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# A simple test for macromolecular heterogeneity in the analytical ultracentrifuge

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A simple check for the presence of heterogeneity in a macromolecular system is proposed, employing comparison of Rayleigh sedimentation-equilibrium patterns for two solutions of the same fringe concentration but differing absolute concentrations. The method is illustrated by application to a bronchial glycoprotein from a cystic-fibrosis patient.

Many macromolecular systems give symmetrical schlieren peaks in a sedimentation-velocity experiment, or constant point-average molecular weights throughout the solution column in a sedimentation-equilibrium experiment. It has been stressed repeatedly in the literature that such criteria alone are insufficient to prove that a system is ideal and homogeneous (see, e.g., Creeth, 1964; Teller, 1965). Much more recently, Gilbert & Gilbert (1980) have show that computer prediction of the shape of schlieren patterns in sedimentation velocity is sufficiently sensitive to prove heterogeneity in a protein preparation that gave just such a single symmetrical peak. Although the method is elegant, it depends on a sophisticated computer program that will not be universally available; for this reason, we wish to show that similar information may readily be obtained, without computer analysis, from a particular combination of sedimentation-equilibrium patterns. Although the method depends on well-known principles, it has the usual advantage of equilibrium over velocity methods in requiring far smaller quantities of material.

The essential problem in sedimentation equilibrium is that heterogeneity‡ and thermodynamic non-ideality produce effects of opposite sign; thus, in a particular experiment on a sample that is heterogeneous, the initial concentration may be such that the effects of association and/or polydispersity virtually completely counteract the effects of thermodynamic non-ideality to give a Rayleigh equilibrium pattern characteristic of an ideal single-solute system.

In the present study we propose a relatively simple test for detecting the presence of non-ideality and heterogeneity. The principle of the method we propose to separate and identify these effects is to compare Rayleigh equilibrium fringe patterns that represent the same initial fringe loading concentrations ($J_0$) but different absolute loading concentrations ($c_0$). This can be achieved by employing cells of differing path length coupled with accurate dilution to 'compensate' $J_0$ for this difference. If the experimental conditions used to obtain each pattern are the same, a single-solute thermodynamically ideal system would give identical 'ideal' fringe patterns (viz. $\mathrm{d}\ln J/\mathrm{d}r^2$ constant throughout and the same for each pattern, where $J$ is the fringe concentration and $r^2$ the square of the radial displacement). On the other hand, the patterns would be different for a self-associating (or polydisperse) thermodynamically non-ideal system, since association and non-ideality are (differing) functions of concentration, whereas polydispersity is independent of concentration. Both patterns can be obtained from the same experiment if a four-hole rotor and a suitable arrangement of balanced wedge or masked multichannel cells are used.

As an example, consider a glycoprotein from the bronchial secretion of a cystic-fibrosis patient (Harding & Creeth, 1982). At an initial loading concentration of 0.2 mg/ml, $\mathrm{d}\ln J/\mathrm{d}r^2$ remains remarkably constant throughout the 3 mm solution column (Fig. 1) in a low-speed equilibrium experiment, a property consistent with an ideal single-solute model for the system. In order to test for non-ideality we used a four-hole rotor as stated above (Beckman An-J) with balanced 12 mm and 30 mm Yphantis-style (Yphantis, 1964; Teller, 1973)

‡ For convenience we use the term 'heterogeneity' in its widest sense to describe any system where the solute species do not have a single value of molecular weight, no matter what the origin of the variation may be. Thus this usage differs from that proposed, for example, by Gibbons (1972).



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multichannel cells (Fig. 2). The stock solution comprised a nominal 3 mg/ml solution of the glycoprotein after dialysis against solvent consisting of 1 M-NaCl in 0.1 M-phosphate/chloride buffer, pH 6.8. A 1 mm column of stock solution was then placed in the solution side of the inner and middle pairs of compartments in the 12 mm cell, and the diffusate in the solvent side. The outer pair was masked and then a 1 mm column of the 2.5-times-diluted solution placed in the solution side of the outer compartment of the 30 mm cell, diffusate being placed in the solvent side. The inner and middle pairs of compartments were then masked.

The corresponding equilibrium patterns at 2228 rev./min obtained in a Beckman model E ultracentrifuge are shown in Fig. 3. It is clearly evident that the patterns for the 12 mm cell ($c_0 = 3.0$ mg/ml) and the 30 mm cell ($c_0 = 1.2$ mg/ml) are different. The 12 mm fringes are nearly parallel and straight, whereas the 30 mm fringes show 'normal' curvature. Thus we have shown, in a *single experiment*, that our sample is both heterogeneous and thermodynamically non-ideal, hence accounting for the observed linearity of the plot in Fig. 1.

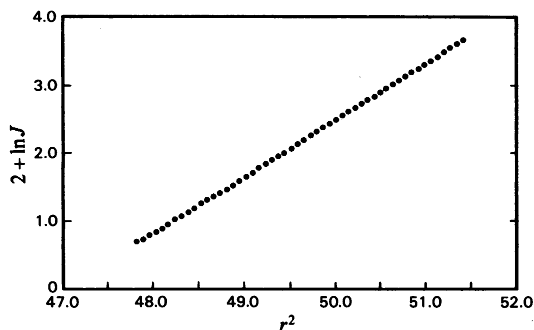


Fig. 1. *Plot of the logarithm of the fringe concentration against the square of the radial displacement for a glycoprotein from a cystic-fibrosis patient* (*Harding & Creeth, 1982*)

The data was obtained from a Rayleigh low-speed equilibrium fringe pattern (equilibrium speed 2231 rev./min; 30 mm cell; 3 mm solution column; loading concentration ~0.2 mg/ml; 1 M-NaCl solvent in 0.1 M-phosphate/chloride buffer, pH 6.8) and processed on a Wang 720 C desk-top computer.

Inspection of Fig. 3 shows that the two 12 mm-cell patterns are qualitatively identical, even though their mean radial positions differ by approx. 8%. The pattern from the 30 mm cell, however, is apparently shorter. This effect arises from two causes: (i) the apparent width of the meniscus is much greater in the cell of longer optical path, and (ii) the fringes at the base of the cell are steeper and accordingly less intense and so are partially lost on photographic reproduction. Photography by the schlieren system, using a flap to mask off the non-radial member of the Rayleigh double slit, confirmed that the column heights in the three experiments were virtually identical.

Although the Yphantis-type cells used here are quite satisfactory for qualitative tests, the differences in mean radial position of the compartments make them less suitable for quantitative comparisons than the 12 mm wedge centrepieces available from the manufacturer. For such work, extrapolation of the fringe patterns to the true radial positions of meniscus and base is mandatory (J. M. Creeth & S. E. Harding, unpublished work).

It should be pointed out that although non-identical patterns are conclusive proof of the presence of heterogeneity, the converse is not necessarily true; identical patterns are not completely diagnostic of homogeneity. Very excep-

Table 1. *Summary of the effects of various combinations of polydispersity, thermodynamic non-ideality and self-association on the simple test illustrated in Fig. 3*

+, Same effect as Fig. 3, namely lower curvature of fringes and lower $J_{base} - J_{meniscus}$ ($J$ = fringe concentration) for the 12 mm cell; –, opposite effect.

| Combination | Effect |
|---|---|
| Polydispersity and non-ideality | + |
| Association and non-ideality | + |
| Polydispersity and association | – |
| Polydispersity, association and non-ideality | +, 0 or – |

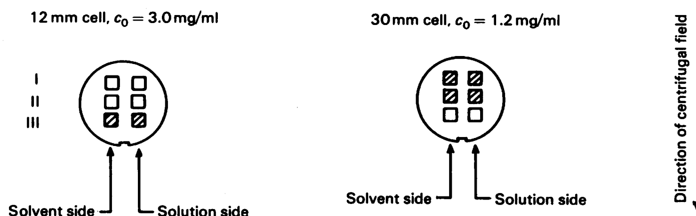


Fig. 2. *Cell filling for the 12 mm- and 30 mm-path-length Yphantis cells*

▨, Masked channel; I, inner channels; II, middle channels; III, outer channels.

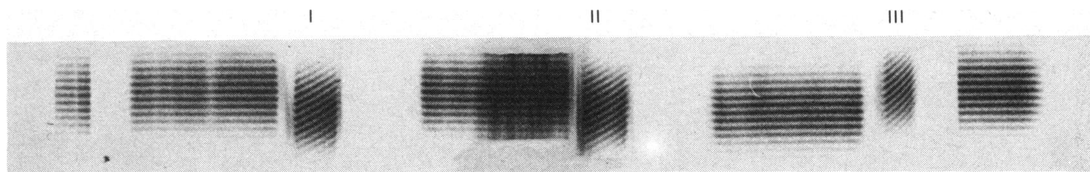


Fig. 3. *Comparison of Rayleigh equilibrium patterns of the same fringe loading concentration but different absolute loading concentrations* ($c_0$)

Patterns I and II: $c_0 = 3.0$ mg/ml, 12 mm-path-length cell; pattern III: $c_0 = 1.2$ mg/ml, 30 mm-path-length cell. The different radial positions of I and II does not appreciably affect their concentration distribution. The solution column of III appears shorter than in I and II for reasons given in the text.

tionally, polydispersity, self-association and thermodynamic non-ideality could in principle combined to produce virtually identical patterns (see Table 1). The remedy, of course, is again to vary the concentration.

In spite of this theoretical limitation, it is suggested that the simple test described should be applied before claims are made concerning the homogeneity of preparations which are potentially non-ideal in the thermodynamic sense.

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