On the Expected Behavior of the Coefficient of Variation with an Inverted Response Curve

The purpose of this white paper is to examine the behavior of the coefficient of variation with an inverted response curve. The coefficient of variation of interest pertains to the uncertainty of the estimated concentration. This behavior is a function of: (1) the measurement noise (e.g. camera noise, spatial and temporal variability in strip behavior during a test flow, …); (2) the uncertainty in the concentration; and (3) the functional shape of the response curve itself.

The Inverted Response Curve

In this discussion, an inverted response curve is one in which the measured system response is inversely related to the input signal e.g. concentration. That is, the higher the concentration, the smaller the response. The following figure shows two notional examples of an "inverted" response curve.

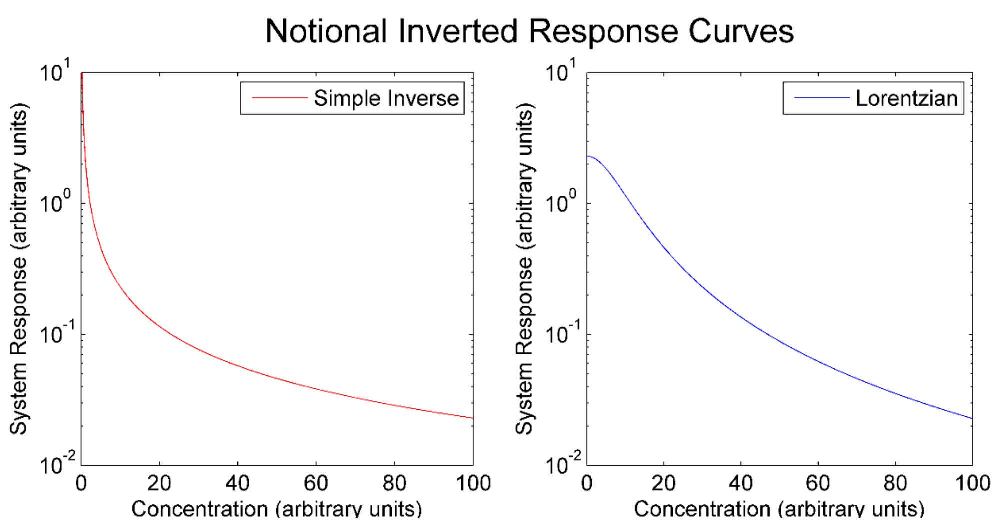

Figure 1: Notional inverted response curves – left panel illustrates a simple inverse relationship, while the right panel illustrates a more realistic Lorentzian relationship.

The simple inverse response curve shown in the left panel above, while “simple”, is in general unrealistic because of the divergence towards infinity as the concentration approaches zero. On the other hand, the Lorentzian response curve shown in the right panel above is well-behaved at both the zero concentration and large concentration limits. Indeed, the Lorentzian curve flattens to zero slope as the concentration approaches zero. This would be consistent for example with a “saturation” behavior in the measured signal. These notional curves will be made more explicit in the following discussion.

A Simple Measurement Noise Model

The coefficient of variation of the estimated concentration depends on a measurement noise model as well as the particular shape of the response curve. For illustration, we consider here a noise model consisting of:

1. α_0 – A constant measurement noise floor due to intrinsic variability in the measurement process. This may include such things as sensor “read” noise and image compression artifacts among others. The essential characteristic is that these noise sources are independent of the measured signal level.

2. $\alpha_1 \cdot S$ – A signal-proportional noise source, where S is the measurement signal. This may include such things as photon “shot” noise, variability of the density of binding sites in the test region of a lateral flow assay strip in the transverse-flow-direction due to variability in the manufacturing process, or variability of the flow itself in the transverse-flow-direction.

3. $\frac{\partial S}{\partial C} \cdot \Delta C$ – Intrinsic uncertainty in the sample concentration, where the derivative is the slope of the response curve, and ΔC is the uncertainty in the concentration. If we

assume this uncertainty is expressed as a constant relative value, we may write $\Delta C = \alpha_2 \cdot C$.

Given these noise components, and the assumption that they are uncorrelated, the total measurement noise is written as the quadrature sum

$$\Delta S = \left(\alpha_0^{\ 2} + \left(\alpha_1 \cdot S \right)^2 + \left(\frac{\partial S}{\partial C} \cdot \alpha_2 \cdot C \right)^2 \right)^{1/2} \tag{1}$$

The Coefficient of Variation (*CV*) of the Estimated Concentration

Using a model for the measurement error as above, the estimated error in concentration is derived using the slope of the response curve as

$$\Delta C = \Delta S \cdot \frac{\partial C}{\partial S} = \Delta S \Big/ \frac{\partial S}{\partial C} \tag{2}$$

(where the last step follows from the chain-rule of differentiation on invertible functions). The coefficient of variation is then simply

$$CV = \frac{1}{C} \cdot \left| \Delta C \right| = \frac{1}{C} \cdot \left| \Delta S \Big/ \frac{\partial S}{\partial C} \right| \tag{3}$$

Substituting the measurement error from expression (1) into (3) and rearranging terms yields

$$CV = \left(\frac{\alpha_0^{\ 2} + \alpha_1^{\ 2} \cdot S^2}{\left(C \cdot \frac{\partial S}{\partial C} \right)^2} + \alpha_2^{\ 2} \right)^{1/2} \tag{4}$$

which shows the explicit dependence of *CV* on the measurement S and the slope $\partial S / \partial C$.

Coefficient of Variation for the Simple Inverse Response Curve

While not realistic, the simple inverse response curve is useful as a pedagogical example. Qualitatively, it is not quite clear how expression (4) behaves in the limit as the concentration goes to zero. In this case, the slope of the response curve approaches infinity, but it is always multiplied by the concentration itself, which approaches zero. So, for example, does the denominator in the first term in expression (4) approach zero or infinity? To answer this question, an explicit calculation of the slope of the response curve must be made.

Let the simple inverse response curve be defined as

$$S(C) \equiv \frac{S_1}{C} \quad \rightarrow \quad \frac{\partial S}{\partial C} = \frac{-S_1}{C^2} \tag{5}$$

where S_1 is a constant and the slope is computed as shown. Substitution of the response curve slope from (5) into expression (4) yields the remarkably simple(!) result

$$CV = \left(\frac{\alpha_0{}^2}{S^2} + \alpha_1{}^2 + \alpha_2{}^2 \right)^{1/2} \tag{6}$$

Therefore, for the simple inverse response curve, we find that in the limit as the concentration approaches zero and the measured signal S approaches infinity, the CV approaches the constant value

$$\lim_{C \to 0} CV = \sqrt{\alpha_1{}^2 + \alpha_2{}^2} \tag{7}$$

On the other hand, in the limit as the concentration becomes large and the measured signal S approaches zero, expression (6) yields

$$\lim_{C \to \infty} CV = \frac{\alpha_0}{S} = \frac{\alpha_0}{S_1} \cdot C \tag{8}$$

and we find that the *CV* becomes linearly proportional to the concentration. Given this limiting behavior, a sketch of the curve describing *CV* as a function of concentration can be drawn as shown in the following figure.

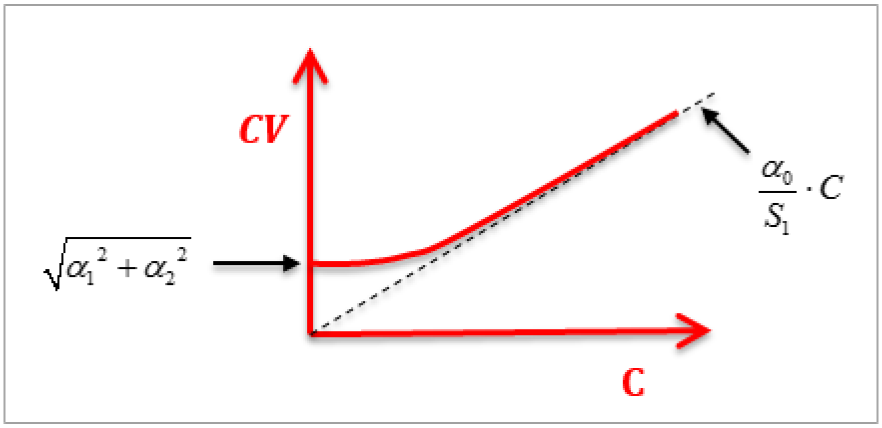

Figure 2: *CV as a function of concentration for the simple inverse response curve, showing the approach to a constant value at low concentrations, and the linear behavior at large concentrations.*

Coefficient of Variation for the Lorentzian Response Curve

A more realistic model for the inverted response curve is given by the Lorentzian function

$$S(C)=\frac{S_0}{1+\left(C/C_0\right)^{\alpha}} \quad\rightarrow\quad \frac{\partial S}{\partial C}=-\alpha\frac{S^2}{S_0C_0}\left(\frac{C}{C_0}\right)^{\alpha-1} \tag{9}$$

where the "spectral slope" $\alpha>1$. This model has no infinities, with the function remaining finite, and its slope flattening to zero when the concentration goes to zero as previously mentioned, and as can be seen now explicitly from expression (9). To explore the behavior of the CV in this limit, note first the following limits:

$$\lim_{C\to 0} S(C)=S_0 \quad \textit{and} \quad \lim_{C\to 0} C\cdot\frac{\partial S}{\partial C}=-\alpha S_0\cdot\left(\frac{C}{C_0}\right)^{\alpha} \tag{10}$$

With some simple manipulation, we then find

$$\lim_{C\to 0} CV = \frac{\sqrt{\alpha_0{}^2 + \alpha_1{}^2 S_0{}^2}}{\alpha S_0 \cdot \left(\frac{C}{C_0}\right)^{\alpha}} \tag{11}$$

and as the concentration goes to zero, the associated *CV* in fact diverges to infinity as $1/C^{\alpha}$ (recalling $\alpha > 1$). Whereas in the simple inverse response curve the measurement diverged to infinity as $1/C$ and the associated *CV* became constant, here we have the measurement remaining finite while the associated *CV* diverges.

In the limit of large concentration, the measurement S goes to zero so that the *CV* expression (4) may first be simplified to

$$\lim_{C\to\infty} CV = \left(\frac{\alpha_0{}^2}{\left(C \cdot \frac{\partial S}{\partial C}\right)^2} + \alpha_2{}^2 \right)^{1/2} \tag{12}$$

Using the Lorentzian slope from expression (9), the denominator in the first term is given by

$$C \cdot \frac{\partial S}{\partial C} = -\alpha \frac{S^2}{S_0} \left(\frac{C}{C_0}\right)^{\alpha} \tag{13}$$

Furthermore, we note from the definition of the Lorentzian in expression (9) that

$$\lim_{C\to\infty} S^2 \propto C^{-2\alpha} \tag{14}$$

so that

$$\lim_{C\to\infty} \left| C \cdot \frac{\partial S}{\partial C} \right| = \lim_{C\to\infty} \left| \alpha \frac{S^2}{S_0} \left(\frac{C}{C_0}\right)^{\alpha} \right| \propto C^{-2\alpha} \cdot C^{\alpha} \to C^{-\alpha} \tag{15}$$

This means that the denominator in the first term of expression (12) goes to zero as the concentration becomes large and that the second term can be neglected. In this limit we now see that the CV diverges again

$$\lim_{C\to\infty} CV = \frac{\alpha_0}{\lim_{C\to\infty}\left|C\cdot\frac{\partial S}{\partial C}\right|} \propto \frac{\alpha_0}{\lim_{C\to\infty} C^{-\alpha}\to 0} \to \infty \qquad (16)$$

Given the divergence of the CV at both low and high concentrations, the expected shape of CV versus concentration should appear qualitatively as shown in the following figure.

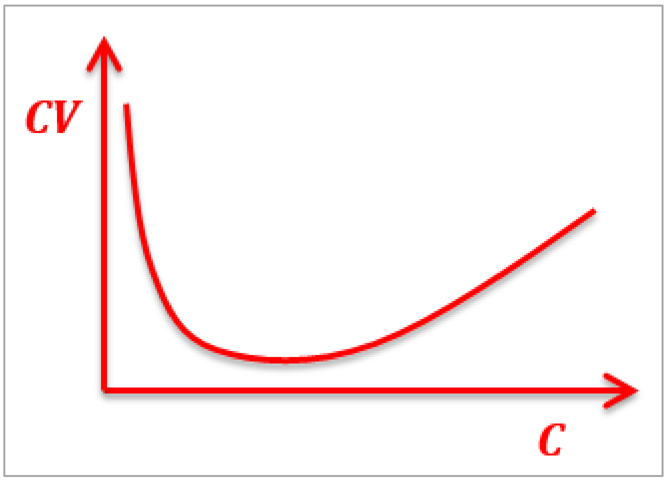

Figure 3: *CV as a function of concentration for the Lorentzian response curve, showing divergence at both small and large concentrations.*