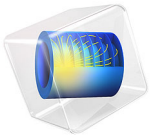

Degradation of DNA in Plasma

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 feature with the Reaction Engineering interface to find the rate constants of three consecutive reactions involved in a DNA degradation process.

Note: This application requires the Optimization Module.

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.

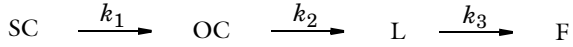

Figure 1: Kinetic model of plasmid DNA interconversion and decomposition. Supercoiled pDNA (SC) converts to an open-circular form (OC), which in turn converts to a linear form (L). The linear pDNA decomposes to form linear fragments (F).

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 three irreversible reactions in Figure 1 translate into these reaction rate expressions:

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

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

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

The rate constants k_1 through k_3 are found 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 unit of the experimental data in the table above is ng/μl (= $1{\cdot}10^{-3}$ kg/m^3). For consistent units the model variable, used in the optimization, is defined as the molar concentration (mol/m^3) times the molar mass (kg/mol).

Results and Discussion

The following rate constants are calculated from the experimental data and proposed reaction mechanism: $k_1 = 9.6{\cdot}10^{-3}$ (1/s), $k_2 = 4.8{\cdot}10^{-4}$ (1/s), and $k_3 = 9.6{\cdot}10^{-4}$ (1/s). In addition, the initial concentration of the SC species is estimated to 9.7 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.

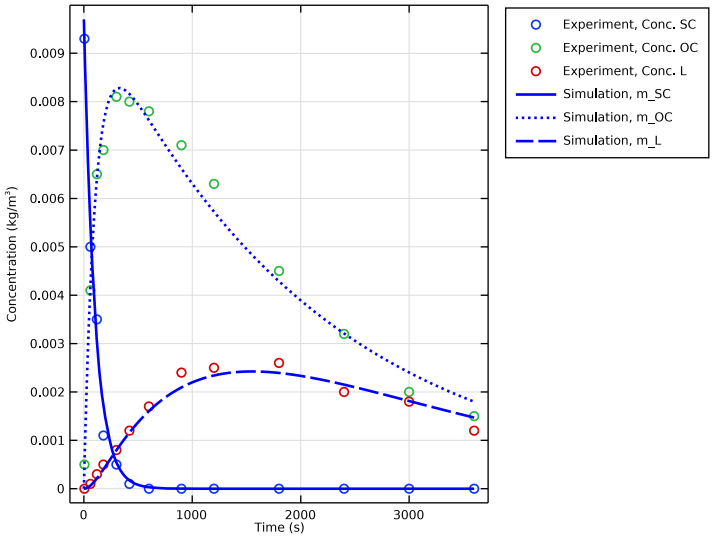

Figure 2: A plot resulting from reading in experimental data and comparing it to simulation results.

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 24.1 and 12.0 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`.

Read variable definitions from a text file.

DEFINITIONS

Variables 1

1 In the **Model Builder** window, under **Component 1 (comp1)** right-click **Definitions** and choose **Variables**.

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

3 Click **Load from File**.

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

The variable expressions are shown in yellow since the model variables `re.c_SC`, `re.c_SC and re.c_SC` are not created yet.

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 the "Liquid".

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

2 In the **Settings** window for **Reaction Engineering**, click to expand 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**.

Parameter Estimation 1

Choose a **Parameter Estimation** feature to optimize the three reaction rate constants in the reactions entered in the interface.

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

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

3 In the **Parameter** table, enter the following settings:

Parameter	Initial value	Scale	Lower bound	Upper bound
k1	1e-3	1e-3		

4 Click **Add**.

5 In the **Parameter** table, enter the following settings:

Parameter	Initial value	Scale	Lower bound	Upper bound
k2	1e-3	1e-3		

6 Click + **Add**.

7 In the **Parameter** table, enter the following settings:

Parameter	Initial value	Scale	Lower bound	Upper bound
k3	1e-3	1e-3		

8 Click + **Add**.

9 In the **Parameter** table, enter the following settings:

Parameter	Initial value	Scale	Lower bound	Upper bound
c_SC_init	10[ng/ul]/M_pDNA	10[ng/ul]/M_pDNA		

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.

Experiment 1

Select an **Experiment** feature to import experimental data to which the simulation will be optimized.

1 In the **Reaction Engineering** toolbar, click **Attributes** and choose **Experiment**.

2 In the **Settings** window for **Experiment**, 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 Click **Import**.

Note that the concentration unit in the imported data file is ng/µl, while the unit of concentration variable in **Reaction Engineering** is mol/m^3. To specify the unit, enter ng/ul in the **Unit** column of the **Experimental Data** section. The mass concentrations equal the molar concentrations multiplied by the molar mass (`M_pDNA`), as defined in the **Variables 1** node above. Enter these variables in the column **Model variables**.

6 In the table, enter the following settings:

Data column	Use	Model variables	Unit	Weight
Time	√	t	1	1
Conc. SC	√	m_SC	ng/ul	1

Data column	Use	Model variables	Unit	Weight
Conc. OC	√	m_OC	ng/ul	1
Conc. L	√	m_L	ng/ul	1

Initial Values 1

The default unit in **Initial Values** feature is mol/m^3. For water, the density is 1000 [kg/m^3], its molar concentration (mol/m^3) is `c_H2O_init = 1000 [kg/m^3]/18 [g/mol]`.

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

In the **Study** node, add an optimization step to finalize the optimization settings.

STUDY 1

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`.

Optimization

1. In the **Study** toolbar, click **Optimization** and choose **Optimization**.
2. In the **Settings** window for **Optimization**, locate the **Optimization Solver** section.
3. From the **Method** list, choose **Levenberg-Marquardt**.
4. In the **Optimality tolerance** text field, type `1.0E-4`.
5. Locate the **Output While Solving** section. Select the **Plot** check box.
6. In the **Study** toolbar, click **Compute**.

Follow these steps to create Figure 2. The experimental and simulation data should match.

RESULTS

Concentrations

1 In the **Model Builder** window, under **Results** click **Experiment 1 Group**.

2 In the **Settings** window for **1D Plot Group**, type `Concentrations` in the **Label** text field.

3 Click to expand the **Title** section. From the **Title type** list, choose **None**.

4 Locate the **Plot Settings** section.

5 Select the **x-axis label** check box. In the associated text field, type `Time (s)`.

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

7 Locate the **Legend** section. From the **Layout** list, choose **Outside graph axis area**.

Experimental Data

1 In the **Model Builder** window, expand the **Concentrations** node, then click **Experiment 1 Data**.

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

3 Click to expand the **Legends** section. From the **Legends** list, choose **Automatic**.

4 Find the **Prefix and suffix** subsection. In the **Prefix** text field, type `Experiment, `.

Simulation Data

1 In the **Model Builder** window, under **Results>Concentrations** click **Global 1**.

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

3 Click **Replace Expression** in the upper-right corner of the **y-Axis Data** section. From the menu, choose **Component 1 (comp1)>Definitions>Variables>m_SC - Mass supercoiled DNA - kg/m³**.

4 Click **Add Expression** in the upper-right corner of the **y-Axis Data** section. From the menu, choose **Component 1 (comp1)>Definitions>Variables>m_OC - Mass open-circular form DNA - kg/m³**.

5 Click **Add Expression** in the upper-right corner of the **y-Axis Data** section. From the menu, choose **Component 1 (comp1)>Definitions>Variables>m_L - Mass linear form DNA - kg/m³**.

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

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

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

9 From the **Legends** list, choose **Automatic**.

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

11 In the **Concentrations** toolbar, click **Plot**.

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

Output the values of the estimated parameters to a table.

13 In the **Results** toolbar, click **Evaluate** and choose **Evaluate All**.

Last plot group is not used and is therefore removed.

Concentration (re)

In the **Model Builder** window, under **Results** right-click **Concentration (re)** and choose **Delete**.