Stoichio-kinetic model discrimination and parameter identification in continuous microreactors

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A B S T R A C T

Kinetics is essential for chemical reactor modelling, in particular to reduce the inherent risks of extrapolation going along with scaling-up. Pharmaceutical industries are especially concerned. However, when chemical systems are very complex, development of good models may lead to prohibitively expensive and time consuming experiments. The aim of this paper is to describe an efficient experimental design strategy for discrimination of stoichio-kinetic models. The proposed methodology is based on model-based experimental design (optimal design), which uses information already acquired on models to determine the best conditions to implement a new experiment with the highest discrimination potential. The combination with microreactor technology is also proposed in this work. The whole procedure for model discrimination is firstly described in detail and then, applied to a numerical study case, consisting of a chemical synthesis carried out in a microreactor. The discrimination procedure efficiently leads to the determination of the single adequate model among the various potential models proposed before the implementation of the designed experiments. It is verified that the procedure does not depend on the set of preliminary experiments and is time-saving when compared to a classical factorial plan.

1. Introduction

The Research & Development process in the pharmaceutical industry follows a long and complex course. First, key molecules responding to target needs are selected. Then, preclinical development comes: studies on toxicity, pharmacodynamics, pharmacokinetics and control of molecule formulation and production feasibility. Following this, clinical trials are conducted. From Phase I to Phase III, key molecule efficiency, side effects and behavior in the body are investigated on humans, starting from typically 20–100 volunteers for Phase I, then 100–500 for Phase II and 1000–5000 for Phase III. The drug is finally submitted to regulatory agencies for evaluation and registration before commercialization. Chemical development and drug production follow each development step and must meet the requirements for the drug by scaling up manufacturing. The complexity of phenomena (chemical reactions, hydrodynamics, heat and mass transfers) limits the amount by which laboratory production methods may be extrapolated, consequently the scaling-up can lead to the unexpected degradation of product quality and formation of by-products.

The development of kinetic models, and more generally of phenomenological models, is a way to ensure reliability in extrapolation and process modelling. However, the complexity of reaction systems

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Nomenclature

ξ_i	Vector of experimental conditions of the ith experiment
t	Vector of time of each sample of the ith experiment
y_m	Vector of model responses
y_{exp}	Vector of measured experimental variables
rss	Residual sum of squares
χ^2	Chi-square statistical distribution
σ	Standard deviation on an experimental measurement
Σ	Covariance Matrix of standard deviations on experimental measurement
θ	Vector of parameters of a model, k when it is kinetic parameters
M	A model structure
$M(\theta)$	A model with a parameter set θ
ϕ_{GM}	Gauss–Markov criterion
M_F	Fisher information Matrix
Φ	Parameter estimation error covariance matrix
Ω	Model prediction error covariance matrix
$dy/d\theta$	Matrix of sensibility of model response y

involved, coupled with the fact that the number of species that can be followed up by analytical methods can be limited, generally means that several potential stoichio-kinetic models may be proposed. As a consequence, the experimental efforts (and the associated costs) to discriminate among the different stoichio-kinetic models and to correctly identify kinetics model parameters can be prohibitive, particularly in pharmaceutical chemistry.

In addition, the search and the selection for several potential stoichio-kinetic models, for example proposing various side reactions (i.e. different stoichiometric schemes), or including mass or heat transfer properties, is of crucial interest, since this could reduce the risks during the project development by giving tools to forecast eventual misfires. For example, a highly toxic by-product molecule can be in negligible quantity at lab-scale, but can appear to be predominant at semi-industrial one. In this case, the toxicological assessment will lead to stop the project, but in a downstream step, meaning after substantial investments. To avoid such configurations, a methodology based on the comparison of different stoichio-kinetic models should be absolutely implemented as it offers the advantage to check at the different stages of development which side effect could appear, and then to propose different associated models. In this way, the production of a toxic by-product can be anticipated at earlier stage of development. To reach this objective, the use of advanced experimental tools and strategies is required to rationalize experimental data collection.

Experimental design strategies are interesting tools to face this challenge. They propose mathematical tools to find the best experiments to discriminate between several stoichio-kinetic models, thus reducing the experimental efforts and the risks during the project. Simultaneously, microstructured reactors become an alternative to classical batch reactors for kinetic data acquisition. The combination of experimental design strategy with microfluidic tools thus opens new perspectives in chemical reaction characterization. This is the answer proposed in this work, to face the challenges described above.

The advantages of microfluidic tools are reported in numerous works (Hartman and Jensen, 2009; Hessel, 2009; Jähnisch et al., 2004; Sinton, 2014). The low handled volumes guarantee economy of products, particularly critical for advanced pharmaceutical molecules, and a higher safety than in batch systems. The perfect control of the operating conditions (mixing, heat and mass transfer) is also a key point for kinetic studies. Indeed, unexpected phenomena (hot spots, side reactions) are limited, and the experimental window is enlarged to operating conditions inaccessible for batch reactors (short residence time, high concentration). Reliability and repeatability of experiments are also improved. Besides, flow chemistry and potential on-line analysis enable a partial automation of experimental procedure and a quick screening of operating conditions.

Experimental design is the second component of the strategy proposed in this work. The main feature of all the design procedures developed for stoechio-kinetic models, and generally for nonlinear models, is an optimization problem. The optimal design, also named model-based design, is based on criteria using information already acquired on the models. The nature of the criterion used depends on the experimental goal. The two main kinds of optimal design are devoted either to accurate estimation of model parameters or to model discrimination. The approach of model-based experimental design has been successfully applied in literature for some applications in heterogeneous catalysis in tubular reactors: hydrogenation of isooctane and oxidation of o-xylene (Froment, 1975), dehydrogenation of 1-butene (Dumez et al., 1977), synthesis of methanol from gas (Buzzi-Ferraris and Forzati, 1984; Schwaab et al., 2008, 2006), and oxidation of methanol (Galvanin et al., 2015). Other applications have been presented in bioengineering: in fermentation (Galvanin et al., 2007; Strigul et al., 2009; Ternbach et al., 2005), in enzymatic catalysis and in pharmacokinetics (Dette et al., 2005; Donckels et al., 2009; Galvanin et al., 2013; López-Fidalgo et al., 2008; Tommasi, 2009). Finally, a little number of applications have been published in fine chemistry: they concern the works of Atkinson et al. (1998) on reversible esterification, of Issanchou et al. (2003, 2005) on the alkaline synthesis of *n*-amylacetate in liquid–liquid batch reactors, and of Mathieu et al. (2013) on the iodination of tyrosine on a multi-reactor experimental test. Both types of optimal design have been developed separately, since Box and Lucas (1959) for parameter estimation and Hunter and Reiner (1965) for model discrimination, or jointly since Hill et al. (1968), developing hybrid criteria or multiobjective optimization.

As its predecessors, the global objective of this work is to highlight the potential of optimal experimental design for comparison and selection of stoichio-kinetic models from a reduced number of adequate experiments. However, a particular attention will be here paid to some underestimated aspects of the construction of models. Firstly, the fact that the number of measurable species in a reactional system is limited stays a usual issue in industrial applications and has some consequences. The control of the identifiability of model parameters and of the discernibility of the competitive models becomes then essential and yet, these two structural properties of models are rarely evaluated in most of the works. This lack of information also limits the insight into the system that the experimenter can have, thus implying that the use of numeric tools becomes appreciated. For these reasons, the presented methodology will take into account the issues of discernibility and identifiability before focusing on the discrimination of various competitive stoichio-kinetics models, cases commonly encountered in fine chemistry. The other novelty of the optimal design proposed in this paper is to identify the experimental windows where the potential for model comparison is the best, and this before to emphasize on model parameter determination precisely.

All these points will be illustrated with a specific numerical study case, which will be described in Section 2. Then, the experimental design strategy for model discrimination will be introduced in Section 3. The last section (Section 4) will discuss the results obtained from the strategy applied on the study case; it will be also verified that the procedure does not depend on the set of preliminary experiments and is time-saving when compared to a classical factorial plan.

2. Study case

2.1. *Description of the problem*

To illustrate purposes, a study case classically encountered in fine chemistry is chosen. It consists of an organic synthesis involving a well-characterized main reaction and an unknown by-product reaction which leads to an unidentified impurity.

Main reaction

$$\mathbf{1} + \mathbf{2} \rightarrow \mathbf{3}$$

By-product formation

Model A	*Model B*	*Model C*	*Model D*
$\mathbf{1} + \mathbf{2} \rightarrow \mathbf{4}\boldsymbol{a}$	$\mathbf{2} + \mathbf{3} \rightarrow \mathbf{4}\boldsymbol{b}$	$2 \times \mathbf{2} + \mathbf{3} \rightarrow \mathbf{4}\boldsymbol{c}$	$\mathbf{3} \leftrightarrows \mathbf{4}\boldsymbol{d}$

Fig. 1 – Different reaction mechanisms proposed for the production of the species 3 and the formation of a by-product 4i (i = a, b, c, d).

Depending on experimental conditions, a significant amount of impurity can be produced or not.

Let's assume that after some preliminary experiments, four propositions are postulated to explain and describe how this impurity can be produced. They are shown in Fig. 1 where species **1** and **2** are the reagents, **3** is the target molecule, and **4i** (i = a, b, c, d) are the possible by-products.

These models are classical stoichio-kinetic models for reactions taking place in homogeneous phase: *model A* suggests a competitive reaction to form **4a**. For *model B* and *model C*, a consecutive competitive reaction takes place, with a different stoichiometry for the two models. Finally, *model D* corresponds to a reversible consecutive dissociation of the target product **3**. The challenge is here that species **4i** will be considered as unmeasurable.

The aim of the methodology presented below will be to determine, in an efficient way, which model is the true one.

2.2. *Establishment of the set of equations*

The first step in the optimal experimental design procedure consists in establishing the set of equations characteristic of the system under test, which is based on mass balances inside the microreactor coupled with the stoichio-kinetics laws. For that, the following assumptions are made:

- The microreactor behaves as a purely isothermal plug-flow reactor. The input reagents are perfectly mixed. Then, the concentration and yield profiles are directly determined from intrinsic kinetics (they are only dependent on residence time, or microreactor length).
- Only the concentrations of the species **1**, **2** and **3** are measured and will be thus considered in the optimal design procedure as the experimental responses. The fact that the impurities **4i** are not measured adds clearly a difficulty, but it reproduces a classical configuration in fine chemistry industry.
- For any case, the reaction rates are expressed from Eq. (1):

$$r_i = \exp\left(\kappa_i + \frac{E_i}{T}\right) \times \prod_k^{\text{reagents}} [C_k]^{n_{k,i}} \quad \text{with} \kappa_i = \exp\left(k_i^0\right), E_i = \frac{E_{ai}}{R} \tag{1}$$

Where κ_i and E_i are the kinetic parameters of the reaction i which correspond to the reparametrized frequency factor (k_i^0) and activation energy (E_{ai}) from the Arrhenius law, C_k the molar concentration of a reagent k and $n_{k,i}$ its associated kinetic order for the reaction i, taken as the stoichiometric coefficient of the reagent k for the reaction i. T is the medium temperature and R the ideal gas constant.

Table 1 – Experimental domain.

Temperature (K)	Residence time (s)	$[2]_0/[1]_0$
340–450	20–1000	0.2–5

Based on these assumptions, the following mass balances can be written, enabling to describe the variation of the concentration $[C_k]$ of each chemical component k along the microreactor:

$$\frac{d[C_k]}{dz} \times \langle u_z \rangle = \frac{d[C_k]}{dt} = \sum_i^{\text{reactions}} \nu_{k,i} \times r_i \tag{2}$$

Where z is the axial position along the microreactor, t the residence time in the microreactor, u_z the mean flow velocity along the axial direction z, and r_i the rates of the reactions involving the species k with a stoichiometric coefficient $\nu_{k,i}$.

2.3. *Generation of the experimental data and operating domain*

In this paper, the "experimental" data are numerically generated with a predetermined stoichio-kinetic model. *Model A*, which is a strictly competitive model, has been chosen for this task. Let us note that this implies that the experimental design strategy implemented is supposed to lead to the elimination of the three other models (*models, B, C* and *D*). A random error, following a normal law with zero mean and a given standard deviation σ, has been then added to generate the experimental errors observed on experimental measurements.

In this study, it will be considered that the experimental parameters that are modified for the experimental data procedure are residence time (t), temperature (T) and initial stoichiometric ratio of the species **1** and **2** ($[2]_0/[1]_0$). The ranges considered are reported in Table 1. The measured experimental variables will be the output concentrations of the species **1**, **2** and **3**, and it will be assumed that no information is available on the by-product produced during the synthesis.

3. Experimental design strategy

3.1. *Description of the overall process*

The aim of the optimal experimental design strategy is to find the best stoichio-kinetic model among several models in competition, while optimizing the number and the quality of the experiments used. The process that has been implemented to achieve this goal is composed of successive steps depicted in Fig. 2.

From the preliminary experiments (step A1), some stoichio-kinetic models can be proposed (step A2). Then the parameters of all models are identified (step B) and the model accuracy compared (step C). If several models remain valid, the experimental design step (step D) generates the best experiment to discriminate the remaining models. Once this experiment is performed (step E), the iterative process (B-C-D-E-B-. . .) continues until only one model allows fitting all the experiments, or if no new experiment allows model discrimination anymore. Each step will be now described in detail.

3.1.1. *Steps A1 and A2—the preliminary steps*

The first step consists of carrying out several preliminary experiments. They give information on the system and thus

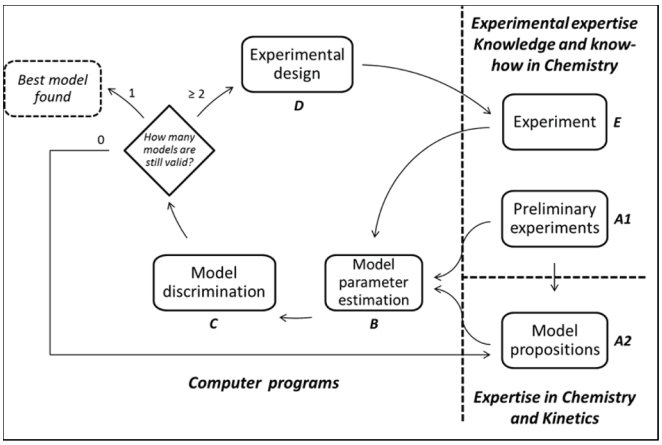

Fig. 2 – Sequential procedure of experimental design for model discrimination.

enable different statements to be made, related for example to the experimental parameters influencing the reactions, or to the variation of the species concentrations. Depending on the complexity of the chemical system, several stoichio-kinetic models can then be proposed. Once the models are selected, their properties should be verified in terms of identifiability and discernibility, and this before making any additional experiments. This procedure is detailed in Section 3.2. Finally, the preliminary experiments can be used to make a first identification of the model parameters.

3.1.2. *Step B—model parameter identification*

The iterative process starts with this step. The process for estimating the kinetic model parameters (κ_i and E_i) is implemented after each new experiment. The parameters are determined by minimizing a classical sum of square estimator called the Gauss–Markov estimator Φ_{GM}, given in Eq. (3).

$$\phi_{GM}(\boldsymbol{\theta}) = \frac{\left[\mathbf{y}_m(\boldsymbol{\theta},\xi,t) - \mathbf{y}_{exp}(\xi,t)\right]\left[\mathbf{y}_m(\boldsymbol{\theta},\xi,t) - \mathbf{y}_{exp}(\xi,t)\right]^T}{\sigma(\xi,t)^2}$$

Where y_m and y_{exp} represent the model and experimental responses respectively (i.e. the concentrations $[C_k]$), θ the kinetic model parameters, ξ the experimental variables, and t the residence time. $\sigma(\xi,t)$ is the standard deviation of the experimental response; even if the expression σ strictly depends on each sample, it will be considered as a constant. The use of σ reduces the influence of noisy data and normalizes data dimensions (necessary if, for example, temperature and concentration are both experimental responses).

3.1.3. *Step C—model discrimination*

The Gauss–Markov residual, namely the minimized value of ϕ_{GM} once the parameters are identified, can be directly used to evaluate model adequacy. Indeed, assuming that the experimental errors follow a zero mean normal distribution, the Gauss–Markov residual is a sample of the χ^2 distribution with a degree of freedom corresponding to the total number of samples minus the number of model parameters (Dumez et al., 1977). So the χ^2 statistic distribution can be used to evaluate the adequacy of each model, comparing the residual sum of squares with the χ^2 sample value. A model is considered inadequate if its residual rss (Residual Sum of Squares) is significantly higher to its associated χ^2 value.

3.1.4. *Step D—experimental design*

Once the inadequate models are removed, new experiments are required to be able to discriminate between the models still in competition. The basic idea of optimal design methodology is to use the information already acquired about the models, to look for the experimental conditions where the model responses are the most different between each model. In this way, some of the remaining models will possibly not fit with experimental data anymore. In practice, a mathematical criterion ϕ is used to compare model responses. Hunter and Reiner (1965) proposed the simplest criterion shown in Eq. (4).

$$\phi_{m1,m2}(\xi,t) = \left[\mathbf{y}_{m1}(\xi,t) - \mathbf{y}_{m2}(\xi,t)\right]\left[\mathbf{y}_{m1}(\xi,t) - \mathbf{y}_{m2}(\xi,t)\right]^T \quad (4)$$

Where y_{m1} and y_{m2} are the responses of two different models, m_1 and m_2, to discriminate.

However, many extended criteria have been developed since, to take into account specific experimental or mathematical considerations like experimental error or model prediction error (Hunter and Reiner, 1965; Atkinson et al., 1975; Buzzi-Ferraris and Forzati, 1983; Schwaab et al., 2006, 2008; Donckels et al., 2009). More details are given in Section 3.3.

3.1.5. *Step E—implementation of the new experiment and iteration*

The experiment proposed by the experimental design methodology is then carried out. After, the iterative process starts again, including the steps of model parameter identification and model evaluation. In this way, it is expected that thanks to the new experiment, some models could be removed. Finally, the procedure continues until just one model still fits with experimental data or until it is not possible to discriminate between the remaining models.

This sequential aspect of optimal design strategy offers interesting possibilities. Indeed, if after some iterations, it appears that the set of proposed models is not satisfying, modified or new models can be added to the initial base of models. This constitutes a powerful tool which enables to use the information acquired after each new experiment to propose into the selection procedure more accurate models if needed.

3.2. *Identifiability and discernibility properties of models*

The validation of these mathematical properties is an essential step before implementing any experimental design strategy, as it can become critical, particularly when the number of measured species is limited compared to the total number of species involved (this is a common situation for industrial applications as it is often difficult to get quantitative analysis data for all components).

This validation consists in checking that kinetic parameters of all the proposed models are identifiable and then that all the models are discernable. These two properties are defined below.

Definition 1. Given a model structure $M(.)$ and the associated space of parameters Θ, the model $M(\theta)$ with $\theta \in \Theta$ will be identifiable only if the proposition (5) is valid (Walter and Pronzato, 1994)

$$\forall(\theta,\theta') \in \Theta^2 \quad M(\theta) = M(\theta') \Rightarrow \theta = \theta' \quad (5)$$

Definition 2. Given two model structures $M_1(.)$ and $M_2(.)$, and their associated space of parameter Θ_1 and Θ_2 respectively, the models $M_1(\theta)$ and $M_2(\theta')$ with $\theta \in \Theta_1$, and $\theta' \in \Theta_2$, will be discernable only if the proposition (6) is valid (Ollivier, 1990)

$$\forall\theta \in \Theta_1 \forall\theta' \in \Theta_2 \quad M_1(\theta) \neq M_2(\theta') \tag{6}$$

In other words, a non-identifiable model could provide the same response with several (even an infinity) sets of parameters, and two non-discernable models will give the same response and could not be differentiable.

Several methods have been developed to verify identifiability and discernibility. The most used methods are the linearization method (Grewal and Glover, 1976), the similarity transformation method (Vajda and Rabitz, 1989) and the Taylor series development method (Pohjanpalo, 1978). A detailed description of all these latter is also given by Ollivier (1990) and by Walter and Pronzato (1993). In this work, the Taylor series approach is used because of its simplicity. The model response can be then represented with a Taylor series expansion in a time interval $[0, t_T]$ as following in Eq. (7).

$$y_m(t, \theta) = \sum_{n=0}^{\infty} \frac{a_n(\theta)}{n!}(t)^n \tag{7}$$

With:

$$a_n(\theta) = \frac{d^n[y_m(t=0, \theta)]}{dt^n} \tag{8}$$

Where $a_n(\theta)$ are the Taylor coefficients: the evaluation of the nth derivative of the model response with time, at zero time and for the set of model parameters θ.

Now, a sufficient condition to prove the identifiability of a model $M(\theta)$ is to demonstrate the proposition (9):

$$\forall(\theta, \theta') \in \Theta^2 \quad a_n(\theta) = a_n(\theta') \Rightarrow \theta = \theta', \quad \text{for} n = 0, 1, 2, \ldots, n_{\max} \tag{9}$$

Where $n_{\max}$ is generally a low integer to keep low calculation needs.

In a similar way, the sufficient condition to show the discernibility between two models $M_1(\theta)$ and $M_2(\theta')$ is the demonstration of proposition (10):

$$\forall\theta \in \Theta_1 \nexists\theta' \in \Theta_2 \quad a_{n,1}(\theta) = a_{n,2}(\theta') \Rightarrow \theta = \theta', \quad \text{for } n = 0, 1, 2, \ldots, n_{\max} \tag{10}$$

Once the structure properties of all the models are checked, the kinetic parameters can be identified. The model adequacy can be then evaluated, the models selected, and the step of optimal experimental design implemented to find a new experiment if necessary.

3.3. *Criteria for optimal design strategy*

3.3.1. *Criteria*

The first design criterion has been proposed by Hunter and Reiner (1965) for two model discrimination. It is based on a comparison of the model responses with a quadratic criterion (Eq. (4)) which is maximized depending on the experimental variables (residence or sampling time mostly). This enables the residence time where the two models have the most divergent response to be found. So if one of the models follows the experimental response at this residence time, the other cannot, and is thus rejected.

This design criterion has been modified and improved by several other research groups afterwards. Hunter and Reiner (1965) have themselves modified the latter criterion by taking into account the experimental error (Eq. (11)).

$$\phi_{m1,m2}(\xi, t) = \frac{[\mathbf{y_{m1}}(\xi, t) - \mathbf{y_{m2}}(\xi, t)][\mathbf{y_{m1}}(\xi, t) - \mathbf{y_{m2}}(\xi, t)]^T}{\sigma(\xi, t)^2} \tag{11}$$

The advantage of this criterion is to avoid proposing experimental conditions for which the experimental errors are too important. For that, it uses the variance of the experimental error σ^2.

The next criterion has been proposed by Buzzi-Ferraris and Forzati (1983):

$$\phi_{m1,m2}(\xi, t) = \frac{[\mathbf{y_{m1}}(\xi, t) - \mathbf{y_{m2}}(\xi, t)][\mathbf{y_{m1}}(\xi, t) - \mathbf{y_{m2}}(\xi, t)]^T}{(2\boldsymbol{\Sigma}(\xi, t) + \boldsymbol{\Omega_{m1}}(\xi, t) + \boldsymbol{\Omega_{m2}}(\xi, t))} \tag{12}$$

Where $\boldsymbol{\Omega}$ represents a covariance matrix on model prediction errors, and $\boldsymbol{\Sigma}$ the covariance matrix on experimental response errors. It is common to consider $\boldsymbol{\Sigma}$ as a diagonal matrix of the σ_i^2 of each experimental response i.

This criterion includes the uncertainty on model prediction, which is estimated using $\boldsymbol{\Sigma}$ and the model prediction error covariance matrix $\boldsymbol{\Omega}_i$ for each model i. Details on estimation of $\boldsymbol{\Omega}$ are given later in this section. The idea of this criterion is to remove regions of the experimental domain in which the model predictions are too poor to enable discrimination of the two models.

Schwaab et al. (2008) and Donckels et al. (2009) simultaneously proposed to modify the Buzzi-Ferraris criterion. The approach consists in including the expected model prediction covariance matrix Ω associated to the new experiment (that is predicted with the actual estimation of the model parameters), before the experiment is performed. The experimental responses and the associated matrix Ω are "anticipated" using information already acquired on models, explaining thus why it has been called the "anticipatory approach" by Schwaab et al. (2008) and Donckels et al. (2009). These authors have shown that using the predicted values of the model prediction uncertainty, the number of experiments necessary to discriminate between the models can be reduced and the resulting experiments often enable a better estimation of model parameters at the same time (Alberton et al., 2011).

All the latter criteria have been developed to compare two models. When more than two models are in competition, the classical method consists in calculating a global criterion, function of the pairwise criteria. For that, Buzzi-Ferraris and Forzati (1990), Schwaab et al. (2006) and Donckels (2009) have proposed three strategies: (i) to calculate and maximize the average or the sum of all the pairwise criteria, that was firstly proposed by (Dumez et al., 1977), (ii) to maximize the highest pairwise criteria, (iii) to maximize the smallest of the pairwise criteria. In the present study, the criterion in Eq. (13) will be chosen: the designed experiment performed will be then the one associated to the highest pairwise criterion. By this way, it is almost sure that at least one model will be eliminated after the experiment, or in other words, the designed experiments that cannot discriminate any models are eventually avoided.

$$\Phi_G(\xi, t) = \max_{i,j}\left[\phi_{i,j}(\xi, t)\right] \tag{13}$$

3.3.2. *Comparison of the different criteria*

In this work, the Buzzi-Ferraris criterion (Eq. (6)) will be used because, as shown by Donckels (2012), this is the most conservative of the four approaches. In other words, it always succeeds to find the best model. To demonstrate that, Donckels (2012) have applied 150 times the discrimination procedure, with 5 different series of initial experiments, repeated 30 times each and for all the criteria. The succeed of the strategy was 100% for Buzzi-Ferraris criterion, 89% for the anticipatory one, 85% for the modified Hunter & Reiner one and 97% for the original Hunter & Reiner one.

3.3.3. *Calculation of parameter estimation error covariance matrix* $\boldsymbol{\Phi}$*, and model prediction error covariance matrix* $\boldsymbol{\Omega}$

There are different methods to calculate the parameter estimation error covariance matrix $\boldsymbol{\Phi}$. The Monte-Carlo methods are the first well-known methods. The idea is to use the model parameters evaluated from experimental data to generate a sufficient amount of synthetic data. Gaussian noise is added, which is close to the real experimental error variance. Then, all these data enable the estimation of the mean and covariance of the model response. The Jackknife and Bootstrap methods are the most common Monte-Carlo methods (Efron, 1981; DiCiccio and Romano, 1988).

Another way to estimate $\boldsymbol{\Phi}$ is by its statistical estimation. The last works on experimental design applied to non-linear models have used this method for the estimation of $\boldsymbol{\Phi}$ (Buzzi-Ferraris and Forzati, 1983; Sedrati et al., 1999; Issanchou et al., 2003; Schwaab et al., 2008; Donckels et al., 2009). The more efficient an estimator is, the smaller its matrix $\boldsymbol{\Phi}$ will be. But there is a lower limit to the Φ value, characterized by the Cramer-Rao inequality: $\boldsymbol{\Phi} \geq \boldsymbol{M_F}(\theta^*)^{-1}$ where $\boldsymbol{M_F}$ is called the Fisher Information Matrix. This inequality asymptotically tends to be an equality if the estimator is efficient and without bias (like the Gauss–Markov estimator which is used in this work). While it is very difficult to estimate $\boldsymbol{\Phi}$, $\boldsymbol{M_F}$ remains easier to calculate with some hypothesis detailed by Issanchou (2002). Then, $\boldsymbol{M_F}$ is used as an approximation of $\boldsymbol{\Phi}$. The general definition of $\boldsymbol{M_F}$ is:

$$\boldsymbol{M_F} = E\left\{\left[\frac{\partial}{\partial\theta}\ln f_y(y|\theta)\right]\left[\frac{\partial}{\partial\theta}\ln f_y(y|\theta)\right]^T\right\} \tag{14}$$

Where $f_y(y|\theta)$ is the density function of the model response y, with θ fixed, and E the mathematical expectation.

For nonlinear models with a Gaussian noise, $\boldsymbol{M_F}$ can be calculated following Eq. (15) (Donckels et al., 2009):

$$\boldsymbol{M_F} = \sum_{\text{experiments}}^{\text{samples}} \frac{d\mathbf{y}_m}{d\boldsymbol{\theta}}\boldsymbol{\Sigma}^{-1}\frac{d\mathbf{y}_m}{d\boldsymbol{\theta}}^T \tag{15}$$

Where $d\mathbf{y}_m/d\boldsymbol{\theta}$ is the sensitivity of the model to its parameters. This equation is also an approximation of the hessian of the Gauss–Markov estimator, thus increasing the interest of the use of this estimator for parameter identification. Indeed, numerical tools for hessian calculation can be used in a first approximation.

In this work, the statistical method is preferred because Monte-Carlo methods need a very large amount of calculation while $\boldsymbol{M_F}$ is easy to calculate. Moreover, using the Gauss–Markov estimator, a good approximation of $\boldsymbol{\Phi}$ is ensured with $\boldsymbol{M_F}$.

The Buzzi-Ferraris and the anticipatory criteria require the knowledge of the model prediction error covariance matrix $\boldsymbol{\Omega}$. The matrix $\boldsymbol{\Omega}(\xi^*, t^*)$ of one new experiment with experimental conditions ξ^* and at time t^*t^* is given by Donckels et al. (2009):

$$\boldsymbol{\Omega}(\xi^*, t^*) = \frac{d\mathbf{y}_m(\xi^*, t^*)}{d\boldsymbol{\theta}} \times \boldsymbol{M_F}^{-1} \times \frac{d\mathbf{y}_m(\xi^*, t^*)}{d\boldsymbol{\theta}}^T \tag{16}$$

Where $\boldsymbol{M_F}$ is the Fisher Information Matrix calculated from all the existing experiments and $d\mathbf{y}_m/d\boldsymbol{\theta}(\xi^*, t^*)$ the model sensibility matrix at (ξ^*, t^*) If there are many samples $t_k{}^*$ in one experiment, the model prediction error covariance matrix $\boldsymbol{\Omega}_m$ used for the Buzzi-Ferraris criterion becomes (Donckels et al., 2009):

$$\boldsymbol{\Omega}_m(\xi^*, \mathbf{t}^*) = \sum_{k}^{\text{samples}} \frac{d\mathbf{y}_m(\xi^*, t_k{}^*)}{d\boldsymbol{\theta}} \times \boldsymbol{M_F}^{-1} \times \frac{d\mathbf{y}_m(\xi^*, t_k{}^*)}{d\boldsymbol{\theta}}^T \tag{17}$$

Where $\mathbf{t}^*$ is the vector of residence times $t_k{}^*$ of the new experiment.

For the anticipatory approach of Schwaab et al. (2008) and Donckels et al. (2009), the $\boldsymbol{M_F}$ expression is modified as $\boldsymbol{M_F}{}^*$:

$$\boldsymbol{M_F}{}^* = \boldsymbol{M_F}(\boldsymbol{\xi_E}) + \boldsymbol{M_F}(\boldsymbol{\xi_{E+1}}) \tag{18}$$

Where $\boldsymbol{M_F}(\boldsymbol{\xi_E})$ is the common Fisher Information Matrix and $\boldsymbol{M_F}(\boldsymbol{\xi_{E+1}})$ the Fisher Information Matrix of experiment $E+1$, but calculated with model parameters identified from experiments 1 to E. $\boldsymbol{M_F}(\boldsymbol{\xi_{E+1}})$ represents the expected information content of the new experiment $E+1$.

Another family of criteria has been developed (Box and Hill, 1967; Reilly, 1974) and used (Sedrati et al., 1999) Without going into details, these methods are based on information theory and the notion of "entropy", and proposed a Bayesian approach. However, various research groups (Dumez et al., 1977; Atkinson, 1978) have shown that there is no real systematic difference between experimental plans designed with Box and Hill's criterion or with Hunter and Reiner's one. Besides, several drawbacks of the "entropy" approach have been highlighted in details in Buzzi-Ferraris and Forzati, (1983), Buzzi-Ferraris and Manenti (2009) and Froment and Mezaki (1970). As a consequence, the criteria derived from Hunter and Reiner's approach remain more interesting because of the complexity of the entropy approach.

3.4. *Numerical methods*

As kinetic laws and associated reactor models are generally strongly nonlinear, parameter identification and design criterion optimization require adapted optimization algorithms. For the first parameter identification, an evolutionary algorithm (Ardia et al., 2011) is used as the robustness of this family of algorithm tends to limit the problems associated with parameter initialization. Then, for parameter identification following the new experiments, the Levenberg–Marquardt method is used (Marquardt, 1963; More, 1978). Indeed, the parameters are known and their values do not change significantly with new experimental data, so a more efficient but less robust optimization algorithm is more appropriate. For the optimization of Buzzi-Ferraris criterion, the evolutionary algorithm is also used.

Table 2 – Experimental conditions for the initial generated "experiments".

Experiment	Temperature (K)	Residence time (s)	$[2]_0/[1]_0$	$[3]_0$ (mol L^{-1})
1	340	510	1.1	0.5
2	340	1000	1.1	0.5
3	395	20	1.1	0.5
4	395	510	1.1	0.5

Reactor models also generally induce the resolution of differential equations systems. For that, an integrated package of the software *R*, solving ordinary differential equations, is used (Petzold, 1983). The software proposes many classes of solving methods included in a program which automatically chooses the best method depending on the system of differential equations.

4. Results and discussion

The methodology proposed in Section 3.1 is now applied on the study case presented in Section 2.

Four preliminary experiments are carried out (Table 2), enabling a first estimation of the kinetics parameters for each model. The estimated parameters are the Arrhenius-like parameters proposed in Eq. (1), namely κ_i and E_i. Fig. 3 presents the profiles of concentration for species **2** and **3**, obtained for each model. The generated "experimental" data are also reported in the figure.

Firstly, it is important to note that, even if species **3** is a product, its initial concentration is not set at zero. This is due to the fact that the parameter identification of *model D* needs $[3]_0$ non equal to zero. It comes from kinetic parameters of the reverse reaction, giving **3** with **4d**, that cannot be estimated without this condition. This shows that the issues related to parameter identification and model discrimination can lead to operate in experimental conditions uncommon for synthesis applications (for example with a non-null initial concentration of the main product). For illustration purpose, the test of *model D* identifiability is detailed in annexes. Identifiability of the other models as discernibility between models are not problematic and consequently not detailed in the paper.

As experiments 1 and 2 and experiments 3 and 4 only differ by the residence time (see Table 2), the concentration values of the different species are plotted on the same graph. From Fig. 3, it can be observed that *model A* and *model D* give similar profiles, both close to the "experimental" concentrations obtained from the preliminary experiments, whereas *model B* and *model C* do not fit at all. The challenge of the design methodology is then to find which new experiments are able to discriminate between *model A* and *model D*. For illustration purposes, all the models are kept for the first designed experiment, even if it is already clear that *model B* and *model C* are not adequate.

Fig. 4 illustrates the effect of each designed "experiment" on the adequacy of each model. As the adequacy test is based on the comparison between the Gauss–Markov residual rss and the χ^2 distribution, the ratio between the two is represented on the figure. The 5th experiment corresponds to the first designed "experiment". The inadequacy of *model B* and *model C* is shown again. All the models are taken into account and therefore the discriminatory criterion (Buzzi-Ferraris one) leads to the elimination of the worst model, according to Eq. (7). Thus, this experiment heightens discriminatory potential of the bad models (*B* and *C*). This is exactly what is shown in Fig. 4 with the increase of rss/χ^2 of *model B* and *model C* and stagnation of rss/χ^2 of *model D*, for experiment n°5 (first designed "experiment"). Once *model B* and *model C* are eliminated by the discrimination process, the designed "experiments" n°6 and n°7 enable the elimination of *model D* and thus the validation of *model A*, the true one in this case as it has been considered for "experimental" data generation.

Table 3 – "Experimental" conditions of the new designed experiments.

Experiment	Temperature (K)	Residence time (s)	$[2]_0/[1]_0$	$[3]_0$ (mol L^{-1})
5	395.6	1000	2	0.5
6	395.1	193	0.2	0.5
7	395	172	0.2	0.5

The experimental conditions associated with the new "experiments" are reported in Table 3. It can be observed that the reaction temperature remains almost the same, thus meaning that temperature is not a key experimental parameter to discriminate between models.

Experiment n°5 (the first designed one) is found by the methodology for its potential to discriminate between *model B* and *model C*. It corresponds to the maximal residence time (1000 s) and maximal stoichiometric ratio. The relevancy of such a result is easy to demonstrate. As the limiting reagent is **1**, when it is completely consumed (in other words, for a long residence time), some of the species **2** remains. Then if the species **1** participates in the side reaction as described by *model A*, all the reactions will stop, whereas if **1** does not participate as described by *model B* or *model C*, then the side reaction will continue. In other words, the two kinds of models cannot give the same response to this experiment; that is why it should be decisive. For *model D*, the equilibrium of the side reaction is compatible with the two behaviors.

As reported in Table 3, experiments n°6 and n°7 are close in terms of operating conditions and completely different from the experiment n°5: the residence time is short and the stoichiometric ratio is at the minimum, with the species **1** in excess. These conditions are defined by the design procedure to discriminate between *model A* and *model D*, as the other models (B and C) have already been removed. Fig. 5 shows that the concentration of **3** is the determining response for the discrimination. Concerning the result of 193 s for the optimal residence time, the initialization value for the residence time implemented in the algorithm is set at 20 s to give priority to the shortest times, now the difference in model responses on concentration of **3** is constant after 193 s (Fig. 5(b)). Thus the discrimination criterion stays constant within residence time from this value, and the algorithm keeps this value for the optimum residence time. Concerning the choice of the optimal stoichiometric ratio, for *model A*, the species **3** is not consumed, so for a very low stoichiometric ratio, the profile corresponds to an increase of the concentration of **3**, stopped by the disappearance of **2**. For *model D*, the low initial concentration of **2** significantly reduces the influence of the main reaction on the global kinetics; the main reaction cannot initiate the production of **3**, as for *model A*; the reversible reactions lead the production/consumption of **3**.

These findings show that the methodology leads to efficiently discriminate the different models and to choose the

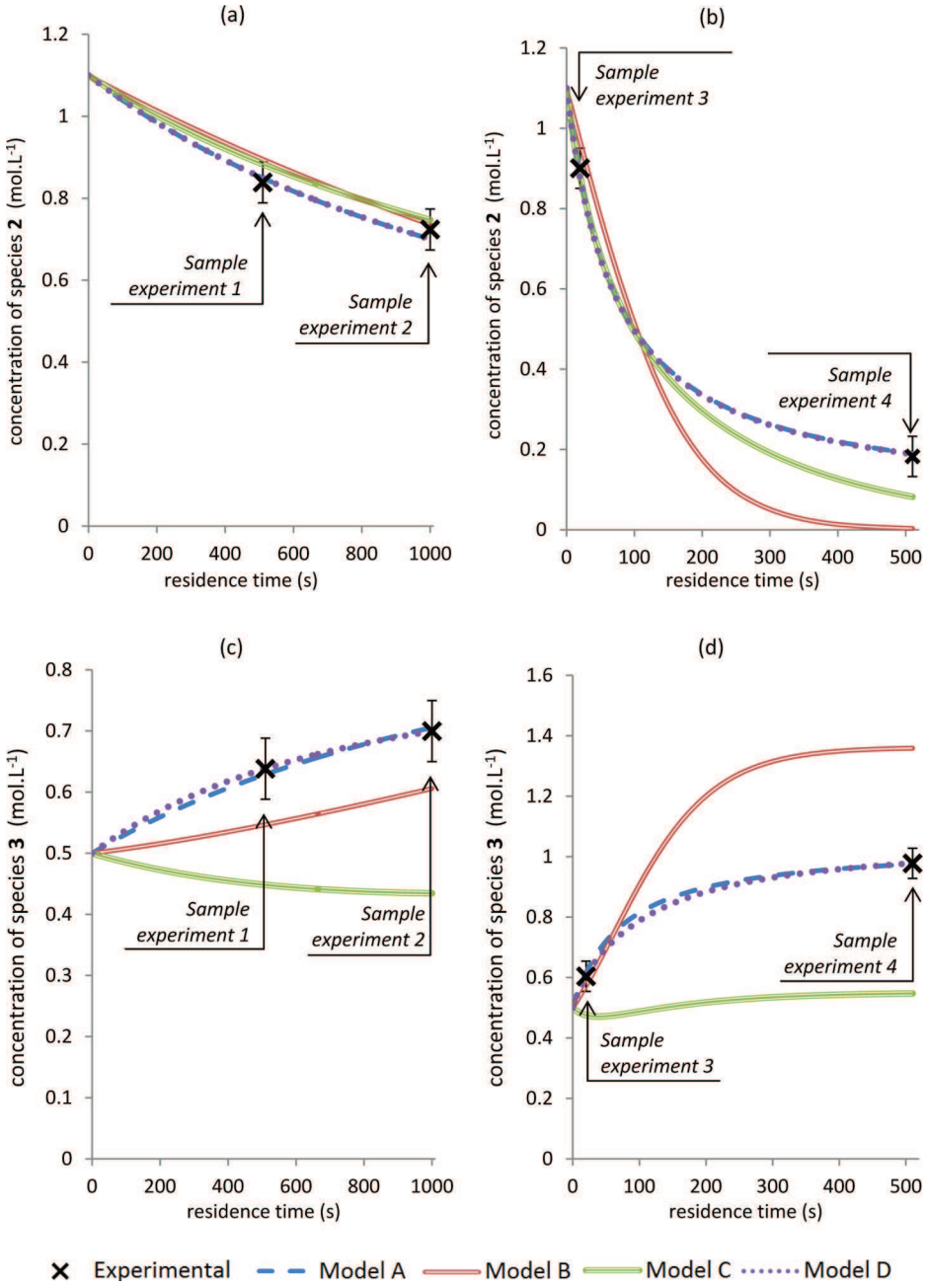

Fig. 3 – Concentration profiles of species 2 and 3 calculated for each model and "experimental" data. As "experiments" 1 and 2 just differ in residence time, the same profiles are used ((a) and (c)). As "experiments" 3 and 4 just differ in residence time, the same profiles are used ((b) and (d)).

more adequate one, while implementing a reduced number of relevant experiments.

In order to verify that the set of initial experiments does not influence the results of the discrimination process, the strategy has been repeated with other sets of preliminary experiments (Table 4).

The results obtained are globally similar for all trials. *Model B* and *model C* are rejected after 4 "experiments" and *model D* after 5–7 "experiments". When the four models are included in the procedure, a high stoichiometric ratio is always proposed by the strategy. When only *model A* and *model D* are still in competition, the minimal stoichiometric ratio is always proposed. On the contrary, for the residence time and the temperature, the results are not so distinct. It appears that there are two interesting domains for residence time (20–150 s and 1000 s), and a temperature always high but varying for each "experiment".

These findings clearly demonstrate that the efficiency of the experimental design strategy (i.e. both in terms of model discrimination and number of required experiments) is not much influenced by the preliminary data acquisition.

At last, the potential of the optimal design strategy implemented in terms of time saving will be illustrated. For that, let's assume that the experimenter will define "experiments" following a logical plan as a factorial plan. Note that there is theoretically no real interest in using such plan for experimental design with nonlinear models; since it is optimized for the choice of experiments to model phenomena throw linear and polynomial models .The objective is here to show what happens for model discrimination if experiments are implemented regularly on the experimental range without taking

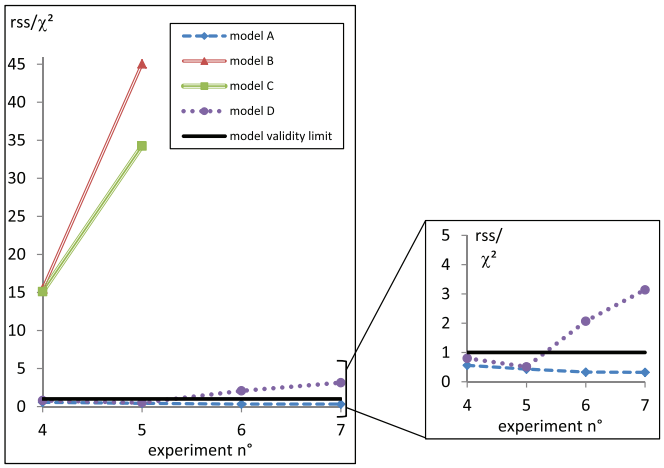

Fig. 4 – Adequacy test based on χ^2 distribution (see Section 3.1). An adequate model has test value under 1 which tends to decrease. 1st validity test is made after the 4 first "experiments".

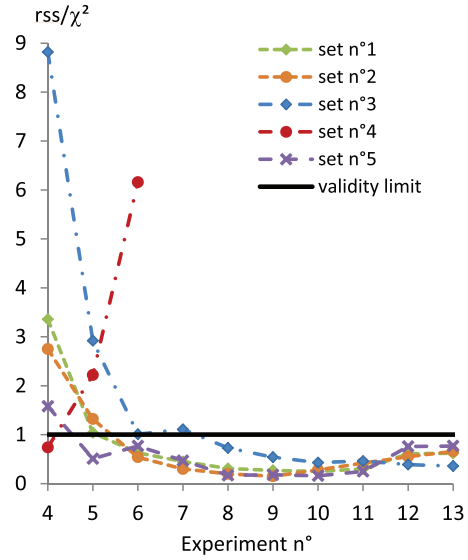

Fig. 6 – Validity test of *model D*, based on χ^2 distribution (Cf. Section 3.1), after each experiment, for the first half of each set of factorial plan. A valid model has test value under 1 and tending to decrease. 1st validity test is made after the 4 first experiments.

into account information on models. The sets of factorial designs proposed correspond to series of experiments at 3 levels for each experimental variable (the maximum, minimum and average value on the range, see Table 1). For comparison purpose, five sets of factorial plans have been tested, each one being modified to have the same initial experiments that the five sets of designed experiments of the previous section. Then, the next experiments follow rigorously a factorial plan. These plans are presented in Appendix B. Each plan is composed of 27 (3^3) experiments. Even if each one leads to the selection of *model A* over the other, more than 4 times more experiments are needed compared to the optimal designed experiments.

For purposes of comparison with the optimal design procedure, after each experiment of the factorial plan, the model parameters of all the models are estimated, and the adequacy test is done. Fig. 6 shows the evolution of the adequacy test for *model D*, the most difficult model to eliminate, for the first half of the sets of factorial plans. Most of the sets cannot lead to the elimination of *model D* after 13 experiments, being twice more than for optimal design. Just one set of experiments, which makes an exception, needs 6 experiments as for designed experiments. This is due to the sequencing of this set which gives by chance the relevant experiments, i.e. the ones close to the designed experiments, at the beginning of the set. This comparison with factorial plan confirms that experimental design strategy enables to discriminate various stoichio-kinetics models while reducing experimental efforts.

5. Conclusion

The combination of model-based experimental design strategy with the use of microfluidic tools appears clearly as a promising answer to the specific needs of fine chemistry and pharmaceutical industries, namely: (i) to obtain strong and reliable stoichio-kinetic models for rapid scaling-up and reduction of extrapolation deviation and associated risks, (ii)

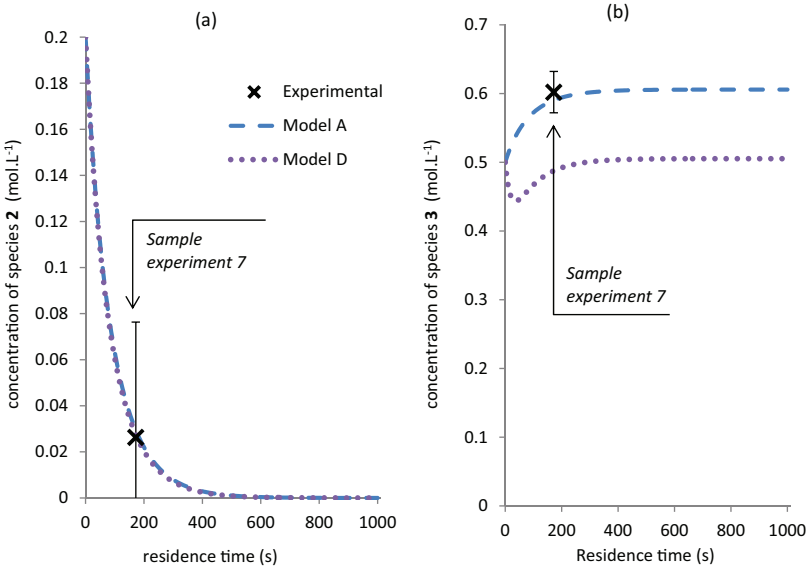

Fig. 5 – Concentration profiles of species 2 and 3 calculated for each model and in the conditions of the "experiment" n°7.

Table 4 – Different sets of preliminary "experiment" to begin the discrimination procedure.

Set	Exp.	Temperature (K)	Residence time (s)	$[1]_0/[2]_0$	$[3]_0$ (mol L^{-1})
2	1	340	20	1.1	0.5
	2	395	20	1.1	0.5
	3	450	20	1.1	0.5
	4	340	510	1.1	0.5
3	1	340	20	1.1	0.5
	2	395	20	1.1	0.5
	3	450	20	1.1	0.5
	4	340	510	1.1	0.5
4	1	395	20	1.1	0.5
	2	340	20	1.1	0.5
	3	340	1000	1.1	0.5
	4	450	20	1.1	0.5
5	1	340	20	1.1	0.5
	2	395	20	1.1	0.5
	3	450	20	1.1	0.5

Table 5 – Experimental conditions of the new designed "experiments" for each set of preliminary experiments. * indicates that "experiments" are chosen for discrimination between *models* A, B, C and D, ** for *model* A and *model* D only.

Set	Exp.	Temperature (K)	Residence time (s)	$[1]_0/[2]_0$	$[3]_0$ (mol L^{-1})
2	5*	387.5	1000	2	0.5
	6**	416	20	0.2	0.5
	7**	450	1000	0.2	0.5
3	5*	450	20	2	0.5
	6*	393.9	20.1	2	0.5
4	5**	442	20	0.2	0.5
	6**	412	20	0.2	0.5
5	4*	362	1000	2	0.5
	5**	450	150	0.2	0.5

without requiring prohibitive experimental efforts, (iii) taking in account the fact that only a limited number of species are analytically measured (unidentified products, analytical method complexity), (iv) reduced consumption of chemicals and enlargement of the experimental window in terms of operating conditions. Indeed, model-based design enable to select the most relevant experimental sets for model discrimination, which are not instinctive for a chemist, because often far from the optimal conditions regarding the chemical yield or conversion.

The objective of this work was to highlight the interest of model-based experimental design strategy for stoichio-kinetic models discrimination. For that, a numerical study case was considered, consisting in a reactional system classically encountered in pharmaceutical industries, involving an impurity which is not measured; various models could be thus initially postulated to describe how this impurity is produced. The strategy implemented allowed to check the issues about structural properties of models due to the lack of measurement information, and to consequently adjust the experimental conditions to assure the correct estimation of model parameters. Then, based on the use of advanced criteria, the strategy lead to an efficient selection of the most accurate model with a reduced number of experiments, while determining at the same time the parameters of the kinetics laws that may be directly integrated in the reactor engineering step for scale up operations.

In the future, the efficiency of the present strategy will be demonstrated with experimental study cases for which assuming monophasic plug-flow microreactors remains valid. In a second time, the strategy will be extended to more complex cases, especially those involving coupled phenomena like heat or mass transfers.

Appendix A. identifiability test of *Model D*

The rigorous test of identifiability of *Model D* with the Taylor series method is shown below; the system (A.1) represents the exact formulation of *Model D*.

$$\begin{cases} \dfrac{d[1]}{dt} = \dfrac{d[2]}{dt} = -k_1[1][2] \\ \dfrac{d[3]}{dt} = +k_1[1][2] - k_2[3] + k_3[4] \\ \dfrac{d[4]}{dt} = +k_2[3] - k_3[4] \\ [1]_0 \neq 0; [2]_0 \neq 0 \\ [3]_0 = c \\ [4]_0 = 0 \\ y_m = ([1], [2], [3]) \\ k_i = \exp\left(\kappa_i + \dfrac{E_i}{T}\right) \end{cases} \tag{A.1}$$

The identification test will be executed considering temperature kinetic parameters k_i for simplicity matters. Indeed,

once the k_i are identifiable, it is trivial to demonstrate that experiments at two different temperatures enable to identify the κ_i and E_i of the reparametrized Arrhenius law (Eq. (A.2)) from the k_i

$$k_i = \exp\left(\kappa_i + \frac{E_i}{T}\right) \tag{A.2}$$

Initially the initial concentrations of the species **3** is considered set at zero. In this case, and if we do not consider the temperature influence, the system (A.3) represents the a_n coefficients of the Taylor series associated to *Model D* with $n = 0,1,2$. It is clear than it is impossible to identify k_3 since the parameter does not appear in the system. Consequently *Model D* is not identifiable in these conditions.

$$\begin{cases} a_0(k) = \begin{pmatrix} [\mathbf{1}]_0 \\ [\mathbf{2}]_0 \\ [\mathbf{3}]_0 \end{pmatrix} \\ a_1(k) = \begin{pmatrix} \frac{d[\mathbf{1}]}{dt}(t=0) \\ \frac{d[\mathbf{2}]}{dt}(t=0) \\ \frac{d[\mathbf{3}]}{dt}(t=0) \end{pmatrix} = \begin{pmatrix} -k_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 \\ -k_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 \\ +k_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 \end{pmatrix} \\ a_2(k) = \begin{pmatrix} \frac{d^2[\mathbf{1}]}{dt^2}(t=0) \\ \frac{d^2[\mathbf{2}]}{dt^2}(t=0) \\ \frac{d^2[\mathbf{3}]}{dt^2}(t=0) \end{pmatrix} \\ = \begin{pmatrix} {k_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 \\ {k_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 \\ -{k_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 - k_1k_2 \times [\mathbf{1}]_0[\mathbf{2}]_0 \end{pmatrix} \end{cases} \tag{A.3}$$

Now to solve the identifiability problem, *Model D* has to be modified. Three choices are available:

- Reject the model. This solution is nevertheless damaging if the model well fit with experimental data
- Add a new model response, thus meaning that the species **4** should be measured.
- Change the initial conditions on one of the species **3** or **4**, making it non zero.

In this paper, as the measurement of **4** will be kept impossible, only a change of the initial concentration of the species **3** will be proposed. It is why $[\mathbf{3}]_0$ has been set at $0.5\,\mathrm{mol\,L^{-1}}$.

Now if $[\mathbf{3}]_0 \neq 0$, the system (A.4) represents the a_n coefficients of the Taylor series associated to *Model D* with $n = 1,2$ ($n = 0$ is useless):

$$\begin{cases} a_1(k) = \begin{pmatrix} \frac{d[\mathbf{1}]}{dt}(t=0) \\ \frac{d[\mathbf{2}]}{dt}(t=0) \\ \frac{d[\mathbf{3}]}{dt}(t=0) \end{pmatrix} = \begin{pmatrix} -k_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 \\ -k_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 \\ +k_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 - k_2 \times [\mathbf{3}]_0 \end{pmatrix} \\ a_2(k) = \begin{pmatrix} \frac{d^2[\mathbf{1}]}{dt^2}(t=0) \\ \frac{d^2[\mathbf{2}]}{dt^2}(t=0) \\ \frac{d^2[\mathbf{3}]}{dt^2}(t=0) \end{pmatrix} \\ = \begin{pmatrix} {k_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 \\ {k_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 \\ -{k_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 + {k_2}^2[\mathbf{3}]_0 - k_1k_2 \\ \times [\mathbf{1}]_0[\mathbf{2}]_0 + k_2k_3 \times [\mathbf{3}]_0 \end{pmatrix} \end{cases} \tag{A.4}$$

Then, considering two sets of parameters k, and p from Θ, the proposition (9) can be demonstrated in Eq. (A.5):

$$\begin{cases} a_1(k) = a_1(p) \\ a_2(k) = a_2(p) \end{cases}$$

$$\Rightarrow \begin{cases} -k_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 = -p_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 \\ +k_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 - k_2 \times [\mathbf{3}]_0 = +p_1 \times [\mathbf{1}]_0[\mathbf{2}]_0 - p_2 \times [\mathbf{3}]_0 \\ {k_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 = {p_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 \\ -{k_1}^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 + {k_2}^2[\mathbf{3}]_0 - k_1k_2 \times [\mathbf{1}]_0[\mathbf{2}]_0 + k_2k_3 \times [\mathbf{3}]_0 \\ = p_1^2 \times ([\mathbf{1}]_0 + [\mathbf{2}]_0)[\mathbf{1}]_0[\mathbf{2}]_0 + {p_2}^2[\mathbf{3}]_0 - p_1p_2 \times [\mathbf{1}]_0[\mathbf{2}]_0 + p_2p_3 \times [\mathbf{3}]_0 \end{cases}$$

$$\Rightarrow \begin{cases} k_1 = p_1 \\ k_2 = p_2 \\ k_3 = p_3 \end{cases} \tag{A.5}$$

Finally with $[\mathbf{3}]_0 \neq 0$, *Model D* becomes identifiable with kinetic parameters k_i, this is why it is set at $0.5\,\mathrm{mol\,L^{-1}}$.

Appendix B. Factorial plans

(1): Temperature (K)
(2): residence time (s)
(3): initial molar ratio $[2]_0/[1]_0$

Exp	Set 1			Set 2			Set 3			Set 4			Set 5		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
1	**340**	**20**	**1.1**	**510**	**340**	**1.1**	**395**	**20**	**0.2**	**340**	**1.1**	**510**	**340**	**20**	**1.1**
2	**395**	**20**	**1.1**	**1000**	**340**	**1.1**	**340**	**20**	**2**	**395**	**1.1**	**510**	**395**	**20**	**1.1**
3	**450**	**20**	**1.1**	**20**	**395**	**1.1**	**340**	**1000**	**0.2**	**450**	**1.1**	**510**	**450**	**20**	**1.1**
4	**340**	**510**	**1.1**	**510**	**395**	**1.1**	**450**	**20**	**0.2**	**340**	**0.2**	**510**	340	510	1.1
5	395	510	1.1	1000	395	1.1	450	1000	2	395	0.2	510	395	510	1.1
6	450	510	1.1	20	450	1.1	450	510	1.1	450	0.2	510	450	510	1.1
7	340	1000	1.1	510	450	1.1	340	1000	1.1	340	2	510	340	1000	1.1
8	395	1000	1.1	1000	450	1.1	395	1000	1.1	395	2	510	395	1000	1.1
9	450	1000	1.1	20	340	0.2	450	1000	1.1	450	2	510	450	1000	1.1
10	340	20	0.2	510	340	0.2	340	20	0.2	340	1.1	20	340	20	0.2
11	395	20	0.2	1000	340	0.2	340	20	1.1	395	1.1	20	395	20	0.2
12	450	20	0.2	20	395	0.2	340	510	1.1	450	1.1	20	450	20	0.2
13	340	510	0.2	510	395	0.2	340	510	0.2	340	0.2	20	340	510	0.2
14	395	510	0.2	1000	395	0.2	395	510	0.2	395	0.2	20	395	510	0.2
15	450	510	0.2	20	450	0.2	450	510	0.2	450	0.2	20	450	510	0.2
16	340	1000	0.2	510	450	0.2	450	20	1.1	340	2	20	340	1000	0.2
17	395	1000	0.2	1000	450	0.2	395	1000	0.2	395	2	20	395	1000	0.2
18	450	1000	0.2	20	340	2	450	1000	0.2	450	2	20	450	1000	0.2
19	340	20	2	510	340	2	395	20	1.1	340	1.1	1000	340	20	2
20	395	20	2	1000	340	2	395	20	2	395	1.1	1000	395	20	2
21	450	20	2	20	395	2	450	20	2	450	1.1	1000	450	20	2
22	340	510	2	510	395	2	340	510	2	340	0.2	1000	340	510	2
23	395	510	2	1000	395	2	395	510	2	395	0.2	1000	395	510	2
24	450	510	2	20	450	2	450	510	2	450	0.2	1000	450	510	2
25	340	1000	2	510	450	2	340	1000	2	340	2	1000	340	1000	2
26	395	1000	2	1000	450	2	395	1000	2	395	2	1000	395	1000	2
27	450	1000	2	20	340	1.1	395	510	1.1	450	2	1000	450	1000	2

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