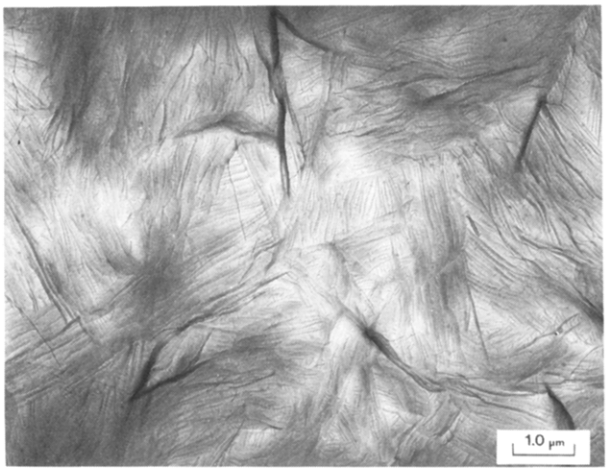


Figure 13. Electron micrograph of an unstained thin film of HDPE exposed directly to the electron beam.

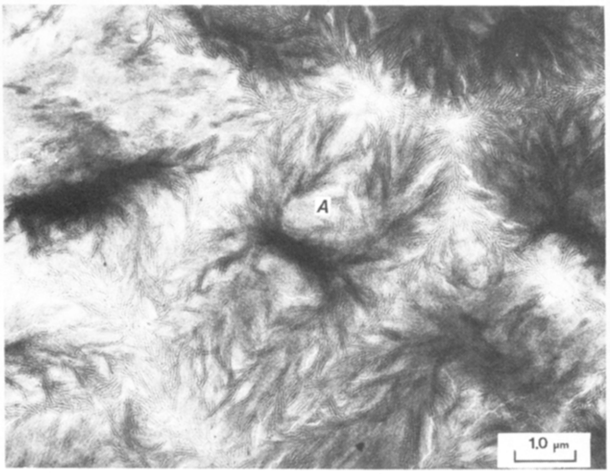


Figure 14. Electron micrograph of a thin film of HDPE stained with RuO_4.

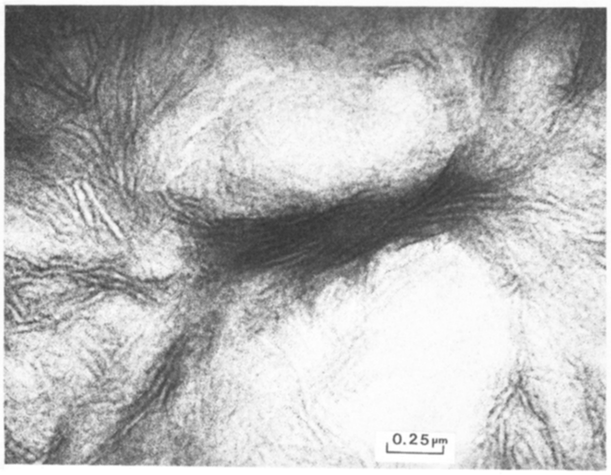


Figure 15. Electron micrograph of the spherulite nucleus seen in Figure 14 (designated by the letter A).

TEM, the original spherulitic structure was manifest (Figure 14) and the film remained stable to the beam. The mechanism for this stabilization may be similar to that of the many fixing agents (including OsO_4) used in the stabilization of biological specimens (i.e., cross-linking). In connection with this we note that polystyrene film stained with RuO_4 for 4 h was found not to dissolve in toluene but to break up into small fragments.

Since diffusion of RuO_4 vapor in the amorphous region would be expected to occur more readily than that in the crystalline regions, the dark stained lines in Figures 14 and 15 are probably the amorphous areas between crystallites. (This view is supported by the work of Kanig[43,44] and Voigt-Martin,[45] who have used chlorosulfonic acid and uranyl acetate as a means for staining bulk HDPE samples).

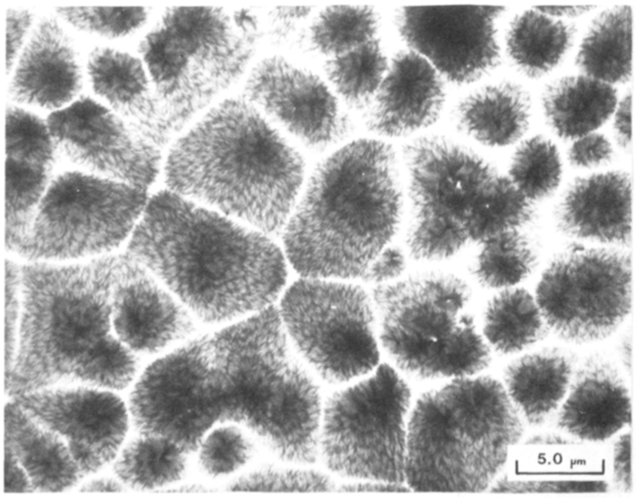


Figure 16. Electron micrograph of a nylon 11 film stained with RuO_4 vapor.

It can be seen from Figure 14 that nucleation occurred at several points and crystal growth proceeded in a radial fashion from each nucleus until the growth fronts from the neighboring structures impinged. Figure 15 is a magnified image of the nucleus seen in Figure 14 (designated by the letter A), illustrating a "wheat sheath" of radiating crystalline fibrils. Note that the central bundle of parallel lamellae appeared to twist in the center of the nucleus. As they began to grow outward they diverged, twisted, and branched to form an overall spherulitic structure that was radially symmetric. The lamellar thickness in the nucleus ranged from approximately 70 to 140 Å and up to 300 Å outside the nucleus.

Figure 16 is an electron micrograph of a nylon 11 film stained with RuO_4 vapor. Spherulites approximately 5 μm in diameter can be seen with a crystal growth structure noticeably different from that of the HDPE film observed in Figure 14. The action of the stain also stabilized the nylon 11 film to electron beam damage.

4. Conclusions

The effect of RuO_4 on small molecules that contain oxidizable moieties is the basis for comment on the relative effect of the stain on various polymers. Ruthenium tetraoxide has been shown to stain polymers that contain an ether, alcohol, aromatic, amine, or unsaturated moiety in their unit structure. Consequently, polymers that contain one or more of these oxidizable moieties should be stained by RuO_4. The polymers shown to be stained by RuO_4 vapor were PS, PPO, PVME, PVA, PET, PEO, PEOPO, PECH, PVFor, PB, PVP, PAA, SAN, PC, PPES, PVMK, HDPE, LPE, aPP, iPP, nylon-11, H-Film, cotton, wood, and 5 min Epoxy. Examples of other polymers that may be stained are poly(phenyl methacrylate), poly(vinyl butyl ether), poly(2-chloro-1,3-butadiene), poly(hexamethyleneadipamide) (nylon 66), and all the polycarbonates, polyamides, polyoxides, cellulose derivatives, polydienes, and polystyrenes. Polymers not affected by the stain were PMMA, PVC, PVF_2, and PAN.

The role of RuO_4 diffusion rate in staining is beyond the scope of this paper, although it could be of considerable practical importance. For example, slow diffusion of RuO_4 could give a light stain even though the tetraoxide may be highly reactive on various polymers.

Detailed structural features of blends of PS/PMMA, ABS, and SAN/PS films and, in addition, the semicrystalline polymers HDPE and nylon 11 were significantly enhanced by RuO_4 staining before their examination in the TEM. The associated electron micrographs demonstrate that RuO_4 is extremely useful as a staining agent for many saturated and unsaturated polymers, improving image contrast in the TEM and stabilizing these films toward electron beam damage.

The staining technique makes available a method to observe phase behavior of polymer blends, the structure of spherulites, and the influence of solvent and temperature on the morphology and growth of phase-separated particles and spherulites.

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^{13}C NMR Studies of Solid Urea–Formaldehyde Resins Using Cross Polarization and Magic-Angle Spinning

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ABSTRACT: Experimental ^{13}C NMR data are presented for the cross polarization magic-angle spinning (CP/MAS) experiment applied to urea–formaldehyde polymers. The effect of static magnetic field is demonstrated with spectra presented at three magnetic field strengths—1.41, 2.35, and 4.70 T. The effect of residual dipolar coupling of ^{13}C to ^{14}N is largely suppressed at high fields, in contrast to the low-field case, allowing resolution of individual carbon types in these polymers. Spectra deconvolutions have been carried out on complex peaks, and tentative peak assignments and corresponding structural conclusions have been made on the basis of comparisons with solution data.

Introduction

The chemical shift in a ^{13}C NMR experiment is a sensitive probe of chemical structure, and the relatively large chemical shift range makes ^{13}C NMR an attractive tool for analyzing complicated materials such as those of synthetic polymers and resins. Limitations in solubility of many of these materials—especially cured resins—often preclude the use of liquid-state NMR. The technique of solid-state ^{13}C NMR with cross polarization and magic-angle spinning (CP/MAS)[1] provides a powerful technique to overcome