Application of Electrochemically Produced Aluminum Hydroxide Gel for Prepurification of Recombinant Synthetic Green Fluorescent Protein Produced in Tobacco Leaves

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The use of recombinant proteins has increased greatly in recent years, as also have increased the number of techniques and materials used for their production and purification. Among the different types of bioreactors being studied, 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. In the present work, the adsorption to electrochemically produced aluminum hydroxide gel was applied as a prepurification step for recombinant synthetic green fluorescent protein (sGFP), also referred to as enhanced green fluorescent protein, produced in Nicotiana benthamiana *leaves. Removal efficiencies of 99.7% of chlorophyll, 88.5% of phenolic compounds, and 38.5% of native proteins from the* N. benthamiana *extracts were achieved without removing sGFP from the extracts. As electrochemical preparation of aluminum 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.* © 2011 American Institute of Chemical Engineers *Biotechnol. Prog.*, 27: 1029–1035, 2011
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Introduction

When downstream processing (DSP) of recombinant proteins is compared with different expression systems—animal, microbial, or vegetal—basically the same purification steps are observed in all cases.[1] The principal differences in production processes when using plant-based bioreactors are the extraction and clarification steps.[2] The principal differences between the processes cited above are in the extraction and clarification steps, which are most complex in the case of using plant-based bioreactors. However, the greatest disadvantage of such expression systems is the high concentrations of chlorophyll and phenolics in the extracts, which tend to precipitate and denature the recombinant protein also in the extract, besides causing a fouling of the chromatographic column resins during the purification steps.[3] As a consequence, the need to clean the column chromatographic resin more frequently, or to change it, will result in the higher cost of the desired final product.[4] Therefore, when high purity of the product is needed and chromatographic steps are indispensable, prechromatographic steps should be used to remove the chlorophyll and phenolics from the extracts, consequently prolonging the life of the chromatographic resins.[5]

Interactions between proteins and phenolic compounds can be divided into four groups based on the type of interaction.[6] The hydroxyl group of phenolic compounds can either form a hydrogen bond with the oxygen in peptidic bonds or interact ionically with basic residues (pK_a above 9) of proteins. Also, the aromatic ring in phenolic compounds is hydrophobic and can interact with hydrophobic residues of proteins. The strongest bond, however, is the covalent coupling of the oxidized form of phenolic compounds—quinone—with the $-SH$ and $-NH_2$ residues of the proteins. In chlorophyll, the interaction mechanisms are mainly hydrophobic and to a lesser extent hydrogen bonding.[7]

Although, relatively little work has been done in this area, recently it has been receiving more attention. Platis and

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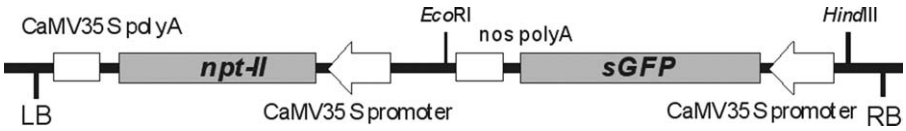

Figure 1. Schematic representation of t-DNA region of the pCambia 2300-SGFP vector.

The sGFP and npt-II genes are under transcriptional control of the CAMV35S promoter (35-S) and the nos and CaMV35S terminators, respectively. LB and RB: right and left borders of the t-DNA.

Labrou[8] optimized an aqueous two-phase system (ATPS) containing polyethyleneglycol, which removed 89% of the phenolics, while 95% of recombinant protein was recovered. Another interesting approach has been developed by Glatz and coworkers,[9,10] where the ATPS in conjunction with two-dimensional electrophoresis has been used to predict the protein removal from the transgenic plant extracts; however, no investigation on chlorophyll and phenolics removal was performed. Holler and Zhang[11] added polyvinylpolypyrrolidone (PVPP) to the extracts, to remove phenolics from tobacco leaf extracts, a procedure similar to the one described by Andersen and Sowers.[12] However, the authors did not mention the efficiency of phenolics removal by this technique. Woodard et al.[13] efficiently removed phenolics (98%) from transgenic *Lemna minor* extracts by using a hydrophobic styrene–divinylbenzene matrix with Amberlite IRA-402 anion exchange resin similar to the one described by Jervis and Pierpont.[14] Although several approaches with high removal efficiencies have been found in the literature, all the mentioned techniques needed to use acidic pH (5 for ATPS and around 4 for PVPP and Amberlite IRA-402 removals), which usually denatures the proteins.

Electrocoagulation (formation of insoluble aluminum hydroxide gel by electrochemical dissolution of aluminum electrodes) is a relatively inexpensive separation technique based on the electrochemical formation of aluminum hydroxide gel (Eq. 1) whose superficial characteristics can be easily manipulated by the pH of the process.[15–18]

$$2Al^{3+}_{(aq)} + 6H_2O + 6e^- \rightarrow 2Al(OH)_{3_{(s)}} + 3H_{2_{(g)}} \qquad (1)$$

Its main advantage over other techniques is that it can be operated under neutral to slightly basic pH conditions so that practically no protein undergoes denaturation or activity loss. The observed high efficiency and selectivity of electrocoagulation are the result of a constant pH during the whole process of gel particle formation, thereby resulting in homogenous material, which is usually not the case when low-cost adsorbents are used.[15,19] Electrochemically produced aluminum hydroxide gel is also more efficient in selectively removing the desired compounds than gel produced by dissolving the aluminum salts, as the pH during the electrocoagulation is constant, whereas at aluminum salt dissolving techniques this parameter is dependent on the salt added.[20,16,21] Also, the electrochemically produced gel contains less bound water and is therefore more shear resistant and more readily filterable.[22] The cost for energy, chemicals, and disposal of metallic residue of electrocoagulation can be up to five times lower than the one using chemical precipitation.[23]

In the present work, transgenic *Nicotiana benthamiana* leaves expressing synthetic green fluorescent protein (sGFP) were used to evaluate the potential of electrochemically produced aluminum hydroxide gel in the removal of chlorophyll and phenolics from the plant leaf extracts. The removal of native proteins from the extract was also evaluated, as removing them earlier in the DSP reduces the burden of chromatographic columns, thereby lowering final product cost.

Our previous studies with electrocoagulation[18] with pure components showed that a protein (bovine serum albumin) and a phenolic compound [(+)-catechin] interact with the gel produced by different mechanisms, independently of the pH used. The amount of proteins removed is proportional to the amount of gel present in the solution (Eq. 2), whereas the phenolics removal mechanism is the result of the distribution of the compounds between the aqueous and the aluminum hydroxide gel phase (Eq. 3).

$$N_{i,eq} = \frac{n_{i,Al(OH)_3}}{m_{Al(OH)_3}} \qquad (2)$$

$$K_i = \frac{c_{i,Al(OH)_3}}{c_{i,H_2O}} \qquad (3)$$

In this work, aluminum hydroxide gel was produced separately and added to the fresh extracts afterward. The optimum removal of chlorophyll and phenolics is achieved if pH 8.0 is used during the electrocoagulation procedure, and therefore only the quantity of gel added to the extract was optimized here.

Materials and Methods

Materials

Transgenic *N. benthamiana* was obtained by *Agrobacterium tumefaciens*-mediated transformation of leaf tissues. The sGFP gene expression cassette was cloned at the *Hind*III and *Eco*RI restriction sites of the binary vector pCambia2300 (Figure 1). Competent *A. tumefaciens* GV3101 cells were transformed by electroporation using a Gene Pulser electroporator (Bio-Rad). Selected clones were grown overnight at 27°C in Luria-Bertani (LB) medium and inoculated on leaf explants cultivated on Murashige and Scoog (MS) medium[24] containing 2.0 mg/L benzilaminopurine (MS-BAP). After two days, explants were transformed into selection medium (2.0 mg/L MS-BAP, 200 mg/L timetin, and 100 mg/L kanamycin). Regenerated plants were transplanted to soil and analyzed by polymerase chain reaction (PCR), using sGFP-specific primers. Leaf samples from F1 plants were mounted on a slide and GFP fluorescence evaluated on an Axiovert fluorescent microscope with the proper filter set (480 nm excitation and 520 nm emission wavelengths). Leaves showing sGFP fluorescence were collected, frozen in liquid nitrogen, and lyophilized in Alpha 1-4 plus freeze dryer (Christ, Germany) for 24 h. The sodium chloride (purity $\geq$ 98%) used was from Merck (Germany) and all other chemicals used were from Sigma and of at least analytical grade. Aluminum electrodes used in all the experiments were of the 1100 type

(purity $\geq$ 99%). High-purity water prepared with a Milli-Q system (Millipore) was used in all the experiments.

Extraction procedure

N. benthamiana lyophilized leaves were mixed with extraction buffer containing 10 mmol/L of tris(hydroxymethyl)aminomethane (TRIS) and 5 mmol/L of sodium metabisulfite (MBS) in a 1:100 solid-to-liquid ratio. Extraction was carried out at 25°C for 2 min using an immersion blender. MBS was added to prevent oxidation of phenolic compounds to quinones by peroxidase, thereby preventing covalent coupling of quinines with proteins.[25] After extraction, suspensions were centrifuged at 12,000*g* for 20 min at 25°C and filtered through a 3-μm filter paper.

Electrochemical preparation of suspensions of aluminum hydroxide gel

An acrylic cell having internal dimensions of 40 × 20 × 80 mm^3 and two aluminum electrodes (40 × 80 × 1 mm^3) set 20 mm apart was used[18] in all the experiments. A volume of 40 mL of solution containing 200 mmol/L of NaCl and 100 mmol/L of TRIS at pH 8.0 was added to the cell. Strong buffer solution was used to maintain the pH constant during the aluminum hydroxide gel formation, resulting in more homogenous material. Another reason for using such strong buffer systems was to complex the chlorine ions liberated during the electrocoagulation. The difference in potential between the electrodes was maintained at 5.0 V (60 Hz) for 2 h. At given conditions, 0.044 mg (0.001 mg/mL) of dry aluminum hydroxide gel was formed per hour.

Adsorption of the N. benthamiana leaf components on aluminum hydroxide gel

The tobacco leaf components (chlorophyll, phenolics, and native proteins) were removed by mixing the suspensions of aluminum hydroxide with the extracts in 0.25, 0.33, 0.50, 0.75, and 1.00 v/v ratios, separately. The resulting suspensions were agitated for 1 h, and the coagulate was removed by centrifugation at 12,000*g* for 20 min at 25°C. As a result the clarified extract was obtained.

Determination of chlorophyll, phenolics, and total protein in the extracts

Concentrations of the compounds in the extracts were measured according to the methods of Arnon[26] for chlorophyll, Price and Butler[27] for phenolic compounds, and Bradford[28] for protein concentration. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (15% acrylamide gels) under reducing conditions was carried out using a Protean II System (Bio-Rad). The gels were stained with Coomassie blue and a low molecular weight standard protein mixture (GE Healthcare) was used as a molecular mass protein marker. All the spectrophotometric measurements were done in a DU 650 spectrophotometer (Beckman).

Determination of sGFP in the extracts

Fluorometric measurements were done according to Robic et al.[29] by using an F-4500 fluorescence spectrophotometer (Hitachi, Japan) set to a 480-nm excitation wavelength with an excitation slit opening of 5 nm and a 513-nm emission wavelength with a 20-nm emission slit opening. As a reference sGFP(S65T) was used, which was prepared as described in our previous publication.[29] One unit of sGFP fluorescence was defined as the fluorescence of 1 ng/mL of sGFP in 50 mmol/L sodium phosphate buffer pH 7.00.

Determination of $Al(OH)_3$ produced during electrocoagulation

To determine the quantity of aluminum hydroxide in the suspension produced by electrocoagulation, the gel was separated from the solution by filtration on a 0.22-μm membrane filter and then dried at 100°C for 30 min and its mass was determined by an analytical balance.

Isoelectric focusing (IEF) electrophoresis and densitometric analysis of the bands

Sample preparation was based on the procedure described by Laukens et al.[30] A volume of 400 μL of the liquid phase, obtained as described in the coagulation procedure, was treated with 1,600 μL of 10% (m/v) trichloroacetic acid and 0.07% (v/v) β-mercaptoethanol in acetone and incubated overnight at −20°C. After centrifugation (15,000*g* at 4°C for 10 min), the supernatant was discarded, 1,600 μL of acetone containing 0.07% (v/v) β-mercaptoethanol was added to the pellet, and the sample was stored for 1 h at −20°C. The sample was then centrifuged again (15,000*g* at 4°C for 10 min), the pellet was air dried at room temperature, and 300 μL of rehydration buffer [8 mol/L urea, 2% (m/v) 3-((3-Cholamidopropyl)dimethylammonio)-1-propanesulfonate (CHAPS), 50 mmol/L dithiothreitol (DTT), 0.2% (v/v) Bio-Lyte 3/10 ampholyte (Bio-Rad), and 0.002% (m/v) bromophenol blue] was added to it.

The samples were applied on 7 cm immobilized pH gradient strips (pH 3–10) by 12 h passive ingel rehydration and subsequently focused in the Protean IEF cell (Bio-Rad) according to the following protocol: 1 h at 250 V with linear gradient, 2 h at 4,000 V with linear gradient, and 10,000 V/h with rapid gradient.

The strips obtained were treated for 1 h with 20% trichloroacetic acid to remove the ampholytes from the gels and precipitate the proteins inside the strips. Subsequently, the bands were visualized by staining with 1% (m/v) Coomassie blue in 40% methanol and destained with 40% methanol. The strips (and SDS-PAGE gels) were scanned on a GS-800 calibrated imaging densitometer (Bio-Rad) at 36.3 μm per pixel. Image analysis was performed with Quantity One 4.6.7 (Bio-Rad) software using the following settings: background method, disk; background radius, 100; band sensitivity, 10.0; bandwidth, 2.5; band minimal density, 0.0; band filter, 4.0; band shoulder, 1.0; and band size, 5.

Results and Discussion

Extraction of chlorophyll, phenolics, total protein, and sGFP from N. benthamiana leaves and their removal from the extracts by aluminum hydroxide gel

The levels of phenolics extracted from transgenic *N. benthamiana* leaves (Table 1) were two times lower than the ones obtained with *Nicotiana tabacum*[8,11] and about three times higher than the ones obtained with *L. minor*.[13] In terms of total protein, the concentrations in the *N. tabacum* and *L. minor* extracts, the levels were three and two times lower,

Table 1. Concentrations of Chlorophyll, Phenolics, Total Protein, and sGFP in Transgenic *N. benthamiana* Extracts and Their Efficiencies of Removal as a Function of Quantity of Aluminum Hydroxide Gel Added

V_{GEL}/V_{EXT} (mL/mL)	Chlorophyll Concentration (mg/L) and Removal (%)		Phenolics Concentration (mmol/L) and Removal (%)		Total Protein Concentration (mg/mL) and Removal (%)		sGFP Concentration (μg/mL) and Removal (%)	
0	4.17	0	0.71	0	0.46	0	4.73	0
0.25	0.75	82.1	0.36	50.2	0.38	17.5	4.68	1.0
0.33	0.46	89.4	0.26	64.5	0.35	25.2	4.73	0
0.50	0.17	96.2	0.16	76.7	0.34	26.3	4.67	1.2
0.75	0	99.7	0.08	88.5	0.29	38.4	4.73	0
1.00	0	99.7	0.08	89.0	0.24	47.4	4.20	19.1

V_{GEL}: volume of aluminum hydroxide gel suspension and V_{EXT}: volume of tobacco leaf extract.

All results represent an average of two independent experiments. The maximal standard deviations of 3, 4, 1, and 1% for chlorophyll, phenolics, total protein, and sGFP removal were obtained, respectively.

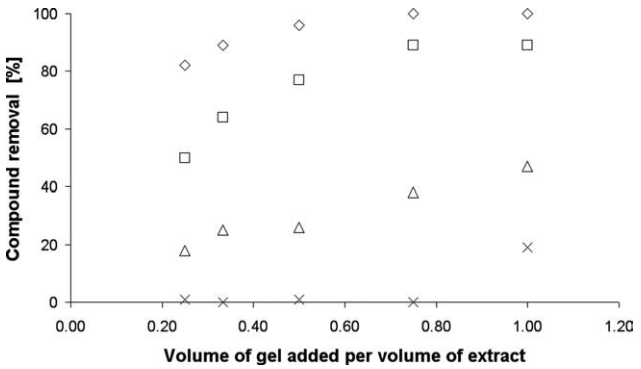

Figure 2. The efficiencies of chlorophyll (◇), phenolics (□), total protein (△), and sGFP (×) removal from *N. benthamiana* extracts by the addition of aluminum hydroxide gel suspensions at pH 8.0.

The maximal standard deviations of 3, 4, 1, and 1% for chlorophyll, phenolics, total protein, and sGFP removal were obtained, respectively.

respectively. Therefore, the burden of phenolic compounds in *N. benthamiana* and *N. tabacum* is about six times higher than that in the *L. minor* extracts, as the ratio of phenolics to total proteins in the extracts of this aquatic plant is six times lower. Unfortunately, the authors did not report the concentrations of chlorophyll present in the extracts and therefore this comparison is not possible. However, the lower ratio of phenolics to total protein in the *L. minor* plant makes it a more suitable host for recombinant protein production.

The suspension of aluminum hydroxide gel [0.35 mg/mL of $Al_2(OH)_3$] was added to the extracts shortly after the extraction procedure. The quantity of gel suspension added was varied to verify if it affects the removal of chlorophyll, phenolics, total protein, and sGFP (Figure 2). It was determined that until a 1:1.33 (0.75) v/v of aluminum hydroxide-to-extract ratio was obtained, the sGFP had not been removed from the extracts. Under this condition, high efficiencies of chlorophyll and phenolics removal were observed (Table 1) with a practically complete removal of chlorophyll. Also, the levels of phenolics obtained were lower than the ones observed with the soybean seed extracts.[31] Soybean seeds are considered one of the most promising plant expression systems for recombinant protein production (along with canola, corn, and tobacco) as they contain the lowest levels of phenolics.[5] Moreover, a considerable amount of native *N. benthamiana* leaf proteins was removed (38.4%), resulting in a sGFP purification factor of 1.6, which is on the same level as the typical purification factors obtained with ammonium sulfate.[5] The purification factors and recovery found in the literature obtained with ATPSs are as high as 4 and 100%,[8] and 2 and 90%.[32] When comparing these results with the ones obtained in the present work (purification factor 1–6 and recovery 100%), we conclude that the method developed here has a potential to be used as a recombinant protein prepurification step. As the aluminum hydroxide is practically insoluble in water, and therefore does not represent the concern of interfering with the chromatographic purification steps, as 22 μm filtration is usually applied before the first chromatographic step, which effectively removes the aluminum hydroxide gel from the solution. However, to establish the generic application of the proposed method, further optimizations and alternative hosts need to be considered.

Analysis of chlorophyll, phenolics, and protein removal

The addition of aluminum hydroxide gel affected the removal of the chlorophyll and phenolics differently from the removal of total proteins. To fully appreciate the results obtained, we verified the relationships between the quantity of compounds removed and the quantity of gel in solutions. Equation 4,

$$c_{i,0}V = n_{i,Al(OH)_3} + c_{i,H_2O}V \tag{4}$$

which represents the ratio of the quantity of compound in the aqueous phase to that in the aluminum hydroxide gel phase is substituted into Eqs. 2 and 3, Eqs. 5 an 6 are obtained.

$$\frac{c_{i,H_2O}}{c_{i,0}} = 1 - \frac{N_{i,eq.}}{c_{i,0}}\frac{m_{Al(OH)_3}}{V} \tag{5}$$

$$\frac{c_{i,H_2O}}{c_{i,0}} = \frac{1}{1 + K_i\frac{m_{Al(OH)_3}}{V}} \tag{6}$$

These two models, although based on different removal mechanisms, relate the mass of the aluminum hydroxide added to the quantity of the compound removed. In Eq. 5, the compound removed is proportional to the amount of gel in the solution, whereas in Eq. 6, the mechanism of compound removal is its distribution between the aqueous and the aluminum hydroxide gel phases.

Good agreement was obtained when the equilibrium two-phase model for chlorophyll ($R^2 = 0.991$) and phenolics ($R^2 = 0.973$) and the stoichiometric model for total protein ($R^2 = 0.923$) were used (Figure 3). In chlorophyll and phenolics removal, K_i values of 73.45 and 15.98 were obtained,

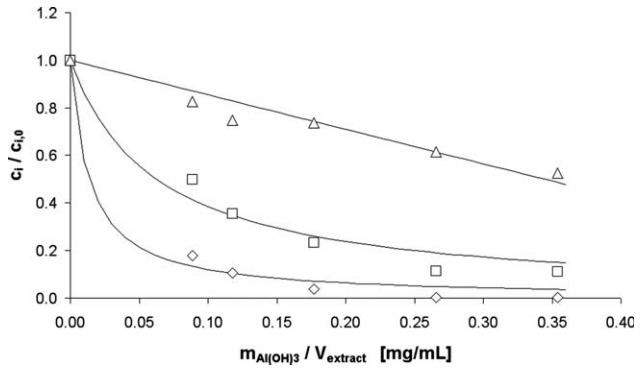

Figure 3. Experimental and modeled values for chlorophyll (◇), phenolics (□), and total protein (△) removal from *N. benthamiana* extracts by the addition of aluminum hydroxide gel suspensions at pH 8.0.

The maximal standard deviations of 3, 4, 1, and 1% for chlorophyll, phenolics, total protein, and sGFP removal were obtained, respectively.

respectively. An $N_{i,eq.}$ of 3.16 was obtained for the stoichiometric model in protein removal. Generally speaking, the removal of the compounds is therefore fairly predictable, which makes optimization of the process relatively simple. However, the removal of the sGFP was not predictable, as the protein was removed only when higher quantities of aluminum hydroxide gel were added. This is probably the result of its low affinity toward the gel, whereas the other components of *N. benthamiana* extract interact more readily.

*Selectivity of the method for the proteins in the extract based on their molecular masses and p**I**'s*

To determine the efficiency of native protein removal from *N. benthamiana* according to their molecular mass and p*I*, the clarified extracts were analyzed by SDS-PAGE (Figure 4) and IEF electrophoresis (Figure 5). The results of the SDS-PAGE indicate that the proposed method does not selectively remove proteins based on their size. This conclusion was made based on visual estimation of the SDS-PAGE gels, as well as densitometric analysis of the bands, which showed that the proportion of the bands was the same before and after the clarification (Figure 4). By comparing the quantity of protein fractions in the *N. benthamiana* extract, based on their p*I*, before and after the addition of aluminum hydroxide gel, one can observe that, in general, the acidic proteins (p*I* below 6.10) were preferentially removed with aluminum gel (Figure 6). However, the p*I* of the protein is not the only parameter for its removal efficiency, as the removal of protein fraction with p*I* 7.41 was as high as 72%. Also, the acidic sGFP (pI = 5.51) was only removed when quantities higher than 1:1.33 (0.75) v/v of gel per tobacco extract were added. The results suggest that complex interactions occur between the proteins and aluminum hydroxide gel. Therefore, the removal efficiencies for the specific protein are not straightforward and should be determined on case by case study.

Conclusions

In the present work, the application of electrocoagulation in the field of biotechnology, or more specifically DSP of recombinant proteins produced in green plant tissue as bioreactors, was evaluated. We successfully developed an

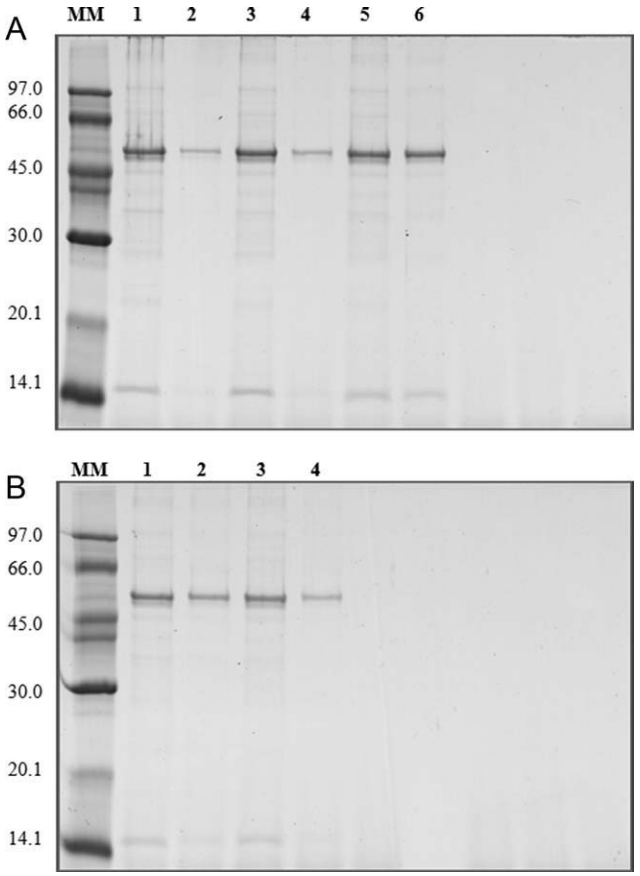

Figure 4. Electrophoresis of extracts (extraction conditions: 10 mmol/L TRIS, 5 mmol/L MBS, pH 8.0) and clarified extracts treated with electrochemically produced aluminum hydroxide gel (gel produced at 5 V for 2 h in 200 mmol/L NaCl, 100 mmol/L TRIS buffer) of *N. benthamiana* in distinctive proportions of "extract:gel suspension."

All the extracts were diluted in the same proportion with the pure electrocoagulation buffer (no gel present) to visualize more clearly the effect of the clarification step. Gel A: MM, molecular mass marker; (1) extract "4:1"; (2) clarified extract "4:1"; (3) extract "3:1"; (4) clarified extract "3:1"; (5) extract "2:1"; and (6) clarified extract "2:1." Gel B: MM, molecular mass marker; (1) extract "1.33:1"; (2) clarified extract "1.33:1"; (3) extract "1:1"; and (4) clarified extract "1:1."

electrocoagulation method for the clarification applicable to these systems, as practically all the chlorophyll and a majority of the phenolics were removed from the *N. benthamiana* leaf extracts. Another appealing attribute of this technique is its protein prepurification as 38.5% of the native proteins were also removed. Moreover, the pH at which the removal occurs is slightly alkaline, in contrast to the methods using acidic pH recently proposed by other authors.[8,11,13] Therefore, when using electrochemically produced aluminum hydroxide gel, the risk of denaturation or loss of activity of the desired protein is greatly reduced. The process seems to be more efficient in removing acidic proteins with p*I* below 6.10, although p*I* is not the only criterion, as demonstrated by the low removal of acidic sGFP and the high removal of protein with p*I* of 7.41. Also, the method presented is considered cost effective, as the price of the aluminum used in the study is lower than the NaCl, the price of the transformer and other control equipment was less than 100 USD, and the gel could also alternatively be removed by sedimentation (data not shown). Another advantage of the methodology is

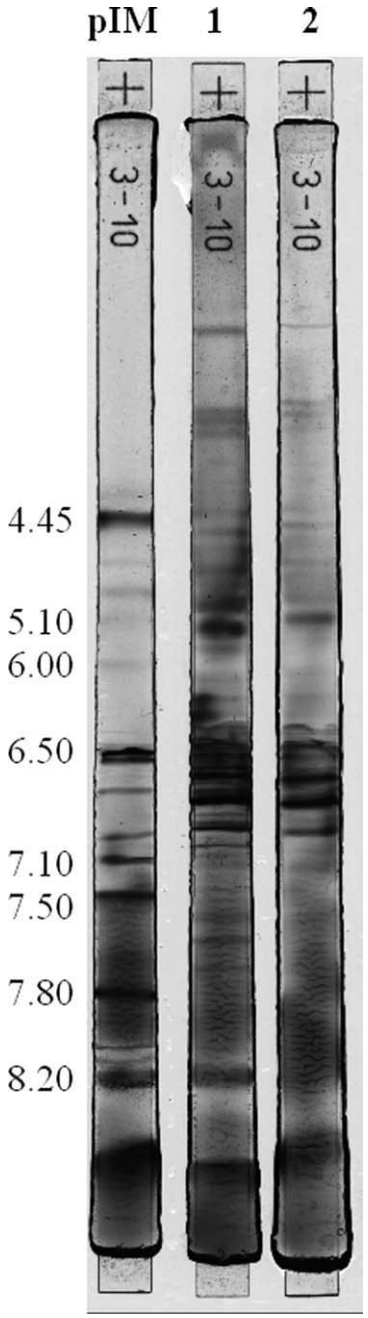

Figure 5. Isoelectric focusing PAGE of the *N. benthamiana* extracts (obtained with TRIS 10 mmol/L, MBS 5 mmol/L, pH 8.0) before and after the addition of aluminum hydroxide gel (produced in TRIS 100 mmol/L, NaCl 200 mmol/L, pH 8.0 for 2 h at 5.0 V).

The aluminum hydroxide gel-to-extract volume proportion used was 1:1.33 (0.75). pIM: isoelectric point marker; (1) extract diluted only with electrocoagulation buffer and (2) clarified extract.

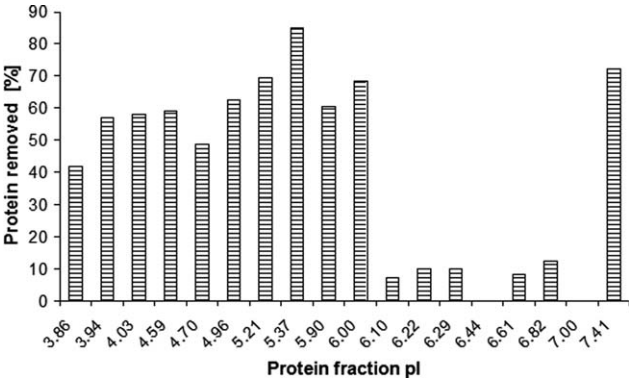

Figure 6. Efficiency of protein fraction removal from *N. benthamiana* extract (10 mmol/L TRIS, 5 mmol/L MBS, pH 8.00 extraction buffer) with aluminum hydroxide gel at pH 8.0 (aluminum hydroxide gel obtained with 100 mmol/L TRIS, 200 mmol/L NaCl for 2 h at 5.0 V and 60 Hz) according to their p*I*.

The aluminum hydroxide gel-to-extract volume proportion used was 1:1.33 (0.75).

its high reproducibility. All experiments exhibited relative standard deviations that were low and below 5%, where the value also includes analytical errors. Therefore, this study contributes toward solving a bottleneck in the use of green plant tissues as bioreactors for recombinant protein production.

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Notation

c_{i,H_2O} = molar concentration of component i in the aqueous phase, mol/L
$c_{i,Al(OH)_3}$ = molar concentration of component i in the aluminum hydroxide phase, mol/L
$c_{i,0}$ = initial molar concentration of component i, mol/L
K_i = equilibrium constant for component i between the aqueous and the aluminum hydroxide phase
$m_{Al(OH)_3}$ = mass of aluminum hydroxide gel formed during the process, mg
$n_{i,Al(OH)_3}$ = moles of component i in the aluminum hydroxide phase, mol
$N_{i,eq.}$ = equivalent capacity of the aluminum hydroxide gel for component i, mol/mg
V = total volume of solution during electrocoagulation, L

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