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THERMALLY STABILIZED VERY THIN (0.02–0.3 mm) POLYACRYLAMIDE GELS FOR ELECTROPHORESIS

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SUMMARY

A simple technique and device for the preparation of gels are described that complement the conventional "pouring" technique, which fails in the preparation of bubble-free gels with thicknesses less than about 0.4 mm. Included are methods for processing and manipulation, design of a thermostating platen and gel casting device, and chemical treatment of glass and polyester substrates to which the polyacrylamide gel adheres during processing. The gel is cast directly between the glass cover plate and the thermostating platen, and this assembly provides isothermal conditions in the gel layer during the electrophoresis. Further, techniques for introducing spacers, stacking gel, sample slot forming and sample application have been developed and applied to DNA sequencing and protein sodium dodecyl sulphate electrophoresis. The results obtained indicate that the technique is simple to use, has many analytical applications (DNA sequencing, sodium dodecyl sulphate–polyacrylamide gel electrophoresis, isoelectric focusing) and has various advantages.

INTRODUCTION

The theoretical justification for the use of very thin gels for zone electrophoresis can be deduced from simple models and equations. The main advantages of thinner gels are as follows: (1) small temperature gradient across the gel thickness; the efficient heat transfer, in combination with gel thermostating, eliminates distortions caused by thermal effects; (2) lower Joule heating per unit electrical field and surface area, permitting the application of higher field strengths, resulting in improved resolution and substantially shorter running times; (3) significantly shorter staining (fixing) and de-staining times; and (4) less sample is needed (higher sensitivity).

The use of very thin (< 0.2 mm) polyacrylamide gel has been mentioned previously[1], but its wider application has been limited by problems in the preparation of larger bubble-free gels and in their handling.

In this paper, a simple technique and device for the preparation of bubble-free gels less than 0.3 mm thick are described that complement the conventional "pouring" technique, which fails in the preparation of thinner gels, because the elimination of gas bubbles trapped in the thin layer is very difficult. Thin gels up to

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1000 mm long, 300 mm wide and 1.5 mm thick had been prepared using this technique. A procedure for the treatment of polyester and glass surface was developed, which makes it possible to polymerize the gel on a transparent support to which it adheres during all subsequent processing steps, including scanning and storage. An inexpensive glass thermostating platen was designed that maintains the gel layer under isothermal conditions during the electrophoresis.

Techniques for introducing spacers, stacking gel, sample slot forming and sample application have been developed for thin gels and applied to DNA sequencing and protein sodium dodecyl sulphate (SDS) electrophoresis in a vertical system.

MATERIALS AND EQUIPMENT

Gel electrophoresis

Polyacrylamide solutions for protein separation were prepared as described elsewhere[2,3]. A modified Laemmli system was used in the discontinuous and phosphate buffer in the continuous method, in both instances varying the ionic strength. The gels and buffers for DNA sequencing will be described elsewhere[4].

Spacers were made of PTFE and sample combs of PTFE, polyester or stainless steel treated so as to be hydrophobic. Sample application was performed by means of thin-walled glass capillaries for X-ray analysis (A. Müller Glas- und Vakuumtechnik, Berlin, G.F.R.). Power supplies with a range of 0–2000 V are suitable.

Device for gel casting*

A sliding technique was developed for preparing very thin gels. It consists of a flat, horizontal supporting bed provided with guiding studs on each side, between which a flat plate, to be coated with the gel, is laid down. Next to this plate, leaving a gap, a dummy plate having the same thickness is placed. PTFE spacer strips are laid over the plates next to their outer edges, and a third plate is placed on the spacers, so that it covers the dummy plate and a few millimetres of the plate that is to be coated. The cover plate can slide over the spacers; this sliding motion is constrained to one direction by the guiding studs. A slow horizontal sliding motion permits the gel solution to be spread evenly between the bottom and the top plate. Between the guiding studs, the top and bottom plates are accessible and can be clamped firmly together after the space between them has been filled with gel solution. A minimum of manipulation is required. On the device shown in Fig. 1, 200 mm wide gels up to 600 mm long can be cast. Plates of standard width that fit between the guiders are used, but otherwise they can be of any desired length or thickness. The top plate may have one or more cut-outs of any shape at its sides for bringing the gel into contact with buffer solutions.

Hydrophobic treatment (gel repel coat) of at least one of the surfaces in contact with gel solution speeds up the sliding step and decreases the risk of trapping an air bubble.

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* Available soon commiercially.

Thermostating

An inexpensive glass thermostating platen was designed and constructed. For efficient transfer of heat from the gel the platen is used in place of one of the two glass plates in the gel mould. In protein separation the platen cools the gel, whereas in DNA sequencing it heats it.

The water circulating and thermostating unit was a Thermomix–Frigomix combination from B. Braun, Melsungen, G.F.R.

Gel supports

To make handling possible, thin gels need a suitable support (desirable properties: electrical insulator, high thermal conductivity, transparent, inert to staining dyes, resistant to chemicals, dimensional stability, flexibility and the possibility of cutting out certain parts of the gel). The following materials were found to be suitable supports: nylon net, cellophane, polyester, polystyrene and glass. A process for surface treatment of the last three materials was developed by which the polymerized gel adheres covalently to them during all subsequent processing steps. Thin polyester sheets and glass plates were found to be the most convenient materials for gel supports.

For the surface treatment, the relatively non-hazardous γ-methacryloxypropyltrimethoxysilane (Wacker Chemie, Munich, G.F.R.) was found to be a suitable coupling agent amongst many tested, as used in the semiconductor industry.

The following procedure is applied to glass and polyester surfaces (Hostaphan BN 100 or BN 180 polyester sheet from Kalle Wiesbaden, G.F.R.) to enhance adhesion of the polyacrylamide gel:

(1) The surface is washed (glass with acetone, polyester with ethanol or isopropanol) and dried.

(2) Solutions are prepared: (A) 3 ml of water + 0.3 ml of glacial acetic acid; (B) 100 ml of ethanol + 0.3 ml of the silane, stirring well; (C) A and B are mixed. The whole of step 2 should not take longer than 2 min and solution C should be used immediately because the silane is quickly hydrolysed.

(3) Solution C is applied to the surface to be treated (dip, spray or paint on with lint-free paper) which, after standing for 2 min, is dried with lint-free paper.

(4) The plate is baked at 120°C (100°C with polyester) for 15 min, resulting in a dry and spotless surface.

(5) The treated polyester or glass plates can be used immediately or stored. Prior to use the surface is wiped with lint-free paper soaked in ethanol to remove excess of the silane and dried.

Prior etching of the polyester sheet in NaOH (as was reported to be necessary, in the process described in ref. 1) is not needed in the above process.

With the longer running times (*ca.* 3 h) and higher gel temperatures (50–60°C) encountered in DNA sequencing, the gel adhered to the polyester sheet less strongly.

This inconvenience was not observed with glass plates as the gel support.

METHODS

Preparation of very thin gels

The method is shown schematically in Fig. 1. First, a few drops of 5% glycerine solution are deposited on one face of the thermostating platen and a polyester sheet, treated on the top side, is rolled on to it using a rubber roller (the sheet can also be placed on the glass cover, resulting in even better heat transfer from the gel). Two PTFE spacers are placed on the polyester. Located next to the platen, the glass cover plate rests on a dummy block, the thickness of which is very nearly the same as that of the platen. The glass cover extends about 5 mm over the platen edge. About two thirds of the prepared gel solution (roughly three times the calculated ideally minimum amount of the solution needed should be prepared) is poured on to the polyester in the cut-out of the cover plate. The solution is drawn by capillary forces below the cover plate, quickly filling the 5 mm wide strip. The cover is then carefully slid over the thermostating platen, forming a thin layer of solution between the polyester sheet and the glass cover. More gel solution is poured in front of the cover when needed. Any trapped bubbles are removed by sliding the cover plate slowly backwards, until the bubbles are released. When a bubble appears at the edge of the cover, it can be removed with the help of a thin stainless-steel strip.

The sample comb is inserted below the cover plate either at the start or at the end of the sliding motion. The plates are then firmly clamped and transferred to a location for horizontal polymerization. If difficulties with comb insertion are feared, additional narrow PTFE spacers (about 0.1 mm thicker than the desired gel thickness determined by the normal spacers) are placed on the outside next to the normal spacers. The additional spacers are removed after sliding and comb insertion.

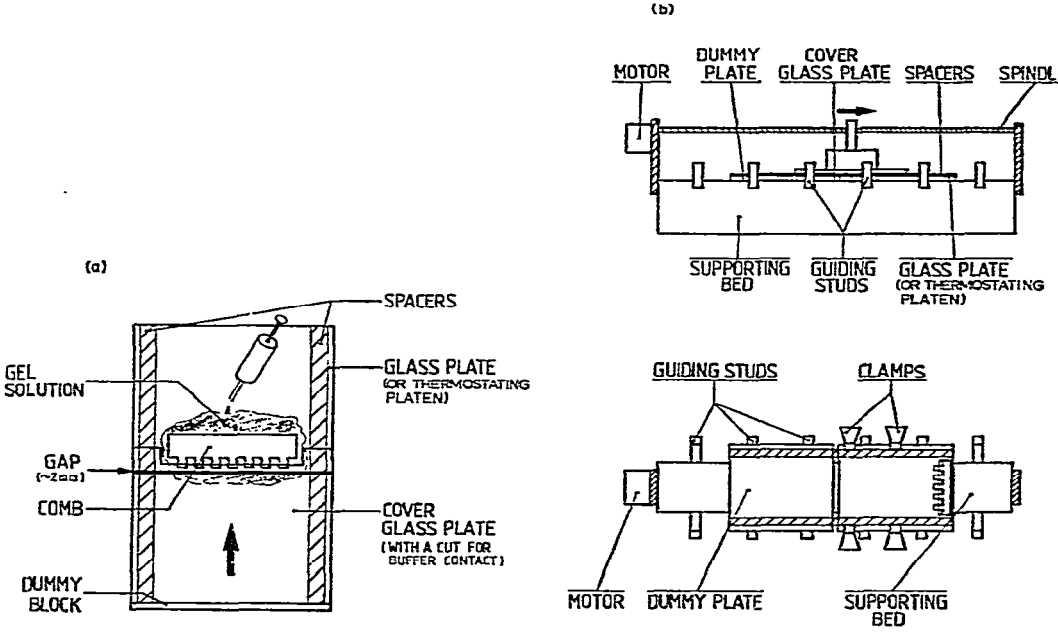

Fig. 1. Preparation of gels by sliding technique (schematic): (a) principle; (b) gel casting device.

The gel solution is held in place by capillarity and does not leak out. To prepare a gel 200 × 200 mm with this technique takes about 1 min.

A simple device for the preparation of thin gels was designed (see above), providing for guiding of the cover plate and an easy means of rapid clamping, limiting the manipulation to the minimum.

To cast a stacking gel, a cone-shaped stacking PTFE spacer is carefully inserted (at an angle, to avoid introducing bubbles) in the separation gel solution, instead of the comb above, and clamped. After polymerization, the stacking spacer is removed and stacking gel solution poured using, *e.g.*, a Raven Pipetman. If a bubble appears, it is removed with the aid of a thin stainless-steel strip. The sample comb is inserted, the plates are re-clamped and the stacking gel is polymerized in either a vertical or horizontal position. After removal of the stacking spacer, the stacking space may be flushed with buffer and any remnants of gel removed with the aid of the stainless-steel strip. After flushing, the buffer is sucked out of the stacking space with a filter-paper.

The gel preparation is facilitated when one of the surfaces in contact with the gel solution is treated to make it hydrophobic. The usual gel repellant agents give satisfactory results.

The thin gels polymerized on the polyester foil (or glass plate) rest directly on the thermostating platen and their temperature can be optimally regulated during the run, in either a vertical or a horizontal running system.

Safety precautions for work with the acrylamide monomer must be observed during the whole process.

Vertical system

As described in the previous section, the gel is cast directly between the thermostating platen and the glass cover plate. This assembly can be clamped in any conventional vertical system of appropriate size and the platen connected to a thermostating bath unit. After filling the buffer in the buffer chambers, the sample comb is carefully removed and remnant gel rim left behing the comb on the cover plate is scratched away using a spatula. The sample slots are flushed with the buffer (using a 1-ml Raven Pipetman or Pasteur pipette).

The sample is most conveniently applied by means of glass capillaries (*e.g.*, thin-walled glass capillaries for X-ray analysis from A. Müller), which can be washed and re-used many times. Very thin metal capillaries on syringes have been developed for this purpose, but proved to be two orders of magnitude more expensive, the inner diameter is smaller and their maintenance requirements are greater.

To ensure uniform sample deposition, the filled capillary is placed in a solid holder prior to insertion into the sample slot. The sample is dispensed by regulation of air pressure, using a syringe with micrometer control or an air pump[4] and silicone-rubber connecting tubes, although the "mouthpiece method" works as well with less viscous samples. Samples of 0.2–6 μl have been applied in this way.

Although 0.05 mm thick gels were used for protein separation, it is the step of sample application (its viscosity and the availability of thin-walled glass capillaries) that limits at present the practical thickness range of the very thin gels to 0.1–0.2 mm in the vertical method.

In protein separations, the voltage applied to a 10% gel (200 × 200 mm) was

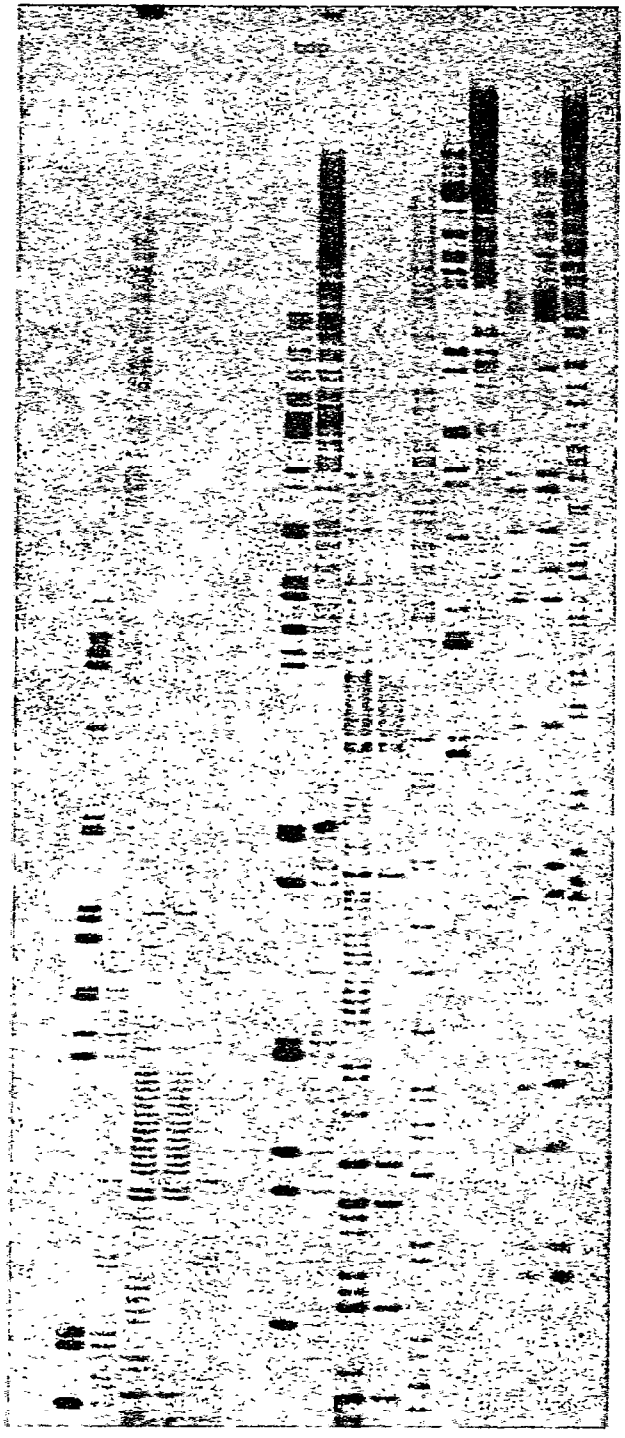

Fig. 2. Thermostated (55°C) DNA sequencing gel, thickness 0.2 mm, size 200 × 400 mm, E = 50 V/cm, I = 12 mA (from ref. 4).

in the range 300–1000 V, the applied power was 3–20 W and the running time was 20–60 min at a gel temperature of 10°C. For gels (200 × 400 mm) used in DNA sequencing[4], the applied voltage was 1800–2000 V, the power was 20–30 W and the running time was about 50 min at a gel temperature of 55°C.

Horizontal system

The horizontal system offers the advantage of simple application of the sample, and therefore gels thinner than 0.1 mm are practicable. Tests have been made in our laboratory on 0.05–0.1 mm thick gels with very encouraging results. The voltage applied to a 10% gel (100 × 100 mm) was in the range 400–500 V, the applied power was 5–10 W and the running time was 30–80 min at a gel temperature of 6°C. The forming of sample slots is at present the limiting factor in our laboratory for the use of the very thin gels in a horizontal system. Isoelectric focusing, in which sample slots are not needed, is relatively easy on thin gels[1].

RESULTS AND DISCUSSION

DNA sequencing

A DNA-sequencing autoradiograph obtained on a 0.2 mm thick thermostated gel (200 × 400 mm) is shown in Fig. 2. Gels of thickness down to 0.14 mm were tested with similar results. The bands are straight in both the vertical and horizontal directions and the autoradiograph is suitable for analysis by computer. The band sharpness and the number of resolved bases are higher than those with the sequencing pattern of the same sample obtained by conventional techniques.

The method and results of the sequencing will be reported elsewhere[4].

Protein separation

Standard protein markers (low-molecular-weight range) from Bio-Rad Labs. (Richmond, CA, U.S.A.), separated in a SDS continuous phosphate buffer system on a 0.13 mm thin gel (200 × 200 mm) are shown in Fig. 3. The gel and sample pockets were formed as described above. A 2-μl volume of sample solution (0.15 μg of each protein) was loaded into the 5 mm wide sample pocket.

Good results were obtained in the discontinuous system with Laemmli-type buffers. With 3 μl of sample solution (0.15 μg of each protein) the observed band width was comparable to that in the continuous system above, in agreement with observations by other workers[5–7].

Discussion

The results obtained indicate that the technique of using thermally stabilised very thin (0.1–0.2 mm) gels is very simple in practice, has many analytical applications (DNA sequencing, SDS–polyacrylamide gel electrophoresis (PAGE), isoelectric focusing), and brings all the expected advantages as listed in the Introduction. Manipulations during gel preparation are easier and take less time than in the conventional techniques. Gels of 3–40% concentration were prepared without difficulty using the technique described.

In DNA sequencing, the application of thermostated very thin gels[4] improves

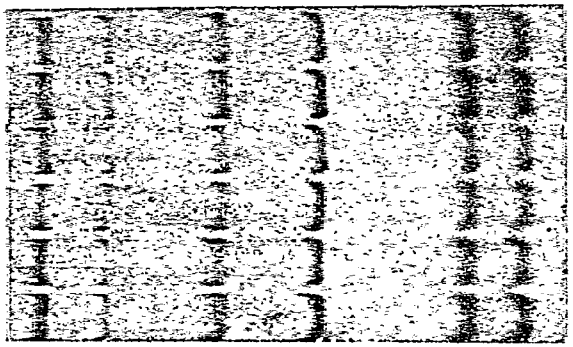

Fig. 3. SDS–PAGE continuous phosphate buffer, gel thickness 0.13 mm, size 200 × 200 mm, Bio-Rad low-molecular-weight protein standard, temperature 10°C, $E = 40$ V/cm, $I = 20$ mA.

the resolution, as predicted by theoretical considerations, and virtually eliminates thermal pattern distortions.

Typical running, staining and destaining times for protein separation on a conventional 1 mm thick gel are compared in Table I with those for a 0.1–0.15 mm thin thermostated gel. The total time requirement is an order of magnitude shorter for the very thin gel.

TABLE I

COMPARISON OF TYPICAL RUNNING, STAINING AND DESTAINING TIMES FOR SDS–PAGE ON A CONVENTIONAL (1–1.5 mm THICK) AND A VERY THIN (0.15 mm) POLYACRYLAMIDE GEL (SIZE 200 × 200 mm)

Gel thickness (mm)	*Time (min)*			
	Run	*Fix + stain*	*Destain*	*Total*
1–1.5	360	120	180	660
0.15	60	10	15	85

The lowest protein load applied in our test runs was 0.01 μg of protein per band, clearly visible after destaining on the 0.1 mm thin gel used in the discontinuous SDS separation run.

In several tests broad protein bands were observed when the thin gels were run at a high field strength ($\gtrsim$ 50 V/cm) without previous optimization of ionic strength, sample load or stacking voltage. Further study of the factors (temperature, sample load, field strength, ionic strength, current density, gel concentration) that affect the resolution and band width with very thin gels will be necessary in order to optimize the technique for different applications, and to extend similar investigations made with thicker gels. According to our experience the geometric scaling effects agree very well with physico-chemical predictions for a uniform layer, neglecting surface effects.

Variation of pore size and thickness on the thin gels will be further evaluated, although no effect attributable to a variation in thickness has been observed during the tests with standard glass plates.

There is interest in applying more sensitive detection methods to the very thin gel technique, and to investigate gel supporting structures further.

CONCLUSION

Very thin polyacrylamide gels (0.02–0.2 mm thick) are prepared with the aid of a gel casting device. The gel is cast directly between the glass cover plate and a thermostating platen, which maintains the gel layer under isothermal conditions during electrophoresis. To make handling possible, a chemical procedure for the treatment of glass and polyester surfaces was developed that makes it possible to polymerize the gel on a transparent support to which it adheres during subsequent processing. The sample is most conveniently applied by means of glass capillaries which are simply washed and re-used many times. To ensure uniform sample deposition, a regulating sample dispensing system is used. Gels of 3–40% concentration can be prepared without difficulty using the technique described. The results obtained indicate that the technique is simple to use, has many analytical applications (DNA sequencing, SDS–PAGE, isoelectric focusing) and has various advantages.

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