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A METHOD FOR THE RAPID ELUTION OF PROTEINS FROM "MASHED GEL" AFTER STARCH GEL ELECTROPHORESIS

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SUMMARY

An improved method for the elution of serum proteins from starch gel sections through secondary electric fields after electrophoresis is described. By using gel sections that had been mashed to an appropriate extent in the second run, almost complete recovery of the proteins was obtained rapidly, except for γ-globulin. The effect of "mashed gel" on the recovery of albumin was investigated by varying the degree of mashing.

INTRODUCTION

Various methods have been described for the recovery of proteins from starch gel after electrophoresis[1]. Of these methods, a procedure involving a second stage of electrophoresis applied to gel sections that have been cut out after the primary electrophoresis is the most efficient, and is used generally. Although many devices have been made for this procedure[2–8], the yield has not been satisfactory.

The method described in this paper concerns the elution of serum proteins from starch gel sections by means of a second electrophoresis, and has been devised in order to obtain higher recoveries of proteins in a short time. The distinctive feature of the technique is the use of gels that have been mashed to an appropriate extent in the second run instead of the gel sections that are usually employed[3–8], starch gel cut into pieces of 4–5 mm along one side[2], or completely disintegrated gel produced with a homogenizer[9]. The apparatus used was devised for the application of "mashed starch gel" and is simple and easy to operate. Although this apparatus resembles that of Sulitzeanu and Goldman[9] for the elution of substances from polyacrylamide gel, it differs mainly in the use of an arrangement for maintaining the elution temperature constant, in the use of a glass filter instead of filter-paper layers and in the use of agarose gel instead of Sephadex.

EXPERIMENTAL

Apparatus

The apparatus described consists of two parts and is shown in Fig. 1. Part 1 is

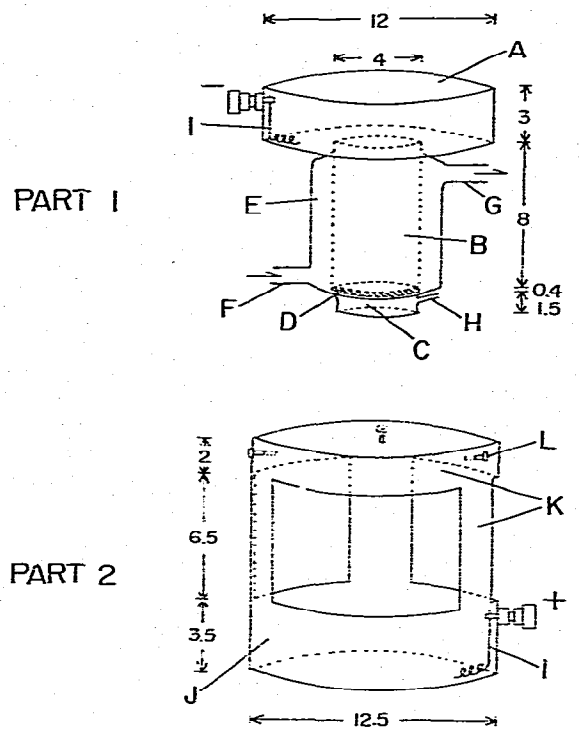

Fig. 1. Apparatus for electrophoretic elution of proteins from "mashed gel". Dimensions are given in centimetres. A, Upper electrode vessel; B, gel column; C, dialysis column; D, glass filter disc; E, jacket for circulating cold water; F, cooling-water inlet; G, cooling-water outlet; H, side-arm; I, platinum electrode; J, lower electrode vessel; K, frame equipped with bolts (L) for supporting upper electrode vessel. Dialysis membrane under dialysis column is not shown.

made of hard glass and is composed of the gel column (B), which is continuous with a hole at the centre of the bottom of the upper electrode vessel (A) containing a platinum electrode, and also with a dialysis column (C). A glass filter disc* (D) divides the gel column from the dialysis column. The gel column is surrounded by a jacket (E) for circulating cold water. A side-arm (H) projects from the side of the dialysis column. Part 2 is made of Plexiglass and is composed of the lower electrode vessel (J), equipped with a platinum electrode and a frame (K) for supporting the upper electrode vessel of part 1.

Preparation of "mashed gel"

The device for mashing starch gel (Fig. 2) consists of a stainless-steel cylinder (A) with a threaded end, a stainless-steel rod (B), a brass net cut out in a circle (C) and a brass cap (D). The net is held in the inside of the cap and the cylinder is screwed into the cap until the net is fixed. Then gel sections containing a substance to be eluted are introduced into the cylinder and the "mashed gel" is prepared by pushing out with the rod through the net.

* G3 glass filter disc (medium type), pore size 20–30 μm (Sibata Chemical Apparatus Mfg. Co. Ltd., Tokyo, Japan).

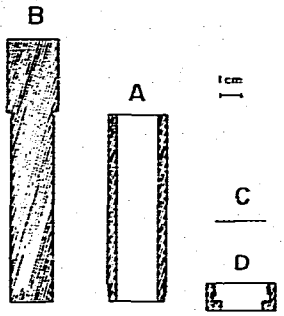

Fig. 2. Device for mashing starch gel. A, Cylinder for introducing gel sections; B, rod for pushing out gel sections; C, net cut out in a circle; D, cap of cylinder.

Electrophoretic elution

To the "mashed gel" is added one third of its volume of buffer solution in order to suspend the gel. Under the dialysis column is stretched the dialysis membrane (Visking cellulose membrane), which is fixed by a rubber band. Buffer solution is poured through the side-arm until the dialysis column is full and then the side-arm is stoppered. Next, the "mashed gel" suspension is placed on the glass filter disc of the gel column, taking care that no air bubbles remain, and is covered with a disc of filter paper. A 1 % agarose (Behringwerke agarose) solution, prepared by dissolving agarose in heated buffer solution is cooled to 40°, poured so as to form a layer 3–5 mm thick and left to induce gelation. To the upper and lower electrode vessels are added 120 and 200 ml of buffer solution, respectively, and the apparatus is assembled by placing part 1 on part 2 (see Fig. 1). In doing so, the bottom surface of the dialysis membrane is arranged so as to be slightly immersed in the buffer of the lower electrode vessel. The electrophoretic elution is conducted with circulation of cooling water in the jacket. On completion, the solution containing the eluted substance in the dialysis column can easily be sucked out through the side-arm by using a hypodermic syringe equipped with polyethylene tubing.

RESULTS AND DISCUSSION

Example

After conducting starch gel (14.8 ‰) electrophoresis of human serum (5.1 ml) with Smithies' continuous buffer system[10], five gel sections corresponding to A–E shown in Fig. 3 were cut from the non-stained main gel. For each fraction, 20 ml of gel were collected and each of the gels was subjected to separate electrophoretic elution. The "mashed gel" was prepared with a net of eighty 1-in. meshes. For the suspension of "mashed gel", the gel buffer (0.03 *M* H_3BO_3 – 0.012 *M* NaOH, pH 8.78) used for the primary electrophoresis was employed. For solutions in the electrode vessels and others, the bridge solution (0.3 *M* H_3BO_3 + 0.06 *M* NaOH, pH 8.05) used for the initial run was employed. The elution was carried out at a current of 125 mA (105–110 V) for 20–35 min at room temperature; the current density was 10 mA/cm², with the anode in the lower and the cathode in the upper electrode vessel. By circulating tap water (15°) at the rate of 3 l/min through the jacket, the temperature of the "mashed gel" suspension was maintained below 26°. Recoveries of the proteins were

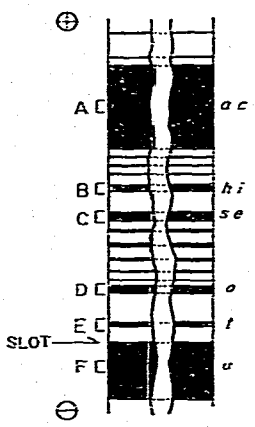

Fig. 3. Pattern obtained by starch gel electrophoresis of human serum. Small letters represent zones: a, Albumin; c, α_1-antitrypsin; h, haptoglobin; i, ceruloplasmin; s, transferrin; e, α_1-glycoprotein + 4.6S-postalbumin; o, macroglobulin; t, β-lipoprotein; u, γ-globulin.

measured by the micro-Kjeldahl method, with the sum of the nitrogen contents in the "mashed gel" suspension after elution and in the eluate being taken as 100. The "mashed gel" suspension and eluate are contaminated with trace amounts of nitrogen which may include protein, originating from the gel. These amounts were determined previously in a blank experiment, and were deducted from the apparent values. The blank test consisted of electrophoresis of a starch gel slab to which nothing had been added, electrophoretic elution of the "mashed gel" prepared from appropriate sections, and determination of nitrogen contents in the "mashed gel" suspension after the elution and in the eluate obtained.

The recovery of the serum proteins is shown in Table I (A–E). When the elution was conducted for only 35 min, ac-, hi- and se-components (for explanation of zones, see caption to Fig. 3) were recovered almost completely, each from the respective "mashed gel". At this time, the recoveries of o- and t-components are 86% and

TABLE I

PROPORTIONS OF HUMAN SERUM PROTEINS RECOVERED FROM "MASHED STARCH GEL"

For conditions of elution, see text. Figures in parentheses are proportions with the cathode in the lower electrode vessel.

Section of gel	*Zone*	*Nitrogen in eluate (%, w/w) after*					
		20 min	*35 min*	*40 min*	*80 min*	*120 min*	*200 min*
A	ac	84	100				
B	hi	68	98				
C	se	63	98				
D	o	37	86				
E	t	34	81				
F	u			22 (16)	28 (9)	37 (7)	47

81%, respectively. The sections cut off from the main gel should preferably be rapidly eluted. Gel sections left for longer than necessary showed a slight decrease in recovery. Soluble starch existing in the eluates can be removed according to need by the usual methods[1,11,12].

Effect of mashing the gel

The effect of mashing the gel on recovery was investigated. The mesh size of the net used for the production of "mashed gel" was changed from 8 to 270 inch meshes, and the elution of ac-components from 20 ml of gel sections was conducted for 25 min under the same electrical conditions as above. With the net of 80 or 100 inch meshes, as seen in Fig. 4, the recovery reached a maximum and was 100%; with smaller or larger meshes, the recovery was lower.

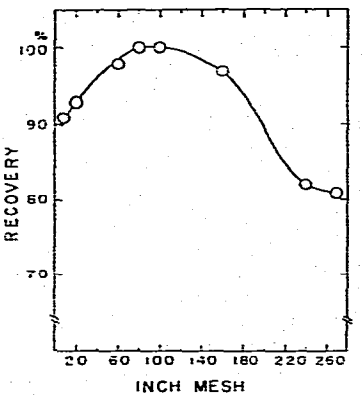

Fig. 4. Influence of mesh size (number of mesh/inch) for preparing "mashed gel" on the recovery of ac-components. Elution was conducted for 25 min; for other conditions, see text.

In the present method, complete disintegration of gel sections with a homogenizer is unfavourable. Gel homogenized to a paste state was prepared after adding the gel buffer to 20 ml of gel sections at the same rate as above, and the elution of ac-components was carried out under the same current density at 105–110 V for 25 min. The recovery was 81%. The same recovery of ac-components was obtained with the "mashed gel" prepared with the net of 270 inch meshes (see Fig. 4). In both instances a marked electroendosmotic flow was noted during the elution, which resulted in a considerable decrease in the amount of buffer in the dialysis column. The decrease in recovery with net sizes of greater than 160 inch meshes is due mainly to the increase in both electroendosmotic flow and adsorption to the gel of the components.

Elution of γ-globulins

The u-component corresponding to γ-globulin was eluted from the gel section F shown in Fig. 3 by the same procedure as described under *Example*. Further, the elution of this component was also carried out with the cathode in the lower and the anode in the upper electrode vessel, as it was thought that during the elution some of the component would migrate to the cathode side by the electroendosmotic flow. The recovery in each instance was measured by the micro-Kjeldahl method, with the sum

of nitrogen in the "mashed gel" before elution being taken as 100. Trace amounts of nitrogen contained in the "mashed gel" and eluate, originating from the gel, were determined previously by a blank test, and were deducted from the apparent values. Good recovery of γ-globulin could not be obtained, as shown in Table I (F), *i.e.*, when the anode was the lower electrode vessel, only a 47% yield was obtained after elution for 200 min, and with the cathode in the lower electrode vessel, the recovery became poorer and decreased inversely with increase in the elution time to more than 80 min. In the latter case, by prolonging the elution time the "mashed gel" layer in the gel column moved upwards a little with the passage of time, and there was a marked increase in the amount of buffer in the dialysis column due to electroendosmotic flow.

As Gordon[3] had demonstrated the superiority of 0.02 *M* phosphate buffer at pH 8.3 in the second electrophoresis of starch gel sections, the elution of the serum proteins was attempted with this buffer for 20 min (current density 10 mA/cm², 110 V). The result was slightly inferior to those shown in Table I, (A–E).

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