THE DIFFUSION OF IONS IN AGAR GEL SUSPENSIONS OF RED BLOOD CELLS

Thesis by

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ABSTRACT

A method was developed for measuring the self-diffusion coefficient of solutes in agar gel suspensions of red cells. The purpose was to investigate the influence of the red cell on the diffusion of ions which are important in blood solute transport.

The capillary diffusion method was employed. The diffusion coefficient was calculated from the initial and final concentration of tracer in the capillary. The results are discussed in terms of a modified Maxwell equation for the average conductivity in a granular medium in which a discontinuous boundary condition is employed to account for the observed partition coefficients.

The results indicate the ratio of the diffusivity of solute in the red cell suspension to that in the suspending medium varies considerably with the ion. This ratio is greater for sodium than for chloride over the range of red cell volume fractions investigated. An augmented diffusional mechanism for bicarbonate appears to exist in the red cell suspension. Depleting the intracellular ATP has little effect on the diffusion of sodium in the red cell suspension.

TABLE OF CONTENTS

I. INTRODUCTION

The purpose of this investigation was to develop a reliable method for measuring the diffusivities of solutes in blood, and to use this method to determine the diffusivities of certain important blood solutes. The emphasis of this study was directed towards the non-gaseous blood solutes.

This investigation is of both practical and academic interest. The practical aspect lies in the engineering realm of designing heart-lung or artificial kidney devices. The academic aspect deals more with the influence of the concentration inequalities of certain ions inside and outside the red blood cell and the red cell itself on the transport of those ions through blood. What effect the active transport mechanism, if it exists, has on the diffusion of ions peculiar to that transport mechanism is also of interest.

An important goal of the engineer in designing a device such as the artificial kidney is to construct the device in such a way that its criteria of performance, set in collaboration with the medical profession, will be met. A detailed knowledge of the fluid flow through and the mass transfer in the device is necessary to achieve this goal. When part of this detailed knowledge is not available, due to an unusually complicated flow pattern or lack of pertinent experimental data, a useful,

workable device can still be constructed. Since this empirical development is not amenable to rigorous evaluation and optimization, experimental investigations dealing with the various gaps in our knowledge of such devices and leading to well-defined models are important. One necessary piece of information in the mass transport regime is the value for the various diffusivities of pertinent solutes in blood. This research work is concerned with that aspect of the design problem.

II. BACKGROUND AND THEORETICAL CONSIDERATIONS

A. Cell Theories.

According to the membrane theory, the cell is essentially a sac of aqueous solution (25). The cell membrane is semi-permeable, passing some substances at various rates, and some not at all. The osmotic behavior of the cell has been one of the main arguments in favor of the theory. A cell placed in a solution of a certain solute may either shrink, remain the same in size, or swell. If it shrinks or swells, it may revert after a time to its original shape. The initial shrinking or swelling indicates that the osmotic pressure of the solution is either greater or less than that of the cell. If the cell returns to its original shape, this is an indication that the cell membrane is permeable to the solute; the rate of return gives a qualitative indication of the magnitude of the membrane permeability to that particular solute. There are various theories to explain how substances cross the membrane (9, 47).

One of the unusual characteristics of the red cell-plasma system is the unequal concentrations of certain ions inside and outside the cell. At thermodynamic equilibrium the chemical potential of species able to penetrate the cell membrane is the same inside and outside the cell. Although it would not be

unusual to expect the ionic concentrations also to be equal, the charge of the ionic species and the high concentration of charged hemoglobin molecules complicate the situation. A theory, called the Gibbs-Donnan Equilibrium, was derived to deal with this special case (12). This theory, although successful in explaining the intracellular osmotic pressure, the potential difference across the membrane, and the unequal distribution of many ions, fails in certain important cases.

The concentration of sodium and potassium ions inside and outside the red blood cell could not be explained in terms of the Gibbs-Donnan theory. In human blood, the ratio of the concentration of potassium inside the red blood cell to the potassium concentration in the plasma is about 30/1. On the other hand, the ratio of the sodium ion concentration inside the red blood cell to that of the plasma is about 1/6. The first explanation for this unusual distribution was that the sodium and potassium concentrations in the red blood cell were set at its birth, and did not change throughout the life of the cell. The red blood cell membrane itself was thought to be impermeable to sodium and potassium. This view held until the use of radioactive tracers conclusively showed that the red blood cell membrane is permeable to these cations (15).

A new explanation then emerged showing that the concentration difference was maintained by an energy consuming process. The age of active transport unfolded.

Much time and energy has been expended on this concept. It is the basis of good research work and a multitude of journal publications. Briefly, the process seems to depend on glucose metabolism. The active uptake of potassium and expulsion of sodium from the red cell appear to be linked, although not in a one to one ratio (30). The detailed transport mechanism appears to involve the sodium and potassium dependent ATPase enzyme. The cell membrane, only slightly permeable to potassium and sodium, enables the pumping mechanism to maintain the relatively high potassium and low sodium concentration inside the red blood cell. It is not an equilibrium but a steady-state system. The theory is consistent with many observations, such as the loss of potassium and the gain of sodium by the red blood cells upon cold storage or incubation in a glucose deficient media, yet the theory is not entirely satisfactory for a number of reasons.

A relatively new cell theory, not yet widely accepted, but gaining momentum, is invading the biological literature. Troshin (42), one of the main Russian proponents of the new theory, has written a delightful book on the subject. He labels this new concept the sorptional theory. In the United States, Ling (24) is the champion of the theory which he calls association-induction hypothesis. He has also written a detailed, theoretical book in which he attempts to substantiate his hypothesis. The two theories agree that a semi-permeable membrane is

not the cause of the concentration difference of species inside and outside the cell.

According to Troshin, the cell is a separate phase, like a coacervate drop. It is enclosed in an envelope, the cell membrane, but this is not a semi-permeable membrane. The cell has no selective ion permeability, passing both cations and anions. Because it is a separate phase, or because a good portion of the water is bound to the cell colloids and therefore is unavailable for dissolution of solutes (4), the concentration of unbound solutes is less inside the cell than outside. The key word is "unbound". This concept could qualitatively explain the low concentration of sodium inside the red blood cell. For solutes which can be adsorbed to the colloids of the cell, the picture may be quite different. There is generally a point at a sufficiently small external concentration of the solute where the internal concentration is greater than the external. At a sufficiently high external concentration, when the amount adsorbed reaches saturation and can no longer counterbalance the cell solute deficiency due to a lower apparent solubility, the external concentration will then exceed the internal concentration of solute. The first explantion would presumably apply to the distribution of potassium, the second to sodium. A lower inside than outside concentration of cations may also be

due in part to the Gibbs-Donnan Equilibrium. These results have been observed in non-living systems, such as suspensions of coacervate drops.

Ling (24) postulates that the interior of the cell is highly structured. The cell colloids are arranged in a precise configuration, which electrostatically favors the potassium ion over the sodium ion in the cell matrix. He presents calculations which indicate that active transport as an energy consuming process cannot maintain the observed concentration difference of sodium and potassium ions inside and outside the red cell.

The various theories present a number of possibilities. The cell may be a sac of aqueous solution, its membrane quite impermeable to cations. The cell may be a separate phase exhibiting a true partition coefficient for a solute between it and the surrounding media. It also may be that the cell is a very concentrated aqueous solution, much of its water being bound by the cell colloids.

Since the various theories on the physical state of the living cell are quite different, explanations based on the various theories of certain experimental observations might be expected to differ considerably. This is definitely the case. For example, it has been observed, using radioactive tracers, that the leakage of cations into or out of the red blood cell is very slow. Depending on which theory applies, this could be due to a slightly permeable membrane, a high

diffusional resistance of a phase boundary, or slow adsorption-desorption kinetics.

As illustrated in the foregoing, several concepts of the physical state of the red blood cell are available. The particular view adopted will govern the interpretation of the results presented in this report. Some of the hypotheses, due to lack of pertinent supportive data, will not allow a complete explanation of the results. In other cases, the data from this work will help strengthen some hypotheses and refute others.

B. A General Non-Convective Diffusion Equation for Solute Transfer in Blood.

A general non-convective mass balance on solute i in a given volume element leads eventually to

$$\frac{\partial}{\partial t}(n_i) = -\nabla \cdot J_i + R_i \qquad (1)$$

RATE OF CHANGE IN A STAGNANT ELEMENT — NET DIFFUSION FROM THE ELEMENT — NET CHEMICAL REACTION IN THE ELEMENT

Consider the set of species n_{ik}, $k = 1, \ldots, r$, where n_{i1} refers to the concentration of the unreacted or unbound form of solute species i, and the $n_{i2}, \ldots, n_{ir}$, refer to the concentration of reacted or bound solute species i. For example, if the solute species i were the sodium ion, n_{i1}

would refer to the concentration of free, hydrated sodium ion in solution, whereas the n_{ik}'s ($k = 2, \ldots, r$), would refer to the concentration of sodium reacted with or electrostatically bound to other molecular species, particulate matter, or solids in the solution. By summing over all the various chemical forms of sodium, and noting that

$$\sum_{k=1}^{r} R_{ik} = 0 \tag{2}$$

the result is an overall mass balance on species i, namely

$$\frac{\partial}{\partial t}(n_t)_i = -\nabla \cdot (J_t)_i \tag{3}$$

where

$$n_t = n_{i1} + N_i \tag{4}$$

$$N_i = \sum_{k=2}^{r} n_{ik} \tag{5}$$

and

$$J_t = J_{i1} + \sum_{k=2}^{r} J_{ik} \tag{6}$$

It is instructive to rewrite equation (3), following the notation of Spaeth and Friedlander (37), as

$$\frac{\partial}{\partial t}(n_{il} + N_i^*) = -\nabla\cdot(J_t)_i + \frac{\partial}{\partial t}(N_i^* - N_i) \qquad (7)$$

NET RATE OF CHANGE OF $(n_t)_i$ ASSUMING CHEMICAL EQUILIBRIUM IN THE ELEMENT

NET DIFFUSION INTO THE ELEMENT

RATE OF CHANGE OF DISPLACEMENT FROM CHEMICAL EQUILIBRIUM IN THE ELEMENT

where N_i^* is the equilibrium value of N_i.

Equation (7) is a general equation which can now be used to describe the transport of solutes in a stagnant solution. In order to solve this equation, the following must be known:

1. The free solute concentration, n_{il}
2. The equilibrium relationships, N_i^*
3. The diffusive flux, $(J_t)_i$
4. The displacement from equilibrium, $(N_i^* - N_i)$

The membrane theory maintains that the red blood cell is essentially a sac of aqueous solution, with both the sodium and potassium ions existing inside the red blood cell in their free or uncombined state. The sorptional theory, on the other hand, describes the red blood cell as a separate phase, with a good portion of the interior water bound to the cell colloids

and therefore unavailable for dissolution of solutes. The sodium ion exists mainly as the free ion dissolved in the unbound water or fluid portion of the cell. The bulk of the potassium ion in the cell is bound to the cell colloids.

If the first theory is correct, the form of the diffusion equation to use is

$$\frac{\partial n_i}{\partial t} = -\nabla \cdot J_i \qquad (8)$$

as the term $N_i = 0$. This should be valid for both the sodium and the potassium ion. Although it would also be the equation to use for the sodium ion should the second theory be true, it would not hold for the potassium ion.

Equation (1) can be rewritten as follows

$$\frac{\partial n_{ii}}{\partial t} = -\nabla \cdot J_{ii} + \left[\text{NET RATE OF DESORPTION OF SPECIES } ii\right] \qquad (9)$$

If

c_v = the concentration of vacant sites for potassium on the colloids,

c_o = the total number of moles of potassium that could be adsorbed,

$\hat{c}_a$ = the number of moles of potassium that are adsorbed,

c_a = the concentration of potassium in the fluid, the rate of adsorption of potassium ion is

$$r_a = k_1 c_a \hat{c}_v \tag{10}$$

while the rate of desorption of potassium is

$$r_d = k_2 \hat{c}_a \tag{11}$$

and the net rate of desorption is

$$r_n = -\left[k_1 c_a \hat{c}_v - k_2 \hat{c}_a \right] \tag{12}$$

Then

$$\frac{\partial n_i}{\partial t} = -\nabla \cdot J_i - \left[k_1 n_i \hat{n}_v - k_2 \hat{n}_i \right] \tag{13}$$

C. Theory of Diffusion in a Heterogeneous Media.

Solving the general diffusion equation requires a relationship between the diffusive flux and the concentration gradient. For a homogeneous medium, Fick's First Law is usually employed:

$$J_i = -D_i \nabla n_i \tag{14}$$

where D_i is the diffusivity of species i in the medium of interest and n_i is the concentration of species i in that medium. For a heterogeneous medium, this form would be valid only for the unlikely case in which the diffusivity of species i was the same in both the dispersed and continuous phase. Otherwise both D_i and n_i in Fick's relationship must be redefined. Since the unsteady state term in the general diffusion equation is based on the total or average concentration of species i, it is convenient to similarly define n_i when modifying Fick's expression. For a system such as blood, if n_i is the average concentration of species i in a small volume element which nevertheless contains many red cells, an average diffusivity can be defined by the expression

$$J_i = - D_i^* \nabla n_i \tag{15}$$

D_i^* is now some function of the volume fraction of the dispersed phase, the diffusivity of species i in the dispersed and continuous phases, and the geometry of the particulate dispersed phase.

A theory of heat conduction through a granular medium with spherical dispersed particles was developed by Maxwell (26). Fricke (13) developed a theory for electrical conduction in a heterogeneous medium which took into account the non-sphericity of the particulates. He applied his results to blood, showing how the measured values of the conductivity could be related to the conductivities of the plasma and the red blood cell, once the volume fraction and shape of the red blood cells were known.

Consider a spheroid with half axes a and b, where a is the axis of symmetry. If the diffusivity of species i through the continuous phase is D_{1i}, its diffusivity through the dispersed phase D_{2i}, and its average diffusivity through a suspension of dispersed volume fraction α is D_i, then

$$D_i = D_{1i}\left\{\frac{1+XR}{1-R}\right\} \tag{16}$$

where

$$R = \alpha\left\{\frac{D_{2i}/D_{1i}-1}{D_{2i}/D_{1i}+X}\right\} \tag{17}$$

and

$$X = - \frac{(D_{2i}/D_{1i} - 1) - (D_{2i}/D_{1i})\beta}{[(D_{2i}/D_{1i}) - 1] - \beta} \qquad (18)$$

The shape factor, β , is given by

$$\beta = \frac{1}{3}\left[\frac{2}{1+(D_{2i}/D_{1i}-1)\frac{1}{2}M} + \frac{1}{1+(D_{2i}/D_{1i}-1)(1-M)}\right](D_{2i}/D_{1i}-1) \qquad (19)$$

where

$$M(a<b) = \frac{\phi - \frac{1}{2}\sin 2\phi}{\sin^3\phi} \qquad \cos\phi = a/b$$

$$M(a>b) = \frac{1}{\sin^2\phi'} - \frac{1}{2}\frac{\cos^2\phi'}{\sin^3\phi'}\log\left(\frac{1+\sin\phi'}{1-\sin\phi'}\right) \qquad (20)$$

$$\cos\phi' = b/a$$

For the case of spheres, X reduces to 2, and we are left with the expression

$$D_i = D_{1i}\left\{\frac{1 + 2\alpha\left[\frac{D_{2i}/D_{1i} - 1}{D_{2i}/D_{1i} + 2}\right]}{1 - \alpha\left[\frac{D_{2i}/D_{1i} - 1}{D_{2i}/D_{1i} + 2}\right]}\right\} \qquad (21)$$

Fricke experimentally verified the theory, using data on conductivity in dog blood. Agreement was excellent over the hematocrit range from 10 to 90. It is possible, therefore, knowing D_i, D_{1i}, α, and the shape of the dispersed phase particulates, to calculate the value for D_{2i} (see Figure 1).

In the case of blood, D_{2i} is the average diffusivity of the solute species i in the red blood cell. This again is a function of the shape of the red blood cell, the diffusivity of species i through the cell envelope or membrane and its diffusivity through the cell interior, and the thickness of the membrane and interior. A membrane resistance of the dispersed phase particles does not invalidate the theory. Maxwell (26) developed an expression for the average diffusivity through spherical composite particles.

The expression given in equation (21) is only valid for substances whose concentration is the same in the dispersed and continuous phase at equilibrium. For blood, this condition is generally true for all non-electrolytes (47), but not for ions. If a substance exhibits a partition coefficient K in a particular heterogeneous medium or an active transport mechanism is operative, the boundary conditions in the derivation leading to an expression for the average diffusivity must be changed. The derivation for a dispersed phase of spherical particles which takes into account the concentration discontinuity at the phase

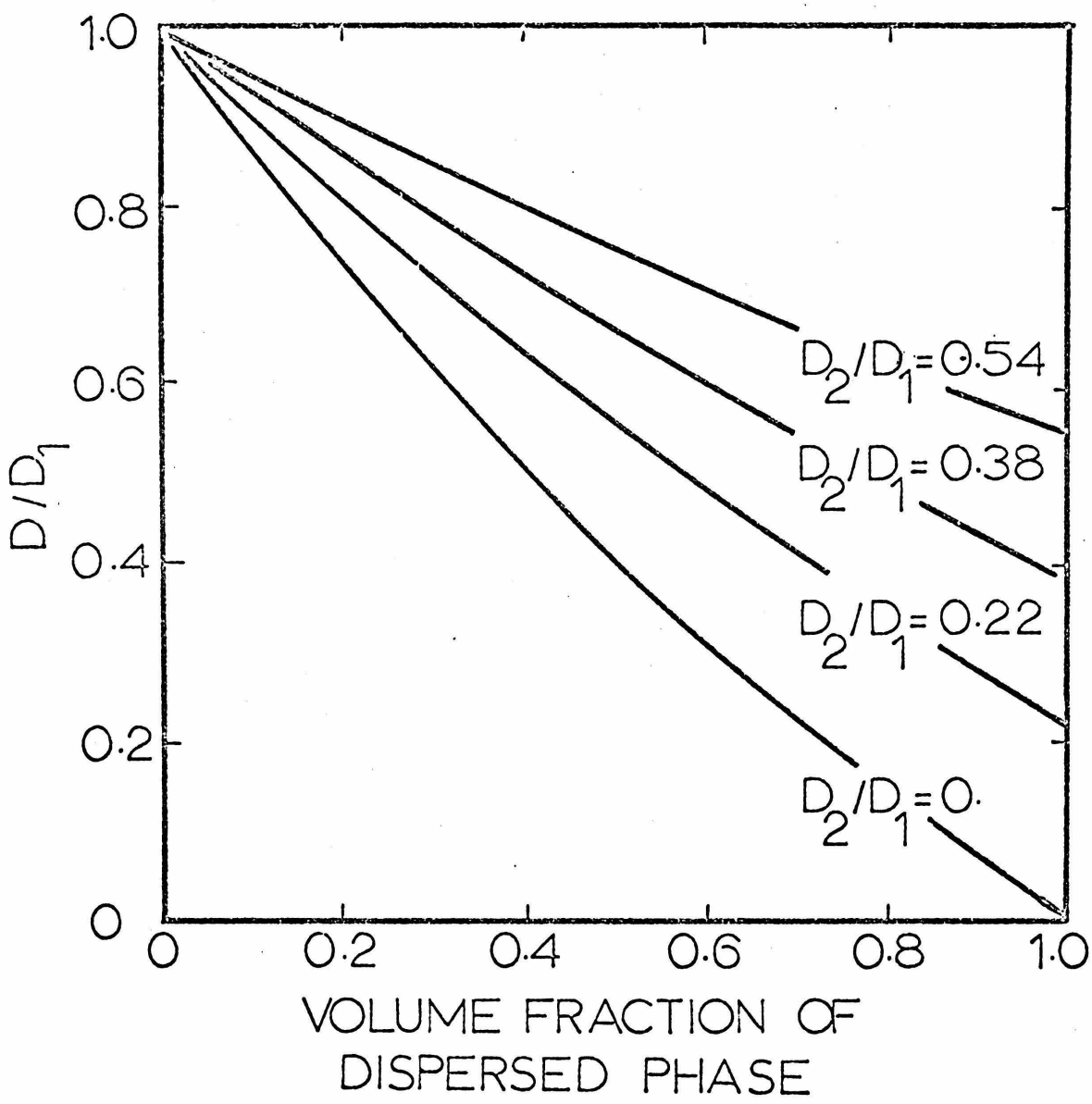

Figure 1. Solutions to Maxwell's Equation.

boundary is given in Appendix I-1. The average diffusivity in this case is

$$D = D_1 \left\{ \frac{1 + 2\alpha \left[\frac{KD_2/D_1 - 1}{KD_2/D_1 + 2} \right]}{1 - \alpha \left[\frac{KD_2/D_1 - 1}{KD_2/D_1 + 2} \right]} \right\} \qquad (22)$$

D. Previous Investigations.

Many studies have been made on the exchange of sodium and potassium between red blood cells and plasma. / The rates vary significantly depending on the type of red blood cell (human, rabbit, dog, etc.) and the experimentalist. The results of these investigations have strengthened the membrane theory. The experimentally determined exchange rates are very slow. Practically all the sodium in rabbit erythrocytes is exchanged for plasma sodium in 15 minutes (16), while the equivalent time for human red cells appears to be as long as 22 hours (47). The time for half-exchange of potassium is about 35 hours for human red blood cells (29). Other research work indicates this figure should be 18 hours for complete exchange. In any case, it is significantly different from the time of 500 msec for 90 percent saturation of the red cell with urea (20).

The efflux of chloride from human cells occurs by a first-order process with a half time of about 0.2 seconds (41). This value agrees with that found by Mook (11) for the choride-

bicarbonate exchange. Since Whittam (47) states that in the chloride-bicarbonate exchange, it is not known which ion is rate limiting, the half time for bicarbonate is no more than 0.2 seconds. Whittam also mentions that this half time for chloride exchange is at least 70 times greater than that for water.

The conclusions generally drawn from such experiments are that the cell is enclosed in a membrane with very special properties. The membrane allows small, lipid soluble molecules like urea to pass relatively rapidly, and also exhibits a high permeability to anions. The permeability of the cell membrane for cations, however, is extremely low. In certain instances, the permeability rates can be described at least in part by the adsorptional kinetics (47).

The intracellular diffusion coefficients of substances are in general thought to be very low (19), but Ling (24) refers to work which indicates the intracellular diffusion coefficient is only slightly less than the extracellular diffusion coefficient for potassium diffusing in an axon.

There is not an extensive literature dealing with the diffusion of substances in blood. Spaeth and Friedlander (37) carried out one of the first detailed studies of gas transport in flowing blood. In their work, they calculated diffusion coefficients by means of the theory of transport through hetero-

geneous media based on diffusion coefficients measured in plasma and in concentrated hemoglobin solutions. They assumed a negligible red blood cell membrane resistance to their diffusing species. The diffusion coefficients thus calculated were used in their calculations of convective diffusion rates.

Stein (38) more recently verified the use of the heterogeneous media theory for the diffusion of oxygen through red blood cell suspensions. He suspended out-dated human red blood cells in an agar gel, and studied the diffusion of oxygen through this red blood cell-gel mixture by means of an oxygen electrode. His results could be explained by calculations based on the Fricke theory. By measuring the average diffusion coefficient of oxygen through the mixture and the diffusion coefficient of oxygen through the plain gel, he could then calculate the diffusion coefficient of oxygen through the red blood cell. The calculated value agreed within experimental error with the diffusion coefficient of oxygen through a hemoglobin solution of about the concentration of hemoglobin in the red blood cell. Unfortunately, the experimental errors were quite large.

Most of the methods used to study the diffusion of gases in blood are of limited versatility. The method used by Stein is severely limited by the number of measuring electrodes available. The wetted wall column used by Hershey et al. (18)

is limited to the study of gaseous diffusion. The various approaches taken by Spaeth and Friedlander (37), Buckles et al. (2), and Collingham et al. (7) are in general limited by the solute resistance of the permeable membrane material used in the experimental apparatus.

III. EXPERIMENTAL APPARATUS AND PROCEDURE

A. Theory.

1. The Capillary Diffusion Method.

The capillary diffusion method was chosen for this investigation. There are a number of reasons for using this particular approach. The apparatus is uncomplicated and easy to operate. Results obtained by this method are both accurate and precise. The number of substances which can be investigated with this method is limited only by the availability of suitable tracers. The low volume requirements are a decided asset. These combined advantages made it an ideal method for the present investigation.

When tracer amounts of a solute diffuse through a salt solution, and the concentration of a tracer is negligible compared to the total concentration of other ions in the solution, the diffusion coefficient of tracer is constant along the diffusion path. The measured diffusion coefficient is then the so-called "differential" diffusion coefficient. This special case of tracer diffusion is sometimes called self-diffusion (44).

By applying Fick's First Law, equation (3) can be rewritten as

$$\frac{\partial n_i}{\partial t} = D_i \nabla^2 n_i \qquad (23)$$

where $N_i = 0$. The diffusion coefficient is not a function of n_i, in the case of self-diffusion. To solve this equation, the boundary conditions for the capillary tube configuration must be known. With the tracer initially in the capillary tube of length 1, the boundary conditions are

1. $n_i = n_{io}$ $\quad t = 0,\ 0 \leq x \leq 1$
2. $\partial n_i / \partial x = 0$ $\quad t \geq 0,\ x = 1$
3. $n_i = 0$ $\quad t \geq 0,\ x = 0$

The solution of equation (23), given the above boundary conditions, is

$$\frac{\bar{n}_i}{n_{io}} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 \pi^2 D_i t / 4l^2\right] \qquad (24)$$

In equation (24), $\bar{n}_i$ is the average concentration of tracer in the capillary at time t.

When the value Dt/l^2 exceeds 0.2, only the first term in the expansion is necessary. In the present study, the first two terms were used.

The above equation can only be used if the radioactive tracer in use has a relatively long half life. For sodium-22, with a half life of 2.6 years, it is quite satisfactory. But for a tracer like potassium-42, with a half life of 12.4 hours, the equation must be modified. Since the radioactive decay can be

described by first order kinetics, the original equation must be written as

$$\frac{\partial n_i}{\partial t} = D_i \nabla^2 n_i - k^* n_i \qquad (25)$$

where k* is the first order decay constant for the radioactive species. The same boundary conditions still apply. Solving equation (25) leads to

$$\frac{\bar{n}_i}{n_{i_0}} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[-\left\{\frac{\pi^2 D_i (2n+1)^2}{4l^2} + k^*\right\} t\right] \qquad (26)$$

The boundary condition $n_i = 0$ at $x = 0$ was maintained experimentally as follows: First, the open end of the capillary tube was in contact with a bath of sufficient volume so the tracer concentration in it due to tracer loss by diffusion from the capillary tube was negligible throughout the experiment; secondly, the bath was stirred, fast enough to prevent accumulation of tracer at the mouth of the capillary but slow enough to prevent scooping out a large portion of fluid from the capillary. Wang (44) describes a test which can be used to determine if the scooping out is important. His results showed that at modest stirring rates using capillaries with an approxi-

mate diameter of 0.5 mm and lengths ranging from two to four centimeters, the scooping out was negligible during a sufficiently long run. The work by Castleden and Fleming (6) showed little change in the measured diffusivity between modest stirring and no stirring conditions.

2. An Inquiry Concerning D_i.

The average diffusivity D_i, defined in equation (23) and determined experimentally by the capillary diffusion method, is based on the average concentration of species i. When species i exhibits a partition coefficient in a heterogeneous system, the value of its diffusivity in the medium depends on which concentration, the average or continuous phase, is used in the calculation.

For example, consider the cylinder shown in Figure 2, which is capped at the top and bottom by an infinitesimally thick porous plug. The top porous plug is in contact with a large, stirred volume of salt solution of concentration c_1. The bottom porous plug is in contact with a similar large, stirred volume of salt solution of concentration c_2. The cylinder is filled with a mixture of the salt solution and a dispersed phase. The salt is soluble in and can diffuse through the dispersed phase. However, there is a partition coefficient K for the salt between the dispersed and continuous phase which is not equal to one.

At steady-state there will be some constant flux J of salt through the system. But depending on which concentrations are used, the same flux J will lead to different values of the diffusion coefficient. Two ways of expressing the concentration will be examined.

The steady-state expression for diffusive flux through this system is

$$J = D \frac{\Delta c}{l} \tag{27}$$

The first approach is to base the diffusion coefficient on the salt bath concentrations, c_1 and c_2. Then

$$D = \frac{Jl}{c_1 - c_2} \tag{28}$$

On the two-phase side of each porous plug the concentration of salt in the continuous phase is the same as the concentration of salt in the solution in contact with the porous plug. But because of the partition coefficient, the average salt concentration on either side of the porous plug is not the same.

Consider a heterogeneous phase in the cylinder of dispersed

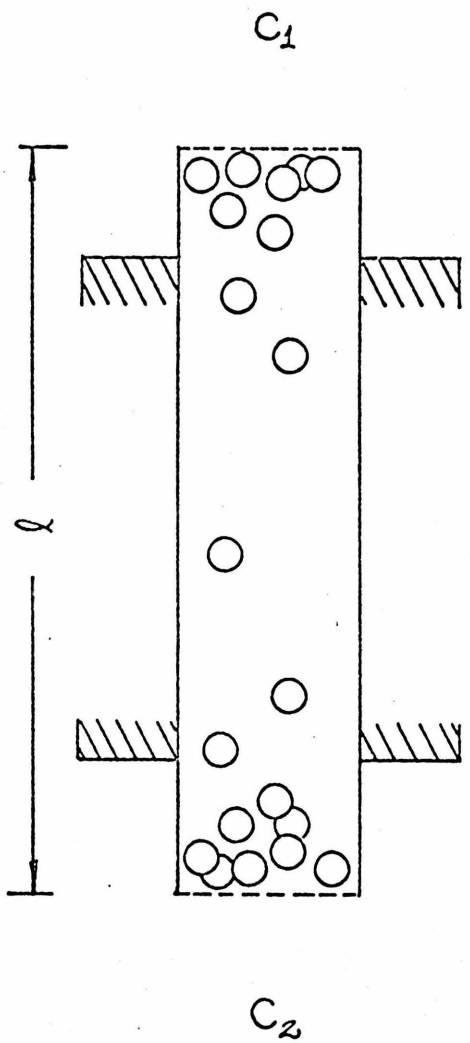

Figure 2. Diffusion in Heterogeneous Media.

phase volume fraction α. The average concentration "inside" the cylinder is

$$C^* = (1-\alpha) C_o + \alpha C_i \tag{29}$$

where

c_o = the concentration of the salt in the continuous phase,

c_i = the concentration of the salt in the dispersed phase.

Since $c_i = Kc_o$, equation (29) can be rewritten as

$$C^* = (1-\alpha) C_o + \alpha K C_o \tag{30}$$

or

$$C^* = [1-\alpha+\alpha K] C_o \tag{31}$$

For the special case where K = 1, $c^* = c_o$.

Therefore, basing the diffusion coefficient on the average concentration inside the porous plug, we have

$$J = D^* \left[\frac{(1-\alpha+\alpha K)C_1 - (1-\alpha+\alpha K)C_2}{l} \right] \tag{32}$$

or

$$J = D^* \left[1-\alpha+\alpha K\right] \frac{C_1 - C_2}{l} \tag{33}$$

Therefore

$$D^* = \frac{Jl}{(C_1-C_2)\left[1-\alpha+\alpha K\right]} \tag{34}$$

Comparing equations (28) and (34), the result is

$$D^* \left[1-\alpha+\alpha K\right] = D \tag{35}$$

This gives the relationship between the diffusion coefficient based on the average concentration and the diffusion coefficient based on the continuous phase concentration. Generally, steady state diffusion measurements involving the diffusion of gases through liquids are based on the continuous phase concentration. Unsteady state, capillary diffusion measurements involving radioactive tracers are based on the average concentration.

If the cylinder shown in Figure 2 is completely filled with the dispersed phase ($\alpha = 1$), the equation (35) reduces to

$$D^* K = D \tag{36}$$

In this case D is the diffusion coefficient based on the apparent concentration difference across the cylinder. From equation (32) it is clear that D* is based on the actual concentration difference across the medium in the cylinder. Thus D* in this case is the actual diffusivity in the dispersed phase.

Recall the modified Maxwell equation for the ayerage diffusivity in a granular medium in which there is a disconuity in the concentration at the boundary of the dispersed and continuous phases.

$$D = D_1 \left\{ \frac{1 + 2\alpha \left[\frac{KD_2/D_1 - 1}{KD_2/D_1 + 2} \right]}{1 - \alpha \left[\frac{KD_2/D_1 - 1}{KD_2/D_1 + 2} \right]} \right\} \tag{22}$$

Substituting $\alpha = 1$ in this expression leads to

$$D = D_2 K \tag{37}$$

A comparison of equations (36) and (37) indicates that the average diffusivity in the modified Maxwell equation is based on the continuous phase concentration and is related to the diffusivity measured in the capillary method by the expression

in equation (35). To test this conclusion, it is worthwhile to examine any reported literature dealing with the diffusion in heterogeneous systems.

Wang (46) carried out a number of diffusion experiments using the capillary tube diffusion method to investigate the self-diffusion coefficient of water in protein solutions. The protein he used was ovalbumin, in weight concentrations up to 24.5 percent.

Tanford (39) states that rigid macromolecules, those which possess internal bonding which allows little or no latitude in the position of one part of the molecule relative to another, can be considered as a solid particle. Osmotic and hydrodynamic experiments suggest that ovalbumin can be considered as a rigid macromolecule, of almost spherical shape.

Thus, for the case of water diffusing through a protein solution, the diffusivity of water through the protein "phase" should be zero. The protein "phase" consists of the protein macromolecule plus its bound water.

To plot the data in a characteristic Fricke plot, the measured diffusivities must be corrected for the bound tagged water, i.e., the non-diffusible water. The protein weight fraction must also be converted to the volume fraction of "protein phase".

Define H as the hydration of the protein in solution, expressed as grams of bound water per gram of protein. Let

c_p be the concentration of protein in grams of anhydrous protein per cc of solution, and w be the weight fraction of protein in grams of anhydrous protein per gram of solution. If the density of the protein solution is designated as ρ, then

$$C_p = \rho w \tag{38}$$

The volume fraction, ϕ, of hydrated protein can now be written as

$$\phi = C_p (\bar{V}_p + H/d_o) \tag{39}$$

where $\bar{V}_p$ is the apparent specific volume of the anhydrous protein in its aqueous solution, and d_o is the density of pure water. Wang (45) gives a justification for this approach. The solution density ρ is found in Figure 3, obtained from data of Goldstick (14) for the density of a general protein solution.

The equation to be used in describing the self-diffusion of water in protein solutions is

$$\frac{\partial C_t}{\partial t} = D\nabla^2 C_d \tag{40}$$

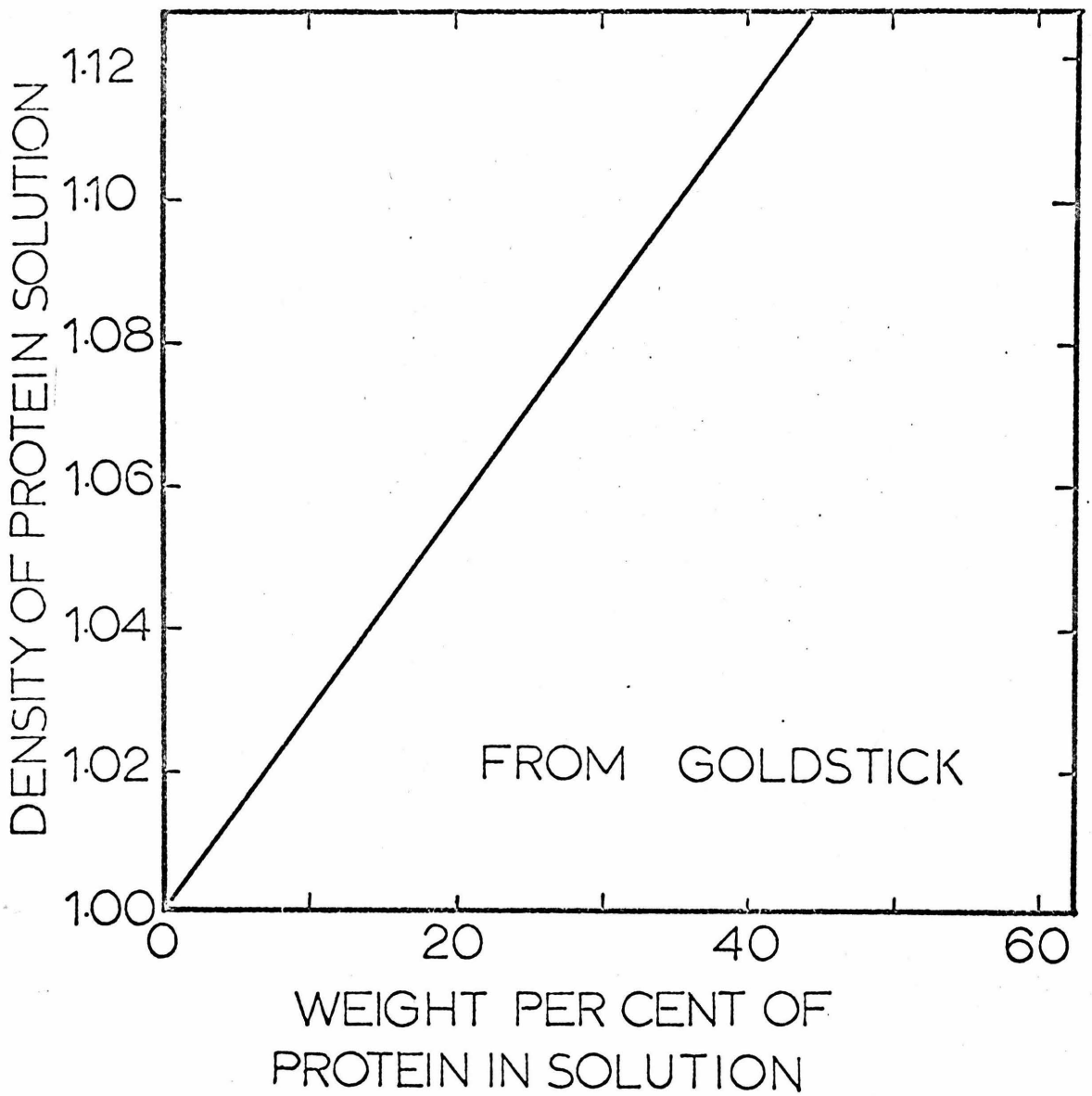

Figure 3. Weight Percent versus Density of a Typical Protein Solution.

where c_t is the total concentration of water, and c_d is the concentration of diffusible water. However, the data obtained from an experiment of this type are described by an equation of the form

$$\frac{\partial c_t}{\partial t} = D^* \nabla^2 c_t \tag{41}$$

where c_t is the amount of water (in grams or moles, etc.) divided by the total volume. In order to solve equation (40) and obtain the actual diffusivity D, an expression relating c_d to c_t is necessary. This is obtained as follows

$$C_t = (1-\alpha) C_s + \alpha C_p \tag{42}$$

where c_s is the concentration of water in the continuous phase and c_p is the concentration of water in the protein phase. By setting

$$C_s = \frac{1}{\xi} C_p \tag{43}$$

equation (42) can be rewritten as

$$C_t = \left[1 - \alpha + \frac{\alpha}{\xi}\right] C_s \tag{44}$$

Since the water in the protein phase is non-diffusible water, and all the water in the continuous phase is diffusible,

$$C_d = (1-\alpha) C_s \tag{45}$$

Therefore

$$C_t = \left[\frac{1-\alpha+\alpha\Xi}{1-\alpha}\right] C_d \tag{46}$$

and equation (40) can be rewritten as

$$\frac{\partial C_t}{\partial t} = \frac{D}{\left[\frac{1-\alpha+\alpha\Xi}{1-\alpha}\right]} \nabla^2 C_t \tag{47}$$

From equation (41)

$$D^* \left[\frac{1-\alpha+\alpha\Xi}{1-\alpha}\right] = D \tag{48}$$

and the true diffusion coefficient can be obtained by multiplying the measured diffusion coefficient by the factor

$$\left[\frac{1-\alpha+\alpha\xi}{1-\alpha}\right]$$

The quantity ξ is merely the grams of water in the protein phase divided by the grams of water in the continuous phase. The point should be stressed that the corrected diffusivity is based on the amount of diffusible water per total volume of protein solution; it is not based on the concentration of diffusible water in the continuous phase per unit volume of continuous phase. It is an average, not a continuous phase concentration.

The corrected data of Wang et al. (46) are plotted in Figure 4. The dashed line in Figure 4 is the Fricke plot for a zero diffusivity of water through the protein phase.

The experimental results are in excellent agreement with the theoretical. The good correlation is also a strong verification for the value 0.18 grams bound water per gram anhydrous protein (45). It appears from this work that the average concentration of the diffusing species is the concentration to use when determining the diffusivity which will subsequently be subjected to a Fricke analysis.

The theoretical Fricke curve in Figure 4 has been corrected for the non-sphericity of the protein macromolecules. The axial ratio used to make this correction was a/b = 2.6. The

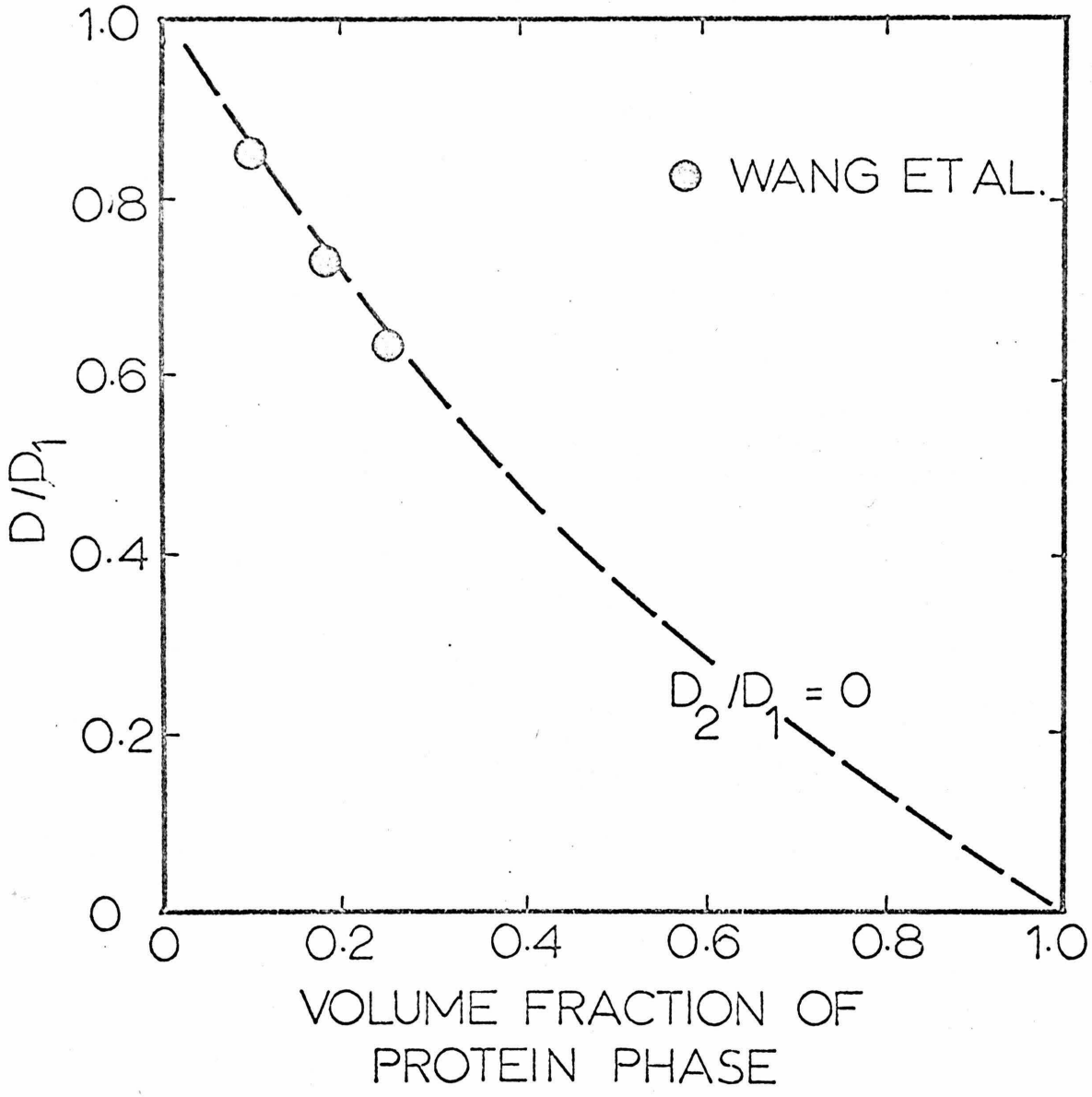

Figure 4. The Self-Diffusion Coefficients of Water in Protein Solutions.

corrected curve does deviate slightly from the curve for a spherical particle. According to Wang (45), the equilibrium assumption used in this analysis between bound and free tagged water is valid.

Unsteady-state gaseous diffusional studies have also been carried out on heterogeneous systems. Goldstick (14) investigated the unsteady-state diffusion of oxygen through protein solutions. One detailed study was carried out using the protein BSA. The protein solution was held in a small cylinder which had as its bottom an oxygen electrode. The electrode functioned as an oxygen sensor. The top of the cylinder was open to a gas atmosphere of a particular oxygen partial pressure.

Wet gas of a known oxygen partial pressure was passed over the protein solution until the system was equilibrated. The oxygen partial pressure in the gas stream was then suddenly changed, and held at this new value during the remainder of the run. The oxygen concentration at the sensor surface which formed the lower boundary of the protein solution was continuously recorded during the experiment.

The equation Goldstick used to describe the process was

$$\frac{\partial p}{\partial t} = D \frac{\partial^2 p}{\partial x^2} \qquad (49)$$

The average concentration of oxygen in the solution is given by the expression

$$C^*_{O_2} = C_{O_2}(1-\alpha) + \alpha C_{O_2 P} \qquad (50)$$

where c_{O_2} is the concentration of oxygen in the continuous phase α is the volume fraction of the protein phase, and $c_{O_2 P}$ is the concentration oxygen in the protein phase. It is reasonable to assume that $c_{O_2 P} = 0$, in which case

$$C^*_{O_2} = C_{O_2}(1-\alpha) \qquad (51)$$

Since

$$C_{O_2} = k P_{O_2} \qquad (52)$$

where k is the Henry's law constant for oxygen, equation (51) can be rewritten as

$$C^*_{O_2} = k P_{O_2}(1-\alpha) \qquad (53)$$

Multiplying through equation (49) by $k(1-\alpha)$ gives

$$\frac{\partial(kP(1-\alpha))}{\partial t} = D \frac{\partial^2(kP(1-\alpha))}{\partial x^2} \qquad (54)$$

or

$$\frac{\partial C_{O_2}^*}{\partial t} = D \frac{\partial^2 C_{O_2}^*}{\partial x^2} \qquad (55)$$

In this case also, the measured diffusivity is based on the average concentration of oxygen.

Again it is assumed that the diffusivity of oxygen through the dispersed phase is zero. Goldstick reports the protein concentrations in weight percent, and they must be corrected to volume percent of hydrated protein. In the case of BSA, H is now 0.34 grams bound water per gram anhydrous protein. The data are plotted in Figure 5. In this case also, the diffusivities based on the average concentration are in agreement with the theoretically predicted curve. The data tend to fall somewhat below the predicted curve. This is probably because the calculated volume of the protein phase is lower than the actual excluded volume.

Two sets of independent experimental results indicate the

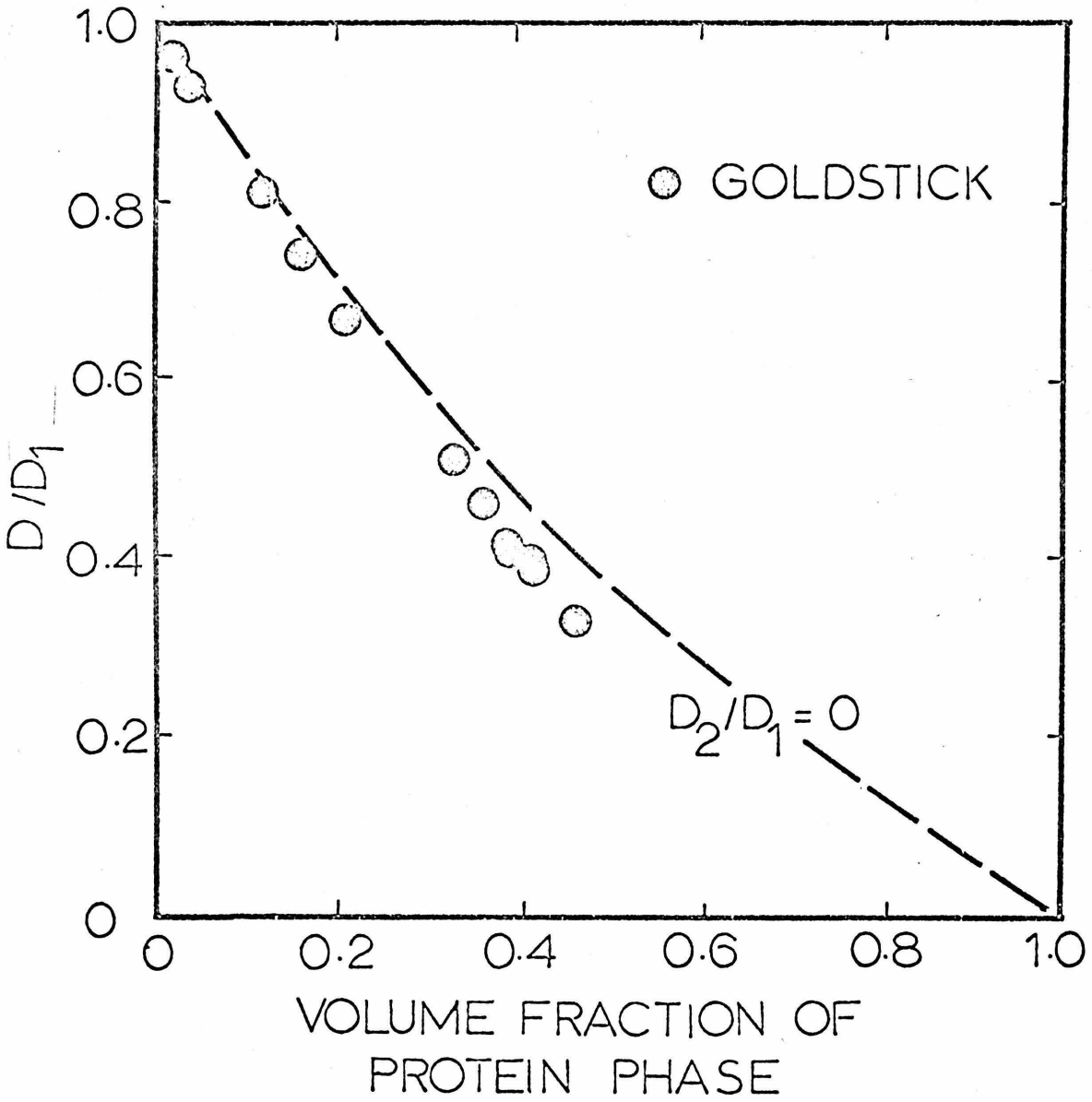

Figure 5. Diffusion Coefficients of Oxygen in Protein Solutions.

average concentration should be used to determine the diffusivity which corresponds to the average diffusivity in the heterogeneous media theory. In both examples cited, the concentration of diffusing species was not continuous across the phase boundary between the continuous and the dispersed phase. The conclusion is that the heterogeneous media theory, derived for a continuous boundary condition, is also valid for a discontinuous boundary condition if the measured diffusion coefficient is based on the average concentration of diffusing species in the medium. This is not in agreement with the previous conclusion based on a rederivation of the Maxwell equation taking into account the discontinuous boundary condition. For the examples just given, the measured diffusivities should be multiplied by the factor $(1-\alpha)$ before they correspond to the diffusivity predicted by the rederived Maxwell equation for which $KD_2/D_1 = 0$. The reason for this obvious inconsistency is not known.

Since Fricke (13) has shown the heterogeneous media theory to be independent of the size of the suspended particles, the small size of the protein molecule should not be a complicating factor. Bull and Breese (3) investigated the conductivity of protein solutions. Their experimental results can be fitted by the Maxwell equation if it is assumed that about 0.65 grams of water are bound per gram of protein. Since the conductance

measurements yield an excluded volume, this somewhat high value for the amount of bound water is not unusual. Because the conductivities of protein solutions are consistent with the Fricke theory, diffusivities in protein solutions would also be expected to be consistent with the theory.

3. Bound Diffusing Species.

At this point it may be wise to ask the question, if part of the sodium in the red blood cells is bound, how will this affect the measured diffusion coefficient? For according to Troshin, who quotes numerous authors, from 5 to 20 percent of the cell's sodium (including erythrocytes, muscle fibers, and egg cells) is not diffusible.

Again, the equation to be used is

$$\frac{\partial c_t}{\partial t} = D\nabla^2 C_d \qquad (40)$$

where

c_t = the total concentration of sodium,

c_d = the concentration of diffusible sodium.

Now, if c_s stands for the concentration of sodium in the continuous phase and c_{drbc} stands for the diffusible concentration of sodium in the red blood cell, then

$$C_d = (1-\alpha) C_s + \alpha C_{d-rbc} \tag{56}$$

Let $c_{d-rbc} = \gamma c_{rbc}$, where c_{rbc} is the concentration of sodium in the red blood cell, and $c_{rbc} = \xi c_s$.

Then

$$C_d = (1-\alpha) C_s + \alpha\gamma C_{rbc} \tag{57}$$

or

$$C_d = (1-\alpha) C_s + \alpha\gamma\xi C_s \tag{58}$$

and finally

$$C_d = (1 - \alpha + \alpha\gamma\xi) C_s \tag{59}$$

Now,

$$C_t = (1-\alpha) C_s + \alpha C_{rbc}$$

or

$$C_t = \left[1 - \alpha + \alpha\xi\right] C_s \tag{61}$$

Therefore

$$C_s = \frac{C_t}{\left[1 - \alpha + \alpha\xi\right]} \tag{62}$$

and

$$C_d = \left[\frac{1 - \alpha + \alpha\gamma\xi}{1 - \alpha + \alpha\xi}\right] C_t \tag{63}$$

Substituting this into equation (40) gives

$$\frac{\partial C_t}{\partial t} = D\left[\frac{1-\alpha+\alpha\gamma\xi}{1-\alpha+\alpha\xi}\right] \nabla^2 C_t \tag{64}$$

or

$$\frac{\partial C_t}{\partial t} = D^* \nabla^2 C_t \tag{41}$$

where D^* is the experimentally measured diffusion coefficient.

Therefore, the actual diffusion coefficient is

$$\mathcal{D} = \left[\frac{1-\alpha+\alpha\xi}{1-\alpha+\alpha\delta\xi}\right]D^* \tag{65}$$

Assume for the moment that the average concentration of diffusing species must be used to obtain a measured diffusion coefficient which is consistent with the heterogeneous media theory. A similar argument applies if the continuous phase concentration should be used. The measured diffusivities will be expected to correspond to a curve derived from the Fricke theory. Since

$$\mathcal{D} = \mathcal{D}_1\left\{\frac{1+2\alpha\left[\frac{D_2/D_1-1}{D_2/D_1+2}\right]}{1-\alpha\left[\frac{D_2/D_1-1}{D_2/D_1+2}\right]}\right\} \tag{21}$$

it follows that

$$D^* = \mathcal{D}_1\left\{\frac{1+2\alpha\left[\frac{D_2/D_1-1}{D_2/D_1+2}\right]}{1-\alpha\left[\frac{D_2/D_1-1}{D_2/D_1+2}\right]}\right\}\left\{\frac{1-\alpha+\alpha\delta\xi}{1-\alpha+\alpha\xi}\right\} \tag{66}$$

The effect of the bound fraction on the diffusion coefficient depends on the amount that is bound. Since the presence of a bound fraction may not always be known in advance, it is worthwhile to know if its presence can be inferred from the measured diffusivities.

As an example, take sodium diffusing through blood where $\xi = 1/6$. Assume that $D_2/D_1 = 0.54$ and that σ, the fraction of free, diffusible sodium in the red cell is 0.6. The Fricke curve is first determined for this system based on the actual D_2/D_1 ratio, and is given by the solid line in Figure 6. The expected experimental data based on $\sigma = 0.6$ are then determined from this curve, given by the circles in Figure 6. Finally, the experimental data are fitted by another Fricke curve given by the dashed line.

Although the complete range of experimental data cannot be fitted by a Fricke plot, a sizeable portion can. In fact, from the example, the data taken up to a hematocrit of 60 would not allow the conclusion that a non-diffusible fraction was present. This would give a value for D_2 lower than the actual value. A non-diffusible fraction present would cause an underestimated value of D_2.

At higher values of σ, the effect is not nearly as pronounced. For instance, at a hematocrit of 50, in the same system where now $\sigma = 0.8$, $D^* = 1.03D_m$, whereas if $\sigma = 0.9$, $D^* = 1.01\ D_m$. That is to say, if 90 percent of the

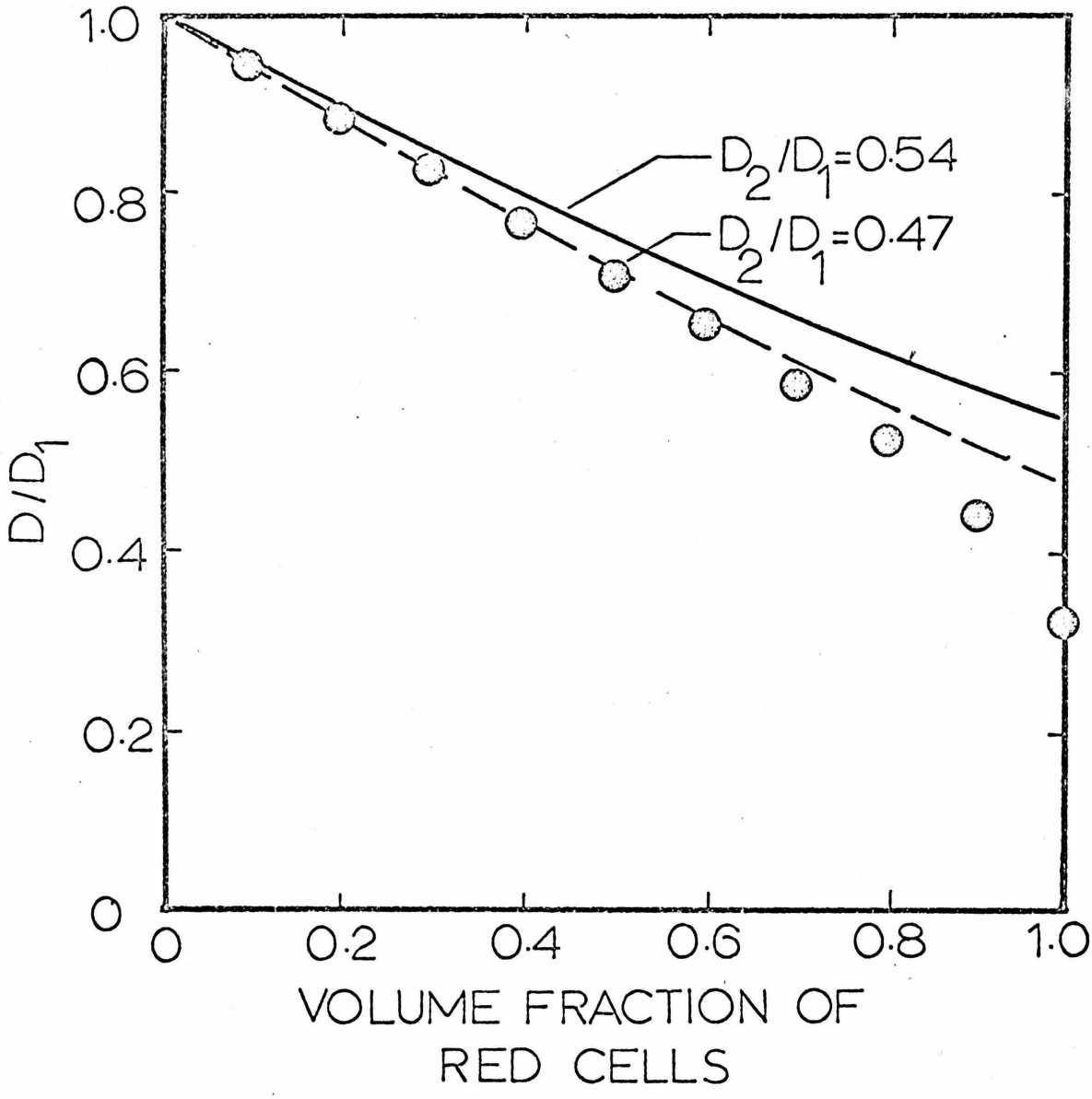

Figure 6. Effect of Slightly Bound Species on the Diffusion of those Species in Blood.

sodium in the red blood cell was diffusible, the measured diffusivity and the actual diffusivity would only differ by one percent.

The effect is small because the concentration in the dispersed phase is so much less than that of the suspending medium. This means that the amount that is non-diffusible is a very small fraction of the total at the low hematocrits.

For a species which is highly concentrated in the dispersed phase, e.g., potassium, quite different results might be expected. As found previously,

$$D = D^* \left[\frac{1 - \alpha + \alpha\xi}{1 - \alpha + \alpha\sigma\xi} \right] \qquad (65)$$

Note that, whatever the species in question, $D_m \leq D^*$, as $0 \leq \sigma \leq 1$.

As an example, consider potassium diffusing through blood with $D_2/D_1 = 0.54$ but with $\sigma = 0.4$ and $\xi = 25$. In Figure 7, the dashed line is a Fricke plot corresponding to $D_2/D_1 = 0.54$. The circles represent the expected experimental data with the given σ and ξ. The solid line represents the Fricke plot for $D_2/D_1 = 0$.

B. Experimental Procedure.

1. The Capillary Tube Apparatus

Self-diffusion coefficient measurements using the capillary

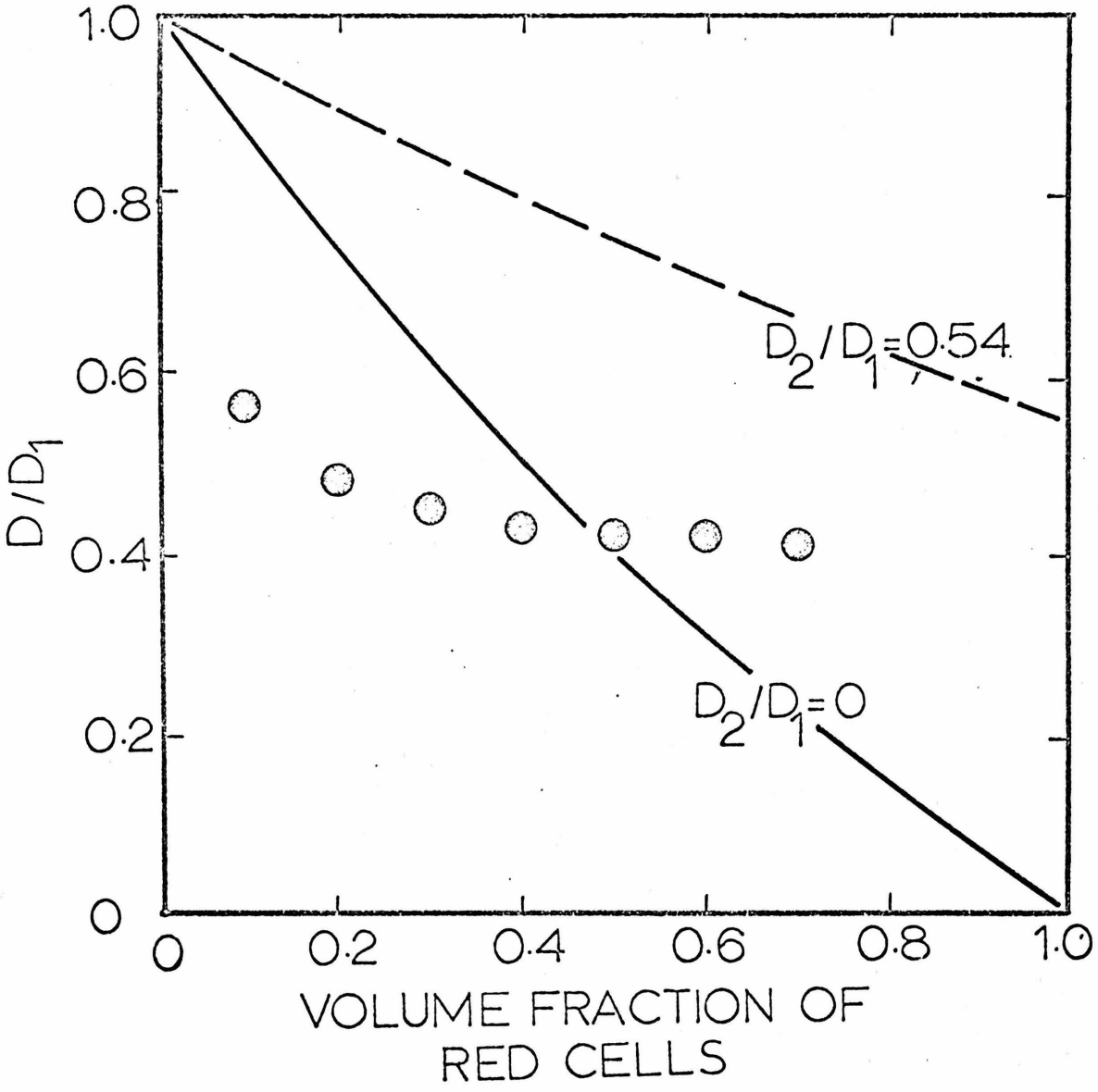

Figure 7. The Effect of Strongly Bound Species on the Diffusion of those Species in Blood.

tube method were first made by Anderson and Saddington (1). Their method has since been analyzed, perfected, and extensively used by Wang (43, 44).

Following Witherspoon and Saraf (31, 32), a special capillary of variable length was used with an internal diameter of about 0.10 cm. The capillary tube was a 50 μl gas-tight syringe manufactured by the Hamilton Co., Inc., Whittier, California. It was modified by grinding flat the needle end of the syringe at the zero mark (Figure 8). The tight, fitting Teflon tip of the plunger effectively contained any liquid in the capillary. The plunger allowed a convenient choice of capillary length up to about 7 cm. An optimum length of about 3 cm was chosen based on the scooping-out effect investigated by Wang. If the length was too short, the scooped-out portion of the capillary was too large a fraction of the total capillary volume. However, if the length was too long, too much time was required to obtain a reasonable tracer loss. In calculating the diffusion coefficient, it was convenient to use as few terms in the series expansion as possible. Shorter experimental times were preferred to minimize the biological decay of the fluid in the capillary.

A most critical dimension was the length of the working portion of the capillary. This was found by adding the length of the glass barrel to the length of the spacer, and then sub-

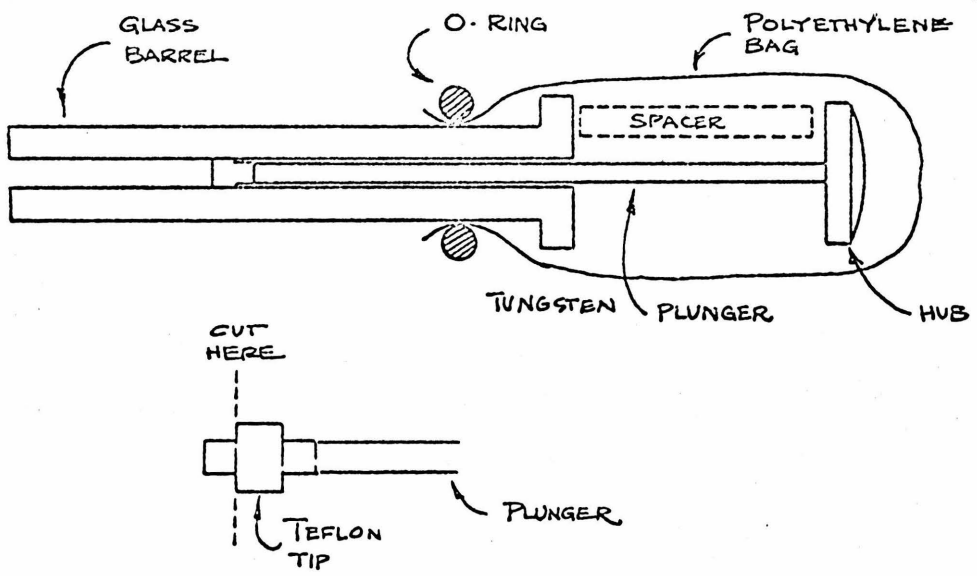

Figure 8. The Capillary.

tracting from this sum the distance from the tip of the plunger to the hub. Although the latter distance was difficult to obtain, measurements made at different times were quite reproducible.

The Teflon tip first obtained from the Hamilton Co. fitted snugly along its whole length and was used as obtained. Teflon is subject to cold flow, and the tight fitting Teflon tip loosened in time. Immersing the tip in boiling water rectified this tendency, but not indefinitely. When new tips were ordered, they were shaped somewhat differently, as shown in Figure 8 and had to be modified by cutting off the portion beyond the raised part. It was necessary to grind a small portion of the tungsten plunger since it fitted very deeply into the Teflon tip. Thus the fluid in the plunger end of the capillary was not exposed to a complete Teflon surface, but this did not seem to affect the results. The inner surface of the capillary tube was coated at times with the preparation Siliclad, but it also seemed to have no effect on the results.

2. Radioactive Counting Apparatus.

Following Thompson (40) and Sachs (30), both the sodium-22 and the potassium-42 were counted in a well type scintillation counter. Sodium-22, with a half life of 2.6 years, emits gamma radiation with a peak energy of 1.274 Mev. Potassium-42 has a half life of 12.4 hours. Its strong gamma radiation (18%) is 1.53 Mev. It also emits a strong beta radiation.

The counting apparatus consisted of a Baird Atomic Model 810B Well Scintillation Detector. A two dram polyvial fitted snugly into the well. The detector was powered by a Model 409A High Voltage D.C. Supply made by the John Fluke Mfg. Co., Inc., Seattle, Washington. The output from the detector was fed into a Baird Atomic Model 250 Amplifier-Analyzer. The amplified signal was finally passed to a Baird Atomic Model 132 Multiscaler II, which recorded the counts per minute. A Baird Atomic Model 960R Timer was used in conjunction with the Model 132 Multiscaler. The power source for the Model 132 was a CV-1 Constant Voltage Transformer manufactured by the Sola Electric Co., Chicago, Illinois.

The beta emitters were counted in a Beckman LS-100 Liquid Scintillation System.

3. The Diffusion Run.

The capillary tube was filled by first depressing the plunger until the Teflon tip protruded slightly from the ground end. This end was then immersed in the tagged solution, and shaken to dislodge the bubbles which commonly formed on the Teflon tip. The plunger was then pulled up until the capillary was slightly over-filled with respect to its final volume. The tagged solution was completely wiped off the sides and tip of the capillary. During this procedure some of the liquid right in the top of the capillary tube was lost, which dropped the

liquid level in the capillary below the ground face. The spacer was then placed between the glass barrel and the hub of the plunger, and the plunger depressed until the hub contacted the spacer. This set the capillary volume. At this point the fluid protruded out slightly from the end of the capillary tube. This could be left to counteract the initial scooping-out when the capillary was placed in the bath solution. The slight excess was removed using the corner of an absorbent paper. In this way, the liquid was absorbed slowly, and the process was stopped at the exact point where the excess liquid disappeared. A small polyethylene bag was placed over the plunger end of the capillary tube and secured to the glass barrel by means of a rubber "O"-ring (Figure 8).

The filled capillary tube was then inserted into a Teflon cylinder (Figure 9) which was subsequently filled with the bath fluid and placed in a water bath.

Three samples were taken before the run was started to determine the initial concentration of tracer in the capillary.

The cylinder was positioned so the end containing the capillary tube was down. The top of the cylinder was then unscrewed, and the prewarmed bath solution poured slowly down the side of the cylinder from a flask until the cylinder was almost full. The timer was started the moment the bath liquid came in contact with the open end of the capillary. The un-

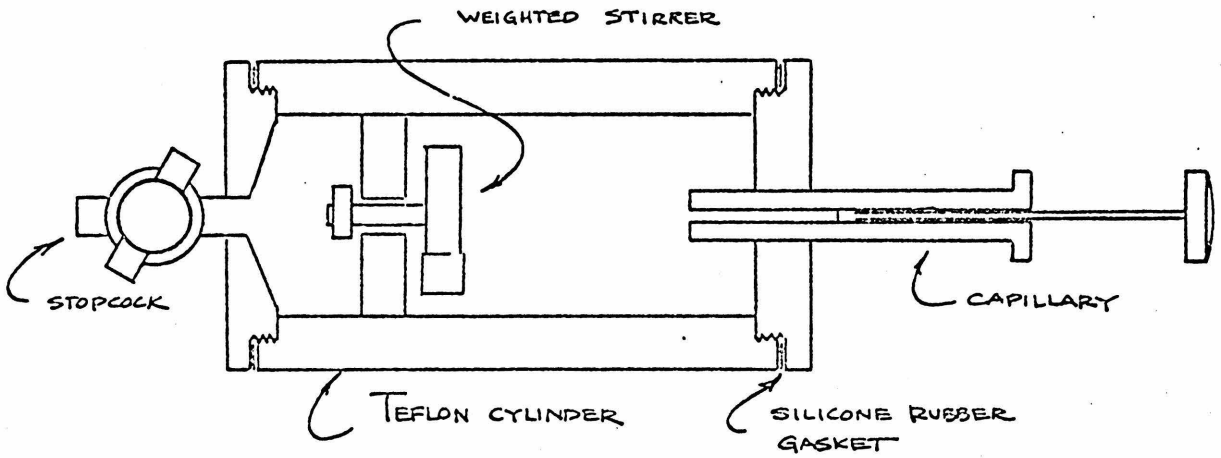

Figure 9. The Diffusion Apparatus.

screwed top, with the stopcock open, was then replaced. A 10 ml syringe, fitted with a long needle, containing the bath solution, was inserted through the stopcock into the cylinder and completed the filling process. The stopcock was closed, the cylinder was inserted into its holder and lowered into a water bath.

The cylinder was rotated horizontally; the capillary was parallel to the lab bench. A weighted stirrer inside the cylinder remained stationary as the cylinder rotated, thus stirring the bath fluid.

The bath fluid was the same composition and concentration as the continuous phase in the capillary tube, but did not contain the tagged species. Therefore, the only concentration gradient in the system was in the concentration of tracer in the capillary.

At the end of the run, the cylinder was removed from its holder and positioned stopcock downward over a flask. The stopcock was opened and the syringe carefully removed. The sides of the capillary were wiped dry, and as much as possible of the excess liquid on the ground end of the syringe was removed. Care was taken not to remove any liquid in the capillary tube itself. The contents were then ejected into an appropriate counting vial. To the vial was also added approximately one ml of water which was used to rinse the end of the capillary. This insured complete removal of all tracer. A larger volume of

rinsing fluid was found unnecessary. The timer was stopped just as the capillary was removed from the Teflon cylinder.

4. Preparation of Blood.

To produce a suspension of any given hematocrit, the red cells were first separated from the plasma by centrifugation and then recombined with the plasma in the proper proportions. In packing the cells they were centrifuged for 20 minutes at 2500 rpm in a Universal Model UV centrifuge made by the International Equipment Co. The original hematocrit was observed directly by using graduated centrifuge tubes. The plasma was decanted and the buffy coat always discarded.

Rabbit blood was obtained fresh from the Biology Division of the California Institute of Technology. The anticoagulant used was ACD. Citrated human blood was obtained from Hyland Laboratories, Los Angeles, California.

When the red blood cells were to be suspended in an artificial plasma such as Earles' Solution, they were first washed one or two times in an isotonic salt solution and then twice in the artificial plasma. The stored blood was kept in a refrigerator at about 6°C.

5. Blood-Gel Mixture.

When the apparatus was originally designed, it was hoped that the slowly rotating, horizontal capillary tube would keep the red blood cells essentially stationary, but this did not turn out

to be the case. To avoid the problem of cell motion, a gel suspension was prepared using a technique developed by Stein (38) for another geometry.

One gram of agar was dissolved in 90 ml of Earles Solution A (Appendix II-2) by heating the solution to boiling. After cooling the solution to 43°C in a water bath, 10 ml of Earles Solution B (Appendix II-2) were added. The solutions could not be combined beforehand due to the formation of a precipitate on heating. A volume of packed red blood cells was also brought to 43°C. A certain amount of each was added to a vial containing the radioactive material, also in a 43°C water-bath, to give a desired hematocrit. The contents were stirred by shaking the vial and allowed to equilibrate for a certain length of time. Samples were taken from the vial for the experiment. When using the blood-gel mixture, no excess liquid was left on the end of the capillary.

A sample was drawn into the syringe and allowed to cool and gel for 5 minutes. It was then brought to the correct volume by means of the spacer, and the excess gel protruding from the capillary was neatly removed by a double-edged razor blade.

The bath solution in the cylinder consisted of 10 ml of Earles Solution B in 90 ml of Earles Solution A.

6. Radioactive Counting Procedure.

The well scintillation detector used in counting sodium and potassium was calibrated using a Cesium 137 source. The battery of instruments was allowed to warm up for approximately one hour. The pulse-height selector knob on the Multiscaler was turned to its maximum value. The high voltage supply was set at 850 volts, positive polarity. The lower level on the Amplifier-Analyzer was set at 6.62 (66.2 volts) and the "Integral-Differential" switch set in the "Differential" position. The window was set at 2.00 (4.00 volts). The cesium sample was placed in the well, and counts were taken over one minute intervals. The coarse and fine gain settings were varied until a maximum cpm reading was obtained. This completed the calibration procedure.

To count a radioactive sample, the lower level was set at 0.50 (5.00 volts) and the "Integral-Differential" switch moved to the "Integral" setting. The sample was placed in the well and counted for a given amount of time, generally 10 minutes. A background count, with no sample in the well, was taken at the beginning and end of a series of sample counts. A sample was generally counted twice to insure reproducibility of the count. The samples were always less than one microcurie.

IV. EXPERIMENTAL DATA AND ANALYSIS

A. Sodium Diffusion.

1. The Diffusion of Sodium in Potassium Chloride Solutions.

Wang (44) made a detailed study of the self-diffusion of sodium-22 in a potassium chloride solution, to determine how the measured self-diffusion coefficient varied with increasing solute concentration. It was decided to test the results of the present apparatus against his data.

An appropriate volume of radioactive sodium-22 solution was evaporated to dryness and redissolved in a small volume of 0.100 M potassium chloride solution. This solution was used in the capillary tube. The bathing solution used in the cylinder was the 0.100 M potassium chloride solution. The water temperature bath used in these experiments was maintained at 25°C.

The first few runs lasted 48 hours. With this length of run all terms after the first in the series expansion solution to the diffusion equation could be dropped. The average self-diffusion coefficient of sodium-22 through 0.100 M potassium chloride solution, based on three runs, was 1.320 ($\pm$ 0.013) x 10^{-5} cm^2/sec.

Since the goal was eventually to use blood in the apparatus, a shorter run was desirable. With this in mind, three runs

were made with the same system, but for only 29 hours. This necessitated including the second term in the series expansion solution. The average self-diffusion coefficient for the three short runs was 1.310 ($\pm$0.013) x 10^{-5} cm^2/sec. Since shortening the run time to 29 hours had no effect on the diffusion coefficient determinations, the duration of the run was further reduced to 24 hours for the remainder of the experiments.

The result obtained by Wang for the self-diffusion coefficient of sodium-22 through a 0.100 M KCl solution was 1.30 ($\pm$0.037) x 10^{-5} cm^2/sec. This result was in good agreement with that found in the present investigation, which indicated the capillary diffusion apparatus was performing well. Wang used glass capillary tubes with an internal diameter of 0.5 mm, whereas a 50 μl syringe with an internal diameter of 1.0 mm was used in this work. The volume of his diffusion bath was one liter, whereas the volume of the diffusion bath in this work was approximately 80 ml. One concern was that the small bath volume would invalidate the boundary condition of zero tracer concentration at the mouth of the capillary. The shorter run time would be favorable from this standpoint. The results which were obtained indicated the boundary condition was held throughout the diffusion run.

2. The Diffusion of Sodium in Sodium Chloride Solutions.

A few determinations of the self-diffusion coefficient of sodium-22 in 0.100 M sodium chloride solutions at 25°C were also made. Averaging the results of four runs gave a value of 1.344 (±0.012) x 10^{-5} cm^2/sec. These were 48 hour runs.

Mills (28) measured the self-diffusion of sodium in sodium chloride solutions at 25°C using the capillary tube apparatus. Extrapolating back his results to 0.10 M NaCl gave a value for the self-diffusion coefficient of approximately 1.31 x 10^{-5} cm^2/sec.

Castleden and Fleming (6), using the same technique, obtained a self-diffusion coefficient for sodium diffusion in a 0.10 M sodium chloride solution of 1.25 (±0.03) x 10^{-5} cm^2/sec.

The data of Slade et al. (35), although not obtained in a capillary tube apparatus, confirmed the data of Mills. Their measurements gave a value for the self-diffusion coefficient of sodium in a 0.10 M NaCl solution of 1.305 x 10^{-5} cm^2/sec. The diffusivities obtained in this study were definitely higher than those reported in the literature.

It was soon discovered that the distance between the mouth of the capillary and the stirrer was a critical factor in obtaining reliable results. The stirrer could not be kept close to the mouth of the capillary. Once the stirrer was properly positioned at the far end of the cylinder, a value for the dif-

fusion coefficient of sodium in 0.100 M NaCl at 25°C, based on two 24-hour runs, was found to be 1.316 (±0.00) x 10^{-5} cm^2/sec. This result was much more satisfactory.

3. The Diffusion of Sodium in Rabbit Plasma.

Fresh rabbit blood was diluted approximately 6:1 with ACD solution (30 ml ACD to 200 ml blood), and immediately centrifuged. The plasma was decanted and the red blood cells discarded. A small portion of the plasma was added to a vial containing the radioactive sodium. The remainder was used as the bathing fluid in the capillary apparatus. Since the plasma tended to deteriorate rapidly at 37°C, the runs were carried out at 25°C. Based on five runs, the average self-diffusion coefficient of sodium in the plasma was found to be 1.031 (± 0.030) x 10^{-5} cm^2/sec.

The specific gravity of the plasma at 25°C was 1.025. The ratio of the viscosity of plasma to that of water at 25°C was 5/3 (1.667). If the expected diffusivity of sodium in the plasma is calculated based on a viscosity correction of the sodium diffusivity in water, the equation to be used is

$$D_P = D_{H_2O} \frac{\eta_{H_2O}}{\eta_P} \qquad (67)$$

Therefore,

$$D_p = (1.316)(1.67) \times 10^{-5} \text{ cm}^2/\text{sec}$$
$$= 0.79 \times 10^{-5} \text{ cm}^2/\text{sec}$$

and the actual is considerably higher than the expected. The actual diffusivity ratio is

$$D_p / D_{H_2O} = 1.031 / 1.316$$
$$= 0.78$$

This high ratio may be peculiar to sodium, which would suggest a sodium diffusion augmentation mechanism in the plasma, or may be due merely to the poor ability of a viscosity correction to predict diffusion behavior in fluid systems such as plasma.

Spaeth (36) compiled the available data on the diffusivities of oxygen through water and plasma. From these data it appeared as though the viscosity correction should be valid, and in light of this the results of the diffusion of sodium in plasma found in this study become quite interesting. However, the evidence is not conclusive.

Stein (38) also obtained measurements of the diffusion of oxygen through water and plasma. From his work, a diffusivity ratio of 0.75 was obtained. If his results are correct, the con-

clusion follows that there is nothing peculiar in the diffusion of sodium through plasma. Unfortunately, he did not give viscosity data for the plasma he used, so a more concrete conclusion can not be formed.

4. The Diffusion of Sodium in Rabbit Blood.

The determination of the diffusion of sodium through rabbit blood was only slightly more involved than the determination of the diffusion of sodium through plasma. A sample of blood was centrifuged and the plasma decanted. A given volume of packed red blood cells was added to a certain volume of plasma to give a desired hematocrit. This blood sample was then placed in a vial containing the radioactive sodium and eventually drawn up into the capillary tube. Plasma was used as the bath fluid; the presence of red cells in the plasma bath was not considered necessary.

The capillary tube was set in a horizontal position and rotated at approximately 10 rpm. Calculations, taking into account both the settling velocity and the centrifugal force on the red cells, indicated this configuration and rotational speed would minimize the red blood cell movement.

The results of this experiment indicated the convection problem had not been completely eliminated (Figure 10). Except for the case of packed cells, this method does not appear valid for measuring the diffusivities of solutes in blood. Only one approach remained; the cells must in some way be immobilized.

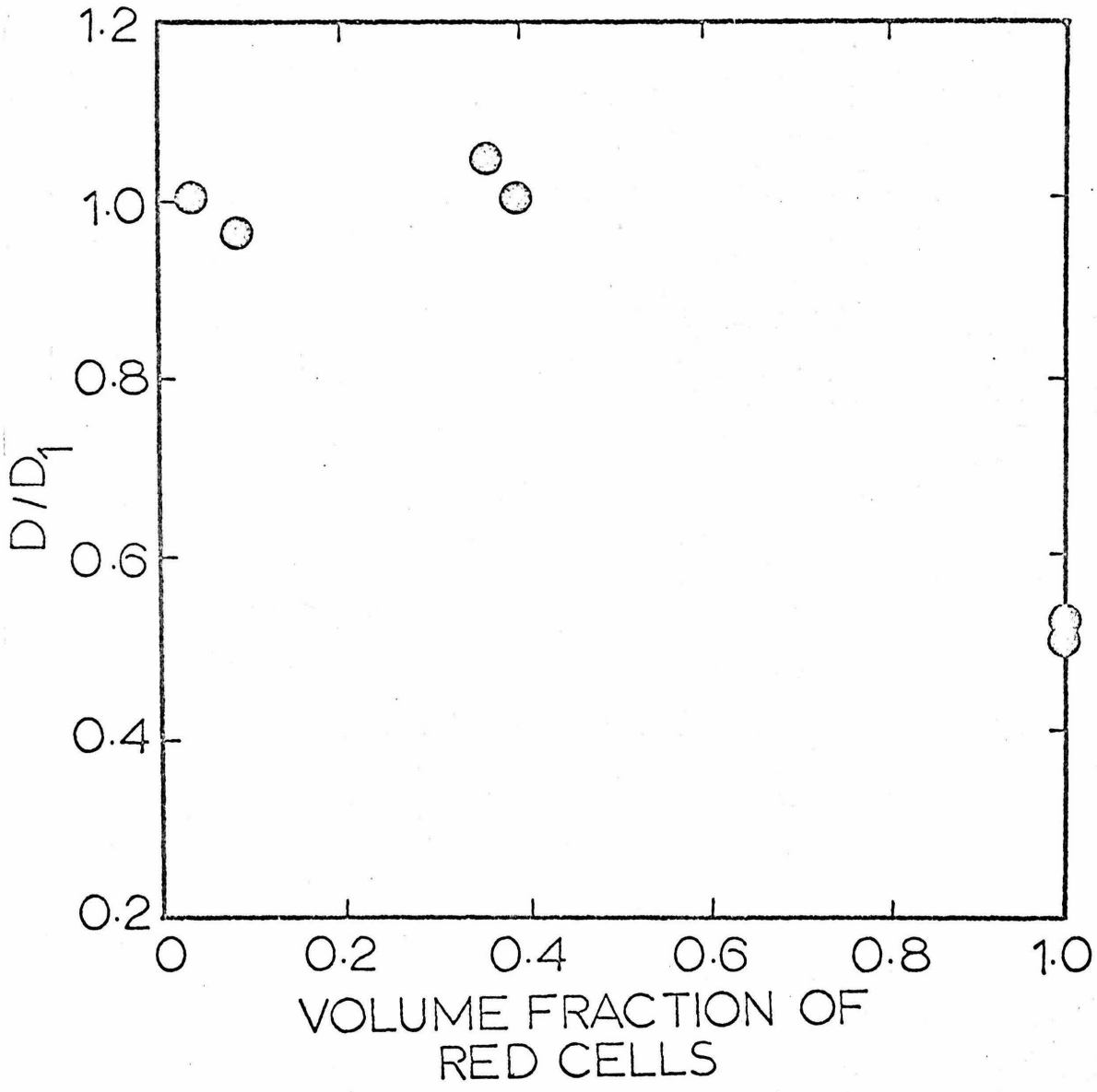

Figure 10. Diffusion Coefficients of Sodium in Rabbit Blood at 25° C.

The work of Stein (38) indicated an agar gel would be an effective solution to this problem. Since the liquid used to make the gel solution must be heated to the boiling point of water to dissolve the gel, plasma could no longer be used. Earle's Solution was chosen as the artificial plasma. An artificial plasma has the advantage over regular plasma of being chemically reproducible.

5. The Diffusion of Sodium in an Agar Gel.

The preparation of the gel has already been described. The hot gel solution was drawn into the capillary and allowed to set before the run was initiated. The liquid in the Teflon cylinder was Earle's Solution.

The results for the diffusion of sodium through the agar gel at 25°C are given in Table I. The two sets of experiments were made at different times of the year with slightly different capillary lengths. The results evidence good reproducibility and negligible variation in time. The overall average value for the self-diffusion coefficient of sodium through the one percent agar gel (w/v) was $1.254\ (\pm 0.025) \times 10^{-5}\ cm^2/sec$. This is the value for D_1 which will be used in the succeeding calculations.

6. The Diffusion of Sodium in an Agar Gel Suspension of Rabbit Red Cells.

As was stated earlier, the rabbit blood was obtained fresh, by means of a small incision in the ear of the rabbit. The rabbits were New Zealand Whites, and were kept by the Biology

Table I. The Diffusion Coefficients D_1 of Sodium in a One Percent Agar Gel at 25°C.

Date	D x 10^5 (cm^2/sec)
Oct. 1968	1.255
"	1.250
"	1.264
April 1969	1.235
"	1.254
"	1.266
"	1.254
"	1.258
Average value	1.254

Division of the California Institute of Technology. Some of the experiments were made with the blood the day it was collected, others with red blood cells stored for a few days in Earle's Solution at 6°C.

The diffusional data obtained in this phase of the investigation are plotted in Figure 11. These experimentally determined diffusion coefficients are based on the average concentration of tracer in the capillary as defined in equation 23. The unrevised experimental diffusion coefficients presented in this work are always of this form. They were calculated by means of equation 24 from the initial and final concentrations of tracer in the capilary.

The scatter in the data is quite pronounced. Referring to the data in Table VI (Appendix III-1), D_2 is calculated as 0.54 ($\pm$0.10) x 10^{-5} cm^2/sec. This is the effective diffusivity of sodium through the rabbit red blood cell based on the average concentration of sodium in the red cell-gel mixture.

Kreuzer (22) determined the value of the diffusion coefficient of oxygen through red blood cells as 0.43 ($\pm$0.07) x 10^{-5} cm^2/sec at 37°C. Keller (21) determined the diffusivity of oxygen through a concentrated hemoglobin solution (33 weight percent) as 0.86 ($\pm$0.21) x 10^{-5} cm^2/sec at 25°C. Therefore, the lack of reproducibility in this work is not extremely large compared with the deviations presented by other investigators.

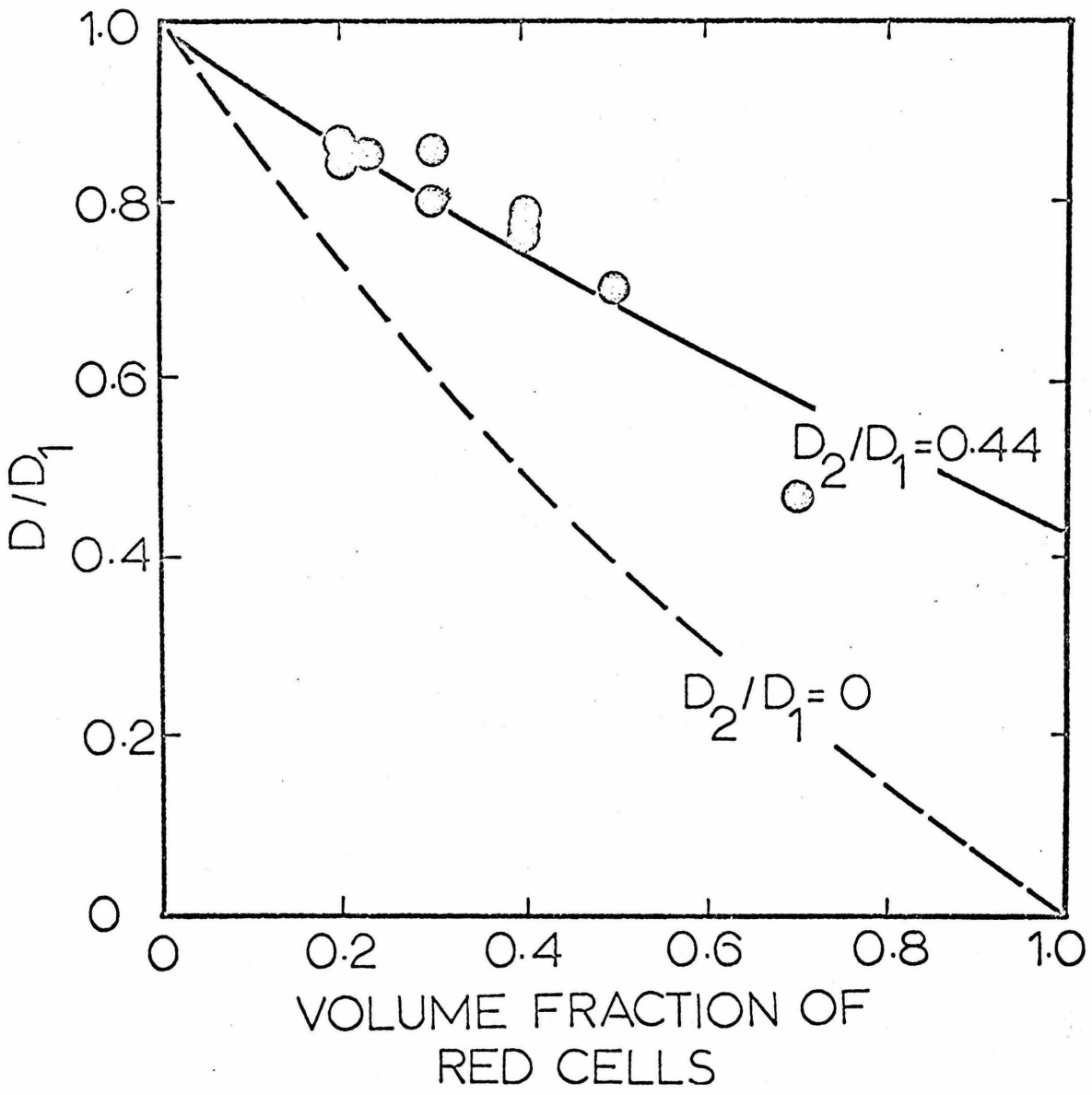

Figure 11. Diffusion Coefficients of Sodium in Agar Gel Suspensions of Rabbit Red Cells at 25° C.

However, based on the excellent reproducibility of results obtained in this investigation on the diffusion of sodium through salt solutions and the agar gel before the rabbit blood work, and that done afterwards on the diffusion of ions through human red blood cell-gel mixtures, the lack of precision here stands as an enigma. No single reason seems convincing.

The striking aspect of this work is the value 0.54×10^{-5} cm^2/sec for the diffusivity of sodium through the red cell. This diffusivity is slightly more than half that through plasma, and much higher than expected. All studies dealing with the transport of sodium across the cell membrane, as discussed in Chapter II, indicate the membrane is very impermeable to this ion. Because the membrane appears so impermeable to sodium, the average diffusivity of sodium through the red cell (membrane plus interior) would be expected to be orders of magnitude less than the diffusivity of sodium in plasma.

The obvious answer is that the results should be interpreted differently. The modified Maxwell equation discussed earlier indicated that the diffusion coefficients should be based on the concentration of diffusible species in the continuous phase. Revising the data accordingly, the results given in Figure 12 are obtained. To obtain these diffusivities based on the concentration of sodium in the continuous phase, it must be assumed that the ratio of the concentration of tagged sodium in the red

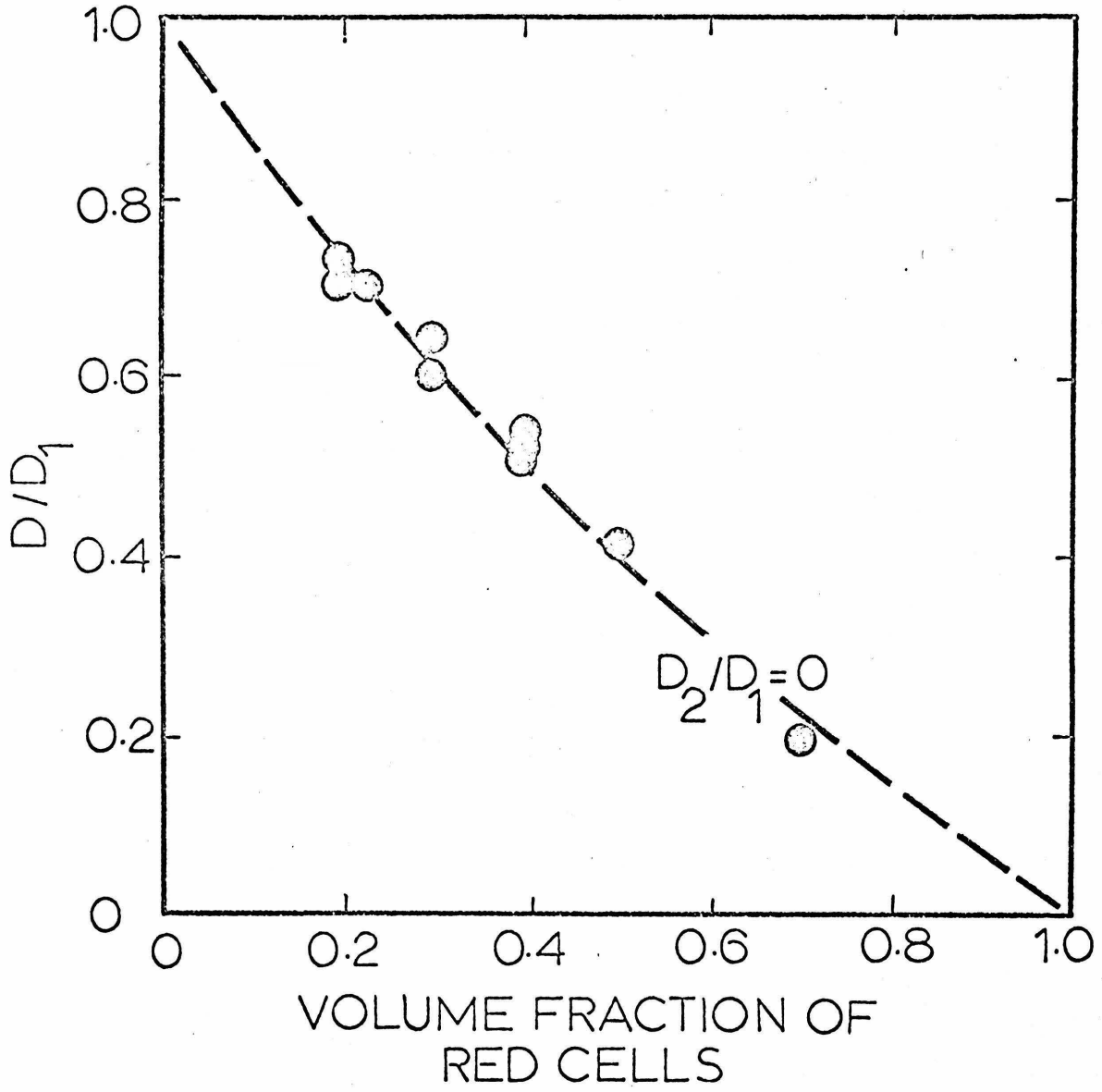

Figure 12. Diffusion Coefficient of Sodium in Agar Gel Suspensions of Rabbit Red Cells at 25°C, Based on the Assumed Equilibrium Sodium Concentration in the Continuous Phase.

cell to that in the gel remains the same throughout the run. This ratio is 1/6. Figure 12 indicates the value for D_2K is essentially zero. The actual diffusivity of sodium through the rabbit red cell is six times the value for D_2 in the D_2/D_1 curve which best fits the data in Figure 12, but because of the experimental error and the uncertainty in the red cell shape factor, a precise value for the diffusion coefficient of sodium through the red cell can not be obtained. The value does appear to be much less than D_1, the diffusivity of sodium through the gel.

This second approach is consistent with the expectation expressed above of a relatively low diffusivity of sodium through the red cell. This approach is strictly valid only if the concept of the physical state of the cell held by Troshin is correct, where the concentration discontinuity of sodium between red cell and plasma is the result of a phase solubility. If, on the other hand, this concentration discontinuity is the result of an active transport mechanism, this approach may be only approximately valid.

The second approach is not without its shortcomings, however. As discussed previously, the diffusion coefficients based on the continuous phase concentration for the self-diffusion of water and the diffusion of oxygen in protein solutions are not consistent with those predicted from the Fricke theory. The calculated diffusion coefficients are consistent with the theory when based on the average concentration.

Recall the work reported earlier on the diffusion of sodium in rabbit blood. The observed diffusivity through the packed cells was 0.527 (±0.014) x 10^{-5} cm^2/sec. This agrees well with the effective diffusivity of sodium through the rabbit red cells based on the average sodium concentration found in the red cell-gel work.

By assuming a reasonable void fraction in the packed red blood cells of six percent, the value of D_2 in the red blood cell-gel experiment is recalculated as 0.50 x 10^{-5} cm^2/sec. The Fricke analysis must now be used to obtain D_2 in the packed red blood cell-plasma run. It is now calculated as 0.50 x 10^{-5} cm^2/sec. The agreement is still excellent.

This consistency in the value of D_2 over the full range of hematocrits, for two different red cell environments, is a strong argument in favor of the value for the diffusivity of sodium in the red cell as 0.5 x 10^{-5} cm^2/sec. This consistency would not be expected if the analysis was not correct.

If the high effective diffusivity of sodium through the red blood cell really exists, it is most interesting. There are two factors which could contribute to this phenomenon. One is the actual diffusivity of sodium through the red blood cell and the other is a surface diffusion of sodium around the red cell. The present analysis only allows the statement that there is an effective diffusivity of sodium through the red cell. It does not give any information concerning the actual diffusional mechanism.

It has been shown by a previous discussion that if a portion of the sodium is reversibly bound in the red blood cell, it would give an observed diffusivity through the red cell which is less than the actual diffusivity. Therefore, the high diffusivity observed for sodium in the red cell is not an artifact caused by any internal binding of sodium. In fact, it does appear as though the whole of the cell sodium is exchangeable (47), and this exchange is essentially complete in 15 minutes for rabbit blood (16).

To determine what effect the active transport mechanism might have on the sodium diffusional behavior, four experiments were completed. The red cells used in three of these experiments were incubated for 24 hours at 25°C in a dextrose deficient Earle's Solution. This procedure depleted the intracellular ATP, and halted the active transport mechanism. The ratio of intracellular to extracellular sodium should be much closer to one for these cells than for fresh red cells. One experiment used red cells which had been incubated in a dextrose-deficient media at 25°C for 17 hours, then in Earle's Solution for 5 hours at 37° C to restore the ATP.

The data are given in Figure 13 along with the average result for fresh rabbit cells. Depleting intracellular ATP appears to have no discernible difference on the behavior of sodium diffusion in the suspension. Incubating the cells in a dextrose deficient media would not be expected to appreciably

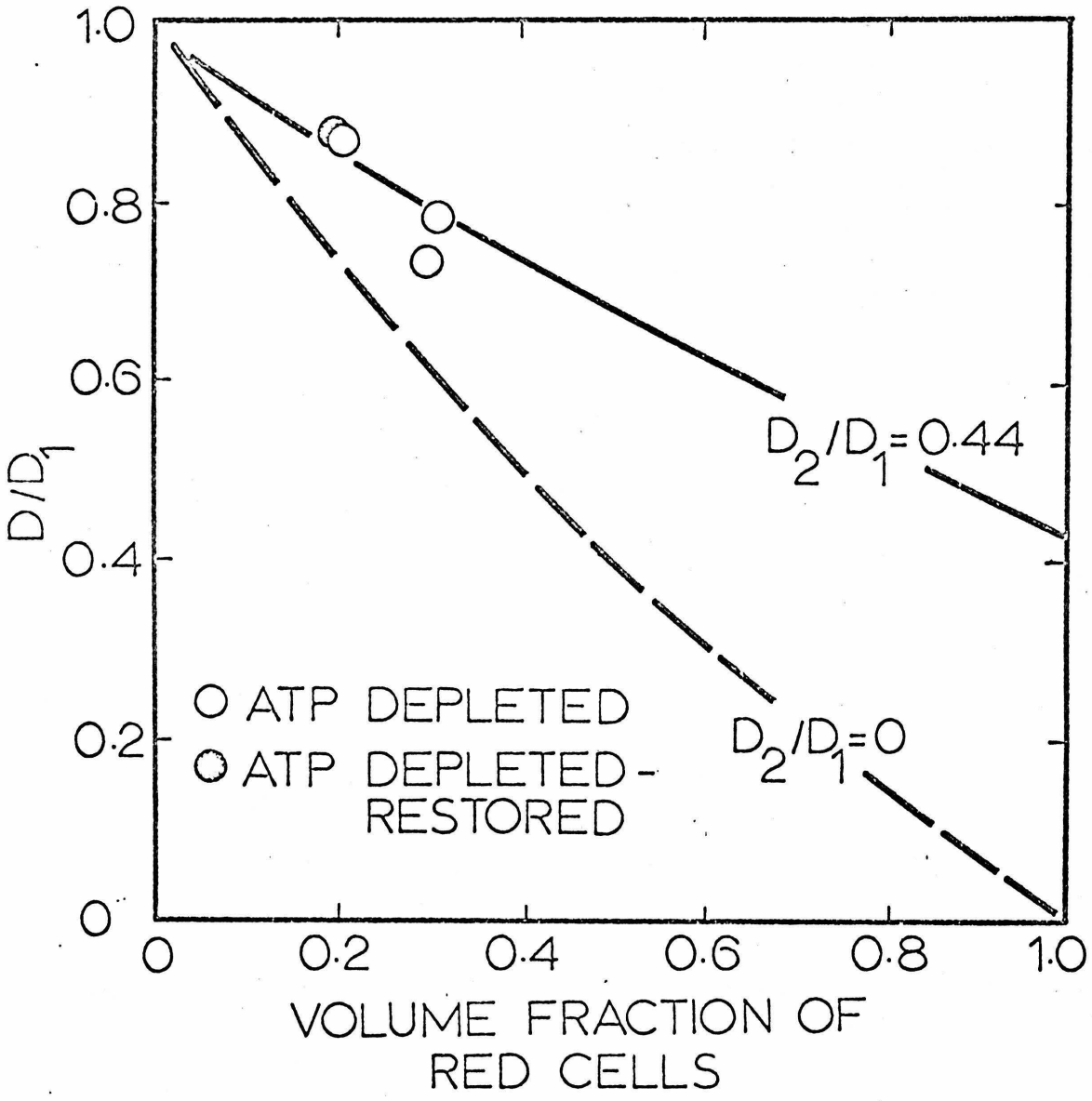

Figure 13. Diffusion Coefficient of Sodium in Agar Gel Suspensions of Rabbit Red Cells with Depleted and Restored Levels of ATP at 25° C.

increase the diffusion coefficient of sodium in the red cell. If the diffusion coefficient of sodium inside and outside the cell remains the same, changing the ratio of intracellular to extracellular sodium should, according to the modified Maxwell equation, change the average diffusivity of sodium through the medium. This does not seem to be the case. The results are more consistent with the assumption that the Maxwell equation holds regardless of any concentration discontinuity at the phase boundary.

Because of the method used in determining the diffusivities in this investigation, care must be taken in the application of these results. The sodium ion diffusivities calculated here are based on that fraction of the total sodium ion concentration which is exchangeable with the added radioactive sodium. If, for instance, a sizeable portion of the red blood cell sodium was essentially nonexchangeable with the added radioactive sodium over a 24-hour period, using this diffusivity and the total amount of sodium in blood to calculate expected sodium fluxes would give erroneous results. Since it does appear as though the sodium is completely exchangeable, the applications should be straightforward.

7. The Diffusion of Sodium in an Agar Gel Suspension of Human Red Cells.

Human blood was obtained from Hyland Laboratories, Los Angeles, California, for this work. The blood was approximately

two days old when it was obtained.

Experiments were made at 25°C for hematocrits of 20, 30, and 50. The results based on the average concentration of sodium in the red cell-gel medium are given in Figure 14. The results are well described by a curve derived from the Fricke theory for $D_2/D_1 = 0.35$. The calculated value for the effective diffusivity of sodium through human red blood cells, based on these results, is $0.44\ (\pm 0.02) \times 10^{-5}$ cm^2/sec. This is somewhat lower than the value obtained in the rabbit blood, but not enough to suspect the diffusional mechanism is not the same in both cases.

The diffusivities based on the concentration of sodium in the continuous phase, assuming $\xi = 1/6$, are plotted in Figure 15. In this form, the data are consistent with the assumption of zero diffusivity of sodium through the red cell. Since the cells were cold stored prior to the diffusion run, the value of ξ could easily have been somewhat larger than 1/6. This might in part account for the experimental values falling below the line for $D_2 = 0$.

Li and Gainer (23) have presented an equation which predicts the diffusivity of a solute through a protein solution. Their equation is

$$\frac{D_{as}}{D_{ab}} = \left(\frac{M_b}{M_s}\right)^{1/2} \left(\frac{V_s}{V_b}\right)\left[1 - \frac{\Delta H_M}{\Delta H_{vap}}\right] \qquad (68)$$

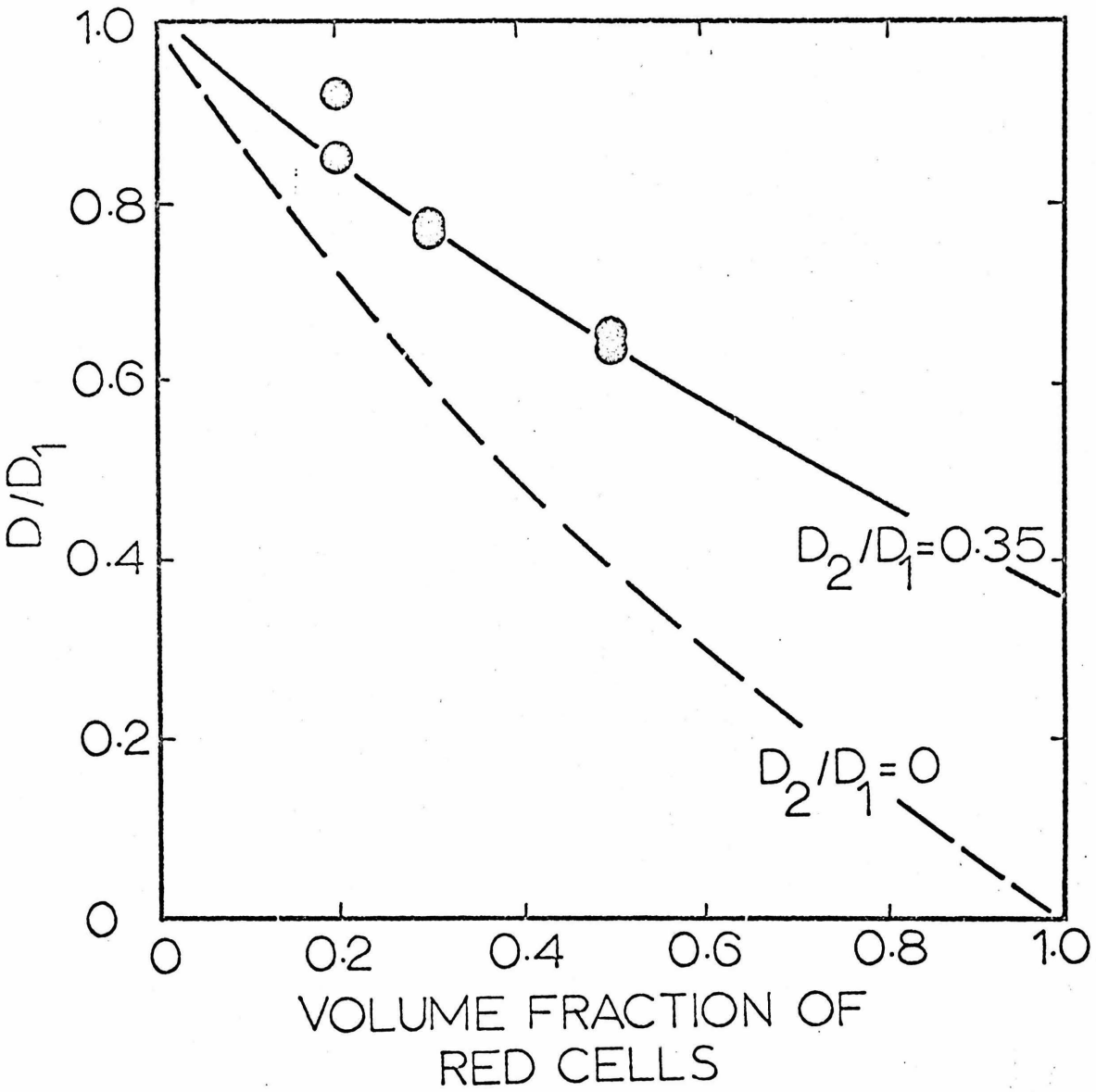

Figure 14. Diffusion Coefficients of Sodium in Agar Gel Suspensions of Human Red Cells at 25° C.

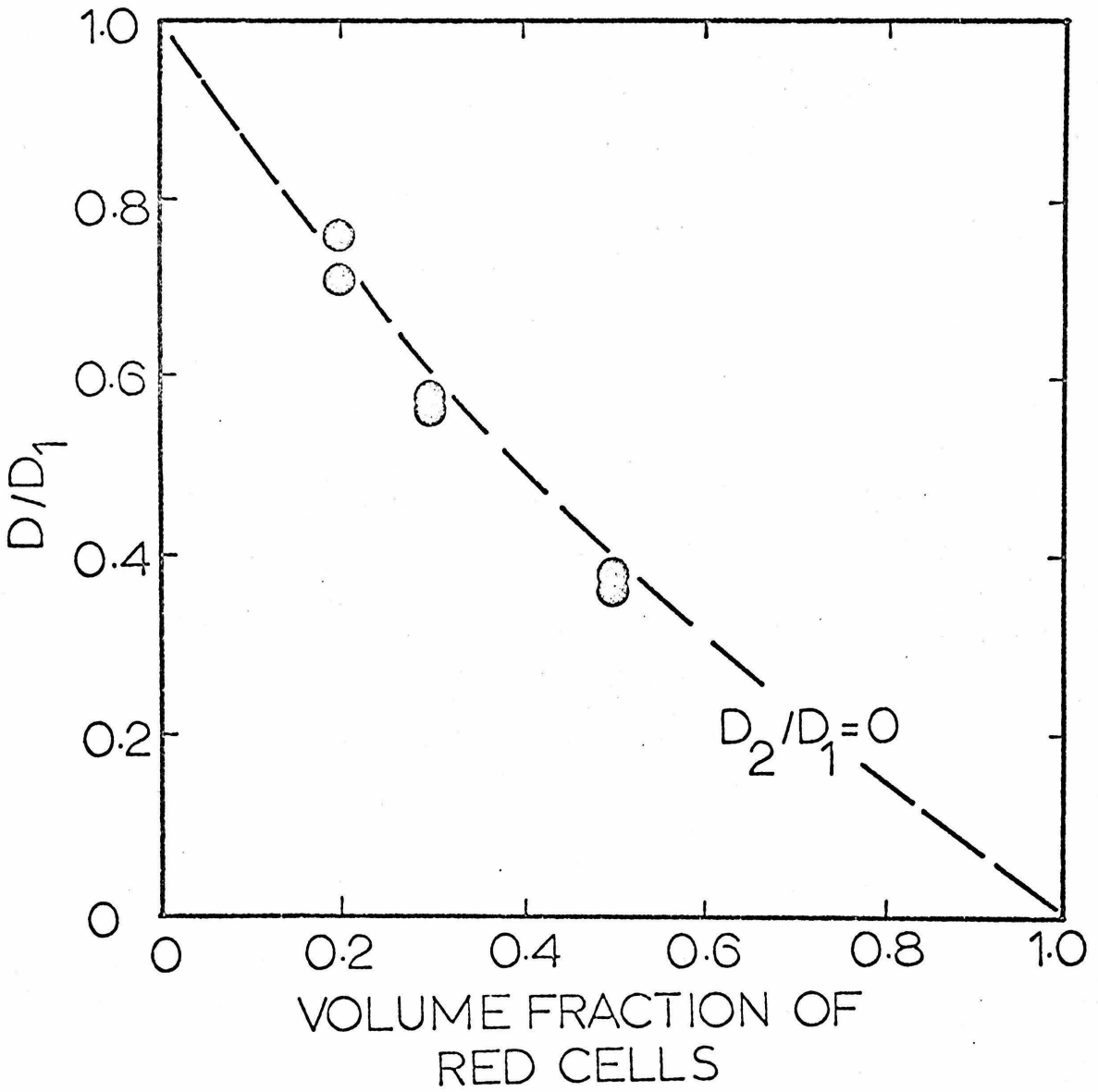

Figure 15. Diffusion Coefficients of Sodium in Agar Gel Suspensions of Human Red Cells at 25° C, Based on the Assumed Equilibrium Concentration of Sodium in the Continuous Phase.

where

D_{as} = diffusion coefficient for a solute diffusing through a polymer solution,

D_{ab} = diffusion coefficient for a solute diffusing through pure solvent,

A = solute,

B = solvent,

S = polymer solution (solvent B plus polymer),

M = molecular weight,

V = molar volume,

ΔH_m = heat of mixing of the polymer with the solvent,

ΔH_{vap} = heat of vaporization of the pure solvent.

This indicates that for a given dilute polymer solution, the ratio of the diffusivity of any solute through the given polymer solution to that through pure solvent is always the same, since it is only a function of the properties of the polymer and the solvent. It may be worthwhile to extend this idea to the limit of the red blood cell, which is no longer a dilute polymer solution, to see what information can be obtained.

The diffusivity of sodium "through" the human red cell is 0.44×10^{-5} cm^2/sec. Its diffusivity through a 0.100 M sodium chloride solution is 1.316×10^{-5} cm^2/sec. The ratio of these two diffusivities is 0.33.

Stein (38) measured the diffusion of oxygen through packed

human red blood cells. His result for the diffusion of oxygen through water was 2.03×10^{-5} cm^2/sec. At relatively low oxygen partial pressures, he obtained three results for the diffusion of oxygen through packed red cells which averaged out to a value of 0.53×10^{-5} cm^2/sec at $25^{\circ}C$. With 100 percent oxygen above the sample, he obtained two results which averaged out to 0.80×10^{-5} cm^2/sec. This gave a diffusivity ratio of 0.26-0.39, which brackets the sodium results presented in this study. This supports the idea that, if the calculated effective diffusivity of sodium through the red cell is not an artifact, the diffusion mechanism is probably not surface diffusion, but actual diffusion through the red cell.

All the sodium experiments were made with the same batch of blood. The first experiment was made with the blood approximately three days old, the last on approximately the expiration date. The results indicate the aging of the blood had no effect on the diffusional behavior of sodium through the red blood cell-gel mixture.

8. The Diffusion of Sodium in an Agar Gel Suspension of Fixed Human Red Cells.

Fixed, osmotically inactive human red cells were kindly supplied by Dr. Meiselman of the Thomas Laboratory at the California Institute of Technology. The cells were washed twice in Earle's Solution prior to use. The shape of the fixed cells appeared normal when viewed under the microscope.

To test whether the cells slowly adsorbed radioactive sodium, a dilute solution of the cells was mixed with a solution of radioactive sodium. Immediately after mixing, a sample was taken and centrifuged. A one ml aliquot was removed from the supernatant. The dilute suspension containing the radioactive species was allowed to sit with occasional stirring for 24 hours at room temperature. After this time, another sample was removed and centrifuged. Again, a one ml aliquot was taken from the supernatant. The two aliquots were counted in the well scintillation counter. The count rate in the initial sample was 8524 cpm, while that in the final sample was 8529 cpm. The results were negative.

The measurement of the volume fraction presented a problem. The fixed cells, when packed, formed a fairly solid mass, which could not be drawn up in a syringe. An accurate volume of packed cells could not be measured, and the volume fraction of cells in the capillary could not be determined in the manner used with fresh red cells. To circumvent this problem, the hematocrit was determined after the run was started.

A two dram polyvial was partially filled with a radioactive sodium solution. The solution was evaporated to dryness, and the vial placed in a 43°C water bath. A certain volume of hot gel solution (agar gel-Earle's Solution) was added to the vial. The cells were washed, packed, and heated to 43°C. A volume of warm, packed cells was added to the gel solution and thor-

oughly mixed. Samples were taken and the diffusion run was initiated. A 100 ml volumetric flask was partially filled with warm (45-50°C) water, to which was added 0.5 ml of the hot gel-fixed cell suspension. The flask was allowed to cool to room temperature, then filled to the mark. The pipet delivering the suspension was completely rinsed with warm water into the flask to offset the volume loss of the suspension on cooling to room temperature. Since the dilution was 200:1, the contents of the flask could be introduced directly into a standard hemacytometer. In this work the Bright Line Counting Chamber, manufactured by the American Optical Company, Buffalo, New York, was used. The volume of the red cell was assumed to be 85 μ^3.

The results are presented in Figure 16. The curve predicted from the Maxwell equation for zero diffusivity is given as a reference. The data lie somewhat above this line.

The void fraction of these fixed cells in a packed condition is approximately 40 percent, and it was probably closer to 50 in this investigation. The gel solution was significantly diluted when the packed cells were added, and the value of the diffusion coefficient for sodium in the suspending medium was larger than that of 1.254×10^{-5} cm^2/sec used to calculate the diffusion coefficient ratios in Figure 16. Also, rinsing the pipet possibly overcompensated for the volume decrease on cooling, making

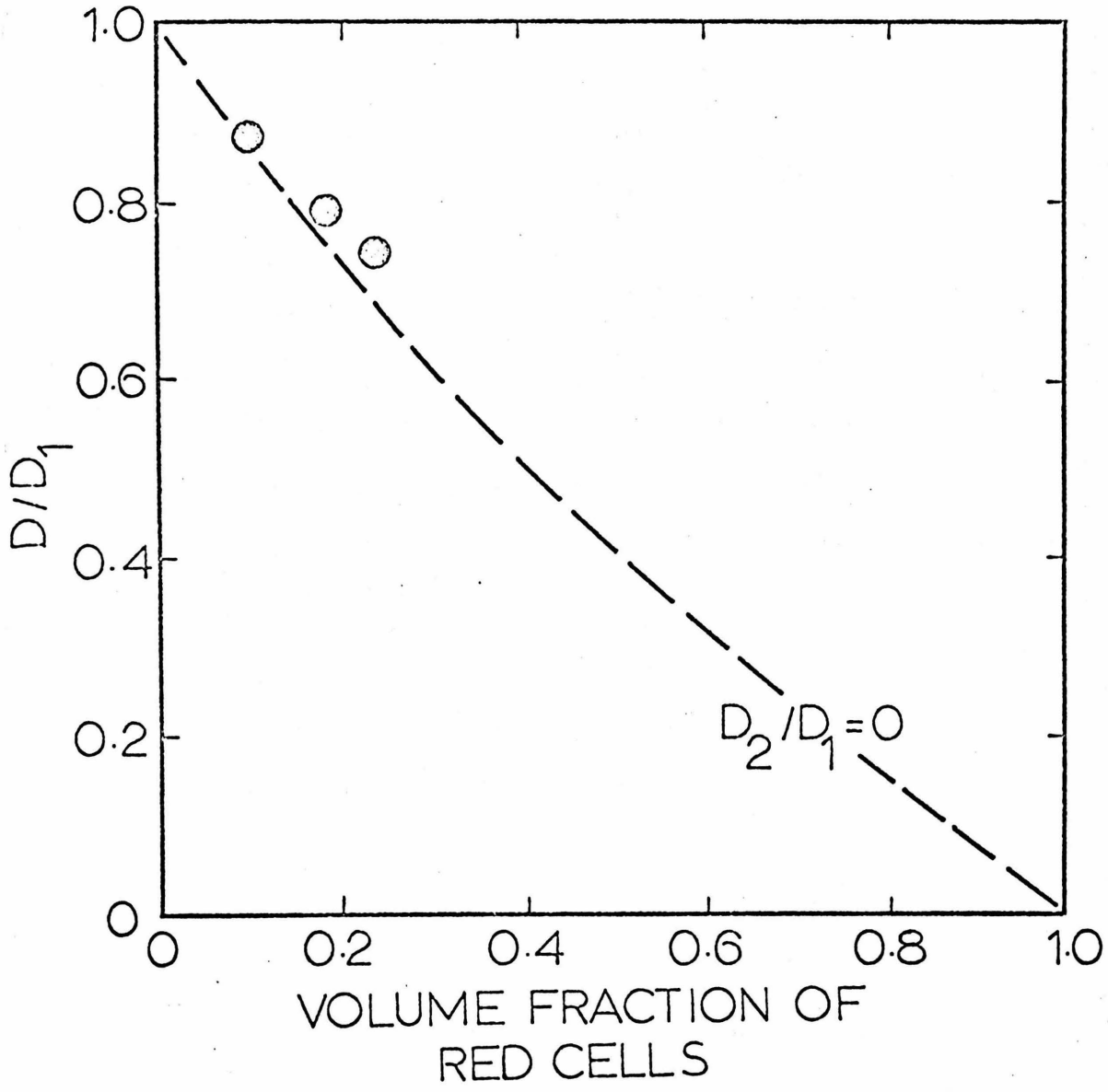

Figure 16. Diffusion Coefficients of Sodium in Agar Gel Suspensions of Fixed Human Red Cells at 25°C.

the measured hematocrits too high. Both effects just stated would tend to drop the results closer to the curve for zero diffusion through the dispersed phase.

Since the fixed red cell is a gelled mass of protein, the diffusion of sodium through it is expected to be zero. The results, based on the average concentration of sodium in the suspension, are close to the curve predicted by the Maxwell equation for zero diffusivity through the dispersed phase. A comparison of these results with those in Figure 14, for the diffusion of sodium through an agar gel suspension of human red cells is interesting. It is obvious that the washout of tracer is greater for the suspension of fresh cells than it is for the suspension of fixed cells.

Fixing the red cells should poison the active transport mechanism, which would tend to equalize the intracellular and extracellular sodium concentration. Thus, the discrepancy between the sodium diffusional data with fixed and fresh red cells would not be in opposition to expectations based on the modified Maxwell equation.

9. The Diffusion of Sodium in an Agar Gel Suspension of Glass Spheres.

A series of experiments were made on the diffusion of sodium through a gel suspension of glass spheres. The spheres were the 29 μ Superbrite Glass Beads manufactured by the 3M Company, Minneapolis, Minnesota. By adding a known weight

of glass spheres to a known volume of hot gel, the volume fraction of spheres could be calculated. The gel was one percent (w/v) agar gel in Earle's Solution (Appendix II-2). The glass spheres settled rapidly in the hot gel, and the gel-glass sphere suspension containing the radioactive tracer had to be shaken vigorously immediately prior to filling the capillary. The void fraction for the packed spheres was 40 percent.

The results are plotted in Figure 17. The Fricke curve for zero diffusivity of sodium through the spheres is also given in Figure 17. In this case it seems as though the only explanation for the observed phenomena is surface diffusion of the sodium ion. The shape of the curve is quite peculiar. A glass sphere volume fraction of five must be exceeded to obtain an enhanced diffusion. This behavior is not predicted by previous analyses (26, 33, 34). The reason for this apparently anomalous behavior is not understood. Possibly, a certain critical distance between the spheres must be attained for the surface diffusion mechanism to operate.

B. Potassium Diffusion.

1. The Diffusion of Potassium in an Agar Gel Suspension of Human Red Cells.

The short half life of potassium-42 made the experiments more difficult than those previously described. The rate of decay of the radioactive potassium had to be accounted for to obtain meaningful results.

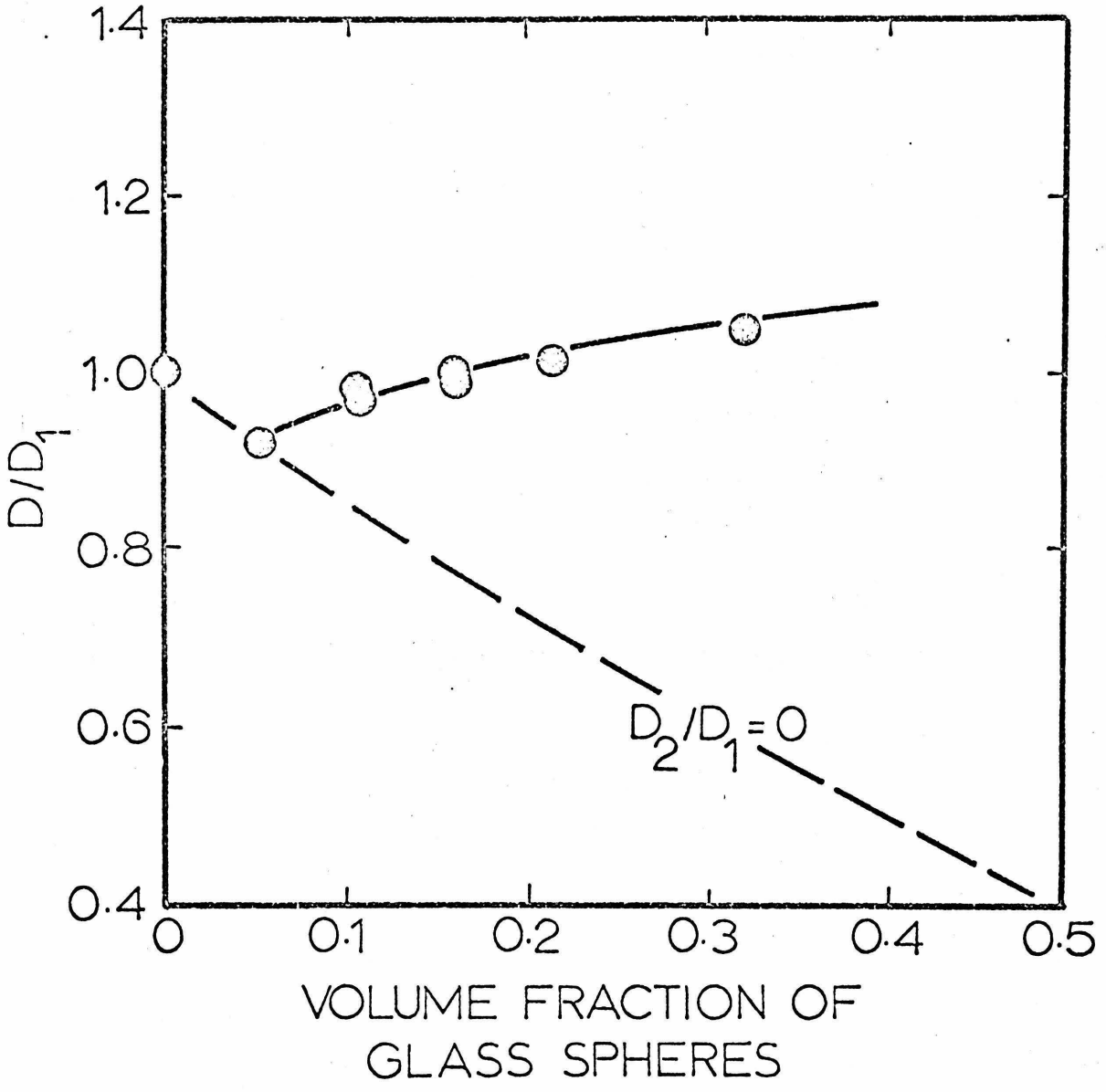

Figure 17. Diffusion Coefficients of Sodium in Agar Gel Suspensions of 29 μ Glass Beads at 25° C.

One millicurie of radioactive potassium, as KCl, was ordered per shipment from the International Chemical and Nuclear Corporation, Nuclear Science Division, West Mifflin, Pennsylvania. The liquid sample was diluted to five milliliters on delivery. For a given run, one milliliter of this hot solution was diluted to give a final solution concentration of four millicuries per liter. One-half milliliter of this solution was evaporated to dryness and used in the experiment. The potassium experiments were carried out in exactly the same way as the sodium experiments. Three initial samples were taken before the run was initiated. The run was concluded 24 hours later. In this case, however, not only was the time recorded when the experiment started and when it finished, but also when each vial was counted in the well scintillation counter. Each vial was counted three or four times. Knowing the decay constant for potassium-42 and the time between the beginning of the run and the counting of each initial sample vial, the counts per minute in the capillary at the beginning of the experiment could be calculated. The counts per minute in the capillary at the end of the experiment from the final sample vial were similarly calculated. From these corrected count rates, the diffusivity was calculated, using that form of the diffusion equation which took into account the radioactive decay.

To solve for the diffusivity, an accurate value for the

radioactive decay rate was necessary. Merrit and Taylor (27) recently determined the value for the half life of potassium-42 to be 12.358 hours. This number was accurate enough for this investigation. The decay constant, calculated from the half life, is 1.5580×10^{-5} sec^{-1}.

A potassium sample was counted for 10 minute intervals over a period of a few hours. From a plot of the counts per minute versus time, the decay constant was calculated, which was found to be 1.571×10^{-5} sec^{-1}. This is in excellent agreement with the literature value.

A two-run average gave a value for the diffusion coefficient of potassium with the gel of 1.890×10^{-5} cm^2/sec. Two experiments in the red blood cell-gel mixture at a hematocrit of 20 gave diffusivity ratios of 0.564 and 0.540 respectively (Table X, Appendix III-1). These diffusivities are based on the average concentration of radioactive potassium in the red cell-gel medium. Referring to Figure 16, it is observed that this value is below the line for zero diffusivity through the red blood cell.

As indicated in Chapter II, if a significant amount of reversible binding of the potassium in the red cell occurred, the observed results would not be surprising. However, such results do not unambiguously show penetration of potassium into the red cell. The results could be described by postulating the added radioactive potassium does not penetrate the red blood cell but

only adsorbs to the cell surface. In terms of the membrane cell theory with a low permeability of the red cell membrane to potassium, somewhat similar results might be expected. Since the cell potassium is effectively contained by the membrane, only the plasma potassium would be diffusible. Thus, the diffusivity ratio predicted by the Fricke analysis for no diffusion through the dispersed (red blood cell) phase should result. But since a potassium adsorption mechanism is operative, the experimental value would be even lower. This is an explanation. Based on numerous results of potassium uptake by red cells, however, it is probably not an adequate explanation.

If the red cell is covered by a membrane which is very impermeable to potassium, but not completely impermeable over the time scale of the diffusion run, a somewhat different explanation than that of the previous discussion would result. During the diffusion run, the red cells would act as sinks for the radioactive potassium. The ratio of the diffusivity of potassium through the red cell to that through the plasma would still be much less than one, or essentially zero on the Fricke curve because the membrane is only slightly permeable to potassium. Since the radioactive potassium is in a sense being irreversibly bound by the red cell, similar data to that reported in this investigation should result.

In the light of Raker's work (29), this description seems quite plausible. He believes the cell membrane is quite impermeable to potassium and calculates a half time of exchange of approximately 35 hours. Since in this work the experiment lasted only 24 hours, and the final potassium concentration (on a non-decay basis) was greater than half the original concentration, the red cells should, on the average, have acted as a sink for potassium throughout the run. Analyzing this would involve solving an equation of the form

$$\frac{\partial c_t}{\partial t} = D\nabla^2 (c_t - x(t)) - k^* c_t \qquad (69)$$

where

c_t = total concentration of potassium,

k^* = radioactive decay constant for potassium,

$x(t)$ = amount of bound potassium.

Information about the complicated term $x(t)$ is not available and this equation can not be solved.

The potassium experiments were started within an hour from the time the blood-gel mixture was added to the vial containing the radioactive potassium. If the above explanation is correct, equilibrating the red blood cell-gel mixture with the radioactive potassium for a longer period of time before beginning the run should trap more radioactive potassium in the

red blood cells. This would leave less outside the red blood cell to diffuse and thereby give a lower diffusion coefficient.

A special run was made to test this hypothesis. After the appropriate volumes of packed red blood cells and gel solution were added to the vial containing the radioactive potassium, it was allowed to equilibrate for 17 hours at 43°C. After this equilibration time, a 24-hour run was initiated. The hematocrit was again 20. A diffusivity ratio of 0.52 was obtained. This is not significantly lower than the previous results. The expectations were not fulfilled.

Since an equilibration time prior to the diffusion run did not appreciably change the results, it is probable that the red cells did not act as sinks for the radioactive potassium during the whole experiment. Therefore, it is not unreasonable to recalculate the diffusion coefficients in terms of the concentration of potassium in the continuous phase. When this was done for the sodium work, diffusivities were obtained which, according to the Fricke analysis, indicated the effective diffusivity of sodium in the red cell was at least two orders of magnitude less than that in the gel. As similar results were expected for potassium, it was easiest to calculate the concentration difference of potassium between the red cell and the gel which would give the expected diffusivity.

At a hematocrit of 20, the ratio of the diffusion coefficient in the heterogeneous medium to that in the continuous phase for $D_2 = 0$ is 0.73. To make the diffusion coefficient based on the continuous phase concentration of potassium correspond to this value, when the experimental values based on the average concentration of potassium are 0.54 and 0.56, a value for ξ of three was found necessary. Thus, to make the potassium results consistent with the sodium results, a value for the ratio of the concentration of potassium inside the red cell to that outside was found to be three. This is surprising since the expected value is 25. Possibly, only a fraction of the total amount of potassium in the cell is exchangeable with the tagged potassium.

Since the assumption that the average diffusivity in the Fricke analysis was based on the average concentration of diffusing species and the resulting substantial diffusion of sodium in the red cell could not be entirely ruled out, the consistency of the sodium and potassium results was investigated from this point of view.

It seems reasonable that, if the red blood cell membrane is quite impermeable to potassium, it will also be quite impermeable to sodium. Red cell influx and efflux studies with potassium and sodium seem to verify this. But if this is true, the same qualitative behavior should occur for both sodium and potassium. The red cells should also act as a sink for the added radioactive sodium, giving diffusion results which would lie below

the Fricke curve for zero diffusivity through the red cell. The effect should not be nearly as pronounced for sodium as for potassium, since the amount of potassium in the red cell is so much greater than sodium, but it should nevertheless be qualitatively the same. Obviously, it is not. Possibly, it is a surface diffusion of sodium which causes the difference. This seems reasonable, and bears further inquiry.

Dennis (10), studying the diffusion of salts in silica gels, obtained results which indicated quite strong surface diffusion of the salts through the silica gel, since the diffusion coefficients in the gels were greater than those in water.

The enhanced diffusion was found for both sodium and potassium. Not only are both ions involved in surface diffusion, but also the amount of enhancement for both ions is essentially the same.

Cremers and Laudelout(8) studied the diffusivities of many cations through clay suspensions. They too observed surface conductivities of the cations in the clay suspensions. Again when a surface diffusion of cations was observed, both sodium and potassium (as well as other cations) were involved. In this case also, the surface diffusivities for sodium and potassium are almost the same.

From these results it is probable that, if a cation surface diffusion mechanism exists, it works for both sodium and potassium with almost equal efficiency. This leads to the con-

clusion that, if the red cell exhibits a surface diffusion for the sodium ion, it would also exhibit a surface diffusion for the potassium ion.

Postulating a surface diffusion for potassium also would not preclude the observed results. Since the red cell is a much better sink for the added potassium than for the added sodium, the sink effect for potassium might well outweigh the surface diffusion effect and still give the observed results. If a red cell surface diffusion for the sodium and potassium is postulated, and the red cell membrane is assumed to be relatively impermeable to both ions, recognizing the high ratio of internal to external potassium and the low ratio of internal to external sodium, qualitatively the sodium and potassium results are consistent with one another.

The diffusivity data for potassium can also be analyzed in terms of equilibrium binding, as was done for the water diffusing through the protein solution. Since the equilibration time prior to the experiment made little difference in the result, this may be a valid approach. As found earlier

$$D^* = \left[\frac{1-\alpha+\alpha\delta\xi}{1-\alpha+\alpha\xi}\right] D \qquad (65)$$

where D^* is the measured diffusivity based on the total amount

of potassium in the system, D is the expected diffusivity based on the diffusible potassium, and σ is the fraction of red cell potassium which is diffusible. The hematocrit is α and ξ is the ratio of red cell to plasma potassium. Thus

$$C_{d\text{-}rbc} = \sigma\, C_{rbc}$$

$$C_{rbc} = \xi\, C_s$$

If $c_{d\text{-}rbc} = c_p$, it follows that $\sigma = 1$. From the value of $D^*/D = 0.62$, the value for ξ is calculated to be four. This again is rather low, unless there are two fractions of potassium in the red cell.

Basing the potassium diffusional data on either the averaged or continuous phase concentration gives results which seem equally consistent. In either case, the ratio of internal to external potassium is surprisingly low.

Storing the cells at 6°C would tend to decrease the internal concentration of potassium. This may, in part, explain the results. Also, some early work by Hevesy and Hahn (16) indicates that for rabbit, dog and rat erythrocytes, only 30-35 percent of the total cell potassium is exchanged for potassium of the plasma. This might be true of human red cells also. The two effects working together would be consistent with the observations.

C. Chloride Diffusion.

1. The Diffusion of Chloride in an Agar Gel Suspension of Human Red Cells.

The radioactive chloride (chlorine-36) was obtained from the Nuclear Chicago Corporation, Des Plaines, Illinois. It was received as NaCl and diluted to a workable activity of one μ c/ml. The chloride experiments were done in exactly the same manner as the sodium experiments.

The samples were counted in a Beckman LS-100 Liquid Scintillation System. The gel was ejected from the syringe into a vial, and the tip of the syringe washed with one ml of water. The washings were added to the vial. The capped vial was allowed to sit for a day before 10 ml of the fluor solution was added (Appendix II-2).

The results of the chloride phase of this investigation are plotted in Figure 18. As in the case of sodium, the chloride results can also be fitted by a curve predicted from the Fricke theory. The effective diffusion coefficient of chloride through the red cell based on the average concentration of chloride in the medium, is 0.34×10^{-5} cm^2/sec. Strangely enough, the effective diffusivity of chloride through the red blood cell is less than that for sodium. On the other hand, the diffusivity of sodium through the gel is less than that for chloride. As stated previously, the ratio of the diffusivity through the red cell to

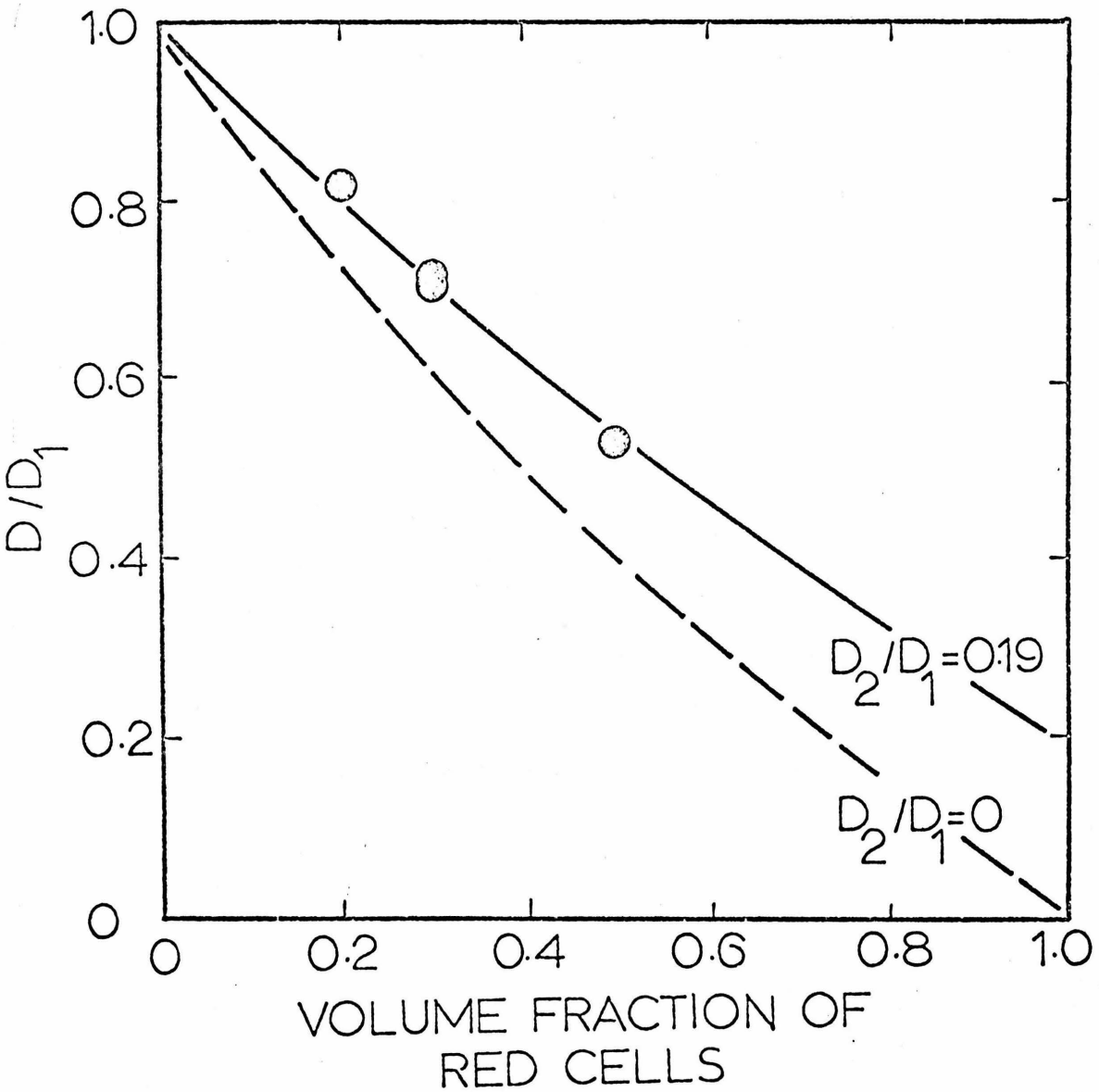

Figure 18. Diffusion Coefficients of Chloride in an Agar Gel Suspension of Human Red Cells at 25° C.

that through the continuous phase for various species should be the same. For sodium it is 0.35, but for chloride it is only 0.19. They are definitely different.

Of course, if this is a surface diffusion phenomenon, the ratio would not be expected to be the same for all species. But if there is an actual diffusion through the red blood cell, the results do look somewhat strange. A second look may help to clear some of the confusion.

The conclusion that the diffusivity ratios should be the same was based on the assumption that the diffusing species did not interact in any way with the protein solution. But according to Ling's fixed charge hypothesis (24), this may not be so for the red blood cell. Under his hypothesis it is not impossible to conceive a negatively charged ion experiencing a force which retards its diffusion through the red cell relative to a positive ion, or vice versa. Thus, the possibility that actual diffusion through the red cell occurs for both sodium and chloride can not be ruled out.

It is evident that at least for the chloride ion, a substantial value for the effective diffusion coefficient in the red cell does exist. The work of Harris and Maizels (17) indicates that for this investigation, in which cold stored cells were used, the ratio of the internal to external chloride concentrations was approximately 0.9. The diffusion coefficients recalculated on the basis of the continuous phase concentrations are given in

Figure 19. The difference in the two methods is slight. The chloride ion is not involved in the active transport mechanism and its partition coefficient is close to unity. The application of the heterogeneous media theory should be straightforward; the conclusions should be meaningful.

As presented earlier, the half time for exchange of chloride in the red cell is 0.2 seconds. The permeability constant corresponding to this half time is 2×10^{-4} cm/sec. This constant is defined by the equation

$$\frac{dc_{in}}{dt} = P\frac{A}{V}(C_{out} - C_{in}) \qquad (70)$$

where dC_{in}/dt is the rate of increase in the concentration of solute in the cell, C_{out} is the outside or plasma concentration, A is the area of the cell membrane and V is the cell volume.

If the diffusivity of chloride across the membrane is much smaller than its diffusivity in the interior of the cell, the effective diffusivity through the red cell may be quite low. The derivation given in Appendix I-2 gives an approximate analysis of how the diffusivity across the membrane influences the average diffusivity across the whole cell.

This analysis indicates that, based on the results presented here, a lower limit for the permeability of the red cell mem-

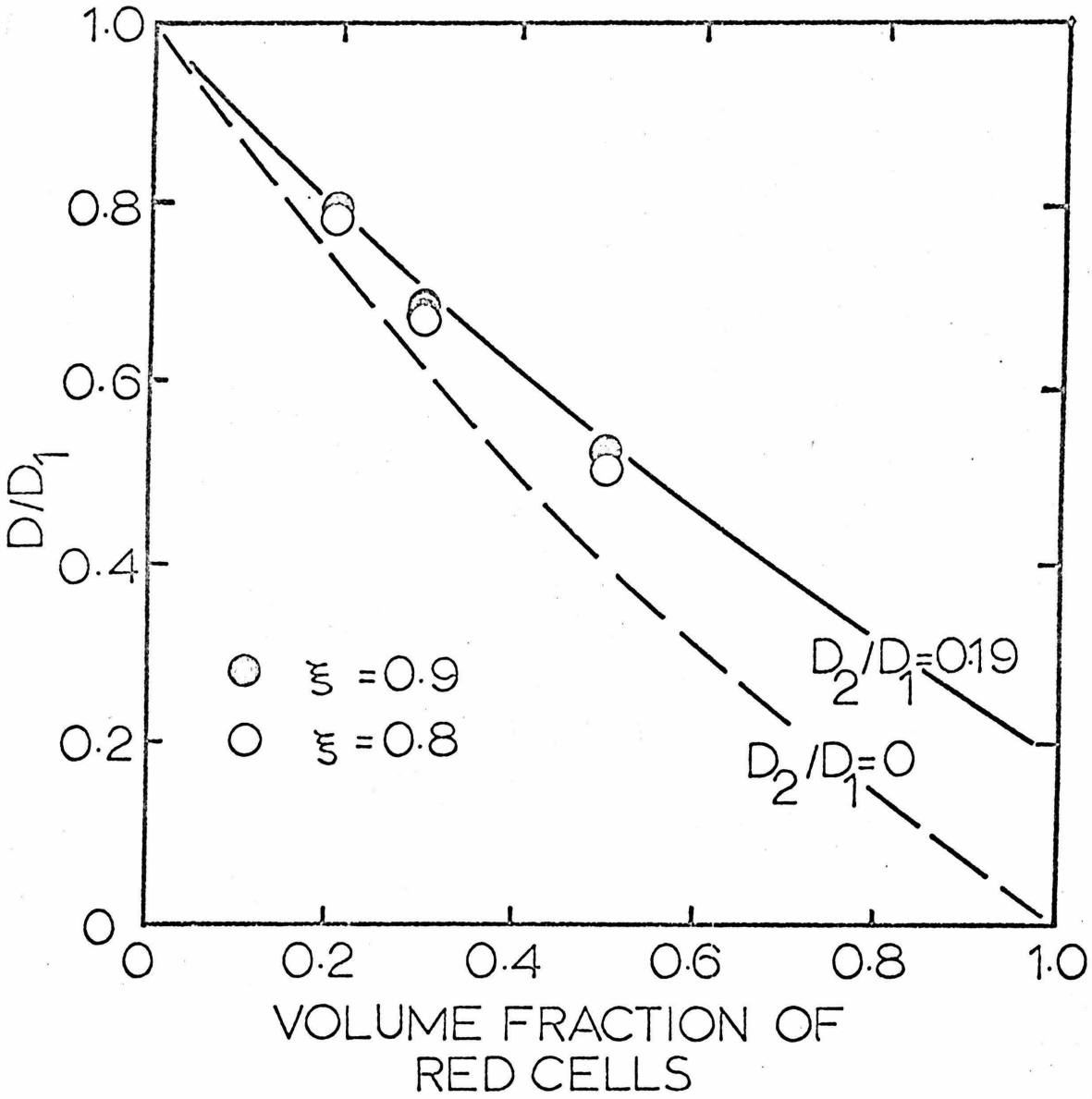

Figure 19. Diffusion Coefficients of Chloride in an Agar Gel Suspension of Human Red Cells at 25°C, Based on the Assumed Equilibrium Concentration of Chloride in the Continuous Phase.

brane to chloride is 10^{-2} cm/sec. This is two orders of magnitude higher than expected (2×10^{-4} cm/sec), based on chloride equilibration times.

As an extra precaution against any bacterial growth, streptomycin and penicillin were added to the Earle's Solution used in the chloride investigations (Appendix II-2). Although these antibacterial agents had not been used in the earlier work, test runs showed no change in the results when streptomycin and penicillin were used.

D. Bicarbonate Diffusion.

1. The Diffusion of Bicarbonate in an Agar Gel Suspension of Human Red Cells.

Radioactive bicarbonate (Carbon-14), as an aqueous solution of sodium bicarbonate, was obtained from the Nuclear Chicago Corporation, Des Plaines, Illinois. The solution as received was diluted to an activity of 0.1 mc/ml.

Because of the equilibrium

$$NaH\overset{*}{C}O_3 \rightleftharpoons NaOH + \overset{*}{C}O_2\uparrow \qquad (71)$$

the radioactive sample could not be evaporated to dryness as was done with the previous radioactive compounds. Approximately 0.01 ml of the 0.1 mc/ml solution was added to the two

ml of hot gel red blood cell mixture and allowed to equilibrate. The samples were then taken for the diffusion experiment.

Since the vial containing the radioactive sample was not in equilibrium with radioactive carbon dioxide, there was a continual loss of radioactive carbon dioxide during the time the samples were taken. Thus, the initial concentration in the capillary at the beginning of the diffusion run was unknown. The loss from one vial to the next was substantial.

In the calculation of the diffusion coefficients, the initial concentration of bicarbonate was obtained by subtracting the average decrease in the sample vial concentration from the final sample vial concentration. Since the drop in the sample concentration between the first and second and the second and third was not generally consistent, the calculated value for the initial concentration of tracer in the capillary was only approximately correct. The determined diffusion coefficients are not accurate, but certain important trends can be observed in the results.

The vials were treated in the same manner as those in the previous chloride experiments. They were counted in the Beckman LS-100 Liquid Scintillation System. The mouths of the vials were coated with a high vacuum grease to minimize the loss of radioactive carbon dioxide.

Table II gives the results for four experiments which could be analyzed. The diffusion coefficients are presented based

Table II. The Diffusion Coefficients of Bicarbonate in a One Percent Agar Gel Suspension of Human Red Cells at 25° C.

Volume Fraction of Red Cells	$D \times 10^5$ (cm^2/sec)
0	1.20
0	1.13
0.30	1.34
0.50	1.58

on the average concentration of bicarbonate ion. The conclusion from these results is that the red cell enhances the diffusion of bicarbonate in the medium, since the diffusion coefficients for $\alpha > 1$ were larger than those for $\alpha = 1$. Since the loss of carbon dioxide when $\alpha > 1$ would not be expected to be greater than that when $\alpha = 1$, the inaccuracies in the individual diffusion coefficients should not be a factor in the observable trend of increasing D with increasing α.

Spaeth and Friedlander (37), in their work on the convective diffusion of carbon dioxide in blood, found experimental transport rates which were higher than those predicted by the local equilibrium model. They assumed the ratio of the diffusion coefficient of carbon dioxide in the red cell to that in the plasma was the same as the ratio for oxygen. Since the local equilibrium model which incorporated the assumed average diffusion coefficient predicted results lower than those experimentally obtained, they also suspected the presence of an augmented diffusional mechanism for carbon dioxide in blood. The results presented here appear to confirm their suspicion. Although the general augmentation mechanism is expected to be similar to that for oxygen in hemoglobin solutions (21), the inaccuracy in the bicarbonate results found in this investigation prevents an analysis of the detailed mechanism of augmentation. A method similar to the one used by Stein (38) would be better for further investigation of this phenomenon.

V. SUMMARY AND CONCLUSIONS

Interest in the transport of solutes in blood has been greatly stimulated by the successful development of artificial organs. In view of this, the aspect of the transport process dealing with the influence of the red cell on the diffusion process was investigated. This study was limited to the non-convective diffusion of sodium, potassium, chloride and bicarbonate ions.

A method was developed for measuring the diffusion coefficient of solutes in agar gel suspensions of red cells. The capillary diffusion method was used to measure the self-diffusion coefficients. The diffusion coefficient was calculated from the initial and final concentrations of tracer in the capillary. The method is limited only by the availability of suitable tracers.

The diffusion of sodium in an agar gel of fixed red cells and 29 μ glass beads was also investigated. The diffusional loss of tracer in the capillary was found to be greater for viable than for fixed cells. The glass beads exhibited a surface diffusion mechanism for sodium.

The results of this study indicate the ratio of the diffusivity in the red cell suspension to that in the agar gel varies considerably with the ion. This ratio, except for the bicarbonate ion, decreased as the volume fraction of red cells increased. The bicarbonate results indicated a diffusion augmentation

mechanism is operative in a red cell suspension. At any hematocrit, the diffusivity ratio for chloride was less than that for sodium. Depleting intracellular ATP appeared to have no significant effect on the sodium diffusion in the red cell suspension.

A modified form of the Maxwell equation for the average conductivity in a granular medium was derived to account for the discontinuous concentration at the continuous-dispersed phase boundary. The average diffusion coefficient in the modified form was shown to be based on the concentration of diffusing species in the continuous phase. The capillary diffusion method gave diffusion coefficients which were based on the average concentration of diffusing species in the heterogeneous medium. The factor $(1-\alpha+\alpha\xi)$ was used to convert the measured diffusion coefficients to coefficients based on the concentration of diffusing species in the continuous phase. This assumes the ratio of the concentration of diffusing species in the dispersed phase to that in the continuous phase was constant during the experiment. This equilibrium assumption is questionable for unsteady-state experiments in which the diffusion coefficient in the dispersed phase is much less than that in the continuous phase.

Using the modified Maxwell equation to interpret the sodium diffusional data for rabbit and human red cells gave results which indicate the effective diffusion coefficient of sodium

in the red cell is much less than that in plasma. Sodium efflux rates from the red cell would predict this behavior.

The modified Maxwell equation could not explain the data found by other workers on the self-diffusion of water and the diffusion of oxygen in protein solutions. The diffusion coefficients were based on the average concentration of diffusing species in the medium. Although partition coefficients of unity were not expected in either case, the data were consistent with the unmodified form of the Maxwell equation. In all cases of sodium and chloride diffusion, the measured diffusion coefficients could be fitted by a curve derived from the unmodified Maxwell equation. Using the Maxwell equation to determine D_2 for sodium diffusion in agar gel suspensions of rabbit red cells and for sodium diffusion in rabbit blood at hematocrits close to 100 gave consistent results. Changing the partition coefficient for sodium by depleting the intracellular ATP in the rabbit red cells did not significantly change the diffusional behavior of sodium in the suspension.

Thus, in numerous instances, the measured diffusion coefficients appear more compatible with the unmodified Maxwell equation, despite a partition coefficient other than unity. In either case, a substantial diffusion coefficient for chloride in the red cell and augmented diffusion for bicarbonate in the presence of red cells appear to exist. Either approach is equally consistent with respect to potassium. For engineering

applications, it is probably best to proceed according to the modified Maxwell equation.

The method developed in this report has certain interesting applications. Perhaps the most important will be to determine what effect certain substances in the red cell suspension have on the transport of solutes in the suspension. The effect of gaseous pollutants, pesticides, and various drugs on solute transport in blood would be an important contribution to some pertinent problems being faced today. The effect of physical disturbances of the red cell on the transport process can also be investigated. The method is also useful for studying diffusion in other heterogeneous systems, such as clay suspensions.

APPENDICES

APPENDIX I

1. The Modified Maxwell Equation

In the derivation of the average conductivity in a granular medium given by Maxwell (5), one of the boundary conditions is that the temperature is continuous at the boundary between the continuous and dispersed phases. When concentrations are used instead of temperatures, this boundary condition signifies the partition coefficient is unity. For systems in which the partition coefficient is not equal to unity, the derivation should not be valid. To determine the effect of a partition coefficient, Maxwell's result will be rederived for a discontinuous boundary condition.

If a temperature gradient is imposed on a homogeneous medium of conductivity k, the temperature distribution in the medium is given by

$$\sigma = Vr\cos\theta \qquad (72)$$

where σ is the temperature at any position, and V is the temperature gradient in the medium. If a sphere of radius a and conductivity k' is inserted into the medium, it will distort the temperature distribution in the medium immediately surrounding

the sphere. The temperature in the medium is now

$$v = Vr\cos\theta + \frac{B}{r^2}\cos\theta \tag{73}$$

whereas the temperature in the sphere is

$$v' = Ar\cos\theta \tag{74}$$

A and B are unknown constants, and r, θ are spherical polar coordinates. These satisfy Laplace's equation, v' is finite as $r \to 0$ and $v \to Vz$ as $r \to \infty$, as required. The boundary conditions are

1. $v = \beta v'$ at $r = a$
2. $k\,\partial v/\partial r = k'\,\partial v'/\partial r$ at $r = a$

$0 \le \theta \le \pi$

There is a discontinuity of temperature at the boundary, but no buildup of heat there. These give, from equations (73) and (74)

$$\begin{aligned} \beta A a^3 &= Va^3 + B \\ k(Va^3 - 2B) &= k' A a^3 \end{aligned} \tag{75}$$

Solving for A and B, gives

$$\mathcal{V} = V_z \left[1 + \frac{a^3}{r^3} \frac{\beta k - k'}{2\beta k + k'} \right] \tag{76}$$

$$\mathcal{V}' = \frac{3kV_z}{2\beta k + k'} \tag{77}$$

Defining

$$\frac{k'}{\beta} = k'' \tag{78}$$

equation (76) becomes

$$\mathcal{V} = V_z \left[1 + \frac{a^3}{r^3} \frac{k - k''}{2k + k''} \right] \tag{79}$$

The temperature distribution in the discontinuous media can be reduced to that of a continuous media (5) if an effective conductivity is defined for the dispersed phase.

If n spheres of radius a and effective conductivity k'' are now inserted into the medium, the temperature at large distances due to the spheres is

$$v = V_z \left[1 + \frac{n a^3}{r^3} \frac{k - k''}{2k + k''} \right] \tag{80}$$

The volume containing the n spheres is then replaced by a large sphere of radius b and conductivity k°. Here $na^3 = \alpha b^3$, where α is the volume fraction of small spheres. By equation (79), the temperature at great distances due to this sphere is

$$v = V_z \left[1 + \frac{b^3}{r^3} \frac{k - k^\circ}{2k + k^\circ} \right] \tag{81}$$

Since equations (80) and (81) must be equal, equating the two leads directly to the result

$$k^\circ = k \left\{ \frac{1 + 2\alpha \left[\frac{k''/k - 1}{k''/k + 2} \right]}{1 - \alpha \left[\frac{k''/k - 1}{k''/k + 2} \right]} \right\} \tag{22}$$

The result is independent of the radius of the small spheres.

This derivation implies that if the equilibrium concentration in the dispersed phase is less than that of the continuous phase $\frac{1}{\beta} < 1$, then the average diffusive flux through the system

for a given diffusivity in the dispersed and continuous phase will be less than for the situation where $\frac{1}{\beta} = 1$. A partition coefficient less than unity causes the effective diffusion coefficient in the dispersed phase to be less than the actual diffusion coefficient. This can best be illustrated by considering the following example.

Consider a membrane of thickness l separating two salt solutions of different concentrations, C_1 and C_2. There is a partition coefficient $K<1$ for the salt between the membrane and the solution. The diffusivity of salt in the membrane is D.

The actual flux of salt across the membrane is

$$J = D\,\frac{KC_2 - KC_1}{l}$$

$$= DK\left[\frac{C_2 - C_1}{l}\right] \qquad (82)$$

If the partition coefficient is unity, the flux of salt across the membrane would be

$$J' = D\left[\frac{C_2 - C_1}{l}\right] \qquad (27)$$

Since $K<1$, the presence of a partition coefficient reduces the flux through the membrane by the factor K relative to what it would be if $K=1$. The opposite is true if $K>1$.

APPENDIX I

2. The Effect of the Red Cell Membrane on the Diffusion of Chloride through the Red Cell

To determine how the diffusivity across the red cell membrane influences the average diffusivity across the whole cell, the red cell can be approximated as a three-layered diaphragm in a diaphragm diffusion cell. The average diffusivity across all three layers can be calculated knowing the thickness of and the diffusivity in each layer.

If the flux through the diaphragm is J and the area of the diaphragm is A, it follows that

$$\begin{aligned} AJl_3 &= D_3 \Delta c_3 A \\ AJl_2 &= D_2 \Delta c_2 A \\ AJl_1 &= D_1 \Delta c_1 A \end{aligned} \tag{83}$$

$$\begin{aligned} V_1 dc_1 + \frac{D_1 A}{l_1}(c_1 - c_j)dt &= 0 \\ V_3 dc_3 + \frac{D_1 A}{l_1}(c_j - c_1)dt &= 0 \end{aligned} \tag{84}$$

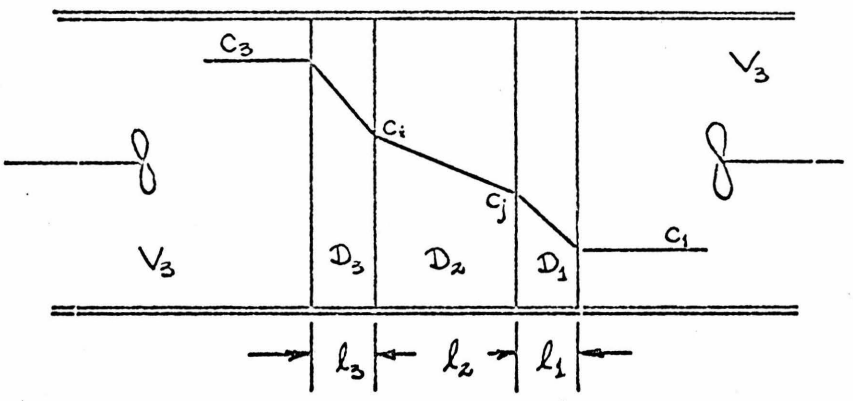

Figure 20. Diaphram Diffusion Cell.

$$V_1 dc_1 + \frac{D_2 A}{l_2}(c_j - c_i)dt = 0$$
$$V_3 dc_3 + \frac{D_2 A}{l_2}(c_i - c_j)dt = 0 \qquad (85)$$

$$V_1 dc_1 + \frac{D_3 A}{l_3}(c_i - c_3)dt = 0$$
$$V_3 dc_3 + \frac{D_3 A}{l_3}(c_3 - c_i)dt = 0 \qquad (86)$$

Combining, gives

$$dc_1 - dc_3 = \frac{D_1 A}{l_1}\left(\frac{1}{V_1} + \frac{1}{V_3}\right)(c_j - c_1)dt$$
$$dc_1 - dc_3 = \frac{D_2 A}{l_2}\left(\frac{1}{V_1} + \frac{1}{V_3}\right)(c_i - c_j)dt \qquad (87)$$
$$dc_1 - dc_3 = \frac{D_3 A}{l_3}\left(\frac{1}{V_1} + \frac{1}{V_3}\right)(c_3 - c_i)dt$$

which leads to

$$dc_1 - dc_3 =$$

$$\frac{D_2 A}{l_2}\left(\frac{1}{V_1} + \frac{1}{V_3}\right)\left[\frac{-dc_1 - dc_3}{\frac{D_3 A}{l_3}\left(\frac{1}{V_1}+\frac{1}{V_3}\right)dt} + c_3 - \frac{dc_1 - dc_3}{\frac{D_1 A}{l_1}\left(\frac{1}{V_1}+\frac{1}{V_3}\right)dt} - c_1\right]dt \qquad (88)$$

Let

$$\alpha = \frac{D_1}{l_1}, \quad \beta = \frac{D_2}{l_2}, \quad \gamma = \frac{D_3}{l_3}, \quad \xi = A\left(\frac{1}{V_1} + \frac{1}{V_3}\right)$$

then

$$dc_1 - dc_3 = \beta\xi\left\{-\frac{dc_1 - dc_2}{\gamma\xi dt} + c_3 - \frac{dc_1 - dc_3}{\alpha\xi dt} - c_1\right\}dt \quad (89)$$

$$= \beta\xi(c_3 - c_1)\,dt - \beta\left[\frac{\alpha+\gamma}{\alpha\gamma}\right](dc_1 - dc_2) \quad (90)$$

Let

$$\omega = \left[\frac{\alpha+\gamma}{\alpha\gamma}\right]^{-1}$$

then

$$dc_1 - dc_3 = \beta\xi(c_3 - c_1)dt - \frac{\beta}{\omega}(dc_1 - dc_3) \quad (91)$$

and

$$\frac{dc_1 - dc_3}{(c_1 - c_3)} = -\left[\frac{\omega\beta\xi}{\omega+\beta}\right]dt \quad (92)$$

Therefore

$$\ln\frac{\Delta c_f}{\Delta c_o} = -\left[\frac{\omega\beta\xi}{\omega+\beta}\right]t \quad (93)$$

For the diaphragm cell with a single diaphragm, the final equation is

$$\ln \frac{\Delta c_f}{\Delta c_0} = - \frac{\bar{D}}{l} \xi t \qquad (94)$$

Thus, for the three-layered diaphragm

$$D = \frac{\omega\beta}{\omega+\beta} (l_1 + l_2 + l_3) \qquad (95)$$

and finally

$$D = (l_1 + l_2 + l_3) \left\{ \frac{\alpha\beta\gamma}{\alpha\beta+\alpha\gamma+\beta\gamma} \right\} \qquad (96)$$

For the red cell

$$\alpha = \gamma \quad , \quad l_1 = l_3$$

therefore

$$D = (2l_1 + l_2) \left\{ \frac{\alpha\beta}{2\beta+\alpha} \right\} \qquad (97)$$

or

$$\mathcal{D} = (2l_1 + l_2)\left\{\frac{D_1 D_2}{2D_2 l_1 + D_1 l_2}\right\} \tag{98}$$

For the case when

$$l_2 \gg l_1$$

$$\mathcal{D} = l_2\left\{\frac{D_1 D_2}{2D_2 l_1 + D_1 l_2}\right\} \tag{99}$$

Let

$$\mathcal{D} = \times D_2$$

then

$$\frac{D_1}{D_2} = \frac{l_1}{l_2}\left(\frac{2\times}{1-\times}\right) \tag{100}$$

Now D_2, the diffusion of chloride through the interior of the red cell, is probably less than the diffusion of chloride through agar gel. By assuming them to be equal, a lower limit on $\times$ is found to be 0.2 from Figure 18.

Thus

$$\left(\frac{2x}{1-x}\right) = \frac{1}{2}$$

and

$$\frac{D_1}{D_2} = \frac{1}{2}\,\frac{l_1}{l_2}$$

Take for the thickness of the red cell $l_1 = 50\,\text{Å}$, and for the interior $l_2 = 5\times10^{4}\,\text{Å}$. Then

$$\frac{1}{2}\,\frac{l_1}{l_2} = \frac{1}{2}\,\frac{50}{5\times10^{4}} = 5\times10^{-4}$$

and

$$\frac{D_1}{D_2} = 5\times10^{-4}$$

Since

$$D_2 \cong 1\times10^{-5}\ \text{cm}^2/\text{sec}$$

$$D_1 \cong 5\times10^{-9}\ \text{cm}^2/\text{sec}$$

$$P = \frac{D_1}{l_1} = 1\times10^{-2}\ \text{cm/sec}$$

This permeability is two orders of magnitude higher than the expected value (2×10^{-4} cm/sec), which is based on equilibration times for chloride.

APPENDIX II

1. Hemolysis Analysis

The hemolysis analysis was a slight modification of the method used by Sachs et al. (30), where the percent hemolysis is given as the ratio of the absorbance of the supernatant before complete induced hemolysis divided by the absorbance of the total volume after induced hemolysis. The initial sample must be separated into two parts for this; one part centrifuged for the supernatant and the other hemolysed. The absorbance was recorded at 540 mμ with a Bausch and Lomb Spectronic 20 colorimeter.

Sachs measured the absorbance of the solutions directly, but this does not give valid results because the absorbance of hemolysed blood is not linear with increasing dilution. The hemoglobin in this work was converted to acid hematin. A five ml sample of blood was diluted to 50 ml with distilled water. A 20 ml aliquot of this was again diluted to 50 ml with 1.0 N NCL. The absorbance of this solution was linear with increasing dilution.

Red cells were kept in Earle's Solution for 24 hours at 25°C. The amount of hemolysis was found to be approximately 0.3 percent. This slight hemolysis should not significantly affect the experimental results.

APPENDIX II

2. Solutions

Earle's Solution A

sodium chloride	6.8	grams
potassium chloride	.400	grams
calcium chloride	.200	grams
magnesium sulfate	.100	grams
sodium monophosphate	.140	grams
dextrose	1.00	grams
distilled water	700	ml

Earle's Solution B

sodium bicarbonate	2.200	grams
penicillin G	2	million units
streptomycin sulfate	2	grams
distilled water	300	ml

Earle's Solution is formed by adding seven parts of A to three parts of B.

Liquid Scintillation "Cocktail"

PPO	6	grams
toluene	1	liter

Mix two parts of PPO solution to one part of Triton X-100 (Rohm and Haas, Philadelphia, Pennsylvania). Ten ml of resulting solution will dissolve one ml of aqueous sample.

APPENDIX III

1. Data

Table III. Diffusion Coefficients of Sodium in a 0.100 M KCl Solution at 25°C.

	$D \times 10^5$ (cm^2/sec)
48-hour runs	1.298
	1.320
	1.310
	1.325
	1.280
Average	1.313 ± 0.024
24-hour runs	1.330
	1.301
	1.307
Average	1.312

Table IV. Diffusion Coefficients of Sodium in 0.100 M NaCl Solutions at 25°C.

$D \times 10^5$ (cm^2/sec)
1.316
1.316

Table V. Diffusion Coefficients of Sodium in Rabbit Plasma at 25°C.

$D \times 10^5$ (cm^2/sec)
1.071
0.980
1.064
1.021
1.017
Average 1.031 ± 0.030

Table VI. Diffusion Coefficients of Sodium in Agar Gel Suspensions of Rabbit Red Cells at 25°C.

Volume Fraction of Red Cells	$D \times 10^5$ (cm^2/sec)
20	1.096
"	1.056
23	1.073
30	1.021
"	1.075

40	0.978
"	0.960
"	0.988
50	0.885
70	0.586
ATP depleted prior to run	
20	1.089
30	0.916
"	0.976
ATP depleted-restored prior to run	
20	1.082

Table VII. Diffusion Coefficients of Sodium in Agar Gel Suspensions of Human Red Cells at 25°C.

Volume Fraction of Red Cells	$D \times 10^5$ (cm^2/sec)
20	1.151
"	1.064
30	0.968
"	0.956
50	0.792
"	0.817

Table VIII. Diffusion Coefficients of Sodium in Agar Gel Suspensions of Fixed Human Red Cells at 25°C.

Volume Fraction of Red Cells	D x 10^5 (cm^2/sec)
10	1.097
19	.998
24	.934

Table IX. Diffusion Coefficients of Sodium in Agar Gel Suspension of Glass Spheres at 25°C.

Volume Fraction of Glass Spheres	D x 10^5 (cm^2/sec)
5	1.159
10	1.221
"	1.228
15	1.260
"	1.245
20	1.275
30	1.320
60	1.579

Table X. Diffusion Coefficients of Potassium in Agar Gel Suspensions of Human Red Cells at 25°C.

Volume Fraction of Red Cell	D x 10^5 (cm^2/sec)
0	1.894
0	1.887
20	1.021
20	1.064
Potassium equilibrated for 17 hours	
20	0.983

Table XI. Diffusion Coefficients of Chloride in Agar Gel Suspensions of Human Red Cells at 25°C.

Volume Fraction of Red Cells	D x 10^5 (cm^2/sec)
0	1.785
"	1.794
"	1.752
20	1.457
30	1.257
"	1.268
50	0.938

NOMENCLATURE

a = radius (cm)

A = area (cm^2)

c_i = concentration of species i (moles/l)

d_o = density of water (g/cc)

D_i = diffusivity of species i (cm^2/sec)

H = hydration of protein in solution (gm/gm)

J = diffusional flux (moles/cm^2 sec)

k^* = radioactive decay rate (sec^{-1})

k = thermal conductivity (cm^2/sec)

K = partition coefficient

ℓ = length (cm)

n_{ik} = concentration of species i in the form (moles/l)

N_i = total concentration of reversibly bound species i (moles/l)

P = partial pressure (atm)

r = radius (cm)

R = net chemical reaction of species i

t = time (sec)

T = temperature (^oC)

$\mathcal{T}$ = temperature

V = volume (cm^3)

NOMENCLATURE

$\bar{V}_p$ = specific volume of protein

x = distance (cm)

α = volume fraction

ξ = partition coefficient

γ = fraction of diffusible species in a phase

η = viscosity (gm/cm sec)

Subscripts:

d = diffusible

i = species i

k = reaction with species i

p = protein

rbc = red cell

s = continuous phase

t = total

1 = continuous phase

2 = dispersed phase

Superscripts:

$*$ = based on average concentration

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PROPOSITIONS

PROPOSITION I

The Interpretation of Adsorption of Metal Cation versus pH Data

This proposition will deal with a method of interpreting metal ion adsorption data. The method was presented by Dr. T. W. Healy in a seminar given in the W. M. Keck Laboratories at the California Institute of Technology during the summer of 1969. The method is shown to be valid only under certain conditions.

The adsorption of iron (III) on quartz surfaces was one topic discussed by Dr. Healy. His results, plotted as the percent adsorption of iron (III) on quartz versus pH, showed a very strong pH dependence. A typical curve is given in Figure 21. The results were significant in that the large increase in percent adsorption of iron (III) over a narrow pH range occurred at the pK_1 value for iron. Other experimental results showed no drastic change in the surface properties of quartz over the pH range of interest.

At pH values far below the pK_1 value for iron (III), the predominant iron (III) species in solution is the free (aquo) ion. As the pH approaches the value for pK_1, the concentration of the first hydrolysis product of iron (III), $Fe(OH)^{+2}$, becomes

significant. Since the sharp rise in the percent adsorption versus pH curve occurred at the pK_1 value for iron (III), Dr. Healy concluded that the predominant iron species involved in the adsorption mechanism was $Fe(OH)^{+2}$. He also felt the percent adsorption versus pH data for other metal cations could be used to determine the principal hydrolyzed species involved in the adsorption process.

O'Melia and Stumm (1), investigating the coagulation of silica sols by iron (III), observed stoichiometric behavior. The minimum concentration of iron (III) necessary to induce coagulation was proportional to the total surface area of the silica particles. The adsorption data they obtained was in accord with a curve predicted by the Langmuir equation. They showed that if $K\Gamma_m S(1-\theta) \gg 1$ in their rearranged form of the Langmuir equation, then the equation is consistent with the stoichiometric relationship they observed.

The Langmuir equation is

$$\frac{C_A}{S} = \frac{\Gamma_m K C_e}{1 + K C_e} \qquad (1)$$

where C_e and C_A are the concentrations of residual and sorbed species (moles/liter), respectively; Γ_m is the sorption capacity (moles/m^2); S is the surface area of the sorbent (dispersed phase) in the suspension (m^2/l) and K is a constant

(the equilibrium constant for the adsorption-desorption reaction in the Langmuir model).

If in the adsorption of iron (III) on quartz, $Fe(OH)^{+2}$ is the principal sorbate, then C_A is the concentration of adsorbed $Fe(OH)^{+2}$ and C_e is the concentration of $Fe(OH)^{+2}$ in solution. If the total concentration of iron originally added is C_t, and the concentrations of the other hydrolysis products of iron (III) are assumed negligible in the low pH range, then

$$C_t = C_A + C_e + C_o \qquad (2)$$

where C_o is the concentration of the free (aquo) ion.
We know that

$$Fe^{+3} + H_2O = Fe(OH)^{+2} + H^+ \quad : K_1 \qquad (3)$$

Thus

$$C_t = C_A + C_e + C_e \frac{[H^+]}{K_1} \qquad (4)$$

Rearranging the Langmuir equation to

$$C_e = \frac{C_A / S\Gamma_m}{K\left[1 - C_A/S\Gamma_m\right]} \qquad (5)$$

Equation (4) now becomes

$$C_t = C_A \left\{ 1 + \left[\frac{[H^+]}{K_1} + 1 \right] \frac{1}{\left(1 - \frac{C_A}{S\Gamma_m}\right) KS\Gamma_m} \right\}, \qquad (6)$$

Since C_A/C_t is the percent iron (III) adsorbed, we have

$$C_A/C_t = \left[1 + \left[\frac{[H^+]}{K_1} + 1 \right] \frac{1}{KS\Gamma_m(1-\theta)} \right]^{-1} \qquad (7)$$

where

$$\theta = C_A / S\Gamma_m \qquad (0 \leq \theta \leq 1) \qquad (8)$$

This gives an expression for the percent iron (III) adsorbed versus pH. For stoichiometric behavior, $KS\Gamma_m(1-\theta) \gg 1$ If this is true,

$$\left[\frac{[H^+]}{K_1} + 1\right] \frac{1}{KS\Gamma_m(1-\theta)} << 1$$

For values of the pH in the neighborhood of pK_1, and from equation (7), $C_A/C_t = 1$ (100 percent). This is not consistent with the data Dr. Healy presented.

From O'Melia and Stumm (1), for iron (III) interacting with Min-U-Sil 5

$\Gamma_m = 2.3 \times 10^{-6}$ moles/m^2

$K = 10^7$ l/mole

$S = 168$ m^2/l (20 g/l of Min-U-Sil 5)

and $KS\Gamma_m = 400$. For $\theta = 0.4$, $KS\Gamma_m(1-\theta) = 240$. From equation (7), where $K = 6.8 \times 10^{-3}$, we find that for $C_A/C_t = 0.5$, $[H^+] = 1.63$. The rise in the curve of percent iron (III) adsorbed in Min-U-Sil 5 versus pH for a silica concentration of 20 g/l should occur at a pH much lower than the pK_1 value for iron (III).

It is obvious that the plot of C_A/C_t versus pH is very sensitive to S. If S were 1 m^2/l in the above example, instead of 168 m^2/l, $KS\Gamma_m(1-\theta) = 1.4$. At $[H^+] = K_1$, the value for C_A/C_t is now 0.42. This would qualitatively agree with Healy's results. The method proposed by Dr. Healy

is only valid if $KS\Gamma_m(1-\theta) = 1$. Then equation (7) becomes

$$C_A/C_t \cong \left[1 + \left\{ \frac{[H^+]}{K_1} + 1 \right\} \right]^{-1} \qquad (9)$$

and a curve similar to that in Figure 21 will result.

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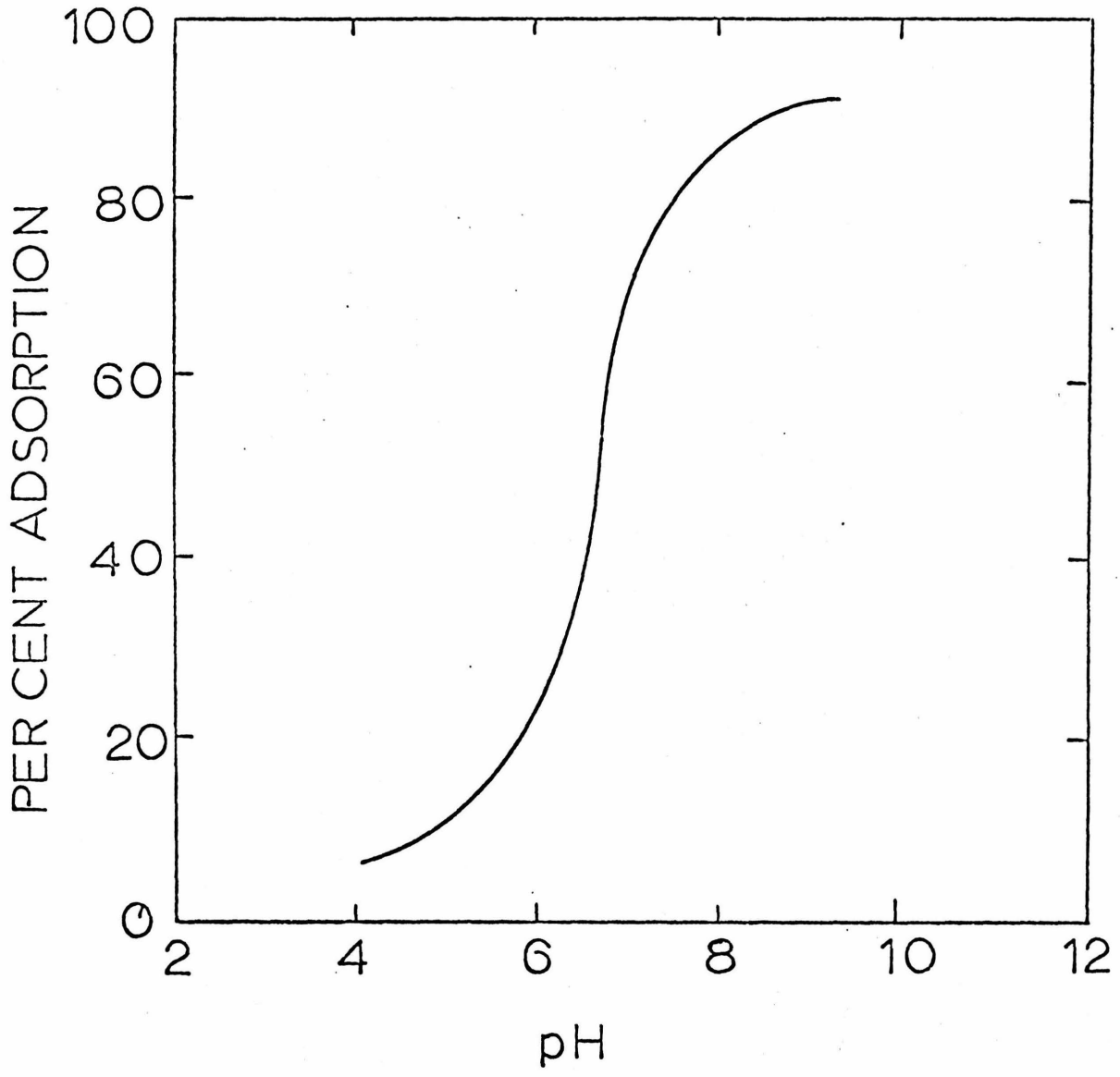

Figure 21. Typical Per Cent Adsorption on Silica as a Function of pH Curve for a Metal Cation.

PROPOSITION II

A Method for Stripping Interfering Gaussian Peaks from Complex Envelopes by an On-line Computer with Limited Storage Capacity

This proposition describes a method for stripping overlapping peaks from a complex envelope which can be used in on-line computer analysis of data acquired from any system generating Gaussian curves as a function of time.

Several methods have been suggested for separating the interfering peaks (2). In general, the most accurate methods employ a nonlinear least-squares technique. Such techniques are relatively easy to use on the large off-line computer, but must be simplified before they can be used on a small on-line computer. A simple, yet efficient, means for analyzing multiple peak envelopes has been devised to meet this problem.

Mathematical Treatment

For the case of multiple peak envelopes, the determination of the beginning and end of the envelope by the computer has been previously described (6). The envelope is analyzed by fitting the curve with the number of individual peaks corresponding to the number of pairs of inflection points detected within the envelope. In this procedure, individual peaks must be first represented by a suitable mathematical model. Many can be adequately described as Gaussian or modified Gaussian curves. The initial choice of

parameters of the assumed Gaussian peaks comprising the envelope (e.g., peak position, mean value, and standard deviation) are obtained from the envelope. Corrections to the initial choices are calculated, and the area and position of the peaks in the envelope are computed.

A brief mathematical description of the method is given below. If the peaks in the chromatogram are assumed to be Gaussian in shape, then the envelope can be described by

$$A = \sum_{j=1}^{n} \bar{A}_j \exp\left[-(T-\bar{T}_j)^2/2\sigma_j^2\right] \qquad (1)$$

where

A = the total absorbance value at T,

$\bar{A}_j$ = the maximum absorbance of individual peak j,

T = the elution time,

$\bar{T}_j$ = the value of T at ,

σ_j = the standard deviation of the peak j,

n = the number of interfering peaks.

Equation (1) can be rewritten as

$$A = A(T; \beta_1, \beta_2, \cdots, \beta_k) \qquad (2)$$

where β refers to the parameters $\bar{A}_j$, $\bar{T}_j$, and σ_j. The experimental data points are denoted as

$$(\hat{A}_i, \hat{T}_i) \; ; \; i = 1, \cdots, k. \qquad (3)$$

Let A_i^* denote the predicted value obtained for A when, for the ith data point, the value for the independent variable is substituted into equation (2). The estimates of $\beta_1, \beta_2, \cdots, \beta_k$ will be denoted by $b_1, b_2, \cdots, b_k$. Thus

$$A_i^* = A(\hat{T}_i; b_1, b_2, \cdots, b_k) \tag{4}$$

For the conventional least squares analysis of the problem, the unknown parameters are varied until

$$\phi = \sum_{i=1}^{k} [\hat{A}_i - A_i^*]^2 \tag{5}$$

is a minimum (4). Since the function A in equation (2) is non-linear in the parameter, equation (4) is expanded in a Taylor series about the unknown parameters. The Taylor series is truncated after the linear terms and substituted into equation (5). Corrections to the initial choices of the parameters are found by setting the partial derivative of ϕ with respect to the corrections to the parameters equal to zero. New estimates for the parameters are found, and the process is repeated until the corrections become negligible. Since the initial estimate of the parameters must be quite good to ensure convergence, more sophisticated approaches have been devised (1, 5). However, for the purposes of envelope analysis by an on-line computer with limited storage capability, a simple, yet efficient, approach which utilizes a minimal amount of fast memory is imperative.

The simplest approach is to dispense with the iteration procedure in the least squares analysis and assume the initial estimates of the parameters, once obtained, are sufficiently accurate. This, however, has been found to give unacceptable errors in the area determinations.

The next approach is to use the truncated Taylor series expansion of equation (4) to obtain corrections to the initial estimates of the parameters. This method appears to work very well. For this simplified analysis the assumption is made that

$$\hat{A}_i = A_{ic}^* \tag{6}$$

where

$$A_{ic}^* = A_i^* + \Delta A_i^* \tag{7}$$

The quantity ΔA_i^* is a correction to A_i^* obtained from the truncated Taylor series expansion of A. Therefore,

$$\hat{A}_i = A_i^* + \sum_{j=1}^{k} \Delta\beta_j \frac{\partial A}{\partial \beta_j} \; ; \; i = 1, \ldots, k \tag{8}$$

where A_i^* and the partial derivatives $\partial A / \partial \beta_j$ are evaluated at $\hat{T}_i$ and b_j, the initial estimates for β_j. The quantity $\Delta\beta_j$ is the correction to the initial estimate of β_j.

Satisfactory results were obtained when only the initial estimates of $\overline{A}_j$ and $\overline{T}_j$ were corrected. Thus, in this dis-

cussion, $k = 2n$, and a convenient choice of experimental points is the value of A and T at each inflection point in the envelope.

The initial choices of the parameters $\bar{A}_j$ and $\bar{T}_j$ are

$$\bar{A}_j^i = (\hat{A}_{2l} + \hat{A}_{2l-1})/2 \quad ; \quad l = 1, \cdots, n$$
$$\bar{T}_j^i = (\hat{T}_{2l} + \hat{T}_{2(l-1)})/2 \quad ; \quad l = 1, \cdots, n \tag{9}$$

The assumption is also made that each peak in a given envelope has the same σ. This assumption appears to be justified in the case of high resolution chromatography of body fluids, as near-neighbor peaks have almost identical standard deviations (6). Therefore, the choice of σ^i is

$$\sigma^i = (\hat{T}_{2m} - \hat{T}_{2m-1})/2 \tag{10}$$

where $2m-1$ and $2m$ are the data points on either side of the envelope maximum.

The corrections to $\bar{A}_j^i$ and $\bar{T}_j^i$ are found by solving the set of linear, simultaneous, algebraic equations given in equation (8). The equations are solved by means of a process of elimination, an efficient method for a small, on-line computer (3). A flow diagram for the stripping routine is given in Figure 1. Thus, the position of peak j in the envelope is

$$\bar{T}_j = \bar{T}_j^i + \Delta\bar{T}_j \tag{11}$$

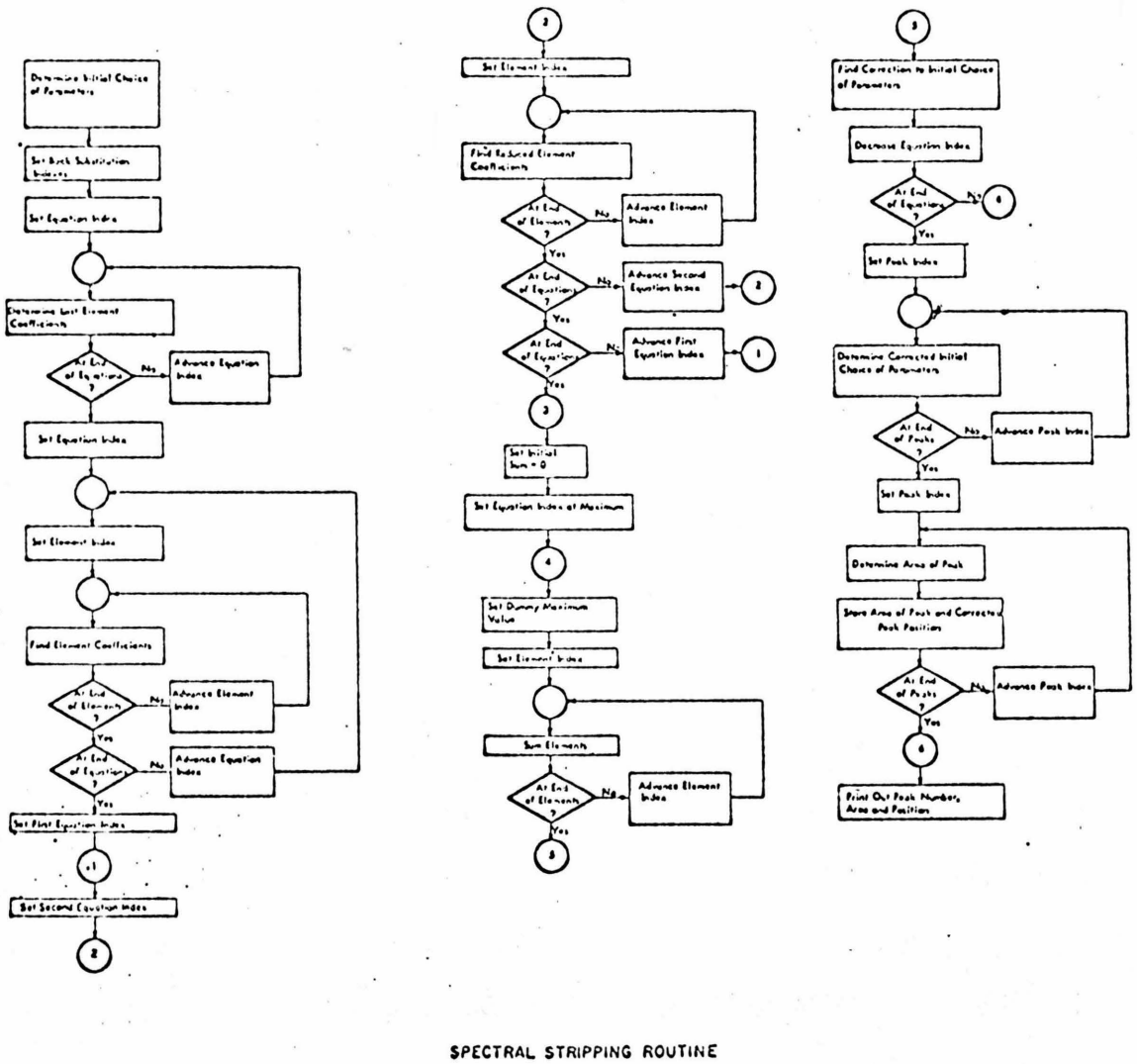

Figure 1. Flow Diagram of the Stripping Routine.

and the area S of the peak is

$$S_j = 2.5066\,(\bar{A}_j^{i} + \Delta\bar{A}_j)\,\sigma_j^{i} \qquad (12)$$

Results

For evaluation purposes, a computer program was written in FOCAL language and added to an existing program used to analyze data from a high-pressure ion-exchange column. The program was written in such a way that the stripping routine was used only when more than one pair of inflection points were found between any two consecutive minima on the chromatogram. A Digital Equipment Corporation Model PDP-8/I digital computer (Maynard, Mass.) equipped with a 8K-12 bit core memory and a 32K-12 bit disk storage was used to evaluate the program. This computer system was coupled to an ASR 33 Teletype for input/output.

Table 1 shows the computer analysis of four envelopes comprised of Gaussian curves. For each envelope, 500 data points were calculated. The computer analyzed the synthetic data and printed out the position of the envelope maximum. It also printed out the position of the peaks comprising the envelopes and their respective areas. The computer, using this method, was able to analyze up to three Gaussian peaks in an envelope with an error less than 5%, based on the peak areas.

Table 1. Computer Analysis of Synthetic Data

Data					Computer Analysis				
Peak No.	$\bar{A}$	$\bar{T}$	σ	S	Peak No.	Infl. Pt.	Maximum	Peak Position	Area
1	150	150	10	3760	1	142		150	3714
2	250	170	10	6266		155			
					2	160		170	6309
							167		
						180			
						133			
1	100	150	20	5013	1	161		150	5006
						177			
2	150	190	20	7520	2	199		190	7488
						212			
3	700	230	20	10026	3	249	222	230	10145
						100			
1	100	120	10	2507	1	131	119	119	2510
						171			
2	200	180	10	5013	2	191	181	180	5115
						198			
3	100	200	10	2507	3	208		200	2393
1	100	150	20	5013	1	131		151	4974
						159			
						184	199		
2	200	200	20	10026	2	217		200	10564
						242			
3	100	250	20	5013	3	270		249	4891

Summary

A computer program that is capable of effective and efficient peak stripping of complex envelopes has been developed for use with a small on-line computer. The program determines both the area and position of each interfering peak in the envelope. Envelopes with up to three interfering peaks can be analyzed with this method, yielding errors based on the area determination of less than 5%.

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PROPOSITION III

The Relationship Between the Distribution Coefficient and the Gradient Substance in Gradient Elution Chromatography

This proposition derives a theoretical relationship between the distribution coefficient and the concentration of the gradient substance in gradient elution chromatography.

In chromatography, mass is transferred between a flowing fluid and a fixed phase. If only the elution positions of the peak maxima are considered, a simple mathematical model of this system can be derived. This model, presented by Drake (1) and others (2, 5, 6, 7), is obtained by integrating the overall differential mass balance equation

$$\int_0^{\bar{V}} \frac{dv}{\lambda(v,z)+\epsilon} = A\int_0^Z dz = AZ = V \qquad (1)$$

where

A = total cross-sectional area of the column,

v = eluent volume,

$\bar{v}$ = elution volume of peak maximum,

V = geometric volume of the column,

z = height of the column measured from the entrance,

Z = total column length

ϵ = void fraction

λ = distribution coefficient

This simplified approach neglects the dispersion in the column.

The complexity of equation (1) resides in the term $\lambda\ (v, z)$. Since, in the case of gradient elution chromatography, λ is a function of the concentration of gradient substance (4), the exact relationship is worth seeking.

If we let R stand for the fixed ion in the resin, A for the counter ion and B for the gradient substance (also a counter ion), then we assume that for the exchange reaction

$$A^{a-} + aRB \rightleftharpoons R_aA + aB^- \qquad (2)$$

we have

$$K' = \frac{[R_aA]\,[B]^a}{[RB]^a\,[A]} \qquad (3)$$

Since λ is defined as the ratio of the concentration of the sample ion in the stationary phase to that in the mobile phase,

$$\lambda = \frac{n}{c} = \frac{[R_aA]}{[A]} = K\,\frac{[RB]^a}{[B]^a} \qquad (4)$$

We assume that the concentration of B in the resin compared to that in the flowing fluid can be described by a Freundlich isotherm. Thus,

$$[RB] = [B]^m \qquad (5)$$

In view of the discussion by Diamond and Whitney (3), this should be a reasonable assumption, at least for strong anion-exchange resins. Therefore,

$$\lambda = K' \frac{[B^m]^a}{[B]^a} = K' B^{a(m-1)} \qquad (6)$$

Since the concentration of gradient substance versus time in the column can be determined, and can in general be predicted based on the type of gradient generating device employed (4), equation (1) can now be successfully integrated.

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