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Computational Modeling for the Diffusion of Biosensor from Collagen Matrix into Aqueous Solution

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Abstract

The collagen fibrils can act as a substrate carrying a biosensor that can detect the presence of date rape drugs in a drink. Vitamin B12 (VB12) was chosen as analogous to methyl red (MR), the indicator of gamma-hydroxybutyric acid, a commonly used date rape drug. VB12 is chosen since it is a larger molecule than MR, but has a similar diffusivity constant. Diffusion of the biosensor from the collagen matrix into the aqueous solution was performed experimentally and modeled using COMSOL Multiphysics 4.4. The concentration profiles obtained for the experimental and COMSOL diffusion models were similar, however, COMSOL provided more precise values. While took about five days to completely diffuse the VB12 into aqueous solution; visually and experimentally the steady state value was reached in about one hour. Using the initial concentration of MR and the minimum concentration needed for a reaction between the drug and indicator, the diffusion of the indicator from collagen matrix into the aqueous solution was modeled on COMSOL for different drink glasses. It took just a minute to produce a color change in a shot glass while it took the longest time to indicate the reaction between the drug and the indicator was with a pint glass at 32 minutes.

Keywords: collagen; COMSOL; vb12, GHB; rohypnol; date rape; diffusivity; diffusion-coefficient; unsteady state; transmittance; concentration profiles.

Introduction

Collagen is a structural protein found in all animals and humans. It is not soluble in water and its surface charge chemistry helps it to hold up to hundred times its mass in water. This unique surface charge chemistry makes it very useful in biochemical and environmental applications. Bovine raw corium was used to make these collagen dispersions. Once the collagen paste was made, collagen dispersions were subjected to freezing, freeze-drying and sometimes even cross linking. The new freeze-dried material possessed the characteristics of the old material but also had 99% void space, unlike the original material [1].

Collagen has several applications in biotechnological and environmental areas. One of the notable environmental applications includes filtration processing. The positive charges of collagen dispersions are attracted to the negative ends of polar water molecules, thereby, agglomerating the dirt and other unwanted particles in the waste water or sludge [2].

One of the other applications of a collagen matrix in drug delivery was investigated to pursue a safe drinking environment. Odorless, almost colorless date rape drugs have been slipped into the drink without the knowledge of the person drinking it. The perpetrators choose unsuspecting victims, who become helpless once they consume the alcohol. The forgetful effect will led the perpetrators take advantage and try to rob, rape and even harm the victim since the victim would not be able to remember anything. The most common types of these date rape drugs include the gamma-hydroxybutyric acid (GHB) and flunitrazepam (rohypnol). Therefore, the prospect for the use of a collagen matrix in helping to identify the presence of drug rape drugs in a drink was researched [3].

Collagen matrices are extremely porous and additionally, the pore size can be altered in order to carry and deliver a biosensor [4]. One aspect of a biosensor is that it can indicate the presence of date rape drug in the drink. In order to achieve this goal, Vitamin B12 (VB12) was chosen as a substitute to

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the biosensor. VB12 has 1355.4 *g/mol* molecular weight and is a larger molecule than the GHB and rohypnol indicators. The common GHB and rohypnol indicators are methyl red (MR), bromocresol green (BCG) and bromocresol purple (BCP) [5].

Computational modeling was done for the rate of dispersion of the VB12 biosensor from collagen matrix into water in a graduated cylinder using COMSOL Multiphysics 4.4. If the model works with the VB12 indicator, it is potentially a substitute that can work for the GHB and rohypnol indicators.

Materials and methods

Bovine Corium Processing

The collagen paste was made from Type *I* Bovine Corium. Corium is the dermis layer of the hide that contains a high amount of collagen. In order to get a well dispersed paste, the raw corium was mixed with water and milled using zirconium milling balls for 7 to 10 days. The resultant dispersed collagen paste was washed by a centrifuge that operated at low temperatures. After the paste was centrifuged, the fats and oils were decanted.

The viscous phase that contained all of the collagen content was then blended into a solution with water and acetic acid. The formulation of the collagen dispersion in percentages was 0.1-0.75% collagen paste, 94.25-94.9% water and 5% acid. Comparatively the obtained dispersed collagen had better pore size and mechanical properties. Since, the collagen was mixed into solution with acid, it is considered to be sterile for a long time.

Blending of biosensor VB12 into dispersed collagen

The VB12, which acts as a substitute to the biosensor was mixed with the collagen dispersion and then the solution was frozen for a day before it was placed in the freeze-dryer for about two days. After three days, the VB12 and collagen solution was removed from the freeze-dryer and a matrix with VB12 and collagen was obtained.

The initial number of moles of VB12 present in the matrix was found by measuring the amount of VB12 in grams and a specific volume of $9.9*10^{-8}$ m^3, the initial concentration of the VB12 in mol/m^3 were used to calculate the concentration of VB12 by equation "1" below.

$$C_{vb12} = \frac{mol_{vb12}}{V} \quad (1)$$

Where C_{vb12} = concentration of VB12
mol_{vb12} = number of moles of VB12
V = initial volume of the VB12

The area of the graduated cylinder was calculated from the diameter of the graduated cylinder. The relationship between the diameter and area of the graduated cylinder was found using equation "2."

$$A = \frac{\pi D^2}{4} \quad (2)$$

Where A= area in square meters
D = diameter of the graduated cylinder

Furthermore, the molar flux of VB12 in water was determined in order to obtain the diffusivity of the biosensor in water. The diffusivity of VB12 in water was necessary to model the diffusion of the biosensor into the solution using COMSOL Multiphysics 4.4. Molar flux is the rate of moles flow per unit area. The diffusion constant was experimentally found by placing a sample of the VB12 and collagen matrix in a graduated cylinder filled with water.

The molar flux was computed using the values of concentration of VB12 and area, according to equation "3." [6]

$$N_{AZ0} = \frac{mol}{A*t} \quad (3)$$

Where N_{AZ0} = molar flux in mol/ (m^2*sec)
mol = number of moles of VB12
A = area
t = time (assumed to be one second)

Since, the molar flux and the initial concentration were calculated; the diffusivity was obtained by using the equation "4." [6]

$$N_{AZ0} = Ca0\sqrt{\frac{\mathcal{D}_{ab}}{\pi*t}} \quad (4)$$

Where N_{AZ0} = molar flux in m^2/sec
$Ca0$ = initial concentration
$\mathcal{D}_{ab}$ = diffusivity of VB12 in water
t = time.

One of the assumptions made in this equation was that the interfacial area was not changing as a function of time. Now that the experimentally obtained diffusivity was deduced, the concentration profile for the diffusion of VB12 into a finite body of fluid was schematically obtained.

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Concentration profile for the diffusion of VB12 into finite fluid

To experimentally acquire the concentration verses time profile, first a library of percent transmittance verses concentration data was created for known concentrations. Percent transmittance data was collected using a spectrophotometer. Then percent transmittance verses time was plotted for VB12 of unknown concentrations. Correlating the percent transmittance for different times to the library of percent transmittance verses concentration, a plot of concentration verses time was plotted for the unknown concentrations of VB12. The diffusion took about one hour to reach steady state visually.

Once the concentration profile for the diffusion of VB12 was obtained experimentally, it was modeled on COMSOL Multiphysics 4.4. Since it is a two dimensional unsteady state diffusion problem, a 2D axisymmetric, time dependent and transport in diluted species model was used. The height and radius of the graduated cylinder was 17.5 cm and 1.4 cm respectively. These were inputted into COMSOL for modeling. Additionally, to represent the initial concentration of the collagen matrix carrying the biosensor, a second rectangle was created. Using the experimentally acquired values of diffusivity ($9.0*10^{-8} m^2/sec$) and the initial concentration ($368.9 mol/m^3$) were inserted in the diffusion and initial values under the transport of diluted species tab. Finally, an extra fine mesh was added and the surface concentration after a period of time was evaluated by COMSOL.

At time equals 60 minutes, the 3D COMSOL model for the surface concentration is shown in "Figure 1." COMSOL presented more precise values. Despite visually reaching equilibrium after 60 minutes, according to the COMSOL model, the concentration of VB12 was still diffusing out."

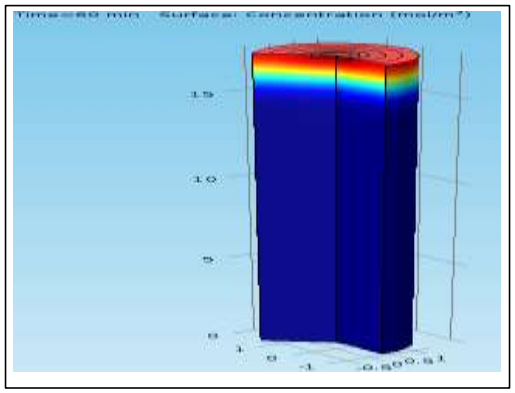

Figure1: COMSOL model for change in concentration at 60 minutes

Hand calculation for one dimensional, unsteady change in concentration of VB12 versus height of the graduated cylinder in which the diffusion occurs.

Calculations were done by hand using Microsoft Excel to obtain the concentration profiles in moles per cubic meter as a function of height in meters for a specific time of 60 minutes. Additionally, the initial mole fraction, x_{a0}, of the VB12 (0.00588) was small compared to the total mole fraction. Therefore, it was inferred that $\psi = 1$ and $\varphi = 0$ [6]. The temperature and pressure were assumed to be constant and also that the molar average velocity in the gas phase does not depend on the radial coordinates.

After the hand calculation was made, it was compared to COMSOL's calculation by making a 1D unsteady state plot with the same length, diffusivity, initial concentration, time period (60 minutes). The program also yielded a concentration profile with respect to the x axis.

COMSOL modelling to infer the minimum amount of time to react with drug indicator for various drink glasses

The concentration of MR, the indicator of GHB and rohypnol was calculated by using its density and molecular weight in Equation "5."

$$C_A = \rho * MW \qquad (5)$$

Where C_A = concentration of MR in mol/m^3
ρ = density of MR in g/m^3
MW = molecular weight in g/mol

The initial concentration was used when modelling for the minimum time required in reacting with the drug indicator. Then the minimum amount of concentration of the indicator that was necessary to react and result in a color change was referenced. Using the initial concentration of the indicator as starting initial values, different geometries for drink glasses were created. These included a shot glass, a wine glass, a pint and a cappuccino glass. The simulated model of each of these glasses is shown in "Figure 2." Each of the images in "Figure 2" was calculated after 30 minutes of diffusion.

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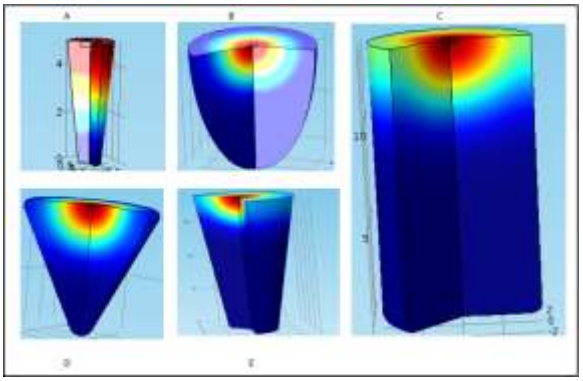

Figure2: COMSOL model for shot glass (A); wine glass (B); cappuccino glass (c); martini glass (D) and pint glass (E).

The minimum time that took to show a color change by reacting with the drug was inferred for each individual drink glass.

Results and discussion

The initial concentration of the VB12 in the collagen matrix was calculated as 368.9mol/m^3. The molar flux then obtained was 0.062 $mol/(m^2*sec)$. Since the diffusion is governed by Fick's second law of diffusion, equation "4" deduced the diffusivity as $9.0*10^{-8} m^2/sec$. The experimentally deduced diffusion coefficient was compared to the reference. Additionally, the diffusion constants for the other drug indicators (MR, BCP and BCG) were also referenced.

Table 1: Comparison table for Diffusion co-efficient

(m^2/sec)	VB12	MR	BCP/BCG
Reference	$2.3*10^{-10}$	$6.3*10^{-7}$	$3.3\text{-}3.4*10^{-10}$
Experimental	$9.0*10^{-8}$	NA	NA

Comparing, the experimental diffusivity of VB12 to that of methyl red, the main indicator of GHB in a drink, was found to be similar. However, BCP and BCG were in the order of 10^{-10} m^2/sec, which is similar to that of the reference diffusivity of VB12. From "Table 1", it can be inferred that while modelling for concentration profiles for the diffusion of the other indicators such as BCG, BCP or MR, the diffusion coefficient is the only thing that needs to be changed without altering any other parameters on COMSOL.

Concentration versus time plot

To get the concentration versus time plot experimentally, a library was created with percent transmittance verses concentration. This is represented in "Figure 3." Percent transmittance data was able to be collected only for very small concentrations because of the sensitivity of the spectrophotometer. Therefore, the concentrations ranged from $7.6*10^{-5}$ to $9.1*10^{-5}$ *mol/ L*.

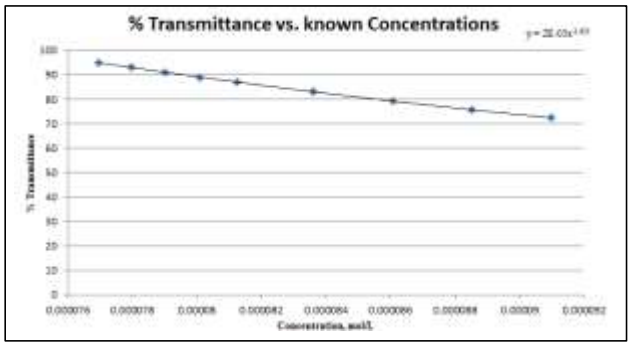

Figure3: Percent transmittance vs. known concentration

Then the percent transmittance for the VB12 and collagen matrix was measured at different time intervals as seen in "Figure 4."

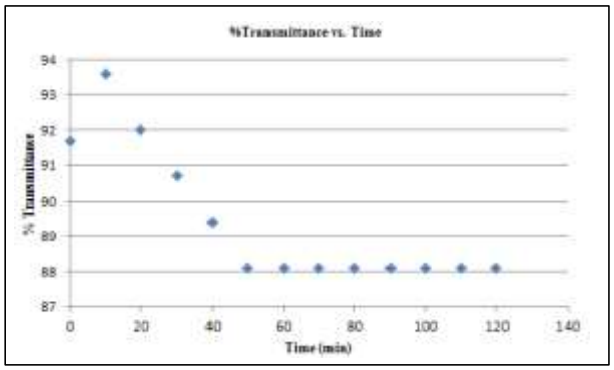

Figure4: Percent transmittance vs. time

"Figure 4" shows that the percent transmittance peaked during the first 10 minutes before steadily dropping until it reached a constant value around 60 minutes of time.

Since percent transmittance corresponds to concentration in an inverse relationship, there was an initial drop in concentration at the beginning before steadily increasing between 10 and 60 minutes as shown in "Fig. 3."

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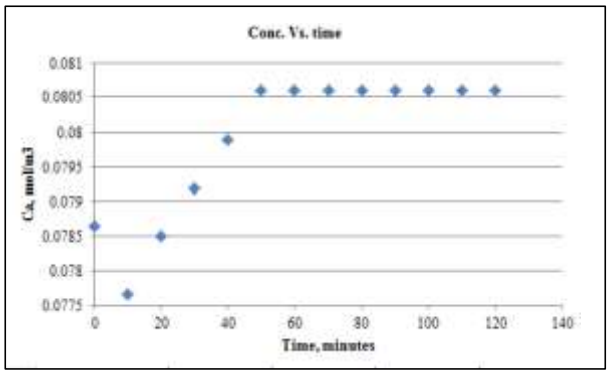

Figure5: Percent concentration of VB12 diffusing vs. time

The concentration leveled out in roughly 60 minutes. Therefore, it takes about 60 minutes for VB12 to diffuse entirely throughout the graduated cylinder visually. However, the experimental procedure was based on the assumption that there was no air in initial collagen matrix when placed in the solution and therefore there was no void space. Additionally, it was assumed that the collagen was immiscible in water and would stay as a solid.

After experimentally figuring the concentration profile for the VB12 diffusing out into water, the diffusion of VB12 was modeled on COMSOL Multiphysics. The concentration profile for the VB12 diffusing out into the water was represented in "Figure 6" below.

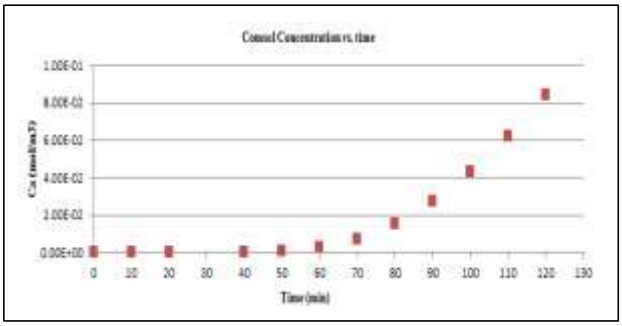

Figure6: COMSOL concentration vs. time plot

Some differences can be accounted due to the sensitivity of the spectrophotometer was not able to measure low concentrations that diffused into water in first 20 minutes. Additionally, the collagen matrix with the biosensor was not completely dry. Another reason was that the percent transmittance readings were taken for a small amount of solution taken out from the graduated cylinder. However, COMSOL evaluates the surface concentration over time for the height of 17.5m. Therefore, COMSOL has more accurate data compared to experimental values.

Concentration versus height 1D plot

Hand calculations were done to verify the original diffusion model that was computed. These calculations were done for a one dimensional, unsteady state diffusion, resulting in a concentration profile. The assumptions that were made was the height at the top of the cylinder was equal to zero and the interfacial surface where the concentration $x_a = x_{a0}$ (initial concentration) occurs at the top of the cylinder. The height of 17.5m is at the bottom of the graduated cylinder, where the concentration $x_a = 0$. After the surface concentration change with respect to height was found on Microsoft Excel, the model was rerun on COMSOL with 1D and same diffusivity and other parameters. Both concentration profiles were plotted and depicted below in "Figure 7.

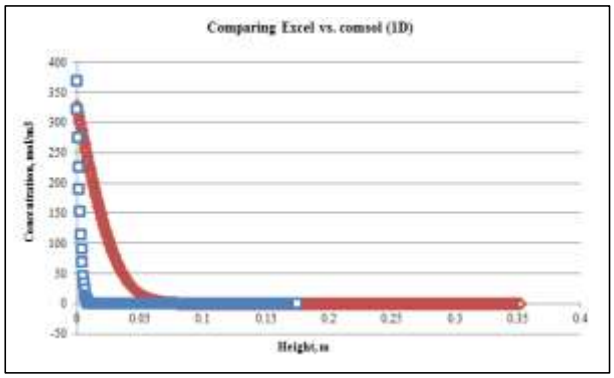

Figure7: Microsoft Excel vs. COMSOL

The change in concentration with respect to height of the cylinder was evaluated at exactly 60 minutes for both COMSOL and Microsoft Excel. In "Figure 7," the red line represents the Excel data and the blue line represents the COMSOL data. It can also be noted that both data are similar with varying values at the top of the cylinder. A reason for the discrepancy is because COMSOL assumes to have continuous flow in the cylinder when Excel considers a non-continuous flow.

Minimum reaction time with drug indicator for different drink glasses

The last goal was to determine the reaction time necessary to react with the minimum amount of indicator that could indicate the presence of the drug in the drink by a color change. The initial concentration of MR, the GHB indicator that was diffused was calculated by using Equation "5." The

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concentration was found to be about 3000 *mol/m*^{3}. Then the referenced minimum concentration of MR needed to produce a change of color in drink was 6.31*10^{-5}*mol/m*^{3}. Using these parameters, COMSOL's diffusion model was designed for a shot glass, a wine glass, a cappuccino glass, a martini glass, and a pint glass. "Table 2" illustrates the time required for each type of glasses.

Table 2: Minimum reaction rate for various drink glasses

Glasses	Min. concentration of indicator (*mol/m*^{3})	Min. time to react (*minutes*)
Shot Glass	6.31e^{-5}	1.03
Wine Glass	6.31e^{-5}	7
Cappuccino	6.31e^{-5}	11
Martini Glass	6.31e^{-5}	15
Pint Glass	6.31e^{-5}	32

It can be inferred from "Table 2" that it takes the least amount of time for a shot glass size to react, while it takes the most amount of time for a pint glass size to react. Further studies need to be done in order to reduce the minimum reaction time between the drug and the indicator.

Conclusion

Collagen is a protein present in all animals. It has various uses in biotechnological and environmental applications. One of the major uses of collagen in drug delivery is that the fibrils can act as substrates carrying the biosensor, which when released into solution or drink, will have a potential reaction resulting in a possible color change. VB12 was used to represent the biosensor MR or BCG or BCP. It took about 60 minutes to diffuse all the initial concentration of 368.9 *mol/m*^{3} of VB12 in water experimentally. A COMSOL model was created to observe the diffusion of VB12 into water in order to compare it to the experimental data. Both the concentration profiles with respect to time were similar with differences indicating that the COMSOL data was more accurate than the experimental data. The reason can be attributed to the sensitivity of the spectrophotometer used in determining the concentration of the biosensor samples experimentally.

Hand calculations were also done for a one-dimensional diffusion for the same conditions. However, the change in concentration was studied as a function of height of the cylinder. This was again modeled on COMSOL using 1D. The concentration change with respect to height was similar in both cases. However, again COMSOL had more precise values. A major reason as to why COMSOL was more accurate is because it assumed continuous flow and Excel model does not consider the system to have a continuous flow.

Further for MR, the GHB indicator, the initial concentration of about 3000 mol/*m*^{3} was calculated. The minimum concentration of MR needed to produce a reaction between the GHB and itself was about 6.31*10^{-5}*mol/m*^{3}. Using these parameters, COMSOL modeled different drink glasses, with the shortest time it took for the indicator to diffuse throughout the glass was in a shot glass with about 1 minute of diffusion time needed. However, it took a pint glass the longest to diffuse with about 32 minutes of diffusion time needed. Future research is needed to create a way to reduce the reaction time between the drug and the indicator in each drink glass.

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