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The Science and Practice of Pharmacy

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atoms. It is possible, however, to study the details of molecules without lenses, by means of diffraction experiments. Of the three types of radiation, X-rays have proved to be the most useful and fruitful for studying molecular structure.

Crystalline State

Atoms and molecules tend to organize themselves into their most favorable thermodynamic state, which under certain conditions results in their appearance as crystals. This form is characterized by a highly ordered arrangement of the molecules, associated with which is a three-dimensional periodicity. The repeating three-dimensional patterns, ideally depicted as *lattices*, are essential for X-ray structural analysis.

X-ray Diffraction

In 1912 von Laue and two of his students, Friedrich and Knipping, carried out an experiment with X-rays that opened the door to crystallographic structural analysis. They allowed a beam of nonhomogeneous X-rays to pass through a crystal of copper sulfate pentahydrate; they recorded, by means of photographic plates, the diffracted X-ray beam. A diagram of the experiment is shown in Figure 13-4.

The results showed that X-rays, which had been discovered by Roentgen less than two decades earlier, had wave characteristics (wavelength: approximately 1 Å). As a crystal is composed of a regular array of atoms with interatomic separations of the angstrom (Å) range, they were able to show that the diffraction pattern obtained on the plates was due to the crystal acting as a three-dimensional diffraction grating towards the X-rays.

This discovery led Bragg to make use of X-rays for the study of the internal structures of crystals. He considered that X-rays are reflected from planes of atoms within the crystal lattice. The reflections from a particular family of planes will occur only at a particular angle of incidence and reflection. The essential condition for reflection is diagramed in Figure 13-5. In this figure the *crests* of the two incident waves will stay in phase if the thickened portion of the path (as shown in the diagram) of one wave is an integral multiple (n) of the wavelength (λ). The condition for reflection is given by the well-known Bragg equation:

$$\frac{\lambda}{2} = d_{nh,nk,nl} \sin \theta$$

The equation is satisfied only when $n = 1, 2, 3, \ldots$ If n is not a whole number, there will be destructive interference between the diffracted waves.

In any crystal there are an infinite number of families of planes that can be constructed. These planes usually are de-
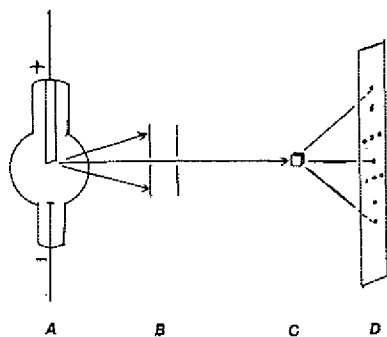

Figure 13-4. Diagram of Laue experiment:(*A*) x-ray tube, (*B*) lead slits, (*C*) crystal, (*D*) photographic plate.
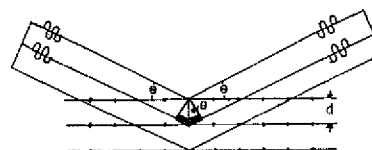

Figure 13-5. Bragg condition for reflection.

noted by their Miller indices (*hkl*), as shown in Figure 13-6. These indices dictate the spacing between the planes (d_{hkl}) for a particular crystal. Because the highest value of θ that is theoretically possible to measure is 90° (reflected beam comes back along the incident beam's path), the number of planes (highest order) that one is capable of orienting in a diffracting position is limited by the wavelength of the radiation.

The planes that are accessible for a particular wavelength (X-ray) can be brought into a diffracting position by the proper orientation of the crystal relative to the collimated beam. In turn, many sets of planes can be recorded on a photographic plate by the movement of the crystal, when each of the planes will come into its diffracting position. In diffraction photographs, in which the crystal has been oscillated about an axis relative to the incident radiation, the various spots on the film arise from reflections from different planes; each spot can be indexed, according to the Miller indices of the respective plane, by its location on the film. The spacing between the various spots enables one to derive the distances and angles between the primitive translations—that is, the unit-cell dimensions.

In most cases little information can be gleaned from a knowledge of the unit-cell dimensions alone. To learn about the crystal and molecular structure, it is necessary to consider the intensities of the Bragg reflections.

Application of X-ray Diffraction

MOLECULAR WEIGHT—The measurement of the unit-cell parameters provides a means of accurately determining molecular weights of compounds. The density of a crystal can be obtained by means of flotation in mixtures of suitable liquids, the density of which may be altered by dilution until it matches that of the crystal.

The density (g/cm³) is proportional to the molecular weight of the material in the unit cell.

The relationship is

$$\text{Mol wt} = \frac{Density \times V_{cell} \times N_a}{Z}$$

where N_a is Avogadro's number (6.023×10^{23}) and Z is the number of molecules in the unit cell. The unit-cell volume (V_{cell}) can be measured to a very high degree of accuracy. The number of molecules in the unit cell (Z) must be a whole number, with values of 1, 2, 4, and 8 being the most common among organic materials. When there is a high degree of solvation, it is necessary to approximate the amount of liquid bound by another means.

IDENTIFICATION OF MATERIALS—Every compound that is crystalline will give a characteristic X-ray diffraction pattern. These patterns can be very useful for identification
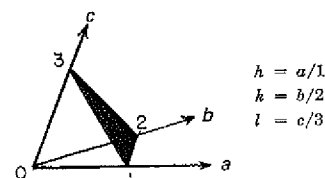

Figure 13-6. Crystal axes intercepted by a crystal plane.

purposes, and also for quantitative analysis of solid mixtures (see Chapter 34). They also have been used to a great extent by the pharmaceutical industry for the identification and classification of polymorphic and solvated forms of drugs. The *powder method*, in which the specimen is ground to a fine powder containing minute crystals oriented in every possible direction and a large number with their Bragg planes in correct orientation for reflection, is a valuable technique when quick comparisons of different forms are to be made and also when quantitative work is done. An example of such a comparison between the hydrated and anhydrous form of theophylline is shown in Figure 13-7.

Extraction of quantitative information from diffraction patterns permits measurements of the physical and chemical stability of solid dosage forms. The kinetics of phase transformations are obtained easily by following the disappearance and/or appearance of various diffraction maxima corresponding to certain solid states as a function of time. One easily can visualize how this can be accomplished for theophylline hydrate by looking at the patterns in Figure 13-7.

STRUCTURE DETERMINATION—The body of substances of medicinal value whose structures were elucidated primarily by X-ray diffraction techniques is quite large. They range in molecular size from penicillin to vitamin B_{12}, and on up to the globular proteins. The structural determinations, in most instances, have played a major role in uncovering the secrets associated with the biological functions of the various molecules. A photograph of the ribonuclease molecule as determined by the X-ray studies of Kartha, Bello, and Harker is shown in Figure 13-8. This enzyme catalyzes the hydrolysis of phosphodiester bonds in RNA chains.

There also are large numbers of macromolecules of biological importance that do not form three-dimensional crystals in the usual sense, but will form fibers. The bundles of molecules in the fiber are aligned with respect to one another in a somewhat crystalline manner. These materials give X-ray diffrac-
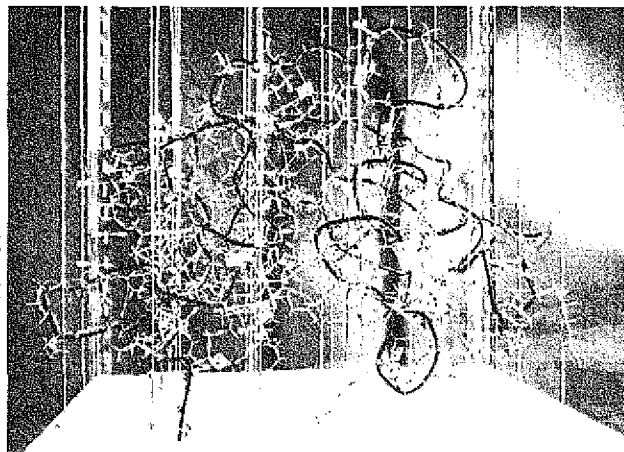

Figure 13-8. Model of bovine ribonuclease derived from X-ray data. The snakelike tube marks the backbone of the protein. (Courtesy Dr G Kartha.)

tion patterns which have proved very useful in deriving molecular information. By fitting models to the X-ray pattern, many valuable biological polymers have had their secrets exposed. The two best examples are the α-helices of keratin and the double helix of deoxyribonucleic acid.

In recent years X-ray studies have been coupled with computer graphic and quantitative structure–activity relationship (QSAR) approaches in computer-assisted drug design (CADD) (see Chapter 28 for a more detailed discussion).

INTRAMOLECULAR BONDING AND CONFIGURATIONS—The precise determination of a crystal structure enables the bond lengths and angles between the various atoms to be determined accurately. This information is extremely valuable in the further understanding of how various chemical substituents influence the valence states and configurations of a molecule. With such knowledge, structure–activity relationships, which are of fundamental interest to the medicinal chemist, have much more depth. The observed bond orders also serve as experimental criteria by which theoretical models can be judged. It also is possible to compare quantum mechanical calculations relating drug interaction with actual observation.

Intramolecular steric effects, which tend to distort molecules, are unraveled easily by the scrutiny of their structures. It is possible to distinguish between repulsive and attractive effects of substituents. The torsional angles about various bonds can be calculated from the atomic positions and are extremely helpful in correlating NMR data to structure.

In recent years the combination of X-ray and neutron-diffraction studies has enabled information on the bonding and nonbonding electrons within a molecule to be delineated clearly. Neutron-diffraction experiments enable atomic nuclei in a crystal to be positioned accurately; on the other hand, X-rays locate the electron clouds. Both types of data can be combined to calculate three-dimensional electron density maps with the inner-core electrons around each atom subtracted; this makes the unshared pairs and bonding electrons clearly visible. The atomic positions derived from neutron data are used for phases in calculating electron density maps with the X-ray data.

Refer to Chapter 34 for additional information on the physical methods discussed in this chapter.
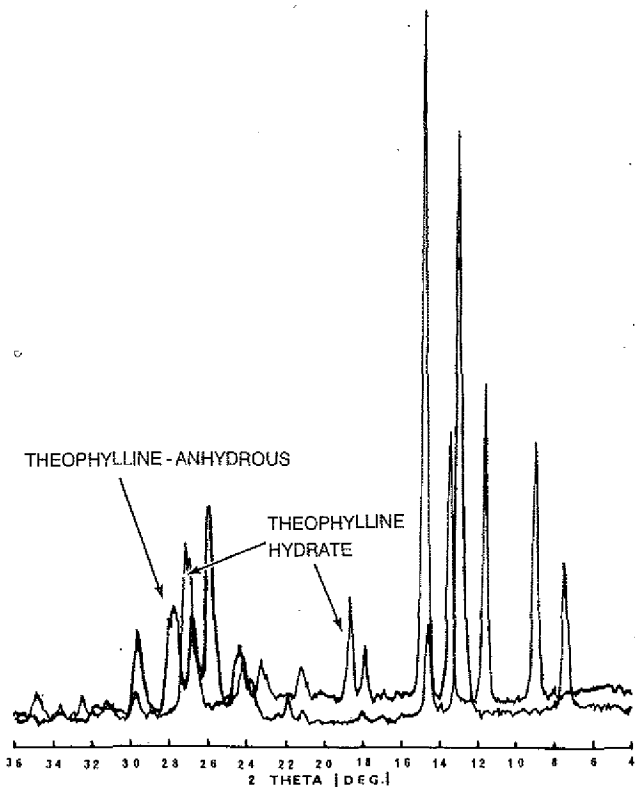
Figure 13-7. A tracing of the powder-diffraction patterns of theophylline monohydrate and an anhydrous form.

THEOPHYLLINE - ANHYDROUS

THEOPHYLLINE HYDRATE

2 THETA (DEG.)

STATES OF MATTER

The aim of this section is to discuss both generalities and specifics, most of which are not related explicitly to dosage

forms, because the latter will be discussed in other chapters. Some of the principles should be useful to have in mind when dosage forms and their manufacture and processing are studied by the product-development pharmacist. It should be noted that due to the range of subjects covered by the section title it was necessary to take an eclectic approach in developing mostly qualitative discussions. The goal has been not to produce a difficult, in-depth section, but rather one that presents a mostly macroscopic overview of the significant states of matter.

Normally, matter exists in one of three states: solid, liquid, or gas. Although it is not pharmaceutically important, two other states of matter exist: the plasma state, in which matter exists as a hot gaseous cloud of atoms and electrons; and a more speculative state, possibly having only a momentary existence, is one which has characteristics of a superdense supermetal. The latter transient state is produced when material is subjected to very high pressures such as those used to make diamonds when compressing graphite.

To avoid the pitfalls of semantics, there is no need to call attention to other systems of classification, because for all practical purposes it is convenient to think only of the three most obvious states. These states are actually a continuum, with two common factors determining the position on the *scale of states.*

The first factor is the *intensity of intermolecular forces* of all kinds: solids have the strongest forces, and gases have the weakest. The other common factor is *temperature.* Obviously, as the temperature of a substance is raised, it tends to pass from a solid to a liquid to a gas. When the phrase "as temperature is increased" is used, it should be remembered that this is a relative phrase. Even at what is called room temperature, some of the effects of a temperature increase are present because room temperature is far above absolute zero.

SOLVATES AND HYDRATES—During the process of crystallization, some compounds have a tendency to trap a fixed molar ratio of solvent molecules in the crystalline (solid) state. These are called *solvates.* When water is used as the solvent, *hydrates* may be formed. Some recent pharmaceutical examples include gallium nitrate ($Ga(NO_3)_3 \cdot 9H_2O$) and nafarelin acetate, where each decapeptide contains 1–2 molecules of acetic acid and 2–8 molecules of water.

As a point of historical interest, note that Lavoisier, the great "father of modern chemistry," thought of heat as a type of matter; the view even as late as the 18th century was that the three states of aggregation differ only with respect to how much heat they contain. Thus, although not all are satisfied with this phraseology, the term *enthalpy* (or *heat content*) is still used in thermodynamics.

Thinking further back to the ancient Greek philosophers and their original four elements (earth, air, fire, and water), note again the great significance attached to heat. Although the ancient philosophers' concepts of the nature of matter were not correct, they did recognize heat as an integral part of the scheme of things, and nothing could be truer. Heat, a vital form of energy, the mirror of molecular motion, is *the* form of energy of greatest importance to mankind.

As alluded to above, there is no clear line of demarcation between the states of matter, but the following arbitrary division may make the approach this section takes more coherent.

Changes of State

As a solid becomes a liquid and then a gas, heat is absorbed and the *enthalpy (heat content)* increases as the material passes through these phase changes. Thus, the enthalpy of a liquid is greater than that of its solid form, and the enthalpy of a gas is greater than that of its liquid form, because heat is absorbed when melting and vaporization occur. The *entropy* (a measure of the degree of total molecular randomness) also increases as materials go from solid to liquid to gas.

It is the balance of enthalpy, entropy, and temperature that determines if changes proceed spontaneously. Obviously, if systems tend to settle to states of lowest energy, it means that enthalpy and entropy considerations may counteract each other. Much of thermodynamics is concerned with explaining and quantitating the changes which systems undergo.

Latent heat is heat absorbed when a change of state takes place without a temperature change, as when ice turns to water at 0°. This example is one in which the heat required to produce the change of state is designated the *heat of fusion.* The counterpart, the *heat of vaporization,* is used when a change of state from liquid to gas is involved.

As molecules of a liquid in a closed, evacuated container continually leave the surface and go into the free space above it, some molecules return to the surface, depending on their concentration in the vapor. Ultimately, a condition of *equilibrium* is established, and the rate of escape equals the rate of return. The vapor then is saturated and the pressure is known as the *vapor pressure.*

Vapor pressure depends on the temperature, but not on the amounts of liquid and vapor, so long as equilibrium is established and both liquid and vapor are present. Heat is absorbed in the vaporization process, and therefore the vapor pressure increases with temperature. As the temperature is raised further, the density of the vapor increases, and that of the liquid decreases. Ultimately, the densities equal each other and liquid and vapor cannot be distinguished. The temperature at which this happens is called the *critical temperature,* and above it there can be no liquid phase.

A very important process that involves a change of state from liquid to vapor and back to liquid is that of *distillation.*

Solids also have vapor pressures that depend on temperature. When a solid is converted directly into gas, it is said to *sublime.* Sublimation pressures of solids are much lower than those of liquids at any given temperature. When a solid is transformed directly into a liquid, two types of melting may be distinguished. In the first type, *crystalline melting,* a rigid solid becomes a liquid, during which procedure two phases are present—the bulk of the solid or its inner parts are not really changing. The second type is *amorphous melting.* This involves an intermediate plastic-like condition that envelops the whole mass; the viscosity decreases and a state of liquidity follows. Crystalline melting involves more definite melting points and latent heats than does amorphous melting.

Sublimation

All solids have some tendency to pass directly into the vapor state. At a given temperature each solid has a definite, though generally small, vapor pressure; the latter increases with a rise in temperature. *Sublimation* is the term applied to the process of transforming a solid to vapor without intermediate passage through the liquid state. In pharmaceutical manufacturing the process commonly includes also the condensation of the vapor back to the solid state.

A solid sublimes only when the pressure of its vapor is below that of the triple point for that substance. The *triple point* is the point, having a definite pressure and temperature, at which the solid, liquid, and vapor phases of a chemical entity are able to coexist indefinitely. If the pressure of vapor over the solid is above that of the triple point, the liquid phase will be produced before transformation to vapor can proceed.

Figure 13-9 depicts a phase diagram illustrating the principle involved. The line *OA* indicates the melting point of the solid form of a substance at various pressures; only along this line can both solid and liquid forms exist together in equilibrium. To the left only the solid form is stable; to the right only the liquid form remains permanently. The line *OB* shows the vapor pressure of the liquid form of the substance at various temperatures. It is called the *vapor-pressure curve*

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