Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

The Recovery of Serum Proteins by Elution Convection After Gel Electrophoresis

J. Broome[a]; Esther M. Jordan[a]
[a] Current, Inc., Pennsylvania

To cite this Article Broome, J. and Jordan, Esther M.(1966) 'The Recovery of Serum Proteins by Elution Convection After Gel Electrophoresis', Separation Science and Technology, 1: 2, 319 — 328
To link to this Article: DOI: 10.1080/01496396608049449
URL: http://dx.doi.org/10.1080/01496396608049449

SEPARATION SCIENCE, 1(2 & 3), 319–328 (1966)

The Recovery of Serum Proteins by Elution Convection After Gel Electrophoresis

J. BROOME and ESTHER M. JORDAN
CURRENT, INC., PHILADELPHIA, PENNSYLVANIA

Summary

The recovery of serum proteins by elution convection has been determined. Depending on the precise conditions, the recoveries ranged from 40% to nearly quantitative. The resolution was considered sufficient to justify this technique as a useful single-step fractionation procedure.

With the advent of modern gel electrophoretic techniques (*1–3*), the analysis of complex protein mixtures is no longer a formidable exercise. The concept of employing similar high-resolution systems for preparative work is clearly most attractive. Previous methods have relied upon either column electrophoresis (*4–6*) or the elution of material from selected portions of the gel matrix after its physical disruption or by electrophoretic means (*7,8*).

The recovery technique here employed eliminates many of the difficulties associated with the above-mentioned systems (*9,10*). It has the additional advantage that the equipment is commercially available. Hence it was considered of interest to examine its potential as a single-step fractionation method when applied to normal human serum. No attempt was made to optimize conditions for the separation of a specific component; instead, quite general techniques were employed.

MATERIALS AND METHODS

Chemicals were of reagent grade, except for Cyanogum 41, which was the "analytical grade for electrophoresis" supplied by the E-C Apparatus Corporation. *Serum* was obtained from healthy volun-

teers and centrifuged after 60 minutes. It was used the same day it was taken. *Analytical electrophoresis* was conducted essentially as described by Peacock et al. (*11*).

Preparative Electrophoresis

An EC480* cell containing a 5% Cyanogum 41 gel was employed throughout. The sample was applied either as a fluid in four 23 × 4 mm slots at the upper face of the gel, or photopolymerized above the whole width of the gel. To construct a suitable gel for the latter method, the following procedure was found optional. The cell was assembled and supported at about 45° on the front edge of the outer cooling plate. One hundred milliliters of a 10% solution of Cyanogum 41 in the chosen buffer (containing 0.1 ml tetramethylethylenediamine and 0.1 g ammonium persulfate to catalyze polymerization) was poured into the tank. When this had gelled to form a plug, the tank was set vertically to receive the gel proper (5% Cyanogum 41 in the same buffer containing the same proportions of catalysts) which was added to within 3 cm of the top of the rear cooling plate. This was immediately overlaid with a 2 to 3 mm layer of water so that a level gel surface would result. After polymerization, the top of the gel was drained and rinsed with a little of the sample gel solution (1 part serum, 1 part buffer, 3 parts water containing 5% Cyanogum 41, 5% sucrose, 0.1% TMED, and 5 to 10 ppm of riboflavin). A fresh quantity of the latter solution was introduced to give about 20% more than the required sample volume (to allow for oxygen inhibition), which was again overlaid with water. Without disturbing the tank, the sample-containing layer was then irradiated with a fluorescent lamp some 8 cm distant until gelled. Throughout the above manipulations it is important not to feed coolant to the tank.

Two buffer formulations were used for preparative electrophoresis: first, Peacock's tris-EDTA-borate pH 8.4 (*11*), and second, a glycine-borate buffer (prepared by bringing a solution 0.06 *M* with respect to glycine and 0.02 *M* to sodium hydroxide to pH 8.8 with boric acid). For either, electrophoresis was carried out at 300 V without a pre-run. The higher pH buffer necessitated reducing the time by about 15% for an equivalent distance of migration.

After electrophoresis a guide strip was excised, stained in amido

* E-C Apparatus Corp., Philadelphia, Pa.

black 10B, and cleared either for a densitometric trace or to provide a visual check on the run.

Elution

An EC730* elution cell fitted with convection ports was employed. It was initially operated in accordance with the manufacturer's instructions supplemented with published observations on the technique (*10*). At first, Peacock's buffer was used, but this was later replaced by a pH 9.3, $I = 0.02$, sodium glycinate buffer. It was found to be important to ensure that convection paths, other than those desired, were minimized. In practical terms, the buffer level in the tank was set no higher than the top of the gel; the gel slab was carefully trimmed so that it fitted the horizontal dimension of the grid exactly and provided an effective seal to the top of the tubules; similarly, the dialysis sleeve had to be perfectly free of creases or other irregularities that might impair contact with the sides of the convection channels.

For some runs additional cooling was provided by inserting a coil (3 turns, 12-cm diameter) of stainless-steel tubing (thin-walled 9-mm OD) between the buffer pump and the buffer inlet connection on the electrode. This coil was immersed in a bath maintained at about 5° (it is probable that a temperature in the region of 12° is optimal). When this coil was employed, the power input was raised to 12 to 15 W.

Protein Estimation

The biuret method of Gornall et al. (*12*) was used. The concentration of all the components was increased by a factor of $2\frac{1}{2}$, so that equal volumes of sample and biuret reagent (instead of 1 part sample and 4 parts reagent) were mixed, and after 30 minutes the extinction was read at 540 nm. Human serum albumin was employed as the standard.

Concentration of Protein Solutions

Eluted material was concentrated fourfold for analytical electrophoresis with Curtain's (*13*) polyacrylamide pellets. In our hands they were more conveniently prepared by gelling the acrylamide

* E-C Apparatus Corp., Philadelphia, Pa.

solution in the analytical cell instead of in glass tubes. After cutting the slab into strips $3 \times 3 \times 100$ mm, they were exhaustively washed, dried by exposure to the atmosphere for 48 hours, and finally desiccated in a current of warm dry air.

Thermal Measurements on the Elution Cell

Thermal measurements were made by cutting a slot in a gel slab to fit the bulb and stem of an accurate thermometer. This was inserted in a grid chamber the top of which was drilled to accept the thermometer. Equilibrium temperatures of both the gel (T_g) and the cooling bath (T_b) at various power levels (P) were determined. The results were expressed as a thermal resistance, $\theta = (T_g - T_b)/P$, in degrees per watt. No great precision is claimed for this technique. Also, it was not applicable to the estimation of the thermal resistance of the tubules. However, it enabled the area for empirical assessment to be defined.

RESULTS AND DISCUSSION

Since gel electrophoresis was employed as the initial step in a fractionation procedure, it was clearly desirable to employ as large a sample as possible. As the loading was increased, using the standard technique, the resolution was notably impaired compared with analytical runs. This was found to be a consequence of two distinct effects. First, very high molecular weight material and colloidal aggregates were retained at the floor of the sample slot, preventing the rapid ingress of the soluble proteins to the gel during electrophoresis. This gave rise to a tail extending from the sample slot to the final position of the band. The second effect was frank overloading, leading to a general broadening of the bands.

These phenomena occurred at different loading levels. Albumin tailed visibly when 150 μl of serum per square centimeter was applied. However, the systems investigated would tolerate up to 200 μl of serum per square centimeter before gross overloading became apparent. Both effects could be minimized by polymerizing the sample in an acrylamide gel about 1 cm thick above the whole surface of main gel. In this way approximately 1.5 ml of serum (200 μl/cm^2) could be run without too great a sacrifice in resolution. Under these conditions the colloidal material was dispersed throughout a centimeter of gel instead of congregating at the sur-

face. Although this necessitated a comparatively wide sample application line, the effective width could be reduced to approach that of an elution tubule by using a diluted buffer in this sample gel (*14*). Unfortunately, some of the soluble proteins were retained in the sample gel—an effect that became more noticeable as the sample

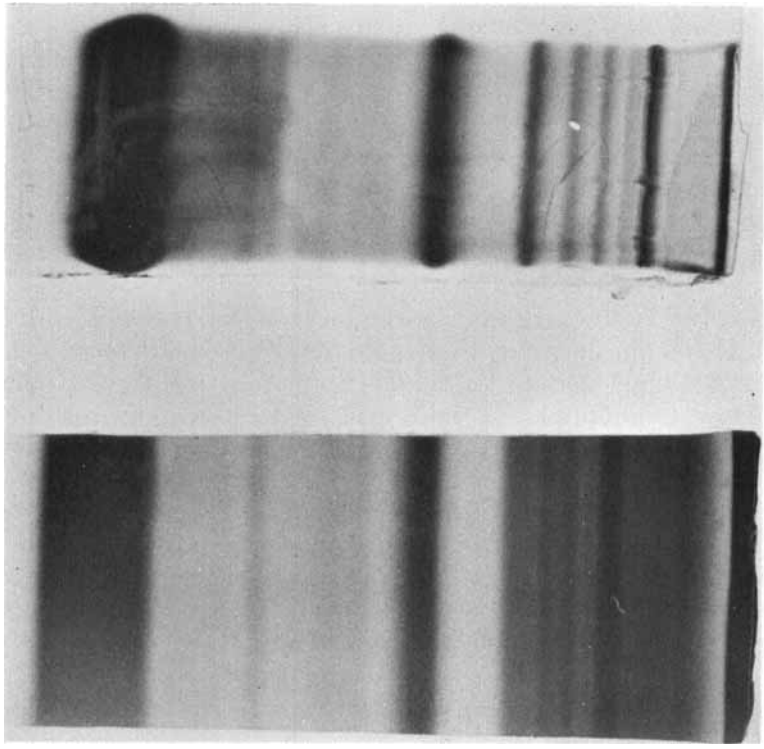

FIG. 1. Preparative gels after staining (upper) serum sample loaded in a conventional slot at 150 μl/cm^2 and run in Peacock's buffer. The albumin tail is clearly apparent. Lower: Serum at 200 μl/cm^2 photopolymerized in situ before electrophoresis (glycine-borate buffer), showing a substantial quantity of protein retained at the origin. The direction of migration (anodic) is toward the left in both examples.

load increased. Presumably this indicates that the reagents used to initiate polymerization react with protein (*15*), and must, at least in part, account for the lower recoveries using this technique. Nonetheless, adequate resolution was achieved by this technique, as illustrated in Fig. 1.

The buffers employed were largely governed by considerations of the method of protein determination rather than resolution. Tris-based buffers yield amine complexes with divalent copper and so interfere with the biuret reaction. This could be allowed for by subtracting a buffer-blank value, but a more sound approach was to employ a buffer system that was inert toward the biuret reagent. Hence later runs were conducted in a glycine buffer, both for separation and elution. It should be noted that if a gel contains a low-molecular-weight component, this is not evenly distributed throughout the buffer volume on elution, and substantial quantities can be found in the tubules, as though it were nondialyzable. When Peacock's buffer was used for separation and glycine for elution, the tris from the former gave high biuret values, and a base line, corresponding to the protein-free fractions, had to be inserted to enable the true recovery to be assessed.

Clearly a prerequisite for maximum recovery is that no soluble protein remain on the gel after elution. This was determined by staining the gel slab. Since the electrophoretic mobility of a given protein is markedly lower in the gel compared with its free mobility, the time required for complete elution will be governed largely by the rate of movement of the slowest protein component through the gel. Since in a given apparatus the distance between the electrodes is fixed and electrophoretic velocity is proportional to the applied voltage gradient (all other factors being constant), it is convenient to employ volt-hours as the unit for expressing the movement of a given band. Hence it is valid simply to multiply the po-

TABLE 1

Total Recoveries by Elution Convection of Serum Proteins Following Gel Electrophoresis under a Variety of Different Operating Conditions

Total protein, mg	Elution buffer	Elution, volt-hr	Cooling	Sample	Recovery, %
21	Tris, pH 8.4	300	Tank	Liquid	101
36	Glycine pH 9.2	120	Tank	Liquid	68[a]
36	Glycine pH 9.2	230	Tank	Liquid	96
36	Glycine pH 9.2	240	Tank	Liquid	68
72	Glycine pH 9.2	315	Tank	Gel	48
72	Glycine pH 9.2	410	Tank and buffer	Gel	37[a]
72	Glycine pH 9.2	450	Tank and buffer	Gel	41[a]

[a] Material still detectable on gel slab after elution.

tential difference between the electrodes by the duration of the run. This value is given in the second column of Table 1.

The resolution attainable with any separation based on an electrophoretic mechanism is limited ultimately by random diffusion. It follows that the performance of the equipment is dependent on its capacity to dissipate ohmic heat per unit temperature rise of the material undergoing separation (*10*). Therefore, to assess the proper input power to the elution cell, its thermal resistance was roughly determined. As supplied by the manufacturer, it yielded a value of 1°/watt; i.e., under the recommended electrical conditions, the gel temperature would rise approximately 6 to 8° above that of the coolant. When the additional cooling coil was inserted in the buffer circuit, the thermal resistance fell to 0.3 to 0.4°/watt. In theory it should be possible to increase the input power by a factor of $2\frac{1}{2}$ with a consequent increase in efficiency and reduction in time. However, two circumstances complicated this analysis.

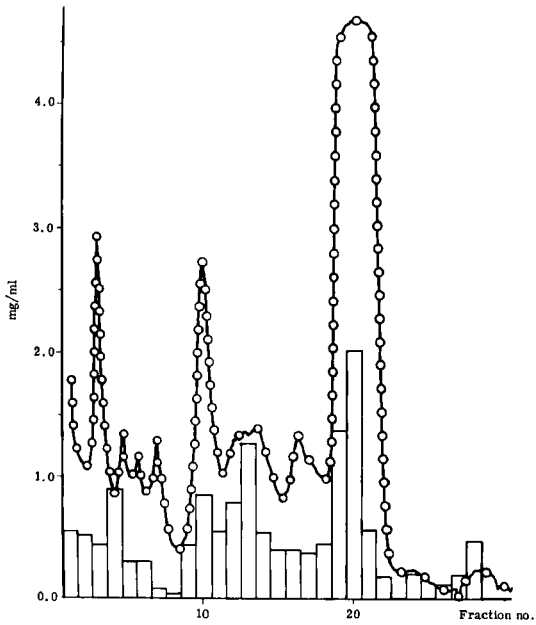

FIG. 2. Comparison of an elution diagram (columns) with the densitometric trace (0-0-0) of the gel from which it was derived. The gel was run (slot loading) and eluted in Peacock's buffer. Protein concentrations of the fractions were determined with the biuret reagent and the gel was stained prior to densitometry.

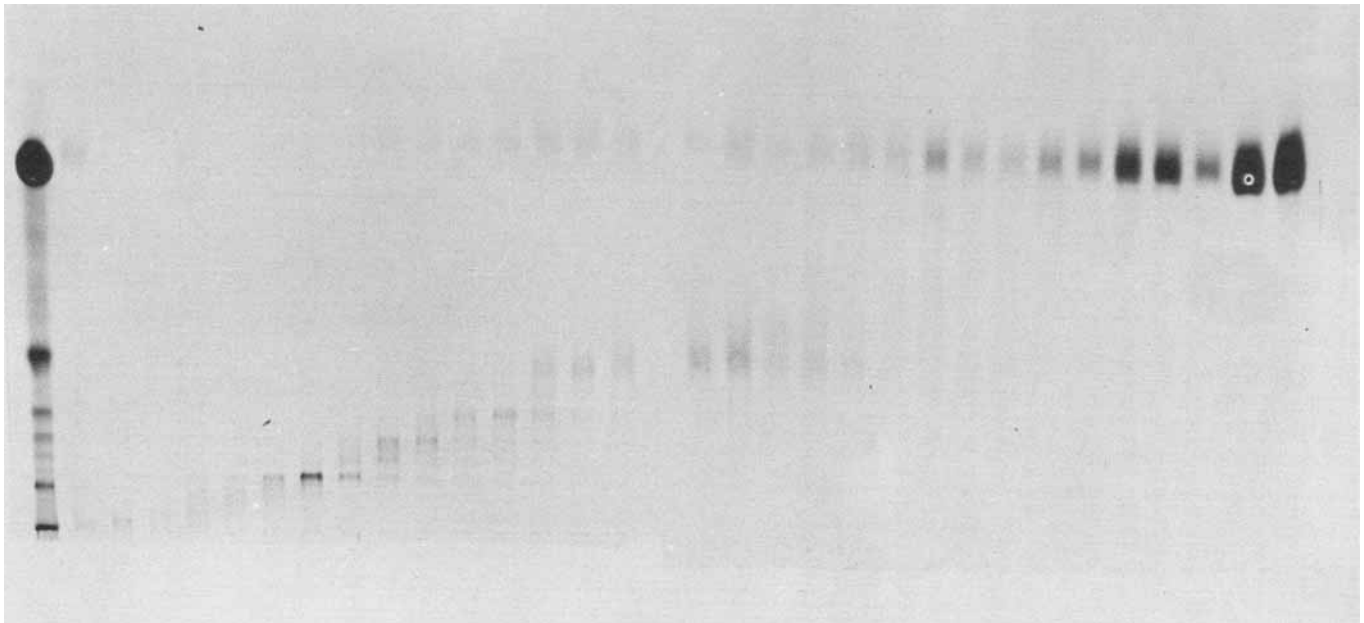

FIG. 3. The distribution of serum components among fractions after recovery, as demonstrated by gel electrophoresis. The original serum sample is shown on the left. The content of the individual tubules appears in sequence, starting with the least-mobile fractions on the left. The preparative run (and elution) was conducted in borate-glycine buffer using the photopolymerized gel technique. Peacock's buffer was employed for the final analytical separation.

First, the effect of temperature gradients in the tubules has been neglected. If a vigorous thermal current is set up in this part of the apparatus, it can interfere drastically with the concentration process. Since the tubules lack the stabilizing influence of the gel slab, the temperature of the latter is not the factor limiting power input. Second, the temperature of the coolant has a much greater effect on protein mobility when tightly coupled with the cell by the use of the metal heat exchanger. As shown in Table 1, this effect was responsible for the poor recovery of slow-moving components when a coolant temperature of 5° was used, even though the volt-hr figure was nearly doubled. Hence a moderate coolant temperature (10 to 12°), together with a modest increase in power (12 to 18 watts), was considered optimal, the lower coolant temperature being reserved for thermosensitive compounds.

An indication of the resolution obtainable is given in Fig. 2, where the biuret values of each fraction can be compared with a densitometric trace of the original gel. The major peaks are clearly apparent with little diffusion evident. A further test of resolution consisted of submitting each fraction to analytical electrophoresis after concentration. The result of such a run is shown in Fig. 3. When allowance is made for the width of the tubule (3 mm) in relation to the length of gel run (100 mm), the separation must be considered acceptable. This illustration also shows the tailing effect of high loading on albumin.

In conclusion, this recovery technique is useful as a single-step procedure applicable to unprocessed human serum containing proteins in the 10-mg range. Undoubtedly, future developments can be expected to increase its area of application.

Acknowledgments

We should like to express our thanks to Dr. S. Raymond for stimulating discussion and sound advice. The skilled technical assistance of Miss Elaine Hislop and Mr. Harold Sherman is also gratefully acknowledged.

REFERENCES

1. O. Smithies, *Biochem. J.*, **71**, 585 (1959).
2. S. Raymond, *Clin. Chem.*, **8**, 455 (1962).
3. L. Ornstein, *Ann. N.Y. Acad. Sci.*, **121**, 321 (1964).

4. A. M. Altschul, W. J. Evans, W. B. Carney, E. J. McCourtney, and H. D. Brown, *Life Sci.*, **3**, 611 (1964).
5. P. H. Duesberg and R. R. Rueckert, *Anal. Biochem.*, **11**, 342 (1965).
6. T. Jovin, A. Chrambach, and M. A. Naughton, *Anal. Biochem.*, **9**, 351 (1964).
7. J. Moretti, G. Boussier, and M. Jayle, *Bull. Soc. Chim. Biol.*, **40**, 59 (1958).
8. A. H. Gordon, *Biochim. Biophys. Acta*, **42**, 23 (1960).
9. S. Raymond, *Science*, **146**, 406 (1964).
10. S. Raymond and E. M. Jordan, *J. Separation Sci.*, **1**, 95 (1966).
11. A. C. Peacock, S. L. Bunting, and K. G. Queen, *Science*, **147**, 1451 (1965).
12. A. G. Gornall, C. S. Bardawill, and M. M. David, *J. Biol. Chem.*, **177**, 751 (1949).
13. C. C. Curtain, *Nature*, **203**, 1380 (1964).
14. H. Hoaglund and A. Tiselius, *Acta Chem. Scand.*, **4**, 957 (1950).
15. P. Bernfeld and J. Wan, *Science*, **142**, 678 (1963).

Received by editor March 31, 1966
Submitted for publication May 12, 1966