Enzyme Purification by Electrodecantation

By B. G. WINCHESTER, M. CAFFREY AND D. ROBINSON

Department of Biochemistry, Queen Elizabeth College, Campden Hill Road, London W.8, U.K.

(Received 5 August 1970)

1. Electrodecantation has been applied to the initial fractionation from crude material of pig kidney β -D-glucosidase and sheep testicular *N*-acetylglucosaminidase. 2. The isoelectric points determined by isoelectric focusing were pH 4.9 for the β -D-glucosidase and pH 6.3 for the *N*-acetylglucosaminidase. 3. Electrodecantation of pig kidney extract and sheep testicular extract was carried out at pH 4.9 and 6.3 respectively. 4. A six- to ten-fold increase in specific activity could be obtained with good recoveries after a single cycle of electrodecantation. 5. The technique has also been used to purify further an extracellular *Bacillus subtilis* protease preparation. 6. Attempts to use electrodecantation for the concentration of very dilute enzyme solutions resulted in considerable loss of activity. 7. The limitations and potential use of the technique in laboratory-scale enzyme preparation are discussed.

Isolation procedures for enzymes are facilitated by an initial fractionation of the crude tissue extract or extracellular fluid, which is the source of the enzymic activity. Precipitation by ammonium sulphate or by organic solvents such as acetone or ethanol is widely employed for this purpose. A more selective initial fractionation can be achieved by exploiting a specific physical property of the protein, electrophoretic immobility at its isoelectric point. If a mixture of polyelectrolytes at a buffered pH is subjected to electrodialysis between two semipermeable membranes, then charged molecules will migrate towards one or other of the electrodes until a membrane is encountered. The concentrated layer, which forms adjacent to the membranes, will be of a higher density than the rest of the solution and will sink or be decanted to the bottom of the membrane compartment. However, molecules that are isoelectric at this pH will not migrate and this isoelectric fraction will tend to be displaced upwards. In this manner it is possible to separate the isoelectric from the charged polyelectrolytes. Thus an initial fractionation can be effected if the isoelectric point of the enzyme which it is desired to purify is known.

Polson & Largier (1960) have discussed the principles of this technique and have described a multimembrane technique. Its use for the separation of serum proteins has been reported (Fleetwood & Milne, 1967).

The present paper is concerned with partial purification of some enzymes by multimembrane electrodecantation in an apparatus of this type.

MATERIALS AND METHODS

Enzyme assays. β -D-Glucosidase (β -D-glucoside glucosylhydrolase EC 3.2.1.21) was determined fluorimetrically (Price & Robinson, 1966) by using a Locarte LFM/5 fluorimeter. The corresponding fluorimetric assay for *N*-acetylglucosaminidase (β -2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucosylhydrolase, EC 3.2.1.30) was also used (Leaback & Walker, 1961). One unit of activity was defined as that amount necessary to catalyse the production of 1 μ mol of 4-methylumbelliferone/min under the conditions of assay. The turbidimetric method due to Di Ferrante (1956) was employed to determine hyaluronidase activity. Proteolytic activity was measured by the Anson (1937) procedure by using ox haemoglobin (Koch-Light Ltd., Colnbrook, Bucks., U.K.) from which low-molecular-weight material had been removed by prolonged dialysis against water. One unit of activity was defined as that amount of enzyme necessary to catalyse the production of an E_{280} reading of 1 after 1 h in the soluble fraction after adding 3 ml of trichloroacetic acid to a reaction mixture containing 0.25 ml of haemoglobin, 0.9 ml of 0.1 M-sodium phosphate buffer, pH 7.0, and 0.1 ml of sample. A Unicam SP.500 spectrophotometer was used for this assay and other spectrophotometric measurements. Protein was measured by the Folin method (Lowry, Rosebrough, Farr, & Randall, 1951) as modified for use with the Technicon AutoAnalyzer.

Determination of isoelectric points. Isoelectric points of enzyme components in starting materials were determined by isoelectric focusing on an Ampholine type 8100 column (LKB Instruments Ltd., London S.E.20, U.K.) as described by Vesterberg & Svensson (1966). pH gradients were established by using the appropriate ranges of synthetic ampholytes, Ampholines (LKB

Instruments Ltd.). Electrofocusing took place for at least 48 h at 300 V and 5°C. Fractions (2.4 ml) were collected by using a BTL Chromapump and Chromafac (Baird and Tatlock Ltd.) with a rate of pumping of 60 ml/h. The column effluent was monitored continuously for E_{280} with a Uvicord II absorptiometer (LKB Instruments Ltd.) and the pH and enzyme activity of each fraction were measured. All pH measurements were made at 5°C with a Pye model 290 pH-meter and Pye-Ingold spear combined electrode, type 401-M5.

Gel electrophoresis. Starch gels were prepared in 5 mm-sodium phosphate buffer, pH 7.0, by the method of Smithies (1955) by using partially hydrolysed starch (Connaught Medical Laboratories, Toronto, Canada). Electrophoresis was carried out for 4 h at 100 V and 20–25 mA, with a buffer concentration of 40 mM in the electrode compartments. Glycosidase activity was detected on the gel surface with the appropriate fluorogenic substrate (Robinson, Price & Dance, 1967). The gel and buffer systems of Ornstein & Davis (1964a,b) were employed to examine glycosidase fractions by polyacrylamide-gel electrophoresis. Protease fractions were analysed by polyacrylamide-gel electrophoresis with the gel and buffer systems described by Reisfeld, Lewis & Williams (1962). Protein bands were detected by staining with Amido Black 12B (G. T. Gurr Ltd., Romford, Essex, U.K.).

Enzyme sources. 1. β -D-Glucosidase. Pig kidneys were obtained immediately after slaughter (T. Wall and Sons Ltd., Willesden, London N.W.10, U.K.) and kept frozen at –15°C until required. A small sample was taken from each kidney and was analysed by starch-gel electrophoresis at pH 7.0 to determine whether it contained one or two glucosidase components (Abrahams & Robinson, 1969). Kidneys containing a single β -D-glucosidase component isoelectric at pH 4.9 were used. The isoelectric point was determined by isoelectric focusing. Typically, 1028 g of kidneys was homogenized in 2 litres of 50 mM-sodium acetate buffer, pH 4.9, at 5°C. After standing for 2 h at 5°C the homogenate was centrifuged for 20 min at 6000 rev./min in the 6 × 250 ml angle head of the MSE High-Speed 17 refrigerated centrifuge (5°C). The supernatant was filtered through muslin and centrifuged again under the same conditions. After diluting the final supernatant (1.7 litres) to 2 litres with the acetate buffer, the pH was adjusted to 4.9 by the addition of 50 mM-acetic acid. This material was fed directly into the decanter. It had a specific β -D-glucosidase activity of 10.77 m-units/mg of protein and contained 4.8 mg of protein/ml. A partially purified kidney β -D-glucosidase prepared by removing inactive protein by agglutination at pH 5.0 and freeze-drying (Abrahams & Robinson, 1969) was also used as a starting material. In this case an 8% (w/v) solution of the material was dialysed against several changes of the appropriate isoelectric buffer at 4°C for 12–18 h. The non-diffusible material was applied directly to the decanter after adjustment of the pH. It had a specific β -D-glucosidase activity of 9 m-units/mg of protein.

2. *N*-Acetyl- β -glucosaminidase. Frozen sheep testes were obtained from Gland Supplies Ltd., Manchester, U.K. A 100% (w/v) homogenate of this tissue was prepared in 30 mM-sodium phosphate buffer, pH 6.3, at 5°C, with an MSE Atomix homogenizer. After standing for 20 h at 5°C the homogenate was passed through muslin

and then centrifuged for 20 min at 7000 rev./min in the 6 × 250 ml angle head of the MSE High-Speed 17 refrigerated centrifuge. The supernatant was diluted to 2 litres with the above-named phosphate buffer and its pH readjusted to 6.3. This material, which had a specific activity of 2.2 m-units/mg and contained 20 mg of protein/ml was introduced directly into the decanter.

In one experiment, material that had been partially purified by ammonium sulphate fractionation by the procedure of Dorfman (1955) was used.

3. *Bacillus subtilis* proteases. Superase, a mixture of bacterial extracellular proteases derived from *B. subtilis*, was a gift from Pfizer Ltd., Sandwich, Kent, U.K. A 1% (w/v) solution was dialysed against 50 mM-sodium borate buffer, pH 8.5, at 5°C, until the conductivity of the diffusate was the same as that of the buffer. The pH of the non-diffusible material was readjusted to 8.5 before introduction into the decanter. It contained 0.93 mg of protein/ml and had a protease activity of 14.1 units/mg.

Multimembrane electrodecanter. The multimembrane electrodecanter (Quickfit and Quartz, Stone, Staffs., U.K.) comprises an electrodecanter cell unit, a buffer-circulating system, a glycol heat-exchanger and refrigeration unit, a power pack and a control unit. The electrodecanter cell unit (Fig. 1a) consists of three separation cells, arranged alternately with four cooling compartments and has an electrode compartment at each end. All these compartments are separated by a semi-permeable membrane of Visking cellulose sheet. Cooled (5°C) buffer at the pH of the isoelectric point of the protein to be purified is circulated through the cooling and electrode compartments. It is re-mixed before re-entering the glycol heat-exchanger. This circulating buffer provides cooling during electrodialysis and also removes and mixes electrolysis products, which might alter the pH in the separation compartments. Convection currents are minimized by inclining the electrodes towards each other at the top, thereby creating a temperature gradient from the bottom to the top of the cell.

Each separation cell (Fig. 1b) has three functional ports and contains a pack of 22 cellulose membranes each 0.8 mm apart. The capacity of each cell is 600 ml. A variable-speed peristaltic pump is used to pump off isoelectric material from the top port of the last separation cell when they are used in series or from the top of each cell when they are used in parallel. Decanted material can be removed from the port in the base of the separation cells by using a similar pump of lower speed range. The sample is thus drawn into a separation cell through the sample port at a rate equal to the combined rate at which isoelectric and decanted material are removed. The speed of these pumps can be altered during the run. Decanted and isoelectric fractions are collected separately in vessels standing in ice-water.

Mode of operation. The electrodecanter can be used either in a continuous flow or in a static batchwise manner. In the continuous-flow method the three separation cells, which are separated by cooling sections, are connected in series (Fig. 1a). They are filled with the appropriate buffer and the pump system is also primed with buffer. After the power (usually 300 V and 2.5 A) is switched on the cell temperature is brought to 5°C by adjustment of the temperature of the glycol in the heat-exchanger. The sample is then drawn into the bottom of separation

Fig. 1. (a) Diagrammatic representation of electrodecantation cell unit; (b) cross-section of individual separation cell.

cell 1 by pumping off buffer from the top of cell 3. A pumping rate of 400 ml/h was commonly used for drawing off this isoelectric material, which was collected as discrete fractions. Simultaneously material is pumped out of the decanting port at the base of cell 1 at a slower rate (100–200 ml/h). This decanted fraction is returned to and mixed with the starting material until all the sample has been introduced into the decanter. This stage is reached when the volume of the isoelectric fraction equals the original volume of the starting material. Buffer is now drawn into the sample port of separation cell 1 and the decanted material from cell 1 is collected as discrete fractions. This process is continued until the E_{280} or the enzymic activity of the isoelectric effluent or both reach zero. The pumps are then switched off and electrodecantation is allowed to continue in a static manner, usually overnight. After the cells have been isolated from one another the contents of each cell are collected separately in several fractions, by draining through the decanting ports. The volume, pH, protein content and enzymic activity of the cell, isoelectric and decanted fractions are then determined. If necessary it is possible to collect isoelectric and decanted fractions from any of the other cells concurrently. In certain experiments only one or two cells in series were employed.

In static batchwise operation the separation cells are isolated from one another and the buffer in the cells is replaced completely by the buffered sample. After electrodecantation for 4–24 h at 5°C and 300 V and 2.5 A,

the cells are emptied either by draining from the decanting port or by upward displacement with 10% (w/v) sucrose through the isoelectric port. The distribution of the enzymic activity and protein among the fractions obtained is then determined.

The following buffer systems were employed: (a) 50 mM-succinic acid adjusted to pH 4.9 with 50 mM-NaOH; (b) 50 mM-acetic acid adjusted to pH 4.9 with 50 mM-sodium acetate; (c) 200 mM-boric acid adjusted to pH 8.5 with 50 mM-sodium metaborate; (d) 30 mM-sodium phosphate buffer, pH 6.3, 6.5, 6.9 and 7.0. Unless otherwise stated all reagents were of AnalaR grade.

RESULTS AND DISCUSSION

β -D-Glucosidase. Choice of buffer is an important factor for successful operation. Initial experiments indicated that marked pH changes occurred in the solutions when electrodecantation was carried out in succinate buffers. An increase of 0.4 pH unit was observed in 50 mM-sodium succinate buffer after 5 h at 300 V and 2 A at 5°C. Sodium acetate-acetic acid offers an alternative buffer system for this pH range. Partially purified pig kidney β -D-glucosidase was dialysed against 50 mM-sodium acetate buffer, pH 4.9, and processed in the electrodecantation in this buffer. Cells 1 and 2 were used in series and isoelectric material was collected as discrete fractions from

Table 1. *Distribution of β -D-glucosidase activity after electrodecantation of pig kidney extract in sodium acetate buffer, pH 4.9*

A solution (1250 ml) of pig kidney extract, specific β -D-glucosidase activity 0.0104 unit/mg, was processed at 400 ml/h at 300 V and 2.5 A. Total recoveries of enzymic activity and protein were 59 and 55% respectively.

Fraction	Volume (ml)	Activity recovered (%)	Specific activity (unit/mg)
Isoelectric	4280	75.2	0.0115–0.0349
Cell 1 Bottom	200	4.01	0.0023
Middle	200	4.69	0.0023
Top	200	5.77	0.0019
Cell 2 Bottom	200	2.96	0.0037
Middle	200	2.18	0.0027
Top	200	5.20	0.0036

the top of cell 2. Table 1 gives the results of this experiment. Under these conditions 75% of the recovered activity was found in the isoelectric fraction with a maximum threefold purification. In Fig. 2 the activity, protein content and pH of the isoelectric fractions are recorded as a function of the volume collected. The labile nature of pig kidney β -D-glucosidase in dilute protein solution could account for the relatively low recoveries in these experiments.

The same conditions were applied to the purification of β -D-glucosidase from a 50% (w/v) homogenate of pig kidneys that contained a single component isoelectric at pH 4.9. This homogenate was processed continuously in 50 mM-sodium acetate buffer, pH 4.9, with removal of decanted material from the bottom of cell 1 at 200 ml/h during the run. Collection of decanted material was started as soon as signs of precipitation appeared; failure to do this resulted in the appearance of most of the β -D-glucosidase in the decanted fraction. The first 600 ml of isoelectric material consisted of displaced buffer containing negligible activity. The following material, which was collected as one large fraction, contained 70% of the original activity. It had a specific activity 11-fold that of the starting material (Table 2).

If an isoelectric fraction obtained by electrodecantation at one pH is subjected to electrodecantation at a different pH then proteins in this fraction should be decanted to the bottom of the cell to form a concentrated solution. An attempt was made to concentrate the pig kidney fraction isoelectric at pH 4.9 by continuous electrodecantation at pH 7.0 in 30 mM-sodium phosphate buffer by using two cells in series. The β -D-glucosidase was efficiently removed from the isoelectric fraction but there was no effective concentration in the decanted material because of the considerable (58%) loss of activity that occurred in the process. Fig. 3 illustrates the concentration of protein and β -D-glucosidase in cell 1 at the end of the run.

Fig. 2. Analysis of isoelectric fraction obtained by electrodecantation of pig kidney extract in sodium acetate buffer, pH 4.9. Two separation cells were used in series and isoelectric material was collected at 400 ml/h. □, β -D-Glucosidase concentration (m-units/ml); ■, protein (μg/ml);, pH.

The efficiency of the electrodecanter as a concentrator was clearly demonstrated with a saturated solution of ox haemoglobin (isoelectric at approx. pH 6.9) at pH 4.9, by using the electrodecanter in a continuous flow with three cells in series. Only 1% of the haemoglobin was not decanted and was carried over into the isoelectric fraction. Removal of decanted material during the run from one, two or three cells did not significantly alter this proportion.

N-Acetyl- β -glucosaminidase. The testis is a relatively rich source of glycosidases and a highly purified preparation of hyaluronidase (EC 3.2.1.35) has been obtained from bovine testes (Borders & Raftery, 1968). In the course of a similar preparation from sheep testes an examination by isoelectric focusing of a water-soluble extract revealed that there are several enzymic components with *N*-acetyl- β -glucosaminidase activity in this tissue (Fig. 4a). The two major components have isoelectric points of 6.0 and 6.3 at 5°C.

The extract prepared as described in the Materials

Table 2. *Distribution of β -D-glucosidase activity after electrodecantation at pH 4.9*

Pig kidney extract (1100 ml), specific β -D-glucosidase activity 0.00696 unit/mg, was processed at 300 V and 2.5 A. Isoelectric and decanted material were collected at 400 and 200 ml/h respectively. Total recoveries of enzymic activity and protein were 103 and 77% respectively.

Fraction	Volume (ml)	Specific activity (unit/mg)	Activity recovered (%)
Isoelectric	945	0.07745	69.5
Decanted	720	0.000692–0.01133	21.0
Cell 1 Bottom	200	0.00203	9.0
Middle	200	0.00229	
Top	140	0.00234	
Cell 2 Bottom	200	0.000103	1.0
Middle	200	0.000799	
Top	195	0.000915

Fig. 3. Distribution of protein and β -D-glucosidase in the separation cell after concentration of pig kidney fraction isoelectric at pH 4.9 by further electrodecantation at pH 7.0. $\square$, β -D-Glucosidase activity (m-units/ml); $\blacksquare$, protein (mg/ml).

Fig. 4. Isoelectric focusing of sheep testicular material over pH range 5–8. (a) Crude extract, approx. 10 mg of protein; (b) fraction isoelectric at pH 6.3, 4.2 ml applied to the column. $\square$, *N*-Acetyl- β -glucosaminidase activity; $\circ$, pH; —, E_{280} .

and Methods section was processed in the electrodecanter at 5°C in 30 mM-sodium phosphate buffer, pH 6.3. Three electrodecanter separation cells in series and the continuous mode of operation were employed. Ten isoelectric fractions were collected from the top of cell 3 and decanted material was collected from the bottom of cells 1 and 2 until there was no deposit at the bottom of these cells. At the end of the run the distribution of *N*-acetyl- β -glucosaminidase activity among the isoelectric, decanted and cell fractions was investigated (Table 3). Under these conditions there was no loss of enzymic activity but 50% of the protein was not recovered in solution. Considerable precipitation was observed on the cell membranes and walls and this precipitate was not all removed upon draining the cells. All the isoelectric material had an appreciably

higher specific activity than the starting material, the purification factor ranging from 5.8 to 10.25. Material of a comparable increased specific activity was also obtained from the top of cell 3, which suggested that if the run had been extended further *N*-acetyl- β -glucosaminidase could have been collected in the isoelectric fraction. It would, however, have been very dilute. During the run, the pH of the isoelectric fraction decreased to pH 6.2 and that of the decanted material to between pH 6.0 and 6.2.

To concentrate the testicular extract isoelectric at pH 6.3, its pH was adjusted to pH 4.9 and it was

Table 3. *Distribution of N-acetyl- β -glucosaminidase activity after electrodecantation of sheep testicular extract at pH 6.3*

Of a soluble extract of sheep testes, specific *N*-acetyl- β -glucosaminidase activity 0.0022 units/mg of protein, 21 was processed at 200 V and 3 A. Isoelectric material was collected from cell 3 at 400 ml/h and decanted material from cell 1 at 100 ml/h. Total recoveries of enzymic activity and protein were 107 and 49.5% respectively.

Fraction	Activity (units)	Volume (ml)	Activity recovered (%)	Specific-activity range (unit/mg)
Isoelectric	59.01	6455	62.6	0.0124–0.0225
Decanted	23.12	750	24.5	0.0009–0.0060
Cell 1	1.365	600	1.45	0.0008–0.0048
Cell 2	4.126	600	4.26	0.0045–0.0111
Cell 3	6.593	600	7.0	0.0064–0.0224

Table 4. *Concentrations of sheep testicular extract isoelectric at pH 6.3 by further electrodecantation at pH 4.9*

Material (600 ml) isoelectric at pH 6.3 was subjected to static electrodecantation at 200 V and 3 A for 18 h in 50 mM-sodium acetate buffer, pH 4.9. Total recoveries of enzymic activity and protein were 56 and 30% respectively.

Cell contents	Volume (ml)	Protein (mg/ml)	Specific activity		Purification relative to original homogenate
			(unit/mg)	(unit/ml)	
Bottom	50	0.45	0.0216	0.00971	9.8
	50	0.50	0.0266	0.01329	12.1
	50	0.46	0.0424	0.01952	19.3
	50	0.48	0.038	0.01828	17.3
	200	0.33	0.0216	0.00715	9.8
Top	250	0.28	0.0120	0.00335	5.45
Starting material	600	0.885	0.0124	0.01789	5.64

subjected to static electrodecantation in 50 mM-sodium acetate buffer, pH 4.9. Although considerable precipitation and loss of activity occurred, it was possible to achieve an overall purification of up to 20-fold for some fractions (Table 4).

To investigate the resolution of the electrodecantation the heterogeneity of the pH 6.3 isoelectric fraction was investigated by isoelectric focusing and polyacrylamide-gel electrophoresis. The higher resolving power of isoelectric focusing (Fig. 4b) showed that this isoelectric fraction contained several protein components, which are isoelectric within a range of approx. 1 pH unit. pH changes during the electrodecantation run would contribute to a broadening of the protein band removed. Analysis of the starting material, decanted material and isoelectric fraction by polyacrylamide-gel electrophoresis confirmed that the isoelectric fraction was not homogeneous (Fig. 5). However, there are considerably less protein components in the isoelectric material than in the decanted and starting material.

The resolution of the electrodecantation was investigated further by processing partially purified testicular material (Dorfman, 1955) at pH 6.5, the

reported isoelectric point of bovine testicular hyaluronidase (Malmgren, 1953). A single cell was used for these experiments and decanted material was not generally collected during the run. Electrodecantation at 0.2 pH unit away from the isoelectric point of the *N*-acetyl- β -glucosaminidase decreased the proportion of this enzyme recovered in the isoelectric fraction from 67 to 43%. In other experiments at pH 6.5, the proportion fell to 37%. In contrast 52.5% of the recovered hyaluronidase activity was found in the isoelectric fraction. Although the purification factor for hyaluronidase in this particular experiment was only 1.6, in the other runs three- to four-fold increases in the specific activity of hyaluronidase were obtained in single step processes.

B. subtilis proteases. Superase, a crude preparation of extracellular proteases derived from *B. subtilis*, was shown by electrofocusing over the pH range 3–10 to contain at least five protein components with proteolytic activity (Fig. 6a). As the major protease peak (III) appeared to be heterogeneous at this resolution, a similar sample was electrofocused over the pH range 5–10 (Fig. 6b). This resolved the main protease component into

two components (III and IV) isoelectric at pH 8.15 and 8.5 respectively. Neither of these components is inhibited by 1 mM-EDTA or inactivated at 60°C. In this respect they resemble the bacterial alkaline proteases, which have a serine residue in the active site. In contrast peak VI is partially inhibited by 1 mM-EDTA (approx. 55.5%) and partially inactivated at 60°C (approx. 74%). Peaks I and II were also slightly thermolabile.

To separate peaks III and IV from the other protease components, a 1% (w/v) solution of Superase was subjected to electrodecantation at pH 8.5, the isoelectric point of component IV. Boric acid (20 mM) adjusted to pH 8.5 with 50 mM-

sodium metaborate was used in the electrodecanter. Table 5 summarizes the results of this experiment. The protease activity of the isoelectric fraction was uninhibited by 1 mM-EDTA. Analysis of the isoelectric fraction by polyacrylamide-gel electrophoresis showed that it contained a single band of protein components (Fig. 5).

Electrodecantation offers a selective process of separation of high-molecular-weight ampholytes based on their isoelectric points. These can now be measured rapidly and accurately by isoelectric focusing. The method can be applied to the purification of minor enzymic components, e.g. the

Fig. 5. Polyacrylamide-gel electrophoresis of processed material. Sheep testis: (a) crude extract, i.e. starting material (the pattern for decanted material was indistinguishable from this); (b) fraction isoelectric at pH 6.3 (concentrated by electrodecantation). Bacterial proteases: (c) starting material; (d) material decanted at pH 8.5; (e) fraction isoelectric at pH 8.5.

Fig. 6. Isoelectric focusing of Superase; (a) pH range 3–10, with 6 ml of a 1% (w/v) solution applied to the column; (b) pH range 5–10, with 3 ml of a 5% (w/v) solution applied to the column. Both solutions were dialysed against 1% (w/v) glycine before analysis. □, Protease activity; ○, pH; —, E_{280} .

Table 5. Distribution of protease activity after electrodecantation of Superase at pH 8.5

A 1% (w/v) solution (1110 ml) of Superase, specific protease activity 14.1 units/mg, was processed at 200 V and 3 A. Isoelectric and decanted material was collected at 400 and 100 ml/h respectively.

Fraction	Volume (ml)	Activity recovered (%)	Specific activity (units/mg of protein)
Isoelectric	2805	61	21.4–49.7
Decanted	535	18	5.9–11.3
Cell 1	580	4.5	6.1–23.0
Cell 2	600	8.8	2.9–23.7
Cell 3	600	7.25	4.7–57.0

isolation of the β -D-glycosidases described here, or to the further removal of contaminants from a partially purified enzyme, e.g. as seen in the further purification of Superase. Significant increases in specific activity with good total recoveries are possible but in general concentration of isoelectric material by further electrodecantation is disappointing. There is danger of loss of enzyme activity when in dilute solution and some method of stabilization is required. In this respect the addition to the collection vessels of reversibly binding ligands such as substrate or competitive inhibitor which can be removed subsequently by electrodialysis might be useful. Alternatively an inert protein could be used to stabilize the isoelectric fraction. If concentration by electrodecantation was carried out subsequently at the isoelectric point of the inert protein, enzyme concentration and the removal of stabilizing protein might be achieved in the one operation. Careful choice of buffer is necessary both from the point of economy, since approx. 25–30 litres are used in each run, and because of the pH change that may occur with some systems during passage through the electrodecanter.

Quantities of 0.5–1 litre of 2% (w/v) crude enzyme protein/h may be processed and the only limitation on continuous operation in this manner is that after some time the membranes become blocked with precipitated material, which interferes mechanically with electrodialysis.

The main advantages over traditional methods of fractionation by salt or organic solvent precipitation are the relatively mild nature of the treatment, the ease with which different isoelectric fractions might be obtained from the same starting material, the selectivity of isoelectric separation and the fact that there is no need for the subsequent removal of chemical precipitating agents.

The competent technical assistance of Mr W. J. Martyka is gratefully acknowledged. This work was supported by the Ministry of Technology, the Science Research Council, BDH Chemicals Ltd. and Quickfit and Quartz Ltd.

REFERENCES

- Abrahams, H. E. & Robinson, D. (1969). *Biochem. J.* **111**, 749.
- Anson, M. L. (1937). *J. gen. Physiol.* **20**, 561.
- Borders, C. L. & Raftery, M. A. (1968). *J. biol. Chem.* **243**, 3756.
- Di Ferrante, N. (1956). *J. biol. Chem.* **220**, 303.
- Dorfman, A. (1955). In *Methods in Enzymology*, vol. 1, p. 166. Ed. by Colowick, S. P. and Kaplan, N. O. New York: Academic Press Inc.
- Fleetwood, J. G. & Milne, G. R. (1967). In *The Protides of the Biological Fluids*, vol. 15, p. 455. Ed. by Peeters, H. Amsterdam: Elsevier Publishing Co.
- Leaback, D. H. & Walker, P. G. (1961). *Biochem. J.* **78**, 151.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Malmgren, H. (1953). *Biochim. biophys. Acta*, **11**, 524.
- Ornstein, L. & Davis, B. J. (1964a). *Ann. N.Y. Acad. Sci.* **121**, 321.
- Ornstein, L. & Davis, B. J. (1964b). *Ann. N.Y. Acad. Sci.* **121**, 404.
- Polson, A. & Largier, J. F. (1960). In *A Laboratory Manual of Analytical Methods in Protein Chemistry*, p. 163. Ed. by Alexander, P. & Block, J. London: Pergamon Press.
- Price, R. G. & Robinson, D. (1966). *Comp. Biochem. Physiol.* **17**, 129.
- Reisfeld, R. A., Lewis, W. J. & Williams, D. E. (1962). *Nature, Lond.*, **195**, 281.
- Robinson, D., Price, R. G. & Dance, N. (1967). *Biochem. J.* **102**, 525.
- Smithies, O. (1955). *Biochem. J.* **61**, 629.
- Vesterberg, O. & Svensson, H. (1966). *Acta chem. scand.* **20**, 820.