# ENANTIOMERIC CRYSTALLIZATION FROM DL-ASPARTIC AND DL-GLUTAMIC ACIDS: IMPLICATIONS FOR BIOMOLECULAR CHIRALITY IN THE ORIGIN OF LIFE

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**Abstract.** Amino acids in living systems consist almost exclusively of the L-enantiomer. How and when this homochiral characteristic of life came to be has been a matter of intense investigation for many years. Among the hypotheses proposed to explain the appearance of chiral homogeneity, the spontaneous resolution of conglomerates seems one of the most plausible. Racemic solids may crystallize from solution either as racemic compounds (both enantiomeric molecules in the same crystal), or less commonly as conglomerates (each enantiomer molecule separate in different enantiomeric crystals). Only conglomerates can develop a spontaneous resolution (one of the enantiomeric molecule crystallizes preferentially, the other one remains in solution). Most of natural amino acids are racemic compounds at moderate temperatures. How can we expect a hypothetical spontaneous resolution of these amino acids if they are not conglomerates? In this paper we show how DL-aspartic and DL-glutamic amino acids (racemic compounds), crystallize at ambient conditions as true conglomerates. The experimental conditions here described, that allows this 'anomalous' behaviour, could be also found in natural sedimentary environments. We suggest that these experimental procedures and its natural equivalents, have a potential interest for the investigation of the spontaneous resolution of racemic compounds comprising molecules associated with the origin of life.

**Keywords:** amino acids, chirality, crystallization, enantiomer, origin of life, resolution

## 1. Introduction

A chiral object can exists either in the 'right-handed' or 'left-handed' enantiomorphic forms, which are mirror images of one another. Usually these chiral objects are optically active (they can rotate the plane of polarized light). It is well known that crucial organic molecules associated with life, as amino acids, are chiral. Amino acids monomers occurring in proteins have only the L-configuration, therefore proteins are composed of monomers units which are 'enantiomerically pure'. The origin of this selective chirality of amino acids has remained a fundamental enigma since the time of Pasteur, some 140 years ago. Chirality and optical activity are regarded as a principal criterion for life, both on Earth and elsewhere in the universe. The fundamental questions of how molecules of a unique chirality arose in Nature puzzle scientists, thus numerous theoretical and experimental studies addressing these questions have appeared in the last years (for a review

see Bonner, 1991). The spontaneous resolution on crystallization is a plausible hypotheses proposed to explain the abiotic origin of molecular chirality in nature. Although the spontaneous resolution mechanism over a period of time will produce an equal number of opposing resolutions, leaving a net racemic condition, much work has been done on this subject (Kondepudi *et al.*, 1990; Buhse *et al.*, 1999; for a review see Bonner, 1991).

An equimolar mixture of two enantiomers (L and D) whose physical state is unspecified or unknown is called a racemate or racemic modification. Crystalline racemate or racemic modification may belong to one of two different classes: in the first case the crystalline solid is a conglomerate, i.e., a mechanical mixture of crystals of the two pure enantiomers, where each enantiomeric molecule separate into its own enantiomeric crystal; the second case corresponds to the formation of a racemic compound (by far most frequently observed type) in which the two enantiomeric molecules are present in equal quantities. This occurs in the same well-defined arrangement within the crystal lattice, i.e., both enantiomeric molecules are found in the same 'racemic' crystal. This is a case of polymorphism and as such, the nature of the racemate (racemic compound or conglomerate) depends mainly on pressure and temperature (Jacques *et al.*, 1991, p. 140). Simple comparison of X-ray difractograms or infrared spectra of racemates with their corresponding enantiomers, constitutes a excellent test to determine the nature of the racemate. If the racemate is a conglomerate, its X-ray diffraction pattern and infrared spectrum will be superimposed on that of the enantiomers. On the other hand, if we are dealing with a racemic compound, they will be different because crystals of the enantiomers and the racemic compound have different organization, which gives rise to differences in diffraction and vibration modes.

In the crystallization process of conglomerates any of the two enantimeric molecules may crystallize preferentially, while the other one will remain in solution. We call this phenomenon 'spontaneous resolution', through which a real separation of the two enantiomeric molecules occurs. Resolution, while purely accidentally, can ultimately be rendered irreversible, for example, as a consequence of: 1) the runoff of the mother liquor away from the crystalline deposit; 2) through chemical transformation of the enantiomer remaining in solution in presence of a small quantity of an appropriate reagent; 3) the enantiomer remaining in solution during the slow crystallization of a racemic conglomerate, rapidly convert into the other enantiomer before crystallization is complete, thus a 'second order asymmetric transformation' may occur, with the entire racemate crystallizing as a single enantiomer. The spontaneous resolution of conglomerates as a mechanism of separation of the enantiomeric molecules was early and widely championed as one of the most plausible explanation for the origin of optical activity on Earth (Bonner, 1972), an idea which is still supported today. Spontaneous resolution would appear to be the most likely terrestrial mechanism for producing the prebiotic homogeneity necessary for the emergence of life. Spontaneous resolution could only be expected from conglomerates, however they are relatively rare and only 250 cases have been

reported (Jacques *et al.*, 1991, p. 53). In fact, most of the protein amino acids form, at moderate temperature, racemic crystals which never develop spontaneous resolution. Thus, how can we expect a hypothetical spontaneous resolution of natural amino acids to explain the origin of molecular chirality if they are not conglomerates?

At this point it is important to know under which experimental and natural conditions we could find amino acids in the form of conglomerates or racemic compounds (polymorphic phenomena) (Jacques *et al.*, 1991, p. 140). We here establish an experimental system with natural equivalents, under which DL-aspartic and DL-glutamic amino acids (racemic compounds at room conditions), crystallize as true conglomerates.

Aspartic and glutamic acids are among the most abundant amino acids formed in experiments simulating the primitive Earth and have been found to be key amino acids for thermal copolymerization (Fox, 1995). They are also present in the Murchison meteorite (Cronin *et al.*, 1988) and form part of the repetitive sequence calculated for the original ancestral molecule of Ferrodixine (Eck, 1966), which is believed to be one of the first proteins formed on Earth (Hall and Rao, 1971).

## 2. Experimental Procedure

We prepared two different solutions of 1000 mL from doubly distillated water by dissolving 10 g of aspartic acid in one of them and 10 g of glutamic acid in the other one (DL-sigma). The resulting solutions were constantly stirred and heated to 80 °C during 30 min to ensure complete dissolution of the solute. Solutions were not buffered. Samples of 50 mL of each solution were transferred to a 100 mL cylindrical glass vessel and left for crystals to grow. The initial concentration was such that cooling alone did not produce crystals. Thus evaporation of solution was necessary. These evaporation experiments were performed attempting to mimic possible primitive earth conditions in off-shore or laguna-type environments. A parallel experiment was conducted with another set of similar samples of solution but with a porous material partially immersed in the solutions (initially 2 cm into the solution, 8 cm out of solution). We used as porous materials both a refractory porous brick (insulating fire brick TC-26 from Thermal Ceramics Company, 100 × 10 × 10 mm prisms), and sheets (100 × 20 mm) of Whatman paper grade 3 Chr. The latter is used in chromatographic techniques as a reference material with homogeneous texture and porous size, although this paper, made of cellulose, is chiral and related forms of cellulose as wool and cotton have been used by seeding for partial optical resolution of DL-aspartic copper complex (Harada, 1968). In our case the porous materials used allow capillary rise, concentration by evaporation and crystallization of the amino acids in a narrow upper zone of the materials. The evaporation experiments mimic a sedimentary environment such as a playa where one may expect capillary rise of saline ground water. The experiments were car-

ried out at room conditions, without control of temperature and relative humidity (variation interval of both parameters, 18–24° and 35–55%) for a few days until crystallization occurred. All crystals were collected and, if necessary, filtered and dried before total evaporation of solutions (8–14 days for free solutions and 3–4 days for porous media). All experiments have been performed in at least 6 parallel independent series and repeated several times along months. Morphology of crystals were studied using a binocular lens. X-ray diffraction patterns and infrared spectra were used for enantiomer versus racemic phase identification.

## 3. Results and Discussion

When aspartic and glutamic amino acids were crystallized from free solutions, the result was always crystals of racemic compounds DL-aspartic and DL-glutamic amino acids (Figures 1 and 2). When aspartic and glutamic amino acids were crystallized from solutions inside both porous media, brick and paper, the result was always crystals of conglomerates D- and L-enantiomeric aspartic or glutamic amino acids (Figures 1–3). Prismatic crystals were the predominant morphology in both DL-aspartic and DL-glutamic racemic compounds that indicates low supersaturation level, whereas efflorescences, whiskers and crusts were produced in both conglomerates (D- and L-enantiomeric aspartic or glutamic acids), all these characteristic morphologies of high level of supersaturation (Sunawaga, 1987).

This is a typical case of polymorphism, in which two phases Dcrystal + Lcrystal and DLcrystal are involved. Because racemic crystals are more stable than enantiomeric conglomerates, the free energy change for the 'reaction':

Dcrystal + Lcrystal – Racemic compound

is always negative. For this the enantiomer D- and L-phases are metastable with respect racemic LD phase (Jacques *et al.*, 1991, p. 97). The crystallization process in free solution agrees with the thermodynamic model, i.e., giving rise to the more stable racemic compounds phases. However in the porous media the metastable enantiomeric phases occur as if the racemates were conglomerates. Why crystallize the not 'correct' thermodynamic phases from these porous media?

It is well documented that crystals resulting from high supersaturation may be different in habit to those formed from lower supersaturation, furthermore, the former may give rise to metastable phases. This is because at high supersaturation level, thermodynamic criteria compete with kinetic criteria and in many cases the less stable thermodynamically polymorphs phase may be crystallized if it is kinetically the most favorable. Therefore there is a relationship between the supersaturation threshold (defined as the maximum supersaturation that can be reached for a solution before crystallization occurs) and the appearance of metastable phases. The value of the maximum supersaturation depends not only

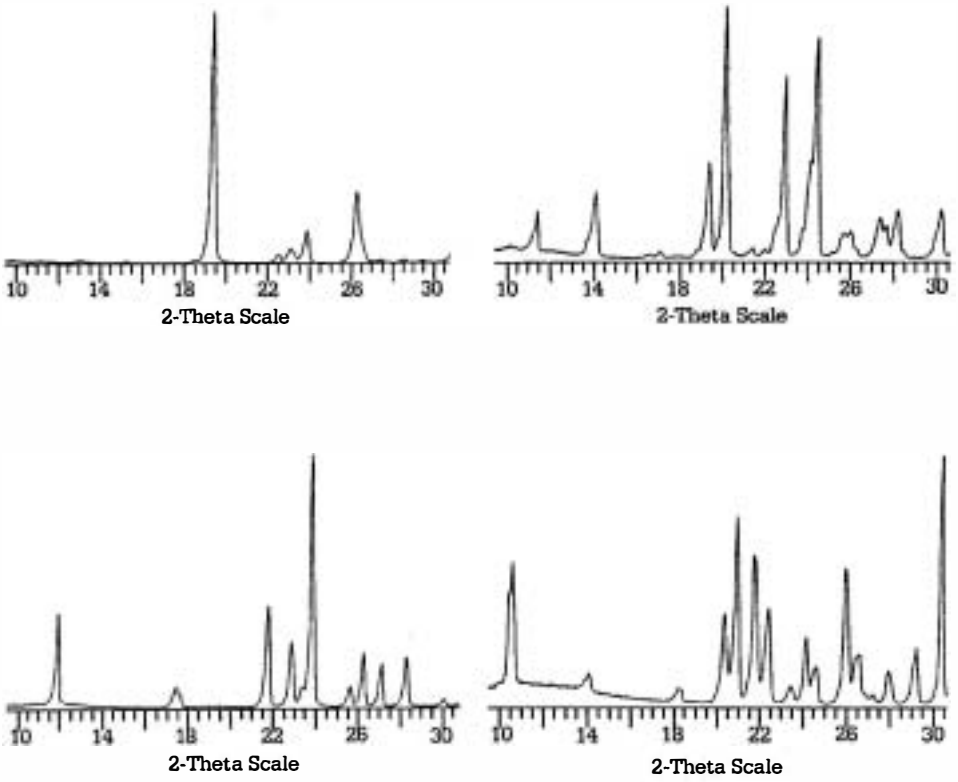


*Figure 1.* X-ray diffraction patterns of aspartic amino acid (left) and glutamic amino acid (right). Above from samples obtained in free solutions, which correspond to DL-racemic phases. Below from samples obtained in porous materials, which correspond to D- and L-enantiomeric phases.

on the nature of the solute and solvent, but also on the temperature, stirring and mechanical shocks, the thermal history, the total mass of solution etc. (Khamskii, 1969; Jong, 1978). In an evolving system (our case), the kinetic of crystallization depends on the rate at which the system moves from equilibrium, i.e., there is a relationship between: 1) the maximum supersaturation which can be attained before crystallization begins, and 2) the value of the supersaturation gradient in time (Prieto, 1990). In turn this depends on the transport properties of the medium in which crystallization takes place. Moreover the limited particle mobility in a porous media involves a wide metastability range (high supersaturation level), as compared to the crystallization behaviour in free solutions (Prieto, 1994).

In short, the crystallization technique used in this study based on the capillary rise, concentration and supersaturation by evaporation of solution through the porous structure, enable the system to go far from the equilibrium in a non-linear process (Ortoleva, 1994). This allows reaching of the unstable region where crystallization results at high level of supersaturation and metastable phases can be

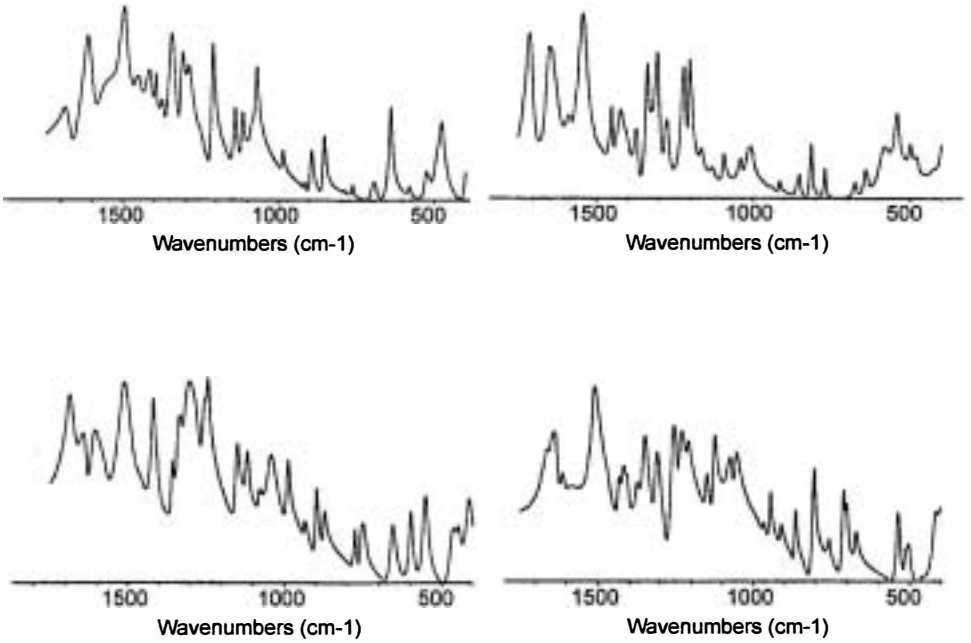


*Figure 2.* Infrared spectra of aspartic amino acid (left) and of glutamic amino acid (right). Above from samples obtained in free solutions, which correspond to DL-racemic phases. Below from samples obtained in porous materials, which correspond to D- and L-enantiomeric phases.

expected. More examples and discussion of crystallization processes in natural and artificial porous media can be found in the literature (Lopez-Acevedo, 1997).

Thus, the crystallization of D- and L-enantiomeric phases of aspartic and glutamic amino acids is an 'anomalous' behaviour that may serve as a first insight into the problem of crystallization of metastable phases in nature. The general principle that the kinetically most favourable phases will form, as metastable, rather than those thermodynamically more favorable which involves the greatest reduction in free energy, has been recognized by chemists for some time as the Ostwald Rule (Putnis and McConnell, 1980).

The proposed amino acid crystallization method in a heterogeneous porous material as a brick, allows the simulation in short-time period of a sedimentary media in which crystallization occurs based on capillary rise and concentration. The less 'realistic' porous medium, the Whatman paper, has a porosity and pore-size distribution higher and far more uniform allowing that experimental data can be more exactly reproducible in any place and easily comparable.

According to Kondepudi and Nelson (1985), and Avetisov *et al.* (1991), the kinetic behaviour of a hypothetical unstable, far from equilibrium, racemic system which pass through a bifurcation point may randomly divaricate into a condition of enantiomeric purity. This is because such far from equilibrium bifurcations endow the system with a pronounced sensitivity to the slightest factors, which might

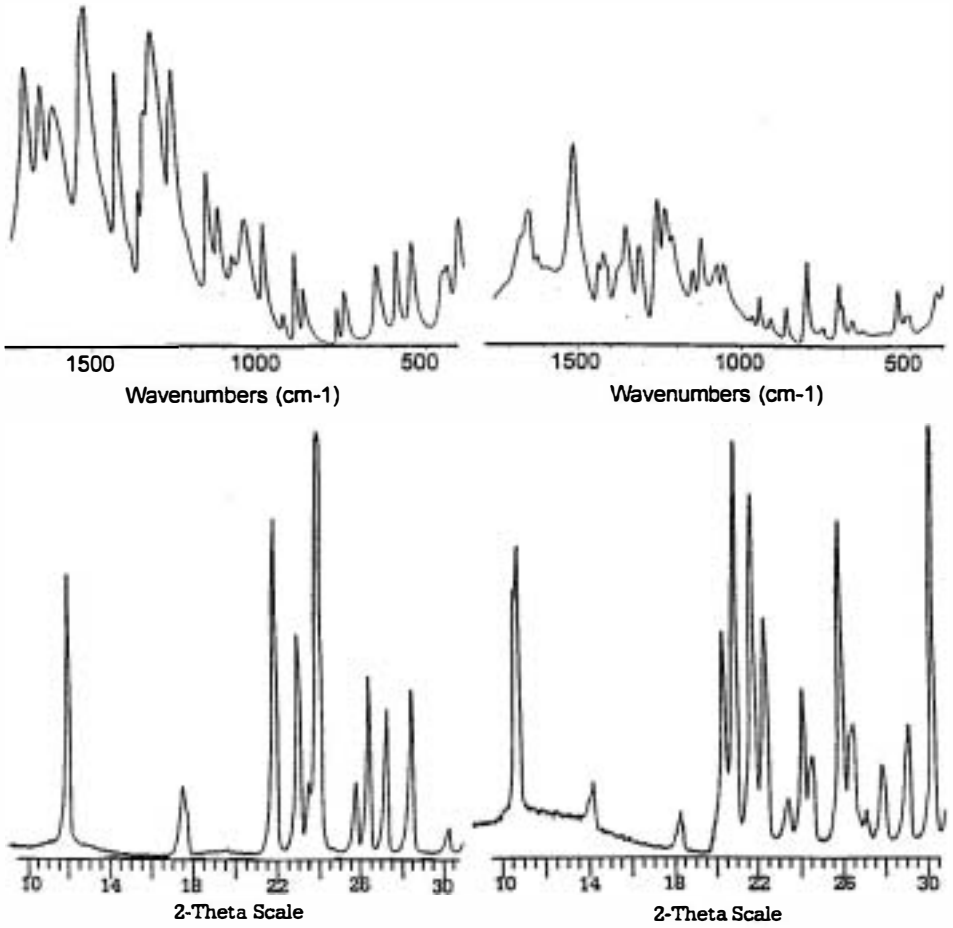


*Figure 3.* Infrared spectra (above) and X-ray diffraction patterns (below) of pure commercial crystals of L-aspartic acid (left) and L-glutamic acid (right) used as control.

induce the non-random selection of a preferred molecular chirality (Nicolis and Prigogine, 1981). We proposed before (Viedma, 2000) that systems that allow the reaching of high levels of supersaturation, as this porous-solution system does, trigger to go far from equilibrium where these speculations find its best place to be tested.

Finally, we may conclude that if the possibility of separating two enantiomers by direct crystallization of their mixture implies that the racemate behaves as a conglomerate, then the resolution of the two natural amino acids studied in this work may be more easily achieved in a porous medium than in free solution. This may have happened more easily in a colloidal suspension, in a playa, a sand bar, or another sedimentary porous media than in a open lagoon or stationary mass of free water.

In short, if we look for spontaneous resolution we must search for systems where amino acids avoid early crystallization, if they are racemic compounds, and therefore can go far from equilibrium where conglomerate behaviour can be expected.

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## References

Avetisov, V. A., Goldanskii, V. I. and Kuz'min, V. V.: 1991, Handedness, Origin of Life and Evolution, *Physics Today* **44**, 33–41.

Bonner, W. A.: 1972, in C. Ponnamperuna (ed.), *Exobiology*, North-Holland, Amsterdam, pp. 170–234.

Bonner, W. A.: 1991, The Origin and Amplification of Biomolecular Chirality, *Origin of Life and Evolution of the Biosphere* **21**, 59–111.

Buhse, T., Kondepudi, K. and Hoskins, B.: 1999, Kinetic of Chiral Resolution in Stirred Crystallization of D/L-Glutamic Acid, *Chirality* **11**, 343–348.

Cronin, J. R., Pizarello, S. and Cruikshank, D. P.: 1988, in J. F. Kerridge and M. S. Matthews (eds), *Meteorites and the Early Solar System*, Univ. Arizona Press, Tucson, pp. 819–857.

Eck, R. V.: 1966, Evolution of the Structure of Ferredoxin Based on Living Relics of Primitive Amino Acid Sequences, *Science* **152**, 363–366.

Fox, S. W.: 1995, Thermal Synthesis of Amino Acids and the Origin of Life, *Geochimica et Cosmochimica Acta* **59**, 1213–1214.

Hall, D. O. and Rao, K. K.: 1971, Role for Ferrodoxins in the Origin of Life and Biological Evolution, *Nature* **233**, 136–138.

Harada, K.: 1968, Optical Resolution of DL-Aspartic Acid Copper Complex by the Use of Biopolymers, *Nature* **218**, 199.

Jacques, J., Collet, A. and Wilen, S.: 1991, Enantiomers, Racemates and Resolutions, Krieger Publishing Company, Florida, 447.

Jolles, P. (ed.): 1998, *D-Amino Acids in Sequences of Secreted Peptides of Multicellular Organisms*, Birkhauser, Basel, pp. 159–188.

Jong, E. J.: 1978, in E. J. Jong and S. J. Janccic (eds), *Industrial Crystallization*, Elsevier, Amsterdam.

Kondepudi, D. K. and Nelson, G. W.: 1985, Weak Neutral Current and the Origin of Biomolecular Chirality, *Nature* **314**, 438–441.

Kondepudi, D. K., Kaufman, R. J. and Singh, N.: 1990, Chiral Symmetry Breaking in Sodium Chlorate Crystallization, *Science* **250**, 975–976.

Khamskii, E. V.: 1969, *Crystallization from Solutions*, Consultants Bureau, Plenum Publishing Corp., New York.

Lopez-Acevedo, V., Viedma, C., Gonzalez, V. and La Iglesia, A.: 1997, Salt Crystallization in Porous Construction Materials II. Mass Transport and Crystallization Processes, *J. Crystal Growth* **182**, 103–110.

Ortoleva, P. J.: 1994, *Geochemical Self-Organization*, Oxford University Press, New York, p. 411.

Nicolis, G. and Prigogine, I.: 1981, Symmetry-Breaking and Pattern Selection in Far-From Equilibrium Systems, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 659–663.

Prieto, M., Putnis, A. and Fernandez-Diaz, L.: 1990, Factors Controlling the Kinetics of Crystallization: Supersaturation Evolution in a Porous Medium. Application to Barite Crystallization, *Geological Magazine* **127**, 85–95.

Prieto, M., Putnis, A., Fernandez-Diaz, L. and Lopez-Andres, S.: 1994, Metastability in Diffusing-Reacting Systems, *Journal of Crystal Growth* **142**, 225–235.

Putnis, A. and McConnell, J. D. C.: 1980, *Principles of Mineral Behaviour, Blackwell Scientific Publications*, Oxford, p. 257.

Sunagawa, A.: 1987, *Morphology of Crystals*, Part B. Tokyo, Terra Scientific Publishing Company, p. 743.

Viedma, C.: 2000, Formation of Peptide Bonds from Metastable Versus Crystalline Phase: Implications for the Origin of Life, *Origins of Life and Evolution of the Biosphere* **30**, 549–556.