

The Morse code effect: A crystal–crystal transformation observed in gel-grown lead (II) oxalate crystals

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ABSTRACT

This paper reports on an unusual crystal–crystal transformation phenomenon, which we have called the *Morse Code Effect*, based on the change in appearance of lead(II) oxalate crystals grown in agarose gels.

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1. Introduction

The growth of crystals in gels has a long history, but dates from the work of Liesegang [1]. The ring patterns named after Liesegang were first observed in photographic quality gelatin and were produced by diffusing together soluble ionic species in the gel to form insoluble colloidal precipitates. The precipitates formed usually had a distinctive band or ring structure. A secondary process, which Liesegang must have regarded as an unwelcome interference, was the occasional appearance of crystals in the bands. This secondary process is called *Ostwald ripening* [2], and an example from our own work is shown in Fig. 1. This shows crystals of copper(II) benzoate trihydrate growing in silica gel, among and, at the expense of, the Liesegang rings, which formed initially.

Ostwald ripening is explained by an extension of the *Kelvin equation*, [3] which was originally developed to explain why droplets of a liquid have a greater vapour pressure compared to the flat liquid surface. In the extended form of the equation, small particles of compound precipitated in solution have a greater solubility than larger particles. Thus, the smaller particles tend to dissolve for the benefit of the larger particles, and as shown in Fig. 1, the larger crystals grow at the expense of the colloidal precipitate.

In modern times, the value of crystallizing sparingly soluble salts by gel growth has become widely recognized since the publication of the monograph by Henisch [4]. In this paper, we report on the crystallization of lead (II) oxalate in agarose gel, and how it has enabled us to witness an apparent phase transition in slow motion, many weeks after an initial crystallization took place. The mechanism appears to rely on an Ostwald ripening process.

The crystallization of lead(II) oxalate has received considerable attention over the last fifty years. Part of the motivation is the toxicity of soluble lead salts, and the observation that precipitation of the highly-insoluble oxalate salt is a common defence mechanism used in nature to remove lead from the environment. For example, Sayer et al. report that the fungus *Aspergillus Niger* can transform pyromorphite ($\text{Pb}_5(\text{PO}_4)_3\text{Cl}$), the most stable lead mineral under a wide range of geochemical conditions, into lead oxalate dihydrate, $\text{PbC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ [5]. The fungus then converts the hydrate into the anhydrous form, PbC_2O_4 .

Important to our considerations here is the work of Grases et al. [6]. In their studies of lead(II) oxalate crystalline growth in solution, they observed the growth of needle-like acicular crystals within two hours of mixing reactant solutions of lead nitrate and sodium oxalate (both solutions 0.2 M, final pH 5.6), but on standing these crystals transformed into a polyhedral form. Both forms analysed as PbC_2O_4 . The nature of this transformation was indeterminate, but Grases et al. suggested, on the basis of kinetic evidence, that it may arise due to a surface nucleation process.

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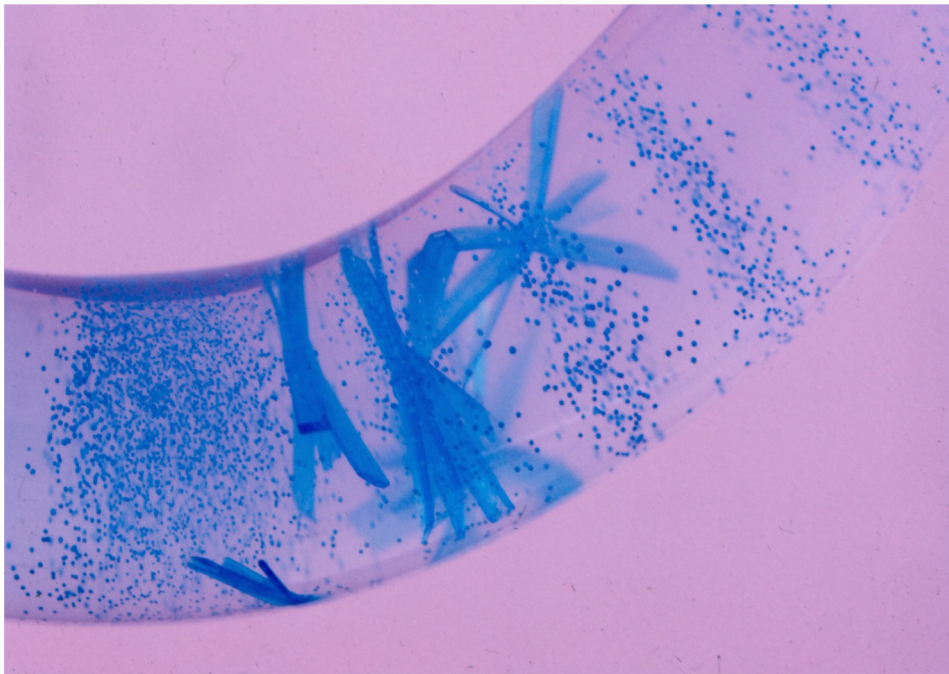


Fig. 1. Crystals of copper(II) benzoate trihydrate growing in a silica gel by double diffusion in a U-tube. The gel was formed by neutralising sodium silicate solution (density: 1.04 g/cm³) with 0.1 M sulphuric acid following Henisch [2]. The reactants: 0.1 M copper(II) sulphate and sodium benzoate solutions were each placed in opposite arms of the tube. The bands of fine precipitate – the Liesegang rings – were slowly invaded by the crystals, apparently by an Ostwald ripening process.

In this paper, we report on an ongoing transformation between fine needle-like lead(II) oxalate crystals to a polyhedral form, in situ, in an agarose gel. Agarose is a relatively “soft” gel, compared to silica gel, and we had previously used this gel successfully for protein crystallization. For reasons which will become apparent, we have called this transformation the ‘Morse code effect’.

2. Experimental

Lead (II) nitrate, Pb(NO₃)₂, and oxalic acid were supplied by Aldrich Co. and were used without further purification. Stock solutions of 0.1 M of lead(II) nitrate and oxalic acid were prepared using 0.22 μm filtered distilled water. The agarose gel used was electrophoresis grade supplied by Life Technologies Inc. This was a low melting point agarose having a low gelling temperature ≤ 30 °C for a 2% (w/v) solution. Prior to use a 1% agarose gel was prepared by dissolving 0.5 g of agarose in 50 mL of filtered (0.22 μm)

distilled water. The agarose solution was kept under sterile conditions at 80 °C for 20 min to minimize bacterial growth. Lead nitrate solution was mixed with 0.1 M acetate buffer and water according to the volumes shown in Table 1 under the heading ‘Lower Gel’ and placed in a small 7.5 mm diameter glass tube, previously sterilized. The tube was placed in a water bath at 80 °C and 400 μL of 1% agarose gel was transferred to the tube. When the bath temperature was reached, the tube was removed, vortexed for 20 s and then placed in the bath for a further 5 min. The tube was then sealed with parafilm and placed in a cold room to gel.

In a separate experiment a mixture of oxalic acid solution, agarose and water were mixed together according to the values shown under the heading ‘Upper Gel’ (Table 1). This mixture was warmed gently in a Bunsen flame until the first signs of boiling were observed (slight bumping). The mixture was then vortexed and then 2 mL of the mixture were transferred to the tube containing the lower gel [see above]. The tube was then quickly sealed with parafilm and placed in a cold room to set. In later experiments it

Table 1
Upper and lower gel compositions per tube.

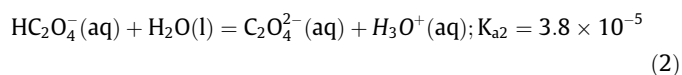
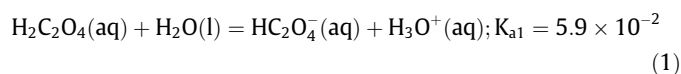
		Volumes in μL				
Upper gel	Composition tube	A	B	C	D	E
	1% Agarose	400	400	400	400	400
	Water	1100	1200	1300	1400	1500
	0.1 M Oxalic acid	500	400	300	200	100
	Total volume	2000	2000	2000	2000	2000
Interface region	Initial pH [*] , **	2.26	2.29	2.34	2.41	2.53
	Observation	Polyhedra	Polyhedra	Polyhedra		
	0.1 M Lead(II) nitrate	100	200	300	400	500
	Acetate buffer [*]	250	250	250	250	250
	Total volume	2000	2000	2000	2000	2000
Lower gel	Initial pH [*] , **	4.70	4.70	4.70	4.70	4.70
	Observation	Needles	Needles	Needles		

^{*} For the lower gel the pH is set 0.1 M acetate buffer pH 4.7.
^{**} For the upper gel the initial pH calculated as described in the text.

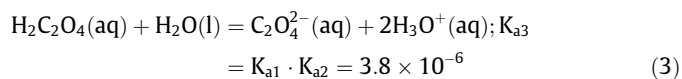
was found better practice to include a barrier gel of 0.2% agarose to prevent the hot mixing of gel layers. A volume of 200 μL was found to be sufficient to prevent mixing of gel layers.

As can be seen from Table 1 the concentrations of the reactants were arranged to vary from low lead – high oxalate (tube A) to high lead – low oxalate (tube E).

To avoid the problem of the intrusive effects of inserting a pH electrode into the gel, the initial pH of the oxalic acid gel (upper gel) was calculated using an iterative method based on the oxalic acid equilibria:



Adding these equilibria yields



The equilibrium can then be written:

$$K_{a3} = \{[\text{C}_2\text{O}_4^{2-}][\text{H}_3\text{O}^+]^2\} / [\text{H}_2\text{C}_2\text{O}_4] = [\text{X}] \cdot [2\text{X}]^2 / [\text{F} - \text{X}] = 4\text{X}^3 / (\text{F} - \text{X}) \quad (4)$$

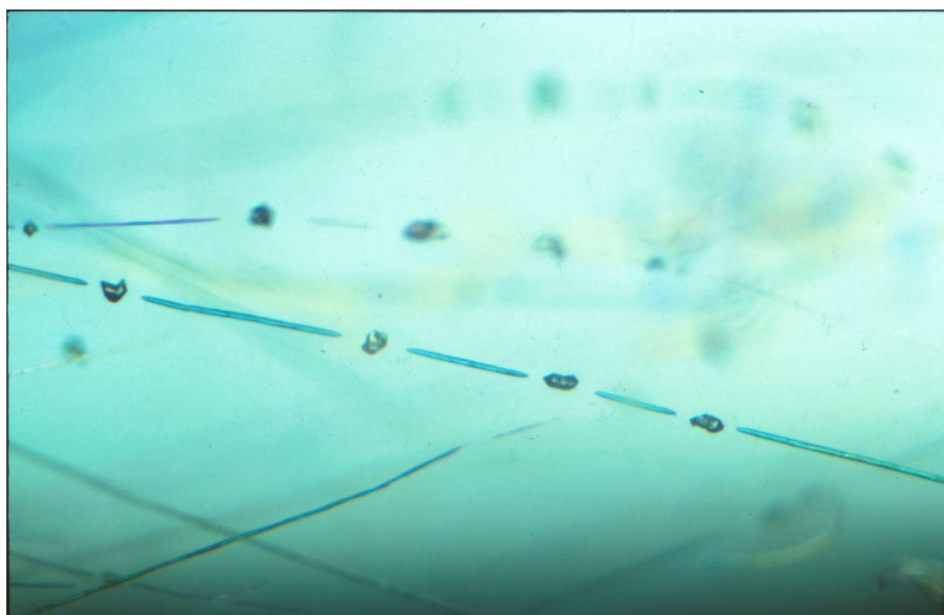


Fig. 2. Tube A after three weeks. The development of the Morse code effect is evident.



Fig. 3. Tube B after one week. At the center of the spray pattern a polyhedron is forming. Note that it is not in contact with its needle arms.

where X is the change in the formal concentration of oxalic acid, $[\text{H}_2\text{C}_2\text{O}_4]_{\text{F}}$ denoted by the symbol, F . At equilibrium, $[\text{H}_2\text{C}_2\text{O}_4] = [\text{H}_2\text{C}_2\text{O}_4]_{\text{F}} - X = F - X$. Eq. (4) rearranges to give

$$X = \{1/4K_{\text{a}3}(F - X)\}^{1/3} \quad (5)$$

This equation may now be solved by the *method of successive approximations* [5], by writing it as an iterative equation:

$$X_{i+1} = \{1/4K_{\text{a}3}(F - X_i)\}^{1/3} \quad (6)$$

With $i = 0, 1, 2..$ with $0 \leq X_i < F$, the iteration converges rapidly within four or five cycles. The initial pH with $\text{pH} = -\log_{10} [\text{H}_3\text{O}^+] = -\log_{10} [X_i]$ was obtained after four iterations starting with $X_0 = 0.00 \text{ M}$.

3. Results and discussion

Needle-like crystals appeared in the lower lead-rich gels of tubes A and B within 24 h after the addition of the upper gel. The needle axes of these crystals were roughly parallel to the tube axis. At the interface between upper and lower gels small, poorly formed octahedral crystals appeared, frequently showing twinning. In tubes C and D crystal growth occurred in the upper gel. In tube C similar irregular polyhedra were visible just below the interface, giving way to fine particles of white precipitate in the upper gel. This also occurred in the upper gels of tubes D and E but with much higher densities of precipitate.

In tubes A and B the needle form crystals extended through the lower gel in an unusual manner, showing apparent discontinuous growth. Needle growth appeared to be interrupted at intervals along the needle axes by small spaces containing one or more polyhedra which were not, however, joined to the needles. In some cases this gave rise to the “Morse Code” pattern: the *dots* are the polyhedra, and the *dashes* the needles (Fig. 2). In other cases it appeared that the polyhedra formed the origin of a “spray” of needles (Fig. 3). Furthermore, re-examination after 3 weeks indicated that the needles were disappearing, being replaced by small polyhedra some of which coalesced with one another. In other tubes a change of morphologies was also evident. In tube E, for example,

the precipitate in the upper gel re-dissolved and was replaced by small needle shaped crystals.

It was noted that the polyhedral forms appeared in the gels with high oxalate-low lead concentration ratios. This is evident from inspection of the crystals formed in the gel interface region of tubes A, B and C. It is also apparent, from the changes which develop in tube E with time, that low the low oxalate-high lead ratio favour the needle forms.

According to Table 1, the pH of the crystallization lies in the range $2.2 \leq \text{pH} < 4.70$. In the interface region between the two gels, the oxalate speciation diagram, Fig. 6, indicates that the dominant oxalate species in the interface region is $\text{C}_2\text{O}_4^{2-}$ [6,7]. Comparing our results to those of other workers, Khunur et al. [8] used a working pH of 4.8, while Grases et al. adjusted their working pH to 5.6.

Khunur et al. [8] found that this pH favours the growth of needles of lead(II) oxalate dehydrate, $\text{Pb}(\text{C}_2\text{O}_4) \cdot 2\text{H}_2\text{O}$, but did not observe the polyhedral form. Their crystallizations were conducted in silica gel by double diffusion in ‘U’ tubes following Henisch’s method [2]. Their findings are in contradiction to those of Grases et al., who had found that both needle and polyhedral forms analysed as PbC_2O_4 .

Turning now to the factors governing the transformation from needle to polyhedral forms, at first sight it appears to be due to a simple determinant, that is the oxalate-lead (Ox:Pb) concentration ratio, with a high Ox:Pb ratio favouring polyhedra, and a low ratio favouring the needles. However, this cannot be the full picture as is evident from Figs. 3 and 5. Both Figures show the needle crystals branching, sometimes at 90° . Fig. 3 also shows a remarkable spray, with a polyhedron set at the centre of the bouquet. Also, the needles and polyhedra are not in contact, yet there clearly is an intimate relation between the two. And the needles are slowly transforming into polyhedra. Since the polyhedra appear at the expense of the needles, it appears that the former are at least in relative terms, the equilibrium form. It is possible that the polyhedra represent the final stage of evolution of the crystallites of PbC_2O_4 , that is, it’s *Wulff* structure [9,10].

The needles shown in Figs. 2–4, frequently exhibit rounded ends when in proximity to their nemeses, the polyhedra. Given

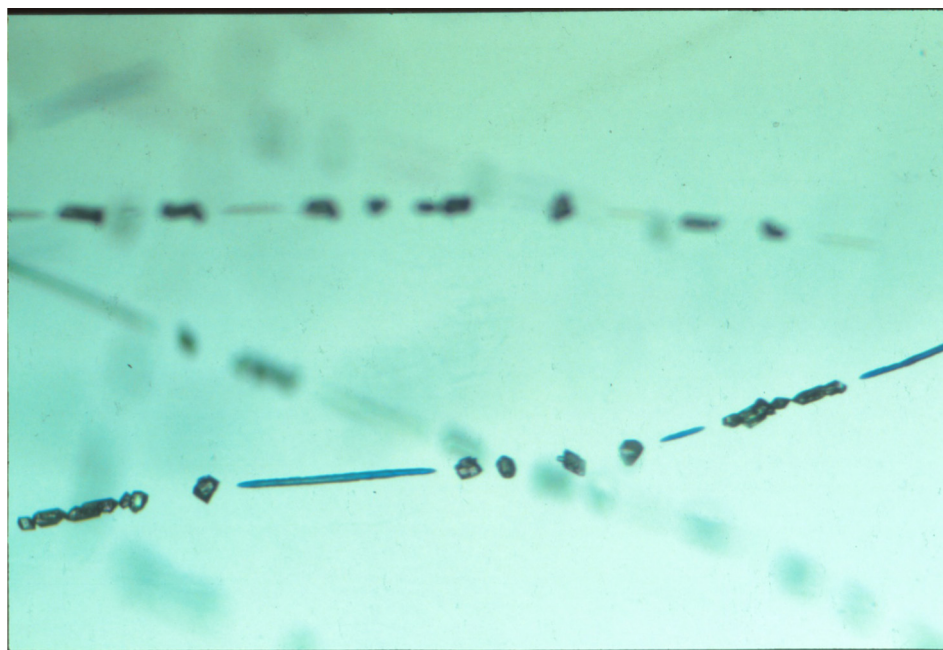


Fig. 4. Tube B after three weeks. The polyhedra are beginning to coalesce.

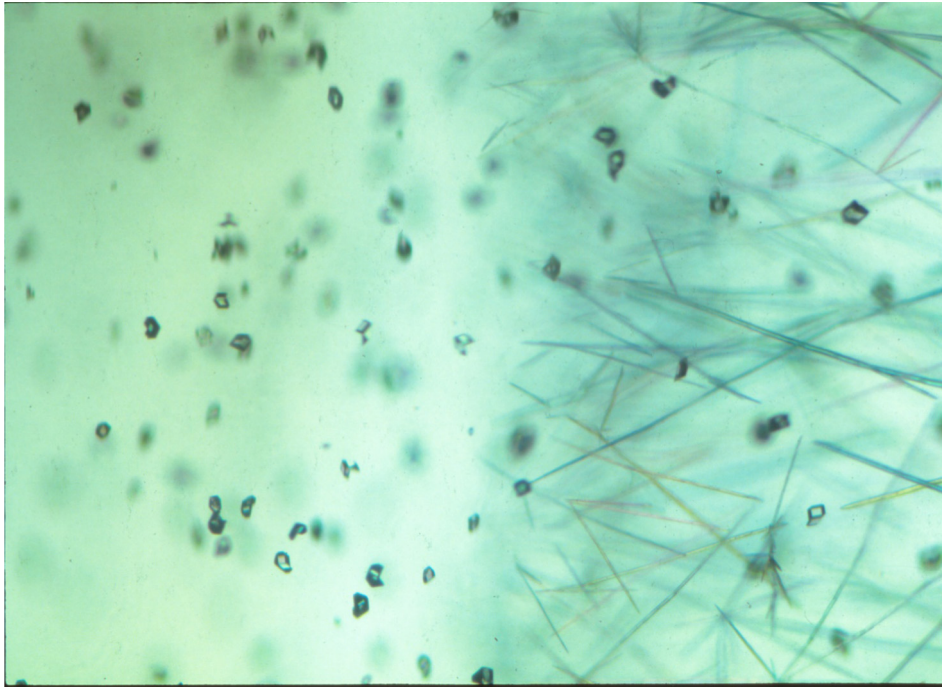


Fig. 5. Tube E.

the small widths of the needles at their rounded ends or tips, it appears that dissolution at the tips may occur in preference to remaining body of the needle, by a. Such a process of evaporation is described by the extended form of the Kelvin equation [9]:

$$RT \ln \left(\frac{C}{C_0} \right) = \frac{2\gamma}{r} \cdot V_m \quad (7)$$

The concentration, C , can now be interpreted as the dissolution concentration of PbC_2O_4 at the tip of the needle, radius, r , and C_0 as the equilibrium concentration at the surface of the Wulff structure. The

question arises here is how to interpret $\frac{\gamma}{r}$. For a Wulff structure this ratio is a constant for all faces [9], but it is clear for dissolution to occur at the tip, followed by transport to the polyhedron, and deposition on its surfaces,

$$(\gamma_N/r_N) > (\gamma_W/r_W) \quad (8)$$

where the subscripts: N , and W , stand for needle tips and Wulff structures respectively. As evident from Figs. 2–4, since $r_N \ll r_W$, then the inequality 8 would imply that $\gamma_N \gg \gamma_W$.

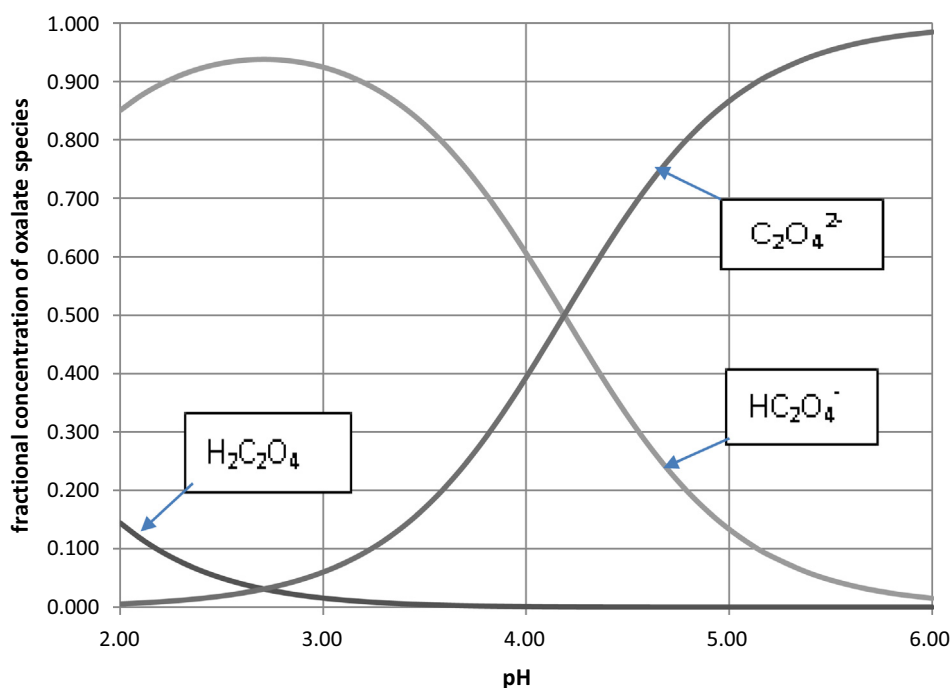


Fig. 6. Variation of fractional concentrations of oxalate species with pH in aqueous oxalic acid solution.

4. Conclusion

There are two aspects of this work that break new ground. Evidence has been presented of a solid phase transition in progress. This is the transition from the needle-like acicular crystals to a polyhedral form. And this transition has been captured, in slow motion and in situ, in an agarose gel.

Although the observations made in this paper can be explained by appeal to classical crystallization theory, in particular, Wulff's theorem [9], Ostwald ripening and the Kelvin equation, the origin of the spray bouquet in Fig. 3 is confounding, as the originator of the spray appears to be a polyhedral crystal. While this polyhedron may have formed on a foreign particle, given the precautions taken to purify the solvent, this appears unexplained. Another question arises as to why the polyhedra formed in Fig. 4 appear to prefer to coalesce together rather than grow bigger separately. It is possible that the gel itself exercises a channeling effect on the solution, but at this time there is no satisfactory explanation for this coalescent growth.

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References

- [1] R.E. Liesegang, *Naturr. Wachenschr.* 11 (1896) 353.
- [2] W. Ostwald, *Lehrbuch der Allgemeinen Chemie*, Leipzig, Germany, vol. 2, (part 1), 1896; W. Ostwald, *Zeitschrift für Physikalische Chemie*, vol. 22 (1897), pp. 289–330.
- [3] Thomson (Lord Kelvin), *Phil. Mag.* 42 (448) (1871) 448–449.
- [4] H.R. Hensch, *Crystals in Gels and Liesegang Rings*, Cambridge University Press, 1988.
- [5] J.A. Sayer, J.D. Colter-Howells, C. Watson, S. Hillier, G.M. Gadd, Lead mineral transformation by fungi, *Curr. Biol.* 9 (13) (1999) 691–694.
- [6] F. Grases, J. Ruiz, A. Costa-Bauza, Studies on lead oxalate crystalline growth, *J. Colloid Interface Sci.* 155 (1993) 265–270.
- [7] D.C. Harris, *Quantitative Analytical Chemistry*, second addition, W.H. Freeman and Company, 1987.
- [8] R.M. Cornell, P.W. Schindler, Photochemical dissolution of goethite in acid/oxalate solution, *Clays Clay Miner.* 35 (1987) 347–352.
- [9] Wulff, Zur Frage der Geschwindigkeit des Wachstums und der Auflösung der Krystallflagen, *Zeitschrift für Krystallographie und Mineralogie* 34 (5/6) (1901) 449–530.
- [10] C. Herring, *Konferenz über Struktur und Eigenschaften fester Oberflächen* Lake. Geneva (Wisconsin) USA, 29. September bis 1 Oktober 1952, *Angewandte Chemie* 65 (1953) 34.