



Model created in COMSOL Multiphysics 6.4

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](#).

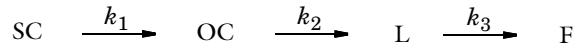


Figure 1: Kinetic model of plasmid DNA interconversion and decomposition. Supercoiled pDNA (SC) converts to an open-circular form (OC), which in turn converts to a linear form (L). The linear pDNA decomposes to form linear fragments (F).

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:

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

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

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

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 I: EXPERIMENTAL CONCENTRATION DATA.

Time (s)	c_{SC} (ng/ μ l)	c_{OC} (ng/ μ l)	c_L (ng/ μ l)
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 table above is [ng/ μ l], while the concentration unit in Reaction Engineering is [mol/ m^3]. The mass concentration [ng/ μ l] is converted to the molar concentration [mol/ m^3] by dividing 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/ μ l.

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.

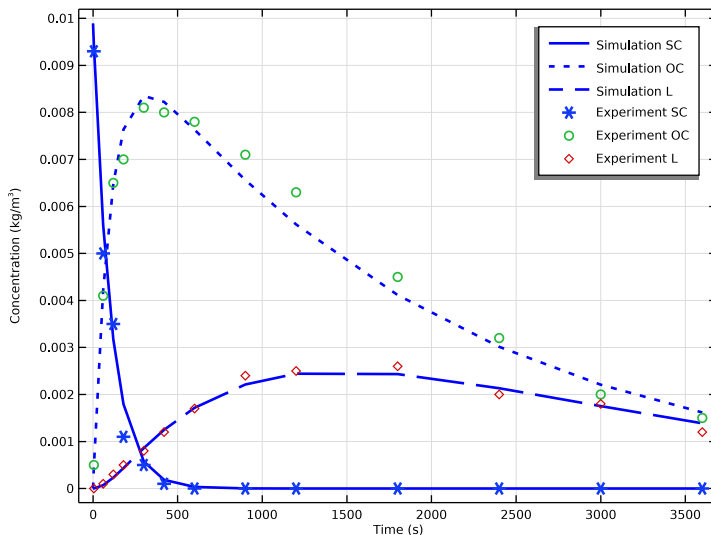


Figure 2: Experimental concentration data compared to simulation results.

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  **OD**.
- 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 $SC \Rightarrow OC$.
- 4 Locate the **Rate Constants** section. In the k^f text field, type k_1 .


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** checkbox.


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** checkbox.

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 $OC \Rightarrow L$.
- 4 Locate the **Rate Constants** section. In the k^f text field, type k_2 .

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 $L \Rightarrow F$.
- 4 Locate the **Rate Constants** section. In the k^f text field, type k_3 .

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** checkbox.

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.

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 **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 Click  **Import**.
- 6 Locate the **Data Column Settings** section. In the table, click to select the cell at row number 2 and column number 1.
- 7 In the **Model expression** text field, type `re.c_SC*M_pDNA`.

8 In the **Column name** text field, type SC.

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

9 In the **Unit** text field, type ng/ μ l.

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

11 In the **Model expression** text field, type $re.c_{OC} * M_{pDNA}$.

12 In the **Column name** text field, type OC.

13 In the **Unit** text field, type ng/ μ l.

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

15 In the **Model expression** text field, type $re.c_L * M_{pDNA}$.

16 In the **Column name** text field, type L.

17 In the **Unit** text field, type ng/ μ l.

STUDY I


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 I** 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 **Study** 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	Initial value	Scale	Lower bound	Upper bound	Unit
k1 (Forward rate constant)	1e-3[1/s]	1e-3	0		l/s
k2 (Forward rate constant)	1e-3[1/s]	1e-3	0		l/s
k3 (Forward rate constant)	1e-3[1/s]	1e-3	0		l/s
c_SC_init (Initial concentration)	10[ng/u1]/M_pDNA	10[ng/u1]/M_pDNA	0		mol/m ³

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 **ID 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** checkbox.
- 5 Select the **y-axis label** checkbox. In the associated text field, type Concentration (kg/m³).

Simulation Data



- 1 In the **Model Builder** window, expand the **Concentration (re)** node, then click **Global I**.
- 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** checkbox.
- 8 Clear the **Solution** checkbox.
- 9 Clear the **Expression** checkbox.
- 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** checkbox.
- 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 I

Parameter Estimation

- 1 In the **Model Builder** window, under **Study I** click **Parameter Estimation**.
- 2 In the **Settings** window for **Parameter Estimation**, click to expand the **Output** section.
- 3 Select the **Plot** checkbox.
- 4 In the **Study** 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**.