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APPLICATION OF IMPULSIVE DETERMINISTIC SIMULATION OF BIOCHEMICAL NETWORKS VIA SIMULATION TOOLS

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Abstract. In order to understand the possible behaviour of biochemical networks, deterministic and stochastic simulation methods have been developed. However in some cases, these methods should be broaden. For example, if the biochemical system is subjected to the unexpected effects causing abrupt changes in the network, the ordinary simulation algorithms cannot capture these impulsive expressions.

In this study, we select the simulations tools, specifically, COPASI and Systems Biology Toolbox for MATLAB among alternatives that enable us to represent the impulsive changes in the system via impulsive or adaptive deterministic simulation algorithms. Accordingly, we compare these tools by applying the two major impulsive scenarios, namely, impulses for fixed times and fixed states, based on their accuracies and computational demands. We evaluate our results for small and large systems, respectively.

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1. Introduction

There are mainly two approaches of simulation to describe the activation of biochemical systems in vitro. These are the deterministic and stochastic simulation algorithms. These methods are based on different mathematical backgrounds and take into account distinct dynamics. For instance, the deterministic approach considers the network as a set of ordinary differential equations in which every equation accounts for the rate of changes in the concentrations of species in the system. On the other hand, the stochastic approach suggests a random error term coming from the Brownian motion in the description of the system's activity and it is based on the chemical master equations dependent on the change in molecular numbers of species of the network [21]. But these algorithms have not been analyzed yet under impulses.

The impulsive expressions account for the possible abrupt changes in the biochemical systems [2]. This can be done under different scenarios. For

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instance, we can define a jump function which increases or decreases the state vector at fixed time intervals, fixed states or autonomously.

In this study, we include impulses in the deterministic simulation of biochemical networks by using the most popular simulation tools. Because in the literature, the simulation tools are widely applied to generate (mainly deterministically) and estimate the model parameters of distinct dimensional systems and to visualize them by various graphical interfaces. But their performances under impulses have not been yet assessed both in terms of computational time and accuracy of the estimates. Hereby, in this work, we initially describe our impulsive functions that are applied in small and realistically large networks. Then, we evaluate the capacity of the selected tools for such calculations. And finally, we present comparative analyses under two main impulses' scenarios. Accordingly in the following section, we briefly describe the simulation tools which we choose in these analyses and describe their algorithms that are suitable for impulsive deterministic simulations. In this section, we also introduce our impulse scenarios. In the application part, we present the selected networks, namely, Lotka-Volterra, PKC, L-L, MAPK-ERK and JAK-STAT pathways and state their importance in biological senses. Here, we further report our findings after the analyses. Finally, in Conclusion part, we summarize the outputs and discuss future works.

2. Simulation Tools and Impulses

In the simulation of the biochemical networks, a number of tools have been developed. Among many alternatives, Cellware [5], COPASI [6], Dizzy [12], Dynetica [20], E-CELL [18], GENESIS [4], Jarnac combined with JDesigner [13, 14], System Biology Toolbox [17] and Virtual Cell [16] are the most widely used tools and freely available for academic purposes. But none of these tools has the highest capacity under the criteria such as the simplicity of the usage, supports from platforms, variety of the algorithms in simulation as well as inference and finally the compatibility of the Systems Biology Markup Language (SBML). Hence the users need to choose the most appropriate tool with respect to their aims. In this study, we select COPASI and Systems Biology Toolbox for MATLAB (SBT) for the simulation. The reason is that they both have the highest number of algorithms for different purposes, are compatible with SBML and both offer documents such as tutorials and manuals. Moreover, these tools can perform impulsive simulations under distinct algorithms. Thereby in the selection of the appropriate algorithms in both tools, we define the following three main criteria.

(1) *The method can handle stiff or nonstiff systems:* Specifically, when the numerical methods for solving the differential equations are numerically unstable and when the step-size is not extremely small, then it is considered as a stiff case. Otherwise, the system is accepted as a non-stiff manner. We choose the algorithms having stiff property.
(2) *The method is implicit or explicit:* The explicit methods are easy to implement, whereas, do not guarantee the numerical stability. On

the other hand, implicit methods are computationally expensive but guarantee stability.

(3) *The simulation method can be adaptive or non-adaptive:* If the algorithm dynamically varies the step-size during the simulation, it is called as the adaptive method, otherwise, we describe it as non-adaptive. For the impulsive calculation, we use the adaptive algorithm.

In Table 1, we classify all the supported deterministic simulation algorithms in COPASI and Systems Biology Toolbox according to our listed criteria. From this clustering, we see that all algorithms for both tools are adaptive. Hereby, in our analyses, we mainly choose the more appropriate algorithms in the calculation with respect to other criteria. Thereby, in the calculation, we use COPASI with LSODA since it is the only method which can satisfy our condition. On the other hand, in the simulation via SBT, we select the Ode113 method since it is explicit and adaptive. But this method is nonstiff. As we also compare the performance of both COPASI and SBT in terms of the computational demand, we give priority to the explicit/implicit feature with respect to the stiff/nonstiff property.

TABLE 1. Deterministic simulation methods available within COPASI and SBT.

Program	Method Name	Algorithm	Stiff/Nonstiff	Implicit/Explicit
COPASI	LSODA	Adams&BDF	Nonstiff and Stiff	Implicit
SBT	Ode45	Runge-Kutta, Dormand-Prince(4,5) pair	Nonstiff	Explicit
SBT	Ode23	Runge-Kutta(2,3) pair of Bogacki&Shampine	Nonstiff	Explicit
SBT	Ode113	Adams-Bashforth-Moulton	Nonstiff	Explicit
SBT	Ode15s	NDFs (BDFs)	Stiff	Implicit
SBT	Ode23s	Rosenbrock	Stiff	Implicit
SBT	Ode23t	Trapezoidal rule	Moderately Stiff	Implicit
SBT	Ode23tb	TR-BDF2	Stiff	Implicit

Accordingly, in the comparative analyses of COPASI and SBT, we consider the following impulses scenarios with the given impulsive functions.

i. Systems with impulses at fixed moments

$$\Delta y = f(t,y), \quad t \neq t_k \tag{1}$$

$$\Delta y = I_k(y), \quad t = t_k \tag{2}$$

where $y = (y_1, \ldots, y_d)$ is the state vector as the function of time t with each component referring to a species j. Thereby, j takes value from 1 to d for totally d types of species. Likewise $f(t,y)$ denotes the change in the concentration with respect to the time t. In Equation 1, we use the notation of the difference, rather than the differential equation where the chemical master equations are based on [19], since the toolboxes work in discrete time. When the time is equal to t_k, then the impulse function $I_k(y)$ increases or decreases the concentrations of species at fixed moments.

ii. Systems with impulses at variable moments

$$\Delta y = f(t, y), \quad t \neq \tau_k(y) \tag{3}$$

$$\Delta y = I_k(y), \quad t = \tau_k(y) \tag{4}$$

where $y = (y_1, \ldots, y_d)$ stands for the state vector whose components show the species j. Hence similar to the previous scenarios, j has a range from 1 to d. d is the total number of species in the system. Likewise the expression in Equation 3 implies the differences in concentrations with respect to the time t. When the time equals to $\tau_k(y)$ which is a variable, then the impulse function, $I_k(y)$, increases or decreases the concentrations of species at variable moments.

3. Application

In the implementation and the comparison of COPASI and SBT with/ without impulses under the given scenarios based on their computational time and accuracies, we select five systems from small to large dimensions and under various conditions. As the small system, we choose the Lotka-Volterra model which is typically used for comparative studies of simulations for its simplicity. This system is defined by 2 species with 3 reactions. As the moderate network, we take the PKC pathway composed of 14 species with 20 reactions. Finally as the large networks, we consider three pathways, namely, L-L, MAPK-ERK and JAK-STAT pathways. The L-L pathway is defined by 33 reactions with 35 species. The MAPK-ERK pathway is presented by 66 reactions with 51 species and the JAK-STAT pathway is described by 67 reactions with 37 species.

Below we firstly describe the biological background of each system shortly and then, represent the initializing conditions for running their deterministic simulations via COPASI and SBT.

3.1. **Lotka-Volterra Model.** The Lotka-Volterra, also known as the prey-predator model, is one of the well-known population models involving a prey and a predator which we consider as rabbit (P_1) and fox (P_2) for our case as an example. In this model, these two species are subjected to the three events, specifically, rabbit reproduction, rabbit-fox interaction, i.e., the death of the rabbit due to the fox, and the death of the fox due to the natural causes as shown in Table 2 [21].

Mathematically, this model can be expressed with two differential equations (Table 3) where each of them accounts for the rate of change in the concentration of species. Moreover, in order to define the full dynamics of the system, we need to know initial numbers of species together with the reaction constants. In our case, we take 4 preys and 10 predators and simulate the system for 100 days. Thereby, the inputs of the deterministic simulation can be represented by the initial state at $t = 0$ as $y(0) = (4, 10)$. Here, the time interval is $t \in [0, 100]$ and the reaction constants for the first, second and the third reactions are $k_1 = 1$, $k_2 = 0.1$ and $k_3 = 0.1$, respectively [2].

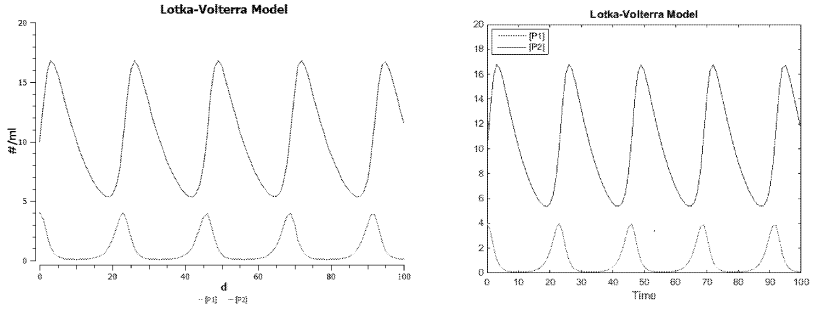
Finally, we simulate the Lotka-Volterra model by considering without impulses as seen in Figure 1 and with impulses as shown in Figure 2. For the impulsive effect, we add 5 preys to the systems when the number of preys becomes greater than 4.

TABLE 2. Reactions, reaction rates and reaction constants of the Lotka-Volterra model for the species P_1 and P_2. [.] denotes the concentrations for the given species.

Reactions	Reaction Rates	Reaction Constants
$R_1 : P_1 \to 2P_1$	$k_1[P_1]$	$k_1 = 1$
$R_2 : P_1 + P_2 \to 2P_2$	$k_2[P_1][P_2]$	$k_2 = 0.1$
$R_3 : P_2 \to \emptyset$	$k_3[P_2]$	$k_3 = 0.1$

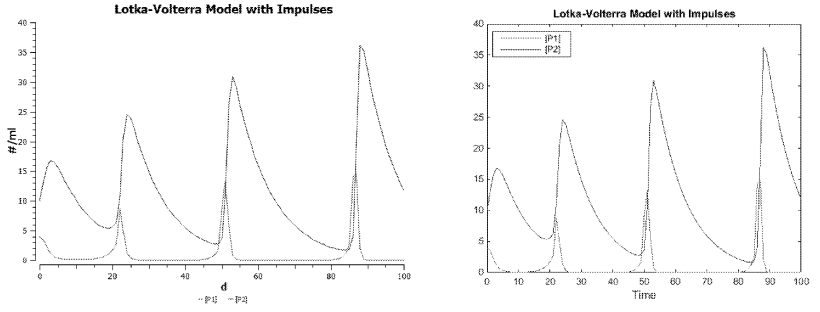
TABLE 3. Ordinary differential equations (ODEs) and initial amounts of the species for the Lotka-Volterra model.

Ordinary Differential Equations (ODEs)	Initial Amounts (in Numbers)
$d[P_1]/dt = k_1[P_1] - k_2[P_1][P_2]$	$[P_1](0) = 4$
$d[P_2]/dt = k_2[P_1][P_2] - k_3[P_2]$	$[P_2](0) = 10$



(a) (b)

FIGURE 1. Deterministic simulation of the Lotka Volterra model by using (a) COPASI and (b) SBT.



(a) (b)

FIGURE 2. Deterministic simulation of the Lotka Volterra model by using (a) COPASI and (b) SBT with impulses.

3.2. **PKC Pathway.** The Protein Kinase C (PKC) signal transduction pathway is involved in major neural functions such as the synaptic long-term potentiation (LTP) and the depression (LTD) [8]. More specifically,

the pathway accounts for the activation of the inactive PKC (PKCi) and ends up with the concentration of the active PKC (PKCa). The model is retrieved from the database of Quantitative Cellular Signaling and it considers a generic mammalian with 14 species. In the PKC pathway, the concentration of the active PKC can be obtained by summing the concentrations of computational intermediates. For this system, we apply two impulsive scenarios for the concentration of PKCi since it is the major substrate of the pathway. In the first case, we decrease the concentration of PKCi by 0.2 when it becomes less then 0.9 while in the second case, we increase the amount of PKCi by 0.005 in every 50-minute. In Figure 3, we present the underlying differences of the PKCi simulation results via COPASI and SBT with/without impulses.

On the other hand, in order to compare the accuracy of the tools, we consider the changes in species under impulses based on their changes in the directions (i.e., increasing or decreasing sides). In order to decide on which direction can be biologically more plausible when the impulses are based on an increasing effect of the species, we think the species having reactions with the PKCi protein on the product sides. Since they can be more likely accumulated in the environment or they can be more than needed in the system, the direction of the activations for the associated species can be seen at the upward side. On the other side, to evaluate the decreasing direction in the level of species, we control the species located in the reactant sides of the reactions which are related to PKCi. We consider that if they are observed more often on the reactant sides in the quasi reaction list of the system, their concentration levels can reduce at the end of the calculation.

Hence from the results, we observe that the directions of the species after the firing of impulses are the same in both tools. Moreover, the activation of PKC is affected in the same direction when the impulses are seen in increasing and decreasing way. Therefore, we consider that both tools can capture the directional information of impulses accurately.

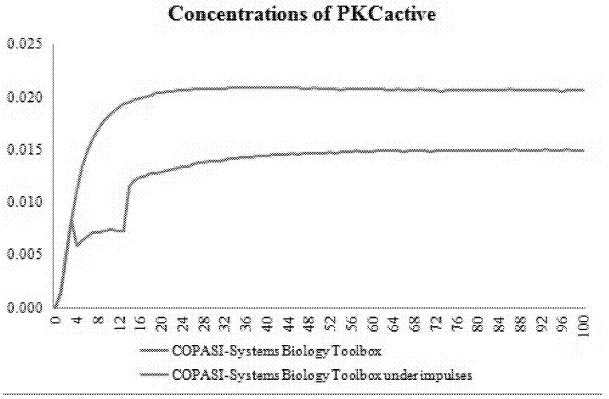


FIGURE 3. Comparison of the differences of the PKCi simulation results via COPASI and SBT with/without impulses.

Finally in order to detect whether these underlying results are valid for different initial conditions, we restart the simulation under two more scenarios too. In the first plan, we initialize all the concentrations of the proteins in the system with 100 units. Then, we set the hazard [21] of each reaction h_j $(j = 1, \ldots, d)$ to 100 and if the reaction is the first-order reaction, the reaction constant is calculated as $k_j = h_j(y;k)/100 = 1$. If the reaction is the second-order, then the reaction constant is taken as $k_j = h_j(y;k)/100^2 = 0.01$. Similarly, if the reaction is the third-order, the associated reaction constant is found as $k_j = h_j(y;k)/100^3 = 0.0001$. In the reaction list of the PKC pathway, there are 14 first-order reactions and 6 second-order reactions whose reaction rates are set to $k_j = 1$ and $k_j = 0.01$, respectively. Hence in order to assess the effect of impulses in decreasing or increasing directions, after simulating the system without impulses, we run it by adding 50 units of concentrations to PKCi in every 25-second and then repeat the simulation by decreasing 50 units of concentrations from it in every 25-second. The outcomes under the new conditions are listed in Table 5 and the associated computational time is given in Table 4 for the comparison. From the findings we observe that similar to the previous condition, both tools are successful in detecting the direction of impulses and produce accurate results.

3.3. **L-L Pathway.** The lysis-lysogeny (L-L) pathway is a crucial regulatory mechanism which separates the population of the Escherichia coli (E.coli) cell between lytic and lysogenic outcomes [3]. In this pathway, the proteins CII, CIII and N are the key proteins to find the number of lysogens produced in an infected cell population. The description of this system includes the genetic mechanisms, which consist of the operator/promoter bindings, transcription initiation, transcription initiation of translation, translation and the initiation of mRNA degradation, coupled protein dimerization and degradation reactions. Hereby, the associated reaction list is defined by 33 reactions with 35 species. In the simulation of this system with impulses, we add 10 units of concentration to two core proteins, CII and CIII, in every 100-second due their importance in the regularization of the cell. Then, we repeat the simulation by decreasing 10 units of concentration from the same proteins in every 100-second. Finally, we check the similarity of the results between COPASI and SBT. From the analyses based on the increasing effect, we find 2 species showing the opposite direction and 9 species having the same direction, resulting in, 24 invariant genes. On the other side if we evaluate the results of the decreasing effect, then, we detect only 1 and 10 species having the opposite and the same direction, respectively, with the same 24 invariant genes. Hence, we see that both tools indicate very similar outputs under impulses and the cause of the opposite directional species cannot be explained via our validation criteria.

On the other hand in order to assess the findings under different conditions, we equate the initial number of molecules of each species to 100 and assign the reaction rates of the first (totally 23 reactions) and the second order (totally 10 reactions) reactions as $k_j = 1$ and $k_j = 0.01$, respectively, similar to other selected pathways. Here, we aim to investigate the influence of the initialization on the findings of impulses. Under this second condition

of the system, we control the effects of impulse when we increase the amount of concentrations by 50 units in CII and CIII proteins at every 100-second and decrease them 50 units for the same proteins at every 100-second. As seen in Table 5, we observe that when the effect of impulses is in the increasing direction, 7 species show the same direction of changes, 14 species have opposite directions and 15 species do not indicate any change. On the other side, when the effect of impulses is in the decreasing direction, 5 and 16 species have the same and opposite directions, respectively, and the remaining the same 15 species are invariant from the impulses. Under this condition and among the species showing opposite directions, 2 species can be biologically validated by the outputs of SBT. Lastly, we still detect that both COPASI and SBT are almost invariant to the direction of the impulse although they can capture the absolute changes in the amount of concentrations for each species and the calculated differences are observed at the incremental level. Finally in Table 4, we present the computational time of both tools to compare their efficiencies in the calculations. In Figure 4, we plot the underlying differences in the simulation results of the L-L pathway via both tools with/without impulses.

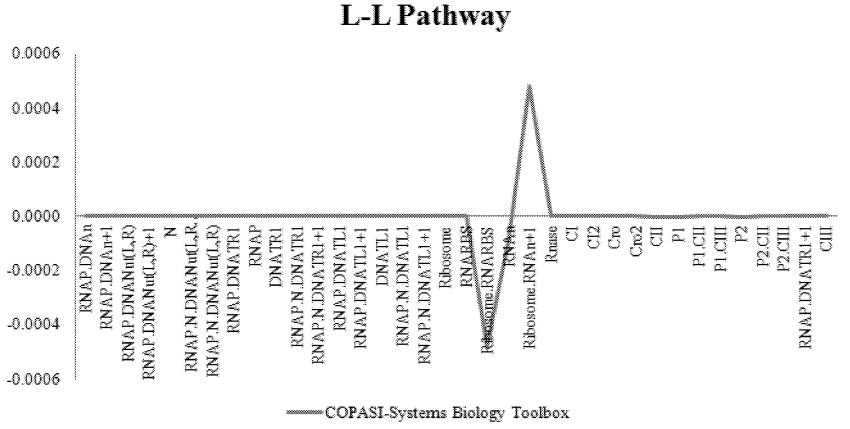


FIGURE 4. Comparison of the differences in simulation results of the L-L pathway via COPASI and SBT with/without impulses.

3.4. **MAPK-ERK Pathway.** The MAPK (mitogen-activated protein kinase), also known as ERK (extracellular signal-regulated kinase), pathway is a popular signaling pathway which governs the cellular growth of all eukaryotes from the cell reproduction to the death [11]. Moreover, this pathway has a crucial role in various diseases such as the cancer, immunological, inflammatory and the degenerative syndromes [9]. Therefore, understanding the dynamics of the pathway can be done via simulations and sheds light on the drug developments. In our assessment, we add fixed-time impulses and run the simulation again. Accordingly, we add 10 units of concentrations to both RKIP and ERK.p2 species, which are the major two proteins

controlling the inhibitory regulation of the system, in every 100-second. In Figure 5, we plot both tools simultaneously without impulses. It is seen that, there is almost no difference between tools under this condition. In Figure 6, we draw RKIP and active ERK, denoted by the ERK.p2 species, with/without impulses for illustration. From the plots, we observe that COPASI and SBT indicate distinct equilibrium points for large systems and under impulses. Furthermore, they show differences in their computational time (Table 4). On the other hand, in order to compare their accuracies, we consider the changes in species under impulses based on their changes in the directions (i.e., increasing or decreasing sides). From the outputs, we see that among 51 species used in the description of the system, 27 of them indicate the same directions after the effects of impulses and 8 of them show opposite directions. Finally, the remaining 16 species are not affected by the impulses. These results are also listed in Table 5. In order to decide on which direction can be biologically more plausible, we apply the same rules as described previously, for only those 8 species. We see that the tools have some minor differences. SBT looks for a solution which decreases the effect of the impulse, whereas, COPASI searches for an optimal solution. But, as we are interested in the long term behaviour in the deterministic simulation, the effect of the impulses cannot be observable.

To detect whether the directions of impulses are effective, we repeat the simulations under very sharp decreases. For the MAPK-ERK system, we decrease RKIP by 20 units of concentrations when it hits 30 units and decrease ERK.p2 by 130 units when it hits 170 units. We observe that among 51 species, 33 of them indicate the same directions while almost the same 8 of them show the opposite directions and the remaining 10 species become irrelevant from impulses. Hereby, these results show that both tools are almost insensitive to the direction of impulses and when the system has such changes, they push to the system to the closest equality points and turn it into a steady-state position.

Additionally to evaluate the system under different initial conditions, we restart the simulation by setting the amount of concentrations for all substrates to 100 units and initial hazards of all reactions that are used to compute the reaction rates to 100. Then by calculating the reaction rates inversely proportional to the amount of concentrations in each reaction, we equate the reaction rates to $k_j = 1$ for the first-order reactions, $k_j = 0.01$ for the second-order reactions and $k_j = 0.0001$ for the third-order reactions, as similarly implemented for other pathways. In our reaction list, since 23 of the reactions are the first-order and the remaining 43 are the second-order, the reaction rates are set to $k_j = 1$ (for the first-order reaction) and $k_j = 0.01$ (for the second-order reaction) for the MAPK-ERK system. In order to assess the impulsive effects in decreasing or increasing directions, after simulating the system without impulses, we run it initially by adding 50 units of concentrations to the RKIP and the ERK.P2 proteins in every 100-second and then repeat the simulation by decreasing the same amounts from the same proteins. The outcomes under the new conditions are listed in Table 5 and the associated computational time is represented in Table 4 for comparison. From the outputs in Table 5, we observe that under the

increasing effects of impulses, most of the species, (i.e., 17 common species) have opposite directions via COPASI and SBT. But according to our accuracy rules, we see that 6 of them can produce more biologically validated results in COPASI and 5 of them can be biologically validated in SBT. On the other side, when we evaluate the outcomes of the decreasing effect of impulses, we find that 7 species in COPASI and 4 species in SBT can be biologically confirmed among 20 species. On conclusion from this analysis, we cannot explain the cause of opposite directions in both tools via our evaluation rule.

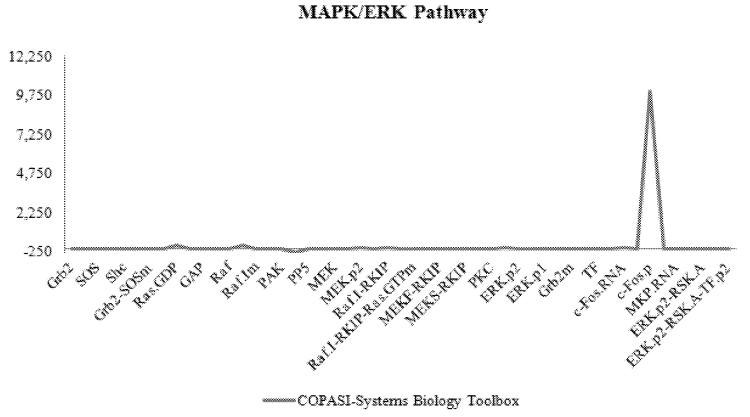
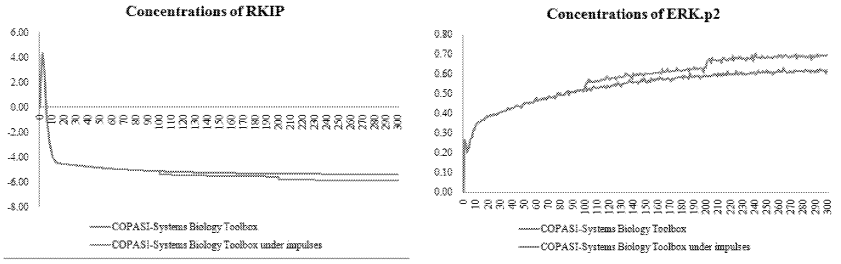


FIGURE 5. Comparison of the differences in simulation results of the MAPK-ERK pathway via COPASI and SBT with/without impulses.



(a) (b)

FIGURE 6. Comparison of the differences in simulation results of RKIP and ERP.p2 via COPASI and SBT with/without impulses.

3.5. **JAK-STAT Pathway.** The Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway transmits information coming from extracellular polypeptide signals with transmembrane receptors, which

causes the DNA transcription and the activity in the cell. The system is mainly composed of a receptor, Janus kinase (JAK) and the Signal Transducer as well as Activator of Transcription (STAT) [1].

The selected model includes 64 reactions and 38 species. The complete list of reactions, the species, their initial concentrations and their associated reaction constants can be found in [7].

In Figure 7, we represent the comparative simulation of the JAK-STAT pathway without impulses via COPASI and SBT. From Table 4, it is seen that COPASI is computationally less demanding than SBT when the system becomes more complex.

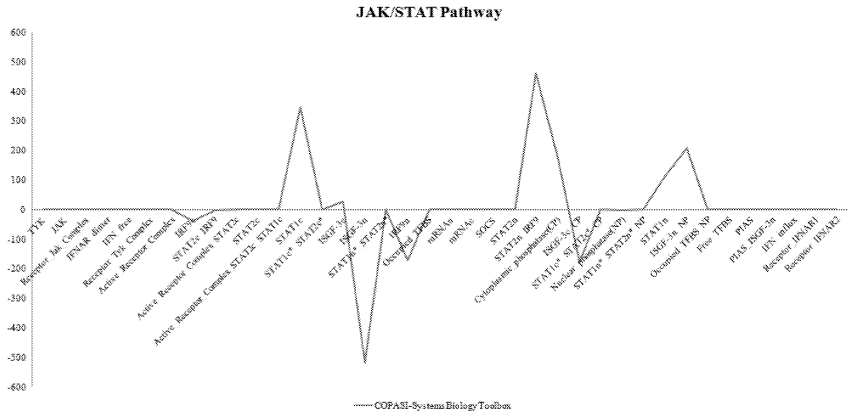


FIGURE 7. Comparison of the differences in simulation results of the JAK-STAT pathway via COPASI and SBT with/without impulses.

Finally in order to evaluate the effect of impulses in this pathway, we add 10 units of concentrations to JAK, which is the main component of the system, in every 100-second similar to the previous conditions. Then as performed for the MAPK/ERK pathway, we check the changes in the level of concentrations either in increasing or decreasing directions without considering their magnitudes. From the outputs of COPASI and SBT, we find that among 38 species, 4 of them indicate the same directional changes while none of them shows opposite directions. Furthermore, the remaining 34 species do not show any change in both tools. On the other side, when we decrease 20 units of concentrations from JAK while its concentration becomes less than 0.2 units, we observe that the same 4 species have the same directions and none of them shows opposite sides in the change of concentrations. Moreover, the remaining 34 species do not display any fluctuations in COPASI, whereas, they represent changes in SBT. Finally in the assessment of biologically plausible directions against impulses, we implement the same criteria as stated beforehand and detect that the outputs of COPASI and SBT can be validated biologically [7].

Lastly in order to assess these findings under different initials, we set all amounts of concentrations of the species to 100 units and assign the reaction

rates with respect to the order of reactions under constant initial hazards for each reaction as performed other systems. Thereby, we have 45 first-order reactions with $k_j = 1$, 15 second-order reactions with $k_j = 0.01$ and 1 third-order reaction with $k_j = 0.0001$. In the JAK-STAT pathway, there are 3 reactions which show the external stimulus of the system. We set these associated reaction rates to 1 as well since they are reversible reactions in the system whose reversible reaction order is one. Then as performed for the MAPK-ERK pathway, we extend the simulation results by adding 50 units of concentrations to JAK in every 100-second as the sharp impulse to the system and by decreasing the same amount of concentrations from JAK under the same condition to evaluate the effect of directions in impulses. The findings of these new conditions are presented in Table 5 and the associated computational time is listed in Table 4. Here, similar to previous pathways, we investigate whether the cause of opposite directions in both tools can be explained via our evaluation rule. From the outputs, we observe that only 1 species found by SBT and 3 species detected by COPASI indicate biologically relevant directions.

4. Conclusion

In this study, we have selected the simulations tools, specifically, COPASI and System Biology Toolbox for MATLAB (SBT) among alternatives that enable us to represent the impulsive changes in the system via impulsive deterministic simulation algorithms. We have compared these tools by applying two major impulsive scenarios and various initial conditions based on their accuracies and computational demands. We have observed that COPASI is computationally less demanding that SBT, whereas, there is almost no difference in the accuracy of the tools if they are initialized with biologically validated values. On the other hand when we changed the initial settings of the systems, in particular, for large systems, we have seen differences between the simulation results of both tools. Hereby regarding our assessment rule, we have found that COPASI can produce more biologically relevant outputs. But the variation between the tools for large networks and arbitrary initials can also imply that COPASI and SBT are, indeed, more suitable for the regular deterministic simulations, i.e., without impulsive effects, of the biological systems.

On the other hand, in our study, the applications of the impulses are limited under two main scenarios. But they can be extended as the impulses under random times, random states or predefined functions. Under these conditions, the user may need to develop the codes of the impulsive functions and insert them to the tools as a special package. Such implementations are available in SBT. Furthermore, we think to extent this work by analyzing the capacities of the tools in the representation of the bifurcation graphs. Since these graphs enable the researchers to see stable and unstable species in the system, this type of visualization can be also helpful for observing the effect of impulses in the network.

TABLE 4. Comparison of COPASI and SBT according to their computation time (in second). For all pathways, the biologically relevant initials are denoted by Condition 1 and the initials based on the order of reactions are shown by Condition 2.

Size of System	Condition	Network Model	Program	Time
Small	1	Lotka-Volterra	COPASI	1
		Lotka-Volterra	SBT	1
		Lotka-Volterra with Impulses	COPASI	1
		Lotka-Volterra with Impulses	SBT	1
Moderate	1	PKC	COPASI	1
		PKC	SBT	3
		PKC with Impulses	COPASI	1
		PKC with Impulses	SBT	3
Moderate	2	PKC	COPASI	1
		PKC	SBT	3
		PKC with Impulses	COPASI	1
		PKC with Impulses	SBT	3
Large	1	L-L	COPASI	1
		L-L	SBT	4
		L-L with Impulses	COPASI	1
		L-L with Impulses	SBT	6
Large	2	L-L	COPASI	1
		L-L	SBT	4
		L-L with Impulses	COPASI	1
		L-L with Impulses	SBT	6
Large	1	MAPK/ERK	COPASI	1
		MAPK-ERK	SBT	24
		MAPK-ERK with Impulses	COPASI	1
		MAPK-ERK with Impulses	SBT	28
Large	2	MAPK/ERK	COPASI	1
		MAPK-ERK	SBT	24
		MAPK-ERK with Impulses	COPASI	1
		MAPK-ERK with Impulses	SBT	28
Large	1	JAK-STAT	COPASI	1
		JAK-STAT	SBT	244
		JAK-STAT with Impulses	COPASI	1
		JAK-STAT with Impulses	SBT	390
Large	2	JAK-STAT	COPASI	1
		JAK-STAT	SBT	22
		JAK-STAT with Impulses	COPASI	1
		JAK-STAT with Impulses	SBT	28

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TABLE 5. Comparison of COPASI and SBT results based on directional similarities/dissimilarities under impulses and for Condition 1 (system-specific changes) and Condition 2 (the initials based on the order of reactions) under increasing/decreasing impulses.

Pathway	Total Number of Species	Condition	Same Direction	Opposite Direction	No Change
L-L	35	1 (Increasing)	9	2	24
		1 (Decreasing)	10	1	24
		2 (Increasing)	7	14	15
		2 (Decreasing)	5	16	15
MAPK-ERK	51	1 (Increasing)	27	8	16
		1 (Decreasing)	27	8	16
		2 (Increasing)	18	17	16
		2 (Decreasing)	14	20	17
JAK-STAT	38	1 (Increasing)	4	0	34
		1 (Decreasing)	4	0	34
		2 (Increasing)	9	14	15
		2 (Decreasing)	6	16	16

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