

Device for eluting proteins from starch gel by freezing and thawing

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If starch gel is frozen and thawed, its structure is so changed that most of the water and protein can be extracted by centrifugation (Smithies, 1955) or by

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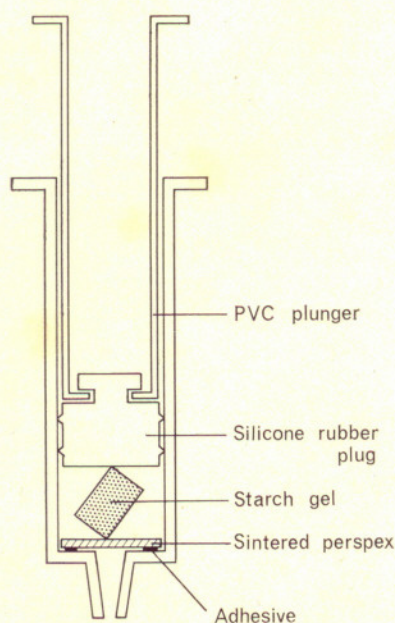


FIG. 1. The device is based upon a 10 ml disposable syringe made of polyvinyl chloride, but having a close-fitting plug of artificial rubber attached to the end of the plunger. The minimum quantity of perspex cement (4% perspex in chloroform) is placed round the perimeter of the end of the barrel (inside), using a Pasteur pipette. A disc of sintered perspex (Fisons Scientific Instruments Ltd, Loughborough) is cut to fit inside the barrel, and is then forced down with the plunger until it lies in contact with the adhesive. The plunger is then withdrawn, and the syringe laid aside for 24 hours to harden. Alternative adhesives might be tried with advantage.

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squeezing. This is conveniently done in the device illustrated here. Portions of the completed gel are cut out, placed in the barrel of the syringe, and then frozen by keeping at -20° for two hours. After allowing to thaw on the bench for two hours, fluid is expressed, and the syringe and gel matrix are then washed as often as desired by drawing up water or a suitable buffer solution and re-expressing after an interval of 10 minutes to 24 hours. The recovery of haemoglobin was virtually complete after two rinses, but serum albumin proved more difficult, only some 80 to 90% being extracted after three rinses.

The advantages of this device are that it is convenient, at least for small numbers, and that it is easy to rinse the gel matrix. Only about 75% of the water contained in the gel is recovered at the first expression or centrifugation, and rinsing is essential if recoveries are to be quantitative. The chief disadvantages are shared with any procedure for extraction by freezing and thawing—the sample is contaminated with polysaccharides, and even some protein, derived from the gel itself.

Bocci (1963) described the use of metal-glass syringes fitted with filter paper discs to extract protein by the freezing and thawing process, from starch gel expanded with Pevikon C870. Filters of this kind have proved too weak to stand up to the pressures used in expressing gels made of starch alone. Also, if the gel matrix is to be rinsed by aspirating buffer, the filter must be firmly attached to the end of the syringe barrel. A demountable system of screw clamps can be used instead of simply glueing the porous disc in place, but it is more expensive.

REFERENCES

- Bocci, V. (1963). *Nature (Lond.)*, **197**, 491.
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