Modelling Diffusion Across Red Blood Cells of Different Sizes and Elongations

Table of Contents

1 Introduction

I first learned about red blood cells in biology class. There, I learned that red blood cells are biconcave which helps maximize their surface area for diffusion and certain diseases such as anemia or cycle cell disease cause red blood cells to become deformed from their traditional shape, negatively impacting the transport of oxygen in vessels. I hope through this report to investigate two modifications to red blood cells: their size (assuming the same proportions) and their elongation by "squishing" the red blood cells from one side.

2 Determining a Relationship for Diffusion

2.1 Deriving a Formula

Fick's first law provides an equation for diffusion as follows.

$$J = -D\frac{\Delta C}{\Delta x}$$

J is the number of molecules transferred per second and unit area ($m^{-2}s^{-1}$). ΔC is the concentration gradient measured in molecules per unit volume (m^{-3}); Δx is the distance over which diffusion occurs, membrane thickness in this case. D (m^2s^{-1}) is the diffusion coefficient, which is dependent on the properties of the system, ie. temperature and the diffusing substance (Dickson, n.d.; Khan Academy, n.d.). Based on the definition of J, the following equation can be written:

$$\frac{dC_{RBC}}{dt} = \frac{J \cdot A}{V}$$

$$\frac{dC_{RBC}}{dt} = \frac{DA}{Vd}\left(C_{Surroundings} - C_{RBC}\right)$$

d is the membrane thickness. The negative sign from Fick's first law is excluded since the direction of molecule transfer is known to be into the red blood cell in this case. Also, I will assume $C_{Surroundings}$ is constant since it is saturated with the gas. Therefore, this is a first order linear differential equation. To solve this, I can use the separation of variables method (Simon Fraser University, n.d.-a).

$$\frac{1}{\left(C_{Surroundings} - C_{RBC}(t)\right)}dC_{RBC} = \frac{DA}{Vd}\,dt$$

$$\int \frac{1}{\left(C_{Surroundings} - C_{RBC}(t)\right)}dC_{RBC} = \int \frac{DA}{Vd}\,dt$$

$$-\ln\left|\left(C_{Surroundings} - C_{RBC}(t)\right)\right| + c_1 = \frac{DA}{dV}t + c_2$$

Since the gas is diffusing into the RBC, $C_{Surroundings} > C_{RBC}$.

$$-\ln\left(C_{Surroundings} - C_{RBC}(t)\right) + c = \frac{DA}{Vd}t$$

$$C_{RBC}(t) = C_{Surroundings} - e^{-\frac{DA}{Vd}t + c}$$

$C_{RBC}(0) = C_0$ Therefore, $c = \ln(C_{Surroundings} - C_0)$

$$\boxed{C_{RBC}(t) = C_{Surroundings} - (C_{Surroundings} - C_0)\left(e^{-\frac{DA}{Vd}t}\right)}$$

Since all of the constants are positive, having a larger surface area to volume ratio would lead to a more vertically stretched function - faster diffusion.

Note: concentration here is taken as number of molecules per unit volume rather than number of moles.

2.2 Determining Constants

I am going to use the ideal gas law to estimate the initial oxygen concentration, C_0, in the RBC and the concentration of the surroundings, $C_{Surroundings}$. The ideal gas model assumes that the oxygen molecules have negligible volume and no intermolecular forces between the molecules. While it is not a perfect assumption here, it should provide a reasonable estimate. Alveolar air (surroundings) has a partial pressure for oxygen of 104 mmHg (Lumen Learning, n.d.), and deoxygenated blood has a partial pressure of 40 mmHg (Rhodes et al., 2022). I am assuming the typical body temperature of 37 °C.

$$\begin{aligned} C_{Surroundings} &= \frac{N}{V} = \frac{P}{K_B T} \\ &= \frac{(104 * 133.322)\ \text{Pa}}{1.38\ \text{JK}^{-1} \times (37 + 273.15)\ \text{K}} \\ &\approx 32.39547 \text{ molecules m}^{-3} \end{aligned}$$

$$\begin{aligned} C_0 &= \frac{(40 * 133.322)\ \text{Pa}}{1.38\ \text{JK}^{-1} \times (37 + 273.15)\ \text{K}} \\ &\approx 12.45980 \text{ molecules m}^{-3} \end{aligned}$$

The diffusion coefficient, D, for oxygen in blood is $1.62 \times 10^3\ \mu m^2 s^{-1}$ at 37 °C (Goldstick, 1976). The membrane thickness, d, of an RBC is 5-10 μm (Faghih & Sharp, 2019), so I am going to take the value to be 7.5 μm.

Note: from the boxed equation above, D and d must be in terms of μm as that is going to be the units of volume and area, concentrations can stay as m^{-3} to ensure that $C_{RBC}(t)$ is in the same unit.

3 Determining a Function to Model an RBC

3.1 Using points to model an RBC

To determine a function's ability to model an RBC, I first need to have a set of known points that can be used to test its validity.

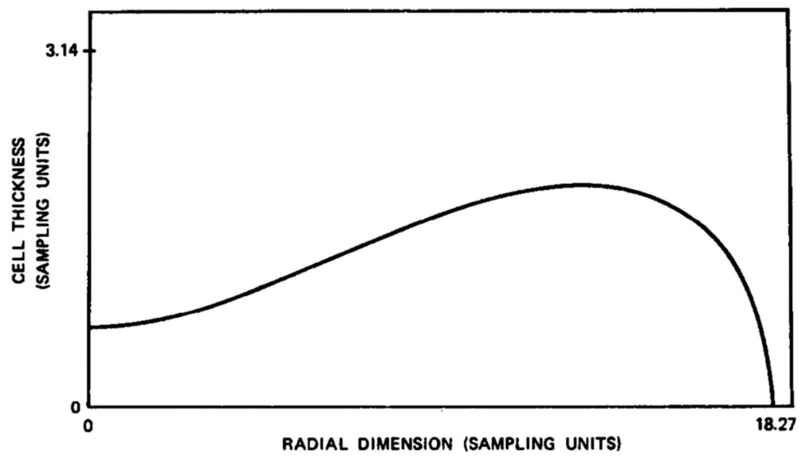


Figure 1: RBC profile obtained via a hologram. (Evans & Fung, 1971).

Figure 1 shows an RBC profile which used holography (an experimental method) to obtain a profile for an RBC (Evans & Fung, 1971). Using a Python script I coded (see Appendix), I was able to obtain a set of points to model this image. The points are scaled so that the x-intercept occurs at 1. Since the function will be dilated later on, it does not matter what the initial width and height are.

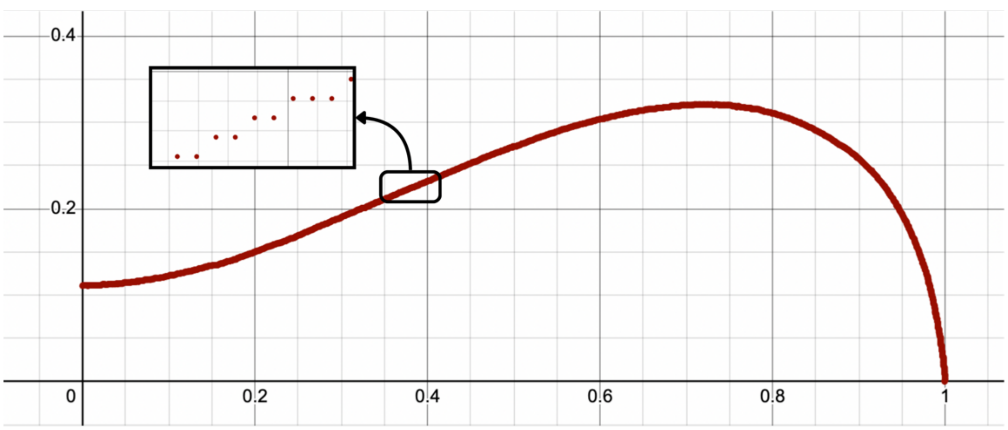


Figure 2: Graph showing the points obtained from the image in figure 1. While it looks like a line, they are in fact just many points (Created in Desmos).

3.2 Using a Quartic Function

I am going to use 5 data points which represent x-intercepts, the maxima, and the local minimum of an RBC to determine a function. The maxima and x-intercepts are reflected to represent the other side of an RBC. The maxima had to be estimated as there was a cluster of points which all had the maximum height.
x-intercepts: $(\pm1, 0)$
Maxima: $(\pm0.720, 0.321)$
Minimum: $(0, 0.111)$

With n+1 distinct points, a polynomial can be constructed of at most degree n (University of Cambridge, 2020). Since I have 5 points, I can construct a polynomial of at most the 4th degree, a quartic: $f(x) = a_0x^4 + a_1x^3 + a_2x^2 + a_3x + a_4$. The polynomial can be solved using an augmented matrix.

$$\left[\begin{array}{ccccc|c} 1 & 1 & 1 & 1 & 1 & 0 \\ 1 & -1 & 1 & -1 & 1 & 0 \\ 0.720^4 & 0.720^3 & 0.720^2 & 0.720 & 1 & 0.321 \\ (-0.720)^4 & (-0.720)^3 & (-0.720)^2 & -0.720 & 1 & 0.321 \\ 0 & 0 & 0 & 0 & 1 & 0.111 \end{array}\right]$$
$$\sim \left[\begin{array}{ccccc|c} 1 & 0 & 0 & 0 & 0 & -1.07 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0.96 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0.111 \end{array}\right]$$

The matrix was solved using the Casio CG50 graphics display calculator (GDC). The resulting polynomial is thus $f(x) = -1.07x^4 + 0.96x^2 + 0.111$. This is a symmetric function as it is an even function, $f(x) = -f(x)$.

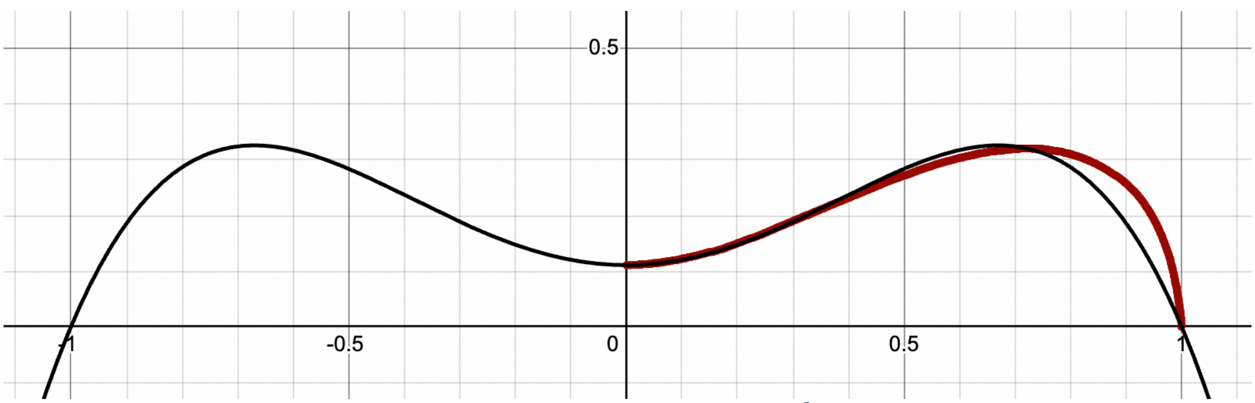


Figure 3: Graph showing the quartic function fitted onto the RBC points. The R^2 correlation is 0.815 (Created in Desmos).

From figure 3, the quartic is not a very good fit for the function, yielding a coefficient of determination, R^2, of only 0.815. The function does not fit with the points in many locations as seen in figure 3. Using interpolation to find the coefficients while manually setting the ones of x^3 and x to 0 to ensure an even function, yields a much better $R^2$of 0.957 (in Desmos). However, the fit does not visually appear to match the points, and it also falls apart in several locations as seen in figure 4. Therefore, a different method is required.

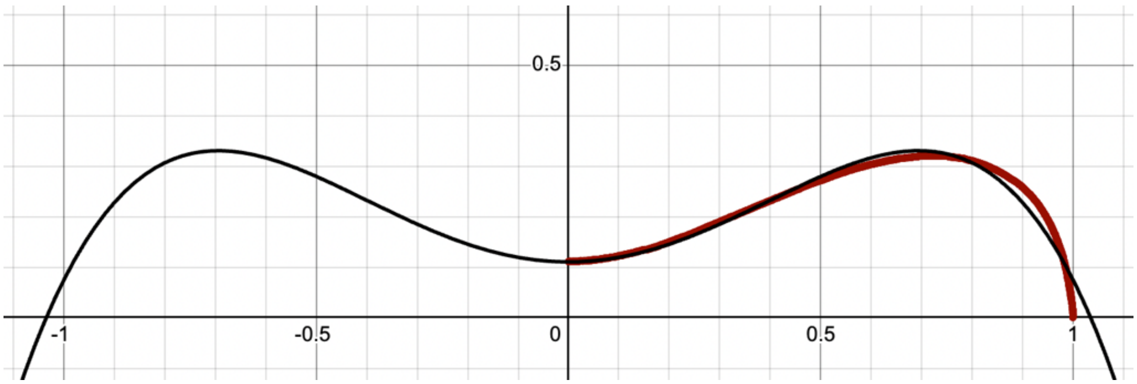


Figure 4: Graph showing best fit quartic function for the points where the coefficients are calculated with technology (Created in Desmos).

3.3 Using a Unique Function

After doing more research, I found that an RBC can be modelled with the following equation (Evans & Fung, 1971; Nayanajith et al., 2012; An et al., 2022).

$$f(x) = \sqrt{1 - a_0x^2}(a_1 + a_2x^2 + a_3x^4)$$

a_0 is wholly dependent on half the width of the RBC, for this case it is 1. Determining the other coefficients with Desmos' line interpolation, the following function is obtained.

$$f(x) = \sqrt{1 - x^2} \cdot (0.11786 + 0.873326x^2 - 0.380179x^4), \mathrm{D}: \{x \in \mathbb{R} | -1 \leq x \leq 1\}$$

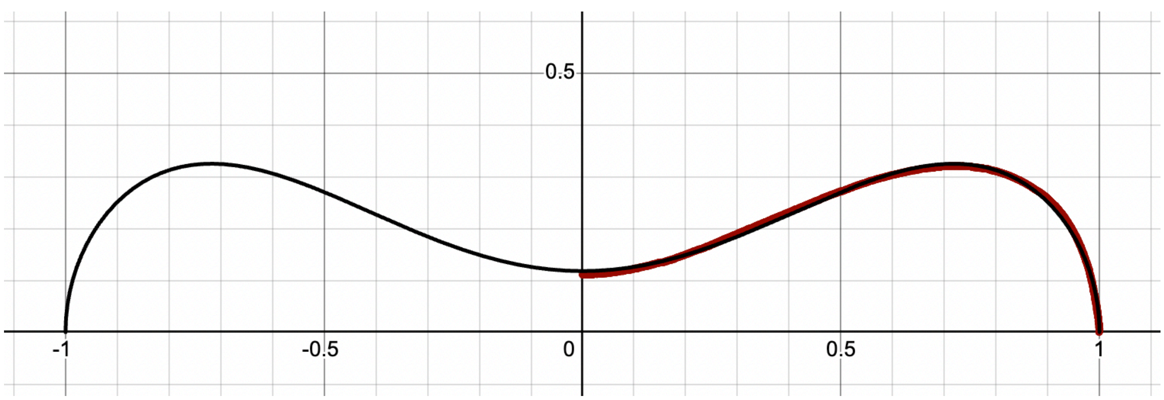


Figure 5: Graph showing the function f(x) on the experimental points (Created in Desmos).

As seen in figure 5, this function has an excellent fit. The R^2 value is 0.9957 indicating a very strong fit. This function will be the one used from here on.

4 Volume and Area for Circular RBC

4.1 Determining Dilation Factors for Circular RBC

To find the maxima of the function, I can use its derivative,

$$f'(x) = \frac{-x \cdot (0.11786 + 0.873326x^2 - 0.380179x^4)}{\sqrt{1-x^2}}$$
$$+\sqrt{1-x^2} \cdot (1.746652x - 1.520716x^3)$$
$$= \frac{x(1.900895x^4 - 4.140694x^2 + 1.628792)}{\sqrt{1-x^2}}$$

Using the quadratic formula, the non-zero x-intercepts are $x^2 = \frac{4.140694 \pm \sqrt{4.760696526}}{3.80179}, x = \pm 0.72, \pm 1.29$. Only the first two zeros are within the domain of the function D: $\{x \in \mathbb{R} | -1 \leq x \leq 1\}$. Therefore, the maxima occur at $y = 0.32508$.

The x-intercepts of f(x) are $(\pm 1, 0)$ as seen in the domain, from the $\sqrt{1-x^2}$ factor in f(x).

If f(x) is the function representing the RBC, I can dilate it with df(kx) where d is the vertical dilation and k the horizontal one. Using the maxima and the x-intercepts, I can determine the dilation factors. Half the width of a typical RBC is 3.91 µm (7.82 µm is the full width) and half its height is 1.29 µm (Faghih & Sharp, 2019). Therefore $k = \frac{1}{3.91}$ and $d = \frac{1.29}{0.32508}$. I am going to use other widths of 5, 6, 7, 8, 9, 10 (half widths: 2.5, 3, 3.5, 4, 4.5, 5). Microcytes (abnormally small RBCs) have a width of less than 6 µm and macrocytes (abnormally large RBCs) have a width greater than 9 µm (Lynch, 1990). The height's dilation factor, d, will be determined so as to maintain the same ratio between d and k as that for the typical RBC.

Table 1: Table showing the horizontal and vertical dilation factors of f(x) in the form of df(kx). The horizontal d and k were calculated as follows: $k = \frac{1}{half\ width}, d = \frac{\frac{half\ width}{3.91} \times 1.29}{0.32508}$.

Half width (µm)	k (horizontal) dilation factor	d (vertical) dilation factor
2.5	0.40000	2.53725
3	0.33333	3.04470
3.5	0.28571	3.55215
3.91 (Typical)	**0.25575**	**3.96825**
4	0.25000	4.05959
4.5	0.22222	4.56704
5	0.20000	5.07449

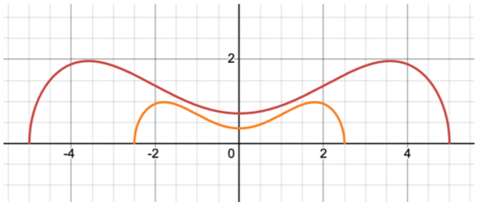


Figure 6a: Graph showing df(kx) for the smallest and largest half widths of 2.5 and 5 μm (Created in Desmos).

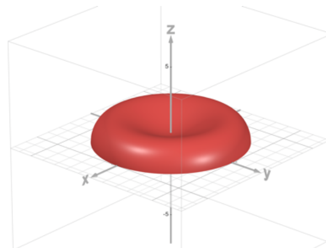


Figure 6b: Graph showing df(kx) revolved around the y-axis for the half width of 5 μm (Created in Desmos 3D).

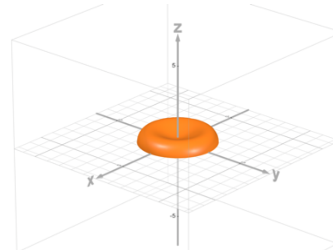


Figure 6c: Graph showing df(kx) revolved around the y-axis for the half width of 2.5 μm (Created in Desmos 3D).

Figure 6: Graphs of the smallest and largest dilations of df(kx), the half widths of 2.5 and 5 μm. Figures 6b and 6c are on the same scale and were created by substituting $\sqrt{x^2+y^2}$ for x in df(kx) and equating the whole function to z.

From figure 6, by dilating the function to keep the same proportions, I am essentially obtaining different sizes of the typical RBC.

4.2 Finding Volumes of the Circular RBCs

The disk method of calculating the volume, learned in HL math, cannot be used here since the function is not easily invertible: the function fails the horizontal line test, indicating that it is not invertible unless it is made piecewise (restricting its domain). A better method that can be used is the shell method which states that the volume of revolution of h(x) about the y-axis on the interval $x \in [a_1, a_2]$ is given by $V = 2\pi \int_{a_1}^{a_2} xh(x)dx$, assuming that h(x) is continuous and non-negative on the interval $[a_1, a_2]$. This method is better suited in this case since it does not require inverting the function (note: this method uses x-limits of integration unlike the disk method).

Since the original function, f(x) should be revolved on the interval of $[0,1]$, df(kx) should be revolved on the interval $\left[0, \frac{1}{k}\right]$. Therefore, the volume is given by:

$$V = 2\pi d \int_0^{\frac{1}{k}} x \cdot f(kx)\, dx$$

I can simplify the equation using a substitution.

$u = kx, \dfrac{du}{dx} = k$

$\text{If } x = \dfrac{1}{k}, u = 1$

$\text{and if } x = 0, u = 0$

Therefore,

$$V = \frac{2\pi d}{k^2} \int_0^1 uf(u)\, du$$

Since u is a "dummy variable", it can be replaced with x. (bprp calculus basics, 2022)

$$
\begin{aligned}
V &= \frac{2\pi d}{k^2}\int_0^1 xf(x)\,dx \\
&= \frac{d}{k^2}V_0
\end{aligned}
$$

So now, I only need to calculate V_0 rather than integrating the volume for every dilated form of f(x).

$$
\begin{aligned}
V_0 &= 2\pi\int_0^1 xf(x)\,dx \\
&= 2\pi\int_0^1 x\sqrt{1-x^2}\cdot(0.11786+0.873326x^2-0.380179x^4)\,dx
\end{aligned}
$$

To solve this integral, I can use substitution to simplify the square root term.

$u = 1 - x^2, \dfrac{du}{dx} = -2x$

If $x = 1, u = 0$

and if $x = 0, u = 1$

$$
\begin{aligned}
V_0 &= 2\pi\int_0^1 \sqrt{1-(1-u)}\cdot[0.11786+0.873326(1-u)-0.380179(1-u)^2]\cdot\left(\frac{-1}{2}\right)\frac{du}{dx}dx \\
&= -\pi\int_1^0 \sqrt{u}\cdot(0.11786+0.873326(1-u)-0.380179(1-u)^2)\,du \\
&= -\pi\int_1^0 \left(0.611007\sqrt{u}-0.112968u^{\frac{3}{2}}-0.380179u^{\frac{5}{2}}\right)du \\
&= -\pi\left[0.407338u^{\frac{3}{2}}-0.0451872u^{\frac{5}{2}}-0.10862257142u^{\frac{7}{2}}\right]_1^0 \\
&\approx 0.79648
\end{aligned}
$$

The final areas calculating using $V = \frac{d}{k^2}V_0$ can be found in table 2. V is of course only half the volume since f(x) only represents half of an RBC. Therefore, the volumes were all multiplied by 2.

Table 2: Calculated volumes of the circular RBC.

Half Width (μm)	Volume (μm^3)
2.5	25.26091
3	43.65085
3.5	69.31593
3.91 (typical)	**96.64050**
4	103.46867
4.5	147.32160
5	202.08725

4.3 Finding Surface Areas of the Circular RBCs

To find the surface area of $y = f(x)$ revolved around the y-axis between the bounds a and b, I can use the following formula $A = 2\pi \int_a^b x\sqrt{1 + \left(\frac{dy}{dx}\right)^2}\,dx$ (Math with Professor V, 2020).

$$A = 2\pi \int_0^{\frac{1}{k}} x \sqrt{1 + \left[\frac{d}{dx}\big(df(kx)\big)\right]^2}\, dx$$

Using the chain rule to simplify the derivative of df(kx):

$$A = 2\pi \int_0^{\frac{1}{k}} x\sqrt{1 + (dkf'(kx))^2}\, dx$$

Now, a substitution can be used to write the area in terms of f'(x) rather than f'(kx).

$u = kx, \frac{du}{dx} = k$

If $x = \frac{1}{k}, u = 1$

and if $x = 0, u = 0$

$$A = 2\pi \int_0^1 \frac{u}{k^2}\sqrt{1 + \big(dkf'(u)\big)^2}\, du$$

Since u is a substitution variable, it can be replaced with x:

$$A = \frac{2\pi}{k^2}\int_0^1 x\sqrt{1 + \big(dkf'(x)\big)^2}\, dx$$

While this cannot be simplified to remove the a and b from within the integral, at least now the bounds are constants independent of a and b, and it is in terms of f'(x). f'(x). was calculated earlier, but the expression is very complex. Therefore, I am going to use numerical methods to solve it. The integrals were calculated in Desmos. Like for the volume, the areas were doubled to represent a full RBC.

Table 3: Calculated surface areas of the circular RBC.

Half width (μm)	Surface area (μm^2)
2.5	55.59095
3	80.05097
3.5	108.95826
3.91 (typical)	**135.98080**
4	142.31283
4.5	180.11467
5	222.36380

5 Volume and Area for Elliptical RBC

5.1 Determining Dilation Factors for Elliptical RBC

Since I intend to create an elliptical, or stretched, RBC, I need to modify f(x) to become a three-dimension graph, a multivariable function. To do this, I will substitute $\sqrt{x^2 + y^2}$ for x in f(x).

$$f(x,y) = \sqrt{1 - \sqrt{x^2 + y^2}^2} \cdot \left(0.11786 + 0.873326\sqrt{x^2 + y^2}^2 - 0.380179\sqrt{x^2 + y^2}^4\right)$$

Now, I can dilate f(x,y) to af(bx, cy) which will allow me to dilate so as to not obtain a circular RBC, see figure 7.

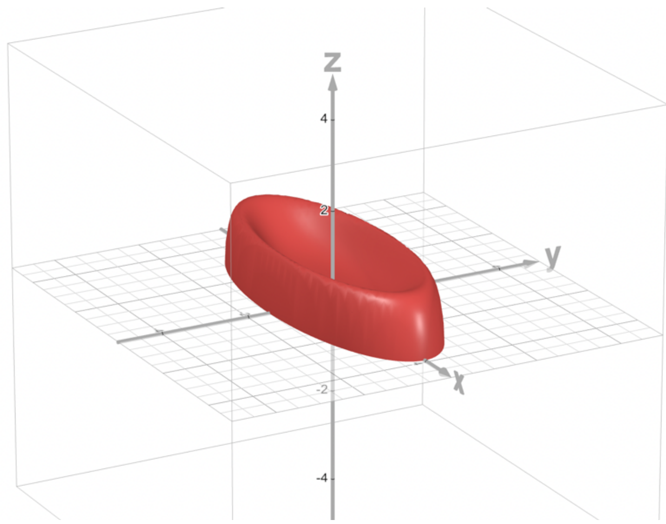


Figure 7: Graph showing the dilated version of f(x) for the RBC of half width 1.5 µm (Created in Desmos 3D).

Elliptocytes have a length to width ratio of up to 2 (Quigley et al., 2007). Half the typical width of a circular RBC is 7.82 µm, as was used earlier. $7.82 / 2 = 3.91$, therefore, I will use the widths of 7.82, 7, 6, 5, 4, 3 µm (half widths: 3.91, 3.5, 3, 2.5, 2, 1.5 µm). The dilation factors of z and x will be the same as those used of the typical RBC used in section 4, only now the vertical dilation factor becomes a and the horizontal one b: $a = 3.968$, $b = 0.256$.

Table 4: Values of the c dilation factor of af(bx,cy) for elongated RBC. c was calculated as follows: $c = \frac{1}{half\ width}$.

Half width (µm)	c dilation factor
3.91 (Typical)	**0.25575**
3.5	0.28571
3	0.33333
2.5	0.40000
2	0.50000
1.5	0.66667

5.2 Finding Volumes of the Elliptical RBCs

To compute the volume of the multivariable function, I can use a double integral to integrate the area over an interval, the formula is as follows. The limits of integration are determined by the area which encompasses the volume (Simon Fraser University, n.d.-b).

$$V = \iint_R f(x,y)\, dx\, dy$$

Since f(x) has an x-intercept at 1, f(x,y) lies above a circle of radius 1, $x^2 + y^2 = 1$. For af(bx,cy), the circle becomes dilated as follows:

$$(\mathrm{bx})^2 + (\mathrm{cy})^2 = 1$$
$$\mathrm{x} = \pm\frac{1}{\mathrm{b}}\sqrt{1-\mathrm{c}^2\mathrm{y}^2}$$

The y-intercepts of $\mathrm{x} = \pm\frac{1}{\mathrm{b}}\sqrt{1-\mathrm{c}^2\mathrm{y}^2}$ are $\pm\frac{1}{\mathrm{c}}$. Therefore, the formula becomes:

$$\mathrm{V} = \int_{-\frac{1}{\mathrm{c}}}^{\frac{1}{\mathrm{c}}}\int_{-\frac{1}{\mathrm{b}}\sqrt{1-\mathrm{c}^2\mathrm{y}^2}}^{\frac{1}{\mathrm{b}}\sqrt{1-\mathrm{c}^2\mathrm{y}^2}}\big(\mathrm{af}(\mathrm{bx},\mathrm{cy})\big)\,\mathrm{dx}\,\mathrm{dy}$$

Next, substitution can be used to simplify the integrals.

$\mathrm{u} = \mathrm{bx}, \frac{\mathrm{du}}{\mathrm{dx}} = \mathrm{b}$

If $\mathrm{x} = \pm\frac{1}{\mathrm{b}}\sqrt{1-\mathrm{c}^2\mathrm{y}^2}, \mathrm{u} = \pm\sqrt{1-\mathrm{c}^2\mathrm{y}^2}$

$$\mathrm{V} = a\int_{-\frac{1}{\mathrm{c}}}^{\frac{1}{\mathrm{c}}}\left(\int_{-\sqrt{1-\mathrm{c}^2\mathrm{y}^2}}^{\sqrt{1-\mathrm{c}^2\mathrm{y}^2}}\big(\mathrm{f}(\mathrm{u},\mathrm{cy})\big)\cdot\frac{1}{\mathrm{b}}\mathrm{du}\right)\mathrm{dy}$$

$\mathrm{n} = \mathrm{cy}, \frac{\mathrm{dn}}{\mathrm{dy}} = \mathrm{c}$

If $\mathrm{y} = \pm\frac{1}{c}, \mathrm{n} = \pm 1$

$and\ if\ n^2 = c^2y^2$

$$\mathrm{V} = \mathrm{a}\int_{-1}^{1}\left(\int_{-\sqrt{1-\mathrm{n}^2}}^{\sqrt{1-\mathrm{n}^2}}\big(\mathrm{f}(\mathrm{u},\mathrm{n})\big)\cdot\frac{1}{\mathrm{b}}\mathrm{du}\right)\cdot\frac{1}{\mathrm{c}}\mathrm{dn}$$
$$= \frac{\mathrm{a}}{\mathrm{bc}}\int_{-1}^{1}\int_{-\sqrt{1-\mathrm{n}^2}}^{\sqrt{1-\mathrm{n}^2}}\mathrm{f}(\mathrm{u},\mathrm{n})\,\mathrm{du}\,\mathrm{dn}$$

Since u and n are “dummy” variables:

$$\mathrm{V} = \frac{\mathrm{a}}{\mathrm{bc}}\int_{-1}^{1}\int_{-\sqrt{1-\mathrm{y}^2}}^{\sqrt{1-\mathrm{y}^2}}\mathrm{f}(\mathrm{x},\mathrm{y})\,\mathrm{dx}\,\mathrm{dy}$$

Since the double integral now represents the volume of the typical, circular RBC, it is expected that the integrals simplify to V_0. To simplify it, I am going to switch to polar coordinates. Since, as explained, the domain of integration is a circle of radius 1, r is over the interval $[0,1]$, and θ over the interval $[0,2\pi]$. dx dy must be replaced with r dr dθ (Houston Math Prep, 2020).

$$\mathrm{V} = \int_{-1}^{1}\int_{-\sqrt{1-\mathrm{y}^2}}^{\sqrt{1-\mathrm{y}^2}}\mathrm{f}\left(\sqrt{\mathrm{x}^2+\mathrm{y}^2}\right)\mathrm{dx}\,\mathrm{dy}$$
$$= \int_{0}^{2\pi}\int_{0}^{1}\mathrm{f}(\mathrm{r})\cdot\mathrm{r}\,\mathrm{dr}\,\mathrm{d\theta}$$

Since $\mathrm{f}(\mathrm{r})$ is independent of θ, the integral can be split (University of Maryland, 2017).

$$
\begin{aligned}
V &= \int_0^{2\pi} d\theta \cdot \int_0^1 f(r) \cdot r\, dr \\
&= [\theta]_0^{2\pi} \cdot \int_0^1 f(r) \cdot r\, dr \\
&= [\theta]_0^{2\pi} \cdot \int_0^1 f(r) \cdot rdr \\
&= 2\pi \int_0^1 xf(x)\, dx \\
&= V_0
\end{aligned}
$$

As expected, the integrals simplify to V_0, therefore the volume is $V = \frac{a}{bc}V_0$. Using the value of V_0 calculated in section 4.2, the volumes of the elongated RBC can be calculated.

Table 5: Calculated volumes of the elongated RBC.

Half width (μm)	Volume (μm^3)
3.91	96.64050
3.5	86.50684
3	74.14872
2.5	61.79060
2	49.43248
1.5	37.07436

5.3 Finding Surface Areas of the Elliptical RBCs

The surface area of f(x,y) over a bounded region R can be found with the following formula that also relies on a double integral (Hartman et al., n.d.).

$$A = \iint_R \sqrt{1 + (g_x)^2 + (g_y)^2}\, dx\, dy$$

g_x or $\frac{\partial g}{\partial x}$ and g_y or $\frac{\partial g}{\partial y}$ are the partial derivatives of g(x,y). When taking the partial derivative with respect to a variable, the other variable is considered to be a constant. They can also be defined as (London's Global University, 2016):

$$g_x = \lim_{h \to 0} \frac{g(x+h, y) - g(x)}{h}$$

The partial derivative with respect to y can be similarly defined with first principles. The bounded region R has the same limits of integration as those used for volume. f(x,y) is dilated to af(bx,cy).

$$A = \int_{-\frac{1}{c}}^{\frac{1}{c}} \int_{-\frac{1}{b}\sqrt{1-c^2y^2}}^{\frac{1}{b}\sqrt{1-c^2y^2}} \sqrt{1 + \left[\frac{\partial}{\partial x}\big(af(bx, cy)\big)\right]^2 + \left[\frac{\partial}{\partial y}\big(af(bx, cy)\big)\right]^2}\, dx\, dy$$

a is a constant, and so it can be factored out of the partial derivative. To simplify the derivative further, I am going to need to use the chain rule. Using $u = bx$ and $v = cy$ I can rewrite $\frac{\partial}{\partial x}\big(f(bx, cy)\big)$ as $\frac{\partial}{\partial x}\big(f(u, v)\big)$. The chain rule applied to multivariable functions is as follows (Imperial College, 2005).

$$\frac{\partial f}{\partial x} = \frac{\partial f}{\partial u}\frac{\partial u}{\partial x} + \frac{\partial f}{\partial v}\frac{\partial v}{\partial x}$$

$\frac{\partial v}{\partial x} = 0$ since y is taken as a constant for the partial derivative with respect to x. Therefore $\frac{\partial f}{\partial x} = \frac{\partial f}{\partial u}b$. Similarly, it can be shown that $\frac{\partial f}{\partial y} = \frac{\partial f}{\partial v}c$. So the area becomes:

$$A = \int_{-\frac{1}{c}}^{\frac{1}{c}} \int_{-\frac{1}{b}\sqrt{1-c^2y^2}}^{\frac{1}{b}\sqrt{1-c^2y^2}} \sqrt{1 + [abf_x(bx, cy)]^2 + \left[acf_y(bx, cy)\right]^2}\, dx\, dy$$

I can use a substitution to simplify the integrals even further:

$u = bx, \frac{du}{dx} = b$

If $x = \pm\frac{1}{b}\sqrt{1 - c^2y^2}, u = \pm\sqrt{1 - c^2y^2}$

$$A = \frac{1}{b}\int_{-\frac{1}{c}}^{\frac{1}{c}} \int_{-\sqrt{1-c^2y^2}}^{\sqrt{1-c^2y^2}} \sqrt{1 + [abf_x(u, cy)]^2 + \left[acf_y(u, cy)\right]^2}\, du\, dy$$

If $n = cy, \frac{dn}{dy} = c$

If $y = \pm\frac{1}{c}, n = \pm 1$

$$A = \frac{1}{bc}\int_{-1}^{1} \int_{-\sqrt{1-n^2}}^{\sqrt{1-n^2}} \sqrt{1 + [abf_x(u, n)]^2 + \left[acf_y(u, n)\right]^2}\, du\, dn$$

$$= \frac{1}{bc}\int_{-1}^{1} \int_{-\sqrt{1-y^2}}^{\sqrt{1-y^2}} \sqrt{1 + [abf_x(x, y)]^2 + \left[acf_y(x, y)\right]^2}\, dx\, dy$$

This integral is too complex to solve without a numerical method. Desmos could not compute the integrals, likely due to the fact that the square root causes a lot of gaps in the integrand function. So, to integrate the function, I had to use a python library, Scipy (code in appendix), which has a function called dblquad that allowed me to solve a double integral using an interval subdivision method that allows it to overcome the holes (Oliphant & Woods, 2001).

Table 6: Calculated surface areas of the circular RBC.

Half Width (μm)	Surface Area (μm^2)
3.91	135.98080
3.5	124.67401
3	111.11764
2.5	97.89597
2	85.13708
1.5	73.05222

The surface area for a typical RBC calculated using f(x) in section 4.3 is 135.98080. The python code obtains the same value when rounded, which shows that it is very accurate.

6 Conclusion

6.1 Circular RBC Conclusions

Using the formula derived in section 2, $C_{RBC}(t) = C_{Surroundings} - (C_{Surroundings} - C_0)\left(e^{-\frac{DA}{Vd}t}\right)$, as well as the constants obtained, $C_{Surroundings} \approx 32.39547$ molecules m^{-3}, $C_0 \approx 12.45980$ molecules m^{-3}, $D = 1.62 \times 10^3\ \mu m^2 s^{-1}$, and $d = 7.5\ \mu m$, graphs can be created for each dilated RBC function using the calculated volume and area values. As explained, this equation shows that having a larger surface area to volume ratio leads to faster diffusion.

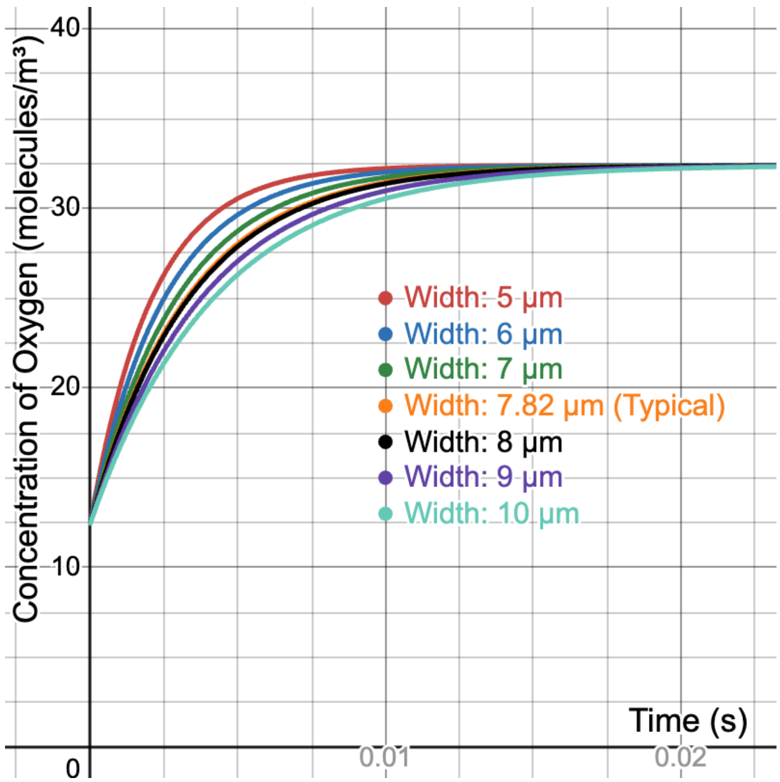


Figure 8: Concentrations of oxygen over time for the circular RBC of various widths (Created in Desmos).

Figure 8 shows the concentrations of the oxygen in the circular RBC over time. All the curves approach the same horizontal asymptote, $y = C_{Surroundings}$, since the surrounding concentration is the same. Also, all curves have the same y-intercept, $y = C_0$, since I am assuming the same initial concentration. The smaller circular RBC gain a higher concentration of oxygen over time than larger circular RBC. Even more, the curves appear to diverge slightly more for smaller RBC indicating that decreasing their size may lead to an exponential increase in the rate of diffusion.

This can be explained by the surface area to volume ratios for the circular RBC, as seen in figure 9.

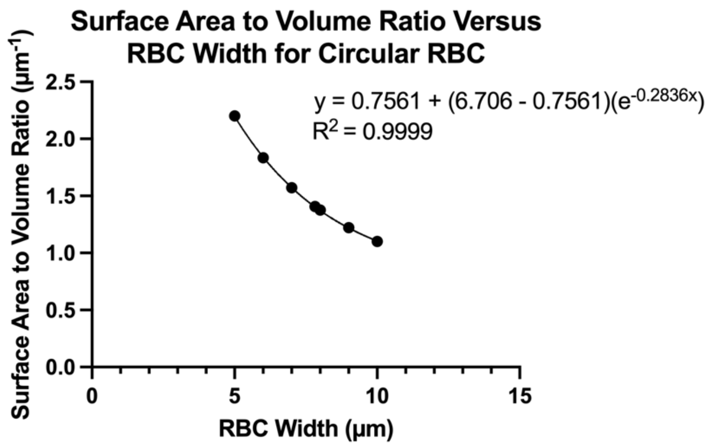


Figure 9: Surface area to volume ratios versus RBC width for the circular RBC (Created in GraphPad Prism).

For smaller circular RBC, the surface area to volume ratio increases exponentially (going from right to left) which explains the diverging of the curves from figure 8.

The graph of figure 8 is slightly misleading because it does not show the actual number of oxygen molecules that the RBC hold, rather the concentrations. By multiplying the concentration function by the volumes, the number of molecules in the RBC can be found, $\mathrm{N_{RBC}(t)} = \mathrm{V}\left[\mathrm{C_{Surroundings}} - (\mathrm{C_{Surroundings}} - \mathrm{C_0})\left(\mathrm{e}^{-\frac{\mathrm{DA}}{\mathrm{Vd}}\mathrm{t}}\right)\right]$, but the volumes need to be converted to m^3 by multiplying them by $(10^{-6})^3 = 10^{-18}$. This is done in figure 10.

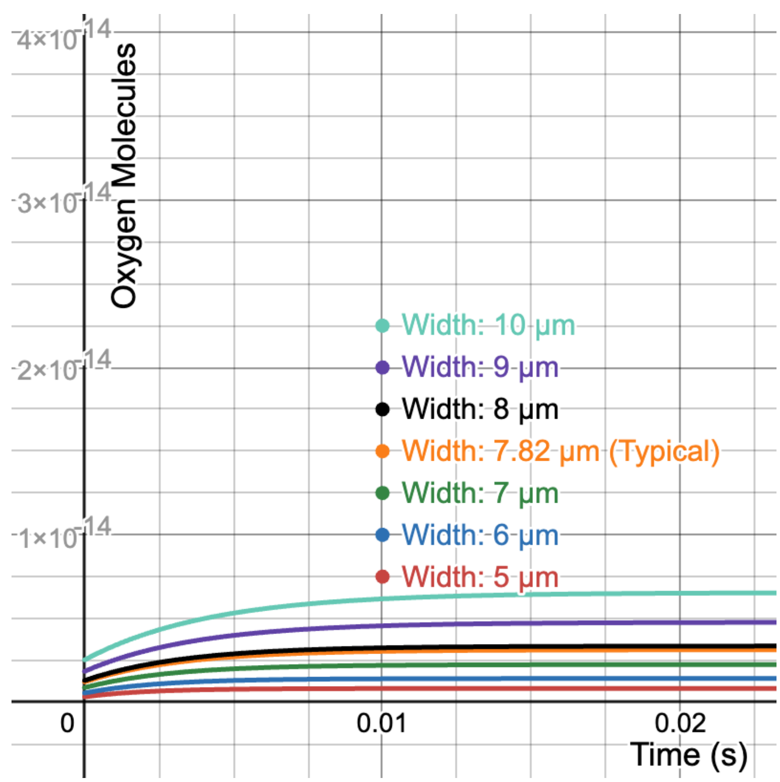


Figure 10: Number of oxygen molecules over time for the circular RBC of various widths (Created in Desmos).

Now, the trend reverses with larger circular RBC gaining more oxygen molecules through diffusion in a given time period despite having a slower rate of diffusion in terms of concentration. Also, all the lines approach different horizontal asymptotes and have different y-intercepts because although they have the same initial and final concentrations, they have a different number of oxygen molecules as a result of their various volumes. The lines diverge for larger circular RBC indicating that the number of oxygen molecules an RBC gains over time likely increases exponentially with volume.

In reality however, abnormally large RBC are associated with a lower RBC count as well as diseases or vitamin deficiencies that come with many negative health effects (National Library of Medicine, 2024).

6.2 Elliptical (Elongated) RBC Conclusions

Doing the same but for the elongated RBC of different widths, the curves in figure 11 are obtained.

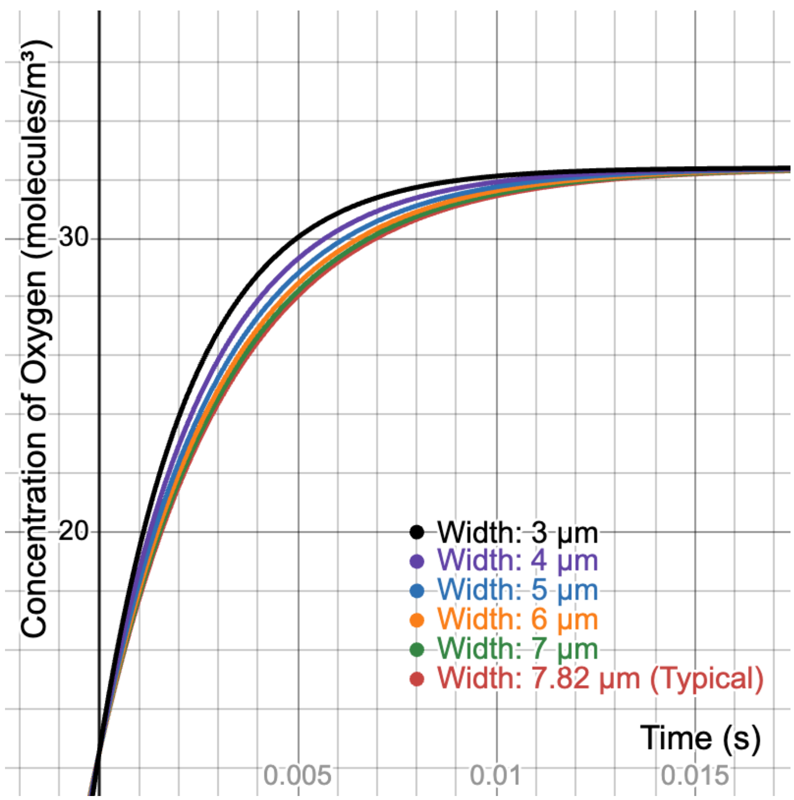


Figure 11: Concentrations over time for the elongated RBC of various widths (Created in Desmos).

The typical RBC, of width 7.82 µm, has the lowest rate of diffusion and the most elongated RBC has the highest rate of diffusion, at least in terms of concentration. Here, the lines are getting much further apart with smaller widths, it is much more exaggerated than for the circular RBC; this shows that diffusion in terms of concentration probably grows exponentially as the RBC shape becomes more elongated.

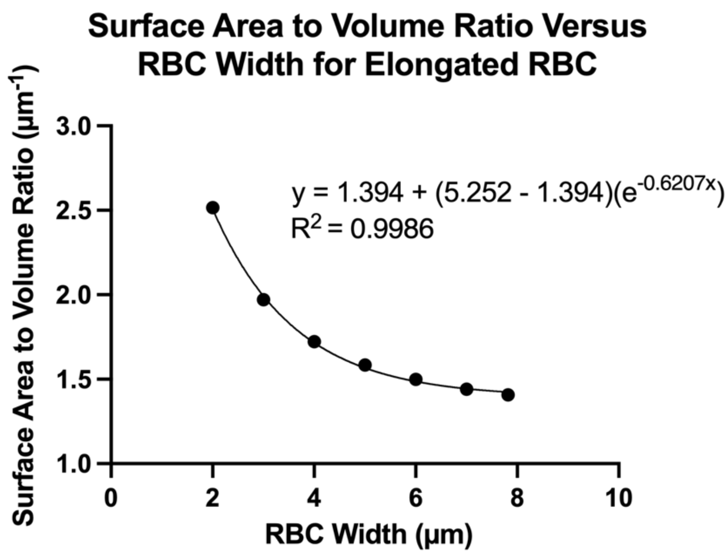


Figure 12: Surface area to volume ratios versus RBC width for the elongated RBC (Created in GraphPad Prism).

From figure 12, as an RBC becomes elongated (the width become smaller) the surface area to volume ratio increases exponentially, even more than for changing the size of the circular RBC (figure 9). This explains the diverging of the curves in figure 11: since the surface area to volume ratios increase exponentially for smaller widths, so does diffusion in terms of concentration.

Like before, by graphing the number of molecules rather than concentration, the trend reverses.

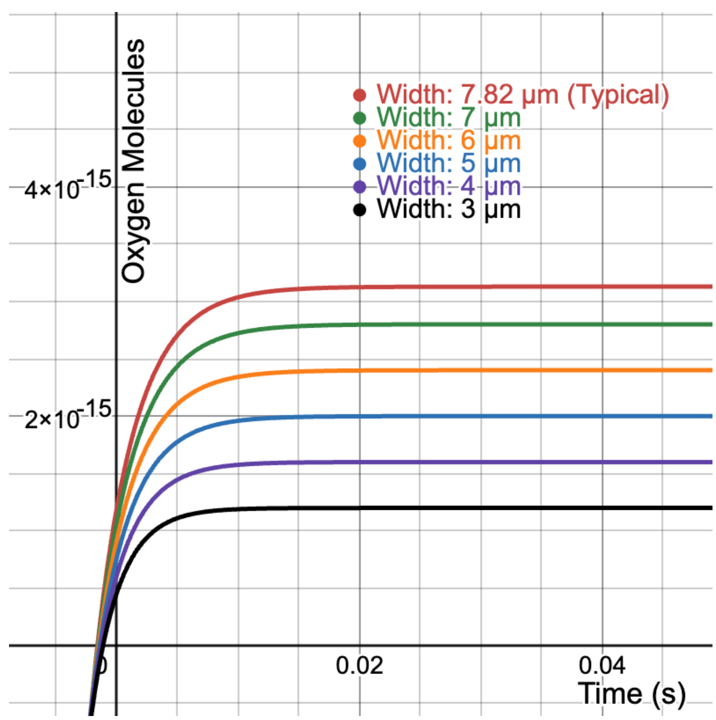


Figure 13: Number of oxygen molecules over time for the elongated RBC of various widths (Created in Desmos).

Figure 13 shows that the typical RBC obtains more oxygen molecules for all times. Interestingly, these curves are evenly spaced, except for the typical RBC but it changes by an uneven increment. This is the result of the volumes changing linearly with RBC width. As determined in section 5.2, half the RBC width, V, is given by $\mathrm{V} = \frac{\mathrm{a}}{\mathrm{bc}}\mathrm{V}_0$. a and b are constant for all the elongated RBC. c was calculated with $\mathrm{c} = \frac{1}{\text{half width}}$ (section 5.1). Therefore, V is directly proportional to c and the asymptotes are all at locations given by $\mathrm{V} \times \mathrm{C}_{\mathrm{Surroundings}}$. That being said, the lines appear to get closer for less elongated RBC during the initial curved portion of the graph.

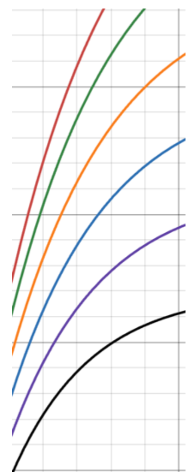


Figure 14: Zoomed in portion of the graph in figure 13.

Therefore, the benefit of the increased volume (which allows for more oxygen molecules to be transferred in a given time) appears to exponentially decay in the initial period before the asymptote. During the asymptote period, when the RBC become full of oxygen molecules, less elongated RBC gain a linear advantage in terms of maximum carrying capacity.

To summarize from figures 11 and 13, elongated RBC have faster diffusion due to a higher surface area to volume ratio, but since their volume is smaller, they gain less oxygen molecules over time. The trend in figure 11 (the graph of concentration) shows that diffusion increases exponentially to elongation as the curves diverge. On the other hand, for the number of molecules in figure 13, it increases linearly after the initial period and during the initial period it exponentially decays with less elongated RBC (figure 14).

7 Evaluation

Overall, the function used to model the RBC is an excellent fit as seen in figure 5, with an incredibly high R^2 correlation of 0.9957. Furthermore, the surface area and volume of the typical RBC are very close to the literature values. The volume found was $96.640\ \mu\mathrm{m}^3$ and the surface area was $135.981\ \mu\mathrm{m}^2$. The literature values are $94 \pm 14\ \mu\mathrm{m}^3$ and $135 \pm 16\ \mu\mathrm{m}^2$ respectively.

The weaknesses of this investigation lie in the underlying assumptions made. The assumptions made in developing the relationships and model are as follows.

- $C_{Surroundings}$ is constant.
- The oxygen molecules in the surroundings and inside the RBC act as an ideal gas.
- Temperature of surroundings and RBC is 37 degrees.
- Volume and area stay constant – no deformation of the RBC.
- Circular RBC maintain the same width to height proportions for all sizes.
- By dilating the functions f(x) and f(x,y), the shape of the RBC is not modified.
- The biconcavity of the RBC still holds as it is elongated.
- The entire RBC volume is used for diffusion.

The assumption that is likely to cause the greatest inaccuracy is the last one. In reality, not all RBC volume is used to store and transport oxygen. The proportion of an RBC's volume used for diffusion also varies with its size. Larger RBC, macrocytic anemia, have been associated with lower levels of hemoglobin - the protein that carries oxygen (Moore & Adil, 2022). Therefore, while it may seem like the volume used for diffusion increases with larger RBC, it may actually be the opposite.

References

An, L., Ji, F., Yin, Y., Liu, Y., & Zhou, C. (2022, December 9). *Modeling of Red Blood Cells in Capillary Flow Using Fluid–Structure Interaction and Gas Diffusion*. MDPI. https://www.mdpi.com/2073-4409/11/24/3987

bprp calculus basics. (2022, May 18). *why “dummy variable” of a definite integral doesn’t matter*. YouTube. https://www.youtube.com/watch?v=PeXyrknSXfU&ab_channel=bprpcalculusbasics

Evans, E., & Fung, Y.-C. (1972, October). *Improved measurements of the erythrocyte geometry*. ScienceDirect. https://www.sciencedirect.com/science/article/abs/pii/0026286272900696?fr=RR-2&ref=pdf_download&rr=91f50e47dfc1aac2

Faghih, M. M., & Sharp, M. M. and prediction of flow-induced hemolysis: a review. (2019, March 7). *Modeling and prediction of flow-induced hemolysis: a review*. Springer Nature Link. https://link.springer.com/article/10.1007/s10237-019-01137-1

Goldstick, T. K., Ciuryla, V. T., & Zuckerman, L. (1976). *Diffusion of oxygen in plasma and blood*. Advances in experimental medicine and biology. https://pubmed.ncbi.nlm.nih.gov/1015403/#:~:text=The%20average%20values%20for%20D,5)%20cm2/sec%20respectively

Hartman, G., et al. (n.d.). *13.5: Surface Area*. Mathematics LibreTexts. https://math.libretexts.org/Bookshelves/Calculus/Calculus_3e_(Apex)/13%3A_Multiple_Integration/13.05%3A_Surface_Area

Houston Math Prep. (2020, April 24). *Double Integrals in Polar Coordinates*. YouTube. https://www.youtube.com/watch?v=PeeC_rWbios&ab_channel=HoustonMathPrep

Imperial College. (2005, January 2). *THE CHAIN RULE IN PARTIAL DIFFERENTIATION*. Department of Mathematics, Imperial College. https://www.ma.imperial.ac.uk/~jdg/AECHAIN.PDF

Khan Academy. (n.d.). *Gases: Fick’s law and oxygen diffusion in salmon*. Khan Academy. https://www.khanacademy.org/test-prep/mcat/physical-sciences-practice/x04f6bc56:foundation-4-physical-processes/e/biological-adaptations-in-response-to-physical-constraints--the-case-of-atlantic-salmon

LibreTexts. (n.d.). *9: Diffusion*. Chemistry LibreTexts. https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Kinetics/09%3A_Diffusion

London's Global University. (2016, October 28). *Partial Differentiation*. London's Global University. https://www.ucl.ac.uk/~zcahge7/files/week4.pdf

Lumen Learning. (n.d.). *Gas Exchange*. Courses Lumen Learning. https://courses.lumenlearning.com/suny-ap2/chapter/gas-exchange/#:~:text=The%20partial%20pressure%20of%20oxygen%20in%20alveolar%20air%20is%20about,in%20the%20alveoli%20remains%20high

Lynch, E. C. (1990). *Chapter 155 Peripheral Blood Smear*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK263/

Math with Professor V. (2020b, September 25). *Calculus 2: Area of a Surface of Revolution (Video #9) | Math with Professor V*. YouTube. https://www.youtube.com/watch?v=mKNBnwnwAkw&ab_channel=MathwithProfessorV

Moore, C. A., & Adil, A. (2022, July 11). *Macrocytic Anemia*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK459295/#:~:text=There%20are%20many%20etiologies%20for%20macrocytic%20anemia%2C,treatable%20and%20some%20that%20that%20are%20life%2Dthreatening.&text=Macrocytic%20anemia%20that%20is%20megaloblastic%20contains%20hypersegmented,peripheral%20blood%20smear%20(PBS)%20(figure%20%22macrocytic%20anemia%22)

National Library of Medicine. (2024, December 9). *Thiamine-responsive megaloblastic anemia syndrome*. MedlinePlus. https://medlineplus.gov/genetics/condition/thiamine-responsive-megaloblastic-anemia-syndrome/

Nayanajith, P. G. H., Gu, Y. T., Saha, S. C., Senadeera, w, & Oloyede, A. (2012, November 25). *Numerical Simulation of Red Blood Cells' Motion, A Review*. ResearchGate. https://www.researchgate.net/publication/235631724_Numerical_simulation_of_red_blood_cells'_motion_a_review

Oliphant, T., & Woods, N. (2001). *_quadpack_py.py*. GitHub Scipy. https://github.com/scipy/scipy/blob/main/scipy/integrate/_quadpack_py.py

Quigley, M., Linfesty, R. L., Bethel, K., & Sharpe, R. (2007, March 15). *Stubby elliptocytes are an invariable feature of leukoerythroblastosis*. American Society of Hematology Publications. https://ashpublications.org/blood/article/109/6/2666/23319/Stubby-elliptocytes-are-an-invariable-feature-of

Rhodes, C. E., Denault, D., & Varacallo, M. A. (2022, November 14). *Physiology, Oxygen Transport*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK538336/

Simon Fraser University. (n.d.-a). *Calculus Early Transcendentals: Integral & Multi-Variable Calculus for Social Sciences*. Simon Fraser University. https://www.sfu.ca/math-

coursenotes/Math%20158%20Course%20Notes/sec_first_order_differential_equations.htm
l

Simon Fraser University. (n.d.-b). *Calculus Early Transcendentals: Integral & Multi-Variable Calculus for Social Sciences*. Simon Fraser University. https://www.sfu.ca/math-coursenotes/Math%20158%20Course%20Notes/sec_VolumeAvgHeight.html

University of Cambridge. (2020, January 15). *Numerical Analysis – Lecture 1*. Department of Applied Mathematics and Theoretical Physics, University of Cambridge. https://www.damtp.cam.ac.uk/user/hf323/L20-IB-NA/Lect01.pdf

University of Maryland. (2017, September 16). *Lecture 17 : Double Integrals*. Department of Mathematics, University of Maryland. https://www.math.umd.edu/~millson/teaching/STAT400fall18/slides/article17.pdf

Yew, A. (2011, April 21). *Numerical differentiation: finite differences*. Department of Mathematics, Brown University. https://www.dam.brown.edu/people/alcyew/handouts/numdiff.pdf

Appendix

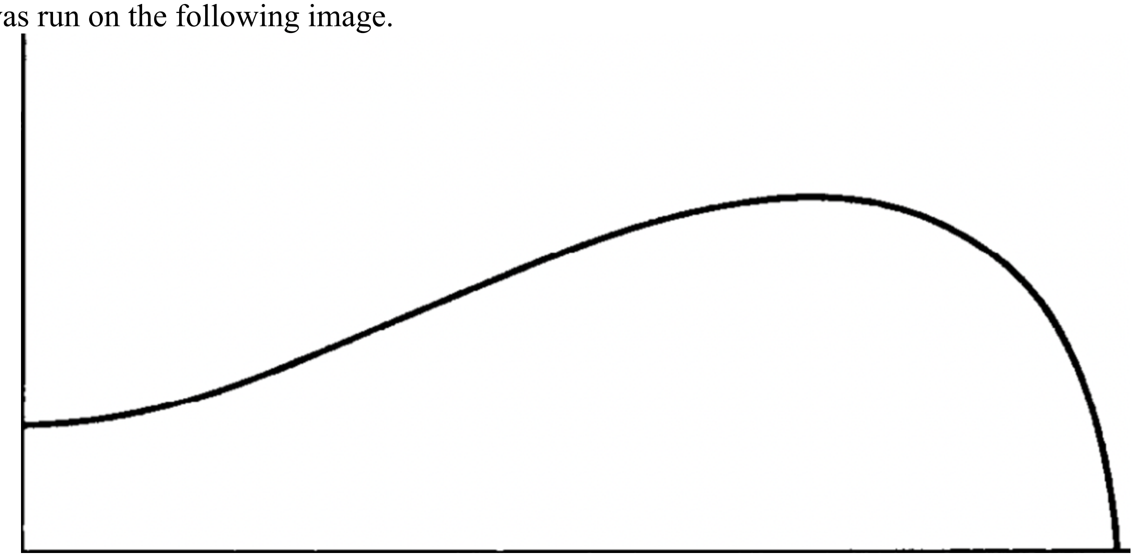
Appendix A

The following is the Python code I used to find the points of an RBC.

```python
import cv2
import numpy as np
import pandas as pd
import matplotlib.pyplot as plt

def extract_contour_points(image_path, width):
    # Load the image
    image = cv2.imread(image_path)

    # Convert to grayscale
    gray = cv2.cvtColor(image, cv2.COLOR_BGR2GRAY)
    binary = cv2.inRange(gray, 50, 255)

    # Find contours
    contours, _ = cv2.findContours(binary, cv2.RETR_EXTERNAL,
cv2.CHAIN_APPROX_NONE)
    contour_image = np.zeros_like(binary)
    cv2.drawContours(contour_image, contours, 0, (255, 255, 255), 2)

    # Extract the largest contour
    contour = contours[0]
    contour_points = contour.squeeze() # Extract points

    image_height = binary.shape[0]
    flipped_points = [(x, image_height - y) for x, y in contour_points]

    # Convert to DataFrame
    df = pd.DataFrame(flipped_points, columns=['X (µm)', 'Y (µm)'])
    df.to_csv("scaled_contour_points.csv", index=False)

    # Removing points drawn as a border to the image. The bounds were
found by first plotting the points without this step.
    x_min1, x_max1, y_min1, y_max1 = 0, 7.5, 0, 177.5
    x_min2, x_max2, y_min2, y_max2 = 0, 1550.5, 0, 9

    df_filtered = df[
        ~(
            (df['X (µm)'].between(x_min1, x_max1) & df['Y
(µm)'].between(y_min1, y_max1)) |
            (df['X (µm)'].between(x_min2, x_max2) & df['Y
(µm)'].between(y_min2, y_max2))
        )
    ]

    # Scaling to have a half width of 1 µm. It does not really matter what
value is as the function is dilated anyways.
    scale_factor = width / (df_filtered["X (µm)"].max() - df_filtered["X
(µm)"].min())
```

```
    df_filtered["Y (µm)"] = df_filtered["Y (µm)"] * scale_factor
    df_filtered["X (µm)"] = df_filtered["X (µm)"] * scale_factor # Scaling proportionally


    # Shift x values so the points start on the y-axis
    df_filtered["X (µm)"] -= df_filtered["X (µm)"].min()

    # Shift y values so the points start on the x-axis
    df_filtered["Y (µm)"] -= df_filtered["Y (µm)"].min()

    # Save to CSV
    df_filtered.to_csv("scaled_contour_points.csv", index=False)

image_path = "imageName.png"  # Update with the correct path
width = 1 # µm
extract_contour_points(image_path, width)
```

It was run on the following image.



Appendix B

This is the Python code I used to calculate the double integrals for surface area.

```
import scipy.integrate as si
import numpy as np

a=3.96825396825
b=(1/3.91)
c=(1/3.91) # Replace 3.91 with the half width

def f(x, y):
    r = np.sqrt(x**2 + y**2)
    return (0.11786 + 0.873326 * r**2 - 0.380179 * r**4) * np.sqrt(1 -
r**2)

# Compute numerical derivatives
def df_dx(x, y, h=1e-7):
    return (-f(x + 2*h, y) + 8*f(x + h, y) - 8*f(x - h, y) + f(x - 2*h,
y)) / (12 * h)

def df_dy(x, y, h=1e-7):
    return (-f(x, y + 2*h) + 8*f(x, y + h) - 8*f(x, y - h) + f(x, y -
2*h)) / (12 * h)

# Define the integrand
def integrand_cartesian(x, y):
    return np.sqrt(1 + (a*b*(df_dx(x, y)))**2 + (a*c*(df_dy(x, y)))**2)

# Computing the integral
surface_area, error = si.dblquad(integrand_cartesian, -1, 1, lambda y: -
np.sqrt(1 - y**2), lambda y: np.sqrt(1 - y**2), epsabs=1e-5, epsrel=1e-5)

# Print the result
print(surface_area * 2 * (1/(b*c))) # Multiply by 2 since the function
represents half of an RBC
```

To calculate the partial derivatives, I used the fourth order formulas which I found gave more precise answers based on the volume of the typical RBC (half width 3.91 μm) calculated in section 4.3 (Yew, 2011).