# EXPERIMENTAL PROFILE MODELLING OF METABOLISM

Youcef Derbal*


## Abstract

The human metabolic network of enzymatic reactions limits the toxicity associated with the exposure to substances such as therapeutic drugs, hormones, carcinogens and vitamins. Modelling metabolism is therefore necessary for the study of drug pharmacokinetics and the assessment of the health impact of foreign and endogenous substances. In particular, predictive metabolic models may provide guidance on the required dosage of therapeutic drugs needed to reach cancerous tumors. Models of metabolic networks may be used to estimate the concentration dynamics of oxidative metabolites which is required for the development of cancer risk models. The proposed approach to the modelling of metabolism, we call experimental profile modelling, relies on a first-order approximation of reaction kinetics with an experimentally driven estimation of its parameters. The modelling strategy is illustrated through the development of a model of estrogen metabolism. This model is shown to yield a satisfactory prediction of the experimental profiles of metabolite concentrations. However, the future availability of new experimental data and the conception of novel model parameter tuning algorithms is expected to enhance the prediction capacity of the model.



## Key Words

Enzymatic reaction kinetics, metabolism, experimental profile modelling, oxidative metabolites, computational modelling


## 1. Introduction

Substances of foreign and endogenous origins are subject to bio-transformations by the human metabolic network of enzymatic reactions. The actions of the metabolic network limits the toxicity associated with exposure to therapeutic drugs, hormones, vitamins and xenobiotic substances. However, the metabolism of hormones such as estrogen is also the source of oxidative metabolites such as catechol estrogens and estrogen quinones believed to be implicated in the formation of DNA adducts leading to mutagenesis and cancer. In addition, the biotransformation of foreign substances, such as therapeutic drugs, have causal implications on drug pharmacokinetics and the delivery of therapeutic drugs in adequate concentrations to target sites such as cancerous tumors. Genetic variants of metabolic enzymes, independently or in interaction with environmental exposures, were shown to have an impact on metabolite concentrations [1], [2]. As an example, studies have exposed statistical correlations between the modulating effects of genetic variations on estrogen detoxification as well as the susceptibility to breast cancer. Beyond the particularities of this example, the interplay between environmental conditions and genetic variants of enzymatic genes have been statistically modeled and widely reported [1]. However, the corresponding assertion of statistical correlations are of limited utility to the development of a robust understanding of the mechanisms underlying the dynamic effectors of metabolism on drug pharmacokinetics and carcinogenesis respectively. Such understanding would require quantitative models of the mechanistic dynamics underlying the working of the relevant metabolic networks. These models would be instrumental in providing quantitative estimates of the effect of various exposure patterns to endogenous and exogenous substances on the distributions of oxidative metabolites, allowing hence an improved risk assessment for diseases such as breast cancer. Metabolic models may also be instrumental in developing a quantitative understanding of pharmacokinetics of drugs and improve their therapeutic index. In particular, accounting for the effect of single nucleotide polymorphism (SNP)-based genetic variations of key metabolizing enzymes such as the cytochrome P450 family would further enhance the utility of quantitative models of metabolism. Indeed, the consideration of SNP modulation of the kinetics of drug metabolizing enzymes may help in adjusting cancer drug dosage for individual patients so as to be sufficient enough to reach the target tumors without causing undue toxicity to healthy tissues.

Computational models of metabolism are relevant, as mentioned above, to different contexts including the study of drug pharmacokinetics [3], [4], cancer epidemiology [5]–[8], synthetic biology [9], and the identification of growth promoting genes that could be targeted by cancer drugs [10]. Flux balance analysis (FBA) is one of the frameworks with increasing use for a variety of metabolic modelling applications [9], [11]–[13]. However, FBA is limited to the determination of steady state fluxes and cannot yield predictions of metabolite concentrations. On the other hand, the development of kinetic models of metabolism faces the challenge that rate constants of enzymatic reactions are either unknown or difficult to determine experimentally.

* Ted Rogers School of Information Technology Management, Ryerson University, Canada; e-mail: yderbal@ryerson.ca
*Recommended by Dr. Momiao Xiong*
*(DOI: 10.2316/J.2014.210-1072)*

The ensemble modelling (EM) approach addresses this challenge with the use of parameterized kinetic models, constrained by reaction thermodynamics and the steady state flux conditions [14]–[16]. The EM method is particularly useful for perturbation analysis of metabolic networks which can be used to explore the identification of enzymatic genes as potential cancer drug targets [15]. However, for a more precise representation of metabolic networks, the application of the EM approach would require novel parameter sampling algorithms with reduced computational burden.

The proposed experimental profile modelling (EPM) is an attempt to circumvent the need for prior knowledge of the rate constants. A linear approximation of the reaction kinetics is put forth whereby the model parameters are tuned based on known experimental concentration profiles of metabolite products at various nodes of a metabolic network. The rational underlying the proposed approximation is based on assumptions relating to the relative ratios between the concentrations of substrates, enzymes and metabolite products. The proposed EPM approach is applied to estrogen metabolism, where a model is developed to predict the concentration of oxidative metabolites known to be implicated in the formation of DNA adducts leading to an increase risk for breast cancer.

## 2. EPM of Metabolism

Enzymatic reactions between a substrate $S$ such as estrogen and enzyme $E$ such as cytochrome P450 (CYP) 1A1 take place in two phases as follows:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \overset{k_3}{\rightarrow} E + P \tag{1}$$

The Michaelis–Menten equation of enzyme kinetics for the above system of reactions provides an approximation of the time-dependent dynamics of product concentration as a function of the substrate and enzyme concentrations. This approximation is given as follows:

$$\frac{d[P]}{dt} = k_3[E]_0 \frac{[S]}{k_m + [S]} \tag{1.1}$$

$$= V_{\max} \frac{[S]}{k_m + [S]} \tag{1.2}$$

$$k_m = \frac{k_2 + k_3}{k_1} \tag{1.3}$$

where $[S]$ and $[P]$ are the concentrations of the substrate and reaction product, respectively. $[E]_0$ is the enzyme concentration and $k_1$, $k_2$ and $k_3$ are the kinetics rate constants. $V_{\max}$ is the maximum rate of the above reaction system under saturating substrate concentration, and $k_m$ is the Michaelis–Menten constant. Equation (1) can be rewritten as follows:

$$\frac{dp(t)}{dt} = V_{\max}\, x(1+x)^{-1} \tag{2}$$

where $p(t) = [P]$, $V_{\max} = k_3[E]_0$ and $x(t) = [S]/k_m$. Under the assumption $[S]/k_m \ll 1$, which is justifiable for a large $k_m$ and a vanishing substrate concentration in the steady state, the time-dependent product concentration may be approximated by the following Taylor series expansion:

$$\begin{aligned} \frac{1}{V_{\max}} \frac{dp}{dt} &= x(1 - x + x^2 + O(x^4)) \\ &\approx x - x^2 + x^3 \end{aligned} \tag{3}$$

Let us assume that the depletion of the substrate, in this case $x$, can be approximated with a vanishing exponential function $A_0 e^{-\alpha t}$, from an initial concentration $A_0$ with a rate $\alpha > 1$. The substrate concentration can be shown to have biphasic dynamics for an enzymatic reaction; however, these dynamics have a coarse appearance of first-order or pseudo-first-order kinetics [17], [18]. Indeed since substrate–enzyme complex often react through unimolecular processes, a first-order kinetics is experimentally observed justifying hence the above assumption of exponential substrate depletion [19]. As a result of the above assumption, the integration of (3) with further term rearrangements will yield the following relation:

$$\begin{aligned} \frac{p(t)}{V_{\max}} &= -\frac{1}{\alpha} A_0 e^{-\alpha t} + \frac{1}{2\alpha} A_0^2 e^{-2\alpha t} - \frac{1}{3\alpha} A_0^3 e^{-3\alpha t} \\ &\quad + A_0^4 \frac{1}{4\alpha} e^{-4\alpha t} - \frac{1}{5\alpha} A_0^5 e^{-5\alpha t} + \ldots + \\ &= -\frac{1}{\alpha} x + \frac{1}{2\alpha} x^2 - \frac{1}{3\alpha} x^3 + \frac{1}{4\alpha} x^4 - \frac{1}{5\alpha} x^5 + \ldots + \end{aligned} \tag{4}$$

By factoring out the rate $\alpha$, the above relation can be rewritten as follows:

$$\frac{\alpha\, p(t)}{2V_{\max}} = -\frac{1}{2} x + x^2 - x^3 - \frac{3}{4} x^2 + \frac{5}{6} x^3 + O(x^4) \tag{5}$$

Given the assumption $x(t) \ll 1$, it can readily be shown that the cumulative contribution of the fourth and subsequent terms in the right-hand side of relation (5) is negligible compared to that of the first three terms. The following approximation can therefore be made:

$$\frac{\alpha\, p(t)}{2V_{\max}} \approx -\frac{1}{2} x + x^2 - x^3 \tag{6}$$

Furthermore, (3) can be rewritten as follows:

$$\frac{1}{V_{\max}} \frac{dp}{dt} \approx \frac{1}{2} x - \left( -\frac{1}{2} x + x^2 - x^3 \right) \tag{7}$$

Using (6) and (7) the product concentration kinetics can then be approximated using the following relation:

$$\frac{d[P]}{dt} \approx k_s[S] - k_p[P] \tag{8}$$

where $k_s = \frac{V_{\max}}{2k_m}$ and $k_p = \alpha/2$. These parameters resulted from the approximation and the associated assumptions

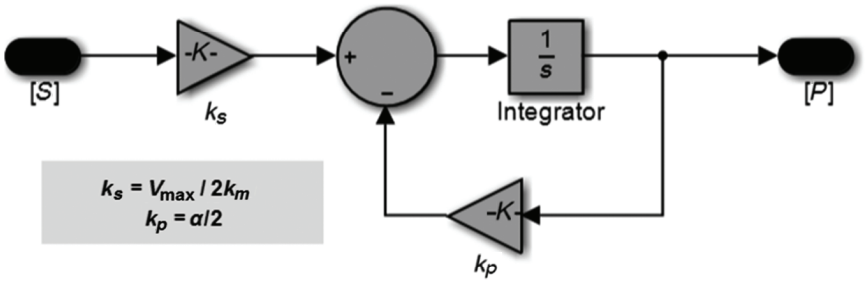


Figure 1. Approximation module of enzymatic reaction kinetics.

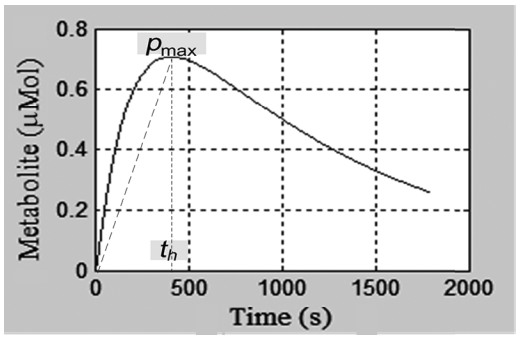


Figure 2. Example profile of metabolite concentration.

detailed above. It may be possible to attribute a physicochemical meaning only in the context of the assumed experimental profile of product concentration. In this respect, $k_s$ is a first-order approximation of the enzyme–substrate catalysis rate, while $k_p$ defines the dynamics of convergence to the steady-state driven by the substrate depletion rate. The above derived first-order approximation of enzyme kinetics is graphically illustrated using a Matlab$^{\text{TM}}$ specified approximation unit module shown in Fig. 1. This approximation of enzymatic reaction kinetics will serve as the elementary building block of the proposed approach to the modelling of metabolic networks. The performance of this approximation will be illustrated by its capacity to yield models of metabolic networks that replicate the experimentally known patterns of metabolite concentration profiles.

The proposed approximation of enzyme kinetics is used to circumvent the need for explicit knowledge of the kinetic rates. The tuning of the model parameters make use of the experimental profiles of metabolite concentrations, which may be available through direct experimentation or curated from the literature. In the case of insufficient availability of experimental data, qualitative knowledge about the metabolites and the catalyzing reactions is used to infirm the tuning of the model parameters. As an example, this tactic is needed for the redox cycle involving catechol estrogens and their quinones associated with estrogen metabolism. The subsystem illustrated in Fig. 1 represents the unit computational module used to internalize the approximation of reaction kinetics. The conservation of mass across a chain of tandem reactions is mediated through the provision of an additional negative feedback to model the depletion of products serving as substrates to the next reactions in a given pathway. The model parameters $k_p$ and $k_s$ are tuned starting from initial values that are heuristically set based on experimental data. In particular, consider the metabolite concentration profile shown in Fig. 2. Assuming a product concentration equal to zero at $t=0$, (8) can be rewritten as follows:

$$\left.\frac{dp}{dt}\right|_{t=0} \triangleq \theta \approx k_s[S]_{\max} \tag{9}$$

As a result, the following approximation can be written:

$$k_s = \frac{\theta}{[S]_{\max}} \tag{10}$$

The derivative $\theta$ of the product concentration at time $t=0$ can be estimated from the experimental data as follows:

$$\theta \approx \frac{p_{\max}}{t_h} \tag{11}$$

where $t_h$ is the time at which the product reaches its maximum value $p_{\max} = p(t_h)$. Combining the relations (10) and (11) leads to the following estimation:

$$k_s = \frac{p_{\max}}{t_h \cdot [S]_{\max}} \tag{12}$$

Since $p(t)$ reaches its maximum value for $t=t_h$ its derivative will be equal to zero. It follows that the evaluation of both sides of (8) for $t=t_h$ will yield the following estimate:

$$k_p = \frac{k_s[S]_{t_h}}{p_{\max}} \tag{13}$$

where $[S]_{t_h}$ is the substrate concentration at time $t=t_h$.

The application of the proposed modelling approach and the underlying approximation of reaction kinetics is illustrated for the example network of enzymatic reactions shown in Fig. 3, resulting in the model of Fig. 4.

The organization of the computational model mirrors the topology of the reaction network, whereby each reaction is represented by its approximation model of kinetics. In addition, in order to provide the means of maintaining reaction mass balance, the output of its model is linearly fed back to its input. The relatively modest experimental

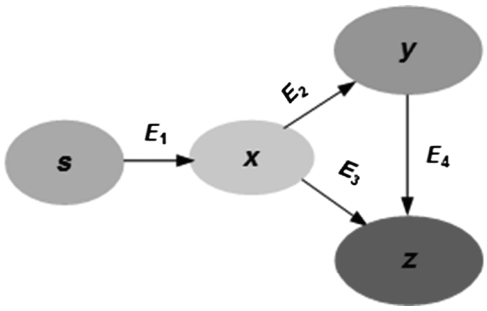


Figure 3. Network of enzymatic reactions. $E_1$, $E_2$, $E_3$ and $E_4$ are enzymes. $S$ is the input substrate and $x$, $y$ and $z$ are the metabolite products of the reactions.

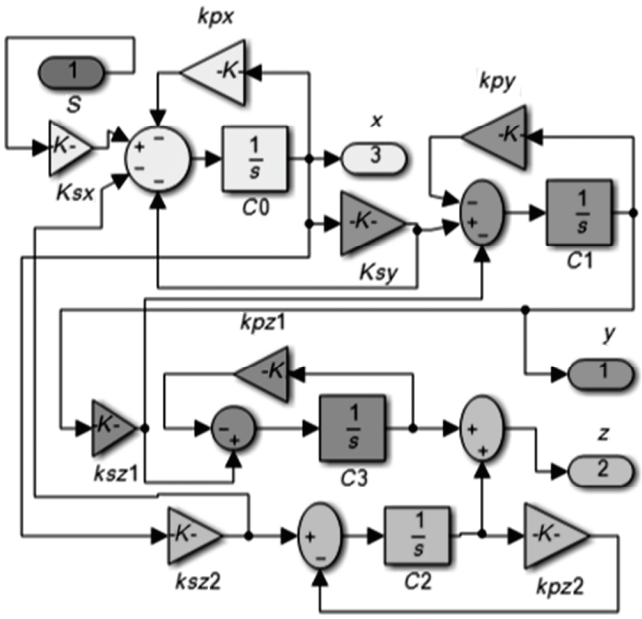


Figure 4. Computational model of the network of enzymatic reactions given in Fig. 3.

data required to carry out the estimation of the model parameters may bring the modelling of large metabolic networks within practical reach. However, this practical feasibility would have to be assessed alongside the verifiable predictive capacity of the resulting models. One actionable step towards this end is to further constrain the model parameters to achieve some desired biological and clinical properties of interest regarding steady-state behaviour or to correlate patterns of metabolite concentrations with the level of enzymatic activities. In this paper, we will limit our attention to exploring these issues in the practical context of estrogen metabolism. A more formal methodology relating to this aspect of the modelling approach is planned for future works.

### 3. Application to Estrogen Metabolism

The risk for female breast cancer has been associated with oxidative metabolites such as catechol estrogens and estrogen quinones believed to be implicated in the formation of DNA adducts leading to mutagenesis and cancer. The enhanced proliferation of cancer in cells expressing estrogen receptors is another source of breast cancer risk putatively associated with prolonged exposure to increased levels of estrogen. The established link between breast cancer and estrogen metabolites have sustained a significant research interest in the study of estrogen metabolism and the development of associated cancer risk models [5], [8], [20]–[23]. Multiple iterations of one particular estrogen metabolic model has been widely reported with proposed improvements related to reaction kinetics, lifetime estrogen exposure and enzyme polymorphism [5], [7], [21], [22], [24]. The pathways of estrogen metabolism are illustrated in Fig. 5, [8], [20], [25]. The Matlab$^{\text{TM}}$ specification of these metabolic pathways is obtained through the modelling of each metabolic reaction using the proposed kinetics approximation, similarly as was done for the illustrative example network of enzymatic reactions given in Fig. 3. The activating enzymes CYP1A1 and CYP1B1 oxidize $E_2$ (17$\beta$-estradiaol – form of estrogen) into catechol estrogens 2-OH$E_2$ and 4-OH$E_4$. These catechol estrogens are further oxidized into semi-quinones ($E_2$-2,3-SQ, $E_2$-3,4-SQ) and ultimately to the quinones $E_2$-2,3-Q and $E_2$-3,4-Q. These quinones are known to react with DNA and lead to DNA depurination. Potential errors associated with the repair of the apurinic DNA sites can lead to mutations and may ultimately trigger the initiation of cancer [8], [26]. Molecular oxygen can also mediate the oxidation of semi-quinones and results in the formation of hydroxyl radicals in the presence of $Fe^{2+}$. Lipid hydroperoxides generated by these hydroxyl radicals can detrimentally modulate CYP towards higher oxidation of catechol estrogens to their quinones [8]. The phase II deactivating enzyme COMT catalyzes the methylation of catechol estrogens into methoxyestrogens limiting hence their transformation to semi-quinones and quinones. The homeostatic balance between activating and deactivating pathways of estrogen metabolism is further protected by the quinones oxidoreductase 1 and 2 ( NQO1 and NQO2) which catalyze the reductive conversion of quinones to their catechol estrogens [8], [20]. The glutathione-$S$-transferase (GST) enzyme provides an additional protection against the DNA damaging quinones by catalyzing their conjugation into GSH-conjugates.

The full quantitative knowledge of the kinetic rates is lacking for many of the enzymatic reactions making up the estrogen metabolizing system detailed above. This makes the estrogen metabolic network an appropriate example for the application of the proposed modelling approach and associated first-order approximation of reaction kinetics. The experimental data used for parameter tuning and model validation are sourced from the dataset used in [27]. The corresponding *in vitro* experiment of estrogen metabolism is run for 30 min with an initial 10 $\mu$mol of $E_2$. The concentrations of the metabolites of interest are recorded for the time points 0, 2, 5, 10, 20 and 30 min, respectively (see Table 1). The metabolite concentrations are expressed in $\mu$mol. It should be noted that since the experimental data were extracted from the graphical illustrations reported in [27], they match their original

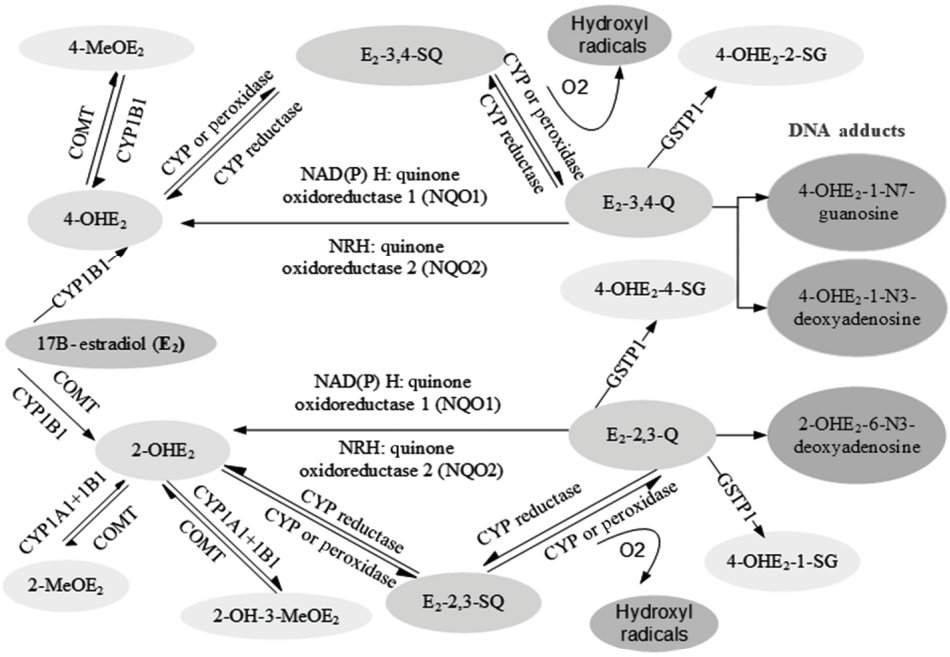


Figure 5. Estrogen metabolic pathways.

Table 1
Metabolite Concentrations ($\mu$mol)

| Time (min) | $E_2$ | 4-$OHE_2$ | 4-$MeOE_2$ | 4-$OHE_2$-2-SG |
|---|---|---|---|---|
| 0 | 10 | 0 | 0 | 0 |
| 2 | 4 | 1.5 | 0.038 | 0.06 |
| 5 | 2 | 1.25 | 1.2 | 0.15 |
| 10 | 0.5 | 0.5 | 1.75 | 0.3 |
| 20 | 0 | 0.125 | 1.75 | 0.4 |
| 30 | 0 | 0.1 | 1.55 | 0.42 |

values only within the margin of the unavoidable parallax error.

The initial values of the model parameters $k_p$ and $k_s$ were estimated using (12) and (13) and the experimental data summarized in Table 1. The estrogen metabolites of interest are the DNA depurinating quinones $E_2$-2,3-Q and $E_2$-3,4-Q. However, it was reported that the contribution of the latter to DNA adducts dominates with 97% compared to 3% for the first quinone [8]. Hence, the model simulation analysis is focused on the pathways associated with the production of the quinones $E_2$-3,4-Q. The $k_s$ parameters associated with the reduction of $E_2$-3,4-Q were estimated using the values of $k_m$ and $V_{\max}$ for the oxidoreductases NQO1 and NQO2 as reported in [20]. On the other hand, the initial values of $k_{pNQO1}$ and $k_{pNQO2}$ which are associated with the depletion rates of $E_2$-3,4-Q, have been set to the rate of estrogen depletion. Furthermore, given the lack of kinetics information about the chain of reactions transforming the catechol estrogens 4-$OHE_2$ into quinones $E_2$-3,4-Q, the parameters for these reactions are initially tuned to satisfy the assumption that the quinones have concentration profiles similar to those of the substrates (catechol estrogens) from which they form. The modulating feedback of the hydroxyl radicals on CYP is another component of the model for which there isn't sufficient knowledge of kinetics. In this case, the parameters associated with the relevant reactions are arbitrarily initialized so as not to impact the concentration of the catechol estrogen 4-$OHE_2$. Multiple iterations of parameter tuning were applied to replicate the concentration time-profile of the various metabolites for which experimental data is available (see Table 1). In particular, the iterative tuning of the model parameters was performed manually using heuristics that rely on the following properties of metabolite concentration profile and their relationships to the model parameters:

1. The *accumulation time* $T_a$; defined as the time for a metabolite concentration to increase from 10% to 90% of its maximum value. The value of $T_a$ is adjustable in an inverse proportion to $k_s$.
2. The *settling time* $T_s$; defined as the time it takes the metabolite concentration to settle within 10% of the steady state level. The value of $T_s$ is adjustable in a direct proportion to $k_p$.
3. The *peak value* $C_p$; defined as the maximum value of the metabolite concentration. The value of $C_p$ is adjustable in a direct proportion to $k_s$.

The tuning of the model parameters starts with a forward-sweep iteration where each reaction model is tuned in a sequence order starting from the estrogen input node of the network along the various pathways leading to the end point reactions associated with the production of the quinones and DNA adducts. For each reaction model in the pathway under consideration, the parameters $k_p$ and $k_s$ are adjusted in increment of 10% or less so as to replicate the experimental concentration profiles of the

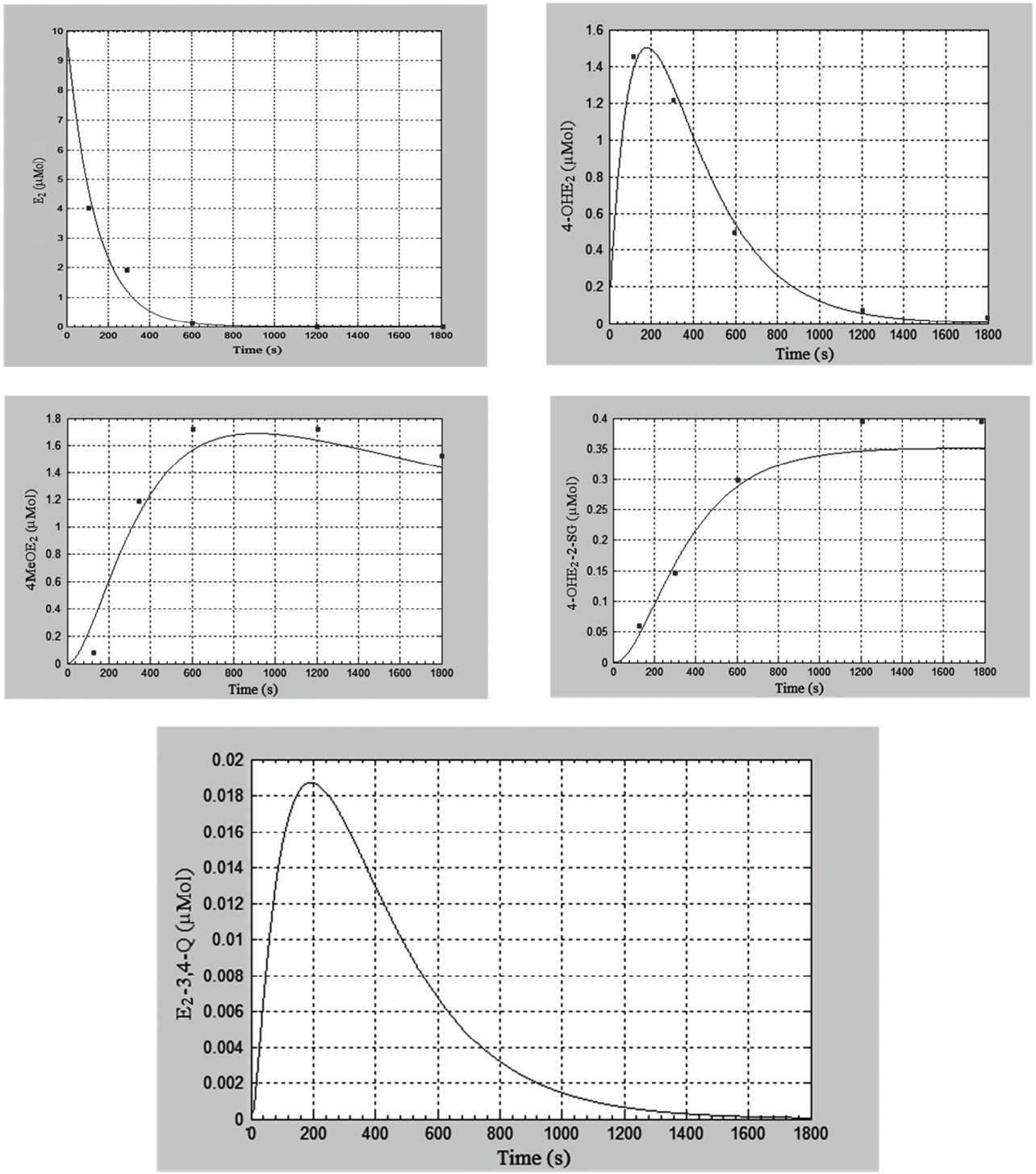


Figure 6. Model predicted metabolite concentrations compared to experimental data (black dots).

relevant metabolites as described by their accumulation times, settling times and peak values respectively. Once the forward-sweep tuning is completed, a reverse-sweep tuning is undertaken whereby the reactions are considered in the reverse order of the forward-sweep. After each iteration round of forward-tuning sweep followed by a reverse-tuning sweep, the metabolite concentrations generated by the model are assessed for their divergence (with respect to $T_a$, $T_s$ and $C_p$) against their experimentally known counterparts. As a general guideline, property value divergence exceeding 15% for more than one reaction would require another iteration round of tuning. If the divergence is localized to a single reaction, the selective adjustment of the specific reaction model parameters might be sufficient to redress the problem. The use of a direct human inspection of the concentration profiles to assist the tuning process was instrumental in enabling a relatively fast convergence to an acceptable set of model parameters. In future works, an algorithmic solution will be sought to implement the heuristic rules of parameter tuning detailed above.

The developed model of estrogen metabolism yielded the metabolite concentration estimates illustrated in Fig. 6. More than a dozen iteration rounds of tuning was needed to achieve a convergence to the set of model parameters associated with the achieved estimates of metabolite concentrations. The simulation results illustrate the structural capacity of the model to internalize the kinetics of estrogen metabolism as informed by the available experimental data. In particular, the model predictions of metabolite concentrations of interest closely match published experimental results. However, given the lack of sufficient experimental data, it is not possible to assert the plausibility of the estimated concentrations for the various metabolites of concern, including the carcinogenic quinones $E_2$-3,4-Q. Nevertheless, given the overall encouraging performance of the model, it is expected that the confidence in its predictions will be significantly enhanced as newly available experimental data are used to further tune its parameters. Indeed , the improvement in metabolic modelling is indispensable to furthering our understanding of cancer risk and initiation associated with carcinogenic metabolites that often defy direct experimental measurements.

For future works, the model may be extended to include the effect of genetic variations and antioxidants on the metabolic activities of enzymes. In particular, the variability of model parameters may be used to capture the modulating effect of enzymatic gene expression known to be caused by SNPs. Similarly, the potential effect of antioxidants may be modelled using added unit reaction modules (see Fig. 1) to neutralize oxidizing species and restore estrogen homeostasis [8].

## 4. Conclusion

The modelling of metabolism is explored using a proposed framework of EPM. The framework relies on a first-order approximation of reaction kinetics with an experimentally driven estimation of its parameters. The model parameters are determined through multiple iterations of tuning guided by a set of heuristic rules that leverage the available experimental data about metabolite concentrations and reaction kinetics. The proposed EPM is applied to the modelling of estrogen metabolism. The resulting model is shown to reproduce the experimental time profiles of metabolite concentrations of interest. However, more experimental data is needed to enhance the overall confidence in its predictions. It is also expected that an algorithmic solution to the parameter tuning process would enhance the practical feasibility of the proposed framework *vis-à-vis* scalability and predictive capacity.

## References


[1] S. Dawling, N. Roodi, R.L. Mernaugh, X. Wang, and F.F. Parl, Catechol-*O*-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant COMT isoforms, *Cancer Research*, *61*(18), 2001, 6716–6722.

[2] I.H. Hanna, S. Dawling, N. Roodi, F.P. Guengerich, and F.F. Parl, Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity, *Cancer Research*, *60*(13), 2000, 3440–3444.

[3] J.L. Staudinger, Disease, drug metabolism, and transporter interactions, *Pharmaceutical Research*, *30*(9), 2013, 2171–2173.

[4] U.M. Zanger and M. Schwab, Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation, *Pharmacology and Therapeutics*, *138*(1), 2013, 103–141.

[5] P.S. Crooke, C. Justenhoven, H. Brauch, S. Dawling, N. Roodi, K.S. Higginbotham, W.D. Plummer, P.A. Schuyler, M.E. Sanders, D.L. Page, J.R. Smith, W.D. Dupont, and F.F. Parl, Estrogen metabolism and exposure in a genotypic-phenotypic model for breast cancer risk prediction, *Cancer Epidemiology, Biomarkers and Prevention*, *20*(7), 2011, 1502–1515.

[6] F.F. Parl, K.M. Egan, C. Li, and P.S. Crooke, Estrogen exposure, metabolism, and enzyme variants in a model for breast cancer risk prediction, *Cancer Information*, *7*(5), 2009, 109–121.

[7] S.C. Philip, D.R. Marylyn, L.H. David, D. Sheila, R. Nady, and F.P. Fritz, Estrogens, enzyme variants, and breast cancer: a risk model, *Cancer Epidemiology Biomarkers & Prevention*, *15*(9), 2006, 1620–1629.

[8] E.L. Cavalieri and E.G. Rogan, Unbalanced metabolism of endogenous estrogens in the etiology and prevention of human cancer, *Journal of Steroid Biochemistry and Molecular Biology*, *125*(3–5), 2011, 169–180.

[9] A.M. Feist and B.O. Palsson, The growing scope of applications of genome-scale metabolic reconstructions using *Escherichia coli, Nature Biotechnology*, *26*(6), 2008, 659–667.

[10] O. Folger, L. Jerby, C. Frezza, E. Gottlieb, E. Ruppin, and T. Shlomi, Predicting selective drug targets in cancer through metabolic networks, *Molecular Systems Biology*, *7*(501), 2011, 501–501.

[11] J.D. Orth and I. Thiele, What is flux balance analysis?, *Nature Biotechnology*, *28*(3), 2010, 245–248.

[12] N.C. Duarte, S.A. Becker, N. Jamshidi, I. Thiele, M.L. Mo, T.D. Vo, R. Srivas, and B.O. Palsson, Global reconstruction of the human metabolic network based on genomic and bibliomic data, *Proceedings of National Acadmic of Sciences: United States of America*, *104*(6), 2007, 1777–1782.

[13] A.M. Feist, M.J. Herrgard, I. Thiele, J.L. Reed, and B.O. Palsson, Reconstruction of biochemical networks in microorganisms, *Nature Reviews Microbiology*, *7*(2), 2009, 129–143.

[14] J.T. Dean, M.L. Rizk, Y. Tan, K.M. Dipple, and J.C. Liao, Ensemble modelling of hepatic fatty acid metabolism with a synthetic glyoxylate shunt, *Biophysical Journal*, *98*(8), 2010, 1385–1395.

[15] T. Khazaei, *et al*, Ensemble modelling of cancer metabolism, *Frontiers in Physiology*, *3*(5), 2012, 1–14.

[16] L.M. Tran, M.L. Rizk, and J.C. Liao, Ensemble modelling of metabolic networks, *Biophysical Journal*, *95*(12), 2008, 5606–5617.

[17] S. Schnell and C. Mendoza, The condition for pseudo-first-order kinetics in enzymatic reactions is independent of the initial enzyme concentration, *Biophysical Chemistry*, *107*(2), 2004, 165–174.

[18] J. Srividhya and S. Schnell, Why substrate depletion has apparent first-order kinetics in enzymatic digestion, *Computational Biology and Chemistry*, *30*(3), 2006, 209–214.

[19] *Biochemistry: the chemical reactions of living cells* (San Diego, California, USA: Harcourt/Academic Press, 2001).

[20] N.W. Gaikwad, L. Yang, E.G. Rogan, and E.L. Cavalieri, Evidence for NQO2-mediated reduction of the carcinogenic estrogen ortho-quinones, *Free Radical Biology and Medicine*, *46*(2), 2009, 253–262.

[21] F.F. Parl, S. Dawling, N. Roodi, and P.S. Crooke, Estrogen metabolism and breast cancer: a risk model, *Annals of the New York Academy of Sciences*, *1155*(2), 2009, 68–75.

[22] J.L. Bolton and G.R. Thatcher, Potential mechanisms of estrogen quinone carcinogenesis, *Chemical Research in Toxicology*, *21*(1), 2008, 93–101.

[23] E. Cavalieri, D. Chakravarti, J. Guttenplan, E. Hart, J. Ingle, R. Jankowiak, P. Muti, E. Rogan, J. Russo, R. Santen, and T. Sutter, Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of

susceptibility and cancer prevention, *Biochimica et Biophysica Acta*, *1766*(1), 2006, 63–78.

[24] D.L. Hachey, S. Dawling, N. Roodi, and F.F. Parl, Sequential action of phase I and II enzymes cytochrome p450 1B1 and glutathione S-transferase P1 in mammary estrogen metabolism, *Cancer Research*, *63*(23), 2003, 8492–8499.

[25] M. Rahman, C. Hayes Sutter, G.L. Emmert, and T.R. Sutter, Regioselective 2-hydroxylation of 17$\beta$-estradiol by rat cytochrome P4501B1, *Toxicology and Applied Pharmacology*, *216*(3), 2006, 469–478.

[26] D.E. Stack, G. Li, A. Hill, and N. Hoffman, Mechanistic insights into the Michael addition of deoxyguanosine to catechol estrogen-3,4-quinones, *Chemical Research in Toxicology*, *21*(7), 2008, 1415–1425.

[27] S. Dawling, D.L. Hachey, N. Roodi, and F.F. Parl, In vitro model of mammary estrogen metabolism: structural and kinetic differences between catechol estrogens 2- and 4-hydroxyestradiol, *Chemical Research in Toxicology*, *17*(9), 2004, 1258–1264.

## Biography

*Youcef Derbal* is an associate professor at the Ted Rogers School of Information Technology Management. He received a Doctorate in Electrical and Computer Engineering from Queen's University (Canada). Prior to joining Ryerson University in 2003, he worked in robotics and automation, telecommunications, and software and computing industries. His research interests include the study of complex adaptive systems and their modelling and simulation.