Salting-out curves of multi-component systems with and without the use of constant final volumes

Salting-out curves have been widely used to examine biological extracts containing mixtures of proteins[1-3]. In general, a salting-out curve is determined by plotting the concentration of protein remaining in solution in a series of tubes containing gradually increasing final concentrations of salt, but all made up to the same final volume, against the concentration of salt[4]. This can be achieved either by adding to a fixed volume of extract a constant volume of salt solution of gradually increasing concentration, or by adding to a fixed volume of extract both saturated salt solution and water in appropriate amounts to give the desired final concentration of salt and at the same time to maintain constant final volumes.

It would be advantageous to be able to add to constant volumes of extract merely saturated salt solution to give the desired final concentration, but without maintaining the final volumes constant. The effect of this modified procedure on the salting-out curves and on the differential curves with respect to salt concentration is examined in this paper.

A preliminary assumption is made that all volumes are additive. Let z denote the salt concentration expressed as a percentage of saturation. Then

$$\frac{z}{100} = \frac{v}{u + v + w} \tag{1}$$

where u is the volume of protein solution of concentration c_0, v the volume of saturated salt solution and w the volume of water mixed together in each tube.

Let $S^{(n)}(z)$ denote the solubility of protein n at salt concentration z and let $c_{\mathrm{nom}}{}^{(n)}$ denote the nominal concentration of protein n (irrespective of salting out). Then

$$\frac{c_{\mathrm{nom}}{}^{(n)}}{c_0{}^{(n)}} = \frac{u}{u + v + w} \tag{2}$$

If $c_{\mathrm{nom}}{}^{(n)}(z) < S^{(n)}(z)$, then $c^{(n)}(z) = c_{\mathrm{nom}}{}^{(n)}(z)$, where $c^{(n)}(z)$ is the actual concentration of protein n in the supernatant of the tube with salt concentration z. But if $c_{\mathrm{nom}}{}^{(n)}(z) \geqslant S^{(n)}(z)$, then $c^{(n)}(z) = S^{(n)}(z)$.

It follows from Eqns. 1 and 2 that

$$\frac{c_{\mathrm{nom}}{}^{(n)}}{c_0{}^{(n)}} = \left(\frac{u + v}{u + v + w} - \frac{z}{100}\right) \tag{3}$$

If we define $z_0{}^{(n)}$ as the value of z for which $c_{\mathrm{nom}}{}^{(n)} = S^{(n)}$, then

$$\frac{S^{(n)}(z_0{}^{(n)})}{c_0{}^{(n)}} = \left(\frac{u + v}{u + v + w} - z_0{}^{(n)}/100\right) \tag{4}$$

Condition A: $w = 0$. Hence Eqn. 4 simplifies to

$$S^{(n)}(z_0{}^{(n)}) = c_0{}^{(n)}(1 - z_0{}^{(n)}/100) \tag{5}$$

Eqn. 5 applies to the point where salting-out of protein n commences.

From Eqn. 3 it follows that if $c_{nom}^{(n)}(z) < S^{(n)}(z)$ and $w = 0$,

$$c^{(n)} = c_0^{(n)}(1 - z/100) \tag{6}$$

and

$$\frac{dc^{(n)}}{dz} = -c_0^{(n)}/100 \tag{7}$$

so that the slope of a plot of $c^{(n)}$ is a constant. The differential coefficient of $c^{(n)}$ with respect to z has a discontinuity at $z_0^{(n)}$. Eqns. 6 and 7 are graphed in Figs. 1a and b.

Condition B: $w \neq 0$ and $u + v + w = K$ (constant). From Eqn. 2 it follows that

$$\frac{c_{nom}^{(n)}}{c_0^{(n)}} = \frac{u}{K} \tag{8}$$

But for any series of measurements u is kept constant, so that u/K may be replaced by a new constant L. If $c_{nom}^{(n)}(z) < S^{(n)}(z)$, $c^{(n)}(z) = c_{nom}^{(n)}(z)$ and it follows from Eqn. 8 that

$$\frac{c^{(n)}}{c_0^{(n)}} = L \tag{9}$$

and

$$\frac{dc^{(n)}}{dz} = 0 \tag{10}$$

Eqns. 9 and 10 are graphed in Figs. 1c and d.

For both conditions A and B if $c_{nom}^{(n)}(z) \geqslant S^{(n)}(z)$, then $c^{(n)}(z) = S^{(n)}(z)$.

Hence for any one protein n the salting-out curves obtained under conditions A and B differ until the protein actually starts to salt out, but are identical while protein is salting out. The same applies to the differential curves derived from the salting-out curves. For a number of proteins, each of which has a salting-out and differential curve similar in shape to those illustrated in Figs. 1a and b (condition A)

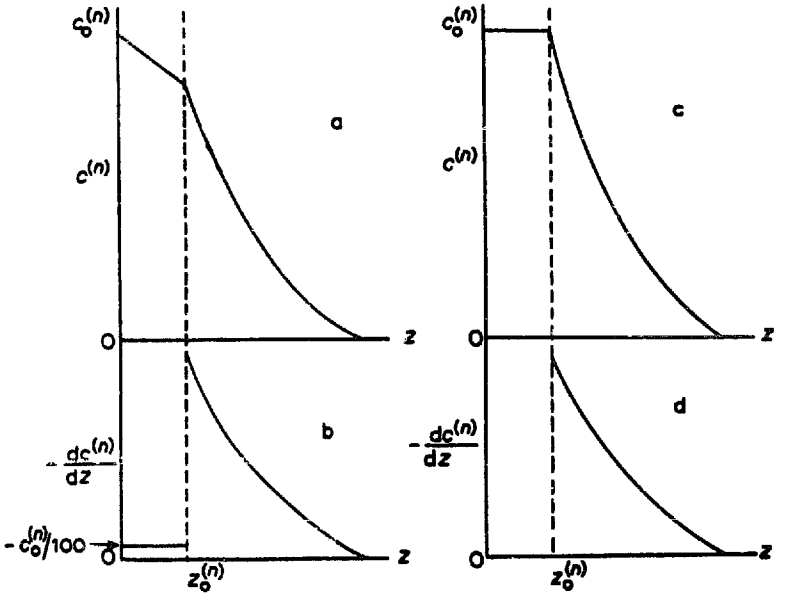

Fig. 1. Plot of (a) concentration $c^{(n)}$ of protein n and (b) differential coefficient of protein concentration ($-dc^{(n)}/dz$) against salt concentration z under condition A; plot of (c) concentration $c^{(n)}$ of protein n and (d) differential coefficient of protein concentration ($-dc^{(n)}/dz$) against salt concentration under condition B.

or in Figs. 1c and d (condition B), the actual salting-out curve and differential curve will be the sum of the individual curves.

The modified procedure (condition A) has been applied to a study of the proteins in saline extracts of normal and pathological human thyroid glands[5].

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A device for rapid collection of cells from radioactive medium

Rapid exchange of metabolites between cells and the medium may make accurate determinations of exchange rates difficult, because of the time required to wash the cells free of radioactive medium. A device, called a cell washer, has been designed which permits fairly accurate, rapid sampling of cells from radioactive medium (Fig. 1). Cells suspended in radioactive medium are separated from the latter as the cell washer is centrifuged and the cells pass through the denser, non-radioactive rinse solution and collect at the tip of the column.

Routinely, 12-cm sections of polyvinyl tubing (Transflex No. 18, Minnesota Mining and Manufacturing Company, Minneapolis, Minn.) are held horizontally and filled with the desired rinse solution by means of a syringe. One end of the tubing is carefully doubled over for a distance of about 1 cm and fastened with a few turns of thin copper wire. Into the other end of the polyvinyl tubing is inserted the tip of an empty, funnel-shaped polyethylene sample holder having an over-all length of 5.5 cm. The sample holders are made by drawing out one end of a piece of polyethylene tubing, inside diameter 0.45 cm, to a tip fine enough so when cut off short it fits tightly into the polyvinyl tubing. The completed cell washer is supported by inserting the polyvinyl tubing into a glass sleeve, 11 cm long, inside diameter 0.635 cm, with the shoulder of the sample holder resting on one end of the tubing. The glass sleeve, in turn, is contained in an 18 × 150 mm test tube. Radioactive cell suspensions up to 3.0 ml are placed in the sample holder and centrifuged horizontally for 3 min at 840 × *g*. The cells, quantitatively collected at the tip of the cell washer, are recovered by slicing off the tip with a blade, removing the wire, and rinsing out the cells with a fine stream of water. Sample chambers are washed and used again while the tubing is discarded. In practice, a number of cell washers are assembled in advance of use.