standing 2 days, but it is probably advisable to make up only sufficient for the day's requirements.

Sodium Acetate. With local ores the essential minimum was 5 ml. of sodium acetate solution. The quantity of sodium acetate necessary would vary with different products and it was decided that a safe addition was 10 ml.

Nitric Acid. It is essential to add sufficient nitric acid to dissolve any precipitate formed with sodium acetate and to destroy color complexes formed with metals other than cobalt. It was found that the nitric acid could be increased to 15 ml. without affecting the color development, when 10 ml. of sodium acetate were used. The presence of certain elements necessitated alterations in procedure. Very high iron, manganese, etc., required more than the standard addition of 5 ml. of nitric acid to clear the precipitate formed with sodium acetate.

Time of Boiling. The final boiling had to be carefully controlled. The solution must be brought to a vigorous boil, nitric acid added, and the boiling continued for from 1 to 2 minutes. The extension of the boiling time to 3 minutes caused little change, but any further extension resulted in a definite bleaching of the cobalt color. On the other hand, any decrease in boiling time prevented the full development of the red color. This confirms the work of previous investigators (1).

Fading of Color on Exposure to Light. Samples permitted to stand for 24 hours in Erlenmeyer flasks fitted with loose covers were practically unchanged. On standing for a further 24 hours there was an observable lessening of color. The flasks were exposed to diffused sunlight for half of this period. The effect of exposure to light, although slight, is apparently more pronounced than experienced by Haywood and Wood. This is probably due to the more intense sunlight prevailing in Africa.

It would appear that when dealing with quantities of cobalt between 0.01 and 0.50 mg. the control of conditions need not be so critical as described by Kidson and Askew (2), who were working with quantities of cobalt as low as 0.0001 mg. While Haywood and Wood (1) report good results with very high cobalt-containing steels, the authors have found the greatest range of usefulness

of this method to lie in the lower concentrations of cobalt in mining and metallurgical products.

SUMMARY

A modification of the nitroso R salt method for cobalt is described which should have wide adaptability for the determination of small quantities of this element in metallurgical products and similar materials. Results compare very favorably in accuracy and precision with those obtained by the longer standard gravimetric procedures, and the recovery of added cobalt is complete.

A comprehensive review of the effect of other elements and of varied conditions is given. The determination is best carried out on a sample or aliquot containing 0.01 to 0.5 mg. of cobalt. Apart from the elements removed with hydrogen sulfide in the course of the determination, this work to date shows that the final color comparison can be made in the presence of at least 1000 times as much iron, 100 times as much aluminum, zinc, titanium, thorium, uranium, zirconium, tungsten, barium, strontium, calcium, and magnesium, and 25 times as much vanadium. Quantities of manganese in excess of 50 times, nickel beyond 25 times, and chromium in excess of 15-fold the cobalt present, interfere and must be removed.

Although details are presented for the final determination of cobalt in the Spekker absorptiometer, the method is adapted for any other means of photoelectric or visual color comparison.

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Radioactive Studies

Utilization of the Radioactive Isotope Dilution Procedures for Special Types of Chemical Problems. Quantitative Determination of the Three Individual Components of Mixtures of Dibenzyl Sulfide, Sulfoxide, and Sulfone as an Illustrative Example[1]

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ALTHOUGH certain highly specialized instruments, such as spectrometers (infrared, mass, etc.) and polarographs enable the analysis of certain individual components of many multicomponent systems, there are still numerous types of mixtures that cannot be quantitatively analyzed by means of these apparatus.

It has been known for some time that the isotope dilution method is applicable in principle to any analytical problem (6). Recently Foster, Rittenberg, and others (1, 2, 7) have begun to use stable isotopes in the determination of the amino acid content of proteins. In the case of radioactive isotopes, the usual analytical errors of 10 to 15% prevented their usage in the isotope dilution method (9). Since recent studies (3, 4) have shown that some of the radioactive tracers most difficult to quan-

titate—namely, long-lived sulfur, arsenic, carbon, and hydrogen—can be analyzed to within 2%, the accuracies of the isotope dilution method, with either stable or radioactive isotopes, are essentially identical. This communication indicates the full scope of the radioactive isotope dilution method as applied to chemical problems and gives a specific experimental example, using S^{35} of 87-day half-life.

GENERAL CONSIDERATIONS

Let P be a nonradioactive compound. Let P^* be the same compound in which a minute fraction of the molecules contain a tracer element in a stable position, and let the radioactivity per milligram of this compound be denoted by $(act/mg)_{P^*}$. Let $P.P^*$ denote the chemically indistinguishable mixture of P and P^*, and let the radioactivity per milligram of this mixture be denoted by $(act/mg)_{P.P^*}$. Let the ratio of the specific activities, $(act/mg)_{P^*}/(act/mg)_{P.P^*}$, be defined as R.

There are two general procedures in applying the isotope dilution method.

PROCEDURE I. The amount of P in a multicomponent system is unknown. If a known quantity of P^* is added to and dispersed uniformly throughout the mixture, the amount of P can be determined, provided that a weighable aliquot (≥ 0.05 mg.) of $P.P^*$

The radioactive isotope dilution method is a powerful tool which is applicable to analytical problems that cannot be solved readily by any other means. There are two general variations to this method; one is best suited to the analysis of the individual constituents of multicomponent systems, and the other is useful in the investigation of mechanisms and/or yields of reactions. As an illustrative example utilizing long-lived radioactive sulfur, S^{35}, quantitative data are presented for the analysis of the three individual components of mixtures of dibenzyl sulfide, sulfoxide, and sulfone. These data show that these analyses, if done in triplicate, can be made with an average error of less than 1% and a maximum error of less than 2%.

can be isolated in a pure state. This relationship is given by Equation 1:

$$mg_P = mg_{P^*} (R - 1) \tag{1}$$

PROCEDURE II. The amount of P^* in a multicomponent system containing other radioactive compounds is unknown. The addition of a predetermined amount of P sufficient to isolate subsequently a weighable quantity of $P.P^*$ in a pure state, followed by complete mixing of the active and inactive species, will enable the analysis to be made. Equation 2 gives this relationship:

$$mg_{P^*} = mg_P \, [1/(R - 1)] \tag{2}$$

For both Procedures I and II, the specific activity of P^* can be predetermined to make $R \gg 1$ and to make each radioactive measurement with a maximum error of 2%; thus the maximum uncertainty of an individual analysis by the radioactive isotope dilution method is 4%.

Procedure I is best suited for any problem involving the analysis for the individual components of a multicomponent system, provided that sufficient P is present to enable the isolation of a weighable amount of PP^*. Thus it cannot be used for the determination of traces of substances.

Procedure II is useful in the investigation of the mechanism and/or yield of reactions. This is most readily shown by considering a specific example—namely, the following hypothetical polymerization:

$$A + P = A_1P + A_2P + A_3P + \ldots = \sum_{1}^{N} A_K P \tag{3}$$

where A polymerizes in the presence of the catalyst, P, to A_K and the polymerization is terminated by the addition of P to the polymer. It is essential that the polymers, $A_K P$, can be obtained in pure form—e.g., be reacting large quantities of A and P and subsequent fractional distillation and/or crystallization. Then the synthesis of P^* and the use of this compound instead of P in reaction 3 enables the determination of the exact amount of each $A_K P^*$ formed, since $(act/mg)_{A_K P^*}$ can be computed stoichiometrically from $(act/mg)_{P^*}$, and $(act/mg)_{A_K P.A_K P^*}$ is experimentally determined.

Methods similar to those outlined above are applicable to any reaction. Unlike Procedure I, Procedure II enables the analysis of traces of substances; in fact, since most radioactive isotopes can be obtained in high millicurie strength, it is possible to analyze quantitatively for less than 0.001 microgram of many compounds—e.g., $A_K P^*$—by the isotope dilution method. It is here that radioactive isotopes come into their own, since the allowable dilution factors are much greater than those of stable isotopes.

Utilizing radioactive sulfur, S^{35}, the authors have used both Procedures I and II; the former is considered in the next section and the latter has been used to investigate the mechanism of mustard gas vesication (5). The procedures are interdependent and the accuracy to be expected from either isotope dilution method is essentially identical.

ILLUSTRATIVE EXPERIMENTAL EXAMPLE

In order to evaluate the radioactive isotope dilution method as a quantitative analytical procedure, an illustrative example of

Procedure I will be considered in detail—the analysis of the three individual components of mixtures of dibenzyl sulfide, sulfoxide, and sulfone. In essence the procedure consists of the following steps for this particular case:

Dibenzyl sulfide, sulfoxide, and sulfone, each containing radioactive sulfur, are synthesized.

The amount of radioactivity per milligram, $(act/mg)_{P^*}$, is determined for each radioactive compound.
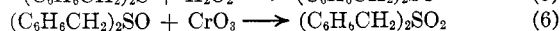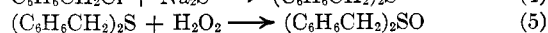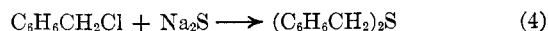
Accurately weighed amounts of each of the radioactive compounds are added to the unknown mixture of dibenzyl sulfide, sulfoxide, and sulfone.

A portion of each substance is isolated in the pure state by fractional crystallizations.

Weighed amounts of the three purified fractions are subjected to radioactive analysis and the $(act/mg)_{P.P^*}$ is computed for each substance. From the reduction in specific activity from $(act/mg)_{P^*}$ to $(act/mg)_{P.P^*}$ together with the known weight of radioactive compound added, the amount of each of the original compounds present in the unknown mixture is calculable by means of Equation 1.

PREPARATION OF THE COMPOUNDS. About 20-gram quantities of nonradioactive dibenzyl sulfide, sulfoxide, and sulfone were prepared by the method described in detail by Shriner et al. (8).

This procedure is based on the following schematic reactions:

$$C_6H_5CH_2Cl + Na_2S \longrightarrow (C_6H_5CH_2)_2S \tag{4}$$
$$(C_6H_5CH_2)_2S + H_2O_2 \longrightarrow (C_6H_5CH_2)_2SO \tag{5}$$
$$(C_6H_5CH_2)_2SO + CrO_3 \longrightarrow (C_6H_5CH_2)_2SO_2 \tag{6}$$

Since the techniques involved in the preparation of dibenzylsulfide on the milligram scale are rather different, the preparation of this compound containing radioactive sulfur is described in detail. The radioactive sulfur used in this preparation was obtained from the Massachusetts Institute of Technology, Radioactivity Center.

Dry barium sulfate (200 mg.) containing about 30 microcuries of S^{35} (87-day half-life) was placed in a platinum boat which was introduced into a long quartz tube. By heating the tube to 800° C. for 4 hours, while passing through a stream of hydrogen, a 98% reduction to barium sulfide was obtained. The trace of hydrogen sulfide formed by the side reaction

$$BaSO_4 + 4H_2 \longrightarrow BaO + H_2S + 3H_2O \tag{7}$$

was caught in a zinc chloride trap. The resulting zinc sulfide precipitate was added to the barium sulfide in the platinum boat.

The boat with contents was transferred to a small gas generator containing 1 gram of 20-mesh zinc. Sufficient 6 M phosphoric acid to cover the boat was now added through a stopcock and the mixture was heated. The issuing gases were dried with phosphorus pentoxide, and the hydrogen sulfide was condensed in a trap chilled by liquid nitrogen. The purpose of the hydrogen was to sweep out all traces of hydrogen sulfide from the generator with a noncondensable gas. The hydrogen sulfide was then stored in an evacuated bulb attached to a vacuum system.

A tube, containing 1.85 millimoles (235 mg.) of benzyl chloride 1.85 millimoles (75 mg.) of sodium hydroxide, 6 cc. of ethyl alcohol, and 1 cc. of water, was attached to the vacuum system by means of a ground joint and frozen down and evacuated. The hydrogen sulfide (0.85 millimole) was vacuum-distilled into this tube chilled with liquid nitrogen. This tube was then sealed and placed on a steam bath for 3 days. The tube was opened and the contents were extracted four times with petroleum ether (b.p. 40° to 60° C.). The petroleum ether solution was placed in a test tube, and the volatile contents were pumped away; the contents of the apparatus were further pumped for 1.5 hours to remove most of the residual benzyl alcohol and benzyl chloride. The dibenzyl sulfide was recrystallized from ethyl alcohol. Yield, 168 mg., 92% based on barium sulfate; m.p. 49° C.

To prepare the dibenzyl sulfoxide, 120 mg. of radioactive dibenzyl sulfide were used.

Yield, 107 mg., 83%; m.p. 134° C.

To prepare the sulfone, 60 mg. of the radioactive sulfoxide were used.

Yield, 45 mg., 70%; m.p. 149° C.

For these last two compounds, Shriner's method (8) scaled down to these quantities was exactly followed.

Thus, about 45 mg. of each compound containing about 10 microcuries of S^{35} were prepared. This quantity of material would allow the analysis in triplicate of at least 100 unknown mixtures of the three dibenzyl compounds.

Table I. Triplicate Radioactive Sulfur Standardizations of the Three Dibenzyl Compounds

a	(C₆H₅CH₂)₂S*	(C₆H₅CH₂)₂S*O	(C₆H₅CH₂)₂S*O₂
ct/mg)P*	169.0	156.5	148.7
	169.6	160.0	148.1
	169.7	158.8	147.2
Av. (act/mg)P*	169.4	158.4	148.0
Av. (act/micromole)P*	36.2	36.4	36.4

a The activity of each sample is computed by the following formula:

$$\text{Activity} = [(d/t)_s - (d/t)_b] \times \frac{10^2}{f} \tag{8}$$

where d is the number (10) of scale divisions traversed by the electroscope fiber in time t in seconds; s and b denote sample and background, respectively; and f is the beta-ray self-absorption correction factor due to the weight of the benzidine sulfate precipitate (see *3* for further details).
With the electroscope used, one unit of activity represents about 10⁻³ microcurie of S³⁵.

Table II. Analysis of Known Mixtures of Dibenzyl Sulfide, Sulfoxide, and Sulfone by Radioactive (S³⁵) Isotope Dilution Procedure

Mixture No.	(C₆H₅CH₂)₂S Weight added *Mg.*	Weight determined by S³⁵ analysis *Mg.* Av.	Individual analysis	Mixture of Nonradioactive Compounds (C₆H₅CH₂)₂SO Weight added *Mg.*	Weight determined by S³⁵ analysis *Mg.* Av.	Individual analysis	(C₆H₅CH₂)₂SO₂ Weight added *Mg.*	Weight determined by S³⁵ analysis *Mg.* Av.	Individual analysis
1	400	406	412 / 395 / 410	400	399	403 / 401 / 392	400	399	409 / 390 / 397
2	400	402	396 / 403 / 406	400	399	403 / 395 / 398	400	397	393 / 406 / 393
3	400	396	389 / 400 / 399	400	401	392 / 404 / 406	400	395	397 / 401 / 390
4	200	199	203 / 197 / 196	400	400	404 / 391 / 404	1400	138₀	1355 / 1402 / 1390
5	400	400	399 / 400 / 402	1400	139₀	1368 / 1408 / 1396	200	203	205 / 199 / 206
6	1400	140₀	1382 / 1415 / 1404	200	201	206 / 193 / 204	400	407	405 / 406 / 410

RADIOACTIVE STANDARDIZATIONS. Approximately 1 mg. of each of the three radioactive compounds was carefully weighed out on a microbalance and oxidized to the sulfate ion by the Carius method. Each sample was washed into its respective 100-cc. volumetric flask. Triplicate 3-cc. aliquots were then subjected to S³⁵ analyses by the procedure described elsewhere in detail (*3*).

Table I shows the reproducibility of these radioactive standardizations as measured by the modified Lauritzen electroscope.

These data show that with care accurate radioactive sulfur analysis can be made. They also are indicative of the purity of the radioactive compounds.

PREPARATION OF RADIOACTIVE STANDARD SOLUTION. About 15 mg. of the radioactive sulfide, sulfoxide, and sulfone were weighed out, respectively, to within 0.05 mg. This mixture was placed in a volumetric flask and made up to 100 cc. with 95% ethyl alcohol. Thus a known volume of this solution contained a predetermined weight of each of the three radioactive compounds. As an additional check, triplicate radioactive analyses were made on 1-cc. aliquots of this solution. The average of the activities obtained was within 0.4% of that computed from the radioactive standardizations of the individual components.

ISOLATION OF PURE PORTIONS OF EACH COMPOUND (P.P*) FOR RADIOACTIVE ANALYSIS. To an acetone solution containing the mixture, *P*, of nonradioactive dibenzyl sulfide, sulfoxide, and sulfone to be analyzed, exactly 10 cc. of the radioactive standard solution, *P**, were added. The solution was well stirred and the acetone and alcohol were evaporated off. The solid mixture was now ready for the isolation of the pure portions of the

three compounds, *P.P**. The procedure used was based on the relative solubility of these compounds in petroleum ether (b.p. 40° to 60° C.) and 95% ethyl alcohol.

Cold petroleum ether was added; the sulfide was partially separated by filtering, since the sulfoxide and sulfone were somewhat insoluble. The remaining solid was dissolved in alcohol; upon cooling, some of the sulfone precipitated while most of the sulfoxide remained in solution. Thus, there now remained a petroleum ether solution of crude sulfide, alcoholic solution of impure sulfoxide, and solid crude sulfone. The petroleum ether and alcohol were respectively evaporated off and the three impure solids were recrystallized three times, the sulfide from 95% ethyl alcohol, the sulfoxide from petroleum ether (b.p. 40° to 60° C.), and the sulfone from 95% alcohol. The melting point of each compound was taken, and if it were not correct further recrystallizations were made.

Six different mixtures containing various known amounts of the three dibenzyl compounds were subjected to the above procedure. Since 200 mg. was the minimum amount of any of the dibenzyl compounds, *P*, in the original mixture, and 20 mg. more than sufficed for radioactive analysis, it was not difficult to obtain the three substances in a highly purified state. Triplicate radioactive sulfur analyses (*3*) were made on weighed amounts (~6 mg.) of each purified component, *P.P**, and the radioactivities were calculated by means of Equation 8.

The experimental results, as computed by Equation 1, are given in Table II. In order to indicate the magnitude of the experimental error, each individual determination has been included in Table II. The greatest deviation found between the amount of dibenzyl compound added and an individual analysis is 3.2% (1400 mg. of dibenzylsulfone added, 1355 mg. found; mixture 4). The average per cent deviation between an individual analysis and the amount of compound added is 1.4. The maximum and average per cent deviations between the average of the triplicate analyses and the weight of dibenzyl compound added are 1.8 and 0.7, respectively.

Thus the accuracy of these data compares favorably with that obtained by using stable isotopes in isotope dilution Procedure II (*2*). It is believed that with care this error of less than 2% could be duplicated in all analyses, utilizing either radioactive isotope dilution Procedure I or II. Since there are numerous analytical problems that cannot be solved readily in any other manner, the radioactive isotope dilution method will ultimately take its place among the other highly specialized analytical techniques.

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