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Application of electric fields for controlling crystallization†

Lee Fiona Alexander and Norbert Radacsi *

This highlight investigates the different aspects of electric fields controlling crystallization, focusing on strong electric fields. Application of both internal and external electric fields, as well as utilizing both alternating and direct currents (AC and DC) are discussed, with an emphasis on protein crystallization. Attention is drawn to the similarities and opposing findings within the papers published in the field to date. It has been demonstrated that the crystallization process can be significantly enhanced by the application of electric fields. Namely, electric fields can reduce the nucleation time, control the location of nucleation, increase the product yield, control the product crystal size, enhance overall crystal quality, control the crystal orientation and control the polymorphism. In addition, recent advances including the use of an electric field for separation of multicomponent mixtures, electric field-assisted crystallization in a continuous flow, and transformation of amorphous material into crystalline are discussed.

Introduction

Crystallization is one of the oldest separation and purification techniques and has not changed in the past centuries significantly, until recently.1 The technique is used abundantly in industry, with practical applications in pharmaceutical, chemical, petrochemical, food industries and biotechnology,2-4 making it a multitrillion-dollar business.5 It is crucial for most industrial crystallization processes to control the crystallization, as the properties of the final product are dependent on the extent of crystallinity, polymorphism, and the magnitude and quality of the final crystals obtained. Furthermore, it is of vital significance in today's industry to have the ability to control the promotion or suppression of crystallisation.6 Electric fields comprise one method of achieving this. They are of great attention due to their tunable strength and direction leading to the possibility of enhanced crystal properties. Understanding the effects of electric fields on the crystallization process can potentially lead to control and enhancement of the crystalline product.

In literature, key topics regarding protein crystallization enhancement via the application of electric fields have been
reviewed by Al-Haq et al. (2007),\textsuperscript{7} Frontana-Urabe and Moreno (2008),\textsuperscript{8} Hammadi and Veesler (2009)\textsuperscript{9} and most recently, by Nanev (2017).\textsuperscript{10}

Considering the methods already established for crystallization purposes, the application of electric fields stands out as a technique that incites a considerable amount of attention among researchers today. Comprehending the growth of crystals and their nucleation mechanisms under the presence of electric fields is fundamental, with regards to its utilization in a practical environment, e.g. for inducing crystallization and controlling the crystal polymorphic outcome,\textsuperscript{2,11,12} improving the quality of protein crystals,\textsuperscript{13-17} separation of components from their suspensions in multicomponent mixtures,\textsuperscript{18} and for crystallization of food systems.\textsuperscript{4}

The aim of this highlight is to provide a helicopter view on all the different aspects of electric fields affecting crystallization, focusing on recent studies made with strong electric fields. The influence of both internal and external electric fields, as well as utilizing both alternating and direct currents (AC and DC), and the similarities and opposing findings are discussed.

**Background**

The effects of an electric field on crystallization from solution is a relatively new and active field of research, with a vast amount of research conducted in the early 21st century. Only a few published papers on the topic were available prior to the research of Taleb et al. (1999).\textsuperscript{19} The electric field effects on crystallization have only in recent times been studied extensively.

**Internal and external fields**

Electric-field-induced crystallization experiments are executed in two different configurations: (1) if the electrodes are immersed in the solution then it is classed as an internal electric field. In this case, the strength of the electric fields used are restricted to small voltages or currents to limit faradic reactions that would be expected to occur at higher applied strengths and (2) if the solution has no contact with the electrodes when the electric field is applied, then it is an external electric field.

**Electric field effects on the thermodynamics of nucleation and crystal growth**

The chemical potential and therefore the chemical potential difference between the liquid and solid are altered by the application of an electric field to a solution or crystalline bulk material. It may, therefore, modify the nucleation work by altering the chemical potential difference.\textsuperscript{20}

The subsequent equation denotes the addition of the electric field, \( E \), to the chemical potential difference.\textsuperscript{20}

\[
\Delta \mu_{c} = \Delta \mu + \epsilon c E^2
\]

where \( \epsilon c \) is a function of the relative permittivities and is defined by:\textsuperscript{20}

\[
\epsilon c = \frac{3 \epsilon_o \epsilon_m (\epsilon_o - \epsilon_m) \mu_s}{2 (\epsilon_o + \epsilon_m)}
\]

\( \epsilon_o \) is the vacuum permittivity, \( \epsilon_c \) and \( \epsilon_m \) are the crystalline material and medium relative permittivity, respectively.

The subsequent equation expresses the nucleation work, \( W^* \), under the influence of an electric field:\textsuperscript{20}

\[
W^* = \frac{16 \mu_s^2 \gamma^2}{3(\Delta \mu + \epsilon c E^2)}
\]

Nucleation is enhanced when \( \epsilon_c > \epsilon_m \), here, \( \epsilon c \) is positive and the chemical potential difference is enlarged.\textsuperscript{20,21} In a situation where \( \epsilon_c < \epsilon_m \) the nucleus creation is impeded by the applied field, and therefore nucleation is inhibited.\textsuperscript{20,21} If \( \epsilon_c = \epsilon_m \), the electric field does not influence nucleation.\textsuperscript{20} A decrease in nucleation work is related to a rise in the nucleation frequency.\textsuperscript{20,22}

By substituting eqn (5) into eqn (1), and noting that the supersaturation is given as \( \Delta \mu = kT \ln S \), where \( S \) is the supersaturation ratio, the resulting equation for the rate of stationary nucleation accounting for the applied electric field is:\textsuperscript{20}

\[
J_s = \frac{1}{\sqrt{\pi}} c_0^* \exp \left( -\frac{B}{(kT \ln S + \epsilon c E^2)^2} \right)
\]

where \( \epsilon c E^2 \) is the change in the electrostatic energy per molecule. Thus, classical nucleation theory conveys that the nucleation rate in the presence of an electric field alters due to the field’s effect directly on the chemical potential difference.\textsuperscript{20}

The theory developed by Kashchiev assumed the electric field to be uniform (before the nucleus is formed) and the same at different distances from the nucleus after it has been formed).\textsuperscript{20,22} Furthermore, the system containing the nuclei is often immersed inside a medium during crystallization, which decreases the electrostatic energy of the system of fixed charges.\textsuperscript{21} Thus, for crystallization processes, the effect of the electrostatic energy to the chemical potential should be considered from the Gibbs and Helmholtz free energy.\textsuperscript{20,24}

From these the following equations are obtained when the electric field is accounted for:\textsuperscript{23}

\[
dG = -5dT + Vdp + \sum \mu dm_i - V_e P dE
\]

\[
dA = -5dT - pdV + \sum \mu dm_i + V_e E dP
\]

where, \( G \) is the Gibbs free energy, \( A \) is the Helmholtz free energy, \( S \) is the entropy, \( T \) is the absolute temperature of the surroundings, \( V_e \) is the volume of the container, \( \mu \) is the chemical potential, \( E \) is the strength of the electric field and \( P \) is the polarization. \( P = \epsilon D \), where \( \epsilon \) is the relative...
permittivity and $D$ is the electric flux density. And then, from the Helmholtz free energy change, the contribution of the electrostatic energy to the chemical potential can be obtained as follows:23

$$\mu_c = \mu_c^{\infty} + \frac{1}{2} \frac{1}{V} \left[ \frac{\partial E}{\partial n} \right]_{T,F,P} E^2$$  \hspace{1cm} (7)

This equation should be used in the case of crystal growth since the relative permittivities are different between the liquid and solid phases.

Koizumi et al.25 discussed the electric field effects on thermodynamics by investigating the effects of crystal growth rate on entropy. They discussed in their study that both the electrostatic energy added to the energy required for step formation and the entropy related to the shape of the step must be considered when calculating the free energy on the crystal surface $F_s$, which is expressed from the Helmholtz free energy:25

$$F_s = U_s - TS_s$$  \hspace{1cm} (8)

where $U_s$ is the energy required for the formation of a step and $S_s$ is the entropy related to the shape of the step. When the effects of the electric field are considered, the energy from the formation of the step $U_{s(\varepsilon)}$ can be derived from the Helmholtz free energy:25

$$U_{s(\varepsilon)} = U_{s(0)} + \frac{1}{2} V \left[ \varepsilon - T \frac{\partial E}{\partial n} \right] E^2$$  \hspace{1cm} (9)

where $U_{s(0)}$ is the energy required for the formation of the step. The electric field changes the entropy related to the shape of the step, which can be derived as:25

$$S_{s(\varepsilon)} = S_{s(0)} - \frac{1}{2} V \frac{\partial E}{\partial T} E^2$$  \hspace{1cm} (10)

Thus, both the entropy related to the shape of step and the energy required to form the step depends on the volume to which the external electric field is applied, the absolute temperature and the magnitude of the applied electric field.

Electric field effects on protein crystallization

The study of electric field effects on protein crystallization was initiated approximately twenty years ago by Aubry’s group.13,19 Since then, this area of research has been widely studied by the teams of Moreno,8,14,26–30 Koizumi,24,31–34 and Veesler.9,35 Conventionally, the majority of experimental investigations have been undertaken using hen egg white lysozyme (HEWL) as a model protein. Four different approaches were taken to study the effects on proteins. Depending on their nature, electric fields can be generated by (static) direct current (DC) or (dynamic) alternating current (AC). Furthermore, defined by the design, electric fields can internal or external in regard to the studied material.

External DC electric field

In 1999, Taleb et al.19 designed two basic devices to study the crystallization of proteins under a direct current external electric field. The study found that the external electric field affects both the size and quality of the produced crystals, resulting in fewer crystals being formed of greater size, in the presence of an electric field than in its absence.19 The crystals developed at the surface of the droplet close to the cathode.19

Altering conditions such as pH, temperature and precipitation agent composition can be utilized along with external electric fields to impact crystalline properties of proteins.19 Taleb et al.19 demonstrated that an external DC electric field had a sizeable impact on the nucleation rate of lysozyme crystallization. Furthermore, the electric field impacted the size and quality of crystals; fewer crystals of greater size were produced, and their quality was enhanced as measured by the detected mosaic spread.

In a subsequent study, an external electric potential of $7.5 \times 10^5$ Vm$^{-1}$ was used. Nucleation did not occur as the protein solution remained liquid phase throughout the experiment.13 For a pH controlled HEWL solution with no NaCl addition, there was a detected increase in protein concentration near to the cathode, which confirms why crystals were favorably found within close proximity of the cathode in the earlier experiment.19 The experiment clearly showed a concentration gradient between the electrodes, resulting from the effects of an external DC electric field.14 It was also demonstrated that the variance in concentration between the electrodes is dependent on the overall charge of the protein, with the effect of the electric field increasing relative to the magnitude of the charge.13 Furthermore, the authors noted that the protein crystalline solubility is not altered by the presence of the field.13

Findings concluded that the presence of the electric field significantly reduced the time to reach equilibrium.13 It was also noted that when crystallizing in a low ionic strength solution and at a pH far from the isoelectric point of the protein, the effects were greatest.13 After four weeks, the protein concentration was much lower when under the influence of an external electric field than in the absence of the electric field.13

Another group36,37 repeated the original experiment of Taleb et al.19 using the SDVD method. The Nanev group,36 using custom made two-dimensional glass cells,36 (Fig. 1) investigated the effect of HEWL crystallization as a function of temperature. In order to aid comparison, the experiments were undertaken using a pair of cells, the first had an applied DC external electric field of $1.5 \times 10^5$ Vm$^{-1}$ and the second cell had no exposure. Other conditions, for example, the temperature and pH, were identical in both cells. The control of temperature decreased convection, and microscopic observation are all benefits of this setup.36

Following Taleb’s work regarding a concentration gradient within the cell, the authors confirmed that it is primarily at
the cathode where nucleation happens and that crystals develop more rapidly at this electrode due to the electric field. Furthermore, their studies also found that at a relatively low temperature the presence of an electric field has a more significant effect on the induction time and nucleation rate. Nanev's studies demonstrated that the orientation of the crystals is greatly influenced by the presence of an external electric field. They found that operating at temperatures less than 5–7 °C, combined with the applied electric field, results in a considerably larger number of growing crystals having a preferred c-axis orientation. Interestingly, this action of the crystals orientating due to the electric field was not observed at 18 °C, irrespective of the applied electric field strength, which was up to 4.0 × 10^5 Vm⁻¹. Nanev assumed that at higher temperatures the presence of an electric field was not orienting the molecules as their thermal motion disturbed and repositioned them. From this, one can deduce that reducing the temperature (kinetic energy of the molecules) facilitates the electric field to control the orientation of the crystals in solution, or crystallization is influenced differently at larger ΔT. Moreover, Taleb et al. have deduced that crystal quality was enhanced for those grown under the influence of an electric field. In opposition to this, Nanev's group reported that in some cases, a rough crystal morphology was evident. The differences are, however, recognizable. Taleb et al. used higher temperatures in their studies combined with a higher protein concentrated solution. The authors stated that this rough crystal morphology could be a consequence of strain that occurs due to the nucleation temperature being low. Potential reasoning for the aforementioned deductions can be accredited to the fact that, in the solution (pH 4.5), HEWL molecules hold a positive charge under conditions of nucleation.

In one of the latest advances regarding the growth of protein crystals by the influence of an external electric field, glucose isomerase crystals at ambient temperature were developed via the microbatch method, established by Chayen et al. and used recently by Rubin et al. Firstly, tests using the microbatch method were conducted with HEWL at four field potentials (1, 2, 4 and 6 kV). Comparisons were made with and without the application of the electric field. These revealed that the lysozyme crystals experienced growth, while the quantity of nucleation was reduced when applied to the field, as well as the number of crystals per well being reduced in relation to the burgeoning electric field intensities (Fig. 2).

Fig. 1 Representation of the growth cell displaying two lysozyme crystals with the c-axis orientation (normal to the glass plate, with respect to all other orientations). Reprinted from ref. 36 with permission of J. of Crystal Growth, Elsevier.

Fig. 2 Glucose isomerase crystals grown under the application of a DC electric field of varying intensities. Each row corresponds to experimental results obtained on different dates. Reprinted with permission from ref. 17 Copyright (2017) American Chemical Society.
oil-growth solution. Sui et al. used a significantly lower DC electric field, up to $10^3 \text{ Vm}^{-1}$, in a microfluidic crystallization setup. They found that the crystals grown in the presence of an electric field diffracted to a higher resolution than crystals grown in the absence of the field. Thus, when performing X-ray diffraction analysis, the signal-to-noise ratio is improved, which can help in determining the crystal structures of proteins and other biological molecules. The improved X-ray diffraction signal-to-noise ratio was also reported by other scientists.

Thus, it can be concluded that applying an electric field to macromolecule crystallization results in a reduction of nucleation sites, and larger crystals of enhanced crystallographic quality being produced.

**Internal DC electric field**

Electric-field-induced protein crystallization was first introduced in a cutting-edge paper in 1976 by Chin et al. Studies on internal DC electric fields on protein crystallization have since been carried out by Mirkin et al., Sazaki et al., Moreno and Sazaki, Nieto-Mendoza et al., Flores-Hernández et al., and Sui et al.

The preliminary work by Mirkin et al. for the purpose of examining the impact of an internal electric field on HEWL and thaumatin crystallization, saw the application of a revised design of the gel-acupuncture arrangement, so as to apply an electric field by use of electrodes within the crystallization medium.

The conditions classically used for HEWL and thaumatin crystallization by the gel acupuncture method were the same as those applied in this experiment.

Mirkin et al. proposed that one of the key benefits of using their technique for the growth of crystals is that less time is required to achieve crystals in comparison to the standard gel-acupuncture setup. Thaumatin crystals were obtained in just five days using their method under the influence of the internal electric field, which was seven days quicker than using the conventional method. Therefore, for both lysozyme and thaumatin, a reduction in both induction time and quantity of crystals forming was detected by Mirkin et al. This supports Taleb’s earlier findings.

With regard to the crystal orientation, Mirkin et al. detected that for every crystal joined to the anode, there appeared to be favored orientation under the application of the electric field towards the c-axis of the lysozyme crystal. It was confirmed by X-ray crystallography that the structure of the thaumatin crystals was not altered by the application of the internal electric field. The study also confirmed that the closer the electrodes were to each other (i.e. the higher the electric field strength was), the faster the crystal growth was.

In the work published by Moreno and Sazaki, tests were carried out at three varying distances between the electrodes to study the impact of the internal electric field. Bubbles of gas were apparent as a result of water electrolysis at currents greater than 3 μA in the initial experiments. To override this issue, the current was reduced to a maximum of 2 μA in the subsequent experiments. To assist with comparisons, every experiment had a reference in which no current was applied. Under the influence of a DC current the authors made a number of observations: (1) nucleation time of HEWL was observed to have reduced for both gel and supersaturated solutions; (2) a smaller quantity of nuclei was favored and as a result, crystals were larger in size; (3) it was only within close proximity of the cathode that HEWL crystals appeared, whereas amorphous precipitation was seen near the anode. These observations are consistent with the prior observations stated for effects on crystallization processes under the influence of an external electric field.

The groups of Nanev and Aubry solely used external electric fields of magnitude $1.5–7.5 \times 10^5 \text{ Vm}^{-1}$ in their investigations. Moreno and Sazaki, however, stated substantial effects could be achieved even when applying electric fields of reduced size. In their methodology, a significantly lower electric field of 0.19 Vm$^{-1}$ was used and the electrodes were placed directly in contact with the solution (internal DC field). From this observation, they concluded that they must account for three different effects: (1) electromigration; (2) redox reactions on the surface of the electrode, but this was discounted as throughout the crystallization process, there was no noticeable production of gas; (3) impact of electric potential produced by the electric field on the chemical potential difference.

Moreno and Sazaki claimed that both the formation of crystals occurring near the cathode, and the reduction in induction time could be interpreted by (1) electromigration; (2) the polarity difference between the electrodes and the protein molecules; (3) interactions among protein molecules and the Cl$^-$ ions within the vicinity of the electrodes. The decline in the number of deposited crystals could also be accredited to the electric potential created by the field. It was also observed that the crystals exhibited a preferred orientation along the c-axis (normal to the glass support, in relation to all other orientations) of the tetragonal HEWL. This effect was also detected by Nanev et al. in the presence of a $1.5 \times 10^5 \text{ Vm}^{-1}$ DC external electric field, and by Mirkin et al. when they made use of an internal electric field.

According to the findings of Moreno and Sazaki, one of the primary benefits of their technique for crystal growth was mainly the control of the crystal quantity. As a result, this method enabled an increase in the size of the crystals and enhanced the diffraction intensity for X-ray crystallography purposes.

A transparent crystallization cell, comprised of two glass plate electrodes coated with conductive indium tin oxide (ITO), was utilized by Gil-Alvaradejo et al. The improved intensity was observed from X-ray diffraction analysis of lysozyme crystals grown at 6 μA, and of ferritin crystals grown between 2–6 μA (both cases DC power was used). Even though the crystal quality was improved, no conformational changes in the 3D protein molecule structures were observed.
A subsequent paper was published in 2013 by Flores-Hernández et al.\textsuperscript{26} exhibiting similar effects and in unanimous agreement to the aforementioned papers. The authors yet again made use of the model protein, lysozyme, but also investigated a more challenging protein, 2TEL-Lys. They created a novel crystallization device (using again ITO-covered-glass-electrodes), combining an electric current with a sitting drop vapor diffusion setup, which, would enable it to be used with an extensive range of proteins. They implemented this device by reengineering their previous device with the reasoning that the batch method is limited to merely simple proteins. Their results indicated that 50 times fewer crystals were obtained around the cathode when grown in the presence of an internal DC electric field than in its absence.\textsuperscript{26} In addition, these crystals were drastically larger in size and exhibited enhanced quality.\textsuperscript{26} This design is said to be straightforward to use and would allow for a smooth scale up for production of larger crystals.\textsuperscript{26}

**Internal AC electric field**

Compared to DC electric fields, there has been a lack of investigation in the area of AC electric field application to crystallization. This could be attributed to the fact that it presents a supplementary variable – the current frequency.\textsuperscript{9} A major advance in 2008, and the initial study carried out to investigate the effects of an AC electric field on protein crystallization, was carried out by Hou and Chang.\textsuperscript{15} They used an internal AC electric field to examine both the frequency and voltage impacts of the electric field. Diminishing Faradaic reactions on the electrodes, thereby enabling greater applied voltages to be used, is the first known benefit of an AC field.\textsuperscript{15} The authors tested HEWL solutions in the presence of various different AC fields for signs of nucleation in 24 hours, and observed three different zones in relation to the applied voltages and frequencies (Fig. 3): (1) the first zone, beneath the solid line, contained no crystals in 24 hours.\textsuperscript{15} With the prolonged application of the field, however, crystals developed at rates and dimensions equipollent to crystals grown in the absence of electric field.\textsuperscript{15} This indicates that in this region, the AC electric field had minimal impact on the crystallization process; (2) crystals were detected in the zone between the solid and dashed lines; (3) the final zone (above the dashed line) was the production of a gel, from which the largest single, and least defected crystals were produced (in comparison to case 2).\textsuperscript{15} The minimum of zone 2 represents the most favorable frequency with the lowest required voltage for crystallization in a 24 hour period (Fig. 3).\textsuperscript{15}

To summarize, Hou and Chang have successfully shown that the voltage and frequency of the applied AC electric field could create reversible gelation.\textsuperscript{15} Eradication of the field then resulted in a small number of high-quality crystals being produced as the induced gel phase was converted. Their investigations thus confirmed enhanced HEWL crystal quality (large single crystals with few defects) when an AC electric field is applied, in agreement with aforementioned studies on external electric fields affecting crystallization.\textsuperscript{16,19} A phase diagram similar to Fig. 3 would be required to choose the most suitable voltage, frequency, and length of exposure for alternative proteins.

Using an alternative protein, thaumatin, Wakamatsu\textsuperscript{45} assessed the effect of applying an internal AC electric field (1.06 × 10³ Vm⁻¹) during crystallization.\textsuperscript{45} The author utilized a transparent cell containing two ITO thin film electrodes and demonstrated that the application of the field encouraged crystallization.\textsuperscript{45} Wakamatsu and Ohnishi have also made use of ITO-coated conductive glass cells to investigate HEWL crystallization in an earlier study.\textsuperscript{46} Utilizing a similar transparent cell, an internal electric field and a low-angle (<8deg) forward light scattering technique, the authors observed electric field-induced aggregate formation from lysozyme solutions.\textsuperscript{47} The analysis of the lysozyme aggregation is detailed in a later paper by the same group.\textsuperscript{48}

**External AC electric field**

There are many examples of research focusing on the use of DC external fields to control nucleation.\textsuperscript{2,11,13,17,19,36,37,49,50} These articles have primarily demonstrated a reduction in the nucleation rate with an applied electric field. Nonetheless, making use of an external AC electric field with suitable frequency to successfully control an increase and a reduction in the rate of nucleation for crystallization processes had not been accomplished until the work of Koizumi et al.\textsuperscript{24} in 2009. The impact on the rates of nucleation under the influence of an external electric field has been examined theoretically by Kashchiev\textsuperscript{20,22} and Isard.\textsuperscript{21} Their analysis proposed that whether the rate of nucleation is increased or reduced, is dependent on the variance in the electrical permittivity between solid and liquid phases.\textsuperscript{26} Koizumi et al.\textsuperscript{24} have examined their theory experimentally, by using an AC electric field on
protein crystallization. These authors suggested that there was a potential of a reverse in the electrical permittivity between the two phases.\textsuperscript{24} They anticipated that the dielectric constant of the HEWL crystals would be greater than that of the medium at frequencies <500 kHz and vice versa for frequencies >500 kHz.\textsuperscript{24} The group has carried out a thorough study in the field over the last decade to understand how AC electric fields could impact protein crystallization. They proved that the rate of nucleation of HEWL increases in the presence of a 1 MHz electric field, however it decreases under the presence of the same field at 10 kHz.\textsuperscript{24} Demonstrating an improvement and impedance on the rate of nucleation of lysozyme crystals, the group effectively succeeded in controlling the nucleation process.\textsuperscript{24} In a later study, using a different compound, they also showed that the rate of nucleation of porcine insulin was found to increase under the presence of an external AC electric field at 3 MHz.\textsuperscript{32} The authors credited this phenomenon to the electrostatic energy which was added to the chemical potentials of each phase, and to the formed electric double layer (EDL) that forms at the interface between the dissimilar phases.\textsuperscript{32} The chemical potential of the solid material was considerably altered in comparison to the liquid, which resulted in a burgeoning nucleation driving force in agreement with the classical nucleation theory.\textsuperscript{32} Koizumi \textit{et al.} have successfully communicated that the observed increase or decrease in lysozyme rate of nucleation was a consequence due to the variance between the electrical permittivity of the liquid and solid phases.\textsuperscript{24,31,32} The authors concluded that the use of an AC field can be a valuable practice for crystallization of proteins, and could subsequently be applied to a vast majority of proteins on the market.\textsuperscript{32}

Following on from this study, another paper submitted by the same group exhibited crystallization experiments at room temperature using the batch method.\textsuperscript{16} They applied a $4 \times 10^4$ Vm$^{-1}$ external AC electric field at a frequency of 1 MHz to the protein solution, and further confirmed by X-ray diffraction rocking-curve measurements, that HEWL crystal quality could be enhanced in the presence of an external AC electric field. Fig. 4 shows the batch setup used in their study. Further investigations by the same group led to the conclusion that there was an optimum frequency to advance the protein crystal quality.\textsuperscript{31}

More recently, Pareja-Rivera \textit{et al.}\textsuperscript{14} demonstrated that it is not only the number of appearing crystals that are influenced by an applied AC field but that their size is also affected subject to the electrical fields’ frequency. Using an AC electric field (2 and 8 Hz) they revealed for both glucose isomerase and lysozyme crystals, that the greater the applied AC frequency, the greater the number of crystals produced (Fig. 5).\textsuperscript{14}

The majority of investigations conducted to date make use of uniform electrodes. Hou and Chang constructed quadrupole and interdigitated Ti/Au electrodes for their research and concluded that the quantity of nucleation sites reduced.\textsuperscript{15} In addition, the number of crystals was enhanced under the application of the nonuniform electric field arising from the patterned electrodes.\textsuperscript{15}

Numerous devices utilizing ITO electrodes have been constructed for enhanced control over nucleation rate and the final crystal quality.\textsuperscript{25,26,52,53} Li and Lakerveld\textsuperscript{51} examined the influence of alternating electric fields on protein crystallization (using insulin and HEWL as model compounds) in microfluidic devices. The authors used ITO-coated glass slides as the electrodes; the bottom of which was ITO-patterned and the top uniformly coated. Eight different patterns in combination with different surface areas in parallel-plate configurations were used in their research. It was demonstrated that depending on the parameters of the design of an electrode (shape, pattern and surface area), both an enhanced and inhibited effect on crystallization could be observed.\textsuperscript{53} Their results further proposed that the location of nucleation was manipulated by the non-uniform electric field brought about by the characteristic electrode designs and by the ITO layer, which acted as a template for nucleation.\textsuperscript{53}

\textbf{Electric-field-assisted protein crystallization in continuous flows.} To date, enhancing control over the location and induction time of protein nucleation has been performed by utilizing electric fields in small batches. To change this, Li and Lakerveld\textsuperscript{54} produced an original piece of apparatus portraying continuous flow. The utilization of continuous flow parallels modern practice in the (bio)pharmaceutical industry of continuous manufacturing. As far as we are aware, within the field of study, this is the premier implementation of applying non-uniform electric fields with the purpose of separating and purifying proteins from solution under continuous flow. Li and Lakerveld\textsuperscript{24} crystallized HEWL protein using a transparent microfluidic device with patterned electrodes in a coplanar configuration (Fig. 6). In their investigations, the researchers used an electric field strength of $5 \times 10^4$ Vm$^{-1}$, and revealed that under continuous flow conditions, a shorter induction time was obtained using this method as opposed to batch crystallization, regardless of whether an electric field was applied. Furthermore, when comparing the application of electric fields, and the control
flow experiments, the resultant yield was higher using the continuous flow conditions than using the batch crystallization method. This higher yield, however, can only be obtained when a longer residence time is applied, which results from the higher secondary nucleation at lower supersaturation. To conclude, this novel setup arrangement of electric field-assisted protein crystallization in continuous mode can produce a greater crystallization yield than both control experiments utilizing no electric fields and batch experiments.

**Further applications of electric fields on crystallization**

**Food industry.** A promising outlook for the application of static DC electric fields is in the food industry, specifically the use of static DC electric fields for food system crystallization (freezing).4 A recent review by Jha et al. communicated that static DC electric fields can enhance heat and mass transfer during the crystallization process.4 The application of a strong DC field during the freezing process \(2 \times 10^{10} \text{ Vm}^{-1}\) promotes nucleation, increases the nucleation rate and decreases the induction time.4 They conveyed that under this application of a static electric field, crystals of ice in the food product were of reduced size.4 This was said to have consequently resulted in smaller damage as a result of freezing.4 It was therefore predicted that both the disruption to the cell and protein denaturing would be reduced, as well as enhancing the preservation qualities of fresh food following a thawing process.4 From this example, we note that static electric fields increased nucleation rates throughout water crystallization,4 yet in general a reduction in the rate of nucleation for proteins has been observed.19,24,29,30,36,37,43 Considering the varied results of applying the static electric fields to different substances, it can be proposed that there is an opportunity for further investigation in order to completely comprehend the nucleation and crystal growth mechanism under the application of an electric field.

Another study from 2019 by Fallah-Joshaqani et al.55 used an external electric DC electric field up to \(9.6 \times 10^5 \text{ Vm}^{-1}\) to...
Electric fields for polymorphism control. By altering the crystallization behavior of a compound, nucleation and growth can be affected in such a way that a different polymorph can be produced under the influence of an electric field.\textsuperscript{2,11,12,59,60} Electric fields can control the polymorphs of glycerin when grown in solutions.\textsuperscript{5,12} It was demonstrated in 2005 by Aber \textit{et al.}\textsuperscript{2} that the application of an intense DC electric field ($6 \times 10^5$ V m$^{-1}$) to supersaturated aqueous glycerine solutions leads to the nucleation of the most stable $\gamma$-glycerine. They showed experimentally that the distribution of the polymorphs had a tendency to move away from $\alpha$-glycerine under the influence of the DC field increasing the quantity of $\gamma$ polymorph when compared to the samples obtained without the electric field (Table 1).\textsuperscript{2} The authors claimed that this was a consequence of the orientation of pre-established groups of glycerin molecules, brought about by the electric field, aiding their formation into a crystalline arrangement.\textsuperscript{2}

A later study in 2013 by Di Profio \textit{et al.}\textsuperscript{12} investigated glycerin crystallization using membrane crystallization technology (which allowed for enhanced control of supersaturation in solution, by adjusting the removal rate of solvent, in the presence of an AC electric field). Experiments were carried out using the apparatus depicted in Fig. 7, by varying the operating conditions: removal rate of solvent, pH, and the use of a pulsed electric field. The setup is comprised of one internal and one external electrode. The former being the cathode, a stainless-steel conductor directly in contact with the glycerin solution, and the latter being the copper wire anode. This setup, combined with the pulsed AC field, delivered polarization that was adequate to prevent electrolysis (which could be present if a highly conductive solution and two internal electrodes were to be used).\textsuperscript{12}

Di Profio \textit{et al.} claimed that, from their experimental work with and without an applied electric field, the factors influencing glycerin polymorphism are: electric field $> pH >$ rate of supersaturation $> $ concentration of solute.\textsuperscript{12} Their findings disprove the classical justification grounded on the ‘self-poisoning’ mechanism and standard ‘cyclic dimer hypothesis’ for the crystallization of $\alpha$ and $\gamma$ polymorphic forms from solution.\textsuperscript{12} They proposed that the rates of nucleation, in relation to the respective progress in the kinetics of both polymorphs affected by differing conditions, have a leading part in shaping the polymorphic outcome.\textsuperscript{12} In opposition to the aforementioned disproval, the authors advocate a molecular nucleation process where open chain dimers can act as the foundations for each polymorphic form in the rate-limiting stage of the multi-step mechanism for nucleation.\textsuperscript{12}

In contrast to Aber \textit{et al.},\textsuperscript{2} who provided evidence that the $\gamma$-polymorph could be preferentially crystallized under application of a DC electric field, Di Profio \textit{et al.}\textsuperscript{12} demonstrated in their study that the application of the electric field consistently gave rise to the $\alpha$ form of the polymorph, even under the circumstances in which only the $\gamma$-polymorph was predicted to crystallize.\textsuperscript{12}

Using a different model compound, Radacsi\textsuperscript{59} explored the impact of a DC internal electric field ($5.6 \times 10^5$ V m$^{-1}$) on...
the polymorphic outcome of isonicotinamide (a highly polar molecule) from solution throughout a cooling crystallization method from 1,4-dioxane (non-polar solvent). The use of the non-polar solvent facilitates crystallization without interacting with the electric field, and also prevents redox reactions and short-circuits (sparks) from occurring. Two experimental arrangements were used in this study as a novel way to produce polymorphs, namely ‘parallel-plate setup’ and ‘parallel-rod setup’ (Fig. 8). The author found, by structural analysis, that the polymorphic form changed under the influence of an electric field. When no electric field was applied to the solution, the isonicotinamide crystals grown were the metastable form I. However, when a homogeneous electric field of $5.6 \times 10^5 \text{ V m}^{-1}$ was applied, the crystals formed on the anode were of the stable form II. Moreover, the author observed that when an inhomogeneous electric field was applied of a potential lower than $5.6 \times 10^5 \text{ V m}^{-1}$, a combination of both polymorphs forms was present. The study found that raising the strength of the field led to the transformation of the polymorphic structure from the metastable form I to the stable form II, and thus had the ability to alter the crystallization kinetics. Additionally, Radaci confirmed that the crystals grew in less time under application of the electric field in comparison to those grown without. The crystal growth rate increased from $8.3 \mu\text{m min}^{-1}$ to $126 \mu\text{m min}^{-1}$. This observation is in agreement with the previous authors who have studied crystallization under an electric field. They concluded, from the recrystallization of isonicotinamide, that the control of crystallization (localized growth, induction time and polymorphism) was attributed to the increased local supersaturation due to electromigration by application of the electric field (likewise established by prior authors).

Adrjanowicz et al. published a paper on DC electric field effects on crystal quality using cooling crystallization. They reported that application of an external DC electric field ($4.0 \times 10^6 - 2.0 \times 10^7 \text{ V m}^{-1}$) resulted in the crystallization behaviors of a subcooled, glass-forming liquid (4-vinyl-propylene).
carbonate) being altered, and thereby stimulating the development of a new polymorphic crystal that could not be formed in the absence of the field. Furthermore, in agreement with Radacsi, their results show that under the influence of an electric field, changes are made in the overall crystallization tendency and a large proportion of a different form of the polymorph is created. The time for crystallization is reduced when the potential is applied, in comparison to its absence. Thus, their results agree with the findings of Radacsi, even though Adrjanowicz et al. used an external DC electric field (while Radacsi used and internal DC electric field).

Furthermore, molecular dynamics simulations by Parks et al. demonstrated that a strong DC field of $1.5 \times 10^9$ Vm$^{-1}$, had the ability to create an innovative paracetamol polymorph. Through simulation, they assessed the influence of an external DC electric field on dissolution and growth rates, the morphology of the crystals and the polymorphic form obtained. From the dynamics they found that the crystal growth rate of the supersaturated nanocrystals was suppressed by application of the field, claiming a 40% reduction in growth rate under the applied intensities. This finding, although contrasting with previous findings, is in accordance with magnetic field crystallization studies of paracetamol. In addition, for paracetamol nanocrystals which are not saturated, the simulations indicated that the electric field could both enhance and impede the rate of dissolution. Finally, the newly created polymorph was found to be metastable under the influence of the electric field, exhibiting improved aqueous solubility and thus it is suspected that this form will have improved bioavailability and show distinctly new characteristics when compared to the already known forms of paracetamol. This is of particular importance in pharmaceutical applications.

The aforementioned studies have shown that an applied electric field has the potential to be used for controlling polymorphic formation in crystallization processes.

**Solid separation from mixed suspension through electric field-enhanced crystallization.** The crystallization process from a multi-component mixture requires multiple steps and usually yields low purity. The concurrent separation of particles and development of crystals, whereby the crystals of two solutes are individually collected from both electrodes, consequently accomplishing in one stage, product retrieval and purification from a multicomponent solution, has been made possible via the presence of an inhomogeneous DC electric field on a mixture suspension.

In their seminal paper, Li et al. studied the impact of a continuous DC electric field on the result of nucleation and polymorphic form of crystals throughout cooling crystallization experiments. Fluid dynamics and the rate of growth of the crystals were observed to change under application of the
This work, to the best of our knowledge, was the first separation technique based on coupling manipulation of particles by application of a strong internal DC electric field, with cooling crystallization for in situ product recovery and separation. Initial experiments carried out showed that in the presence of an internal electric field (above 2 kV), particle movement was induced (see Video in ESI† and Fig. 9). Crystals of isonicotinamide began to move from their suspension in 1,4-dioxane, and attach on the anode. As the potential difference was raised, the number of crystals around the anode also increased. A crystal bridge was created at potential differences higher than 5 kV as the number of crystals participating in the movement was amplified. Even though the crystals connected the anode with the cathode, no short-circuit or reactions were observed (see Video in ESI†). Removal of the field resulted in all the crystals detaching from the anode and falling to the base of the vial.18 The authors claimed that the motion of the particles was primarily related to the interaction of the crystals and the applied field.18 They observed no liquid motion when the experiment was carried out in the presence of the field but in the absence of crystals in the solution.18 They also stated that electrochemical reactions could not be responsible for this phenomenon as there was no gas formation.18

Using this newly discovered phenomenon, phenazine and caffeine (two organic crystalline compounds) were shown to be individually removed from their suspension in 1,4-dioxane (Fig. 10).18 Application of an inhomogeneous electric field to this mixture at 30 °C led to crystals adhering to the surface of both electrodes (phenazine on the cathode, and caffeine on the anode). The suspension was gently cooled to 15 °C under the application of the electric field, in order to immobilize the crystals on the electrode thus allowing recovery of the deposited crystals.18 Solubility had been reduced during the cooling process, enabling the growth of accumulated crystals. Crystals were then removed from the bulk solution by withdrawing the electrodes, allowing them to be separately collected. These crystals were of different color, shape and thickness.18 The purities were found to be greater than 91%,18 which is slightly lower than the 99% purity that can be obtained in theory by a single crystallization process step.6

These results proved that the hybrid of particle manipulation, brought about by the presence of the electric field, and cooling crystallization can be used for separation and recovery of two crystalline substances from their multicomponent solution.18

Although promising, this new and unique technique exhibits limitations in its design. It requires additional work and improvements to be made in the design in order to allow a higher yield to be obtained to prove satisfactory in industry scale-up.

Summary

This highlight was undertaken in order to investigate all the different aspects of strong electric fields controlling crystallization and has underlined the importance of electric fields in significantly enhancing the crystallization process. Substantial progress has been made in investigating electric field-assisted crystallization within the last two decades. The results to date have been very promising, depicting clear advantages of crystallization in the presence of electric fields. Electric fields have been proven to be a valuable manipulation instrument for controlling the crystallization process and the crystal product. The studies have been undertaken by the

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![Fig. 9 Experimental observations of Li et al.\textsuperscript{18} of particle movement when an inhomogeneous static electric field was applied to a suspension of isonicotinamide in 1,4-dioxane. Reprinted from ref. 18 with permission of Angewandte, Chemie, John Wiley and Sons.](image_url)

![Fig. 10 The experimental arrangement of Li et al.\textsuperscript{18} in the presence of an electric field and subsequent cooling crystallisation. Phenazine and caffeine were separately recovered from their suspension in 1,4-dioxane. Reprinted from ref. 18 with permission of Angewandte, Chemie, John Wiley and Sons.](image_url)
application of four primary approaches; the influence of both internal and external electric fields, as well as utilizing both alternating and direct currents (AC and DC). Table 2 summarizes the different types and magnitudes of electric fields applied on various model compounds, using different electrode materials and crystallization method, that are presented in this highlight.

Discussion and challenges
Several factors have been deduced to affect crystallization processes induced by application of an electric field. One such factor is the respective strength of the field, and therefore the efficiency in the ability to control the crystal quantity. It appears that, unsurprisingly, external electric fields involve the application of greater field strengths up to $7.5 \times 10^5$ Vm$^{-1}$ to obtain significant impacts on the kinetics of crystallization, whereas internal fields can generally obtain similar effects at much lower intensities. However, it seems that there is an optimal field strength for these positive effects, and increasing the electric field above a certain limit might decrease or diminish the benefits seen during nucleation.55

The groups of Nanev and Aubry13,19,37 solely used external electric fields of magnitude between $1.5-7.5 \times 10^5$ Vm$^{-1}$ in their investigations, whereas Moreno and Sazaki30 stated that substantial effects could be reached when applying a weak electric field (in their methodology, an electric field of 0.19 Vm$^{-1}$ was used). In addition, Rubin, Owen and Stojanoff17 suggested that the large variance in the magnitude of the external electric field strength stated necessary for lysozyme crystallization (of magnitude $\times 10^5$ Vm$^{-1}$) in comparison to glucose isomerase (100–1000 Vm$^{-1}$) could be explained by the differences detected in the dipole moments, resultant molecular charges, and ratios between the quantity of positive and negative residues.17

Moreover, Taleb et al.13,19 deduced that crystal quality was enhanced for those grown under the influence of an electric field. In opposition to this, Nanev’s group36 reported that in some cases, a rough crystal morphology was evident. The differences are, however, recognizable. Taleb et al. used higher temperatures in their studies combined with a higher protein concentrated solution.19 The authors stated that this rough crystal morphology could be a consequence of strain that occurs due to the nucleation temperature being low.36 Potential reasoning for the aforementioned deductions can be accredited to the fact that, in the solution (pH 4.5), HEWL molecules hold a positive charge under conditions of nucleation.36 From this, one can deduce that reducing the temperature disrupts the orientation of the crystals in solution. Nanev suggested in a recent article that the relation between the electric field effects and the supersaturation ratio should be studied to understand this phenomena.10

Frontana-Uribe and Moreno noted the significant difference occurring between the forming crystals and the inert electrodes in the solution, which is similar to the electrophoresis process, and it causes molecules concentrating around the cathode or the anode.64 While in case of direct electro-crystallization, current flows through the electrolyte between the immersed electrodes in a solution containing small-molecule ions, and electromigration occurs (positively charged ions migrate towards the cathode and negatively charged ions travel towards the anode).64 When an ion reaches the electrode material, it loses its charge, and gas bubbles are released. However, these bubbles are not noticeable, and they dissolve in the solution when the current is low.64

It is also crucial to investigate if the effects of the electric field are the same for different types of molecules. However, it is hard to compare the effects, as different molecules can have substantially different solvent systems, with different properties. Al-Haq et al., 2007 (ref. 7) did not notice any major differences in the nucleation behaviour between HEWL and thaumatin crystallized in the presence of electric fields, except for the location of the crystal growth. While HEWL grew around the cathode, thaumatin crystallized on the surface of the anode.7 They explained the difference with the presence of the electric double layer that is formed around an electrode dipped in a solution.1 et al.18 and Radacsi59 also found different crystallization locations when crystallizing different small molecules under the influence of an electric field. While some chemicals crystallized on the anode, other ones crystallized on the cathode, making it possible to use electric fields for separation of molecules.18

Fallah-Joshaqani et al.55 found opposing nucleation behaviour when crystallizing water and NaCl solution in the presence of the electric field. While the nucleation time was significantly decreased for water at $9.6 \times 10^5$ Vm$^{-1}$, the nucleation time increased for the NaCl solution.55

From the classical nucleation theory by Kashchiev,72 it was claimed that if $\varepsilon_c \approx \varepsilon_m$, the electric field does not influence the nucleation. However, to the best of our knowledge, this theory had not been experimentally proven.20 For the instance of the INA-1,4-dioxane system, as utilized in Radacsi’s research,59 the relative permittivities of INA and that of the medium are $\varepsilon_c = 2.209$ and $\varepsilon_m = 2.25$ respectively.60,65 Being nearly equal, the contribution of the electric field to the chemical potential difference and therefore on the nucleation work is estimated to be less than $10^{-7} kT$. Consequently, the electric field should have no effect on nucleation thermodynamics in the used solution system. In disagreement to this theory, Radacsi found that a strong electric field does affect the nucleation and crystallization of isonicotinamide in 1,4-dioxane.59 Therefore, the main challenge ahead is to understand how electric fields change the nucleation and crystallization kinetics. In order to control the crystalline product quality, it is essential to understand all the process parameters, influencing the crystallization process. The strength of the electric field, as well as its nature (external, internal, DC or AC), is affecting the crystalline product directly. Once the effects of electric fields on crystallization are controlled, the
<table>
<thead>
<tr>
<th>Type of electric field</th>
<th>Method</th>
<th>Material studied</th>
<th>Electric field strength</th>
<th>Electrode material</th>
<th>Solution concentration</th>
<th>Ref no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>External DC</td>
<td>HDVD</td>
<td>HEWL</td>
<td>$2.0 \times 10^3$ Vm$^{-1}$</td>
<td>Metal</td>
<td>20, 30, and 40 mg mL$^{-1}$</td>
<td>Taleb et al., 1999 (ref. 19)</td>
</tr>
<tr>
<td>External DC</td>
<td>SDVD</td>
<td>HEWL</td>
<td>$7.5 \times 10^3$ Vm$^{-1}$</td>
<td>Metal</td>
<td>10 mg mL$^{-1}$</td>
<td>Taleb et al., 2001 (ref. 61)</td>
</tr>
<tr>
<td>External DC</td>
<td>SDVD</td>
<td>HEWL</td>
<td>$4.8 \times 10^3$ Vm$^{-1}$</td>
<td>Metal</td>
<td>20 and 24 mg mL$^{-1}$</td>
<td>Nanev and Penkova, 2001 (ref. 62)</td>
</tr>
<tr>
<td>External DC</td>
<td>HDVD</td>
<td>HEWL</td>
<td>$1.5 \times 10^3$ Vm$^{-1}$</td>
<td>Metal</td>
<td>20 and 24 mg mL$^{-1}$</td>
<td>Nanev and Penkova, 2002 (ref. 37)</td>
</tr>
<tr>
<td>External DC</td>
<td>Laser-induced nucleation Microbatch</td>
<td>Glycine</td>
<td>$6 \times 10^5$ Vm$^{-1}$</td>
<td>Brass</td>
<td>4.1–4.5 mg mL$^{-1}$</td>
<td>Aber et al., 2005 (ref. 63)</td>
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<tr>
<td>External DC</td>
<td>Molecular dynamic simulations</td>
<td>Glucose isomerase</td>
<td>$100$–$1000$ Vm$^{-1}$</td>
<td>Copper</td>
<td>30 mg mL$^{-1}$</td>
<td>Rubin, Owen and Stojanoff, 2017 (ref. 17)</td>
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<tr>
<td>External DC</td>
<td>Protein film organized by external electric field</td>
<td>Paracetamol</td>
<td>$1.5 \times 10^5$ Vm$^{-1}$</td>
<td>N/A</td>
<td>N/A</td>
<td>Parks et al., 2017 (ref. 11)</td>
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<td>External DC bias</td>
<td>Cooling crystallization</td>
<td>4-vinylpropylene carbonate</td>
<td>$4.0 \times 10^5$–$2.0 \times 10^6$ Vm$^{-1}$</td>
<td>Invarsteel plates</td>
<td>N/A</td>
<td>Walter et al., 2018 (ref. 50)</td>
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<tr>
<td>External DC bias</td>
<td>Chemical synthesis</td>
<td>PVD</td>
<td>Maximum $8.0 \times 10^6$ Vm$^{-1}$</td>
<td>Pt and Co/Cr coated tips</td>
<td>N/A</td>
<td>Ganguly et al., 2018 (ref. 57)</td>
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<td>External DC</td>
<td>Chemical synthesis</td>
<td>ZnO</td>
<td>$5 \times 10^4$ Vm$^{-1}$</td>
<td>Stainless steel</td>
<td>N/A</td>
<td>Qi et al., 2018 (ref. 58)</td>
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<td>External DC</td>
<td>Cooling crystallization</td>
<td>Water, NaCl solution, mushroom (extract)</td>
<td>$3.2$–$9.6 \times 10^5$ Vm$^{-1}$</td>
<td>Copper</td>
<td>0.713% w/w NaCl</td>
<td>Fallah-Joshaqani et al., 2019 (ref. 55)</td>
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<tr>
<td>External DC</td>
<td>Microbatch</td>
<td>HEWL, thaumatin</td>
<td>$\sim 2.7 \times 10^5$ Vm$^{-1}$</td>
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<td>Al-Haq et al., 2007 (ref. 7 and 49)</td>
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<td>Internal DC</td>
<td>Microfluidic device</td>
<td>HEWL</td>
<td>Maximum $10^9$ Vm$^{-1}$</td>
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<td>3.85 mg mL$^{-1}$</td>
<td>Sui et al., 2018 (ref. 41)</td>
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<td>Internal DC</td>
<td>Electrophoretic diffusion</td>
<td>Estradiol 17β-dehydrogenase</td>
<td>$1500$–$4000$ Vm$^{-1}$</td>
<td>N/A</td>
<td>0.02–6.9 mg mL$^{-1}$</td>
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<td>Internal DC</td>
<td>Gel</td>
<td>HEWL, Thaumatin</td>
<td>N/A (applied current 0.9–1 μA)</td>
<td>Pt wire Graphite</td>
<td>100; 100 mg mL$^{-1}$</td>
<td>Mirkin et al., 2003 (ref. 43)</td>
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<tr>
<td>Internal DC</td>
<td>Gel</td>
<td>HEWL</td>
<td>N/A (applied current 2.0 μA)</td>
<td>Pt</td>
<td>10 mg mL$^{-1}$</td>
<td>Sazaki et al., 2004 (ref. 29)</td>
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<td>Internal DC</td>
<td>Gel</td>
<td>HEWL</td>
<td>0.19 Vm$^{-1}$</td>
<td>Pt</td>
<td>N/A</td>
<td>Moreno and Sazaki, 2004 (ref. 30)</td>
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<tr>
<td>Internal DC</td>
<td>Gel</td>
<td>HEWL</td>
<td>N/A (applied current 2.0 μA)</td>
<td>Pt</td>
<td>30 mg mL$^{-1}$</td>
<td>Nieto-Mendoza et al., 2005 (ref. 40)</td>
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<td>Internal DC</td>
<td>Crystallization cell where at least one of the electrodes is a sharp tip</td>
<td>HEWL, BPTI</td>
<td>Maximum $10^6$ Vm$^{-1}$</td>
<td>Tungsten wire</td>
<td>25 (HEWL), 20 (BPTI) mg mL$^{-1}$</td>
<td>Hammadil et al., 2007 (ref. 35)</td>
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<td>Internal DC</td>
<td>Gel</td>
<td>HEWL, BPTI, INA</td>
<td>Maximum $5.6 \times 10^5$ Vm$^{-1}$</td>
<td>Cu</td>
<td>17, 20, 25, 30 and 35 mg mL$^{-1}$</td>
<td>Radači, 2012 (ref. 59)</td>
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<td>Internal DC</td>
<td>SDVD</td>
<td>HEWL, 2TEL-Lys</td>
<td>$0.2 \times 10^0$ Vm$^{-1}$</td>
<td>Cu</td>
<td>60 (HEWL), 4.5 (2TEL-Lys) mg mL$^{-1}$</td>
<td>Flores-Hemández et al., 2013 (ref. 26)</td>
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<td>Internal DC</td>
<td>Cooling crystallization</td>
<td>INA, CAF, PHE</td>
<td>Maximum $1.25 \times 10^6$ Vm$^{-1}$</td>
<td>Cu</td>
<td>18 (INA), 13 (CAF), 29 (PHE) mg mL$^{-1}$</td>
<td>Li et al., 2016 (ref. 18)</td>
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<td>Microfluidic device</td>
<td>HEWL</td>
<td>$400$–$600$ Vm$^{-1}$</td>
<td>Graphene</td>
<td>80 mg mL$^{-1}$</td>
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<td>External AC</td>
<td>Batch</td>
<td>HEWL</td>
<td>$6.6 \times 10^5$ and $8.6 \times 10^4$ Vm$^{-1}$</td>
<td>Cu</td>
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<td>Batch</td>
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<td>57 mg mL$^{-1}$</td>
<td>Koizumi et al., 2010 (ref. 31)</td>
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<td>HEWL</td>
<td>$9.0 \times 10^7$ Vm$^{-1}$</td>
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<td>10 mg mL$^{-1}$</td>
<td>Koizumi et al., 2012 (ref. 32)</td>
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Table 2 (continued)

<table>
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<td>Batch</td>
<td>57 mg mL⁻¹</td>
<td>Koizumi et al., 2013, 2015, 2016 (ref. 25)</td>
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<td>Li and Lakerveld, 2018 (ref. 54)</td>
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<td>Batch</td>
<td>40 mg mL⁻¹ (insulin)</td>
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<td>Batch</td>
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<td>1959.9–468.2 mg mL⁻¹</td>
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<td>Internal AC</td>
<td>HEWL</td>
<td>Vacuum diffusion</td>
<td>Li and Lakerveld, 2018 (ref. 54)</td>
<td></td>
</tr>
<tr>
<td>External AC</td>
<td>HEWL, lysozyme</td>
<td>Batch</td>
<td>90 mg mL⁻¹ (lysozyme)</td>
<td>Wakamatsu, 2016 (ref. 45)</td>
</tr>
<tr>
<td>Internal AC</td>
<td>HEWL</td>
<td>Batch</td>
<td>90 mg mL⁻¹ (lysozyme)</td>
<td>Wakamatsu, 2016 (ref. 45)</td>
</tr>
<tr>
<td>Electric field strength</td>
<td>Cu</td>
<td>ITO</td>
<td>4.0 × 10⁴ V m⁻¹</td>
<td>Koizumi et al., 2013, 2015, 2016 (ref. 16, 33 and 51)</td>
</tr>
<tr>
<td></td>
<td>ITO</td>
<td>ITO</td>
<td>1.1 × 10⁵ V m⁻¹</td>
<td>Koizumi et al., 2017 (ref. 53)</td>
</tr>
<tr>
<td></td>
<td>ITO</td>
<td>ITO</td>
<td>3.0 × 10⁵ V m⁻¹</td>
<td>Koizumi et al., 2017 (ref. 53)</td>
</tr>
<tr>
<td></td>
<td>ITO</td>
<td>Cu/Ir</td>
<td>5.6 × 10⁴ V m⁻¹</td>
<td>Li and Lakerveld, 2018 (ref. 54)</td>
</tr>
<tr>
<td></td>
<td>Cu/Ir</td>
<td>Cu/Ir</td>
<td>1.66 × 10⁵ V m⁻¹</td>
<td>Li and Lakerveld, 2018 (ref. 54)</td>
</tr>
</tbody>
</table>

Effects resulting from the application of AC fields on crystallization have also been studied by Hou and Chang, Li and Lakerveld, and a series of publications by Koizumi et al. Each author contributed findings on the effect of the applied electric field on the crystallization process. It has been demonstrated that application of electric fields has the ability to control both an increase and a reduction in the rate of nucleation and crystal size. An increase in the final crystal quality was obtained, improved X-ray diffraction signal-to-noise ratio was observed, and control of crystal orientation and polymorphic form was demonstrated by Li and Lakerveld.

Conclusions

This highlight has underlined the importance of electric fields in significantly enhancing the crystallization process. These fields have proven to be a valuable manipulation instrument for both the dynamics of crystallization and the crystal size distribution. This highlight has shown and confirmed by using an extensive range of conditions and methods, that utilizing electric fields is one successful method of enhancing crystallization through controlling the nucleation rate and location of nucleation.

Crystallization under a DC field has primarily been employed for protein crystallization in both internal or external electrode arrangements. Effects resulting from the application of AC fields on crystallization have also been studied by Hou and Chang, Li and Lakerveld, and a series of publications by Koizumi et al. Each author contributed findings on the effect of the applied electric field on the crystallization process. It has been demonstrated that application of electric fields has the ability to control both an increase and a reduction in the rate of nucleation and crystal size, an increase in the final crystal quality was obtained, improved X-ray diffraction signal-to-noise ratio was observed, and control of crystal orientation and polymorphic form was demonstrated by Li and Lakerveld.

The findings drawn from past studies show in general, positive effects on the behavior of crystal growth under application of an electric field. Table 3 summarizes the observed effects on crystallization induced by an electric field as outlined in this literature review.

As a conclusion, there are several benefits brought by application of the applied electric field in controlling crystallization, which are: reduction of the nucleation time, control of the location of nucleation, control of the product crystal size, enhancement of overall crystal quality, increase of the crystal-line product yield, control of the crystal orientation and next issue is the scale-up. As the crystallization vessel is often metal, the applied high voltage might raise safety concerns. This could be justified by the low current in the system, meaning that the system is non-lethal, and proper design and material use (e.g. glass instead of metal) of the setup, along with safety signs and interlock system, could solve the safety concerns. For industrial applications, the increased costs could be also a concern. However, the power of a high voltage power supply is usually low due to the low current, meaning low operational costs. The increased yield and crystal quality should overcome the financial investments in an electric field-assisted crystallization system. Finally, the scale-up of such systems is still a challenge. If used in a batch system, the applied potential difference might be too high, leading to sparks and safety concerns. Therefore, the application of electric fields would be more realistic and safe in continuous-flow crystallization systems, as was demonstrated by Li and Lakerveld.
Increased X-ray diffraction signal-to-noise ratio Sui et al., 2009; Gil-Alvaradejo et al., 2011

<table>
<thead>
<tr>
<th>Observed effect by application of electric field</th>
<th>Research groups</th>
<th>Ref. number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced nucleation time</td>
<td>Taleb et al., 1999; Nanev and Penkova, 2001, 2002; Mirkin et al., 2003; Moreno and Szakai, 2004; Szakai, Moreno and Nakajima, 2004; Koizumi et al., 2009; Radacsi, 2012; Flores-Hermendez et al., 2013; Adrjanowicz, Pauch and Richard, 2018; Walter et al., 2018; Sui et al., 2018</td>
<td>19, 24, 26, 30, 37, 41, 43, 50, 59, 60, 62, 66</td>
</tr>
<tr>
<td>Increased nucleation rate</td>
<td>Koizumi et al., 2009, 2012; Jha et al., 2017</td>
<td>4, 24, 32</td>
</tr>
<tr>
<td>Enhanced overall crystal quality</td>
<td>Koizumi et al., 2009, 2012; Jha et al., 2017</td>
<td>4, 24, 32</td>
</tr>
<tr>
<td>Less crystals produced, and with an enlarged size</td>
<td>Taleb et al., 1999; Hou and Chang, 2008; Koizumi et al., 2013; Pareja-Rivera et al., 2017; Rubin, Owen and Stojanoff, 2017; Koizumi et al., 2017; Walter et al., 2018</td>
<td>14–17, 19, 25, 50</td>
</tr>
<tr>
<td>Substantial effect of crystalline orientation was induced – Crystals exhibited a preferred orientation along c axis</td>
<td>Taleb et al., 1999; Nanev and Penkova, 2001, 2002; Szakai, Moreno and Nakajima, 2004; Al-Haq, Lebrasseur, Tsuchiy, et al., 2007; Flores-Hermendez et al., 2013; Pareja-Rivera et al., 2017; Owen and Stojanoff, 2017</td>
<td>7, 14, 17, 19, 26, 29</td>
</tr>
<tr>
<td>Polymorphic outcome was successfully controlled</td>
<td>Koizumi et al., 2009, 2015</td>
<td>14, 30, 37, 43, 62</td>
</tr>
<tr>
<td>Electric field had a greater impact on nucleation rate and induction time at lower temperatures</td>
<td>Koizumi et al., 2009, 2015</td>
<td>14, 30, 37, 43, 62</td>
</tr>
<tr>
<td>Using optimized AC frequency to improve the crystal quality for protein crystals</td>
<td>Koizumi et al., 2009, 2015</td>
<td>14, 30, 37, 43, 62</td>
</tr>
<tr>
<td>Increasing charge of the protein led to increasing electric field effect</td>
<td>Koizumi et al., 2009, 2015</td>
<td>14, 30, 37, 43, 62</td>
</tr>
<tr>
<td>Increased X-ray diffraction signal-to-noise ratio</td>
<td>Koizumi et al., 2009, 2015</td>
<td>14, 30, 37, 43, 62</td>
</tr>
<tr>
<td>The position of crystals depended on the polarity of the electric field</td>
<td>Koizumi et al., 2009, 2015</td>
<td>14, 30, 37, 43, 62</td>
</tr>
<tr>
<td>Decreased growth rate</td>
<td>Parks et al., 2017; Koizumi et al., 2017</td>
<td>11, 25</td>
</tr>
<tr>
<td>Promotion or inhibiting of protein crystallization</td>
<td>Li et al., 2016</td>
<td>18</td>
</tr>
<tr>
<td>Separation and recovery of two crystalline substances from their multicomponent solution</td>
<td>Hou and Chang, 2008; Pareja-Rivera et al., 2017; Walter et al., 2018; Li and Lakerveld, 2018</td>
<td>14, 15, 50, 54</td>
</tr>
<tr>
<td>Increased crystallization yield</td>
<td>Ganguly et al., 2018</td>
<td>57</td>
</tr>
<tr>
<td>Transformation from amorphous to crystalline form</td>
<td>Ganguly et al., 2018</td>
<td>57</td>
</tr>
</tbody>
</table>

control the polymorphism. In addition, electric fields can be also used for separation of multicomponent mixtures, polyn-184 meth transformation into crystalline material and crystalliza-185 tion in continuous flows.

**Outlook**

Regardless of the progress, information regarding a thorough and uniform understanding of electric field promoted nucleation is yet to be ascertained. There remain questions to be unanswered regarding the range of crystallizing compounds and appropriate solvents that can be used under these conditions, as well as process engineering design and scale-up from laboratory work and miniature devices. The upcoming decade is likely to see this gap of knowledge converging, and thus promoting several opportunities in areas such as pharmaceutical science, food industry, chemical industry or life science. The knowledge obtained thus far can be tested on more challenging compounds that poorly diffract e.g. membrane proteins, or complex cellular proteins, like ribosomes, which are difficult to crystallize for X-ray analysis. Furthermore, the future could see an inclination in research concentrated on polymorphism in proteins. Recently, results have proved that the hybrid of particle manipulation, brought about by the presence of the electric field, and cooling crystallization can be used for separation and recovery of two crystalline substances from their multicomponent solution. In addition to this, supplementary research could be conducted on merging various crystal growth approaches as advanced methods of controlling crystallization, which would deepen our understanding regarding the effect of the electric field on the process of crystallization. With regards to crystallization in a continuous flow, as this is a novel approach, there is a lot of opportunity for optimization in this area. As an example: flow rate (shear), flow direction, distribution of the electric-field potential, and frequency may each be altered to fully study and comprehend the effects of crystallization under continuous flow. As the emphasis has been placed on model proteins and compounds, it is worthwhile investigating the varied reactions of different materials to electric fields. This would enable the researcher to see if there is a common effect with the model compounds, and therefore allow them to completely comprehend the nucleation and crystal development mechanisms in these circumstances. It is anticipated that numerous advances will arise to further control the
properties of crystals, and thus obtain a set of great quality products in the near future.

Author contributions

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Abbreviations

AC Alternating current
CAF Caffeine
DC Direct current
HDVD Hanging drop vapor diffusion
SDVD Sitting drop vapor diffusion
INA Isonicotinamide
ITO Indium tin oxide
PHE Phenazine
HEWL Hen egg white lysozyme
BPTI Bovine pancreatic trypsin inhibitor
PVDF Poly(vinylidene fluoride)
SDVD Sitting drop vapor diffusion

Conflicts of interest

There are no conflicts to declare.

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