NOTE ON A SIMPLIFIED GEL ELECTROPHORESIS APPARATUS USED IN WHEAT GLUTEN PROTEIN RESEARCH[1]

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Zone electrophoresis on gel supports such as starch, acrylamide, and agar has resulted in optimum separation of component proteins in many systems of diverse origin. In this Note a simplified, inexpensive electrophoresis apparatus is described which facilitates gel preparation, affords direct contact of buffer and gel, and allows higher voltages to be applied, resulting in more rapid mobility of components. Details of the application of the apparatus to the separation of cereal proteins are presented.

A gel electrophoresis apparatus is composed essentially of three parts: a tray or trough which contains the gel medium, buffer chambers containing an electrode system, and a constant voltage source. Most of the apparatus described in the literature employ a current-conducting link between the buffer chamber and the gel. This link may be filter-paper or cloth wicks, sponges, or salt bridges. The apparatus described here eliminates this interconnecting link by using a direct-contact system between the gel and the voltage-buffer supply. Such a system is advantageous because it affords a more constant current condition. Filter-paper wicks, for example, tend to dry out owing to the heat developed during a run. The direct-contact principle has also been used in the elaborate apparatus of Paletta (1) and by Raymond and Nakamichi (2) in their vertical zone electrophoresis apparatus.

Apparatus

The simplified basic apparatus (which does not include the power source and the water-cooled commercial base and cover plates) has three main components: a horizontal tray with connecting buffer wells, spacer blocks, and electrode blocks. Figure 1 is a side-view drawing of the apparatus. All parts are constructed of Plexiglas.[2]

The horizontal gel tray *A* has three sections, a gel bed and two buffer wells, one at each end of the gel bed. A typical tray used in this laboratory is 60 cm. in total length, 7 cm. wide, 0.6 cm. deep along the gel bed, and 3 cm. deep at the buffer wells. Of this total length, 10 cm.

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[2]Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

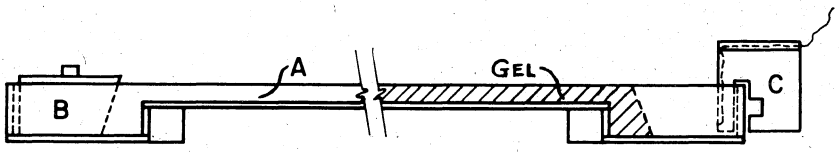

Fig. 1. Side view of gel electrophoresis apparatus. Basic components: A, tray; B, spacer block; C, electrode block. Left half of figure represents apparatus before gel formation; right half shows apparatus ready for use.

is taken up by the two buffer wells. Over-all length was chosen to fit a water-cooled base and cover from an electrophoresis apparatus (EC Apparatus Co. Model EC 451) which was available. Width of the tray and wells may also vary, depending on the available cooling source. Direct contact is made between the tray and the cooled platform during an electrophoresis run. Similarly, the water-cooled cover rests on the gel, covered with Saran.

Two spacer blocks fit closely into the buffer wells, *B*. The blocks, 3 cm. deep, are right trapezoidal prisms having top surface length 4.5 cm. and bottom surface length 3.7 cm. Their width conforms to the width of the tray. The distance between the end of the bed portion of the tray and the 15° face of the block is 0.7 cm. Each block is placed to the rear wall of the buffer well with the sloping face positioned toward the bed of the tray. The fluid gel medium is poured into the tray and open area of the buffer well. After the gel has set, the blocks are carefully removed, leaving a preformed "gel bridge" extending down into the buffer chambers.

The third necessary part of this simplified apparatus is an electrode block, *C*. These blocks (6.2 by 3.2 by 3.1 cm.) are attached to the end of each buffer well by means of a tongue and groove. Each block contains a platinum wire extending across the lower front face. (When in use, the wire must be well covered by buffer.) The wire passes up through the block and is connected by an insulated lead to one of the poles of the power supply. A block of the same size can be used on a wider tray if desired. The polarity of the electrodes can be changed easily by reversing the plug in the power supply socket. Any suitable constant voltage source may be used.

Method and Application

The general procedure used to investigate wheat proteins with this apparatus follows.

The gel is prepared by adding 34.1 g. of hydrolyzed starch[3] to 200 ml. of aluminum lactate-lactic acid buffer (0.008*M*) and bringing the

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slurry to boil over an open flame. After removal from the flame, 30 g. of urea dissolved in buffer to 50-ml. volume is added and swirled into the gelatinized starch (total mixture is 2*M* urea). This procedure is used to prevent possible hydrolysis of urea at elevated temperatures. The hot solution is poured into the tray containing the spacer blocks in the buffer wells. Precoating the tray with a suitable mold release is optional. When the gel has cooled, the spacer blocks are loosened with a razor blade or knife, and are carefully removed. The top gel surface is smoothed by running a wire over the length of the gel and removing the excess. The electrode blocks are then carefully set in place. Aluminum lactate buffer containing urea (2*M*) is placed in the wells. Protein samples dissolved in buffer-urea solution are soaked into filter-paper slips and placed in slits cut into the gel near the positive end. The gel is then covered with Saran film and placed on the water-cooled base (25°). The desired voltage is then introduced.

After the run (usually 2–4 hr., 9–10 v./cm.), the electrode blocks are removed and the buffer is poured from the wells. After the gel is cooled in a refrigerator to make it firmer before cutting, it is removed, sectioned horizontally with a thin wire, and placed in a flat dish. Nigrosin dye (0.5%) is added for 8–10 min. and then washed from the gel with 5% acetic acid solution. The electrophoretic pattern appears within 0.5 hr. Further washing diminishes background color. Clear straight-band patterns are produced.

Wichita wheat gluten protein was resolved into nine components in 4 hr. At least five additional bands were apparent in a 5-hr. run, and numerous other bands can be seen in total wheat protein runs. The electrophoresis results obtained with this apparatus compare very favorably with gel studies appearing in the literature (3). With the use of higher current and potential differences, a 4-hr. run with this equipment will give results similar to those of a 24-hr. run on commercial instruments with paper wicks.

Acknowledgment

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