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Investigations on the use of graphite electrodes using a Hull-type growth cell for the electrochemically-assisted protein crystallization

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Abstract

This paper describes the use of an electrochemical Hull type cell adapted for protein crystallization evaluating three inclination angles (45°, 60° and 90°). For optimization experiments, classical platinum wire electrodes were used and once the best geometry was known, they were replaced with 0.5 mm diameter low-cost graphite automatic pencil leads. Using Pt and graphite, the cell with electrodes fitted at 90° showed the most favorable geometry for promoting the growth of lysozyme crystals with enough size for protein crystallography (between 200–250 μm in solution, and between 500–650 μm in gel). The crystalline quality (mosaicity and $I/\sigma(I)$ ratio) of crystals obtained at different current values, was studied using these graphite electrodes and was compared with those protein crystals grown using platinum wire electrodes in solution as well as in gel experiments. These studies showed that it is possible to efficiently substitute the platinum electrodes by the low-cost graphite electrodes. This cell could be a first approach to a disposable cell for a large-scale use of electrochemically-assisted crystal growth method.

Keywords

Biocrystallization; Lysozyme; Electrochemically-assisted crystal growth; Growth from solutions; Biological Macromolecules; Graphite electrodes

1.0 Introduction

In order to obtain single crystals of high quality for protein crystallography applications, new crystal growth methods have been developed and optimized. The aim of these methods should focus on shorter induction times for the nucleation stage, and on the production of larger crystals for X-ray data collection for higher resolutions. Other studies have considered a diversity of physico-chemical parameters in order to control the nucleation phenomena of proteins, by applying magnetic fields,^{1,2,3} high-pressure,⁴ and external^{5,6,7} and internal^{8,9,10,11,12} electric fields. Electrochemically-assisted protein crystallization (name coined by our group),¹³ technique that uses internal electrical fields, has demonstrated the

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reduction on the time needed for crystallization, near to any of the electrodes, whether the protein is positively or negatively charged.⁸ For example, Cytochrome C was successfully crystallized from non-previously purified solution¹⁴ and thus proving that protein can be concentrated near to one of the electrodes using an internal electric field.¹⁵ The studies on the application of internal electric field, have been also carried out applying alternate current^{16,17,18} or direct current,^{8,19,20} using crystal growth cells constructed in a diversity of designs. However, one disadvantage of this method is the high price of the electrodes, like platinum wires for instance.

Alternatives to the use of noble metals electrodes in electrochemically-assisted protein crystallization have been poorly investigated, thin platinum^{8,9,10,19} and tungsten^{15,20} wires being the most common electrodes used for this type of research. This is due to their electrochemical stability, to their high reduction and oxidation potentials, and to their high conducting properties. So far, graphite electrodes have never been used for batch crystallization method and only a preliminary report was published by our group using the gel-acupuncture method.¹⁰ Instead of using platinum electrodes, it is possible to use for electrochemical applications carbon based electrodes constructed with materials like graphite,²¹ carbon paste²², carbon nanotubes,²³ fullerenes²⁴ and more recently graphene.²⁵ A graphite electrode of 0.5 mm diameter is readily available from the leads of mechanical pencils, and due to its cost is the most suitable material for an electrochemical disposable device or teaching purposes.²⁶ These graphite electrodes should resist the electrochemical potentials and current used in the electrochemically-assisted protein crystallization method.

The use of a Hull cell provides a way of studying the importance of electrochemical parameters for different types of deposits mainly metallic.²⁷ This device is usually constructed by fixing one electrode and moving the other at specific angle. Thus, when electrical current is fixed, the electrode potential and the current density regularly changes along the length of the electrodes; some equations that describes this variables have been published previously.²⁸ The use of this cell allows one to study the quality of electrodeposits obtained at different potential electrode and current density conditions in a sole experiment. This way to have soft changes of the electrical parameters was used to obtain a better insight on the protein electromigration phenomena.¹⁹ The present contribution describes the use of a Hull type cell applied for lysozyme protein crystallization; previous studies only focused on the number of electrodes, but the effect of the electrode angle has not been studied before. In order to explore this approach, three different inclination angles using platinum wire electrodes were used for the geometry optimization experiments, and lately they were substituted by the graphite electrodes. Finally, synchrotron radiation was used to characterize these crystals. The crystal quality was evaluated at different current values, demonstrating the feasibility of the electrode material change.

2.0 Experimental section

2.1. Solutions, Materials and methods

Lysozyme (Seikagaku, Co. Code 100940 six times recrystallized) solution 80 mg/ml was prepared in 200 mM sodium acetate buffer pH 4.5. The precipitating agent, NaCl 80 mg/ml, was prepared using the same buffer solution. For solution batch experiments, the same volumes of both solutions, protein and precipitant, are carefully mixed therefore, due to dilution, the final concentrations are the half of the initials. For experiments in gel, Agarose (Sigma, A4018) was prepared in water using a constant concentration (0.066 % w/v). Equal volumes of precipitating agent (120 mg/ml), agarose hydrogel and protein solution (120 mg/ml) were mixed together (1:1:1), and introduced in to the growth cell. The experiments were performed at constant temperature ($18\text{ }^{\circ}\text{C} \pm 0.1\text{ }^{\circ}\text{C}$) using a thermostated plate connected to a temperature controller (water circulator) device. All experiments were carried out at

controlled current (I) using a galvanostat VIMAR (model FCC-17). For gel experiments with Pt electrodes $I = 1 \mu\text{A} \pm 0.1$ and for solution experiments $I = 2 \mu\text{A} \pm 0.1$, whereas for graphite electrodes in gel $3.5 \mu\text{A} \leq I \leq 6.5 \mu\text{A}$. All photographs were collected by using a stereoscopic microscope Zeiss Stemi SV11 adapted to a digital camera. The software Axio Vision was used for data and imaging processing (provided by Zeiss Co.).

2.2. Cell construction for the electrochemically-assisted protein crystallization experiments

All experiments were performed using an electrochemical cell based on the pioneering design published by our group¹⁹, but with different electrodes material and size geometries. The crystal growth cell (as shown in Scheme 1) was constructed using two pieces of triangular microscope glass cover slides, separated by a 2 mm wide triangle-type polystyrene foil (PS, 0.75 mm thickness). This was then either sealed with epoxy resins glue or heated silicone sealant (commercially available in rods and heating gun kit). A small hole for pressure-release was made in the corner of the PS, used also for filling the cell with a micropipette. The electrodes were inserted into the PS through a hole made with a hot needle, keeping always one of them in a vertical position and the other, fixed at certain angle (90°, 60° and 45°). The electrodes used were platinum wires (99.99 Alfa AESAR Co. 0.2 mm diameter) and graphite rods (0.5 mm diameter graphite of a lead pencil, this term does not refer to the element lead) are commercial lead refills for mechanical pencils. Each electrode hole was sealed with silicone, after fixing the electrodes angles. The crystal growth cell was first filled with ultrapure water (18 mS), and then perfectly cleaned by sonication (1 to 5 minutes). Finally it was rinsed twice with water before use, eliminating water with a syringe. After a careful and slow filling of the cell with the batch solution (protein-gel-precipitant), the growth cell was carefully sealed with silicone. Then, after the silicone dried off, the electrodes were connected to the galvanostat and the circuit was closed by fixing the desired current and polarity of the cell. In all experiments, a control cell was made with dipped electrodes in the batch solution using the same electrodes geometry, but without applying any electric current.

2.3. Synchrotron X-ray diffraction experiments

X-ray data of different lysozyme crystals were collected on the X6A beam line at the National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY, USA. Six crystals were analyzed obtaining high resolution ranging from 1 to 1.3 Å. For each crystal two selected full data sets (1.3 and 1.0 Å resolution) of 180 and 360 oscillation images, were collected with an ADSC Quantum Detector 210 respectively. The X-ray data were integrated and scaled with the HKL 2000 package. The data collection was performed under a flux of nitrogen gas kept at 100 K, using as cryoprotectant 30 % (v/v) PEG-1000 mixed with mother liquor of NaCl (precipitating agent). Higher concentrations of this cryoprotectant were not efficient since the crystals were dissolved and damaged and ice rings were observed for lower concentrations.

3.0 Results and discussion

3.1 Effect of the geometry of the cell and electrode polarities

The electromigration phenomena, which occur during the crystallization of lysozyme under the influence of a small quantity of direct current,¹³ was evaluated using the growth cells shown in Scheme 1. The range of direct current where the crystal growth takes place and water electrolysis is avoided is in the range of some μA and had been previously determined.^{14,19} This value corresponds to the potential region where there is only capacitive current (movement of ions toward the electrodes), and a limited faradaic current due to water electrical decomposition is generated. In these conditions the potential is not

constant and the galvanostat adjusted it constantly in order to apply the desired current value. Thus the charged proteins can respond to it generating a constant flux of biomolecules toward the electrode of contrary charge. In order to be able to compare with previous reports, the cells for the geometry optimization were constructed using Pt electrodes. The experiments were carried out comparing the crystallization efficiency in terms of number of lysozyme crystals and their sizes. In all cases a control experiment without electrical field was also evaluated. The first studies (Figure 1) were carried out in solution, taking into account that 12 hours were needed to complete the crystallization process. In both cells (without and with electric current $I = 2 \mu\text{A}$) the classic tetragonal habit of lysozyme was observed. The experiment, in which electric current was applied, showed a narrower crystal-size-distribution, and larger crystals. In the absence of electricity, the nucleation was massive without homogeneity in the crystal-size distribution. Thus, the effect of the electrode polarity was remarkable, since crystals were bigger around the cathode and smaller near the anode, around which a non favorable crystallization process was observed. At the electrodes tips (Figure 1) the size of the crystals was smaller than the opposite direction, indicating that the electric potential field was not uniform along the cell as is expected for a Hull cell. Here, the electrical current is higher than at other part of the electrodes and the migration effect is so intense that the crystallization is not favored, probably by the presence of a turbulent or convective ions movement.¹⁹ This condition does not favor the crystal growth, but in other parts of the cell the crystals reached a considerable size.

The angle between the electrodes was modified by moving the cathode, though the length of the electrode had to be changed in some cases. Sometimes, this change produced water electrolysis characterized by the appearance of gas bubbles around the Pt cathode, mainly with the more acute angles due to the fact that a higher current density is attained while using shorter electrodes (Figures 1d and 1f). It is well known that this metal is an excellent electrocatalyst for the proton reduction reaction ($2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2$), which can be favored with a small change in the cathodic current density that flows through the electrodes generated by the change in angle. Even if the gas bubbles were obtained after several hours of imposing electrical current, probably a small quantity of gas (H_2 and O_2) is present around the electrodes that remain soluble until generating a bubble. Also as consequence of the smaller anode size and the higher current density on it, a depletion zone was observed near the anode with electrodes at 60° and 45° . However, crystals showed a much better morphology and size distribution when current was applied to the crystallization process using the larger angles cells (90° , see Figure 1b). Using electrical current a preference for the crystallization around the cathode was observed (Figures 1b, 1d and 1f); this phenomenon has already been reported in previous publications.^{8,19} Additionally, rate of crystal growth was also positively affected by the presence of an electric field reducing the time for observing the first crystals. This phenomena, attributed to a faster but controlled transport of protein to the nuclei, was also observed in a modified gel acupuncture cell¹⁰ and with other cell configurations for electrochemically assisted protein crystallization,¹⁹ and seems to be a positive characteristic of experiments in the presence of an internal electrical field. The average size of lysozyme crystals, grown in solution after 12h with and without electric current, is summarized in Table 1. Larger crystal sizes were observed for the cell fixed at 90° between the electrodes without gas bubbles produced by water electrolysis (Figure 2). A considerable decrease of the crystal growth was observed for smaller angles, which demonstrates that the larger the current densities (shorter electrodes) the less favorable the crystallization process.

Figure 3 shows the cathode surface coverage by lysozyme crystals as a function of the angle used in the crystallization cell. For the electrodes at 45° the tip of the negative electrode was completely covered with a homogeneous protein crystalline layer. For the cathode placed at 60° in the cell a less dense deposit is observed all along the electrode. Finally, at 90° on the

surface of the cathode, even at the tip where the current density is greater, small quantities of well-shaped crystals randomly distributed were obtained. The affinity of the protein to Pt has been attributed to possible interactions of disulphide bridges of the protein polypeptide chain with the metallic electrode,⁸ but the density of the deposit can be explained by the fast electromigration phenomena that is occurring at the electrode tip and that is more pronounced in a 45° electrodes cell than in the 90° cell. Some crystals randomly placed along the Pt wire were observed in the experiments without electrical current, but never reached the coverage level observed in the presence of the internal electrical field; this corroborate the affinity of protein crystals with Pt.

Crystallization in solution is highly affected by gravity force and convective transport.²⁹ Due to this fact it was decided to carry out experiments in gel media. Figure 4 shows lysozyme crystallization in gel using different angular electrode configurations with Pt electrodes in the Hull cell. In gel experiments the best current value was 1 μ A, the value of 2 μ A used in solution generated large quantity of bubbles around the electrodes. A plausible explanation to this fact can be attributed to the difference in the protein transport velocity, which in gel provokes a slower crystallization process that requires lesser current but more time. As in solution, the best results using a gel media in terms of size and shorter time for observing the first crystals were observed at 90°. It is worth mentioning that crystals grown in gel media were bigger in size, up to 650 μ m in the longest axis, compared to crystals grown in solution. The results of the experiments in gel are summarized in Table 2.

The effect of the protein migration provoked by the internal electrical field was more remarkable in gel media experiments (figure 4); around the anode there are small lysozyme crystals due to the charge repulsion generated by the positive polarity of the electrode, and the positively charged protein at pH 4.5. It is clear that in solution the protein transport is faster producing over nucleation. On the other hand, gel experiments showed better crystal growth behavior through the crystallization process. In these conditions they were obtained well-defined and isolated crystals with the typical tetragonal habit and a size > 200 μ m, which made them suitable for X-ray crystallography (Figure 4b). For the cells with closer electrodes (smaller angles) the increase in crystals size was less than for the cell with electrodes at 90° (Figure 4d and 4f). Here probably, when a nucleation center around the anode is created in gel media, it might be dissolved due to the smooth decrease of concentration provoked by the migration of protein molecules towards the cathode. This observation can be explained due to the short distance between the electrodes that the proteins have to move to reach the region of high concentration around the cathode, favoring the nucleation-growth stages. The gradient concentration generated by the presence of the electrical field should control the crystallization behavior observed; experiments focused in this point are currently in course and will be published elsewhere. For these reasons, experiments with electrodes at 90° in solution and gel showed reproducibility and larger size of crystals suitable for X-ray crystallographic studies. This geometry was selected for carrying out the experiments using graphite rod electrodes. If the polarity current is inverted the same global effect is observed both in solution and in gel. Just the time required to observe the first crystals was the difference, being slightly larger when the cathode is bigger than the anode, indicating that the current density on the cathode limits the protein migration.

3.2 Replacement of platinum wire electrodes by graphite rod electrodes

Metals like Cu or Fe were not suitable as electrodes because they corrode so readily in the presence of the components of the crystallization process.³⁰ This oxidation of copper or iron electrodes was observed, even at low current values in previous experiments published elsewhere.³¹ This oxidation was clearly evidenced by the blue colour observed around the anode, characteristic for Cu complexes or brownish colour for the Fe compounds. The

graphite rods used as electrode material in the electrochemically-assisted protein crystallization experiments, showed more electrochemical stability than Cu or Fe in this experimental set up (90° Hull type cell), being possible to apply even current values up to 10 μA for some hours without an evident degradation. The use of graphite rods instead of platinum wires was done because graphite leads from mechanical pencils are significantly less expensive and are readily available in stationery stores. Some of them contain organic polymers that give mechanical stability to the leads, but this decreases their conductivity. Several lead brands were tested by measuring the resistance with a multimeter between two points separated by 2 cm. Therefore, the better graphite electrodes were the cheapest graphite rods, which contained almost pure carbon and showed the least electrical resistance.

The same trend in terms of angular dependency for the crystallizations process, previously described, at 90° using Pt electrodes was observed for 0.5 mm diameter graphite electrodes in gel and solution media, but its use required a current higher or equal than 3.5 μA in order for the effect of the electrical current to be notice on the crystallization process (Figure 5). Comparing with 0.2 mm diameter Pt electrodes this current value is more than double, and it is necessary because the 0.5 mm electrodes have more exposed area. At a same current value, the current density (A/cm^2) is lower if the electrode has larger surface compared with other of lesser area and less potential is operating on it, thus, in order to have same current density values, the current applied must be higher for the larger surface electrode.³² Figure 6 shows the crystallization behavior of crystals grown at 6.5 μA of current applied along the crystallization process after 24 hrs in gel media. It was observed that some well-shaped lysozyme crystals were attached to the cathode as occurs with Pt electrodes. It has been reported the existence of organic species like phenols, carboxylic acids, quinones, hydroquinones and lactones as impurities on the surface of carbon-based electrodes.³³ This is particularly important because amino acid residues of lysozyme with acid-base properties like arginine, histidine, cysteine, tirosine, aspartic acid or glutamic acid, can interact via hydrogen bridges with this organic species promoting anomalous adsorption and focus-nucleation due to extremely high local supersaturation and charges attractions between the electrode (negatively-charged) and the protein (positively-charged). Additionally, it was noticed that graphite anodes electrodes faced erosion or degradation when current equal or higher than 6.5 μA were applied for more than 12 hrs, producing a characteristic gray halo around the electrode (See figure 6 anode). For these higher currents at larger times, the halo formed rapidly increases in size and contaminates the growth solution, which turned into a dark gray. However, using current values between 3.5 and 6 μA , graphite electrodes were convenient, effective and with a very low cost, compared to Pt electrodes for crystallization of lysozyme. This proposal could be a first approach for an affordable and disposable device for crystal growth cell using internal electrical field. A second advantage of using graphite electrodes is the reduction of the kinetics of parasitic reactions, mainly the production of molecular hydrogen. From the electrochemical point of view it is well known that Pt electrodes are better electrocatalysts for proton reduction than carbon, which is characterized by a high exchange current value and this factor favors the apparition of gas bubbles into the cell.³⁴

During all the experiments using Pt and graphite electrodes the voltage cell observed at the end of the experiment was higher (50–250 mV) than the initial value, depending on the cell geometry and the crystallization media composition. This fact indicates that, as the crystallization occurs, there is an increment of the system electrical resistance. The protein deposits observed on the electrodes and the loss of soluble and charged protein that behaves as electrolyte at the beginning of the experiment are factors responsible for this voltage cell increment.

Intrigued by the effect of higher current values required for graphite electrodes in gel media on the crystal quality, a systematic study was carried out to test the lysozyme crystals obtained at different applied current values, ranging from 3.5 to 6.5 μA . Current values lower than 3.5 μA did not show any effect of the electrical current and higher than 6.5 μA generated a prominent gas evolution from the graphite electrodes, indicating that the potential of the electrodes was enough to produce water electrolysis. For this quality study, two crystals, both from the same cell used for the growth at a specific current, were taken from a region close to the cathode (1 mm away), and exposed to X-rays; the data shown are the average of the obtained values. This procedure was repeated with each current value tested. It is noteworthy that the crystal quality can be estimated by using three different criteria: 1) low values of mosaicity, 2) higher values of the ratio I/σ (I) and 3) low values of B factor. The first parameter verified in this study was the effect on the mosaicity of the crystals grown at different applied currents during the crystallization process. It is important to remark that mosaicity is defined as the angular measure of the degree of long-range order of the unit cells within a crystal. Lower mosaicity indicates better ordered crystals and hence better diffraction. Mosaicity values calculated in this study were obtained from the statistic analysis of the X-ray diffraction data and are different from the mosaicity determined from the rocking curves. Figure 7 shows the effect on this parameter applying lower and higher values of electrical current to the crystallization process, using graphite leads as electrodes; the mosaicity decreased from 0.84 at 3.5 μA to 0.29 at 6.5 μA . This result clearly indicates that, the higher the current, the higher the quality of the crystals. However, for current values higher than 6.5 μA graphite leads are not suitable to be used, due to the water electrolysis and erosion of the electrodes; all values higher than 7 μA produced a large amount of bubbles inside the cell disturbing the full crystallization process and the solution became gray after few hours. Therefore, there must be a limit in the current applied when graphite electrodes are used.

The analysis of the ratio I/σ (I) as function of the attained resolution limit between 1 to 1.5 Angstroms for the analyzed crystals is shown in Figure 8. This result shows that adequate current condition for obtaining high quality crystals using 90° graphite electrodes by the electrochemically assisted lysozyme crystallization method with the Hull-like cell, is the current value of 6 μA . The higher current at 90 degrees seems to create a homogenous force field, which produces a controlled gradient of concentration and thus better crystals. This trend was also observed, but not as clear as was observed in this study, when ITO electrodes with other cell configuration were used.³⁵

4.0 Conclusions

The use of an electrochemical Hull type cell for protein crystallization was evaluated for two different electrode materials, Pt and C, different electrode geometry and applied current. The best results using this crystallization method were obtained with graphite electrodes fitted at 90°. This geometry produced the largest size and highest quality lysozyme crystals in solution as well as in gel. The crystalline layer of crystals obtained on the negative electrode in the solution experiments was a function of the angle used, showing it to be denser when 45° was used. Higher angles produced better and larger single crystals attached to the cathode and closer to this electrode. Finally, the platinum wire electrodes were successfully replaced by graphite lead of 0.5 mm in diameter, producing crystals with an excellent quality and suitable to be analyzed by X-ray radiation. The quality of crystals obtained using graphite electrodes was function of the used current where the higher the current, the higher the quality of the crystals with a limit of 6 μA . Graphite as electrode material minimized the parasitic electrochemical reactions, as well as reduced the cost of the experiments using the proposed experimental set up. The change to graphite electrodes can help to extent the use of this methodology into the screening protein crystallization conditions, where a large

number of cells need to be constructed and the cost of using Pt electrodes was a restrictive element.

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5.0 References

- Ataka M, Katoh E, Wakayama NI. *J Cryst Growth*. 1997; 173:592–596.
- Sazaki G, Yoshida E, Komatsu H, Nakada T, Miyashita S, Watanabe K. *J Cryst Growth*. 1997; 173:231–234.
- Mirkin N, Moreno A. *J Mex Chem Soc*. 2005; 49:39–52.
- (a) Kadri A, Lorber B, Jenner G, Giegé R. *J Crystal Growth*. 2002; 245:109–120. (b) Charron C, Robert MC, Capelle B, Kadri A, Jenner G, Giegé R, Lorber B. *J Crystal Growth*. 2002; 245:321–333.
- Nanev CN, Perkova A. *J Crystal Growth*. 2001; 232:285–293.
- Taleb M, Didierjean C, Jelsch C, Mangeot JP, Aubry A. *J Crystal Growth*. 2001; 232:250–255.
- Taleb M, Didierjean C, Jelsch C, Mangeot JP, Capelle B, Aubry A. *J Crystal Growth*. 1999; 200:575–582.
- Moreno A, Sazaki G. *J Crystal Growth*. 2004; 264:438–444.
- Sazaki G, Moreno A, Nakajima K. *J Crystal Growth*. 2004; 262:499–455.
- Mirkin N, Frontana-Uribe BA, Rodríguez-Romero A, Hernández-Santoyo A, Moreno A. *Acta Crystallogr D*. 2003; 59:1533–1538. [PubMed: 12925781]
- Al-haq MI, Lebrasseur E, Tsuchiya H, Torii T. *Crystallography Reviews*. 2007; 13:29–64.
- Hammadi Z, Veessler S. *Progress in Biophysics and Molecular Biology*. 2009; 101:38–44. [PubMed: 20025897]
- Frontana-Uribe BA, Moreno A. *Cryst Growth Des*. 2008; 8:4194–4199.
- Pérez Y, Eid D, Acosta F, Marín-García L, Jakoncic J, Stojanoff V, Frontana-Uribe BA, Moreno A. *Cryst Growth Des*. 2008; 8:2493–2496.
- Hammadi Z, Astier JP, Morin R, Veessler S. *Cryst Growth Des*. 2007; 7:1472–1475.
- Hou D, Chang HC. *Appl Phys Lett*. 2008; 92:223902.
- Koizumi H, Fujiwara K, Uda S. *Cryst Growth Des*. 2010; 10:2591–2595.
- Koizumi H, Tomita Y, Uda S, Fujiwara K, Nozawa J. *J Cryst Growth*. 2012; 352:155–157.
- Nieto-Mendoza E, Frontana-Uribe BA, Sazaki G, Moreno A. *J Cryst Growth*. 2005; 275:e1437–e1446.
- Hammadi Z, Astier JP, Morin R, Veessler S. *Cryst Growth Des*. 2009; 9:3346–3347.
- a) Rabah MA, Nabil N, Abdul AA. *Carbon*. 1991; 29:165–171. b) Ranganathan S, Kuo TC, McCreery RL. *Anal Chem*. 1999; 71:3574–3580.
- a) Zittel HE, Miller FJ. *Anal Chem*. 1965; 37:200–203. b) Bauer D, Gaillochet MPH. *Electrochimica Acta*. 1974; 19:597–606. c) Svancara I, Vytras K, Barek J, Zima J. *CRC Crit Rev Anal Chem*. 2001; 31:311–345.
- a) Mao LQ, Yan J, Zhou HJ, Yu P, Su L. *Adv Mater*. 2008; 20:2899–2906. b) Pumera M. *Chem Eur J*. 2009; 15:4970–4978. [PubMed: 19360829]
- a) Szucs A, Loix A, Nagy JB, Lamberts L. *J Electroanal Chem*. 1995; 397:191–203. b) Miranda-Hernandez M, Rincon ME, Gonzalez I. *Carbon*. 2005; 43:1961–1967.

25. a) Wang X, Zhi L, Müllen K. Nano Lett. 2008; 8:323–327. [PubMed: 18069877] b) Bae S, Kim H, Lee Y, Xu X, Park JS, Zheng Y, Balakrishnan J, Lei T, Kim HR, Song YI, Kim YJ, Kim KS, Oezylmaz B, Ahn JH, Hong BH, Iijima S. Nature Nanotech. 2010; 5:574–578.
26. a) Parker RH. J Chem Educ. 2011; 88:1428–1430. b) Goldcamp MJ, Underwood MN, Cloud JL, Harshman S, Ashley K. J Chem Educ. 2008; 85:976–979.
27. a) Sedusky HJ, Mohler JB. Metal Finishing. 1947; 45:59–63. b) Fox J. Metal Finishing. 2011; 109:461–470.
28. West AC, Matlosz M, Landolt D. J Appl Electrochem. 1992; 22:301–303.
29. García-Ruiz JM. Key Eng Mater. 1991; 88:87–106.
30. Pourbaix, M. Atlas of electrochemical equilibria in aqueous solutions. 2. NACE; USA: 1974. English ed
31. BSc Thesis of Andrea E Gutierrez-Quezada, Faculty of Chemistry. UNAM; 2008. Nucleation and Protein Crystal Growth: Ferritin Crystallization under the Influence of an Internal Electric Field.
32. Bard, AJ.; Faulkner, LR. Electrochemical methods. Fundamentals and applications. 2. John Wiley and sons; USA: 2001. p. 11-43.
33. Thorogood CA, Wildgoose GG, Jones JH, Compton RG. New J Chem. 2007; 31:958–965.
34. Roberge, PR. Corrosion Engineering, Principles and Practice. Mc Graw-Hill; USA: 2008. p. 87
35. Gil-Alvaadejo G, Ruiz-Arellano RR, Owen C, Rodríguez-Romero A, Rudiño-Piñera E, Antwi MK, Stojanoff V, Moreno A. Cryst Growth Des. 2011; 11:3917–3922.

Synopsis

The use of an electrochemical Hull type cell with electrodes fitted at 90 degrees promoted the growth of lysozyme crystals with enough size for protein crystallography (200-250 μm in solution, and 500-650 μm in agarose gel). Platinum electrodes were successfully substituted by 0.5 mm diameter low-cost graphite automatic pencil leads; nevertheless higher current values were required to favor the process.

Figure 1.

Lysozyme crystallization in solution after 12 h, [Lysozyme] = 40 mg/mL, [NaCl] = 40 mg/mL in acetates buffer solution pH 4.5, 200 mM at 18 °C. As a reference the Pt electrodes diameter was 0.2 mm. The letter A and C represent anode (+) and cathode (−) respectively.

Figure 2.

Left, magnification of the 90° lysozyme crystallization cell in solution after 12 h (Figure 1b). Right, zoom of the crystals obtained nearby the cathode. [Lysozyme] = 40 mg/mL, [NaCl] = 40 mg/mL in acetates buffer solution pH 4.5, 200 mM at 18 °C. As a reference the Pt electrodes diameter was 0.2 mm. The letter A and C represent anode (+) and cathode (–) respectively.

Figure 3.

Pt cathodes covered with lysozyme crystals after using a cell where $I = 2 \mu\text{A}$ with with electrodes fitted at a) 45° , b) 60° , c) 90° . [Lysozyme] = 40 mg/mL, [NaCl] = 40 mg/mL in acetates buffer pH 4.5, 200 mM, at 18°C . As a reference the Pt electrodes diameter was 0.2 mm.

Figure 4.

Lysozyme crystallization in a gel media after 24 hrs, [Lysozyme] = 40 mg/mL, [NaCl] = 40 mg/mL [Agarose] = 0.066 % w/v in acetates buffer solution pH 4.5, 200 mM at 18 °C. As reference the Pt electrode diameter is 0.2 mm. The letter A and C corresponds to anode (+) and cathode (−) respectively.

Figure 5.

Lysozyme crystallization in solution (left) after 12 h and in gel (right) after 24 h.

[Lysozyme] = 40 mg/mL, [NaCl] = 40 mg/mL [Agarose] = 0.066 % w/v in acetates buffer solution pH 4.5, 200 mM at 18 °C, $I = 6.5 \mu\text{A}$. As reference the graphite electrode diameter is 0.5 mm. A and C indicates respectively anode (+) and cathode (–).

Figure 6.

High magnified images of the electrodes of the cell showed in figure 5 right at the end of the lysozyme crystallization process in gel at 18 °C, $I = 6.5 \mu\text{A}$ for 24 hrs. As reference the graphite electrode diameter is 0.5 mm. Left-hand side image corresponds to cathode (-) and right-hand side image corresponds to the anode (+).

Figure 7.

Effect of the electrical current used for the electrochemically-assisted protein crystallization with graphite electrodes in gel media on the mosaicity obtained from Synchrotron X-ray radiation experiments of the lysozyme crystals. The values are the average of two crystals and the bars represent the dispersion of the two values.

Figure 8.

Effect of the electrical current used for the electrochemically assisted protein crystallization with graphite electrodes in gel media on the $I/\sigma(I)$ ratio vs resolution obtained from Synchrotron X-ray radiation experiments for the lysozyme crystals grown in a Hull-type cell with electrodes positioned at 90° .

Scheme 1.

Construction of the crystal growth cell used for these electrochemically assisted protein crystallization experiments.

Table 1

Comparison of the results obtained for the crystallization of lysozyme in solution after 12 h with and without electrical current.

	Control cells ($I = 0 \mu\text{A}$)	Cells with electrical current ($I = 2 \mu\text{A}$)		
Nucleation	Massive	Controlled in function of electrodes polarity		
Average size of crystals	150–200 μm 545 crystals per cm^2	Angle	Nearby the anode (crystals per cm^2)	Nearby the cathode (crystals per cm^2)
		90°	120–150 μm (186)	200–250 μm (220)
		60°	50–100 μm (114)	150–200 μm (213)
		45°	50–100 μm (127)	100–150 μm (355)
Time required for the appearance of the first crystals	3–4 hours	1–2 hours		

Table 2

Comparison of the results obtained for Lysozyme crystallization in agarose gel after 24 h with and without electrical current.

	Control cells ($I = 0 \mu\text{A}$)	Cells with electrical current ($I = 1 \mu\text{A}$)		
Nucleation	Controlled	Controlled in function of electrodes polarity		
Average size of crystals	150–400 μm 354 crystals per cm^2	Angle	Nearby the anode (crystals per cm^2)	Nearby the cathode (crystals per cm^2)
		90°	300 μm (65)	500–650 μm (127)
		60°	200–250 μm (166)	300–400 μm (191)
		45°	200–250 μm (152)	300–400 μm (186)
Time required for the appearance of the first crystals	12 hours	12 hours		

Table 3

Comparison of the results obtained for Lysozyme crystallization in agarose gel after 24 h with and without electrical current using graphite electrodes.

	Control cells ($I = 0 \mu\text{A}$)	Cells with electrical current		
Nucleation	Controlled	Controlled in function of electrodes polarity		
Average size of crystals	150–250 μm 343 crystals per cm^2	I (1A)	Nearby the anode (crystals per cm^2)	Nearby the cathode (crystals per cm^2)
		3.5	150–200 μm (129)	250–280 μm (220)
		4.5	150–200 μm (120)	280–300 μm (185)
		5.5	150–200 μm (98)	280–300 μm (174)
		6.0	100–150 μm (85)	280–350 μm (150)
		6.5	100–150 μm (83)	280–350 μm (141)
Time required for the appearance of the first crystals	12 hours	12 hours		