

fragments differing in length by only a single base—a resolution of better than 5Å—makes possible the techniques by which Sanger and Gilbert in the mid-1970's revolutionized the determination of the sequence of bases along a DNA molecule. In the early 1970's it had taken Gilbert and his student Allan Maxam several years to work out the base sequence of a DNA strand only 20 bases long. It is now possible, by the methods for which Gilbert and Sanger won the prize, for a single person to sequence a strand of several hundred bases in a day. In 1977 Sanger's Cambridge group performed the first determination of the entire base-sequence code of a complete organism—the 5347-base genetic message of the bacterial virus ϕ X174.

The rapid sequencing method developed by Gilbert and Maxam in 1975 employs four chemicals (not enzymes), each of which breaks DNA at the site of a particular base. When, for example, the chemical that attacks only G sites is applied to a large number of identical DNA strands that had been radioactively labelled at one end defined by a restriction-enzyme cut, the result is a collection of fragments of many different lengths—each corresponding to the distance between the labelled end and some occurrence of G further down the

line. (It is essential to apply the chemical in sufficiently dilute solution that not all G sites are broken.) One can then easily read off all the G sites from an autoradiograph of the gel-electrophoretic separation of these fragments by length. Laying four such autoradiographs side by side, each produced by one of the four chemicals, one gets a complete map of the order of the bases G,T,A and C on the original DNA strands.

Sanger's sequencing technique is similar in spirit and efficiency. An enzyme is used to copy strands of DNA cleaved at known sites. But mixed in with the nucleotide triphosphates that provide the bases for the copy are analogs—fakes that closely resemble the four bases. When, for example, copying takes place in the presence of a small amount of dideoxythymidine, some of the sites where a T should have been copied, will get a counterfeit analog of T that brings the copying process to an abrupt halt. Each of the four base analogs can then produce an autoradiographic map just as is done with the four chemical cleavers of Gilbert and Maxam. The sequencing techniques of Gilbert and Sanger are currently used interchangeably in hundreds of laboratories throughout the world. —BMS

Gel collapse is phase transition

An infinitesimal change in temperature or fluid composition can, under the right conditions, abruptly collapse a gel to 1/500th of its original volume. This appears to be a phase transition in the classic thermodynamic sense. A gel is a crosslinked polymer network that holds a fluid in its interstices—for example, a jellied consommé. Considerable theoretical and experimental work has been done since the 1940's on the sol-gel transition, the phase transition from the liquid to the gel state. But prior to the recent work of Toyochi Tanaka and his colleagues at MIT,^{1,2} it was not known that a phase transition could occur within the gel state itself.

This newly discovered phenomenon is a transition between two gel phases, differing only in the degree of swelling—by as much as two orders of magnitude. It suggests that gels might provide useful mechanochemical engines or artificial muscle fibers. The study of these phase transitions may also shed light on pathological changes of gels in the human body, for example retinal detachment, caused by a sudden shrinkage of the vitreous humor in the eyeball.

Critical opalescence. The first hint of such a phase transition came in 1977, when Tanaka was studying the tem-

perature dependence of thermal density fluctuations in gels by means of laser light scattered by the gel. As he lowered the temperature of the polyacrylamide gel under study, the clear gel developed what appeared to be "critical opalescence"—a sudden clouding, with a strong increase in the intensity of the scattered light. Correlation studies of the noise pattern of the scattered light showed that the characteristic relaxation time of the fluctuations of polymer concentration was diverging to infinity. This is analogous to what one sees when supercritical steam is cooled to its "critical point," the point on the phase diagram where the distinction between the liquid and vapor phases first appears. Above this critical point (647 K at 217 atmospheres) the discontinuous density difference between steam and water vanishes.

The observation of critical phenomena such as critical opalescence thus implies the onset of discontinuous phase transitions. Tanaka told us it was not at first obvious that he had seen a critical phenomenon. But when he added acetone to the gel fluid to raise the temperature at which opalescence occurred, he discovered the discontinuous volume changes that signaled the phase transition ly-

ing below the critical temperature.

The acrylamide gels studied in Tanaka's laboratory consist of long polyacrylamide chains cross-linked by bisacrylamide monomers. It turns out that the thermodynamic behavior of these gels depends crucially on their degree of ionization. On the unadulterated polyacrylamide network, every second carbon has an aminocarbonyl (CONH_2) side chain. If one treats the gel for an extended period with a basic solution, some of the side chains are hydrolyzed to carboxyl (COOH) groups. The degree of conversion depends on the hydrolysis time, reaching a maximum of about 25% carboxyl groups after 60 days. After the hydrolyzing base is washed away and the gel is saturated with a water-acetone mixture, the carboxyl groups liberate hydrogen ions into the gel fluid, leaving fixed negative charges on the polymer network.

When the temperature of such swollen gels is gradually lowered (or equivalently, when the acetone concentration is gradually increased), a discontinuous volume change is seen only in the strongly ionized gels. That is to say, the abrupt shrinkage that signals a phase transition is observed only in gels that had been hydrolyzed for at least six days. Tanaka and his MIT colleagues David Fillmore, Shao-Tang Sun, Izumi Nishio, Gerald Swislow and Arati Shah have recently reported² that a maximally ionized acrylamide gel (hydrolyzed for 60 days) will shrink suddenly by a factor of about 350 when it is cooled through its transition temperature (25°C in a 57% acetone solution). Gels hydrolyzed for lesser times will shrink by smaller factors, and at somewhat higher transition temperatures. Finally, for gels hydrolyzed less than six days, the discontinuous volume change is no longer seen; one has merely an inflection point on a continuous curve of volume against temperature.

Flory-Huggins theory. Tanaka told us that these phase transitions and their dependence on the ionization of the gel can be well understood in terms of the mean-field theory developed for gels by Paul Flory and Maurice Huggins in the 1940's. The first of the mean-field theories was introduced by van der Waals in 1873. All such theories approximate the difficult many-body interactions of fluids, ferromagnets and the like by considering only the interaction of any particle with the averaged effect of all other particles in the system.

With a conventional choice of gel parameters, the isotherms of osmotic pressure against gel volume derived from the Flory-Huggins theory turn out to be monotonic; as the gel expands at fixed temperature, its osmotic pressure (its tendency to suck up fluid) falls,

without discontinuities or wiggles. But what's needed for a phase transition is a "van der Waals loop"—the classic wiggle in the pressure-volume isotherm that describes the liquid-gas transition in water and most other fluids. Maxwell showed that such a theoretical wiggle (a local pressure minimum followed by a local maximum), being unstable, must be replaced by an averaging horizontal line that describes the discontinuous volume change one actually sees at the phase transition.

In 1968, Karl Dusek (Institute for Macromolecular Chemistry, Prague) and D. Patterson (then at the Center for Research on Macromolecules, Strasbourg) found that there did exist gel parameters for which the Flory-Huggins theory would generate van der Waals loops. But it was not clear whether these parameters corresponded to an experimentally realizable gel, and hence a real phase transition.

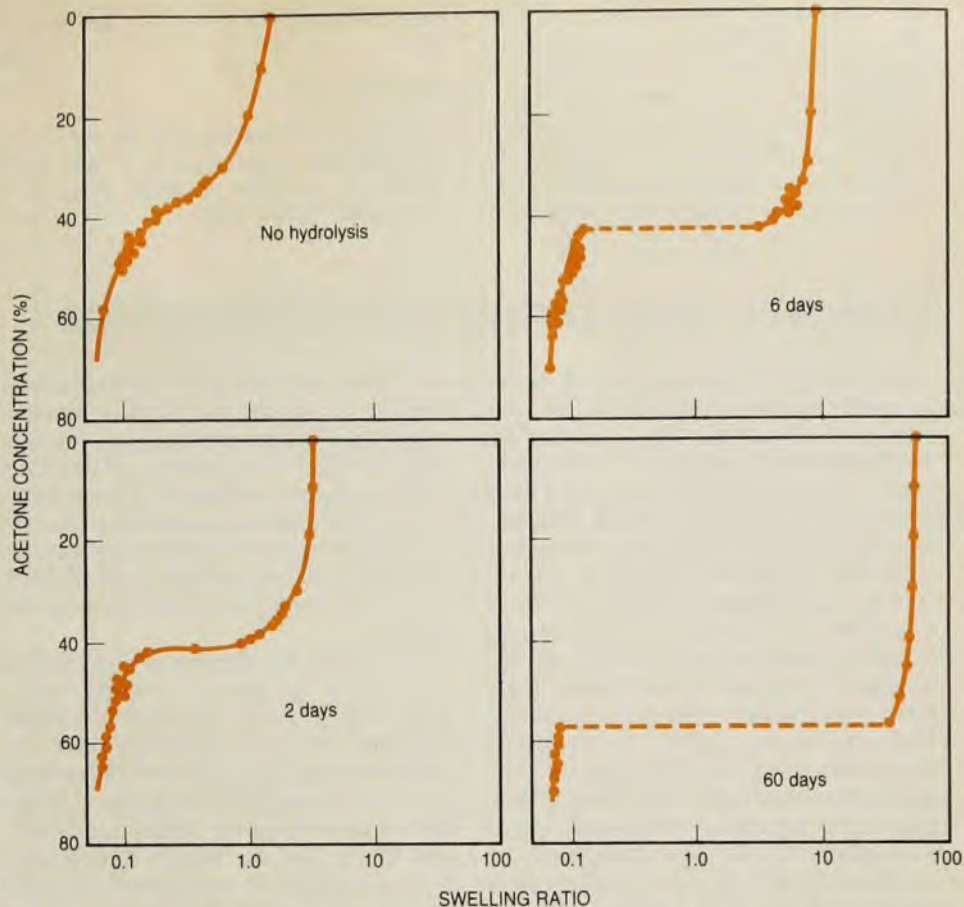
Tanaka told us that he discovered the crucial gel property—ionization—by accident. He was working with old acrylamide gels that had become inadvertently hydrolyzed with age. The MIT group's recent calculations² have explained how these van der Waals loops can be generated by the interplay of the hydrogen ions in the gel fluid with the competing mechanisms that contribute to the osmotic pressure. The net osmotic pressure of the gel is the sum of three terms, contributing with different signs, and different temperature and volume dependence:

- The polymer-polymer affinity contributes negatively to the osmotic pressure; it tends to contract the gel and expel fluid. This term is independent of temperature, but it decreases with increasing volume and increases with acetone concentration.

- The "rubber elasticity" contributes positively or negatively to the osmotic pressure, depending on whether the average polymer strand is stretched or contracted relative to its "normal" length. This contribution increases with temperature.

- The hydrogen ions released by the carboxyl groups into the gel fluid contribute positively; they tend to expand the gel, just as a gas tends to expand its container. As with a gas, this contribution increases with temperature and decreases with increasing volume. It also depends, of course, on the degree of ionization of the gel.

Gel ionization. In the absence of ionization, the shrinking tendency of the polymer-polymer affinity is opposed only by the rubber elasticity. The greater the degree of ionization, the lower is the temperature at which the (temperature independent) polymer-polymer pressure overwhelms the elas-



Shrinkage of polyacrylamide gels as the acetone concentration of the gel fluid is raised (equivalent to lowering the temperature) depends crucially on the hydrolysis time (indicated with each curve). The longer the gel is hydrolyzed, the more ionized it becomes. Swelling ratio (final/initial gel volume) shows discontinuous change, indicating a phase transition, only for gels hydrolyzed at least 6 days. For maximally ionized gels (60 days), the volume shrinks by a factor of 350 at the phase transition. Data from Tanaka's MIT group.

ticity. The MIT calculations show that at sufficiently high ionization the triumph of the polymer-polymer forces occurs at a temperature (or acetone concentration) below a critical point; the isotherms develop van der Waals loops and the gel collapse becomes discontinuous. The more ionized the gel, the lower this collapse temperature falls below the critical point, with corresponding increase of the volume change at collapse.

The dependence of gel phase transitions on ionization, temperature and acetone concentration turns out to be in good agreement with the MIT Flory-Huggins calculation. Additional evidence for the crucial role of the free ions in the gel fluid comes from the fact that the group was able to induce gel collapse by infinitesimal changes in the pH or concentration of sodium or magnesium salts added to the fluid.

Tanaka and his colleagues have also induced collapse with a curious spatial dependence, by subjecting ionized gels to a small external electric field. When a column of gel is suspended between two electrodes, it contracts first at the anode end, forming itself into a narrow-necked bottle shape in a field of a few volts per centimeter. This is the result

of a smoothly varying pressure gradient generated by the field acting on the polymer's fixed negative charges. The discontinuous bottle shape (like a clarinet rather than a Burgundy bottle) results from the discontinuous dependence of volume on pressure at a phase transition. As the field is further increased, the entire gel collapses.

We have been describing "abrupt" volume changes of two orders of magnitude. But for gels of centimeter dimensions these collapses are abrupt only in the sense that they are induced by infinitesimal changes of temperature, fluid composition or applied field. In time they are in fact rather sluggish. The gel takes several days to attain its new equilibrium volume. It is difficult to imagine a mechanochemical engine or an artificial muscle functioning usefully on such a time scale. This time, however, is proportional to the cross-sectional area of the gel. A column of gel one micron in diameter (comparable to a single muscle myofibril) would shrink or swell in about a millisecond.

The relaxation of natural muscle fibers is controlled by the release of calcium ions liberated in response to a nerve impulse. Just as he has done with sodium and magnesium ions in

the laboratory, Tanaka suggests that one might be able to use naturally liberated calcium ions to contract artificial muscle made from gels. But it might be even better, he adds, to contract the artificial muscle by means of a small electric field triggered by the nerve impulse. This might even be an

improvement on Nature, he speculates. —BMS

References

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Literally two-dimensional magnets

The theoretical description of phase transitions remains a challenging problem in statistical physics. Looking for mathematically simple systems, theorists have investigated one- and two-dimensional models. Such models, however present a challenge to experimenters: The world remains—except in very special cases—stubbornly three dimensional. Recent experiments, however, have produced what are apparently truly two-dimensional magnets: manganese stearate films deposited on quartz plates and oxygen adsorbed on exfoliated graphite. Because the substrates are nonmagnetic and have completely different structures from the adsorbed substances, the magnetic ordering can only be two-dimensional. These systems may become useful in investigations of the general properties of phase transitions as well as the specifically two-dimensional theories (*PHYSICS TODAY*, August 1978, page 17, and April 1980, page 17).

Manganese. Melvin Pomerantz of IBM has constructed literally two-dimensional magnets using the Langmuir-Blodgett method to deposit monolayers of manganese stearate onto quartz plates.¹ The Langmuir-Blodgett technique, which is well known among organic chemists, is based on the fact that while polar or ionic molecules are hydrophilic, dissolving readily in water, long-chain hydrocarbon molecules are hydrophobic and are repelled by water while dissolving readily in organic solvents. Molecules that have a polar end on a long organic chain (called "amphiphilic") readily form boundaries between water and organic material (hence their usefulness as detergents) or even air: They will float on water with their polar ends immersed in water and their hydrocarbon tails pointing up out of the water. Irving Langmuir showed (in 1917) that such molecules could be compacted into densely packed monolayers on the water surface. In 1935 Katherine Blodgett transferred such monolayers to a solid substrate by simply dipping a plate of a hydrophilic substance (such as glass) into the water: Nothing happens as the plate is dipped in, but as the plate is removed the polar ends of the molecules stick to it, and if one continues to compress the monolayer film on

the water surface the molecules will deposit uniformly onto the substrate, polar ends on the surface and organic ends sticking up in the air. If one uses a hydrophobic substrate, the organic ends of the molecules adhere to it, so a monolayer forms on insertion; removing the plates produces a bilayer sandwich with the hydrophilic parts in the middle.

One of the substances for which this technique works is stearic acid, $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$; its salts, of course, are similarly amphiphilic and will also form monolayers on water. Pomerantz produced manganese stearate (MnSt_2) by putting drops of stearic acid (dissolved in hexane, which evaporates) onto a solution of manganese chloride (with a pH near 7, or nearly neutral, so manganese ions bind to stearate ions). He then transferred these onto plates, about 1 cm^2 in area, of graphite or quartz, depending on the subsequent experiments.

The first problem, Pomerantz said, was to make sure that the manganese ions were indeed on the plates and arranged in a monolayer with a regular, crystalline pattern. To analyze the composition of the films, he and his collaborators obtained infrared spectra of the films and also used nuclear backscattering and electron-microprobe methods. To obtain the geometry of the packing they used electron-diffraction measurements. The electron-diffraction data show that the manganese ions are arranged in a rectangular grid and that the coherence of the structure apparently extends over fairly long distances. The fact that the arrangement is rectangular, and thus not as symmetric as it could be, means that the theory of the phase transitions is not as simple as one would like, even though the interaction between manganese ions is highly spherically symmetric.

Pomerantz used electron-spin resonance to determine the magnetic state of the manganese stearate films as a function of temperature; his data indicate a transition to a magnetically ordered state below about 2 K. While many of the data are consistent with antiferromagnetic ordering, Pomerantz told us that the state most probably has weak ferromagnetic order-

ing. (In a weak ferromagnet, like in an antiferromagnet, nearest-neighbor spins belong to one of two different sublattices; in weak ferromagnets the spins of the two sublattices are not exactly opposed.) In any case, however, there is apparently a long-range magnetic ordering at low temperatures.

Neutron diffraction is, of course, the ideal tool to investigate magnetic ordering, because neutrons interact with the magnetic moments of the scattering atoms. Other scattering techniques provide only indirect probes of the magnetic ordering of a material. Pomerantz told us that the small amount of material in the manganese stearate samples (about 10^{-7} g/cm^2) apparently precludes using neutron diffraction to study the in-plane ordering: The signal—even from stacks of many quartz plates carrying monolayers—is too weak. However, he added, the signal from multiple layers may be large enough to be useful in investigating the transition from two dimensions to three.

Oxygen. Molecular oxygen is, in some ways, the simplest of magnetic species: The molecule has a net spin of 1, which usually points perpendicular to the axis of the molecule. Several groups have been investigating the low-temperature behavior of molecular oxygen adsorbed on graphite. Among these are Robert Birgeneau and his group at MIT, Stephen Gregory at Cornell, John Stoltenberg and Oscar Vilches at the University of Washington, and John McTague at UCLA and Mourits Nielsen at the Risø National Laboratory in Denmark. In these experiments one generally uses some form of exfoliated graphite, such as Grafoil or ZYX Graphite, which consists of small, nearly parallel flakes of graphite with very consistent crystallographic orientations. The available surface area is extremely large—on the order of square meters per gram—so that even a monolayer of adsorbed molecules has a fairly high volume density. One can thus study, for example, adsorbed oxygen with both neutron and x-ray diffraction as well as with measurements of the heat capacity and magnetic susceptibility. By supplying predetermined amounts of O_2 one can adjust the covering of the graphite to one, two, or more layers of oxygen molecules.

In the bulk, solid oxygen is paramagnetic, but at low temperatures there is a transition to antiferromagnetic ordering, first with short-range order and then, below about 20 K, a long-range order. The situation for oxygen monolayers appears to be more complex.

In 1976 McTague and Nielsen reported² the results of neutron-scattering studies performed with the cold-neu-