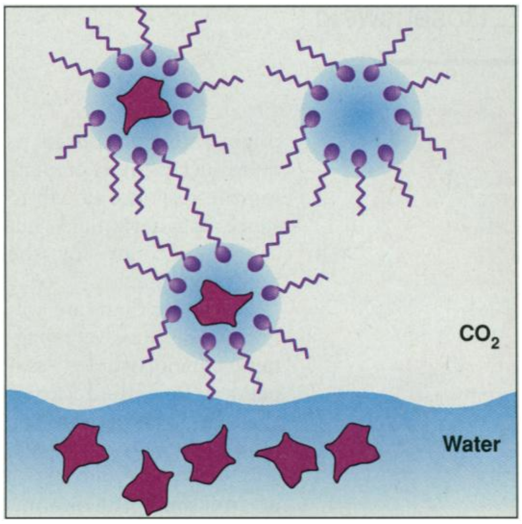
Carbon Dioxide Extraction of Biomolecules

Eric J. Beckman

Although one's instinctive reaction to the mention of carbon dioxide might be to think "greenhouse gas," chemical engineers (and increasingly chemists) have been examining the use of CO_2 (both liquid and supercritical) in environmentally friendly chemical processing for the past two decades. Carbon dioxide can perhaps be considered the organic solvent analog to water, because if it is removed from the environment, used in a chemical process, and returned to the environment in a clean state, there is no penalty. In the case of a compressed gas like CO_2, returning the fluid to the environment in such a state is as simple as lowering the pressure to 1 atm. Because it is relatively nontoxic and nonflammable, CO_2 is not currently regulated by either the Food and Drug Administration (FDA) or the Environmental Protection Agency (EPA). Furthermore, at four cents per pound, CO_2 is probably the least expensive organic solvent one can obtain commercially. And now, the results of a collaboration between several research groups, reported on page 624 of this issue (*1*), help pave the way for use of CO_2 in extraction and purification of biomolecules, an exciting application that had previously been thought untenable.

Given its "green" characteristics, CO_2 elicited considerable excitement among the chemical community in the early 1980s when calculations (from the equation of state) showed that its solubility parameter resembles that of normal alkanes at pressures above 200 bar. Hence, one should be able to simply replace many conventional organic solvents with CO_2. For example, the successful scale-up and commercialization of the CO_2-based coffee decaffeination process by General Foods seemed to validate this concept. However, it soon became clear that CO_2 was not going to simply replace organic solvents in most processes: like alkanes, it simply lacked the solvent power for many applications. Because high-pressure technology is more expensive than analogous processes conducted at atmospheric pressure, use of CO_2 as a solvent was therefore deemed appropriate for those processes where the environmental benefits were high (such as the coffee process) or where the solute in question was both of high value and present in dilute solution, such as biomolecules. Experiments quickly showed, however, that neat CO_2 is a very poor solvent for biomolecules. Although this was not surprising (CO_2 was supposed to be like hexane, after all), what was unexpected was that conventional surfactants, which one would normally use to solubilize such polar solutes in normal alkanes (*2*), were either insoluble or ineffective in CO_2 (*3*). Johnston and colleagues (*1*) subsequently showed that the early calculations of CO_2's solubility parameter that had elicited such excitement in CO_2 technology were perhaps inflated by as much as 20% (owing to the effect of CO_2's quadrupole moment on its equation of state properties), and thus, describing CO_2 as an alkane analog was entirely inappropriate. It seemed to many in the field that the environmental benefits of CO_2 technology could not be applied to those dilute yet high-value solutes that warranted it most.



Dissolving a problem. Proteins (red) that are insoluble in CO_2 can be dissolved with the help of a fluoroether surfactant (purple). The surfactant molecules form micelles that are soluble in CO_2 and carry pockets of water containing the proteins.

Fortunately, beginning in the mid-1980s, researchers began to see that CO_2 was indeed not like hexane where solvent power was concerned, yet it was a good solvent for certain classes of compounds. Work by Krukonis and colleagues at Phasex Corporation, as well as Enick and Brady at the University of Pittsburgh (*4*), showed that CO_2 has significant capacity to solubilize compounds such as fluoroalkyls, fluoroethers, and silicones. Even Consani (*3*), in his study of surfactant solubility (or lack thereof) in CO_2, found that those surfactants with fluoroalkyl hydrophobic tails exhibited higher solubility in CO_2 than those with alkyl tails. These early results inspired others: our group at the University of Pittsburgh found that fluoroalkyl and fluoroether surfactants exhibit high solubility in CO_2, allow a water-soluble dye to be solubilized, and support emulsion polymerization of a water-soluble monomer (*5*). DeSimone and colleagues at the University of North Carolina showed that fluoroacrylate polymers with molecular weights of 1 million can be polymerized homogeneously in CO_2 and that these fluoroacrylate polymers will stabilize dispersion polymerization in CO_2 (*6, 7*). Yet still, the question remained: were these CO_2 soluble surfactants really forming micelles that would absorb water (and hopefully, desirable solutes such as proteins) in spherical pools, or were they merely acting as cosolvents, much like methanol acts as a cosolvent when added to CO_2?

The paper by Johnston and coworkers (*1*) answers this question definitively, and the answer is indeed yes: micelles form in CO_2. This international team of scientists with extensive experience in CO_2 technology has shown, using detailed spectroscopic evidence, that a fluoroether surfactant dissolves in and forms aggregates containing water pools in CO_2. As the amount of water in the system increases, the micelles swell to the point where the solubilized water creates an environment that closely resembles that of bulk water. They have further shown that these micelles will solubilize a protein (see figure), whose solubility in neat CO_2 is essentially zero. Indeed, it has recently been shown that one can extract subtilisin Carlsberg from cell broth into CO_2 using fluoroether amphiphiles, with a modest 10% loss in activity upon recovery through depressurization (*8*).

Will this breakthrough catalyze further research and applications in CO_2 extractions of polar materials? High-pressure processing is still expensive, yet use of highly CO_2 soluble surfactants (such as the fluoroether used in the Johnston work) allows one to lower the operating pressure to levels where economic viability can be attained. A number of pharmaceutical companies are working on applications of CO_2 in extractions and separations, including preparative scale separations. Now that we know how to solubilize highly polar compounds in CO_2, it seems clear that its environmental advantages will lead to commer-

---

The author is in the Department of Chemical Engineering, University of Pittsburgh, Pittsburgh, PA 15261, USA. E-mail: beckman@vms.cis.pitt.edu

cial applications wherever the value of the solute is high, its concentration in the matrix is low, and conventional solvents lead to either recovery problems or difficulties with regulatory agencies. This work successfully brings to an end a decade-long and often maddening search for materials that will form reverse micelles in CO_2 and will hopefully inspire others to enlarge the catalog of CO_2-soluble ampiphiles in the future.

References

1. K. P. Johnston *et al.*, *Science* **271**, 624 (1996).
2. G. A. Ayala, S. V. Kamat, C. Komives, E. J. Beckman, A. J. Russell, *Biotechnology* **10**, 1584 (1992); K. P. Johnston, G. J. McFann, R. M. Memert, *ACS Symp. Ser.*: Supercritical Fluid Science and Technology **1989**, 406 (1989).
3. K. A. Consani and R. D. Smith, *J. Supercrit. Fluids* **3**, 51 (1990).
4. V. J. Krukonis, personal communication; A. Iezzi, P. Bendale, R. M. Enick, M. Turberg, J. Brady, *Fluid Phase Equilibria* **52**, 307 (1989).
5. F. A. Adamsky and E. J. Beckman, *Macromolecules* **27**, 312 (1994); T. A. Hoefling, R. M. Enick, E. J. Beckman, *J. Phys. Chem.* **95**, 7127 (1991).
6. J. M. DeSimone, Z. Guan, C. S. Elsbernd, *Science* **257**, 945 (1992).
7. J. M. DeSimone *et al.*, *ibid.* **265**, 356 (1994).
8. E. Ghenciu *et al.*, paper no. 207g presented at the American Institute of Chemical Engineers Annual Meeting, Miami, FL, 14 to 18 November 1995.

"Negative Viscosity" in a Magnetic Fluid

Ronald E. Rosensweig

Ultrastable magnetic fluids (MFs) attract scientific and technological interest because they remain liquid when highly magnetized, even in the most intense applied magnetic fields. The fluids are distinguished from ordinary fluids by the body and surface forces that arise, yielding new fluid mechanical phenomena (*1*). Ordinarily MFs exhibit the Newtonian rheology of water and other common liquids, with stress proportional to rate of strain, albeit the coefficient of viscosity increases in applied magnetic field. This behavior was believed to be general, but recent theoretical analysis predicts (*2*) and experimental investigation demonstrates (*3*) that under appropriate conditions with a time-varying field, the viscosity of MF exhibits a substantial reduction, that is, a negative viscosity component. The effect is attributable to the generation of asymmetric stress in MF; a corroborating analysis with generalization to elliptically polarized fields is given by Zahn and Greer (*4*). Mere generalization of the Navier-Stokes equations in which equilibrium body and surface forces of magnetic origin are introduced is quite successful in predicting the response of MF to steady magnetic fields but cannot account for the new phenomenon (*5, 6*).

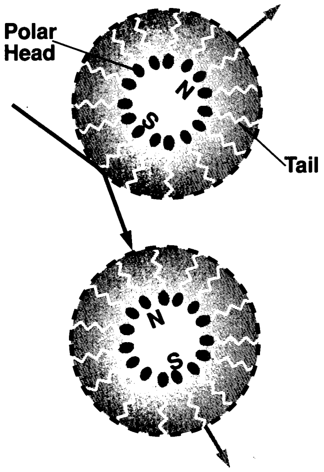


Fig. 1. Magnetic fluid in a colloidal dispersion of single-domain magnetic particles of about 10 nanometers. The sketch illustrates particles stabilized with a molecular coating; alternatively, the particles may be charge stabilized.

Magnetic fluids are colloidal solutions of magnetic nanoparticles suspended in a fluid carrier (Fig. 1). Each particle is a permanent magnetic dipole, and when the particle is not too small, the orientation of the dipole is locked into the crystal axis of the particle. A repulsive force acts as an elastic cushion, preventing the particles from sticking to each other. Thanks to Brownian translational motion, the particles do not settle in gravitational or magnetic fields. By the same token, Brownian rotational motions prevent complete alignment of the dipoles with applied fields. Thus, when the field has a changing direction or magnitude, the magnetization is unable to track the field closely and becomes unequilibrated. This lag process is responsible for the negative viscosity effects.

The earliest observation of an unequilibrated response in MF was reported in 1967 (*7*). A rotating magnetic field applied to MF in a beaker spun the fluid into motion. Soon, another investigator repeated the experiment, placing the beaker on a rotating turntable. Surprisingly, the fluid rotated the "wrong way," that is, the beaker rotated in the direction of the field while the fluid rotated in the opposite direction. For a long time, the mechanism responsible for this behavior was not understood. A magnetic field exerts an orienting influence on each suspended magnetic particle, similar to the effect on a compass needle, and when the particles are spun relative to the carrier fluid, a dissipative viscous coupling results. P. G. deGennes noted from the consideration of symmetry that no velocity field can be generated if the spin field is spatially uniform (*8*). Zaitsev and Shliomis (*9*) contributed analysis imposing a condition of retarded spin at the wall and, through diffusion, a nonuniform distribution of spin in space. Gradients of the spin produce a calculable body force from which the spin-up motion of the fluid ensues. However, the observed flow speed is orders of magnitude larger than predicted. Moreover, the predicted direction of the flow is opposite that of the observed behavior. As it turns out, the problem is not with the theoretical framework, which introduces an equation of angular momentum balance (*10*) and a relation for magnetic relaxation, but with the assumptions made in applying the equation set.

Thus, subsequent theories invoked spatial nonuniformity of the applied field, presence of temperature gradients, and onset of flow instability to reconcile theory with experiment. Finally in 1989, experiments and a new reading of the known relation for magnetic stress (*11*) showed that the direction of fluid rotation in relation to the field's rotation depends on the curvature of the free surface meniscus, whereas a flat meniscus yields no coupling. Thus, the spin-up phenomenon is a surface not a volume effect.

A striking visual display of the asymmetric stress is exhibited by drops of MF (*12*) suspended in another fluid. The drops are produced as a phase-separation product possessing a low value of interfacial tension. Subjected to a rotating magnetic field, the drops first elongate and spin and then develop spiny arms reminiscent of starfish. Depending on experimental conditions of frequency and field intensity, the number of arms undergoes transitions and other dynamics are observed, such as an eel-like form that curls around and attaches to its own flank. The questions these flows raise could keep theorists occupied for years.

For study of the negative viscosity phenomenon, a Poiseuille flow is established in a horizontal capillary tube 1 mm in diameter. A pressure gradient is formed by the difference in MF level between the inlet and outlet of the tube. The tube is put inside a solenoid that provides an alternating magnetic field at frequencies up to 1 kHz with magnetic intensity up to 0.2 T parallel to the flow direction. A MF having viscosity of

---

The author was with Exxon Research & Engineering, Annandale, NJ 08801 USA.

Present address: 34 Gloucester Road, Summit, NJ 07901, USA. E-mail: rhvh41a@prodigy.com