

3.2. DETERMINATION OF THE DENSITY OF SOLIDS

Table 3.2.2.1. Possible substances for use as gradient-column components

Hydrophobic components			Hydrophilic components	
Liquid	Approximate density at 298 K (g ml ⁻¹)	α	Solute	Approximate maximum density of concentrated aqueous solution at 298 K (g ml ⁻¹)
Isooctane (2-methylheptane)	0.69	-0.8	Sodium chloride	1.20
Kerosene	0.79	-0.5 -0.8	Potassium chloride	1.40
<i>m</i> -Xylene	0.86	-0.85	Potassium iodide	1.63
Chlorobenzene	1.10	-1.1	Iron(III) sulfate	1.80
Bromobenzene	1.49	-1.3	Zinc bromide	2.00
Carbon tetrachloride	1.60	-1.9	Zinc iodide	2.39
Methyl iodide	2.28	-2.7	Thallium(I) formate	3.5
Bromoform	2.89	-0.9	Thallium(I) formate-malonate	4.3
<i>s</i> -Tetrabromoethane	2.96	-2.2		
Methylene iodide	3.32	-2.6	Ficoll* (60% w/w in water)	1.25

The density at temperature T K can be computed by substituting the values of the density at 298 K and α in the formula $d_T = d_{298} + 10^{-3}\alpha(T - 298)$. *Trade name for a synthetic high-molecular-weight polysaccharide derivative.

inclusions still remain, or if the sample is truly a mixture, a stable distribution of material will be observed. The density of the material of interest can then usually be obtained by measurement of the appropriate layer, generally the most dense, without further treatment of the sample. This is the only technique by which the homogeneity of the sample can be tested simply. All other methods provide an average density value. A satisfactory technique for removing crystalline powders from the gradient column has not been devised. If a precision of

± 0.002 g ml⁻¹ is adequate, it is simplest to prepare a new wide-range column for each determination in a 10 ml test tube.

Detailed specifications for the preparation of large density-gradient columns are contained in the records of the British Standards Institution (1964). In the experience of the author, for ordinary laboratory use, the procedures described are unnecessarily complicated as is the large scale of the system. The large columns are not suitable for centrifuging and the settling times tend to be many hours. However, if extreme sensitivity (*i.e.* use of a shallow gradient) is required, the large column may be useful, as it was in the original studies of Linderstrom-Lang (Linderstrom-Lang, 1937; Linderstrom-Lang & Lanz, 1938).

In the specific application of this technique to protein crystals, where a gradient of organic liquids is used, it is necessary to have available crystals sufficiently large that they can individually be quickly wiped free of adhering mother liquor with dampened filter paper before insertion. The uncertainty of successful cleaning combined with rapid evaporation of liquid from the pores within the crystal always affect the estimated accuracy of the measurement. An important improvement in the technique has been made by Westbrook (1976, 1985) through the use of concentrated aqueous solutions of the water-soluble polymer Ficoll. This very high molecular weight polysaccharide can be dissolved in water to concentrations of at least 60% by weight. The solutions are very viscous but do provide satisfactory water-based gradient columns. The polymer is both too large to enter the solvent-filled pores of the protein crystals and too high in molecular weight to develop a significant osmotic pressure. An aqueous suspension of crystals can be added directly to the column. This procedure has been adapted for measurements of protein-crystal density under hydrostatic pressures from 1 to 2000 atm (1 atm \equiv 101 325 Pa) (Kundrot & Richards, 1988). The general principle of using high-polymer-based gradients can presumably be extended to other porous materials.

3.2.2.1.2. Suitable substances for columns

Some representative liquids are listed in Table 3.2.2.1; all are readily available. For further information, see Meyrowitz, Cuttitta & Hickling (1959), and for very heavy liquids Sullivan

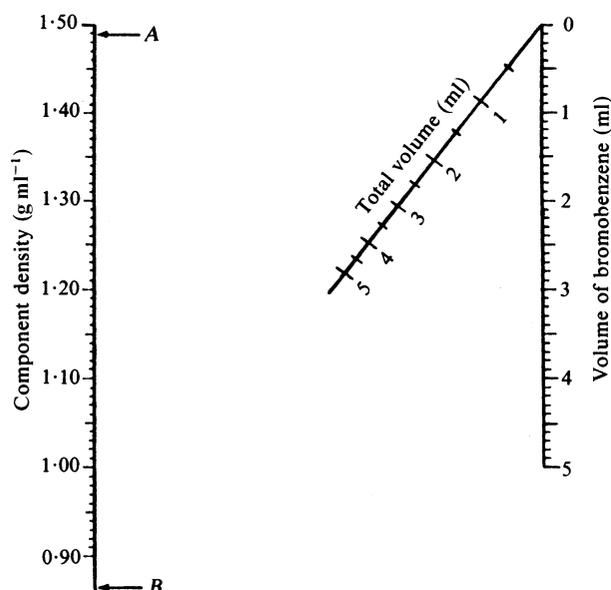


Fig. 3.2.2.1. Nomogram for the preparation of bromobenzene-xylene gradient column components at room temperature. From the desired component density and total volume, the required amount of bromobenzene is read from the chart, the volume difference being made up with xylene. To adapt this chart to any other pair of liquids, it is only necessary to change the component density scale. A uniform scale is drawn up such that the density of the heavy liquid lies at the point *A* while that of the light liquid is at *B*. The volume scales may be multiplied by any constant factor in order to change their range.