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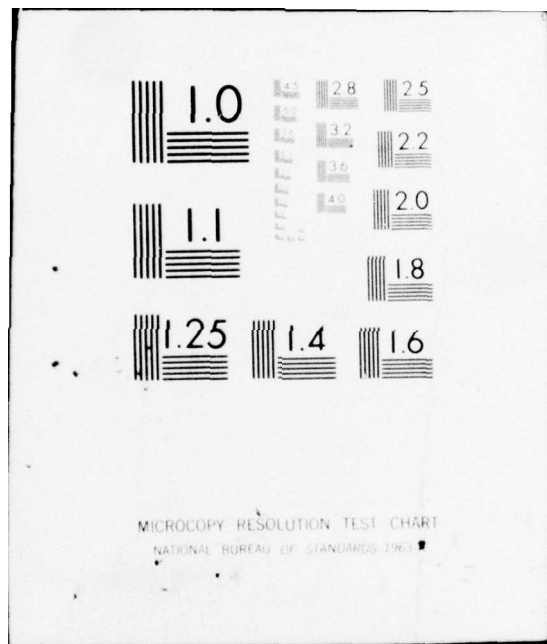
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ROBUST CALIBRATION

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by

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Allied Chemical Corporation

and

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State University of New York at Buffalo

TECHNICAL REPORT NO. 41  
May 1976

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ROBUST CALIBRATION

James J. Tiede and Marcello Pagano\*  
 Allied Chemical Corporation and  
 State University of New York at Buffalo

*is presented*

ABSTRACT

We present a method for fitting radioimmunoassay calibration curves which are used for measuring the concentration of various antigens in vitro. The curves to be fitted are modified hyperbolae on the basis of only a few observations (typically 12 to 16). Previous methods of fitting involved either linearizing the curve and estimating by least squares or fitting directly by nonlinear least squares. Unfortunately, the linearization techniques used are not usually successful in their intent and, furthermore, outliers are quite common due to the large number of sources of error.

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We present the algorithm we devised and used successfully for finding M-estimates (introduced by P. J. Huber) of the radioimmunoassay curves, and demonstrate its superiority to the method of least squares in the presence of outliers and also its similarity to least squares in the absence of outliers.

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## 1. INTRODUCTION

The calibration experiment can be abstracted as follows, [5]: the variable of interest,  $X$ , is difficult or impossible to measure directly, while a related variable,  $Y$ , which is dependent on  $X$ , is fairly easy to measure. A measurement is made on the variable  $Y$  and an estimate is made of the associated  $X$ . Operationally, the relationship between  $X$  and  $Y$  must be quantified. For a given value of  $X$ ,  $Y$  is distributed about a mean function,  $f(X, \beta)$ , with a certain dispersion  $\sigma$ . To determine this functional relationship,  $n$  couplets  $(X_1, Y_1), \dots, (X_n, Y_n)$  are observed at known values of  $X$ , where it is assumed that the measurement errors associated with  $X$  are negligible relative to the errors in  $Y$ . If the form of the function  $f$  is known, then these observations are used to estimate the parameters  $\beta$  and  $\sigma$ . Subsequently, independent observations  $Y_j$ ,  $j = n + 1, \dots, n + m$ , are made at  $m$  unknown values of  $X$ , and the estimated calibration curve  $f$  is employed to make inference on the corresponding unknown values  $X_j$ ,  $j = n + 1, \dots, n + m$ .

The calibration problem occurs in radioimmunoassay, which is a biological technique devised to measure minute concentrations of various biological substances which appear in man [9]. The variable  $X$  refers to the concentration of the substance of interest<sup>\*\*</sup>; a concentration sometimes as small as a picogram per milliliter thus making direct measurement virtually impossible. The variable  $Y$  refers to radioactive counts of bound residue of the assay [6]. A typical clinical radioimmunoassay is shown in Table 1. Here  $n = 14$  (it is usually 12, 14 or 16). In this paper we only concentrate on obtaining the calibration curve so we do not show  $Y_j$ ,  $j = n + 1, \dots, n + m$ ; typically,  $m$  can be anywhere between about 50 and 300.

<sup>\*\*</sup>We thank Dr. Jehuda Steinbach of the Nuclear Medicine Department, Veteran's Hospital, Buffalo, for making the data available to us.

<u>i</u>	<u>Y</u> <u>counts</u>	<u>X</u> <u>concentration</u>
1	7720	0.0
2	8113	0.0
3	6664	2.0
4	6804	2.0
5	4994	5.0
6	4948	5.0
7	3410	10.0
8	3208	10.0
9	4478	20.0
10	2396	20.0
11	1302	50.0
12	1377	50.0
13	1025	100.0
14	1096	100.0

Table 1

One of the most common methods for finding the form of the dependence of Y on X with radioimmunoassay data is the linearization method [4]. Suppose that the count at  $X = 0$  is  $Y_0$ , (usually estimated by taking the average of the two observations at zero concentration) and N is the background noise (usually estimated by placing two "empty" tubes into the counter and taking the average of the resultant counts). Then define a new response variable by  $Y' = (Y - N)/Y_0$ . After making a number of assumptions about the chemical reactions Rodbard and Lewald [4] show that

$$\text{logit } Y' = \log \left( \frac{Y'}{1 - Y'} \right) = \alpha' + \beta' \log X + \epsilon, \quad (1)$$

where the errors,  $\epsilon$ , have zero mean and are heteroscedastic. An example of this fit is given in Figure 1.

This method is not recommended. Some experiments have too small  $Y_0$  or too large N (since they are measured quantities, they are also subject to error) thus leading to  $Y'$  which are not in the interval (0,1). But more disturbing, is, that frequently, as in Figure 2, the data are not approximately colinear so that the fitted straight line is not representative of the data.

## 2. THE HYPERBOLIC CURVE

Since the linearization method does not produce acceptable fits, we chose to inspect the scatter plots of  $Y$  versus  $X$  for a large number of data sets (124 in all, including the substances, Insulin, Renin, TSH, Digoxin, Folic Acid, Vitamin B-12, T3 and Gastrin). The data suggests a modified hyperbola,

$$Y = \alpha + \frac{\beta}{1 + \gamma X^\delta} + \epsilon, \quad (2)$$

where the  $\epsilon$  have mean zero and are homoscedastic. Figures 3 and 4 show both curves (1) and (2) fitted by least squares; the dotted line is curve (1).

The superiority of the modified hyperbola is evident even though both curves usually require four parameters; in curve (1) one must include both zero concentration and background counts as parameters to be estimated. Incidentally, for Vitamin B-12 and Gastrin one may reduce the number of parameters in (2) by setting  $\delta = 1$  and thus obtaining a rectangular hyperbola. If one assumes zero error, the two curves, (1) and (2), are mathematically identical ( $N = \alpha$ ,  $Y_0 = \beta$ ,  $\alpha' = -\log \gamma$ ,  $\beta' = -\delta$ ). Thus the difference between the two methods is the different error structures assumed and the flexibility afforded by curve (2) for estimating zero concentration counts and background noise.

Having decided that curve (2) is superior to curve (1), the question then arises as to a reasonable algorithm for estimating the curve parameters. Usually, an ordinary least squares fit of the modified hyperbola yields a reasonable calibration curve. Testing has shown that an assumption of homoscedasticity for the errors is valid, [7]. Unfortunately though, data sets, such as displayed in Figures 5 and 6, too often occur for which one or two points are obviously in gross error (see also, points 2 and 9 in Table 1). The dotted line in both figures represents the least squares estimation of curve (2). The fit is clearly not acceptable; it is overly influenced by outlying observations. These last mentioned observations could be discarded and the curve refitted by least squares, but the statistical properties of the resulting curve are difficult to evaluate. Furthermore, wishing to find an automatic procedure we prefer to use the following methodology.

## 3. M-ESTIMATES

A way of curtailing the influence of observations which are overly large or small is to turn to robust estimation methods. Andrews [1], [2] reports success with the use of M-estimates, [3], in linear regression. To extend the method to nonlinear regression (see also [8]), consider the least squares estimates of  $\beta$  in model (2), on the basis of observations  $(Y_1, X_1), \dots, (Y_n, X_n)$ . They satisfy,

$$\sum_{j=1}^n f'_i(X_j, \beta) \varphi(Y_j - f(X_j, \beta)) = 0, \quad i = 1, \dots, 4 \quad (3)$$

where, with  $\varphi(z) = z$ ,

$$f(X, \beta) = \alpha + \frac{\beta}{1 + \gamma X^\delta}, \quad \beta = (\alpha, \beta, \gamma, \delta)$$

and  $f'_i$  is the partial derivative of  $f$  with respect to the  $i^{\text{th}}$  component of  $\beta$ . Replacing the above  $\varphi$  by

$$\varphi(z) = \begin{cases} \sin(z/cs) & |z| \leq \pi cs \\ 0 & |z| > \pi cs \end{cases}$$

where  $s$  is a scale estimate, we obtain the sine-estimate, [1], a special case of the M-estimates. We use  $c = 2.1$  and as an estimate of scale

$$s = \text{median} \{ \text{largest } (n - p + 1) |Y_j - f(X_j, \beta)| \},$$

with  $p$  the number of parameters.

To solve equations (3) for the sine estimate since the equations are nonlinear we must rely on an iterative algorithm. The iteratively reweighted least squares algorithm we use to solve equations (3) is similar to the Gauss-Newton algorithm. Denoting the  $k^{\text{th}}$  iterate by  $\beta^{(k)}$  then  $\beta^{(k+1)}$  is the solution to the following normal equations:

$$\sum_{j=1}^n \left( F(\beta^{(k)}) \right)_{ji} w_j^{(k)} \left( v_j - \sum_{i=1}^4 \left( F(\beta^{(k)}) \right)_{ji} \beta^{(k+1)}(i) \right) = 0$$

where

$$\left( F(\beta) \right)_{ji} = \frac{\partial f(X_j, \beta)}{\partial \beta(i)} \quad \begin{array}{l} j = 1, \dots, n \\ i = 1, \dots, 4 \end{array}$$

$$r_j^{(k)} = \left( Y_j - f(X_j, \beta^{(k)}) \right) / s^{(k)} \quad ,$$

$$w_j^{(k)} = \varphi(r_j^{(k)}) / r_j^{(k)} \quad ,$$

$$v_j^{(k)} = s^{(k)} r_j^{(k)} + \sum_{i=1}^4 \left( F(\beta^{(k)}) \right)_{ji} \beta^{(k)}(i) \quad .$$

Without modification, this algorithm failed to converge  $(|\beta^{(k)}(i) - \beta^{(k+1)}(i)| < \epsilon |\beta^{(k)}(i)|, i = 1, \dots, 4)$  in 9 of the 124 data sets. On inspection it was found that these data sets contained too many gross inaccuracies. The calculations took about 50% more time than the ordinary Gauss-Newton method (part of this time is undoubtedly due to the calculation of the sine function; simpler weight functions are available). To obtain starting values we used the following facts: (i)  $f(\infty, \beta) = \alpha$  if  $\delta \geq 0$ , (ii)  $f(0, \beta) = \alpha + \beta$  if  $\delta \geq 0$ , (iii)  $f'(0, \beta) = -\beta$  if  $\delta = 1$ , (iv) use  $\delta = 1$  as a starting value, since, usually  $0.5 \leq \delta \leq 2.5$ .

Figures 5, 6 and 7 show examples of the resultant fitted curve (solid line). Figure 7, which does not have any outliers, also displays the least squares line which is indistinguishable from the robust line. These are examples of the general result we found, if there are outliers, the robust curve is not usually influenced by these values, if there are no outliers then the robust curve is similar to least squares.

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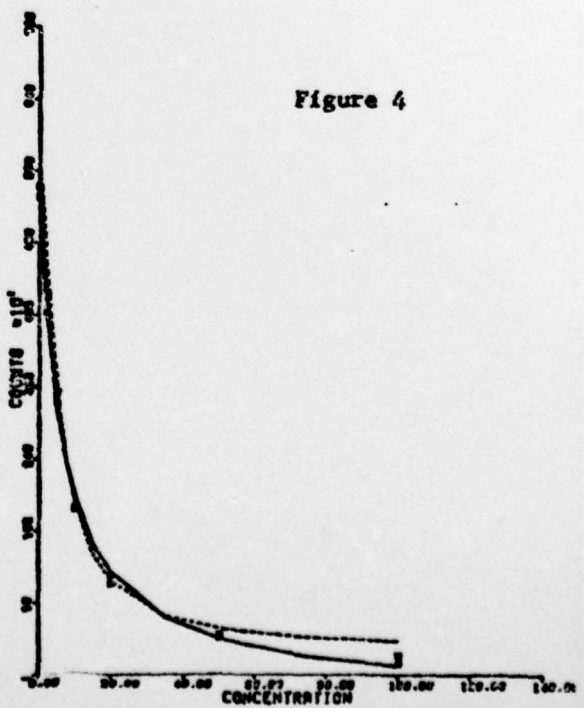
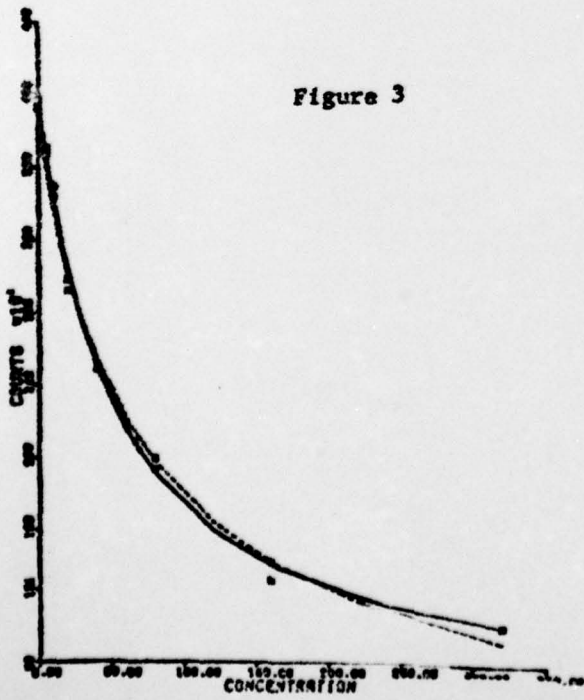
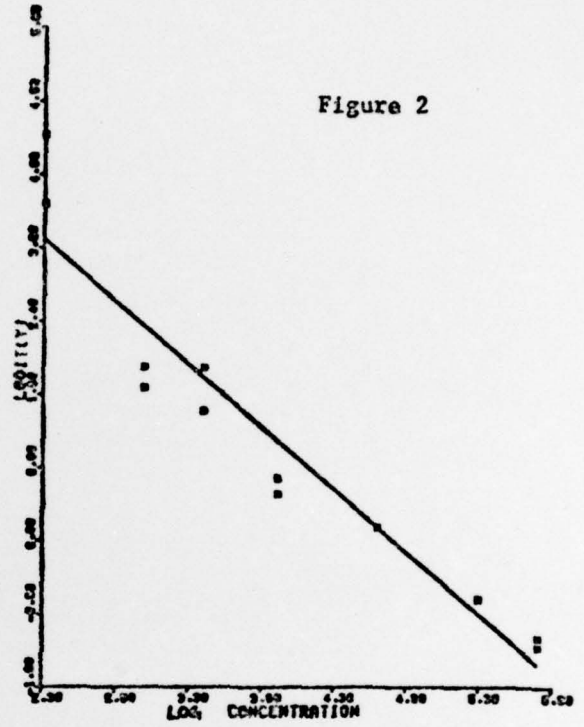
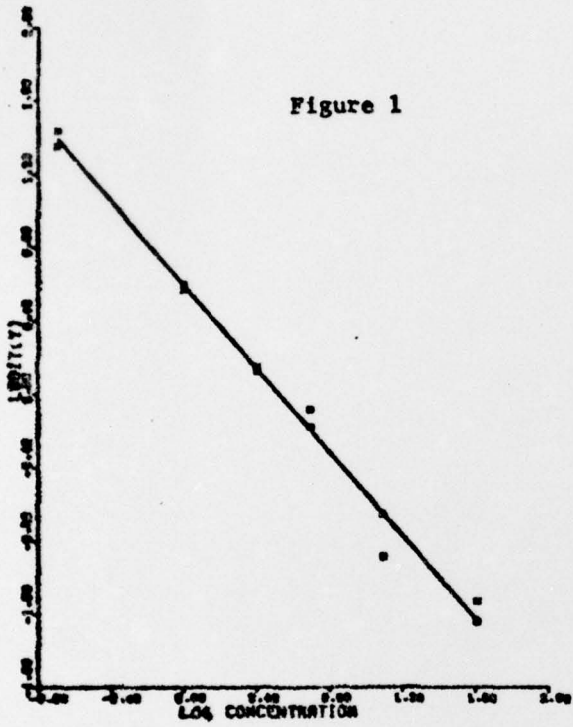


Figure 5

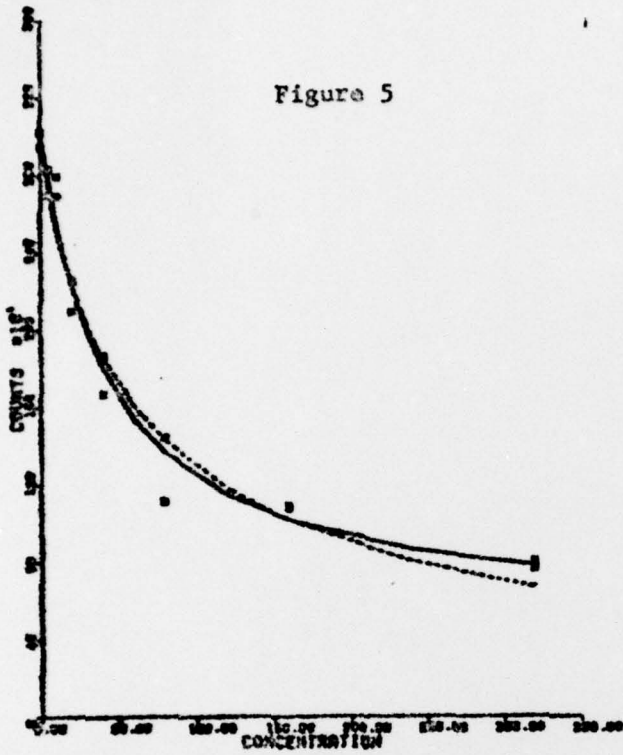


Figure 6

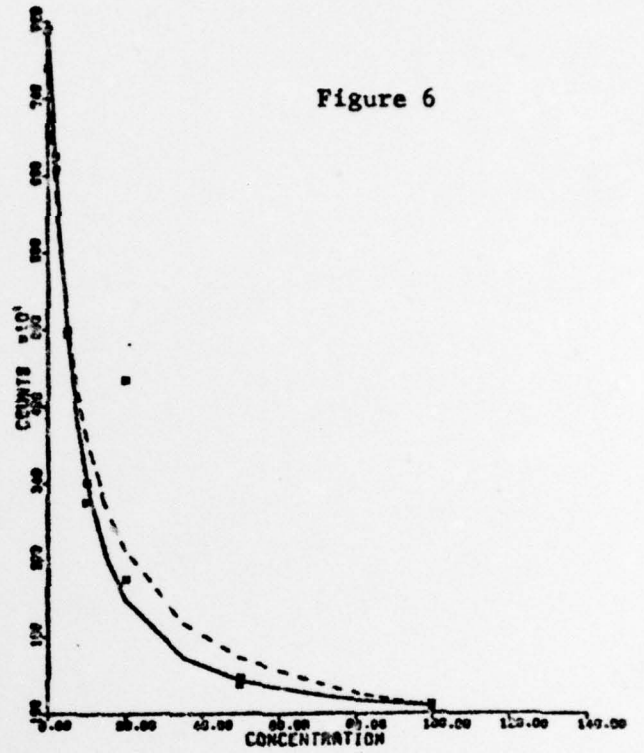
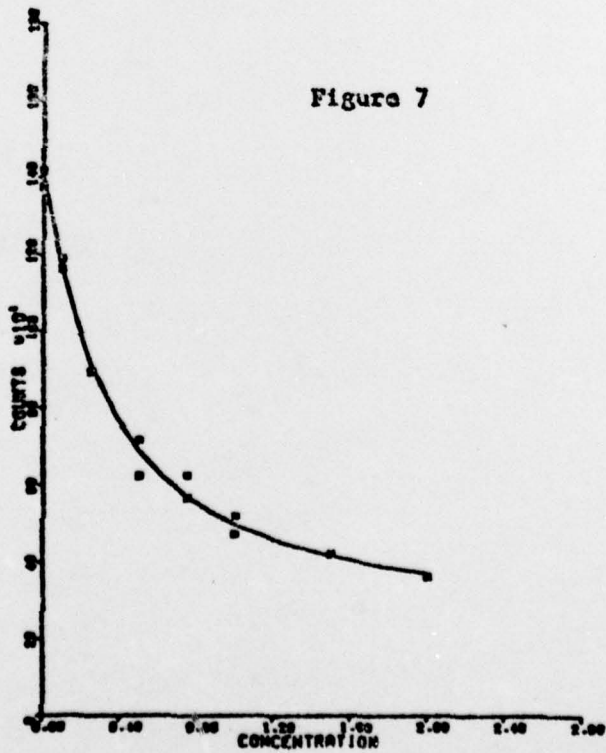


Figure 7



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