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OSMOSEDIMENTATION METHODS: PREPARATIVE AND ANALYTICAL APPLICATIONS

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Sedimentation Phenomena

Mass transfer occurs in every uniform, many-component system submitted to a gravitational or inertial field. The net result of this mass transfer is a lowering of the mass center of the system and the concurrent formation of concentration gradients. The lower-density components move upwards, the higher density components move downwards, until every component reaches a uniform chemical potential, throughout the system[1].

Uniform chemical potentials in gravitational or inertial fields require the existence of concentration gradients, according to the "barometric" equations[2]; in an ideal solution:

$$\frac{d(\ell n\ x_i)}{dz} = \frac{M_i(1-\bar{v}_i\rho)g}{RT} \quad \text{(under gravity)} \qquad (1)$$

and

$$\frac{d(\ell n\ x_i)}{dr^2} = \frac{M_i(1-\bar{v}_i\rho)\omega^2}{2RT} \quad \text{(under centrifugation)} \qquad (2)$$

which describe changes in component *i* mol fraction, x_i as a function of either *z*, the depth within the solution column or r^2, the squared distance from a point within the solution to the centrifugation axis.

Concentration gradients at sedimentation equilibrium have been very useful, throughout this century. Some examples of this assertion are the fundamental work of Jean Perrin[3,4] on the kinetic behavior of the matter and *R* constant evaluation, and the extensive use of equilibrium sedimentation in the determination of macromolecular MW's, MW distributions and virial coefficients[5-10].

However, in most of the actual sedimentation or centrifugation experiments currently performed by chemists, biochemists, molecular biologists, colloid scientists and other workers, sedimentation equilibrium state is not actually reached. There is a large number of non-equilibrium preparative and analytical methods, for polymer and particle fractionation, separation, concentration and characterization which are fast, effective and thus widely used.

Sedimentation Kinetics

Equilibrium sedimentation thermodynamic equations (such as eqs. 1 and 2) apply to every conceivable fluid system, either gas or liquid, particle dispersion, macromolecular or micromolecular solution. However, the concentration gradients predicted by equations 1 and 2 are seldom observed, due to the usually slow kinetics of sedimentation, in liquid solutions and colloidal sols.

We may consider, as a first approximation, that sedimentation kinetics is described by Stokes' law: the rate of vertical displacement of a spherical particle in an inertial or gravitational field is given by the ratio between its buoyant weight and friction with the surrounding fluid[11]. Under gravity, equation 3 holds:

$$\frac{dz_i}{dt} = v_i = \frac{m_i(1-\bar{v}_i\rho)g}{6\pi\eta a_i} = \frac{2a_i^2(\rho_i-\rho)g}{9\,\eta} \qquad (3)$$

where the last equality holds for spherical particles, only.

As a result, sedimentation rates for a substance of a given ρ_i will depend on particle radius a_i and on the fluid medium density and viscosity. In the case of an inertial field, $\omega^2 r$ is substituted for g, in eq. 3.

Using eq. 3 we can verify that particles having, for instance, $\rho_i = 2$, in water, have high sedimentation velocities under gravity, if their radii exceeds 1 µm; otherwise they settle slowly; 1 nm particles settling velocity is negligible[11], of the order of 8 nanometers per hour.

Rate of approach to sedimentation equilibrium has been extensively dealt with, in the literature; fundamental work done by Mason and Weaver[12], Archibald[13], Van Holde and Baldwin[14] led to equations useful for predicting the times taken by a given system to approach sedimentation equilibrium, to a given extent.

If a centrifuge is run so as to satisfy the following inequality:

$$\frac{RT}{M_2(1-\bar{v}_2\rho)\omega^2\bar{r}h} > 0.6 \tag{4}$$

where $\bar{r}$ is the distance between the rotation axis and the middle of the solution column, h is the solution column height, the following equation can be used[14,15]:

$$t_{0.1\%} \doteq 0.7\ h^2/D \tag{5}$$

where $t_{0.1\%}$ is the time required to reach concentrations within 0.1% of the equilibrium values, at any point in the cell.

This shows that a typical macromolecular solute ($D = 10^{-6} - 10^{-7}$ cm^2 s^{-1}) will take many hundred hours to equilibrate in a 1-cm tall aqueous solution column. Many-centimeter or meter-long columns are obviously useless, for this purpose. This explains why we can keep protein and other polymer solutions in containers, without observing the fairly large concentration gradients predicted by the thermodynamic relationships. For instance, dissolved proteins are not concentrated by centrifugation, even though eq. 1 predicts a large concentration gradient for, let say, bovine serum albumin spun at 3000 rpm, at 4°C, in aqueous solution:

$$\frac{d(\ell nc)}{d(r^2)} = 0.036\ \text{cm}^{-2} \tag{6}$$

which means that 32% of the albumin should be found within the lowest 20% of the solution column at this speed. Unfortunately, it will take ca. 9 years for equilibrium attainment in an albumin solution within a standard, 10 cm tall centrifuge tube.

Sedimentation of small particles and of macromolecules gives thus good examples of thermodynamically spontaneous but kinetically restricted phenomena - many conceivable experiments are not feasible because they will occur but in excessively long times.

Osmosedimentation: Early Observations

Porter, from the University of London, in the early twenties, concerned himself with the problem of equilibrium sedimentation in colloidal sols[16]. He was not aware of the kinetic problems involved but made an important contribution: he demonstrated theoretically that osmotic equilibrium across a semipermeable membrane should be concurrent with sedimentation-diffusion equilibrium[17]. To rephrase it, he showed that approach to equilibrium in an osmotic system should lead to a difference in the hydrostatic heads and to concentration gradients, at once.

Thirty years later[18,19] Lang, in Germany, verified (apparently, without knowing about Porter's work) that concentration gradients were formed within an osmometer fitted with a vertical membrane. It seems that Lang's motivation was the solution of an important question of his time: how should osmometers be built, to minimize experimental errors? Lang did not further this work for two reasons, apparently: first, he did not notice that the time scale at which solute was concentrating at the osmometer bottom was much shorter than that usually observed, in sedimentation; second, he believed (although no clues are given for the source of his belief) that he could not use his concentration gradients to calculate molecular-weights.

In 1977, M. Jafelicci Jr., a student from this laboratory, was preparing iron oxide sols. Although these sols did not settle while contained in glass or plastic containers they did so, during dialysis. It seems that similar observations are often made in biochemical laboratories, during protein dialysis. Without knowing about Lang's work we started to examine this phenomenon, from both theoretical and experimental points of view. It was fortunate to have done so without knowing about previous work, in the earlier part of this project - this would probably have prevented us from moving ahead[20].

Osmosedimentation : Theoretical Aspects

Monodisperse systems. A solution under a gravitational or inertial field approaches sedimentation equilibrium by solute and solvent mass transfer, along the field. The denser component moves to the solution containers' bottom, while the lighter component moves upwards. This is often too slow: even a 50 S sedimenting solute will have a vertical displacement velocity of 1.5 x 10^{-7} m s^{-1}, under an inertial field equal to 3000 x gravity[7].

On the other side, if the solution (denser than solvent) is separated from the solvent by a semipermeable membrane, solvent may flow to and from the solution, depending on the liquid column height. Solvent flow to the solution is obviously expected, due to osmotic forces; solvent flow from the solution occurs at points beneath the liquid surface wherein the greater pressure within the solution column leads to reverse osmosis[21].

The height of a dialysis cell needed to generate reverse osmosis under centrifugation can be easily evaluated[21]. Using an arrangement such as depicted in Fig. 1, containing an ideal binary solution of uniform composition:

$$J_{H_2O,x} = -K\Delta\mu_{H_2O,x}(r) \tag{7}$$

and

$$\Delta\mu_{H_2O,x}(r) = RT\,\ell n\, x_{H_2O} + \frac{1}{2}\bar{V}_{H_2O}\,\Delta\rho\omega^2\,(r^2-r_0^2) \tag{8}$$

The horizontal (perpendicular to the membrane) solvent mass current $J_{H_2O,x}$ contains two terms: one depends on x_{H_2O} and is independent of the field; it leads to solute dilution; the other varies from solution top to bottom and contributes to solute concentration.

Using equations 7 and 8 it is possible to calculate that in an ideal, 1% solution of a 130.000-MW solute having $\bar{v} = 0.7$ cm^3 g^{-1} contained in a dialysis cell at 4°C, which top is at a 15 cm distance from the spinning axis (3000 rpm), osmosis occurs from the solution column top down to a depth of ca. 0.4 cm; reverse osmosis takes place from there on.

Bulk liquid movement drags solute to the cell bottom. This will go until a steady concentration gradient is obtained. At this point, solute and solvent chemical potentials are uniform and solute concentration varies along the cell, according to the barometric equation (eq. 2).

Polydisperse systems. When considering polydisperse systems a question arises: is there solute redistribution throughout the cell? That means: do lower-MW solute fractions give smaller concentration gradients than high-MW fractions? Looking at Figure 1

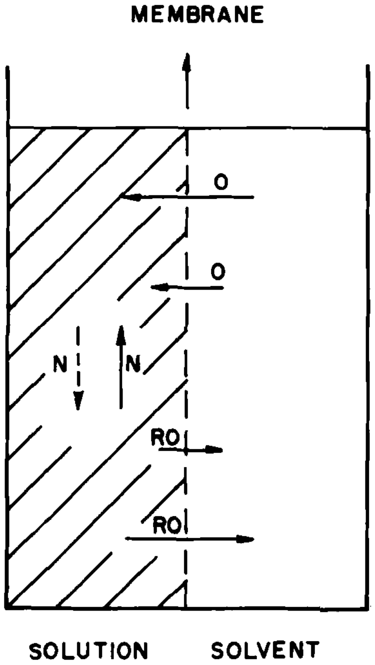

FIGURE 1

Osmotic (O) and reverse osmotic (RO) mass currents in a dialysis cell, under gravity or an inertial field. N are the normal sedimentation currents of the lighter (——→) and denser (--→) components.

one might be tempted to conclude that different fractions should not give different concentration gradients, since solute mass transfer is a result of overall solute dragging by solvent. However, there is some experimental evidence showing that solute fractionation may go on, in some cases. This problem has not been fully elucidated, from a theoretical point of view. Our current working hypothesis is the following: *horizontal* solute concentration gradients are also formed within the dialysis cell, as a result of solvent osmosis and reverse osmosis, as shown schematically in Fig. 2. The ensuing *density* gradients lead to convective instabilities and to another mass transfer mechanism: less dense, more dilute solution moves up, more concentrated solution moves down. One should expect the more dilute convective layers to be richer in lighter, faster-diffusive particles, as depicted in Fig. 3. This would account for solute fractionation, in osmosedimentation; as of this time, this mechanism has no quantitative expression but it is substantiated by some experimental results, given in the following sections. Moreover, fractionation by this diffusion - convection mechanism has been demonstrated, many years ago[22].

Irreversible thermodynamics treatments. Osmosedimentation has been examined using two different approaches based on irreversible thermodynamics formalisms.

First, the rate of approach to equilibrium in a system in contact with temperature and pressure reservoirs, in the absence of chemical reactions, is given by:[23]

$$\frac{dG}{dt} = \sum_{i} \sum_{\alpha A} \psi_{i,\alpha A} \, \Delta\mu_{i,\alpha A} \simeq \sum_{i} \sum_{\alpha A} - K\Delta\mu^{2}_{i,\alpha A} \qquad (9)$$

The rate of decrease of G will thus increase as i) the number of (*i*) components and ii) the number of (αA) phase boundaries both increase. In an osmosedimentation experiment there are more pathes for mass transfer (the αA's), leading to faster mass transfer. Equation 9 allowed us to predict that mass transfer rates in

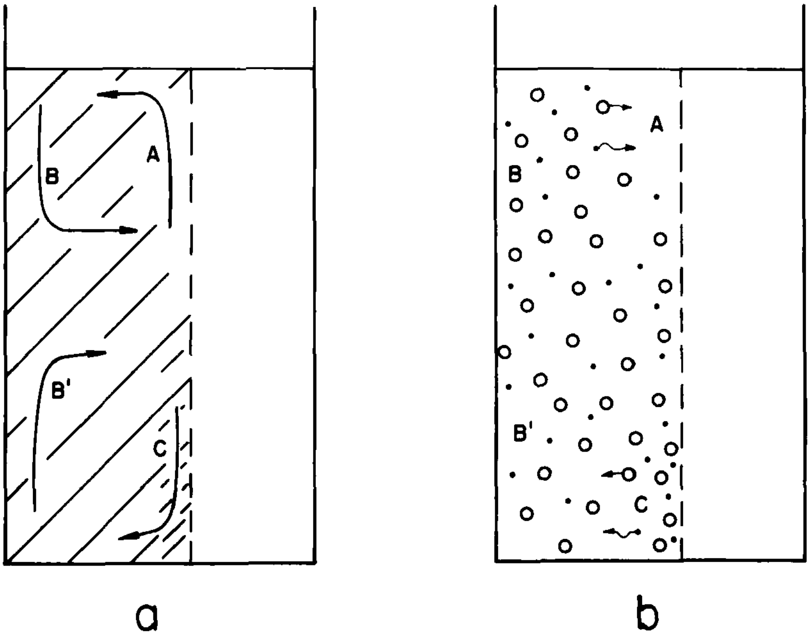

FIGURE 2

a) Convection currents within the solution compartment, in a dialysis cell. Solution in A is diluted by osmotic solvent flow; solution in C is concentrated by reverse - osmotic flow; solution in B and B' is unaltered. Denser fluids move downwards, lighter ones upwards.

b) Lighter (•) particles diffuse from the more concentrated to the more dilute regions, within the solution: from B to A and from C to B'. Heavier (o) particles do also diffuse, but at a lower rate. As a result the more concentrated, denser solution, is enriched in the heavier particles and moves downwards. Less dense solution (as in A) is enriched in lighter particles and moves upwards.

osmosedimentation could be up to 10^5 times greater than in normal sedimentation.

Osmosedimentation has also been treated theoretically, using the linear formalism of non-equilibrium thermodynamics[24]. It was found that solute settles within a dialysis cell with apparent sedimentation coefficients orders of magnitude larger than normal sedimentation coefficients. Moreover, apparent sedimentation coefficients increase with solute concentration, cell height and dialysis cell membrane area. Using a schematic cell such as depicted in Figure 3, we can calculate S_{app}/S ratios as given in Figures 4 and 5.

These calculations show that osmosedimentation works better at higher concentrations, in taller cells - just the opposite of sedimentation. It is rather advantageous for slow sedimenting particles but not much for 100 S - plus solutes.

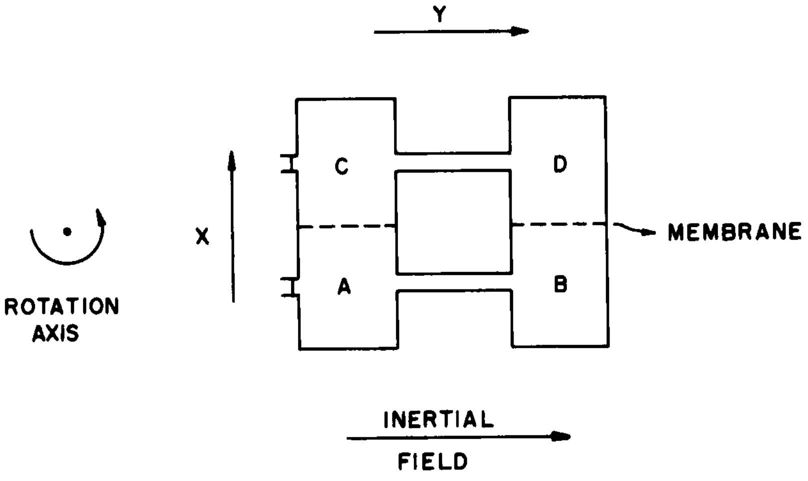

FIGURE 3

Schematic dialysis cell used to evaluate relative efficiencies of osmosedimentation and centrifugation.

Application to Solute and Particle Concentration

Solutes and particles which do not settle under gravity or in a low-speed centrifuge can be made to do so, if they are placed in a dialysis cell, instead of an usual container, such as a beaker, a flask or a centrifuge tube.

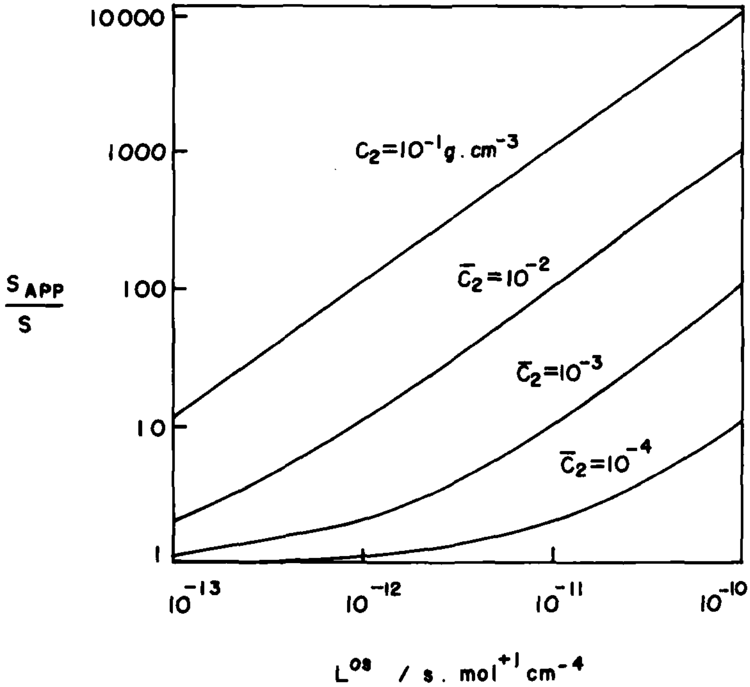

FIGURE 4

S_{app}/S ratios, as a function of membrane permeability to solvent. The S_{app}/S ratio indicates how much faster is the rate of solute vertical displacement, as compared to that in centrifugation.

<u>Settling of a latex, under gravity</u>[21]. Polytetrafluoroethylene (PTFE) latex is made of very small particles, which settle very slowly, under gravity. In an experiment[21], three dialysis cells, each at a given height, were interconnected with tubing. The three cells were filled, on one side, with a detergent-stabilized PTFE dispersion; the other side of the three-cell array was filled with the dispersant medium. The system was allowed to equilibrate, undisturbed. As a result, PTFE concentration in the lowest cell rose very fast and after some time nearly all of the PTFE had transferred from the two upper cells to the lowest one. The rate of particle transfer, in this case, is much greater than

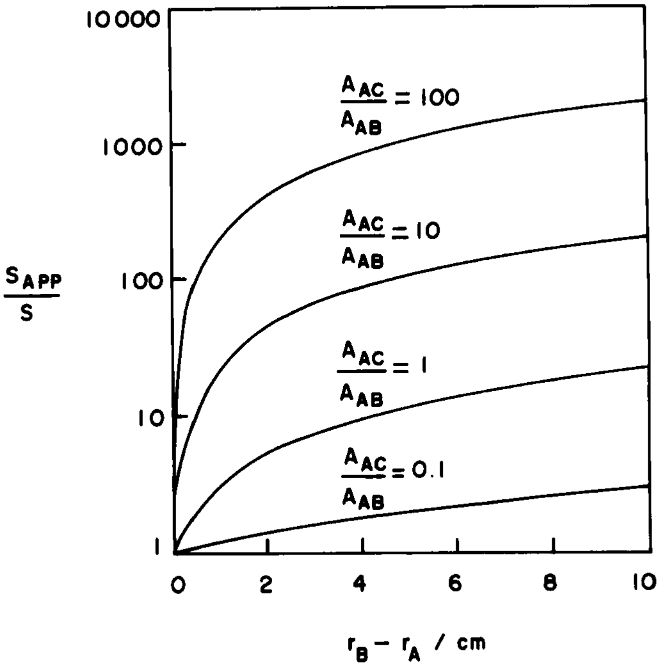

FIGURE 5

S_{app}/S ratio, as a function of cell height.

in a control run, in which the same dispersion was allowed to settle within a piece of glass tubing of the same total height (Figure 6).

Concentration of proteins and polysaccharides, under low centrifugation speeds. Centrifugation of protein and polysaccharide solutions, at low speeds (1-5 x 10^3 rpm), does not generate any appreciable concentration gradients. Many of these solutes (gammaglobulin, serum albumin, dextrans) were osmocentrifuged[25] and thus concentrated at 2000-3000 rpm, in a standard "blood" centrifuge (6-liter, swinging buckets). Results of gammaglobulin and

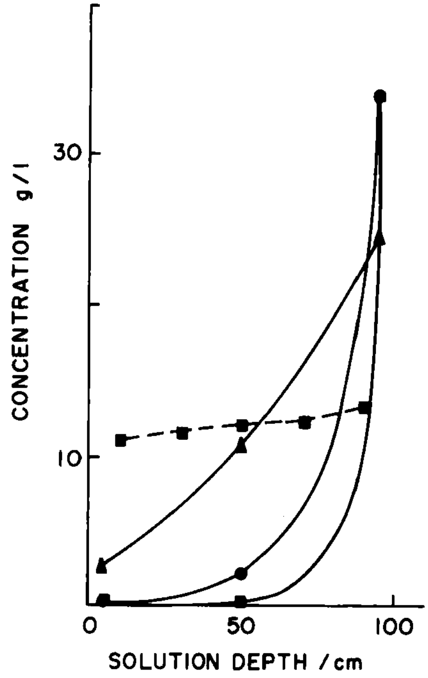

FIGURE 6

PTFE concentrations in a stack of three dialysis cells. Equilibration times were as follows: (▲) 6 h, (●) 20 h, (■) 67 h; t = 24 ± 1°C. The control (---) was PTFE in glass tubing, 67 h.

blue dextran osmocentrifugation are in figures 7 and 8. In the second case > 80% of the solute is recovered from the lowest one-fifth of the dialysis cell solution column, after 8 hours osmocentrifugation, at a concentration greater than 4 times the original concentration.

Osmosedimentation x ultrafiltration. One might be tempted to compare osmosedimentation and ultrafiltration, as methods for solute concentration. We have not approached this question, in a systematic way but a few statements can be made: osmosedimentation takes longer times; on the other hand, it is not plagued by membrane fouling and clogging; no solution stirring is necessary, so that no shearing problems arise; in the case of latex particles, in which caking is a serious problem in both ultrafiltration and centrifugation, osmosedimentation under gravity leads to concentrated dispersion without cake formation.

It should be noted that problems in ultrafiltration tend to grow as the feed solution concentration increases - as opposed to osmosedimentation, which is fairly innefective at low (0.1%) protein concentration but becomes more and more effective as the starting solution concentration increases[25].

Application to Density Gradient Formation

Centrifugation in density gradients is an important separation methodology, widely used in protein, virus, subcellular particles and cell fractionation and characterization. More recently polymer latexes have been examined, also.

Percoll, a modified colloidal silica, and Ficoll, a copolymer of sucrose and epichlorohydrin, have been largely used as density gradient - forming materials for biological separations[26, 27]. Continuous gradients can be obtained by various methods and can be self-generated when Percoll solutions are centrifuged at high g values[26]. This is specially important for isopycnic sedimentation of cellular and subcellular particles. Cells can be

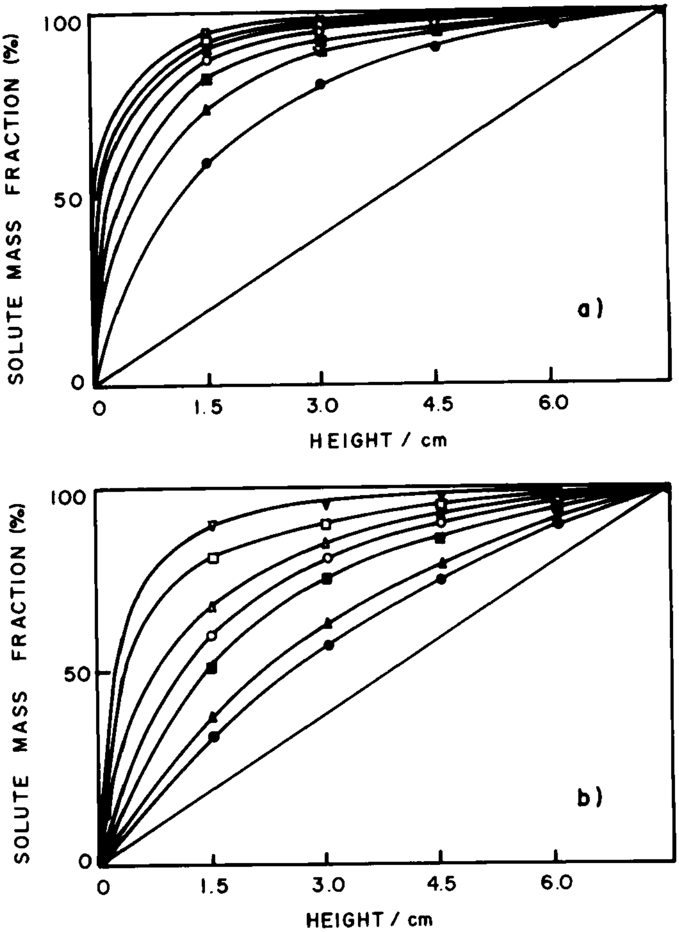

FIGURE 7

Concentration of gammaglobulin solutions, by osmocentrifugation. The ordinates give the fractions of initial solute mass accumulated from the cell bottom to the given height. The straight diagonal line is the result expected in the absence of a solute concentration gradient. Initial solute concentrations: a) 1.0%, b) 0.25%. Spinning time: (●) 4 h, (▲) 8 h, (■) 14 h, (○) 20 h, (Δ) 30 h, (□) 51.5 h and (▽) 103 h.

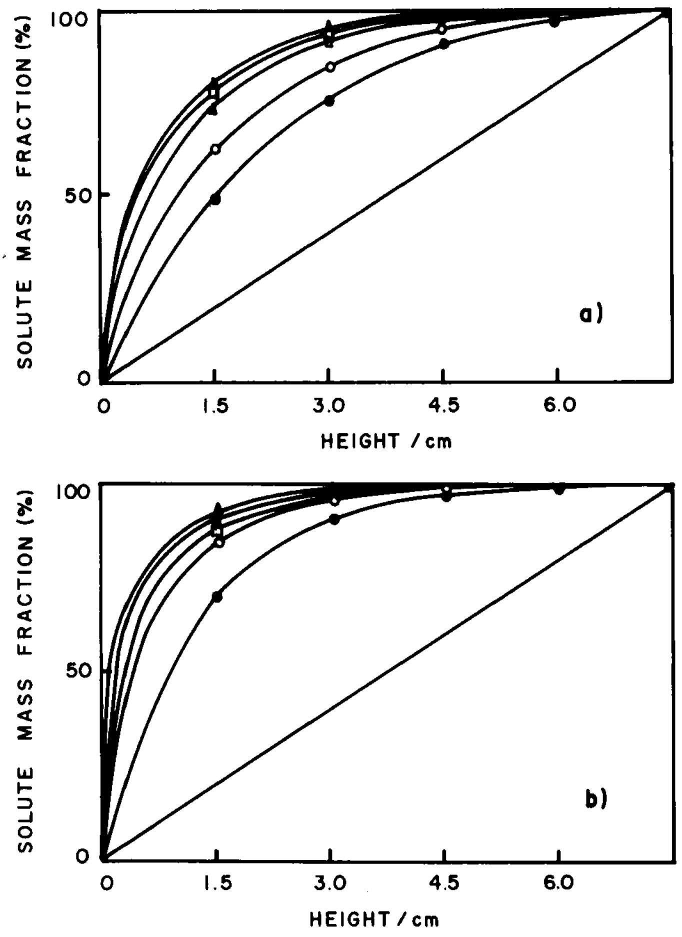

FIGURE 8

Concentration of blue dextran solutions of initial concentration 1.0% at a) 2000 rpm and b) 3000 rpm, during (●) 4 h, (○) 8 h, (Δ) 14 h. (□) 20 h and (▲) 30 h. The ordinates give the fractions of total solute mass contained between the cell bottom and each given height.

isopycnically banded in Percoll by low-speed centrifugation, but Percoll gradients have to be preformed at higher speeds.

Recent work[27], demonstrated that Percoll and Ficoll self-generated density gradients can be obtained by low-speed centri-

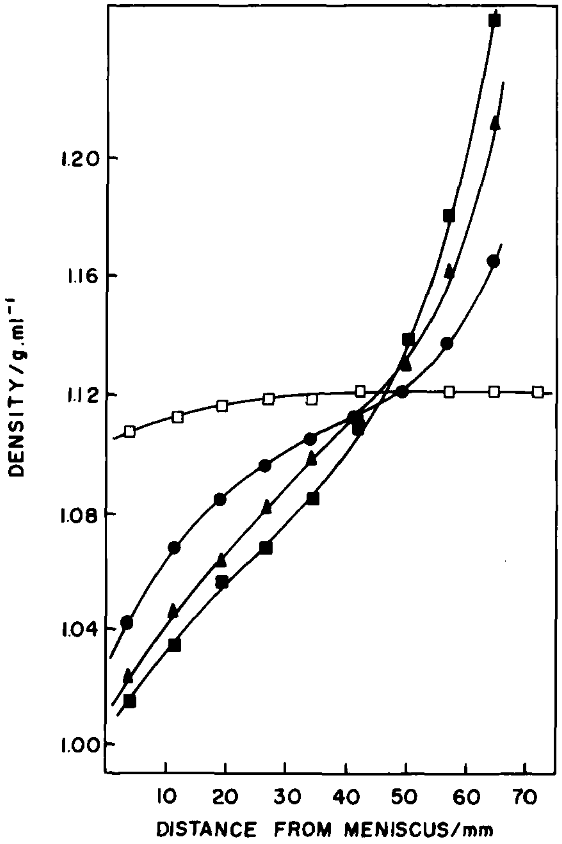

FIGURE 9

Percoll density gradients obtained by osmocentrifugation; starting density, 1.12 g·ml^{-1} in 0.15 M NaCl. Running conditions: 725 g for (●) 10, (▲) 20, and (■) 30 min at 4°C. (□) Control run: 30-min centrifugation in a glass tube.

fugation of their solutions within dialysis cells: useful Percoll gradients can be obtained after 10-30 min centrifugation at 220-2010 g (e.g., figure 9). Ficoll density gradients, which are usually more difficult to self-generate, can be obtained by the same technique. Results summed up in Table I compare the effectivenesses of osmosedimentation and ultracentrifugation in density gradient self-generation. Red cell band formation in a Percoll density gradient can be done in a single step by using dialysis cells as the centrifugation solution container. Osmosedimentation has two intrinsic advantages in density gradient self-generation: i) simpler and larger-scale equipment can be used and ii) a single-step operation replaces two steps.

Solute Fractionation in Osmosedimentation Experiments

The question of solute fractionation in osmosedimentation experiments has been raised in the section dealing with polydisperse systems. At this point we shall give evidence showing that in some cases solute fractionation is observed, although in other cases the mixed solutes sediment, without undergoing any separation.

Fractionation in colloidal silver osmocentrifugation[28]. A colloidal silver dispersion was centrifuged, within a dialysis cell. The cell contents were divided in fractions, which were analysed in the transmission electron microscope. Top fractions were richer in smaller particles, as opposed to bottom fractions (Figure 10).

Fractionation of a dextran solution[29]. Dextran solutions were allowed to equilibrate within 1 m - tall, 1.2 ℓ capacity dialysis cells. Cell contents were withdrawn and subjected to MW determination. Some results are in Table II.

TABLE I

Characterization of Self-Generated Density Gradients

Material	Solvent	Starting density ($g \cdot cm^{-3}$)	Inertial field x time ($10^3 g$ x min)	Slope[a] ($g \cdot ml^{-1} \cdot cm^{-1}$ x 10^3)	Method
Percoll	Water	1.12	6.6	14	Osmocentrifugation
			27	33	Osmocentrifugation
			60	63	Osmocentrifugation
Percoll	0.15 M NaCl	1.12	6.6	11	Osmocentrifugation
			22	34	Osmocentrifugation
			42	34	Osmocentrifugation
Ficoll	Water	1.06	60	3	Osmocentrifugation
Percoll	0.15 M NaCl	1.06-1.13	1000	6	Ultracentrifugation[b]
		1.05-1.14	1000	7.5	Ultracentrifugation[b]

[a] Slope of the density gradient in the middle of the centrifuge tube.

[b] Reference 26.

The results mentioned above show that solute separation occurs, in some cases. However, there is other evidence leading to the opposite conclusion[25]. It was found that if a solute (blue dextran), in a low concentration is centrifuged slowly in a dialysis cell while adding a high concentration of another solute (unlabelled dextran), the rate of blue dextran concentration gradient formation is much enhanced - one solute thus being dragged by the other.

As of the time of this writing the efforts of this laboratory are directed towards elucidating this question: when does fractionation occur in osmosedimentation, and to what extent.

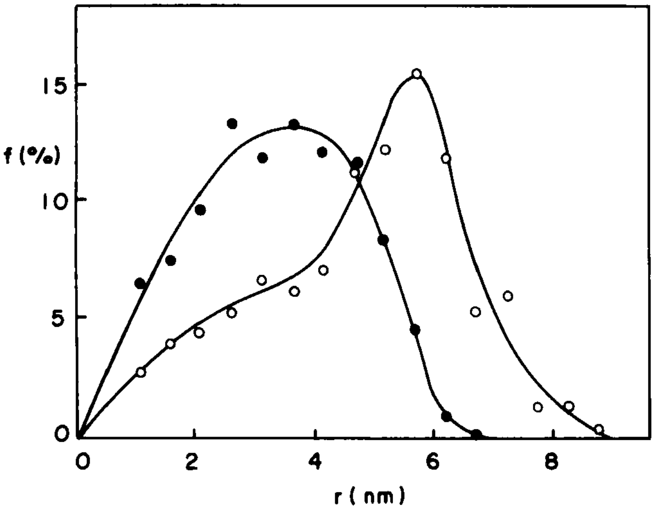

FIGURE 10

Colloidal silver particle size distribution in fractions collected at the top (●) and bottom (○) of a dialysis cell, after 32 h centrifugation at 2000 rpm. Particle size determination was done by transmission electron microscopy. Experimental conditions: $C_{Ag} = 0.154$ g $^{-1}$, in 0.10% sodium cholate; t ≃ 30°C.

Osmosedimentation Equilibrium

The osmosedimentation effect is responsible for the faster rate of solute mass transfer observed within dialysis cells, as compared to usual solution containers. Experiments were performed to verify the attainment of a sedimentation equilibrium state[30,31]. Starting with a solution of uniform concentration in one cell side and solvent in the other, the concentration of the solute changes with time, with the solute center-of-mass being displaced down-

TABLE II

T-2000 Dextran Fractionation by Osmosedimentation.

Fraction	V (ml)	Solute mass (g)	Accumulated solute mass (g)	$\bar{M}_w \times 10^6$
1	114	0.82	0.82	0.28
2	131	0.97	1.79	0.29
3	145	1.21	3.00	0.31
4	146	1.21	4.21	0.36
5	143	1.26	5.47	0.39
6	140	1.25	6.72	0.65
7	153	1.32	8.04	1.92
8	148	1.43	9.47	5.70
Starting solution[a]	1,120		9.50	
Recovered solvent	1,075		0.02	

[a] 1.12 ℓ, dextran in 30% aqueous ethanol; allowed to equilibrate for 7 days at room temperature.

wards. Another starting situation is such that: i) the solution is at the bottom of one cell compartment and ii) the remaining, upper volume of this compartment and the other compartment are filled with solvent (Fig. 11). In this case, solute will be displaced upwards, so as to reach a more uniform concentration distribution, throughout the cell.

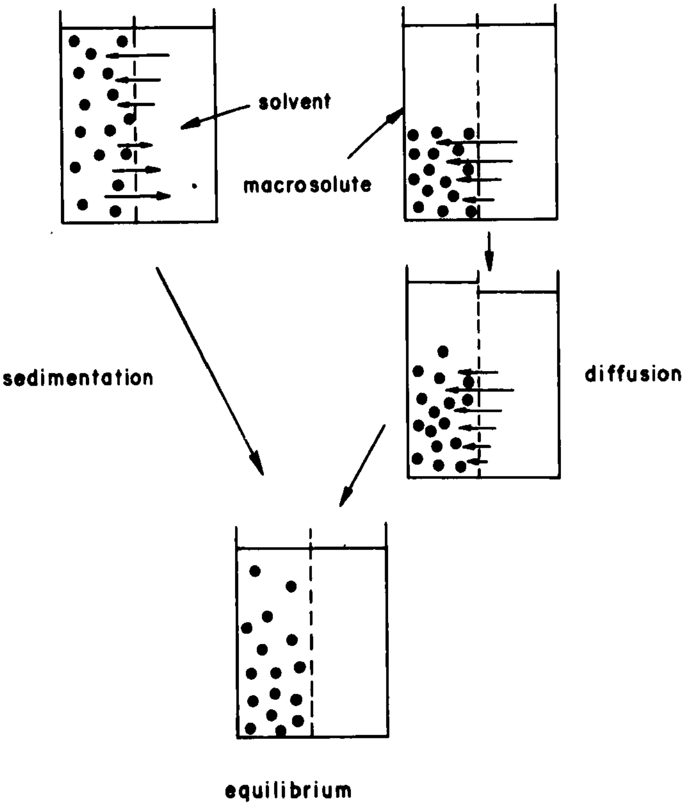

FIGURE 11

Schematics of an experiment to demonstrate attainment of sedimentation equilibrium state, in a dialysis cell.

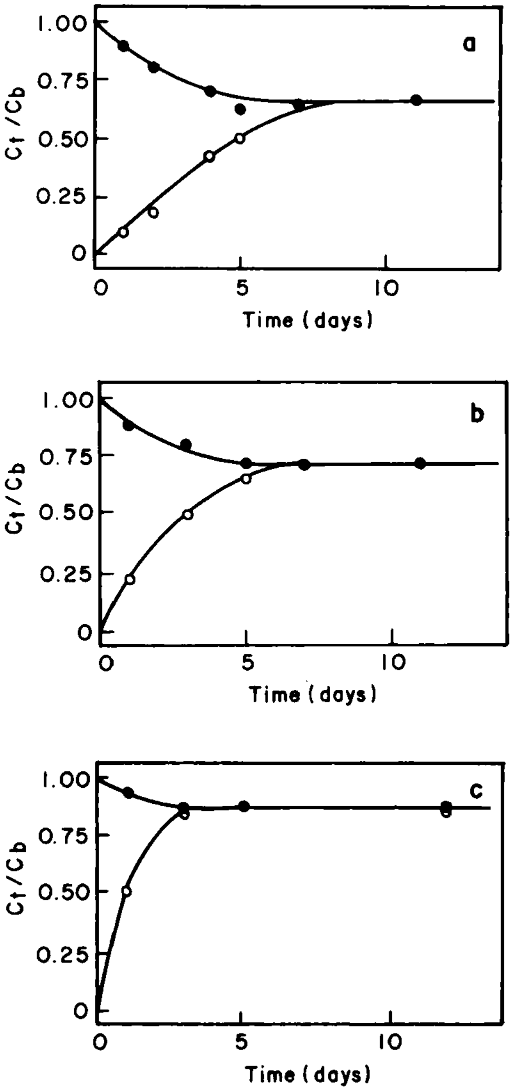

FIGURE 12

Osmosedimentation experiments with silver sols, under gravity, at (300 ± 0.2) K. Each point is an average over 2-4 individual runs. The ratio of concentrations at the top (c_t) and bottom (c_b) of the cell is shown for three different average concentrations: (a) 0.168, (b) 0.320 and (c) 0.615 g ℓ^{-1}. Open (closed) circles refer to experiments in which the solution center of mass was displaced upwards (downwards).

Both starting situations should lead to a same concentration distribution, since this is characteristic of an equilibrium state. This was experimentally verified, as described in figures 12 and 13.

Concentration gradients at the sedimentation equilibrium state can be used to calculate solute MW's, MW-averages and virial coefficients. Some of the data collected thus far is in Table III. In these calculations we assumed, in the case of polydisperse samples, that solute redistribution occurred during equilibration, exactly as in normal sedimentation or centrifugation. Procedures used in these calculations are well-established, in the literature[32].

Membranes for Osmosedimentation Experiments

Solute osmosedimentation rates are highly dependent on the membrane permeability, to solvent. The ideal membrane for osmosedimentation is one with complete solute retention and a minimal resistance to solvent flow. These requirements are the same as those made on ultrafiltration membranes, but there is a difference: osmosedimentation membranes do not have to stand pressure differences greater than ca. 0.1 atm, for which reason they are not required to be as strong as ultrafiltration membranes.

The work on osmosedimentation required, so far, large amounts of membranes, in a variety of sizes and shapes. This led us to develop new procedures for membrane preparation, which are simple, convenient and inexpensive[37-39]. Membranes are cast by spreading solutions of cellulose acetate in a ternary solvent (acetic acid, acetone, water) on a glass sheet; the solution is coagulated by immersion in a water bath, and rinsed to complete removal of free acid. All the preparation steps are done at room temperature. Changes in casting solution composition, spread film thickness and membrane drying time (prior to coagulation) allow the preparation of membranes with a variety of retention-permeability characteristics.

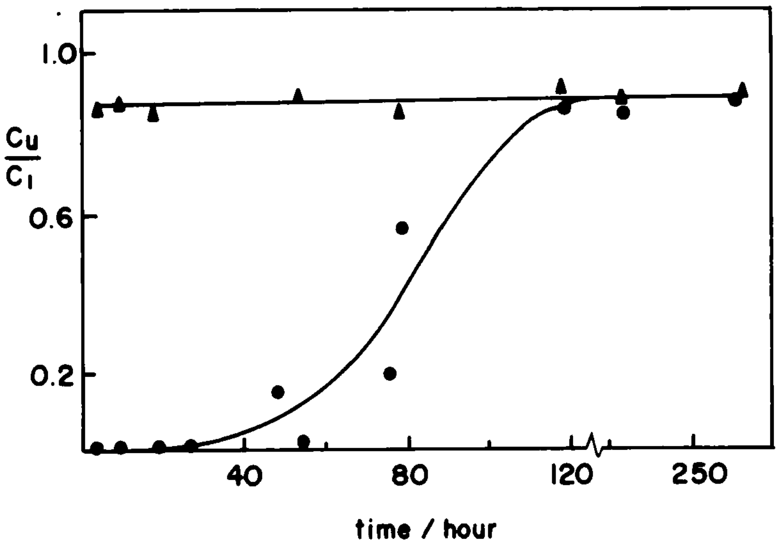

FIGURE 13

Concentration ratio between the contents of top and bottom dialysis cell compartments, as a function of time. (▲) Experiments in which the solution side of the cell was filled with Dextran T-500 solution of uniform concentration, 1.2% w/w; (●) experiments in which the lower half of the solution side of the cell was filled with 2.4% w/w Dextran and the upper half was filled with water. t = 20.0°C.

TABLE III

MW-Determination of Polymer Solutes and Dispersed Particles.

Sample	t/°C	$\overline{M}_w \times 10^{-5}$	Method
Dextran	5.5	5.3 ± 0.4	Osmosedimentation[30]
T-500	20.0	5.3 ± 1.5	Osmosedimentation[30]
in water	40.0	4.9 ± 1.4	Osmosedimentation[30]
		5.17	Gel-permeation chromatography[a]
Dextran	7.1	18.9 ± 4.8	Osmosedimentation[30]
SIGMA in water		18.0	Light scattering[b]
Thyroglobulin	20.0	7.1 ± 0.7	Osmosedimentation[30]
in aqueous 0.1 M KNO_3, pH 5.8		6.69	Sedimentation-diffusion[33]
Colloidal	27.0	5.2 ± 0.25	Osmosedimentation[31]
silver in aqueous 0.10% Na-cholate		5.2 ± 0.47	Electron microscopy[31]
Bovine serum	4.0	0.68 ± 0.06	Osmosedimentation[33]
albumin		0.667	Sed.-dif. equilibrium[34]
Ovalbumin	4.0	0.43 ± 0.06	Osmosedimentation[33]
		0.40 - 0.46	Sed.-dif. equilibrium[35]
Gammaglobulin	4.0	1.59 ± 0.07	Osmosedimentation[33]
		1.49 ± 1.80	Various methods[36]

[a] Data sheet from Pharmacia Fine Chemicals.

[b] Data from Polysciences.

Of course, it is possible to use commercial ultrafiltration membranes, for osmosedimentation. We have tested the Amicon XM100 membranes, which are perfectly suitable. We have also attempted to use semipermeable hollow fibers, in osmosedimentation. They can be used under gravity, but an osmocentrifugation experiment with the Amicon 3P10 fibers was unsucessful, because the fibers ruptured, under centrifugation at 2000 rpm, when they were aligned with the centrifugation radius.

Conclusions

The osmosedimentation effect can now be well understood, on theoretical grounds. Its use allows the easier performance of many hitherto difficult experiments: polymer and particle MW determination, under gravity; protein concentration, by low speed centrifugation, polymer fractionation, by sedimentation, fine particle concentration and fractionation, both under gravity and low speed centrifugation.

Major advantages of the experimental methods thus far developed are i) the use of low cost, high-capacity, sturdy equipment instead of high-speed centrifuges and ii) avoidance of conditions leading to sample degradation: exposure to damaging reagents, high pressures or high shear rates.

At this point, major disadvantages of these methods are the times required in some experiments and the still incomplete modeling of mass transfer within the dialysis cell. The first problem may be solved by developing semipermeable membranes which are less resistant to solvent flow. Both problems are now under study, in the authors' laboratories.

List of Symbols

a_i : radius of particle i
c : concentration

g : gravity
$J_{i,x}$: flux of i along x
K : membrane permeability to solvent
m_i : particle mass
M_i : molar weight
r : distance to centrifuge axis
S : sedimentation coefficient
v_i : rate of vertical displacement
$\bar{v}_i$: partial specific volume
$\bar{V}_i$: partial molal volume
x_i : mole fraction
z : depth within a solution
αA : a separation (e.g., a membrane) between two phases, in a system
η : viscosity
$\psi_{i,\alpha A}$: mass current of component i, across phase separation αA
μ_i : chemical potential
ω : rotor angular velocity
ρ : density

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