

SPECTRAL ANALYSIS OF LASER LIGHT SCATTERED FROM MOTILE MICROORGANISMS

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ABSTRACT The theoretical basis of laser scattering from motile microorganisms is examined. Spectra of swimming particles are compared with spectra arising from brownian motion. For mixtures of motile and resting organisms, that part of the spectrum related to the motile organisms is enhanced when $V_s/|k|D$ is large, where V_s is the mean swimming speed of the motile microorganisms, $|k|$ is the Bragg wave vector, and D is the diffusion coefficient of the nonmotile particles. When the directed motion of swimming microorganisms persists for periods which are much longer than $\tau = (|k|V_s)^{-1}$, the scattering spectrum is given as $S(\mathbf{k}, \omega) \propto \tilde{P}(|\omega - \omega_0|/k)$, where \tilde{P} is the probability distribution obtained by two-dimensional integration over the swimming speed distribution. A computation of scattering from bull spermatozoa, based on published velocity distributions, is investigated in detail.

I. INTRODUCTION

Laser light scattering techniques have recently been used to study various biological materials. Measurements have been made of the translational diffusion coefficients of bovine serum albumin, lysozyme, calf thymus DNA, and a hemocyanin (Dubin et al., 1967; Foord et al., 1970), and the translational and rotational diffusion coefficients of tobacco mosaic virus (Cummins et al., 1969). Changes in the width of the scattering spectrum have been used to monitor the equilibrium helix-coil distribution of polybenzyl-L-glutamate (Ford et al., 1969), and related techniques have been used to measure the rate of intramolecular conformational change in poly(dA-dT) (Yeh, 1970).

Bergé et al. (1967) have shown the feasibility of using laser scattering spectroscopy to study the movement of motile microorganisms. They found that the frequency distribution of the scattered light could be correlated with the viability of the microorganisms. The central scattering peak from live spermatozoa was found to be much wider than that from dead spermatozoa, indicating that movement initiated

by the microorganisms leads to spectral broadening. Similar effects were observed when fish spermatozoa were suspended in solutions having differing salinity.

Laser light scattering data can be accumulated rather rapidly for large particles, and laser techniques might be used to study the manner in which the motility of microorganisms is influenced by factors such as temperature or the concentration of salts and metabolites in the suspension medium. Since the scattering is performed upon collections of particles, the information obtained by this method would necessarily pertain to various collective properties of motion. This is in contrast with the sophisticated cinematographic techniques which have been employed, for example, to learn about the detailed tail movements of individual spermatozoa (see, e.g., Rikmenspoel, 1962; Bishop, 1962). However, in principle the utility of laser scattering should be akin to that of other techniques which provide statistical information about motility such as photoelectric scanning of sperm velocity distributions (Rikmenspoel and van Herpen, 1957; van Herpen and Rikmenspoel, 1969). The information gained from the scattering spectra would be complementary to that obtained from other types of measurements.

The purpose of this note is to examine the theoretical basis of laser scattering from motile microorganisms, and thus to better understand the nature of the information which can be obtained from such experiments. In section II, various general relationships between the frequency spectrum of the scattered light and the translational motion of the scattering particles are reviewed. Although dead microorganisms—which move due to the brownian forces arising from the fluid—scatter light according to a lorentzian frequency spectrum, the scattering spectra of living microorganisms can have very different character. In general, the calculation of these spectra is quite difficult. However, simplification occurs if the directed velocities resulting from the motile activities of the organisms persist for times which are long compared with times characteristic of the relaxation of the laser scattering spectra. For such cases the spectral distributions are related in a simple way to the velocity distributions of the motile particles. This point is discussed in section III, and calculations are performed for some specific examples. Section IV contains additional discussion and brief commentary regarding the manner in which laser light scattering might be used to probe particles which are moving in response to chemotactic agents.

II. SCATTERING SPECTRA (INTRODUCTORY COMMENTS)

General aspects of the theory of laser scattering have recently been reviewed in a number of publications (see, e.g., Cummins et al., 1969; Yeh and Keeler, 1969). As indicated in these studies, when the size of the scattering particle either exceeds or is of the same order as the wavelength of the incident radiation (i.e., θ [3000 Å]), the scattered spectrum may contain contributions due to rotational diffusion. Thus, for large angles the information contained in the scattering spectrum may depend

strongly on the shape of the scattering particles. However, considerable simplification occurs when the angle between the incident and scattered light is small (Pecora, 1968). In this case, the total scattering spectrum $S(\mathbf{k}, \omega)$ can be described as

$$S(\mathbf{k}, \omega) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \exp [i(\omega - \omega_0)t] C_\phi(\mathbf{k}, t) dt, \quad (1)$$

where the "correlation function" $C_\phi(\mathbf{k}, t)$ is given as

$$C_\phi(\mathbf{k}, t) = |A|^2 \left\langle \sum_{i=1}^N \sum_{j=1}^N \exp [-i\mathbf{k} \cdot \mathbf{R}_i(0)] \exp [i\mathbf{k} \cdot \mathbf{R}_j(t)] \right\rangle. \quad (2)$$

In the above expression, $\mathbf{R}_i(0)$ and $\mathbf{R}_j(t)$ are, respectively, the positions of the i th particle at time 0 and j th particle at time t . The joint expectation $\langle \exp [-i\mathbf{k} \cdot \mathbf{R}_i(0)] \exp [i\mathbf{k} \cdot \mathbf{R}_j(t)] \rangle$ generally is taken with respect to an equilibrium or stationary state, and the summation is over all particles of the scattering assembly. The scattering amplitude A is assumed here to be the same for all particles. In equation 1, ω and ω_0 are the frequencies of the scattered and incident light.

The wave vector \mathbf{k} is related to the scattering angle ϕ according to

$$|\mathbf{k}| = \frac{4\pi n_0}{\lambda} \sin(\phi/2). \quad (3)$$

In this expression, λ is the wavelength of the incident light and n_0 is the index of refraction of the solvent. The criterion for applicability of equations 1 and 2 is that $kL/4 \lesssim 1$, where L is the maximum dimension of the particle (Pecora, 1968). In the following, it is assumed that this condition is met; for particles whose maximum dimension is $\sim 10,000$ Å, an appropriate scattering angle would be $\lesssim 15^\circ$ when using a He-Ne laser ($\lambda = 6328$ Å).

Let us consider the correlation function $C_\phi(\mathbf{k}, t)$ and assume that the particles move independently of each other. (In certain cases, achieving this condition would require that scattering experiments be performed on dilutions differing considerably from normal physiological concentrations.) For such a situation, contributions to the sum in equation 2 will arise only from the terms for which $i = j$. If all particles are assumed to be alike, $C_\phi(\mathbf{k}, t)$ is given as

$$\begin{aligned} C_\phi(\mathbf{k}, t) &= N |A|^2 \langle \exp \{i\mathbf{k} \cdot [\mathbf{R}_i(t) - \mathbf{R}_i(0)]\} \rangle \\ &= N |A|^2 \left\langle \exp \left[i\mathbf{k} \cdot \int_0^t \mathbf{v}_i(\tau) d\tau \right] \right\rangle, \end{aligned} \quad (4)$$

where $\mathbf{v}_i(\tau)$ is the velocity of the i th (arbitrary) particle at time τ .

The entire problem now rests in evaluating the expectation on the right-hand side of equation 4. The exponential may be expanded in a Taylor's series and each component of the resulting expression be considered separately. In the absence

of any intrinsic spatial anisotropy, all odd terms of the series vanish so that

$$\begin{aligned} \left\langle \exp \left[i\mathbf{k} \cdot \int_0^t \mathbf{v}(\tau) d\tau \right] \right\rangle &= 1 - \frac{1}{2!} \int_0^t dy \int_0^t dz \langle [\mathbf{k} \cdot \mathbf{v}(y)][\mathbf{k} \cdot \mathbf{v}(z)] \rangle \\ &+ \frac{1}{4!} \int_0^t dx \int_0^t dw \int_0^t dy \int_0^t dz \langle [\mathbf{k} \cdot \mathbf{v}(x)][\mathbf{k} \cdot \mathbf{v}(w)][\mathbf{k} \cdot \mathbf{v}(y)][\mathbf{k} \cdot \mathbf{v}(z)] \rangle \dots \quad (5) \end{aligned}$$

Although isotropic conditions will not always pertain as, for example, when light scattering is used to probe chemotactic response (see section IV, below), equation 5 is appropriate for most applications.

It is now instructive to evaluate equation 5 for the case of brownian particles. Although the result can be derived by other procedures (for example, by solving the diffusion equation), the foregoing analysis clearly points up requirements about the scattering system which are necessary for a lorentzian spectrum to be obtained. In the following section, modifications due to the special kinetic properties of swimming microorganisms are examined.

When the movement of the particles is determined only by brownian forces arising from the random molecular motions of the solvent, particle motion is distributed as a gaussian random variable with zero mean (Chandrasekhar, 1943). The evaluation of the series of equation 5 is easily accomplished for gaussian particles, since all higher order correlations factor into sums of products of pair correlations. One finds (Kubo, 1963)

$$\left\langle \exp \left[i\mathbf{k} \cdot \int_0^t \mathbf{v}(\tau) d\tau \right] \right\rangle = \exp \left[-\frac{1}{2} W(t) \right], \quad (6)$$

where $W(t)$ is defined as

$$W(t) = \int_0^t \int_0^t \langle [\mathbf{k} \cdot \mathbf{v}(y)][\mathbf{k} \cdot \mathbf{v}(z)] \rangle dydz. \quad (7)$$

Because of spatial isotropy and time translational invariance, one may express $W(t)$ as

$$\begin{aligned} W(t) &= \frac{k^2}{3} \int_0^t \int_0^t \langle \mathbf{v}(0) \cdot \mathbf{v}(z - y) \rangle dydz \\ &= \frac{2}{3} k^2 \int_0^t (t - \xi) \langle \mathbf{v}(0) \cdot \mathbf{v}(\xi) \rangle d\xi. \quad (8) \end{aligned}$$

We note that equations 6-8 pertain *only* to particles whose statistical properties are gaussian with zero mean velocity.

In the absence of a motive force originating with the microorganism, the velocity $\mathbf{v}(t)$ could be described by the Langevin equation (Chandrasekhar, 1943). By solving for $\mathbf{v}(t)$ from this equation (which appears in modified form as equation 34,

below) and assuming that the forces arising from the fluid are not correlated with the instantaneous velocity of the particle, one finds

$$\langle \mathbf{v}(0) \cdot \mathbf{v}(t) \rangle = \langle [v(0)]^2 \rangle e^{-\beta t/m}. \quad (9)$$

In equation 9, β is the friction coefficient and m is the mass of the particle. For this form of the velocity autocorrelation function the integral of equation 8 is easily evaluated, yielding

$$W(t) = \frac{2k^2 m^2}{3\beta^2} [e^{-\beta t/m} - 1 + \beta t/m] \langle [v(0)]^2 \rangle. \quad (10)$$

The second important requirement now arises, viz., that for times of experimental relevance the quantity $\beta t/m$ be much greater than 1. In this case equation 10 simplifies to

$$W(t) \approx \frac{2k^2 m t}{3\beta} \langle [v(0)]^2 \rangle, \quad (11)$$

so that from the definition of the diffusion coefficient (Zwanzig, 1965)

$$D = \frac{m}{3\beta} \langle [v(0)]^2 \rangle, \quad (12)$$

one finds from equation 6 that C_ϕ can be expressed as

$$C_\phi(\mathbf{k}, t)_{\text{brownian}} = N |A|^2 e^{-Dk^2 t}. \quad (13)$$

Indeed, the limiting procedure used to obtain equation 11 is appropriate for laser scattering experiments performed on large brownian particles, since experimental resolution times are of the order of 10^{-5} sec or greater (Chen and Polonsky-Ostrowsky, 1969), whereas molecular relaxation times m/β are of the order of 10^{-7} – 10^{-8} sec.

III. SCATTERING SPECTRA FOR MOTILE MICROORGANISMS WITH NONGAUSSIAN VELOCITY DISTRIBUTIONS

Consider, now, calculation of the scattering spectrum of swimming microorganisms. It is immediately apparent that two important modifications must be made. First, the distribution of the velocities of the particles is not a simple gaussian centered about $v = 0$, so that equation 7 is not valid. Second, the correlation between the velocity of a swimming particle at two different times may persist for periods which are long compared with the characteristic measurement time of the spectrometer. In this case the limiting procedure used to derive equation 11 is not applicable.

Let us now assume that the assembly of microorganisms is composed of two populations, one which is swimming and the other which is "resting". The latter might be composed of either dead microorganisms or organisms having temporarily low metabolic activity, but the important point is that in both cases such particles move primarily as a result of the action of brownian forces. As seen either from equation 5 or equation 2, the correlation function C_ϕ must be written as

$$C_\phi(t) = N |A|^2 \{ \alpha C_{\phi 1}(t) + (1 - \alpha) C_{\phi 2}(t) \}, \quad (14)$$

where α is the fraction of the total population in the swimming group. The second term in the above expression pertains to the "essentially brownian" particles, so that $C_{\phi 2}$ is evaluated by the arguments which led to equation 13. On the other hand, the evaluation of the first term $C_{\phi 1}$ is quite different.

To illustrate this point let us consider the simple model that all swimming particles have the same speed, although moving in totally random directions. In this case one can solve for the directed velocity distribution function of the swimming particles, $\bar{P}(v_x)$. This function is needed for computing the expectations in the series given by equation 5, and is calculated from the joint probability distribution $P(v_x, v_y, v_z)$ by integrating over the extraneous variables, i.e.,

$$\bar{P}(v_x) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P(v_x, v_y, v_z) dv_y dv_z. \quad (15)$$

For a model of uniformly speedy particles, the probability distribution for the total velocity is given as

$$P(v_x, v_y, v_z) = \frac{1}{4\pi V_s^2} \delta(|\mathbf{v}| - V_s), \quad (16)$$

where V_s is the swimming speed, $\delta(x)$ denotes the Dirac delta function, and $4\pi V_s^2$ is a normalization factor.¹ Consequently, from equation 15 one finds the simple result

$$\bar{P}(v_x) = \begin{cases} \frac{1}{2V_s} & \text{for } |v_x| \leq V_s \\ 0 & \text{for } |v_x| > V_s \end{cases}. \quad (17)$$

Note that in equation 5 the scattering function is expressed as a series of multiple time correlation functions. Thus, at first glance, it seems necessary to know the

¹ $1 = A \iiint \delta(|\mathbf{v}| - V_s) dv_x dv_y dv_z = \int_0^{2\pi} d\varphi \int_0^\pi \sin \theta d\theta \int_0^\infty \delta(v - V_s) v^2 dv$
 $= 4\pi A V_s^2.$

details by which the swimmers change speed and direction in order to obtain the scattering spectrum. Fortunately, the situation is not quite so difficult; whereas the correlation functions for brownian particles relax in times which are *short* compared with the characteristic time scale of measurement, the correlations of swimming motion may persist for times which are *long* with respect to characteristic measurement times. Indeed, it has been reported that the directed motions of bull sperm persist for several seconds (Gray, 1958; Rikmenspoel et al., 1960). For this case it is necessary only to know the equilibrium velocity distribution of the swimming organisms in order to obtain the spectrum.

Consider, for example, the second term on the right-hand side of equation 5. During the time of measurement the velocity of a particle is almost constant, i.e. $\mathbf{v}(t) \approx \mathbf{v}(0)$, implying (Kubo, 1963)

$$\int_0^t \int_0^t \langle [\mathbf{k} \cdot \mathbf{v}(s)][\mathbf{k} \cdot \mathbf{v}(z)] \rangle dsdz \approx \frac{1}{3} t^2 k^2 \langle v(0)^2 \rangle. \quad (18)$$

The equilibrium correlation function $\langle v(0)^2 \rangle$ must now be evaluated according to equation 17. One finds

$$\frac{1}{3} \langle v(0)^2 \rangle = \langle v_x^2 \rangle = \int_{-\infty}^{\infty} v_x^2 P(v_x) dv_x = \frac{1}{3} V_s^2. \quad (19)$$

Similar approximations are suitable for other terms in the series, so that

$$\begin{aligned} \left\langle \exp \left[i\mathbf{k} \cdot \int_0^t \mathbf{v}(\tau) d\tau \right] \right\rangle &\approx 1 - \frac{t^2 k^2}{2!} \left\{ \frac{1}{3} V_s^2 \right\} + \frac{t^4 k^4}{4!} \left\{ \frac{1}{5} V_s^4 \right\} - \dots \\ &= \sum_{j=0}^{\infty} \frac{(-1)^j (tkV_s)^{2j}}{(2j+1)!}. \end{aligned} \quad (20)$$

But, as can be seen by examining the series expansion of $\sin(x)$, equation 20 can be written more simply as

$$\left\langle \exp \left[i\mathbf{k} \cdot \int_0^t \mathbf{v}(\tau) d\tau \right] \right\rangle_{\text{swimmers}} = \frac{1}{tkV_s} \sin(tkV_s). \quad (21)$$

Is the approximation invoked to derive equation 18 really valid? Typical speeds for bull sperm are approximately 100 μ /sec (van Herpen and Rikmenspoel, 1969), so that $\langle v(0)^2 \rangle \sim 10^{-4}$ cm²/sec². Also, with a He-Ne laser and a scattering angle of $\sim 15^\circ$, one finds from equation 3 that $k^2 \sim 10^8$ cm⁻². The relaxation curve $C_{\phi 2}$ is roughly equal to e^{-1} times its maximum when the quantity $(k^2/2) \langle v_x^2 \rangle t^2$ is equal to 1, indicating that $t \sim \mathcal{O}(10^{-2}$ sec) is an appropriate time scale of measurement. Indeed, this is much less than the reported correlation times for the linear motion of spermatozoa (Gray, 1958).

From equations 21, 13, and 14 one finds the following expression for the correlation function $C_\phi(\mathbf{k}, t)$

$$C_\phi(\mathbf{k}, t) = N |A|^2 \left\{ \frac{\alpha}{ikV_s} \sin(tkV_s) + (1 - \alpha)e^{-k^2Dt} \right\}. \quad (22)$$

Finally, upon taking Fourier transforms, the corresponding frequency spectrum is found to be (cf. equation 1)

$$S(\mathbf{k}, \omega) = N |A|^2 \left\{ \frac{\alpha}{2|k|V_s} H(|\omega - \omega_0| - |k|V_s) + \frac{(1 - \alpha)}{\pi} \frac{k^2D}{(\omega - \omega_0)^2 + (k^2D)^2} \right\}, \quad (23)$$

where

$$H(x) = \begin{cases} 1 & \text{if } x < 0 \\ 0 & \text{if } x > 0. \end{cases}$$

Simple analytical expressions may also be obtained for another illustrative case. When the swimming speed is assumed to be uniformly distributed up to V_s , $P(\mathbf{v})$ is given as

$$P(\mathbf{v}) = \begin{cases} \frac{3}{4\pi V_s^3} & \text{for } |\mathbf{v}| \leq V_s \\ 0 & \text{for } |\mathbf{v}| > V_s, \end{cases} \quad (24)$$

and $\bar{P}(v_x)$ is

$$\bar{P}(v_x) = \begin{cases} \frac{3}{4V_s^3} (V_s^2 - v_x^2) & \text{for } |v_x| \leq V_s \\ 0 & \text{for } |v_x| > V_s. \end{cases} \quad (25)$$

It is interesting to compare the concomitant spectrum with that given by equation 23, in order to see how well laser scattering experiments can resolve basic differences in underlying velocity distributions.

By the same arguments which led to equation 22 and 23, one now finds

$$C_\phi(\mathbf{k}, t) = N |A|^2 \left\{ \left\{ 3\alpha \left[\frac{\sin tkV_s}{ikV_s} + 2 \frac{\cos tkV_s}{(tkV_s)^2} - 2 \frac{\sin tkV_s}{(tkV_s)^3} \right] + (1 - \alpha)e^{-k^2Dt} \right\} \right\}, \quad (26)$$

and

$$S(\mathbf{k}, \omega) = \begin{cases} N |A|^2 \left\{ \frac{3\alpha(k^2 V_s^2 - (\omega - \omega_0)^2)}{4(|k| V_s)^3} + \frac{(1 - \alpha)k^2 D}{\pi[(\omega - \omega_0)^2 + (k^2 D)^2]} \right\} & \text{for } |\omega - \omega_0| < kV_s \\ N |A|^2 \frac{(1 - \alpha)}{\pi} \frac{k^2 D}{[(\omega - \omega_0)^2 + (k^2 D)^2]} & \text{for } |\omega - \omega_0| > kV_s. \end{cases} \quad (27)$$

The expressions given by equation 22, 23, 26, and 27 are plotted in Fig. 1, where they are compared with corresponding expressions for purely brownian motion. α has been taken to be 0.5 for these calculations, as a representative value signifying equal populations of swimming and resting organisms. Clearly, a value of α close to 1 would enhance that part of the spectrum due to the swimming organisms. These calculations indicate, also, that the higher the ratio γ —defined as $\gamma \equiv V_s / |k| D$ —the easier it is to see that part of the spectrum due to the motile particles. For large γ , the spectrum of the swimmers will be broad compared with the spectrum due to the brownian particles, and therefore less of the spectrum will be hidden in the lorentzian peak (see Fig. 1 *b*). Consequently, the smallest possible scattering angles should be used. (For illustrative purposes, a value of γ equal to 10 has been chosen for Fig. 1, corresponding, for example, to $D = 10^{-9}$ cm²/sec, $k = 10^6$ cm⁻¹, and $V_s = 10$ μ /sec).

As indicated in Fig. 1, it appears easier to obtain experimental parameters from frequency spectra rather than from time domain data. Moreover, concerning the question of resolution of basic differences in the underlying velocity distributions, it seems that the general form of the distribution $P(\mathbf{v})$ needs to be known from other measurements in order to comprehend the light scattering data. However, once the general form of $P(\mathbf{v})$ is known, the laser scattering experiments can be employed to measure changes in characteristic parameters.

The latter point is further illustrated in the following example. Consider that $P(\mathbf{v})$ is

$$P(\mathbf{v}) = B \exp [-\frac{1}{2} (|\mathbf{v}| - V_s)^2 / \sigma], \quad (28)$$

a distribution with a spread about the swimming speed V_s . As shown in Fig. 2 *a*, this expression is a good approximation of measured velocity distributions for bull sperm (van Herpen and Rikmenspoel, 1969); equation 21 is a limiting case of this expression. One finds from normalizing $P(\mathbf{v})$ that B is given as

$$B = \frac{1}{2\pi(2\sigma)^{3/2}} \cdot \left[\frac{V_s}{(2\sigma)^{1/2}} \exp [-V_s^2 / 2\sigma] + \frac{\sqrt{\pi}}{2} \left(1 + \frac{V_s^2}{\sigma} \right) \left\{ 1 + \operatorname{erf} \left(\frac{V_s}{\sqrt{2\sigma}} \right) \right\} \right]^{-1}, \quad (29)$$

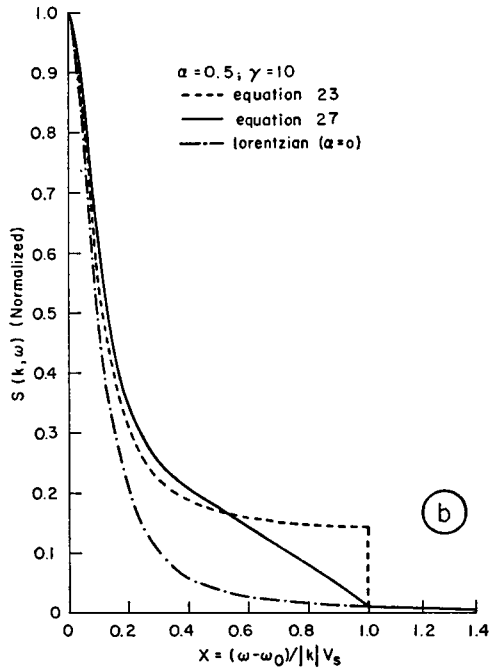
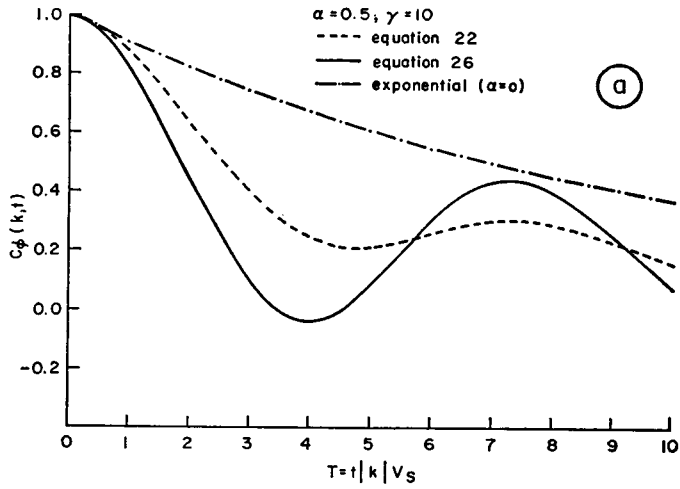


FIGURE 1 Spectra for distributions given by equations 17 and 24. (a) $C_p(k, t)$ as a function of the reduced time variable $T = t|k|V_S$. The scattering assembly is assumed to be composed of equal numbers of swimming and nonswimming particles (i.e., $\alpha = 0.5$); $V_S/|k|D = 10$. The curve for a brownian particle is presented for comparison. (b) The corresponding spectra $S(k, \omega)$, as functions of the reduced frequency variable $X = (\omega - \omega_0)/|k|V_S$ (normalized, so that $S(k, 0) = 1$).

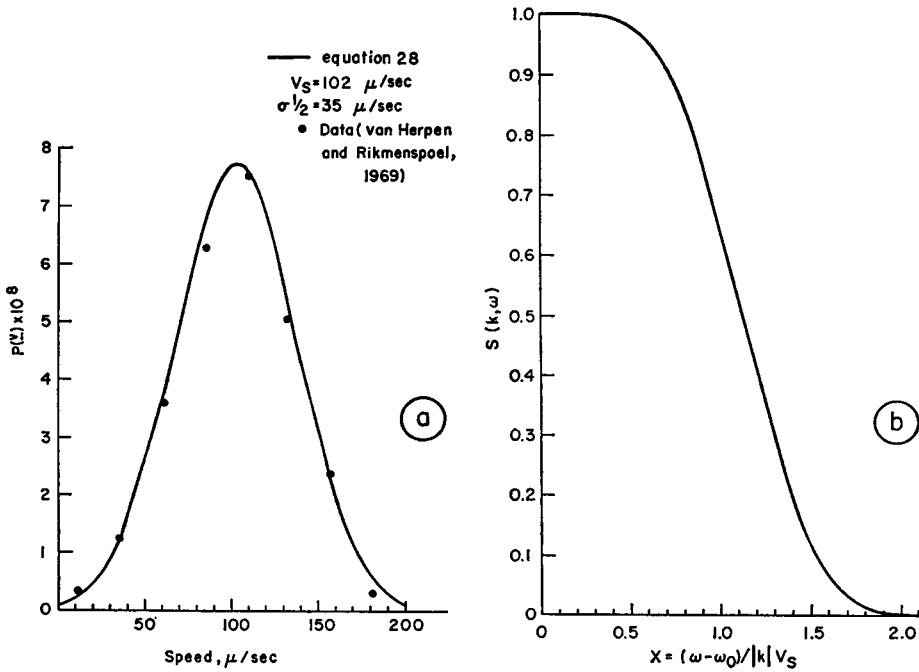


FIGURE 2 Computations for bull spermatozoa. (a) The velocity distribution $P(v)$ for bull sperm which have been diluted into a modified Krebs-Ringer solution. The data have been taken from a publication by van Herpen and Rikmenspoel (1969), and are seen here to be well represented by a truncated gaussian with $V_S = 102 \mu/\text{sec}$ and $\sigma^{1/2} = 35 \mu/\text{sec}$ (see equation 28). (b) The corresponding scattering spectrum $S(k, \omega)$, plotted as a function of the variable $X = (\omega - \omega_0) / |k| V_S$ (cf. equations 32 and 30).

where the error function is defined as $\text{erf}(x) = (2/\sqrt{\pi})(\int_0^x e^{-y^2} dy)$. Thus, $\bar{P}(v_x)$, calculated according to equation 15, is

$$\bar{P}(v_x) = \begin{cases} 2\pi B \left\{ \sigma \exp[-(v_x - V_S)^2/2\sigma] + \sigma^{1/2} V_S \sqrt{\frac{\pi}{2}} \left[1 + \text{erf}\left(\frac{V_S - v_x}{\sqrt{2\sigma}}\right) \right] \right\} & \text{if } v_x > 0, \\ 2\pi B \left\{ \sigma \exp[-(v_x + V_S)^2/2\sigma] + \sigma^{1/2} V_S \sqrt{\frac{\pi}{2}} \left[1 - \text{erf}\left(\frac{V_S + v_x}{\sqrt{2\sigma}}\right) \right] \right\} & \text{if } v_x < 0. \end{cases} \quad (30)$$

It is seen immediately that the velocity correlation functions—which are required for the time domain expressions—are going to be rather complicated functions. If the particle motions were not correlated over long times, such terms would have to be considered when computing scattering spectra. However, it follows from equation 1 and the arguments made prior to equation 18 that in the

present case $S(\mathbf{k}, \omega)$ is given as

$$\begin{aligned} S(\mathbf{k}, \omega) &= \frac{N |A|^2}{2\pi} \int_{-\infty}^{\infty} \exp [i(\omega - \omega_0)t] \langle \exp [i(\mathbf{k} \cdot \mathbf{v})t] \rangle dt \\ &= N |A|^2 \left\langle \frac{1}{2\pi} \int_{-\infty}^{\infty} \exp \{i[\omega - \omega_0 + k v_x]t\} dt \right\rangle \\ &= N |A|^2 \langle \delta(|\omega - \omega_0| + |k| v_x) \rangle, \end{aligned} \quad (31)$$

where $\delta(x)$ is the Dirac delta function. But, since the expectation of the δ function implies

$$\langle \delta(|\omega - \omega_0| + |k| v_x) \rangle = \frac{1}{|k|} \int_{-\infty}^{\infty} \delta(|\omega - \omega_0| + y) \bar{P}(y/|k|) dy,$$

it is seen that $S(\mathbf{k}, \omega)$ is simply related to $\bar{P}(v)$ by

$$S(\mathbf{k}, \omega)_{\text{swimmers}} = \frac{N |A|^2}{|k|} \bar{P} \left(\left| \frac{\omega - \omega_0}{k} \right| \right). \quad (32)$$

Thus, for organisms whose swimming motions are *correlated over long times*, the frequency spectrum is homologous with the reduced distribution function of directed velocities.

The spectrum $S(\mathbf{k}, \omega)$ corresponding to the bull sperm swimming speed distribution of equation 28 may now easily be calculated from equation 32. The result is presented in Fig. 2 *b*. The frequency can be scaled as a function of $|k| V_s$ and the swimming speed can therefore be determined by changing the scattering angle. Further, the ratio $\sqrt{\sigma}/V_s$ can be estimated from the derivative of the spectrum, since the latter is given as

$$\frac{dS(\mathbf{k}, \omega)}{d\omega} \propto - N |A|^2 \frac{(\omega - \omega_0)}{|k|^3} \exp [-(\omega - \omega_0 - k V_s)^2 / 2k^2 \sigma]. \quad (33)$$

In this way, both of the relevant parameters, V_s and σ , can be determined.

IV. FURTHER REMARKS

The calculation of spectra becomes much more difficult for situations where the time scales of motion are not readily separable. In this case a good starting point seems to be the Langevin equation, modified to have the form

$$m \frac{d\mathbf{v}}{dt} = -\beta \mathbf{v} + \mathbf{X}(t) + \mathbf{F}_s(t), \quad (34)$$

where β is a frictional damping term, $\mathbf{X}(t)$ represents the random brownian forces arising from the fluid, and $\mathbf{F}_s(t)$ is the metabolic force exerted by the micro-

organism itself. β is related to $X(t)$ according to (Kirkwood, 1946)

$$\beta = \frac{1}{3k_B T} \lim_{\epsilon \rightarrow 0} \int_0^\infty e^{-\epsilon t} \langle X(0) \cdot X(t) \rangle dt. \quad (35)$$

The solution of this equation would proceed by evaluation of the terms of the series given by equation 5, but would involve several difficulties. In particular, one would need to know the details of a model for the time dependence of the swimming force. Consequently, it might be impossible to utilize all of the information theoretically inherent in the scattering, and one might examine only the various frequency moments of the scattering spectrum. These moments may be related to the equilibrium velocity distribution; for example, the second moment of the spectrum is

$$\int_{-\infty}^{\infty} \omega^2 S(\mathbf{k}, \omega) d\omega = k^2 \langle v_x^2 \rangle. \quad (36)$$

Finally, let us briefly consider an experiment to measure chemotactic response. In this case spatial isotropy does not pertain because the microorganisms will be streaming towards the chemotactic agent. The velocity might now be expressed as

$$\mathbf{v} = \mathbf{v}_d + \mathbf{v}_{sw}, \quad (37)$$

where \mathbf{v}_d is the *nonzero* average velocity due to the chemical signal. Instead of equation 5, one has

$$\left\langle \exp \left[i\mathbf{k} \cdot \int_0^t \mathbf{v}(\tau) d\tau \right] \right\rangle = \exp [i(\mathbf{k} \cdot \mathbf{v}_d)t] \left\langle \exp \left[i\mathbf{k} \cdot \int_0^t \mathbf{v}_{sw}(\tau) d\tau \right] \right\rangle. \quad (38)$$

Consequently, when Fourier transforms are taken according to equation 1, one finds that the scattering spectrum $S(\mathbf{k}, \omega)$ no longer is symmetric with respect to $\omega - \omega_0$. Thus, measurements at different scattering angles should yield the "chemotactic velocity" \mathbf{v}_d , which would be determined from concomitant shifting of the scattering spectrum along the frequency axis.

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