Title
THEORETICAL CONSIDERATIONS ON CELL SHAPE, CONVECTION, AND AN AREA ANOMALY PERTINENT TO DEVELOPING A MOVING BOUNDARY THEORY FOR ULTRACENTRIFUGATION

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ABSTRACT

The intuitive concept that a sector shaped centrifuge cell is free from convection is criticized. Not only is a form of convection present for a single sedimenting species, but a more insidious type occurs in a mixture having an appreciable Johnston-Ogston effect. Rather than striving for convection-free sedimentation, the proposal is to utilize if possible an apparently harmless type of convection occuring in a very thin annulus in order to avoid the convection extending between boundaries in a mixture. The requirement that the concentrations be independent of time meets this condition and yields a hyperbolic cell, which is approximated by a sector cell placed in the rotor backwards. Simultaneously, area measurements and calculations involving the Johnston-Ogston anomaly are simplified because of the time independence.

Hyperbolic centrifugation requires a concept of the sedimentation coefficient based on the velocity per unit field of the net mass transport across any level, rather than the classical velocity per unit field of each

and every particle. This concept of s allows description of centrifugation by the law of conservation of mass on an apparatus level ("macroscopic") which is thus different from the atomistic ("microscopic") theories relating an observed s rate to the molecular weight of a single particle.

In order to show in contrast the theoretical simplification offered by the hyperbolic cell, the classical equations for the sector cell are derived (appendix).

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INTRODUCTION

The original Johnston-Ogston[1] formula was derived for the case of two migrating species in a uniform field in a rectangular cell. The basic principle that is applied is the law of conservation of mass, given that the velocity of the various species present are different in the various phases. The result is that the concentrations cannot be constant throughout the system, but each component present on one side of a boundary must have a different concentration on the other side. Hence the area of each peak seen on a schlieren diagram of the boundary system does not quantitatively correspond to the concentration of any specific component.

Other groups[2,3] have considered the area anomaly to be due to backward flow of solvent, not realizing that: (a) backward flow is just one of the many mechanisms which can alter the sedimentation rate with respect to the cell and the concentrations in the various phases; and (b) that the Johnston-Ogston treatment is on a level entirely different from this, encompassing any and all mechanisms altering s rates and concentrations since each net rate and concentration is given a symbol and not expressed in terms of mechanisms.

Harrington and Schachman[4] have chosen a system which shows a truly tremendous area anomaly - mixtures of TMV (asymmetric fast component) and BSV (nearly spherical slow component). Their experiments indicate that the build-up of area of the slow peak is qualitatively as predicted from the limited Johnston-Ogston formula, but that there was reasonable quantitative agreement only when the slow area, corrected for sector-sedimentation, was in addition extrapolated back to the meniscus. This decrease of area faster than accounted for by the sector-sedimentation implied convection between the fast and the slow boundaries. They pointed out that convection would be predicted from the original Johnston-Ogston formula since the dilution of the fast component as the run progressed would give a smaller slowing of the slow component and hence less of a build-up. The smaller build-up right behind the fast boundary yields a negative density gradient with radius due to concentration of solute, which can apparently exceed the positive density gradient due to compression and hence can convect. The convection between the two boundaries must have been quite fast and efficient, for the schlieren pattern showed only two peaks separated by a baseline region. In a flotation system the Johnston-Ogston formula would predict a greater build-up with time, which would lead to a positive density gradient due to solute since the solute is less dense than the solvent. This is stable and a clear baseline region between the peaks would not be expected if the change in the Johnston-Ogston build-up with time were appreciable.

The sector cell in a centrifugal field thus complicates an already complicated build-up phenomenon by giving a time dependence in addition to that due to radial dilution on the areas measured, and for sedimentation (but not flotation) a convection between the fast the slow boundaries. The sector cell even for a single component must have a certain type of minute convections,

for considering a differential lamina, more particles leave than enter because the field increases with distance from the center of rotation. Thus "holes" must be filled in by solvent. The intuitive idea that by having the walls of the cell radial so that nothing would collide with them and thus yield convection free sedimentation is thus not borne out. Since collision with a wall does not made quantitative sedimentation impossible in the angle preparative ultracentrifuge, for example, it seems plausible to consider redesign of the analytical cell such that concentrations do not have a time dependence. For those runs on unknown mixtures where separation of components is complete enough for only one area measurement to be made on each component, removal of the requirement of extrapolation to the meniscus will be imperative.

On the assumption that a micro eddy type of convection will prevail to yield uniform concentrations, a macroscopic moving boundary theory can be set-up which closely parallels the moving boundary theory for electrophoresis[5]. Some of the more complicated formulae for sector-centrifugation can be derived for reference (see Appendix) and may aid in interpreting existing or future data obtained with sector cells.

HYPERBOLIC CENTRIFUGATION

Derivation of hyperbolic shape.

The equation of continuity in the plateau region of a general cell can be derived as follows (refer to Figure 1):

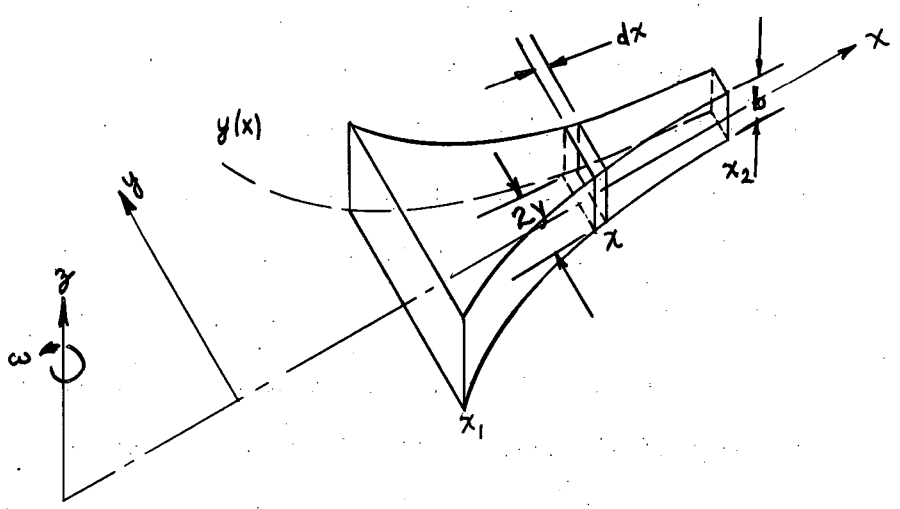

Fig. 1 General cell

Consider the anular lamina at x of height dx

$$(\text{mass/time})_{in} - (\text{mass/time})_{out} = \frac{\partial c}{\partial t} \cdot (\text{vol})$$

$$(\text{mass/time})_{in} = (s\,\omega^2 x)\ (2yb)c$$

$$(\text{mass/time})_{out} = 2b\,\omega^2\ csxy + \frac{\partial(2b\,\omega^2\ csxy)}{\partial x}\,dx$$

Thus,

$$\frac{\partial c}{\partial t}\ (2bydx) = -\frac{\partial(2b\,\omega^2\ csxy)}{\partial x}\,dx \qquad (1)$$

or

$$\frac{dc(t)}{dt} = -\frac{\omega^2\ c(t)\ s(t)}{y}\ \frac{d(xy)}{dx} \qquad (2)$$

where c and s are assumed to be a function of t only, and s has been used as a net volocity to account for all transport. Now impose the condition that the concentration has no time dependence (at points outside of boundary regions). That is $\frac{dc\ (t)}{dt} = 0$; Equation 2 then yields $d(xy)/dx = 0$. Therefore,

$$xy = K, \text{ a constant.} \qquad (3)$$

This is a hyperbolic cell.

Consider the quantity passing through level x_2 (left to right):

$$\bar{v}(x_2)\ 2y(x_2)\ bc(x_2)\ dt$$

and the quantity passing through x_1 (left to right):

$$\bar{v}(x_1)\ 2y(x_1)\ bc(x_1)\ dt$$

If we choose $y(x)$ such that c is a constant, then the quantity contained is constant and

$$\bar{v}(x_2)\ 2y(x_2)\ bc(x_2) = \bar{v}(x_1)\ 2y(x_1)\ bc(x_1)$$

$$\frac{\bar{v}(x_2)}{\bar{v}(x_1)} = \frac{y(x_1)}{y(x_2)}$$

Therefore from Eq. 3, the hyperbolic cell has:

$$\frac{\bar{v}(x_2)}{\bar{v}(x_1)} = \frac{x_2}{x_1}$$

$$\frac{\bar{v}(x_2)}{x_2} = \frac{\bar{v}(x_1)}{x_1} = \text{const.}$$

Hence the quantity

$$s = \frac{\bar{v}(x)}{\omega^2 x} = \frac{\frac{d\bar{x}}{dt}}{\omega^2 x} \qquad (4)$$

will be a constant for any one run, and is to be interpreted as the average velocity of particles at any level in the direction normal to the level per unit field rather than the velocity of any (and all) particles as it is in the sector cell. The s as defined here, then is the velocity per unit field of the net mass transport across any level. Since the sedimentation rate s is a constant, the derivation of Eq. 2, assuming it independent of x, is legitimate and thus the conclusion of a hyperbolic shape is valid.

The tangent to a rectangular hyperbola at the point (x,y) has slope -y/x; hence a sector cell, with slope of side y/x, in reverse, represents

approximating the hyperbola with its tangent. For the dimensions used in the Spinco[6] ultracentrifuge, the largest error in y is less than 1 per cent.

Concept of Convection in Hyperbolic Cell.

Let us consider the microscopic viewpoint for a moment. The instantaneous random velocity of the particles undergoing Brownian motion is many factors of ten greater than the average drift velocity in the centrifugal field, so that with any wall, tremendous collisions are occuring all the time. But now note that in the actual apparatus individual particles are not observed, and that to derive an apparatus theory it is necessary only to integrate expressions set up on the basis of differential elements of volume (or time), for example, which are small (or short) compared to apparatus dimensions (or time). These differential elements of volume contain such large numbers of particles that even a "point" in the coordinate system itself is considered large enough to contain a statistical number of particles. That is, for example, when one says that the concentration at the point (x,y) is c, he means that $c = \lim_{\Delta v \to 0} \frac{\Delta M}{\Delta v}$ where ΔM is the quantity of particles contained in Δv and Δv is to be shrunk not to mathematical zero but to the size of the point in the applied coordinate system which in our case contains a statistical number of particles. It is only in this way that we describe our measurements with time or distance in an apparatus.

Referring to Figure 2, the concentration at each point along the wall which was originally uniform in (a) is increased a very small amount after a very short lapse of time. The resulting increase in density due to concentration is indicated by the cross-hatched area in (b). Simultaneously the density in the center of the cell is decreased due to the attempted radial dilution, and is indicated by stippling. Under the centrifugal field, the hydrostatic leveling force will be tremendous and will result in convection.

But will the convection be of the form in (c) or (d)? It is the premise of this paper that to a first approximation, convection is of the form in (d). The density gradient due to compression, the symmetry of the cell, and the geometry chosen to yield a uniform concentration if convection in (d) occurs, would suggest that the solution would not sustain an extensive circulating radial convection, but would break into micro-eddies. By the macroscopic term level is meant, then, a very thin annulus containing not only a statistical number of particles in Brownian motion, but also a statistical number of micro-eddies such that the concentration is independent of y. It is in this sense that the equation of continuity was set up above.

Preliminary experiments with reversed cells indicate the narrower the cell, the more nearly the data is explained by the assumption of micro-eddies rather than radial convection. The 2° sector in reverse appears to be narrow enough, whereas the 4° sector in reverse is too wide.

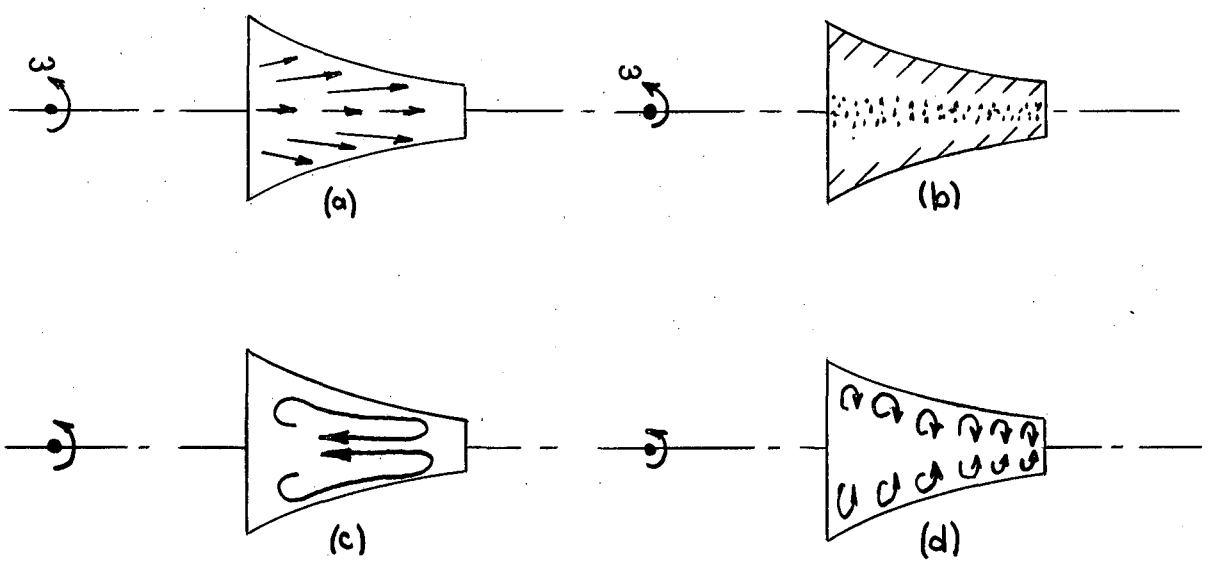

Fig. 2 Convection in hyperbolic cell (see text)

Boundary Definition.

Define a boundary region in a cell as the region in which the concentration depends on distance from the center of rotation x. Define the boundary position $\bar{x}$ as the position of the step in concentration if the material were rearranged in the boundary region to give an infinitely sharp boundary between the two concentrations at the extremes of the boundary region[7] (see Fig. 3).

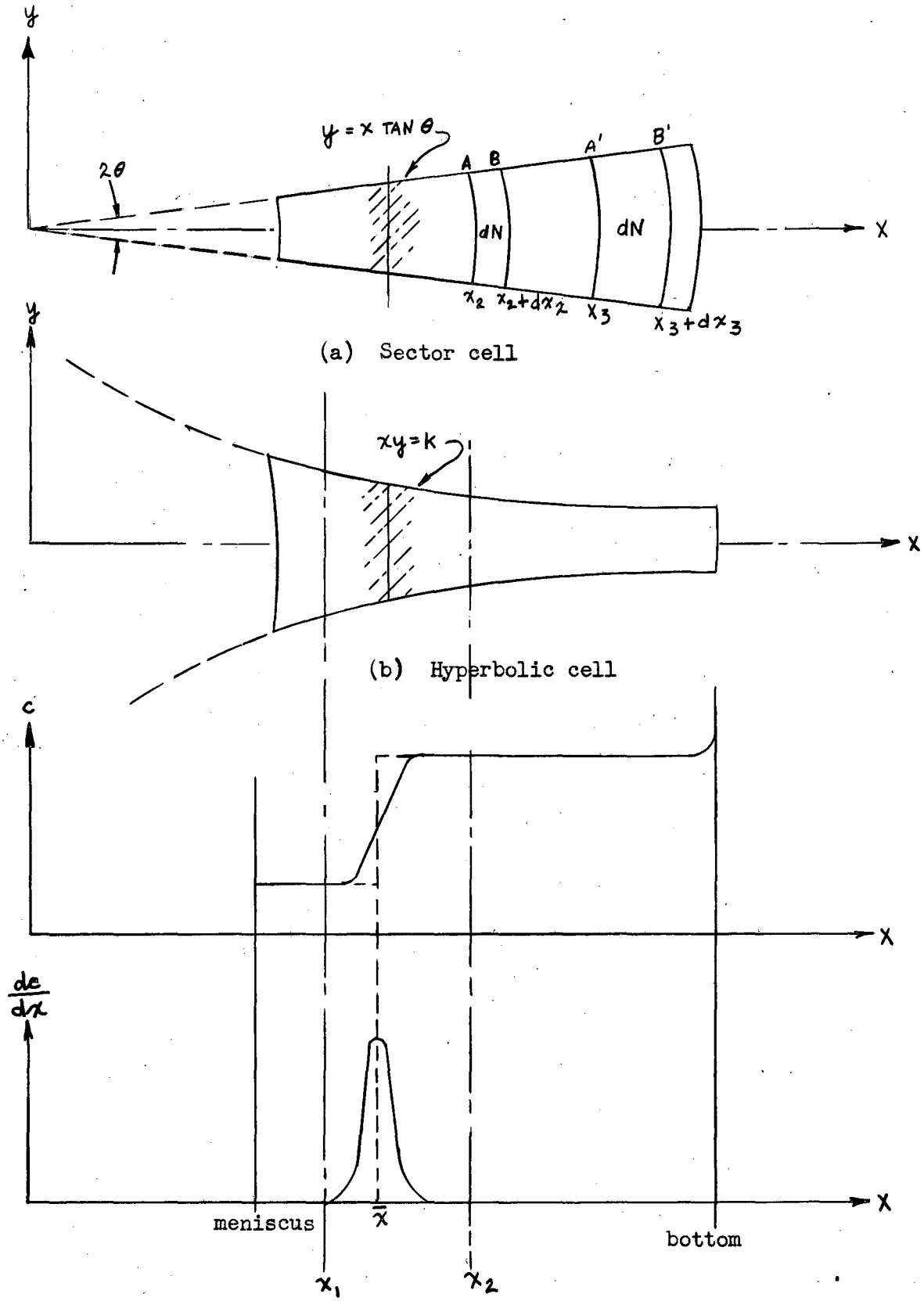

Fig. 3 Boundary position in centrifuge cell

We want to express $\bar{x}$ in terms of some property of the concentration gradient curve, which is what is usually recorded in the schlieren optical systems.

The quantity of material in a lamina of height dx is

$$c(x)\ 2y(x)\ b\,dx$$

The quantity Q_{12} between x_1 and x_2 is

$$Q_{12} = 2b \int_{x_1}^{x_2} c(x)\ y(x)\ dx$$

For the hyperbolic cell $xy = K$, so

$$Q_{12} = zbK \int_{x_1}^{x_2} \frac{c(x)}{x}\, dx \qquad (5)$$

This can be expressed in terms of the concentration gradient by symbolically integrating by parts. Note that this is considered at a particular time t, so that $dt = 0$.

Let

$$u = c(x) \qquad dv = \frac{dx}{x}$$

$$du = dc = \frac{\partial c}{\partial x}\, dx + \frac{\partial c}{\partial t}\, dt \qquad v = \ln x$$

$$= \frac{\partial c}{\partial x}\, dx$$

$$Q_{12} = 2\ bK\left\{\left[c(x)\ \ln x\right]_{x_1}^{x_2} - \int_{x_1}^{x_2} \ln x \left(\frac{\partial c}{\partial x}\right) dx\right\}$$

$$= 2\ bK\left\{c\ (x_2)\ \ln x_2 - c(x_1)\ \ln x_1 - \int_{x_1}^{x_2} \ln x \left(\frac{\partial c}{\partial x}\right) dx\right\} \qquad (6)$$

In terms of $\bar{x}$

$$Q_{12} = 2\ bK\left\{\int_{x_1}^{\bar{x}} \frac{c(x_1)}{x}\, dx + \int_{\bar{x}}^{x_2} \frac{c(x_2)}{x}\, dx\right\}$$

$$= 2\ bK\left\{-c(x_1)\ \ln x_1 + c(x_2)\ \ln x_2 - \ln \bar{x}\left[c(x_2) - c(x_1)\right]\right\} \qquad (7)$$

Equating (6) and (7) yields:

$$\ln \bar{x} = \frac{\int_{x_1}^{x_2} \ln x \left(\frac{\partial c}{\partial x}\right) dx}{\int_{x_1}^{x_2} \left(\frac{\partial c}{\partial x}\right) dx} \tag{8}$$

This can be rewritten in a smipler form if the following very good approximation is made

$$x = x_1 + z = x_1 (1 + z/x_1)$$

$$\ln x = \ln x_1 + \ln(1 + z/x_1) \approx \ln x_1 + z/x_1 \tag{9}$$

Substitution of (9) in (8) yields:

$$\bar{x} \approx \frac{\int_{x_1}^{x_2} x \left(\frac{\partial c}{\partial x}\right) dx}{\int_{x_1}^{x_2} \left(\frac{\partial c}{\partial x}\right) dx} \tag{10}$$

Thus $\bar{x}$ is approximately the position of the center of gravity (first moment) of the concentration gradient curve.

Dole Transformation.

By defining the boundary position as above, a given experimental pattern can be replaced by a diagram composed of homogeneous phases separated by infinitely sharp boundaries. See Figure 4. Such a transformation on the experimental diffuse pattern will be called a "Dole Transformation" in honor of V. P. Dole who first used this concept in his famous moving boundary theory for electrophoresis[5]. The first value of such a transformation comes from the theorem that the boundary position of all the superimposed gradients in a boundary region coincide. This theorem will not be rigorously proven here, but rather taken as a reasonable assumption on the basis of the following reasoning: Suppose that the boundary position of one species did not coincide with that of the species which caused the first to

have a distribution, then since it did coincide at the beginning of the run (when all the boundaries were at the meniscus), it must be getting progressively further away. Since the boundary position is defined on the basis of quantity of material, the relative movement of two boundary positions means a net quantity of material would be transferred, which would alter the s rates in such a way as to oppose the change.

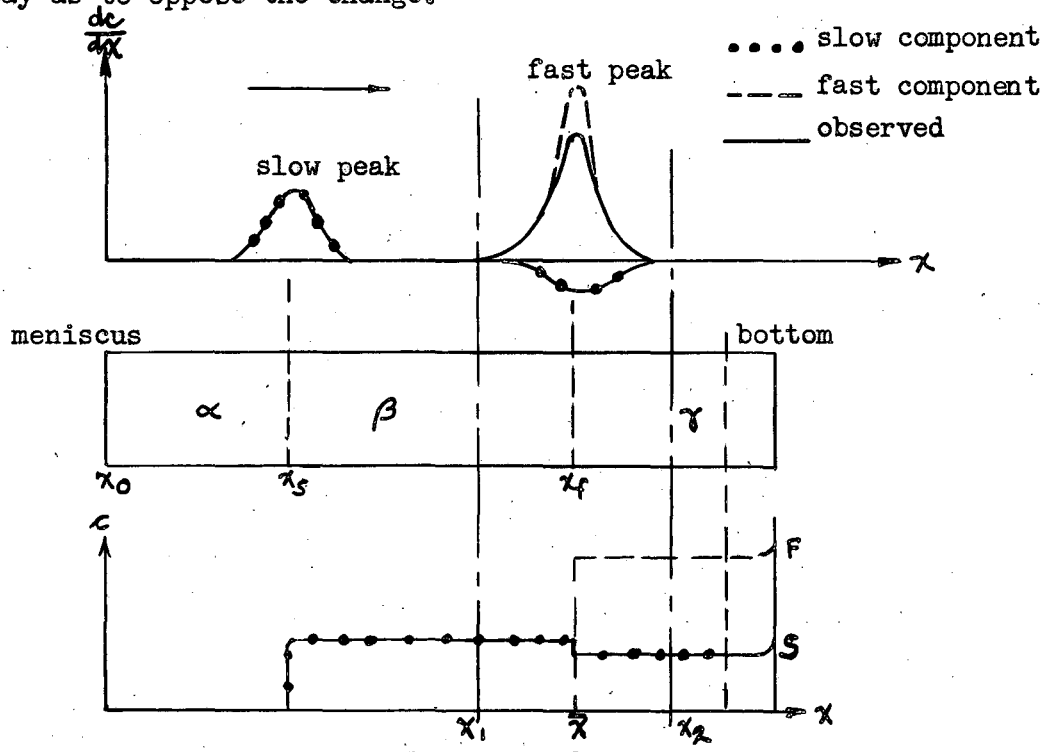

Fig. 4 Dole Transformation

The second value of the Dole Transformation is that in the absence of convection from boundary to boundary, the mathematical treatment of a moving boundary system is greatly simplified because the shape of the boundary need not be considered.

Moving Boundary Equation.

Referring to Figure 4, let c_j^{β} be the concentration of some species j in the β phase, and c_j^{γ} its concentration in the γ phase; and x_1 and x_2 be fixed with respect to the cell. Then the amount per unit time per unit thickness of hyperbolic cell entering the region between x_1 and x_2 from left to

right across x_1 is $(\omega^2 x_1 s_j^{\beta})(2K/x_1) c_j^{\beta}$; and that leaving across x_2: $(\omega^2 x_2 s_j^{\gamma})(2K/x_2) c_j^{\gamma}$. The rate of increase of the quantity contained between x_1 and x_2 is $(\omega^2 \bar{x} s^{\beta\gamma})(2K/\bar{x})(c_j^{\beta} - c_j^{\gamma})$ where $s^{\beta\gamma}$ is the velocity per unit field of the boundary position. Hence by the law of conservation of mass,

$$(\omega^2 x_1 s_j^{\beta})(2K/x_1) c_j^{\beta} - (\omega^2 x_2 s_j^{\gamma})(2K/x_2) c_j^{\gamma} = (\omega^2 \bar{x} s^{\beta\gamma})(2K/\bar{x})(c_j^{\beta} - c_j^{\gamma})$$

$$s_j^{\beta} c_j^{\beta} - s_j^{\gamma} c_j^{\gamma} = s^{\beta\gamma}(c_j^{\beta} - c_j^{\gamma})$$

which can be put into the following useful forms:

$$c_j^{\beta}(s_j^{\beta} - s^{\beta\gamma}) = c_j^{\gamma}(s_j^{\gamma} - s^{\beta\gamma}) \tag{11}$$

$$s^{\beta\gamma} = s_j^{\gamma}\left[1 - \left(\frac{s_j^{\beta} - s_j^{\gamma}}{s_j^{\gamma}}\right)\left(\frac{c_j^{\beta}}{c_j^{\gamma} - c_j^{\beta}}\right)\right] \tag{12}$$

$$\left.\begin{aligned} \frac{c_j^{\beta}}{c_j^{\gamma}} &= \frac{s_j^{\gamma} - s^{\beta\gamma}}{s_j^{\beta} - s^{\beta\gamma}} = 1 - \frac{s_j^{\beta} - s_j^{\gamma}}{s_j^{\beta} - s^{\beta\gamma}} \\ c_j^{\gamma} - c_j^{\beta} &= -\frac{s_j^{\beta} - s_j^{\gamma}}{s^{\beta\gamma} - s_j^{\beta}} c_j^{\gamma} \end{aligned}\right\} \tag{13}$$

Equation 11 is general; 12 and 13 are valid if one does not divide by zero. Because of the fundamental theorem mentioned above, the boundary position for the distribution of each species coincides in any one boundary region with that for any other species, and there is thus only one value of $s^{\beta\gamma}$ for each boundary region. Hence Eq. 11 is general for $j = 1, \ldots, n$ if there are n species classified according to s rates. Therefore at each boundary, in general, there are n equations of the form 11, n - 1 of which are independent, for if n - 1 concentrations are specified, the remaining one (say the solvent) is thus determined.

In analyzing the Dole transformed experimental pattern, s rates are to be used instead of velocities. In the hyperbolic cell in centrifugation

or rectangular cell in a uniform field, areas as read from the plates are to be used for boundary areas. In the sector cell, areas should be extrapolated from measurements from frame to frame to the meniscus for sedimentation or to the bottom of the cell for flotation. It is for these areas that the equations take a form similar to those for hyperbolic centrifugation. (See Eq. 46 in Appendix for t = 0). If only one concentration can be read during a run, then the extrapolation to the meniscus can be done to a first approximation by the square law (this neglects the change in the area anomaly during the run). (Compare again Eq. 46 in Appendix.)

Boundary s Rate.

In the usual ultracentrifuge technique, the initial boundary is the meniscus for sedimenting and the bottom of the cell for floating species. This technique is simpler than for electrophoresis, since in the latter, it is necessary to place an overlying solution in order to be able to introduce the electric field. The centrifuge pattern is always of the descending type, i.e. the boundaries always move into a solution containing protein. In this notation, the α phase is the (developed) supernatant, and a protein species can only disappear across a boundary from a higher numbered phase to a lower, e.g. can be present in γ but absent in β. Suppose then that some species k appears across the $\beta\gamma$ boundary. Then in Eq. 11, $c_k^\beta \equiv 0$ and $s_k^\gamma \equiv s^{\beta\gamma}$. Thus the s rate of a boundary measures the s rate of the appearing species (called a leading species by Svensson[8] in electrophoresis) in the phase containing this species.

Area Anaomaly.

From Eq. 13, it follows that if the s rate of a component is different on the two sides of a boundary, its concentration will also be different. Thus in general, it is to be expected that each boundary is made up of super-

imposed gradients. The failure of an observed area to correspond to the concentration of a particular component is one of the classical anomalies of ultracentrifugation and electrophoresis. It is seen from Eq. 13 to be a natural consequence of the macroscopic law of conservation of mass analysis of a moving boundary system. In particular, Johnston and Ogston[1] have emphasized the importance of the superimposed gradient of the slower species on the boundary of the faster component in a two component mixture. Their formula results if we let j = s, the slow component, $s^{\beta\gamma} = s_f^{\gamma}$ the s rate of the fast component in the γ phase in Eq. 13.

$$\frac{c_s^{\beta}}{c_s^{\gamma}} = \frac{s_f^{\gamma} - s_s^{\gamma}}{s_f\gamma - s_s^{\beta}} \tag{14}$$

which can be rearranged to:

$$c_s^{\gamma} - c_s^{\beta} = -\frac{s_s^{\beta} - s_s^{\gamma}}{s_f^{\gamma} - s_s^{\beta}}\, c_s^{\gamma} = -\frac{s_s^{\beta} - s_s^{\gamma}}{s_f^{\beta} - s_s^{\gamma}}\, c_s^{\beta} \tag{15}$$

Enoksson[2] on the other hand, in his second effect of backward flow has apparently focussed attention on the solvent concentration change at a boundary. Both effects are described by Eq. 11, letting j take all values, including the solvent. Note that the first effect of Enoksson on backward flow is taken into account by referring quantities to the cell, and that when j = solvent <u>net</u> s rates and concentration of solvent are involved. Thus at this level the analysis does not consider the partial flows making up the net flow which are so important to consider from the microscopic viewpoint.

Equation 14 shows that in the hyperbolic cell we have not eliminated the Johnston-Ogston effect, but have removed the time dependence it has in the sector cell. Let us focus our attention on refractive index gradient measurements. Then, the solvent concentration change is included when the refractive

increment is specified, and we need consider for the area anomaly only the protein concentration changes. Hence for a two component system, we can write from Figure 4:

$$\begin{aligned} c_s^{obs} &= c_s^{\beta} = c_s^{\gamma} + (c_s^{\beta} - c_s^{\gamma}) > c_s^{\gamma} \\ c_f^{obs} &= c_f^{\gamma} - (c_s^{\beta} - c_s^{\gamma}) < c_f^{\gamma} \\ c_s^{obs} + c_f^{obs} &= c_s^{\gamma} + c_f^{\gamma} \end{aligned} \tag{16}$$

Hence it is important to determine the relationship between c_s^{β} and c_s^{γ}. This is given by the Johnston-Ogston formula 15, which will be considered in greater detail in the next section.

Deductions About the Johnston-Ogston Effect Using an Assumed s versus c Dependence.

In order to make deductions from Eq. 15, the following assumptions are made:[4] (1) s versus c curve for both the fast and slow species is linear in range used, i.e.,

$$\begin{aligned} s_f &= s_f^o (1 - k_f c_f) \\ s_s &= s_s^o (1 - k_s c_s) \end{aligned} \tag{17}$$

(2) In a mixture, the sedimentation rate is influenced by other species in the same way in which those species influence themselves, i.e.,

$$\begin{aligned} s_s^{\gamma} &= s_s^o (1 - k_s c_s^{\gamma} - k_f c_f^{\gamma}) \\ s_f^{\gamma} &= s_f^o (1 - k_s c_s^{\gamma} - k_f c_f^{\gamma}) \\ s_s^{\beta} &= s_s^o (1 - k_s c_s^{\beta}) \end{aligned} \tag{18}$$

Note that if the k's are constant, $1 - k_s c_s^{\gamma} - k_f c_f^{\gamma}$ can be written linearly in terms of the dilution d if the same mixture is run at several dilutions as

$$\frac{s_s^{\gamma}}{s_s^o} = \frac{s_f^{\gamma}}{s_f^o} = 1 - k_s c_s^{\gamma} - k_f c_f^{\gamma} = 1 - d (k_s c_s^{\gamma} + k_f c_f^{\gamma})_{d=1} \tag{19}$$

Substitution of Eq. 18 into Eq. 15 yields:

$$c_s^{\gamma} - c_s^{\beta} = - \frac{s_s^o}{s_f^o - s_s^o} \frac{k_f c_f^{\gamma} - k_s (c_s^{\beta} - c_s^{\gamma})}{1 - k_s c_s^{\gamma} - k_f c_f^{\gamma}} c_s^{\beta} \qquad (20)$$

We would like to simplify this expression so that predictions of the magnitude of the area anomaly and proper extrapolation plots to infinite dilutions can be set up.

Assume for a first approximation that

$$k_s (c_s^{\beta} - c_s^{\gamma}) \ll k_f c_f^{\gamma} \qquad (21)$$

$$k_s c_s^{\gamma} + k_f c_f^{\gamma} \ll 1 \qquad (22)$$

The resulting value of c_s^{β} can be used to check the validity of the assumption. If it is not good enough then that value can be used in Eq. 20 for a second approximation.

Equation 20 reduces to

$$\frac{c_s^{\gamma}}{c_s^{\beta}} \approx 1 - \frac{s_s^o}{s_f^o - s_s^o} k_f c_f^o$$

$$c_s^{\beta} - c_s^{\gamma} = \frac{s_s^o}{s_f^o - s_s^o} k_f c_f^{\gamma} c_s^{\beta} \qquad (23)$$

This can be rearranged to give

$$k_s (c_s^{\beta} - c_s^{\gamma}) = \left[\frac{s_f^o}{s_f^o - s_s^o} k_s c_s^{\beta}\right] (k_f c_f^{\gamma})$$

Hence approximation (21) will be valid if

$$\left(\frac{s_f^o}{s_f^o - s_s^o} k_s c_s^{\beta}\right) \ll 1 \qquad (24)$$

Assuming that Eq. 24 is valid, the expressions for the fast and slow observed areas can be written from Eq. 16 and Eq. 23 in terms of the infinite dilute s rates, concentrations in the cell, and the k's of the s versus c dependence plots.

$$c_f^{obs} = c_f^{\gamma}\left[1 - \frac{s_s^o}{s_f^o - s_s^o} k_f\, c_s^{obs}\right]$$

$$c_s^{obs} = c_s^{\gamma}\left[\frac{1}{1 - \frac{s_s^o}{s_f^o - s_f^o} k_f\, c_f^{\gamma}}\right] \qquad (25)$$

Therefore we can write

$$\frac{c_f^{obs}}{c_f^{obs} + c_s^{obs}} = \left(\frac{c_f^{\gamma}}{c_f^{\gamma} + c_s^{\gamma}}\right)\left(1 - \frac{s_s^o}{s_f^o - s_s^o} k_f\, c_s^{obs}\right) \qquad (26)$$

$$\frac{c_s^{obs}}{c_f^{obs} + c_s^{obs}} = \left(\frac{c_s^{\gamma}}{c_f^{\gamma} + c_s^{\gamma}}\right)\left(\frac{1}{1 - \frac{s_s^o}{s_f^o - s_s^o} k_f\, c_f^{\gamma}}\right)$$

Note that the ratio

$$\frac{c_f^{obs}}{c_s^{obs}} \approx \left(\frac{c_f^{\gamma}}{c_s^{\gamma}}\right)\left(1 - \frac{s_s^o}{s_f^o - s_s^o} k_f\, c_s^{obs}\right)\left(1 - \frac{s_s^o}{s_f^o - s_s^o} k_f\, c_f^{\gamma}\right)$$

is non linear in $(c_s^{obs} + c_f^{obs})$

The usual deductions[4] concerning the area anomaly follow directly from these equations. It is worthy of note that to a first approximation the error in ascribing a measured area to the initial concentration of one component depends directly on the concentration of the other component, and on the s versus c dependence of the faster component. From a theoretical and practical point of view, there is a definite need to rigorously investigate the limit as $s_s^o \longrightarrow s_f^o$, and to extend the analysis to a multicomponent system.

Extension to Multicomponent System.

In the absence of detailed mathematical theory for the general case, the following method is suggested for obtaining the composition of an unknown mixture in which the components are chemically independent, i.e., are not in some sort of dissociation-association equilibrium.

1. Run the mixture at several dilutions.

2. For each run determine the ratio of area of each peak to the total area of that peak and all the slower peaks.

3. From the data for each run, plot these ratios for each peak versus the area of all the slower peaks. Extrapolate these curves to infinite dilution. They should be linear if the k's are constant according to Eq. 26.

4. Obtain the true composition by successive differences. That is, in a three component system, the true ratio of the fastest component to the total concentration is obtained directly from the plot. The ratio of the sum of the two slower components to the total is obtained by difference. Call this difference A. The composition of the middle component is obtained by multiplying the extrapolated value of the ratio of the middle component to the sum of the middle plus the slowest times A. The slowest component's concentration is obtained by difference.

5. An idea of the magnitude of the k's can be obtained from the slope of the area ratio plots and the infinite dilution s rates using Eq. 26. The infinite dilution s rates can be obtained by extrapolating a plot of measured s rate versus area of that peak and all slower peaks to infinite dilution. This plot will be linear if the k's are constant as seen from Eq. 19.

6. In all unknown studies the aim should be to simplify the mixture as much as is possible before running. In particular when there are lipoproteins present with other proteins, an extreme case of the Johnston-Ogston effect can be present in which relative velocity of two species is reversed in sign on the two sides of a boundary. Thus, s versus total concentration for the lipoprotein species goes below zero due to the density effect in addition to the viscosity effect. This results in pile-up, which has been adequately described by Gofman, Lindgren and Elliott[9].

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APPENDIX

Classical Plateau Concentration Relation in Sector-Centrifugation[10,11].

Referring to Fig. 3 we can draw arcs on the cell such that all the particles dN between A and B at time t_1 are between A' and B' at time t_2. Assume that s is a constant.

$$s = \frac{\frac{dx}{dt}}{\omega^2 x}, \quad \int_{x_2}^{x_3} \frac{dx}{x} = \int_{t_1}^{t_2} \omega^2 s\,dt,$$

$$\ln \frac{x_3}{x_2} = \omega^2 s (t_2 - t_1)$$

$$\therefore \frac{x_3}{x_2} = e^{\omega^2 s (t_2 - t_1)} \tag{27}$$

Since this is true for particles at any x, it will be true for those located at $x_2 + dx_2$ moving to $x_3 + dx_3$. Thus

$$\frac{x_3 + dx_3}{x_2 + dx_2} = e^{\omega^2 s (t_2 - t_1)}$$

$$x_3 - x_2 e^{\omega^2 s (t_2 - t_1)} = dx_2\, e^{\omega^2 s (t_2 - t_1)} - dx_3$$

Substitute Eq. 27

$$\frac{dx_3}{dx_2} = e^{\omega^2 s (t_2 - t_1)} = \frac{x_3}{x_2} \tag{28}$$

Now

$$c\,(x_2,\ t_1) = \frac{dN}{x_2\ 2\vartheta\ dx_2\ b}$$

$$c\,(x_3,\ t_2) = \frac{dN}{x_3\ 2\vartheta\ dx_3\ b}$$

$$\frac{c\,(x_3,\ t_2)}{c\,(x_2,\ t_1)} = \frac{x_2\ dx_2}{x_3\ dx_3} = \left(\frac{x_2}{x_3}\right)^2 \qquad (29)$$

Suppose that at t_1 the concentration everywhere was c_o, i.e. neglect compressibility. Then (27) in (29) gives

$$\frac{c\,(x_3,\ t_2)}{c_o} = e^{-2\omega^2 s\,(t_2 - t_1)} \qquad (30)$$

This is independent of x_3, hence the concentration is everywhere the same (i.e. a plateau exists), decreasing with time.

In moving boundary centrifugation there is no source continually feeding in material, so that when the material originally at the meniscus goes past a point the concentration falls to zero. But until that time, we can consider the boundary region to move down the cell preceded by a plateau region, the area under the curve being proportional to the concentration in the plateau region.

If it were not for the bottom of the cell, the maximum concentration would drop for a standard Spinco[6] cell

$$\left(\frac{x_{\text{meniscus}}}{x_{\text{bottom}}}\right)^2 = \left(\frac{5.95}{7.25}\right)^2 = 0.67$$

or by about one-third in going from the meniscus to the bottom.

Knowing that diffusion occurs when there is a concentration gradient, the concentration distribution in sedimentation as a result of diffusion broadening the boundary region and "back diffusion" broadening the pile-up at the bottom of the cell is indicated in Fig. 5.

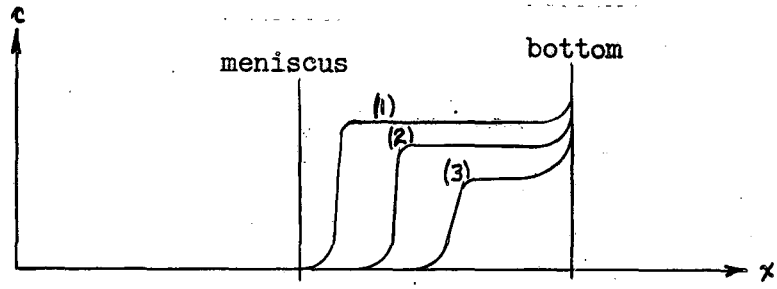

Fig. 5
Concentration Distribution at successive times (1), (2), (3) in sector sedimentation

Boundary Position.

Paralleling the treatment leading to Eq. (8) we can write for the sector cell:

$$\bar{x}^2 = \frac{\int_{x_1}^{x_2} x^2\left(\frac{\partial c}{\partial x}\right) dx}{\int_{x_1}^{x_2} \left(\frac{\partial c}{\partial x}\right) dx} \qquad (31)$$

Thus, locating $\bar{x}$ involves determining the second moment of the concentration gradient curve. Using the approximation

$$x = x_1 + z$$

$$x^2 \approx x_1^2 \left(1 + \frac{2z}{x_1}\right)$$

Equation 31 can be written

$$\bar{x} \approx \frac{\int_{x_1}^{x_2} x\left(\frac{\partial c}{\partial x}\right) dx}{\int_{x_1}^{x_2} \left(\frac{\partial c}{\partial x}\right) dx} \qquad (32)$$

So that to a first approximation on skew areas, the position of the center of gravity (first moment) should be used as the boundary position, rather than the maximum ordinate.

Equation of Continuity for plateau region for Sector-Centrifugation.

The equation for a side of the sector cell is $y = x \tan \theta$ (Fig. 3) so that Eq. (1) reduces to:

$$\frac{\partial c}{\partial t} = - \frac{\omega^2}{x} \frac{\partial (sc\, x^2)}{\partial x} \tag{33}$$

Because of the initial plateau of c, Eq. 30 and the discussion above indicate that the pattern can always be interpreted as boundaries separated by plateaus. Hence c is not a function of x in any phase and therefore s is also not a function of x (for the time that no boundary passes through the level x). Equation 33 then becomes in general

$$\frac{dc}{dt} = - \frac{\omega^2 sc}{x} \frac{d(x^2)}{dx} = -2 \omega^2 sc \tag{34}$$

Integrate Eq. 34 for two special cases:

a. s is independent of c

$$- \int_{c_o}^{c} \frac{dc}{c} = 2\omega^2 s \int_{o}^{t} dt$$

$$c_o/c = e^{2\omega^2 st} = \left(\frac{x}{x_o}\right)^2$$

which is the result obtained previously (Eq. 29).

b. $s = s^o (1 - kc)$

$$- \int_{c_o}^{c} \frac{dc}{c (1 - kc)} = 2\omega^2 s^o \int_{o}^{t} dt$$

$$\frac{c_o}{c} \left(\frac{1 - kc}{1 - kc_o}\right) = e^{2\omega^2 s^o t}$$

This can be rearranged to give:

$$\frac{c_o}{c} = e^{2\omega^2 s^o t} \left[1 - k c_o (1 - e^{-2\omega^2 s^o t})\right]$$

$$\approx e^{2\omega^2 s^o t} e^{-2\omega^2 s^o k c_o t}$$

$$= e^{2\omega^2 s^o (1 - kc_o) t}$$

where e^x has been approximated by $1 + x$ and vice versa.

Rewrite this in terms of x:

$$\frac{dx}{x} = \omega^2 s dt = \omega^2 s^o (1 - kc)\, dt$$

$$= \left[\omega^2 s^o - \omega^2 s^o k c_o e^{-2\omega^2 s^o (1 - k c_o) t}\right] dt$$

Integrate from x_o to x, 0 to t:

$$\ln \frac{x}{x_o} = \omega^2 s^o t - \frac{k c_o}{2 (1 - k c_o)} \left[1 - e^{-2\omega^2 s_o (1 - k c_o)t}\right]$$

$$\approx \omega^2 s^o t - \frac{k c_o}{2(1 - k c_o)} \left[2 \omega^2 s^o (1 - k c_o)t\right]$$

$$= \omega^2 s^o (1 - k c_o)t$$

Therefore:

$$\left(\frac{x}{x_o}\right)^2 = e^{2\omega^2 s^o (1 - k c_o)t} = \frac{c_o}{c} \qquad (35)$$

Hence even when s is a function of c, the square law relationship is a very good approximation for areas.

General Equation for the Average Concentration in β Region in Sector-Centrifugation With or Without Convection.

Referring to Fig. 4 the total mass of the slow component S before sedimentation begins between the meniscus in the cell, x_o, and x_f the position of the fast boundary after time t_1 is: $c_s^o (x_f^2 - x_o^2) \pi b \frac{2\theta}{2\pi}$. Expressed per unit half angle, θ, per unit thickness b of cell, this is $m_s^o = c_s^o (x_f^2 - x_o^2)$. The amount of slow component passing through the level x_f in time dt is $c_s^\gamma (\omega^2 x_f s_s^\gamma) 2x_f \theta b dt$. Expressed per unit half angle θ, per unit thickness b, this is $dm_s = 2\omega^2 x_f^2 c_s^\gamma s_s^\gamma dt$. During the time t from the start of sedimentation, the total amount of slow component passing through this level

(expressed per unit half angle per unit thickness) is

$$m_s \equiv \int_{t=0}^{t=t} dm_s \equiv 2\omega^2 x_f^2 \int_0^t c_s^{\gamma} s_s^{\gamma} dt \qquad (36)$$

Since we would expect no radial convective transport in the γ solution, Eq. 34 can be expected to hold (even though s is a function of c). Thus the integral of Eq. 36 can be expressed as

$$\int_0^t c_s^{\gamma} s_s^{\gamma} dt \equiv -\frac{1}{2\omega^2} \int_0^t \frac{dc_s^{\gamma}}{dt} dt$$

$$\equiv -\frac{1}{2\omega^2}\left[c_s^{\gamma}(t) - c_s^{\gamma}(o)\right]$$

and m_s can be written $m_s \equiv -x_f^2\left[c_s^{\gamma}(t) - c_s^{\gamma}(o)\right]$. The amount remaining behind the x_f level is thus $\Delta m_s \equiv m_s^o - m_s \equiv x_f^2 c_s^{\gamma}(t) - x_o^2 c_s^o$ since $c_s^o \equiv c_s^{\gamma}(o)$. The volume that this quantity of material is distributed in is $\pi(x_f^2 - x_s^2)\frac{2\theta}{2\pi} b$ or expressed per unit half angle per unit thickness: $v^{\beta} \equiv x_f^2 - x_s^2$. The average concentration of the slow component in the β region then is* $\bar{c}_s^{\beta}(t) \equiv \frac{\Delta m_s}{v^{\beta}} \equiv \frac{x_f^2 c_s^{\gamma}(t) - x_o^2 c_s^o}{x_f^2 - x_s^2}$ (37)

From Fig. 4, the following can be written

$$c_s^{obs} \equiv \bar{c}_s^{\beta}$$
$$c_f^{obs} \equiv c_f^{\gamma c} - (\bar{c}_s^{\beta} - c_s^{\gamma}) \qquad (38)$$

where it is assumed that only a simple slow peak occurs as a result of the density inversion convection.

Define corrected areas as the area of a peak multiplied by $\left(\frac{x}{x_o}\right)^2$ where x is the position of that peak.

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* This derivation is similar to that in reference 4.

$$c_s' \equiv \left(\frac{x_s}{x_o}\right)^2 c_s^{obs}$$
$$c_f' \equiv \left(\frac{x_f}{x_o}\right)^2 c_f^{obs} \qquad (39)$$

From Eq. 38 these can be written

$$c_s' = \left(\frac{x_s}{x_o}\right)^2 \bar{c}_s^{\beta}$$
$$c_f' = \left(\frac{x_f}{x_o}\right)^2 (c_f^{\gamma} + c_s^{\gamma} - \bar{c}_s^{\beta}) \qquad (40)$$

Investigate the sum of the two corrected areas $S_{fs} \equiv c_f' + c_s'$ and the difference $D_{fs} \equiv c_f' - c_s'$ using the sector Johnston-Ogston formula Eq. 37. This can be rearranged to

$$\left(\frac{x_f}{x_o}\right)^2 \bar{c}_s^{\beta} - \left(\frac{x_s}{x_o}\right)^2 \bar{c}_s^{\beta} = \left(\frac{x_s}{x_o}\right)^2 c_s^{\gamma} - c_s^{o} \qquad (41)$$

or $$c_s' = \left(\frac{x_f}{x_o}\right)^2 (\bar{c}_s^{\beta} - c_s^{\gamma}) + c_s^{o} \qquad (42)$$

Hence,

$$S_{fs} = c_f' + c_s' = \left(\frac{x_f}{x_o}\right)^2 c_f^{\gamma} + c_s^{o} = c_f^{o} + c_s^{o} \qquad (43)$$

The sum of the corrected areas equals the sum of the true concentrations.

Similarly,

$$D_{fs} = c_f' - c_s' = 2c_f' - (c_f^o + c_s^o)$$

Thus the difference yields an expression containing the sum of the true concentrations rather than their difference and is hence of no value, when combined with Eq. 43 for calculating c_s^o and c_f^o.

Effect of s versus c Dependence.

From Eq. 35, Eq. 37 can be written:

$$\frac{\bar{c}_s^{\beta}\left(\frac{x_s}{x_o}\right)^2}{c_s^{o}} = \frac{\left(\frac{x_f}{x_o}\right)^2 e^{-2\omega^2 s_s^{\gamma} t} - 1}{\left(\frac{x_f}{x_o}\right)^2 \left(\frac{x_o}{x_s}\right)^2 - 1} \qquad (44)$$

and

$$\frac{x_f}{x_o} = e^{\omega^2 s_f^{\alpha} t}$$

$$\frac{x_s}{x_o} = e^{\omega^2 s_s^{\beta} t} \qquad (45)$$

$$\frac{\left(\frac{x_s}{x_o}\right)^2 \frac{-\beta}{c_s}}{c_s^{o}} = \frac{e^{2\omega^2 t (s_f^{\alpha} - s_s^{\alpha})} - 1}{e^{2\omega^2 t (s_f^{\alpha} - s_s^{\beta})} - 1}$$

To simplify this expression and to determine dependence on t, expand exponentials according to $e^x - 1 \approx x\, e^{x/2}$. This yields

$$\frac{\left(\frac{x_s}{x_o}\right)^2 \frac{-\beta}{c_s}}{c_s^{o}} = \frac{s_f^{\alpha} - s_s^{\alpha}}{s_f^{\alpha} - s_s^{\beta}} e^{\omega^2 t (s_s^{\beta} - s_s^{\alpha})} \qquad (46)$$

and

$$\frac{\frac{-\beta}{c_s}}{\frac{-\alpha}{c_s}} = \frac{s_f^{\alpha} - s_s^{\alpha}}{s_f^{\alpha} - s_s^{\beta}} e^{-\omega^2 t (s_s^{\beta} - s_s^{\alpha})}$$

Thus, in the sector cell, the ratio of concentrations of the slow species at the fast boundary is modified over the Johnston-Ogston formula. Not only is there an explicit dependence on t, but due to the dilution during the run the s's will change with t as mentioned before. When t = 0

$$\frac{c_s'}{c_s^{o}} = \frac{s_f^{\alpha} - s_s^{\alpha}}{s_f^{\alpha} - s_s^{\beta}} \qquad (47)$$

Thus Harrington and Schachman[4] recommend extrapolation to the meniscus for data to verify the Johnston-Ogston Formula. They obtained a straight line relationship with negative slope when plotting c_s'/c_s^o against x_s.

Using the same s versus c assumption as before (Eq. 18) Eq. 46 can be written

$$\frac{c_s^o}{c_s'} = 1 - \left\{ k_f\, c_f^{\alpha}(t) - k_s \left[c_s^{\beta}(t) - c_s^{\alpha}(t) \right] \right\} \left[\frac{s_s^o/(s_f^o - s_s^o)}{1 - k_s\, c_s^{\alpha}(t) - k_f\, c_f^{\alpha}(t)} - \omega^2 s_s^o\, t \right.$$

where the c's have the time dependence:

$$c_s^{\gamma}(t) = e^{-2\omega^2 s_s^{\circ}(1 - k_s c_s^{\circ} - k_f c_f^{\circ})t}$$

$$c_s^{\beta}(t) = e^{-2\omega^2 s_s^{\circ}(1 - k_s c_s^{\circ})t}$$

$$c_f^{\gamma}(t) = e^{-2\omega^2 s_f^{\circ}(1 - k_s c_s^{\circ} - k_f c_f^{\circ})t}$$

This equation is quite complicated and shows the tremendous problem of trying to use one area measurement (c_s') in a sector centrifugation run to determine c_s° without foreknowledge of the magnitude of the s versus c k's. If corrected areas are also extrapolated back to the meniscus to remove the time dependence, then Eq. 47 holds and conclusions drawn from the Johnston-Ogston formula should apply.

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