A multipurpose device for protein concentration, dialysis and buffer exchange

M. P. Divya and Y. S. Rajput

A multipurpose device and process for protein concentration, dialysis and buffer exchange suitable for microlitre volumes has been described. The device is simple and can be fabricated from 1.5 ml centrifuge tube. Sample loading and sample recovery are convenient. The chances of blockage of pores of membrane by protein molecules are least and therefore free flow of small molecules across semi-permeable membrane is maintained during protein concentration, dialysis and buffer exchange. Recovery of protein after concentration is quantitative. The described method for protein concentration is efficient, concentrating sample 7.5 to 15 folds in 60 min and especially suited for loading to SDS–PAGE. Dialysis is also efficient and removes about 86% small molecules in 60 min. The device can be repeatedly used.

Dilute protein solutions are required to be concentrated for (i) loading sufficient protein in SDS–PAGE for its easy subsequent detection, (ii) improving storage stability. Dialysis and buffer exchange processes are also routinely used in protein chemistry, biochemistry, molecular biology laboratories and industries involved in the preparation of biochemicals. This encouraged the development of devices for these applications. Methods and devices developed for large volumes are in general not suitable for small volumes; recovery in small volumes always remained questionable. Microcentrifuge tube-based devices for dialysis[1–3] and buffer exchange[1] have been reported. Commercially available devices for sample concentration, dialysis and buffer exchange are also available, wherein a centrifugal force is applied to force the passage of small molecules through an ultrafiltration membrane. These devices are widely used in research laboratories. In the present note, a multipurpose device and process for protein concentration, protein solution dialysis and buffer exchange has been described.

The multipurpose assembly was constructed from a commercially available 1.5 ml microcentrifuge tube (Figure 1 *a*). The tube has a recess inside the lid that forms a chamber for placing the protein sample. The lid (Figure 1 *b*) and tube (Figure 1 *c*) were made from the microcentrifuge tube by cutting the attachment between the lid and the tube. This was followed by the cutting of the tube from the lower end to make a holed-tube (Figure 1 *d*). The hole in holed-tube was used for addition of water-binding solvents/materials or exchange buffer or water into the tube and their subsequent removal after sample concentration/dialysis/buffer exchange. The lid was placed on a horizontal surface with the recess surface upward (Figure 1 *b*). The sample was added to the recessed portion of the lid (Figure 1 *e*) and the dialysis membrane was placed over it (Figure 1 *f*). The holed-tube in inverted position was then placed over the lid so as to make a perfect seal (Figure 1 *g*). Depending on the purpose for which device is to be used, water or exchange buffer or water-binding

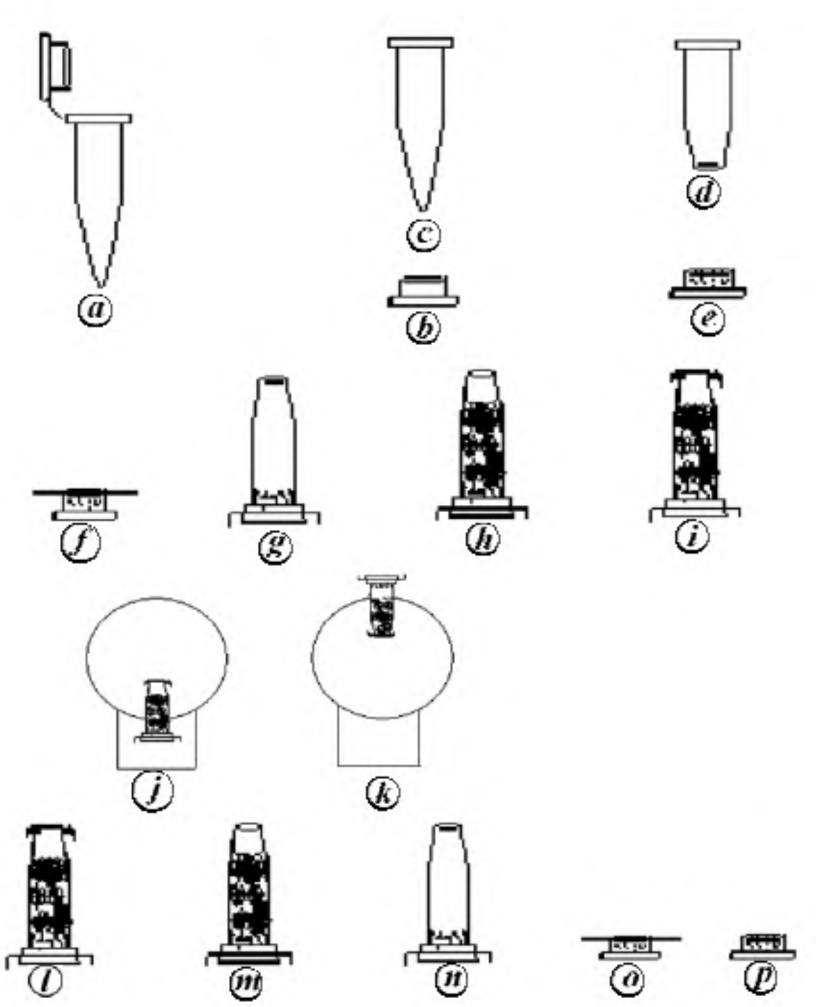

Figure 1. Sequential steps in the preparation of the multipurpose device and its uses.

solvent/material was added through the hole of the tube, leaving some empty space (Figure 1 *h*). The hole in tube was then sealed with parafilm (Figure 1 *i*). The assembly was fixed in a rotary shaker (Figure 1 *j*), which was rotated vertically (Figure 1 *k*). This resulted in the movement of the protein solution in the lid and water-binding solvents/materials, or exchange buffer or water in the holed-tube. During rotation, permeable molecules permeated across the membrane. Whereas for protein concentration, water-binding materials or solvents such as glycerol were added to the tube, water and buffer were added for dialysis and buffer exchange respectively. After rotation, the device was removed from the rotary shaker (Figure 1 *l*) followed by peeling-off the parafilm (Figure 1 *m*). Water binding solvent/material, or exchange buffer or water was then removed (Figure 1 *n*). Subsequently, the holed-tube was detached (Figure 1 *o*). The sample was then recovered from the recessed face of the lid after removal of the dialysis membrane (Figure 1 *p*).

The efficiency of the device for bovine serum albumin sample was checked by placing 20, 40, 60, 80 or 100% glycerol in the holed-tube. After different periods of rotation (15, 30 and 60 min), the sample was recovered, its volume and amount of protein[4] measured. It was found that 100% glycerol was most effective in concentrating protein sample. Also, 150 µl protein sample did reduce to 10 µl in 60 min duration (Figure 2 *a*). When 20, 40, 60 or 80% glycerol was used, nearly twofold reduction in volume could be achieved. There was quantitative recovery at all concentrations of glycerol (Figure 2 *b*). There is the possibility that glycerol could cross the dialysis membrane and contaminate the protein sample. The sample concentrated in this way could be used in SDS–PAGE, since the sample buffer in SDS–PAGE contained glycerol as one of the ingredients to make the sample heavier. The device was also checked for its effectiveness when a solution containing a mixture of proteins was evaluated. About 150 µl of solution containing BSA, alcohol dehydrogenase, carbonic dehydrogenase and lysozyme did reduce to 20 µl in 60 min (Figure 3 *a*) and recovery was quantitative (Figure 3 *b*). Efficiency of the device was also checked with other water-binding materials such as Sephadex G-100 (Figure 4). When 50 mg Sephadex beads were placed in the holed-tube, 150 µl protein sample did reduce to 23 µl in 5 h with quantitative recovery (Figure 4). Since Sephadex cannot cross the pores of the membrane, the sample was concentrated without contaminating the sample with Sephadex. Therefore, such materials have been recommended for use where possible contamination of glycerol is not desired. Compared to Sephadex, glycerol is more efficient in removing water from the protein sample. Therefore, sample concentration through the use of glycerol would be the method of choice, if the sample has to be used for SDS–PAGE. The latter technique is widely used in research laboratories. Efficiency of the device for dialysis was checked by placing 200 µl of 5% potassium dichromate solution in the recessed face of the lid and 1.5 ml distilled water in the holed-tube. Absorbance of the solution was measured

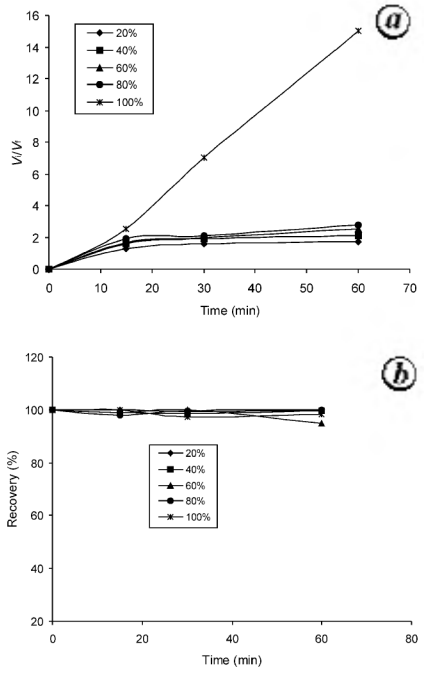

Figure 2. Volume reduction and recovery of protein using glycerol in holed-tube of the multipurpose device. BSA (1 mg/ml) solution (150 µl, initial volume, V_i) was subjected to different concentrations (20, 40, 60, 80 and 100%) of glycerol. At the indicated time, final volume (V_f) and protein content of the recovered sample were measured. ***a***, Volume reduction, V_i/V_f; ***b***, Protein recovery in percentage.

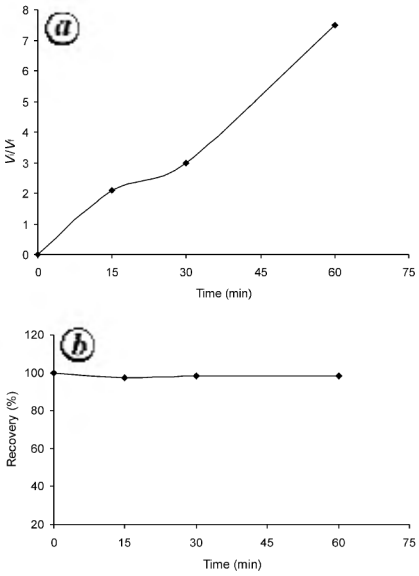

Figure 3. Volume reduction and recovery of a mixture of proteins using 1 ml 100% glycerol in holed-tube of the multipurpose device. A mixture of proteins (150 µl) (250 µg each of BSA, alcohol dehydrogenase, carbonic anhydrase and lysozyme) were dissolved in 1 ml distilled water was placed in lid. At the indicated time, final volume (V_f) and protein content of recovered sample were measured. ***a***, Volume reduction, V_i/V_f; ***b***, Protein recovery in percentage.

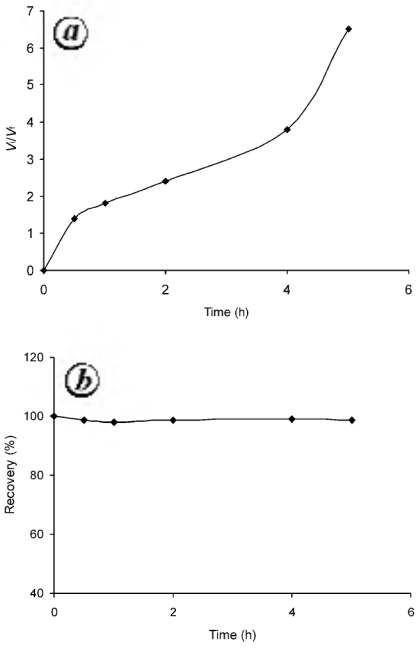

Figure 4. Volume reduction and recovery of protein using 50 mg Sephadex G-100 in holed-tube of the multipurpose device. BSA (1 mg/ml) solution (150 µl, initial volume, V_i) was subjected to concentration. At the indicated time, final volume (V_f) and protein content of the recovered sample were measured. ***a***, Protein concentration (V_i/V_f). ***b***, Protein recovery in percentage.

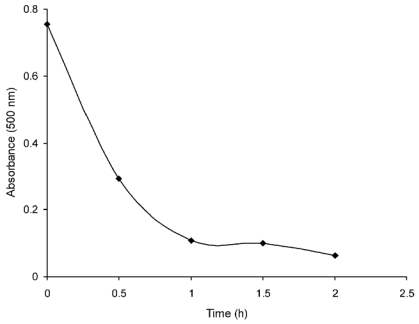

Figure 5. Dialysis of potassium dichromate solution against distilled water. Two hundred microlitres 5% potassium dichromate solution was dialysed against 1.5 ml distilled water. The retentate collected at different time intervals was diluted with water to obtain 4 ml solution and absorbance of the resultant solution was measured at 500 nm.

after 20 times dilution with water at different time intervals. About 60 and 86% potassium dichromate molecules could be dialysed in 30 and 60 min respectively, indicating efficient dialysis (Figure 5). There was not much additional change in absorbance after 60 min. Therefore, it is advised to replace water in the holed-tube after 60 min. Similarly, buffer exchange can be achieved in 60 min by placing the buffer in the holed-tube.

During concentration and dialysis of the protein sample, the device was rotated vertically. During each rotation, buffer/water would strike the membrane. This caused displacement of protein molecules adhering to the membrane and thereby free flow of small molecules across the membrane would be maintained. This is in contrast to the most commonly available assembly for protein concentration/dialysis, wherein centrifugal force was commonly used for pushing out small molecules across the membrane. Because of the unidirectional force, large molecules, if bound to the membrane, would not diffuse easily and result in membrane blockage. Over time, these assemblies in fact work with decreased efficiency. During rotation, water-binding materials in the holed-tube mixed with each rotation. This allowed new water-binding molecules to come in contact with the membrane, thereby facilitating removal of dialysed water molecules.

Methods and devices for dialysis or protein concentration exist for large as well as small volumes. An ultrafiltration membrane has been used for the removal of water as well as small molecules such as salts and therefore effectively works in dialysis and protein concentration. Application of gravitational force (Nanosep device, Pall Corporation; Centriplus, Millipore) or electrical force (Electroelution and Dialysis Tool, Gene Bio Application; Centriluter, Millipore) facilitates removal of permeable molecules in the process of protein concentration. Slide-A-Lyser Dialysis Cassettes (Pierce) or other devices prepared from microtitre plates[5] and microcentrifuge tubes[1–3] are also available for dialysis of samples in microlitre volumes. The commercial devices for dialysis and protein concentration are expensive. The device presented here can be prepared in any laboratory without the involvement of expensive material. The process of dialysis or protein concentration will require a rotator and simple laboratory equipment. Concentration and desalting of peptide and protein samples with a newly developed C18 membrane in microspin format has also been recently developed[6].

The process of protein concentration, dialysis and buffer exchange is simple and efficient and recovery of protein is quantitative. The device and method is suitable for microlitre volumes and can be repeatedly used.

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M. P. Divya and Y. S. Rajput are in the Dairy Chemistry Division, National Dairy Research Institute, Karnal 132 001, India.*
**e-mail: ys_rajput@rediffmail.com*

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S. Sreekumar, C. K. Biju, G. Hari Sankar and P. N. Krishnan

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