NSCM005 - Mathematical Modelling in Biology and Medicine
Assessed Coursework 1

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Numerically Modelling Phagocytosis Using Diffusion

1 An Introduction to Phagocytosis

Phagocytosis is the process by which certain living cells called phagocytes ingest or engulf other cells or particles. The phagocyte may be a free-living one-celled organism, such as an amoeba, or one of the body cells, such as a white blood cell. In some forms of animal life, such as amoebas and sponges, phagocytosis is a means of feeding. In higher animals phagocytosis is chiefly a defensive reaction against infection and invasion of the body by foreign substances. [Phaa]

1.1 The Discovery of Phagocytes

The Russian zoologist Ilya Ilyich Mechnikov was among the first to identify that specialised cells played a vital role in the defense against microbial infections [Kau19]. In 1882 he studied motile (freely moving) cells in the larvae of starfish, hypothesising that they played a vital role in the animals immune system. To test his hypothesis, he inserted small thorns into the larvae; after several hours he noticed that the motile cells had surrounded the thorns [Kau19]. He shared his findings with Carl Friedrich Claus, who suggested the name "phagocyte" (from the Greek words "phagein", meaning "to eat or devour" and "kutos" meaning "hollow vessel" [Dic83]) for the cells that Mechnikov had observed [Ate98]. Over the next few decades Mechnikov continued studying phagocytes and phagocytosis for which he was jointly awarded the 1908 Nobel peace prize in Physiology and Medicine with Paul Ehrlich [FCSG08].

1.2 Biology of Phagocytosis

Phagocytosis is a form of endocytosis, which is a process of actively transporting material from outside a cell to the inside; this is opposite of exocytosis which involves actively transporting material from the inside of the cell to the outside. The significant difference between this active form of transport and passive forms, such as osmosis and diffusion, is active transport pumps material against the concentration gradient using Adenosine triphosphate, (ATP) (energy) whilst passive moves along the the concentration gradient, using ATP (energy). The combination of both active and passive transport allow a cell to move material along or against the concentration gradient which allows the cell more control of the materials that pass in and out of its membrane.

1.2.1 Endocytosis

Endocytosis involves first reshaping the cell membrane to form a cup-shaped structure that engulfs the external material, this cup gradually pinches off to form a internal membrane-bound compartment called a vesicle. This process occurs in a number of different forms, based on vesicle size, cell membrane behaviour and proteins involved but is typically split into four different types [RE17a]. The first two: clathrin-mediated and caveolar-type endocytosis involve relatively small vesicles, typically less than $100nm$. The third type, macropinocytosis, is used for internalising fluid and so is often called 'cellular drinking'. The final type is phagocytosis, which involves internalisation of relatively large solid particles, above $0.5nm$ and up to $20nm$ or even larger.

1.2.2 Steps involved in Phagocytosis

The first stage of phagocytosis is the activation of the phagocyte which is triggered by inflammatory mediators such as bacterial products, complement proteins or inflammatory cytokines. As a result, resting phagocytes produce pattern-recognition receptors that recognize and bind to pathogen-associated molecular patterns either directly or via opsonins. With these receptors active, the chemotaxis of the phagocyte begins. This is the movement of the phagocyte towards an increasing concentration of the activation attractant, until the receptors are able to bind to the target molecule. Figure 1 shows at (A)(B) the binding of receptors on the phagocyte cell membranes to the distinct molecular patterns on the surfaces of the target particles. (C) Binding is followed by actin polymerization at the site of ingestion, which causes extensive deformation of the plasma membrane into pseudopodial extensions. (D) The pseudopodia surrounds the particle completely so that the particle is engulfed in a cup-shaped structure, called the phagocytic cup. Once the particle has been internalized, actin filaments begin depolymerizing from the base of the phagocytic cup, causing the cup to close into a membrane-bound vacuole called the phagosome. Thereafter, the phagosome undergoes maturation, through a series of membrane fusion and fission events with the endosomal compartments, until it fuses with the lysosomes to form a microbicidal phagolysosome. [Phab]

1.3 Phagocytosis in the Immune System

Phagocytosis is a vital mechanism in both the innate immune system, which is responsible for providing an immediate, non-specific response to infections and also in the adaptive immune system, which provides a highly specific but slower response. Although the majority of cells can perform phagocytosis, certain cells perform it as part of their main function; these cells are called 'professional phagocytes'.

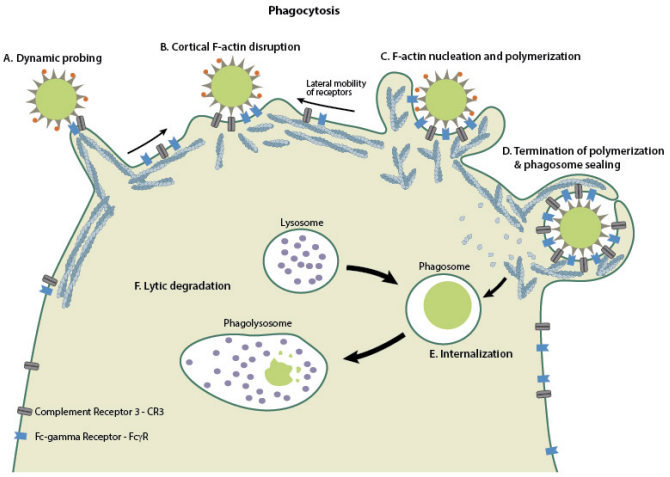


Figure 1: A Schematic depicting the mechanisms behind phagocytosis. [Phab]

1.3.1 Innate immune system

In the innate immune system phagocytosis is one of the first processes responding to infection. Neutrophils, macrophages and monocytes can all be classified as professional phagocytes [Gor16], and play the greatest role in innate immune response to most infections [Mur11]. Neutrophils patrol the bloodstream ready to rapidly migrate to tissues in case of an infection [Mur11]. There they directly kill microscopic organisms, such as bacteria, fungi and viruses through phagocytosis. Macrophages are resident in almost all tissues and are the mature form of monocytes, which circulate in blood and continually migrate into tissues, where they differentiate. Here they form a resting barrier against pathogens through phagocytosis [Mur11].

1.3.2 Adaptive immune system

In the adaptive immune system, professional phagocytes such as dentritic cells reside in tissues and use phagocytosis not as a means to ingest pathogens, but not to kill the microbes, but rather break them down for antigen presentation [Mur11]. This antigen material is presented to T cells and B cells to initiate and shape the adaptive immune response.

2 Modelling Phagocytosis

Here we refer to modelling as the process of modelling a process or mechanism through the use of mathematics and our understanding of the physical concepts involved. There are various methods one could take when building mathematical model, one approach is to include every element of the process; a downside to this is the model can become overly complex. A more common approach is to simplify the process which can reduce the accuracy but if done correctly this accuracy lose is negligible and meaningful insights can still be extracted from the models.

Since phagocytosis is such a vital mechanism in our immune system, developing models that help us understand this complex mechanism allows us to better understand our immune system. One hope is that this increase in knowledge improves our ability to fight and cure infections and viruses.

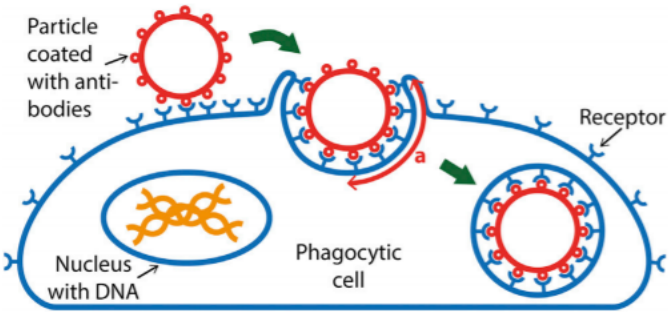


Figure 2: A Schematic depicting the stages of phagocytosis, putting emphasis on the receptor process and visually representing cup size, a. [RE17a]

2.1 Methods used to Model Phagocytosis

Since phagocytosis is such a complex process involving numerous different steps including remodelling of the membrane, the cytoskeleton and intracellular signalling there are different models that focus on different processes. Models from recent work include models which focus on the motion of the receptors, models that examine the fundamental types of forces involved, and models that determine the membrane shape by minimising some energy functional [RE17b]. In this report we will be investigating the models explored by Richards et al. in their report titled 'The Mechanism of Phagocytosis: Two Stages of Engulfment' [RE17a]. In this paper they focus on the movement of the $F_{c\gamma}$ receptors, which recognise particles coated in immunoglobulin. $F_{c\gamma}$ receptors are expressed in white blood cells in four different classes, distinguished by their antibody affinity

2.1.1 Modelling receptor motion

There is an elegant approach which maps the motion of receptors within the membrane to the Stefan problem, which was popularised by Gao et al. [GSF05] when modelling the related problem of endocytosis. The Stefan problem with the archetypal example of melting ice, where the boundary between water and ice continually moves as more ice melts. This is analogues to the cup boundary during engulfment, which increases as receptors move towards the cup, through the process of diffusion. Phagocytosis is considered a more active and controlled process than endocytosis but does share similarities with endocytosis and thus this is an appropriate approach; this gives motivation for the pure diffusion model.

2.1.2 Pure diffusion model

We begin by defining engulfment, a as the arc length from the center of the cup to edge of the cup (cup size), see Figure 2. The bead being engulfed is set to be spherically symmetric with radius R, then engulfment starts from $a = 0$ before phagocytosis begins and monotonically increases to $a = \pi R$ (half the circumference of the bead) upon full engulfment. We consider a circularly-symmetric two-dimensional membrane that first touches the bead at the origin, that is $a = 0$ at time, $t = 0$ and in the next instant engulfment begins.

Due to the assumed circular symmetry, the problem of engulfment can be reduced to receptors moving on a semi-infinite one dimensional line. We paramaterize this line by the distance from the origin, r (with the origin being the point at which the cell first touches the bead), and the receptor density $\rho(r, t)$. Before contact with any bead, the receptor density is independent of r and is given by ρ_0. During engulfment, the receptor distribution is no longer constant, although density is always ρ_0 at infinity. Within the engulfed region (where the distance from the origin is less than the cup size, $r < a$), receptors are attached to the ligands on the bead. As such the receptor density is said to be related to the density on the bead, and thus we assume that ρ in this region is constant and given by ρ_L, where $\rho_L > \rho_0$. We denote the receptor density at the cup edge, $\rho(a(t), t)$, by $p_+(t)$; this variables is also constant.

There are various possible physical processes for the motion of the receptors in the non-engulfed region of the membrane (r≥a). The first we consider is a purely passive process, where receptors move around the membrane by diffusion [GSF05]. Diffusion of ρ can be described by $\partial p/\partial t = D\nabla^2\rho$, where D is the diffusion constant and ∇^2 is the radial part of the Laplacian in cylindrical coordinates. We can described the evolution of a by considering the conservation of receptor property. Receptors move from outside to inside the cup region with flux $-D\rho'_+$, where ρ' is the partial derivative of ρ with respect to r, evaluated at $r = a$. This flux increases the density of receptors in the boundary region from ρ_+ to ρ_L, from which the rate of change of a follows. Hence, our pure diffusion model, for the non-engulfed region of the membrane (r $\geq a$) is identical to the super-cooled one-dimensional Stefan problem;

$$\frac{\partial \rho}{\partial t} = \frac{D}{r}\frac{\partial}{\partial r}\left(r\frac{\partial \rho}{\partial r}\right) \tag{1}$$

$$\frac{da}{dt} = \frac{D\rho'_+}{\rho_L - \rho_+} \tag{2}$$

With initial conditions $\rho(r, 0) = \rho_0$ and $a(0) = 0$.

To find a solution to this system, we need in addition to the initial conditions and the behaviour of ρ at $r = \infty$, to specify one extra condition. We find this extra condition by considering the free energy, in particular by requiring that there is no free-energy jump across the boundary $r = a$. This indicates that all energy from receptor-ligand binding and configurational receptor entropy is used for bending the membrane and engulfment. We then consider three contributions to the free energy

1. The binding between receptors and ligands - This reduces the energy. If each receptor-ligand bond decreases the energy by $-\mathcal{E}k_BT$, then the total binding energy, given that binding only occurs in the bound region ($\rho = \rho_L$), is given by $-\pi a^2\rho_L\mathcal{E}k_BT$. Where $\mathcal{E}$ is the binding energy per receptor-ligand bond, and

2. The curvature of the membrane - The bending energy per unit area is given by $\frac{1}{2}(2H)^2\mathcal{B}k_BT$. Where $\mathcal{B}K_BT$ is the elastic bending modulus, and H is the mean curvature (for a spherical bead, $H = 1/R$). The total bending energy is then $2\pi a^2\mathcal{B}k_BT/R^2$.

3. The receptor entropy - The entropy per receptor (relative to the initial entropy when $\rho = \rho_0$ everywhere) is $-k\ln\frac{\rho}{\rho_0}$, which means that free energy due to entropy is $k_BT\int\rho\ln\frac{\rho}{\rho_0}\,dA$, where the area integral is taken over all space.

k_p is the radius of curvature of the bead (for a spherical bead of radius R, $k_p = 2/R$.
With this interpretation we can write the free energy, $\mathcal{F}$ can be written as

$$\frac{\mathcal{F}(t)}{2\pi k_BT} = \left(-\frac{1}{2}\rho_L\mathcal{E} + \frac{\mathcal{B}}{R^2} + \frac{1}{2}\rho_L\ln\frac{\rho_L}{\rho_0}\right)a^2 + \int_a^\infty \rho\ln\frac{\rho}{\rho_0}r\,dr \tag{3}$$

It follows, requiring no free-energy jump across the cup boundary implies that

$$\frac{\rho_+}{\rho_L} - \ln\frac{\rho_+}{\rho_L} = \mathcal{E} - \frac{2\mathcal{B}}{R^2\rho_L} + 1 \tag{4}$$

From which it follows that ρ_+, the receptor density at the cup edge, is a constant, independent of time. The (numerical) solution for ρ_+ gives the extra condition needed to uniquely solve the system Equation 13 and Equation 14.

This solution is given by

$$\rho(r,t) = \begin{cases} \rho_L & \text{for } r < a \\ \rho_0 - AE_1\left(\frac{r^2}{4Dt}\right) & \text{for } r \geq a \end{cases} \tag{5}$$

$$a(t) = 2\alpha\sqrt{Dt} \tag{6}$$

Where

$$E_1(x) = \int_x^\infty \frac{e^{-u}}{u}\, du \tag{7}$$

and α can be found by solving (numerically)

$$\alpha^2 e^{\alpha^2} E_1(\alpha^2) = \frac{\rho_0 - \rho_+}{\rho_L - \rho_+} \tag{8}$$

After which A is given by $A = (\rho_0 - \rho_+)/E_1(\alpha^2)$.

This purely passive model compared to experimental data, and was found to closely resemble the first stage of phagocytosis, which is thought to mostly be controlled by receptor diffusion and capture. However, comparing to the second stage which is thought to be more active, we see that this model proves to be inaccurate.

Richard et al. [RE17a] over come this by next building a model which considers a purely drift model which is a purely active model, this is found to resemble the second, faster stage of engulfment. Finally they construct a model that uses both diffusion and drift that resembles both stages of the engulfment.

3 Solving the Pure Diffusion Model

Whilst we have provided an analytical solution to our model, it can often be the case that an analytical solution can not be reached or can be too complex. In these cases we can develop so called numerical schemes which provide a solution with a degree of error. In this section we will solve our model analytical and numerically.

We start by setting our parameters to the same values as the paper by Richard et al. [RE17a].

- $D = 1\ \mu m^2 s^{-1}$ diffusion constant
- $\rho_0 = 50\ \mu m^{-2}$, typical receptor density
- $\rho_L = 500\ \mu m^{-2}$, typical ligand density
- $\mathcal{E} = 15$, binding free energy ($15\ k_BT$)
- $\mathcal{B} = 20$, typical value of bending modulus ($20\ k_BT$)
- $R = 2\ \mu m$, radius of the bead
- $L = 50\ \mu m$, approximate circumference of our cells

3.1 Numerical Solution

For our numerical solution we start by splitting both our time, t and space, r variables into discrete, equally spaced elements. For time we construct an array from $t_0 = 0$ to $t_{N_1} = 60$ with each value equally spaced by $\Delta t = \frac{t_{N_1} - t_0}{N_1}$. We define $t_i = t_0 i\Delta t\ :\ i \in [0, N_1]$. Similarly we define $r_0 = 0$, $r_{N_2} = 50$ and $r_i = r_0 i\Delta r\ :\ i \in [0, N_2]$. For our solution we choose N_1 and N_2 such that $\Delta T = 0.01$ and $\Delta r = 2.5 \times 10^{-5}$. This allows us to solve ρ for discrete points, we thus define $\rho(i,j) = \rho(i\Delta r, j\Delta t)$ and $a(i) = a(i\Delta t)$

Now consider our system for $r \geq a$, Equation 13 and Equation 14. We see that both of these equation involve (partial) differentials, a common method to numerical solve differential equation is using 'Finite Difference Scheme'. This Scheme allows us to approximate differentials using Taylor's expansion. We first rewrite Equation 13 as

$$\frac{\partial\rho}{\partial t} = \frac{D}{r}\frac{\partial\rho}{\partial r} + D\frac{\partial^2\rho}{\partial r^2} \tag{9}$$

We then use the following finite difference schemes for the first and second order derivatives

$$\frac{\partial\rho}{\partial t} \approx \frac{\rho(x, t+\Delta t) - \rho(r,t)}{\Delta t} \qquad \frac{\partial^2\rho}{\partial r^2} \approx \frac{\rho(r-\Delta r, t) - 2\rho(r,t) + \rho(x+\Delta r, t)}{(\Delta r)^2} \tag{10}$$

To solve Equation 9, this produces the following numerical solution

$$\rho(r,t+1) = \rho(r,t) + \frac{D\Delta t}{(\Delta r)^2}\left[(\rho(r-1,t) - 2\rho(r,t) + \rho(r+1,t)) + \frac{\rho(r+1,t)-\rho(r,t)}{r}\right] \tag{11}$$

Similarly for Equation 14

$$a(t+1) = a(t) + \frac{D\rho'_+}{\rho_L - \rho_+} \tag{12}$$

Using these two schemes paired with what we know about the system behaviour when $r < a$ ($\rho = \rho_L$), the initial and boundary condition, we can work recursively to numerically ρ over our lattice $[t_0, t_{N_1}] \times [r_0, r_{N_2}]$.

3.2 Comparing Numerical and Analytical Solution

In Figure 3a we can see just how closely the numerical solution resembles the analytical solution provided by Richard et al. [RE17a]. It is worth noting that for the implementation of the analytical solution it was necessary to numerically solve both Equation 4 and Equation 8 so it is not technically a true analytical solution. In both cases we can see the expected $a \propto \sqrt(t)$. As mention previously this captures the first, slower stage of engulfment but not the second, faster stage.

3.3 Extending our Numerical Model

Since we are solving our model numerically, it is quite straight forward to make adjustments to our numerical scheme to solve a slightly different system of equations.

For instance if we consider replacing our diffusion constant, D with a diffusion equation that increases near the cup, i.e. $D = D_0 + D_1 e^{-r/\lambda}$, our sytem of equations then becomes

$$\frac{\partial \rho}{\partial t} = \frac{(D_0 + D_1 e^{-r/\lambda})}{r}\frac{\partial}{\partial r}\left(r\frac{\partial \rho}{\partial r}\right) \tag{13}$$

$$\frac{da}{dt} = \frac{(D_0 + D_1 e^{-r/\lambda})\rho'_+}{\rho_L - \rho_+} \tag{14}$$

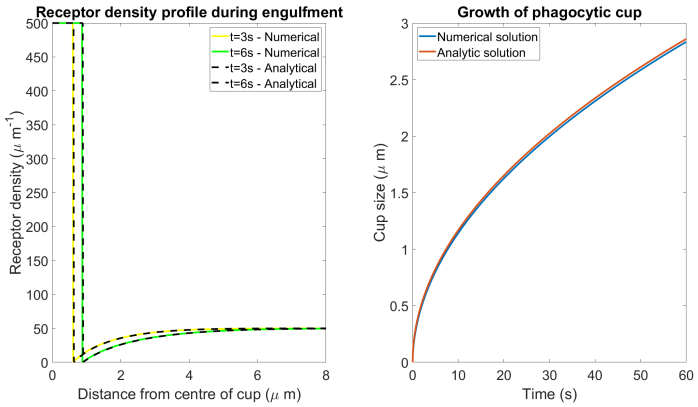
Which we can numerically solve this using

$$\rho(r,t+1) = \rho(r,t) + \frac{(D_0 + D_1 e^{-r\Delta r/\lambda})\Delta t}{(\Delta r)^2}\left[(\rho(r-1,t) - 2\rho(r,t) + \rho(r+1,t)) + \frac{\rho(r+1,t)-\rho(r,t)}{r}\right] \tag{15}$$

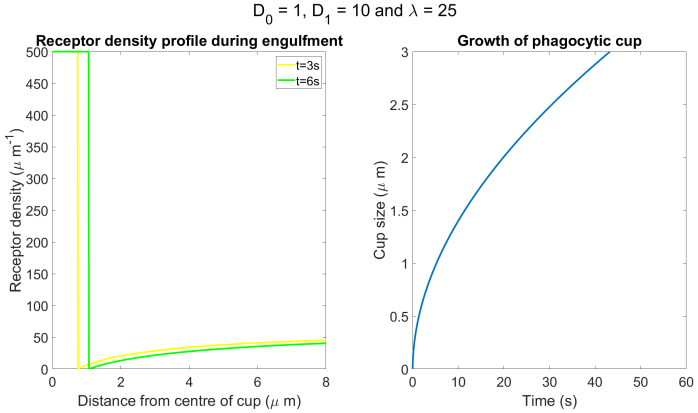
and

$$a(t+1) = a(t) + \frac{(D_0 + D_1 e^{-r\Delta r/\lambda})\rho'_+}{\rho_L - \rho_+} \tag{16}$$

The benefit of including diffusion equation that increases near the cup, is that we can try to replicate the second faster stage of engulfment we see in experimental settings. To make comparisons between our previous numerical scheme, and our extended model, I decided to set $D_0 = 1$. This means we can attribute the differences between the models down to the exponent, $e^{-r\lambda}$. Looking at $D = D_0 + D_1 e^{-r/\lambda}$ we can see that by increasing $D1$ we increase the impact that the exponential, will have on the solution. From experimenting with the model, I found that I could not set $D_1 > 1$ without decreasing to Δt value. However, at $D_1 \leq 1$ the differences between the models was negligible, and so I reduced the time step, $\Delta t = 4.0 \times 10^{-6}$; I decided on setting $E_1 = 10$. This then left λ to be varied. Since the exponent is has a negative sign, we can determine that the bigger $r\Delta r/\lambda$ gets the smaller this exponent will be. To see the biggest impact we could set λ to a very large number, meaning the exponent would almost always equal 1, effectively summing E_0 and E_1. However, we want to see this exponential vary as the the distance from origin, r is varied, we know $r\Delta r \in [0, 50]$. Setting $\lambda = 25$ puts the value of the exponential between 0.15 and 1. With $D_1 = 10$ this increases our D_0 by 150 to 1000%. The results from this extended model with these parameters, $D_0 = 1, D_1 = 10$ and $\lambda = 10$ is shown in Figure 3b. We can see that the cup size now grows at a much faster rate in our plot of $a(t)$, we can also that for the same time values, $t = 3$ and $t = 6$ the receptor density falls further from the centre of the cup. This is due to effective increase of the diffusion constant, which exponentially increases as $r \to 0$. This additonal force can be thought of as the active element of the phagocytosis mechanism.



(a) Results from both the numerical and analytical solution of Equation 13 and Equation 14. Showing both ρ at $t = 1$, $t = 2$ and $a(t)$. (The analytical solutions are distinguishable by their position, the colour black was chosen so the analytical solution could clearly be indicated)



(b) Results from the extended numerical model with $D = D_0 + D_1 e^{-r/\lambda}$

Links to MATLAB code used to produce plots.

(a) Numerical and Analytical Solution Plot

(b) Extension of Numerical Model

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