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A Simplified Method for Preparing Sucrose Gradients

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Numerous sucrose gradients can be prepared simultaneously by diffusion in horizontal centrifuge tubes.

The usual layering method for making sucrose gradients, often using four to six layering steps, can be tedious. The alternative method, using a gradient-forming mixing chamber, apart from being time-consuming, presents difficulties with gradients of small volume, and is rather inefficient with more viscous solutions. Recently a third method, involving several cycles of freezing and thawing of an initially uniform sucrose solution, has been described (Baxter-Gabbard, 1972). However, this rather slow process is unsuitable for the production of steep gradients, and the result is influenced by the presence of salts etc.

The undemanding technique now described allows numerous gradients to be prepared simultaneously. Only two sucrose solutions are needed, and, because of the relatively large density difference between them, the single layering step can be performed rapidly. Maintenance of sterility, where necessary, is straightforward.

The principle of the method is as follows. Several centrifuge tubes are firmly held in a block of Perspex [poly(methyl methacrylate)]. Each tube contains a volume of dense sucrose solution overlain with a less dense one, and is closed with a vented nylon plug. The block is slowly tipped on to its side, some 20s being taken for this (40s for tubes of internal diam. <13mm or for viscous solutions of >60%, w/w, sucrose), and is kept in the horizontal position until a gradient is formed by diffusion across the now shallow layers of liquid. The block is then slowly returned to the upright position. Provided that the correct diffusion time is chosen, longer times being used for tubes of greater diameter and for solutions of greater viscosity, the gradient obtained is approximately linear. However, to compensate for the irregular cross-section of the horizontal sucrose layers and for a small degree of mixing along the tube wall during tipping, it is necessary to use initial sucrose concentrations somewhat higher and lower, respectively, than the maximal and minimal concentrations desired in the final gradient.

Typical results for a range of tube sizes and sucrose concentrations are shown in Fig. 1. Temperature variations between 20° and 28°C have negligible effect.

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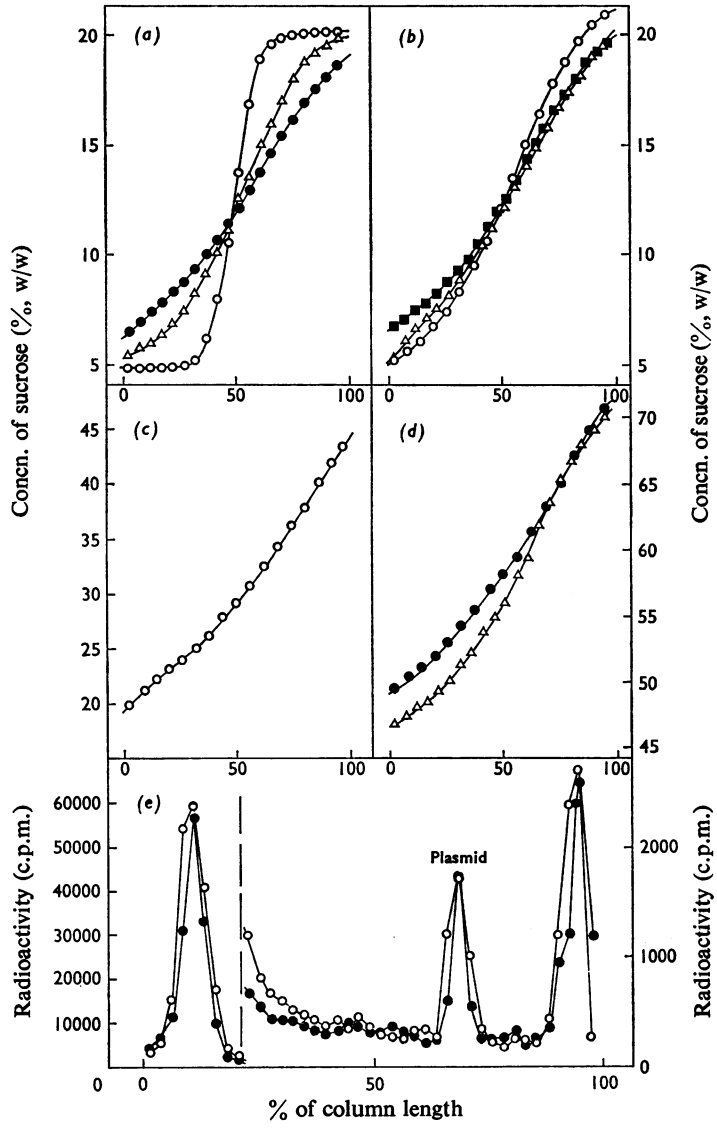

Fig. 1. *Adaptability of the method*

Fractions were collected with an ISCO density-gradient fractionator. Sucrose concentrations were measured at 20°C with a Bellingham and Stanley (London) refractometer. Radioactive fractions were processed and their radioactivities counted as already described (Stone, 1970). (*a*) 4.1ml of 20% and 4.1ml of 5% (w/w) sucrose in 60mm×15mm tubes, allowed to diffuse at 25°C for: ○, 0.5h; △, 2h; ●, 4h. (*b*) Initial sucrose concentrations 22% and 3% (w/w): ○, 2.5ml of each in a 60mm×12mm tube, 1.25h; △, 4.1ml of each in a 60mm×15mm tube, 4h; ■, 22ml of each in a 105mm×25mm tube, 12h. (*c*) ○, 2.5ml of 12% and 2.5ml of 48% (w/w) sucrose in a 60mm×12mm tube, 4h. (*d*) 73% and 42% (w/w) sucrose: ●, 2.5ml of each in a 60mm×12mm tube, 7h; △, 4.1ml of each in a 60mm×15mm tube, 9h. (*e*) Separation of covalently closed circular plasmid DNA (R1; mol.wt. 67×10^6) from fragments of *Escherichia coli* DNA, both labelled with [^{3}H] thymidine (Freifelder *et al.*, 1971), by centrifugation for 1h at 100000*g* through 7.8ml of alkaline (0.8M-NaOH, 0.5M-NaCl) sucrose gradients prepared in 60mm×15mm tubes either by allowing a four-step gradient (20%, 16%, 13% and 10%, w/w) to diffuse vertically for 6h (○), or by the simplified method with 3.9ml of 21% and 3.9ml of 9% (w/w) sucrose (alkaline), 4h diffusion (●).