# APPLICATION OF SPLINES TO THE CALCULATION OF BACTERIAL SWIMMING SPEED DISTRIBUTIONS

G. B. STOCK

*From The Thomas C. Jenkins Department of Biophysics, The Johns Hopkins University, Baltimore, Maryland 21218*

ABSTRACT A new method of extracting information about bacterial speeds from photon correlation spectroscopy is presented. This method has the advantage that an estimation of the translational speed distribution is directly varied so as to achieve a best least-squares fit to the experimental autocorrelation function. The theory of spline approximations to continuous functions is briefly outlined. The importance of the previously disregarded diffusional component of bacterial motion is discussed. Experimental data from *Salmonella* at a low scattering angle is analyzed by this method of spline approximation and the distribution of translational speeds is obtained.

## INTRODUCTION

Efforts to use photon correlation spectroscopy (PCS) to determine the distribution of swimming speeds of bacterial populations have extended over the past five years. Although initial reports (1, 2) were very optimistic and it seemed that a rather simple model of bacterial motion could be used to explain the data obtained, further research (3–5) indicated many important complications and argued against modeling bacteria as spheres undergoing simple translational motion. Among the complications is the fact that at higher angles (>20°) effects due to wobble begin to contribute strongly to the observed autocorrelation functions. Once understood, the contribution due to wobble can be used to gain information about the rotational motions of the bacteria, or it can be avoided by restricting measurements to low scattering angles where these effects are not substantial (5). There remains, however, the basic problem of extracting the bacterial speed distribution from the observed photon autocorrelation functions. Successful application of a simple Fourier sine transformation (1) is barred by truncation problems which render this method effectively unworkable (G. B. Stock, unpublished results). Fitting the autocorrelation functions to power series expansions of speed moments yields, at best, estimates of the first few even moments of the distribution ($<v^2>$ and $<v^4>$) where $v$ is the speed. These moments may be useful in characterizing bacterial speeds, but a direct estimate of the speed distribution itself is often preferable. This is especially true if the distribution happens to be multimodal. The method of splines treated in this paper gives just such a direct estimate of the

bacterial speed distribution, and this estimate can subsequently be used to estimate the odd speed moment $<v>$.

In this method, the bacterial swimming speed distribution $P(v)$ is represented by a smooth piecewise polynomial function (spline). The spline is varied so as to have a corresponding intensity autocorrelation function $G^{(2)}(\tau)$ which most closely approximates (in a least-squares sense) the experimental $G^{(2)}(\tau)$. One advantage of directly varying the approximation for $P(v)$ to achieve a best fit to the experimental autocorrelation data is that information about $P(v)$ obtainable from other sources (e.g. microscopy) can be readily inserted into the problem as constraints on $P(v)$. Examples of this types of information are: (*a*) The speed distribution is positive, i.e., for all $v$, $P(v) \geq 0$; (*b*) The speeds of the bacteria are bounded, i.e., there exists a $v_{max}$ such that for all $v \geq v_{max}$, $P(v) = 0$.

The use of splines to represent $P(v)$ has the further advantage that integrals encountered are analytically soluble. It, however, does not force $P(v)$ into a rigid analytic form unjustified by theoretical considerations.

## THEORETICAL

Prior to a detailed discussion of the method itself, a brief introduction to spline approximation (6) will be given.

Given a strictly increasing sequence of real numbers $(v_0, v_1, v_2, \ldots, v_n)$, the class of functions which are polynomials of degree $\leq k$ on the intervals $(-\infty, v_0], (v_0, v_1]$, $(v_1, v_2], \ldots, (v_n, +\infty]$ and have derivatives of orders 0 to $k-1$ which are continuous everywhere are called splines of degree $k$ with knots at $(v_0, \ldots, v_n)$. Every spline, $P(v)$, of degree $k$ with knots $(v_0, \ldots, v_n)$ has an expansion of the form

$$P(v) = \sum_{i=0}^{k} a_i v^i + \sum_{i=0}^{k} b_i \Delta_k[v; v_i], \tag{1}$$

where

$$\Delta_k[v; v_i] = \begin{cases} 0 & \text{for} \quad v \leq v_i \\ (v - v_i)^k & \text{for} \quad v > v_i. \end{cases} \tag{2}$$

A set of $n - k$ linearly independent basis splines can be generated (7) by using sequentially, from $m = 1$ to $k + 1$, the following recursive relationship for $i = 0$ to $n - m$:

$$\Delta_k[v; v_i, \ldots, v_{i+m}] = \frac{(\Delta_k[v; v_i, \ldots, v_{i+m-1}] - \Delta_k[v; v_{i+1}, \ldots, v_{i+m}])}{(v_{i+m} - v_i)}. \tag{3}$$

The resultant $n - k$ splines, specified as $\Delta_k[v; v_i, \ldots, v_{i+k+1}]$ for $i = 0$ to $n - k - 1$, form a basis of "local support" in that each element is of degree $k$ in the interval $(V_i, V_{i+k+1}]$ and is identically zero outside of this region. A significant simplification in computation is achieved through the employment of such a basis. As an example, consider the set of all linear splines (degree 1) which have knots at $(v_0, v_1, v_2, v_3, v_4)$

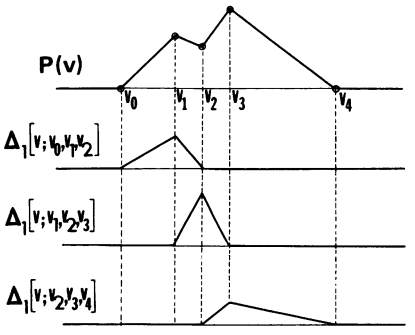


FIGURE 1 A sample spline from the set of splines which are identically zero for $\nu \leq \nu_0$ and $\nu \geq \nu_4$ and have knots at $(\nu_0, \nu_1, \nu_2, \nu_3, \nu_4)$. Also shown are the three basis functions which together constitute a basis of "local support" for this set. The height of the peak of each basis element $\Delta_1[\nu; \nu_i, \nu_{i+1}, \nu_{i+2}]$ is $1/(\nu_{i+2} - \nu_i)$.

and are identically 0 for $\nu \leq \nu_0$ and $\nu \geq \nu_4$. A complete local support basis (Fig. 1) would be composed of three linearly independent basis functions:

$$\Delta_1[\nu; \nu_0, \nu_1, \nu_2], \Delta_1[\nu; \nu_1, \nu_2, \nu_3], \Delta_1[\nu; \nu_2, \nu_3, \nu_4]. \tag{4}$$

Local support bases could also be generated for higher order splines.

## RESULTS AND DISCUSSION

In order to determine the velocity distribution represented by an experimental autocorrelation function $G^{(2)}(\tau)$, $P(\nu)$ is represented by a $k$th order spline on the net of knots $(\nu_0, \nu_1, \ldots, \nu_n)$. In practice, fitting to a first or third degree spline is sufficient and the fitted $P(\nu)$ is relatively independent of the specific net chosen. Knots should of course be more concentrated in regions of changing $P(\nu)$.

The net of knots is chosen, the degree of the spline fit is selected, and then a local support basis is generated. If the basis functions, $\Delta_k[\nu; \nu_j, \ldots, \nu_{j+k+1}]$ are denoted $B_j^k(\nu)$ then any $P(\nu)$ can be written as the expansion $P(\nu) = \sum_{j=0}^{M} c_j B_j^k(\nu)$ where $M = n - k - 1$. At low scattering angles, the field autocorrelation function can be represented as (5)

$$G^{(1)}(\tau) = \int_0^\infty \frac{\sin(q\tau\nu)}{q\tau\nu} P(\nu)\, d\nu, \tag{5}$$

where $q$ is the scattering vector (10). Thus, since the basis is one of local support:

$$G^{(1)}(\tau_i) = \sum_{j=0}^{M} c_j \int_{\nu_j}^{\nu_{j+k+1}} \frac{\sin(q\tau_i\nu)}{q\tau_i\nu} B_j^k(\nu)\, d\nu, \tag{6}$$

and the integrals can easily be evaluated for each $B_j^k(\nu)$ and each $\tau_i$.

$$I_{ij} = \int_{\nu_j}^{\nu_{j+k+1}} \frac{\sin(q\tau_i\nu)}{q\tau_i\nu} B_j^k(\nu)\, d\nu, \tag{7}$$

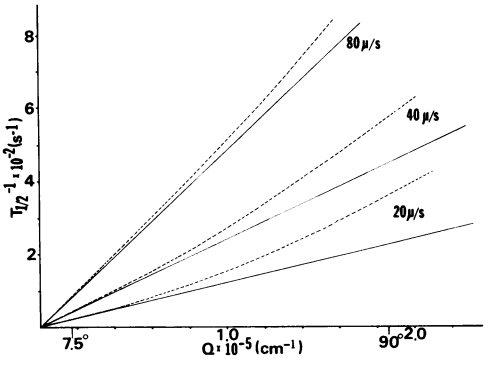


FIGURE 2

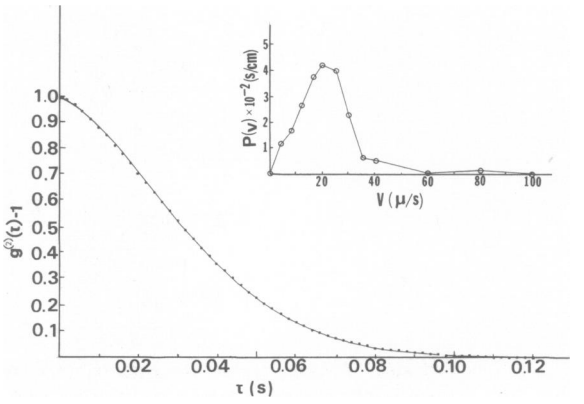


FIGURE 3

FIGURE 2 Graphs of $1/T_{1/2}$ vs. $q$ (scattering vector) for a collection of spheres translating with Maxwellian speed distributions having most probable speeds of 20, 40, and 80 μm/s. The plots are both with (-----) and without (____) concurrent diffusional motion. The results were generated by computer and the diffusion constant used is that measured on a preparation of nonmotile bacteria (*Salmonella typhimurium*) and is equivalent to that of a sphere of diameter 1.2 μm in water at 30°C.

FIGURE 3 Experimental photon autocorrelation function (■), $G^2(\tau) - 1$ normalized to an amplitude of 1, taken at a scattering angle of 7.5° on a preparation of *Salmonella* at 30°C. *S. typhimurium* strain SB3507 (*trpB223*) was grown to mid-log phase in nutrient broth at 35°C, and subsequently centrifuged at 3,000 g into a pellet and resuspended in chemotaxis medium (9) at 30°C. The fitted autocorrelation function (____) has the associated velocity distribution indicated (see inset). This speed distribution is a linear spline fit with 13 knots at the indicated positions (○). $P(v)$ is constrained to be zero at the knots at 0 μm/s and 100 μm/s. The presence of 1.5% dead bacteria in this preparation is implied by this fit ($\beta = 0.015$). Crude estimates of the mean velocity and the fraction of dead bacteria were determined by examination of the sample with a phase microscope and were, respectively, 20 μm/s and <3%.

and thus

$$G^{(1)}(\tau_i) = \sum_{j=0}^{M} c_j I_{ij}. \tag{8}$$

A least-squares fit to the $G^{(1)}(\tau)$ obtained from the experimental $G^{(2)}(\tau)$ (10) can now be performed by varying the parameters $c_j$.

In any preparation of bacteria there is, of course, some fraction of nonmotile organisms. A parameter, $\beta$, was consequently introduced to describe this fraction. In addition, any bacterium moves diffusively whether or not it is also moving actively. This diffuse term is not important for motile bacteria at low angles; but at higher angles, especially when swimming speeds are slow, it can be very important. If $T_{1/2}$, the time for $G^{(1)}(\tau)$ to decay to $\frac{1}{2}$ amplitude, is used as an index of the speed distribution and diffusion is disregarded, the overestimations of $<v>$ from spectral measurements at 90° will be (refer to Fig. 2) 60%, 28%, and 13% for populations of bacteria with Maxwellian speed distributions and most probable speeds of 20, 40, and 80 μm/s,

respectively. This overestimation of speeds which results from neglect of diffusion, is, however, only 5%, 2%, and 1%, respectively, for these same distributions when the spectral measurements are from a scattering angle of 7°. If the translational diffusion constant of a bacterium is $D_T$ and a fraction $\beta$ of the bacteria are nonmotile, then, if diffusion and active translation are assumed to be statistically independent:

$$G^{(1)}(\tau_i) = e^{-D_T q^2 \tau_i}\left(\beta + (1 - \beta)\sum_{j=0}^{M} c_j I_{ij}\right). \tag{9}$$

A fit can now be performed by varying the $c_j$'s and the additional parameter $\beta$. The diffusion constant, $D_T$, can be measured independently on a preparation of nonmotile bacteria. Fig. 3 shows the results of this type of fit to photon correlation data obtained from *Salmonella* under the conditions indicated in the legend. The fit was by a nonlinear least-squares procedure developed by Marquardt (8, 11). This program and others for generation of basis splines are available upon request.

The method of splines described here has been successfully used to extract useful information about bacterial swimming speed distributions from photon correlation data. Although linear splines seem to approximate the data well, it appears that higher order spline fits will serve to smooth the speed distribution function.

An investigation of the errors associated with the estimated $P(v)$ obtained by this method is now in progress. The investigation includes an examination of the sharpness of the minimum that exists in $\chi^2$ space (11) (e.g., cross-correlations between different fitting parameters), the experimental accuracy of the photon autocorrelation functions measured, and the reproducibility of results obtained from different bacterial samples. These and other details and additional experimental results will be presented in a future paper.

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