Isotopes as Indicators

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THE indicator[1] applications of isotopes, once limited to a small number of naturally occurring radioelements, have assumed considerable importance since the discovery of artificial radioactivity and with the development of improved methods of concentrating stable isotopes. In view of the increasing interest being displayed in this type of investigation, especially in the biological sciences, it is perhaps timely to review the principles upon which such applications are based and to present a number of pertinent examples.

All such indicator experiments depend upon the use of some isotope, the properties of which permit its detection and measurement in the presence of other isotopes of the same element. This applies alike to stable and to radioactive isotope indicators, although the latter were employed long before the stable isotopes were available in convenient concentrations. In the case of radioelements, the measurement may require the aid of photographic plates, electroscopes, or counters; with stable isotopes, it is necessary to resort to properties[2] such as gas density or heat conductivity, specific gravity, or refractive index of water produced by combustion if oxygen or hydrogen isotopes are involved, or the mass spectrograph may be employed. Stable isotopes of the relatively light elements only can make good indicators because of the great analytical difficulties entailed in distinguishing between isotopes of high atomic weight. Indicators may be used in an isotopically pure form or mixed with other isotopes of the same element. With radioelements which are detected readily in unweighable quantities, pure isotopes are rarely employed. For stable elements, on the other hand, the analytical procedures are not so sensitive and require a higher concentration of indicator isotope; hence a relatively high concentration is the rule, and even isotopic purity (*3*) may be approached.

Investigations involving indicator isotopes may be divided into several general groups depending on the ratio of distinctive or marked or "tagged" indicator isotope to the ordinary form or forms. One class of applications results from the chemical inseparability of isotopes once mixed, *i. e.*, the isotope ratio remains constant throughout this type of experiment. A second type permits conclusions to be drawn from changes in this ratio, such as may be produced by an exchange or redistribution reaction. Finally there is a third group of investigations in which the ratio is immaterial; but examples of this class are of interest primarily to the biologist, and will not be discussed in great detail. Without pretending to present a complete survey of the field, examples will be cited of how isotopes, both active and stable, have been exploited to yield information of significance in chemistry.

THE ISOTOPE RATIO[3] REMAINS CONSTANT

The earliest indicator work (*1*) belongs to this group, in that an active lead isotope such as radium D or thorium B was mixed with ordinary lead so that the weight of element corresponding to a given activity was fixed. This radioactivity was then the means of performing a microanalytical determination for lead, since chemical processes are incapable of producing any appreciable change in isotope ratio. Thus the small amount of lead present in a saturated solution of lead chromate prepared from the original active solution was revealed by the radioactivity of this solution. Since the weight of lead corresponding to a given activity was known in advance, and the activity of a given volume was measured, the solubility, which was of the order of 10^{-7} molar, could be derived quite simply. Similar measurements of cobalt (*4*), phosphorus (*5*) and other lead (*6*) compounds have since been reported.

A second physical property determined by means of a radioelement is the exceedingly low vapor pressure (*7*) of thorium acetylacetonate measured by the gas saturation method. In this case, of course, the thorium itself is radioactive, and served as a measure of the minute quantities swept along by a stream of nitrogen.

Another potential microanalytical use of radioelements is as a check on the completeness with which small amounts of various elements can be handled. It is evidently possible to determine the limits of error in different analytical operations such as precipitation, electrodeposition, etc., as well as to correct for losses during the manipulation of small amounts of material. Hevesy and Hobbie (*8*) have made effective use of such a procedure in connection with a series of analyses of rocks for their lead content by adding a known amount of radium D immediately after solution of a sample, and then measuring the activity of the final form (lead peroxide in this case) in which the lead was recovered for

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[1] This term was coined by Hevesy and Paneth in 1913 (*1*) in connection with the first use of radioactive isotopes in solubility measurements in which the properties of the radioelement were of secondary importance. Of late the term tracer element has come into use. It seems to the writer that this term should apply only to cases in which the isotope ratio remains constant or is immaterial.

[2] Detailed information concerning the preparation, concentration, and measurement of isotopes and isotopic mixtures may be found elsewhere (*2*).

[3] In dealing with radioactive substances, the quantity of active isotope is never expressed as an actual concentration, which is extremely small, but rather in arbitrary units of activity depending on the measuring device employed. These in turn are proportional to the quantity of radioelement present.

weighing. By comparing the initially added activity with that of the peroxide, any lead deficiency was revealed and could be corrected for.

With radioelements it has been possible to test a number of fundamental laws down to very small concentrations. Thus the mass action law, applied to the solubility of radium sulfate (*9*) in sodium sulfate solutions, was found to hold in 0.1 N solutions of the latter; and the validity of the Nernst electromotive force equation (*10*) was demonstrated down to 10^{-12} N with bismuth using thorium C as indicator. In view of the great sensitivity of detection of radioelements, it is not surprising that adsorption and coprecipitation studies have been made at exceedingly low concentrations (*11*).

Observations of certain chemical properties also have been facilitated by this ease of detection. The earliest examples are the experiments of Paneth (*12*), who proved the existence of polonium and bismuth hydrides. Furthermore, the alpha radiations from polonium, which is not available in weighable quantities, have permitted studies of the chemical properties (*10, 13*) of this element.

Certain diffusion measurements also fall under this heading. By way of illustration, the diffusion of lead (*14*) into thallium may be cited. A lead foil, activated by thorium B, was pressed against a thallium foil, and the diffusion process followed by observing the growth in activity or increase in number of alpha-particle scintillations on the thallium side. By making these observations at time intervals, it was possible to calculate the diffusion coefficient without resorting to laborious analyses.

Corroborative evidence for the existence of an anomalous type of mixed crystals (*15*), in systems of non-isomorphous components such as lead and alkali halides, has been obtained by taking advantage of the effect of radioactive radiations on a photographic plate. A polished surface of the crystal is brought into contact with the plate for a time; and from photomicrographs made after development, it is possible to differentiate between "internal adsorption," which produces spotted exposure, and true incorporation of the lead ions in the alkali halide crystal lattice, which shows up as a uniform blackening of the plate within the boundary of the crystal. Such photographs have been called "radiographs."

The same photographic technic has revealed the existence of colloidal aggregates of atoms, termed radiocolloids (*16*), in solutions of certain radioelements despite the fact that the concentration of the element is insufficient to exceed the solubility product of any compound involved. This phenomenon occurs chiefly with isotopes (as thorium C) of elements (as bismuth) the salts of which are readily hydrolyzed. Similar radiographs have made visible the locale where certain elements concentrate during the formation and treatment of alloys (*17*), and have even served to identify an intermetallic compound (*18*) in the barium–zinc system. Radium, which, though not isotopic, is isomorphic with barium, was used as the indicator for the barium.

Only radioactive isotopes have been mentioned so far because this first group of applications depends on a sensitive method of analysis beyond the range of ordinary analytical chemistry. Obviously no analytical advantage is gained with stable isotopes by substituting one isotope for another.

THE ISOTOPE RATIO IS IMMATERIAL

Whenever the path and distribution of an element in a complicated system is of primary importance, regardless of the mechanism of the process, any variation in ratio of isotopic forms is immaterial. Thus if the phenomenon of self-diffusion is considered, only the local concentration of the marked or tagged isotope is of significance. Such measurements (*19*) have been performed for active lead, gold, and copper diffusing into ordinary lead, gold, and copper, for active lead salts into inactive salts, and for the liquid phase diffusion of deuterium into hydrogen (*20*). The radiograph experiments previously mentioned in which the concentration of certain elements in grain boundaries of alloys was revealed could well be included in this category.

Many examples of this group of applications are to be found in biological researches. Since isotopes allow a distinction to be made between that portion of an element, as phosphorus or calcium, which is newly introduced, and that which was in the system originally, it is obviously feasible to ascertain the distribution and to trace the path of practically all elements in plants and animals. Although these metabolism studies are of great interest, they cannot be discussed here. Surveys of such work can be found in the literature. They involve the use of radioactive indicators (*21*) such as active phosphorus, calcium, sodium, iron, iodine, and many other artificial radioelements, as well as stable isotopes (*22*) of oxygen, nitrogen, hydrogen and potassium.

THE ISOTOPE RATIO CHANGES

By taking into account both the indicator and the total isotope concentrations, it is possible to gain considerable insight into the mechanism of chemical processes as well as the relative strength and equivalence of chemical bonds. Let us illustrate this contention first with single-phase systems. An exchange of bromine atoms between radioactive hydrogen bromide and bromine (and vice versa) in the gas phase has been found (*23*) to occur rapidly at room temperature. The reaction may be written[4]

$$\mathrm{H\overset{*}{Br}} + \mathrm{Br_2} = \mathrm{HBr} + \mathrm{\overset{*}{Br}_2}$$

and could have been demonstrated only by the isotope indicator technic. Judging from the rapid rate at

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[4] "The asterisk indicates a radioactive atom. $\overset{*}{\mathrm{Br}}_2$ means that one of the bromine atoms is radioactive.

which the bromine became radioactive, the reaction seems to proceed by a chain mechanism involving bromine atoms. A photosensitized exchange of hydrogen atoms between methane and molecular hydrogen, demonstrated (*24*) by means of deuterium, illustrates the use of a stable indicator. This reaction was followed with the aid of infra-red absorption measurements.

A great many liquid phase exchanges have been reported. They deal with oxidation-reduction reactions as well as with others, ionic and molecular, in which no valence change takes place. We find (*25*), for example, that radioactive bromine will distribute itself completely between ethyl bromide and dissolved aluminum bromide in about one minute, showing that the bromine atoms in the latter compound are equivalent as regards reactivity. The criterion for complete exchange is that the ratio of active to inactive isotope shall be the same in all components of the system. In other words, at exchange equilibrium the activity of a component should be proportional to the quantity of inactive isotope it contains. In apposition may be mentioned the examples of sodium thiosulfate (*26*) and cupric bromide (*27*) in which the sulfur and bromine atoms were shown by radioactive indicator experiments to be of unequal reactivity. By comparing the rate and extent of exchange of halogen between active sodium iodide (*28*) or aluminum bromide (*25*) and various halides, the greater strength of the aromatic over the aliphatic halogen bond is brought out clearly. The resistance to a redistribution of iodine, which would have resulted in the incorporation of radioactive iodine in the organic molecule, was greater for the aromatic compounds.

A more intimate knowledge of the mechanisms of a number of organic reactions—as the Hoffman (*29*) and Walden (*30*) rearrangements for example—has been obtained by the use of active halogens. Recently Calingaert (*31*) and his collaborators have reported a statistical redistribution of organic radicals between organo-metallic compounds, this random interchange being catalyzed by a wide variety of metal halides and alkyl metal halides. To ascertain whether the catalyst likewise was capable of participating in such an exchange, an equimolecular mixture of radioactive tetraethyl lead and inactive catalyst triethyl lead chloride was prepared, and the activities of the components measured at various time intervals. It was found that within twenty-four hours both compounds had become equally active, proving not only that this particular catalyst at least played an active role in the reaction, but that the chlorine atoms as well as the ethyl groups had taken part in a rapid interchange between the lead compounds. This reaction may be represented as

$$\mathrm{Et_4\overset{*}{Pb} + Et_3PbCl = Et_3\overset{*}{Pb}Cl + Et_4Pb}$$

A number of inorganic exchange reactions involving ions of phosphorus (*32*), arsenic (*33*), and manganese (*34*) have been studied. No significant exchange is manifest in mixtures of $\overset{*}{P}O_4^{-3}$ and PO_3^{-3}, or of AsO_4^{-3} and $\overset{*}{As}O_3^{-3}$, in either alkaline or acid media at boiling temperatures. In the case of the arsenate–arsenite equilibrium, addition of iodine and hydrochloric acid permitted the exchange to take place. This was utilized to measure the rate of the oxidation-reduction reaction

$$\mathrm{H_3AsO_3 + I_3^- + H_2O = H_3AsO_4 + 3I^- + 2H^+}$$

at equilibrium since the velocity of the reaction

$$\mathrm{\overset{*}{As}O_3^{-3} + AsO_4^{-3} \rightarrow AsO_3^{-3} + \overset{*}{As}O_4^{-3}}$$

could be measured despite the fact that no appreciable overall concentration changes were possible under the experimental conditions. This exchange reaction obviously represents the rate of the reversible oxidation of arsenite.

There is no lack of exchange reactions involving stable isotopes in liquid media. Thus the exchange of O^{18} between water (*2e*) and a large number of organic compounds such as acids, aldehydes, ketones, etc., has been demonstrated; and inorganic substances such as sulfates, phosphates, carbon dioxide, and sulfur dioxide undergo a similar reaction. To illustrate the use of deuterium, mention may be made of the hydrogen–deuterium exchanges which occur between esters (*35*) and deuteroalcohol or between many organic substances (*36*) and heavy water. Stable isotope indicators have been used also in studying the mechanism of organic reactions such as ester hydrolysis, polymerization, and esterification. Polanyi (*37*) employed H_2O^{18} in the saponification of amyl acetate to prove that the bond which broke during this reaction was between the AmO— and the CH_3CO— groups instead of adjacent to the amyl radical. This followed from the absence of additional heavy oxygen in the amyl alcohol formed. Had the split involved simply the alkyl radical, the O^{18} would have been incorporated in the amyl alcohol.

Exchange reactions are not limited to homogeneous systems. Bromine atoms are found to distribute themselves between gaseous radioactive bromine and silver bromide (*38*); and they will do likewise (*25*) between either liquid or solid aluminum bromide in contact with hydrogen bromide or free bromide. Exchanges in the gas phase can be induced catalytically. In the presence of oxide (Mn, Fe, Cr, etc.) catalysts, O^{18} will exchange (*39*) between gaseous oxygen and heavy water vapor. Metals will catalyze (*40*) a deuterium–hydrogen interchange between deuterium and hydrocarbons, while an N^{14}–N^{15} exchange occurs (*41*) in mixtures of light and heavy nitrogen at the surface of iron synthetic ammonia catalysts and tungsten filaments. The latter reaction takes place readily only above 450°, which stands in marked contrast to the ready exchange (*3*) of hydrogen isotopes on chromium oxide and nickel at temperatures as low as −190°. The importance of such indicator studies in the field of catalysis is obvious.

A most interesting application of an isotope exchange at the solid–liquid interface involves the determination of the specific surface (*42*) of crystalline powders. Assuming that the exchange is restricted to the surface of a powder, such as silver chloride immersed in a solution containing radioactive sodium chloride ($\mathrm{Na\overset{*}{Cl}}$), it follows that the ratio of the adsorbed to unadsorbed indicator isotope $\mathrm{\overset{*}{Cl}}$ will be equal to the ratio of chloride ions at the surface to the number of chloride ions dissolved. Since this ratio is readily obtained by comparing the initial and final activities of the salt solution, and the amount of dissolved chloride can be regulated, the specific surface follows directly from the expression

$$Sp.\ Surf. = \frac{\mathrm{\overset{*}{Cl}}_{ads.}}{\mathrm{\overset{*}{Cl}}_{left}}\ \frac{(\mathrm{Cl^-})_{soln.}}{\mathrm{wt.\ AgCl}}$$

The specific surfaces of a large number of lead salts (*42, 43*) and of silver halides (*44*) have been measured by this method of Paneth. With fresh precipitates (*45*), the value so obtained is extremely large, but diminishes rapidly, thereby revealing aging processes which are not restricted to external surfaces. They appear also to involve those ions which are at the interior of microscopically visible particles.

This discussion will be concluded by citing an investigation which illustrates an interesting metal catalyzed stable isotope exchange between the gas and liquid phases. Polanyi (*46*) and co-workers have observed a hydrogen–deuterium interchange between molecular hydrogen (heavy) and liquid benzene at room temperatures, catalyzed by platinum black and nickel. This exchange is surprising because it is not accompanied by a simultaneous hydrogenation of the benzene.

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