

# Large scale preparative gel electrophoresis

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## Abstract

This paper describes a piece of gel electrophoresis apparatus designed for the separation and purification of proteins on the multigram scale.

## Introduction

After many decades of inventive effort, electrophoresis has not achieved acceptance in preparative work commensurate with its overwhelming success as an analytical tool. This is presumably because of shortcomings of the available methods and equipment in respect of actual separative performance, convenience, scale, reliability, reproducibility and cost. To overcome these problems a simple and sturdy instrument has been designed, which has been used for the separation and purification of proteins on the multigram scale; it can be further scaled up without introducing any new features likely to degrade separative performance.

The apparatus enables electrophoresis to take place in a horizontal axis, with fractions collected from a chamber or slot at one or both ends of the electrophoresis medium (typically but not necessarily a gel with molecular sieving properties). Distinctive features are the large cross-sectional area of the electrophoresis bed, and the maintenance of uniform conditions throughout that cross-section, by stabilising partitions, appropriate cooling geometry and preventing inhomogeneities (in directions transverse to the electrophoretic axis) of protein concentration in the separating bands.

## Instrumentation

Figure 1a provides a general, isometric view of the apparatus without the upper cooling plate which sits on top of the gel during electrophoresis. Figure 2 is an expanded diagram to indicate how the various components function and also the pattern of coolant (electrode) buffer flow. Between two electrode chambers (1) lies a gel housing (2) divided by vertical partitions (3) into four parallel and equal subdivisions (4). The base of the gel housing (5), its side walls and the upper cooling plate (8) are hollow and equipped with baffles (7) to induce a flow of coolant buffer such that there is an almost even temperature over all surfaces. Coolant buffer flows through these constructions via inlet and outlet tubes (9). All parts are made from perspex (lucite) sheet, and are glued together to form a single watertight unit.

Few dimensions are critical, though the electrode tanks should be larger in cross-section than the gel housing. Actual dimensions of the gel bed are (for each of the four subdivisions of the gel housing) length in electrophoretic axis 7.2 cm, breadth 2.4 cm and depth 8 cm. Also required, but not illustrated, are a casting chamber and a casting lid. The casting chamber should be of internal dimensions identical to the gel housing. The casting lid sits on top of the chamber during the casting process, but is conveniently made longer in the intended electrophoresis direction to allow a choice to be made as to the length of the electrophoresis path. The casting lid bears on its lower surface long, tongue-like projections, the slot formers, typically 0.3 cm thick (in the electrophoresis direction), 1.8 cm wide and 7.6 cm long. There is one for each of the subdivisions of the gel housing, arranged in a single row, accurately aligned, transversely across the casting lid, so that the four subdivisions are identical. When the gel casting process is complete, the casting lid is removed, leaving behind deep cavities in the gel corresponding to the slot formers. These constitute the sample or 'origin' slots.

Polyacrylamide gels, once transferred out of the casting chamber and into

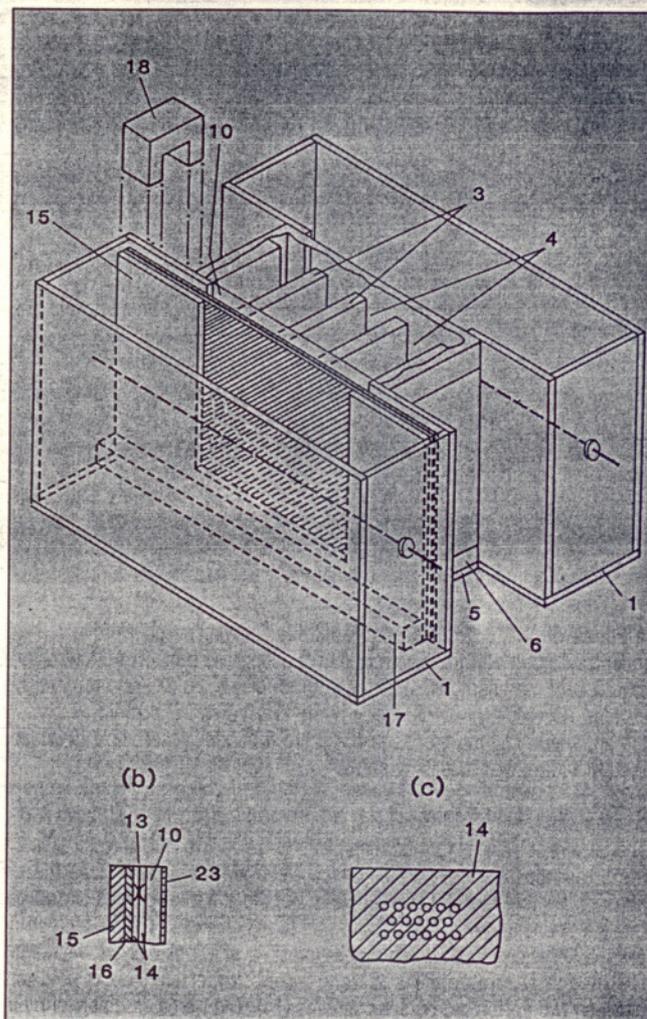


Figure 1(a). General isometric view of the apparatus showing the four-chamber gel housing placed between two electrode tanks. There is a collection slot (10) at each end of the gel housing. The upper cooling plate is not shown.

Figure 1(b). Detail showing formation of the elution slot as the space between the end of the gel and an assembly comprising two perforated perspex screens (14) on each side of a sheet of dialysis membrane (13), a gasket (16) and a pressing plate (15).

Figure 1(c). Detail showing a portion of one of the perforated perspex screens (14).

the gel housing, may be liable to slip out of the gel housing into the collection slot. This is prevented by glueing at each lower corner of each subdivision of the gel housing a 0.2 x 0.2 cm cube of perspex. It will also be apparent that the four subdivisions of the gel housing are independent of each other. In certain variant forms of the apparatus this feature can be exploited to allow side-by-side comparisons of different samples.

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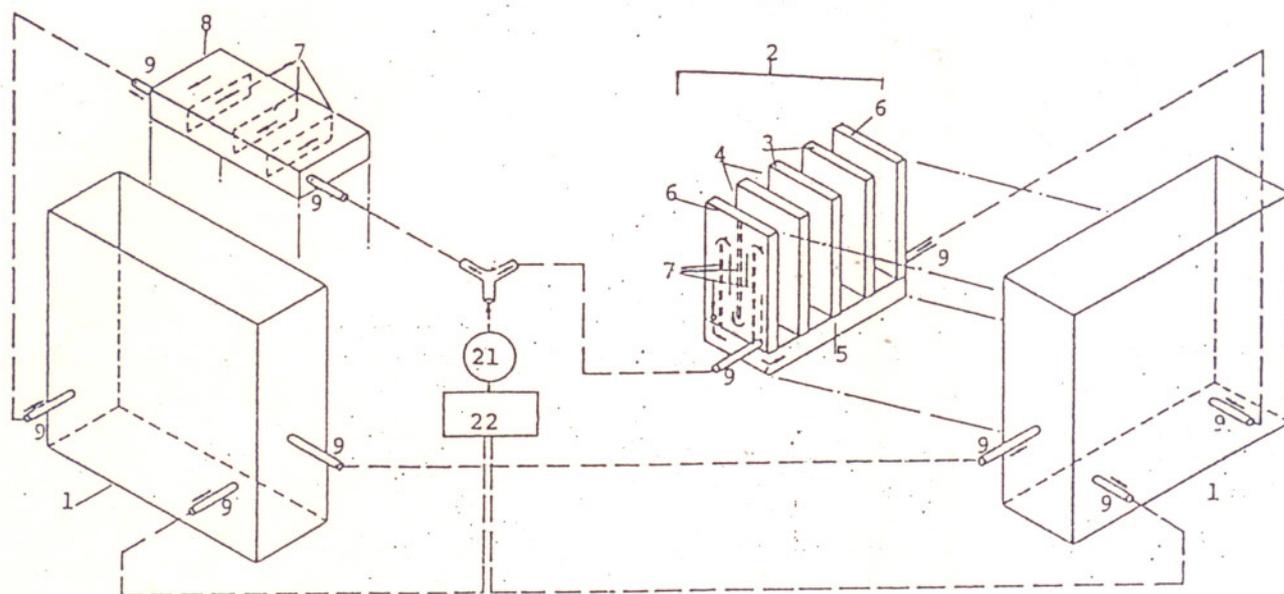


Figure 2. Expanded drawing showing the relationship of various parts to each other, and the pattern of coolant buffer circulation (interrupted lines).

For several distinct reasons the electrode tank buffer should be mixed and recirculated between the electrode tanks during electrophoresis, and also it should cool the ends (faces) of the gel, not only the sides, the top and the bottom. Accordingly, the electrode buffer is used as the coolant throughout the apparatus and the electrode tanks are included in the buffer circulation system (interrupted lines in figure 2).

**Elution slot**

The elution slot (10) is formed by a piece of perspex sheet, shaped as in figure 3, and constituting the side of the electrode tank which faces the gel housing. In use, buffer flows into the slot via the inlet port (11) and out through the outlet port (12). On one side of the elution slot is the face of the electrophoresis gel (2,3), and on the other, a sheet of dialysis membrane (13), which separates the slot from the electrode tank and is held taut between two perforated perspex screens (14). The whole assembly of membrane and support screens, 'pressing plate' (15) and gasket (16), is clamped in place by the retaining bar (17), glued to the floor of the electrode tank, and two U-shaped pieces (18). The U-shaped pieces (18) fit tightly over the wall of the electrode compartment and the above assembly (including pressing plate and gasket), so as to hold the whole assembly firmly against the wall of the electrode chamber.

**Stirring mechanism for elution slot**

The stirring mechanism (figure 4) consists of an L-shaped piece of thin perspex (19) hinged at the angle of the L, and rocked to and fro, against a light spring, by a cam (20) driven by a slow-rotating electric motor. This assembly is attached to the upper cooling plate in such a way that the long arm of the L dips into the elution slot, as deep as possible, while still allowing a full range of movement.

**Ancillary devices**

These are shown only schematically or not at all. They include electrophore-

sis power supply and connections; proportioning peristaltic pump(s) (21) to provide for coolant buffer circulation as in figure 2 and flow of elution buffer through the elution slot; buffer reservoir; refrigeration unit (22); fraction collector; gel casting chamber; and the casting lid carrying the sample slot formers.

**Method of use**

The calculated volume of the chosen acrylamide monomer solution, containing the desired electrophoresis buffer, is prepared, initiators are added, and the mixture is poured into the casting chamber. Typically, this will be 5.5% w/v total monomers (95% acrylamide, 5% *N,N*-methylene-bis-acrylamide) containing 1 mg/ml ammonium persulphate, freshly weighed out, and 1 μ/ml *N,N,N,N*-tetramethylethylene diamine. The casting lid is then lowered into place, carefully excluding bubbles (there should be a slight excess of monomer solution), and the apparatus is then set aside to polymerise. With correctly chosen conditions and at 25°C, polymerisation should occur within 10 min.

After polymerisation, the casting lid is removed and individual slabs of gel are transferred to the gel housing. Pre-electrophoresis is generally thought to be necessary. For this, sample and elution slots, and electrode tanks, are filled with buffer; circulation and elution pumps are set going with eluant flowing to waste; and the electrophoresis current applied for 1.5 h at 7 V/cm with coolant buffer at 4°C.

The sample slots are then aspirated free of buffer, and the sample is introduced in the form of a warm solution in 0.5% agarose, made, for example, by mixing the desired protein solution, pre-warmed to 37°C, with one third of its volume of a 2% agarose solution, previously melted and then swiftly cooled to 37°C. Pipettes and glassware should also be warmed and if the operation is conducted briskly there is little risk of premature setting of the agarose. Low-melting agaroses may be used. Both the agarose and the protein solutions should be made up in the appropriate buffers. The volume applied to each sample slot should be sufficient to bring the level to no closer than 0.5 cm

from the top of the slot. The sample slots are then sealed by filling up with agarose solution, and the upper cooling plate is set in place.

Electrode tanks and elution slots are then filled with fresh buffer, the refrigeration, buffer circulation, elution and fraction collection systems are set going, and the electrophoresis current is switched on. Typical settings would be 7 V/cm with elution buffer flowing at 50 ml/h and coolant buffer at 4°C.

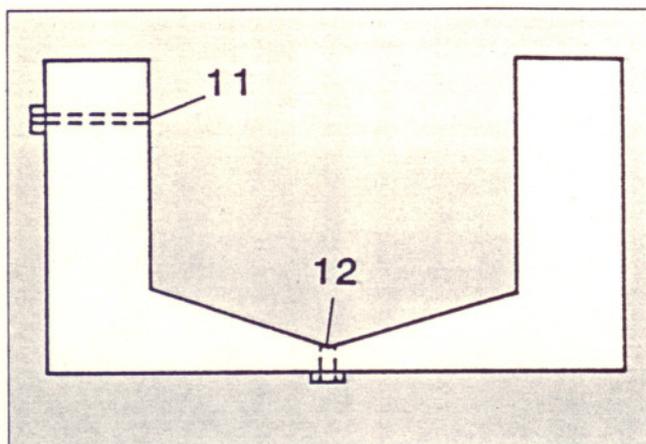


Figure 3. Detail showing the shape of one wall of the electrophoresis chamber, facing the gel housing, and the arrangement of inlet (11) and outlet (12) tubes.

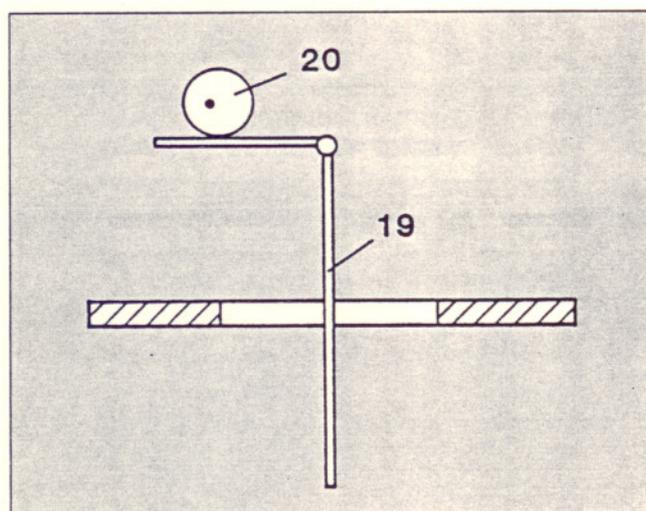


Figure 4. Detail showing stirring device. In use, this is secured to the upper cooling plate (8).

## Discussion

Development of this, and related apparatus, has continued intermittently since 1962. Apparatus based on the prototype, with a gel cross-section of 6.0 x 1.0 cm and intermittent rather than continuous-flow elution, has been used in the purification of ferritin monomer and oligomers [1], and ferritin conjugated to antibody [2,3], lactate dehydrogenase (LDH) isoenzymes [3], alkaline phosphatase [4], urinary glycosaminoglycans [5], and in studies of factors which affect resolution during operation on a preparative scale, that is to say, at high sample load [6]. This large-scale apparatus has been used for experiments with haemoglobin and albumin, and for preparation of plasma cholinesterase iso-forms [7] and isoenzymes of horse-radish peroxidase [8]. In the work on LDH isoenzymes [3] and also that on horse-radish peroxidase isoenzymes [8], collection at both ends of the gel was employed. An improved apparatus, with microprocessor control, has been developed [9], but is customarily used with gel dimensions like the prototype.

Even at 1.0 cm thickness, serious loss of resolution can be caused by temperature inequalities and inhomogeneities of protein concentration in an axis transverse to the electric field, and even greater difficulty is encountered when the cross-sectional area is larger, as in this case. In response, most designs adopt a vertical electrophoresis axis, to combat electrodecantation effects. In such instruments, however, only downward migrating species are recoverable and elaborate arrangements may still be needed to prevent transverse movements of concentrated protein solutions in the sample chamber or the collection slot. Further, there may be trouble arising from channelling of elution buffer in an uncontrolled manner to narrow tracks within the collection chamber, and from the presence of a semipermeable membrane which traps the protein molecules that manage to reach it.

All these problems are avoided in the present design. Electrodecantation in the sample slot is eliminated by use of agarose and in the collection chamber by stirring, which is also effective in preventing protein molecules from piling up on the limiting dialysis membrane.

Heat must be generated within any electrophoresis bed. In a steady state, this heat must be conducted away down a temperature gradient and it follows that temperature inequalities are inevitable. They are usually minimised by using thin gels, but for large-scale work this is impossible and quite significant temperature differences occur between different parts of the gel bed, with consequential variations in the mobility of separating molecules. This problem could be partially alleviated by using low-conductivity buffers and low voltage gradients, but these, in principle, have an adverse effect on resolution except under special conditions [6]. The existence of temperature differences and hence mobility differences transverse to the electrophoresis axis are probably the most important influences damaging the resolution achieved in preparative gel electrophoresis and hence preventing the achievement of results comparable with analytical work in thin gels.

It has been proposed that the temperature gradients should be allowed only parallel to the electric field [10,11], so that though they experience different temperatures during a run, and hence different mobilities over time, all the molecules which constitute one band (or a narrow element of a broader band) are always at the same temperature as each other at any given moment. However, no clear advantage of this principle of end-cooling or 'face-cooling' has yet been demonstrated. There is also an obvious disadvantage in restricting the area available for heat exchange, since the absolute temperature in the middle of the bed may rise too high.

The present design represents a compromise wherein cooling occurs roughly equally in directions parallel to and perpendicular to the electrophoretic field. Justification lies in the results obtained and in the lack of 'bowing' or distortion of coloured protein bands viewed from the side.

Stirring in the collection slot is used in this design primarily to prevent electrodecantation. The results obtained in the present work argue that uniformity of protein concentration throughout the collection slot [10] is indeed beneficial.

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