THE CALCULATION OF PARAMETERS OF THE MOFFITT EQUATION FOR ROTATORY DISPERSION DATA

DELOS F. DETAR

From the Department of Chemistry and the Institute of Molecular Biophysics, Florida State University, Tallahassee

ABSTRACT The least squares calculation of the best values of the parameters of the Moffitt equation and of the Drude equation is examined. It is proved that the least squares evaluation of all three parameters of the Moffitt equation becomes indeterminate as the b_0 term approaches zero. Estimates of low helical content based on the Moffitt relationship are therefore also indeterminate and of dubious value. Both the size of b_0 and the range of wavelengths chosen affect the standard deviations of the parameters. The magnitude of the effects is illustrated by selected examples. The computer program OPTROT is available for evaluating the extent to which data may be correlated by the equations.

The Moffitt equation (1) has proved useful in providing an indication

$$M = a\lambda_0^2/(\lambda^2 - \lambda_0^2) + b_0\lambda_0^4/(\lambda^2 - \lambda_0^2)^2 \qquad (1)$$

of the extent to which a helical conformation of polypeptides is assumed (references 1 and 2).[1] From correlation with other data it has been suggested that a polymer completely in the α-helical form and devoid of side chain chromophores will exhibit a λ_0 of about 210 to 230 and a b_0 parameter of about -600. Other values have also been suggested. At best, estimates about conformation based on the Moffitt relationship must be understood to be semiquantitative, although still useful.

The purpose of this paper is to consider certain important mathematical properties of the Moffitt equation. Our interest in the problem arose from attempts to apply the conventional plotting technique[2] to rotation data for sequence peptide polymers (3). Sometimes, true to expectation, the plots gave a straight line for one value of λ_0 and curved lines for all others. However, straight lines were also sometimes obtained for two λ_0 values, e.g. 180 and 280 mμ, neither of which was very

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[1] $M = \alpha$*(mol. wt.)/(length*concentration*correction*10); length is path length in centimeters, concentration is in grams/milliliter, and correction $= (n^2 + 2)/3$ where n is the refractive index, α is the observed rotation.

[2] The plot of $(\lambda^2 - \lambda_0^2)*M$ vs. $1/(\lambda^2 - \lambda_0^2)$ gives a straight line if the proper λ_0 value is used, and the slope is $b_0\lambda_0^4$ and the intercept $a_0\lambda_0^2$.

close to the presumed "correct" value, and sometimes straight lines resulted from all choices of λ_o.

We therefore developed a computer program, OPTROT, to make possible a thorough examination of a given set of data. It is assumed that the normally distributed error is the scalar error in α, and the program minimizes this error by a least squares procedure. Provision is made for adjusting all parameters of both the Moffitt and the Drude equations[3] and it is also possible to fix λ_o and to find the best values of the remaining parameters.

This approach differs from those previously explored. Sogami, Leonard, and Foster (4) have described a computer program which makes calculations at fixed λ_o values and which minimizes the error in the function $(\lambda^2 - \lambda_o^2)*M$. This results in an involuntary weighting which tends to bias the estimates of the parameters. The method has the merit of examining directly the need for b_o term. Marsh has used a computer approach which also minimizes the error in $(\lambda_2^2 - \lambda_o^2)*M$ (5).

It becomes obvious once one tries to make calculations in which all three parameters are adjusted, that the Moffitt equation is sometimes badly behaved. Even with close initial estimates of the parameters the calculation may diverge, and this usually occurs with data which conform to the Drude equation. Since these data necessarily also conform to the Moffitt equation with b_o small or zero, such behavior is at first sight rather surprising. However, it turns out to be possible to prove that general statistical evaluations of the parameters of the Moffitt equation become indeterminate as b_o approaches 0.

Perhaps it should be emphasized that all valid statistical procedures must give the same estimates of the parameters and of their errors, if based on the same weighting of the data. Thus the F function described by Sogami, Leonard, and Foster (4) becomes indeterminate (0/0) as b_o goes to 0; the $\Sigma(y_i - y)$ term becomes zero for data which conform to the Drude equation (4). The significance of this type of indeterminacy is that a_o, b_o, and λ_o become functionally related as b_o becomes small, and there then exist sets of values of the parameters ranging over many tens of millimicrons of λ_o all of which equally well correlate the data.

The proof is based on a consideration of a special form of the normal equations. General methods are available for obtaining normal equations for any function (6). They are derived from partial differentials of the null function, equation (2). (The constant f is the set of factors relating M and α.)[1]

$$\begin{aligned} F &= \alpha_{\text{obsd}} - \alpha_{\text{calcd}} = \alpha_{\text{obsd}} - a'L - b'L^2 \qquad (2) \\ L &= 1/(\lambda^2 - \lambda_o^2) \\ a' &= a_o\lambda_o^2 f \\ b' &= b_o\lambda_o^4 f \end{aligned}$$

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[3] The form of the Drude equation used in this paper is $M = a_o\lambda_o^2/(\lambda^2 - \lambda_o^2)$ in order to emphasize its similarity to the Moffitt equation.

The partials of F with respect to a', b', and λ_0 are represented by F_a, F_b, and F_{λ_0} and are given in equations (3). The normal equations may be written in

$$\begin{aligned} F_a &= -L \\ F_b &= -L^2 \\ F_{\lambda_0} &= -(2a'\lambda_0 L^2 + 4b'\lambda_0 L^3) \end{aligned} \tag{3}$$

matrix form, equation (4), or they may be expanded as in equation (5). The a_{ij} are further

$$|A| * |C| = |\Sigma F_i F_o| \tag{4}$$

$$\begin{aligned} a_{11}C_1 + a_{12}C_2 + a_{13}C_3 &= \Sigma F_a F_o \\ a_{21}C_1 + a_{22}C_2 + a_{23}C_3 &= \Sigma F_b F_o \\ a_{31}C_1 + a_{32}C_2 + a_{33}C_3 &= \Sigma F_{\lambda_0} F_o \end{aligned} \tag{5}$$

expanded in equation (6). In use the terms a_{ij} are evaluated with preliminary estimates of a', b', and λ_0, and solution gives C_1, C_2, and C_3 which are the respective

$$\begin{aligned} a_{11} &= \Sigma L_i^2 \\ a_{12} &= a_{21} = \Sigma L_i^3 \\ a_{13} &= a_{31} = 2a'\lambda_0 \Sigma L_i^3 + 4b'\lambda_0 L_i^4 \\ a_{22} &= \Sigma L_i^4 \\ a_{23} &= a_{32} = 2a'\lambda_0 \Sigma L_i^4 + 4b'\lambda_0 \Sigma L_i^5 \\ a_{33} &= 4a'^2\lambda_0^2 \Sigma L_i^4 + 16a'b'\lambda_0^2 L_i^5 + 16b'^2\lambda_0^2 L_i^6 \end{aligned} \tag{6}$$

corrections. F_o is the value of F for a given λ and would be zero if there were no errors. The data consist of a set of paired values of α and λ and the summations include all such pairs.

Comparison of equations (5) and (6) with $b' = 0$ shows that the determinant, equation (7), has two rows (and two columns) which are identical except for the

$$\begin{vmatrix} \Sigma L_i^2 & \Sigma L_i^3 & 2a'\lambda_0 \Sigma L_i^3 \\ \Sigma L_i^3 & \Sigma L_i^4 & 2a'\lambda_0 \Sigma L_i^4 \\ 2a'\lambda_0 \Sigma L_i^3 & 2a'\lambda_0 \Sigma L_i^4 & 4a'\lambda_0^2 \Sigma L_i^4 \end{vmatrix} \tag{7}$$

constant term $2a'\lambda_0$; its value is therefore 0. This means that a', b', and λ_0 are not independent.

OPTROT uses a somewhat different set of normal equations in that correction terms are calculated directly for a_0, b_0, and λ_0 rather than for the derived a' and b'. The resulting normal equations do not readily simplify as b_0 goes to 0 and it is therefore not obvious that the singularity exists. Although these two approaches to the best values of the parameters follow different routes, the final result must be the same in both cases, providing consistent weighting factors are used.

If the least squares procedure converges, there is an advantage in adjusting all three parameters, for error estimates are then available for all. Conversely, there are also advantages in following the customary technique of fixing λ_0 at a series of values and then finding best values of a_0 and b_0 for each of these choices of λ_0. If the sum of the squares of the residuals (or of the variance of the α values, which is equivalent) is plotted against the λ_0 values selected, then the best λ_0 value is the one which gives a minimum. The graphical method always converges and it also shows clearly whether there is a well-defined λ_0 value.

To demonstrate numerically the behavior of the Moffitt function, we have generated exact data by substituting $\lambda_0 = 200$ mμ, $a_0 = 250$, and with $b_0 = 0, 50, 100, 200$, and 400. (The units of a_0 and b_0 are 0.1° $-$cm^2/mole.) It was assumed further that the concentration was 0.01 g/ml, the path length 10 cm, the molecular weight 200, and that the refractive index correction was unity at all wavelengths. (This does not affect the general conclusions.) The resulting α values were then used as observed α values in the input data for OPTROT and best values of both Moffitt and Drude parameters calculated. From these best parameter values, the corresponding calculated α values were again obtained so that they could be compared with input. These calculations were made at an effective accuracy in α of about ± 0.00003, an error arising only from arbitrary round off of the data.

Some of the results are shown in Table I.[4] In order to estimate the errors in the parameters, the value of 0.0035° was arbitrarily assumed for the standard deviation of α, unless the actual value was larger. Since the standard deviation of a parameter is directly proportional to that taken for α, the error corresponding to any desired accuracy of α may readily be estimated.

The data in Table I show clearly the uncertainties to be expected. With $b_0 = 0$, the Moffitt adjustment is impossible using only the 5 wavelengths indicated with a circled X (i.e. the sodium line and the mercury lines) because more than 15 places are lost in the attempted solution of the normal equations. This is a consequence of the determinant [cf. equation (7)] going to zero. As b_0 because larger, the fractional error in b_0 becomes smaller, although the absolute error remains about the same. With $b_0 = 400$, the calculated b_0 is 400.01 ± 16.

The plot of the variance of α vs. a series of assumed λ_0 values is another instructive way to examine the data. Fig. 1 illustrates such a plot. The "observed" data here were generated from the Moffitt equation with $a_0 = 250$, $b_0 = 50$, and $\lambda_0 = 200$. The rotation values were comparable to those shown in Table I. However, an error amounting to a standard deviation of 0.005° was introduced by use of a table

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[4] The behavior was also investigated with data generated with $a_0 = 200$, $b_0 = -600$, and $\lambda_0 = 210$, the archetype for the α-helix. As expected, the definitive fit to the Moffitt equation was even more pronounced than the results shown in columns 5 and 6 of Table I.

such as the one in the appendix of Deming (6). This gives a set of α values comparable to those that might be obtained experimentally.

To determine the effect of having rotation data over a restricted range of wavelengths, three sets of calculations were made: (*a*) with the 13 wavelengths from 589 to 250, which were used in Table I; (*b*) with the 5 wavelengths marked with the circled X's in Table I; (*c*) with these five plus 350, 325, and 300.

The presentation in Fig. 1 may be compared with the results of calculation of

TABLE I

TEST OF LEAST SQUARES PROCEDURE FOR FITTING THE MOFFITT EQUATION PARAMETERS TO EXACT DATA DERIVED FROM EQUATION (1), $a_o = 250.0$, $\lambda_o = 200.0$, b_o AS GIVEN

	$b_o = 0$*	$b_o = 100$*	$b_o = 100$	$b_o = 200$*	$b_o = 200$
Moffitt a_o	*c*	250.0 ± 2.5*¶		250.0 ± 3	
Moffitt b_o	*c*	100.0 ± 20		200.0 ± 17	
Moffitt λ_o	*c*	200.0 ± 2		200.0 ± 1	
Drude‡ a_o	250.0 ± 2.5	227.5 ± 8**	190.86 ± 5*		249.1 ± 20‡‡
Drude λ_o	200.0 ± 0.7*||	219.4 ± 1.3**	230.0 ± 5		225.4 ± 2
λ	α_{obsd}§§	α_{obsd}¶¶	α_{calc}||||	α_{obsd}¶¶	α_{calc}
589	0.1629⊗	0.1714⊗	0.1717	0.1799	0.2136
578	0.1700⊗	0.1793⊗	0.1796	0.1885	0.2233
550	0.1905	0.2021		0.2137	0.2513
546	0.1937⊗	0.2057⊗	0.2059	0.2177	0.2558
435	0.3351⊗	0.3710⊗	0.3704	0.4069	0.4570
405	0.4031	0.4552		0.5072	0.5587
400	0.4167	0.4722		0.5278	0.5793
365	0.5363⊗	0.6284⊗	0.6286	0.7204	0.7675
350	0.6061	0.7236		0.8411	0.8823
325	0.7619	0.9477		1.1334	1.1538
300	1.0000	1.3200		1.6400	1.6137
275	1.4035	2.0339		2.6642	2.5481
250	2.2222	3.8025		5.3827	5.4061

* Standard deviation of parameters is based on the standard deviation of 0.0035 for α unless otherwise noted.

‡ The Drude a_o has been calculated in the same units as the Moffitt a_o to permit direct comparison.

§ The Moffitt equation failed to converge.

|| The agreement between "observed" and calculated values of α was within ±0.00005.

¶ Using all points. Using just the five values marked with the ⊗, there are two sets of parameters: $a_o = 156.66 \pm 85$, $b_o = -22.34 \pm 28$, $\lambda_o = 252.95 \pm 63$ as one set, and $a_o = 249.94 \pm 253$, $b_o = 99.84 \pm 613$, $\lambda_o = 200.03 \pm 108$ as the second set. Both sets reproduce the data to better than 0.00003.

** Based on the observed standard deviation of $\alpha = 0.019$.

‡‡ Based on the observed standard deviation of $\alpha = 0.054$.

§§ The calculated values for the Drude equation reproduced these numbers to the fourth decimal place.

|||| Drude parameters from values at the five wavelengths marked with the ⊗.

¶¶ The Moffitt equation reproduced these to the fourth decimal place.

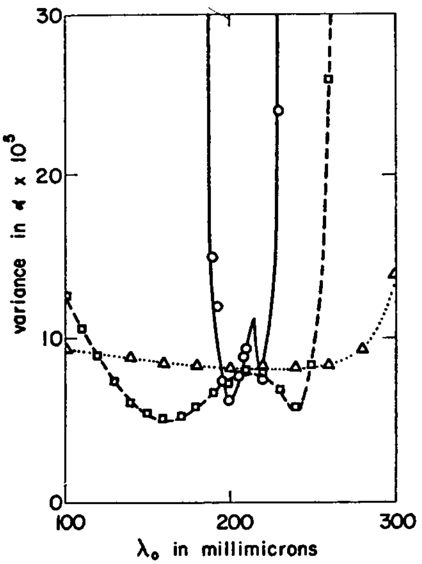

FIGURE 1. Plot of variance of α vs. λ_0 in millimicrons. For each λ_0 the best values of a and b were calculated. ○; Apply to 13λ values as listed in Table I. △; Apply to 5λ values as shown by the ⊗ in Table I. □; Apply to 8λ values, the above 5 plus 350, 325, and 300. See text for equation.

parameter values and error estimates by direct adqustment of all parameters. With 13 points, the calculated standard deviation of λ_0 is ±6 mμ, corresponding roughly to the λ_0 values in Fig. 1 at which the variance is twice the minimum value. With five points, the calculated standard deviation of λ_0 is ±320 mμ, and it can be seen from the curve that the minimum is undefined within the range covered by the curve. Even with eight points, which extend down to 300 mμ, the λ_0 value is essentially undefined. It may also be noted that the minima do not coincide with the correct value of λ_0. The precise form of the curves corresponding to those in Fig. 1 is very sensitive to the particular accidental residual errors in α, and one can easily be led to believe that some given data set corresponds to a better definition of λ_0 than is the case. Of course, the uncertainties in b_0 are large when those in λ_0 are large.

The double minima in the curves shown in Fig. 1 are characteristic of data which either cover too restricted a range of wavelengths or in which the error in α is too large to give a good definition. Direct solution of the Moffitt equation, using α values at three wavelengths, leads in general to two positive values of λ_0 as a result of there being two solutions for λ_0^2. With a sufficient range of data and a sufficiently large value of b_0, one of these turns out to be a false solution. For limited data, both solutions give an equally satisfactory fit. As mentioned above, plots of the function $M(\lambda^2 - \lambda_0^2)$ vs. $1/(\lambda^2 - \lambda_0^2)$ also may reflect these two roots by giving straight lines for two λ_0 values. Incidentally, direct solution for the parameters of the Moffitt equation rather often gives imaginary λ_0 values.

In view of these properties of the Moffitt equation, it is appropriate to ask what significance can be obtained from a calculation of the parameters. If the Moffitt equation is clearly followed, as is the case with poly-Glu(OBl) and poly-Glu(OH), then it is proper to be concerned with error limits in λ_0 and in the b_0 values. While the OPTROT calculation is theoretically superior since it gives relatively unbiased estimates, other methods of calculation are not greatly inferior. Attention can also be directed to the question of whether such an entity as a common b_0 value exists (2).

However, as b_0 becomes smaller, the uncertainty rapidly reaches large values. Some workers have recommended selection of a fixed λ_0 value to be used for all Moffitt calculations. There may be some justification for this approach if one is arbitrarily deciding on a value of 212 in preference to 210 or 214 for example. However, to make such a choice with data which give the shallow plots shown in Fig. 1 is arbitrary almost to the point of futility. Dispersion equations such as the Moffitt and the Drude equations are at best first approximations to complex behavior. We have applied the Drude equation to many dozens of peptide derivatives and find that Drude λ_0 values vary from 150 to 350. There is no obvious reason for supposing that λ_0 values should be any more constant for the Moffitt equation.

The detailed examination of rotary dispersion data requires a heavy amount of computing, and computer program OPTROT makes this feasible. OPTROT consists of about 1500 source cards, including comment cards, written in FORTRAN II for the IBM 709. It uses double precision arithmetic (about 15 places) for all critical calculations since it was found that single precision gave inferior results. The program has many options in which all parameters of either the Moffitt or the Drude equation can be adjusted, or λ_0 values (or all parameters) can be supplied. There is also a search routine which automatically makes calculations at fixed λ_0 values at 10 mμ intervals from 100 to 300 mμ and then at 1 mμ intervals around the λ_0 value which gives minimum in the variance of α. The output can be plotted automatically if desired on the 1401 printer to give results as shown in Fig. 1. Various types of weighting can be used. Since each rotation and each concentration can be assigned its own standard deviation, the user has complete control of the weighting if he so desires. Copies of the program will be provided to within limits of our resources. A user's manual is available.

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