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Short communication

# In-use stability modeling

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ABSTRACT

Experimental design and modeling of in-use stability testing are presented in this paper. In-use open container degradation is considered in terms of time open container or/and the number of instances that the same container is used. Degradation is estimated based on two models, the fixed and the general model. The fixed model estimates in-use degradation for those fixed time points of closed container where the in-used experimental data is collected. The general model estimates in-use degradation for any time point of closed container using the estimated relationship between closed container time and the degradation rate of open container. Data for in-use open container stability does not have to be collected at a closed container time of interest to estimate in-use degradation at this time point as long as this point is within the range of the experiment. Stability of the product in terms of drift from the initial time to the time of interest is calculated as the sum of closed and in-use open containers drifts.



## 1. Introduction

Stability of products is the length of time that they retain their properties and functionality while stored or handled as defined by the manufacture's specifications. During their life span products may change as they age but they are considered to be stable as long as their characteristics remain with the specifications. The change of the performance as products age is called degradation and is usually defined in terms of loss of activity or/and decrease of performance [1–3].

Stability encompasses several stages of product life; i.e., time and events during transportation of products from manufacturer to the end user, the length of time that products are stored at recommended conditions without being used, time and events while products are being used. The last stage is referred to as in-use stability [2]. The above stages may not be all inclusive and all products may not go through all them. There are products that are designed for a single use while others are stored in containers that can be used for a period of time after it is opened. The later group of products and in-use stability will be the focus of this paper.

The kinetics of the chemical reaction of degradation of in-use stability is affected by the same factors of a conventional stability testing. Products usually degrade faster when they are subjected to elevated stress conditions like temperature, humidity, radiation, etc. [4,5]. However, the open container can be more sensitive to the external factors particularly the number of times that a particular container is used. Age of the product is another factor that might affect the degradation rate of the product in the open container. Usually, older products tend to degrade faster when in use.

Stability testing is described in literature mainly in terms of degradation as a function of product age while product is not in use. These are called real time stability tests. Elevated temperatures and humidity are used to accelerate degradation and predict stability at recommended storage conditions by using pre-defined relationships [6–10]. Degradation in real time stability tests is estimated from the stability model as the amount of drift from initial time to stability time [2,6,11]. Confidence interval for the drift is also calculated and both drift and the upper limit of the confidence interval are compared to the specification. Product meets the stability claim when the upper limit of the drift is smaller than the specification.

Experimental designs and statistical modeling of stability data are well publicized [5,8,9] but we are not aware of any paper addressing design and statistical modeling of in-use stability. Thus, the objective of this paper is to provide testing designs and models to estimate in-use stability and use experimental data to demonstrate the utility of the models.

Throughout the paper we will used the terms, 'closed' to refer to the time of stability in an unused container, 'open' to refer to time of in-use stability, and 'event' to refer to the number of instances when a product in an open container is used. Both closed and open are expressed in time units (days, hours, etc.) while event as number.

## 2. Experiments

Data from experiments designed to evaluate the stability of a hematology cell control reagent are used for illustration purposes.

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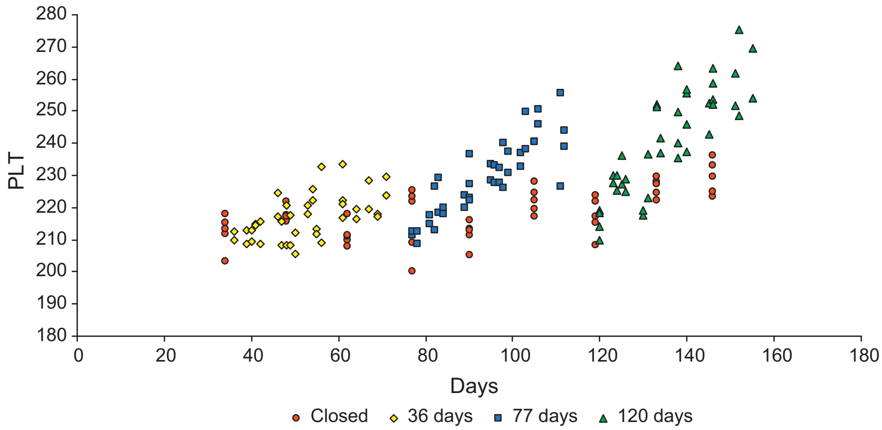


**Fig. 1.** Scatter plot of closed and open container stability experiment.

Vials of the reagent were stored at recommended storage conditions and tested on a hematology analyzer in 5 replicates at 34, 48, 62, 77, 90, 105, 120, 133, and 146 days after manufacturing. A new vial is opened at each testing time point. This constitutes real time closed container stability testing of the product. The in-use stability testing was performed at three different time points of product's life, 36, 77, and 120 days after manufacturing. Vials were open at these time points and tested 20 times in duplicates for a maximum of 35 days.

Number of platelets ($\times 10^3$ cells/$\mu$L) is the measurand in the tests. Scatter plot of closed and open container results are shown in Fig. 1. Let us denote with $t_c$ time of closed container stability ($c$ is the number of closed container time points), $t_o$ is time of in-use open container stability (o is the number of open container time points), and $e$ events ($n$ is the number of instances that the same vial/container is used). Let us also assume that the degradation patterns for both real time and in-use stability are linear.

## 3. Closed container degradation

Model for closed container stability is expressed as,

$$Y_i = \alpha_0 + \alpha_1 t_{ci} + \varepsilon_i \tag{1}$$

where, $Y_i$ is the measurand ($1 \le i \le c$), $\alpha_0$ is the result at time zero, $t_{ci}$ is each time of closed container, $\alpha_1$ is degradation rate, and $(_i$ is the experimental error considered to be normal, independent and identically distributed. Least squares or other methods can be used to estimate parameters of the model and their standard errors [12].

Drift at a target time is calculated as,

$$D_c = \alpha_1 t_{ct} \tag{2}$$

Standard error of the drift is,

$$\mathrm{Se}(D_c) = \mathrm{Se}(\alpha_1) t_{ct} \tag{3}$$

where $t_{ct}$ is the target time and $\mathrm{Se}(\alpha_1)$ is the standard error of the degradation rate. The following parameters, $\alpha_0$, $(_i$, $D_c$, $\mathrm{Se}(D_c)$ are measured as platelet cells/$\mu$L $\times 10^3$, $t_{ci}$ and $t_{ct}$ are number of days, while $\alpha_1$ and $\mathrm{Se}(\alpha_1)$ can be expressed as the change of platelet cells/$\mu$L $\times 10^3$ per day.

Closed container degradation statistics estimates, their standard errors (SE), lower and upper confidence intervals are presented in Table 1. Intervals for all estimates are calculated based on 95% confidence. Degradation rate is statistically greater than zero ($p < 0.001$, $H_0$: $\alpha_1 = 0$) indicating for the presence of a significant degradation during the 146 days of closed container testing. Drift at $t_{ct} = 120$ days is estimated to be $15.96 \times 10^3$ cells/$\mu$L.

## 4. In-use open container fixed model degradation

### 4.1. Model description

Sometimes in-use stability is assessed at only one point of product closed container life. Usually this time point is chosen to be by the end of stability. In this paper we will refer to the statistical model that will describe this degradation pattern as the fixed model since assessment is going to be based on the fixed closed container time that is tested in the experiment.

Degradation of in-use stability can be expressed as a function of open container time (4), number of events (5) or/and both time and events (6).

$$Y_i = \alpha_0 + \alpha_1 t_{ct} + \beta_o t_{oi} + \delta_i \tag{4}$$

$$Y_i = \alpha_0 + \alpha_1 t_{ct} + \beta_e e_i + \delta_i \tag{5}$$

$$Y_i = \alpha_0 + \alpha_1 t_{ct} + \beta_{oe} t_{oi} e_i + \delta_i \tag{6}$$

where $\beta_o$, $\beta_e$, $\beta_{oe}$ are the degradation rates of in-use product, $t_{oi}$ is each time of open container, $\delta_i$ is the experimental error, $o = n$, and $1 \le i \le n$. In-use degradation of $Y_i$ depends on the degradation of closed container for the fixed length of time $t_{ct}$ where the experimental open container data are collected.

Drifts at a target time, event or both are calculated as,

$$D_o = \beta_o t_{ot} \tag{7}$$

$$D_e = \beta_e e_t \tag{8}$$

$$D_{oe} = \beta_{oe} t_{ot} e_t \tag{9}$$

where $t_{ot}$, and $e_t$ are the target time and number of events. Standard errors of the drifts are,

$$\mathrm{Se}(D_o) = \mathrm{Se}(\beta_o) t_{ot} \tag{10}$$

$$\mathrm{Se}(D_e) = \mathrm{Se}(\beta_e) e_t \tag{11}$$

$$\mathrm{Se}(D_{oe}) = \mathrm{Se}(\beta_{oe}) t_{ot} e_t \tag{12}$$

where $\mathrm{Se}(\beta_o)$, $\mathrm{Se}(\beta_e)$, and $\mathrm{Se}(\beta_{oe})$ are the standard errors of the respective degradation rates.

**Table 1**
Closed container degradation statistics.

| Parameter | Estimate | SE | Lower | Upper |
|---|---|---|---|---|
| Intercept ($\alpha_0$) | 206.27 | 2.47 | 201.28 | 211.26 |
| Degradation rate ($\alpha_1$) | 0.13 | 0.0254 | 0.08 | 0.18 |
| Drift at 120 days | 15.96 | 3.05 | 9.82 | 22.10 |

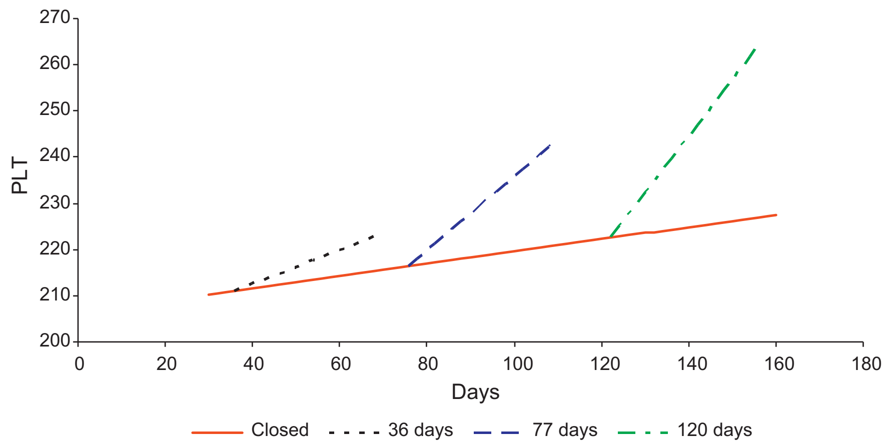


**Fig. 2.** Degradation pattern of closed container and in-use open container.

Drifts parameters, their standard deviations, and $\delta_i$ are measured as platelet cells/μL × $10^3$, while degradations rates and their standard deviations can be expressed as the change of platelet cells/μL × $10^3$ per day.

### 4.2. Experimental results

Estimated degradation patterns of PLT (platelet cells/μL × $10^3$) closed container and in-use open container at three closed times are shown in Fig. 2. Degradation statistics for in-use open container for the three scenarios, time, events, and both time and events are presented in Table 2. All degradation rates are statistically significant ($p$-values <0.001) indicating for a significant degradation during these phases.

Drift of in-use stability depends on the age of closed container. Regardless of the way (time, events, time & events) drift is estimated, its magnitude increases as closed container product gets older. Drift of in-use open container for $t_{ot}$ = 35 days and $e_t$ = 20 events is 15.32 × $10^3$ cells/μL at $t_{ct}$ = 36 in comparison to 34.56 × $10^3$ cells/μL at $t_{ct}$ = 77 and 50.81 × $10^3$ cells/μL at $t_{ct}$ = 120. The 95% confidence intervals of these three drift estimates do not overlap providing additional evidence that they are statistically different from each other (Table 2).

Drift estimate for the time of open container ($t_{ot}$ = 35 days) is greater than the drift estimate for the number of events ($e_t$ = 20 events) when they are considered independently. This indicates that the length of time of open container contributes more to the in-use degradation in comparison to the number of events. Time of open container and events are highly correlated with each other but when considered together multiplicatively the degradation accelerated in comparison to their separate effects. The estimated platelet drift for the multiple effects of time and events is 50.81 × $10^3$ cells/μL at $t_{ot}$ = 120 days. This drift is 8.04 × $10^3$ cells/μL greater than the drift for the effect of time of open container and 11.99 × $10^3$ cells/μL greater than the drift of the effect of number of events.

In-use degradation drift of open container for 35 days and 20 events is greater that the drift of closed container for 120 days. This evidenced the fact that the majority of degradation occurs during the period that the product is in use.

## 5. In-use open container general model degradation

### 5.1. Model description

Estimation of degradation can be more accurate when in-use open container data are collected at different times of closed container during the product stability span. An example of this scenario is the stability testing of a hematology cell control reagent described in Section 2. In this case data can be modeled to estimate degradation and drift for any combination of closed container time and in-use open container time and events regardless if they where tested experimentally or not. We are referring to the statistical model for this data as the general model. The underlying idea of

**Table 2**
Fixed model for open container in-use degradation statistics for 20 events or/and during 35 days after 36, 77, and 120 days of closed container storage.

| Days of closed container | Open container | Parameters | Estimate | SE | Lower | Upper |
|---|---|---|---|---|---|---|
| 36 | Days of open container | Degradation rate ($\beta_o$) | 0.3668 | 0.0462 | 0.2734 | 0.4603 |
| | | Drift | 12.83 | 1.62 | 9.57 | 16.11 |
| | Events during open container | Degradation rate ($\beta_e$) | 0.5873 | 0.0758 | 0.4340 | 0.7406 |
| | | Drift | 11.74 | 1.52 | 8.68 | 14.81 |
| | Days and events | Degradation rate ($\beta_{oe}$) | 0.0218 | 0.0029 | 0.0159 | 0.0279 |
| | | Drift | 15.32 | 2.06 | 11.15 | 19.49 |
| 77 | Days of open container | Degradation rate ($\beta_o$) | 0.8103 | 0.0483 | 0.7126 | 0.9079 |
| | | Drift | 28.36 | 1.69 | 24.94 | 31.78 |
| | Events during open container | Degradation rate ($\beta_e$) | 1.3427 | 0.0819 | 1.1773 | 1.5082 |
| | | Drift | 26.85 | 1.64 | 23.55 | 30.16 |
| | Days and events | Degradation rate ($\beta_{oe}$) | 0.0494 | 0.0034 | 0.0426 | 0.0562 |
| | | Drift | 34.56 | 2.35 | 29.81 | 39.31 |
| 120 | Days of open container | Degradation rate ($\beta_o$) | 1.2221 | 0.0716 | 1.0774 | 1.3669 |
| | | Drift | 42.77 | 2.51 | 37.71 | 47.84 |
| | Events during open container | Degradation rate ($\beta_e$) | 1.9413 | 0.1179 | 1.1773 | 1.5082 |
| | | Drift | 38.82 | 2.36 | 34.06 | 43.59 |
| | Days and events | Degradation rate ($\beta_{oe}$) | 0.0726 | 0.0052 | 0.0622 | 0.0830 |
| | | Drift | 50.81 | 3.61 | 43.52 | 58.11 |

**Table 3**
General model for open container in-use degradation statistics for 20 events or/and during 35 days after 120 days closed container storage.

| Open container | Parameter | Estimate | SE | Lower | Upper |
|---|---|---|---|---|---|
| Days of open container | $\gamma_{o1}$ | 0.2804 | 0.0404 | 0.2005 | 0.3604 |
| | $\gamma_{o2}$ | 0.0124 | 0.0014 | 0.0097 | 0.0151 |
| | Drift | 43.64 | 1.97 | 39.74 | 47.54 |
| Events of open container | $\gamma_{e1}$ | 0.4672 | 0.0689 | 0.3308 | 0.6036 |
| | $\gamma_{e2}$ | 0.0121 | 0.0014 | 0.0093 | 0.0148 |
| | Drift | 39.75 | 1.85 | 36.09 | 43.42 |
| Days and events | $\gamma_{oe1}$ | 0.0172 | 0.0028 | 0.0115 | 0.0228 |
| | $\gamma_{oe2}$ | 0.0122 | 0.0016 | 0.0091 | 0.0153 |
| | Drift | 51.97 | 2.72 | 46.57 | 57.36 |

the general model is that the degradation of in-use open container depends on the age (closed container time) of the product. In addition, we assume that the degradation rate of in-use open container is exponentially related to the closed container time.

Let express in-use degradation rates of the effect of open container time, number of events and both time and events as,

$$\beta_{oi} = \gamma_{o1} \quad \exp(\gamma_{o2} t_{ci}) \tag{13}$$

$$\beta_{ei} = \gamma_{e1} \quad \exp(\gamma_{o2} t_{ci}) \tag{14}$$

$$\beta_{oei} = \gamma_{oe1} \quad \exp(\gamma_{oe2} t_{ci}) \tag{15}$$

where $\gamma_{o1}, \gamma_{o2}, \gamma_{e1}, \gamma_{e2}, \gamma_{oe1}, \gamma_{oe2}$ are the parameters of the models to estimate expressed as functions of the change of platelet cells/μL × $10^3$ per day, while a $(1 \leq j \leq a)$ is the number of open container testing during the closed life of the product.

Degradation of in-use open container can be estimated using models (4)–(6), drifts can be estimated using models (7)–(9), and standard error using models (10)–(12) for a any $t_{ct}$ in the range of the experiment.

### 5.2. Experimental results

All data collected in tests described in Section 2 and shown graphically in Fig. 1 are analyzed according to the general model. Since models (13)–(15) are not linear, special numerical iterative techniques using non-linear least squares or maximum likelihood are needed to estimate the parameters. We used PROC NLMIXED (procedure for fitting non linear mixed models) of SAS 9.2 for estimation [13,14].

Estimates of parameters of (13)–(15) and drifts for $t_{ct}$ = 120 days, $t_{ot}$ = 35 days and $e_t$ = 20 events are presented in Table 3. We choose to use the same criteria target criteria ($t_{ct}$ = 120 days, $t_{ot}$ = 35 days and $e_t$ = 20) as in the fixed model in order to compare the two models and validate the approach of the general model. Drift estimates for days, events and days & events for the general model are very close to the estimates of the fixed model. Their respective confidence intervals overlap indicating that the estimates of fixed and general model are not statistically different.

Estimates of closed and open container drifts for different closed container not tested in the experiment are shown in Table 4. The in-use open container drift is based on both number of days and events. The results of the general model support the same trend observed in the results of the fixed model that the magnitude of in-use drift increases as product ages in closed container.

## 6. Evaluation of stability

Stability drift can be estimated as the sum of drifts of closed container and in-use open container. Drift of in-use open container when time and events effects are multiplicative will be used in the following equations. Stability of separate effects of time of open container and number of events can be treated similarly.

**Table 4**
General model for open container in-use degradation statistics for 20 events during 35 days after different days closed container storage not tested in the experiment.

| Days | Parameter | Estimate | SE | Lower | Upper |
|---|---|---|---|---|---|
| 110 | $D_c$ | 14.63 | 2.79 | 9.00 | 20.26 |
| | $\gamma_{oe1}$ | 0.0172 | 0.0028 | 0.0115 | 0.0228 |
| | $\gamma_{oe2}$ | 0.0122 | 0.0016 | 0.0091 | 0.0153 |
| | $D_{oe}$ | 46.00 | 2.12 | 41.81 | 50.19 |
| 100 | $D_c$ | 13.3 | 2.54 | 8.18 | 18.42 |
| | $\gamma_{oe1}$ | 0.0172 | 0.0028 | 0.0115 | 0.0228 |
| | $\gamma_{oe2}$ | 0.0122 | 0.0016 | 0.0091 | 0.0153 |
| | $D_{oe}$ | 40.71 | 1.81 | 37.13 | 44.30 |
| 90 | $D_c$ | 11.97 | 2.29 | 7.37 | 16.57 |
| | $\gamma_{oe1}$ | 0.0172 | 0.0028 | 0.0115 | 0.0228 |
| | $\gamma_{oe2}$ | 0.0122 | 0.0016 | 0.0091 | 0.0153 |
| | $D_{oe}$ | 36.04 | 1.74 | 32.60 | 39.47 |

$$D_s = D_c + D_o \tag{16}$$

$$\text{Se}(D_s) = \sqrt{\frac{\text{Se}(D_c)df_c + \text{Se}(D_{oe})df_{oe}}{df_c + df_{oe}}} \tag{17}$$

where $D_s$ is the total stability drift, Se($D_s$) is the standard error of this drift, while $df_c$ and $df_{oe}$ are the degrees of freedom of the closed and open container drifts.

Drift estimates for $t_{ot}$ = 35 days and $e_t$ = 20 events, and different days of closed container are presented in Table 5. As in the case of in-use degradation, the estimated stability drifts for fixed and general model for $t_{ct}$ = 120 days are very close and their confidence intervals overlap and the amount of stability drift is reduced as time of closed container decreases.

## 7. Discussions

In this paper we are modeling the in-use stability of products once the container is opened. Product may have other types of in-use stability claims that we are not considering here [2].

In-use stability experiments are usually performed at the same time as the closed container real time stability. The decision on the elements of experimental designs, i.e. number of time points/events number of replicates, etc. are based on the same principles as for the closed container testing [8–10]. However, the decision on the number of open container testing during the closed container life depends on the type of product and purpose of the stability test. Confirmatory stability tests require in-use testing at only one time point of closed container. This time point is decided based on the predetermined specification of the closed and in-use stability. You may also test at only one time point of closed container when the degradation rate of in-use stability does not depend on the age of the closed container. This category of products usually degrade very little (negligible) in closed container but accelerate their degradation significantly once the container is opened.

The fixed model in-use open container stability can be used to analyze data from such experiments. This model enables the esti-

**Table 5**
Stability drifts for 20 events during 35 days of in-use open container after different number of days of closed container.

| Model | Days of closed container | Drift | SE | Lower | Upper |
|---|---|---|---|---|---|
| Fixed | 120 | 66.77 | 3.32 | 60.16 | 73.38 |
| General | 120 | 67.93 | 2.90 | 62.16 | 73.69 |
| | 110 | 60.66 | 2.50 | 55.63 | 65.69 |
| | 100 | 54.04 | 2.38 | 49.24 | 58.84 |
| | 90 | 48.03 | 2.36 | 43.29 | 52.78 |

mation of drifts for that fixed closed container time where the experimental data are collected. To estimate drift for a different closed container time when no in-use open container data are collected you need to assume that the degradation rate of open container is constant and independent on the age of the product in closed container. Further on, you may also need to assume that the closed container time point(s) of interest are very close to the experimental closed container time point so that degradation rates are not significantly different from each other. Consequently, based on these assumptions, the open container degradation estimated from the fixed closed time can be extrapolated for other closed times of interest.

The above assumptions are not necessary when the general model is implemented since the degradation rate of the in-use product in an open container can be estimated from the data at any time point of closed container using the estimated relationship between closed container time and the degradation rate of open container. At least three open container tests during the closed container life are needed to accurately evaluate in-use stability of a product. The closed container time points to start testing in-use container stability should be far apart from each other to be able to discern differences between the degradation rates of open container. This depends on the type of product, the uncertainty of measuring device, as well as the number of replicates [8]. The general model is flexible and provides drift estimates for any combination of closed and open container time and number of events. Data for in-use open container stability does not have to be collected at a closed container time of interest to estimate in-use degradation at this time point as long as this point is within the range of the experiment. Stability of closed or/and in-use container can also be predicted based on a predetermined tolerable drift.

In this paper we are only considering the linear model to describe the pattern of degradation in both closed and in-use container. Products that degrade relatively fast usually exhibit an exponential pattern. The basic approach of fixed and general models is the same regardless the degradation pattern but the functions of the models as well the estimation methodology will be different to accommodate for this pattern.

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