



SEDIMENTING PARTICLES AND SWIMMING MICRO-ORGANISMS IN A ROTATING FLUID

J. O. Kessler*, N. A. Hill**, R. Strittmatter* and D. Wiseley*

*Physics Department, University of Arizona, Tucson AZ 85721, U.S.A.

**Department of Applied Mathematical Studies, University of Leeds, Leeds LS2 9JT, U.K.

ABSTRACT

Experiments and calculations on the trajectories of micron-sized spheres, suspended in a fluid that fills a closed container which rotates about an axis perpendicular to g , relate to the planning and interpretation of clinostat experiments. For low Reynolds number motion, the orbits are nearly circular, the radius being inversely proportional to the rotation rate. The swimming direction of micro-organisms can be affected by light, gravity, vorticity etc. The trajectories of algae swimming in steadily rotating environments have been observed and compared with theoretical predictions for ideal gyrotactic micro-organisms, thus providing some insights into the mechanisms of gravitaxis, gyrotaxis and the behaviour of the cells.

INTRODUCTION

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When gravity is absent, as in free fall, all motions and stresses normally caused by it cease. Consider any system, such as a plant (Sachs, 1877) or a chamber filled with fluid that contains particles (Goldberg *et al.*, 1958), mounted in a device that rotates about an axis perpendicular to the force of gravity, g . Such a device is called a clinostat and its contents are said to undergo clinorotation. When viewed in a frame of reference rotating with the clinostat, g rotates and thus particles suspended in the fluid in the chamber execute circular orbits that follow the rotating g vector. Evidently clinorotation is never equivalent to free fall. Nevertheless, it is useful for the investigation of gravitational interactions with biological systems (see Kessler (1992) and other references in that issue of the Bulletin). Ayed *et al.* (1992) calculated the trajectories of a swimming micro-organism numerically, using a two-dimensional model of a cylindrical *Paramecium* inside a cylindrical test tube that rotated rapidly about its axis, which was horizontal. They included the effects of the side walls of the test tube on the flow of the fluid and concluded that the swimming cell would remain suspended and not come into contact with the walls. They also carried out experiments on the growth rate of *Paramecia* in such a clinostat.

In this paper, we assume that the micro-organisms are sufficiently small that wall effects are negligible and we present: (1) mathematical descriptions of the trajectories of inert sedimenting spheres within rotating fluid when the spheres and the rotation rate are sufficiently small that their motion is dominated by viscosity; (2) theoretical trajectories for ideal gyrotactic swimming micro-organisms in a rotating fluid; and (3) preliminary experimental results on the paths of algae swimming in a cuvette rotating about a horizontal axis.

ROTARY SEDIMENTATION

Suppose that a small sphere of mass m , density ρ , radius a and volume $v = 4\pi a^3/3$ is suspended in water of density ρ_w and viscosity η . The fluid rotates uniformly with constant angular velocity Ω rad s^{-1} about a horizontal axis. Since g is vertical, the sphere's trajectory is confined to a vertical plane perpendicular to the axis of rotation. In this plane, we define an inertial (or laboratory) frame of reference, Oxy , where O lies on the axis of rotation and x and y are the horizontal and vertical coordinates respectively. Relative to Oxy , the angular velocity, Ω , is measured in the positive anticlockwise sense. In complex notation, the coordinates for the sphere's centre are $x + iy = z$, where $i = \sqrt{-1}$. In the inertial frame of reference, the equation of motion (Newton's Second Law) can be written as

$$m \frac{d^2 z}{dt^2} + \lambda \frac{dz}{dt} + (m_w \Omega^2 - i\Omega\lambda) z = -ig\Delta m, \quad (1)$$

where $\Omega \equiv |\Omega|$, $\Delta m = m - m_w$, $m_w = v\rho_w$ and $\lambda = 6\pi\eta a$ is the viscous drag coefficient for a sphere of radius a . The pressure gradient term $m_w\Omega^2$ arises because the fluid is in solid body rotation and provides the necessary centripetal force.

A frame of reference $Ox_{\text{rot}}y_{\text{rot}}$ that rotates with the fluid is defined by

$$q \equiv x_{\text{rot}} + iy_{\text{rot}} = ze^{-i\Omega t} \quad (2)$$

and the equation of motion becomes

$$m \frac{d^2 q}{dt^2} + (2i\Omega m + \lambda) \frac{dq}{dt} - \Omega^2(m - m_w)q = -ig\Delta m e^{-i\Omega t}. \quad (3)$$

We now see that the pressure gradient $m_w\Omega^2$ opposes the centrifugal force $m\Omega^2$ and that the Coriolis acceleration is $2i\Omega(dq/dt)$.

These equations require that the Reynolds number,

$$\text{Re} = \frac{\rho_w a}{\eta} \left| \frac{dq}{dt} \right| \ll 1, \quad (4)$$

and that the sedimenting sphere does not substantially affect the uniform rotation of the fluid. In our experiments, this condition is satisfied since $a \simeq 10^{-3}$ cm, $\eta = 10^{-2}$ g cm⁻¹ s⁻¹, $\rho_w = 1$ g cm⁻³ and $|dq/dt| \simeq 10^{-3}$ cm s⁻¹ so that $\text{Re} = 10^{-4}$. We have also assumed that the sedimenting particle is far from the walls of the container. This is not always true in practice and modifies the observed particle trajectories. Furthermore, if the rotation axis is not perfectly horizontal, the motion of the spheres will not be confined to a plane perpendicular to the axis of rotation, which introduces additional differences between the observed trajectories and this simple theory.

The solution of Eq. (1) is

$$z(t) = Ae^{\sigma+t} + Be^{\sigma-t} - ig(\Delta m/m)(\gamma\Omega^2 - i\alpha\Omega)^{-1}, \quad (5)$$

where $\gamma = \rho_w/\rho$ and

$$2\sigma_{\pm} = -\alpha \pm \frac{[(\alpha^2 - 4\gamma\Omega^2)^2 + (4\Omega\gamma)^2]^{1/4}}{[(\alpha^2 - 4\gamma\Omega^2)^2 + (2\Omega\gamma)^2]^{1/2}} [\alpha^2 - 4\gamma\Omega^2 + 2\Omega\alpha i]. \quad (6)$$

For cases of interest here, $\gamma \simeq 1$, $\Omega \leq 10$ and $\alpha \equiv \lambda/m \simeq 5 \times 10^4$ s $\gg 1$ s. On retaining terms to $O(\Omega^2/\alpha^2)$ in Eq. (6), Eq. (5) simplifies to

$$z(t) = A \exp(i\Omega t + t/\tau) + B \exp(-\alpha t) \exp(-i\Omega t - t/\tau) + (V_{\text{sed}}/\Omega)(1 + i\gamma\Omega/\alpha)^{-1}. \quad (7)$$

The entire 'B-term' is transient and can be ignored, due to the rapid decay of $\exp(-\alpha t)$ because the centrifugal time $\tau = (\alpha m/\Omega^2 \Delta m)$ is very large. The sedimentation speed of the sphere is

$$V_{\text{sed}} \equiv g\Delta m/\lambda = (g/\alpha)(1 - \gamma) \simeq 2 \times 10^{-2} \text{ cm s}^{-1} \quad (8)$$

for the above values of a and η . Setting $z = z(0)$ and $dz/dt = 0$ at $t = 0$, and ignoring the small term Ω/α ,

$$z(t) = [z(0) - R] \exp(i\Omega t + t/\tau) + R, \quad (9)$$

where $R = V_{\text{sed}}/\Omega$. In the rotating frame of reference, this becomes

$$q(t) = z(t)e^{-i\Omega t} = [z(0) - R]e^{t/\tau} + Re^{-i\Omega t}. \quad (10)$$

Rewriting Eq. (10) gives

$$x_{\text{rot}} = [x_{\text{rot}}(0) - R] e^{t/\tau} + R \cos \Omega t \quad (11)$$

and

$$y_{\text{rot}} = [y_{\text{rot}}(0) - R] e^{t/\tau} - R \sin \Omega t. \quad (12)$$

Eqs. (11) and (12) show that, in a rigidly rotating fluid, gravity causes small particles to move in circular orbits of radius R , the centres of which drift very slowly away from the axis of rotation. If initially $z(0) = R$, the outward drift vanishes, but this condition cannot be achieved in practice due to disturbances when starting up the experiments and to Brownian motion. Given the underlying assumptions the solutions Eqs. (4) and (5) are exact and can be used in a wide range of investigations. The theory for large values of Ω and for other less restrictive conditions is given by Herron *et al.* (1975).

SWIMMING MICRO-ORGANISMS

We now consider the trajectories of swimming micro-organisms suspended in a uniformly rotating fluid. Several physical forces may influence their direction of motion. If a cell is bottom heavy, its centre of mass is anterior to its centre of buoyancy so that gravity imposes a torque that tends to orient the cell to swim vertically upwards relative to the fluid. Also, the flow of the fluid about a spherical cell exerts a viscous torque on its surface which is proportional to the vorticity. In the case of a fluid in rigid body rotation, the vorticity has the same value 2Ω , i.e. twice the rotation rate of the fluid measured in rad s^{-1} , throughout the fluid. The balance between the gravitational and viscous torques is known as gyrotaxis. A gyrotactic organism may therefore swim at a non-zero angle θ relative to the vertical (measured in the positive clockwise sense), or it may tumble, if $|\Omega|$ is sufficiently large. The velocity of the cell relative to the laboratory frame of reference is the swimming velocity of the cell relative to the fluid plus the advection by the fluid itself.

Another effect of rotation is that \mathbf{g} appears to be rotating in the rotating frame of reference. If the organism actively senses the direction of gravity, this rotation may cause sensing to cease, lag or generate erroneous signals above some critical value of Ω . There may also be resonances with the flagellar beat rate, or with the axial rotation of the swimming micro-organisms.

In the laboratory frame of reference and relative to the motion of the ambient fluid, the mean swimming velocity of a spherical gyrotactic micro-organism of radius a and density ρ is (Kessler, 1986)

$$\mathbf{V}_{\text{swim}} = -V_c \sin \theta \hat{\mathbf{x}} + V_c \cos \theta \hat{\mathbf{y}}, \quad (13)$$

where $\hat{\mathbf{x}}$ and $\hat{\mathbf{y}}$ are unit vectors in the Ox and Oy directions. The cell's swimming speed is $V_c = F_c/\lambda$, where F_c is the forward thrust generated by the micro-organism, and the swimming direction, θ , is given by

$$\sin \theta = 6\eta\Omega/g\rho h \quad \text{if} \quad |6\eta\Omega/g\rho h| \leq 1, \quad (14)$$

where h is the distance of the cell's centre of mass from its centre of buoyancy. If a cell is not gyrotactic but actively senses gravity (e.g. Häder *et al.* 1995), its direction of swimming can still be described by Eq. (13) but the right-hand side of Eq. (14) requires modification and would represent a sensing function. If $|6\eta\Omega/g\rho h| > 1$, then the cell tumbles with angular velocity

$$d\theta/dt = \Omega - g\rho h \sin \theta/6\eta \quad (15)$$

(Kessler 1986) and there is no equilibrium swimming direction.

The equations for passive spheres can therefore be modified to apply to micro-organisms simply by adding the gyrotactic swimming to gravity. Eq. (1) becomes

$$m \frac{d^2 z}{dt^2} + \lambda \frac{dz}{dt} + (\Omega^2 m_w - i\Omega\lambda) z = -ig\Delta m + iF_c e^{i\theta} \quad (16)$$

and thus $-(V_c/\Omega)\exp(i\theta)$, is added to R in Eqs. (9) and (10). In fact, for most swimming algae $V_c \gg V_{\text{sed}}$, so that

$$R \simeq -\frac{V_c}{\Omega}e^{i\theta} \quad (17)$$

and the trajectories of the cells in the rotating frame become

$$q(t) \simeq z(0) + \frac{V_c}{\Omega}e^{i\theta} - \frac{V_c}{\Omega}e^{i(\theta-\Omega t)}. \quad (18)$$

In the rotating frame of reference, the directions of dq/dt and \mathbf{g} relative to the Oy_{rot} -axis are $(\theta - \Omega t)$ and $-(\Omega t + \pi)$, respectively. The difference is the swimming direction relative to \mathbf{g} in the rotating system and equals

$$\phi = \theta + \pi. \quad (19)$$

In general, because of variability within populations of micro-organisms, θ and ϕ actually represent the means of orientational probability distributions.

GRAVITRON DIAGRAMS

Comparing the swimming direction relative to \mathbf{g} provides a useful tool for analysing the trajectories of both passive, sedimenting particles and actively swimming micro-organisms. We construct so-called "gravitron" diagrams by plotting the trajectories $(x_G(t), y_G(t))$ obtained by integrating the gravitron velocity,

$$V_G(t) = \frac{dq}{dt}e^{i\Omega t} = \frac{dz}{dt} - i\Omega z, \quad (20)$$

with respect to t , i.e.

$$x_G(t) + iy_G(t) = \int_0^t V_G(t') dt'. \quad (21)$$

Eqs. (20) and (21) unambiguously define trajectories in the gravitron diagrams. $V_G(t)$ may be thought of as the velocity measured in the stationary frame of reference minus the component of velocity due to rigid body rotation about O with angular velocity Ω . In these plots, all the trajectories begin at the origin, G , of the gravitron coordinate system, Gx_Gy_G , and ϕ is measured in the clockwise sense from the Gy_G -axis. As an example, the trajectories of sedimenting spheres in the gravitron diagram begin at the origin, G , and move with constant speed, V_{sed} , in the direction of \mathbf{g} ($\phi = \pi$) along the negative Gy_G -axis. However in the rotating frame, the trajectories are circles because \mathbf{g} rotates with respect to the rotating axes, whilst in the stationary frame the trajectories are a complicated combination of vertical sedimentation plus rigid body rotation due to advection by the rotating fluid.

For typical populations of the single celled alga *Pleurochrysis carterae*, which were used in the experiments described below, $h \simeq 10^{-4}$ cm and $\rho \simeq 1$ g cm $^{-3}$ so that for example, when the fluid rotates with angular velocity $\Omega = 1$ rad s $^{-1}$, Eq. (14) implies that $\sin \theta = 0.6$ and thus $\phi = 3.79$ rad so that the cells' trajectories in the gravitron diagram should be straight lines in a 'south-easterly' direction. However, at larger rotation rates (e.g. $\Omega = 10$ rad s $^{-1}$), Eq. (14) has no solution and there is no steady equilibrium swimming direction. In this case the cell tumbles with angular velocity given by Eq. (15). Thus the swimming direction angle θ is found to increase monotonically with time, the details depending on the parameters. The greatest values of $|d\theta/dt|$ occur when $\pi < \theta < 3\pi/2$ and the gravitational and viscous torques add. Thus, in the gravitron plots, the paths spiral away from the origin.

EXPERIMENTS

In these experiments, we tested a new system for acquisition and analysis of data on the trajectories of organisms swimming in fluid rotating about an horizontal axis. We studied the correlations between the angular velocity, Ω of the fluid and the behaviour of the cells. The apparatus consisted of a 1 cm diameter, cylindrical chamber bounded by parallel glass plates spaced 1 mm apart, rotating about a horizontal axis perpendicular to the windows. The relatively narrow spacing between the glass plates served to damp out quickly the swirling motions associated with starting up the rotation of the chamber, to ensure that

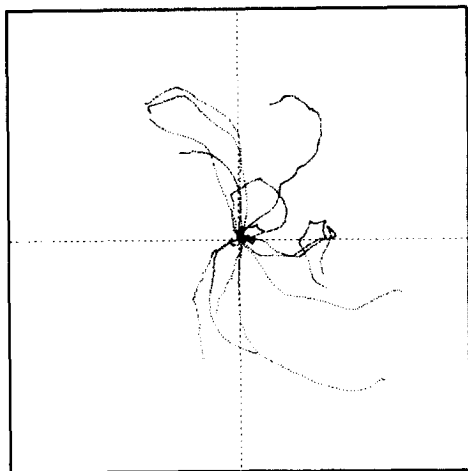


Figure 1: A gravitron plot of non-gravitactic one-day-old *P. carterae* cells when the chamber is rotating with angular velocity $\Omega = 0.7 \text{ rad s}^{-1}$. The cells have no steady preferred orientation and tumble so that their gravitron trajectories spiral outwards from the origin.

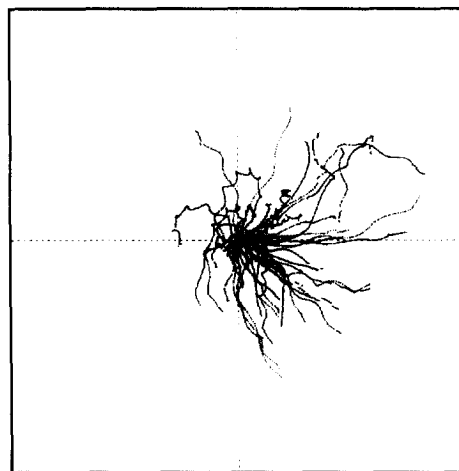


Figure 2: Gravitron plots of trajectories of gravitactic cells when $\Omega = 0.3 \text{ rad s}^{-1}$. At this rotation rate, the preferred swimming direction is at approximately 90° to the direction of gravity, g , but there is a wide spread in the distribution of swimming directions.

the fluid was rotating uniformly when measurements were taken. The chamber was filled with cultures of the swimming coccolithophorid *P. carterae* (van den Hoek et al., 1995), which were observed with a stationary camera attached to a microscope aligned along the axis of rotation so that only trajectories within a radius, r , of less than 1 mm of the axis were studied. The ratio of the centrifugal acceleration to the acceleration due to gravity was $\Omega^2 r/g < 10^{-4}$ and, consequently, centrifugal effects were negligible in these experiments. The movements of the cells were recorded onto videotapes and later digitised by a computer system supplied by the Motion Analysis Corporation, programmed to analyse the statistics of motion in both the inertial (laboratory) and rotating frames of reference. High quality steady rotation was obtained with an electronically controlled platform (Newport Corporation).

RESULTS

Upswimming gravitaxis behaviour in *P. carterae* is essentially absent in the early growth stages of a cell culture but after approximately seven days, upswimming becomes well developed. Whatever occurs is due to the response of the cells' orientation to the apparent rotation of g which affects the intrinsic probability distribution of the cells' swimming velocities. The gravitron plot in Figure 1 illustrates the dynamics of one-day-old cells when $\Omega = 0.7 \text{ rad s}^{-1}$. There is no preferred orientation. The cells tumble and thus their trajectories spiral out from the origin. Figure 2 is a gravitron plot of the tracks of gravitactic (i.e. upswimming) eight-day old cells when $\Omega = 0.3 \text{ rad s}^{-1}$. The distribution of swimming directions is concentrated between $\phi = 45^\circ$ and $\phi = 135^\circ$, with an approximately horizontal mean direction.

Figures 3, 4 and 5 show the trajectories of the same eight-day-old cells when $\Omega = 1.0 \text{ rad s}^{-1}$ and include data for inert micro-spheres that were also suspended in the culture. The micro-spheres are slightly negatively buoyant and sediment in the direction of gravity, g . The tracks in Figure 3 are plotted in the rotating reference frame and the spheres' trajectories are clearly distinguished as ellipses. The slightly elliptical orbits of the sedimenting spherical particles indicate reasonable agreement with the theoretical prediction above of circular orbits. Some preliminary analysis indicates that the deviations from the simple theory above are probably due to hydrodynamic interactions between the particles and the walls of the chamber. Figure 4 is a gravitron plot of the trajectories of just the micro-spheres showing that they travel at a mean

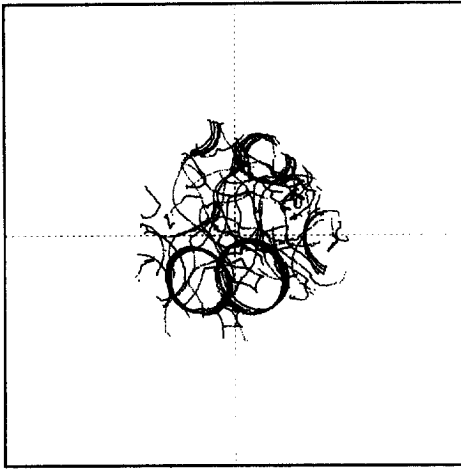


Figure 3: Trajectories of microspheres and swimming cells in the rotating frame of reference when $\Omega = 1 \text{ rad s}^{-1}$. The slightly elliptical trajectories of the sedimenting spheres are readily distinguished from those of the micro-organisms.

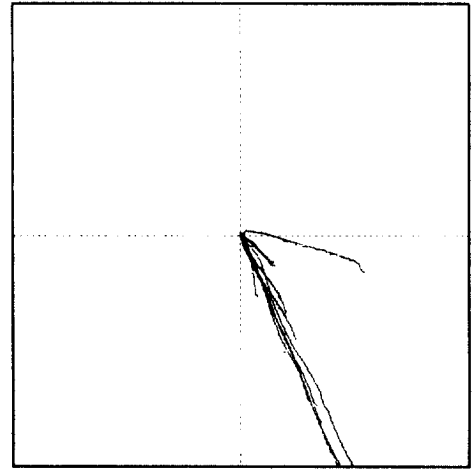


Figure 4: Gravitron plots of the trajectories of only the spheres shown in Figure 3 when $\Omega = 1 \text{ rad s}^{-1}$. The theory for an unbounded fluid predicts that the spheres should move vertically down in the direction of g . The deviations of about 15° from this direction may be due to wall effects or hydrodynamic interactions with other spheres or swimming cells in the fluid.

direction of about 15° away from direction of g . This discrepancy may be due to the proximity of the walls of the chamber. In the final gravitron plot, Figure 5, the trajectories of the swimming cells are superimposed upon those of the micro-spheres in the Figure 4, showing that they travel in almost the same direction as the sedimenting spheres. From this and Figure 2, we tentatively conclude that the behaviour of the cells is somewhat different to what would be expected if they were swimming in directions determined by gyrotaxis alone as in Eqs. (14) and (15) and the discussion in the Examples section above, and that some active reorientation mechanism is playing a role in their response to the uniformly rotating environment.

CONCLUSIONS

We have formulated the basic mathematical description for the motion of inert spheres and swimming micro-organisms suspended in a fluid that rotates about an axis perpendicular to gravity. We have also constructed a chamber in which to study the behavioural responses of swimming micro-organisms in rotating fluids and have demonstrated the utility of gravitron diagrams. Further investigations may help to distinguish between various hypotheses relating to the sensing of gravity, or discover new phenomenology. The preliminary experiments described here were not sufficiently well controlled to provide strong inferences. However, it is apparent that rotation can distinguish in a novel manner between gravitactic and non-gravitactic swimming cells of the alga *P. carterae*, as in Figures 1 and 5. For these gravitactic cells, increasing Ω and thus the apparent rate of rotation of g causes a complicated reorientation response which merits further investigation.

ACKNOWLEDGMENTS

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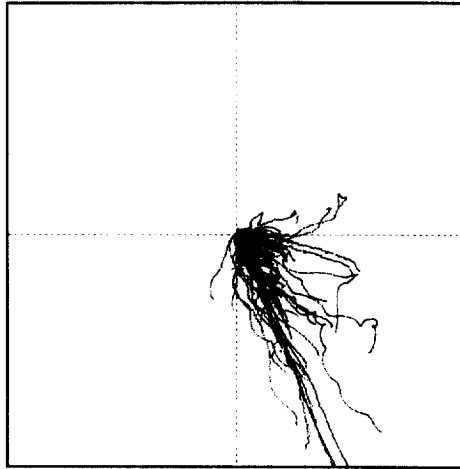


Figure 5: Gravitron plots of the trajectories of both the gravitactic cells and microspheres of Figure 3 when $\Omega = 1 \text{ rad s}^{-1}$. Comparison with Figure 4 shows that the swimming cells tend to move in the same direction as that in which the passive spheres sediment.

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