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Device for miniscale isoelectric focusing of proteins

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Abstract. A simple device is developed for mini-scale electrofocusing of proteins. The main apparatus consists of only two glass tubes joined by a small tubing. No special cooling system, stopcocks, stands, etc., are needed. Even the need for a peristaltic pump for fractionation is eliminated. The apparatus does not require very high voltages and the amount of Ampholines is drastically reduced. The model can be used for analytical as well as semi-quantitative purposes.

Keywords. Isoelectric focusing; enzyme localization; Ampholine gradient; semiquantitative apparatus; analytical apparatus.

Introduction

Electrofocusing of proteins using ampholytes was first described by Svenson (1961a,b). Commercial units available for electrofocusing have disadvantages with regard to size, requirement of a cooling system and large amounts of Ampholines. Various small models have been described in literature (Weller *et al.,* 1968; Godson, 1970; Jackson and Russel, 1984). However, in most of these models, there is no provision for collecting the samples from the bottom which is not only most convenient but will also prevent disturbance of the pH gradient formed.

Materials and methods

Ampholines (pH range 3·5–10) were purchased from LKB Produkter, Sweden. Myoglobin and ferritin were from Sigma Chemical Co., St. Louis, Missouri, USA Partially purified preparations of intracellular glucose (xylose) isomerase and extracellular xylose isomerase from *Chainia* sp. and subtilisin inhibitor from horse gram were from this laboratory. Haemoglobin from a *Bandicoot* sp. was a gift from Ahmednagar College, Ahmednagar. All other chemicals were of analytical grade and were available locally.

Description of the mini-electrofocusing apparatus

The apparatus consists of two pyrex glass tubes (7 mm × 35 cm). The lower ends of these tubes are fitted with two short (2 cm length) flexible silicone tubings. This is to facilitate the use of pinchcocks. These tubes are then connected to a U-tube made of a rigid material (such as polyethylene) of the same inner diameter (7 mm) as the glass tubes (figure 1). The platinum electrode in the heavy electrode solution is made longer (33 cm) in order to minimize resistance during the run. The other platinum electrode is about 11 cm in length.

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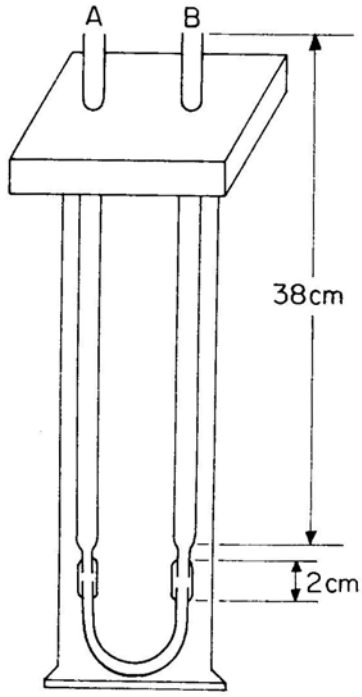

Figure 1. Schematic diagram of the modified electrofocusing set-up.

Solutions used for electrofocusing

Anode solution: Heavy electrode solution-glycerol 13·75 ml, H3P04 (1 M) 4 ml, H20 7·25 ml, total volume 25 ml.

Cathode solution: Light electrode solution-NaOH (1 M) 2·5 ml, H20 7·5 ml, total volume 10 ml.

Heavy density gradient solution: Ferritin 100μg%, myoglobin 250 μg, cytochrome c l00 μg, Ampholine (pH range 3·5–10) 40% solution 0·15 ml, glycerol 3 ml, total volume 5·4 ml with distilled water.

Light density gradient solution: Ferritin 100 μg, myoglobin 250 μg, cytochrome c 100 μg Ampholine (pH range 3·5–10) 40% solution 0·15 ml, total volume 5·4 ml with distilled water.

Electrofocusing

The two arms of the tube assembly were inserted through a thermocole block and held vertically (figure 1). The heavy electrode solution was poured into arm A till it rose to about 1 to 2 cm in arm B. A pinchcock was then fixed on the flexible silicone tube of arm B. The rest of arm A was then filled with the heavy electrode (anode) solution. This forms one of the electrode chambers. A perspex gradient mixer of

11 ml capacity was used for the formation of a linear density gradient in arm B. The light electrode (cathode) solution was then layered over the Ampholine gradient column. The pinchcock was removed and the assembled apparatus was immersed in a cylinder containing cold water at 4°C. Focusing was initiated by inserting the electrodes into the anodic and cathodic solutions and then applying a current of 4 mA at 400 V. Electrofocusing was complete in 27 h as indicated by the drop in amperage to zero. However, the run was continued up to 40 h to ensure the formation of a stable gradient.

Fractionation and collection of the Ampholine gradient

After the run a pinchcock was again fixed to the silicone tubing of arm B. The polyethylene U-tube was then disconnected from arm B. Fractions of 0·15ml (approximately 3 drops) were collected by adjusting the flow rate suitably with the help of the pinchcock. The pH of each fraction was measured using a surface electrode and the individual fractions were assayed for protein or enzyme activity.

Results and discussion

Figure 3 shows a photograph of the apparatus after electrofocusing of ferritin, myoglobin and cytochrome c. The observed pI values of 10·3 for cytochrome c, 6·8–7·3 for myoglobin, 4·6–5·1 for ferritin are close to the values reported in the literature. Intracellular glucose (xylose) isomerase, *Bandicoot* haemoglobin (3 bands), subtilisin inhibitor and extracellular xylose isomerase showed pI values of 4·0, 7·7, 7·85, 7·9; 7·66 and 3·5 respectively (figure 2).

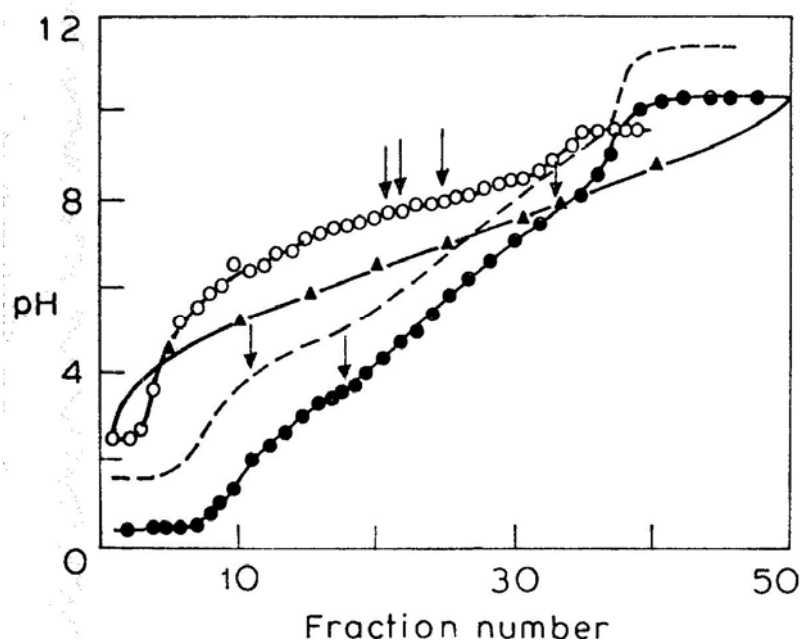

Figure 2. Determination of isoelectric point of (---) intracellular glucose (xylose) isomerase from *Chainia,* (○) Bandicoot haemoglobin (▲) subtilisin inhibitor from horse gram and (●) extracellular xylose isomerase from *Chainia.* Arrows indicate positions of focused proteins in pH gradient.

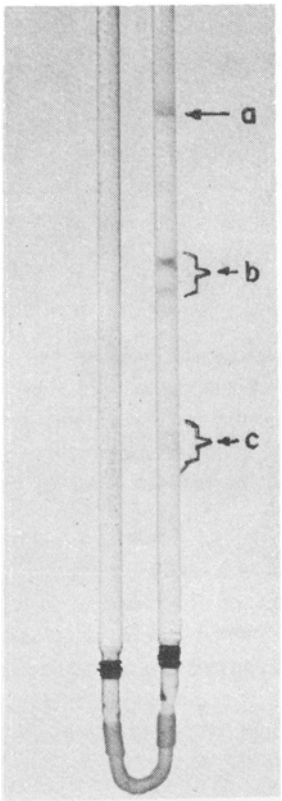

Figure 3 Isoelectric focusing pattern of (a) cytochrome c, (b) myoglobin (two bands) and (c) ferritin (two bands).

The U-tube apparatus described in this communication can be easily fabricated from locally available material. It is the simplest model to operate compared to those described in the literature. Since the entire apparatus is cooled and there is no capillary involved in the system (Jackson and Russel, 1984), very high voltages and consequent heating are eliminated in this model. The U-tube arrangement facilitates removal of fractions from the bottom and dispenses with the need for pumping in sucrose solution or the use of nitrogen to collect fractions from the top. The unit is being routinely used in our laboratory.

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