Matematical Model Characterizing the Release Rates of the Vitamin B12 (VB_{12}) Loaded Collagen Matrices

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ABSTRACT
Collagen fibrils can act as a substrate carrying biosensor that can be used to detect the presence of date rape drugs in a drink. Vitamin B12 (VB_{12}) was chosen as analogous to methyl red (MR), the indicator of gamma-hydroxybutyric acid, a commonly used date rape drug. A mathematical model was developed to describe the release rates of the biosensor from collagen matrix. The model was developed using the partial differential mass transfer equation. It was apparent that the diffusion co-efficient was the parameter that determined the release rates. This diffusivity was directly related to the pore size of the collagen matrix. By controlling the pore size during the collagen matrix preparation techniques, the diffusion-coefficient can be controlled as well. The theoretical model was compared to the experimental data by using Chi-Square hypothesis test. The null hypothesis test using chi-square method proved that the mathematical model cannot be rejected with 99.99% certainty.

KEYWORDS: Collagen nanofibrils; Modelling; Diffusion; Mass transfer; Biosensor; Release Rate.

INTRODUCTION
Collagen is a biodegradable structural protein present in all animals, especially in the flesh and connective tissues of vertebrates, making up about 25% to 35% of the whole-body protein content. Collagen is not soluble in water but due to its surface charge chemistry it can hold up to 500 hundred times its mass in water [1]. This unique surface charge density characteristic makes collagen nanofibrils (CNF) most useful in biotechnological and environmental applications.

The Collagen dispersions were made by using nanofibrils produced form raw Bovine Hide Corium. Collagen dispersions have several applications in environmental and biotechnological fields. The most beneficial environmental application is the use of Collagen as an aid to the filtration process. Collagen dispersion carrying positive charges has affinity to the negative charges of the polar water molecule, thereby helping in agglomeration of the suspended solid particles in sludge or waste [1].

Once these collagen dispersions are made, they can be frozen and freeze dried. The new freeze dried material retains the overall properties of the original frozen material. But the new cryodessicated material has 99% void space and so it is 99% porous, and the remaining material displays a spongy aerogel structure with controllable pore size, excellent mechanical properties and density as low as one thousandth of water. Also, the collagen dispersions can be cross linked, which will secure its shape and pore size [2]. With extensive Dehydrothermal crosslinking, the collagen dispersion is also sterile and therefore can be used in cell cultures especially for organ replication, bone regeneration, and other tissue engineering applications, such as skin replacements. [3].

Moreover, collagen fibrils can act as a substrate carrying a biosensor that can detect the presence of date rape drugs in a drink. Vitamin B12 (VB_{12}) was chosen as analogous to methyl red (MR), the indicator of gamma-hydroxybutyric acid, a commonly used date rape drug. Collagen matrices are extremely porous and additionally, the pore size can be altered in order to carry and deliver a biosensor [4]. This paper addresses the mathematical model that was developed to characterize the release rates of the VB_{12} loaded collagen matrices. The mathematical model was developed using standard mass transport equations. The model was then compared to the experimental data via statistical analysis. The
experimental determination of the release rates of collagen matrices with VB_{12} is clearly described in the Conference Proceedings [5].

MATERIALS AND METHODS

A. Corium Processing

The Collagen dispersion is made from raw fibrillar type 1 bovine corium as a starting material. Corium is the dermis layer of the hide, the skin layer beneath the epidermis. This Corium is made up of connective tissues, which in turn contains 99% Type 1 collagen. The micrograph of the bovine hide collagen before milling is illustrated in “Figure. 1” below.

Figure 1: Collagen nanofibrils before milling

It can be seen from “Fig. 1” that these nanofibrils are not well dispersed. In order to make them dispersed a solution of nanofibrils is ball milled with zirconium media for almost a week or two. The resultant dispersed collagen paste is then strained, washed, and centrifuged at low temperatures [2]. After the centrifugation process, the floating oils and fats are decanted. This method is repeated until no floating oils or fats are found above the clear phase of the dispersion. Now the collagen nanofibrils have dispersed and it can be seen in “Figure. 2”.

Figure 2: Collagen nanofibrils after milling

B. Mathematical Model Development

Before explaining any equations, it is important to understand some basic assumptions for the model that was used in describing the control rates. Here, a microsphere is being “cut” into six shells, and each shell is at r distance. At time t=0, the initial concentration of the biosensor (VB_{12}) at each of these shells was 2.45 mol/m^3. The microsphere and the six different shells is well depicted in “Figure. 3” below.

Figure 3: Microsphere cut into six shells

\[
\begin{align*}
E, r &= 0 \\
D, r &= 0.0004 \\
C, r &= 0.0008 \\
B, r &= 0.0012 \\
A, r &= 0.0016 \\
(S1), \text{ Boundary, } r &= 0.002
\end{align*}
\]
The concentration of the biosensor decreases as time increases at each individual shell. The VB12 gets released at the surface first and then proceeds inward – first 1r, then 2r… until it reaches the center where r is equal to zero. The VB12 release rate is derived from partial differential mass transfer Equations “1”, “2”, “3”, “4” and “5”.

\[
\frac{\partial C_{VB12}}{\partial t} = D^*\nabla^2 C_{VB12}
\]  

(1)

Rewriting for spherical systems,

\[
\frac{\partial C_{VB12}}{\partial t} = D^\ast \left[ \frac{\partial^2 C_{VB12}}{\partial r^2} + \frac{2}{r} \frac{\partial C_{VB12}}{\partial r} \right]
\]  

(2)

In finite difference format,

\[
\frac{\partial C_{VB12}}{\partial t} \approx \frac{C_{VB12,t+\Delta t,r} - C_{VB12,t,r}}{\Delta t}
\]

(3)

\[
= D^\ast \left[ C_{VB12,t,r+\Delta r} + C_{VB12,t,r-\Delta r} - 2C_{VB12,t,r} \right. \\
+ \frac{2}{r} \left. \frac{C_{VB12,t,r+\Delta r} - C_{VB12,t,r}}{\Delta r} \right] \]

(4)

For Excel,

\[
\text{South} \rightarrow \text{Center} \\
= D^\ast \left[ \frac{\text{East} + \text{West} - \text{Center}}{\Delta t} \right. \\
+ \left. \frac{2 \text{East} - \text{Center}}{r} \right] \]

(5)

In the example discussed, the radius of the spherical matrix was 0.002 m and a \( \Delta r \) of 0.0004m was chosen. The time difference \( \Delta t \) was arbitrarily chosen to be 20 seconds and must be chosen to abide by the Crank-Nicholson stability criterion. The diffusivity coefficient was 1.95 \( \text{e}^{-12} \text{ m}^2/\text{sec} \). Using the abovementioned mass transfer equations, the change in concentration versus time at the six different shells were obtained. Then, linest function was used to calculate the statistics for a polynomial concentration model with unknown parameters. This linest function uses least squares curve to produce uncertainty estimates for the fit values. The four unknown polynomial parameters were obtained using linest function. Once these polynomial parameters were known, the equation for concentration was formulated. This is shown in Equation “6”

\[
C_{VB12} = a + br + cr^2 + dr^3
\]

(6)

The formulated equation “6” was multiplied with the volume as shown in Equation “7” and then integrated from 0 to R where R = radius = 0.002m to find the amount remaining in the matrix as shown in Equations “8”.

**Amount Remaining in the matrix**

\[
= \int_0^R C_{VB12} \ast 4\pi r^2 \, dr
\]

(7)

\[
= 4\pi \left[ \frac{a}{3} R^3 + \frac{b}{4} R^4 + \frac{c}{5} R^5 \right. \\
\left. + \frac{d}{6} R^6 \right]
\]

(8)

The initial amount of VB12 was calculated using the Equation “9”.

\[
\text{Initial amount} = C_{\text{initial VB12}} \ast \frac{\pi}{6} Dp^3
\]

(9)

The percent remaining in the matrix was the calculated using Equation “10”

\[
\text{Percent VB12 Remaining in the matrix} = \frac{\text{Amount Remaining in the matrix}}{\text{Initial amount}}
\]

(10)

The percent of VB12 that was diffused over time was obtained using Equation “11”

\[
\text{Percent VB12 Diffused} = 1 - \% \text{VB12 Remaining in the matrix}
\]

(11)

**C. Comparison to Experimental Data**

This percent of VB12 that diffused over time was compared to the experimental diffusion rates. The experimental diffusion rates were obtained by immobilizing the VB12 in the collagen matrix. The release rates were observed by controlling the pore size of the matrix, and the physical characteristic that controlled the release rate was determined. This procedure and results have already been explained in the Conference Proceedings [5]. The transmittance profile for the experimental data was obtained for time t= 0 to 9000 seconds with a dt of 900 seconds.
The transmittance data was converted to concentration profile by taking advantage of the modified Beer’s Law given by the Equation “12”.

\[
A \propto \frac{1}{c_{VB12}}
\]

An even better statistical test of the goodness of fit was done by using the Chi-Square Goodness-of-Fit test. \( \chi^2 \) Is the value of the chi-square random variable with \( k \) degrees of freedom such that the probability that \( \chi^2 \) exceeds this value is \( \alpha \).

From the hypothesized probability distribution, the test statistic is calculated using the Equation “13”

\[
\chi^2_0 = \sum \frac{(O_i - E_i)^2}{E_i} \text{ for } (i = 1 \text{ to } K)
\]

Where \( \chi^2_0 \) follows chi-square distribution with \( k - p - 1 \) degrees of freedom and \( p \) represents the number of parameters of the hypothesized distribution estimated by sample statistics. We would reject the hypothesis that \( \chi \) conforms to the hypothesized distribution if \( \chi^2_0 > \chi_{k-p-1}^2 \).

RESULTS AND DISCUSSION
A. Assumptions to Build the Mathematical Model
Using the partial differential mass transfer equation, the concentration profile at the six shells was obtained. Initial concentration of 2.45 mol/m³ of VB₁₂ was loaded into the collagen matrix. Figure “4” depicts the change in concentration at the different shells with respect to time.

![Figure 4: Concentration versus Time at six shells](image)

It can be seen from Figure. “4” that it takes about 40,280 seconds, which is almost 11.19 hours, to achieve a total diffusion of VB₁₂ at each shell. The diffusion was controlled primarily by the diffusivity of the biosensor which in turn depends on the pore size of the matrix releasing the biosensor. The pore size of the matrix can be controlled via preparation techniques of the matrix, which is described in detail in the Conference Proceedings [5]. Therefore the diffusion co-efficient is the one parameter that the mass transfer depends on.

The theoretical amount of VB₁₂ released over time was plotted and it shows the release rate increased as time increased. An increase in release rate from 88% to 100% was determined within 11.19 hours for a diffusivity of 1.95 \( \text{e}^{-12} \) m²/sec. Again by reducing the pore size via collagen matrix preparation techniques, the diffusivity will be reduced, which in turn gives slower the release rates and vice versa.

![Figure 5: Theoretical Release Rates of VB₁₂](image)

From Figures “6” and “7,” it can be seen that the theoretical release data of VB₁₂ for time \( t = 17250 \) to \( 17700 \) seconds has a linear growth release rate which is in accordance with the higuchi plot of the release data from the experiment [5]. The mathematical model predicts the linear growth of the release rate of VB₁₂ with exacting precision because the considered \( dt \) was only 20 seconds.
To confirm if the mathematical model fits the experimental data, a Chi-Square goodness of fit test was considered.

**Table 1: Chi-Square Goodness of Fit Data**

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>%B12</th>
<th>% in the matrix</th>
<th>% diffused out</th>
<th>%B12</th>
<th>% in the matrix</th>
<th>% diffused out</th>
<th>Predicted</th>
<th>Chi-Square Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.124</td>
<td>0.876</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>900</td>
<td>0.057</td>
<td>0.543</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>1800</td>
<td>0.045</td>
<td>0.555</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>2700</td>
<td>0.034</td>
<td>0.566</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>3600</td>
<td>0.034</td>
<td>0.577</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>4500</td>
<td>0.031</td>
<td>0.588</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>5400</td>
<td>0.028</td>
<td>0.599</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>6300</td>
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<td>0.610</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>7200</td>
<td>0.023</td>
<td>0.621</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>8100</td>
<td>0.020</td>
<td>0.632</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
<tr>
<td>9000</td>
<td>0.017</td>
<td>0.643</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
<td>0.003</td>
<td>0.661</td>
<td>0.003</td>
</tr>
</tbody>
</table>

The $X_0^2$, the observed value of the test statistic calculated for the hypothesized distribution was 0.016. The $X_{ak}^2$ for the sample data with nine degrees of freedom was 0.661. Since $0.016 < 0.661$, we cannot reject the hypothesis that the data comes from a discrete uniform distribution. With 99.99% probability it can be said that the mathematical model fits the experimental data.

**CONCLUSION**

Collagen nanofibrils can be used as a medium to immobilize the biosensor to detect the date rape drug in the drink. Vitamin B$_{12}$ (VB$_{12}$) was chosen as the comparable biosensor to MR, which is the main detector of GHB. Similar diffusion coefficients of VB$_{12}$ and MR make VB$_{12}$ a natural analogy to MR for experimental procedures. A mathematical model was developed to interpret the release rates of VB$_{12}$ loaded collagen matrices. The model was created using the fundamental mass transfer equations for a microsphere “cut” into six different shells at distance apart. A total diffusion of the VB$_{12}$ at each of the shells takes about 11.19 hours with a diffusion coefficient of $1.95 \times 10^{-12}$ m$^2$/sec. Release rate depends on the diffusivity of the biosensor. But the diffusivity of the biosensor depends on the pore size of the collagen matrix. Pore size of the collagen matrix can be controlled by the initial preparation techniques. By creating smaller pores, the diffusion coefficient is made smaller, thereby decreasing the release rate of the VB$_{12}$.

This theoretical model was in agreement with the experimental data. The null hypothesis test using chi-square method proved that the mathematical model cannot be rejected with 99.99% certainty. The $X_0^2$ value was 0.016 and the $X_{ak}^2$ with 9 degrees of freedom was 0.661. Even with a 99.99% probability that the observed value of the hypothesized distribution was higher than chi-square random variable with 9 degrees of freedom, the observed value is less than the predicted random variable, making the model 99.99% in agreement with the experimental data.

**ACKNOWLEDGEMENTS**

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REFERENCES


[2] G. J. Maffia, "Using untreated raw fibrillar type I corium as the starting material; milling to unravel fibers; washing and straining; centrifuging; repeating these steps until no fats are present; and mixing with an acid". United States of America Patent US6660829 B1, December 2003.


