Active suspensions have non-monotonic flow curves and multiple mechanical equilibria

Aurore Loisy, Jens Eggers, and Tanniemola B. Liverpool
School of Mathematics, University of Bristol - Bristol BS8 1TW, UK
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Active suspensions, such as swarms of bacteria and the cytoskeleton of living cells, consist of anisotropic motile units interacting in a passive medium [1–4]. Because these active units self-organize and collectively induce mechanical stresses in the bulk, they may, under shear, reduce the apparent viscosity of the suspension [3, 5–14]. Here we prove that this phenomenon can continue until even a negative apparent viscosity is achieved. The emergence of this regime, observed experimentally in bacteria suspensions [14], is predicted here quantitatively using concepts from liquid crystal theory and shown to be associated with a negative slope in the flow curve. We demonstrate that this latter feature, previously thought to represent mechanically unstable states [15, 16], is peculiar to confined active fluids subject to an imposed strain rate, but is replaced by hysteresis in stress-controlled conditions, thereby showing that for this nonequilibrium system these two ensembles are not equivalent.

The most commonly sought rheological property of complex fluids is the apparent (shear) viscosity, which expresses the macroscopic fluid’s resistance to flow and can be defined through the idealized configuration depicted in Fig. 1a. A thin layer of fluid is confined between two parallel plates of area A separated by a distance L and moving with opposite velocities of equal magnitude V. In order to keep the plates moving at constant speed, an external force F is required to counterbalance internal friction. The apparent viscosity of the fluid is \( \eta_{\text{app}} = \sigma/\dot{\gamma} \) where \( \sigma = F/A \) is the apparent shear stress and \( \dot{\gamma} = 2V/L \) is the apparent shear strain rate. We emphasize that \( \sigma \) and \( \dot{\gamma} \) are defined from a macroscopic viewpoint, they are not equivalent to their local counterparts which may not be uniform across the gap.

Recently, using a highly sensitive rheometer, Lopez et al. [14] were able to measure zero and even negative values of the apparent viscosity in a suspension of E. Coli at steady-state, thereby demonstrating that microscopic bacterial activity can be converted into macroscopic useful mechanical power (\( W = F \cdot \dot{V} < 0 \)). Several theories have been proposed since to rationalize \( \eta_{\text{app}} \leq 0 \), either based on kinetic models [17, 18] or on a generalized Navier-Stokes equation [11], yet agreement with [14] is at best qualitative. Here, using a minimal model of an active liquid crystal (see Methods and Supplementary Information 1), we predict quantitatively the transition to \( \eta_{\text{app}} \leq 0 \) reported by [14] and explain both the non-monotonic evolution of \( \eta_{\text{app}} \) and the decreasing response time of the system with increasing bacterial concentration (Fig. 1b, details below). We show that the steady state with \( \eta_{\text{app}} < 0 \) reached by [14] is a structurally stable mechanical equilibrium, and is associated with a non-monotonic flow curve \( \sigma(\dot{\gamma}) \) (Fig. 1c). As a consequence, if the suspension were to be sheared by tuning the stress rather than the strain rate, experiments would yield different results.

To determine the apparent viscosity of a fluid we have the choice of two ensembles: prescribed \( \dot{\gamma} \) or prescribed \( \sigma \). In passive fluids, both ensembles are equivalent: the steady shear response is characterized uniquely by \( \sigma(\dot{\gamma}) \) or by \( \dot{\gamma}(\sigma) \). This is not generally true in active fluids. Because \( \sigma(\dot{\gamma}) \) can be non-monotonic, sweeping through \( \sigma \) and sweeping through \( \dot{\gamma} \) can yield distinct flow curves (Fig. 2a), and the equivalence can be lost: \( \sigma(\dot{\gamma}) \neq \dot{\gamma}(\sigma) \).

This peculiar property of active fluids may be seen in the limiting cases \( \dot{\gamma} = 0 \) or \( \sigma = 0 \). In the absence of external mechanical forcing, a confined quasi-one-dimensional active liquid film exhibits a spontaneous transition from a homogeneous immobile state to an inhomogeneous flowing state at a critical value of the activity coefficient \( \alpha \) [19]. This coefficient is related to the active stresses in a suspension of particles modelled as force dipoles: the magnitude of \( \alpha \) is proportional to the strength of the force pair and the sign of \( \alpha \) depends on whether the induced flow is extensile (\( \alpha > 0 \)) or contractile (\( \alpha < 0 \)). Crucially, the spontaneous flow transition depends on the mechanical constraint: it occurs at \( \alpha_{e,\sigma} \) for \( \sigma = 0 \), whereas it occurs at a larger value \( \alpha_{e,\dot{\gamma}} > \alpha_{e,\sigma} \) for \( \dot{\gamma} = 0 \) [19] (we assume without loss of generality \( \alpha_{e,\sigma} > 0 \), otherwise the order relations are reversed). The immediate consequence is that \( \sigma(\dot{\gamma} = 0) = 0 \) whereas \( \dot{\gamma}(\sigma = 0) \neq 0 \) for \( \alpha_{e,\sigma} < \alpha < \alpha_{e,\dot{\gamma}} \).

This spontaneous transition corresponds to a pitchfork bifurcation of the system where \( \alpha \) plays the role of the bifurcation parameter. Beyond the bifurcation, two equivalent solutions coexist and the final state is determined by the initial conditions. In the presence of external shear, the symmetry is broken and one may expect the corresponding branch to be selected. By studying numerically an active film under weak applied shear, we find that this is not the case: the system can exhibit two asymmetric stable branches when \( \alpha > \alpha_{c,\sigma} \) if \( \sigma \) is imposed, as shown in Fig. 2b. Similarly, two asymmetric states can coexist when \( \alpha > \alpha_{c,\dot{\gamma}} \) if \( \dot{\gamma} \) is imposed. As such a high level of activity is irrelevant to experiments, discussion of the case \( \alpha > \alpha_{c,\dot{\gamma}} \) is deferred to Supplementary Information 2. This results in the non-equivalence of these two ensembles and in the unconventional properties of the flow curves \( \sigma(\dot{\gamma}) \) and \( \dot{\gamma}(\sigma) \) displayed in Fig. 2a.
L-α consists of anisotropic active units, such as bacteria, dispersed in a passive solvent and modelled as force dipoles of strength \( \sigma \). Sheared active suspension with negative apparent viscosity at steady-state.

The apparent viscosity is defined as \( \eta_{\text{app}} = \sigma / \dot{\gamma} \) where \( \sigma = F/A \) is the macroscopic shear stress and \( \dot{\gamma} = 2V/L \) is the macroscopic shear strain rate. Under certain conditions, the apparent viscosity of the suspension can be negative: macroscopic mechanical power can be extracted from internal activity (\( W = F \cdot V < 0 \)). This regime is characterized by a strongly inhomogeneous flow (thin blue arrows) with local velocity gradients at the walls of sign opposite to that of the applied macroscale velocity gradient. (b) Effect of bacteria volume fraction \( \phi \) on the steady-state apparent viscosity \( \eta_{\text{app}} \) (top) and on the relaxation time \( \tau \) (bottom) of \( E. \text{Coli} \) suspensions subjected to a step change of strain rate from 0 to \( \dot{\gamma} = 0.04 \text{ s}^{-1} \): present theory (lines) and experiments (open symbols) [14]. The different colours, symbols and line styles corresponds to different bacterial strains (ATCC and RP) and oxygen levels. (c) Theoretical flow curves \( \sigma(\dot{\gamma}) \) for \( E. \text{Coli} \) suspensions with \( \eta_{\text{app}} > 0, \eta_{\text{app}} = 0 \) and \( \eta_{\text{app}} < 0 \). The stars in (b) and (c) mark corresponding state points.

![Diagram](image_url)

For \( \alpha < \alpha_{c,\sigma} \), the flow curves are identical and increase monotonically, as in passive fluids. At \( \alpha = \alpha_{c,\sigma} \), a singular point appears at the origin. On the one hand, for imposed \( \dot{\gamma} \), \( d\sigma/d\dot{\gamma}|_{\dot{\gamma}=0} = 0 \) and there exists a unique solution with vanishing apparent viscosity for small applied \( \dot{\gamma} \). On the other hand, for imposed \( \sigma, \) \( d\dot{\gamma}/d\sigma|_{\sigma=0} = \infty \), which corresponds to the bifurcation from a single steady state to bistability. For \( \alpha_{c,\sigma} < \alpha < \alpha_{c,\dot{\gamma}} \), \( \sigma(\dot{\gamma}) \) exhibits local extrema \( \pm \sigma_{m} \) connected by a negative slope \( d\sigma/d\dot{\gamma} < 0 \). The associated solutions are unique and stable when \( \dot{\gamma} \) is imposed (blue square), but are unstable (not shown) when \( \sigma \) is imposed. Instead \( \dot{\gamma}(\sigma) \) has two stable non-equivalent solutions (leftward and rightward green triangles) in the range \([ -\sigma_{m}, +\sigma_{m} ] \). As \( \sigma \) is varied, \( \dot{\gamma}(\sigma) \) changes discontinuously at \( \sigma = \pm \sigma_{m} \) and hysteresis occurs. While these features were identified separately by [16] for the former and by [20] for the latter, here we provide the conceptual framework that shows they are simply different sides of the same coin. At \( \alpha = \alpha_{c,\dot{\gamma}} \), the curve \( \sigma(\dot{\gamma}) \) admits a vertical tangent at the origin \( (d\sigma/d\dot{\gamma}|_{\dot{\gamma}=0} = -\infty) \) which corresponds to the bifurcation for imposed \( \dot{\gamma} \). For \( \alpha > \alpha_{c,\dot{\gamma}} \), \( \sigma(\dot{\gamma}) \) exhibits multiple discontinuous branches which shape depends on the system, see Supplementary Information 2 for more details.

A related, and striking, property of weakly sheared active films is the existence, for \( \alpha_{c,\sigma} < \alpha < \alpha_{c,\dot{\gamma}} \), of a structurally stable mechanical equilibrium with \( \eta_{\text{app}} < 0 \) if (and only if) \( \dot{\gamma} \) is imposed (blue square in Fig. 2). This is the solution reached in the experiments of [14]. If one would tune the applied stress instead, as could be realized with a stress-controlled rheometer, the same system would exhibit hysteresis and at \( \sigma = 0 \), the plates would move.

When an active film is strongly sheared, its rheology can be either Newtonian or strongly nonlinear, as shown in Fig. 3. Which of these two scenarios applies is determined by the magnitude the flow-alignment parameter \( \lambda \), a standard liquid crystal parameter which describes the hydrodynamic coupling between the orientation of the particles and the flow at the continuum scale. For
**FIG. 2.** *Active suspensions can exhibit non-monotonic flow curves and mechanical bistability under weak shear.* Steady-state rheology of a *weakly* sheared active suspension of flow-aligning elongated particles: (a) flow curves in stress-controlled (circles) and strain-rate-controlled (lines) conditions for increasing $\alpha$ (from left to right); (b–c) bifurcation diagrams in stress-controlled (b) and strain-rate-controlled (c) conditions. Only stable solutions are shown. In the absence of applied shear, a pitchfork bifurcation occurs at $\alpha = \alpha_{c,\sigma}$ for $\sigma = 0$ and at $\alpha = \alpha_{c,\dot{\gamma}}$ for $\dot{\gamma} = 0$. In the presence of weak applied shear, two stable branches can also coexist when $\alpha > \alpha_{c,\sigma}$ for imposed $\sigma$ and when $\alpha > \alpha_{c,\dot{\gamma}}$ for imposed $\dot{\gamma}$. These bifurcations correspond to the apparition of singularities ($d\dot{\gamma}/d\sigma|_{\sigma=0} = \infty$ and $d\sigma/d\dot{\gamma}|_{\dot{\gamma}=0} = -\infty$, respectively) in the flow curves. The symbols show the correspondence between the flow curves and the bifurcation diagrams, associated flow profiles are provided in Supplementary Information 2. The stars denote dimensionless quantities, see Methods for their definitions. These results are qualitatively independent of the flow-aligning regime and the particle shape (that is, independent of $\lambda$, here $\lambda = 1.9$).

$|\lambda| > 1$, the particles respond to a large shear flow by aligning at a well-defined angle with respect to the flow direction. Suspensions in this “flow-aligning” regime behave as Newtonian fluids under strong shear. For $|\lambda| < 1$, the particles rotate increasingly and form rolls within the gap as applied shear increases. The flow curves of suspensions in the “flow-tumbling” regime are strongly nonlinear, and are characterized by multiple regions of locally reduced apparent viscosity which coincide with the completion of a half turn by the particles at the centre of the film. These nonlinearities are more pronounced at higher activity (hence the choice of a relatively high value of $\alpha$ in Fig. 3) but their occurrence is independent of the active nature of the particles. Non-monotonic flow curves with multiple local extrema or discontinuities as in Fig. 3 are however peculiar to active systems. Note that the sign of $\lambda$, which is related to the shape of the particles, is of no importance here: Fig. 3 presents results for rod-like particles ($\lambda > 0$), analogous results are obtained for disk-like particles ($\lambda < 0$) in Supplementary Figure 4.

These theoretical predictions have been obtained from generic equations which apply to a broad class of active liquid crystals, such as collections of motile organisms and motor-filament systems [2]. The price to pay for this generality is the introduction of several system-dependent coefficients. Assuming nematic order and homogeneous concentration, the behaviour of active liquid crystals depends on the activity coefficient $\alpha$, the alignment parameter $\lambda$, the bulk fluid viscosity $\eta$, the Frank elastic constant $K$, and the rotational viscosity $1/\Gamma$ (see...
FIG. 3. Flow curves for various types of active suspensions. Under strong shear, active materials in the flow-tumbling regime ($|\lambda| < 1$) exhibit flow curves with multiple regions of reduced apparent viscosity whereas those in the flow-aligning regime ($|\lambda| > 1$) behave as Newtonian fluids. The insets show steady-state profiles of the orientation of the particles. The E. Coli suspensions studied by [14] follow the scenario displayed in the framed panel. The stars denote dimensionless quantities, see Methods for their definitions.

Supplementary Information 1). In the following we will show how these phenomenological coefficients can be extracted from shear experiments to allow testable quantitative predictions.

For that purpose we consider the transient response of a nematic active film of thickness $L$ to applied shear and restrict our analysis to the low-shear Newtonian plateau (small $\sigma$ or $\dot{\gamma}$). By solving the linearised governing equations (Supplementary Information 3) we find that the strain rate response to a step of stress from 0 to $\sigma$, valid for $\alpha/\alpha_{c,\sigma} < 1$, is

$$\frac{\sigma}{\dot{\gamma}}(t) = \eta \left[ \frac{\eta_{\text{app}}}{\eta_{\text{app}}} - \left( \frac{\eta_{\text{app}}}{\eta_{\text{app}}} - 1 \right) \exp \left( -\frac{t}{\tau_{\sigma}} \right) \right]^{-1}, \quad (1a)$$

and that the stress response to a step of strain rate from 0 to $\dot{\gamma}$, valid for $\alpha/\alpha_{c,\dot{\gamma}} < 1$ with $\alpha_{c,\dot{\gamma}} = 4 \alpha_{c,\sigma}$, reads

$$\frac{\sigma}{\dot{\gamma}}(t) = \eta \left[ \frac{\eta_{\text{app}}}{\eta} - \left( \frac{\eta_{\text{app}}}{\eta} - 1 \right) \exp \left( -\frac{t}{\tau_{\dot{\gamma}}} \right) \right]. \quad (1b)$$

The signal of $\sigma/\dot{\gamma}$ initially jumps from 0 to $\eta$, the bulk viscosity of the fluid, owing to the instantaneity of Stokes flow (fluid inertia is neglected in our formulation). It then relaxes to its terminal value $\eta_{\text{app}}$, the apparent steady-
state viscosity, given by
\[
\frac{\eta_{\text{app}}}{\eta} = 1 - \frac{\alpha}{\alpha_{c,\sigma}} \frac{1 - 8}{(1 - 8 \pi^2)} \frac{\xi}{(1 - 8 \pi^2)} + 1 - \frac{8}{\pi^2} \xi + 1,
\]
where
\[
\frac{\alpha}{\alpha_{c,\sigma}} = \frac{\alpha(\lambda - 1)L^2}{2\pi^2 \eta \kappa (1 + \xi)}.
\]
The relaxation time \(\tau\) depends on the constraint, it is related to model parameters by
\[
\frac{1}{\tau_\sigma} = \frac{\tau^2 \kappa}{L^2} \left[ 1 + \xi - \frac{\alpha}{\alpha_{c,\sigma}}(1 + \xi) \right]
\]
for imposed \(\sigma\) and by
\[
\frac{1}{\tau_\gamma} = \frac{\tau^2 \kappa}{L^2} \left[ 1 + \left( \xi - \frac{\alpha}{\alpha_{c,\sigma}} (1 + \xi) \right) \left( 1 - \frac{8}{\pi^2} \right) \right]
\]
for imposed \(\dot{\gamma}\). Here we have introduced \(\kappa = \Gamma K\) and \(\xi = (\lambda - 1)^2/(4\eta \Gamma)\). We note in passing that equation (2) shows that an active suspension behaves, at low applied strain rate, as a Newtonian fluid with \(\eta_{\text{app}} \leq 0\) when \(\alpha/\alpha_{c,\sigma} \geq 1\) (as also visible in Fig. 2c).

The four independent (groups of) parameters \(\eta, \alpha(\lambda - 1), \kappa\) and \(\xi\) control the suspension behaviour. They can all be identified from rheological time traces, as illustrated in Fig. 4a. We applied this idea to the suspensions of \(E.\ Coli\) studied by [14]: using their experimental time signals of \(\sigma(t)/\dot{\gamma}\), we inferred the values of the phenomenological parameters describing their suspensions for bacteria volume fractions \(\phi\) ranging from 0.11% to 2.4% (see Methods).

We obtained \(\xi \approx 0\) (i.e. negligible elastic stresses compared to viscous ones) and \(\eta \approx \eta_s\) where \(\eta_s = 1.4 \times 10^{-3}\) Pas is the viscosity of the solvent (i.e. negligible effect of the bacteria on the bulk viscosity of the suspension), with no significant effect of volume fraction on these two parameters. The parameters \(\alpha(\lambda - 1)\) and \(\kappa\) were found to be positive increasing functions of volume fraction \(\phi\), as shown in Fig. 4b. Since \(E.\ Coli\) are extensile swimmers or “pushers” (\(\alpha > 0\) [21], it follows immediately (Fig. 4b, left panel) that \(\lambda > 1\), i.e., \(E.\ Coli\) behave effectively as flow-aligning rod-like particles (as generally accepted). The dependence of \(\alpha(\lambda - 1)\) on \(\phi\) is approximately linear up to \(\phi \approx 2\%\) (shown with lines), which agrees with the expectation \(\alpha = \alpha_1 \phi\) for a dilute suspension of self-propelled swimmers (for mutually-propelled active units such as cytoskeletal filaments, a quadratic dependence would have been expected instead). A peculiar observation reported by [14] is the shorter response time \(\tau\) at larger volume fraction (Fig. 1b, bottom panel). In the present model, the decrease of the response time with increasing volume fraction arises from the increase of the orientational diffusion coefficient \(\kappa = \Gamma K\) with \(\phi\) (Fig. 4b, right panel). This is consistent with a Frank elastic constant of the form \(K = K_0 + K_1(\phi - \phi_c)^2\) as in conventional nematic liquid crystals, where \(\phi_c\) is the critical volume fraction (smaller than 0.1%) associated to the isotropic-nematic transition, \(K_0\) accounts for mechanical interactions and \(K_1\) for thermodynamic interactions [22].

By fitting laws of the expected form for \(\alpha(\phi)\) and \(K(\phi)\) to each set of experimental data (lines in Fig. 4b), we are in a position to predict the quantitative behaviour of each system in response to shear as a function of volume fraction. The results, presented against the experimental data in Fig. 1b, demonstrate that these simple dependences on volume fraction for \(\alpha\) and \(K\) are sufficient to account for all experimental observations, notably the emergence of a “superfluid” regime (\(\eta_{\text{app}} \sim 0\) with shorter response time, without the need to invoke a transition between a dilute and a semi-dilute regime. Incidentally, the predicted increase of \(\eta_{\text{app}}\) at higher volume fractions, although not clearly seen in the experiments of [14], resemble that reported by [12] (Fig. 3 therein) for a suspension of \(Bacillus subtilis\), an analogous type of rod-like pushers.

We finally comment on the simplifying assumption of nematic order whereas colonies of bacteria are known to form polar phases. Polar terms in the governing equations generally give rise to strong spatio-temporal concentration variations [23] which in turn affects the rheological response. The intrinsic characteristic time of concentration variation is given by \(L^2/D\) where \(D\) is the bacteria translational diffusion coefficient. In the experiments considered here, \(D \approx 7 \times 10^{-11} \text{m}^2/\text{s}^{-1}\) [14], which yields a characteristic time of one hour while the experiments last only 30 seconds. Solving the governing equations numerically for a polar suspension confirmed that polarity has negligible effect over the duration of the experiments (see Supplementary Information 4 for more details).

Geometrical confinement provides a powerful way to control active suspensions [24–27]. The existence of non-monotonic flow curves suggest new control mechanisms, that could find direct application in bacterial energy harvesting [28, 29]. Our results also provide strong support for models of biologically active suspensions as “living liquid crystals” [2, 4, 30] and open the way to a truly quantitative characterization of these systems.

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