Parameter Estimation and Prediction for Time-Dependent Concentration Response Curves for Cytotoxicity Assessment

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Abstract

We propose a model based on the logistic equation and linear kinetics to study the effect of toxicants with various initial concentrations on a cells' population. To efficiently estimate the model's parameters, we design an Expectation Maximization algorithm. The model is validated by showing that it accurately represents the information provided by in-vitro experiments.

Introduction

At the Alberta Centre of Toxicology the effect of various toxicants on growth/death and morphology of human cells is investigated using the xCELLigence Real-Time Cell Analysis High Troughput in vitro assay. The cell index is measured as a proxy for the number of cells, and for each test substance in each cell line, time-dependent concentration response curves (TCRCs) are generated. The toxicants are grouped in clusters, according to the mode of action. The goal of this study is to find a model that could accurately reproduce these curves.

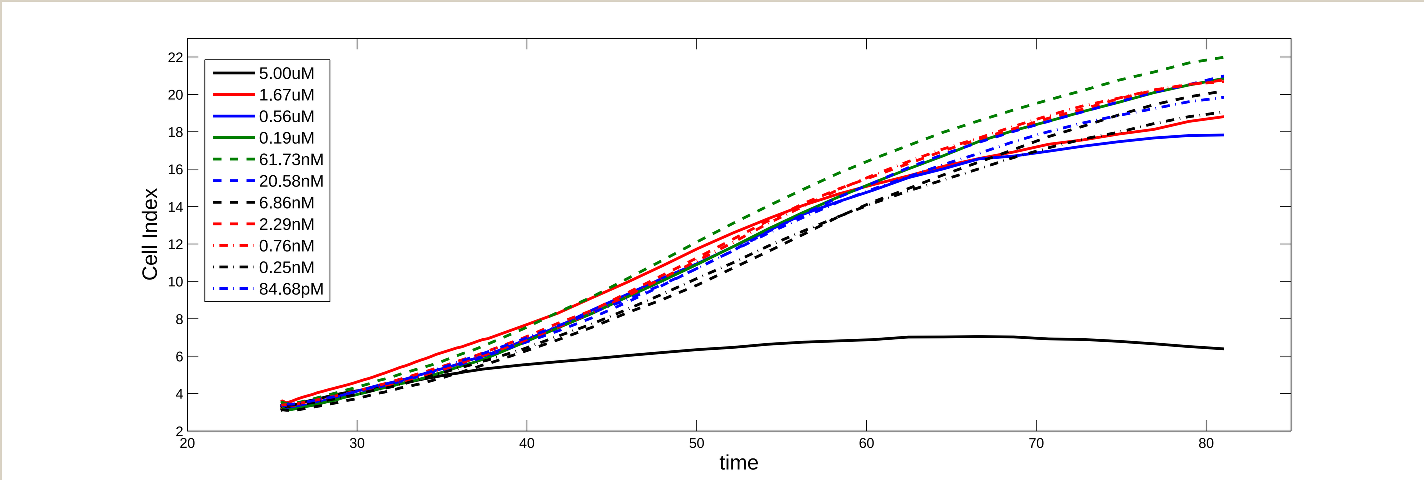

Figure 1: TCRCs for the toxicant PF431396

The Model

$$\frac{dn(t)}{dt} = \beta n(t)(1-\frac{n(t)}{K}) - \alpha C_0(t)n(t),$$
$$\frac{dC_0(t)}{dt} = \lambda_1^2 CE(t) - \eta_1^2 C_0(t),$$
$$\frac{dCE(t)}{dt} = \lambda_2^2 C_0(t)n(t) - \eta_2^2 CE(t)n(t)$$

$n(t)$: cell index at time t
$C_0(t)$: internal toxicant concentration
$CE(t)$: external toxicant concentration
β: cell growth rate
K: capacity volume

λ_1^2, η_1^2: toxicant uptake and input rates from environment
λ_2^2, η_2^2: toxicant uptake and loss rates from cells

To estimate the parameters we write the system in a state-space form. From the experimental data recorded in the TCRCs we get observations, possibly affected by measurement errors, only for the cell index $n(t)$. Using the Euler integration scheme with time step h, we get the discrete state-space system:

$$x_{k+1} = x_k + h\begin{bmatrix} \beta x_k[1](1-\frac{x_k[1]}{K}) - \alpha x_k[2]x_k[1] \\ \lambda_1^2 x_k[3] - \eta_1^2 x_k[2] \\ \lambda_2^2 x_k[2]x_k[1] - \eta_2^2 x_k[3]x_k[1] \end{bmatrix} + v_{k+1}$$
$$y_{k+1} = Cx_{k+1} + w_{k+1}$$

- $x_{k+1} = [n, C_0, CE]^t$ is the state of the system
- y_{k+1} is the observation at time step $k+1$
- v_k and w_k, are uncorrelated, $v_k \sim N(0,Q)$ and $w_k \sim N(0,R)$
- h is the time step; $C = [1 \quad 0 \quad 0]$; $x_1[1] = n(0)$, $x_1[2] = C_0(0) = 0$ and $x_1[3] = CE(0)$ comes from the measured data at $t_0 = 0$

Parameter Estimation

- Using the experimental data corresponding to the negative control (no toxicant) we can estimate β and K using the nonlinear least square method based on the analytic solution of the logistic equation.
- The remaining parameters $\Theta = \{Q, R, \alpha, \lambda_1, \lambda_2, \eta_1, \eta_2\}$ are estimated using the state-space model and the Expectation Maximization (EM) algorithm based on the the unscented filter (UF).
- The UF is an alternative to the extended Kalman filter (EKF) to calculate the filtered values $\bar{x}_i = E[x_i|y_1,\dots,y_i]$, $\bar{P}_i = E\left[(x_i-\bar{x}_i)(x_i-\bar{x}_i)^t|y_1,\dots,y_i\right]$, the predicted values $\hat{x}_{i+1} = E[x_{i+1}|y_1,y_2,\dots,y_i]$, $\hat{P}_{i+1} = E[(x_{i+1}-\hat{x}_{i+1})(x_{i+1}-\hat{x}_{i+1})^t|y_1,\dots,y_i]$ and the smoothed values $x_{i|N} = E[x_i|y_1\dots y_N]$ and $P_{i|N} = E[\left(x_i - x_i^N\right)\left(x_i - x_i^N\right)^t|y_1\dots y_N]$ based on the available observations $y_1 \dots y_N$.
- The state equation is non-linear, so an approximation is needed during the E-step. The likelihood and the conditional likelihood $P(x_1,\dots,x_N,y_1,\dots,y_N|y_1,\dots,y_N)$ are approximated based on a linearization of the state equation.

The EM Algorithm

Initialize the model parameters $\Theta = \{Q, R, \alpha, \lambda_1, \lambda_2, \eta_1, \eta_2\}$
Repeat until the log likelihood has converged
  The E step: compute $\hat{E} = E[\log P(x_1,\dots,x_N,y_1,\dots,y_N)|y_1,\dots,y_N]$
    For k=1 to N
      Run the UF filter to compute $\bar{x}_{k+1}$, $\bar{P}_{k+1}$, $\hat{x}_{k+1}$, $\hat{P}_{k+1}$ and $\bar{P}_{x_k x_{k+1}}$
    For k=N to 1
      Calculate the smoothed values $x_{k|N}$, and $P_{k|N}$
  The M step
    Update the values of the parameters Θ to maximize $\hat{E}$

Model Validation

- We divide the experimental data into a training set (around 70% of the data), and a test set. We use the N observations in the training set to estimate the parameters.
- Once the parameters are estimated, we can predict the future values of x_i, $i = N+1, N+2, \dots$. We validate the model by comparing these predictions with the experimental data in the test set.

Table 1: Estimated Values of Parameters

Toxicant	Cluster	β	K	η_1	λ_1	λ_2	η_2	α
monastrol	X	0.074	18.17	0.209	0.177	0.204	0.5	0.016
HA1100 hydrochloride	I	0.077	21.913	0.143	0.0098	0.0786	0.147	0.351

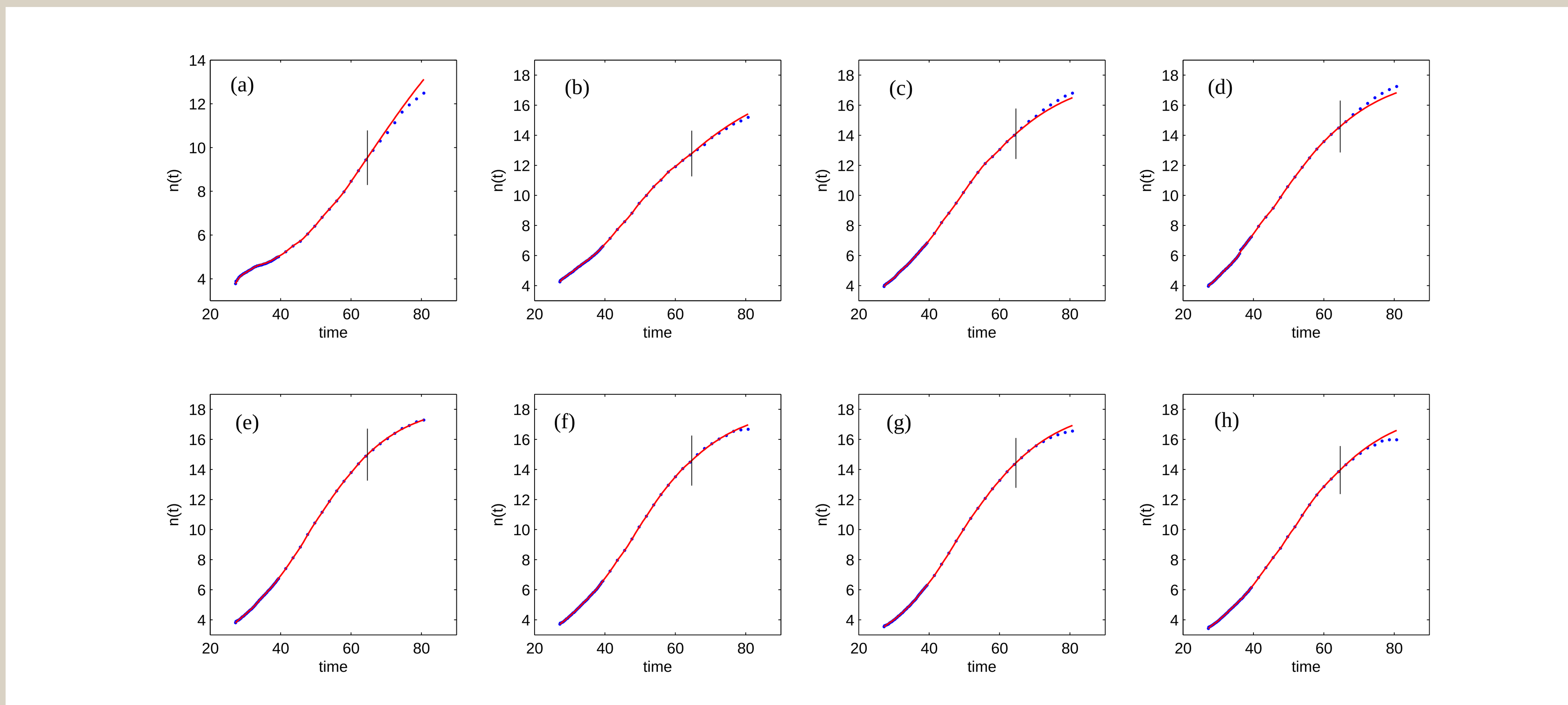

Figure 2: Estimation results for monastrol: dot for experimental data, line for filtered or predicted observations; (a) CE(0)=100.00uM, (b) CE(0)=33.33uM, (c) CE(0)=11.11uM, (d) CE(0)= 3.70uM, (e) CE(0)=1.23uM, (f) CE(0)= 0.41uM, (g) CE(0)=0.14uM, (h) CE(0)=45.72nM

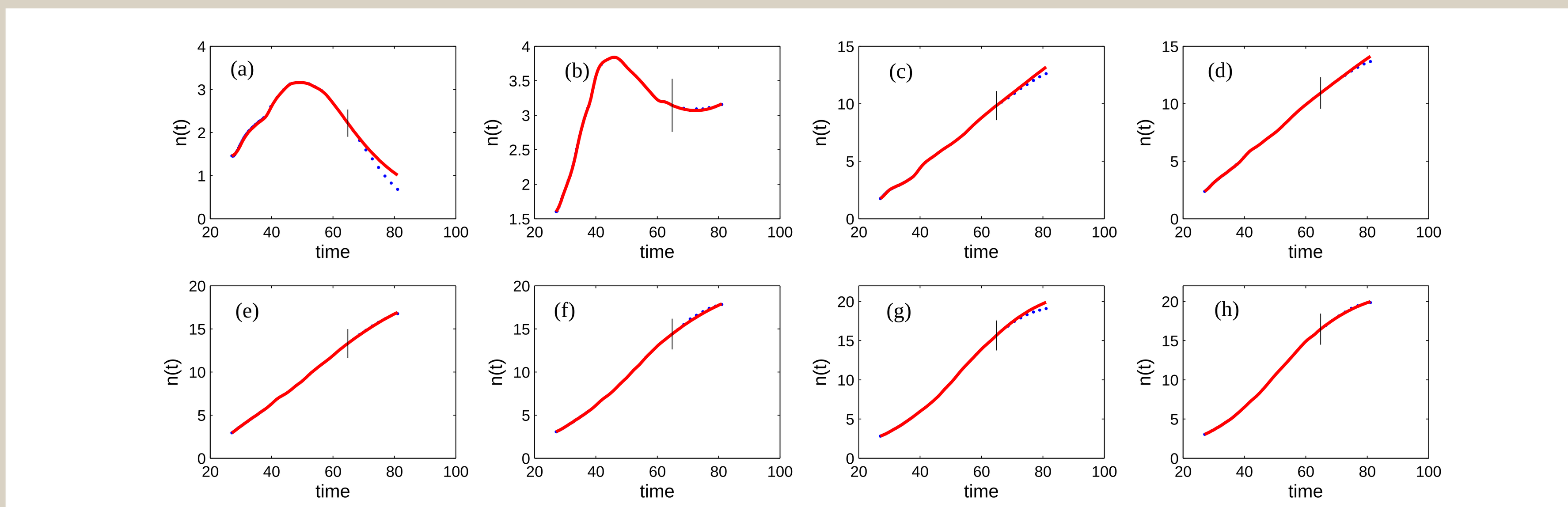

Figure 3: Estimation results for HA1100 hydrochloride: dot for experimental data, line for filtered or predicted observations; (a) CE(0)=1.00mM, (b) CE(0)=0.33mM, (c) CE(0)=0.11mM, (d) CE(0)= 37.04uM, (e) CE(0)=12.35uM, (f) CE(0)=4.12uM, (g) CE(0)=1.37uM, (h) CE(0)= 0.46uM

Conclusions

- The proposed mathematical model is in good agreement with the experimental TCRCs.
- The EM algorithm based on the unscented filter gives accurate predictions of the concentration of toxicant outside the cells.
- The model can be used to determine an appropriate range for the initial concentration of chemicals $CE(0)$ used in the experiments such that both values smaller and larger than the threshold between extinction and persistence are included.

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