Use of freeze–fracture electron microscopy to study the refolding of crystallized chains in block copolymers

Monique Gervais and Bernard Gallot*
Centre de Biophysique Moléculaire, CNRS, 1A, avenue de la Recherche Scientifique, 45045 Orleans Cédex, France

(*Received* 1 *September* 1980; *revised* 5 *November* 1980)

Block copolymers with an amorphous block or polystyrene (PS) of polybutadiene (PB) and a crystallizable block of poly(ethylene oxide) (PEO) or poly(ε-caprolactone) (PCL) exhibit lamellar crystalline structures. In these structures the elementary sheet results from the superposition of two layers: the first contains the amorphous blocks and the second contains the crystallized and refolded PEO or PCL chains. Study of the lamellar structure by freeze–fracture and electron microscopy provides micrographs that show crystallization of the PEO and PCL chains in two superposed layers and confirm the model proposed to explain the results of earlier X-ray diffraction and differential scanning calorimetry studies.

INTRODUCTION

The refolding of crystallized chains in block copolymers formed from a crystallizable block of poly(ethylene oxide) PEO) or poly(ε-caprolactone) (PCL) and an amorphous block of polystyrene (PS) or polybutadiene (PB) was first studied by X-ray diffraction and differential scanning calorimetry[1–4]. Temperature–concentration phase diagrams of the binary systems were drawn, taking each of the systems, block copolymers (preferential solvent of one block), and domains where PEO and PCL blocks crystallize were identified.

Block copolymers polystyrene–poly(ethylene oxide) (SEO), polybutadiene–poly(ethylene oxide) (BEO) and polystyrene–poly(ε-caprolactone) (SCL) in the presence of a preferential solvent for the amorphous block always exhibit a lamellar crystalline structure (*LC*) at temperatures below their melting temperature and for all solvent concentrations where the mesophase exists[1,3,4]. SEO and BEO copolymers in the presence of a preferential solvent for PEO generally exhibit a lamellar crystalline structure (*LCC*) at temperatures below their melting points and for solvent concentrations ranging from zero to a value characteristic of the copolymer studied[2,3]. In these lamellar structures, the elementary sheet is formed by the superposition of the two layers. The first contains the amorphous blocks; the second contains the crystallized and refolded chains; the solvent is localized in the amorphous layer for the *LC* structure (*Figure 1*) and in the crystalline layer for the *LCC* structure (*Figure 2*).

Two types of the crystallized chain refolding have to be considered: firstly, the crystallized chains refold after going through the whole thickness of the crystalline layer; secondly, they can refold after going through only one half of this thickness. Early studies on SEO copolymers provided evidence for the two layer refolding of the crystallized chains.

We will summarize the principal arguments in favour of this model. (a) The lamellar crystalline structure, *LCC*, is observed for SEO and BEO copolymers in a concentrated solution of a preferential solvent for PEO blocks, namely acetic acid and nitromethane for SEO copolymers[2], and acetic and acrylic acid for BEO copolymers[3]. In the *LCC* structure (*Figure 2*) the layer of thickness d_B contains the insoluble amorphous block (PS or PB); the layer of thickness d_A contains the crystallized PEO blocks and the solvent. In order to explain the coexistence of both the crystallized PEO chains and the solvent in a single layer, and the variation of d_A with the solvent concentration (d_A increases with the solvent concentration), the layer of thickness, d_A was assumed to be formed from three lamellae: two outer lamellae containing the crystallized and refolded PEO chains, separated by a layer of solvent. Calculations made on the basis of this hypothesis give the following results.

(1) As the solvent content begins to increase the thickness d_{PEO} of the crystallized chains remains equal to the thickness of the PEO layer in the dry copolymer and the thickness of the solvent layer increases linearly with

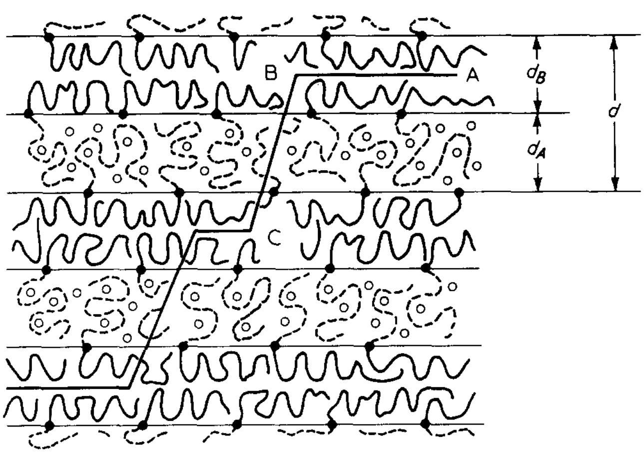

Figure 1 Schematic representation of the lamellar crystalline structure *LC*: *d* = intersheet spacing; d_B = thickness of the layer containing the crystallized chains; d_A = thickness of the layer containing the amorphous chains swollen with solvent. A–B–C = an oblique fracture

0032–3861/81/081129–05$02.00

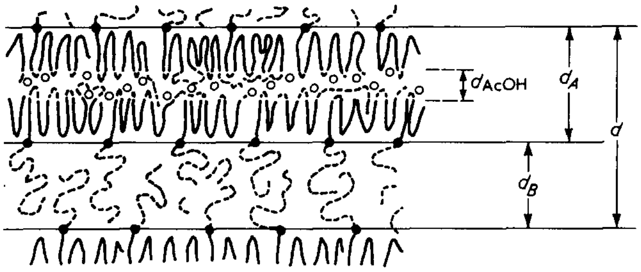

Figure 2 Schematic representation of the lamellar crystalline structure *LCC*[11]: d = intersheet spacing; d_B = thickness of the amorphous layer; d_A = thickness of the layer containing the crystallized chains and the solvent

the amount of solvent C_4 defined as: C_4 = weight of solvent/weight of PEO[2]. The solvent layer progressively separates the crystalline PEO lamellae without dissolving them.

(2) As the solvent content continues to increase, two possibilities exist, depending on the copolymers studied. First, the thickness of the crystallized chains, d_{PEO}, remains constant up to the solvent concentration corresponding to the disappearance of the *LCC* structure. Second, the thickness d_{PEO} varies at several points. For each discontinuity d_{PEO} suddenly decreases while the number (of folds of the PEO chains) increases by one.

Dry SEO and BEO copolymers are about 70% crystalline, but only a small part of the 30% non-crystalline portion is associated with disorder between the crystalline layers[2]. As increasing amounts of solvent are added to the copolymers, the crystallinity, τ, of the PEO blocks decreases and may give rise to discontinuities. The regular decrease of τ corresponds to the swelling of 'loose' PEO situated between the crystalline layers, and the sudden decrease of τ is associated with an increase by one unit of the number of folds of the crystallized PEO chains providing new, disordered PEO, which may be swollen by the solvent[2].

(b) The lamellar crystalline structure, *LC*, is observed for SEO, BEO and SCL copolymers in concentrated solution in a preferential solvent for the amorphous block, namely diethyl phthalate and xylene for SEO and SCL copolymers, and xylene and toluene for BEO copolymers. Discontinuities in the variation of geometrical parameters for the *LC* structure with the solvent concentration or with the crystallization temperature were apparent for some SEO, BEO and SCL copolymers. If the crystallized chains are assumed to fold back after going through the whole thickness of the crystalline layer, discontinuities may be assigned to an increase of half fold of the crystallized chains. If the crystallized chains are assumed to fold back after going through half the thickness of the crystalline layer, discontinuities may be assigned to an increase of an entire chain fold.

The purpose of this paper is to show how the crystallization in two superposed layers of the PCL and PEO blocks can be imaged by freeze-fracture electron microscopy.

EXPERIMENTAL

Materials

Copolymers SEO and SBS (polystyrene-polybutadiene-polystyrene) were prepared by anionic polymerization under high vacuum in tetrahydrofuran dilute solution. The initiator used was cumylpotassium for the SEO copolymer[1] and the dimer dianion of α-methylstyrene/K for the SBS copolymer[5].

SCL copolymer was prepared by a combination of an anionic and a coordination polymerization catalyst[6]. The amorphous polystyrene block was first obtained by anionic polymerization in toluene at room temperature, with s-butyllithium as initiator. After substitution of the OH group by a modified μ-oxo-aluminium-zinc alkoxide, the crystallizable poly(ε-caprolactone) block was produced by coordination of ε-caprolactone into the Al-O bond of the terminal catalyst. The characteristics of the copolymers used in the present study are listed in *Table 1.*

1,2-dihexadecyl-sn-glycero-3-phosphatidylcholine (*DHPC*) was purchased from Fluka (quality puriss).

Freeze-fracture and electron microscopy

A specimen placed in the recess of a small gold disc was first quenched from 25°C to −160°C by immersing in liquid Freon 22 cooled with liquid nitrogen. The frozen sample on its holder was then transferred into the vacuum chamber of a Balzers 301 unit where it was mounted on a precooled stage. It was cleaved with a cold knife at −130°C under high vacuum (2×10^{-6} torr). A replica of the exposed fracture surface was cast: first platinum-carbon was evaporated at 45°C onto the fracture face to accentuate the local topography; evaporated carbon normally produced a replica by binding the isolated patches of the evaporated platinum-carbon[5]. After the replica had been isolated from the sample, it was washed in chloroform and mounted on an uncoated grid. The replica was examined by both transmission in a Siemens Elmiscop 102 electron microscope and reflection in a Jeol 100 C electron microscope equipped with a scanning device.

RESULTS AND DISCUSSION

The technique of freeze-fracture described in the Experimental involves cleavage of frozen samples with a liquid nitrogen-cooled precision ultramicrotome placed inside a vacuum evaporator chamber. The crack induced by the forces exerted on the samples follows the path of least resistance. For example, the fissure generated in biological membranes parted the hydrophobic layer into two lamellae each formed by the aliphatic chains of phospholipids. These results suggest that in the *LC* structure exhibited by the SEO, BEO and SCL copolymers, the fracture would be expected to propagate between the crystallized and refolded PEO or PCL (see *Figure 1*, A and B) similar to the propagation between the aliphatic chains of phospholipids. So the *LC* structure exhibited by the systems copolymer SEO/diethylphthalate or xylene and copolymer SCL/diethylphthalate was studied by freeze-fracture elec-

Table 1 Relative molecular mass and composition of the copolymers studied

Copolymer	PS (wt %)	M_n (PS)[a]	M_n total
SEO 9	29.5	22100	75000
SCL 31	35.0	5600	26000
SBS 362	60.7	31800	47300

[a] PS = polystyrene; M_n = number-average molecular weight

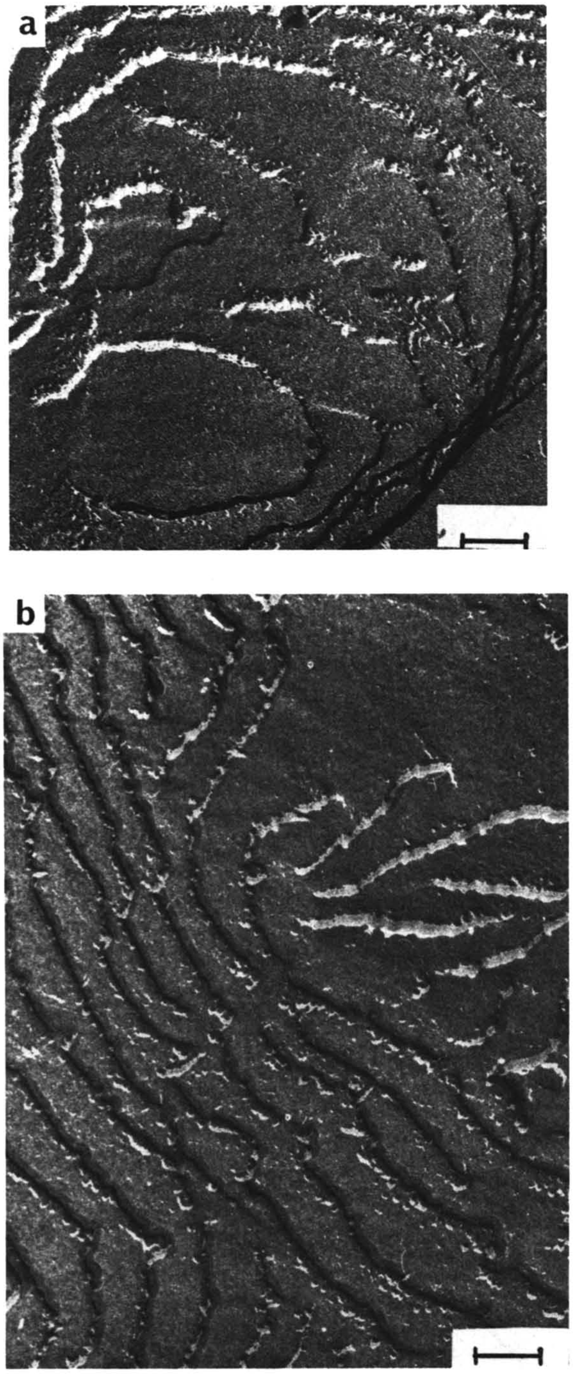

Figure 3 Transmission electron micrographs of freeze–fracture replicas of the *LC* structure, SCL 31/29% diethyl phthalate: (a) x9200, marker = 0.1 μm; (b) x92000, marker = 0.1 μm

tron microscopy. In order to facilitate the interpretation of the micrographs obtained we also submitted two types of systems exhibiting a lamellar structure to freeze–fracture electron microscopy: phospholipid/water system and a copolymer of polystyrene/polybutadiene-polystyrene/preferential solvent of polystyrene system.

Examples of electron micrographs from freeze–fracture replicas of the different systems studied are given in *Figures 3* to *7*.

Figures 3a and *3b* are transmission electron micrographs of freeze fracture replicas of the *LC* structure exhibited by the system SCL/diethylphthalate. *Figures 4* and *5* correspond to freeze–fracture replicas of the *LC* structure exhibited by the SOE/xylene system. *Figure 4*

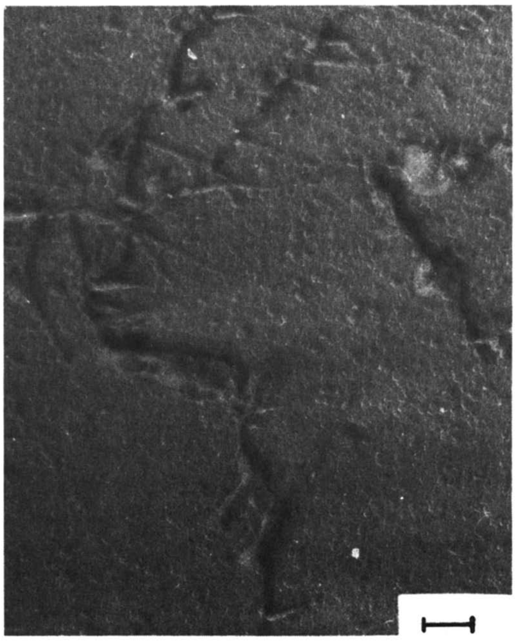

Figure 4 Transmission electron micrograph of freeze–fracture replica of the *LC* structure, SE 09/30% xylene, x70000, marker = 0.1 μm

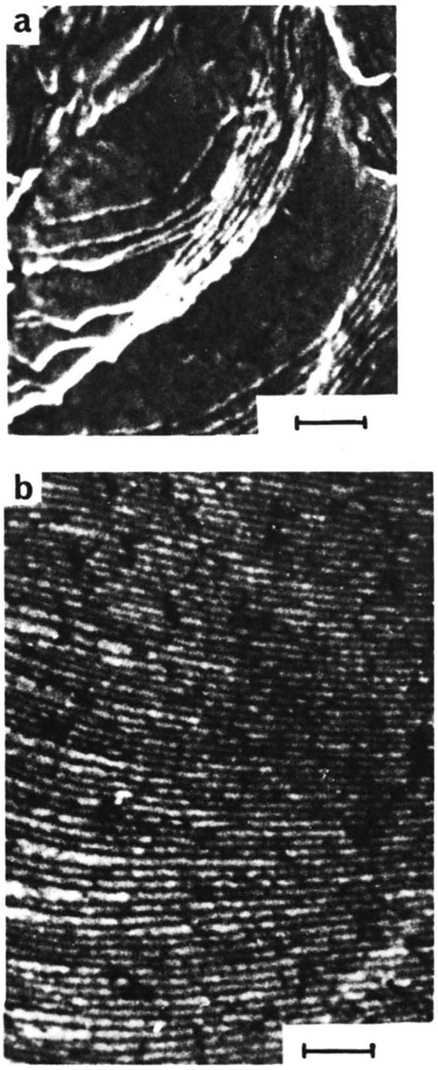

Figure 5 Reflection scanning electron micrographs of freeze–fracture replicas of the *LC* structure, SE 09/30% xylene: (a) oblique fracture, x30000, marker = 0.3 μm; (b) perpendicular fracture, x30000, marker = 0.3 μm

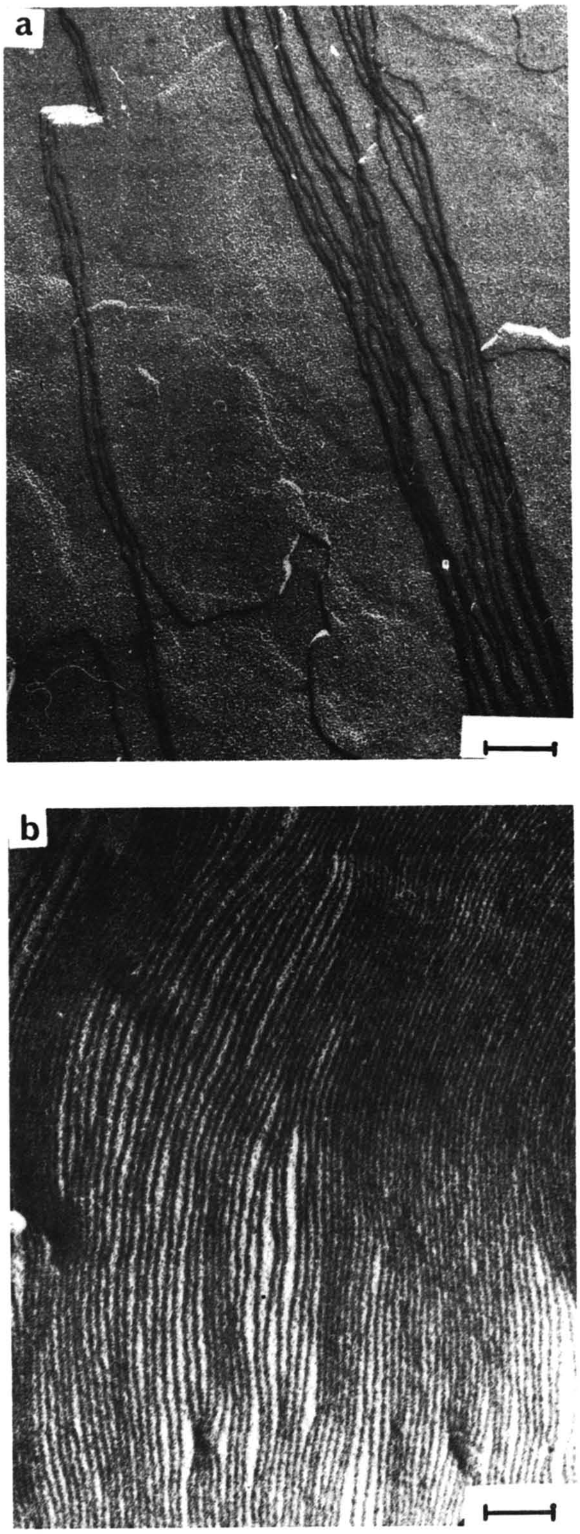

Figure 6 Transmission electron micrographs of freeze–fracture replicas of the *Lβ* structure, DHPC/20% water: (a) oblique fracture, x92000, marker = 0.1 μm; (b) perpendicular fracture, x70000, marker = 0.1 μm

was obtained by transmission electron microscopy and *Figures 5a* and *5b* were obtained by reflection scanning electron microscopy. *Figures 6a* and *6b* are transmission electron micrographs of the lamellar structure *Lβ* of the system phospholipid/water (DPHC/water). *Figure 7* is a transmission electron micrograph of a freeze–fracture replica of the lamellar structure exhibited by the SBS/ethyl methyl ketone system.

Some freeze–fracture electron micrographs of the lamellar *LC* structure of copolymers SCL and SEO (*Figures 3a–5a*) present smooth areas and stairs and are similar to the micrographs obtained with the lamellar structure for the DPHC/water system (*Figure 6a*). Smooth areas correspond to fissure faces and fracture edges (stairs) correspond to the propagation of the crack from one preferred fracture plane to another. As the fracture traverses the frozen specimen, it approaches the lamellar structure layers at different angles and passes more or less rapidly fron one fissure face to another; thus fracture edges are more numerous in some micrographs (*Figures 3b* and *5a*) than in others (*Figures 3a* and *4*). In some instances the fracture continues straight across the layers and the *LC* structure (*Figure 5b*) appears as parallel stripes as for the lamellar structure of phospholipids (*Figure 6b*).

Figures 3 to *5* show that preferred fracture planes exist in the lamellar *LC* structure but it remains to discover precisely the location of such fracture planes. Three possibilities have been envisaged:

(1) the crack propagates at the interface between crystalline and amorphous layers: to do so it must break the copolymer chains;

(2) the crack propagates within the soluble layer between the amorphous polystyrene blocks swollen with solvent;

(3) the crack propagates within the insoluble layer between the crystallized blocks.

In order to choose between these paths, we studied the lamellar structure exhibited by the copolymer SBS 362 swollen with ethyl methyl ketone (EMK, a solvent of the polystyrene) by freeze–fracture and electron microscopy. Unless the polybutadiene chains are broken, the only preferred fracture planes which can be assumed in this system would part the polystyrene chains. The micrographs obtained (*Figure 7*) are quite different from those obtained with the SCL and SEO copolymers. No preferential fracture planes are observed indicating that the crack does not propagate in the swollen polystyrene layer

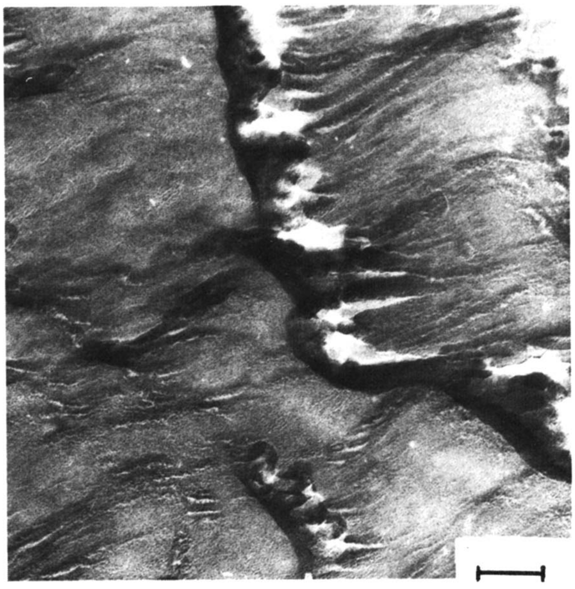

Figure 7 Transmission electron micrograph of freeze–fracture replica of the lamellar structure exhibited by SBS/40% EMC, x92000, marker = 0.1 μm

and that the polybutadiene chains do not break. Thus in the *LC* structure, the fracture can only propagate in the crystalline layer. Lamellar and cylindrical mesophases of the block copolymers are not monocrystals but appear as a grain texture. Such a texture was apparent from electron microscopy[7] and light scattering[8]. In SBS 362/EMK, the crack is assumed to follow the boundaries between the grains where the resistance to fracture is least.

If we assume that, in the *LC* structure, the crystallized PEO or PCL chains go through the whole thickness of the insoluble layer (like the polybutadiene chains in SBS 362/EMK) the micrographs obtained with the *LC* structure should have the same appearance as those obtained for SBS 362/EMK (*Figure 7*). This is not the case and consequently it would suggest that the crystalline chains cannot go through the whole thickness of the insoluble layer before folding. Thus, the existence of preferred fracture planes in the *LC* structure (*Figures 3* to *7*)[5] is additional evidence that in block copolymers with an amorphous and a crystallizable block, the PCL and PEO chains refold in two superposed layers during crystallization.

CONCLUSIONS

A freeze-fracture study of the *LC* structure of copolymers SEO and SCL was performed for various solvent concentrations but no change in the appearance of the electron micrographs was observed. Parallel studies by X-ray diffraction of the effect of the solvent concentration on the *LC* structure of SEO and SCL copolymers showed that addition of solvent to the dry copolymers does not produce discontinuities in the structural parameters. We can thus conclude that in dry SEO and SCL copolymers the PEO and the PCL chains also refold in two layers.

We have studied only two types of crystallizable block but with very different characteristics: PEO takes up a helical conformation and crystallizes in a monoclinic lattice[9], while PCL takes up a planar all-*trans* conformation and crystallizes in an orthorhombic lattice[10]. Thus, the folding in two layers during crystallization is probably a rather general property of copolymers with an amorphous and a crystallizable block.

REFERENCES

1 Gervais, M. and Gallot, B. *Makromol. Chem.* 1973, **171**, 157
2 Gervais, M. and Gallot, B. *Makromol. Chem.* 1973, **174**, 193
3 Gervais, M. and Gallot, B. *Makromol. Chem.* 1977, **178**, 1577
4 Herman, J. J., Jerome, R., Teyssie, Ph., Gervais, M. and Gallot, B. *Makromol. Chem* 1981, **182**, 997
5 Douy, A. and Gallot, B. *Makromol. Chem.* 1973, **165**, 297
6 Herman, J. J., Jerome, R., Teyssie, Ph., Gervais, M. and Gallot, B. *Makromol. Chem.* 1978, **179**, 1111
7 Lewis, P. R. and Price, C. *Polymer* 1972, **13**, 20
8 Stein, R. S. *Polym. Lett.* 1971, **9**, 747
9 Tadokoro, H., Chatani, Y., Yoshihara, T., Tamara, S. and Nurahashi, S. *Makromol. Chem.* 1974, **73**, 109
10 Chatani, Y., Okita, Y., Tadokoro, H. and Yamashita, Y. *Polym. J.* 1970, **1**, 556
11 Gallot, B. 'Liquid crystalline structure of block copolymers' in 'Liquid crystalline order in polymers', (Ed. A. Blumstein) Academic Press, New York, 1978, p 191