

TEM Measurement of Lamellar Thickness in Polyethylene and Cycloparaffins by Staining with RuO₄

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Edge-on crystals of PE were prepared from a solution by epitaxial crystallization on the (001) face of NaCl, and consequently the electron beams could be introduced parallelly to the fold surface of PE lamellar crystal in transmission electron microscopy. Edge-on crystals also grew on KBr and KCl, having the same orthorhombic crystal form and orientation as those on NaCl. The edge-on crystals of PE were stained with RuO₄ to observe the morphology. It was found that the PE edge-on crystals are composed of several parallelly stacked lamellae, the thickness of which is nearly the same as that of single crystals grown sporadically from a solution at the same temperature. Edge-on crystals of cycloparaffins with regular sharp folds and those of normal paraffins were also epitaxially grown on NaCl, and stained with RuO₄. The thickness of the lamellae in PE edge-on crystals and the thickness of monomolecular layers in edge-on crystals of cycloparaffins and normal paraffins were estimated by measuring the distance between the adjoining fine striations due to RuO₄. Each of the striations corresponds to the interfacial region between lamellae or monomolecular layers with its image contrast enhanced by RuO₄ staining.

KEY WORDS: RuO₄ staining/ Lamellar thickness/ Polyethylene/ Cycloparaffin/ Normal paraffin/ Epitaxy/

1. INTRODUCTION

Electron microscopy is one of the useful methods for investigating the fine structure of materials^{1,2}. Observation of the structure is, however, limited by the radiation-induced damage and insufficient image contrast^{3,4}. To overcome these problems, some staining agents containing heavy atoms have been used in transmission electron microscopy [TEM]. The staining agents are, for example, as follows: uranyl acetate⁵, lead acetate⁶, lead nitrate⁷, potassium permanganate^{8,9}, phosphotungstic acid¹⁰, osmium tetroxide [OsO₄]^{11,12} and ruthenium tetroxide [RuO₄]¹³. These agents have usually been used for staining of biomaterials. Staining with OsO₄ is limited to the polymers with some unsaturated bonds¹⁴. Since Vitali *et al.* reported the staining method using RuO₄ for saturated and unsaturated polymers¹³, RuO₄ has been used as a good staining agent for visualizing the domain structure in multiphase polymeric systems¹⁵⁻¹⁷. A systematic study on the RuO₄ staining was carried out for a number of polymers and showed that RuO₄ can stain the aliphatic polymers^{18,19}. For example, lamellar morphologies in the melt-crystallized PE, isotactic polypropylene and some copolymers were

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observed using the RuO_4 staining technique^{18,19}. In these cases, amorphous regions are selectively oxidized and crosslinked, and strong image contrast is expected from the high scattering efficiency of heavy element Ru for electron beams.

In this report, epitaxial crystallization of PE on NaCl is introduced to prepare its edge-on crystals. Electron beams can be incident upon the edge-on crystals in the direction parallel to their fold surfaces. To observe the stacked lamellar morphology in the edge-on crystals of PE and to measure the lamellar thickness, the RuO_4 staining method is used. Edge-on crystals of cycloparaffins with regular sharp folds and of normal paraffins are also prepared, and stained with RuO_4 .

2. EXPERIMENTAL

Epitaxial crystallization of PE on a newly cleaved (001) surface of NaCl was reported at first by Willems²⁰ and later by Fischer²¹. Using the procedure similar to theirs, edge-on crystals of PE ($M_w=13,600$: NBS Reference Material #1482) and cycloparaffins, $(\text{CH}_2)_{60}$, $(\text{CH}_2)_{72}$ and $(\text{CH}_2)_{120}$ were epitaxially grown at constant

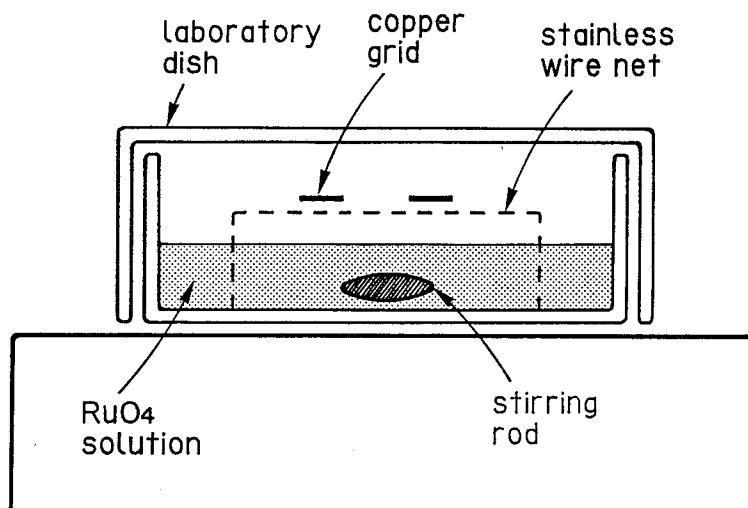


Fig. 1. Schematic representation of our apparatus for RuO_4 staining.

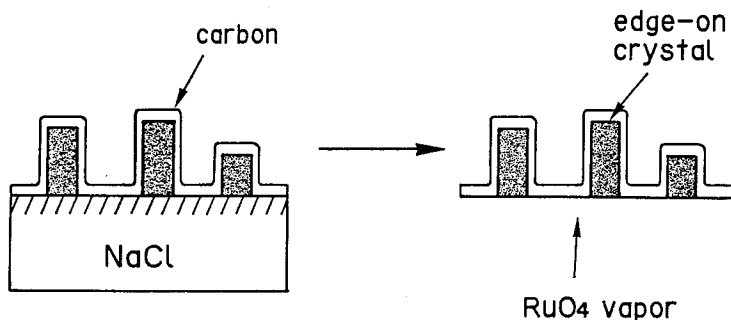


Fig. 2. Preparation procedure of edge-on crystals stained with RuO_4 .

temperatures from respective 0.01 wt% *p*-xylene solutions on a fresh (001) surface of NaCl. The cycloparaffins mentioned above were synthesized as described in our previous paper²²⁾. In the case of normal paraffins, epitaxial crystallization on NaCl from dilute solutions was not successful. Thus, edge-on crystals of normal paraffins, $C_{44}H_{90}$ and $C_{50}H_{102}$ were prepared by vapor deposition onto (001) of NaCl: a normal paraffin was evaporated and deposited onto NaCl under vacuum (10^{-3} Pa) by use of a quartz crucible. The samples thus prepared on NaCl were coated with carbon under vacuum. The samples reinforced with the carbon film were floated off on the water surface by dissolving the NaCl substrate, and then mounted on a Cu grid for TEM. Schematic representation of our apparatus for RuO_4 staining is shown in Fig. 1. This experiment was done in a draft chamber, because RuO_4 is a harmful agent and easily sublimates at room temperature. The solid RuO_4 of about 0.2–0.4 grams was dissolved in 10 ml of distilled water. The samples mounted on a Cu grid were laid on the stainless wire net and exposed to the RuO_4 vapor for about 1 hour. The surface which had been in contact with NaCl was exposed to the RuO_4 vapor as shown in Fig. 2, because the other surface was covered with vapor-deposited carbon. TEM was carried out with a JEOL JEM-200CS at an accelerating voltage of 200 kV.

3. RESULTS AND DISCUSSION

3.1 Edge-on Crystals of PE

Figures 3(a) and 3(b) show crystals of PE grown at 80°C on (001) of NaCl

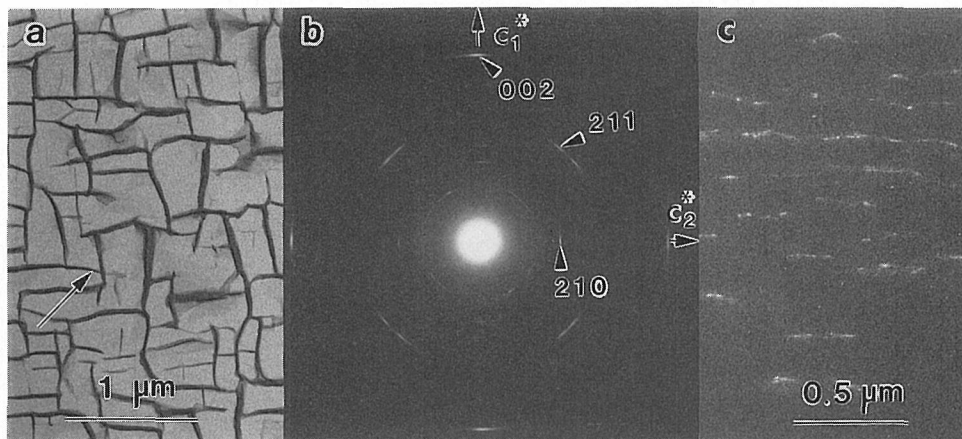


Fig. 3. (a) Edge-on crystals of PE.

They were grown on a newly cleaved (001) surface of NaCl from a solution at 80°C for 3 minutes. The arrow indicates the [100] direction of NaCl, and the specimen was shadowed with Pt-Pd in this direction.

(b) Corresponding ED pattern.

(c) 002 dark-field image.

The 002 reflection used for dark-field imaging is on the meridian in (b). The molecules of PE are arranged normal to the longer side of each rod-like crystal, namely in the vertical direction of the figure (c).

and the corresponding selected-area electron diffraction [ED] pattern, respectively. The rod-like crystal blocks are grown in $[110]$ and $[1\bar{1}0]$ of NaCl. Any fine details are not recognized in the crystal blocks in Fig. 3(a). The ED pattern (Fig. 3(b)) shows that the c -axis, the molecular axis of PE is aligned in the $[110]$ and $[1\bar{1}0]$ of NaCl. Figure 3(c) is the dark-field image taken by using the 002 reflection indicated by the arrow in the meridional direction of Fig. 3(b). The crystal blocks running in the horizontal direction are observed in Fig. 3(c). This clearly demonstrates that the molecular axis of PE is normal to the long axis of the block, and is also parallel to the surface of the substrate. This shows that these rod-like blocks are the plate-like PE crystals with their edges on the substrate: the edge-on crystals. The morphologies and ED patterns of PE edge-on crystals grown on KBr and KCl were the same as those of edge-on crystals on NaCl. The (110) of PE is the principal contact plane on (001) of NaCl, as concluded from the appearance of the reflections 002, 210, 310, 211, 311, etc. in Fig. 3(b).

Figure 4(a) shows the edge-on crystals of PE stained with RuO_4 which were grown on (001) of NaCl at 80°C . A few fine "dark" striations due to RuO_4 can be seen as running parallelly side by side between two thick striations: RuO_4 permeated into the edge-on crystal block (fine striations) and also was deposited rather heavily on the lateral sides of the block (thick striations). The parallel fine striations due to staining indicate that an edge-on crystal block of about 20–40 nm in width consists of several lamellar crystals. The averaged distance of 13 nm between adjoining fine striations was regarded as the thickness of lamellae in the blocks: it should be the lamellar thickness of PE crystals grown at 80°C in a p -xylene solution. This value (13 nm) is in good agreement with the long period measured by small-angle X-ray scattering of the single crystal mat, which was

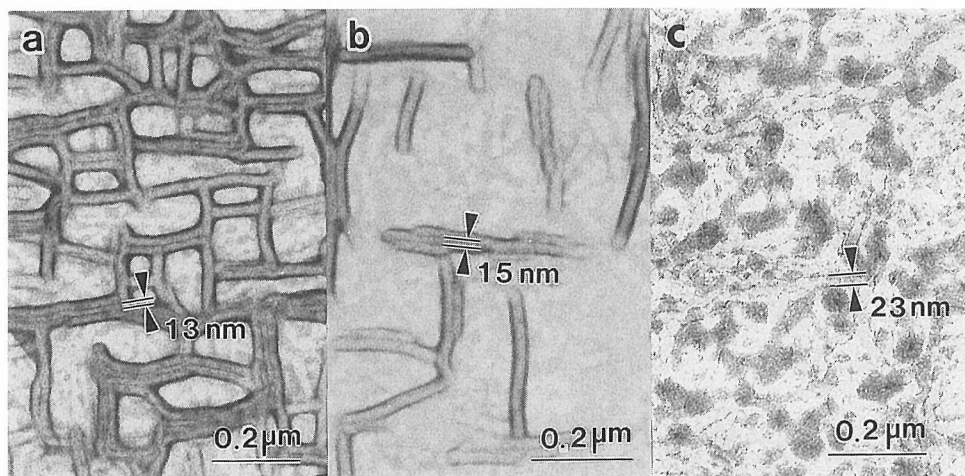


Fig. 4. Edge-on crystals of PE stained with RuO_4 .
 (a) Grown on NaCl at 80°C from a solution.
 (b) Grown on NaCl at 85°C from a solution.
 (c) Thickened by annealing on NaCl at 120°C for 30 minutes.

made by sedimenting single crystals grown at 80°C from a dilute *p*-xylene solution of PE²³⁾. The edge-on crystals grown at a higher temperature, say at 85°C, were also stained by using the same method, and are shown in Fig. 4(b). The lamellar thickness estimated by measuring the averaged distance between the fine dark striations is approximately 15 nm, which also agrees well with that of single crystals grown at 85°C²³⁾. PE molecules begin to move markedly in the solution-grown crystals when the crystals are annealed above 110°C. The edge-on crystals grown at 80°C were annealed on NaCl at 120°C for 30 minutes before carbon coating, and subsequently stained with RuO₄. Figure 4(c) straightforwardly shows that the thickness of the lamellae in the edge-on crystals increased to 23 nm by annealing. ED patterns of PE edge-on crystals annealed on NaCl revealed that the molecular orientation is maintained, probably by the effect of the substrate. A detailed discussion as to the results mentioned above will be presented in the near future with the information about the thickness of crystalline core in a PE edge-on lamella.

The stacked lamellar morphology of solution-grown PE crystals were observed by staining with RuO₄. Now a question arises whether fine structures can be also visualized by staining with RuO₄ in other materials, such as cycloparaffins with regular sharp folds and normal paraffins with terminal methyl groups arranged in a crystallographic register. These materials will be examined in the following sections.

3.2 Edge-on Crystals of Cycloparaffins

The crystal structures of some cycloparaffins including (CH₂)₂₄, (CH₂)₂₆, (CH₂)₃₄ and (CH₂)₃₆ were analyzed^{24~26)}. A series of monoclinic cycloparaffins such as (CH₂)₆₀ and (CH₂)₇₂ have crystal structures similar to that of (CH₂)₃₆²⁶⁾,

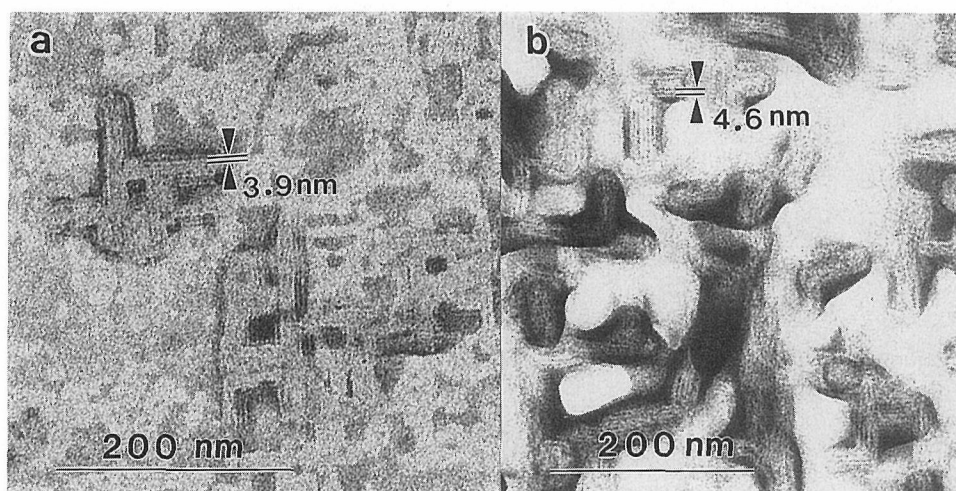


Fig. 5. Edge-on crystals of cycloparaffins stained with RuO₄.
 (a) (CH₂)₆₀ grown at 40°C on NaCl from a solution.
 (b) (CH₂)₇₂ grown at 45°C on NaCl from a solution.

Table 1. The lattice constants of a series of monoclinic cycloparaffins^{a)}.

	cycloparaffin			
	(CH ₂) ₃₆	(CH ₂) ₄₈	(CH ₂) ₆₀	(CH ₂) ₇₂
<i>a</i> (nm)	1.033	1.033	1.033	1.033
<i>b</i> (nm)	0.824	0.819	0.817	0.817
<i>c</i> (nm)	4.63	6.20	7.73	9.26
β (°)	119.4	118.7	118.6	118.8
Z ^{b)}	4	4	4	4

a) The lattice constants of these cycloparaffins were calculated from the data reported by Trzebiatowski *et al.*²⁶⁾ The molecular length is approximately equal to $c/2$. The *b*-axis is the unique axis. The space group is Ia. See Refs. 22) and 27).

b) Z: the number of molecules in the unit cell.

except for the difference in their stem length²⁷⁾ (there exists another monoclinic modification of (CH₂)₇₂²⁸⁾). The conformation of the fold is -t(ggtgg)t- in crystals of such monoclinic cycloparaffins²⁶⁾. All the atoms of folds are located in the crystallographic register on their lamellar surfaces: namely these cycloparaffins have regular sharp folds. The cycloparaffin crystals of (CH₂)₆₀ and (CH₂)₇₂ stained with RuO₄ are shown in Figs. 5(a) and 5(b), respectively. From ED experiments and dark-field imaging by using the reflection corresponding to the 002 reflection of PE, it was concluded that the crystals shown in Fig. 5 are edge-on crystals of the cycloparaffins and their molecular axis in their crystal blocks is oriented normal to the long axis of the block and parallel to the substrate surface²⁷⁾. The fine dark striations due to RuO₄ are recognized in both figures in Fig. 5. The distance between the adjoining striations in these edge-on crystals was found to be practically equal to $c/2$ (see Table 1). The experimental results of RuO₄ staining for (CH₂)₆₀ and (CH₂)₇₂ with a crystallographically regular fold-surface demonstrated that the interfacial region between adjoining monomolecular layers which are closely in contact with each other at their regular fold-surfaces could be stained with RuO₄. In our previous study on epitaxial crystallization of (CH₂)₆₀ on NaCl, it was found that the side surfaces of (CH₂)₆₀ edge-on crystals are not perpendicular to the NaCl surface²⁷⁾. Hence the edge-on crystals of a series of monoclinic cycloparaffins are expected to be inclined: their side surfaces make an angle β (Table 1) with the substrate surface. If RuO₄ were to permeate deeply into the interfacial regions of the edge-on crystals from top to bottom, we could not observe the fine striations due to RuO₄. Thus, it is concluded that RuO₄ permeated "lightly" into the interfacial region between adjoining monomolecular layers of the cycloparaffins.

A cycloparaffin with a long chain-perimeter, (CH₂)₁₂₀ crystallizes in two types of crystal form²⁹⁾: they are orthorhombic and monoclinic forms both of which can be usually found in the edge-on crystals grown on alkali halides and in the single crystals. The straight stems of (CH₂)₁₂₀ are packed in the orthorhombic form in

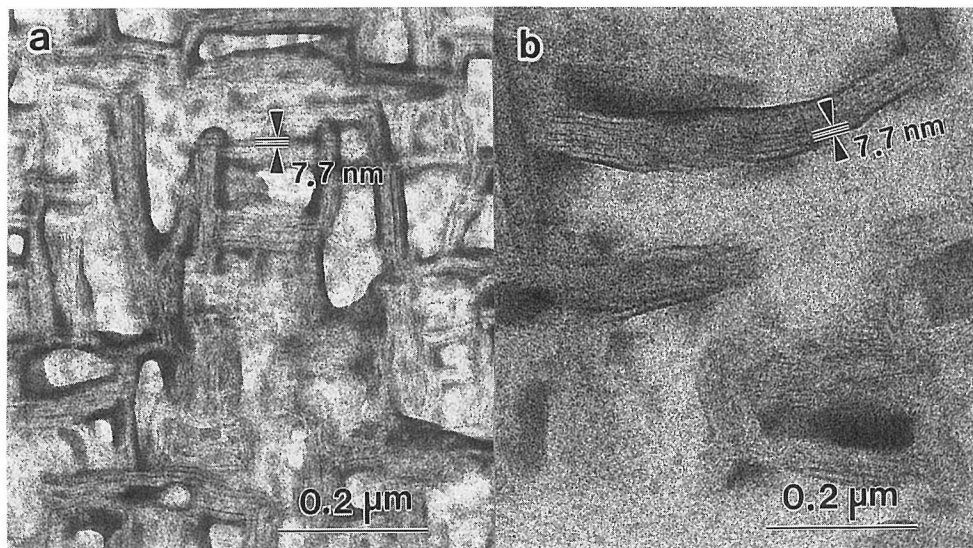


Fig. 6. Edge-on crystals of $(\text{CH}_2)_{120}$ stained with RuO_4 .

(a) Monoclinic $(\text{CH}_2)_{120}$ grown at 60°C on NaCl from a solution.

(b) Orthorhombic $(\text{CH}_2)_{120}$ prepared by annealing the monoclinic $(\text{CH}_2)_{120}$ on NaCl at 110°C for 60 minutes.

the same way as in orthorhombic PE, and in the monoclinic form as in monoclinic PE. The edge-on crystals of $(\text{CH}_2)_{120}$ were grown on NaCl predominantly in the monoclinic form and on KBr and KCl in the orthorhombic form. The monoclinic form of $(\text{CH}_2)_{120}$, as grown on NaCl , was transformed into the orthorhombic form by annealing. These two types of crystal form in $(\text{CH}_2)_{120}$ edge-on crystals were stained with RuO_4 as shown in Figs. 6(a) and 6(b). The molecular axis in the rod-like crystal block is set normal to the rod axis parallel to the substrate surface, for both types of crystal form of $(\text{CH}_2)_{120}$. The fine striations parallel to the longer side of edge-on crystals of $(\text{CH}_2)_{120}$ can be clearly seen in both figures in Fig. 6 and the distance between the adjoining striations should correspond to the thickness of monomolecular layer, roughly to the straight stem length. The thickness measured in Fig. 6 is about 7.7 nm for both types of crystal form, and this value is practically equal to the stem length of $(\text{CH}_2)_{120}^{29}$.

3.3 Edge-on Crystals of Normal Paraffins

Normal paraffin crystals also show a lamellar morphology. The linear zigzag methylene chains with the same length are aligned exactly perpendicular to the surface of orthorhombic normal paraffin platelets. The terminal methyl groups of normal paraffins are arranged in a crystallographically well-defined array on the lamellar surfaces. Two kinds of orthorhombic normal paraffins with different chain length, i.e., $\text{C}_{44}\text{H}_{90}$ and $\text{C}_{50}\text{H}_{102}$ were vapor-deposited on (001) of NaCl in a vacuum. Their well-defined edge-on crystals were obtained as shown in Figs. 7(a) and 7(b). To produce crystals with disordered or indefinitely bounded interfacial regions between monomolecular layers, 1:1 molar mixture of two normal

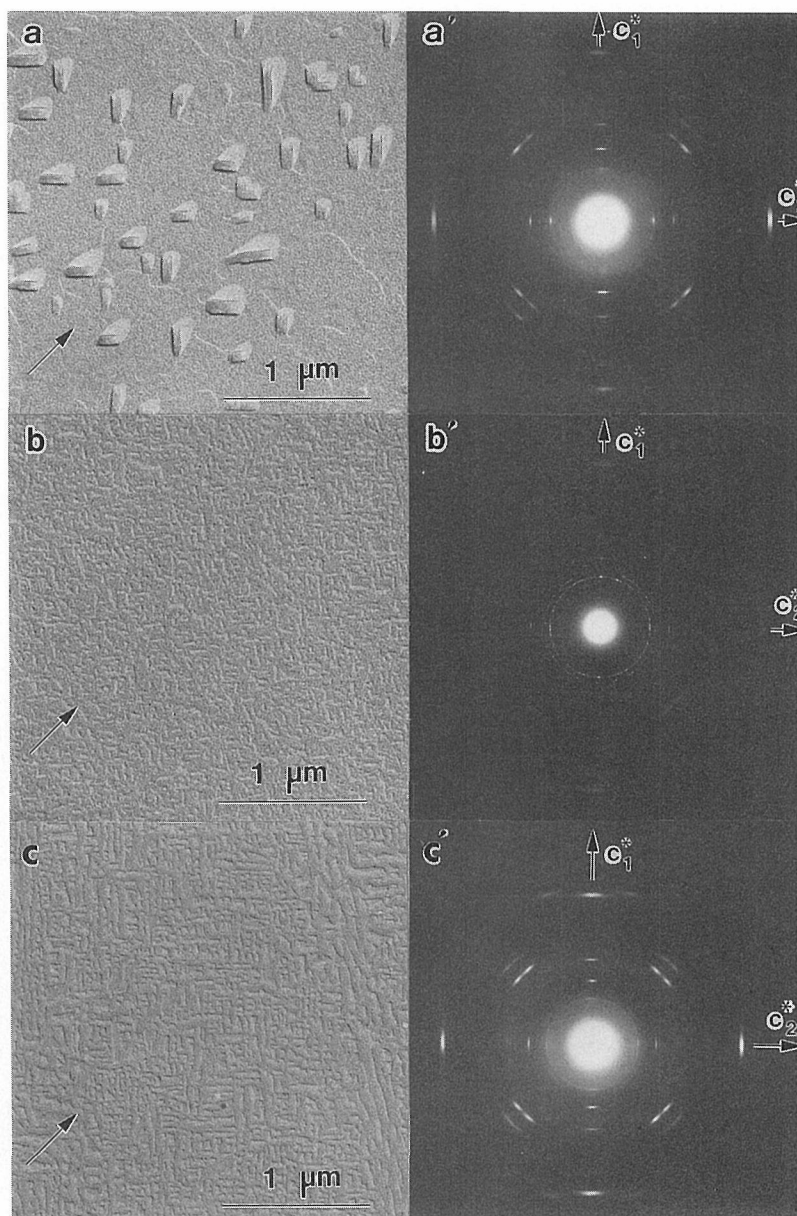


Fig. 7. Edge-on crystals of normal paraffins. They were grown epitaxially on NaCl by vapor-deposition. The arrow indicates the $[100]$ direction of NaCl, and the specimen was shadowed with Pt-Pd in this direction.
 (a) $\text{C}_{44}\text{H}_{90}$, (b) $\text{C}_{50}\text{H}_{102}$, and (c) mixed crystal of $\text{C}_{44}\text{H}_{90}$ and $\text{C}_{50}\text{H}_{102}$ (1:1 mole ratio).
 (a'), (b') and (c') are ED patterns corresponding to (a), (b) and (c), respectively.

paraffins differing in chain length, $C_{44}H_{90}$ and $C_{50}H_{102}$, were first co-crystallized from the melt and subsequently the resulting mixed crystals were vapor-deposited on (001) of NaCl under vacuum (see Fig. 7(c)). In all figures of Fig. 7, fine details were not recognized in the edge-on crystal blocks.

Figures 7(a'), 7(b') and 7(c') show the ED patterns of orthorhombic normal paraffins, $C_{44}H_{90}$, $C_{50}H_{102}$, and a mixed crystal of $C_{44}H_{90}$ and $C_{50}H_{102}$, respectively. The lattice constants of two pure normal paraffins are shown in Table 2³⁰⁾. The ED patterns in Fig. 7 show the same orientation of chains as that in PE edge-on crystals: the molecular axis of normal paraffins is oriented parallel to $[110]$ and $[\bar{1}\bar{1}0]$ of NaCl and the contact plane of normal paraffins is (110). Hence, it was confirmed that the rod-like crystals observed in Fig. 7 are edge-on crystals. The $00l$ reflections of $C_{44}H_{90}$ and $C_{50}H_{102}$ were recognized in the vicinity of the centers of ED patterns in the original negatives of Figs. 7(a') and 7(b'), in two directions perpendicular to each other. The corresponding morphologies of normal paraffins stained with RuO_4 are shown in Fig. 8. The parallel fine striations are running along the longer side of the rod-like crystal blocks in all figures of Fig. 8. In the crystals of normal paraffins, each monomolecular layer has the surfaces composed of terminal methyl groups at both of its sides. In the interfacial region between adjoining monomolecular layers, the surfaces are in contact

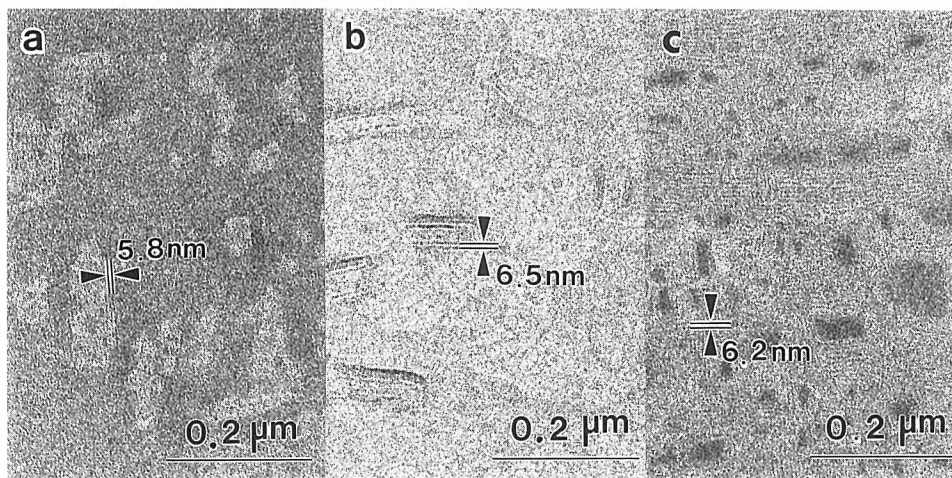


Fig. 8. Edge-on crystals of normal paraffins stained with RuO_4 .
(a) $C_{44}H_{90}$, (b) $C_{50}H_{102}$ and (c) mixed crystal of $C_{44}H_{90}$ and $C_{50}H_{102}$ (1:1 mole ratio).

Table 2. The lattice constants of orthorhombic normal paraffins³⁰⁾.

normal paraffin	Z^*	a (nm)	b (nm)	c (nm)
$(CH_2)_{44}$	4	0.742	0.496	11.55
$(CH_2)_{50}$	4	0.742	0.496	13.07

* Z : the number of molecules in the unit cell.

with each other in a crystallographic register. The distance between the adjoining striations in Figs. 8(a) and 8(b) represents the thickness of monomolecular layer of each pure normal paraffin, and corresponds to $c/2$ in Table 2, namely to the molecular length. The distance between the striations in Fig. 8(c) is, however, estimated at about 6.2 nm, which is the value between those measured in Figs. 8(a) and 8(b). This suggests that the C₅₀H₁₀₂ molecules are not perfectly straight in every monomolecular layer which is to be made up of C₅₀H₁₀₂ and C₄₄H₉₀, and consequently methyl groups are arranged in a rather disordered way in the interfacial regions of the mixed crystal.

4. CONCLUDING REMARKS

The RuO₄ staining method was introduced in this study to examine the morphologies of edge-on crystals of PE, cycloparaffins and normal paraffins epitaxially grown on NaCl. Fine dark striations due to RuO₄ were observed in the edge-on crystal blocks of all the materials studied here. The striations in the blocks were running side by side parallelly to their long dimension. In the case of PE, the distance between the striations corresponded to the lamellar thickness. Thus RuO₄ permeated effectively into the interlamellar regions in the PE edge-on crystals. As for the other materials, the thickness of monomolecular layer measured here corresponded directly to the molecular length. In the case of normal paraffins shown in Fig. 8, the width of striations themselves was not notably changed regardless of the degree of regularity of their interfacial regions. The reason seems to be that the disordered interfacial region in the mixed crystal is not wide enough to permit RuO₄ to permeate deeply into the region.

REFERENCES

- 1) M.G. Dobb, D.J. Jonson and B.P. Saville, *J. Polym. Sci., Polym. Symp.*, **58**, 237 (1977).
- 2) M. Tsuji, S. Isoda, M. Ohara, A. Kawaguchi and K. Katayama, *Polymer*, **23**, 1568 (1982).
- 3) D.T. Grubb, in "Development in Crystalline Polymers", (edited by D.C. Bassett), Applied Science Publishers, New York, chap. 1, (1983).
- 4) R.D. Heidenreich, in "Fundamentals of Transmission Electron Microscopy", Interscience, New York, Chap. 1, (1964).
- 5) M.L. Watson, *J. Biophys. Biochem. Cryst.*, **4**, 475 (1958).
- 6) G. Millongig, *J. Biophys. Biochem. Cryst.*, **11**, 736 (1961).
- 7) E.S. Reynolds, *J. Cell. Biol.*, **17**, 208 (1963).
- 8) A.M. Lawn, *J. Biophys. Biochem. Cryst.*, **7**, 197 (1960).
- 9) J.S. Sutton, *J. Ultrastructure Res.*, **21**, 244 (1968).
- 10) C.E. Hall, M.A. Jakas and F.O. Schmitt, *J. Appl. Phys.*, **16**, 459 (1945).
- 11) K. Kato, *J. Electron Microsc.*, **14**, 220 (1965).
- 12) K. Kato, *Polym. Eng. Sci.*, **7**, 38 (1967).
- 13) R. Vitali and E. Montani, *Polymer*, **21**, 1220 (1980).
- 14) S.Y. Hobbs, *J. Macromol. Sci., Rev. Macromol. Chem.*, **C19**, 221 (1981).
- 15) J.S. Trent, J.I. Scheinbein and P.R. Couchman, *Macromolecules*, **16**, 589 (1983).
- 16) D.E. Morel and D.T. Grubb, *Polym. Commun.*, **25**, 68 (1984).
- 17) S. Chen, T. Cao and Y. Jin, *Polym. Commun.*, **28**, 314 (1987).
- 18) H. Sano, T. Usami and H. Nakagawa, *Polymer*, **27**, 1497 (1986).
- 19) T. Cao, S. Chen and Y. Jin, *Polym. Commun.*, **29**, 66 (1988).

- 20) J. Willems, *Discuss. Faraday Soc.*, **25**, 111 (1958).
- 21) E.W. Fischer, *Kolloid. Z.Z. Polym.*, **159**, 108 (1958).
- 22) K.J. Ihn, M. Tsuji, S. Isoda, A. Kawaguchi, K. Katayama, Y. Tanaka and H. Sato, *Macromolecules*, **23**, 1781 (1990).
- 23) B. Wunderlich, "Macromolecular Physics", chap. 3, Academic Press, New York, (1976).
- 24) P. Groth, *Acta Chem. Scand., Sect. A*, **33**, 199 (1979).
- 25) H.F. Kay and B.A. Newman, *Acta Crystallogr., Sect. B.*, **24**, 615 (1968).
- 26) T. Trzebiatowski, M. Dräger and G.R. Strobl, *Makromol. Chem.*, **183**, 731 (1982).
- 27) K.J. Ihn, M. Tsuji, S. Isoda, A. Kawaguchi, K. Katayama, Y. Tanaka and H. Sato, *Makromol. Chem.*, **190**, 837 (1989).
- 28) G. Lieser, K.-S. Lee and G. Wegner, *Kolloid & Polym. Sci.*, **266**, 419 (1988).
- 29) K.J. Ihn, M. Tsuji, A. Kawaguchi and K. Katayama, in preparation.
- 30) R. Biostelle, in "Current Topics in Material Science" (edited by E. Kaldis), vol. 4, chap. 8, North-Holland, New York, (1980).