MathematicS

MathS In A.

In Action

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Volume 13 (2024), p. 33-47.

https://doi.org/10.5802/msia.40

MathematicS In Action *est membre du*
Centre Mersenne pour l'édition scientifique ouverte
http://www.centre-mersenne.org/
e-ISSN : 2102-5754

MathematicS In Action
Vol. 13, 33-47 (2024)

Modeling vaccine degradation

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Abstract

This expository paper is an introduction to the mathematical modelling of vaccine degradation and to its industrial applications, including the study of vaccine stability and the so called "WHO last mile" program.

1. Introduction

1.1. Industrial context

A central question in the vaccination process is to know whether the vaccine is efficient at the time of administration, or whether it has been too degraded to be efficient. Namely, to inject a degraded vaccine may not protect the patient, which may be worse than not to vaccine. This central question may be declined in several subquestions.

- In perfect conditions, namely in a highly controlled environment, what is the lifespan of a vaccine? May it be conserved at 5 degrees Celsius during 2 years for instance? What temperature specifications ensure its correct preservation?

  This first point refers to legal issues. A vaccine company has to prove that the concentration of active molecules in given conditions remains higher than some baseline concentrations. It is also crucial for the company to have an idea of the stability of its product as early as possible in the development process, long before clinical trials.

  The question of vaccine conservation may also lead to challenging practical problems, as is the case for RNA type vaccines against Covid-19, which have to be conserved at very low temperatures, and thus require specific freezers.

- In real world conditions, what is the vaccine's tolerance with respect to unexpected thermal excursions?

  During long travels, vaccines may be exposed to delays, or to an accidental heating. The World Health Organisation (WHO) has developed guidelines, which must be fulfilled by all vaccines companies to ensure that vaccines are still valid after a long journey [3, 2, 4].

  Another question is the so called "last mile problem" [8, 9]. When vaccines are transported in remote areas (e.g. in limited cold chain technologies countries), many refrigeration-related incidents may occur, especially at the very end of the travel. During the last miles, the probability of a break in the cold chain is higher and the vaccines may

Keywords: Modelling, Vaccine, Chemistry.
2020 *Mathematics Subject Classification:* 00X99.

be exposed to high temperatures. They may be degraded as they reach the target population and it is crucial for the healthcare workers to know whether they administered an active vaccine or not.

A related important industrial challenge is to add visual indicators that would allow healthcare workers to visually control the quality of the vaccine they are about to administer. Visual indicators should change colours when the vaccine is no longer within its specifications. This requires to compare the evolution of visual indicators with the degradation of vaccines and to carefully design indicators.

- The understanding and prediction of the degradation of a vaccine may also be used to monitor an industrial process.

  Do samples of a newly produced vaccine behave according to their specifications? Does a change in the formulation of an industrial process change the lifespan of the vaccine? Are two given batches comparable, as far as degradation is involved? This last question also refers to the detection of industrial counterfeiting.

To have indications of the stability of a product at early stages of its development is also a precious indications to the drug industry since it may avoid large wastes of time and efforts. Note that vaccine stability and quality is a critical issue which is controlled by various national and international organisation, including the WHO (World Health Organisation) or the ICH (International Conference of Harmonization [1]), with many related legal aspects.

A natural approach to address all these questions is to develop mathematical models of the vaccine degradation [6]. Such models, once parametrized and validated, may predict the long time shelf stability, the evolution of a vaccine during an unexpected thermal excursion, or monitor the quality of a production. As a consequence, mathematical approaches to investigate vaccine stability are currently developed by several teams and industries worldwide [13, 16].

1.2. **Specificities**

The general principle of vaccine stability analysis is to accelerate the degradation through heating. Vaccines are heated, up to 35, 45, 60 degrees or even more. Of course, if the temperature is too high, new degradation mechanisms may arise, which limits the range of possible temperatures. It is also usually not possible to go below 0 degrees, which would imply a change of phase.

Let us now detail the specificities of vaccine degradation modelling. The first main problem is that the biochemical degradation mechanism is unknown. It may even be non unique. Vaccines are usually complex combinations of excipients and biological products, and many different mechanisms may arise. Some of the possible degradation mechanisms include protein unfolding, classical chemical reactions with some of the excipients, protein polymerisation, and reactions at the surface of the container.

The second main characteristic is the low number of experimental stability data generated for regulatory purposes [1]. Each data requires time and has a non negligible cost, therefore the number of data ranges from ten to a few dozen.

The third difficulty is the high error margin on experimental measures. For instance when attenuated viruses are involved, the data is the logarithm of the number of attenuated viruses, which may change by several order of magnitudes. Usually the error on this logarithm is ± 0.3, which means that the concentration of active substance is known up to a factor 2.

These three difficulties, well described previously in [6] create the specificity of this modelling problem, which is therefore very different from fitting problems arising in chemistry or physics. Usually, in chemistry we may have access to different byproducts of the reaction and may have many time points and many different measures. In physics the precision is usually much higher.

These three difficulties are at the centre of the difficulty of the modelling approach and of its limitations.

2. Methodology

2.1. Modeling

Let $\phi(t)$ be the concentration of the vaccine at time t, or more precisely the concentration of some of the active substance(s) of the vaccine, or even of a measure related to the concentration of the vaccine. Note that ϕ may also be a vector, containing several concentrations, of different vaccine components. However, in general, $\phi(t)$ is only a scalar.

The evolution of ϕ is modelled through an ordinary differential equation

$$\frac{\mathrm{d}\phi}{\mathrm{d}t} = F(\phi(t), T(t), \theta) \tag{2.1}$$

where $T(t)$ is the temperature at time t, F is the degradation model and θ are the various parameters of the model.

Let $\mathrm{Model}(\phi_0, T, \theta, t)$ denotes the value at time t of the solution of (2.1) with initial data ϕ_0, temperature profile T (usually constant in time) and set of parameters θ.

The data consist of a set of concentrations $y_{i,j}$ which are measured at time $t_{i,j}$, starting from initial concentrations ϕ_i^0 after an evolution at temperature T_i. Each experiment i has its own initial condition ϕ_i^0 and temperature T_i, and measures of the evolution of the concentration are made at several later times $t_{i,j}$, which may be different from one experiment to another. Note that the temperature T_i may depend on time. Usually there are few different temperatures, namely between two and four, and few data (a dozen or a few dozen).

2.2. Best fit and Bayesian approach

It is then classical to try to fit the data with the model by trying to minimise a distance function of the form

$$J(\theta) = \sum_i \sum_j d\Big(\mathrm{Model}(\phi_i^0, T_i, \theta, t_{i,j}), y_{i,j}\Big).$$

The function J measures the difference between the predictions of the model, with parameters θ, and the observed data. In this formula, d may be the usual Euclidian distance, or a relative error, or the difference between logarithms, or more complex formulas, depending on the nature of the error model.

The problem reduces to a classical nonlinear fitting problem, namely to find the best possible set of parameters θ_b, solution of the minimisation problem

$$\theta_b = \mathrm{argmin}_\theta\, J(\theta).$$

The search of the minimum of J is a classical optimisation problem, which can be solved using classical optimisation algorithms like the gradient method, stochastic methods, or a combination of both.

Let us now detail a Bayesian approach. Let D denote the set of data and let $p(\theta|D)$ be the probability of the set of parameters θ, knowing the data D. Then, using the classical Bayes' law,

$$p(\theta|D)p(D) = p(D|\theta)p(\theta), \tag{2.2}$$

where $p(D)$ is the probability density function to observe this set of data, $p(D|\theta)$ is the probability to observe this set of data if the set of parameters is θ and $p(\theta)$ is the probability to have this set of parameters θ. Usually we have no a priori on θ, leading to the choice $p(\theta) = 1$. On the

contrary, $p(D|\theta)$ is given by the error model. In the simplest case, assuming a Gaussian error (an assumption which may be checked),

$$p(D|\theta) = Z_0^{-1} \exp\Big(-\frac{J(\theta)}{2\sigma_{exp}^2}\Big) \tag{2.3}$$

where σ_{exp} is the standard deviation of the experimental method and Z_0 a normalisation constant. This leads to

$$p(\theta|D) = Z_0^{-1} \exp(-J/2\sigma_{exp}^2). \tag{2.4}$$

This last formula gives a probability measure on the parameters' space. This allows us to define an averaged prediction, together with prediction confidence intervals. For instance, within this Bayesian approach, the average prediction at time t with initial concentration ϕ_0 and storage temperature profile T is

$$\int \text{Model}(\phi_0, T, \theta, t) p(\theta|D)\, \mathrm{d}\theta. \tag{2.5}$$

This integral may be approximated by constructing a sequence of set of parameters θ which follow the law $p(\theta|D)$. The first method is to use the classical Metropolis Hastings algorithm, which precisely constructs such a sequence of set of parameters θ_i. However, despite its simplicity, this algorithm may be delicate to tune, because, depending on its internal parameters, it may be imprecise or take a long time before ensuring a good sampling of $p(\theta|D)$. This method is thus often replaced by the second one, which is the approximation of (2.4) by a Gaussian. This easier method, which appears to be precise enough in most cases, consists in replacing J by its second order approximation near the best fit θ_b in (2.4). Let $\widetilde{\theta} = \theta - \theta_b$. Then, using that $DJ(\theta_b) = 0$ since θ_b is precisely a minimum of J, we have

$$J(\theta) = J(\theta_b) + \frac{1}{2} D^2 J(\theta_b).\widetilde{\theta}.\widetilde{\theta} + O(\|\widetilde{\theta}\|^3)$$

This leads to

$$p(\theta|D) = Z_1^{-1} \exp\Big(-\frac{\nabla^2 J(\theta_b).(\theta-\theta_b).(\theta-\theta_b)}{4\sigma_{exp}^2}\Big) \tag{2.6}$$

where Z_1 is a normalization constant. It is then straightforward to construct a sequence of set of parameters θ_i which sample this Gaussian law. In both cases, (2.5) is approximated by

$$\frac{1}{N} \sum_{i=1}^{N} \text{Model}(\phi_0, T, \theta_i, t)$$

provided N is large enough.

Note that in practice, it is very delicate to evaluate σ_{exp} since biochemical experiments are usually a very complex combination of delicate manipulations, depending on the context and even on the operator. Only approximate values of σ_{exp} are available. If no value of σ_{exp} is available, only the "best fit" approach may be followed.

2.3. **Model library**

The leading idea is to first design a series of models, which describe the main degradation mechanisms which may be encountered. This leads to a "model library" which can be tried when studying a new set of data. In general, we have no or limited indications of the bio chemical mechanism(s) which occur and lead to the degradation of the product. Confronted with a new set of data, one of the common approaches is to try all models available in one's "model library". Some will not fit, and others will. Models which do not fit may be dismissed, and the other(s) may be kept for further studies, a point detailed below.

Thus a first important issue is to design pertinent models of vaccine degradation, of the form (2.1). In general, it is not possible to completely describe the degradation process, since many unknown biochemical reactions may be involved at the same time. Many reactions may

be combined, in a parallel or sequential way, and in general there is too few data to be able to describe the complex underlying reaction networks.

One intuitive approach is to assume that there is only one main reaction driving the degradation process; whether one assumes that a single reaction plays a preponderant role amongst all mechanisms occurring at the same time; or that a few reactions of similar kinetics are concomitant.

It is also very important to frame the relation between vaccine degradation and temperature within a thermodynamic context. To this end, it is assumed that prefactors of these reactions must follow the Arrhenius law

$$C = C_0 e^{-Ea/RT}$$

where E_a is the activation energy of the reaction, $R = 8.32$ is the usual constant and T is the temperature. Including Arrhenius law within prefactors ensures that the reaction speed correctly changes with temperature.

Let us give a list of such possible mathematical models:

- First order kinetics
$$\frac{\mathrm{d}\phi}{\mathrm{d}t} = -Ke^{-Ea/RT}\phi \tag{2.7}$$
where $\phi(t)$ is the concentration of the vaccine. In this case the unknowns are the prefactor K and the activation energy E_a and $\theta = (K, E_a)$. In this case ϕ decreases exponentially. Note that Arrhenius law is encoded in (2.7).

- Second order kinetics
$$\frac{\mathrm{d}\phi}{\mathrm{d}t} = -Ke^{-Ea/RT}\phi^2.$$

- n^{th} order kinetics
$$\frac{\mathrm{d}\phi}{\mathrm{d}t} = -Ke^{-Ea/RT}\phi^n.$$
In this case the unknowns are the prefactor K, the activation energy E_a and the order $n \geq 0$.

- Parallel reactions. In this case, the product may be decomposed in two parts, each with a first order degradation: $\phi = \phi_1 + \phi_2$, with
$$\frac{\mathrm{d}\phi}{\mathrm{d}t} = -K_i e^{-Ea_i/RT}\phi_i.$$
The unknowns are K_0, K_1, Ea_0, Ea_1 and the initial decomposition of ϕ in $\phi_1(0)$ and $\phi_2(0)$.

The model library may also contain more complex models like the so called Finke Watsky or Sestak-Berggren (including Prout-Tompkins) models. These models are more "qualitative". Note however that the temperature dependency is always encoded in Arrhenius law.

- Fintke Watsky's model [10]: it is a model of aggregation, of the form
$$\dot{\phi}_1 = -K_1 e^{-Ea_1/RT}\phi_1 - K_2 e^{-Ea_2/RT}\phi_1\phi_2,$$
$$\dot{\phi}_2 = K_1 e^{-Ea_1/RT}\phi_1.$$

- Sestak's family of models [12, 14], including Sestak Berggren and Proutt Tompkins: models of the form
$$\dot{\phi} = -Ke^{-Ea/RT}\phi^n(1-\phi)^m \log(1-\phi)^p.$$
These models correspond to more complex degradation processes, e.g. reactant diffusion or growth at the interfaces.

2.4. **Selection of the model**

As mentioned above, to study a new set of experimental data, the general strategy is to try all the models of the "model library". The first step is to fit them through the data.

The quality of the fit of a given set of experimental data by a given model may then be analysed using classical statistical tests. For instance, if the standard deviation σ_{exp} of the experimental measurement process is known, we may use Fisher's test to compare this standard deviation σ_{exp} with the standard deviation of the differences between the experimental data and their corresponding fits. It is also possible to test whether the average of these differences is close to 0 or not (test on averages). Note that the measurements methods can be complex (e.g. infectious titer) and that the experimental standard deviation σ_{exp} is often poorly evaluated.

Some of the models of the "model library" will fail to pass all these statistical tests (for instance: test that the variance of the prediction errors is smaller than σ_{exp}, test that the average of the prediction errors is zero). However, in general, there is not only one model which fits the data, but several ones. The error margins are so large and the number of data so small, that usually many different models may be coherent with the experimental data. Moreover, often, the experimental data are concentrated on areas of low degradations, in particular in the case of marketed products. There are often few data with a high degradation, making it difficult to differentiate between first order and second order kinetics (e.g. the difference between these two models is more important for small concentration, where first order goes faster to 0 than second order). Thus, classical statistical tests like Student test or Fisher test often do not help in the choice of a single model.

To lift the indeterminacy between two or more models, optimal design methods may be used. These methods consist in trying to find the best set of experiments which differentiate between two or more models (see next section). Once the corresponding experiments are fulfilled, the corresponding experimental points may be added, in order to improve the knowledge of the degradation mechanism, and possibly to single out one single model.

In many cases, however, the modeler will have the choice between several models. The mathematical approach by itself is not sufficient, and expertise of biologists or drug designers needs be added. Sometimes, biochemical experts will know that the degradation follows a second order dynamic, or is a polymerization reaction, or has some given characteristics, which will complete the mathematical analysis. The modeler will also rely on its experience to guide his choice, which in many cases will be somehow empirical.

2.5. **Optimal experimental design**

Taking into account the cost and time needed to obtain new experimental data, it is advantageous to use technics of optimal experimental design when we want to accurate predictions or to differentiate between various models.

2.5.1. *Reduction of the variance of a prediction*

To improve a prediction is often crucial, for instance to know whether the confidence interval of a prediction remains within a given domain, and thus whether it is safe or not to administer the drug. One of the common ways to improve a prediction is to decrease its variance, as much as possible. This approach is developed by Vanlier et al. [15] in a Bayesian framework, an approach that we sum up here.

Let y be the list of the current experiments, namely of liste of N triplets time / temperature / experimental data $(t_{i,j}, T_i, \phi_i^{exp})_{1\leq i\leq N}$, and let us add a "simulated" new experiment y_N (simulated by the chosen model), namely a triplet time / temperature / measure (t, T, ϕ), or a list of $N' \geq 1$ new "simulated" experiments. We would like to choose the experimental design (t, T)

of the new experiment in order to minimize the variance of a prediction $z(\theta)$ (for instance the value of ϕ at a given time and given temperature).

Assume that we know the new experiments y_N, including the experimental data ϕ. Then as proved in a study by Vanlier et al. [15], using a Bayesian approach,

$$p(\theta|y, y_N) = Z^{-1} p(\theta|y) p(y_N|\theta)$$

for some normalization constant Z. If we want to make a prediction $z(\theta)$, then its expectation is

$$E(z|y, y_N) = \int P(\theta|y, y_N) z(\theta)\, \mathrm{d}\theta = Z^{-1} \int p(\theta|y) p(y_n|\theta) z(\theta)\, \mathrm{d}\theta.$$

In order to compute this quantity, we introduce a sample $(\theta_j)_{1\leq j\leq M}$ of the probability distribution $p(\theta|y)$. This sample may be for instance obtained through a Metropolis Hastings algorithm. We then have

$$E(z|y, y_N) \sim \frac{\sum_{i=1}^{M} p(y_N|\theta_i) z(\theta_i)}{\sum_{i=1}^{M} p(y_N|\theta_j)}.$$

Note that the experimental data ϕ of the triplet y is not known, therefore we need to average over ϕ, using our present knowledge, namely $p(\theta|y)$. This leads to

$$E(z) \sim \frac{1}{M} \sum_{r=1}^{M} \sum_{i=1}^{M} \frac{G(\theta_i, \theta_r)}{\sum_{k=1}^{M} G(\theta_k, \theta_r)} z(\theta_i)$$

where

$$G(\theta_i, \theta_r) = \exp\left(-\frac{(y(\theta_i) - y(\theta_r))^2}{2\sigma_{exp}^2} \right),$$

where $y(\theta_i)$ is the prediction of the data at the times and temperatures $(t_i, T_i)_{1\leq i\leq N}$ given by the set of triplets y, assuming that θ_i is the set of parameters of the model.

The variance of the prediction is then

$$\mathrm{Var}[z] = E[z^2] - (E[z])^2.$$

The idea is to find the experimental design y_N which minimise the variance of the prediction, which may be done using classical minimisation algorithms (gradient type, or genetic type). Using this approach, it is possible to design new experiments, namely, to find out pairs of time and temperature, so that to minimise the variance of the prediction z.

2.5.2. *Discrimination between two models*

Usually there are relatively few available experimental data, therefore several models may be compatible with the data, and there is no statistical test to rule out all the models but one. Often two or more models appear likely, both from a mathematical point of view (correct fit of the experimental data) and from a biochemical point of view (various possible degradation mechanisms). To single out one model, it is then necessary to design further experiments. We thus use technics from optimal design, or more precisely T-optimal design strategies.

T-optimal design has been introduced in 1975 by Atkinson and Fedorov (see the history in Braess [5]). Braess later introduced a symmetric approach to discriminate between several models, an approach we will now briefly describe.

Assume that we want to discrimine between two models F_1 and F_2. As in the previous section, let us denote the experiments already conducted by y and let us try to add N' new experiments d_{new}, at times and temperatures $(t_j, T_j)_{N+1\leq j\leq N+N'}$, starting from an initial concentration ϕ^0. We may allow repeated experiments or not.

A first asymmetric approach is to assume that the model F_1 is true, and to fix a set of parameters θ_1^b for F_1 (for instance to take the best possible fit of the experimental data by the

first model) and then to take

$$d_{new} = \text{argmin}_d \; J_{1,2}(d) \tag{2.8}$$

where

$$J_{1,2}(d) = \min_{\theta_2} \sum_{j=1}^{N+N'} \Delta_{1,2}(\theta_1^b, \theta_2, j), \tag{2.9}$$

and where

$$\Delta_{1,2}(\theta_1, \theta_2, j) = \Big|\text{Model}_1(\phi^0, T_j, \theta_1, t_j) - \text{Model}_2(\phi^0, T_j, \theta_2, t_j)\Big|^2.$$

Another possibility is to use a Bayesian approach in the choice of θ_1^b, and to define $J_{1,2}$ as

$$J_{1,2}(d) = \min_{\theta_2} \sum_{j=1}^{N+N'} \int \Delta_{1,2}(\theta_1, \theta_2, j) P_1(\theta_1|y)\, \mathrm{d}\theta_1, \tag{2.10}$$

where $P_1(\theta_1|y)$ is the probability for the model 1 to have parameters θ_1 knowing the previous experiments y.

This approach can be generalized by N models $F_1, \ldots, F_N$. Singling out the first model F_1, we may define

$$J_1(d) = \sum_{2 \le l \le N} J_{1,l}(d) \tag{2.11}$$

and search for a design d_{new} which minimizes J_1.

To symmetrize this approach we may introduce non negative numbers $p_{i,j}$ with $\sum_{i<j} p_{i,j} = 1$ and define

$$J_{sym}(d) = \sum_{1 \le l_1 < l_2 \le N} p_{l_1,l_2} J_{l_1,l_2}(d). \tag{2.12}$$

The numbers p_{l_1,l_2} may be interpreted as the relative importance we set on model l_1 with respect to model l_2. A reasonable starting point is to take all the $p_{i,j}$ equal.

2.6. **Use of the model**

Once one model is selected and fitted, it is possible to predict the concentration of the vaccine at any arbitrary times and temperatures, assuming an arbitrary initial concentration. It is also possible to predict the degradation if the temperature depends on time and follows an arbitrary excursion profile.

A first application is the control of the quality of an industrial production. Experiments may be done on a sample of the production, which may be heated at various temperatures. These experiments may be compared to the prediction of the model, thanks to classical statistical tests (Fisher, Student, etc.). These tests will indicate whether the degradation of the sample departs from the normal one, which would indicates an abnormal production.

Similarly, it is possible to compare different samples to investigate whether they follow the same degradation process or not. For this, we first model one of the samples, and then compare the outputs of this model with the results of the second sample, as in the previous paragraph. This approach may be used for instance when an excipient is changed to predict whether this change of formulation has an impact on the degradation rate.

The model may also be used to predict the evolution of a vaccine which undergoes a thermal excursion (last mile problem). Using an arbitrary time/temperature excursion profile, or data from an actual excursion, it is possible to predict the evolution of the vaccine with time, and in particular to predict whether the concentration at administration time is above or below the expected vaccine dose range.

3. Example: model of the "Typhim Vi" vaccine

3.1. Industrial context

The industrial context is a little peculiar, since in general, mathematical modeling will not be sufficient to single out one model. It is often necessary to add informations coming from biochemistry and from the empirical knowledge of vaccine specialists. Consequently the mathematical prediction cannot be completely taken as granted.

Predictions and computations must thus always be interpreted by the end users which usually are biologists or chemists. It is therefore crucial to provide them with quality indicators of the modelling process. These qualitative indicators must prevent them to blindly trust the computations and rather help them to assess the quality of their experimental data, their number and the number of different temperatures taken into account.

This leads to the empirical notion of "level of confidence". If a single model satisfies all the statistical tests, is coherent with what we know of the biochemical degradation process and if the parameters values of the model are coherent with known empirical values for these parameters (e.g. E_a should be between 10 and 500 kJ/mol), then it may be used to predict complex thermal excursions or its behavior over a long period of time or at high temperature. If several different models go through all the tests, then it is reasonable to trust their predictions as long as they remain close (which will probably not be the case over a longer period of time or for high temperature), but of course not when they are different. In this later case, optimal experimental design technics must be used to try to single out only one model, and to gain confidence in it.

3.2. Application to the "Typhim Vi" vaccine

We will now illustrate this approach on the "Tyhpim Vi" vaccine, a vaccine against typhoid fever developed by Sanofi Vaccines, a French pharmaceutical company.

3.2.1. *Modelling*

The available experimental raw data are shown in Figure 3.1. It includes data on quality attributes of typhoid vaccine after storage at 5 degrees for longer time, i.e., up to 3 years, and data at higher temperatures, namely 25 and 37 degrees only for small times (a few weeks). These sets of data were requested by the regulatory agencies. The figure 3.2 shows how various models (order 1, order 2, order n, parallel reactions, Finke Watsky, Sestak) fit this set of data. The distances $J(\theta_b)$ of the various models are comparable, between 0.10213 for the order 2 and 0.11977 for Sestak. As a consequence, Fisher test does not reject any of these models. Modellers often use the BIC (Bayesian Information Criterion) to reject models. The BIC is defined by

$$\mathrm{BIC} = -2\log(L) + k\log(N)$$

where L is the likelihood (namely $J(\theta_b)$), N the number of data and k the number of parameters of the model. It is often assumed [7] that the models should be chosen amongst the smaller BIC, and if the BIC of two models differ by more than 4 then the model with the highest BIC can be rejected (this criterion is of course purely empirical, see for instance [7]).

In our cases, the various BIC range from -63.6 for the order 2, to -47 for the parallel reactions. Using the "rule of 4", we may forget Finke Watsky, Sestak and parallel reactions. It only remains order 1, order 2 and order n. The order n models gives an exponent $n = 0.16$.

In order to discriminate between these three models, we followed the strategy detailed in Section 2.5.2. We looked for the best possible experiments to be done within two weeks, using possible temperatures 5, 25, 37, 65 and 80 degrees Celsius. The algorithm indicates that we should do experiments at temperatures 65 and 80 degrees Celsius as late as possible. We thus set up experiments at these two temperatures, leading to the experimental data of Figure 3.3.

Figure 3.4 shows the fits of this new enlarged set of data by the various models. Order 2 and order n achieve similar best distances $J(\theta_b)$, namely 0.128, and the exponent of order n is close to 2. Order 1 is close, but with a slightly larger distance. We therefore choose the order 2 model (fit shown on Figure 3.5) which was described for saponification reaction known as the polysaccharide degradation mechanism [11].

3.2.2. *Shelf life*

Let us imagine that we want to predict the concentration of vaccine after 5 years at 5 degrees Celsius. We assume that the experimental data suffer an error of 20 percents. Then, using the Bayesian approach, we get a 95 percents confidence interval of this prediction of $[0.1038, 1.06]$. To decrease this confidence interval we followed the strategy of Section 2.5.1. If we want to get the result before one year, using temperatures 5, 25, 37, 65 and 80, and using only ten experiments, then the algorithm indicates that we should do experiments at 80 degrees Celsius at days 345, 350, 355, 360, 365, at day 5 for temperatures 5, 25 and 37 degrees Celsius and at day 365 for temperatures 5 and 25 degrees Celsius. The optimal experimental design is not obvious and combines information at high temperatures and long times, at low temperatures and long times and initial data (these latest data allowing to precise the initial concentration ϕ_0). After all these experiments the standard deviation of the variance is predicted to decay by 25 percents.

3.2.3. *Application to quality control*

This modelling process has been used as an element of proof by the Sanofi company to be authorised by the FDA to improve the industrial process of vaccine production without any impact on product stability. More precisely, the degradation of vaccines obtained by the initially approved industrial process has been modelled using the current approach. Then the degradation of vaccines obtained by a modified industrial process was compared to the prediction of the previous model. The true degradation and the predicted ones appeared close, which gave a theoretical argument to demonstrate that the change of the industrial process does not alter the degradation rate. Such a theoretical argument has been taken into consideration by the FDA to authorise the change of the industrial process (Figure 3.6).

3.2.4. *The last mile problem*

We now illustrate the application of the current approach on the last mile problem. Using the model, it is possible to predict the evolution of the vaccine concentration during a thermal excursion, as illustrated in Figure 3.7 for an arbitrary thermal excursion profile.

During transport, it is possible to measure on a regular basis the temperature, which provides the exact time and temperature data when a thermal excursion occurs. At the time of injection, we therefore know the complete thermal history of a given vaccine. The mathematical model allows to predict the vaccine concentrations, together with an uncertainty interval, and therefore to evaluate whether the vaccine can still be administered or not or not. This application is of course of considerable interest to monitor vaccination in areas which are difficult to reach.

Acknowledgements

The authors would like acknowledge Manojkumar Patel from the Sanofi Global Hub for editorial assistance.

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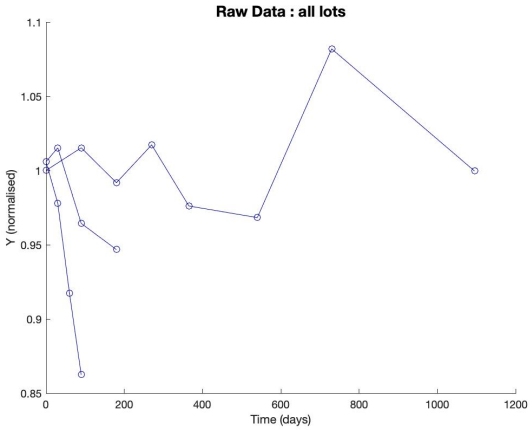

Figure 3.1. Experimental raw data of the O-acetyl content (typhoid vaccine quality attribute measurement - see [6] for experimental details) are presented as the percent of the initial titer at 5° C. These raw data were acquired after isothermal storage (5, 25, or 37° C) at different storage time.

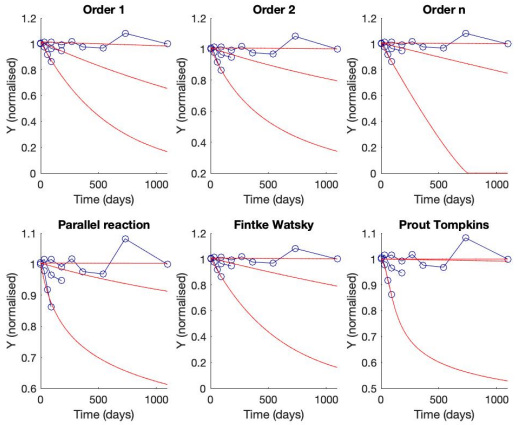

Figure 3.2. Experimental O- Acetyl - Typhim stability data (blue circles and lines) are adjusted to different models (red lines). Good fits for the experimental data are obtained for Fintke Watsky model.

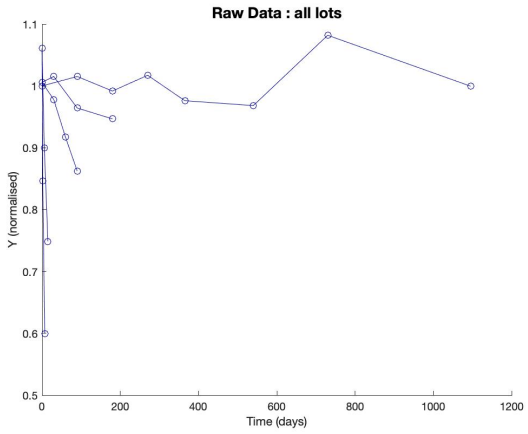

Figure 3.3. Data from the initial experimental design (ICH protocol [1]) are completed experimentally by new data generated using optimal design of simulated experiments (at higher temperatures - 65°C and 80°C) in order to improve the quality of the data set.

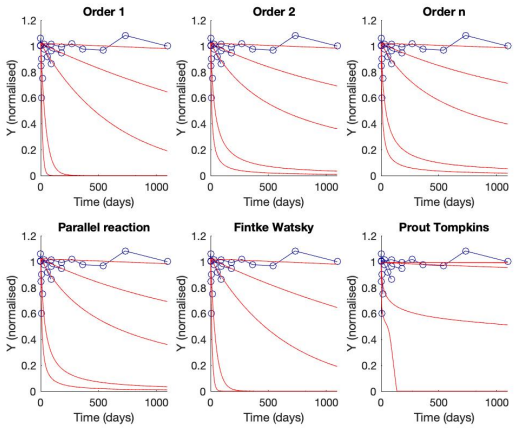

Figure 3.4. Experimental O-Acetyl - Typhim stability data (blue circle and line) generated using optimal design of simulated experiments, are fitted by different models (red lines).

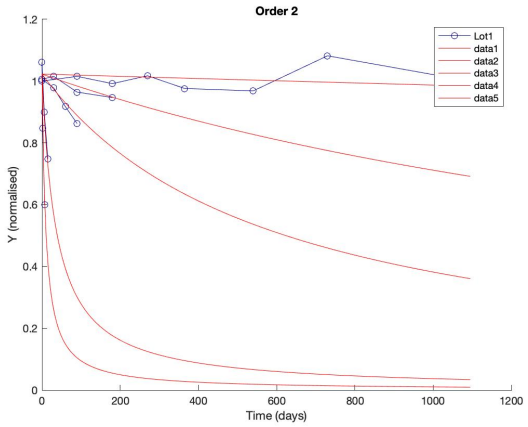

FIGURE 3.5. With the experimental application of the optimized design of experiments (65° C and 80°C), the order 2 model was discriminated unambiguously, and the n value of the n^{th} order model is 2.00.

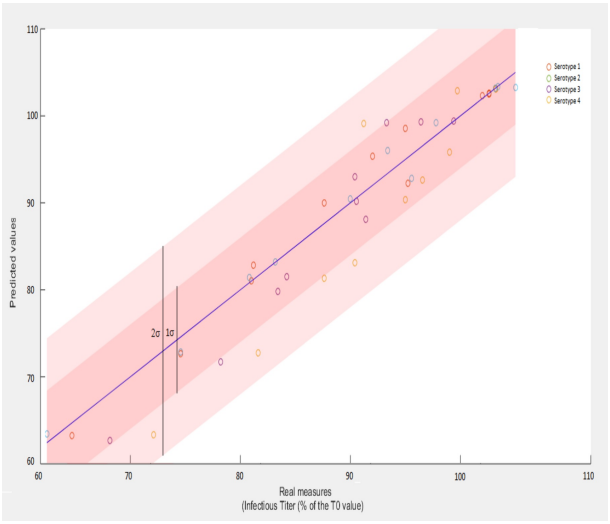

FIGURE 3.6. Typhoid vaccine batch comparison. To verify the potential impact of process modification on process stability, a modeling and batch comparison statistical approach was performed. Based on the parameter values of the selected model to calculate the predictive data, it was shown that all the experimental data are included within $2\sigma_{exp}$ of the model predictions, with a distribution well equilibrated around the diagonal, which indicates batches homogeneity before (batches 1, 2, 3) and after (batches 4, 5 and 6) process changes.

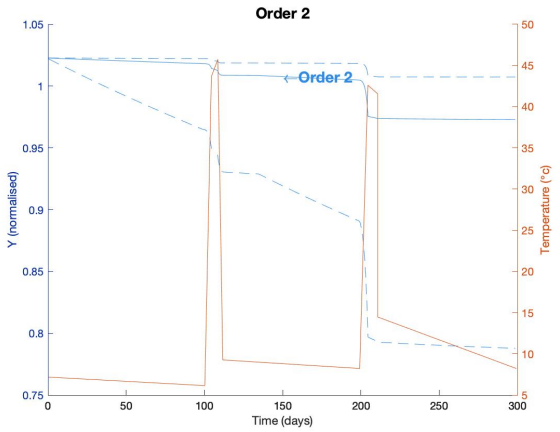

Figure 3.7. Prediction of a thermal excursion (arbitrarily temperature profile, with two "accidents" at days 100 and 200). The experimental data are normalized by the initial concentration of the best fit of the model. Because of experimental errors, normalized experimental data may be larger than 1. Confidence interval of the predictions in dotted lines.