

Spontaneous Chiral Segregation in Bidimensional Films

Pierre Nassoy, Michel Goldmann, Othman Bouloussa, and Francis Rondelez

Laboratoire de Physico Chimie des Surfaces et Interfaces, Section de Physique et Chimie, Institut Curie 11, rue Pierre et Marie Curie, 75231 Paris Cedex 05, France

(Received 3 January 1995)

We have monitored spontaneous chiral segregation in Langmuir films of a racemic mixture of *D* and *L* myristoyl-alanine. Following monolayer deposition, we observe first a strong chiral discrimination, followed at longer times by a spatial segregation between *D* and *L* domains. Evidence for discrimination has been obtained by surface pressure/area per molecule measurements, whereas segregation is inferred from optical observations using epifluorescence microscopy and from an analysis of the lattice symmetry using x-ray diffraction.

PACS numbers: 68.55.-a, 64.75.+g, 68.10.-m

The separation of molecules that are mirror images of each other is a long-standing area of research in chemistry and may play a crucial role in biology and pharmacology [1]. As far back as 1848, Pasteur was able to isolate pure enantiomers from an optically inactive racemic mixture of ammonium sodium tartrate through straightforward recrystallization [2]. However, his success was due to very fortunate circumstances. To date less than 1% of referenced chiral species have been resolved by this method, and mixtures of *D* and *L* molecules most often solidify as either racemic compounds (i.e., homogeneous solid phase in which the two enantiomers coexist in the same unit cell) or random solid solutions. An attractive alternative, initially proposed by Arnett [3], is to enhance the chiral characteristics by confining the molecules in a two-dimensional space. The initial appeal of this suggestion was subdued by Andelman and de Gennes [4], who investigated the phase diagrams of chiral amphiphiles in monolayers and concluded that spontaneous chiral segregation should only occur in the case of electrostatic interactions between the headgroups. The result of this conflicting situation was that few experimentalists have tried to tackle the problem. Several groups have reported differences in the surface pressure-area isotherms between the pure enantiomeric amphiphiles and their enantiomer mixtures in Langmuir monolayers spread at the air-water interface [5]. However, this indicates mere chiral discrimination, i.e., different interaction energies between *D-D* (or *L-L*) and *D-L* pairs, but not chiral segregation. Similarly, the spectacular images of spirals [6] or curved hooks [7] observed by epifluorescence microscopy are not necessarily associated with chiral segregation, since straight nonchiral dendrites have been observed in the liquid/solid coexistence region of a pure enantiomeric monolayer [8]. The issue was not resolved any better by the few microscopic structural investigations undertaken on monolayers transferred onto solid substrates. Using atomic force microscopy, Eckhardt *et al.* [9] have observed a chiral lattice in a racemic mixture, indicating possible segregation. However, their interpretation has been questioned by Viswanathan, Za-

sadzinski, and Schwartz and Peterson *et al.* [10], who were able to observe a similar chiral lattice even in monolayers of nonchiral molecules, in agreement with a theoretical prediction by Selinger *et al.* [11].

In this paper we report the observation of a spontaneous chiral segregation in 2D Langmuir monolayers of a racemic mixture of simple chiral amphiphiles (*D* and *L* myristoyl-alanine). The evidence is derived from a combination of several complementary techniques. A distinction is made between the chiral discrimination, which occurs as soon as the floating film is compressed into its condensed phase, and the spatial segregation between *D*-rich and *L*-rich domains, which occurs at longer times.

The myristoyl-alanine molecule (MAA) is derived from myristic fatty acid by replacing its carboxylic headgroup with a single-chiral-center amino acid [5]. The *D*- and *L*-MAA moieties have been synthesized by coupling *D*- and *L*-alanine [Sigma, purity(TLC) > 99%] to the activated myristoyl chloride and characterized by infrared spectroscopy. Their melting points were found to be identical, equal to $93 \pm 1^\circ\text{C}$. The racemate *DL*-MAA was obtained in the same way by using an optically inactive alanine sodium salt as the starting material. The melting point was $110 \pm 1^\circ\text{C}$ [12]. The monolayers were spread onto an ultrapure acidified water subphase ($p\text{H } 2$) in a Langmuir trough. The surface pressure Π was measured as function of the deposited area per molecule A , with the Wilhelmy technique, using filter paper for the hanging plate. The monolayer was examined optically by epifluorescence microscopy (resolution $\sim 1 \mu\text{m}$), using the same apparatus as in Ref. [8] and NBD-hexadecylamine as the fluorescent dye. Structural information was obtained by grazing incidence x-ray diffraction (GID) on the D24 beam line at LURE, Orsay, using the setup described in Ref. [13]. All the experiments have been performed at 19°C .

Figure 1 shows the Π - A curves for *L*- and *DL*-MAA. The isotherm of the *L* compound exhibits a plateau between 60 and $30 \text{ \AA}^2/\text{molecule}$, indicating a coexistence region between liquid expanded (LE) and liquid condensed (LC) phases [14]. The observed transition pres-

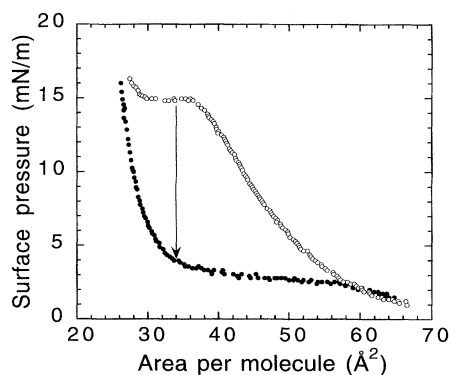


FIG. 1. Surface pressure vs area per molecule isotherms in monolayers of L (●) and DL (○) myristoyl-alanine (Mala) on acidified (pH 2) pure water at $19^\circ C$. Compression rate = $1.8 \text{ \AA}^2/\text{molecule min}$. The arrow indicates the pressure drop observed at fixed area on the LE-LC plateau of DL-MALA monolayers (see text for details).

sure of $2.2 \pm 0.2 \text{ mN m}^{-1}$ is consistent with the detailed phase diagram reported by Bouloussa and Dupeyrat [5]. For area per molecule A between 60 and 90 \AA^2 , the monolayer is in the homogeneous LE state. For A lower than 30 \AA^2 , the monolayer enters into its homogeneous LC state, characterized by its low compressibility. The close-packed area value, as determined by extrapolation of the steep portion of the curve to zero pressure, is $29 \pm 1 \text{ \AA}^2$. It is significantly larger than the 19 \AA^2 usually measured for carboxylic acids. Since molecular models give $28 \pm 1 \text{ \AA}^2/\text{molecule}$ for the alanine head, this suggests that the close-packing area is imposed by the chiral head-group. The stability of the enantiomeric film with respect to dissolution in the subphase is very good, since a material loss of less than $2\%/h$ is detected at 15 mN m^{-1} . All these results also apply to D -Mala, confirming that the physical properties of pure L or D monolayers are unaffected by the mirror symmetry.

The isotherm of the racemic mixture presents both similarities and differences with those of the pure enantiomers. In the low density regime ($A > 60 \text{ \AA}^2$), corresponding to the monophasic LE state, the L - and DL -Mala isotherms can be superimposed; and they are stable for the 2 h duration of the experiment. In the LE-LC plateau region, however, the behavior of the racemic mixture is strikingly different. As shown by the data of Fig. 1, which have been obtained by compressing the monolayer from the LE phase at a rate of $1.8 \text{ \AA}^2/\text{molecule min}$, the width of the coexistence region is reduced by more than 20 \AA^2 and the transition pressure is concomitantly increased to 15 mN m^{-1} . Even more importantly, this state is metastable, at fixed area, the pressure on the plateau quickly drops at a rate of $3 \text{ mN m}^{-1} \text{ min}^{-1}$ during the first 3 min and stabilizes at 2 mN m^{-1} , which is exactly the value measured previously for the pure enantiomers. We have checked that this effect is not caused by the mechanical relaxation sometimes observed in monolay-

ers at large compression rates or by the film desorption, which we estimate to be about $0.08 \text{ \AA}^2/\text{molecule min}$ at 15 mN m^{-1} , corresponding to a pressure variation of less than 1 mN m^{-1} over a 1 h experiment.

The images of the monolayer obtained by fluorescence microscopy emphasize the differences in behavior between L - and DL -Mala, with respect to both the shape of the domains observed in the LE/LC coexistence region and their evolution at long times. For the enantiomeric monolayer, it was shown in Ref. [8] that compression leads to dendritic, solidlike, LC domains. It was observed in particular that they are very stable mechanically: Their shape can resist to large shear stresses up to a limiting value, at which they break into pieces with sharp edges. In contrast, in the racemic monolayer, compression results in the formation of isotropic, circular, LC domains of a typical size of a few 100 \mu m [Fig. 2(a)]. They easily deform into ellipses under surface shear flow, evidencing a mechanical behavior consistent with a highly plastic, amorphous state. Moreover, they undergo a dramatic evolution with time. If compression is stopped, a dark and irregular ring appears at the periphery of each domain after 1 min [Fig. 2(b)]. At even longer times (~ 1 h), the inside of the domains begins to decompose into curved needles, with an optical contrast similar to that of the ring [Fig. 2(c)].

The structures of the monolayers have been characterized at nanoscopic scales by GID. Figure 3(a) displays a typical in-plane diffraction scan for the racemic monolayer spread at the air-water interface. The area per molecule is $A = 30 \text{ \AA}^2$, corresponding to a surface pressure $\Pi = 2 \text{ mN m}^{-1}$. The scan is taken 1 h after stopping the compression, at which point the needles described above have formed and the monolayer has reached its equilibrium state. For comparison, the scan for the pure enantiomer is also shown. We observe immediately the strong similarities between the two scans, with a sloping background due to the diffuse scattering by the water subphase and three peaks at seemingly identical positions. This is ascertained more precisely in Fig. 3(b) which shows separate scans performed in the specific range of Q and over longer counting time periods in order to improve the signal-to-noise ratio. The peaks' positions are at $Q_{xy} = 1.158 \pm 0.001$, 1.316 ± 0.001 , and $1.510 \pm 0.002 \text{ \AA}^{-1}$ for the enantiomer (E), and $Q_{xy} = 1.156 \pm 0.003$, 1.316 ± 0.002 , and $1.520 \pm 0.005 \text{ \AA}^{-1}$ for the racemic (R). The error bars are derived from the curve fitting. The peaks of the two sets of data can be indexed as (10), (01), and (-11) , respectively, corresponding to an identical 2D oblique unit cell with $a = 5.615 \pm 0.005 \text{ \AA}$, $b = 4.935 \pm 0.005 \text{ \AA}$, and $\gamma = 104.8 \pm 0.4^\circ$. Since the peaks are resolution limited, the coherence length is greater than 400 \AA , consistent with a crystalline phase. It should be stressed here that Friedel's law [15] states that diffraction is incapable of distinguishing between the two enantiomeric forms of the same

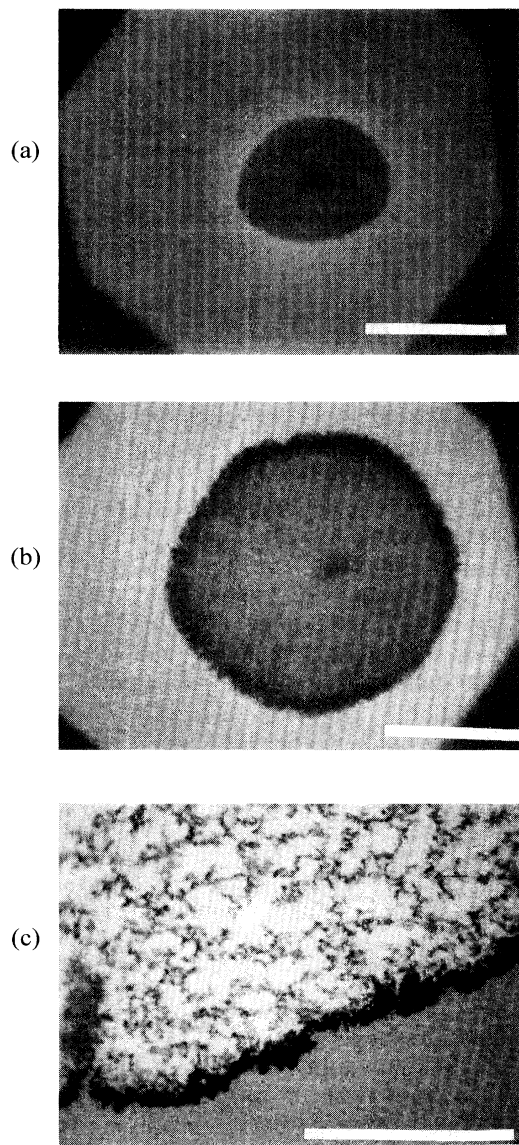


FIG. 2. Fluorescence images of the condensed domains observed in monolayers of a 50/50 mixture of *D*- and *L*-MALa: (a) during compression at $A = 35 \text{ \AA}^2/\text{molecule}$ and $dA/dt = 2 \text{ \AA}^2/\text{molecule min}$; (b) 1 min after stopping the compression at $A = 32 \text{ \AA}^2/\text{molecule}$; (c) after 1 h. The monolayer contains 1% fluorescent probe (NBD-HDA). The bar length corresponds to $200 \mu\text{m}$. Note that the image (c) has been taken at a higher magnification than the images (a) and (b).

crystal, as we have indeed observed by comparing scans obtained with the pure *D* and the pure *L* enantiomer.

Our results allow two stages in the evolution of the condensed phases of the racemic monolayer to be distinguished.

(i) At short times, immediately following monolayer formation, the Π - A measurements show that there is a chiral discrimination of MALa in the condensed phase,

since the LE/LC transitions of the *L*- and *DL*-MALa monolayers occur at markedly different surface pressures: The fact that the racemic mixture requires more compression to condense is consistent with the increased entropy of mixing for multicomponent liquids. On the other hand, epifluorescence microscopy observations show no sign of surface density heterogeneities in the growing LC domains: Their low optical contrast suggests a weak partitioning of the fluorescent dye between the LE and LC phases and the presence of an important free volume in the LC phase. In the pure LE phase no chiral discrimination is observed, and all surface pressures for *L*- and *DL*-MALa are superimposed.

(ii) At long times, the chiral segregation into *D*-rich and *L*-rich domains appears progressively. Π - A measurements indicate that it takes approximately 1 h for the surface pressure to stabilize and reach the thermodynamic conditions characteristic of pure enantiomers. Evidence that the racemic mixture is segregated in the final state is based on our GID observations of an *oblique* unit cell and *identical* to the one observed for the pure *D*- or *L*-MALa 2D crystals. This fact excludes the possibility of having a racemic compound: Indeed, the mirror-image symmetry operation required by a homogeneous *D*- and *L*-MALa equimolar mixture would impose the unit cell to be at least rectangular. Such symmetry has indeed been observed in monolayers of racemic dipalmitoylphosphatidylethanolamine [16] which do not phase separate. Moreover, the positions of the first-order diffraction peaks for a rectangular cell of double area containing one *D*- and one *L*-MALa molecule are calculated to be at 1.299 and 1.317 \AA^{-1} , respectively. This is clearly incompatible with our observation of three peaks at different locations. The possibility of a solid solution can also be rejected, since it would imply a hexagonal cell with a broad peak at 1.12 \AA^{-1} corresponding to a random mixture. Our epifluorescence observations support this x-ray evidence for spontaneous chiral symmetry breaking and the formation of *D*- and *L*-rich domains. The gradual appearance of a dark ring at the periphery of the LC domains and of curved spines in their interior reveals the formation of organized regions within the monolayer. The dye is expelled from these presumably *D*-rich and *L*-rich domains more efficiently than in the initial metastable state. Experiments are currently underway to grow larger crystals in order to be able to probe their handedness by optical methods.

In many aspects, we have performed the 2D analog of the Pasteur experiment, since we have induced a spontaneous spatial segregation in a monolayer initially containing a homogeneous mixture of optical antipodes through a sudden change of the thermodynamic parameters (compression in the liquid condensed state). The segregation leads to domains formed of *D*-rich and *L*-rich regions. However, we are not yet able to identify them visually and *a fortiori* isolate them, as Pasteur did.

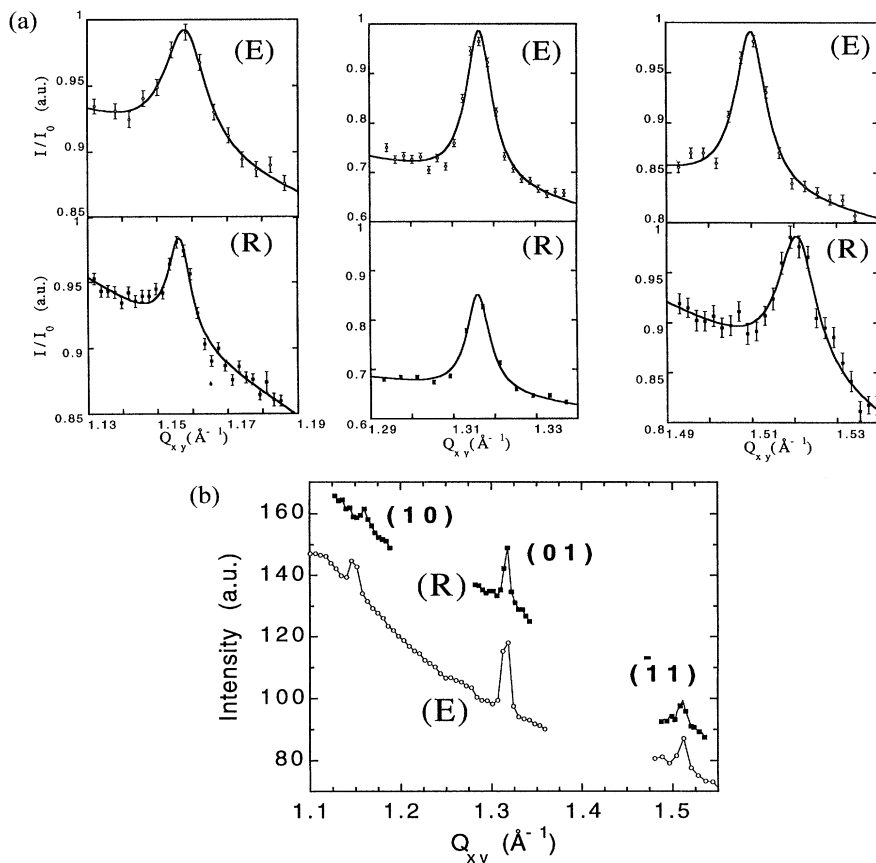


FIG. 3. (a) Extended in-plane diffraction scan for *DL*-MALA monolayer (noted *R*) at $30 \text{ \AA}^2/\text{molecule}$ and 2 mN m^{-1} , 1 h after stopping the compression (■), and for *L*-MALA monolayer (noted *E*) at 5 mN m^{-1} and $30 \text{ \AA}^2/\text{molecule}$ (○). The sloping background is due to scattering by the bulk aqueous subphase; (b) first order (10), (01), and $(\bar{1}1)$ diffraction peaks from *L*-MALA (top) and *DL*-MALA (bottom) monolayers. The solid lines are Lorentzian fits to experimental data.

We gratefully acknowledge helpful discussions with D. Andelman, R.F. Bruinsma, C. Caroli, R. Klein, and J. Malthête. This work has been supported by an industrial grant from the Direction des Recherches, Techniques and Environment of Elf.

- [1] J. Jacques, A. Collet, and S.H. Wilen, *Enantiomers, Racemates and Resolutions* (Wiley, New York, 1981).
- [2] L. Pasteur, C. R. Acad. Sci. **26**, 535 (1848).
- [3] M.V. Stewart and E.M. Arnett, in *Topics in Stereochemistry*, edited by N.L. Allinger, E.L. Eliel, and S.H. Wilen (Wiley, New York, 1982).
- [4] D. Andelman and P.G. De Gennes, C. R. Acad. Sci. **307**, 233 (1988); D. Andelman, J. Am. Chem. Soc. **111**, 6536 (1989).
- [5] E.M. Arnett, N.G. Harvey, and P.L. Rose, Acc. Chem. Res. **22**, 131 (1989); O. Bouloussa and M. Dupeyrat, Biochim. Biophys. Acta **938**, 395 (1988); M. Dvolaitzky and M.A. Guedeau-Boudeville, Langmuir **5**, 1200 (1989).
- [6] H.M. Mc Connell, Annu. Rev. Phys. Chem. **42**, 171 (1991).
- [7] D.P. Parazak, J.Y.J. Uang, B. Turner, and K.J. Stine, Langmuir **10**, 3787 (1994).
- [8] S. Akamatsu, O. Bouloussa, K. To, and F. Rondelez, Phys. Rev. A **46**, 4504 (1992).
- [9] C.J. Eckhardt, N.M. Peachey, D.R. Swanson, J.M. Takacs, M.A. Khan, X. Gang, J.H. Kim, J. Wang, and R.A. Uphaus, Nature (London) **362**, 614 (1993).
- [10] R. Viswanathan, J.A. Zasadzinski, and D.K. Schwartz, Nature (London) **368**, 440 (1994); I.R. Peterson, R.M. Kenn, A. Goudot, P. Fontaine, F. Rondelez, W. Bouwman, and K. Kjaer (to be published).
- [11] J.V. Selinger, Z.G. Wang, R.F. Bruinsma, and C.M. Knobler, Phys. Rev. Lett. **70**, 1139 (1993).
- [12] The large difference between the melting points of the racemate and of the enantiomers can be taken as evidence that spontaneous segregation does not occur in 3D.
- [13] M. Goldmann, P. Nassoy, F. Rondelez, A. Renault, S. Shin, and S.A. Rice, J. Phys. II (France) **4**, 773 (1994).
- [14] We use "liquid condensed" as a generic term to describe any phase exhibiting in-plane positional (crystal) or orientational (hexatic phase) ordering.
- [15] G. Friedel, *Leçons de cristallographie* (Berger Levrault, 1926). Anomalous scattering is the only experimental case where Friedel's law is broken [see the recent paper by R. Colella, Acta Crystallogr. **A50**, 50 (1994)].
- [16] C. Böhm, H. Möhwald, L. Leiserowitz, J. Als Nielsen, and K. Kjaer, Biophys. J. **64**, 553 (1993).

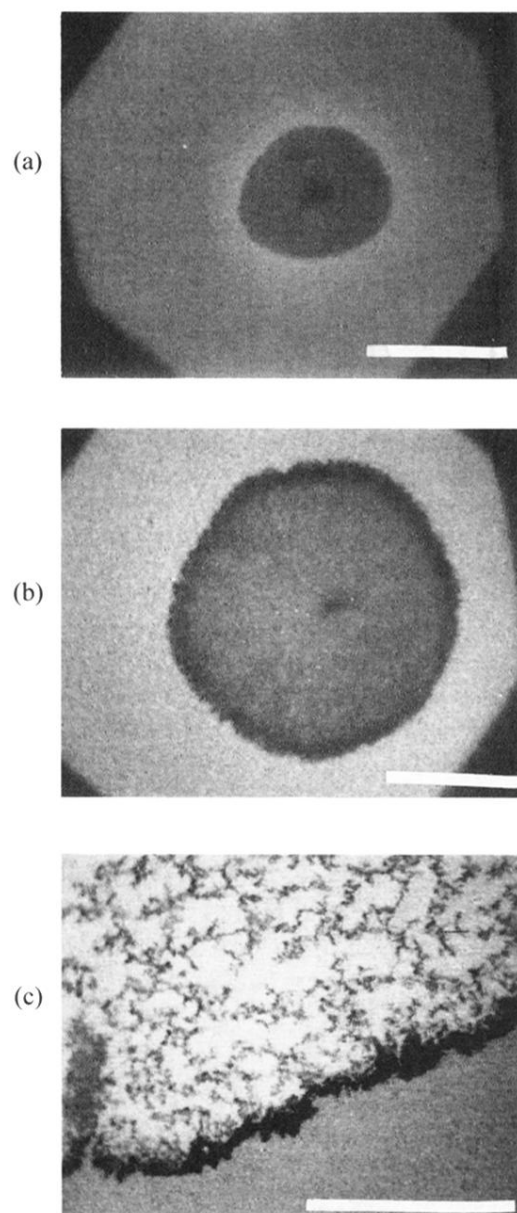


FIG. 2. Fluorescence images of the condensed domains observed in monolayers of a 50/50 mixture of *D*- and *L*-MAla: (a) during compression at $A = 35 \text{ \AA}^2/\text{molecule}$ and $dA/dt = 2 \text{ \AA}^2/\text{molecule min}$; (b) 1 min after stopping the compression at $A = 32 \text{ \AA}^2/\text{molecule}$; (c) after 1 h. The monolayer contains 1% fluorescent probe (NBD-HDA). The bar length corresponds to 200 \mu m . Note that the image (c) has been taken at a higher magnification than the images (a) and (b).