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THE INTERDEPENDENCE OF CHEMICAL PURITY AND SCIENTIFIC ADVANCEMENT¹

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Speaking to an audience which is composed of exponents of several scientific disciplines is a challenging task, for one is faced with the dual problem of saying something of interest for all and of avoiding the pitfall of expatiating upon a specialized topic. It has been customary for past presidents of the Academy to base their speeches on some phase of their research. I have taken the liberty to deviate somewhat from this pattern. As an instructor in a principally four-year liberal arts institution, my primary research interest has been at the undergraduate level. It is the belief of our staff that the research interests of a professor at this level should be geared to the fulfillment of the needs of the undergraduate student, and to create an atmosphere of enthusiasm with regard to scientific research, scholarship, and professionalism.

The research in our department has led us from time to time to use different aspects of chromatography. The great magnitude of scientific advances accomplished during the last thirty years through chromatographic techniques has served to stimulate my interest in the relationship of scientific advances to the attainment of pure materials, and the concomitant dependence of attaining pure materials to the minutiae of methods for purification of materials. Continued pursuit of this interest soon made it apparent that the history of chemistry is replete with major break-throughs in understanding nature as a consequence of the attainment of pure materials. Such break-throughs have been made possible as a result of the inventiveness of man in developing separation processes. However, the spectacular aspects of the feats have overshadowed the minutiae of the method. In nature, most materials are mixtures, few are pure substances. In spite of this, chemists tend to be more interested in pure substances than in mixtures, because from pure substances the architecture of matter can be elucidated. As man has been able to purify matter and establish to his satisfaction that he has a pure substance, his vision regarding the make-up of the physical world has been extended.

I

Ideally, a pure material consists of identical molecules. However, the more we learn about purification, and the inevitable contamination that goes with it, the more we realize that absolute purity is probably attainable only in theory. Therefore, practitioners of science in their quest for chemical purity have settled for operational criteria of purity—that is, the continued fractionation of a sample until the properties being measured, as criteria of purity, are invariable. Further-

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more, the scientist recognizes that the purity of a material is an entirely relative concept and differs according to the goal in view. Thus, the object is to reduce impurities to the point of no effect. In actual practice we seek "effective" purity.

In his Priestley Medal Address, Dr. W. O. Baker (1966), Vice-President of research for Bell Telephone Laboratories, said, "Yet only a beginning is being made, wherein the total substance being handled can be said to consist of one particular set of elements and molecules. This is so whether these substances are pieces of germanium for an integrated circuit, or volumes of helium for a helium-neon laser, or a filament of molybdenum whose super-conductivity is being probed, or a polymer whose ablation characteristics upon re-entry of a space vehicle into the atmosphere are sensitive to impurities. Even then, we know that in a mass of volume containing about 10^{22} atoms, one is extraordinarily successful to have less than 10^{12} impurities of one kind or another".

As the last quarter of the eighteenth century approached, there were no consistent chemical ideas regarding a pure substance. Up to this time, different substances had been confused with one another and identical substances were known under a variety of names and considered to be different. During this period, Lavoisier (1789) introduced the concept of elements as the ultimate residues of chemical analysis. Further, by unravelling the secrets of the composition of air and water and by analyzing the processes of combustion and respiration, Lavoisier was able to banish the phlogiston doctrine from chemical thought and establish a conceptual scheme of revolutionary importance. Lavoisier's work generally established the axiom that only studies with pure substances would yield unambiguous information in the further development of chemistry.

The genius of Lavoisier's work was focused around the discovery that air was a mixture. This major break-through was accomplished by the use of an experimental technique for handling gases which had been reported forty-eight years earlier by Stephen Hales (1727). Hales, who has been referred to as the father of plant physiology, developed the "pneumatic trough," with which pure gases could be collected over water and retained for subsequent study. Here we see an example of one of the most significant characteristics of scientific exploration, which is the way a discovery in one area leads to new developments in another. Hales, the plant physiologist, invented a technique which was used by Lavoisier the chemist, to obtain pure materials. As a result of obtaining pure materials, Lavoisier's important features of chemical thought appeared.

By the middle of the nineteenth century, many organic compounds had been discovered. Thus, the chemist found himself in a dilemma to explain the existence of such an enormous number of compounds formed from essentially carbon, hydrogen, oxygen, and nitrogen. One major problem involved an adequate concept for explaining the existence of more than one particular chemical substance with the same molecular formula. For, at this time, it was an axiom of physical chemistry that substances of identical composition must necessarily have the same properties.

The theory of organic molecular structure which was being forged at this time described different substances having the same molecular formula as isomers. However, the theory of molecular structure failed to account for the isomerism exhibited by tartaric acid and racemic acid. Both of these have the same molecular formula and their chemical identity called for the same structural formula. Yet, in solution, they showed different effects on plane polarized light. Pasteur (1848) recognized that something had been overlooked. It was impossible for molecules to behave differently to polarized light, if they were truly identical in other ways. With this premise in mind, Pasteur conducted his classical work on the fractionation of racemic acid into its two isomeric components. These isomers were identical both chemically and physically, except for their behaviour toward plane polarized light. Pasteur recognized that the cause of the remarkable phenomenon must

reside in the structure of the molecule. He drew the correct conclusion that the molecule of such a compound is itself asymmetrical. From this work we may legitimately regard Pasteur as the Founder of Modern Stereochemistry.

II

The practitioners of chemistry have always been plagued with a major problem of resolution of a system into its pure components, so that the components may be studied and characterized. How the constituents of a mixture can be separated depends on their nature. H. G. Cassidy (1946) of Yale University has described separation processes as a sorting of molecules. He states that, "What we actually do is to provide an environment such that the molecules can become sorted under some driving force, and then we give sufficient time for this to be accomplished". Some understanding of the properties of substances (i.e. volatility, solubility, adsorption affinity, etc.) has led to the invention of techniques for the sorting of molecules.

The powerful practical desiderata for the solution of problems in the laboratory stimulates the craftsman to the design and development of sorting techniques. The craftsman recognizes the need and a *posteriori* reasoning calls forth the invention. Any invention of a new purification technique, when produced, opens new fields for scientific research, scientific theory, and industrial development. Such inventions in turn have put new problems and new powers into the hands of men of science.

When man theorizes about matter, he is doing it on the basis of what he sees with his five senses or with the aid of instruments which extend his senses; therefore, he is not only limited by the instruments, he is also limited by the purity of the material which he is observing. The purity of the material has been predetermined by the techniques which were utilized to obtain the materials. If the scientist has impure materials, he will offer explanations which are based upon observations made on impure materials. Recall the Phlogiston Theory, where the gulf between observations and theory remained unbridged for almost one hundred years. During this period several experiments were performed, any one of which, logically interpreted, could have led to the downfall of the misleading "Phlogiston Theory". What was lacking, however, was the recognition that air was not a pure substance but a mixture. The discovery that air was a mixture was made through use of the invention developed for handling gases. An interesting comment regarding this invention has been made by E. J. Holmyard (1931): "One of the most familiar objects of a modern chemical laboratory is the pneumatic trough, at which gases are collected by the displacement of water or, less frequently, mercury. So simple is the device that, having once seen it in use, we are apt to take it purely as a matter of course and rarely regard it as a supreme achievement of the inventive genius. Perhaps this indifference is only natural, but what an immensity of labor lies behind the trite instruction of the textbook: 'Collect the gas over water at the pneumatic trough.' Indifference before even the dullest chemical experiment cannot survive a knowledge of the work which made the experiment possible. Every chemical we use, every piece of apparatus we take, and every experiment we perform, hide a romance."

Clear ideas and precise conceptions are the prerequisites for the development of knowledge of the internal constitution of matter. But an idea, which is simply a working hypothesis, is generated from facts of nature. The facts are discoveries about nature. The methods for discovering facts are inventions of man. Progress in understanding nature is related to the inventiveness of man in developing techniques for obtaining facts. Scientists operate in a somewhat cyclic manner. Observations generate an idea. Generally a *posteriori* rationalization leads to instruments, techniques, or "black-boxes," which are needed to get more facts. More facts lead to more ideas. *Ad infinitum*—we are constantly asking questions

about nature and seeking answers. The more fertile the scientist is in developing methods for making discoveries, the more answers he is able to obtain about nature.

Science has constantly been fraught with the problems of designing or inventing techniques for obtaining pure materials. Preconceived ideas generate the force for new laboratory techniques and instrumentation to make ideas tenable through experimentation. The development of separation techniques are based on a need to solve a problem which could not be solved by procedures at hand. Often, what have appeared to be unsolvable problems have become solvable by an advance in experimental technique. The cogency of this statement is borne out by the state of understanding reached during the twentieth century regarding the molecular behavior of living systems. These advances in man's knowledge have been gained largely through the great innovation in separation techniques known as chromatography.

While it is true that the earlier chemist performed phenomenal feats by fractional crystallization, extraction, and distillation, it has only been in the last three decades, through the various techniques of chromatography, that undreamed of "log-jams" in purification of materials have been broken. The techniques of purification had scarcely advanced beyond the best achievements of the ancients prior to the advent of chromatography. Evidence points to the fact that the techniques of extraction and distillation were used in ancient Mesopotamia about 3000 B.C. in man's efforts to isolate the fragrant constituents of plant materials. It is fully recognized that many advances have been made in the classical processes of sorting molecules. Prolific study continues today on the design of equipment and the theory of the classical processes of separation. However, these methods have now been supplemented by methods of chromatography, which have enormously increased the chemist's ability to separate chemical compounds and to test the purity of compounds separated.

III

Claude Bernard (1865) who has been described as the father of physiology and who is considered to be one of the fathers of biochemistry wrote "Everytime that a new and reliable means of experimental analysis makes its appearance, we invariably see science make progress in the questions to which this means of analysis can be applied." In his writings Bernard advocated that the biological sciences must borrow the tools and processes of physics and chemistry, and he foretold the molecular biology of today. For, in the broadest sense, molecular biology is a study of biological events in terms of the established principles of physics and chemistry. In 1967, we find that many of the unanswerable questions which faced Bernard, one hundred years ago, have been answered during the relative short time span of the last thirty years. The barriers to subjecting biological phenomena to the same type of experimental laws as chemical and physical phenomena have been removed somewhat since Bernard's day. These advancements have been made by improvement in the means of investigation. The understanding of the fine structure of matter (whether living or non-living) has at each step of the way been limited by the tools available.

The twentieth century revolution in the separation and purification of materials is an outgrowth of the discovery made by Michael Tswett (1906), a Russian botanist. Tswett was able to separate plant pigments by percolating a petroleum-ether extract of plant leaves through a column of powdered calcium carbonate. The plant pigments were separated into a variety of yellow and green bands on the adsorbent calcium carbonate. From these zones individual pigments were isolated. Tswett called the process chromatography and recognized it as an adsorption phenomenon.

Twenty-five years elapsed before Tswett's adsorption process was recognized

and exploited by chemists. The possibilities of Tswett's method were fully realized when Kuhn and Lederer (1931) successfully resolved crystalline carotene, the yellow pigment of carrots, into its components. These workers showed that material which for a century had been thought to be a pure substance was in reality a mixture of two compounds.

The work of Kuhn and Lederer gave impetus to the use of Tswett's chromatographic technique as a valuable laboratory tool. The technique was rapidly appropriated by chemist and biochemist, for no other satisfactory technique was available for the elucidation of metabolic pathways, and for the separation of natural products, such as vitamins, carotenoids, and sterols.

The study of natural products and metabolic pathways must overcome several major obstacles. One such major obstacle is the fact that these materials occur in very low concentrations in highly complex mixtures. The detection, separation, and identification of the individual components are accordingly almost impossible by the classical methods of organic chemistry, such as crystallization to constant melting point for solids and distillation to constant boiling point for liquids. These classical methods pose a problem, because most substances of biological origin are rather unstable, easily altered or destroyed by these traditional techniques. Tswett's chromatographic method has offered a delicate and sensitive purification technique for such materials.

Since 1931, the applications of chromatography have been manifold. Literally thousands of papers have been written describing investigations in which some aspect of the technique has been used. Phenomenal feats have been accomplished, in working with complicated mixtures of natural materials, whereby individual substances were isolated in pure form and thereby pure substances were offered for study relative to their chemical structure. The application of the technique of chromatography to the problems of biology has permitted some insight into the uniqueness of the life process in terms of the chemical events that operate within living cells. The initial thrust to a new departure in purification techniques, given by Tswett in 1906, but which gained momentum only around 1931, has served to open broad vistas of experimentation and theory.

Despite the broad applicability of adsorption chromatography as a technique, it has its limitations, as do all procedures. The biochemists were in need of a technique for the separation of mixtures of amino acids from protein hydrolysates. For this task, which involved the separation of compounds soluble in water, Tswett's adsorption chromatographic technique was not applicable. As late as 1941, the only methods of separating amino acids by protein chemists were the countercurrent-liquid extraction and ester-distillation procedures. The exigencies of a new separation technique to answer the problems facing the protein chemist gave birth to "partition chromatography." As stated earlier, it is typical of the experimental method that the scientist, through reasoning, arrives at a preconceived idea of an invention—a new way to apply known physical phenomena—based upon prior observation of the nature of materials. This operational method of invention is borne out by the introduction of the first innovation in the chromatographic techniques by Martin and Synge (1941). These workers applied the chromatographic technique to separation of compounds soluble in water. This technique, called "partition chromatography," differs from Tswett's adsorption chromatographic technique in that the various components of a mixture are distributed between a polar liquid phase held by a porous solid, such as silica gel, cellulose, or starch, and a less polar moving phase.

As an "offshoot" from partition chromatography in columns, Consden, Gordon, and Martin (1944) introduced paper partition chromatography. In this chromatographic system, a sheet of filter paper, which binds in their cellulose fibers a certain amount of water, serves as the stationary phase, and a solvent soaking along the filter paper serves as the mobile phase. Notably in biochemistry, which requires

analyses of separation on a microgram scale, paper chromatography has been outstanding in simplicity and in general usefulness. The techniques of Martin and his co-workers have further extended the scope of chromatography and rendered yeoman's service for the present understanding of the chemistry of life. The use of partition chromatography and related techniques enabled Frederick Sanger and his colleagues (1956) to arrive at an illuminating understanding of the structure of a protein—the hormone insulin. They had discovered the precise order of the amino-acid sequence in the chains that make up the bovine insulin molecule. The success of Sanger's work opened a new chapter in protein chemistry. Other workers have been prompted to attack the structure of larger proteins. As a result of the development of chromatographic techniques, a stream of significant discoveries regarding the structure of proteins are issuing continually from the laboratories of the world.

Another useful chromatographic technique has been ion-exchange chromatography. Biochemists are profiting from ion-exchange chromatography through its use in probing the structure and composition of proteins and nucleic acids. Other types of chromatography are not generally applicable to molecules of such large size and lability. Suitable ion exchangers did not become available for handling large molecules until the late nineteen-fifties. W. H. Stein and Stanford Moore (1961) have reported the complete analysis of the structure of ribonuclease, an enzyme that digests ribonucleic acid. The amino acids from hydrolyzed ribonuclease were identified and measured quantitatively by using a column of an ion-exchange resin coupled with a photometer.

The chromatographers have been truly protean in their interests and promethean in their accomplishments. Without the isolation of biological materials by chromatographic procedures, it would have been impossible for the knowledge in these areas to develop as rapidly as it did. Practical demands in the laboratory for the solution of a particular problem have led to several other variations of chromatography in addition to the ones cited above.

IV

Two frontiers in purification of materials under development at the present time involve the techniques of zone refining and gel-permeation chromatography. The age-old technique of crystallization has been considered in a new light through the modern technique of zone refining. W. G. Pfann (1952) discovered, on passing a rod of germanium through a short furnace, causing a molten zone to move along the specimen, that the impurities were carried forward in the molten zone; by repeating the process a number of times, a high degree of purification was achieved. Germanium and silicon of extreme purity were prepared by this technique. A high level of purity in germanium and silicon made it possible for the electronics industry to develop a miniature lightweight conductor of electricity, the transistor. The technique has since been used for a wide range of metals and also for both organic and inorganic compounds. It is thought that zone refining can give a one-hundred-per-cent-pure product if used on material which is already of high purity.

Gel-permeation chromatography is a molecular size-separation process. Certain materials, such as crystalline zeolites, cross-linked polystyrene and a three-dimensional network of a cross-linked polysaccharide, can be used to separate mixtures of molecules of different sizes and shapes. The key property of these materials is a very open structure with a network of channels running throughout. Zeolites can now be prepared which will absorb one kind of molecule but reject others. These substances are being used to separate and purify liquids and gases. During the last several years, gel-permeation chromatography has become the accepted technique for polymer characterization and also of great help to the biochemist in structural determination of large molecules.

A novel development, during the last five years, has been the use of cellulose acetate or agar (Bautz and Hall, 1962; Bolton and McCarthy, 1962), complexed with deoxyribonucleic acid as column packings. This technique appears to be akin to gel-permeation chromatography. The immobilized DNA retains the ability to form specific hybrids with complementary RNA. Columns of the complexes have been shown to be potentially useful in determining base sequences in codons and for the purification of sRNA.

Obviously, time will not permit continued illustrations of the interdependency of chemical purity and scientific advancement, but it does become readily apparent that new knowledge in chemistry depends upon new or improved techniques for purification of materials. Often the scientist in his work loses sight of the value of the separation techniques employed in his laboratory. These techniques serve only as a means to an end—"Getting on with the job."

Newly acquired knowledge often leads to interesting new applications. However, as more insight into natural phenomena is achieved, we soon find that the procedures and the equipment necessary to answer the current questions are not always in existence. After having observed the nature of materials, then through reasoning we arrive at a preconceived idea of an invention, a way to make use of known differences in physical properties. The development of a calvacade of methods and techniques has gone into the drawing of the present understanding of nature.

Scientific advancement depends upon inventiveness and creativity in coupling the facts of physical observation and experimentation with a device which will allow for the gathering of more facts. As it has been said, "Every scientific advance is an advance in method."

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