Hyperpolarized Gases Set NMR World Spinning

Chemical applications of laser polarization include improved NMR sensitivity

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C&EN West Coast News Bureau

The noble gases, first-year chemistry students learn, usually don't do a heck of a lot. Their electron shells completely filled, the far right elements on the periodic table are contentedly stable and inert, rarely bonding with their atomic and molecular neighbors.

But that electronic inertness exposes the far weaker magnetic properties of the nuclei of some isotopes of the noble gases, particularly helium-3 and xenon-129, which can then be exploited.

With a decades-old technique known as optical pumping, helium and xenon nuclei can be hyperpolarized—their spins collectively flipped in the "up" or "down" position—which greatly enhances their nuclear magnetic resonance (NMR) signals.

In the past several years, advances in hyperpolarized gas research have led to the high-resolution imaging of things thought impossible or impractical with NMR and magnetic resonance imaging (MRI), such as void spaces like lungs, material surfaces, and human blood and tissue. For example, a group led by chemistry professor Alexander Pines at the University of California, Berkeley, and Lawrence Berkeley National Laboratory has been developing chemical applications that enhance NMR sensitivity.

In recognition of the field's new momentum, a group of scientists gathered in Les Houches, France, in October for the first international meeting on "Perspectives of MRI Using Polarized Gases." Gordon D. Cates, a physics professor at Princeton University, says the conference "was kind of a strange and astonishing experience for me. [The field] has grown up very quickly."

In addition to Cates, his Princeton colleague William Happer—who actually developed the optical pumping methods for ^{3}He and ^{129}Xe—and the Berkeley researchers, groups exploring the capabilities of this enhanced NMR technology include those of radiologist Robert D. Black, formerly at the University of Virginia and now president of Magnetic Imaging Technologies Inc. in Durham, N.C.; biophysical chemist Scott D. Swanson and physics professor Timothy E. Chupp at the University of Michigan, Ann Arbor; radiologist Mitchell S. Albert at Harvard's Brigham & Women's Hospital; and radiologist Allan G. Johnson at Duke University.

Optical pumping techniques had their genesis in the 1950s, when French physicist Alfred Kastler did his Nobel-Prize-winning research demonstrating that bombarding certain atoms with circularly polarized light transferred angular momentum to the atoms, altering the spins of their electrons and nuclei.

Initially, scientists wanted to create hyperpolarized states for high-energy physics experiments to study the structure of nuclei, explains Angelo Bifone, a physicist at the Institute of Cancer Research at the University of London. Later, it was recognized that hyperpolarized gases held great potential for enhancing NMR signals.

Typically, the populations of spin polarization obtained using traditional NMR techniques are very small and result in faint, hard-to-read signals. But optical pumping can polarize nearly all of the atoms in a sample and increase NMR signal sensitivity by up to five orders of magnitude.

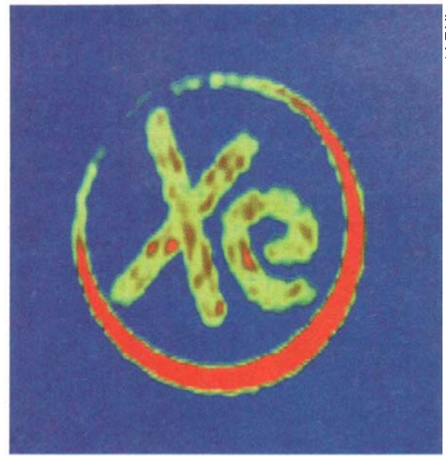
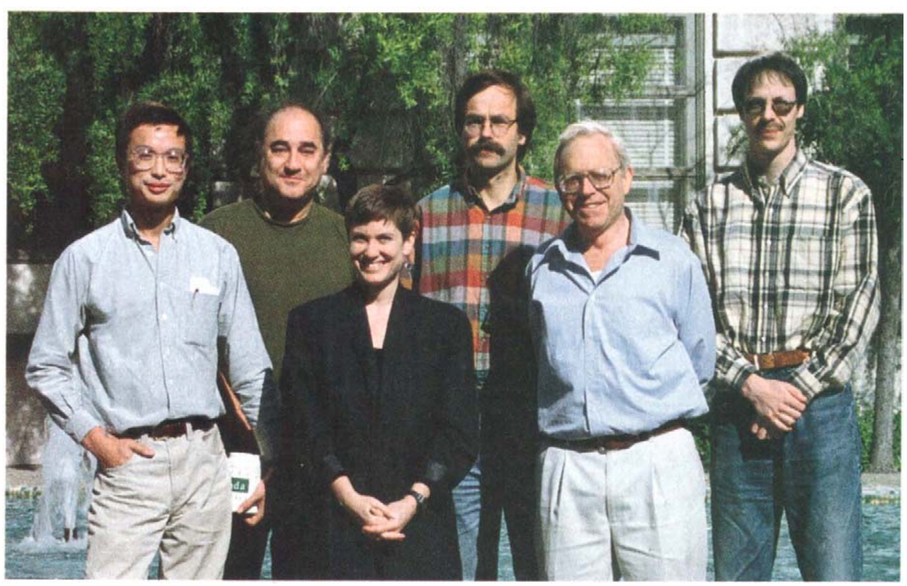


Image courtesy of LBNL

An NMR image of a cavity in the shape of the chemical symbol for xenon filled with hyperpolarized xenon.

Hyperpolarization is a two-step process: First, the valence electrons of an alkali metal, usually rubidium, are optically pumped with circularly polarized laser light. The angular momentum of the rubidium electrons is then transferred to the nucleus of the noble gas atoms through dipole-dipole interactions—like tiny bar magnets influencing each other, Cates explains. "The spins will tend to orient each other in a favorable way."

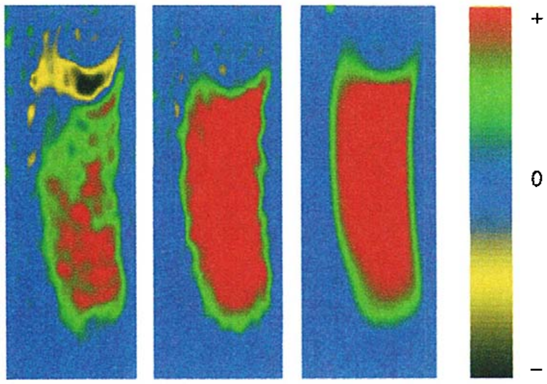
Curiously, the nuclei can retain their hyperpolarized states for more than an hour before their interactions with container walls and other atoms gradually cause the spin orientations to relax into their ground state. "It's fun for students to walk around with a cell of hyperpolarized gas in their pocket for awhile, put it



LBNL photo

From left, Yi-Qiao Song, Pines, Rebecca Taylor, Toomas Room, Navon, and Stephan Appelt have been extending hyperpolarized gas research.

Spin polarization-induced nuclear Overhauser effect (SPINOE)



Source: *Science,* **271,** 1848 (1996)

Three NMR images show a tube of hyperpolarized xenon and liquid benzene; the xenon gradually transfers its polarization to benzene over time.

in an NMR machine, and boom, get a whopping signal from a bunch of polarized spins," Cates says.

One of the technologies driving the field is the development of high-powered diode lasers, he says. "The number of watts per dollar you can get is quite impressive," Cates says.

Also, Chupp's group at Michigan has made major advances in standardizing techniques for mass-producing the hyperpolarized gases.

"The medical applications seem to be the most promising at the moment," Bifone says.

Cates has been working with Happer on the fundamental problems of hyperpolarized gases since 1987. They realized that the heightened NMR signals produced by these gases might enable them to obtain an image of a void space, like a lung. Fortuitously, both helium and xenon can be inhaled by animals.

Teaming up with a group led by associate professor of chemistry Arnold Wishnia at the State University of New York, Stony Brook, the Princeton group produced the first image of the ex vivo lungs and heart of a mouse using hyperpolarized ^{3}He [*Nature,* **370,** 199 (1994)].

The scientists then obtained ^{3}He images of human lungs, and most recently, took images of lungs using ^{129}Xe, Cates says.

Duke's Johnson and Harvard's Albert have also performed seminal experiments imaging lungs and other void spaces. Michigan's Chupp and Swanson also are looking at hyperpolarized xenon as a way to study brain function. The information it will provide will be similar to positron emission tomography (PET), "but we hope it will have a higher spatial resolution," Swanson says.

In their lab, they have found that xenon is quickly transported from the lungs to the heart and into the brain tissue of rats. "We have established the necessary condition that this polarization survives in a small animal," Swanson says. "However, human implementation involves a lot more work. I would say it's very promising right now."

Xenon has the advantage of being soluble in tissue, while helium tends to stay in the gas phase. Therefore, helium may

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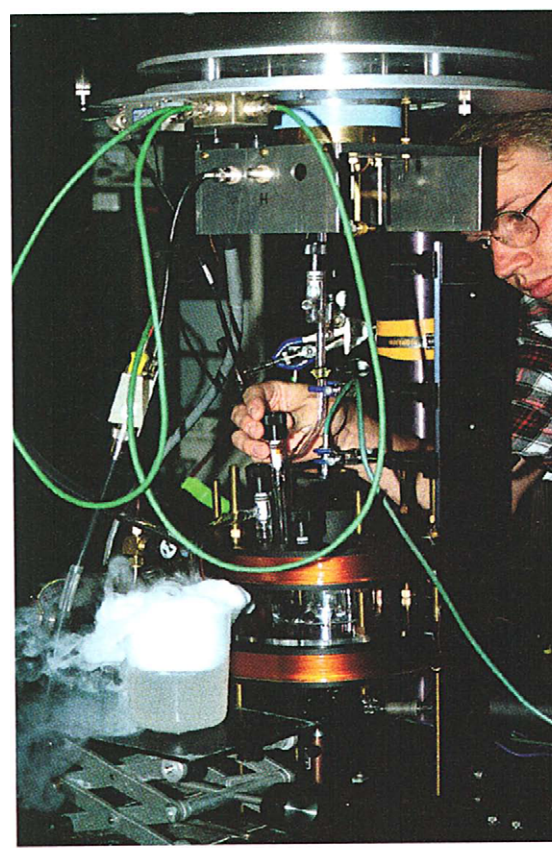
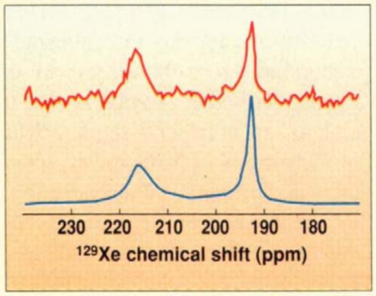


Pines's graduate student, Thomas Meersman, performs an experiment with hyperpolarized xenon at Lawrence Berkeley National Laboratory.

Laser-polarized xenon sharpens spectrum



Source: *Proc. Natl. Acad. Sci. U.S.A.*, 93, 12932 (1996)

Two ^{129}Xe NMR spectra in fresh human blood. The top spectrum was obtained using normal xenon, and the lower spectrum with a laser-polarized xenon/saline solution. The resonance at 216 ppm represents xenon in red blood cells, while the signal at 192 ppm is attributed to xenon in the saline/plasma mixture.

have advantages in void spaces, while xenon may be more appropriate for tissue and blood imaging.

"Helium is almost certainly not much good for tissue, but gives beautiful images of lungs," Cates agrees.

However, gases remain hyperpolarized for only a few seconds inside an animal, where they meet up with a host of molecules such as oxygen. "Even xenon, which is very soluble in fatty tissues and blood, still takes too long—it relaxes before a signal can be taken," Pines says.

Recently, Gil Navon, chemistry professor at Tel-Aviv University in Israel, along with Pines and colleagues at Berkeley, made steps in solving that problem.

First, they dissolved the hyperpolarized xenon in a variety of solutions. "The trick is first get the xenon into a biocompatible solution, and second, the depolarization of xenon in the solution has to be long," Pines says. After being dissolved in solutions such as saline or Fluosol, a blood substitute, xenon retains its hyperpolarization long enough to be injected into blood and produce an NMR spectrum [*Proc. Natl. Acad. Sci. U.S.A.*, 93, 12932 (1996)].

For a long time, scientists have known that xenon binds to hemoglobin, producing a very characteristic NMR chemical shift. Consequently, the xenon still in the original solution can be clearly differentiated from the xenon inside red blood cells.

The mechanism of how xenon diffuses through red blood cells is still unknown, the scientists say. Red blood cells have channels through which water can move in and out; researchers are investigating whether xenon goes through water channels or through other special channels.

Another recent series of experiments by Pines's group at Berkeley holds promise for both medical and material applications.

They have exploited a unique combination of nuclear effects. After first hyperpolarizing xenon, they found they could transfer this hyperpolarization to protons in other solutions such as benzene. The mechanism of this nuclear-nuclear polarization transfer is the nuclear Overhauser effect (NOE), normally associated with electronic to nuclear spin transfer. Dubbed SPINOE (spin polarization-induced NOE), this method provides a way to polarize protons without the need for traditional radiofrequency irradiation used in NMR [*Science,* **271,** 1848 (1996)].

With SPINOE, the scientists should be able to tell where the xenon is binding in macromolecules and proteins, Pines says. "The results are very encouraging, because xenon binds to particular sites in some molecules." This ability to observe the chemical shift of an enhanced signal could be extremely useful in NMR spectroscopy of surfaces and proteins. "We believe this is going to be another breakthrough," Navon says.◀

Epothilone Epiphany: Total Syntheses

Compound shares Taxol's mechanism of action and is more potent in in vitro tests

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The first total syntheses of the promising anticancer agent epothilone A have now been achieved. Epothilone A and a sister compound, epothilone B, have been shown to kill tumor cells with greater potency than the approved antitumor drug paclitaxel (Taxol). They accomplish the job by the same molecular mechanism as paclitaxel: binding to cell structures called microtubules.

The first published total synthesis of epothilone A was carried out by chemistry professor Samuel J. Danishefsky, coworkers at Sloan-Kettering Institute for Cancer Research (New York City), and one of his graduate students at Columbia University [*Angew. Chem. Int. Ed. Engl.,* **35,** 2801 (1996)].

In papers in press in *Angewandte Chemie,* chemistry professor K. C. Nicolaou and coworkers at Scripps Research Institute, La Jolla, Calif., and the University of California, San Diego, describe two additional syntheses of the compound. A third group, that of Dieter Schinzer at the Technical University of Braunschweig, Germany, also is very close to completing a synthesis.

"New anticancer agents that function by microtubule stabilization and avoid some of the difficulties of Taxol, such as multidrug resistance, are of great current interest," says chemistry professor Larry E. Overman of the University of California, Irvine, who specializes in medicinal chemistry and complex molecule total synthesis. "In vitro data suggest that epothilone A is such an agent. The Danishefsky and Nicolaou groups' concise total syntheses are milestones in epothilone A research and pave the way for access to analogs not available from fermentation. Both syntheses are beautifully designed and capable of providing structurally diverse analogs."

The Danishefsky team included (top photo, from left) Meng, Bertinato, Sorensen, Kamenecka (front), Balog, and Su. Nicolaou coworkers are (bottom photo, from left) Sarabia, Ninkovic, Yang, Vourloumis, He, and Vallberg.

The epothilones are bacterial natural products that have antifungal properties and are also possible anticancer agents, having shown activity against breast and colon tumor cell lines in the in vitro antitumor screening program of the National Cancer Institute. Their cytotoxic activity and efficacy against drug-resistant cells surpass that of paclitaxel. Preliminary in vivo studies, not yet published, have also been promising.

Paclitaxel inhibits cell division by acting on the cell's internal skeleton, which is made of threadlike microtubules that assemble and disassemble at specific points in the cell cycle. In 1979, Susan B. Horwitz and coworkers at Albert Einstein College of Medicine, Bronx, N.Y., showed that paclitaxel blocks the ability of microtubules to disassemble, thus preventing normal cell division. Another approved anticancer drug, the analog Taxotere, shares this mechanism.

The epothilones likewise block the disassembly of microtubules, inhibiting cell division, and bind to the same receptor site on microtubules that paclitaxel uses. The epothilones are also partially water-soluble and thus should be more easily formulated than pacli-