APPLICATION OF DYNAMIC GLOBAL SENSITIVITY ANALYSIS IN COMPLEX SYSTEMS

by

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ABSTRACT OF THE THESIS

Application of Dynamic Global Sensitivity Analysis in Complex Systems

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One of the major problems of complex mathematical models that are used to approximate systems and processes is the lack of precise parameter values. This often leads to a high degree of uncertainty in the simulated processes, which in most cases is an undesirable constraint. The uncertainty in parameter values can be addressed using sensitivity analysis, which is the study of how output variations can be apportioned to different sources of variation in the input parameters.

The first part of this work consists of the application of time-varying global sensitivity analysis techniques in a mathematical model of human endotoxemia. In general, biological systems contain a large number of components that interact with each other, making the application of sensitivity analysis a valuable tool to decipher the most critical dynamics of the system. Through sensitivity analysis the parameters or components that have little effect on the model but are experimentally observed to be significant for the system, are identified. The

results imply the need for better parameter estimation, after further experimentation, or model modifications that will capture the experimentally observed system dynamics.

In the second part of this work, the complexity of how interactions between the different unit operations of a continuous tablet manufacturing flowsheet simulation affect the overall product quality is studied. Both quantitative and qualitative results reveal how different uncertain variables of a process dynamically affect an output through the use of time-varying global sensitivity indices. Thus the most important and critical parameters for a certain output are identified at different time points. Such an approach of global sensitivity analysis is not only used to draw significant conclusions about the interactions between specific uncertain inputs to outputs, but also points out necessary correlations that the model fails to capture.

Through this work it is shown that sensitivity analysis should have an important part during the development and validation of a computational model in any scientific field. It allows the quantitative and qualitative investigation of variation and perturbation effects on the system behavior and correlation with experimental data.

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1. INTRODUCTION

Due to the complexity of real-life processes, physical experimentation is time-consuming, expensive or impossible, and for this reason scientists more and more turn to mathematical and computational models that simulate and approximate those processes. Complex mathematical models are defined by a series of equations, a large number of input factors, parameters and variables are developed to approximate systems and processes of varying complexity from different scientific fields.

The inputs of a process, however, are often subject to various sources of uncertainty like errors in measurement, lack of data, and poor understanding of the underlying mechanisms. Further, some models may have natural intrinsic variability in the system such as stochastic events. Those uncertainties produce uncertainty in the response of the model and an evaluation of the confidence in the model is good modeling practice. Sensitivity analysis as a whole provides an understanding of how changes and variations in the inputs affect the model responses, increasing the confidence in the model and its predictions.

In this work, the broad applicability of sensitivity analysis is demonstrated through two case studies in two different scientific fields. In chapter 2 the concept of sensitivity analysis is introduced and different sensitivity analysis approaches found in literature are presented. In chapter 3, the application of a dynamic approach for sensitivity analysis is described in a mathematical model of human endotoxemia and in chapter 4 the dynamic sensitivity analysis is applied to a case study of a continuous tablet manufacturing flowsheet model. Finally, in chapter 5 the conclusions of this research are discussed.

2. SENSITIVITY ANALYSIS

2.1. Introduction

Sensitivity analysis is defined by Saltelli (1) as "the study of how the variation in the output of a model can be apportioned, qualitatively or quantitatively, to different sources of variation, and of how the given model depends upon the information fed into it". Another definition for sensitivity analysis is (2) the systematic investigation of the model responses to perturbations in the model quantitative factors (i.e. inputs/parameters) or variations in the model qualitative factors (i.e. structure, connectivity, etc).

There are two types of sensitivity analysis methods. In local methods the inputs are varied one parameter at a time within a small interval around a nominal value, and the effect of this variation in the output is calculated with partial derivatives. In global methods, more than one input factors are varied simultaneously over a larger parameter space, around a nominal value based on our knowledge base of the parameters. Using sampling based approaches an input parameter vector is created and the effects of individual inputs and interactions between inputs are calculated on the model output.

2.1.1. Uses of sensitivity analysis

Before, during and after model development, sensitivity analysis can be used for a number of applications (1). The first and most fundamental reason is to determine whether the model resembles the system or processes we are trying to build. The

quantitative capabilities of sensitivity analysis identify which factors mostly contribute to the output variability of the model and therefore require additional research or experimentation to strengthen their knowledge base or which factors or model components are insignificant and can be eliminated from the final model. Additionally, sensitivity analysis can identify if there exists a region in the space of input factors for which the model variation is maximum, what are the optimal regions within the space of the factors that can be used in a model calibration and finally certain sensitivity analysis methods can identify the existence of factor interactions with each other or within interacting groups and if the model has a strong dependence on a non-influential factor.

2.1.2. Goals of sensitivity analysis

Sensitivity analysis can be used prior to the model calibration, to determine and identify a set of parameters that will be important during the calibration procedure, since the difficulty of the model calibration against various types of data increases as the number of processes to be modeled becomes larger (1). Sensitivity analysis can also ensure that the model response to its input factors is accounted for, that the model does not have any discrepancies by exhibiting strong dependence on non-influential factors and that the model predictions yield a realistic and sensible range of results.

Model identification can be aided with the use of sensitivity analysis, since in order to describe available evidence the most appropriate model structures and specifications can be identified. Mechanism reduction, which is closely related, can be used in determining a subset of input factors accounting for most of the output variance.

This way the most insignificant factors in a complex model can be identified and eliminated from the final model for a simpler model.

Another capability for sensitivity analysis is that it can determine if there is some region in the space of input parameters for which the model variation is maximum or divergent. In general sensitivity analysis can assist the modeler in deciding whether the model performs as expected from the process and when that is not provided the case, it provides guidance on where to concentrate to solve the problem.

2.2. Local sensitivity analysis

In local sensitivity analysis the inputs are varied one parameter at the time around the nominal value (1).

A time-dependent system might have the following initial-value problem form:

$$\frac{d\mathbf{y}}{dt} = f\left(\mathbf{y},\mathbf{k}\right),\ \mathbf{y}\left(0\right) = \mathbf{y}^0 \qquad \text{(Equation 1)}$$

Where y is the vector of variables, **k** is the vector of system parameters and $\mathbf{y^0}$ is the array of initial values. A Taylor series expansion can express the effect parameter changes will have on the solution:

$$y_i\left(t,\mathbf{k}+\Delta\mathbf{k}\right) = y_i\left(t,\mathbf{k}\right) + \sum_{j=1}^{m}\frac{\partial y_i}{\partial k_j}\Delta k_j + \frac{1}{2}\sum_{l=1}^{m}\sum_{j=1}^{m}\frac{\partial^2 y_i}{\partial k_l \partial k_j}\Delta k_l k_j + \ldots \qquad \text{(Equation 2)}$$

The partial derivatives $\partial y_i / \partial k_j$ are called first-order local sensitivity coefficients and form the sensitivity matrix $\mathbf{S}(t) = \{s_{ij}\} = \{\partial y_i / \partial k_j\}$ partial derivatives $\partial^2 y_i / \partial k_l \partial k_j$ are called second-order sensitivity coefficients and so on.

In general, the first-order local sensitivity coefficient $s_{ij}(t)$ describes the effect a perturbation of the j^{th} input parameter around its nominal value has on the i^{th} output parameter at time t.

2.2.1. Indirect method

Slightly changing one parameter at a time by a small value $k_j + \Delta k_j$ and rerunning the model is the simplest method to calculate local sensitivities (1). Using this finite-difference approximation the sensitivities can be approximated by:

$$s_{ij}(t) \approx \frac{y_i(k_j + \Delta k_j, t) - y_i(k_j, t)}{\Delta k_j}, j = 1, ..., m \qquad \text{(Equation 3)}$$

Using this method, calculation of local sensitivities requires at least m+1 simulation runs of the model. The accuracy of the calculated sensitivities depends on the parameter change Δk_j. Large parameter changes (>5%) would cancel the assumption of local linearity in non linear models, a small parameter change on the other hand would produce high round-off errors. Therefore a trial-and-error approach is employed to find the appropriate percentage of parameter change.

2.2.2. Direct method

If we differentiate Equation 1 with respect to k_j we get the following system of sensitivity differential equations:

$$\frac{d}{dt}\frac{\partial \mathbf{y}}{\partial k_j} = \mathbf{J}\frac{\partial \mathbf{y}}{\partial k_j} + \frac{\partial \mathbf{f}}{\partial k_j} \qquad \text{(Equation 4)}$$

Or in matrix form:

$$\dot{\mathbf{S}} = \mathbf{JS} + \mathbf{F} \qquad \text{(Equation 5)}$$

Where $\mathbf{J} = \{\partial f_i / \partial y_l\}$ is the Jacobian matrix and $\mathbf{F} = \{\partial f_i / \partial k_j\}$ is the parametric Jacobian and the initial condition is a zero vector.

For the numerical calculation of local sensitivities, the decoupled direct method (DDM) developed by Dunker (3) has been proven to be the best general method.

2.2.3. Limitations of local sensitivity analysis

The DDM and other local sensitivity analysis techniques have been applied in various instances in systems biology for the analysis of signal transduction pathways and the identification of influential parameters (4, 5). However, the uncertainty and large range of biological inputs, the possible interactions between parameters and the inability of local sensitivity analysis methods to study multiple parameters at a time pose as limitations in the use of local sensitivity analysis methods in systems biology. With the use of global

sensitivity analysis methods it is possible to investigate the effect of simultaneous parameter variations.

2.3. Global sensitivity analysis

2.3.1. Sampling based methods

In sampling based methods, Monte-Carlo techniques are used to create samples and analysis is performed to explore the mapping between the uncertain inputs and the outputs of the model.

The model under analysis can be represented by the following vector:

$$\mathbf{y} = [y_1, y_2, ..., y_m]$$

and the corresponding input can be represented by the following vector:

$$\mathbf{x} = [x_1, x_2, ..., x_k]$$

where m is the number of outputs and k is the number of inputs.

Sampling-based sensitivity analysis approach involves five steps:

1) The most important step is the definition of the distributions D_1, D_2,..., D_k that characterize the assessed uncertainties of the inputs of **x**. If the analysis is exploratory then the distributions can be developed by selecting a distribution type (normal, uniform, lognormal, etc.) and minimum, maximum, median values

until the representation of the uncertainty in the parameter under consideration is adequate. In biological modeling there is often lack of information and uniform distributions can be used.

2) Use a sampling method (random sampling, Latin hypercube Sampling, etc.) to generate N samples of input vectors $\mathbf{x}_1$, $\mathbf{x}_2$,…, $\mathbf{x}_N$ from the distributions from step 1. Random sampling is the simplest way to generate a sample but a large number of samples might be required to sample the entire range appropriately, however Latin hypercube sampling (LHS) has been shown to be more efficient than random sampling (6).

3) The model is evaluated N times for each of the input vectors, a set of model outputs are obtained $\mathbf{y}(\mathbf{x}_i), i = 1, 2, ..., N$ and the results are stored.

4) The uncertainty of the model outputs is quantified and displayed either as a scalar (mean, variance, etc.) or as a function by plotting the probability density function (PDF) and the cumulative density function (CDF).

5) The effects of the individual parameters on the model outputs are determined by the mapping between uncertain inputs and the uncertainty in the outputs. Quantification is provided when regression and correlation analysis is used, however when non-monotonicities are present in the model then regression and correlation based indices are not accurate. These measures can also be used to

study the sensitivity to dynamic model outputs by calculating at multiple time-points.

2.3.2. Screening methods

When a model contains a very large number of input factors or needs a long time to be evaluated then an initial screening has to be performed (1). This screening method is economical and identifies the few significant factors among a large number of input factors. However the economy of the screening methods has the drawback that the extracted sensitivity measures rank the parameters by their order of importance only qualitatively. For this reason the few identified significant factors can later be studied with another global sensitivity analysis method in greater detail to quantify their effect in the model outputs.

The Morris method (7-9) is the most robust, widely used and popular out of all the screening methods. The two measures of importance used in the Morris screening method are μ which estimates the overall effect of the factor on the output and, and σ that estimates the interaction effects in which the factor is involved. σ is used to detect factors involved in interaction with other factors or whose effect is non-linear while μ detects input factors with an important overall influence on the output. A graphical representation of the two sensitivity measures plotted against each other, σ plotted versus μ, can help interpret the results by comparing where the plotted values are located and provide a relative measure of importance.

Morris screening method

The input factor vector **x** of dimension k with the components x_i takes p values from the set {0,1/(p-1),2/(p-1),…,1}. If Δ is a predetermined multiple of 1/(p-1) then the elementary effect of the i^{th} factor at point **x** is defined as:

$$d_i = \frac{\left[y\left(x_1,...,x_{i-1},x_i+\Delta,x_{i+1},...,x_k\right) - y\left(\mathbf{x}\right)\right]}{\Delta} \quad \text{(Equation 6)}$$

with **x**+Δ having such a value that it is still in the allowed region of experimentation for each of the factors. A distribution of elementary effects for input *i*, F_i, can be generated by sampling **x** from the possible input values. If random sampling is performed for *r* elementary effects from each of the distributions F_i, then $n=2rk$ runs are needed.

To assess the importance of a factor, the mean μ and standard deviation σ of the sample are used. A factor with a high overall importance on the output will have a high mean μ while a factor with a nonlinear effect or interacting with other factors will have a high standard deviation σ.

In the simplest application of the Morris method, the model will be evaluated twice (for **x** and for **x**+Δ), for each elementary effect. Thus for the generation of a sample with size *r*, $n=2rk$ runs are required. The factor *r* is typically in the order of 10.

Generally, the four steps involving the application of the Morris method are the following:

1. The base value **x*** is randomly chosen for **x** with each component being sampled from the set $\{0,1/(p-1),\ldots,1-\Delta\}$.
2. One or more of the components of **x*** are increased by Δ so that that the new vector $\mathbf{x}^{(1)}$ still has values belonging to the set of possible input values.
3. The elementary effect of the i^{th} component of $\mathbf{x}^{(1)}$ is calculated when the i^{th} component has been changed by Δ:

   If $\mathbf{x}^{(1)}$ has been increased by Δ:

$$d_i\left(\mathbf{x}^{(1)}\right)=\frac{y\left(x_1^{(1)},\ldots,x_{i-1}^{(1)},x_i+\Delta,x_{i+1}^{(1)},\ldots,x_k^{(1)}\right)-y\left(\mathbf{x}^{(1)}\right)}{\Delta} \quad \text{(Equation 7)}$$

   If $\mathbf{x}^{(1)}$ has been decreased by Δ:

$$d_i\left(\mathbf{x}^{(1)}\right)=\frac{y\left(\mathbf{x}^{(1)}\right)-y\left(x_1^{(1)},\ldots,x_{i-1}^{(1)},x_i-\Delta,x_{i+1}^{(1)},\ldots,x_k^{(1)}\right)}{\Delta} \quad \text{(Equation 8)}$$

4. A new defined vector $\mathbf{x}^{(2)}=\left(x_1^{(1)},\ldots,x_{i-1}^{(1)},x_i\pm\Delta,x_{i+1}^{(1)},\ldots,x_k^{(1)}\right)$ is the vector that is produced from the previous step. Another new vector $\mathbf{x}^{(3)}$ that differs from $\mathbf{x}^{(3)}$ for only one component *j* is created: $x_j^{(3)}=x_j^{(2)}\pm\Delta$.

   For this factor j, in the case of $\Delta > 0$, the estimated elementary effect is:

$$d_j\left(\mathbf{x}^{(2)}\right)=\frac{y\left(\mathbf{x}^{(3)}\right)-y\left(\mathbf{x}^{(2)}\right)}{\Delta} \quad \text{(Equation 9)}$$

   In the case of $\Delta < 0$, the elementary effect is:

$$d_j\left(\mathbf{x}^{(2)}\right)=\frac{y\left(\mathbf{x}^{(2)}\right)-y\left(\mathbf{x}^{(3)}\right)}{\Delta} \quad \text{(Equation 10)}$$

This step is repeated for k+1 input vectors $\mathbf{x}^{(1)}$, $\mathbf{x}^{(2)}$,…, $\mathbf{x}^{(k+1)}$ so that two consecutive vectors differ by only one component. Any component i of the base vector **x*** has been increased by Δ at least once and the k+1 consecutive vectors define a trajectory in the parameter space.

A matrix **B***, called the orientation matrix is then formed with size $(k+1)\times k$, having the vectors $\mathbf{x}^{(1)}$, $\mathbf{x}^{(2)}$,…, $\mathbf{x}^{(k+1)}$ as rows.

The orientation matrix can be constructed by:

$$\mathbf{B}^{*}=\left(\mathbf{J}_{k+1,1}\mathbf{x}^{*}+(\Delta/2)\left[\left(2\mathbf{B}-\mathbf{J}_{k+1,k}\right)\mathbf{D}^{*}+\mathbf{J}_{k+1,k}\right]\right)\mathbf{P}^{*} \quad \text{(Equation 11)}$$

Where **B** is usually a $(k+1)\times k$ lower triangular matrix of 1s, $\mathbf{J}_{k+1,k}$ is a $(k+1)\times k$ matrix of 1s, **D*** is a k-dimensional diagonal matrix with either +1 or -1 as elements with equal probability and **P*** is a $k\times k$ random permutation matrix in which each column contains one element equal to 1 and all other elements equal to 0 with no two columns having a 1 in the same position.

2.3.3. Variance based methods

Variance based methods quantify the amount of the total output variation explained by the uncertainty in the input factors (1). They are considered to be the best sensitivity analysis techniques because they are model independent and do not depend on relationship assumptions between the model input and outputs. Variance based methods not only quantify the main effects of individual inputs but can also be used to investigate the total effects of all possible interactions between one parameter and all the other parameters. The total effect is a more appropriate measure for the effect of a factor on the output because it takes into account all the possible interactions that factor is involved in.

There are three types of variance based methods used to calculate the effects: correlation ratios, Sobol and Fourier amplitude sensitivity test (FAST). The correlation ratios have been shown to produce similar results to the Sobol and FAST first-order sensitivity indices (10) however Sobol and FAST are more efficient. The FAST method was initially developed by Cukier et al. (11) and it was extended by Saltelli et al. (12) producing the extended FAST (eFAST) which also has the capability to calculate total effects. In the method of Sobol (13) the output variance is decomposed to terms of increasing dimensionality and a Monte Carlo method is used to calculate the effects. The method of Sobol has a simpler implementation than eFAST but was further optimized by Saltelli (14) to be more efficient than before and comparable to the computational cost of eFAST.

However, when the model has a large number of input parameters then the use of these methods can be computationally expensive and another method should be used beforehand to reduce the number of parameters under investigation.

Variance based method of Sobol

The Sobol (1, 13) approach begins by defining the input factor space of the integrable function *f(*x*)* by an *n*-dimensional unit hypercube I^n. The function is decomposed into summands of increasing dimensionality by equation 12:

$$f(x) = f_0 + \sum_{s=1}^{n} \sum_{i_1<\ldots<i_s}^{n} f_{i_1\ldots i_s}\left(x_{i_1},\ldots,x_{i_s}\right) \quad \text{(Equation 12)}$$

Equation 12 upon expansion becomes:

$$f(x) = f_0 + \sum_i f_i(x_i) + \sum_{i<j} f_{ij}(x_i, x_j) + \ldots + f_{12\ldots n}(x_1, x_2, \ldots, x_n) \quad \text{(Equation 13)}$$

In order for equation 13 to hold, f_0 is required to be a constant and the integrals of every summand between unit limits, over any of its variables must be equal to zero:

$$\int_0^1 f_{i_1\ldots i_s}\left(x_{i_1},\ldots,x_{i_s}\right)\mathrm{d}x_k = 0 \text{ for } k = i_1,\ldots,i_s \quad \text{(Equation 14)}$$

From equation 14 it follows that the members of equation 12 are mutually orthogonal and therefore they can be expressed as integrals of *f(*x*)*:

$$\int f(x)\mathrm{d}x = f_0 \quad \text{(Equation 15)}$$

$$\int f(x)\mathrm{d}x_k\big|_{k\neq i} = f_0 + f_i(x_i) \quad \text{(Equation 16)}$$

$$\int f(x)\mathrm{d}x_k\big|_{k\neq i,j} = f_0 + f_i(x_i) + f_j(x_j) + f_{ij}(x_i, x_j) \quad \text{(Equation 17)}$$

The expansion can continue for higher order terms.

If it is assumed that $f(\mathbf{x})$ is square integrable then all $f_{i_1\ldots i_s}$ in [a] are also square integrable. Squaring both sides of equation 12 and integrating over I_n generates:

$$\int f^2(x)\mathrm{d}x - f_0^2 = \sum_{s=1}^{n}\sum_{i_1<\ldots<i_s}^{n}\int f_{i_1\ldots i_s}^2 \mathrm{d}x_{i_1}\ldots dx_{i_s} \qquad \text{(Equation 18)}$$

In equation 18 the left hand side and right hand side can be rewritten as equations 19 and 20:

$$D = \int f^2(x)\mathrm{d}x - f_0^2 \qquad \text{(Equation 19)}$$

$$D_{i_1\ldots i_s} = \int f_{i_1\ldots i_s}^2 \mathrm{d}x_{i_1}\ldots dx_{i_s} \qquad \text{(Equation 20)}$$

$$D = \sum_{s=1}^{n}\sum_{i_1<\ldots<i_s}^{n} D_{i_1\ldots i_s} \qquad \text{(Equation 21)}$$

D is called total variance and $D_{i_1\ldots i_s}$ are called partial variances. As shown from equation 21 the total variance is equal to the sum of partial variances.

Taking the ratio between the partial variances $D_{i_1\ldots i_s}$ and the total variance D the total variance generates the Sobol indices which are defined as:

$$S_{i_1\ldots i_s} = \frac{D_{i_1\ldots i_s}}{D} \qquad \text{(Equation 22)}$$

The term $S_{i_1\ldots i_s}$ provides the fraction of the total variance on the output which is due to one factor or a combination of factors. $S_i=D_i/D$ which is called the first order sensitivity index is the contribution of x_i to the output variation, while S_{ij} for $i\neq j$ is the variation due to x_i and x_j which cannot be explained by the sum of the first order sensitivity indices S_i and S_j and is the variance which is due to the interaction between those factors.

The total sensitivity index (1, 15) of a factor is the sum of all sensitivity indices involving that factor. A set of *k* factors **x** can be partitioned into two factor subsets **w** and **v**, where **v** contains only factor x_i and **w** the remaining $x_{\sim i}$ factors. If we decompose *f(***x***):*

$$f(\mathbf{x}) = f_0 + f_1(\mathbf{v}) + f_2(\mathbf{w}) + f_{12}(\mathbf{v},\mathbf{w}) \qquad \text{(Equation 23)}$$

with

$$\int f_1 d\mathbf{v} = \int f_2 d\mathbf{w} = \int f_{12} d\mathbf{v} = \int f_{12} d\mathbf{w} = 0 \qquad \text{(Equation 24)}$$

and

$$D_{\mathbf{v}} = \int f_1^2 d\mathbf{v} \qquad D_{\mathbf{w}} = \int f_2^2 d\mathbf{w} \qquad D_{\mathbf{vw}} = \int f_{12}^2 d\mathbf{v} d\mathbf{w} \qquad \text{(Equation 25)}$$

The total variance can be written as:

$$D = D_{\mathbf{v}} + D_{\mathbf{w}} + D_{\mathbf{vw}} \qquad \text{(Equation 26)}$$

The total effect of **v** in the output is defined as:

$$D_{\mathbf{v}}^{tot} = D_{\mathbf{v}} + D_{\mathbf{vw}} = D - D_{\mathbf{w}} \qquad \text{(Equation 27)}$$

Therefore the total sensitivity index for $\mathbf{v} = x_i$ is defined as:

$$S_{T_i} = \frac{D_i^{tot}}{D} = \frac{D - D_{\sim i}}{D} = 1 - \frac{D_{\sim i}}{D} \qquad \text{(Equation 28)}$$

Which is equivalent to:

$$S_{T_i} = S_i + S_{i(\sim i)} = 1 - S_{\sim i} \qquad \text{(Equation 29)}$$

Therefore the total sensitivity index is the sum of all sensitivity indices involving the parameter under investigated. This equation describes the total variance in the output of a factor *i* both individually and in all interactions with other factors.

It should be noted that the use of S_{T_i}, in order to investigate the overall effect that a factor has on the output variable, is much more reliable than using the first-order sensitivity indices. The influence a parameter has not only depends on the first order sensitivity indices but also depends on the interactions of all the parameters and that is why methods such as Sobol, which can also compute the total order sensitivity indices, are being used regularly.

If the S_{Ti} is high then p_i is an influential parameter, if S_i and S_{Ti} are both small then p_i is not an influential parameter neither alone nor by its interaction with another parameter. If both the first-order S_i and total-order S_{Ti} sensitivity indices are similar then there are no interaction between p_i and another parameter. Finally, very different first-order S_i and total-order S_{Ti} imply high interactions of p_i with other parameters.

The Sobol method is able to calculate the first-order, all higher-order sensitivity indices and the total sensitivity indices to quantitatively determine the interaction between parameters. However, as the number of indices to be calculated is increased so does the computational cost and calculation time. The use of total order sensitivity indices S_{T_i}, for the investigation of the overall effect of a factor gives better results than simply using the first-order sensitivity indices.

2.4. Conclusions

In computationally expensive models with a very large number of parameters, screening methods can be first used to identify the subset of parameters that mostly

control the output variability (1, 9). The advantage of screening methods is their low computational cost with the trade-off of only providing a qualitative sensitivity measure and ranking the parameters by the order of importance i.e. they do not quantify how much more important is one parameter from another. The Morris method, which uses the mean and the standard deviation of local sensitivity measures to quantify the global importance of input parameters (16-20), is the most effective of the screening methods which is in good agreement with results from the Sobol method.

After the initial screening with the Morris method, identifying whether a parameter is influential or not, not only depends on the first order sensitivity indices but also depends on the interactions of all the parameters and that is why methods such as Sobol, which can also compute the total order sensitivity indices, have gained popularity.

In the next parts of this work the sensitivity analysis technique is applied dynamically to two complex mathematical models from different scientific fields. It allows the quantitative and qualitative investigation of variation and perturbation effects on the system behavior and correlation with experimental data and is shown that sensitivity analysis should have an important part during the development and validation of a complex mathematical model in any scientific field.

3. SENSITIVITY ANALYSIS OF A HUMAN ENDOTOXEMIA MODEL

3.1. Introduction to Systems Biology

Systems biology is the study of biology at the system level and the understanding of the structure and dynamics of various cellular functions (21). Understanding the system properties may have a significant impact on the future of medicine.

The first step to get an understanding of the gene regulatory networks and biochemical interactions in any biological system is to determine the interconnections of genes and proteins. Further steps include the identification of all the genes and proteins involved in a process in an organism however this is not sufficient for an understanding of biological system structure and dynamics.

Systems biology is about designing a biological system using an engineering system design approach. Robustness is achieved through negative feedback and feed-forward control, multiple system components with equivalent functions for backup and redundancy, structural stability through intrinsic mechanisms, and subsystems that are physically or functionally insulated so that in the event of failure in one component system-wide failure does not spread to other subsystems

A thorough system-level understanding of a dynamically interacting biological system can be performed through metabolic analysis, sensitivity analysis, dynamic

analysis methods such as phase portrait and bifurcation analysis, and by identifying essential mechanisms underlying specific behaviors.

3.2. Human endotoxemia model

In this section, global sensitivity analysis techniques are applied to an indirect response model of human endotoxemia taken from the literature and developed by Foteinou et al. (22). A brief description is presented below.

Upon the administration of an endotoxin stimulus (Lipopolysaccharides – LPS) to a human subject, LPS binds and activates the receptors (R). The activated ligand–receptor complex stimulates the production rate of gene transcripts of receptor proteins and triggers the activation of an intracellular signal (DR*), which in turn directly stimulates a pro-inflammatory response (P). It is hypothesized that the pro-inflammatory response (P) stimulates the energetic response (E) and the anti-inflammatory response (A). The anti-inflammatory response (A) is the immunoregulatory signal that aims to restore homeostasis in the system, and is stimulated by the activation of the pro-inflammatory (P) and the energetic response (E). Furthermore it inhibits the production rate of the pro-inflammatory (P) and energetic responses (E). The described elements of the network of interactions of the indirect response model are shown in Figure 1.

The indirect response model of human endotoxemia is illustrated in Figure 1 and the model equations are presented (Equations 30-37):

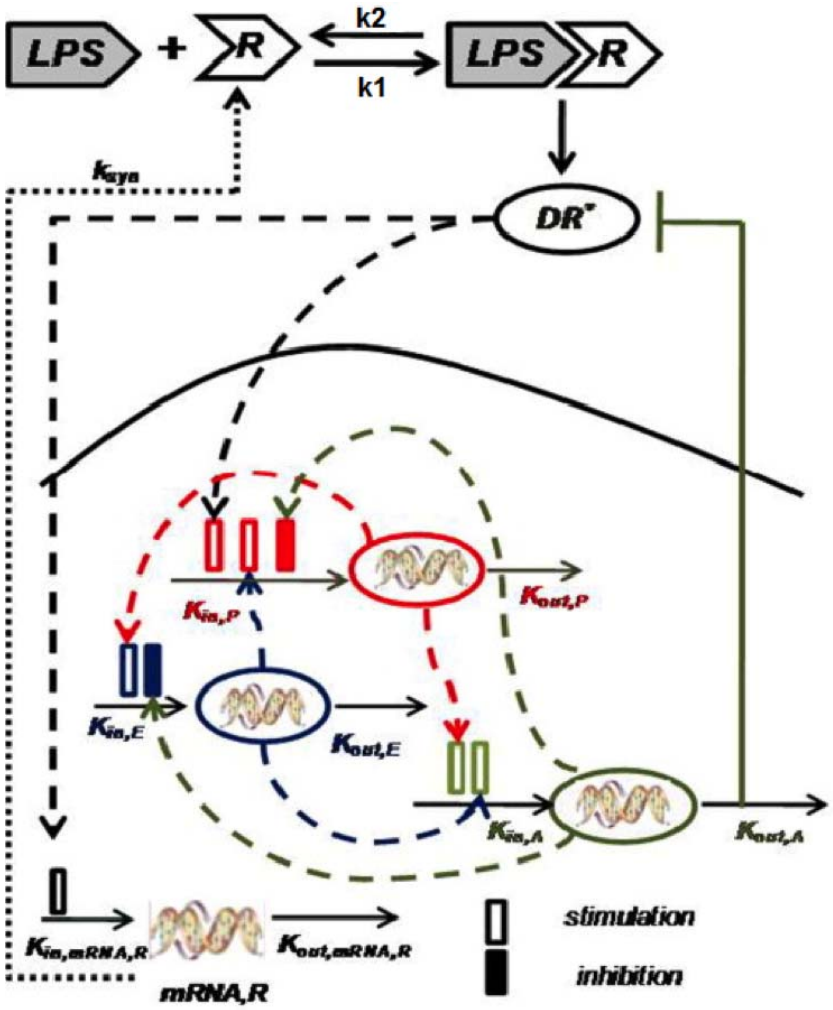

Figure 1. Overall structure of the indirect response model developed by Foteinou et al. (22).

- The inflammatory stimulus (LPS) right after administration, is eliminated with a first order elimination rate $k_{lps,2}$ and a logistic-type function with growth rate $k_{lps,1}$. The parameters $k_{lps,1}$ and $k_{lps,2}$ are estimated accordingly so that if there are no complications the LPS decays within two hours of administration.

$$\frac{dLPS}{dt} = k_{lps,1} \cdot LPS \cdot (1 - LPS) - k_{lps,2} \cdot LPS \qquad \text{(Equation 30)}$$

- The LPS binds to the TLR4 receptor (R) and the dynamics depend on the association (k_1) / dissociation (k_2) parameters as well as the rate of synthesis of new receptors k_{syn} through translation of receptor mRNAs.

$$\frac{dR}{dt} = k_{syn} \cdot mRNA,R + k_2 \cdot \left(LPSR\right) - k_1 \cdot LPS \cdot R - k_{syn} \cdot R \qquad \text{(Equation 31)}$$

- The mRNA gene transcripts of the receptor have a production rate ($K_{in,mRNA,R}$) and a degradation rate ($K_{out,mRNA,R}$) but are indirectly stimulated by the activated signaling complex DR*.

$$\frac{dmRNA,R}{dt} = K_{in,mRNA,R} \cdot \left(1 + K_{mRNA,DR^*} \cdot DR^*\right) - K_{out,mRNA,R} \cdot mRNA,R \qquad \text{(Equation 32)}$$

- The dynamics of the receptor-ligand complex (LPSR) depend on the association (k_1) and dissociation (k_2) rates of the complex as well as the rate of formation of the activated signaling complex, DR*.

$$\frac{d\left(LPSR\right)}{dt} = k_1 \cdot LPS \cdot R - k_3 \cdot \left(LPSR\right) - k_2 \cdot \left(LPSR\right) \qquad \text{(Equation 33)}$$

- The activated signaling complex (DR*) decays with rate k_4 and is proportional to the receptor-ligand complex with a rate constant k_3. In addition, the bistability in the healthy resolution of the system, which is present for a large inflammatory stimulus, is provided through the use of a non-linear Hill type function with parameter k_c.

$$\frac{dDR^*}{dt} = k_3 \cdot \frac{LPSR}{A} - k_4 \cdot DR^* + k_c \cdot \left(\frac{\left[DR^* \right]^5}{1 + \left[DR^* \right]^5} \right) \quad \text{(Equation 34)}$$

- The pro-inflammatory response (P) is indirectly stimulated by the activated signaling complex (DR*) and the energetic response variable (E) and is inhibited by the anti-inflammatory signaling component (A).

$$\frac{dP}{dt} = \frac{K_{in,P}}{A} \cdot \left(1 + K_{P,DR^*} \cdot DR^*\right) \cdot \left(1 + K_{P,E} \cdot E\right) - K_{out,P} \cdot P \quad \text{(Equation 35)}$$

- The anti-inflammatory signal (A) is stimulated by the activated pro-inflammatory response (P) and the energetic response variable (E) and decays with rate $K_{out,A}$

$$\frac{dA}{dt} = K_{in,A} \cdot \left(1 + K_{A,P} \cdot P\right) \cdot \left(1 + K_{A,E} \cdot E\right) - K_{out,A} \cdot A \quad \text{(Equation 36)}$$

- The energetic response (E) is stimulated indirectly by the pro-inflammatory response (P) and indirectly counter-regulated by the anti-inflammatory component (A)

$$\frac{dE}{dt} = \frac{K_{in,A}}{A} \cdot \left(1 + K_{E,P} \cdot P\right) - K_{out,E} \cdot E \quad \text{(Equation 37)}$$

Using parameter estimation techniques the parameter values are estimated from the experimental data and are listed in Table 1:

	Parameter	value		Parameter	value		Parameter	value
1	$k_{lps,1}$	4.5	**9**	$K_{in,mRNA,R}$	13.467	**17**	$K_{in,E}$	0.05
2	$k_{lps,2}$	6.79	**10**	$K_{out,mRNA,R}$	0.211	**18**	$K_{out,E}$	0.234
3	k_{syn}	0.02	**11**	$K_{P,E}$	25.191	**19**	$K_{in,A}$	0.256
4	k_1	3	**12**	k_4	0.33	**20**	$K_{out,A}$	0.86
5	K_{P,DR^*}	15.717	**13**	k_c	3	**21**	$K_{A,E}$	2.291
6	$K_{E,P}$	3.644	**14**	$K_{in,P}$	0.093	**22**	K_{mRNA,DR^*}	13.467
7	k_2	0.04	**15**	$K_{out,P}$	2.428			
8	k_3	2	**16**	$K_{A,P}$	0.022			

Table 1. Estimated values of the parameters based on self-limited response data (Taken from Foteinou et al (22))

More details on the model development can be found in the publication by Foteinou et al (22). For the implementation of the sensitivity analysis, the free software SimLab, (Simulation Laboratory for Uncertainty and Sensitivity Analysis, JRC, Italy, 2006) was used.

3.3. Application of global sensitivity analysis

The model consists of 22 uncertain parameters and 8 output variables. The application of a variance based method typically requires about 15 hours on a typical personal computer. This is a small time compared to the time needed to perform sensitivity analysis on more complex models. For this reason it was decided not to implement an initial screening with the Morris screening method.

All input variables for this model include empirical parameters calculated through parameter estimation methods. Such parameter estimation methods produce parameter sets with high uncertainty, which is addressed with the application of sensitivity analysis.

The uncertainty in the input parameters is represented by the assigned distributions. The input parameter probability distribution functions that were chosen for the Sobol variance based method were intuitive.

- Typically the distribution function for each of the 22 inputs was selected as a normal distribution with a mean value equal to the nominal value from the parameter set. The standard deviation of the normal distributions was equal to 10% of the mean value.

Following the selection of the 22 input factor distributions, 1440 input vectors were generated using quasi-random numbers and the Sobol LP_T sampling schemes which have been shown to perform better than other sampling methods. Two 60 by 22 random input matrices are generated and 60(22+2)=1440 input vectors are created. Each one of the 1440 input parameter vectors contains 22 uncertain input parameters and is used as an input vector for model evaluation. The model is simulated for the 24 hours required for the endotoxin stimulus to be resolved in the system.

The Sobol first and total order sensitivity indices are calculated for each time point (1 hour), capturing a dynamic profile and the effect of each input parameter on a certain output can be quantified through the progression of the simulation.

Performing a variance based sensitivity analysis to this model sheds light to any interactions between input parameters that might affect the model outputs. From the obtained results, qualitatively, the first order sensitivity indices are high for the first 4 hours of the simulation and become small after hour 5, when the system has resolved the inflammatory stimulus. On the contrary the total order sensitivity indices are small for the

first 4 hours of the simulation and become larger when the system has resolved the inflammatory stimulus. This observation can lead to the conclusion that in this case study, input parameters directly affect the resolution of the inflammation during the first 4 hours, later however interactions between the input parameters are what drive the system to its homeostasis.

Figures 2-7 show the time-dependent profile of first (S_i) and total order (S_{Ti}) sensitivity indices for the LPS-receptor activated complex (LPSR), receptor (R) and anti-inflammation (A) responses. The cumulative area plots only include the parameters that are found significant for the specific outputs. The other parameters have very small sensitivities, which are negligible compared to the ones that are plotted. The sensitivity indices for the rest of the outputs have similar profiles and they are not shown.

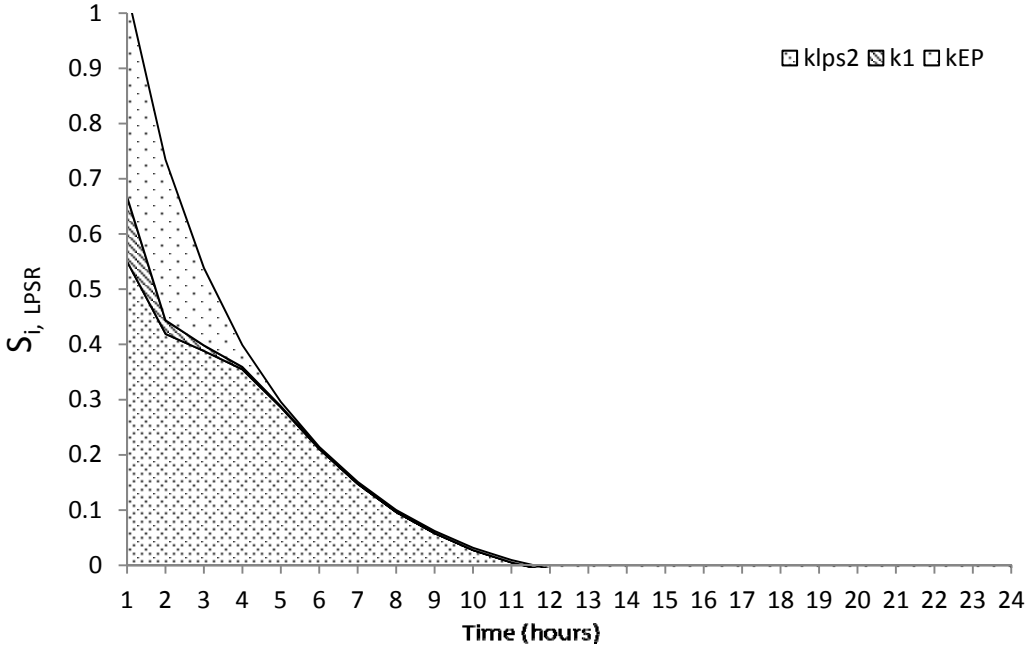

Figure 2. Cumulative area plots of the dynamic first (S_i) order sensitivity indices for the LPS-receptor activated complex (LPSR) output

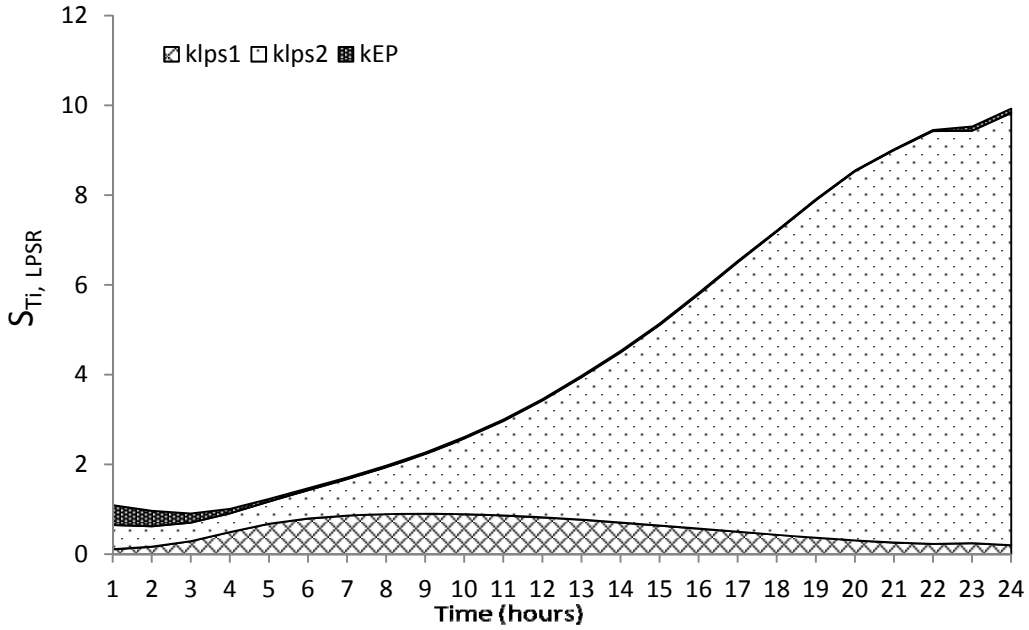

Figure 3. Cumulative area plots of the dynamic total (S_{Ti}) order sensitivity indices for the LPS-receptor activated complex (LPSR) output

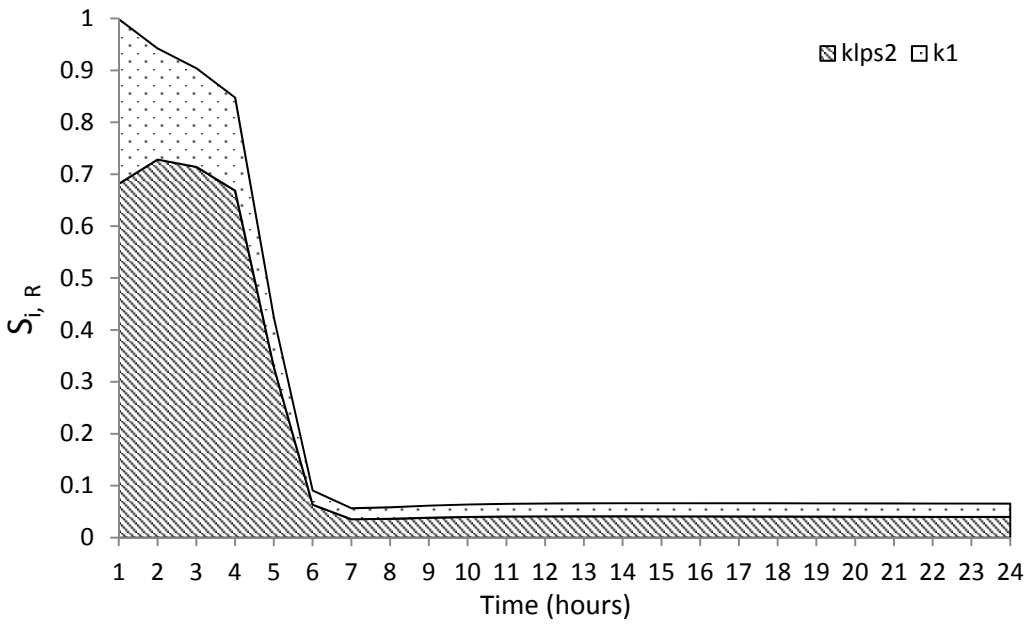

Figure 4. Cumulative area plots of the dynamic first (S_i) order sensitivity indices for the receptor (R) output

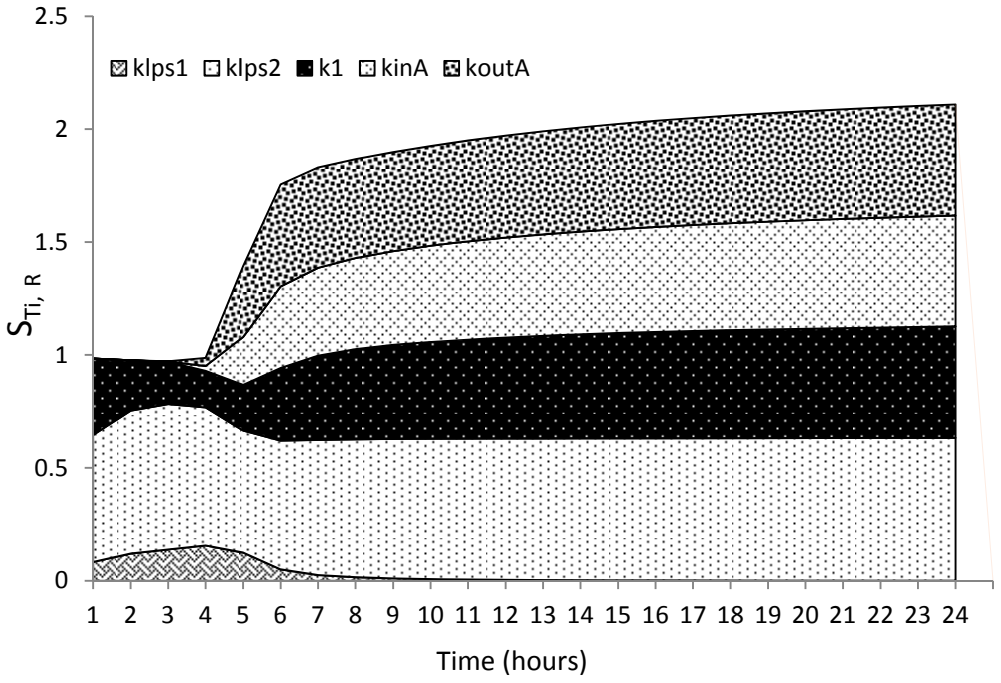

Figure 5. Cumulative area plots of the dynamic total (S_{Ti}) order sensitivity indices for the receptor (R) output

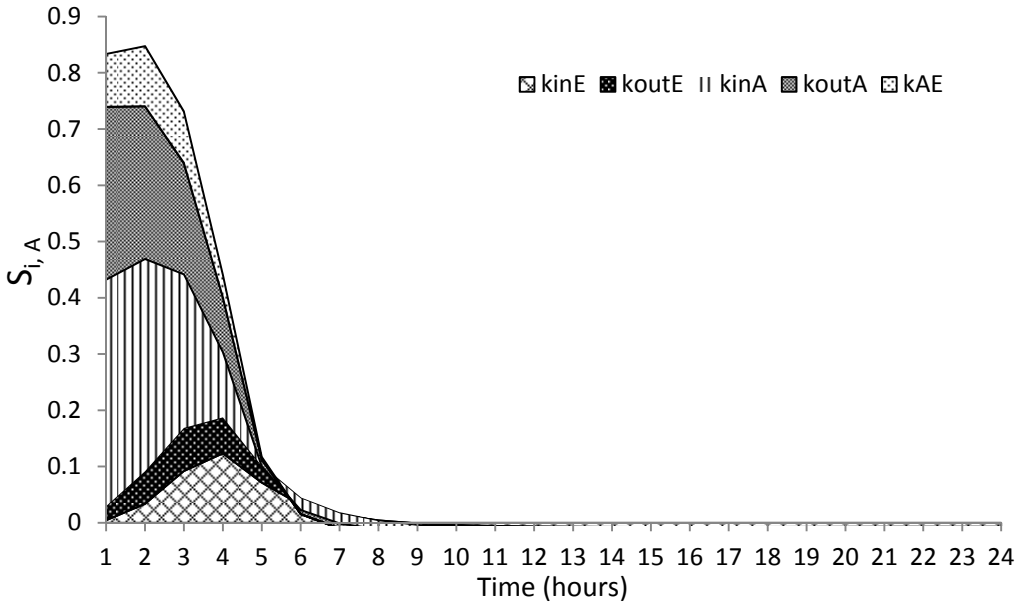

Figure 6. Cumulative area plots of the dynamic first (S_i) order sensitivity indices for the anti-inflammation (A) output

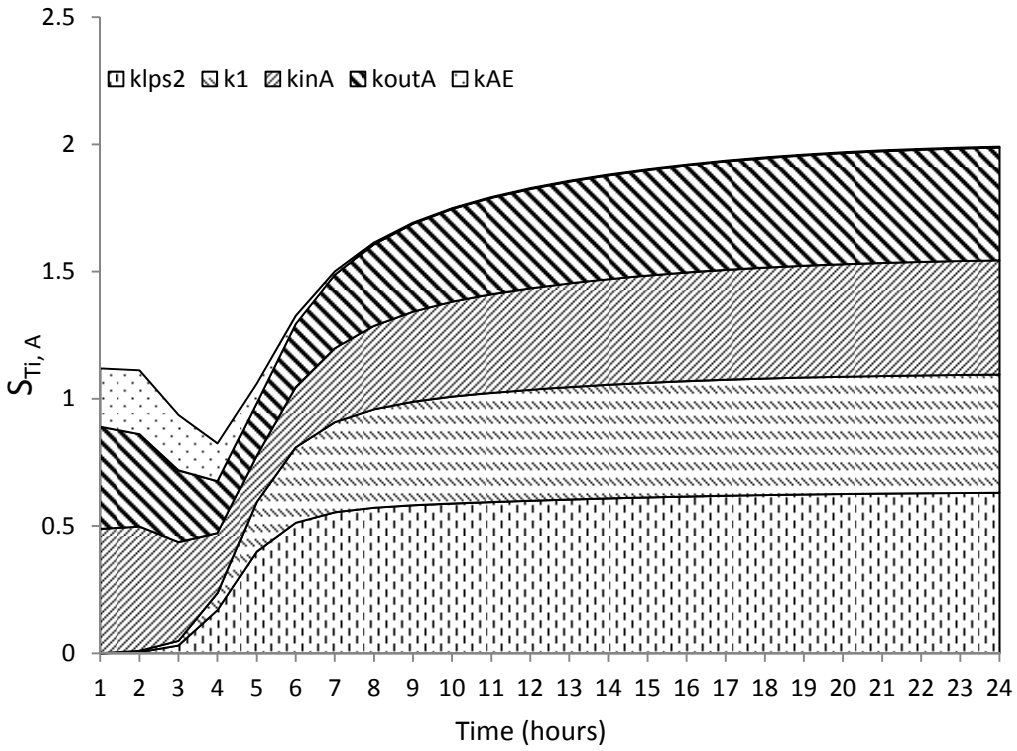

Figure 7. Cumulative area plots of the dynamic total (S_{Ti}) order sensitivity indices for the anti-inflammation (A) output

Through the implementation of the Sobol sensitivity analysis method, the first and total order effect that the input parameters have on any of the model outputs are quantified. Some of the parameters are found to be more significant than others. However a few parameters have no effect during the dynamics of the model. These parameters are the dissociation of the ligand-receptor interaction (k_2), the kinetic parameter of the pro-inflammatory response (k_{AP}) and the Hill function parameter (k_c).

This observation raises a question of possible redundancy of these parameters in the model. In order to either confirm or refute this assumption, there is a need to investigate if the performance and accuracy of the model is affected in the scenario that any of these inputs are removed from the model.

To test this hypothesis the three parameters are set to 0 and the modified model responses are qualitatively compared to the original ones. The original model is capable of capturing different biologically relevant scenarios regarding the clearance if the inflammatory stimulus: a self-limited response, a persistent infectious response, and a persistent non-inflammatory response.

Modifications of the original model were tested with a smaller number of input parameters than the original model. The different parameter deletion scenarios were implemented and suggest that k_c is an important parameter for the model since it models the bistable response of the system in different biological scenarios, something which was not captured with the sensitivity analysis.

3.4. Sensitivity analysis of initial conditions

The parameter k_c models the bistable behavior of the system when the initial endotoxin stimulus is near a critical value and the inflammatory response cannot be resolved. For this reason another application of sensitivity analysis is performed, this time including the initial value of the LPS as an uncertain parameter.

The initial value of the LPS was selected to be sampled from a uniform distribution between the values of 1 and 4 and all other distributions used for the uncertain parameters (Table 1) were the same as the previous study i.e. normal distribution with a mean value equal to the nominal value from the parameter set. The standard deviation of the normal distributions was equal to 10% of the mean value.

Following the selection of the 22+1 input factor distributions, 1500 input vectors were generated using quasi-random numbers and the Sobol LP_T sampling schemes. Two 60 by 23 random input matrices are generated and 60(23+2)=1500 input vectors are created. Each one of the 1500 input parameter vectors contains 23 uncertain input parameters is used an input vector for model evaluation. The model is simulated for the 24 hours to compare with the results of the previous case study.

In order to visualize the results in the simplest possible way, the area-under-the curve of the dynamic profiles of first and total order Sobol sensitivity indices was calculated. In the following Figure 8, the heat map contains the area under the curve values of the dynamic first order Sobol sensitivity indices. The following heat map is an accurate representation that provides a quick correlation between outputs and inputs. With a quick look one can determine which parameters have no effect on the output.

From studying the heat map, it can be seen that the parameter k_c is now found to have a significant effect on some of the outputs. This proves that in order to have a more accurate sensitivity analysis application, it is required to include a sampling range in which the simulations would be bistable.

In almost all the responses, the decay rate of the activated complex DR* (parameter k_4) controls the dynamics of the model outputs of the pro-inflammatory (P), anti-inflammatory (A) and energetic responses. This would be expected and is mechnistically correct since the activated complex is what activates the pro-inflammatory response and in turn activates the anti-inflammatory response.

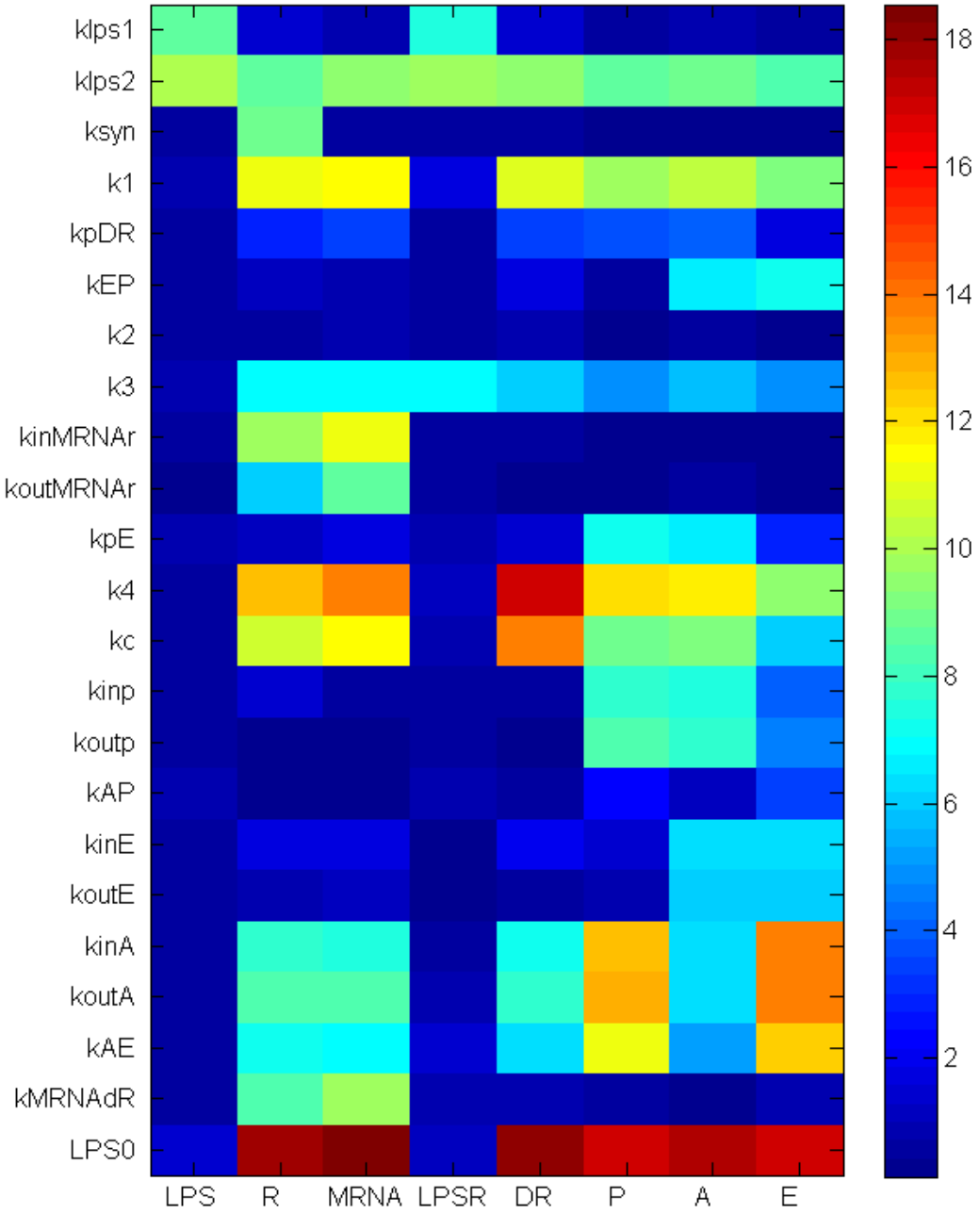

Figure 8. Heat map containing area under the curve values of the dynamic first order Sobol sensitivity indices.

Parameter k_2 is still found to have no effect on the model responses. Despite its important mechanistic role in the model, this is probably the issue of an incorrectly defined parameter set. The model can be recalibrated, a new parameter set that captures the same dynamics can be found but the parameters will have to have sensitivity indices in agreement with the experimental observations.

3.5. Conclusions

In many dynamic systems, the sensitivity of the transient behavior of the system is of particular interest and the most straightforward approach is to calculate the sensitivity indices along the output trajectory (23). Time-varying Sobol sensitivity indices were used in this work in order to extend the global sensitivity analysis for a dynamic process. The sensitivity indices are calculated for the time points of interest and provide an understanding to which parameters are the most important at those time points and need particular attention during parameter estimation.

By using this approach, changes in the effect of one parameter over an entire time interval were assessed and the input parameters that mostly control an output variable were identified.

Some input parameters were found to a larger effect in the output than others. However a few parameters had no effect during the dynamics of the model. A few case studies of biologically relevant scenarios, eliminating the insignificant parameters, were tested to validate model accuracy. Two of the three parameters were found to have higher

sensitivities in the other biologically relevant case studies. To verify this observation additional sensitivity analysis was performed. The initial value of LPS was used as an uncertain parameter, and scenarios that capture the observed bistability when large inflammatory stimulus is administered were investigated.

Despite this investigation, parameter k_2 was still found to be redundant. This however is mechanistically incorrect since the parameter k_2 is essential for the model because it describes the dissociation rate of the ligand-receptor complex. The original model however is able to capture the expected dynamics of the process because the other elements of the specific differential equation, i.e. the new receptors produced from mRNA transcripts, are able to account for all the required unbound receptors during the inflammatory stimulus resolution.

The low sensitivity of some parameters, despite their essential mechanistic role in the model, raises the concern of incorrectly defined parameter sets. A new set of parameters would have to be estimated, such that the new sensitivities indices would be in agreement with the experimental observations.

4. SENSITIVITY ANALYSIS OF CONTINUOUS TABLET MANUFACTURING

4.1. Introduction

Currently, tablet manufacturing in the pharmaceutical industry is performed in batch mode where the process control is based on the human operator's knowledge and experience. This leads to added variability in the properties of the resulting tablets, which would be minimized if there was more knowledge on how the product attributes are affected by some of the material properties and the process parameters. The transition to a continuous manufacturing process can be successful when the effects of material properties, operating parameters and environmental conditions on the product quality are well understood for each sub-process.

In sensitivity analysis the uncertainty in a complex model is quantified and the inputs and initial conditions that are critical to the outputs are identified. Applying global techniques like the Morris screening method and the Sobol variance based method in a dynamic fashion for the developed flowsheet simulation does not only identify the most significant variables and parameters, but can also assess the model form. In the case of an integrated process in which unit operations interacts with each other, a variation of a parameter in one operation most likely will affect the quality of the output stream in another process. In addition, the model parameters which are developed from noisy experimental data, uncertain input streams and control or design variables will cause a

high uncertainty in the whole process and the application of sensitivity analysis will benefit the final design in being more robust and realistic. Global sensitivity analysis techniques in flowsheet simulation models have been applied in the past for flowsheet simulation of solid processes (24) and bio-manufacturing processes in individual unit operations as well as an extension to the entire flowsheet process (25).

In the present work, the complexity of how interactions between the different unit operations of the tablet manufacturing flowsheet simulation affect the output resembles multi-compartment models in systems biology, in which complex interactions of variables in different compartments affect the overall output. This challenge has been tackled in systems biology (26, 27) and both quantitative and qualitative results show how different variables of a process dynamically affect an output. Time-varying sensitivity indices are used in order to extend the global sensitivity analysis for a dynamic process. The time-varying sensitivity indices are calculated for time points that are of interest to the modeler and provide insight to which parameters are the most important at those time points, how long after the perturbation is completed the outcome is still influenced by the perturbation and which parameters are the most critical for a certain output. By using this approach, changes in the effect of one parameter over an entire time interval can be assessed and the output variables and compartments that are most sensitive to a perturbation can be identified. By accomplishing this analysis for the flowsheet simulation, the unit operation that requires particular attention and control during the process can be identified.

The first step for the application of sensitivity analysis to a model is the use of the available data and knowledge on each one of the input parameters to assign probability

density functions that represents our knowledge. If the nominal value for a parameter is known then normal distributions are assigned, setting the nominal value as the mean and selecting a standard deviation according to how uncertain that parameter is. Monte Carlo techniques with the use of random or pseudo-random numbers are used to sample from the input parameter distributions, an input matrix is generated and multiple model output evaluations are performed. The results of these evaluations are used to assess the influence or relative importance of each input parameter on the output variable (1, 28). The model is evaluated at different time points and sensitivity indices are calculated at every time point of interest giving a dynamic character to the sensitivity analysis. Different scenarios, such as process startup and process steady state can be investigated as well as parameter perturbations during each scenario and the quantification of the effect the perturbation has on the product quality.

4.2. Continuous tablet manufacturing model

Direct compaction (Figure 9) is the simplest method to produce pharmaceutical tables with the least number of steps and unit operations. In this case study (29), Acetaminophen tablet production is simulated, with the following formulation: 3% Acetaminophen (API), 96% Avicel (Excipient) and 1% MgSt (lubricant). The system has three feeders, one for each ingredient of the tablet, which feed the ingredients to the mixer where they are blended. The mixed powder exits the mixer and is sent to a hopper which feeds the tablet press through the feed frame forming the acetaminophen tablets. The dynamic flowsheet model is built in gPROMS, setting the appropriate material

properties and parameter values. For the implementation of the sensitivity analysis, the free software SimLab developed at the Joint Research Centre (Simulation Laboratory for Uncertainty and Sensitivity Analysis, JRC, Italy, 2006) was used.

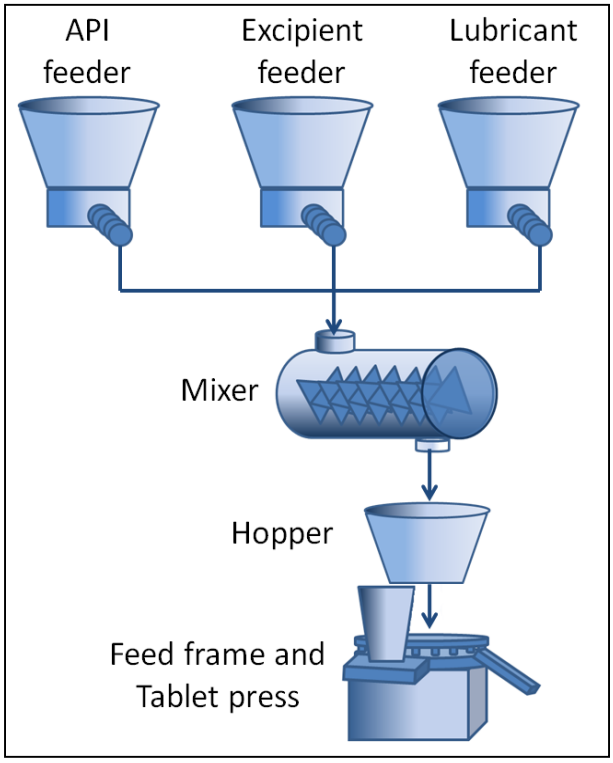

Figure 9. Model of direct compaction (29)

Following is a description of the equations of the unit operations used.

4.2.1. Feeders

The purpose of a feeder in a continuous manufacturing processing line is to supply raw materials in consistent and desired flowrates to the next processing unit operation. The typical gravimetric feeder comprises of a hopper that can hold up to a certain amount of powder material, which is fed to the next unit operation through a

rotating screw. The loss in the weight of the material contained in the feeder over a period of time calculates the output feedrate. The desired output feedrate can be achieved by controlling the rotating screw rotation rate or by using different screw designs or sizes for materials with different powder properties, however only the rotation rate can be modified during a continuous operation and a potential change in the feeder screw type or size would require the unit operation to be offline. The change in total weight can capture any material accumulation in the feeder because the exact amount of material provided to the next unit operation is monitored.

A first order delay differential equation (Equation 38) can be used to describe the model and capture the feeder dynamics. The parameters of this model consist of the process gain parameter (K), the time constant (τ), and the time delay factor (θ). Throughout the process of feeding, the material is assumed to retain its original particle size distribution and bulk density.

$$F_{out}(s) = \frac{Ke^{-\theta s}}{\tau s + 1} \qquad \text{(Equation 38)}$$

4.2.2. Mixers

Currently there are several modeling approaches for powder mixing processes and different models are used for a specific use. In this case a multi-dimensional population balance model (30-32) is used to model blending processes that accounts for *n=2* solid components (active pharmaceutical ingredient and excipients), two external coordinates

(axial and transverse directions in the blender) and one internal coordinate (size distribution due to segregation).

The equation (Equation 39) is shown below:

$$\frac{\partial F(n,z_1,z_2,r,t)}{\partial t}+\frac{\partial}{\partial z}\left[F(n,z_1,z_2,r,t)\frac{dz_1}{dt}\right]+\frac{\partial}{\partial z}\left[F(n,z_1,z_2,r,t)\frac{dz_2}{dt}\right]+\frac{\partial}{\partial r}\left[F(n,z_1,z_2,r,t)\frac{dr}{dt}\right]$$
$$=\Re_{formation}(n,z_1,z_2,r,t)-\Re_{depletion}(n,z_1,z_2,r,t)$$

(Equation 39)

where z_1 is the spatial coordinate in the axial direction, z_2 is the spatial coordinate in the radial direction, r is the internal coordinate that depicts particle size and $n=2$ to indicate presence of two components (Active Pharmaceutical Ingredient and excipient). $\frac{dz_1}{dt}$ and $\frac{dz_2}{dt}$ represent the axial and radial velocity respectively.

4.2.3. Hoppers

Hoppers, an essential and supplementary component of tablet presses, are unit operations that collect powder from an upper opening and feed the material through a bottom orifice to the next unit operation of the process. Hopper model development has been performed since the 1920s and through stress, velocity and density analysis of the flowing material (33-35), it has been shown that in steep-walled hoppers, the outflow is independent of the contained material height but depends on the outlet diameter and mean particle diameter (36).

A carefully designed hopper (height, angle and outlet diameter) will take into account the properties of the processed raw material and will operate in a regime where all the material within the hopper will move towards the exit orifice with a constant flowrate and material stagnation is avoided. A feeding screw or a tablet press feedframe regulate and control the flow rate with which tablets or capsules are produced.

The mass balance on the hopper system is the following (Equation 40):

$$\frac{d\dot{m}}{dt} = F_{in} - F_{out} \qquad \text{(Equation 40)}$$

Where $\dot{m}$ is the mass holdup inside the hopper.

The height of the material inside the hopper can be correlated to the mass holdup through the following relations (Equation 41):

$$\dot{m} = H \cdot A(H) \cdot \rho \qquad \text{(Equation 41)}$$

Where H is the height of the material inside the hopper, A is the area of the hopper and ρ is the bulk density of the material, which we can consider to be constant.

4.2.4. Tablet Press

The rotary tablet press which is most commonly used in the pharmaceutical industry and has been studied extensively in the past (37-40), is the next and final unit operation in the tablet manufacturing production line. Small amounts of blended material, after passing through a hopper, enter a small chamber of rotating blades called the “feed

frame" that fill the dies of the tablet press and are compressed to tablets of predefined size and shape.

Because the purpose of this flowsheet model is to optimize and capture the dynamics of the process without being too computationally intensive, in this work the tablet press is a simple empirical model based on the Heckel equation (38, 41) where the compression force of the powder is related to the porosity of the produced tablets with the following equation (Equation 42):

$$\ln\frac{1}{\varepsilon} = kP + A \qquad \text{(Equation 42)}$$

Where ε is the tablet porosity, P is the compression force, and k and A are empirical material parameters, calculated through experimentation. Dissolution and bioavailability, two properties directly affecting the quality of a produced tablet, are highly correlated to the tablet porosity.

The average residence time of the material in the feed frame is correlated to the die disc speed (x_1) and the feed frame speed (x_2) from the following equation (Equation 43):

$$\text{Average residence time} = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 \qquad \text{(Equation 43)}$$

4.3. Sensitivity analysis application

To successfully apply the methodology of dynamic sensitivity analysis, the model will have to be evaluated multiple times at multiple time points. The computational time

and expense to evaluate the model multiple times at multiple time points and calculate the sensitivity indices for these time points for every single input parameter with regards to the output parameters is enormous in the scope of this study. Additionally the mathematical complexity of the integrated process of direct compaction sets a very important limitation. Therefore it is important to come up with a shortlist of important factors for this case study. A screening method is a computationally cheap experiment requiring a small number of model evaluations helping to identify which parameters are important. It can be efficiently applied to methods containing up to hundreds of inputs factors.

4.3.1. Screening based sensitivity analysis

As a first step, the first two levels of the direct compaction model were isolated from the rest of the direct tablet compaction model. The sub-model contains three feeders and the mixer; a feeder for the active pharmaceutical ingredient, a feeder for the excipient and a feeder for the lubricant as well as the multi-dimensional population balance model of the mixer. The feeders only have the particle size distributions and bulk densities as input parameters, however the mixer has a large number of parameters since for each of the bins that the mixer is discretized in, there are axial, radial and backward fluxes as input parameters. In this case study the mixer is discretized in 36 compartments giving a total of 108 input parameters. In total the 3-feeder/1-mixer sub-model contains 114 input parameters, which are the bulk densities and the mean particle sizes of the active pharmaceutical ingredient, excipients and lubricant and axial/backward/radial flux coefficients for each of the 36 compartments in which the mixer is divided. The bulk

densities and the mean particle size distributions can be found in the references and the flux coefficients are calculated through DEM simulation.

The Morris screening sensitivity analysis technique was used because the 3 feeder-1 mixer model contains a large number of input factors. Due to its economy in comparison with other sensitivity analysis methods, it requires fewer runs than alternative methods and provides us with a qualitative measure of importance. Through this method the average and standard deviation of the elementary effects evaluated at various time points, in this case from time 0 until the system reaches a steady state at 1200 seconds. This approach has been shown to identify the same inputs as influential as the Sobol method, which will be used extensively in this work to quantify the importance of input parameters (20).

Initially the sampling scheme had to be chosen and the parameter distributions selected in order to avoid negative values that were to be sampled. The 114 input parameters included bulk densities of the active pharmaceutical ingredient, the excipient and the lubricant, mean particle sizes of the active pharmaceutical ingredient, the excipients and the lubricant and 108 mixer fluxes (36 forward, backward and radial fluxes). The fluxes values were initially selected as the mean fluxes of particles that were simulated in a 6x6 finite element mixer.

	output
1	mixer output RSD
2	mixer output API concentration
3	mixer output average bulk density
4	API feeder output feed flow
5	excipient feeder output feed flow
6	lubricant feeder output feed flow

Table 2. Outputs for Morris screening sensitivity analysis

	input	mean value	distribution	SD/ bounds	units
1	API bulk density	600	normal	60	kg/m^3
2	excipient bulk density	325	normal	32.5	kg/m^3
3	lubricant bulk density	160	normal	16	kg/m^3
4	API mean particle size	3.00E-05	normal	3.00E-06	m
5	excipient mean particle size	2.00E-04	normal	2.00E-05	m
6	lubricant mean particle size	2.00E-05	normal	2.00E-06	m
7-114	mixer flux coefficients	varies	uniform	$[0.95f_c,1.05f_c]$ or [0,10.00E-05]	-

Table 3. Inputs for Morris screening sensitivity analysis

The input parameter distribution functions that were chosen for the initial Morris screening method included crude pdf's that were intuitively selected (Table 3).

- For the bulk densities and the mean particle sizes, normal distributions with a mean equal to the nominal bibliographic values and a standard deviation equal to 10% of the nominal value were selected.

- For the 108 mixer fluxes, uniform distributions were selected in order to avoid possible negative values in the sampling, which would affect the simulation results.
    - The uniform distributions that were selected for non-zero flux values had a range from 95% of the experimental flux value to 105% of the experimental flux value.
    - Many of the 108 mixer fluxes included fluxes with zero values. For these fluxes, uniform distributions with a range from 0 to 10^{-5} were assigned.

A sample of 1150 input vectors was generated from the 114-dimensional input space and the model was then evaluated for each of the input samples producing 1150 model outputs. The model was evaluated using gPROMS from t = 0 until t = 1200 seconds, the time the system reaches a steady state, reporting the output values every 20 seconds. The following figure (Figure 10) shows the 1150 model output ensembles. The outputs of the relative standard deviation, API concentration and average bulk density show considerable variation in the output due to the uncertainty introduced in the input parameters. It can also be seen from the output ensembles that the feed flows for API feeder, excipient feeder and lubricant feeder do not change despite the perturbations in the corresponding bulk densities and mean particle sizes. An explanation for this could be that the perturbations are too small to see an effect on the feeder output feed flows.

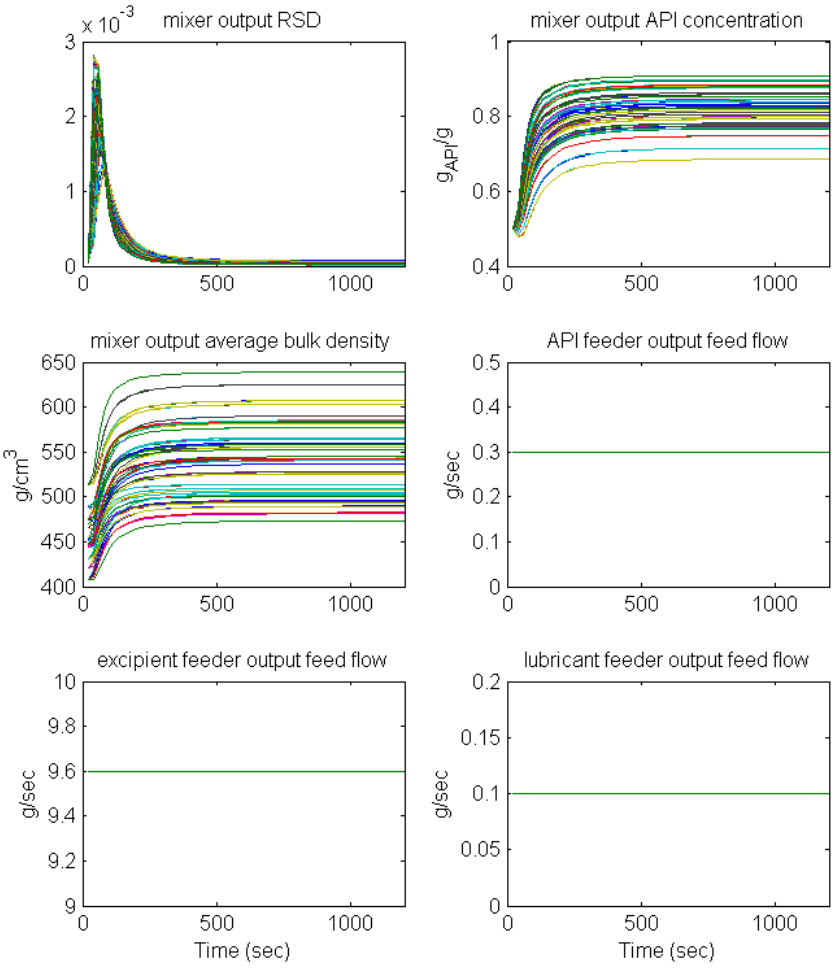

Figure 10. : Ensemble of 1150 model evaluations sampled for the Morris screening method

Following the model evaluations, the Morris method was applied to the simplified 3-feeder, 1-mixer model using the input parameter distributions described above. A design matrix was constructed and the model was evaluated for each of the $r(k+1) = 10(114+1) = 1150$ input vectors where k is the number of parameters, p is the number of levels of each parameter, and r, the number of repetitions. Previous studies (20) have suggested that good results can be obtained using $r = 10$ and $p = 4$ and for this reason these values were used.

The following figure (Figure 11) shows the results of applying the Morris method to our model. Since the perturbations have no effect in the feed flows of API feeder, excipient feeder and lubricant feeder, then it was not needed to further apply the Morris method to these outputs. Only the mixer output RSD, mixer output API concentration and mixer output average bulk density were further investigated since from the model ensembles we can see the variation of every model run caused from the perturbations in the input factors. The scatter plot is showing the mean of the elementary effect against the standard deviation for each parameter for each of the inputs. Therefore it can clearly be identified that there are some parameters with a significant relative effect compared to other uncertain parameters.

The Morris screening method was applied to a wide range of time points (40, 200, 400, 600, 800, 1000 sec) to try and investigate the dynamic nature of the process. In all time points, for all of the outputs there same parameters were qualitatively identified as significant. However for the time point selected to be displayed is 400 seconds because at that time the model is very close to steady state and the sensitivity analysis results that we extract can represent the overall behavior.

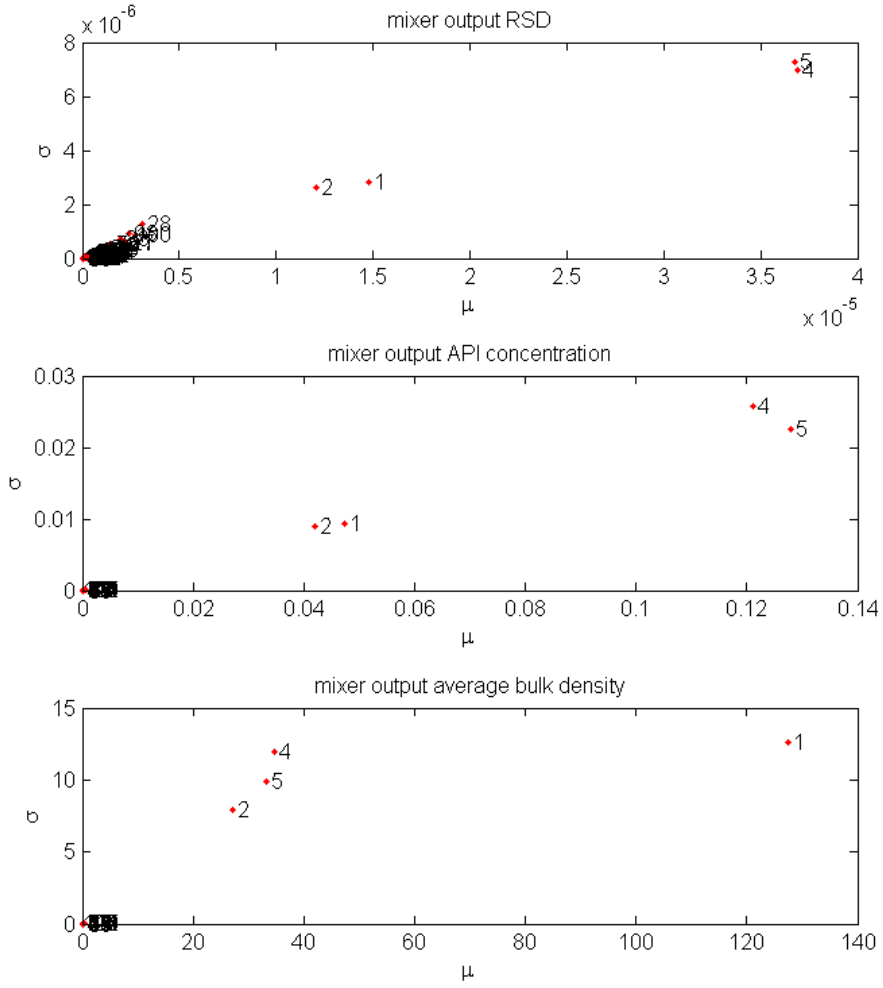

Figure 11. The mean μ, in the x-axis, vs the standard deviation σ, in the y-axis, of the elementary effects for each input in regards to the outputs of mixer output relative standard deviation, mixer output average bulk density at 400 seconds.

The parameters found to be important are the bulk density of API (parameter 1), bulk density of excipient (parameter 2), mean particle size of API (parameter 4) and the mean particle size of the excipient (parameter 5). From the results it is shown that the Morris method identifies the 108 mixer flux parameters as insignificant. In this case, this does not mean that these parameters are redundant but it denotes that for the outputs we chose to consider in this analysis, which are in their majority bulk output stream

properties, these parameters are not significant compared to the rest of the uncertain input parameters. The absence of the lubricant related input parameters (bulk density of lubricant -parameter 3 and mean particle size of lubricant-parameter 6) denotes a parameter unimportance in the relative standard deviation (output 1), API concentration (output 2) and average bulk density (output 3) output which can be explained from the small amount (1%) of lubricant used in the formulation, compared to the larger amounts of the API (3%) and the excipient (96%).

By utilizing the Morris screening method the mixer input flux parameters were found to be insignificant to the model output streams, therefore before proceeding to the next step of the analysis their values can be fixed to the previously calculated values.

4.3.2. Variance based sensitivity analysis

Through the initial screening with the Morris screening method, 4 input parameters were identified as important relative to the mixer model parameters, whose effects were deemed insignificant in this case study. Additionally, parameters directly related to the lubricant i.e. the bulk density of lubricant and the mean particle size of lubricant, were found to have no effect on the model outputs mainly because of the small percentage of lubricant used in the powder formulation. However if we continue using the lubricant related parameters as uncertain inputs in the rest of the sensitivity analysis study, it will not affect the computational time and expense significantly.

The next step is the application of the methodology for dynamic sensitivity analysis to the whole integrated process of direct compaction. The outputs for which the

global sensitivity analysis is performed are listed in Table 4 and include final properties of the produced tablets as well as intermediate product properties.

All possible inputs for this case study include parameters, design variables and process conditions. Material properties for the three ingredients (particle size distributions, bulk densities), model parameters (axial, radial and backward fluxes for each of the 36 mixer compartments, Heckel model parameters, empirical parameters of feeder model), design variables (height, aperture diameter of the hopper) and operating process variables (mixer rpm, feed frame rotation rate, tablet press compaction force). After the initial Morris screening method it is decided that the 108 mixer flux parameters are fixed to their calculated values and the global sensitivity analysis is conducted for the 11 input parameters listed in Table 5. Material properties, design variables and operating process variables are included in the analysis to investigate how uncertainty in the material properties and perturbations in the control of the process will affect the overall product quality.

	output
1	mixer total output flowrate
2	mixer output bulk density
3	mixer output RSD
4	mixer output API concentration
5	hopper output density
6	hopper output RSD
7	hopper output API concentration
8	hopper mean residence time
9	hopper mass holdup
10	hopper height
11	feed frame mean residence time
12	tablet porosity
13	tablet API concentration

Table 4. Outputs for Sobol global sensitivity analysis

	input	mean value	distribution	SD/ bounds	units
1	API bulk density	600	normal	50	kg/m^3
2	excipient bulk density	325	normal	35	kg/m^3
3	lubricant bulk density	160	normal	10	kg/m^3
4	API mean particle size	3.00E-05	normal	5.00E-06	m
5	excipient mean particle size	2.00E-04	normal	2.00E-05	m
6	lubricant mean particle size	2.00E-05	normal	5.00E-06	m
7	mixer rpm	-	uniform	[5-15]	rpm
8	Die disc speed	-	uniform	[0.509-1]	-
9	Feed frame rotation rate	-	uniform	[0.33-1]	-
10	Tablet press compression force set point	-	uniform	[8-12]	KPa
11	Hopper aperture diameter	-	uniform	[0.05-0.06]	m

Table 5. Inputs for Sobol global sensitivity analysis

The uncertainty in the input parameters is represented by the assigned distributions (Table 5). The input parameter probability distribution functions that were chosen for the Sobol variance based method were found in the literature. Unlike the Morris method where the assigned distributions were intuitive, in this scenario the distributions have to be based on the typical operating and design condition ranges of the processes, to test the model capabilities in a realistic scenario.

- For the bulk densities and the mean particle sizes, normal distributions were assigned with a mean equal to the nominal values with a standard deviation equal to values found in literature.
- For the design and operating process variables, uniform distributions were assigned with values based on the typical industrial operating ranges.

Following the selection of the 11 input factor distributions, 1040 input vectors were generated using quasi-random numbers and the Sobol LP_T sampling schemes. Two 80 by 11 random input matrices are generated and 80(11+2)=1040 input vectors are created. Each one of the 1040 input parameter vectors contains 11 uncertain input parameters and is sent to gPROMS for model output evaluation. The flowsheet is simulated for a total of 1500 seconds with data recorded every 10 seconds.

Calculations for the Sobol first and total order sensitivity indices are performed for each time point, capturing a dynamic profile for the sensitivity indices. From the calculations, first and total order sensitivity profiles are obtained, from which the effect

of each input parameter on a certain output through the progression of the simulation can be quantified.

Most outputs have dynamics that are affected from one or two parameters throughout the simulation. However, performing a variance based sensitivity analysis to this model would provide insight to any possible interactions between input parameters that might affect the model outputs. In particular, total sensitivity indices are found to be very similar, both qualitatively and quantitatively, to the first order indices. This observation can lead to the conclusion that in this case study there are no significant interactions between input parameters affecting the model outputs.

An easy way to visualize the results is through an intensity plot of the effects of inputs to each output. In the following figure (Figure 12), the total order Sobol sensitivity indices are displayed at 1000 seconds, when the system has reached steady state. The first order sensitivity indices (S_i) are very similar to the total order sensitivity indices (S_{Ti}), showing that there are no interactions between input parameters, therefore the following intensity plot is considered to be an accurate overall representation of the input parameter effects on the outputs as calculated using the global sensitivity analysis procedure.

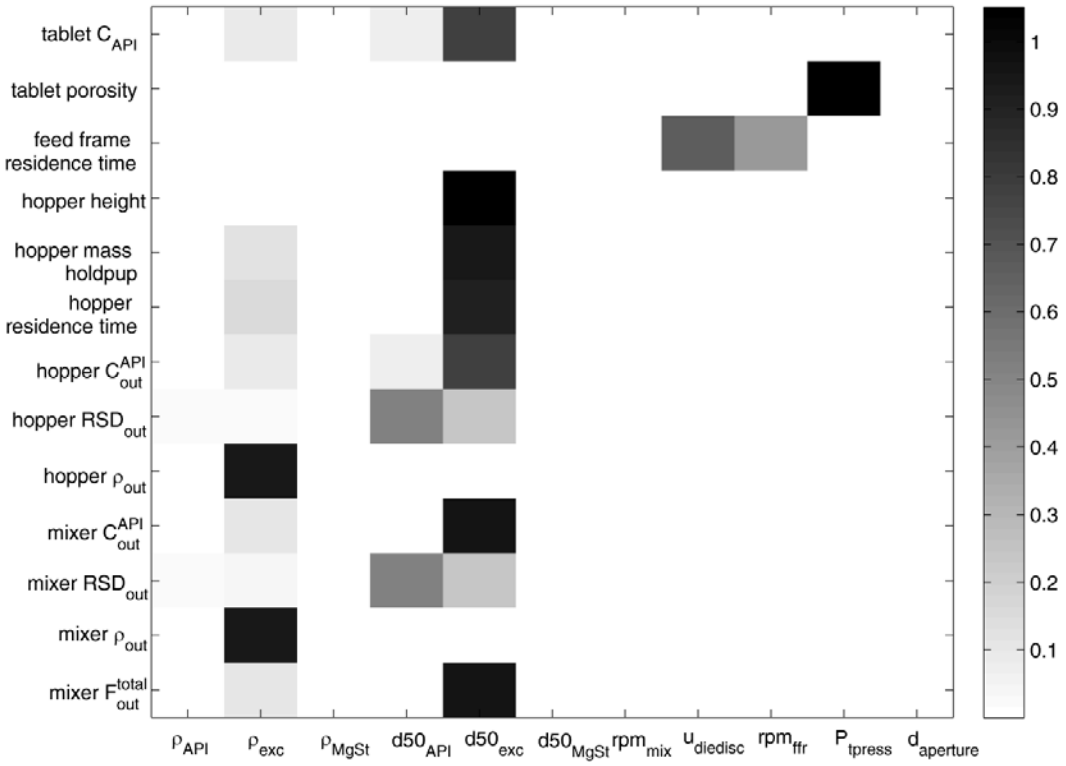

Figure 12. Intensity plot of total sensitivity indices of inputs (Table 5) to outputs (Table 4) at steady state

From this heat map it can be seen that certain outputs depend on a single input while some inputs have no effect on any of the studied outputs. For example, tablet porosity only depends on the compaction pressure something that can be expected since the current flowsheet model uses a tablet press equation, which only takes the compaction pressure into account. In this occasion such an application of sensitivity analysis can assist in the identification of missing or unwanted correlations.

Sensitivity analysis is also performed to validate if the developed model is accurate and agrees with experimental evidence. In the total order sensitivity profile

(Figure 13) of the relative standard deviation of the mixer output stream, the effect of the mixer rotation rate is found to be insignificant, contrary to experimental evidence. The mean particle size and bulk densities of the API and the excipient are found to be significant, overshadowing the effect of the mixer rotation rate. This could be an example of either an improperly selected input parameter distribution for the mixer rotation rate, or a model that does not properly capture the significance of the mixer rotation rate.

The fluctuations in the sensitivity indices that are evident in the initial stages of the simulation are a result of the transition time needed until the system within the mixer reaches steady state conditions. As the system reaches steady state the fluctuations disappear.

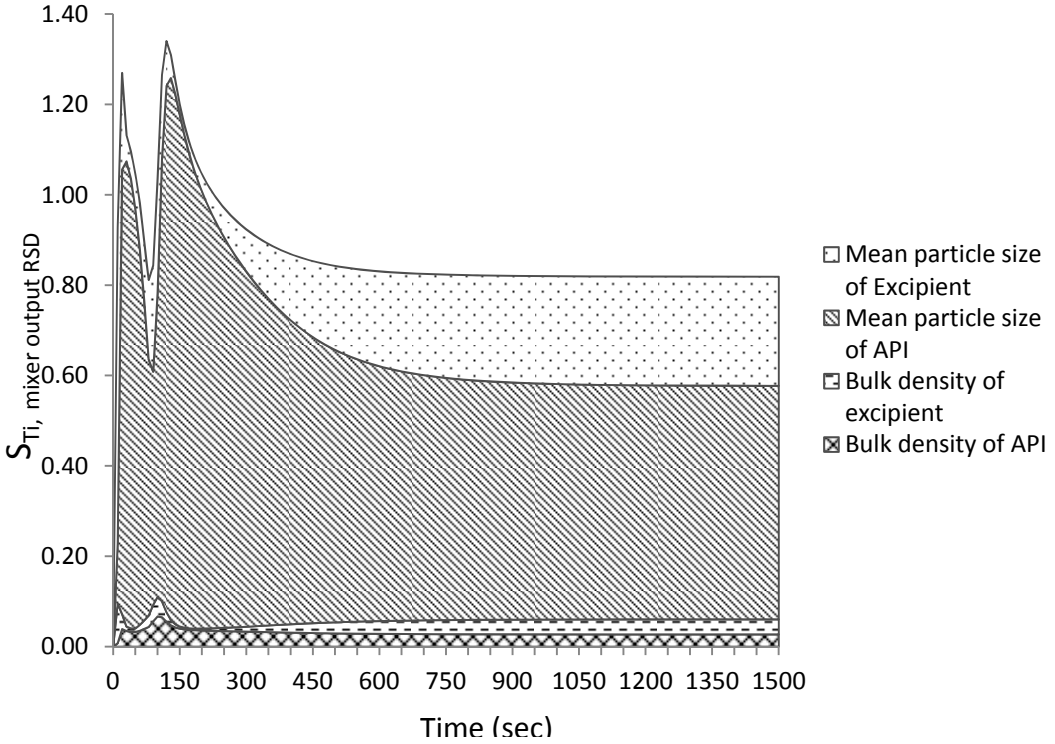

Figure 13. Dynamic sensitivity analysis profile of mixer output Relative Standard Deviation

The attribute that is perhaps the most representative of the produced tablet quality is the final API concentration. The dynamic sensitivity analysis profile of the final API concentration (Figure 14) reveals that the most significant parameter for this output is the mean particle size of the excipient. This can be explained from the high concentration of the excipient in the tablet powder formulation (96%). Between 1200-1500 seconds the system has reached steady state and the variations in the sensitivity indices are likely due to numerical noise that is often encountered in flowsheet simulations.

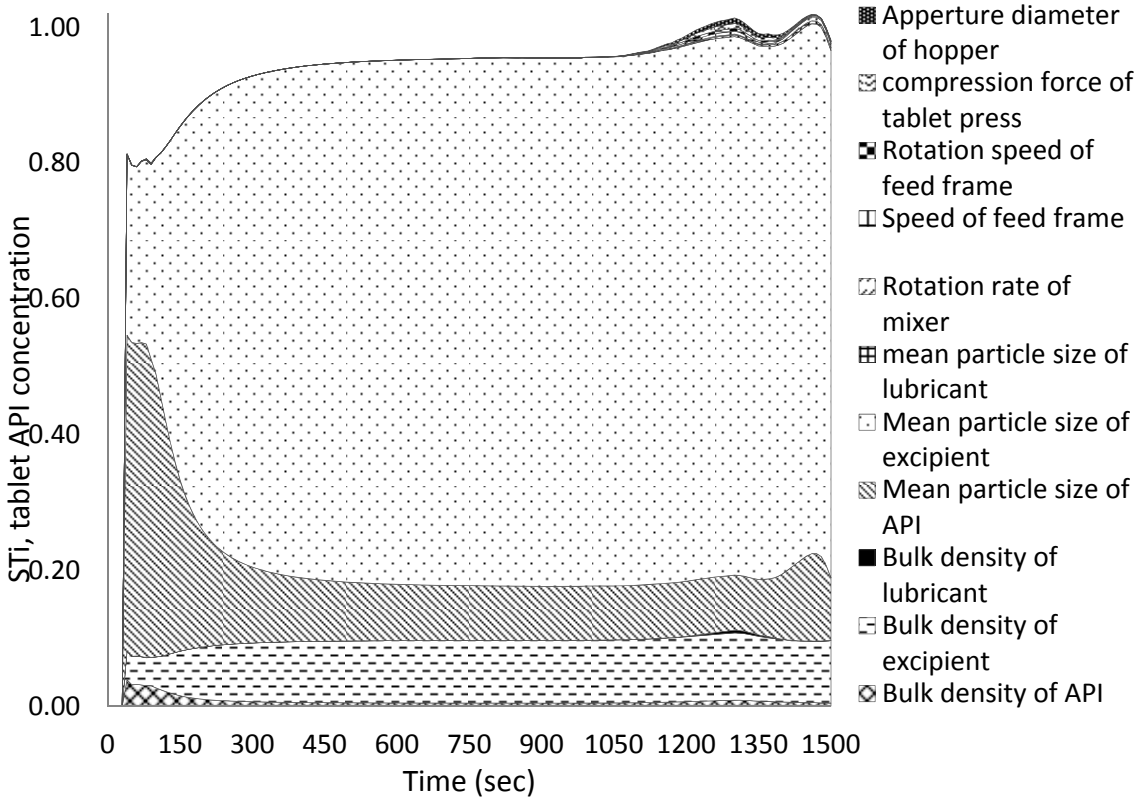

Figure 14. Dynamic Sensitivity Analysis for tablet API concentration

The dynamical effect of the API feeder refill at time t=1000 sec is studied as the second scenario in the case study, and comparison is performed with the results obtained from the first scenario. The simulation is performed for 2200 seconds, time needed for the system to resume steady-state operation after the feeder refill perturbation.

For most output parameters the dynamic sensitivity profiles are similar to the first scenario, however some other profiles are very different (Figure 15), identifying new critical parameters for the tablet API concentration. Comparing the dynamic sensitivity profiles for the tablet API concentration obtained from the two scenarios, one can see that process control parameters such as the mixer rotation rate and feed frame speed overshadow the effect of material properties in the tablet API concentration. These two

inputs control the mean residence time of the material inside the mixer and the feed frame, therefore affecting the time necessary for the tablet API concentration to reach a new steady state. These results also point out possible points of particular attention for process control during a feeder refill in the process.

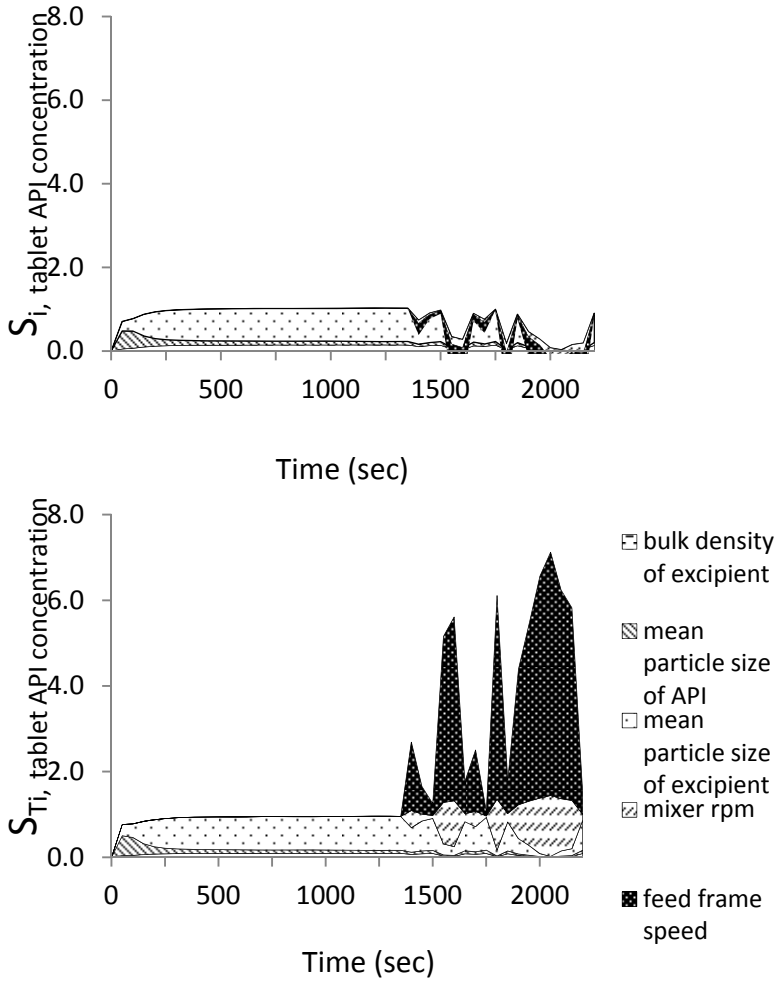

Figure 15. (a) First order S_i indices, (b) total order S_T indices for tablet API concentration after an API feeder refill at t=1000sec

Finally, when the first order and the total order indices are compared, there is an obvious large difference in the sensitivities for the mixer rotation rate and feed frame

speed, verifying that the interactions with other parameters are the cause for the variation in the tablet API concentration. The difference between the values of first order S_i and total order sensitivities S_{Ti} is a measurement of how much the variation caused by a parameter is through interactions with other parameters. The total order sensitivity indices S_{Ti} can be greater than 1 because the various interactions are counted multiple times (1).

5. CONCLUSIONS

Biological systems usually contain a large number of components that interact with each other and systems biology with the integration of most of these components into a single model uses mathematical modeling to define and analyze the structure of such a system. Sensitivity analysis should be an important part during the development and validation of a computational systems biology model. It allows the quantitative and qualitative investigation of variation and perturbation effects on the system behavior.

In the first part of this work, the application of time-varying global sensitivity analysis techniques is performed in a mathematical model of human endotoxemia. Through sensitivity analysis the parameters or components that have little effect on the model but are experimentally observed to be significant for the system, are identified. The obtained results imply the need for better parameter estimation, after further experimentation, or model modifications that will better capture the experimentally observed system dynamics.

The results of sensitivity analysis of biological systems can enhance our understanding of the system by confirming hypotheses which have been observed experimentally or even suggest new mechanisms that can be verified through a new set of experiments. Additionally in the future, sensitivity analysis can be used in computational models to suggest potential critical points of control in the system, identifying components that can potentially be targeted by drugs for therapeutic intervention.

In the second part of this work, the complexity of how interactions between the different unit operations of a continuous tablet manufacturing flowsheet simulation affect the overall product quality is studied. Both quantitative and qualitative results reveal how different uncertain variables of a process dynamically affect an output through the use of time-varying global sensitivity indices. Thus the most important and critical parameters for a certain output are identified at different time points. Such an approach of global sensitivity analysis is not only used to draw significant conclusions about the interactions between specific uncertain inputs to outputs, but also points out necessary correlations that the model fails to capture and identify points of process control during unavoidable perturbations in the process.

6. REFERENCES

1. Saltelli, A., K. Chan, and Scott, *Sensitivity analysis*. 2000: Wiley.
2. Nestorov, I.A., Sensitivity Analysis of Pharmacokinetic and Pharmacodynamic Systems: I. A Structural Approach to Sensitivity Analysis of Physiologically Based Pharmacokinetic Models. *Journal of Pharmacokinetics and Pharmacodynamics* 27: 577-596, 1999.
3. Dunker, A.M., The Decoupled Direct Method for Calculating Sensitivity Coefficients in Chemical-Kinetics. *Journal of Chemical Physics* 81: 2385-2393, 1984.
4. Liu, G., M.T. Swihart, and S. Neelamegham, Sensitivity, principal component and flux analysis applied to signal transduction: the case of epidermal growth factor mediated signaling. *Bioinformatics* 21: 1194-202, 2005.
5. Yue, H., M. Brown, J. Knowles, H. Wang, D.S. Broomhead, and D.B. Kell, Insights into the behaviour of systems biology models from dynamic sensitivity and identifiability analysis: a case study of an NF-kappaB signalling pathway. *Mol Biosyst* 2: 640-9, 2006.
6. Helton, J.C. and F.J. Davis, Latin hypercube sampling and the propagation of uncertainty in analyses of complex systems. *Reliability Engineering & System Safety* 81: 23-69, 2003.
7. Campolongo, F., J. Cariboni, and A. Saltelli, An effective screening design for sensitivity analysis of large models. *Environmental Modelling & Software* 22: 1509-1518, 2007.
8. Morris, M.D., Factorial Sampling Plans for Preliminary Computational Experiments. *Technometrics* 33: 161-174, 1991.
9. Saltelli, A., *Sensitivity analysis in practice : a guide to assessing scientific models*. 2004: Wiley.
10. McKay, M.D., Nonparametric variance-based methods of assessing uncertainty importance. *Reliability Engineering & System Safety* 57: 267-279, 1997.
11. Cukier, R.I., H.B. Levine, and K.E. Shuler, Nonlinear sensitivity analysis of multiparameter model systems. *Journal of Computational Physics* 26: 1-42, 1978.
12. Saltelli, A., S. Tarantola, and K.P.S. Chan, A quantitative model-independent method for global sensitivity analysis of model output. *Technometrics* 41: 39-56, 1999.
13. Sobol, I.M., Global sensitivity indices for nonlinear mathematical models and their Monte Carlo estimates. *Mathematics and Computers in Simulation* 55: 271-280, 2001.
14. Saltelli, A., Making best use of model evaluations to compute sensitivity indices. *Computer Physics Communications* 145: 280-297, 2002.
15. Homma, T. and A. Saltelli, Importance measures in global sensitivity analysis of nonlinear models. *Reliability Engineering & System Safety* 52: 1-17, 1996.
16. Campolongo, F., A. Saltelli, and J. Cariboni, From screening to quantitative sensitivity analysis. A unified approach. *Computer Physics Communications* 182: 978-988, 2011.
17. Campolongo, F., A. Saltelli, and J. Cariboni, Screening important inputs in models with strong interaction properties. *Reliability Engineering & System Safety* 94: 1149-1155, 2009.
18. Saltelli, A., M. Ratto, S. Tarantola, F. Campolongo, and E. Commission, Sensitivity analysis practices: Strategies for model-based inference. *Reliability Engineering & System Safety* 91: 1109-1125, 2006.
19. Saltelli, A., S. Tarantola, and F. Campolongo, Sensitivity analysis as an ingredient of modeling. *Statistical Science* 15: 377-395, 2000.
20. Campolongo, F. and A. Saltelli, Sensitivity analysis of an environmental model an application of different analysis methods. *Reliability Engineering & System Safety* 57: 49-69, 1997.

21. Kitano, H., Systems biology: A brief overview. *Science* 295: 1662-1664, 2002.
22. Foteinou, P.T., S.E. Calvano, S.F. Lowry, and I.P. Androulakis, Modeling endotoxin-induced systemic inflammation using an indirect response approach. *Mathematical Biosciences* 217: 27-42, 2009.
23. Ingalls, B.P. and H.M. Sauro, Sensitivity analysis of stoichiometric networks: an extension of metabolic control analysis to non-steady state trajectories. *Journal of Theoretical Biology* 222: 23-36, 2003.
24. Schwier, D., E.U. Hartge, J. Werther, and G. Gruhn, Global sensitivity analysis in the flowsheet simulation of solids processes. *Chemical Engineering and Processing* 49: 9-21, 2010.
25. Chhatre, S., R. Francis, A.R. Newcombe, Y.H. Zhou, N. Titchener-Hooker, J. King, and E. Keshavarz-Moore, Global Sensitivity Analysis for the determination of parameter importance in bio-manufacturing processes. *Biotechnology and Applied Biochemistry* 51: 79-90, 2008.
26. Marino, S., I.B. Hogue, C.J. Ray, and D.E. Kirschner, A methodology for performing global uncertainty and sensitivity analysis in systems biology. *Journal of Theoretical Biology* 254: 178-196, 2008.
27. Hu, D. and J.M. Yuan, Time-dependent sensitivity analysis of biological networks: coupled MAPK and PI3K signal transduction pathways. *J Phys Chem A* 110: 5361-70, 2006.
28. Chan, K., A. Saltelli, and S. Tarantola. *Sensitivity Analysis Of Model Output: Variance-Based Methods Make The Difference*. [cited; Available from: citeulike-article-id:7751791
http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.23.7700
29. Boukouvala, F., V. Niotis, R. Ramachandran, F.J. Muzzio, and M.G. Ierapetritou, An integrated approach for dynamic flowsheet modeling and sensitivity analysis of a continuous tablet manufacturing process. *Computers and Chemical Engineering* European Symposium of Computer Aided Process Engineering 21 Special issue: 2nd Review, 2011.
30. Boukouvala, F., A. Dubey, A. Vanarase, R. Ramachandran, F.J. Muzzio, and M. Ierapetritou, Computational Approaches for Studying the Granular Dynamics of Continuous Blending Processes, 2 – Population Balance and Data-Based Methods. *Macromolecular Materials and Engineering* n/a-n/a, 2011.
31. Ramachandran, R., C.D. Immanuel, F. Stepanek, J.D. Litster, and F.J. Doyle, A mechanistic model for breakage in population balances of granulation: Theoretical kernel development and experimental validation. *Chemical Engineering Research & Design* 87: 598-614, 2009.
32. Ramachandran, R. and P.I. Barton, Effective parameter estimation within a multi-dimensional population balance model framework. *Chemical Engineering Science* 65: 4884-4893, 2010.
33. Savage, S.B., The mass flow of granular materials derived from coupled velocity-stress fields. *British Journal of Applied Physics* 16: 1885, 1965.
34. Savage, S.B. and M. Sayed, Gravity flow of coarse cohesionless granular materials in conical hoppers. *Zeitschrift für Angewandte Mathematik und Physik (ZAMP)* 32: 125-143, 1981.
35. Weir, G.J., A mathematical model for dilating, non-cohesive granular flows in steep-walled hoppers. *Chemical Engineering Science* 59: 149-161, 2004.
36. Nedderman, R.M., U. Tüzün, S.B. Savage, and G.T. Houlsby, The flow of granular materials--I: Discharge rates from hoppers. *Chemical Engineering Science* 37: 1597-1609, 1982.

37. Hein, S., K.M. Picker-Freyer, and J. Langridge, Simulation of Roller Compaction with Subsequent Tableting and Characterization of Lactose and Microcrystalline Cellulose. *Pharmaceutical Development and Technology* 13: 523-532, 2008.
38. Jain, S., Mechanical properties of powders for compaction and tableting: an overview. *Pharmaceutical Science & Technology Today* 2: 20-31, 1999.
39. Michaut, F., V. Busignies, C. Fouquereau, B.H. de Barochez, B. Leclerc, and P. Tchoreloff, Evaluation of a rotary tablet press simulator as a tool for the characterization of compaction properties of pharmaceutical products. *Journal of Pharmaceutical Sciences* 99: 2874-2885, 2010.
40. Sinka, I.C., J.C. Cunningham, and A. Zavaliangos, The effect of wall friction in the compaction of pharmaceutical tablets with curved faces: a validation study of the Drucker-Prager Cap model. *Powder Technology* 133: 33-43, 2003.
41. Seitz, J.A. and G.M. Flessland, Evaluation of the physical properties of compressed tablets I. Tablet hardness and friability. *Journal of Pharmaceutical Sciences* 54: 1353-1357, 1965.