Keratin Biomaterial Diffusivity Determination in a 1D-Like Channel using COMSOL Multiphysics® and MATLAB®

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INTRODUCTION: Keratin protein biomaterials (KTN) are used in various tissue engineering applications including liquid infusions and gel implants[1,2] (Fig. 1). Random movement of molecular species is characterized by Fick's laws of diffusion (and velocity-dependent convection) with the characteristic diffusivity coefficient (D) of solute in each solvent. Determining D is vital towards a better understanding of the kinetics and transport of KTN.

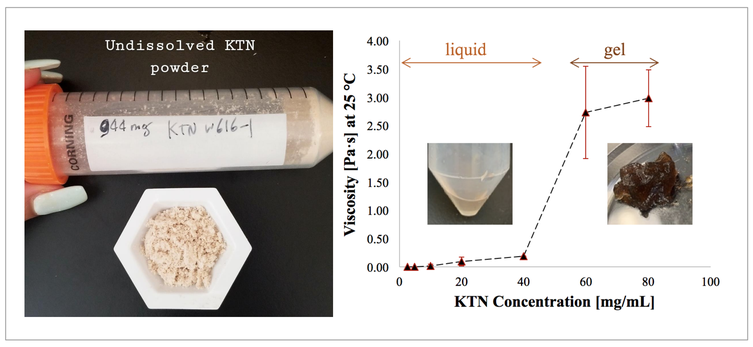

Figure 1. Keratin biomaterials

- Convection (diffusion and advection) equation

$$\frac{\partial c}{\partial t} + \nabla \cdot \mathbf{J} + \mathbf{u} \cdot \nabla c = R$$

where,
t = time [s]
c = concentration [mol/m^3]
J = mole flux [mol/m^{2}s] vector
u = velocity [m/s] vector
R = residual concentration flow rate [mol/m^{3}s]
D = diffusion constant or diffusivity [m^2/s]

- Fick's 1st law of diffusion

$$\mathbf{J} = -D\,\nabla c$$

EXPERIMENTAL AND COMPUTATIONAL METHODS: KTN (as solute) was dissolved in 10 mM NaOH solvent at 2, 4, and 8 mg/mL (or 0.02, 0.04, and 0.08 mol/m^3 using M_r = 98 kg/mol) and separately tested for diffusion across a narrow 1D-like channel (Fig. 2). 10 mL of solvent was first loaded, then 1 mL of KTN solution at one corner. Solutions were collected at time points 5, 10, and 20 min, and at the bottom at positions 5, 20, 50, 75, and 95 mm of the 105-mm span. Protein concentrations were quantified spectrophotometrically using standards. Concentrations (c) were nondimensionalized by dividing using the equilibrium concentrations (c_{eq}), and positions (x) with the total channel length (L). A MATLAB script was developed using method of lines to solve Fick's 2nd law (convection equation at u = 0 in one dimension) partial differential equation (PDE) for c and iterating D to match the experimental values. COMSOL Multiphysics was employed (Table 1) to model the geometry and simulate diffusion using Transport of Diluted Species interface of the Chemical Species Transport module. Parametric estimation study for inverse modeling was also done for determination of D.

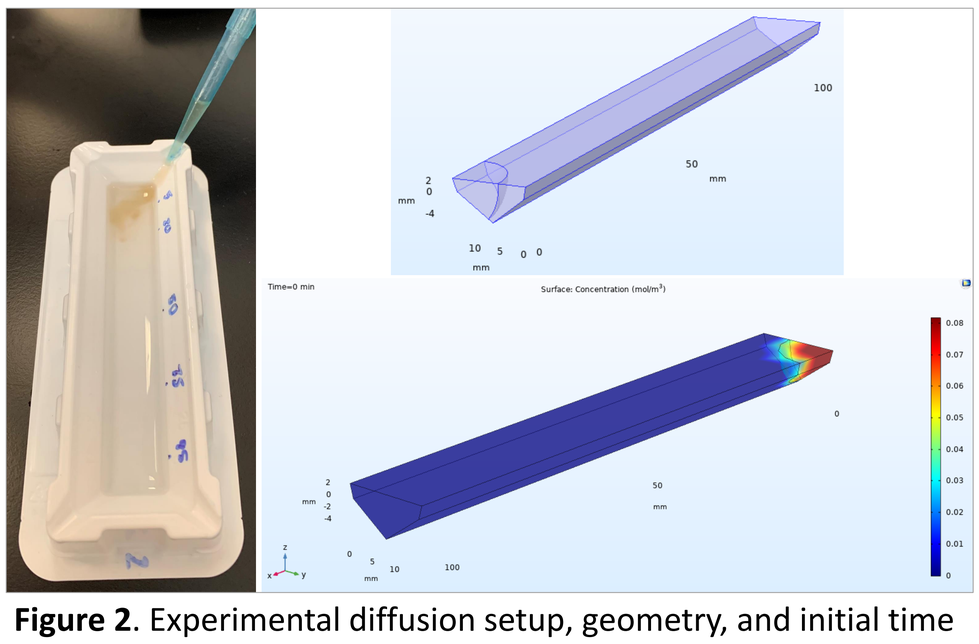

Figure 2. Experimental diffusion setup, geometry, and initial time condition at COMSOL simulation.

RESULTS: D values typical of biomacromolecules (such as 10^{-8} to 10^{-10} m^2/s) without the advection component (u = 0 m/s) expectedly showed a very slow diffusion simulation (Fig. 3), which did not match the experimental data. Observed KTN concentrations close to the region of KTN delivery produced high variability (Fig. 4) but the general trend followed the general diffusion kinetics, towards c/ceq = 1 (at t = ∞). It was found that the diffusivity coefficient of KTN in 10 mM NaOH is 1×10^{-6} m^2/s, based on MATLAB (Fig. 4) and verified using COMSOL (Fig. 5). This relatively high D (leading to high J (Fig. 6)) may also be driven by KTN acid to NaOH electrodynamic interactions and possibly unintended advection.

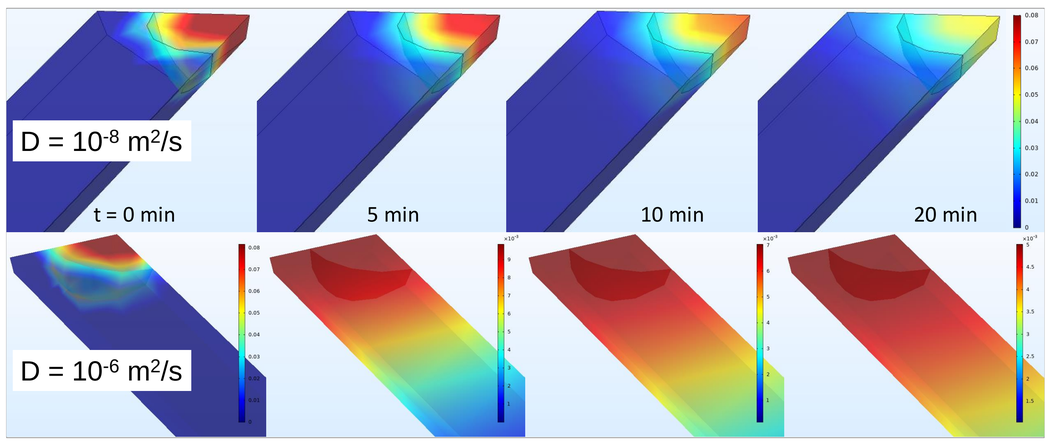

Figure 3. Simulation at different values of D.

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%% Method of Lines with D = diffusivity: Fick's 2nd Law of Diffusion
% by Prof. Roche C. de Guzman
% solute = KTN; solvent = 10 mM NaOH
clear; clc; close('all');
%% Given
xi = 0; xf = 105; dx = 1; % x range and step size = dx [mm]
xL = 0; xU = 105/11; % initial value x lower and upper limits [mm]
ti = 0; tf = 20; dt = 1/10; % t range and step size = dt [min]
ci = 0; % initial concentration value [mg/mL]
cLU = 8; % initial concentration value within x lower and upper limits [mg/mL]
D = 1e-2; % diffusivity [cm^2/s] D = 1e-6 [m^2/s]
%% Calculations
% D Einstein
    De = (1.38064852e-23*298.15)/(6*pi*50e-3*5e-10);
% SI units
    xi = xi/1e3; xf = xf/1e3; dx = dx/1e3; xL = xL/1e3; xU = xU/1e3; % [m]
    ti = ti*60; tf = tf*60; dt = dt*60; % [s]
    D = D/1e4; % [m^2/s]
% Independent variables: x and t
    X = xi:dx:xf; nx = numel(X); T = ti:dt:tf; nt = numel(T);  % x and t vectors and their sizes
    [x,t] = meshgrid(X,T); x = x'; t = t'; % x and t matrices
% Dependent variable: c
    c = ones(nx,nt)*ci; % temporary c(x,t) matrix with rows: c(x) and columns: c(t)
    % Initial values and Dirichlet boundary
        I = find((X>=xL)&(X<=xU)); % index of lower and upper limits
        c(I,1) = cLU; % c at t = 0 for lower and upper limits
    % System of ODEs
        f = @(t,Y) F(t,Y,D,nx,dx); % system of ODE fxs
        Yi = c(:,1); % vector of initial values
        [~,Y] = ode45(f,T',Yi); % solve for c
        c = Y'; % concentration matrix
    % Nondimentionalized
        xtot = xf-xi; xk = xU-xL; x = x/xtot; X = X/xtot;
        ceq = ((xk/xtot)*cLU)+(((xtot-xk)/xtot)*ci);c = c/ceq;
%% Results
% Animation
    for j = 1:nt
        plot(X,c(:,j)); xlabel('x/L'); ylabel('c/c_{eq}');
        title(['Concentration Versus Distance at Time = ' num2str(round(T(j),3)) ' s']) ;
        grid('on'); axis([0 1 0 max(max(c))]); drawnow;
    end
% Graph
    figure; plot(X,c(:,5i),'r-'); hold('on'); plot(X,c(:,101),'g-'); plot(X,c(:,nt));
    xlabel('x/L'); ylabel('c/c_{eq}'); legend({'5 min' '10 min' '20 min'});
%% Custom fx
function Yi = F(~,Y,D,nx,dx)
    c = Y; % assign concentration as Y
    Yi = zeros(nx,1); % temporary derivatives
    for i = 1:nx-2 % position counter
        Yi(i+1) = D*(c(i+2)-2*c(i+1)+c(i))/dx^2; % solve for derivatives
    end
    Yi = [Yi(2); Yi(2:nx-1); Yi(nx-1)]; % derivatives with zero-flux boundary
end
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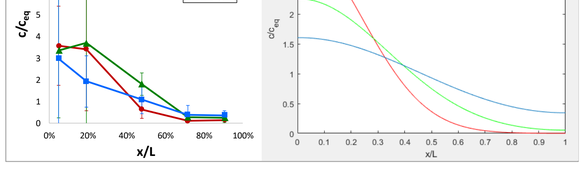

Figure 4. Experimental (left) and MATLAB model (top & right) of KTN c.

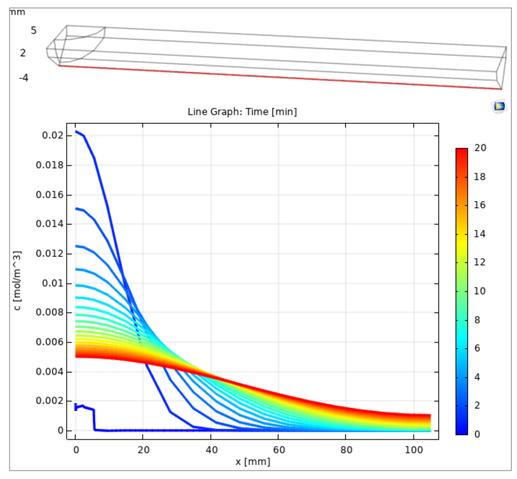

Figure 5. 1D (cut plane from the channel bottom) time-dependent response of concentration.

Name	Expression	Value	Description
cn_KTN	c_KTN/MW_KTN	0.081633 mol/m^3	
the1	atan(Wh/Whe)	1.2925 rad	angle 1
the2	atan((H-Wh)/Wh)	0.62025 rad	angle 2
Whe	2 [mm]	0.002 m	extra half width
Wh	(W/2)-Whe	0.007 m	half width
H	12 [mm]	0.012 m	height
c_KTN	8 [mg/ml]	8 kg/m^3	initial keratin concentration
V_KTN	1 [ml]	1E-6 m^3	initial volume
t_KTN	20 [min]	1200 s	keratin delivery time
D_KTN	1e-6[m^2/s]	1E-6 m^2/s	keratin diffusivity in 10 mM NaOH
L	105 [mm]	0.105 m	length
MW_KTN	98 [kg/mol]	98 kg/mol	molecular mass of KTN
T	25 [degC]	298.15 K	temperature
W	18 [mm]	0.018 m	width

Table 1. Parameters.

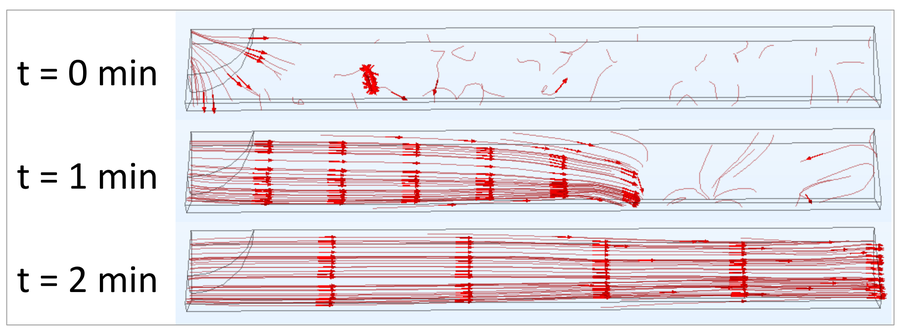

Figure 6. Diffusive mole flux vector streamlines.

CONCLUSIONS: KTN diffusivity was obtained using a simple experimental approach and is now utilized to model different KTN-based systems, but requires further verification using more accurate instrumentations.

REFERENCES:

1. de Guzman R.C. *et al.*, Bone regeneration with BMP-2 delivered from keratose scaffolds, Biomaterials, 34, 1644-1656 (2013).
2. de Guzman R.C. *et al.*, Binding interactions of keratin-based hair fiber extract to gold, keratin, and BMP-2, PLOS ONE, 10, e0137233 (2015).

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