One Dimensional Study of Calcium Distribution in a Hepatocyte cell in presence of Buffer by Finite Volume Model

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Abstract- Calcium is ubiquitous second messenger which controls vital functions of almost all eukaryotic cells. Hepatocyte cell is parenchymal cell of liver. The regulation of calcium concentration in a hepatocyte cell is still not well understood. A model is proposed in this paper to study the distribution of calcium concentration in a hepatocyte cell in presence of buffers. The parameters like concentration of buffer, diffusion coefficient of calcium etc. have been incorporated in the model in the form of boundary value problem in one dimension. The boundary conditions have been formed to incorporate bio physiological facts of the problem. The finite volume method has been implemented to obtain the solutions. The program is developed on MATLAB 2014a to obtain numerical results and they are used to study the effect of buffers on calcium concentration in hepatocyte cell in steady state case. The information can be generated by developing such models of calcium concentration in hepatocyte cell for proper health care of liver.

Index Terms— Calcium, Buffer, Hepatocyte cell, Finite volume method.

I. INTRODUCTION

The largest internal gland in human body is a liver. It secretes many essential hormones required for digestion, metabolism etc. Almost all the activities necessary for fine coordination among internal organs are performed by liver. Therefore tissues of the body cannot survive without proper working of liver. The proper working of liver depends upon the fine coordination of calcium level in hepatocyte cell. Intracellular calcium signaling regulates varieties of function performed by hepatocyte cell. The calcium binds with many proteins and modifies their enzymatic properties. Thus cell need to keep calcium concentration in range from 0.1µM to at most 1µM [1]. The source influx of calcium and buffers play an important role in this calcium regulation in the cell. Buffers are large proteins that soak up nearly 99 % of calcium. The buffers associate with calcium ions to reduce the calcium concentration in the cell [2].

The attempts have been made in the past to study calcium distribution in various cells like neuron [3], myocyte [4], oocytes [5], astrocyte [6], fibroblast [7] and acinar cells [8] under various conditions. However, very little attention has been paid to the study of calcium distribution in hepatocyte cell. In the present work a finite volume model is proposed to study steady state one dimensional calcium concentration distribution in hepatocyte cell. The parameters like buffer, source influx and diffusion coefficient of calcium has been incorporated in the model. The study has been carried out in presence of exogenous buffers and endogenous buffer. In the next section the mathematical formulation has been presented and solution is obtained by implementing finite volume method.

MATHEMATICAL FORMULATION

The calcium released from source ER binds with the buffers present in cytosol of cell. If there are n buffers present in the cytosol of hepatocyte cell then association and dissociation of calcium ion (Ca2+) with buffer (Bi) is governed by following reaction diffusion equation [2].

\[
\begin{align*}
& k_i^+ \quad \text{Ca}^{2+} + B_i \rightarrow \text{CaB}_i \\
& k_i^- \quad \text{CaB}_i \rightarrow \text{Ca}^{2+} + B_i
\end{align*}
\]

Here, Ca 2+ is free calcium ion binds with ith buffer Bi to form calcium bound buffer CaBi.

Using law of mass action and Fick’s law of diffusion the change in calcium concentration with respect to time is given by following reaction diffusion equation,

\[
\frac{\partial [\text{Ca}^{2+}]}{\partial t} = D_\text{ca} \nabla^2 [\text{Ca}^{2+}] + \sum R_i
\]

Where, \( R_i = -k_i^+ [\text{Ca}^{2+}] [B_i] + k_i^- [\text{CaB}_i] \) \( \sum R_i \) D_\text{ca} is diffusion coefficient of free calcium (200-300µm²/s), \( k_i^+ \) is association rate constants and \( k_i^- \) is dissociation rate constant for given ith buffer. Square bracket represents concentration of species enclosed in it.

We assume that total buffer concentration remains conserved. Therefore total concentration of ith buffer \([B_i]_T\) (50-150 µM) is given by,

\[
[B_i]_T = [B_i] + [\text{CaB}_i]
\]
Using it in equation (3) gives,
\[
\frac{\partial [Ca^{2+}]}{\partial t} = D_{Ca}V^2[Ca^{2+}] + \sum_i R_i'
\]
Where, \(R_i' = -k^+_i[Ca^{2+}][B_i] + k^-([B_i]_T - [B_i])\)

By assuming single buffer species in excess and at equilibrium setting reaction term equal to zero gives concentration of excess buffer \([B]_\infty\) as,
\[
[B_i] = [B_i]_\infty = \frac{k_i}{k_i + [Ca^{2+}]_\infty}, \text{ Where, } k_i = k^+.
\]

Using it in Eq. (4) we get,
\[
\frac{\partial [Ca^{2+}]}{\partial t} = D_{Ca}V^2[Ca^{2+}] - k^+ [Ca^{2+}][B]_\infty
\]
\[+ k^- ([B]_T - [B])_\infty\]

The buffer term of Eq. (5) can be put in term of equilibrium calcium concentration \([Ca^{2+}]_\infty\) as follows,
\[
k^- ([B]_T - [B])_\infty = k^+ [Ca^{2+}]_\infty [B]_\infty
\]
Thus Eq. (5) can be written in the form,
\[
\frac{\partial [Ca^{2+}]}{\partial t} = D_{Ca}V^2[Ca^{2+}]
\]
\[- k^+ [B]_\infty ([Ca^{2+}]_\infty - [Ca^{2+}]_\infty)\]

For one dimensional unsteady state case Eq. (5) reduced in the form,
\[
\frac{\partial [Ca^{2+}]}{\partial t} = D_{Ca} \frac{\partial^2 [Ca^{2+}]}{\partial x^2}
\]
\[- k^+ [B]_\infty ([Ca^{2+}]_\infty - [Ca^{2+}]_\infty)\]

The parameter values for exogenous buffers (EGTA, BAPTA) and endogenous buffer are given in the Table 1.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>(k^+)</th>
<th>(k^-)</th>
<th>(K = k^- /k^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGTA buffer</td>
<td>1.5 (\mu M) s(^{-1})</td>
<td>0.3 s(^{-1})</td>
<td>0.2 (\mu M)</td>
</tr>
<tr>
<td>Endogenous buffer</td>
<td>50 (\mu M) s(^{-1})</td>
<td>500 s(^{-1})</td>
<td>10 (\mu M)</td>
</tr>
<tr>
<td>BAPTA buffer</td>
<td>600 (\mu M) s(^{-1})</td>
<td>100s(^{-1})</td>
<td>0.17 (\mu M)</td>
</tr>
</tbody>
</table>

** AUXILIARY CONDITIONS **

Calcium channels are located on the membrane of endoplasmic reticulum. Initially before opening the mouth of calcium channel the equilibrium calcium concentration is 0.1 \(\mu M\). This gives initial condition, \([Ca^{2+}]_T = 0.1 \mu M\)

The calcium releasing channels are scattered near apical part of hepatocyte cell. It is assumed that calcium is released from source kept near left boundary. With this assumption first boundary condition is set at node 1 located at \(x=0\).

\[
\lim_{x \to 0^-} -D_{Ca} \frac{d[Ca^{2+}]}{dx} = \sigma_{Ca}
\]

Where, \(\sigma_{Ca}\) represents flux of calcium incorporated on left boundary.

The calcium concentration near to basal part of hepatocyte cell is assumed to attain background equilibrium concentration 0.1 \(\mu M\) and length of hepatocyte cell is 15 \(\mu m\). Thus, second boundary condition can be framed as,

\[
\lim_{x \to 15^-} [Ca^{2+}] = [Ca^{2+}]_\infty = 0.1 \mu M
\]

** SOLUTION **

The hepatocyte cell is discretized into discrete control volumes, as shown in Fig. (1) to apply finite volume method[11].

![Fig.1. One dimensional discretization of hepatocyte cell.](image)

The control volumes are the subintervals of the problem interval in one dimensional problem and the nodes are centers of those subintervals. To discretized the region between two boundaries uniformly 30 equidistance nodal points are considered separated by equal distance \(\delta x\). Where, node 1 and node 32 represents the boundary nodes. The control volume is considered around each node. Let \(G\) be a general nodal point in a control volume and \(W\) and \(E\) are its neighboring nodes to west and east respectively. Also west side face and east side face of control volume are kept at \(x_w\) and \(x_e\) respectively.

Eq. (8) can be written in the form,
\[
D_{Ca} \left( \frac{d}{dx} \left( \frac{dY}{dx} \right) \right) - k^+ [B]_\infty (Y - Y_\infty) = 0
\]

Where \(y\) is taken in lieu of \([Ca 2+]\) for convenience. Rearranging Eq. (12) in the general form, we get,
\[
\left( \frac{d}{dx} \frac{dY}{dx} \right) - a(Y - Y_\infty) = 0
\]

Where, \(a = \frac{k^+ [B]_\infty}{D_{Ca}}\).

Integrating Eq. (13) over the control volume as shown in Fig. 1 gives [34],
\[
\int_{A_{\delta}} \left( \frac{d}{dx} \frac{dY}{dx} \right) dV - a \int_{A_{\delta}} (Y - Y_\infty) dV = 0
\]

For one dimensional domain we consider \(\Delta V = \delta x\). Thus Eq. (17) can be written as,
\[
A_e \left( \frac{dY}{dx} \right)_{e} - A_w \left( \frac{dY}{dx} \right)_{w} - aY_G A\delta x + aY_\infty A\delta x = 0
\]
As regular structured grid is considered we have
\[ A_w = A_r = A \]
and therefore,
\[ \frac{Y_E - Y_G}{\delta x_{GE}} - \frac{Y_G - Y_W}{\delta x_{WG}} = a Y_G \delta x + a Y_w \delta x = 0 \]  
(16)
Rearranging Eq. (16) gives,
\[ \frac{1}{\delta x_{WG}} Y_W + \frac{1}{\delta x_{GE}} Y_E + aY_w, \delta x \]
(17)
As nodes are separated uniformly we have, \( \delta x_{WG} = \delta x_{GE} = \delta x \). The general form of equation for the interior nodes is given by,
\[ a_G Y_G = a_W Y_W + a_E Y_E + S_u \]  
(18)

Where,
\[ a_w = \frac{1}{\delta x}, a_r = \frac{1}{\delta x}, a_G = a_w + a_e - S_G \]
\[ S_G = -a \delta x, S_u = a C_o, \delta x \]  
(19)
Now the source is kept at node 1 that is at west face of control volume. Therefore the left boundary condition is incorporated by substituting \( Y_W = \sigma_{Ca} \), thus we get,
\[ a_w = 0, a_r = \frac{1}{\delta x}, a_G = a_w + a_e - S_G, S_G \]
(20)
Also the right boundary of cell is kept at equilibrium concentration. Therefore the left boundary condition is incorporated by substituting \( Y_E = Y_b = 0.1 \mu M \), thus we get,
\[ a_w = \frac{1}{\delta x}, a_r = 0, a_G = a_w + a_e - S_G, S_G \]
(21)

Using Eqs. (19), (20), (21) in (18) we get a system of algebraic equations as follows,
\[ PY = Q \]  
(22)
Here \( Y = Y_1, Y_2, ..., Y_{12} \) gives the calcium concentrations at nodes from 1 to 32, \( P \) is system matrix and \( Q \) is system vector.

The numerical solution is obtained by employing Gauss elimination method to solve Eq. (22) by means of program developed in MATLAB R2014a.

Fig. 2. shows the spatial change in calcium concentration in presence of EGTA buffer, endogenous buffer and BAPTA buffer respectively. From the figure it is clear that different types of buffers have different effects on calcium concentration profile in the cell. The calcium concentration is maximum at \( x=0 \) where source influx is present. The calcium concentration falls down gradually in case of EGTA buffer, but it falls down sharply in case of endogenous buffer and more sharply in case of BAPTA buffer. This is due to fact that, BAPTA buffer is fast buffer having large binding rate and EGTA buffer is slow buffer having small binding rate. The endogenous buffer is faster than EGTA buffer and slower than BAPTA buffer. The calcium concentration attains background concentration 0.1 \( \mu M \) beyond 10 \( \mu M \) from the source influx.

Fig. 3 shows the spatial change in calcium concentration when concentration of EGTA buffer is 50, 100, 150 \( \mu M \) respectively. From figure it is clear that with increase in concentration of EGTA buffer the concentration of free calcium decreases at each nodal point of hepatocyte cell. As EGTA binds with free calcium to form calcium bound buffer, it reduces amount of free calcium. This is why increase in concentration of buffer leads to decrease in concentration of free calcium. At the mouth of source channel the concentration of calcium is 0.8 \( \mu M \). The rate of decrease in calcium concentration is increases with increase in buffer concentration. The calcium concentration uniformly decreases towards basal part of the hepatocyte cell and attains background equilibrium concentration 0.1 \( \mu M \).
Fig.3. Spatial calcium concentration with different concentration of EGTA buffer in hepatocyte cell

Fig.4 shows the spatial change in calcium concentration when the value of diffusion coefficient is 200, 250, 300μm²/s. Diffusion coefficient is defined as amount of diffusing substance transported from one part to other part of domain per unit area per unit time. This shows that for higher value of D calcium ions moves fast from apical to basal region of cell. As more amount of calcium is transported for D = 300μm²/s the less amount of free calcium accumulates in the space. Therefore concentration of calcium is decreases with increase in value of diffusion coefficient, i.e amount of free calcium is inversely proportional to diffusion coefficient.

The spatial change in calcium concentration when the value of source amplitude sigma is 1, 2 pA respectively is shown in Fig.5. The characteristic amplitude of current passing through a channel has unit pico amperes (pA). The open channel permits the passage of ions, which is measured as current. The increase in value of source amplitude release more amount of calcium into cytosol.

Thus it leads to increase in concentration of free calcium. From figure it is observed that the concentration of calcium is 0.8 and 1.6μM respectively for 1, 2 pA source amplitude at the mouth of point source. Then afterwards it decreases uniformly up to 0.1μM in presence of EGTA buffer. The appropriate experimental results are not available for comparison; however the results obtained by proposed model are in agreement with the biological facts.

II. CONCLUSION

The proposed finite volume model has been successfully implemented to study the effect of different types of buffers, source amplitude and different rates of diffusion coefficient on the spatial calcium concentration in a hepatocyte cell. From the results it is concluded that, calcium concentration decreases sharply for fast buffers especially for endogenous and BAPTA buffer in comparison with exogenous EGTA buffer. The calcium concentration in the cell is inversely proportional to diffusion coefficient. The variation in calcium concentration in the cell is directly proportional to the source influx. The finite volume method has proved to be quite versatile in obtaining the interesting relationships of calcium concentration in the cell with the type, quantity of buffers, influx rate and diffusion coefficient. The results obtained can be of great use to biomedical scientist for development of new protocols for treatment and diagnosis of liver diseases.

REFERENCES


