A SIMPLE METHOD FOR THE LARGE-SCALE PREPARATION OF SUCROSE GRADIENTS

K.L. BAXTER-GABBARD
Indiana University School of Medicine, Center for Medical Education, Indiana State University, Terre Haute, Indiana 47809, USA

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1. Introduction

In our laboratory the purification of avian reticuloendotheliosis virus, Strain Twiehaus (REV, strain T), by preparative sucrose gradient centrifugation imposed a demand of as many as 100 gradients within a week's time. The preparation of the gradients by Sage pump gradient makers or other similar devices proved to be tedious and time-consuming for so many gradients. This problem was solved by a practical application of Archimedes' Principle of Buoyancy applied to liquids. It is hoped that this simple method will be useful to other investigators confronted with similar problems.

2. Materials and methods

Buffered sucrose solutions were dispensed into cellulose nitrate tubes (0.5 in. X 2 in. or 1 in. X 3.5 in.) supported in an upright position in test tube racks of appropriate dimensions. The sucrose solutions were then alternately frozen at -20° in a freezer and thawed at 4° in a refrigerator. Five-drop fractions were collected from the bottom of the tubes after puncturing with a 20 gauge vacutainer-type hypodermic needle. The refractive index of each fraction was determined using an Abbe-31 Bausch and Lomb refractometer at 28° and each refractive index was then extrapolated to percent sucrose in each fraction. The International Scale (1936) of Refractive Indices of Sucrose Solutions at 28° was used as the reference for determining percent sucrose with respect to refractive index. The buoyant density (g/ml) associated with the percent sucrose at a given temperature of a fraction is determined by using the viscosity and density tables for sucrose published in [1].

3. Results and discussion

The effect of each cycle of freezing and thawing on the gradient formation using beginning sucrose solutions of 10%, 20%, 30% and 40% is demonstrated in fig. 1. With each additional cycle of freezing and thawing the steepness of the gradient is increased, i.e., the range of the percent sucrose is increased. Tube volume with respect to diameter and length was found to have little or no effect on gradient formation. The data presented in fig. 1 was compiled from studies using 4.5 ml gradients contained in 0.5 in. diam. X 2 in. length.

The addition of buffers, salts, detergents, etc., to the sucrose solutions tend to add to the freezing point depression effect, just as does sucrose. However, the temperature of -20° should not prevent the freezing of any sucrose solution containing concentrations of buffers and salts normally used in biological experiments. While this paper reports a simple method, it is important that each investigator should monitor his individual type of sucrose gradient for variables differing from those presented in these data by refractive index of the collected fractions.

The rate of freezing has no influence on the formation of the gradients. The purpose of slowly thawing at 4° is to reduce any convection effects which may be produced by a warmer external temperature such as room temperature. Freezing cellulose nitrate tubes in a solvent–dry-ice bath had obvious detrimental effects to the tube itself, and therefore cannot be used in this procedure.

There appear to be 3 factors responsible for the formation of gradients in the method described. The main factor operative in this method is Archimedes' Principle of Buoyancy applied to liquids. The other 2

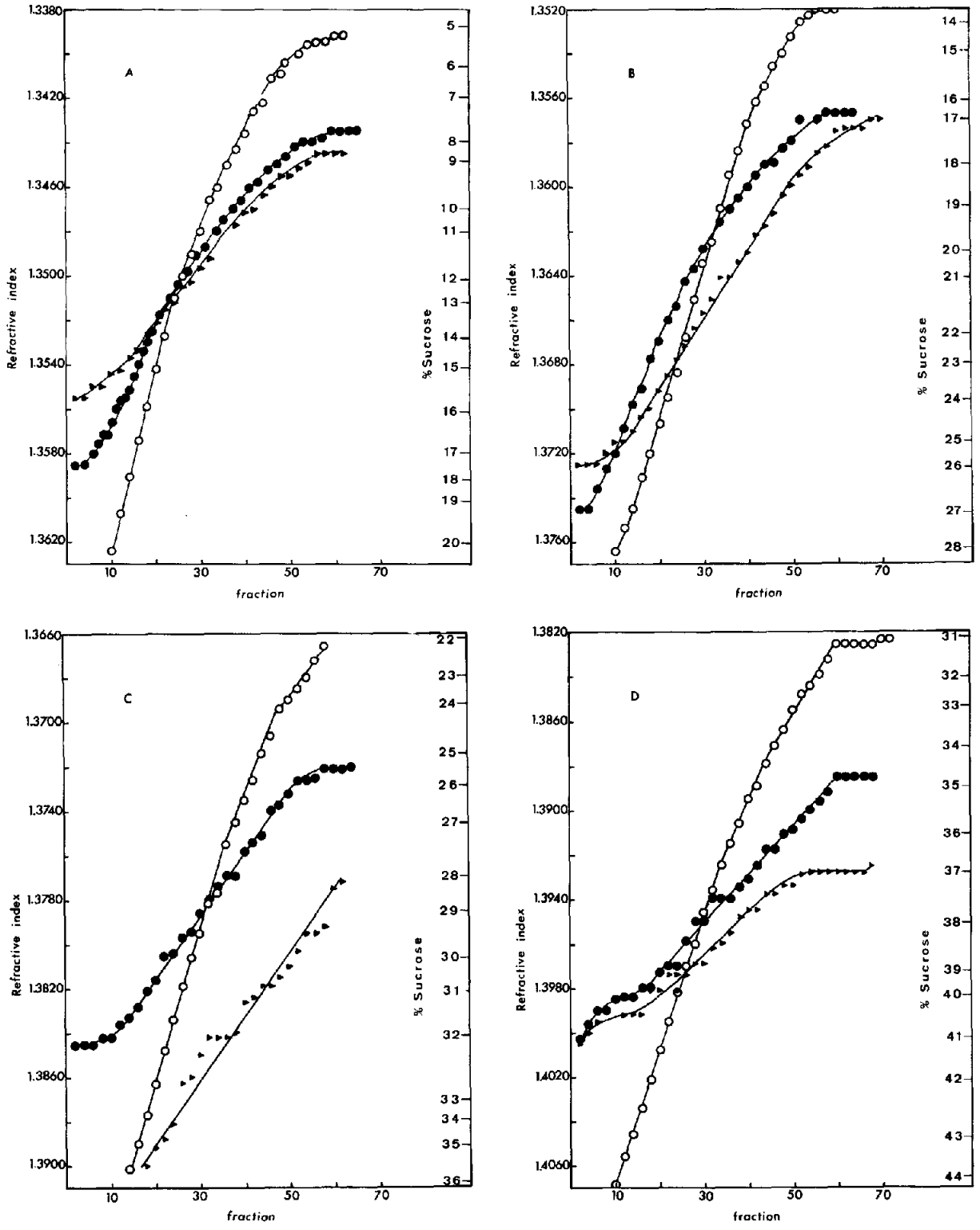

Fig. 1. A, B, C and D represent starting solutions of 10%, 20%, 30% and 40% sucrose, respectively, containing 0.1 M NaCl and 0.1 M Tris-HCl, pH 6.5. (▲–▲–▲), (●–●–●) and (○–○–○) represent graphic plots of gradient formation with 1 cycle, 2 cycles and 3 cycles of freezing at −20° and thawing at 4°, respectively.

factors which undoubtedly exert some effects are freezing point depression contributed by sucrose, salts and buffers, and the aggregation of hydrated sucrose molecules at low temperatures, thus effecting the concentration of the heavier and larger aggregates toward the bottom of the tube.

References

[1] Handbook of Biochemistry, Selected Data For Molecular Biology, ed. Herbert A. Sober (The Chemical Rubber Co., Cleveland, Ohio, 1968).