

Using a microcomputer to convert percent response values to probits

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Abstract

Certain relationships between dose/concentration and percent response/binding in various biochemical systems are sigmoidal rather than linear. This sigmoid relationship is considered linear only over the 20–80 percent range. Appropriate analysis of such data over nearly the entire response/binding range requires the conversion of the percent response data to probits or probability units, producing a linear relationship. While tables of probits are available, the author has incorporated the probability function for probit conversion into a commercially available spreadsheet program. Also included is the Rohlf and Sokal approximation for the area under the normal distribution curve (conversion of probits to cumulative percent). These functions allow use of a spreadsheet to automate the creation of dose-response curves, or standard curves as part of chemical assays (i.e., radioimmunoassay).

Introduction

In the study of drugs such as muscle relaxants, evaluation of the log dose-response curve is essential in determining the response of a study population to the drug. In this type of analysis the measured response would be the percent diminution in muscle strength resulting from a given dose of the study drug. The resulting log dose-response relationship is sigmoidal and considered linear only over the 20–80 percent response range.

A similar relationship exists when analyzing plasma concentrations of a substance by radioimmunoassay (RIA). With RIA, there is a sigmoid relationship between the log concentration of the substance and the percent binding of the substance, or its radio-tagged equivalent, to the antibody.

Appropriate analysis of such data over nearly the entire response/binding range requires the conversion of the percent response data to probits or probability units. The resulting log dose-probit re-

sponse or log concentration-probit percent binding relationships are linear. The subsequent analysis of this linear relationship, using the least squares linear regression equation, permits calculation of values for the ED50 or ED95 of the muscle relaxant or, for drug analysis by RIA, construction of a standard curve.

One example of this type of quantal dose-response analysis and an accompanying computer program written in basic has been published [1]. The probit conversion section of this very complete but lengthy program doesn't arithmetically convert percent to probit but rather assigns a probit value from a table of probit transformations. The probit values from an appended table [1] are sequentially loaded into a one-dimensional array and the percent response, after being rounded to the nearest integer value, is assigned the probit value occupying that sequential location in the array.

In our work with RIA, we were particularly interested in automating the calculations necessary

to convert the raw counts per minute from the scintillation counter to the plasma concentrations of the synthetic narcotic, alfentanil. We have previously used a commonly available spreadsheet (Lotus 1-2-3®) implemented on an IBM PC® compatible microcomputer to automate the process for determining the standard curve and plasma concentrations of indocyanine green. However, in that original application, the measurement (transmittance) is converted by a standard logarithmic transformation to a quantity (absorbance) which is linearly related to the plasma concentration of the substance. When the plasma concentration or log concentration is related to percent binding in a sigmoidal fashion, as it is with RIA, a probit conversion becomes necessary prior to determining the standard curve. With complete automation of the data analysis, in addition to saving time, the investigator is not able to exercise personal bias and judgment in the determination ('eyeball') of the standard curve or subsequent plasma concentrations from the graph paper plot.

Converting percent to probits

Although the technique of assigning the appropriate probit value to the nearest whole number percent response is usually accurate enough for most applications (after entering the table of probit values), the impetus for developing these programs was the desire to obtain the maximum accuracy and efficiency in the conversion. A convenient approximation to the probit value for any given percent response value is [2]:

$$\text{probit } (p) = t - (c_0 + c_1t + c_2t^2) / (1 + d_1t + d_2t^2 + d_3t^3)$$

where $t = \sqrt{\ln(1/p^2)}$

and $p =$ percent, given as the decimal equivalent, $0.00 < p \leq 0.50$

$$\begin{array}{ll} c_0 = 2.515517 & d_1 = 1.432788 \\ c_1 = 0.802853 & d_2 = 0.189269 \\ c_2 = 0.010328 & d_3 = 0.001308 \end{array}$$

In setting up this conversion three factors must be taken into consideration:

- The integer percent must be converted to its decimal equivalent in the range $0.00 < p \leq 0.50$
- The approximation is accurate over this range with the error (as a function of p) $< 4.5 \times 10^{-4}$
- The inflexion point for the percent vs. probit curve occurs at 0.50 (50 percent) and a probit value of 0.0000 (Fig. 1).

The plotted values for this relationship are symmetrical around the inflexion point (Fig. 1) and therefore, the conversion can be expanded over the range $0.00 < p < 1.00$ (1-99%) by making the following entries into a spreadsheet (using the mathematical conventions of Lotus 1-2-3®): (see also Table 1)

Cell Address	Entry	Remark
C1-	e.g 1	- Enter the percent value to be converted to probit
C2-	7	
C3- ...	50	
D1	@IF(C1 > 50, (100-C1)/100, C1/100)	- Convert percent value entered to its decimal equivalent and places all values into the range $0.00 < p \leq 0.50$
E1-	@SQRT(@LN(1.0/D1^2))	- Calculate the value for t as given above
F1-	+ E1-((2.515517 + 0.802853 * E1 + 0.010328 * E1^2)/(1.0 + 1.432788 * E1 + 0.189269 * E1^2 + 0.001308 * E1^3))	- Calculate probit value (point of symmetry, 0.0000) in the range $0.00 < p \leq 0.50$
G1-	@IF(C1 > 50, 5.0 + F1, 5.0-F1)	- Convert the calculated probit values to the conventional probit scale with the point of

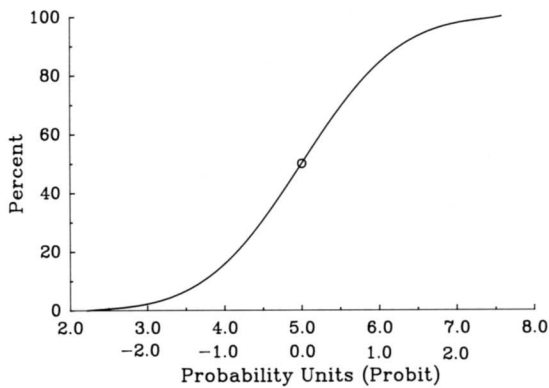


Fig. 1. The relationship between probability units (probits) and the cumulative percentage for the area under the curve of a normal distribution. The inflexion point (point of symmetry) is at the probit value of 5.0000 (or 0.0000) and 50 percent (0.50 decimal equivalent).

symmetry represented
by the probit value of
5.0000

Using the /COPY command, the entries for cells D1–G1 can be copied down the respective columns to fill the necessary number of rows in the worksheet as determined by the number of percent values that need to be transformed.

Conversion of probits to cumulative percent

For completeness, and as a method of error analysis (*vide infra*), the equations of the Rohlf and Sokal approximation to determine the area under a normal distribution curve (reverse transformation of probit to cumulative percent) is presented in spreadsheet format. This is a more detailed description than that previously included in a basic program to simulate quantal dose-response experiments [3]. For this approximation the calculated probit values are likewise symmetrical around a probit value of 0.0000 corresponding to a decimal percent value of 0.50 (Fig. 1).

With appropriate adjustments for a calculated probit value of 5.0000 corresponding to a cumu-

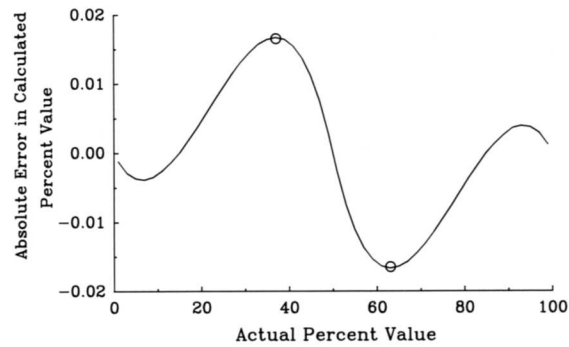


Fig. 2. The errors resulting from performing 'closed-loop' error analysis (see text). The maximum error (○) resulting from the calculated approximations in the conversion from percent to probit and back to percent is $< \pm 0.017$ and occurs at 37 and 63 percent, respectively.

lated (area under the curve) value of 50 percent the entries into the spread sheet again in Lotus 1-2-3® format are:

Cell Address	Entry	Remark
A1-	e.g 2.6732	– Enter the probit values to be converted to percent
A2-	3.5239	
A3- ...	5.0000	
B1-	@ABS(A1-5.0)	– Converts probit value in A1 to \pm probability units with a central (50 percent) value of 0.0000
C1-	@EXP(-(B1^2/2.0))/ (@SQRT(2.0*@PI))	– First subcalculation of Rohlf and Sokal approximation
D1-	+ 1.0/(1.0 + B1 * 0.2316419)	– Second subcalculation
E1-	+ D1 * (0.3193815 + D1 * (- 0.3565638 + D1 * (1.781478 + D1 * (-1.821256 + D1 * 1.330274))))	– Final calculation of cumulative percent in

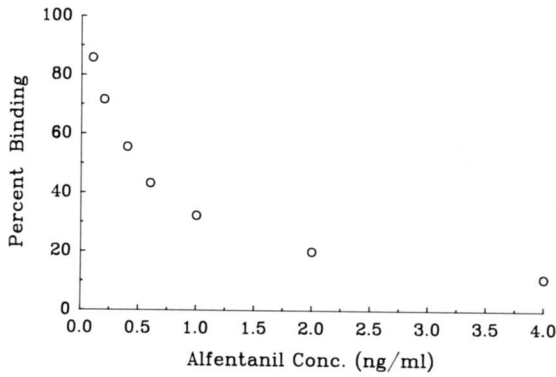


Fig. 3. A plot of a standard curve for the radioimmunoassay of the alfentanil concentration versus the percent binding of alfentanil to the antibody. Of note is the curvilinear relationship.

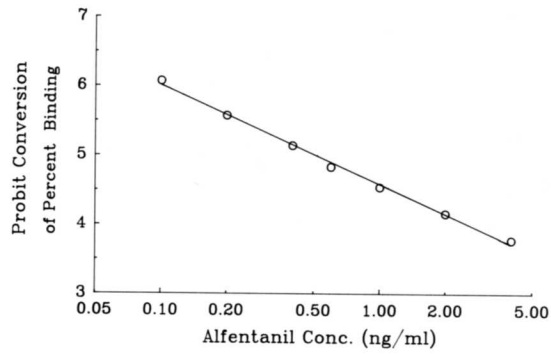


Fig. 4. The same standard curve as in Fig. 3 but with the abscissa (alfentanil concentration) changed to a log scale versus the probit conversion values of the percent binding of the alfentanil to the antibody on the ordinate. The linear regression for the standard curve is $\log [\text{alf}] = -0.6977 \star \text{Probit} + 3.1956$; $r^2 = 0.9965$.

F1- decimal equivalent
 @IF (A1 > 5.0, (1.0 - (C1 * E1)) *
 100.0, (C1 * E1) * 100.0)
 - Convert calculated
 decimal percent to
 integer percent values
 over the full range (1-
 99%)

Verifying accuracy of conversions

In order to check the accuracy of these approximations, a 'closed-loop' analysis was performed. First, the integer percent values (1-99%) were converted to probit values using the first set of equations. These calculated probit values were then converted

Table 1. Probit conversion for alfentanil RIA standard curve using Lotus 1-2-3®.

Lotus column		C	D	E	F	G
Conc	Net CPM	% B/Bo	Frct% 0-50	Subroutine	Prob 0-50	Probit
NSB	0.0					
0.0	2541.5	100.00	0.0000			
0.1	2180.3	85.79	0.1421	1.9754	1.0709	6.0709
0.2	1819.5	71.59	0.2841	1.5865	0.5704	5.5704
0.4	1411.6	55.54	0.4446	1.2733	0.1391	5.1391
0.6	1099.5	43.26	0.4326	1.2945	0.1693	4.8307
1.0	820.6	32.29	0.3229	1.5037	0.4593	4.5407
2.0	510.0	20.07	0.2007	1.7922	0.8390	4.1610
4.0	281.1	11.06	0.1106	2.0985	1.2235	3.7765

Linear regression analysis for the standard curve

$\log [\text{Alf}] = \text{Probit} \star \text{X Coefficient} + \text{Constant}$

Regression Output:

Constant		3.1956		
Std Err of Y Est		0.0360		
R Squared		0.9965		
No. of observations		7.0000		
Degrees of freedom		5.0000		
X Coefficient(s)	-0.6977			R =
Std Err of Coef.	0.0185			0.9983
				R ² =
				0.9965

to percent values using the second set of equations. Finally, the difference (absolute error, entered percent minus calculated percent) between these two sets of values were calculated and displayed (Fig. 2). This technique of program evaluation permitted debugging the spreadsheet entries as well as determining the maximum error that can be expected using either approximation alone or in combination (worst case scenario). For these types of analyses a maximum absolute error in calculated percent of ± 0.017 occurring at 37 and 63 percent, respectively, should be very acceptable.

Summary

The author has outlined an accurate and convenient way to calculate the approximations of a probit value from a percent as well as converting probit values to cumulative percents. This is easily accomplished using almost any one of the commercially available spreadsheet packages.

Furthermore, as has been previously described [4], many of the other advantages of using a spreadsheet can be exploited. First, the spreadsheets perform calculations very rapidly as they are written in machine language. Second, many useful utilities and mathematical operands that are included in these spreadsheets, including linear regression analysis, can be applied to the resultant data, already entered or derived without the need for data reentry or transfer. Finally, utilization of the commercially available software eliminates the tedious job of entering and debugging a lengthy program written in basic.

This program was used to aid in the determination of the plasma alfentanil concentrations in a recent study [5]. The values and calculations performed using Lotus® are given in Table 1. The original data for the standard curve for the determination of plasma alfentanil concentrations using the radioimmunoassay is characterized by a curvili-

near relationship (Fig. 3). There is a marked improvement in the linearity of this relationship when the concentrations of alfentanil are plotted on a logarithmic scale (abscissa) and a probit conversion is applied to the percent binding (ordinate) (Fig. 4). The resultant linear regression used in the subsequent calculation of the unknown plasma concentrations of alfentanil is also plotted.

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References

1. Tallarida RJ, Murray RB. Manual of Pharmacological Calculations with Computer Programs. New York, Springer Verlag 1981; 86 and 127.
2. Handbook of Mathematical Functions with Formulas Graphs and Mathematical Tables. Edited by Abramowitz M. and Stegun IA. Washington, D.C., Govt Print Off 1964; 933.
3. McGilliard KL. A microcomputer program for simulation of quantal dose-response experiments. Trends Pharmacol Sci 1986; 7: 53-54.
4. Dorey PG. Using a computer spreadsheet in pharmacology problem solving. Trends Pharmacol Sci 1984; 5:6.
5. Henthorn TK, Avram MJ, Krejcie TC. Alfentanil clearance is independent of the polymorphic debrisoquin hydroxylase. Anesthesiology 1989; 71: 635-639.

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