Parameter Estimation and Prediction for Time-Dependent Concentration Response Curves for Cytotoxicity Assessment
Cristina Anton
Department of Mathematics and Statistics, MacEwan University

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 Throughput in vitro assay. The 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.

The Model
The state equation is non-linear, so an approximation is needed during the E-step.

\[
\frac{dx(t)}{dt} = \beta n(t) \left( 1 - \frac{n(t)}{K} \right) - \alpha C_0(t)n(t), \quad n(t): \text{cell index at time } t
\]

\[
\frac{dC(t)}{dt} = \lambda C(t) E(t) - \eta_1 C(t), \quad C(t): \text{internal toxicant concentration}
\]

\[
\frac{dE(t)}{dt} = \lambda E(t) C(t) n(t) - \eta_2 C(t) E(t) n(t) \]

\[
\frac{dx_{k+1}}{dt} = x_k + h \left[ \begin{array}{c}
\frac{bx_1}{1 + bx_1^2} \\
\frac{bx_2}{1 + bx_2^2}
\end{array} \right] + \nu_{k+1}
\]

\[
y_{k+1} = Cx_{k+1} + \bar{w}_{k+1}
\]

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

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 \( x_i, \quad i = N + 1, N + 2, \ldots \quad \) We validate the model by comparing these predictions with the experimental data in the test set.

Parameter Estimation
Using the experimental data corresponding to the negative control (no toxicant) we can estimate \( \beta \) and \( K \) using the nonlinear least square method based on the analytic solution of the logistic equation.
The remaining parameters \( \Theta = \{ Q, R, \alpha, \lambda, \eta_1, \eta_2 \} \) are estimated using the state-space model and the Expectation Maximization (EM) algorithm based on the unscented filter (UF).

The UF is an alternative to the extended Kalman filter (EKF) to calculate the filtered values \( x_1 = E[x_1|y_1, \ldots, y_n], \quad P_1 = E[(x_1-x_1)(x_1-x_1)'|y_1, \ldots, y_n], \)
the predicted values \( x_1 = x_1 + P_1 \)
and the smoothed values \( x_{1:N} = E[x_1|y_1, \ldots, y_n] \) and \( P_{1:N} = \left[ E[(x_1-x_1')(x_1-x_1)']|y_1, \ldots, y_n \right] \) based on the available observations \( y_1, \ldots, y_n \).

The state equation is non-linear, so an approximation is needed during the E-step. The likelihood and conditional likelihood \( P(x_1, \ldots, x_N|y_1, \ldots, y_N|y_{1:N}) \) are approximated based on a linearization of the state equation.

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 \( E(0) \) used in the experiments such that both values smaller and larger than the threshold between extinction and persistence are included.

Acknowledgments
This is joint work with Jian Deng, Yau Shu Wong, Stephen Gabo and Yile Zhang from University of Alberta, Weiping Zhang from Alberta Health, Dorothy Yu Huang from Alberta Centre for Toxicology, Canada and Can Jin from ACEA Biosciences Inc, San Diego, California, USA.