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Review

Electrophoretic recovery of proteins from polyacrylamide gel

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Abstract

Polyacrylamide gel electrophoresis, which can effectively resolve a mixture of proteins into individual constituents with a simple apparatus, has been widely and routinely used for analytical purposes. However, its application for preparative purposes is limited owing to the lack of a universal method that permits the recovery of general proteins from the gel in high yields. In early days, the electrophoretically separated protein in gel was recovered by extraction or solubilization of the excised gel, and later by electrophoretic elution. Regarding the electrophoretic recovery of proteins, a number of methods have been reported, which can be divided into two categories: (1) "electroelution", which recovers the protein of interest from excised gel electrophoretically, and (2) "continuous elution" of applied proteins from a preparative-scale gel during electrophoresis, where the electrophoretically separated proteins that migrate through the gel into a buffer stream at the gel bottom are fractionated consecutively. Characteristic features of the electroelution method reside in its simple requirements for the elution apparatus and that microgram amounts of protein can be quantitatively recovered in the concentrated form. The major drawbacks of electroelution are that the method requires manipulations for locating the protein in the gel, followed by the excision and re-electrophoresis of the relevant part of gel for eluting the protein. The continuous elution method, on the other hand, has advantages in its high loading capacity of sample protein and easy monitoring of the elution process. This method, however, requires expensive equipment and gives low concentrations of the recovered protein, the purity of which is sometimes poor. For those who are trying the electrophoretic recovery of protein from polyacrylamide gel for the first time, the electroelution method would be the first choice in view of the easy manipulation, the small amount of protein required for loading and the satisfactory recovery yield with least expense without using of any costly equipment.

Contents

1. Introduction ... 146
2. Method of recovering protein from polyacrylamide gel ... 146
3. Electroelution of protein from polyacrylamide gel ... 147
   3.1. Factors affecting the recovery of protein ... 147
   3.2. Procedures prior to electroelution ... 149
      3.2.1. Locating protein of interest ... 149
      3.2.2. Pretreatment of excised gel ... 150
   3.3. Electroelution methods ... 152

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3.3.1. Entrapment of the eluted protein with semipermeable membranes 152
3.3.1.1. Electroelution in dialysis tubing 152
3.3.1.2. Vertical-type electroeluter 152
3.3.1.3. Horizontal-type electroeluter 153
3.3.1.4. Overpass-type electroeluter 153
3.3.2. Entrapment of the eluted protein by a discontinuous conductivity gradient system 154
3.3.3. Entrapment of the eluted protein by a steady-state stacking buffer system 155
3.3.4. Entrapment of the eluted protein by a steady-state stacking buffer system with high concentration of leading ion 155
3.4. Monitoring the electroelution process 156
3.5. Post-electroelution treatments of recovered protein 157
4. Continuous elution of protein from preparative-scale polyacrylamide gel electrophoresis 157
5. Commercially available devices for electrophoretic elution 159
6. Conclusion 159
Acknowledgement 160
References 160

1. Introduction

A wide variety of electrophoretic separation systems are known in terms of supporting media (free solution, paper, starch, agarose, polyacrylamide, etc.), buffer (pH, presence/absence of dissociating agents, etc.), and apparatus (vertical/horizontal, tube, slab, capillary, etc.). Among these systems, polyacrylamide gel electrophoresis (PAGE) is most widely used for the analysis of proteins and peptides owing to its simple operation, high resolution and excellent reproducibility, in addition to flexibility to prepare gels of desired polyacrylamide concentrations in the presence of various additives.

A number of buffer systems, the major factor which defines the resolution of PAGE, have been reported for non-dissociating PAGE under alkaline [1,2], neutral [3–5]and acidic conditions [6]. Two-dimensional PAGE [7,8] and isoelectric focusing (IEF) in polyacrylamide gel [9,10] are also in popular use. Buffer systems for PAGE containing dissociating agents such as urea [11,12] and sodium dodecyl sulfate (SDS) [12–15] are routinely used.

Recent progress in the microanalysis of proteins has made the "analytical" and "preparative" realms borderless, as microgram amounts of protein recovered from a single piece of gel after PAGE can suffice for the analysis of amino acid composition, sequence and peptide mapping [16–19]. Further, proteins with high M_r and those of membranes with minimal solubility in an aqueous solution, which are difficult to separate by high-performance liquid chromatography (HPLC), are often isolated by SDS-PAGE and then subjected to microanalysis [18]. In this context, the recovery of proteins from polyacrylamide gel is of increasing importance. In this review, methods for the electrophoretic recovery of proteins from polyacrylamide gel, including principles, advantages and disadvantages, and updated information on related techniques are described.

2. Method of recovering protein from polyacrylamide gel

A simple and reliable method that can recover microgram amounts of proteins from polyacrylamide gel, preferably in the biologically active form, is essential for the characterization of scarce proteins that are often the subject of research today.

In initial approaches, the protein of interest in the gel after PAGE was extracted under spontaneous diffusion by shaking the excised and minced gel pieces in an appropriate buffer [20–23]. Although this method is simple and suitable for recovering low-M_r proteins [24], it is poor in yield and reproducibility and takes a long time, even for recovering traces of protein [23]. Moreover, the presence of SDS in elution buffer is

essential for efficient elution, although SDS is well known to denature proteins [23]. The methods of recovering proteins by solubilizing polyacrylamide gel in 30% H_2O_2 at 50°C [25] and using chemically depolymerizable cross-linkers [26–29] give rise to large amounts of contaminants derived from the gel, and the recovered protein inevitably requires further purification.

According to a number of reports, two of the most effective methods for recovering proteins from polyacrylamide gel are (1) "electroelution", which recovers the protein of interest from the excised gel electrophoretically, and (2) "continuous elution" of applied proteins from a preparative-scale gel during electrophoresis, where the electrophoretically separated proteins that migrate through the gel into a buffer stream at the gel bottom are fractionated consecutively. Both of these methods can recover the proteins in high purity with good yield and reproducibility.

3. Electroelution of protein from polyacrylamide gel

Electroelution, a method for recovering protein from excised gel after PAGE, is becoming increasingly popular owing to the excellent recovery and reproducibility without using any expensive apparatus. In this section, practical electroelution methods are described in detail.

3.1. Factors affecting the recovery of protein

In the electroelution of proteins from the gel after PAGE, the recovery efficiency depends on (1) the gel concentration and the buffer system (pH, ionic strength, presence/absence of dissociating agents) of PAGE, (2) the buffer system for electroelution and the applied voltage and elution time and (3) the nature of the individual protein to be recovered, e.g., p*I* and M_r. The optimum conditions of PAGE and subsequent electroelution for one protein are not necessarily valid for another protein, so that the proper conditions for each particular protein should be determined individually in preliminary experiments. The principle for determining the gel concentration and the buffer system of PAGE have been reviewed [20,30,31].

Generally, the buffer system for electroelution is consistent with or modified from the PAGE buffer. The buffer system for PAGE can be classified into those with non-dissociating and dissociating conditions. Under non-dissociating conditions, where the protein of interest can probably retain its biological activity, the intrinsic nature of the protein (p*I*, M_r, hydrophobicity, etc.) defines the net charge and solubility of the protein in the given buffer milieu. Therefore, the use of electroelution buffers at pHs around the p*I* of the analyte protein should be avoided, because the electrophoretic migration of the protein slows appreciably at the p*I* owing to the loss of net charge, and further the protein sometimes precipitates within gel matrices, resulting in a low protein yield even after prolonged electroelution. For the efficient electroelution of protein, the pH of the electroelution buffer should differ by at least 1 pH unit from the p*I* of the protein as long as the biological activity of protein is conserved under the applied conditions. In our experience, PAGE in alkaline [2], neutral [5] and acidic buffer systems [6] in combination with each recommended buffer system for electroelution [5] can recover essentially all proteins. It is known in PAGE under non-dissociating conditions that the higher the polyacrylamide gel concentration, the greater becomes the adsorption of proteins to gel matrices, and a long distance migration of proteins through the gel matrix lowers the protein yield [32]. Thus, embedding the excised gels in polyacrylamide gel prior to the electroelution for the purpose of reducing convection and lateral current flow is not recommended [24]. For the electroelution of high-M_r proteins, the use of a large-pore separating gel that can be prepared by reducing the N,N′-methylenebisacrylamide concentration is effective [33].

In an IEF gel, the protein migrates across the pH gradient toward its p*I* point and is concentrated to a very sharp band in the gel, where the protein frequently precipitates. This precipitation can be circumvented by reducing the

amount of protein loaded or by adding urea [34] or other detergents [35]. For electroelution from an IEF gel, the procedure is essentially the same as that for a non-dissociating PAGE gel [36], although the treatment of the excised IEF gel before electroelution sometimes improves the protein yield effectively (see Section 3.2.2).

In SDS-PAGE, proteins are fundamentally denatured and solubilized by forming an SDS–protein complex [37,38]. All SDS–protein complexes have essentially the same surface density of negative charge and migrate in the polyacrylamide gel towards the anode with mobilities reflecting the size of the protein exclusively rather than the intrinsic charge of the native protein. Although the renaturation of recovered proteins after SDS-PAGE has been described [39,40], the method works only for some exceptionally rigid proteins. Thus, a combination of PAGE and electroelution under non-dissociating conditions is recommended for the recovery of biologically active proteins, especially those which consist of subunits.

Recent progress in the microanalysis of proteins revealed serious problems in the artifacts of protein modifications caused during the electrophoresis–electroextraction/electroblotting processes. For instance, proteins after PAGE often show modifications such as the destruction of tryptophan, histidine and methionine residues by the attack of free radicals and oxidants derived from gel polymerizing agents [41,42]. The Asp–Pro bond in protein is susceptible to acid hydrolysis and this reaction can proceed even during the contact of polyacrylamide gels with acidic staining and destaining solutions [42]. Reagents contaminated with aldehydes that react to NH_2 groups in protein, and those contaminated with NH_2-containing compounds that induce noise in the amino acid sequence analysis should not be used. Attention should be paid to the quality of urea, which is frequently employed in IEF gels, to avoid the carbamylation of NH_2 groups in the protein with contaminating cyanate [42]. These undesirable effects of contaminants can be minimized by the use of highly purified gel electrophoresis-grade reagents. The pre-electrophoresis of polyacrylamide gel and the incorporation of antioxidants such as sodium thioglycolate and glutathione (reduced form) in the cathode buffer are also effective [42,43], although pre-electrophoresis sometimes sacrifices the resolution.

The migration velocity of a protein in PAGE is proportional to the applied voltage, and therefore a higher voltage is preferable for faster electroelution, if the effect of Joule heat is permissible. The Joule heat generated during electroelution gives rise to thermal denaturation of protein [44], leading to the clogging of proteins within gel matrices [45], which interferes with efficient electroelution. The heat can sometimes enhance the proteolytic digestion of analyte protein by the trace amounts of proteinases contaminated in the sample loaded [42]. These effects of Joule heat on the denaturation and enzymic digestion of protein and the disturbance in the progress of protein elution due to convection can be minimized by operating the electroelution procedure in the cold.

Electroelution buffers at low concentrations permit operation at high voltages with least generation of heat, but care must be taken to avoid a pH shift of the buffer, especially in prolonged electroelutions [44]. In electroelution with buffers at low concentrations, the pH can be kept constant by circulating the buffer solution of both electrodes [42] or by using auxiliary reservoirs containing concentrated buffer solution [44].

The addition of SDS to the electroelution buffer increases the protein yield effectively [40] by solubilizing the fixed/stained protein in the gel [46] and accelerates the protein migration with the negative charge appended. SDS in the electroelution buffer is essential to recover proteins that are hardly soluble in aqueous solution [47], of course. In the electroelution methods that employ semipermeable membranes for entrapping the eluted protein, the incorporation of SDS in the electroelution buffer prevents both the permanent adsorption of protein on the membrane and the escape of protein through membrane pores effectively by covering the membrane surface with SDS micelles [24,42]. In general, SDS at concentrations of 0.02–0.1% is

often included in the electroelution buffer (see Section 3.3.1). It should be noted, however, that buffers containing SDS easily form SDS precipitate in cold operation [42].

3.2. Procedures prior to electroelution

It is an important prerequisite for the successful recovery of highly purified protein by electroelution that the protein of interest should be discretely separated from other contaminating proteins by PAGE. Thus, the separation conditions for the PAGE procedure, including the buffer system, gel concentration and amount of proteins loaded, must be determined carefully [20,30,31].

3.2.1. Locating protein of interest

It is crucial for the successful recovery of an analyte protein to identify its precise location in the gel and to cut it out exactly. However, it has to be kept in mind that the locating procedure should not affect the protein quality or interfere with the subsequent electroelution.

Staining the proteins in gels with dyes such as Coomassie Brilliant Blue (CBB), of which the sensitivity is 200–300 ng of protein [42], facilitates the exact manipulations in dissecting the relevant part of gel containing the analyte protein. However, the fixing/staining procedure decreases the protein yield [48], so that prolonged contact of the gel with acidic fixing/staining solutions must be avoided. After brief staining with 0.25% CBB R-250 in 50% methanol containing 10% acetic acid [49], followed by rapid destaining if necessary, the protein bands in the gel are revealed over a light box and the relevant part of the gel containing the protein of interest is cut out with a scalpel. For IEF gels, CBB G-250 is recommended [49]. It should be noted that staining dyes such as CBB and Amide Black do not interfere with the analysis of proteins for amino acid composition and the amino-terminal end [42,50]. As already mentioned (see Section 3.1), the Asp–Pro bond in protein is susceptible to acid hydrolysis and thus minimum contact of the gel with acidic solutions for fixing, staining and destaining is recommended [42].

The location of protein in an unstained gel is often determined by interpolating from the bands in guide strips which are stained. However, the interpolation sometimes leads to inaccurate results owing to swelling and shrinking of the guide strips in the fixing, staining and destaining processes, so that corrections are necessary for the exact location of the protein. In a slab gel, three guide strips are necessary for accurate interpolation, one from the centre lane and one from each side. This method permits the successful dissection of the homogeneous protein band from the gel when contaminants are not in close proximity to the analyte protein, preferably more than 3 mm apart. The guide strips should be stained as quickly as possible while keeping the unstained gel in the cold (except SDS-PAGE gel) in order to minimize spontaneous diffusion of proteins. For rapid staining of guide strips, the recently commercialized Rapid CBB staining kit, with which the staining/destaining procedures are complete within 1 h, is useful in terms of sensitivity and speed.

For the accurate location with the minimum deterioration of protein, the protein of interest in an SDS-PAGE gel can be located under mild conditions by exposing the gel to 0.25–1 *M* potassium chloride [40,51] or 4 *M* sodium acetate [52] to deposit SDS and SDS–protein complex, although not sensitive. It is also known that heavy metal ions such as copper and zinc can reveal protein bands in both gels with and without SDS [53,54]. Rapid and sensitive detection of proteins can be performed within 5 min by exposure to 0.3 *M* $CuCl_2$, where proteins in the gel are stained translucent and pale blue in the presence and absence of SDS, respectively. The stained protein bands are easily detectable by placing the gel over a black sheet, and the copper ion bound to protein can be reversibly removed by immersing the gel in 0.25 *M* Tris–HCl (pH 9.0) containing 0.25 *M* EDTA [53]. According to Lee et al. [53], the sensitivity of this method with 2 mm thick gel is 10–80 and 80 ng of protein in the presence and absence of SDS, respectively.

If the protein of interest is available in a highly purified form, its band in the gel after PAGE with crude protein preparations can be easily identified by co-electrophoresis with a tracer protein that has been labelled with fluorescence [55,56] or radioactivity [57]. Although the tracer method permits direct detection of the relevant protein in the gel without contact with deteriorating chemicals, the pertinent purified protein for labelling that meets the required purity is difficult to procure.

3.2.2. Pretreatment of exercise gel

In general, no treatment of gel is required before electroelution unless the gel has been exposed to extreme pH conditions such as the CBB R-250 staining dye bath including 50% methanol and 10% acetic acid [49]. In practice,

Table 1
Electroelution methods

Method	Sample and amount	PAGE gel	Elution buffer	Electric power
Membrane				
Dialysis tube	– –	+SDS	0.1 *M* Sodium phosphate (pH 7.4)–0.1% SDS	20–200 V (100 mA)
Vertical	Haemoglobin/DNP-albumin Fluorescent-labelled globulin, etc. 7–20 mg (ca. 2 ml)	–SDS, 7.5% T	12.5 m*M* Tris–96 m*M* glycine (pH 8.3)	25–35 mA per tube (140–180 V)
Vertical	Fluorescent-labelled B-tubulin dimer, etc. ca. 1 mg	+SDS, 5–10% T	2.5 m*M* Tris-glycine (pH 8.3)–0.1% SDS	1–2 mA per tube (350–400 V)
Horizontal	Stained membrane proteins (M_r 3000–19 000)	+SDS, 8.75% T	25 m*M* Tris–192 m*M* glycine (pH 8.3)–0.1% SDS	10 V/cm (70 mA)
Overpass	Radiolabeled H-2K[k] (M_r 12 000–68 000)	+SDS, 5–20% T gradient	50 m*M* NEM[b]-acetate (pH 8.5)–0.001% SDS	1 W (4–7 mA)
Overpass	Radiolabeled protein (M_r 65 000) 1 μg–1 mg	+SDS	50 m*M* NH_4HCO_3–0.1% SDS [50 m*M* Tris-acetate (pH 7.8)–0.1% SDS[c]]	50 and 80 V
Discontinuous conductivity gradient	Stained ATP-citrate lyase and stained proteins 0.1–0.5 μg (two sliced gels)	+SDS	25 m*M* Tris–75 m*M* glycine (pH 8.8)–1 m*M* DTE[d]–40% glycerol Trap: 2 *M* NaCl	4 mA per tube
Steady-state stacking	Radiolabelled human growth hormone	–SDS, 6% T	Multi-phasic zone electrophoresis buffer system 2950.0.X	6–7 mA/cm^2, then 3–3.5 mA/cm^2
Steady-state stacking with Sephadex G-25	Retinol-binding protein 0.3–4 mg (1–5 ml)	+SDS, 15% T	L[e]: 23 m*M* NEM–10 m*M* HCl (pH 8.0) T[f]: 30 m*M* Tris–500 m*M* 6-ACA[g] (pH 9.1)–0.1% SDS S[h]: 3 m*M* Tris–50 m*M* 6-ACA (pH 9.1)–0.1% SDS)	4.0 mA
Steady-state stacking with high concentration of leading ion	Unstained albumin, etc. (ca. 0.5 ml gel)	+/–SDS	For alkaline gels, L: 378 m*M* Tris–124 m*M* HCl (pH 8.5)–10% sucrose T: 2.5 m*M* Tris–10 m*M* glycine (pH 8.5)	250–300 V

[a] IEP = immunoelectrophoresis.
[b] NEM = N-ethylmorpholine.
[c] Buffer for cold operation.
[d] DTE = dithioerythritol.
[e] Leading buffer.
[f] Trailing buffer.
[g] 6-ACA = 6-aminocaproic acid.
[h] Sample reservoir buffer.

after brief staining in the acidic dye bath, excised gel pieces are immersed in the electroelution buffer and equilibrated for a few minutes before elution. However, the gel after extensive staining under acidic conditions is treated successively with 1 *M* NaOH for 1 min and 0.1 *M* NaOH containing 0.1% SDS for 30 min, followed by equilibration in the electroelution buffer [58]. The IEF gel requires no pretreatment as long as the protein is kept soluble in the gel [36]. When the protein is precipitated within the IEF gel, the protein requires solubilization before electroelution by exposure to extremely alkaline conditions for 5 min at 0°C [45,59], although permanent denaturation of protein is inevitable even after immediate neutralization.

It has been reported that the efficiency of protein recovery can be improved by mincing the gel into small pieces [42,47,59]. The improvement was ascribed to the decrease in the protein migration distance within the gel and the increased gel surface area. However, we use the

Elution time and temperature	Volume collected (μl)	Recovery (%)	Purity	Other application	Ref.
2–8 h	–	≤80	–	Electrodialysis	63
1.5–4 h, 4°C	3000–3500	63–68	Proved by IEP[a]	Concentration	65
1 h	200	80–85	Single band by SDS-PAGE	Concentration	56
2–8 h	200–800	79–97	Single band by SDS-PAGE	Concentration Electrodialysis	24
2–3 h	100	87–93	Single protein by sequencing	Concentration Electrodialysis	47
3–5 h (soaking), 12–16 h (elution), 20–24 h (electrodialysis), RT/4°C	150–200	50–90	Single band by SDS-PAGE, etc.	Concentration	42
1–2 h (soaking) 0.33–2 h (elution), RT	≤100	85–95	Single band by SDS-PAGE	–	46
≥2 h, 0–4°C	ca. 1000	70	–	Concentration	45
3.75 h	2000	84–95	Pure by amino acid analysis	Concentration	74
3–6 h, 4°C–RT	100–500	≥90	Single band by PAGE, etc.	–	5

excised gel piece without any further reduction in size, as we could not confirm any improvement in protein recovery by mincing the gel [5].

3.3. Electroelution methods

One of the most important features that characterize the differences in electroelution methods resides in how the eluted protein is captured. The principle and application of each method are summarized in Table 1.

3.3.1. Entrapment of the eluted protein with semipermeable membranes

The electroelution devices that capture the eluted protein with semipermeable membranes can be classified into four major types (Table 1 and Figs. 1–3), three of which are commercially available. The semipermeable membrane for capturing the protein is required to be chemically inert and to have a low protein adsorption capacity. The membrane can be selected from a wide variety of commercially available membranes such as cellulose tubing, Spectra/Por and BT membranes [24], depending on the M_r of the protein to be entrapped and the elution apparatus used [24]. It should be taken into account that some dialysis membranes have common characteristics of adsorbing proteins more or less and also pore sizes are enlarged under an electric field [60]. To minimize the protein adsorption on the membrane, low concentrations of SDS are often incorporated into the electroelution buffer (see Section 3.1). For achieving the efficient recovery of protein, the entrapment membrane after electroelution is routinely flushed with the solution in the trapping chamber by vigorous pipetting, otherwise a reversed current is applied for a short period (30 s) before collecting the protein [61,62]. It is an absolute requirement for recovering pure proteins that the membrane should be washed thoroughly before use [42,47].

3.3.1.1. Electroelution in dialysis tubing. The electrophoretic recovery of proteins from SDS-PAGE gels can be simply accomplished by using dialysis tubing [61,63]. The dialysis tubing containing excised gel pieces is filled with electrode buffer, which generally incorporates SDS, placed in a flat-bed electrophoresis chamber so as to be kept parallel to the electrodes and submerged in the electrode buffer. After electroelution, the inner solution containing the eluted protein is centrifuged to remove gel debris and the supernatant is collected. This simple method of eluting protein works well only for gels containing large amounts of protein, and significant loss of protein occurs due to adsorption on the large surface of the membrane when small amounts of proteins are treated [64]. This method usually recovers low concentrations of eluted protein with a relatively large volume of electrode buffer in the dialysis tubing.

3.3.1.2. Vertical-type electroeluter. A simple vertical apparatus for the electroelution of protein can be constructed very easily with least expense, e.g., by fixing a coarse nylon mesh between a pair of glass tubes and a semipermeable membrane at the bottom end using tightly fitted Tygon tubing (Fig. 1) [5,56,65–67]. Electroelution with this laboratory-made apparatus can be performed by classical cylindrical disc gel electrophoresis after placing the excised gel on the supporting nylon mesh. The protein that is eluted from gel and migrates through the electroelution buffer with a current flow is entrapped by the membrane and retained inside the lower unit of the compartment, from which the protein is recovered after removing the upper unit. The vertical-type electroeluter has the advantages of easy operation with reliable recovery of small amounts of proteins from the excised gel. Special attention should be paid to avoid the entrapment of air bubbles underneath the semipermeable membrane and supporting mesh throughout the course of assembling the apparatus and electroelution, because air bubbles interrupt the current flow.

Ihara et al. [68] applied a commercially available Centricon concentrator module to the entrapment of eluted protein, facilitating the simultaneous concentration and buffer exchange of the protein solution in the same vessel to minimize possible loss of the protein.

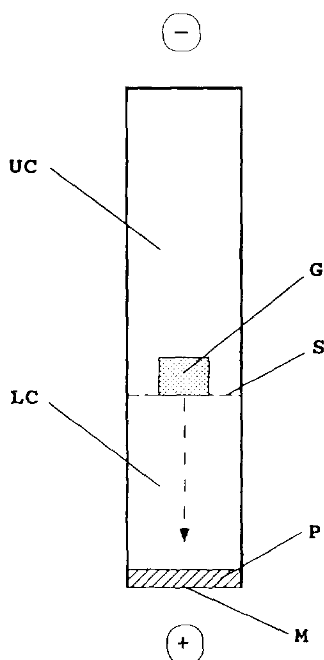

Fig. 1. Schematic representation of the vertical-type electroeluter. Gel pieces (G) containing the protein of interest are placed on the support (S) and held in the upper compartment (UC). With a current flow, the protein is eluted from the gel and migrates downwards as indicated by the arrow, and is finally entrapped by the semipermeable membrane (M) as a concentrated layer (P) inside the lower compartment (LC). All subsequent figures are illustrated assuming that the protein migrates towards the anode under the conditions used.

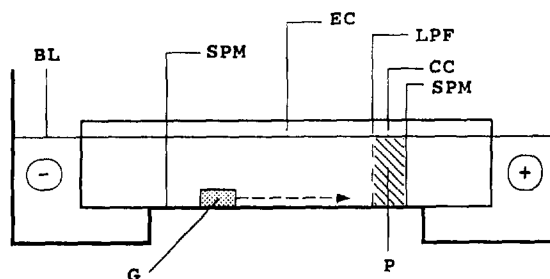

Fig. 2. Schematic representation of the horizontal-type electroeluter. With a current flow, protein is eluted from the excised gel (G), which has been placed in the elution compartment (EC), and migrates through the large-pore filter (LPF) toward the collection compartment (CC), where the eluted protein (P) is captured by the small-pore membrane (SPM). The arrow indicates the direction of protein migration and BL the buffer level.

3.3.1.3. Horizontal-type electroeluter. The horizontal electroelution apparatus was introduced first by Tuszynski et al. [48] and developed by Jacobs and Clad [24] with the same principle, but with a much more practical design (Fig. 2). An open-top plastic vessel lacking a pair of side walls is partitioned with a pair of semipermeable membranes. The space between the membranes is further divided into elution and collection compartments with a coarse filter that traps gel particles. After placing the device in a flat-bed electrophoresis chamber, the buffer is poured into the compartments so as to cover the gel and electroelution is performed. The eluted protein that migrates from gel into the collection compartment through the filter is captured by the semipermeable membrane. A characteristic feature of this apparatus, which permits high yields of protein, is the use of two specific kinds of membranes that show very low protein adsorption. One membrane can retain macromolecules larger than M_r 5000, whereas the other membrane, the pore size of which is 2 μm, excludes gel debris effectively. The open-top structure of this apparatus eliminates the possibility of air bubble entrapment, and allows easy monitoring of the elution process and prompt withdrawal of the recovered protein solution from the collection compartment.

3.3.1.4. Overpass-type electroeluter. The overpass-type electroeluter, which was originally developed for the electrophoretic concentration of protein [44], consists of a bridge-type vessel having a wide and a narrow cup at both ends which can bridge the electrode buffer solution in separate reservoirs of the anode and cathode (Fig. 3). Both ends of the apparatus are sealed with semipermeable membranes before introducing the gel piece and buffer. The gel is placed on the membrane of the larger cup and electroelution is performed. The protein eluted is entrapped on the membrane of the smaller collecting cup [42,44,47,69]. Hunkapiller et al. [42] semiquantitatively recovered microgram

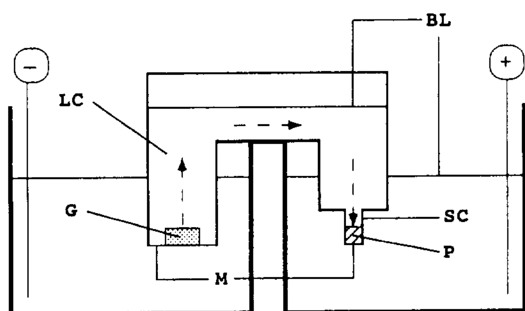

Fig. 3. Schematic representation of the overpass-type electroeluter. The excised gel (G) is placed on the semipermeable membrane (M) in the larger cup (LC). The protein eluted from the gel moves with a current flow to the smaller cup (SC) and is captured as a concentrated layer (P) by the semipermeable membrane. The arrow indicates the direction of protein migration and BL the buffer level.

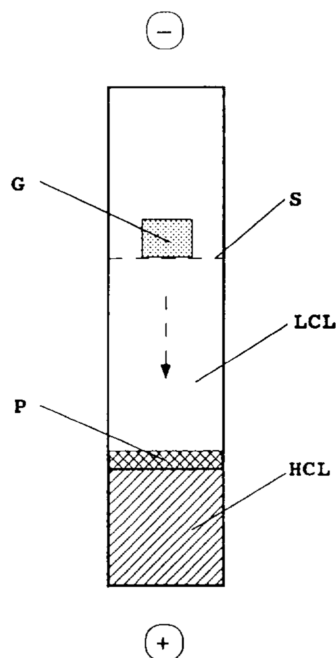

Fig. 4. Schematic representation of protein entrapment by a discontinuous conductivity gradient system. With a current flow, the protein is eluted from the gel (G) which is placed on the support (S) and migrates in the direction shown by the arrow through the low-conductivity buffer layer (LCL), and is stacked into a thin layer (P) at the interface between LCL and the high-conductivity layer (HCL).,

amounts of proteins of M_r 2000–250 000 from SDS gels in the presence and absence of reducing agents such as dithiothreitol [69]. This method is useful not only for eluting proteins from the gel but also for concentrating electrolytic macromolecules, especially when non-ionic solutes such as sucrose are to be eliminated simultaneously [44]. If it is not a matter of removing such non-ionic solutes, the electroelution can be quickly achieved by placing the excised gel just above the smaller collecting cup [60]. The collection of eluted protein should be carried out very carefully so that the dilution of protein due to re-mixing with elution buffer may not occur [47].

3.3.2. *Entrapment of the eluted protein by a discontinuous conductivity gradient system*

Capturing the electroeluted protein with a high-conductivity solution was originally reported by Otto and Snejdárková [70], and improved upon by Strålfors and Belfrage [46]. With the current flow, the protein eluted from gel migrates rapidly in the low-conductivity buffer layer (Fig. 4). When the protein enters the high salt concentration layer, the migration of protein promptly slows and essentially stops, as the contribution of protein as electrolyte becomes negligible compared with those of surrounding ions. As a result, the protein is stacked and concentrated at the interface between the low- and high-conductivity layers.

One of the major advantages of protein entrapment with a high-conductivity solution is that the membrane can be excluded from the electroelution system. Hence all the problems that accompany to the use of membranes in electroelution, including protein adsorption, pore clogging and leakage of proteins through the membrane, are eliminated. This method has the further advantage that the small peptides that cannot be retained on membranes are recovered successfully. It should be noted that the protein entrapped at the buffer interface is occasionally salted out by contact with high concentrations of salts and the biological activity of the protein is sometimes lost, although the protein can be readily recovered as a precipitate by centrifuga-

tion. Long-term electroelutions in this system may lead to the significant decreases in yield owing to a decreased salt ion concentration in the high-conductivity zone.

3.3.3. Entrapment of the eluted protein by a steady-state stacking buffer system

The buffer system for steady-state stacking of protein, first introduced by Ornstein [1] and Davis [2], has been widely and routinely used for concentrating proteins in PAGE. The principle of this buffer system is detailed elsewhere [30,71]. The buffer system involving a pair of ions, referred to as the leading and trailing ions, whose electrophoretic mobilities are higher and lower than the analyte protein, respectively, is effectively applied to the electroelution for capturing the eluted protein (Fig. 5). The lower compartment of the electroelution apparatus is filled with buffer containing the leading ion and the upper compartment with buffer containing the trailing ion. The protein, which has been driven out of the gel by the trailing ion in the upper compartment, migrates between the leading and trailing ions in the lower compartment. A sharp moving boundary formed between the leading and trailing ions is observed, which contains a concentrated band of eluted protein. Several similar methods have been reported [45,72–74]. The apparatus used for this method is similar to the vertical-type electroeluter (see Section 3.3.1.2). However, the lower and upper buffers should be overlaid carefully so that the interface may not be disturbed because the buffer compositions are different. Careful considerations are necessary to determine the combination of leading and trailing ions in the buffer system [75]. It is noted in this system that a loss of protein is possible owing to adsorption, denaturation and clogging at the membrane surface [73,76].

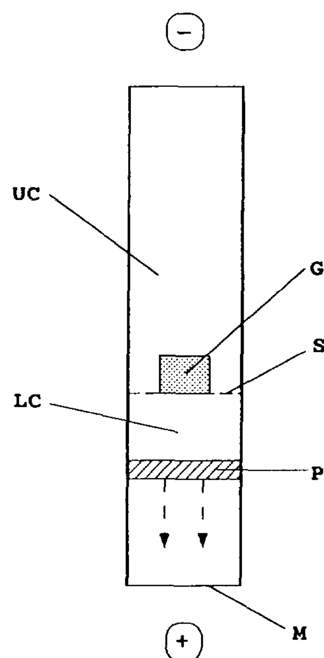

Fig. 5. Schematic representation of protein entrapment by a steady-state stacking system. The excised gel (G) is placed on a supporting mesh (S) and is held in the upper compartment (UC), which is filled with the trailing ion buffer. The protein eluted from the gel migrates with a current flow and is stacked as a sharp zone (P) which can be seen between the leading and trailing ions in the lower compartment (LC). The direction of protein migration is indicated by the arrow. In the case of the steady-state stacking with a concentrated leading ion buffer according to Hashizume et al. [5], the semipermeable membrane (M) is used to secure the protein recovery.

3.3.4. Entrapment of the eluted protein by a steady-state stacking buffer system with high concentration of leading ion

An improved method for protein electroelution from polyacrylamide gel was reported [5] that employs high concentrations of leading ion in the steady-state stacking buffer system. An advantage of this method resides in the ameliorated capacity of the capturing protein in the lower buffer, which permits a prolonged elution time with least pH shift. A longer period of electroelution at higher voltages is theoretically preferable for the complete elution of protein from the gel. In practical electroelution with the steady-state stacking buffer system (see Section 3.3.3) at high voltages, the eluted protein usually rushes down to clog the membrane and results in a decreased protein yield, and also the pH shifts in the elution buffer often damage the biological activity of protein in prolonged elution. However, in electroelution with the steady-state

stacking buffer system containing a high concentration of leading ion, the voltage gradient is kept low in the lower buffer layer and a sufficient elution time is afforded with the least pH shift by the strong buffering capacity of the lower buffer. Another advantage of this method is that the protein precipitation that frequently takes place at the interface of a high salt concentration buffer in the diascontinuous conductivity gradient system (see Section 3.3.2) can be avoided. Electroelution by this method is performed in the same manner as described for the steady-state stacking method (Fig. 5), except that different compositions of the leading and trailing ion buffers are used [5]. After electroelution, the upper unit is removed and the protein solution in the lower compartment is fractionated drop by drop via the pinhole opened in the bottom membrane. Although this system was originally developed for recovering biologically active proteins from alkaline PAGE gels, similar systems for neutral and acidic gels have been established [5]. This system is applicable to the electroelution from agarose–polyacrylamide composite gel in the same manner [77]. A successful electroelution of albumin from SDS-PAGE gel with a 94% recovery was demonstrated with an alkaline buffer system (Fig. 6). A 2.5 times higher concentration of the protein can be recovered in the peak fraction with a total yield of 96% when the lower compartment of the elution apparatus is packed with Sephadex G-25 that is equilibrated with the leading ion buffer, as reported by Öfverstedt et al. [73].

The above electroelution method successfully permits the direct transfer of eluted protein on to a poly(vinylidene difluoride) (PVDF) membrane by overlaying PVDF membrane on the cellulose membrane (Table 2). Thus, the amino acid sequence analysis of protein can be determined directly by using a single or several excised gel pieces.

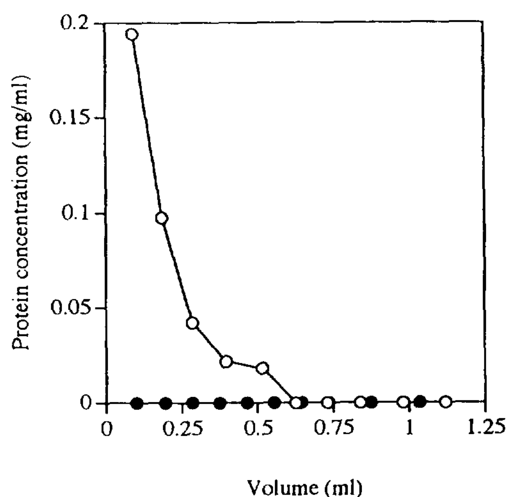

Fig. 6. Electroelution of protein from the gel after SDS-PAGE. Each lane of a mini-slab gel (8% T; 80 × 80 × 1 mm) was loaded with a 3.56-μg aliquot of bovine serum albumin (BSA) and SDS-PAGE [14] was performed. The gel piece containing the BSA band was dissected from each lane, and ten gel pieces in total were electroeluted with the alkaline buffer system according to Hashizume et al. [5] for 4.5 h at 250 V. The recovered BSA solution in the lower compartment was fractionated dropwise via the pinhole opened in the bottom membrane and fractions were assayed for protein. The protein yields in the first three fractions combined and all fractions were 31.0 μg (87.0%) and 33.4 μg (93.7%), respectively. The results for the gel (○) loaded and (●) unloaded with BSA are shown.

3.4. Monitoring the electroelution process

Monitoring the electroelution process is essential for the successful recovery of proteins, especially for determining the end-point. Except for proteins that are coloured or that can be stained with dyes [61], the elution process is usually monitored by observing the migration of the tracking dye; the running front of the gel containing dye is cut out after electrophoresis and used for monitoring the process [5]. Alternatively, the tracking dye can be readily incorporated into blank gel pieces by a brief incubation in dye baths such as those with bromophenol blue (for alkaline gels), methylene blue (for neutral gels) or methyl green and pyronine Y (for acidic gels) [78]. Preferably, the monitoring is performed separately in a concurrent run with the gel pieces containing only dye in order to avoid permanent binding of the dye to the eluted protein [5].

Table 2
Amino acid sequence analysis of albumin after the electroelution on to PVDF membrane

Sample gel	Leading ion buffer	Residue detected (pmol)		
		1st Asp	2nd Thr	3rd His
Unstained	Buffer A[a]	25.3	4.08	6.18
	Buffer A + 20% methanol	41.7	6.32	12.4
	Buffer B[b]	15.5	0	0
Stained[c]	Buffer A + 20% methanol	34.2	4.57	3.08
Unstained[d]	–	18.7	3.90	3.51

After SDS-PAGE (8% T; 80 × 80 × 1 mm) [14] of bovine serum albumin (BSA), ten pieces of excised gel with or without staining were subjected to each electroelution condition where the leading ion buffers described in the table and the trailing buffer composed of 2.5 m*M* Tris–10 m*M* glycine (pH 8.5) [5]. The lower compartment was sealed with double-layered membranes, i.e., inner Immobilon-P membrane (Millipore) for capturing protein and outer cellulose membrane for supporting Immobilon P membrane. Electroelution was performed at 250 V for 8 h for an unstained gel and 12 h for a stained gel. After soaking, each PVDF membrane was analysed with a Model 471 A protein sequencer (Applied Biosystems). BSA loaded for electroelution was ca. 150 pmol.

[a] 378 m*M* Tris–124 m*M* glycine (pH 8.5) containing 10% sucrose.
[b] 2.5 m*M* Tris–10 m*M* glycine (pH 8.5).
[c] Stained with 0.2% Coomassie Brilliant Blue R-250 in 40% methanol–10% acetic acid for 15 min, and then destaining with 7.5% acetic acid–5% methanol for 10 min. The gels were equilibrated shortly with buffer A containing 0.1% SDS and electroeluted.
[d] Unstained gel pieces were crushed in water and extracted. After centrifugation, the protein was recovered by using a ProSpin (Applied Biosystems).

3.5. Post-electroelution treatments of recovered protein

The protein recovered after electroelution usually requires treatment before being subjected to the final analysis, e.g., removal of undesirable contaminants, buffer change, further concentration. Small molecules such as Tris and glycine are effectively removed by dialysis and electrodialysis [24,42]. Gel filtration is useful for a rapid buffer change with simultaneous removal of these small molecules [45] and linear polyacrylate which is derived from the gel [31,45]. SDS, which often interferes with chemical and enzymatic treatments of the protein, can be eliminated by precipitation, extraction with organic solvents and ion-retardation column chromatography [40,79–82]. Furth [83] reviewed methods for removing detergents, including SDS, from the gel. Recently, Simpson et al. [84] introduced reversed-phase chromatography for the removal of SDS and CBB-related materials from protein. Carrier ampholyte can be removed from the IEF gel by bifunctional ion-exchange chromatography [85].

Concentration of the recovered protein is generally performed by lyophilization employing volatile buffers such as 50 m*M* ammonium hydrogencarbonate [42] and N-ethylmorpholine acetate (pH 8.5) [47], although lyophilization frequently leads to significant losses of proteins, especially when small amounts of proteins are treated. The Centricon concentrator is useful for concentrating small volumes of protein solution, but the ultrafiltration and hollow-fibre methods are only effective for larger volumes [86].

4. Continuous elution of protein from preparative-scale polyacrylamide gel electrophoresis

In continuous electroelution (Fig. 7), the sample proteins loaded on the top of the gel migrate

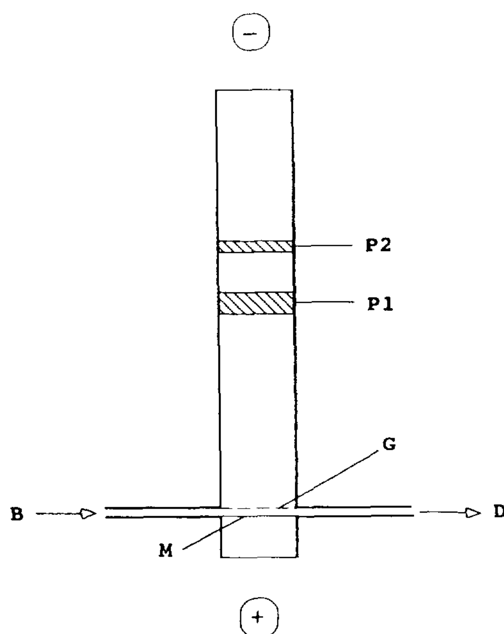

Fig. 7. Schematic representation of the continuous elution of proteins during electrophoresis. The proteins (P1 and P2) are electrophoretically separated on the polyacrylamide gel which is supported by the grid (G). The separated proteins migrate into the buffer stream (indicated by open arrow) running between the grid and semipermeable membrane (M). The eluted protein that is entrapped with the buffer flow (B) is delivered to the fraction collector via the monitoring detector (D).

downwards with a current flow and are separated from each other according to the usual gel electrophoresis. As soon as they leave the gel, the proteins are entrapped within a buffer stream which is running via the gel edge, and then delivered to the fraction collector through monitoring detector. A number of continuous electroelution methods have been devised [66,87–95], and some typical examples are given in Table 3.

Advantages of the continuous elution method reside in its high capacity for loading proteins (50–1000 mg) with simple collection and easy monitoring of eluted proteins on-line. The tedious procedures accompanying the electroelution method, such as location, excision and electrophoretic elution of the protein of interest, are eliminated in this method. The elimination of the staining process can avoid the denaturation of proteins.

A major disadvantage of continuous electroelution, on the other hand, is its inferior resolution as compared with ordinary gel electrophoresis. The poor resolution is ascribed to the re-mixing of the electrophoretically separated proteins in the processes of their entrapment and delivery in the flowing buffer. A slow buffer flow results in diffusion and re-mixing of proteins that have just been separated, whereas a rapid flow leads to dilution of protein concentration. In order to attain a sharp resolution with a higher concentration of the required protein, preliminary experiments are necessary to optimize the elution conditions, including the concentration and size of the separation gel, applied voltage and flow-rate of buffer [31,90], and it is a tedious and time-consuming task to find these optimum conditions. Another drawback of the continuous elution method is that it requires expensive apparatus.

Continuous elution is especially useful for routine work to recover large amounts of specific proteins from partially purified samples under fixed electrophoretic conditions.

Table 3
Continuous elution methods

Protein and amount	Gel feature	Elution conditions	Buffer flow-rate (ml/h)	Recovery (%)	Purity	Ref.
Albumin/α-globulin 50–60 mg	−SDS, 8% T 400 × 45 mm	50 mA (75 V) 22 h	18	–	–	90
Proteins (M_r 14 460–92 500) 1–300 μg	+/−SDS, 5–7.5% T 1–2.5 × 50 mm	0.5–0.7 mA 1–5 h	0.9–1.5	80–90	Single band by SDS-PAGE	93
Lysozyme/ovalbumin 8–14 μg	+SDS, 12% T 4–6 × 15 mm	7.5 V/cm 2 h	1.5–6	>70	Single band by SDS-PAGE	94,95

5. Commercially available devices for electrophoretic elution

Characteristic features of commercially available devices for the electrophoretic recovery of proteins are listed according to manufacturers' instruction manuals in Table 4. Exact comparisons of the devices are difficult owing to descriptions based on different criteria from one manufacturer to another, but Harrington [96] directly compared some of the commercially available electroelution devices. As every device has its own advantages and disadvantages, as described in Sections 3 and 4, it is necessary for investigators to consider which device is most suitable for their purpose, depending on the characteristics of proteins of interest and the requirements in subsequent use.

6. Conclusions

The development of microanalytical methods in the recent years has allowed structural analy-

Table 4
Commercially available devices for recovering protein from polyacrylamide gel

Method	Commercial name	Sample loaded	Elution conditions	Volume collected (μl)	Recovery (%)	Ref.
Membrane overpass	Electro-eluter/ Concentrator[a,b] Model EE-04[b,e]	2–5 ml[a]	–	200–500[a]	75–95[c]	42,69
	Little Blue Tank[b,d] Model NA-1710[b,e]	–	1–4 h[d]	200[d]	80–100[d]	44,47
Membrane horizontal	Bio-Trap[b,f] (Elutrap[b,f])	(20 ml for concentration)	10 V/cm 1–8 h	200–800	80–97	24
Membrane vertical	Model 422[g]	ca. 0.8 ml	8–10 mA per tube 3–5 h	400–600	70–100	56,65,66
	Centrilutor[h]	≤0.5 ml[i] (1–100 μg)	100–400 V 1–4 h	50	≥90	68
Discontinuous conductivity gradient	HSB-eluter/E51[i] Maxyield-NP[j] Model SBE-06[e]	0.5 ml (as solid gel)	100 V[i] 0.33–2 h	20–100[i]	ca. 100[j]	46,70
Steady-state stacking with high concentration of leading ion	GE200 SixPac Gel Eluter[k]	≤0.5 ml	50 V 2 h	100–300	≥80	97
Continuous	Model 491/ Prep Cell[g]	(50 mg protein)	10 W (40 mA) 4–8 h	–	–	90
	Model 230A/ HPEC[l]	0.001–0.1 ml (25–500 μg)	300 V, 1–5 h (0.5–1.5 mA)	20–500	–	31,87,90
Continuous with pulse elution	Bio-Phoresis III[i]	(25 mg protein)	15–20 mA 4.5–5 h	2500–6000	–	–

[a] CBS Sci.
[b] Can be used for concentration purposes.
[c] Tyler Research Institute.
[d] ISCO.
[e] Nihon Eido.
[f] Schleicher and Schüll.
[g] Bio-Rad Labs.
[h] Amicon.
[i] Biometra.
[j] Atto.
[k] Hoefer.
[l] Applied Biosystems.

ses of proteins with microgram amounts of sample [17] and it has even become possible to clone the gene encoding the protein by using the DNA probe deduced from the amino acid sequence of the protein. Proteins are usually purified by a combination of chromatographic techniques such as ion-exchange, gel filtration, affinity, hydrophobic and reversed-phase chromatography. However, we often encounter the difficulty that the protein of interest cannot be completely purified even by using varieties of modern HPLC techniques, especially when only a trace amount of the analyte protein is contained in the sample. In such a case, PAGE provides an invaluable method to isolate the required protein from crude protein samples. Indeed, PAGE has been popularly and routinely used for determining the purities of peptides and proteins owing to its high resolution and excellent reproducibility and the easy preparation of gels at desired polyacrylamide concentrations in the presence and absence of additives such as urea and SDS. The recovery of proteins from polyacrylamide gel after PAGE for the preparative purposes is therefore of increasing importance, especially for purifying proteins available in low abundance which are often the subject of research today.

A number of methods for eluting proteins from polyacrylamide gel have been explored in order to improve the purity and yield of recovered proteins. The data published to date indicate that two methods are useful in practice with regard to the purity and yield of recovered proteins, i.e., the "electroelution" and "continuous elution" methods. In this review, these methods have been described in detail, including the principles, advantages and disadvantages, together with suggestions on important aspects to which attention should be paid, especially focusing on "electroelution", which is the most convenient approach for those who are trying for the first time to recover proteins from polyacrylamide gel with the least amount of protein and minimal expense. The recovery of nucleic acids can be similarly performed electrophoretically, as reviewed by Duro et al. [98], and applications to carbohydrates are also expected in the near future [99].

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References

[1] L. Ornstein, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321.
[2] B.J. Davis, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
[3] D.E. Williams and R.A. Reisfeld, *Ann. N.Y. Acad. Sci.*, 121 (1964) 373.
[4] H.W. Taber and F. Sherman, *Ann. N.Y. Acad. Sci.*, 121 (1964) 600.
[5] S. Hashizume, M.A. Rashid, M. Shoji and K. Kuroda, *Electrophoresis*, 5 (1984) 30.
[6] R.A. Reisfeld, U.J. Lewis and D.E. Williams, *Nature*, 195 (1962) 281.
[7] S. Raymond, *Ann. N.Y. Acad. Sci.*, 121 (1964) 350.
[8] P.H. O'Farrell, *J. Biol. Chem.*, 250 (1975) 4007.
[9] N. Catsimpoolas, *Sep. Sci.*, 5 (1970) 523.
[10] P.G. Righetti, *Isoelectric Focusing: Theory, Methodology and Applications*, Elsevier, Amsterdam, 1983.
[11] R.A. Reisfeld and P.A. Small, *Science*, 152 (1966) 1253.
[12] R.T. Swank and K.D. Munkres, *Anal. Biochem.*, 39 (1971) 462.
[13] K. Weber and M. Osborn, *J. Biol. Chem.*, 244 (1969) 4406.
[14] U.K. Laemmli, *Nature*, 227 (1970) 680.
[15] D.M. Neville, *J. Biol. Chem.*, 246 (1971) 6328.
[16] T.E. Kennedy, K. Wager-Smith, A. Barzilai, E.R. Kandel and J.D. Sweatt, *Nature*, 336 (1988) 499.
[17] J.E. Shively, R.J. Paxton and T.D. Lee, *Trends Biochem. Sci.*, 14 (1989) 246.
[18] R.J. Simpson, R.L. Moritz, G.S. Begg, M.R. Rubira and E.C. Nice, *Anal. Biochem.*, 177 (1989) 221.
[19] L.D. Ward, G.E. Reid, R.L. Moritz and R.J. Simpson, *J. Chromatogr.*, 519 (1990) 199.
[20] L. Shuster, *Methods Enzymol.*, 22 (1971) 412.
[21] D. Bray and S.M. Brownlee, *Anal. Biochem.*, 55 (1973) 213.
[22] C. Bernabeu, F.P. Conde and D. Vazquez, *Anal. Biochem.*, 84 (1977) 97.
[23] B.D. Hames, in B.D. Hames and D. Rickwood (Editors), *Gel Electrophoresis of Proteins*, IRP Press, Oxford, 2nd ed., 1990, p. 101.
[24] E. Jacobs and A. Clad, *Anal. Biochem.*, 154 (1986) 583.
[25] R.W. Young and H.W. Fulhorst, *Anal. Biochem.*, 11 (1965) 389.
[26] J.N. Hansen, *Anal. Biochem.*, 116 (1981) 146.
[27] H.S. Anker, *FEBS Lett.*, 7 (1970) 293.
[28] G. Baumann and A. Chrambach, *Anal. Biochem.*, 70 (1976) 32.

[29] P.B.H. O'Connell and C.J. Brady, *Anal. Biochem.*, 76 (1977) 63.
[30] A. Chrambach, *J. Mol. Cell. Biochem.*, 29 (1980) 23.
[31] A. Chrambach and D. Rodbard, *Science*, 172 (1971) 440.
[32] G. Kapadia and A. Chrambach, *Anal. Biochem.*, 48 (1972) 90.
[33] H. Hirano and B. Wittmann-Liebold, in B. Wittmann-Liebold (Editor), *Methods in Protein Sequence Analysis*, Springer, Berlin, 1989, p. 42.
[34] N. Ui, *Biochim. Biophys. Acta*, 229 (1971) 567.
[35] L.M. Hjelmeland and A. Chrambach, *Electrophoresis*, 2 (1981) 1.
[36] T. Suzuki, R.E. Benesch, S. Yung and R. Benesch, *Anal. Biochem.*, 55 (1973) 249.
[37] R. Pitt-Rivers and F.S.A. Impiombato, *Biochem. J.*, 109 (1968) 825.
[38] J.A. Reynolds and C. Tanfold, *Proc. Natl. Acad. Sci. U.S.A.*, 66 (1970) 1002.
[39] F. Hanaoka, J.L. Shaw and G.C. Mueller, *Anal. Biochem.*, 99 (1979) 170.
[40] D.A. Hager and R.R. Burgess, *Anal. Biochem.*, 109 (1980) 76.
[41] J.J. Koziarz, H. Köhler and T.L. Steck, *Anal. Biochem.*, 86 (1978) 78.
[42] M.W. Hunkapiller, E. Lujan, F. Ostrander and L. E. Hood, *Methods Enzymol.*, 91 (1983) 227.
[43] M. Moos, Jr., N.Y. Nguyen and T.-Y. Liu, *J. Biol. Chem.*, 263 (1988) 6005.
[44] W.B. Allington, A.L. Cordry, G.A. McCullough, D.E. Mitchell and J.W. Nelson, *Anal. Biochem.*, 85 (1978) 188.
[45] N.Y. Nguyen, J. DiFonzo and A. Chrambach, *Anal. Biochem.*, 106 (1980) 78.
[46] P. Strålfors and P. Belfrage, *Anal. Biochem.*, 128 (1983) 7.
[47] A.S. Bhown, J.E. Mole, F. Hunter and J.C. Bennett, *Anal. Biochem.*, 103 (1980) 184.
[48] G.P. Tuszynski, C.H. Damsky, J.P. Fuhrer and L. Warren, *Anal. Biochem.*, 83 (1977) 119.
[49] C.R. Merril, *Methods Enzymol.*, 182 (1990) 477.
[50] N. LeGendre and P. Matsudaira, *BioTechniques*, 6 (1988) 154.
[51] L. P. Nelles and J.R. Bamburg, *Anal. Biochem.*, 73 (1976) 522.
[52] R.C. Higgins and M.E. Dahmus, *Anal. Biochem.*, 93 (1979) 257.
[53] C. Lee, A. Levin and D. Branton, *Anal. Biochem.*, 166 (1987) 308.
[54] J.K. Dzandu, J.F. Johnson and G.E. Wise, *Anal. Biochem.*, 174 (1988) 157.
[55] D.N. Talbot and D.A. Yphantis, *Anal. Biochem.*, 44 (1971) 246.
[56] R.E. Stephens, *Anal. Biochem.*, 65 (1975) 369.
[57] D. Rickwood, in B.D. Hames and D. Rickwood (Editors), *Gel Electrophoresis of Proteins*, IRP Press, Oxford, 2nd ed., 1990, p. 346.
[58] X.C. Chen, H. Kasai and T. Okuyama, *Bunseki Kagaku*, 34 (1985) 137.
[59] N.Y. Nguyen and A. Chrambach, *J. Biochem. Biophys. Methods*, 1 (1979) 171.
[60] H. Ochiai, K. Jin, N. Kakihara and T. Hanafusa, *Seikagaku*, 59 (1987) 1164.
[61] J.B.C. Findlay, in E.L.V. Harris and S. Angal, *Protein Purification Applications*, IRL Press, Oxford, 1990, p. 83.
[62] M. le Maire, S. Deschamps, J.V. Møller, J.P. Le Caer and J. Rossier, *Anal. Biochem.*, 214 (1993) 50.
[63] C. McDonald, S. Fawell, D. Pappin and S. Higgins, *Trends Genet.*, 2 (1986) 35.
[64] B.D. Hames, in B.D. Hames and D. Rickwood (Editors), *Gel Electrophoresis of Proteins*, IRP Press, Oxford, 2nd ed., 1990, p. 102.
[65] D. Sulitzeanu, M. Slavin and E. Yecheskeli, *Anal. Biochem.*, 21 (1967) 57.
[66] U.J. Lewis and M.O. Clark, *Anal. Biochem.*, 6 (1963) 303.
[67] N. Weliky, D.H. Leaman, Jr., and B. J. Kallman, *Anal. Biochem.*, 67 (1975) 507.
[68] S. Ihara, H. Suzuki and M. Kawakami, *Anal. Biochem.*, 166 (1987) 349.
[69] J.A. Smith, in J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach and W. Strober (Editors), *Current Protocols in Immunology*, Wiley, New York, 1991, Unit 8.8.
[70] M. Otto and M. Snejdárková, *Anal. Biochem.*, 111 (1981) 111.
[71] T.M. Jovin, *Biochemistry*, 12 (1973) 871.
[72] H. Wachslicht and A. Chrambach, *Anal. Biochem.*, 84 (1978) 533.
[73] L.-G. Öfverstedt, G. Johansson, G. Fröman and S. Hjertén, *Electrophoresis*, 2 (1981) 168.
[74] L.-G. Öfverstedt, J. Sundelin and G. Johansson, *Anal. Biochem.*, 134 (1983) 361.
[75] T.M. Jovin, M.L. Dante and A. Chrambach, *Multiphasic Buffer Systems Output, PB 196085–196091, 259309–259312, 203016*, National Technical Information Service, Springfield, VA, 1970.
[76] P.H. Duesberg and R.R. Rueckert, *Anal. Biochem.*, 11 (1965) 342.
[77] M.A. Rashid and S. Hashizume, *Anal. Biochem.*, 127 (1982) 334.
[78] I. Mendel-Hartvig, *Anal. Biochem.*, 121 (1982) 215.
[79] O.H. Kapp and S.N. Vinogradov, *Anal. Bochem.*, 91 (1978) 230.
[80] W.H. Konigsberg and L. Henderson, *Methods Enzymol.*, 91 (1983) 254.
[81] D. Wessel and U.I. Flügge, *Anal. Biochem.*, 138 (1984) 141.
[82] T. Ratajczak, M.J. Brockway, R. Hähnel, R.L. Moritz and R.J. Simpson, *Biochem. Biophys. Res. Commun.*, 151 (1988) 1156.
[83] A.J. Furth, *Anal. Biochem.*, 109 (1980) 207.
[84] R.J. Simpson, R.L. Moritz, E.E. Nice and B. Grego, *Eur. J. Biochem.*, 165 (1987) 21.
[85] G. Baumann and A. Chrambach, *Anal. Biochem.*, 69 (1975) 649.
[86] M. Ben-David and A. Chrambach, *Endocrinology*, 101 (1977) 250.

[87] T.M. Jovin, A. Chrambach and M.A. Naughton, *Anal. Biochem.*, 9 (1964) 351.
[88] S. Hjertén, S. Jerstedt and A. Tiselius, *Anal. Biochem.*, 11 (1965) 211.
[89] S. Hjertén, S. Jerstedt and A. Tiselius, *Anal. Biochem.*, 11 (1965) 219.
[90] A.H. Goredon and L.N. Louis, *Anal. Biochem.*, 21 (1967) 190.
[91] A.D. Brownstone, *Anal. Biochem.*, 27 (1969) 25.
[92] S. Hjertén, S. Jerstedt and A. Tiselius, *Anal. Biochem.*, 27 (1969) 108.
[93] D.G. Sheer, D.K. Yamane, D.H. Hawke and P.-M. Yuan, *BioTechniques*, 9 (1990) 486.
[94] D.H. Shain, J. Yoo, R.G. Slaughter, S.E. Hayes and T.H. Ji, *Anal. Biochem.*, 200 (1992) 47.
[95] D. Ruggiero-Lopez, P. Louisot and A. Martin, *Anal. Biochem.*, 212 (1993) 247.
[96] M.G. Harrington, *Methods Enzymol.*, 182 (1990) 488.
[97] H. Ahokas, *Nucleic. Acids Res.*, 15 (1987) 6759.
[98] G. Duro, V. Izzo and R. Barbieri, *J. Chromatogr.*, 618 (1993) 95.
[99] P. Jackson, *Anal. Biochem.*, 216 (1994) 243.