A convenient migration chamber for electrophoresis in solid gels*

Reproducible electrophoretic conditions are provided by a conveniently designed migration chamber, which was constructed at the Instrument Service Shop of Montana State University. This apparatus eliminates many of the difficulties encountered in making and handling both starch and acrylamide gels. The apparatus has been extensively used in our laboratory for routine electrophoretic analysis of milk proteins in starch gel and has the following advantages over comparable units: (1) Gel solutions are poured directly into the assembled unit while it is in the vertical position and the gel is not exposed or removed until it is ready for staining. (2) No filter paper wicks or salt bridges are used, thus eliminating any changes in the buffer or gel composition due to evaporation or contamination. (3) Samples are conveniently applied through a sample introduction opening in the apparatus. (4) Removal of the gel for staining after completion of electrophoresis is greatly simplified without the danger of tearing or breaking the gels, which are fragile. (5) The need for slicing the gel or covering the samples with liquid petrolatum is eliminated. (6) No sponge support for flexible gels such as acrylamide is needed to support the gel in vertical position. (7) Leakage of the buffer from the upper buffer vessel onto the surface of the vertical gel is eliminated. (I found this leakage to be troublesome when the apparatus reported by RAYMOND[3] was used.) (8) The gel thickness can be easily varied between 3, 5 or 9 mm simply by changing the gel frame in the apparatus.

Fig. 1a shows the various components of the unassembled unit while Fig. 1b presents a profile of the assembled unit.

Assembling the unit

The gel frame, 4, together with the back cooling plate, 6, are held tightly against the front cooling plate, 3, with the aid of two clamps, 7, on both sides of the migration chamber. The gel is formed into the shape, 19, on the gel frame, 4. The dimensions of the gel are 10 × 30 cm. The thickness of the gel may be varied between 3, 5 or 9 mm by using a separate frame for each. Both the front and the back cooling plates are made of 1.5 cm thick block of plexiglass with internal channels, 17, 37, for circulation of cooling water. These channels are formed by milling grooves 0.50 in. wide and 1/8 in. deep on one face and cementing a cover plate over the grooves. Inlet, 28, connects to the main supply of cooling water, while outlet, 29, allows the passage of exhaust water. The borders of the two cooling surfaces on the cooling plates, which come in direct contact with the gel, are lined with a 2 mm thick rubber gasket, 34, to prevent leakage of the liquid gel from the assembled unit. The front cooling plate, 3, has two oblong openings, 32 and 38, at both ends measuring 2.5 × 14 cm. These two openings face the two buffer vessels, 1 and 2, which are cemented at both ends of the front cooling plate, 3. Openings 32 and 38, when exposed, provide direct contact of the gel ends with the buffer in the buffer vessels, thus eliminating the need for filter paper wicks. Before introducing the liquid gel into the apparatus, the two openings, 32 and 38, are plugged tightly with two gel end plugs, 11 and 14, which are lined with rubber at the side facing the gel ends. The two gel end plugs, 11 and 14, are secured tightly in place with the thumb screws, 16, on the two gel end

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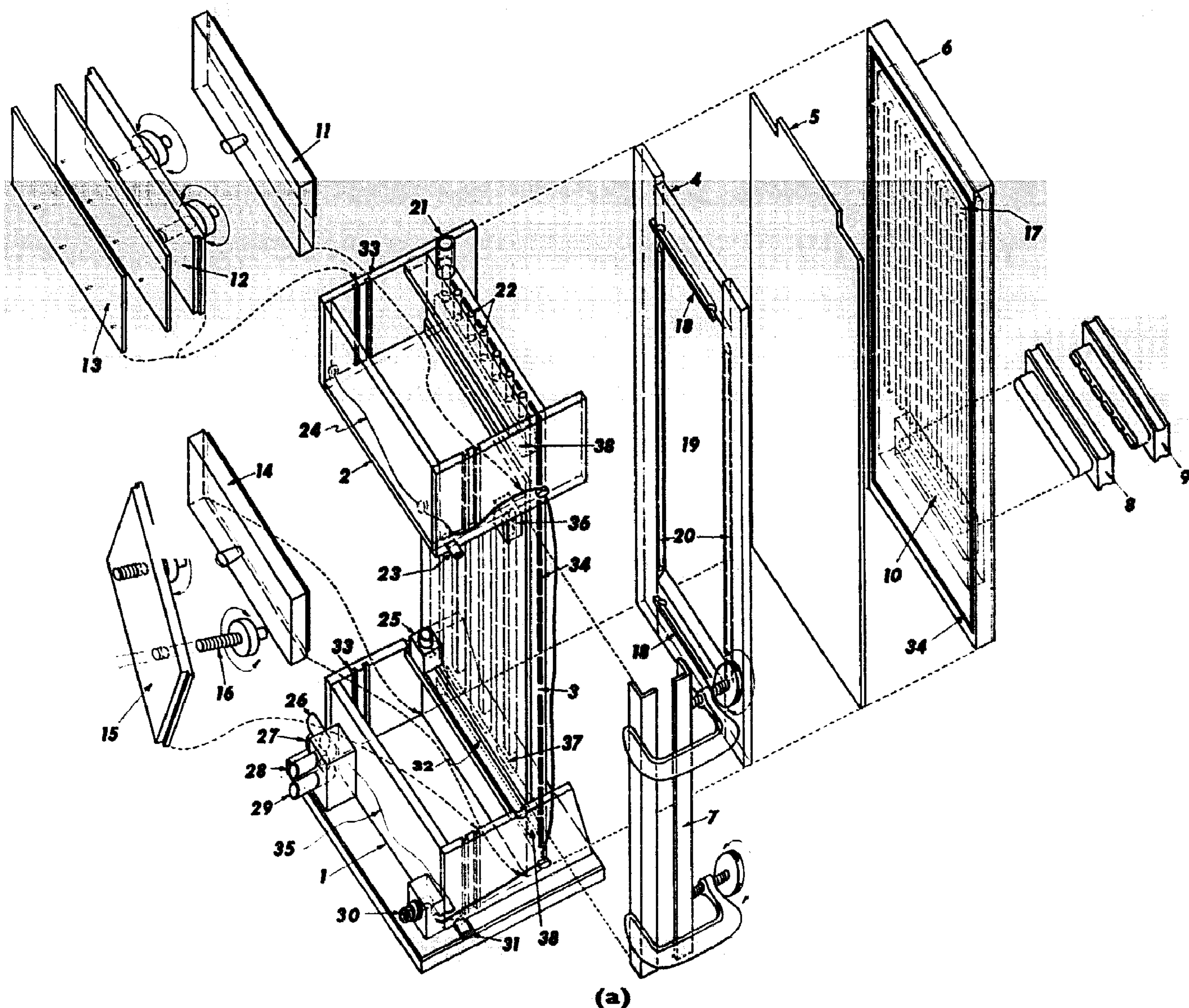
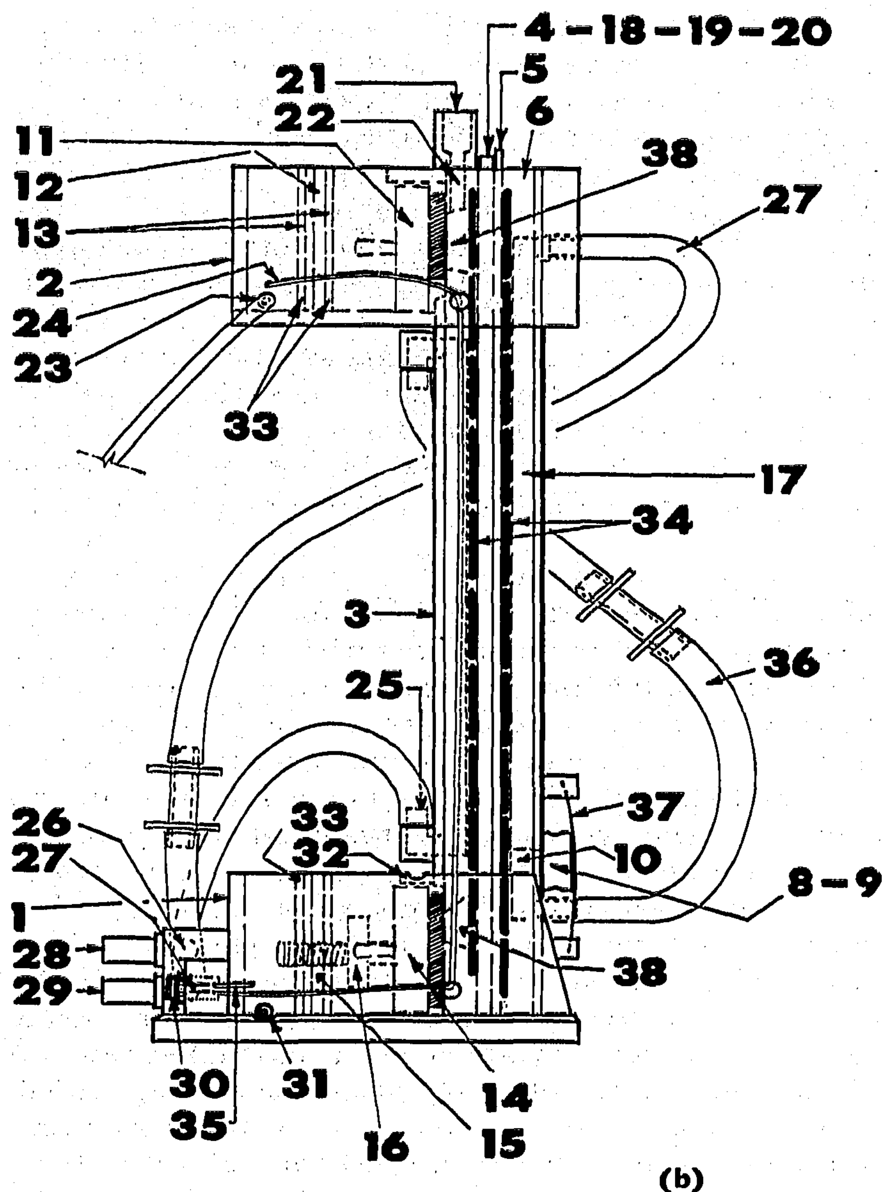


Fig. 1. Diagram of vertical electrophoresis migration chamber: (a) unassembled, (b) assembled unit. Components: 1 and 2 = buffer vessels; 3 = front cooling plate; 4 = gel frame; 5 = gel frame support for gel removal; 6 = back cooling plate with an opening, 10, for sample application; 7 = apparatus clamp; 8 = sample opening plug; 9 = sample slot former; 11 and 14 = plugs for enclosure of openings at both ends of front cooling plate where contact between buffer and gel is made; 12 and 15 = supports for plugs 11 and 14; 13 = buffer vessel partitions; 16 = thumb screws on supports 12 and 15 for holding plugs 11 and 14 tightly in place; 17 and 37 = cooling channels for circulation of water into cooling plates; 18 = gel retainers on gel frame, 4; 19 = space where gel is formed on gel frame; 20 = side channels on gel frame to hold gel in place; 21 = inlet for introducing liquid gel; 22 = air escape vents; 23 and 31 = outlets for emptying buffer vessels; 24 and 35 = platinum electrodes; 25, 26, 27, 28, 29 and 36 = inlets and outlets for cooling water circulation; 30 = electric outlet for connection to the power supply; 32 and 38 = oblong openings at both ends of front cooling plate to expose the gel ends to the buffer; 33 = grooves on sides of buffer vessels to hold either the buffer partitions, 13, or plug supports, 12 and 15, in place; 34 = rubber gaskets on sides of cooling plates.



(b)

plug supports, 12 and 15. The back cooling plate, 6, has an opening for introducing the sample, 10, measuring 12 × 2.5 cm at one of its ends. This opening could be plugged either with the sample opening plug, 8, or with the slot former, 9, before introducing the gel. Plug 8 or slot former 9 are held tightly in place with strong adhesive paper.

When the apparatus is completely assembled the space, 19, on the gel frame, 4, is completely enclosed and safeguarded from leakage. The gel frame, 4, has a bar handle, 18, at each end for holding the gel ends in place. On both sides of the gel frame there are two 0.60 cm deep grooves, 20, to secure the sides of the gel in place on the frame.

Use

Before assembling the unit, the two sides of the cooling plates which come in direct contact with the gel should be covered with a very thin film of liquid petro-

latum. This prevents the surface of the gel block from sticking to the surface of the cooling plates. The back cooling plate, 6, can be assembled with the sample introduction opening, 10, at the upper end of the vertical unit for downward migration of proteins or in the lower most position for upward migration. Before introducing the liquid gel, a funnel is placed into the gel inlet, 21, and the liquid gel is poured directly into the unit while it is in the vertical position. The air displaced by the gel escapes through the vent holes, 22. When using starch gel it is very important to insure that the gel temperature is within the range of 25–30°. If the temperature is higher than that the gel shrinks after setting, allowing condensate to form between the cooling surface and the surface of the gel. With acrylamide gel the gel setting temperature should be $22 \pm 1°$. Drastic changes in temperature during gel setting cause gel shrinkage and localized sweating which are some of the major causes of irregular and defective electrophoretic patterns. Starch gel should be allowed to set for a minimum of 12 h and a maximum of 24 h for optimum performance. Acrylamide gels require 20–30 min for setting.

For sample application the sample opening plug, 8, or the slot former, 9, is carefully removed and the samples are applied either in liquid form in the slots or in the form of dry filter paper inserts previously impregnated with the samples by a technique reported by the author[2]. The sample plug is again secured in place. The gel end plugs, 11 and 14, and their support plates, 12 and 15, are carefully removed to expose the gel ends facing the buffer vessel, 1 and 2. The buffer partitions, 13, are then placed in the grooves, 33, in the buffer vessels and the buffer removal outlets, 23 and 31, are closed by tightening the pinch cocks on the tygon tubing connected to them. The buffer (500 ml) is placed in each of the buffer vessels and the power supply is connected to the migration chamber through the electric socket, 30. The proper potential gradient is applied through the two platinum electrodes, 24 and 35. The electrodes are made of two 5-mil platinum wires stretched across the buffer vessels. The electrophoresis is either conducted in the cold room at 2° or cooling water at $15 \pm 1°$ for starch gel, or $22 \pm 1°$ for acrylamide gel, is circulated through the cooling plates. Fluctuations in the temperature of cooling water should be avoided, otherwise localized sweating of the gel occurs, leading to defective electropherograms.

At the termination of electrophoresis the buffer is emptied through the outlets, 23, 31, on the buffer vessels. The two clamps, 7, are loosened and removed and the back cooling plate is carefully removed. The gel frame with the gel block stretched across it is then carefully removed after supporting the back of the gel with the gel plate support, 5. Staining and destaining of the gel is carried out while the gel is on the frame. After destaining the gel assumes a solid consistency which makes it easy to remove and handle.

Results

Typical electropherograms obtained through the use of the above discussed migration chambers are presented in Fig. 2. In Fig. 2a patterns 1 and 2 represent K-casein prepared and purified by the method of ZITTLE AND CUSTER[4]. Pattern 3 represents a γ-rich casein fraction prepared by a technique previously reported[1]. Pattern 5 represents α_s casein prepared and purified by the method of ZITTLE AND CUSTER[4]. Pattern 6 represents whole casein prepared from pooled milk. Fig. 2b presents five samples of whole casein prepared from the milk of individual cows.

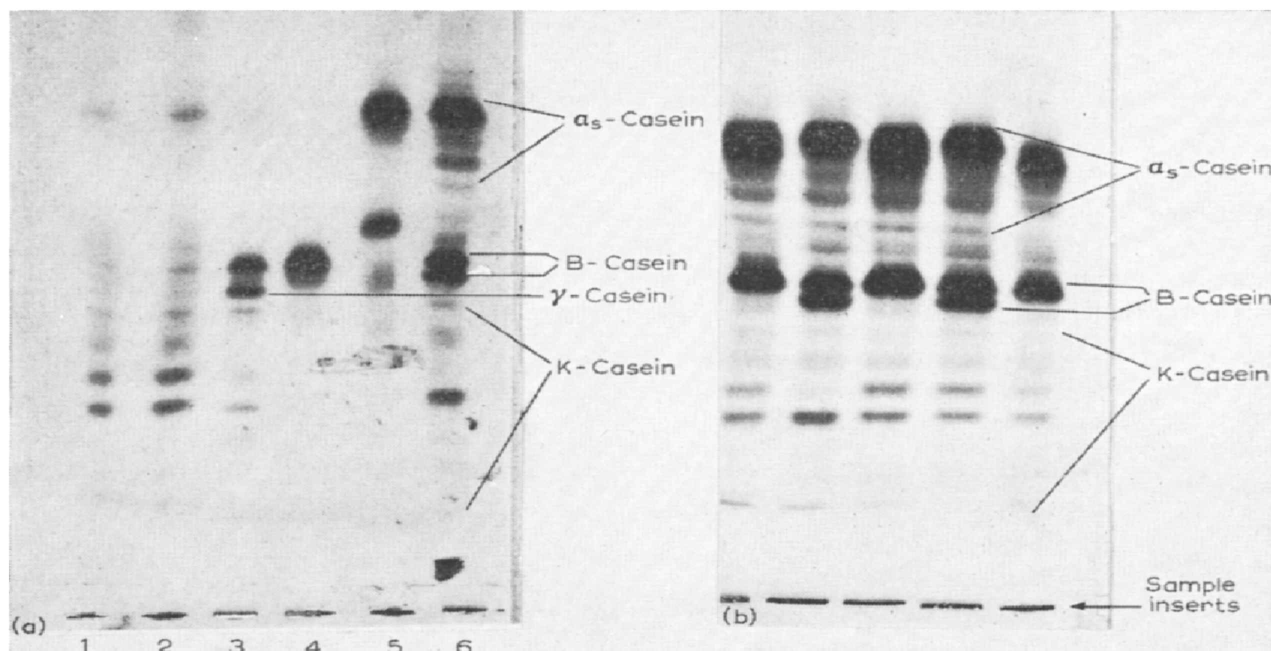


Fig. 2. Starch-gel electropherograms of casein and its components. (a) 1 and 2 = K-casein; 3 = γ-rich casein fraction; 4 = B-casein; 5 = α_s-casein and 6 = whole casein. (b) Five samples of whole casein from individual cows.

The genetic variants of α_s, B-, and K-caseins are clearly demonstrated in these patterns. The starch gel technique used for preparation of these electropherograms has been previously reported by the author[2].

Consistently reproducible starch gel electropherograms have been obtained in the author's laboratory using the above discussed migration chamber.

Agricultural Products Utilization Biochemistry, Department of Animal and Range Sciences and Instrument Service Shop, Montana State University, Bozeman, Mont. (U.S.A.)

A. M. EL-NEGOUMY
HUGH VIA

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The influence of Tween 60 on the microelectrophoretic patterns of human serum on nitrocellulose and acetylcellulose membranes

In a preliminary note[1] on the microelectrophoresis of human serum on nitrocellulose membranes[2] it was shown that a pretreatment of the membranes with polyglycol sorbitol monostearate (Tween 60) was necessary for successful separations