7.5.2 Electric Fields

Electric fields have mostly been applied to crystallization of biomacromolecules such as proteins in order to alter the nucleation and growth kinetics of proteins. Large and high quality protein crystals are needed for structural studies based on X-ray diffraction. However, spontaneous nucleation of large biomolecules is often accompanied with a long induction time even in the presence of high supersaturation. This is supported by the fact that the nucleation mechanism of proteins in solution is itself not fully understood yet. Consequently, electric-field-assisted protein nucleation is at its beginning in terms of understanding. However, it has been widely observed that electric fields of various forms (*e.g.*, external, internal, alternating current, direct current, *etc.*) can have a positive effect on protein nucleation. A recent review of electric-field-assisted protein crystallization has been provided by Nanev.[291] While the majority of studies employing this PI concept focused on generating single crystals for X-ray studies in small batch system with electrodes, protein crystallization as a separation and purification process (*e.g.*, for the manufacture of biopharmaceuticals) suffers scale-up issues (ineffective and unpredictable at best) of the electric-field-assisted batch protein crystallization. Thus, continuous crystallization offers PI opportunities to employ electric-field-assisted protein crystallization as a separation and purification method of proteins. Similarly to ultrasound, continuous crystallization allows for the application of the external field over a smaller volume. Furthermore, when continuous crystallization is executed in a flow channel, a large surface-to-volume ratio provides flexibility to design electrodes with a large contact area with a supersaturated solution. Li and Lakerveld[292] extended the electric-field-assisted protein crystallization from a conventional batch configuration to a configuration based on continuous flow by constructing a microfluidic device with two co-planar electrodes that were separated by a gap in the center of the flow channel (Figure 7.19).

It was demonstrated that the use of electric fields can deliver a higher and more consistent yield compared to control experiments in the flow channel without any electric field due to increased nucleation. In general, such intensified protein crystallization processes in flow hold great potential to replace or supplement conventional chromatography-based separation and purification processes in industry. In addition to applications related to protein crystallization, electric fields have also been used for electrochemically induced crystallization[293,294] and crystal separation[295] of smaller molecules. While the former is often based on a pH shift to change the solubility of an organic molecule with an acidic or basic functional group to create supersaturation, the latter is centred on the notion that particle movements in strong electric fields are based on specific properties such as size and shape, which can be exploited for *in situ* particle separation during crystallization. However, the translation of such concepts towards continuous crystallization is still in its infancy.