Introduction

Today the production of biopharmaceuticals is mainly based on two bioreactors: Chinese hamster ovary cell (CHO) and \textit{Escherichia coli}. When roughly comparing the prices of production of these two systems with the production of plant made (PMBs) the one can see, that the price of production in crops (Table 1), is about 1000-fold lower as in the case of CHO cells commonly applied in industry today. Among the different types of “green” bioreactors being studied today, there is a general consensus among scientists that production in green plant tissues such as leaves is more feasible. However, the presence of chlorophyll and phenolic compounds in plant extracts, which can precipitate and denature the proteins besides damaging separation membranes and gels, makes this technology impracticable on a commercial scale. Electrochemically produced aluminium hydroxide gel can be used to adsorb these compounds, and pre-purify recombinant synthetic green fluorescent protein (sGF) produced in \textit{Nicotiana benthamiana} leaves. Removal efficiencies of 99.7% of chlorophyll, 88.5% of phenolic compounds, and 38.5% of native proteins from the \textit{N. benthamiana} extracts were achieved without removing sGF from the extracts. Since electrochemical preparation of aluminium hydroxide gel is a cost-effective technique, its use can substantially contribute to the development of future production platforms for recombinant proteins produced in green plant tissues of pharmaceutical and industrial interest.

Challenges in electrochemical pre-purification of recombinant proteins from green plant tissues

The use of recombinant proteins has increased greatly in recent years, as have the number of techniques and materials used for their production and purification. The principal advantage of using plants as bioreactors is the cost of the recombinant protein production, which is about 1000-fold lower as in the case of CHO cells commonly applied in industry today. Among the different types of "green" bioreactors being studied today, there is a general consensus among scientists that production in green plant tissues such as leaves is more feasible. However, the presence of chlorophyll and phenolic compounds in plant extracts, which can precipitate and denature the proteins besides damaging separation membranes and gels, makes this technology impracticable on a commercial scale. Electrochemically produced aluminium hydroxide gel can be used to adsorb these compounds, and pre-purify recombinant synthetic green fluorescent protein (sGF) produced in \textit{Nicotiana benthamiana} leaves. Removal efficiencies of 99.7% of chlorophyll, 88.5% of phenolic compounds, and 38.5% of native proteins from the \textit{N. benthamiana} extracts were achieved without removing sGF from the extracts. Since electrochemical preparation of aluminium hydroxide gel is a cost-effective technique, its use can substantially contribute to the development of future production platforms for recombinant proteins produced in green plant tissues of pharmaceutical and industrial interest.

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The main advantage of the process described in the original article lies in its highly predictable and reproducible—the characteristic that will benefit in process validation. The reason for this high reproducibility lies within the process of production of the aluminum hydroxide gel. More specifically its constant diameter and its superficial charge which can be easily controlled by pH of the solution during aluminum hydroxide gel production (Table 2; for more information, see ref. 4). In addition, extraction buffer contained sodium metabisulfite preventing quinine formation and therefore preventing covalent bonding between protein and phenolic compounds, what results in higher phenolics removal and therefore preventing covalent bonding between protein and phenolics if no reducing agent is used during the extractions step. No effect on the chlorophyll removal with the addition of the reducing agent was observed.

Additionally, the removal of phenolics, chlorophyll, and proteins diminished with the lower superficial charge of the aluminum hydroxide gel. This proves, that the aluminum hydroxide acts as an ionic exchanger, which superficial charge can be easily manipulated by the pH of electrocoagulation (Table 2).

The electrocoagulation therefore has its potential not only as clarification step but also in substituting the ionic exchange resins in chromatographic columns. Not only in plant based bioprocesses but generally speaking in all bioprocesses. The companies producing the biopharmaceuticals or industrial proteins could therefore produce its own “resin” without being dependent on the continuously more expensive chromatographic resins, which, in many cases, show lot to lot variabilities which can impact the specific process. This is due to the fact that each protein interacts “individually” with the resins in terms of specific and not specific interactions.

**Conclusions**

In the future the development of predictable first process steps in producing the PMBs will be necessary. This “predictable processes” cannot be achieved by experimental design and process parameter optimization, as is the case of the current researches, but by developing and formulating predictable mathematical models, that take into account the actual phenomenon that is the driving force of the separation itself. This can be achieved by in-depth characterization of the processes employed and in parallel characterization of plant extracts and interaction of its components with the “process” material as well as the interaction of the plant components among them self. Additionally the electrocoagulation can also be applied in place of ionic exchange chromatography, however additional research and development is needed.

### References

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### Table 1. Cost of production of recombinant proteins

| Expression system | Cost (USD/kg) |
|-------------------|---------------|
| Chinese hamster ovary cells (CHO) | 300 |
| Transgenic chicken eggs | 1-2 |
| Transgenic goat milk | 1-2 |
| Microbial fermentation | 1.00 |
| Transgenic plants | 0.10 |

Cost of production using corn seeds at expression of 1% of recombinant protein.

### Table 2. Zeta potential and diameter of aluminum gel particles formed by electrocoagulation in solutions of 200 mmol/L NaCl at different pH values

| Electrocoagulation pH | Particle diameter (μm) | Zeta potential (mV) |
|-----------------------|------------------------|---------------------|
| 7.5                   | 597                    | 21.3                |
| 8.0                   | 632                    | 13.8                |
| 8.5                   | 659                    | 7.1                 |
| 9.0                   | 646                    | 5.1                 |
| 9.5                   | 636                    | 3.6                 |

Validation could lead to high financial losses to the company performing such tasks. Here again, the reproducible and robust extraction and clarification step, besides being cost effective, will be required.

When summarizing all three areas of challenge one could conclude, that the main challenge lies in the first processing steps which has to be well characterized, predictable and robust, besides being of low cost. However, no process encountered in the literature concerning the clarification (pre-purification) step did take in account the predictability of the process in consideration.