An agarose-gel zone electrophoresis technique for *in-vivo* protein binding studies of radio-active isotopes

In-vitro studies of protein binding of isotopes[1–4] can be made on micro-amounts of serum. Activity can then be localised by auto-radiography or by use of a simple counting system. Following tracer dosage, however, serum samples have a much lower activity and as a result greater quantities must be separated if the protein bound isotope is to be localised accurately. A simple apparatus using agarose gel was designed to effect this and is described in this note. Briefly, it enabled 3 ml or more of serum to be separated on a single run with a resolution comparable with that of paper. Moreover, a separate apparatus is described which permits simple and efficient recovery of protein from the gel.

Methods and results

Initial experiments were carried out with 2 % w/v agar blocks (Difco-Noble Agar*), 2 cm deep and with a serum load of 3 ml. At a field strength of 20 V/cm the usual effects of overheating were observed. Cooling the gel showed some improvement but increased curvature of the protein bands. At a field strength of 1 V/cm without cooling, adequate separation with a little band curvature was obtained after 16–20 h electrophoresis. Best results were obtained using barbiturate buffer pH 8.6[5]. When the ionic strength of the buffer was uniform throughout the system, some shrinkage of the gel occurred. This was found to be reduced by increasing the strength of buffer in the electrode compartment. The volume used for each electrode compartment was 750 ml of barbiturate buffer (0.075 ionic strength).

This volume was found to be sufficient to prevent the products of electrolysis migrating into the gel. The buffer compartments are constructed so that the buffer level is above the surface of the gel. This ensures full hydration of the gel and effectively prevents desiccation during electrophoresis.

Of the commercial agars tried, agarose "Seravac"** was found to give best results. Little electro-end-osmosis occurred and the translucency of the gel enabled unstained albumin bands to be clearly visible. No precipitation of beta lipoprotein was observed[6].

The apparatus was made from "perspex" and its design is shown in Fig. 1. The bridge compartments are filled with molten agarose (0.75 % w/v in barbiturate buffer, ionic strength 0.05). Before the agarose has set, secondary lint bridges are inserted along the full length of the compartments connecting the agarose bridges to the electrode chambers. When the central compartment is filled with agarose, uniform contact between the central block and bridges is made through slots along the full length of the bridge compartment.

Molten 0.75 % agarose (in barbiturate buffer) is cooled to 45–48°, and poured into the central compartment. The grooved "perspex" tray and slot former are placed in position. Air bubbles are removed by gentle flaming. For a serum load of 3.5 ml, 320 ml of agarose are used for the block and the slot former dimensions are 14 cm × 2 mm × 4 cm. After the agarose has completely set the slot former is removed. Serum, 4 ml at 45° is mixed with 2 ml of molten triple strength agarose (2.25 % in barbiturate buffer, ionic strength 0.1), and incubated for ten minutes

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** Seravac Laboratories, P.T.Y., Ltd., Maidenhead, England.

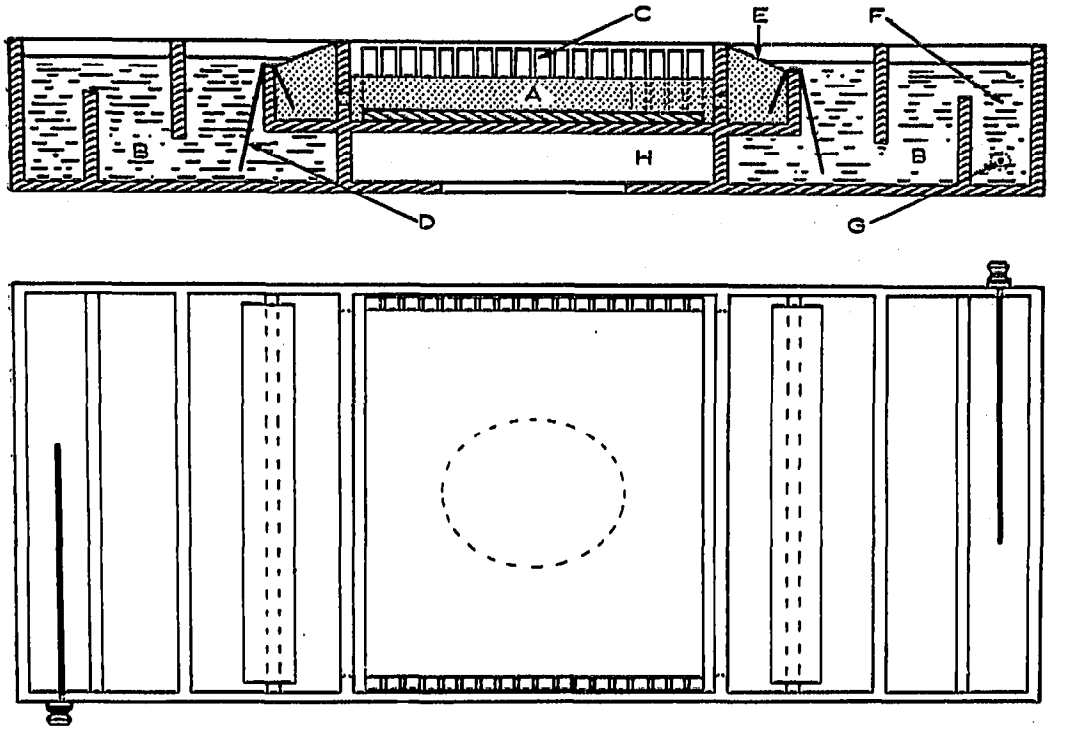


Fig. 1. Plan and cross section of electrophoresis tank. A = agarose; B = buffer; C = grooved agar block tray; D = lint bridges; E = agarose bridges; F = electrode chamber; G = platinum electrode; H = air space.

at 45°. Filling the slot is simplified by applying the mixture from a warm syringe. The buffer compartments are then filled to the level of the agarose bridges and a current of 80 mA applied from a constant current d.c. power supply.

Adequate separation is obtained in 16–20 h. In this time the leading albumin edge will have travelled roughly 7 cm. A run of this length will separate albumin, α_2-, β_1-, β_2- and γ-globulins. The α_1-globulin is usually present as an asymmetry in the trailing edge of the albumin band. When required this fraction can be more clearly separated, either by decreasing the slot width or using "Oxoid"* barbitone acetate buffer with a longer run. Pre-albumin bands are sometimes demonstrated.

After completion of the run the agarose block is removed intact by slicing round the tray, followed by lifting the tray complete with block from the central compartment. The position of the visible bands such as the albumin, start line, etc., is noted in relation to the numbered grooves on the tray. The run is checked by taking a thin section from the block and staining it with ponceau S. The block is then sectioned along the numbered grooves using a ground hack-saw blade.

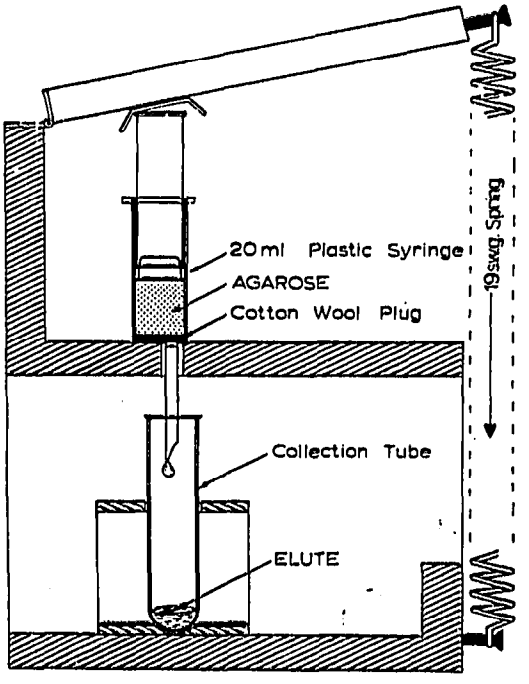
Initially freeze-thawing and centrifugation were used to extract the protein fractions. The yields, however, were poor, and a simple pressure extraction system was devised which gives much better recovery.

The agarose sections are transferred to a 20 ml plastic syringe. The syringe contains a thin, washed cotton wool plug above the nozzle. After insertion of the plunger, the syringe is transferred to the apparatus shown in Fig. 2. Pressure is then exerted upon the syringe plunger by connecting the arm to strong springs which are

* Oxoid Division, Oxo Ltd., London, S.E.1.

secured to the base of the apparatus. The steady pressure causes the extrusion of the protein dissolved in buffer solution. The pressure is maintained for 16-20 h. After 20 h pressure a column of agarose (0.75 % w/v) originally 10 ml in volume is reduced to a thin, tough disc of agarose roughly 1 mm thick. Cellulose acetate electrophoresis of a recovered serum showed uniform loss regardless of molecular size and gave an almost identical scan compared with the same serum directly submitted to electrophoresis. It would seem that selective adsorption does not take place during extraction despite increasing concentration of agarose as extraction nears completion. Further experiments with extracted fractions submitted to electrophoresis showed little change of electrophoretic mobility and no evidence of protein denaturation.

The recovery of added protein from different concentrations of agarose is shown in Fig. 3. The mean recovery for 0.75 % agarose (w/v) is 76 %, the recovered protein being estimated by a Biuret technique (modified), and checked by U.V. absorption at 280 mμ.



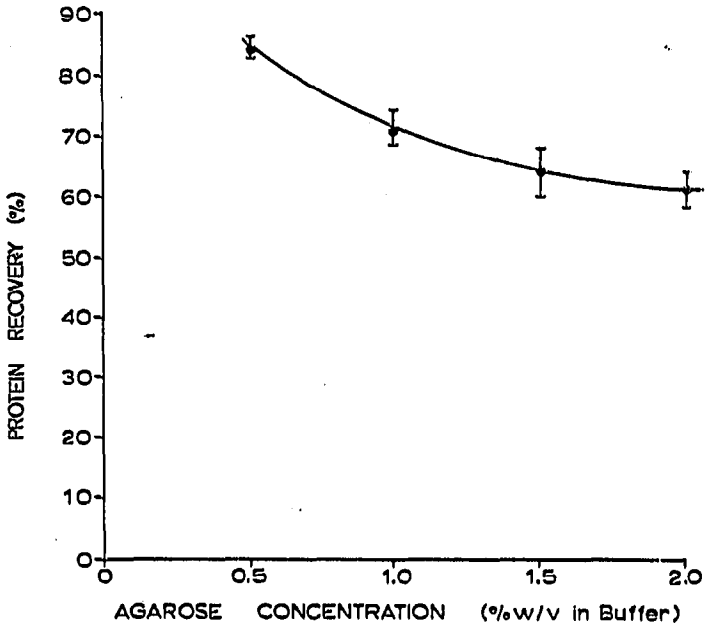


Fig. 2. Pressure extraction apparatus. The above unit is repeated n times according to the number of agar slices to be extracted.

Fig. 3. Effect of increasing agarose concentrations on protein recovery.

Discussion

This note shows that agarose blocks of 2 cm depth or more can be used for electrophoretic separation of serum proteins without cooling. The field strength must be low, *i.e.* about 1 V/cm, and the bridge and buffer compartments designed to ensure full hydration of the block.

The separation and resolution of such a system (see Fig. 4) does not compare with more sophisticated micro-electrophoresis techniques[7], but is adequate for preparative work or projects involving detection of small quantities of protein bound isotope. In particular, the system has proved successful in the study of *in vivo* binding of iodine-131 metabolites following administration of tracer dosage ^{131}I. Results

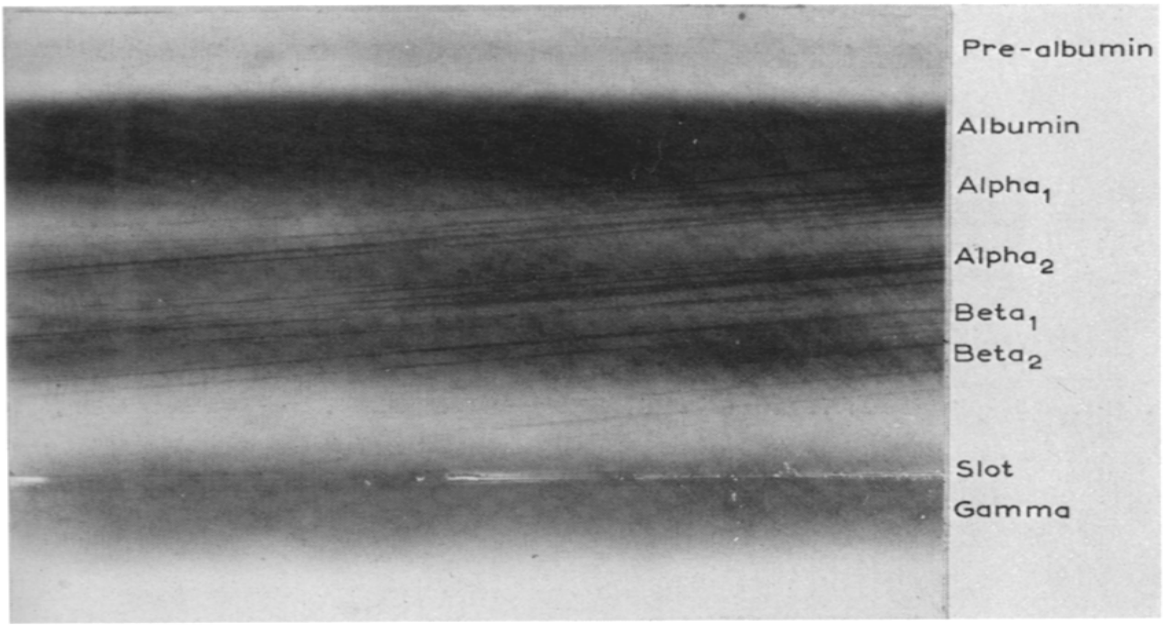


Fig. 4. Agarose blocks (11.5 cm × 15 cm × 1.5 cm) with a 2 ml serum load after electrophoresis for 16 h at a field strength of 0.9 V/cm. β-Lipoprotein is present as an unstained band between the β_1- and β_2-globulin. It should be noted that photography of such thick blocks is difficult.

obtained on hyperthyroid patients were similar to those reported[8-9] where micro-techniques were used, the level of activity in such samples being relatively high, *i.e.* as much as 250 mμC per ml of serum. Separation of 3 ml of serum containing 0.02 mμC per ml, showed binding in the pre-albumin, albumin and the inter-α_1, α_2 fraction (or T.B.G. fraction).

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