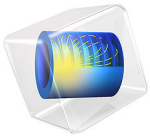

Degradation of DNA in Plasma

Introduction

Gene therapy is one biotechnology example of a clinical application where it is possible to produce proteins *in vivo*, using the body's own mechanisms for protein production. Major issues in gene delivery involve the transport of plasmid DNA (pDNA) to target sites and the conversion between different forms of pDNA.

This example uses the Parameter Estimation interface to find the rate constants of three consecutive reactions involved in a DNA degradation process, as well as the initial concentration of the pDNA.

Model Description

pDNA can be used to express proteins in the human body, proteins that can have therapeutic effects. pDNA exists in three forms — a supercoiled form (SC), an open-circular form (OC), and a linear form (L) — each with varying protein-expression rates. These pDNA-forms interconvert and degrade with time, which means a patient's therapy benefits from knowledge about the distribution of pDNA-forms over time.

The protein expression rate for the SC form is greater than the one for the OC form, which in turn is significantly greater than that for the L form. The kinetic model in this study assumes that the pDNA-forms interconvert and decompose according to the mechanism in Figure 1.

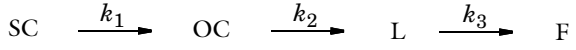

Figure 1: Kinetic model of plasmid DNA interconversion and decomposition. Supercoiled pDNA (SC) converts to an open-circular form (OC), which in turn converts to a linear form (L). The linear pDNA decomposes to form linear fragments (F).

This example proposes a set of irreversible reactions in which an SC-form pDNA converts to the OC form and then to the L form. Then the L-form decomposes into a number of linear fragments, collectively denoted as F.

The reaction rate expressions in three irreversible reactions in Figure 1 are:

$$r_1 = k_1 c_{\mathrm{SC}}$$

$$r_2 = k_2 c_{\mathrm{OC}}$$

$$r_3 = k_3 c_{\mathrm{L}}$$

The rate constants k_1 through k_3 will be determined by parameter estimation, making use of the experimental data summarized in the table:

TABLE 1: EXPERIMENTAL CONCENTRATION DATA.

Time (s)	c_{SC} (ng/μl)	c_{OC} (ng/μl)	c_L (ng/μl)
5	9.3	0.5	0
60	5.0	4.1	0.1
120	3.5	6.5	0.3
180	1.1	7.0	0.5
300	0.5	8.1	0.8
420	0.1	8.0	1.2
600	0	7.8	1.7
900	0	7.1	2.4
1200	0	6.3	2.5
1800	0	4.5	2.6
2400	0	3.0	2.0
3000	0	2.1	1.8
3600	0	1.5	1.2

The concentration unit for the experimental data in the able above is [ng/μl], while the concentration unit in Reaction Engineering is [mol/m^3]. The mass concentration [ng/μl] is converted to the molar concentration [mol/m^3] by divided the former with the plasmid DNA molecular weight M_pDNA ($1.95 \cdot 10^6$ [g/mol]).

Results and Discussion

The following rate constants are calculated from the experimental data and proposed reaction mechanism: $k_1 = 9.5 \cdot 10^{-3}$ (1/s), $k_2 = 5.2 \cdot 10^{-4}$ (1/s), and $k_3 = 1.0 \cdot 10^{-3}$ (1/s). In addition, the initial concentration of the SC species is estimated to 9.9 ng/μl.

Figure 2 shows the experimental values in the same plot as the simulation results. Clearly, the assumptions of the kinetic model are in agreement with the experimental findings.

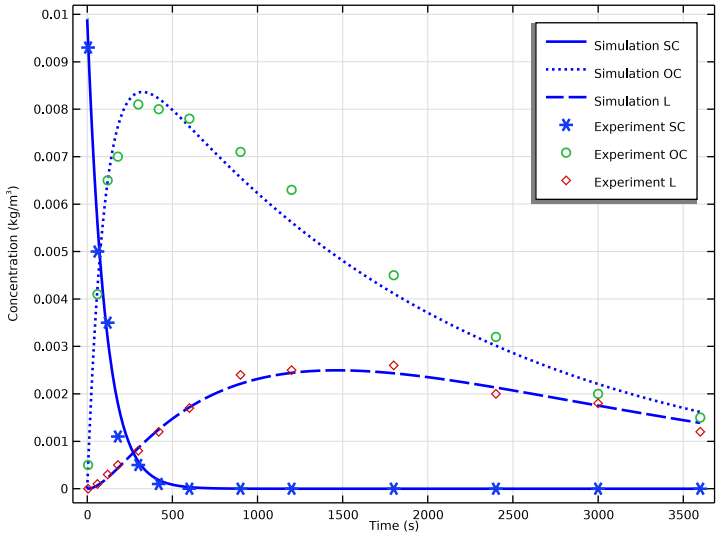

Figure 2: Experimental concentration data compared to simulation results.

The estimated rate constants show that the supercoiled pDNA rapidly transforms into the open-circular form with a half-life of approximately **1.2 minutes**:

$$t_{1/2} = \frac{\ln 2}{k}$$

The open-circular and linear pDNA decay with half-lives of **22** and **11** minutes, respectively. As mentioned, the supercoiled pDNA has the highest protein-expression rate of the three forms. However, because the SC form has a half-life of only **1.2 minutes**, it is likely that it decomposes during transport to the therapeutic target sites. These findings imply that you have to find ways to hinder the relatively fast decay of SC.

Reference

1. B.E. Houk, G. Hochhaus, and J.A. Hughes, "Kinetic modeling of plasmid DNA degradation in rat plasma," *AAPS Pharmsci*, vol. 1, no. 3, pp. 15–20, 1999.

Application Library path: `Chemical_Reaction_Engineering_Module/Ideal_Tank_Reactors/dna_degradation`

Modeling Instructions

From the **File** menu, choose **New**.

NEW

In the **New** window, click **Model Wizard**.

MODEL WIZARD

1 In the **Model Wizard** window, click **0D**.

2 In the **Select Physics** tree, select **Chemical Species Transport>Reaction Engineering (re)**.

3 Click **Add**.

4 Click **Study**.

5 In the **Select Study** tree, select **General Studies>Time Dependent**.

6 Click **Done**.

GLOBAL DEFINITIONS

Read model parameters from a text file.

Parameters 1

1 In the **Model Builder** window, under **Global Definitions** click **Parameters 1**.

2 In the **Settings** window for **Parameters**, locate the **Parameters** section.

3 Click **Load from File**.

4 Browse to the model's Application Libraries folder and double-click the file `dna_degradation_parameters.txt`.

Start by entering the reaction properties in the **Reaction Engineering** interface.

REACTION ENGINEERING (RE)

The main fluid for DNA degradation in plasma consists of water. Set the **Phase** to "Liquid".

1 In the **Model Builder** window, under **Component 1 (comp1)** click **Reaction Engineering (re)**.

2 In the **Settings** window for **Reaction Engineering**, locate the **Mixture Properties** section.

3 From the **Phase** list, choose **Liquid**.

Reaction 1

1 In the **Reaction Engineering** toolbar, click  **Reaction**.

2 In the **Settings** window for **Reaction**, locate the **Reaction Formula** section.

3 In the **Formula** text field, type `SC=>OC`.

4 Locate the **Rate Constants** section. In the k^{f} text field, type `k1`.

Species: SC

1 In the **Model Builder** window, click **Species: SC**.

2 In the **Settings** window for **Species**, locate the **Chemical Formula** section.

3 Clear the **Enable formula** check box.

Species: OC

1 In the **Model Builder** window, click **Species: OC**.

2 In the **Settings** window for **Species**, locate the **Chemical Formula** section.

3 Clear the **Enable formula** check box.

Reaction 2

1 In the **Reaction Engineering** toolbar, click  **Reaction**.

2 In the **Settings** window for **Reaction**, locate the **Reaction Formula** section.

3 In the **Formula** text field, type `OC=>L`.

4 Locate the **Rate Constants** section. In the k^{f} text field, type `k2`.

Reaction 3

1 In the **Reaction Engineering** toolbar, click  **Reaction**.

2 In the **Settings** window for **Reaction**, locate the **Reaction Formula** section.

3 In the **Formula** text field, type `L=>F`.

4 Locate the **Rate Constants** section. In the k^{f} text field, type `k3`.

Species: F

1 In the **Model Builder** window, click **Species: F**.

2 In the **Settings** window for **Species**, locate the **Chemical Formula** section.

3 Clear the **Enable formula** check box.

Species 1

The species are dissolved in water. Add water as a solvent (the solvent water does not affect the final result).

1 In the **Reaction Engineering** toolbar, click **Species**.

2 In the **Settings** window for **Species**, locate the **Name** section.

3 In the text field, type `H2O`.

4 Locate the **Type** section. From the list, choose **Solvent**.

Enter the initial values for the species in the system.

Initial Values 1

1 In the **Model Builder** window, click **Initial Values 1**.

2 In the **Settings** window for **Initial Values**, locate the **Volumetric Species Initial Values** section.

3 In the table, enter the following settings:

Species	Concentration (mol/m^3)
H2O	`c_H2O_init`
SC	`c_SC_init`

COMPONENT 1 (COMP1)

Add a **Parameter Estimation** interface to optimize the three reaction rate constants, and the initial concentration of pDNA.

Global Least-Squares Objective 1

1 In the **Model Builder** window, right-click **Component 1 (comp1)** and choose **Parameter Estimation**.

2 In the **Settings** window for **Global Least-Squares Objective**, locate the **Experimental Data** section.

3 Click **Browse**.

4 Browse to the model's Application Libraries folder and double-click the file `dna_degradation_experiment1.csv`.

5 Locate the **Data Column Settings** section. In the table, click to select the cell at row number 2 and column number 1.

6 In the **Model expression** text field, type `re.c_SC*M_pDNA`.

7 In the **Variable name** text field, type `SC`.

Note that the concentration unit in the imported data file is ng/µl. Enter unit ng/ul in the **Unit** field.

8 In the **Unit** text field, type `ng/ul`.

9 In the table, click to select the cell at row number 3 and column number 1.

10 In the **Model expression** text field, type `re.c_OC*M_pDNA`.

11 In the **Variable name** text field, type `OC`.

12 In the **Unit** text field, type `ng/ul`.

13 In the table, click to select the cell at row number 4 and column number 1.

14 In the **Model expression** text field, type `re.c_L*M_pDNA`.

15 In the **Variable name** text field, type `L`.

16 In the **Unit** text field, type `ng/ul`.

STUDY 1

Solve the model to get a solution with the initial values, without optimization.

Step 1: Time Dependent

1 In the **Model Builder** window, under **Study 1** click **Step 1: Time Dependent**.

2 In the **Settings** window for **Time Dependent**, locate the **Study Settings** section.

3 In the **Output times** text field, type `0 3600`.

4 In the **Home** toolbar, click **Compute**.

Parameter Estimation

Now add a **Parameter Estimation** study step.

1 In the **Study** toolbar, click **Optimization** and choose **Parameter Estimation**.

Select the parameters to be estimated and provide an initial guess. The parameter c_SC_init will be used to estimate the initial concentration of the species SC. Also provide scales for the estimated parameters. Prescribing scales for the estimation parameters increases the efficiency of the optimization procedure. A good starting point is to use scales of the same order as the initial values.

2 In the **Settings** window for **Parameter Estimation**, locate the **Estimated Parameters** section.

3 Click **Add** four times.

4 In the table, enter the following settings:

Parameter name	Initial value	Scale	Lower bound	Upper bound
k1 (Forward rate constant)	`1e-3[1/s]`	`1e-3`	0	
k2 (Forward rate constant)	`1e-3[1/s]`	`1e-3`	0	

Parameter name	Initial value	Scale	Lower bound	Upper bound
k3 (Forward rate constant)	`1e-3[1/s]`	`1e-3`	0	
c_SC_init (Initial concentration)	`10[ng/ul]/M_pDNA`	`10[ng/ul]/M_pDNA`	0	

5 Locate the **Parameter Estimation Method** section. In the **Optimality tolerance** text field, type `0.0001`.

Use **Output While Solving** to visualize the impact of the optimization on the model. Prepare a plot for this by modifying the default plot created in the last computation.

RESULTS

Concentration (re)

1 In the **Model Builder** window, under **Results** click **Concentration (re)**.

2 In the **Settings** window for **1D Plot Group**, click to expand the **Title** section.

3 From the **Title type** list, choose **None**.

4 Locate the **Plot Settings** section. Select the **x-axis label** check box.

5 Select the **y-axis label** check box. In the associated text field, type `Concentration (kg/m<sup>3</sup>)`.

Simulation Data

1 In the **Model Builder** window, expand the **Concentration (re)** node, then click **Global 1**.

2 In the **Settings** window for **Global**, type `Simulation Data` in the **Label** text field.

3 Locate the **y-Axis Data** section. In the table, enter the following settings:

Expression	Unit	Description
`re.c_SC*M_pDNA`	`kg/m^3`	`SC`
`re.c_OC*M_pDNA`	`kg/m^3`	`OC`
`re.c_L*M_pDNA`	`kg/m^3`	`L`

4 Click to expand the **Coloring and Style** section. Find the **Line style** subsection. From the **Line** list, choose **Cycle**.

5 From the **Width** list, choose **2**.

6 From the **Color** list, choose **Blue**.

7 Click to expand the **Legends** section. Find the **Include** subsection. Select the **Description** check box.

8 Clear the **Solution** check box.

9 Clear the **Expression** check box.

10 Find the **Prefix and suffix** subsection. In the **Prefix** text field, type `Simulation ` .

Add a table with the experimental data, and then plot that data together with the data from the simulation. Remember the unit of the experimental data. Divide the data with 1000 to get it in kg/m^3, which is the same as the data plotted from the simulation.

Table 1

1 In the **Results** toolbar, click  **Table**.

2 In the **Settings** window for **Table**, locate the **Data** section.

3 Click  **Import**.

4 Browse to the model's Application Libraries folder and double-click the file `dna_degradation_experiment1.csv`.

Experimental Data

1 In the **Model Builder** window, right-click **Concentration (re)** and choose **Table Graph**.

2 In the **Settings** window for **Table Graph**, type `Experimental Data` in the **Label** text field.

3 Click to expand the **Preprocessing** section. Find the **y-axis columns** subsection. From the **Transformation** list, choose **Linear**.

4 In the **Scaling** text field, type `1/1000`.

5 Locate the **Coloring and Style** section. Find the **Line style** subsection. From the **Line** list, choose **None**.

6 Find the **Line markers** subsection. From the **Marker** list, choose **Cycle**.

7 Click to expand the **Legends** section. Select the **Show legends** check box.

8 From the **Legends** list, choose **Manual**.

9 In the table, enter the following settings:

Legends
`Experiment SC`
`Experiment OC`
`Experiment L`

The plot to use for **Output While Solving** is prepared. Time to solve the model.

STUDY 1

Parameter Estimation

1 In the **Model Builder** window, under **Study 1** click **Parameter Estimation**.

2 In the **Settings** window for **Parameter Estimation**, click to expand the **Output While Solving** section.

3 Select the **Plot** check box.

4 In the **Home** toolbar, click **Compute**.

RESULTS

Concentration (re)

1 In the **Concentration (re)** toolbar, click **Plot**.

2 Click the  **Zoom Extents** button in the **Graphics** toolbar.

Objective Probe Table 2

The values of the estimated parameters are found in table **Objective Probe Table 2**.