Specifications and Criteria for Biochemical Compounds (1971)

Pages
33

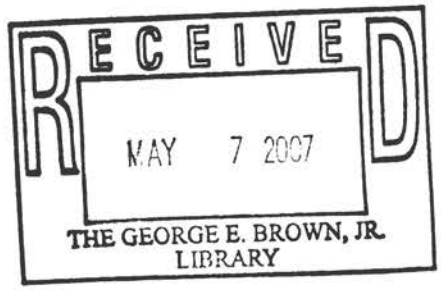
Size
8.5 x 11

ISBN
030936406X

Committee on Specifications and Criteria for Biochemical Compounds; National Research Council

This symposium was organized by members of the Committee on Specifications and Criteria for Biochemical Compounds, National Academy of Sciences, National Research Council. The papers reflect the views of the individual contributors. Publication was aided by contributions from:

BOEHRINGER MANNHEIM CORPORATION
J. T. BAKER CHEMICAL CO.
FRIENDS MEDICAL SCIENCE RESEARCH CENTER, INC.
INTERNATIONAL CHEMICAL AND NUCLEAR CORPORATION
PFANSTIEHL LABORATORIES, INC.



SPECIFICATIONS AND CRITERIA FOR BIOCHEMICAL COMPOUNDS

A Symposium Sponsored by the

Committee on Specifications and Criteria

for Biochemical Compounds

National Academy of Sciences

National Research Council

Preface, Martin Paul, Executive Secretary, Division of Chemistry and Chemical Technology NAS-NRC, Washington, D. C.....1

Introduction, R. W. Von Korff, Chairman, NRC Subcommittee on Enzymes; Director of Biochemical Research, Maryland Psychiatric Research Center, Baltimore, Maryland.....1

Impact of Specifications and Criteria on the Quality of Research Biochemicals, Arthur G. Holstein, President of Pfanstiehl Laboratories, Inc., Waukegan, Illinois.....2

Specifications and Criteria for Enzymes, George Gorin, Professor, Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma.....4

Discussion.....8

Problems in the Commercial Preparation of Isotopic Biochemicals John C. Leak, Senior Production Chemist, International Chemical & Nuclear Corporation, Irvine, California.....9

The Preparation, Containment and Characterization of High-Purity Chemicals, Morris Zief, Senior Research Chemist, J. T. Baker Chemical Company, Phillipsburg, New Jersey.....13

General Discussion.....25

PREFACE

Martin Paul

The National Research Council, the host for this meeting, has had a program on Specifications and Criteria for Biochemical Compounds for some time. This project originated in an attempt to improve the quality of commercial biochemicals. Our most recent publication in this area is the book, "Specifications and Criteria for Biochemical Compounds".

We are greatly indebted to the National Institute of General Medical Sciences for the financial support which has made this work possible. Over 100 persons have participated in this effort on a volunteer basis. Concern with the purity of biochemicals, the development of advanced instrumentation and the demands of clinical laboratories have made the production of high quality biochemicals even more important than before.

We are indebted to Mr. Robert A. Harte, Executive Secretary of the American Society of Biological Chemists, who made arrangements for space and equipment at this meeting.

INTRODUCTION

R. W. Von Korff

On behalf of the Committee on Specifications and Criteria for Biochemical Compounds, Division of Chemistry and Chemical Technology, National Academy of Sciences, National Research Council, I welcome you to the first symposium sponsored by this committee.

The program of this committee was initiated when Dr. Sam R. Hall indicated the long-felt need of biochemists for more knowledge concerning the chemicals used in their studies. At the January 1955 meeting he brought the problem to the attention of the NIH Biochemistry Study Section. As a result a joint committee was established by the American Society of Biological Chemists and the Division of Biological Chemistry of the American Chemical Society.

The first publication of the committee was issued as a looseleaf edition in 1960, followed by a supplement in 1963. In 1967, the second revised edition was issued as a hardback copy containing specifications and criteria for nearly 400 amino acids, carbohydrates, carotenoids, coenzymes, lipids, nucleotides and porphyrins.

Late this year, the committee will issue a revised and enlarged 3rd edition containing data on nearly 500 substances. This edition will have an improved format and will contain some criteria for metabolic intermediates and a discussion of plans for criteria of radioactive compounds. A new subcommittee has been formed to report criteria for biogenic amines and related compounds in subsequent editions.

The various subcommittees have had extensive cooperation from many manufacturers of biochemicals. In the area of enzymes with which I am most familiar, many producers anticipated committee recommendations and criteria requirements, then worked to upgrade their products.

All of us have been concerned on occasion whether particular problems were due to an inadequate reagent, standard or methodology. The work of this committee is designed to eliminate the uncertainties concerning standards for the biochemicals routinely used in the laboratory.

One of our major problems has been a general lack of awareness by chemists of these publications on Criteria and Specifications for Biochemical Compounds. We hope this symposium will help to acquaint more of you with this work. Participation by any individual engaged in the production or use of biochemicals is encouraged.

I. IMPACT ON SPECIFICATIONS AND CRITERIA ON THE QUALITY OF RESEARCH BIOCHEMICALS

Arthur G. Holstein

Compendia such as the United States Pharmacopoeia and the National Formulary have been primarily oriented to meet the needs of the pharmaceutical, food, and related industries. They have not been geared to meet the rapidly growing and expanding requirements of the research laboratories seeking commercially available biochemical compounds. The specifications that are adequate for industry unfortunately are not stringent enough to meet the requirements of research laboratories.

As a producer of carbohydrates and other special chemicals for biochemical use, I can assure you the work of the Committee on Specifications and Criteria for Biochemical Compounds has been a tremendous help in establishing guidelines for manufacturing products to meet the specialized demands of the research market. Although industrial laboratories may be competent in manufacturing biochemicals from natural materials or by synthesis, knowledge about the end-use of these products is often lacking. Each committee helps to determine optimum criteria based on the highest grades of commercially available materials; standard methods of assay are then established.

Care is taken to set standards as high as possible within economic feasibility. For example, galactose and maltose are common sugars produced in commercial quantities for the pharmaceutical industry. It is often unnecessary for these sugars to be chromatographically free of trace materials as long as all other specifications are met. The cost of these two sugars can then be kept reasonably low, if expensive reprocessing to remove trace contaminants can be avoided.

Prior to 1945 the identity of a biochemical compound was based on elemental analysis, specific rotation, index of refraction, preparation of derivatives, biological activity, etc. In addition, general tests included heavy metals, residue on ignition and determination of common anions (chloride, sulfate). Instrumentation was limited to polarimeters, pH meters and colorimeters.

New instrumentation has initiated revolutionary changes. Minor and trace constituents can now be rapidly assessed by gas-liquid or thin-layer chromatography, UV-visible and IR-spectrophotometry. Atomic absorption and flame-emission photometry are sensitive and selective for many trace metals. These techniques are gradually being incorporated into the Criteria Specifications.

A product may be marketed in good faith and with the understanding that the material meets the needs of most of the research market. However, the product may not be suitable for all applications, as the following example shows. Certain laboratories that were aware we offered methyl 2,3-isopropylidene-5-tosyl β-D-ribofuranoside asked that we make available the intermediate, methyl 2,3-isopropylidene-D-ribofuranoside essential for its production. When the intermediate was offered, several laboratories found it useful. However, one group complained that it was contaminated with 15% of the α-anomer. The pure methyl β-furanoside was required. Isolation of this methyl β-furanoside before conversion to the isopropylidene derivative was not essential in our work, since the 5-tosyl derivative was readily crystallizable. The complaint was understandable. Their end product was not readily crystallizable and they needed the pure β anomer to proceed to the next step of synthesis. If we had been informed of this need, we could have isolated methyl β-D-furanoside and converted the pure anomer to the isopropylidene compound. Establishment of improved communication between research laboratories and industry will greatly help industry to meet the needs of research laboratories.

The Subcommittee on Carbohydrates has been fortunate in that problems in establishing specifications have been modest by comparison with the problems of the other subcommittees. For each carbohydrate, specifications have been established based on the highest purity which can be produced economically.

Industry is recognizing the value of the published criteria in the preparation of specifications for purchasing departments. One pharmaceutical

house is using the criteria for amino acids as a basis for purchasing all amino acids.

Standardized reference material frequently is essential for certain assays. This problem has been submitted to the Bureau of Standards. Expansion of NBS clinical standards [W. W. Meinke, Anal. Chem., 43 (6) 28A (1971)] to other biochemical compounds points the direction for the future. At present the research scientist can assure himself of high purity chemicals by referring to the NAS-NRC Criteria and Specifications.

II. SPECIFICATIONS AND CRITERIA FOR ENZYMES

George Gorin

The purity of enzymes for biochemical research and clinical analysis is crucial. At one time, investigators had to isolate enzymes from natural sources and measure the degree of purification, but the situation has changed. A survey of recent American biochemical journals shows that forty-five of the first one hundred enzymes mentioned had been purchased. Only two authors stated that they had tested the purity of the enzymes. It is readily apparent that a critical evaluation of criteria and specifications for enzymes is much needed.

When data for high-purity enzyme preparations are available for reference, the specific activity, i.e. the activity per unit mass, provides information about the purity. Unfortunately, neither the activity nor the mass are easily measured with accuracy. In routine work, the concentration of enzymes in solution is usually estimated from absorbance measurements, at or near 280 nm. However, determination of the specific absorptivity involves measuring the absorbance and mass, consequently the need for an accurate value of the latter is not obviated. It should be desirable to apply other criteria of purity as well; however, at the present time, accurate information on other, apposite characteristics is generally lacking.

The International Union of Biochemistry (IUB) has recommended that the activity of enzymes be measured whenever possible with reference to the amount of substrate n_S consumed per unit time[1]. The equation for activity, Λ may accordingly be written as:

$$(1) \quad \Lambda = -dn_S/dt = -V\, d[S]/dt$$

in which V is the volume of solution and [S] the molar concentration of substrate. This equation is not limited to a particular set of units; n might be expressed in mol, mmol or μmol, t in sec, min, etc. However, the IUB further recommended that μmol and min be the units preferably used, and that the particular magnitude of activity equalling 1 μmol/min be called 1 "enzyme unit" (U). These recommendations have been widely followed.

To determine Λ, finite time intervals are necessarily involved and a difficulty arises if the rate changes with time. In many enzyme-catalyzed reactions Λ does remain constant for a considerable period. This is rationalized, in the simple Michaelis-Menten-Haldane mechanism of enzyme action, on the basis that the enzyme is "saturated" with substrate and the rate is, accordingly, independent of the substrate concentration, or kinetically of "zero order". Then, finite changes in n and t can be substituted for the differentials:

$$(2) \qquad \Lambda = -\Delta n_S/\Delta t = -V\,\Delta[S]/\Delta t.$$

In some cases, however, this condition is not, and possibly cannot be, realized. Then, the conventional prescription is that one should refer to the initial rate of reaction, defined as:

$$(3) \qquad \Lambda_o = \left| -dn_S/dt \right|_{t=o}$$

In principle, to evaluate Λ_o one should know the explicit function relating n_S and t, but this seldom is the case. Often, the initial rate is estimated by plotting the data and drawing a straight line through the first one or two points, but such procedures are liable to serious error (cf. Gorin[2]).

The specific activity λ is given by:

$$(4) \qquad \lambda = \Lambda/m_E = v/c_E$$

in which m_E is the mass of enzyme (the symbol m is used instead of n because the quantity of enzyme is usually expressed in mass and not molar units); c_E is the concentration of enzyme and v is the rate of change of substrate concentration, $-d[S]/dt$. If Λ is measured in U, m_E in mg, c_E in mg/ml, and v in [μmol/(ml-min)], λ will be in U/mg, as recommended by the IUB.

The equation that relates the enzyme concentration to the absorbance A is:

$$(5) \qquad \log I_o/I = A = ac_E d;$$

I_o is the intensity of a monochromatic light beam which impinges perpendicularly on the solution, I is the transmitted intensity, d is the thickness of solution traversed by the beam and a is the specific absorptivity[3]. Usually, d = 1 cm, c_E is expressed in mg/ml and the proper units for a then are cm^2/mg.

If the activity and the absorbance of an enzyme sample are determined without making a direct measurement of mass, information about the purity is provided by the "activity/absorbance" ratio R, defined by the following expression:

$$(6) \qquad R = (\Lambda d)/(AV) = (vd)/A = \lambda/a.$$

The following example illustrates the significance of the quantity R. Consider a hypothetical enzyme having the following characteristics: specific activity, 100 U/mg; reciprocal absorptivity, 1/a, 0.70 mg/cm^2; then R = 70. If a particular sample of enzyme exhibits an R value of 35 and the impurity has the same absorbancy as the enzyme, the purity is 50%. If the absorptivity of the impurity is not known, the percent purity cannot be deduced.

The enzyme concentration may also be determined by other means, e.g. by the biuret test[4]. The ratio (activity/biuret color) also constitutes a criterion of purity; a value lower than that of "pure enzyme" is prima facie evidence for the presence of impurities. But the purity cannot be deduced from that ratio. Since the latter responds only to "biuret-active" impurities, it is, in general, less informative than the ratio R which is lowered by all absorbing impurities.

The third edition of "Specifications and Criteria for Biochemical Compounds"[5] describes criteria for forty-two enzymes. Usually, the information given includes: an equation for the reaction catalyzed by the enzyme; the reciprocal absorptivity; directions for determining and calculating the activity; and the specific activity "of a preparation believed to be of the highest purity (at the time of publication)."

In some cases, the specific activity may be constant only in a narrow range of enzyme concentrations. The assay should then be conducted within closely specified limits [cf. the assays for α-amylase (EC 3.2.1.1) and β-amylase (EC 3.2.1.2)[5]].

Many enzymes can be assayed with more than one substrate; moreover, the composition of the medium and the temperature may be varied. If these factors are not kept constant, of course the observed activities in the different conditions will not correspond to equal concentrations of enzyme. The ratio of the activities can be determined, but this must be done empirically. Such ratios can then be used to compare the results from different assay procedures. Often the enzymic activities reported in the literature are expressed in arbitrary units that may differ from U not only in magnitude but even in dimension(s). The problem of interconverting units of this kind has been discussed by Bowers and McComb[6].

"Purity" has no absolute meaning. For example, terms such as "pure gold," "pure air," "pure color" and "pure species" acquire meaning only as a consensus develops concerning the attributes of the "pure entity" in question and methods for determining them. "Purity" may be defined as the extent to which the attributes approach those of the pure material by definition.

The concept of chemical purity is a special case, that is based on very narrow and demanding criteria[7]. The characterization of chemical compounds usually involves determination of their molecular structures.

The difficulty of such characterization naturally increases with the number of atoms in the structure. Enzymes therefore constitute a problem of extraordinary complexity.

Enzymes, in addition to having large molecular weights, possess other unusual structural features. They contain a greater variety of constituent units than most other polymers, and the units are arranged in definite sequence[8]. As a consequence, usually, certain conformations will be favored over a random arrangement; in turn, the catalytic activity will depend critically on the conformation. So far, comparatively little is known about the relation between the structure and the properties measurable in bulk. Consequently, it is not possible to resolve in decisive fashion the question of whether a given enzyme is a collection of "identical" molecules. For pertinent reviews, see Colvin, et al.[9] and Heremans[10].

A gradual but continuous evolution has been taking place in the methods used for enzyme purification and characterization as well as in the prevailing views concerning purity[10]. Many enzymes that had previously been regarded as homogeneous have been resolved recently into two or more isoenzymes[11].

Improvement in the prevailing standards of purity might be facilitated by having "reference" samples. The U. S. Bureau of Standards has "standard reference samples" for many materials, but not for enzymes[12]. The activity of enzymes decreases more or less rapidly with time, and it may therefore be difficult to establish reference samples having a known and certified activity. On the other hand, "reference standards" for two enzymes, chymotrypsin (EC 3.4.16.4) and trypsin (EC 3.4.16.3), are available from the National Formulary[13]. The feasibility of establishing a reference for lysozyme (EC 3.2.1.17) is under study[2].

As purity is more and more closely approached, the significance of assay tests decreases, because it is limited by experimental errors. At the same time, tests for contaminants become more significant. In practice, high-purity is usually best determined by a judicious combination of assay and impurity tests.

REFERENCES

1. International Union of Biochemistry. Recommendations (1964) on the Nomenclature and Classification of Enzymes. Amsterdam: Elsevier (1965).

2. Gorin, G., Federation Proc. 30, 1132 Abs. (1971).

3. Lothian, G. F., Absorption Spectrophotometry (3rd ed.) London: A. Hilger (1969).

4. Gornall, A. G., Bardawill, C. J. and David, M. M., J. Biol. Chem. 177, 751 (1949).

5. National Academy of Sciences -- National Research Council, Committee on Biological Chemistry. Specifications and Criteria for Biochemical Compounds, 3rd ed.(1972).

6. Bowers, G. and McComb, R. B., Stand. Methods Clin. Chem. 6, 31 (1970).

7. Timmermans, J., The Concept of Species in Chemistry. New York: Chemical Publishing Co. (1963).

8. Dickerson, R. E. and Geis, I., The Structure and Action of Proteins. New York: Harper & Row (1969).

9. Colvin, J. R., Smith, D. B. and Cook, W. H., Chem. Revs. 54, 687 (1954).

10. Heremans, J. F. In: Protides of the Biological Fluids (Proc. 17th Colloquium, 1969), edited by H. Peeters. Oxford: Pergamon Press (1970).

11. Vesell, E. S. (ed.), Ann. N. Y. Acad. Sci. 151, 5 (1968).

12. National Bureau of Standards. Catalog of Standard Reference Materials (NBS Special Publication 260, July 1970 ed.). Washington, D. C.: U. S. Government Printing Office (1970).

13. National Formulary XIII. Washington, D. C.: American Pharmaceutical Association (1970).

DISCUSSION

Question: Gel electrophoresis is useful in detecting protein impurities. Why are not appropriate electrophoretic data published in the criteria for enzymes?

Dr. Gorin: While it may be desirable to employ some additional criteria these should be minimized for practical reasons.

Dr. Von Korff: Several enzyme producers have repeatedly stressed that they are selling activities and that the specific activity of the desired enzyme and of any contaminating enzymes adequately defines their products.

Dr. Rawat: Data for enzyme contaminants should appear on the label to warn the researcher and perhaps save him time and money. Coenzymes should be labeled as to contaminants. On occasion,we have found ethanol in NAD and observed action with alcohol dehydrogenase without addition of substrate.

Dr. Gorin: I agree, however, no reference work and/or label can take into account all the problems that may be encountered by individual investigators.

III. PROBLEMS IN THE COMMERCIAL PREPARATION OF ISOTOPIC BIOCHEMICALS

John C. Leak

INTRODUCTION

The commercial preparation of isotopic biochemicals poses several unique problems. Products containing stable isotopes are generally handled as ordinary research grade chemicals whereas such factors as radiation control, stability, storage, and the price of the isotopic raw material must be considered in a discussion of "commercial" radioisotope preparations. Recently attention has been focused on this subject[1,2,3].

RADIATION CONTROL

Radiation control is a problem common to the manufacture of radiochemicals. The work area, personnel, and products must be considered so that products containing different isotopes are handled in different areas. Protective clothing, shielding and remote handling make common laboratory operations difficult. Health physics supervision, film badges, bioassays, area monitoring, and extensive record-keeping are additional requirements. A chemist exposed to radiation must often be removed from the work area. Packaging and shipping of products require constant vigilance and a competent staff to eliminate errors in measured quantities, cross-contamination of isotopes and improper labeling.

CHEMISTRY

The preparation of radioactive products involves small scale reactions that limit standard methods of purification. Pressure reactions and controlled addition of solid material are all troublesome under these conditions.

Biological syntheses sometimes produce a multitude of products. Dilution of the specific activity by "substrate pools" may reduce the usefulness of the desired product. An example is the biosynthesis of Vitamin-D-^{14}C from labeled acetate. Only a small fraction of the radioactivity is incorporated into the desired product since intermediates in the pathway from acetate to Vitamin-D accumulate in substantial amounts.

Chlorination with ^{36}Cl and the synthesis of organometallics involving acetylene-^{14}C are typical "limited reagent" problems. Straightforward approaches such as bubbling an excess of a gas through the reaction mixture are obviously uneconomical. Invariably, changes in stoichiometry are necessary to avoid an excess of labeled reactants. An excess of alcohol or acylating agent in esterifications or acylations must be avoided when the alcohol or acylating agent is labeled.

Since barium carbonate is the starting point for all carbon-14 synthesis, there are obvious limitations to the raw materials available for synthetic work. Simple intermediates such as potassium cyanide, urea, acetylene, sodium acetate, sodium formate, methanol, and methyl iodide do not allow for great flexibility in the synthesis of complex molecules.

Pilot runs often uncover unforeseen difficulties. Such "tracer" runs are a necessary expense in that they give valuable working experience, but generally give unreliably high yields. The use of commercially available reagents in a pilot run will generally give better results than labeled reactants prepared by dissimilar methods.

In a multi-step synthesis, purification of intermediates at each step is often impractical because of the small scale, inherent instability, and time restrictions imposed by delivery requirements. Multi-step work is dictated by the few practical means of labeling but each step drastically reduces the probability of success. Stoichiometry in a fourth or fifth step is sometimes little more than "guess work". Oxidation, reduction, and halogenation reactions can be affected greatly by accumulated byproducts; low yields of end products result.

Frequently unlabeled reagents are unavailable or contain impurities which produce labeled byproducts that are difficult to remove from the product. For example, pure oxalacetic acid is unavailable. When commercial material is transaminated with pure labeled glutamic acid, five or six byproducts are produced. These byproducts reduce the yield of ketoglutaric acid and complicate the purification procedure. Precursors such as alkyl halides often contain impurities. In the preparation of radioactive fatty acids these impure halides inhibit the reaction or form byproducts that are removed only with difficulty.

The variety of labeled products dictate a need for personnel experienced in many areas of synthetic and analytical chemistry. The final burden falls on the quality control section where the need for broad knowledge is most acute.

PURITY

As it is virtually impossible for a commercial supplier to provide extensive biological testing, problems peculiar to certain specific uses of radiochemicals arise. Nevertheless, commercial suppliers are expected to provide answers for supposedly anomalous "in vivo" results. The following complaints are typical: (1) calcium oxalate-^{14}C isn't readily soluble in water; (2) sodium palmitate-1-^{14}C is insoluble in benzene; (3) a sample bought several months ago contains radiochemical impurities. Many complaints are found to be related to factors other than the radiochemical. A recent example involved the use of ATP in an enzyme system. The system was considered to be poisoned by the labeled reagent. Investigation proved that the buffer was at fault. With fresh buffer the system functioned as expected.

In a number of instances the normally accepted purity criteria do not suffice. Often a labeled product diluted to a specific activity lower than that we normally stock is requested. In many cases, it is impossible to obtain a non-labeled diluent of chemical purity matching that of the

labeled reagent; for example, unlabeled S-Adenosylmethionine is commonly available at a purity level of about only 80% and must be purified before use as a diluent. Glucose-UL-^{14}C at a purity of 99.6% was inadequate for one customer's requirements. We therefore supplied material with a purity greater than 99.75%. Acetic anhydride [^{14}C] or [^{3}H] requires frequent purification to remove a non-volatile impurity which accumulates with age and produces high blanks in steroid analysis.

Trace quantities of labeled impurities are more easily detected than unlabeled materials. Sometimes special purification methods must be developed to remove trace impurities without excessive loss of product. Chromatography is useful for the detection of impurities and for removal from a radiochemical. Paper, thin-layer, gas, and column chromatography are used extensively. Care must be taken not to introduce impurities resulting from oxidation of the sample on the surface of paper and thin-layer chromatograms during sample application, development, drying or elution of the chromatogram. The skillful gas chromatographer will guard against pyrolysis on injection of the sample and avoid introduction of impurities from the column packing. Isotope fractionation has been observed in GLC purifications[4,5,6].

Chromatographic systems can produce artifacts which appear to be impurities. The paper or thin-layer chromatography of some amine salts, employing solvent systems containing organic acids, often produce "ghost" spots or trailing. The sodium salts of fatty acids may produce an additional spot when developed in an alcohol-ammonia system. Certain hydroxy acids when developed in an acidic system are slowly converted to the faster moving lactone, producing a streak rather than a symmetrical spot on a chromatogram. More than one system should be used to check the purity of radiochemicals.

STORAGE

Storage of radioactive materials has caused many problems. Decomposition, minimal at one temperature, may be rapid at another. ATP-γ-P^{32} for example, decomposes rapidly in a solution at 0° C, but when frozen, the decomposition rate is minimal. Microorganism attack can rapidly destroy a high-activity product, especially in a very dilute solution often used for storage. The disappearance of the absorption spectra of tritium-labeled uridine stored in a solution has been attributed to hydration of the molecule. Heating the solution may partially restore the spectra. The pH of a solution may greatly affect the rate of formation of decomposition products. For example, sodium pyruvate in solution forms a dimer. Dimer formation is minimized when pyruvic acid is stored in solution at pH 1. Dilute solutions of high specific activity radiochemicals can change in concentration because of loss of material from solution onto the walls of the container (e.g., high specific-activity fatty acids). Solutions of phosphoric acid-^{32}P can leach silicates from

the walls of glass containers. Users of labeled biochemicals should be aware that a product must be checked before use, particularly if it has been stored for prolonged periods. Data sheets supplied by some manufacturers indicate recommended storage conditions, methods of analysis and shelf life. These sheets should be consulted by a user of isotopic materials unfamiliar with the properties of a particular substance.

ECONOMICS

The price of radiochemicals has consistently decreased over the years, while labor, materials, and overhead have consistently increased. Nucleoside diphospho sugars originally selling for $6,000 per millicurie are now half that price. S-Adenosyl-L-methionine-methyl-^{14}C has decreased from $2,500 to $900 per millicurie. Manufacturers of labeled biochemicals must maintain high purity standards. Development of new products, improvement of manufacturing methods, modification of packaging and storage conditions and investigation of new methods for purity determination add to the cost of the labeled biochemicals. Investigators are demanding higher and higher specific activities, further restricting the scale of work. Such compounds require more frequent checking and repurification with concomitant losses due to accelerated rates of decomposition. Most commercial suppliers realize 80% of their sales from 50-60 compounds, maintaining 300-400 items in a pure form in inventory to make up 20% of sales. The cost of a production run is seldom recovered, and auditors frown on slow-moving inventory.

Commercial suppliers are often faced with custom syntheses which are expensive. Not only must the method of synthesis be proven before a "hot run" is performed, but in most cases the end product is not one of universal appeal and useless as an inventory item. Many of the custom synthesized products are proprietary items or radiochemicals of interest to very few researchers. Frequently manufacturers receive angry reactions from an investigator who cannot understand why we quote in excess of $1,000 for 0.1 mC_i of a custom-labeled polycyclic. The research community should understand that generally it is as costly to manufacture 0.1 mC_i of a product as it is to manufacture a 1 mC_i quantity.

SUMMARY

It is the intention of suppliers to offer a broad line of labeled biochemicals at reasonable cost and high purity and to develop new products as the need arises. We have internal product research, but a technological gap will always exist between the academic world and industry. We solicit and accept your criticisms, but we also need your aid.

REFERENCES

1. Chem. and Eng. News Ed., p. 24, May 3 (1971).

2. Gordon, B. E., Chem. and Eng. News Ed., p. 3, August 23 (1971).

3. Merrill, E., Presentation at San Juan, Puerto Rico, A.C.S. joint meeting, New York and Puerto Rico Sections, April (1971).

4. Lee, E. and Rowland, F., Anal. Chem., 36: 2181 (1964).

5. Bruner, F., Cartoni, G. and Possanzin, M., Anal. Chem., 41: 1122 (1969).

6. West, D. and Marston, A., J. Am. Chem. Soc., 86: 4731 (1964).

IV. THE PREPARATION, CONTAINMENT, AND CHARACTERIZATION OF HIGH-PURITY CHEMICALS

Morris Zief

INTRODUCTION

The modern age of high-purity chemicals started in 1952, the year in which zone melting, an efficient operation that dramatically purified metals, inorganic and organic compounds was introduced[1]. In the same year gas-liquid chromatography (GLC) became available to characterize organic materials[2]. The high efficiency particulate air (HEPA) filter was released for commercial utilization by the Atomic Energy Commission in 1952. This filter is the fundamental unit in laminar flow hoods and clean rooms for the removal of airborne contamination in the handling and packaging of high-purity reagents. These developments upgraded the four key parameters for the production of high-purity reagents; preparation, handling, containment and analysis.

Ordinarily a representative sample of a reagent-grade chemical is submitted to a control laboratory. If the sample meets the pre-determined specifications, the entire batch is packaged without further analysis. Trace impurity contamination from the container is not evaluated when the counter-clockwise sequence of preparation, analysis and containment is normal practice (Fig. 1). In contrast, high-purity liquids and solids sensitive to air are promptly packaged in suitable containers and allowed to equilibrate with the container surface prior to final lot analysis. Clockwise advance from preparation to analysis (Fig. 1) is an important concept for particularly heat-sensitive biochemicals. Urea and cholesterol, for example, are partially decomposed during the flame-sealing of ampoules containing them. Unless these chemicals are analyzed after flame-sealing, the purity of the materials is not assured[3]. Analysis after packaging becomes meaningful for "ultrapure" chemicals containing less than 500 ppm. of impurities (i.e., >99.95% pure)[4].

The minimum assay specifications of typical reagents available in 1971 are listed in Table 1. The purification of calcium carbonate from 98.0 to 99.99 percent represents significant reduction (200-fold) in the impurity content from 20,000 ppm to 100 ppm. Benzene is also available at the same level of purity. The lower assay for cholesterol reflects the difficulties in the purification and analysis of this more complex reagent. The difficulty in assessing the absolute purity of cholesterol illustrates that as a general rule assays are reported more frequently for inorganic reagents that can be analyzed by high accuracy, classical quantitative methods.

Table II compares the specifications for a few selected cations in high-purity reagents. Note the dramatic reduction of lead (>1,000,000-fold) from 100,000 to <0.1 ppb. in phosphorus pentoxide. Dissolution of the purified pentoxide in water (sp. resistance 18 megohm/cm at 25°C) affords phosphoric acid suitable for lead determinations in lunar rocks[5]. This phosphoric acid is an excellent intermediate for the preparation of phosphates free from trace metals. Ultrapure phosphates are necessary for critical studies on nutrition and antiviral vaccines. Extremely low trace-element content in chemicals, particularly in acids, is necessary for many areas of biochemical research. Cation impurities in common acids are now controlled in the low part-per-billion range. Blanks are sufficiently low for the detection of new trace elements required for studies on body chemistry or for the evaluation of injurious environmental pollutants.

In 1967 research workers at the J. T. Baker Chemical Co. initiated the development of a new generation of ULTREX™ ultrapure chemicals[6,7] to provide reagents for the life sciences. These products are produced by advanced purification methods, tested with improved analytical definition and packaged in low-contamination containers. Table III, shows that the purities of glycine and creatinine exceed the National Research Council specifications. Concentrations of eighteen metals are recorded on a certificate of analysis provided with each material. Direct assay of the major component in addition to cation analyses helps to guarantee purity.

PREPARATION

Several purification techniques employed in the ULTREX program are summarized in Table IV. High-purity reagents traditionally have been prepared by utilizing liquid-solid or liquid-vapor phase transitions[8]. The unequal distribution of impurities in the phases at equilibrium results in purification. Often a combination of these techniques is more effective than a single technique alone.

Benzene, listed in Table I, is such an example. Fractional distillation affords a material shown by GLC to be 99.82 percent pure[9]. Purification to an ultrapure sample (99.99 percent) employs multicycle

applications of the liquid-solid phase transition via progressive freezing[10]. Freezing techniques are particularly appropriate for the purification of lipids that possess low melting points and are stable above their melting points.

Organic materials that can be volatilized without decomposition can be obtained 99.95 percent pure by preparative gas-liquid chromatography. Although heat-sensitive biochemicals such as amino acids and carbohydrates can be converted to trimethylsilyl ethers for analysis by GLC, preparative GLC via these derivatives is not practical. The success that GLC has attained for volatile compounds can be predicted for preparative liquid chromatography[11] of labile enzymes, carotenoids and carbohydrates.

Ultrapure metals are readily available through zone refining. By reacting these metals with ultrapure acids, a large variety of ultrapure inorganic salts can now be obtained with a minimum of subsequent purification. Ultrapure gases react with acids in similar fashion. For example, ultrapure ammonia is added to ultrapure acetic acid to give high-purity ammonium acetate.

The history of the starting material is essential in many cases in defining purity. Ultrapure lithium carbonate, for example, is prepared from native lithium ore without depletion of ^{6}Li. When lithium intermediates do not contain the natural isotopic ratio of ^{6}Li and ^{7}Li, a product such as lithium carbonate will have a molecular weight different from that prepared from naturally occurring raw materials.

HANDLING

Transfer, subdivision, drying and other manipulations may result in contamination. Although handling is an integral part of the other parameters (Fig. 1) it merits special consideration. The transfer of liquids perhaps is the most important handling problem. Rubber products require particular attention since a variety of chemicals are incorporated into rubber as accelerators, stabilizers, catalysts, driers, plasticizers and pigments. These chemicals contain alkali metals, cobalt, copper, iron, lead, antimony, tin and zinc added as ZnO[12]. All rubber items, therefore, must be scrupulously investigated or avoided when trace elements must be controlled. A good substitute for rubber tubing is Teflon^{R} FEP (fluorinated ethylene-propylene) that is resistant to chemical attack and possesses adequate flexibility.

Trace impurities are also introduced by airborne contaminants during the handling of uncovered samples[13]. Airborne contamination is eliminated by transferring chemicals in specially designed vertical laminar-flow hoods provided with HEPA (defined on page 13) filters that remove 99.97 percent of all particles and bacteria greater than 0.3 micron in diameter[14,15].

In research on unidentified dietary factors, Schwarz maintained his animals in an all-plastic isolation system furnished with a laminar-flow hood to remove dust and microorganisms[16]. By controlling trace element contamination from the environment as well as dietary ingredients, he discovered that tin is the tenth essential trace element to be recognized[17]. When 1 ppm of tin was present in the diet, growth was enhanced. The problem of establishing new standards of purity for inorganic chemicals in dietary research is obvious when chemicals of 99.9 percent purity, by definition, contain 1000 ppm of impurities.

Chow and McKinney found that Teflon beakers exposed to ordinary laboratory air for eight days acquired 2-4 μg of lead[18]. Lead contamination from ordinary air exceeded that from purified air by a factor of ten.

CONTAINMENT

If a container interacts with its contents, efforts to achieve high-purity may be wasted. Aging studies on packaged acids are necessary to control the impurity content in acids for trace element studies. A preliminary comparison of concentrated acids showed that leaching is related to the water content of the acid. Leaching decreases in the following order: 37% hydrochloric acid > 70% nitric acid > 98% sulfuric acid > 100% acetic acid. Copper increased by a factor of 2.7, sodium and boron by 12, aluminum and silicon by 3, in concentrated hydrochloric acid stored for 13 days in water-washed borosilicate ampoules at 50°C. Water washes, cold or warm, do not provide satisfactory pretreatment. Detergents have been found to contribute to trace impurities. In general, the best leaching agent for a container is the acid which is to be stored in it. Special acid pretreatment affords borosilicate containers that do not significantly alter the original content of the packaged acid. Warm concentrated nitric acid is a good general purpose cleaning agent for glass as well as plastic. A five or six-hour contact time at 60°C. followed by copious washes with water (sp. resistance, 18 megohm/cm. at 25°C) and drying at 50°C *in vacuo* is satisfactory.

ANALYSIS

The characterization of a high-purity chemical, equilibrated with the container, can be divided into three phases - direct assay, analysis of trace impurities and physical measurements. Direct determination of the major component in an ultrapure compound (> 99.95% pure) requires a method reliable to five parts in ten thousand. Precision gravimetry, weight titrimetry and coulometry are preferred techniques for inorganic and organic chemicals. Within the past ten years precision gas-liquid chromatography, differential scanning calorimetry (DSC) and phase solubility analysis (PSA) have been applied to organic reagents. Coulometry and phase solubility analysis are representative of newer analytical methods included in the 13th. edition of the National Formulary[19]. The extensive upgrading of N.F.

analytical tests points the direction of advance for other official specifications. Barnard and co-workers are evaluating techniques that the industrial supplier of fine chemicals can feasibly adopt for the practical analysis of high-purity chemicals[10,20,21].

The quantitative measure of purity via melting-point behavior is an important breakthrough for biochemical materials. In the past, complicated apparatus, large samples and prolonged time-temperature curves made melting methods unattractive. Differential thermal analysis provides a technique for rapidly comparing the purities of thermally stable samples. In 1964, the differential scanning calorimeter became available for determining purity. This instrument measures the thermal energy per unit time transferred to or from the sample as a function of the scanning temperature. By application of the Van't Hoff equation, the molar impurity content of the sample can be calculated. Joy and coworkers found that the available technique afforded a practical upper limit of 99.95% absolute DSC purity[21]. Cholesterol (Table I) is routinely assayed in our laboratory by this technique.

When thermal instability of a compound precludes DSC analysis, equilibration with a solvent can yield absolute purity measurements by phase solubility analysis (PSA) and will detect as little as 0.05 percent of an impurity. DSC and PSA provide convenient methods for the determination of total impurity even when the identity of the impurities is unknown. When cholesterol (Table I) was assayed by these methods, good agreement was obtained between these basically quite different techniques. The purity of the cholesterol was confirmed by thin-layer chromatography (TLC), a third simple technique for assay. Densitometric analysis of TLC chromatograms can extend the limit of detection to 0.05 percent.

Trace cation and anion content must be specified in high-purity reagents. The current limits of detection for anions (low ppm.) do not approach the limits (ppb.) for cations. Therefore, trace anion detection has been receiving major attention in our laboratory.

Classical physical measurements such as melting point and optical rotation are used for many reagents while conductivity, optical absorbance, and particle size distribution are currently used for some reagents. Foreign matter such as dust, paper, etc. in high-purity reagents can be regulated by particulate matter analysis. Specifications for particulate matter in ultrapure reagents are likely in the future. At present, certificates of analysis for some ULTREX reagents describe the particulate matter content after solution of the reagent in water or acid. For example after solution in hydrochloric acid, ULTREX lithium carbonate was found to contain 10 ppm. of particulate matter.

Recognition and evaluation of the contamination problems inherent in the preparation of ultrapure reagents will contribute to improved organic and inorganic chemicals for research and analytical applications.

Thus far, inorganic acids and salts as well as low-molecular weight organics have been the purest chemicals prepared in our program. In many biochemical systems the critical parameter may reside in the purity of simple salts. For example, trace elements in sodium acetate used as a buffer and ammonium sulfate as a precipitant may inhibit enzymes. The preparation of antiviral vaccines may depend on ultrapure potassium citrate. The evaluation of trace element requirements in plant and animal nutrition depends on ultrapure inorganic phosphates. The availability of adequately characterized high-purity chemicals is the sine qua non for trace element research in a variety of biochemical disciplines.

REFERENCES

1. Pfann, W. G., Trans. AIME, 194, 747 (1952).

2 James, A. J. and Martin, A. J. P., Biochem. J., 50, 679 (1952).

3 Joy, E. F., Bonn, J. and Barnard, A. J., Jr., Thermochim. Acta, 2, 57 (1971).

4. Jannke, P., Kennedy, J. K. and Moates, G. H., in M. Zief and W. R. Wilcox, eds., Fractional Solidification, Vol. 1, pp. 463-473, Dekker, New York (1967).

5. Tatsumoto, M. and Rosholt, J. H., Science, 167, 461 (1970).

6. Zief, M., American Laboratory, 1 (10), 55 (1969).

7. Zief, M., Industrial Research, 13 (4), 36 (1971).

8. Perrin, D. D., Armarego, W. L. F. and Perrin, D. R., Purification of Laboratory Chemicals, Pergamon Press, Oxford, (1966).

9. Instra-Analyzed Solvents, Catalog 700, J. T. Baker Chemical Co., Phillipsburg, New Jersey (1970).

10. Gruden, J. R. and Zief, M., in M. Zief and R. Speights, eds., Methods and Techniques of Ultrapurity, Dekker, New York (1972).

11. Bombaugh, K. J. and Levangie, R. G., J. Chromatographic Science, 8, (10), 560 (1970).

12. Robertson, D. E., Anal. Chem., 40, 1067 (1968).

13. Thiers, R. E. in D. Glick, ed., Methods of Biochemical Analysis, Interscience Publishers, Inc., New York, 1957, Vol. 5, pp. 374-409.

14. Contamination Control Handbook, NASA, Cr-61264, February (1969).

REFERENCES (Cont'd)

15. Paulhamus, J. A. in M. Zief and R. M. Speights, eds., Methods and Techniques of Ultrapurity, Dekker, New York (1972).

16. Schwarz, K. in C. F. Mills, ed. Trace Element Metabolism in Animals, E. and S. Livingstone, Edinburgh, 1970, p. 25.

17. Schwarz, K., Milne, D. B. and Vinyard, E., Biochem. Biophys. Res. Comm., 40 (1), 22 (1970).

18. Chow, T. J. and McKinney, C. R., Anal. Chem., 30, 1499 (1958).

19. The National Formulary, Thirteenth Edition, American Pharmaceutical Association, Washington, D. C., (1970).

20. Barnard, A. J., Jr., Joy, E. F., Little, K., and Brooks, J. D., Talanta, 17, 785 (1970).

21. Joy, E. F., Bonn, J. and Barnard, A. J., Jr., Thermochim. Acta., 2, 57 (1971).

22. Mader, W. J., Organic Analysis, Volume II, Interscience Publishers, Inc., New York, 1954.

Figure 1 The Parameters of Ultrapurity

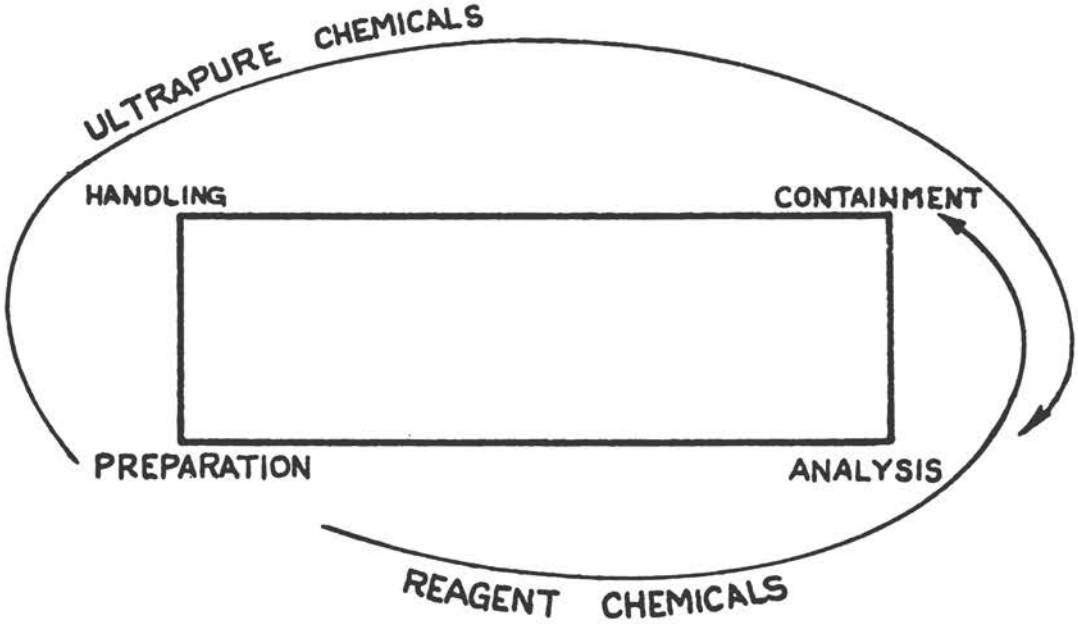


TABLE I

MINIMUM ASSAY OF REAGENTS

	Calcium Carbonate	Sodium Chloride	Benzene	Cholesterol
Rosin[1]	~99.8	~99.9	-	-
U.S.P.[2]	98.0	99.5	-	-
A.C.S.[3]	99.95[a]	-	-[c]	-
'Baker Analyzed'™ [4]	99.0	99.5	99.0	-
Analar[5]	99.5	99.9[b]	-	-
ULTREX[6]	99.99	99.99	99.99	99.8

(1) Reagent Chemicals and Standards, J. Rosin, D. Van Nostrand Co., Inc., Princeton, N. J., Fifth Edition (1967).

(2) The United States Pharmacopoeia, 18th Revision, United States Pharmacopieial Convention, Inc., Bethesda, Maryland 20014 (1970).

(3) Reagent Chemicals, American Chemical Society Specifications, 4th Edition, American Chemical Society Publications, Washington, D. C. (1968).

(4) 'Baker Analyzed' Reagent, Catalog 700, J. T. Baker Chemical Co., Phillipsburg, New Jersey (1970).

(5) Analar Standards for Laboratory Chemicals, Analar Standards Ltd., London (1967).

(6) ULTREX, ultrapurity chemicals, Catalog 700, J. T. Baker Chemical Co., Phillipsburg, New Jersey (1970); typical lot values.

(a) Chelometric standard grade. Other grades do not have an assay requirement.

(b) After ignition.

(c) 'Instra-Analyzed' Reagent.

TABLE II

MAXIMUM LIMITS OF SELECTED CATIONS

	Lithium Carbonate			Phosphorus Pentoxide		Hydrochloric Acid		Hydrofluoric Acid	
	Fe	Pb	Sr	Fe	Pb	Fe	Pb	Fe	Pb
	parts per billion								
A.C.S.[1]	20,000	20,000[3]	-	-	100,000[3]	200	1,000[3]	1,000	50[3]
Resin	20,000	20,000[3]	-	-	50,000[3]	1,000	1,000[3]	1,000	2,000[3]
'Baker Analyzed'	20,000	20,000[3]	-	-	50,000[3]	100	500[3]	1,000	50[3]
Analar	-	-	-	-	-	400	800[3]	1,000	1,000
ULTREX[2]	300	50	2,000	-	<20[4]	7	1	5	1

(1) See Table I for references to grades of chemicals.

(2) Typical lot values.

(3) Heavy Metals (as Pb).

(4) By DC spectrography; by mass spectrometry, <0.1.

TABLE III

SPECIFICATIONS FOR GLYCINE AND CREATININE

	Glycine		Creatinine	
	A[1]	B[2]	A	B
	maximum, parts per million			
Volatile matter	2000	40	3000	100
Ash (sulfated)	1000	90	500	100
Heavy metals (as Pb)	20		30	
Pb, spectrographic		<0.2		<0.2
Ammonium	—	10		
Assay	—	99.9%	—	99.9%

(1) Specifications and Criteria for Biochemical Compounds, National Research Council (1967).

(2) ULTREX, ultrapurity chemicals, Catalog 700, J. T. Baker Chemical Company, Phillipsburg, N. J. (1970); typical lot values.

TABLE IV

PURIFICATION TECHNIQUES

Complexation

Crystallization

Fractional Distillation

Fractional Solidification:

- Column Crystallization
- Progressive Freezing
- Zone Refining

Ignition

Ion Exchange

Preparative Liquid Chromatography

Membrane Filtration

Preparative Gas Chromatography

Quartz Still Distillation

Solvent Extraction

Sublimation

General Discussion

Dr. Harris: (New England Nuclear Corporation)

I would like to call attention to a specific problem. I believe that the publishers of scientific information should demand that authors supply criteria for the determination of purity. Of 600 articles we examined, investigators specified a particular technique for purity measurement in only nine instances. Many of the measurements were copied verbatim from data sheets supplied by the various suppliers. Perhaps this Committee or the individual investigator should insist that criteria of purity is a sine qua non for publication.

Dr. Von Korff:

Implementation of your suggestions would place pressure on the manufacturer to do a better job but in the last analysis the investigator has the ultimate responsibility for establishing the purity of the chemicals he uses.

Dr. Holstein:

With better tools we find that melting point and other accepted methods of characterization are inadequate. Certain sugars have their own idiosyncracies. In the case of fructose, for example, publication C-440 of the Bureau of Standards recommends a 4 percent solution for specific rotation measurements. When determinations were carried out in our laboratory in accordance with the published criteria, our fructose samples failed to pass the test for specific rotation. A 10 percent solution, however, gave acceptable results. We recrystallized fructose until we had the purest sample available. This material was then submitted to six laboratories for testing. The results convinced us to revise the test to use a 10 instead of a 4 percent solution. We have found that the higher concentrations afford more reliable values.

Dr. Mills:

What is the feasibility of a manufacturer indicating suitable criteria for specific products?

Dr. Holstein:

We publish this type of information. Data should be provided for all products that meet NRC specifications. The method of assay should also be listed.

Question:

Do different methods of assay by various suppliers contribute to varying results?

Dr. Holstein:

I can only speak for the Carbohydrate Division. We contacted every known manufacturer and described the degree of purity needed. We then tried to obtain their concurrence with our recommendations. For the new publication, firms such as Pfizer, for example, were contacted in regard to compounds such as ascorbic acid. We asked the producers to tell what they could reasonably do to supply quality products. Specifications more rigid than any thing previously seen in the N. F. or the U. S. P. were submitted by these firms.

Dr. Von Korff:

In partial answer to Dr. Mill's question, each criteria sheet for enzymes specifies the details of the assay method. Methods were selected for reproducibility by many investigators so that comparable results can be obtained in different laboratories. The problem is more difficult in the case of specifications that require an assay method for each impurity. Some companies specify a maximum amount of an impurity as a percentage of the major component. Our subcommittee requests that impurities be expressed in International Units. We plan to use the method reported for each enzyme to assay for foreign activities, e.g., the pyruvate kinase procedure to test lactate dehydrogenase for any contaminating pyruvate kinase*.

Question:

What effect does storage of chemicals in polyethylene containers have on reagent grade materials? Does the Committee have any recommendation on packaging?

Dr. Von Korff:

Polyethylene containers can be a source of trouble when sensitive fluorimetric methods are employed. I believe that Dr. Zief should handle this question.

Dr. Zief:

Polyethylene is an excellent container material. One must consider, however, that a manufacturer may use scrap polyethylene, polyethylene with

---

*Dr. H. U. Bergmeyer of the Boehringer Corp. notes that it is important to assay for contaminating activities either under conditions used to measure the principal activity or those under which the enzyme is used, since the ratio of activities will not be constant under various conditions.

varying amounts of metal catalysts and mold release agents. Polyethylene containers, therefore, must be washed thoroughly to leach the inner surface. A wash with concentrated nitric acid followed by rinsing with deionized water and drying at 50°C in-vacuo is a good general procedure for preparing a clean container.

Chemicals should be refrigerated under a blanket of argon. As a rule, solids are very slightly changed in polyethylene containers. Liquids are more difficult to store. If the liquid freezes, storage in the solid state is preferable. Ultrapure standards are stored at the Bureau of Standards in the solid state whenever possible.

Dr. Von Korff:

With reference to the gentleman's question on polyethylene we have frequently seen a heavy precipitate form in polyethylene bottles containing 1 N NaOH. This is not seen in Teflon bottles. Do you have any idea why the precipitate forms?

Dr. Zief:

I am familiar with a heavy precipitate in glass bottles but not in polyethylene containers. We have packaged 50 percent solutions of sodium hydroxide in polyethylene bottles for several years and have never encountered any complaints. Polyethylene has received excellent ratings as a container for bases such as sodium hydroxide.

Question:

How can suitable criteria for "standards" be developed?

Dr. Zief:

We have found many "standards" which were actually grossly impure with respect to anion content. Calcium carbonate, an important clinical standard, is a good example. We purchased a sample of calcium carbonate that was guaranteed to be 99.99% pure. Emission spectrographic analysis indeed showed that the total of all impurities was less than 100 parts per million. Upon titration of an aliquot, the assay indicated 79% calcium carbonate! We then ran a nitrogen analysis which suggested that calcium nitrate had been incompletely converted to the carbonate and that nitrate was still present.

Inorganic salts must be defined by cation and anion analysis. Biochemists should realize that the criteria of 4, 5, 6, or 7-nine purity established by the electronics industry is satisfactory for metals but not for organic and inorganic salts. It is important to realize that the designation 4-nine purity refers only to emission spectrographic characterization.

Dr. Von Korff:

If there are no further questions, I would like to thank all the speakers and those who contributed to the discussions. I believe it is obvious that considerable work remains to be done in the area of Specifications and Criteria for Biochemical Compounds.