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devoted to the application of microtechniques in all branches of science

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Briefs

The American Microchemical Society. An Informal History. DAVID B. SABINE* AND HERBERT K. ALBER,† **185 Old Broadway, Hastings-On-Hudson, New York 10706 and †36 South Brookside Road, Springfield, Pennsylvania 19064.*

A short history of the Society is given.

Microchem. J. **24**, 265–274 (1979).

Measurement of Protein in Serum and Rodent Embryos by Spectrophotometry at 215 and 225 nm. JAMES B. LABORDE, DENZIL L. TULLIS, JOHN F. YOUNG, AND JOSEPH F. HOLSON, *Division of Teratogenesis Research, National Center for Toxicological Research, Jefferson, Arkansas 72079.*

The Waddell quantitative method for total protein estimation was slightly modified and applied to sera and embryo from rodents and compared to the Lowry method. It was not necessary to employ the Kjeldahl method to determine an instrument correction factor; reference sera were used to establish a standard curve. The results were reproducible from day to day with a high degree of accuracy.

Microchem. J. **24**, 275–286 (1979).

PEG Determinations in Urine, Plasma, and Various Protein Solutions. ANTONIA TY, *Department of Pediatrics, CMDNJ–New Jersey Medical School, Newark, New Jersey 07103.*

Polyethelene glycol is determined in a variety of biological fluids. A stable, accurate, and sensitive method is described which is useful when PEG is used for protein purification, and in physiologic function and pharmacologic mechanism studies.

Microchem. J. **24**, 287–290 (1979).

Microdetermination of Total Sulfur and Inorganic Sulfate in Biological Materials. S. U. KHAN AND G. F. MORRIS, *Chemistry and Biology Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario K1A 0C6, Canada* AND M. HIDIROGLOU, *Animal Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario K1A 0C6, Canada.*

Barium chloranilate colorimetry is used for the determination of total sulfur and inorganic sulfate in biological materials. The method permits the carrying out of about 40 determinations per day.

Microchem. J. **24**, 291–297 (1979).

BRIEFS

The Application of Triphenylmethane Dyes to Visualization of Selected Aliphatic Compounds in Thin-Layer Chromatography. ZBIGNIEW KWAPNIEWSKI AND ROMUALD CICHOŃ, *Department of Analytical Chemistry, Technical University, Cracow, Poland.*

Selected triphenylmethane dyes were used as new visualizing agents in thin-layer chromatography of higher fatty acids, higher fatty alcohols, and higher aliphatic amines.

Microchem. J. **24**, 298–299 (1979).

Automatic Apparatus for the Determination of Carbon and Hydrogen in Organic Compounds. MICHEL HOUDE AND JACQUES CHAMPY with the technical collaboration of RENÉ FURMINIEUX, *Rhone-Poulenc Industries, Centre de Recherches des Carrières, Laboratoire de Microanalyse, 69190—Saint-Fons, France.*

Determination of carbon and hydrogen in organic compounds is achieved by an original technique of coulometric titration in nonaqueous medium. The apparatus is wholly automatic and monitored by a computer making possible the analysis of 23 samples without intervention.

Microchem. J. **24**, 300–309 (1979).

The Use of Iodate and Periodate with Iodide in the Potentiometric Titration of Arsenite, Sulfide, and Sulfite with Mercury (II). H. KHALIFA, N. T. ABDEL-GHANI, AND M. S. RIZK, *Faculty of Science, Cairo University, Giza, Egypt, A.R.E.*

The reductant is added to a known excess of standard iodate or periodate, followed by acid. Excess standard iodide is added, followed by titration of the unreacted iodide with mercury (II) potentiometrically using a silver amalgam indicator electrode.

Microchem. J. **24**, 310–315 (1979).

Oxidation of Organic Substances with Compounds of Trivalent Manganese. XIII. Oxidation of Pyruvic Acid with Manganese(III) Sulfate in a Medium of Sulfuric Acid and with Hexaquomanganese(III) Ions in Noncomplexing Medium of Perchloric Acid. J. BAREK, A. BERKA, AND A. HLADÍKOVÁ, *Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia.*

The oxidation of pyruvic acid was studied and was found to give acetic acid and carbon dioxide and was studied for the analytical application on both the semimicro and micro levels.

Microchem. J. **24**, 316–322 (1979).

The Oxidation of Organic Substances by Compounds of Trivalent Manganese. XIV. Oxidation of Malonic Acid by Manganese(III) Sulfate in Sulfuric Acid Medium and with Hexaquomanganese(III) Ions in Noncomplexing Perchloric Acid Medium. J. BAREK, A. BERKA, AND A. HLADÍKOVÁ, *Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia.*

The oxidation was studied and the optimum conditions found for analytical use of the reaction, including those for the determination of malonic acid.

Microchem. J. **24**, 323–332 (1979).

Thin-Layer Chromatographic Separation of Aldrin, Dieldrin, γ-Hexachlorocyclohexane, Malathion, Ethyl-Parathion, Pentachlorophenol, and of Chlordane, Dieldrin, γ-Hexachlorocyclohexane, Malathion, Ethyl-Parathion, and Pentachlorophenol from Each Other. M. A. KHAN AND J. PAUL, *Chemistry Department, University of Bridgeport, Bridgeport, Connecticut 06602.*

Thin-layer separation of pesticides is described.

Microchem. J. **24**, 333–340 (1979).

Use of the Vanadium(V)–Xylenol Orange Mixture as an Improved Reagent for the Spectrophotometric Determination of Traces of Hydrogen Peroxide. CHIYO MATSUBARA AND KIYOKO TAKAMURA, *Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.*

The reaction in the vanadium(V)–xylenol orange–hydrogen peroxide system was studied by spectrometry. On the addition of hydrogen peroxide to the reagent, the absorption peak of the complex decreases significantly and the decrease is proportional to the concentration of hydrogen peroxide.

Microchem. J. **24**, 341–349 (1979).

Microdetermination of Copper with 4-*S*-Benzyl-1-*p*-chloro-phenyl-5-phenyl-2,4-isodithiobiuret (BPPTB). B. K. DESHMUKH AND R. B. KHARAT, *Department of Chemistry, Nagpur University, Nagpur 440010, India.*

BPPTB forms a bis complex with copper (II), which is easily extractable in chloroform. The values of the equilibrium constants have been evaluated by different methods and the analytical applications studied. Copper can be separated from a large number of ions which generally are present.

Microchem. J. **24**, 350–359 (1979)

BRIEFS

Ultramicrodetermination of Nitrogen in Organic Compounds. XI. Removal of Interference from Iodine for the Sealed-Tube Method. KEIKICHI MIYAHARA AND NORIKO KAMEYAMA, *Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan.*

The interference from iodine was investigated and attempts to minimize it are discussed. Use of tungsten trioxide prepared by heating in a current of air at 800°C for 1 hr is recommended.

Microchem. J. **24**, 360–366 (1979).

Application of the Reaction between 2,2′-Diquinoxalyl and Tin(II) or Titanium(III) to Kinetic Determination of Copper and Iron. R. BARANOWSKI, I. BARANOWSKA, AND ZB. GREGOROWICZ, *Department of Analytical and General Chemistry, Silesian Technical University, Gliwice, Poland.*

The usefulness of the established method was proved for the determination of copper(II) and iron(III) in the concentration range of $10^{-4}-10^{-5}$% with a sensitivity of 0.04 μg/ml.

Microchem. J. **24**, 367–377 (1979)

***N*-*p*-Chlorophenylcinnamohydroxamic Acid: A New Reagent for the Microgram Determination of Vanadium(V).** R. D. ROSHANIA AND Y. K. AGRAWAL, *Analytical Laboratories, Pharmacy Department, Faculty of Technology and Engineering, M.S. University of Baroda, Kalabhavan, Baroda-390001, India.*

Vanadium(V) is extracted from a chloroform solution of the reagent with 4–8 *M* HCl. The bluish violet extract has maximum absorbance at 545 nm and obeys Beer's law with a sensitivity of 0.008 μg V/ml.

Microchem. J. **24**, 378–388 (1979).

MICROCHEMICAL JOURNAL 24, 265–274 (1979)

The American Microchemical Society

An Informal History[1]

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Short accounts covering specific activities and events have appeared from time to time but with the approaching 45th anniversary of the foundation of the American Microchemical Society, the authors feel that the time has come for a résumé of its formation and activities.

At the 89th meeting of the American Chemical Society in New York City in the spring of 1935, two sessions on chemical microscopy were scheduled. They were largely sponsored by Chamot and Mason of Cornell University (*3*), Benedetti-Pichler and Spikes of New York University (*2*), and others who had studied under Friedrich Emich and Fritz Pregl in Graz, Austria. Papers were read by Benedetti-Pichler, Mason, Niederl, Schneider, and others and attendance was so great that the sessions had to be transferred to a larger room and an extra session added. So many people were interested that they agreed that the time had come for the formation of an independent group devoted to chemical microscopy, microanalysis, and general microtechniques. All those interested in such a move were asked to leave their names and addresses with Dr. Frank L. Schneider.

That fall, they all received notices calling for an informal meeting at the Washington Square Branch of New York University. An impressive crowd assembled. There is no authentic list of charter members. In fact the first meetings were quite informal and anyone who joined during the first year rightfully can be called a charter member.

Microchemistry was not a new discipline. Small-scale procedures date back almost as far as recorded history but the eighteenth century marks the beginning of true microanalytical methods. An abbreviated account of the historical development of microchemistry has recently appeared (*9*). Emich is recognized as the founder of classical microchemistry, developing the techniques for all sections of the field but giving preference to inorganic microanalysis (*5*). The other Graz pioneer, Pregl, concentrated on procedures for organic elementary analysis, for which he received the Nobel Prize in 1923 (*11*).

[1] For reprints, contact the Secretary of the American Microchemical Society.

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Microchemistry, as defined by Benedetti-Pichler, "deals with the development, correlation, and systematization of the methods for handling small quantities of materials, and for the observation of their properties. . . . the upper limits of the size is reasonably defined by the statement that the quantity of material taken should be so small as to prevent the use of traditional methods of working. The lower limit is determined by the progress in microtechnique. . . ." (*10*).

H. W. Hermance established a microchemical laboratory at the Bell Laboratories in 1931. This was probably the first industrial laboratory designed exclusively for microanalysis (*8*). But it wasn't until men like Benedetti-Pichler and Niederl, Power, Cheronis, and Schneider offered courses at New York University, Fordham University, Brooklyn College, and other colleges that it really "caught on" in this country.

The group that met in the fall of 1935 formed a society to promote the teaching and practice of microanalysis and related methods. The first name selected was the New York–New Jersey Section of the Microchemical Society, in anticipation of the formation of a nationwide organization. When that failed to materialize, the name Metropolitan Microchemical Society was chosen in 1938 and used for years until the geographical distribution of the membership belied the limitations of the name. The American Microchemical Society seemed more appropriate and was adopted in 1963.

A constitution was written providing for a Chairman, Vice-Chairman, Secretary–Treasurer, and Executive Committee of two. As the Society grew, obvious changes had to be made. The Vice-Chairman became the Chairman-Elect, the duties of the Secretary–Treasurer were divided between two people according to their respective functions, and the Executive Committee was enlarged to three, the immediate past Chairman automatically becoming the third member. Other changes were made from time to time as they became necessary and a complete revision of the constitution was adopted in 1966. Another revision is presently in progress.

Three classes of membership were provided for: regular, life, and honorary. To the latter, Drs. L. T. Hallett, W. R. Kirner, and C. W. Mason were elected when the Society was formed. During the 1956–57 season, the following regular members were elevated to honorary membership: H. K. Alber, J. F. Alicino, A. A. Benedetti-Pichler, H. N. Blume, A. Elek, R. A. Harte, H. W. Hermance, A. F. Knoll, J. A. Kuck, F. A. Meier, D. Price, J. R. Rachele, G. E. Royer, D. B. Sabine, W. Saschek, F. R. Swift, H. V. Wadlow, T. J. Walsh, and L. K. Yanowski. In 1961, at The Pennsylvania State University Symposium, Drs. Feigl, Kofler, Körbl, and Lieb were elected honorary members. In 1965, G. Ingram and in 1970 A. Steyermark were also so honored. By paying a stated lump

sum, a regular member could become a life member but honorary membership was conferred only by a vote of the Society at a meeting. The total membership is now 160.

Since the objectives of the Society were to emphasize the advantages of microtechniques, several special committees were appointed to meet separately on nights other than the regular meeting night, to explore possible applications. Several members joined the Committee on Standardization of Microchemical Apparatus, which was already functioning within the American Chemical Society and the American Society for Testing and Materials, with representatives of several manufacturers participating. Actual Society Committees were the ones on Functional Groups and the Survey Committee, a sort of catch-all. The latter studied the uses of microtechniques from all angles. In other words, it "surveyed" the entire field of analysis, including microscopy. These committees which reported to the regular meetings were enthusiastically attended and were very effective. They became inactive in a few years except for the one on standardization which, headed by Steyermark, spent more than 20 years successfully standardizing microchemical apparatus.

The objectives of the Society were early achieved. Courses in microtechniques were initiated all over the country. Full acceptance, in a manner of speaking, was attained when Erle Stanley Gardner, the famous author, always checked with a member before allowing his detective, Perry Mason, to use microchemistry to convict the murderer in his books "The Case of. . . ." series (*7*).

For many years, the Secretary prepared and sent to all members a summary of the year, giving a brief outline of the lectures given at each meeting. This was a very popular feature and appealed to members from distant parts of the country and even overseas. They stated that, although they were unable to attend the meetings, this feature alone was worth the payment of dues. This practice was finally dropped under the pressure of wartime conditions.

From the very first, all members indicated that they wanted the Society to be completely independent. By refusing to affiliate with the New York Academy of Sciences, they lost the advantage of a permanent meeting place but the consensus was that the advantage was not worth sacrificing independence. Meeting places were not hard to come by but did require ingenuity and planning. They met at New York University both in Washington Square and at the Heights, Columbia University, City College of New York, Fordham University, Bell Laboratories, or any other suitable place that did not call for a fee. Later, arrangements were made with the American Museum of Natural History and meetings were held there for nearly 20 years.

A dinner for the speaker, to which all members were welcome, was held

at a nearby hotel before the meeting. These get-to-gethers were valuable for the exchange of ideas in an informal way.

When most members came from New York and Westchester County, the New York City colleges and the Museum were convenient and quite satisfactory. However, over the years, the "center of gravity" shifted. More and more members came from New Jersey and the Philadelphia area and fewer from New York and Westchester County. So a sort of compromise was reached—meetings are now held in a hotel in northern New Jersey. This has proven convenient for most members of both groups.

After the first few years, the initial meeting of the year was traditionally a plant trip. This included a tour of their laboratories, a dinner (frequently with the compliments of the company), and a speaker from that company. Hoffmann-La Roche Inc., Squibb Institute for Medical Research, Lederle Laboratories, Geigy Corporation (now Ciba-Geigy Corporation), and Bell Laboratories are a few of those that have been toured—many of them more than once.

Gradually the custom grew that the last meeting of the year was "Ladies Night" and a speaker was chosen to talk about something that would interest nonchemists. For many years, this was held at Fordham University. The members and their guests ate at a restaurant nearby, then went over to the Chemistry Building for the meeting. As this was also election night, the incoming and outgoing officers met afterwards in the office of the Head of the Department to discuss plans for the coming year, accompanied by suitable libations. Later, when voting was done by mail, this latter custom faded, but the plant trips and Ladies Night are still popular features of the year's program.

In 1944, one of the most popular members, the Rev. Francis W. Power, S.J. of Fordham University, died after a short illness. He was always enthusiastic about the Society, an able microchemist, and a good speaker with a keen sense of humor. It was he who made the famous remark that "Microchemistry is an art as well as a science." He was the fourth Chairman and would have certainly been reelected—probably for many more terms—but before the balloting started, he pleaded with the members not to vote for him and further suggested that no one ever serve more than one term. In deference to his wishes he was not reelected and his plea for one-term chairmen has become an unwritten rule.

A memorial was certainly in order but the Society was too young to have funds to spare and too few in membership for any significant contributory effort. Mr. E. M. Marshall, a man in his 70s but an active and deeply interested member of the Society solved the problem. "Mac" was a man of many talents—geochemist, mineralogist, geologist, microscopist, and microchemist whose hobby was wood carving. He volun-

teered to carve Johann Becher's "Creed of a Chymist" (*1*) on a piece of black walnut that he had. On May 16, 1946, the carving was presented by Leo Yanowski, Chairman of the Society, to the Rev. Robert I. Gannon, S.J., President of the University who accepted it for Fordham University where it was hung above the stairs in the old chemistry building. When the John Mulcahy Hall replaced the old building, the plaque was moved to it with the rest of the Department and now hangs on a third floor wall.

Membership in the Society was tantamount to a course in microtechniques. The majority of members joined primarily to learn, and meetings were always well attended. Speakers were selected to demonstrate basic and/or new techniques. The programs also included members who had developed new approaches and nonmembers whose work was particularly important, including Nobel Prize winners like Melvin Calvin, Harold Urey, Peter Debye, and Wendell M. Stanley.

Two other features were "gadget night" and "author meets the critic." The first was truly a gadget session: there is no other word which so well describes it. As many as a half dozen members would talk about something unusual they used or did that was worth reporting but did not warrant publication. The others, as its name implies, were rapid fire exchanges between those present.

During the 1945–46 season, the Society initiated a series of annual symposia to further the knowledge and uses of microtechniques and to stimulate interest in microanalysis and microscopy. The programs were especially designed both to introduce microchemistry, microanalysis, microtechniques, and microscopy to the neophyte and new tools to the specialist. They, too, were successful from the start. Programs of outstanding speakers drew large audiences of interested chemists, as well as exhibits by different equipment companies showing their new appliances.

Sixteen of these annual symposia were held and they were more and more popular. In 1959, the New York and North Jersey Analytical Groups of the American Chemical Society joined with the American Microchemical Society and the Society for Applied Spectroscopy to form the Eastern Analytical Symposium. The advantages of greater exposure and more industrial support were obvious.

So the 1960 Symposium was the last of the independent microchemical symposia of the Society except for a special one on April 18, 1963 to honor Dr. Donald D. Van Slyke on his 80th birthday. Speakers were Dole and Kirk, with panel discussions on the uses of the Van Slyke apparatus, including microanalysis led by Steyermark, medical research led by Sendroy, and clinical analysis led by Natelson. The Symposium was well attended and one of the most successful ever held by the Society.

Although the norm was eight meetings a year, extra meetings were called for special purposes—for example to take advantage of the tempo-

rary presence in this country of famous microchemists, such as Fritz Feigl, Wolfgang Kirsten, Karl Linderstrøm-Lang, Ronald Belcher, Wolfgang Schöniger, and many others.

As with other scientific societies, World War II accelerated activities and growth. The development of microtechniques was essential in nuclear chemistry because of the paucity of sample material. Many members were involved in the Manhattan Project, studying nuclear fission, notably Cefola at Stagg Field in Chicago and Schwob at Los Alamos, New Mexico.

As the Society grew strong both in membership and financially, Cheronis, Ma, and Benedetti-Pichler felt that a journal was needed. These three men, Cheronis in particular, persisted and in 1957, after much debate in Society meetings, launched the *Microchemical Journal,* "devoted to the application of microtechniques in all branches of science." It was successful from the start and subscriptions came in from all over the world.

The disastrous motor accident that took the life of Cheronis in 1962 rocked the Society to its foundation. Cheronis had just retired to his farm in Illinois where he planned to work on the amino acid content of vegetable proteins. He hoped to hybridize corn—the mainstay of so many diets in the poorer countries of the world—to make the protein more complete biologically. Specifically, he sought to raise the lysine content to a life-supporting level. He was a very able and competent scientist and might have achieved his goal had he lived.

Cheronis was very popular. His enthusiasm was contagious and his drive and strong personality was a decisive factor in the life of the Society. Donations in his memory poured in from all over and the Society established the Cheronis Scholarship which was awarded to an especially promising student. But his tragic death nearly ended the Journal. However, Academic Press assumed publication with Al Steyermark as Editor-in-Chief, and the *Microchemical Journal* continued without missing a single issue.

At the same time, at Steyermark's recommendation, the Society established an educational fund. For the first few years, the funds were allowed to accumulate so that now a perpetual fund has been written into the by-laws. A committee makes these awards to students whose work indicates promising futures. The first award was presented in 1967. In recent years, five awards of $500 each have been made annually.

Under the sponsorship of the International Union for Pure and Applied Chemistry (IUPAC), international microchemical symposia were held at Graz in 1950 and at Vienna in 1955. A number of Society members attended but the Society as an entity did not participate. Donations by

American microchemists were used for a bust of Emich and for the establishment of the Emich and Pregl Awards.

As a continuation of this international project, a symposium, under the sponsorship of the International Union of Pure and Applied Chemistry, was organized by the American Microchemical Society and was conducted at the Pennsylvania State University in August 1961, with Al Steyermark as General Chairman, H. J. Francis, Jr., as Program Chairman, and J. Y. Steel in charge of arrangements. Approximately 570 persons, representing 19 countries, attended and nearly 100 papers were read. At this meeting, the Austrian Society for Analytical and Microchemistry presented Cheronis with the Emich Award and Steyermark with the Pregl Award (*12*). These Symposia were so successful that they were repeated in 1965, 1968, and 1973, under the same leadership and at the same place. The ones in 1961 and 1965 were supported in part by grants from the National Institutes of Health. The 1968 affair was a workshop meeting. All four were of six days duration and were all well attended.

In 1974, the American Microchemical Society, the Association of Analytical Chemists, Inc., the Division of Analytical Chemistry of the American Chemical Society, and the Society for Applied Spectroscopy formed the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS) along the lines of the Federation of American Societies for Experimental Biology.

After the fourth meeting of the FACSS it was obvious that microchemistry was not getting the attention and publicity for which the Society had originally been formed. So they voted to withdraw from the FACSS after the 1978 meeting but to continue participation in the Eastern Analytical Symposium. This latter group had given tangible results beneficial to the objectives of the Society.

In January 1964, Benedetti-Pichler retired after teaching in New York colleges for more than 30 years. He eagerly anticipated a series of "agrichemical" experiments he had planned on his farm in South Carolina, but he died suddenly from a heart attack in December of that year.

Benedetti-Pichler was widely known, liked, and admired all over the world and contributions in his memory started flowing in immediately. A motion by Alber in meeting called for a committee to take charge of the donations and plan a fitting and permanent memorial. A revolving committee for it was written into the by-laws.

The first committee, headed by Sabine with Yanowski, Cefola, and Hoffmann designed a bronze plaque featuring a profile in bas-relief of Benedetti-Pichler. The inscription reads: "The A. A. Benedetti-Pichler Award, presented to. . . . in recognition of his outstanding contributions to microchemistry" with the proper date. After the initial cost of the

mold, sufficient funds remained for a permanent annual award, unless in any particular year, the committee felt that there was no suitable candidate. The time and place of the presentation was also left to the discretion of the committee. The recipient would be required to deliver a paper exemplifying the principles of Benedetti-Pichler's teaching practices. Although the presence of the recipient was desirable, the award could be accepted by proxy. It is the only comprehensive American Award in microchemistry and has become one of the most coveted. The scientists receiving the Benedetti-Pichler Award are listed in Table 1.

The suggestion made by Power in the very early days, that Chairmen serve only one term has had a double effect that could not have been foreseen when proposed: The governing of the Society has never been controlled by a clique; and bringing a great diversity of experience, personality, capability, and administrative ability to the Chair has been an absolute benefit, greatly strengthening the Society, accelerating its growth, and creating an influence in the discipline inversely proportional to its size. It has also resulted in a long list of members who have served in that capacity. Table 2 lists the names of Chairpersons since the beginning.

CONCLUSION

What does the future hold? According to a survey made by the American Chemical Society in 1978 (*4*) the largest number of jobs available are for analytical chemists. Every researcher needs to know what he is working with and what he has made. Microanalysis will become more

TABLE 1
RECIPIENTS OF THE BENEDETTI-PICHLER AWARD IN MICROCHEMISTRY

J. F. Alicino	1966
F. L. Schneider	1967
W. Kirsten	1968
B. B. Cunningham[a]	1969
W. C. McCrone	1970
L. T. Skeggs	1971
L. C. Craig[a]	1972
No award given	1973
E. Sawicki	1974
P. Zuman	1975
T. S. Ma	1976
G. H. Morrison	1977
J. Jordan	1978
A. Steyermark	1979

[a] Deceased.

TABLE 2
CHAIRPERSONS OF THE AMERICAN MICROCHEMICAL SOCIETY

W. F. Whitmore[a]	1935–6
B. L. Clarke	1936–7
W. F. Spikes	1937–8
F. W. Power[a]	1938–9
A. F. Knoll	1939–40
D. Price[a]	1940–1
J. A. Kuck	1941–2
D. B. Sabine	1942–3
A. Steyermark	1943–4
R. A. Harte[a]	1944–5
L. K. Yanowski[a]	1945–6
F. R. Swift	1946–7
F. A. Meier[a]	1947–8
A. A. Benedetti-Pichler[a]	1948–9
W. A. Hynes[b]	1949–
B. Littman-Rosenfeld	1949–50
J. F. Alicino	1950–1
N. S. Kuettel[a]	1951–2
M. Cefola	1952–3
J. S. Wiberly	1953–4
C. W. Pifer	1954–5
G. J. Hillsdorf	1955–6
H. V. Wadlow	1956–7
A. G. Mistretta	1957–8
H. K. Alber	1958–9
G. Gustin	1959–60
H. J. Francis, Jr.	1960–1
E. R. Hoffmann	1961–2
E. Stehr	1962–3
A. Mowitz	1963–4
J. Grodsky	1964–5
L. N. Brancone	1965–6
J. Y. Steel	1966–7
C. W. Nash	1967–8
K. Fleischer	1968–9
D. Green	1970–71
J. Kobliska	1971–2
T. Kielty	1972–3
F. J. Scheidl	1973–4
F. Joy	1974–5
J. Sidun	1975–6
M. Myers	1976–7
C. Paralusz	1977–8
L. C. Klein	1978–9

[a] Deceased.
[b] Died before taking office.

important in future years—especially in biological chemistry as samples for analysis become smaller and smaller. As Hans T. Clarke has said, "The elaboration by Pregl of methods of microanalysis and the manipulation of organic compounds on a milligram scale was one of the most influential factors in the development of biochemistry" (*6*).

Microchemical analysis as a discipline may seem to disappear from the literature but it will do so in name only. It has been incorporated into all processes of instrumentation and analytical procedures. Uniformity of sample and handling of trace materials are still microchemistry per se.

This paper is not nor is it intended to be a history of microchemistry. That has been well covered in "A History of Analytical Chemistry" (*9*). It is the story of the American Microchemical Society and its influence on the development of microanalysis in the United States.

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Measurement of Protein in Serum and Rodent Embryos by Spectrophotometry at 215 and 225 nm

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INTRODUCTION

Spectrophotometry has been used for the quantitative determination of protein concentrations since 1940 (*11*). Spectrophotometric studies of serum protein have shown that a broad ultraviolet spectrum exists with a maximum of 290 nm and a minimum in the region of 250 nm (*1, 13*). Characteristically, absorbancy in this region can be correlated with the presence of aromatic amino acids, namely phenylalanine, tryptophan, and tyrosine, and with the sulfur-containing amino acid, cystine (*1*).

Warburg and Christian's method (*16*) for estimating protein concentrations utilized a 280/260 nm absorbancy ratio. The utility of this procedure is limited by its insensitivity to protein concentrations less than 100 μg/ml. Additionally, because individual proteins contain different aromatic amino acids, the values obtained for equal concentrations of different proteins will vary (*8*).

Protein measurement by the method of Lowry *et al.* (*6*) is based on the principle of reacting Folin phenol reagent with the aromatic amino acid components of protein molecules, and measuring the absorbance at 500 to 750 nm. The Lowry method is 10 to 20 times more sensitive than the ultraviolet procedure at 280 nm, and 100-fold more sensitive than the biuret method (*4, 5*), which depends on the complexing of copper by polypeptides, and observations of the peak absorbance at 540 nm.

Westley and Lambeth (*19*) described a quantitative analytical procedure based on the biuret reaction utilizing an absorption maximum of 446 nm. This method is useful in the range of 0.05–1.0 mg protein per 0.5 ml, but requires dialysis, precipitation, centrifugation, and color development. Waddell (*15*) described an ultraviolet spectrophotometric procedure for estimating microgram quantities of plasma proteins by multiplying a conversion factor times the difference in the protein absorbance at 215 and at 225 nm. The accuracy and sensitivity of this procedure has been endorsed by Bendixen (*2*) for estimating protein concentration in serum and cerebrospinal fluid, and by Murphy *et al.* (*8*) for monitoring

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chromatographic separation of guinea pig brain protein. In this paper, we are reporting on the use of Waddell's procedure for estimation of total protein concentration in rodent embryonic tissue. In the fields of developmental biology, a precise, accurate, sensitive, rapid, and nondestructive procedure is needed in the determination of minute quantities of uncharacterized rodent embryonic protein. The Waddell method as used in our lab fulfills these needs more fully than the Lowry method.

A statistical analysis of the accuracy, precision, and reproducibility of this procedure is presented, as well as a comparison of the results obtained in embryos using the Waddell and Lowry methods.

MATERIALS AND METHODS

Spectrophotometry

Absorbance measurements at 215 and 225 nm were made in 1-cm matched cuvettes with a Cary 118C spectrophotometer equipped with a constant water temperature (25°C) regulator. A slit setting of 0.1 mm and an absorbance range of 2.0, 1.0, or 0.5 were used. All samples were read against physiological saline (0.9% w/v NaCl).

Preparation of a Standard Curve for use in the Waddell Protein Determination

One vial of lyophilized reference serum (Scale II—High Control Range, Technicon Instruments Corporation, Tarrytown, New York) was volumetrically dissolved in 1 liter of physiological saline. This resulted in a mean total protein value of 760 μg/ml. Quadruplications of each of seven reference serum standard solutions (3.8, 7.6, 15.2, 22.8, 38, 76, and 114 μg/ml) were prepared in 10-ml volumes. Either a Centaur micropipet with disposable tip (Centaur Chemical Co., Danbury, Conn.) or an appropriate glass volumetric pipet was used to dispense the required volume of reference serum accurately. Preparation of standard solutions was repeated on 3 consecutive days by each of two laboratory technicians.

All standard solutions were spectrophotometrically scanned (230 to 210 nm) on the day of preparation. The absorbance at 225 nm was subtracted from the absorbance at 215 nm, and the difference was used to prepare the calibration curve.

Known "Unknown" Protein Standard

To assess the percentage accuracy[1] and precision[2] of the Waddell method, three commercially available clinical serum control standards

[1] Percentage accuracy = $[(\bar{x} - y)100]/y$, where $\bar{x}$ is the mean measured value and y is the stated value of the commercial control sera.

[2] Precision (coefficient of variation) = $SD/\bar{x}$ (100), where SD is the standard deviation and $\bar{x}$ is the mean of a set of measurements.

were used: Validate, Validate-A, and Calibrate 3 (General Diagnostics, Morris Plains, N.J.), whose mean assigned values for total protein (g/dl) were 6.4, 4.8, and 8.0, respectively. Quadruplicate samples of three different dilutions of each of these standards were volumetrically prepared and spectrophotometrically read (see Standard Curve). The mean protein concentration of each quadruplicate, the standard deviation of the concentration, and the coefficient of variation were determined.

Rat Embryos

Five nulliparous Sprague–Dawley rats weighing from 222 to 239 g (mean weight 230 g at time of mating) were used. Animals were caged individually in rooms which were maintained at 22 ± 1°C and approximately 45% relative humidity. A light/dark cycle of 12 hr was maintained. Rats in proestrus, as determined by vaginal lavage, were placed with a male for approximately 17 hr (3:30 PM–8:30 AM). The presence of sperm in vaginal smears indicated that successful mating had occurred, and the day that sperm were observed was considered Day 0 for designating gestational age of embryos. On the morning of the 12th day of gestation (8:15–9:00 AM), each pregnant female was sacrificed by overdosing with ether. A laparotomy was performed and the uterus was removed. The embryos were dissected free of surrounding membranes, blotted dry, and placed in preweighed polypropylene test tubes. Embryos were weighed to 0.01 mg using a Mettler H64 balance.

Two milliliters of saline were volumetrically pipetted into each test tube containing an embryo. The test tubes were placed in an ice-bath and the embryos were sonicated for 30 sec using a Sonifer Cell Disruptor, model W-350 (Branson Sonic Power Co., Danbury, Conn.) equipped with a micro-tip. A power output control setting of 4 was used. Either the embryo protein determinations were performed on the day of sonication, or the homogenate was kept frozen until the determinations were made.

Embryo Protein Determination

A 1:40 dilution of each embryo homogenate was prepared in quadruplicate by dispensing 250 μl of homogenate with a Centaur micropipet into a 10-ml volumetric flask and bringing up to volume with saline. All samples were spectrophotometrically scanned from 230 to 210 nm. The absorbance at 225 nm was subtracted from the absorbance at 215 nm.

Lowry Method for Protein Determination

Reagent A was prepared by dissolving 20 g Na_2CO_3 and 0.2 g Na K tartrate in 0.1 *N* NaOH. Reagent B consisted of 5 g $CuSO_4 \cdot 5H_2O$ dissolved in 1 liter of distilled water. Folin–Ciocalteau phenol reagent was diluted 1:1 with distilled water prior to use. A daily preparation of Reagent C consisted of 50 parts of Reagent A and 1 part of Reagent B.

One milliliter of sample was pipetted into a test tube and diluted to 2 ml with distilled water. Five milliliters of Reagent C was rapidly dispensed into each test tube, followed by the rapid admixture of 0.5 ml Folin–Ciocalteau reagent. Absorption was read after 30 min at 660 nm.

Comparison of the Waddell and Lowry Protein Methods

To compare the Waddell and Lowry protein methods, four replicates of four different concentrations of Calibrate 3 were prepared on 2 different days. A different vial of the lyophilized serum control was used each day. In addition, four replicates of five embryos were assayed for total protein on each day. The embryos were from two litters, and all samples were prepared by one individual. Each sample was divided into two aliquots: One aliquot was analyzed by the Waddell protein method in our laboratory; the other was analyzed in an associated laboratory by personnel experienced with the Lowry protein method. All samples prepared in 1 day were simultaneously analyzed.

RESULTS

The mean values of the Δ absorbance (absorbance at 215 nm minus the absorbance at 225 nm), the standard deviation of the mean, and the coefficient of variation of the mean of the Δ absorbance obtained by the analysis of Scale II—High Control Range serum protein are shown in Table 1. Regression analysis resulted in a slope of 0.0066, an intercept of 0.0006, and a correlation coefficient of 0.9991. The coefficient of variation of the absorbancy was less than 5% between 15.2 and 114 μg/ml. However, the coefficients of variation of the Δ absorbance for 3.8 and 7.6 μg/ml were 17.0 and 8.1, respectively, although the standard deviation was only ±0.004 of an absorbance unit. There was no significant technician effect on any given day or from day to day.

The results obtained from the analysis of three serum control standards

TABLE 1
CALIBRATION DATA FOR TOTAL PROTEIN IN SCALE II—HIGH CONTROL RANGE SERUM

μg/ml	$\bar{x}$ of Abs.[a] (215–225)	Standard deviation	Coefficient of variation
3.8	0.026	0.004	17.0
7.6	0.052	0.004	8.1
15.2	0.100	0.005	4.6
22.8	0.150	0.005	3.6
38.0	0.252	0.008	3.0
76.0	0.501	0.015	2.0
114.0	0.754	0.020	2.7

[a] Mean of 24 analyses of each concentration (four replicates on 3 days by two technicians).

(Validate A, Validate, and Calibrate 3) are presented in Table 2. The slope and intercept values obtained from the calibration data in Table 1 were utilized to calculate a protein concentration for each serum control sample. Examination of the coefficient of variation and the percentage accuracy indicates that the precision for Validate A was >98% with an accuracy >93%. Both the precision and accuracy for Validate were >96%. Results obtained with Calibrate 3 indicate a precision >97%, with an accuracy of at least 94%.

The calibration data for Scale II—High Control Range serum (standard curve) and Calibrate 3 obtained by correlation analysis are summarized in Table 3. There were no statistically significant differences between slopes or intercepts of Calibrate 3 on 3 different days. However, there was a significant difference between the slope of Scale II—High Control Range serum and the slopes of Calibrate 3.

The results obtained by the Waddell and by the Lowry methods are compared in Table 4. The within-day mean Δ absorbance values for Calibrate 3 protein concentrations indicate a precision of 96% with the Waddell procedure as compared to a precision of 92% with the Lowry procedure.

Data presented in Table 5 show that the precision in estimating the total protein in 12-day-old rat embryos by the Waddell method was consistently better within litter (96–98 and 91–98%) and between litters (91–98%) as compared to the Lowry Method (88–97 and 86–97%) and (86–97%), respectively. In only two embryos, 4 and 5 of litter 2, was the

TABLE 2
DETERMINATION OF TOTAL PROTEIN IN THREE SERUM CONTROL STANDARDS

	Stated y[a] (μg/ml)	$\bar{x}$ calculated[b] (μg/ml)	Standard deviation	Coefficient of variation	$\frac{(\bar{x}-y)\%}{y}$[c]
Validate A	4.8	5.2	0.08	1.5	6.8
	24.2	23.2	0.37	1.6	−4.0
	48.8	47.1	0.23	0.5	−2.8
Validate	6.4	6.6	0.26	3.9	3.4
	32.0	32.0	0.15	0.5	0.0
	64.0	62.6	0.79	1.3	−2.0
Calibrate 3	8.0	7.5	0.19	2.5	−6.0
	40.0	37.8	0.55	1.5	−5.6
	80.0	76.1	1.04	1.4	−4.9

[a] Mean of mean of the assigned value by General Diagnostics.
[b] Mean of four replicates.
[c] Accuracy.

TABLE 3
COMPARISON OF CALIBRATION DATA FOR SCALE II—HIGH CONTROL RANGE SERUM AND CALIBRATE 3 OBTAINED BY CORRELATION ANALYSIS

	N	Slope	Intercept	Correlation coefficient (r)
Scale II—high control range serum[a]	24 × 7	0.00660	0.0006	0.9991
Calibrate 3[b]	4 × 5	0.00626	0.0041	0.9982
	4 × 5	0.00640	−0.0005	0.9987
	4 × 5	0.00647	−0.0038	0.9978

[a] Indicates 24 replications of 7 concentrations.
[b] Standards analyzed on 3 different days; four replications of five concentrations.

precision <95% with the Waddell method, while the precision with the Lowry method was <95% in 6 of 10 embryos. The precision in the Lowry procedure surpassed that of the Waddell procedure only in embryos 4 and 5 of litter 2.

Data summarizing the variance of total embryo protein, the coefficient of variation, and the range of embryonic protein content per litter are presented in Table 6. Additionally, the variance of the mean embryo weight per litter, the coefficient of variation, and the embryo weight range per litter are presented. The variance in embryonic protein was comparable to the variance in embryonic weights.

TABLE 4
COMPARISON OF THE WADDELL METHOD WITH THE LOWRY METHOD FOR THE DETERMINATION OF TOTAL PROTEIN IN CALIBRATE 3 ON 2 DIFFERENT DAYS

	Waddell method				Lowry method		
	Concn (μg/ml)	$\bar{x}$ of Abs.[a] (215–225)	SD[b]	CV[c]	$\bar{x}$ Abs.[a] (660)	SD[b]	CV[c]
Day 1	60.0	0.398	0.002	0.6	0.168	0.005	2.8
	40.0	0.254	0.001	0.2	0.110	0.002	2.3
	20.0	0.122	0.004	3.2	0.050	0.002	4.1
	8.0	0.052	0.002	3.3	0.018	0.001	5.4
Day 2	60.0	0.404	0.001	0.2	0.178	0.002	1.0
	40.0	0.245	0.001	0.5	0.111	0.003	2.9
	20.0	0.123	0.002	1.8	0.055	0.002	3.9
	8.0	0.051	0.002	3.8	0.021	0.002	8.0

[a] Mean of four replicates.
[b] Standard deviation.
[c] Coefficient of variation.

TABLE 5
COMPARISON OF THE WADDELL METHOD WITH THE LOWRY METHOD FOR THE DETERMINATION OF TOTAL PROTEIN IN DAY 12 SPRAGUE–DAWLEY RAT EMBRYOS

		Waddell method			Lowry method		
	Embryo number	$\bar{x}$ μg protein/ embryo[a]	SD[b]	CV[c]	$\bar{x}$ μg protein/ embryo[a]	SD[b]	CV[c]
Litter 1	1	1107.2	0.46	3.3	914.4	0.40	3.5
	2	1267.2	0.55	3.5	1038.4	1.60	12.4
	3	1480.8	0.27	1.5	1156.0	0.99	6.9
	4	1476.8	0.41	2.2	1217.6	0.85	5.6
	5	1420.8	0.40	2.3	1190.4	0.49	3.3
Litter 2	1	1104.0	0.32	2.3	737.6	0.86	9.4
	2	1351.2	0.51	3.0	904.8	1.60	14.1
	3	1147.2	0.38	2.6	797.6	0.42	4.2
	4	732.8	0.48	5.2	470.4	0.27	4.6
	5	1048.0	1.12	8.6	730.4	0.63	6.9

[a] Mean of four replicates.
[b] Standard deviation.
[c] Coefficient of variation.

DISCUSSION

The Waddell protein method is simple and well suited to the estimation of total rodent embryonic protein. Since embryo weight and embryo protein concentration are important determinants in embryonic accumulation of agents which bind to proteins, the Waddell procedure would significantly aid researchers who perform mechanistic or biochemical developmental studies in embryos. The many technical difficulties such as the adherence of embryo to the weigh paper, the removal of excess embryonic fluid, etc., encountered in attempts to weigh embryos of early gestational age accurately precludes the direct use of embryonic weights for correlating agent concentration to embryo weight, so the Waddell method of determining protein concentration is indirectly superior.

Spectrophotometry in the far ultraviolet range (240–200 nm) recently has been used to measure total protein in plasma (*2, 15*), serum (*9*), serum fractions (*3, 13, 14*), cerebrospinal fluid (*2, 10, 15*), and other biological materials (*1, 8, 12, 17*). Undoubtedly, instrumental refinements, operational simplicity, accuracy, and availability of a variety of spectrophotometers have stimulated interest in using absorbance measurements in the 240- to 200-nm region for protein determination. However, the selection of optimum wavelengths in the far ultraviolet region remains arbitrary and is compounded by the fact that no peak maximum exists for proteins in this region.

TABLE 6
DAY 12 MEAN EMBRYO TOTAL PROTEIN AND MEAN EMBRYO WEIGHT

Number of embryos	Total embryo protein (μg)				Embryo weight (mg)			
	$\bar{x}^a$	Standard deviation	Coefficient of variation	Range	$\bar{x}^b$	Standard deviation	Coefficient of variation	Range
12	1099.83	175.78	15.98	910.94–1380.63	23.98	3.62	15.11	18.74–29.02
13	1285.76	153.40	11.93	1053.37–1495.78	25.26	2.88	11.41	21.07–29.25
14	1187.35	130.65	11.00	910.94–1413.97	22.63	3.25	14.37	15.28–27.78
14	1331.93	126.30	9.48	1062.46–1607.90	23.76	2.54	10.68	18.68–27.99
13	1259.42	185.37	14.72	804.88–1486.69	23.07	3.98	17.25	14.08–29.61

[a] Mean of mean of four replicates for each embryo.
[b] Mean of embryo weight per litter.

Goldfarb *et al.* (*3*) have shown that absorption curves for bovine albumin and other proteins in the region of 220 to 200 nm increased sharply with decreasing wavelengths and, indeed, did not possess a peak maximum in this region. Data obtained from the shoulder of an absorption curve, rather than a peak, may appear to have no meaningful interpretation. However, an absorption maximum is desirable since the shape of the curve is also typical of protein structure (*3*). Therefore, a suitable specific wavelength in the region below 240 nm would best be empirically determined.

Many of the reported methods which utilize the shorter ultraviolet wavelengths for quantitative protein studies compare the sensitivity of one method to another. Webster (*17*) presented comparative data on the sensitivity, accuracy, and precision of protein estimations at several wavelengths, namely 280, 215 minus 225, 210, and 191 nm using bovine serum as a standard and seven commercial proteins of unknown purity for analysis. His results indicated that measurements in the 225- to 191-nm range were more sensitive, less variable, and more accurate than measurements at 280 nm. However, precision at 280 nm was slightly better than precision in the lower wavelengths region.

The Waddell ultraviolet spectrophotometric method (*15*) for protein estimation was selected for our studies with embryos because of its simplicity and nondestructive nature. The utilization of this method for total protein estimation gave the opportunity to evaluate conveniently three parameters: accuracy, precision, and sensitivity. The minimization of absorbance error from nonproteinaceous material was achieved by using a difference value rather than absorbance at a single wavelength.

Human or bovine albumin was not used as a protein standard in this study for the following reasons: (i) accurate weighing of albumin is difficult due to possible contamination by moisture; (ii) it is difficult to prepare protein standards from albumin accurately because of its adherence to glass; and (iii) since the qualitative protein profile of rodent embryos is unknown, a calibration reference serum was used as a standard from which to estimate total embryonic protein. The serum is composed of a number of distinct proteins in addition to albumin and, therefore, may be a more accurate representation of the protein in embryos.

The estimation of total protein by the Waddell method without the use of known serum protein standards must employ an instrument absorbance calibration factor. This factor can be calculated using solutions in which the protein concentration has been determined by the Kjeldahl method (*7*), a complex chemical procedure which determines the nitrogen content of the protein. Waddell (*15*) found this calibration factor to be 144 for human serum albumin, while Bendixen (*2*) determined a calibration factor of 144 for protein in serum and cerebrospinal fluid and 147.5 for albumin.

Our use of commercially standardized reference sera to develop a standard curve for protein estimation removes the need for an instrument absorbance calibration factor as previously mentioned (*2, 15*). Therefore, this modification greatly simplifies the Waddell method because it is no longer necessary to employ the Kjeldahl procedure.

In addition to modifying the Waddell method for use in our laboratory, we compared our modification to the Lowry method. The accuracy and reproducibility of the Waddell method when using reference serum total protein depends on the stability of the spectrophotometer as well as on the precision of the reference serum prepared by laboratory personnel. By the use of the calculated slope and intercept (Table 3), a precision of >96% and an accuracy of >93% were demonstrated in predicting total protein values in three different serum reference standards in the range of 4.8 to 80 μg/ml. In addition, a precision of >96% was achieved in the 8 to 60 μg/ml protein range as compared to a precision of >92% by the Lowry method. The overall precision for estimating total protein in day 12 Sprague–Dawley rat embryos by the Waddell method was greater than 91% while that of the Lowry method was greater than 86% (Table 5).

The total protein values for all embryos obtained by the Waddell procedure invariably were higher than the protein values obtained by the Lowry method. However, there are qualitative and quantitative differences in aromatic amino acids in the composition of various proteins, possibly explaining the lower total protein values obtained by the Lowry method. A distinctive characteristic of protein structure is the presence of various amino acids united by peptide bonds. The amino acid composition of the protein molecule only slightly influences variability in absorbance when peptide bond absorption is used for protein determination (*12*). The basis of the Lowry procedure is to measure the aromatic amino acid components of protein (*18*) in contrast to peptide bond absorption (*12*).

The higher precision and greater accuracy of the Waddell method make this procedure more attractive and reliable than the Lowry method for the estimation of total protein in serum and in rodent embryos. In addition, there is no destruction of sample and no requirement for chemical additives or color development.

The Waddell method, as described in this communication, would be applicable to studies attempting to correlate the level of chemically or biologically active agent to protein concentration in rodent embryos of early gestational age. This embryo protein determination method has the advantage over embryo weights because slight changes of protein concentration can be readily measured and correlated to the concentration of a chemical agent being studied. For instance, if total protein concentration is slightly greater in lighter weight embryos, then drug binding to the proteins should be greater than in heavier weight embryos with lower

protein concentration. In addition, a subtle physiological alteration in the embryo, although not evident by embryo weight, could cause changes in protein concentration, thus influencing the extent of exposure of the embryo to the agent. Thus the utility of the Waddell method for measuring minute quantities of uncharacterized rodent embryonic protein would be appreciated.

SUMMARY

The Waddell quantitative method for total protein estimation was slightly modified and applied to sera and rodent embryos, and also compared to the Lowry method. It was not necessary to employ the Kjeldahl method to determine an instrument correction factor; reference sera were used to establish a standard curve. The results from this simplified Waddell method were reproducible from day to day with a high degree of accuracy. Comparative data obtained by total protein determination in three commercially available reference sera demonstrated a sensitivity in the modified Waddell method of 4.8 μg/ml with a precision of >96% and an accuracy of >93%. The overall precision in the determination of the total protein by the method in Day 12 rat embryos was >91% as compared to >86% in the Lowry method.

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PEG Determinations in Urine, Plasma, and Various Protein Solutions

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INTRODUCTION

In order to obtain a picture of the urinary protein spectrum in various diseases, it is necessary first to concentrate the samples until their protein content approaches that of plasma. Dialysis against concentrated solution of synthetic high-molecular weight polymers as polyethylene glycol (PEG) is a very rapid procedure recommended for clinical work (*3*). In this study the urines were concentrated, then separated into various protein fractions by PEG precipitation (*7*) and analyzed by SDS–polyacrylamide gel electrophoresis. The molecular weights of albumin and other proteins differed from known markers in some instances. Since some PEG remains after fractionation possibly altering protein size, attempts were made to measure PEG in these preparations. The PEG method for γ-globulin of C.E. Childs described in this journal (*1*) was used and similar erratic results were obtained when albumin was present. In view of this problem and the widespread use of PEG in fractionation of plasma proteins the procedure was adopted for use with albumin, plasma, urine, and the commonly available protein solutions used in man.

MATERIALS AND METHODS

Reagents

PEG. Carbowax 4000, Union Carbide Corporation, N.Y.

Proteins. Albumin, plasma protein fraction (PPF), a commercially available 5% albumin solution, γ-globulin, and antihemophilic factor for human use were obtained from the hospital pharmacy or blood bank.

Iodine solution (0.1 N). Iodine–iodide solution, Fisher Chemical Company.

TCA (10%). Ten grams of reagent grade trichloracetic acid made up to 100 ml with distilled water.

$BaCl_2$ (5%). Five grams of reagent barium chloride made up to 100 ml with 1 *N* HCl.

Apparatus. Beckman spectrophotometer with cuvettes.

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Procedure

Triplicate determinations are performed on all standards and samples. To 1.0 ml of the protein solution add 3.0 ml distilled water and 4.0 ml of 10% TCA. Mix vigorously and let stand for 15 min. Centrifuge at a minimum of 4000 *g* in a swing out type rotor for 10 min. The supernatant though clear should be filtered through a Whatman No. 42 or equivalent. Turbidity or visible particles interfere with the subsequent colorimetric assay. Add 1 ml of 5% $BaCl_2$ solution to 4 ml of the protein free filtrate, then 0.5 ml of 0.1 *N* iodine solution, mix well, and allow to stand for 15 min. A slightly turbid barium–iodide complex of PEG is formed with a maximum absorbance at 535 nm. Blank solutions consist of H_2O and the protein solution at the same concentration as the sample known to be free of PEG. Standards of 2.5, 5.0, 10.0, 20, and 40 μg of PEG per ml in the PEG-free protein solutions are treated exactly as the unknown sample. Micrograms of PEG per milliliter are taken from standard curves of absorbance versus PEG concentration of the original solution in μg/ml.

If protein solutions without PEG are not available the blank solution can be bovine albumin (Miles laboratory) at the desired protein concentration.

Protein determinations on all samples are performed by the Biuret method (*4*).

RESULTS AND DISCUSSIONS

An accurate and reproducible method of PEG analysis for most protein solutions has been developed. A linear PEG concentration, optical density relationship over the range of 2.5–40 μg, is shown in Table 1. The barium–iodide–PEG complex is stable for at least 2 hr making precise timing during the determination unnecessary. The reaction of polyglycols with heterpoly inorganic acid (silicotungstic acid and phos-

TABLE 1
ABSORBANCE OF PEG AT VARYING CONCENTRATIONS[a]

PEG (μg/ml)	Absorbance (A_{535} nm)	Percentage PEG recovered with 10% ISG
2.5	0.053 ± 0.003	98.7
5.0	0.105 ± 0.005	99.2
10.0	0.212 ± 0.007	99.0
20.0	0.426 ± 0.008	99.3
40.0	0.858 ± 0.012	98.9

[a] PEG assay was done in the presence and absence of immune serum globulin (ISG). These results are the average of 40 determinations.

TABLE 2
PEG RECOVERY IN VARIOUS PROTEIN SOLUTIONS

Sample[a]	Protein (mg/ml)	Percentage PEG recovered
Plasma	60	99.1
Urine[b]	0	98.4
Urine	27	98.8
Albumin	250	88.0
Albumin	200	99.2
Plasma protein fraction	50	99.6
γ-Globulin	165	99.4
Anti-hemophilic factor	25	98.5

[a] The samples were obtained from commercial sources or prepared as described. PEG was added at 2.5 and 25 μg/ml, respectively, and each recovery was performed 12 times.

[b] Urine gives a turbid solution on addition of $BaCl_2$ which is removed by centrifugation and does not affect the assay.

phomolybdic acid) in the presence of heavy metal cations used by Shaffer and Critchfield (*8*) and Oliver and Preston (*6*) is tedious, time consuming, and involves digestions with concentrated sulfuric acid. The turbidimetric method of Hyden (*2*) modified by Malawer and Powell (*5*) is insensitive below 3000 μg/ml. The method of Childs which is described in the procedure was limited to γ-globulin. PEG has been determined in urine, plasma, and commercial protein solutions used in humans. The principal modification of Childs' procedure is the protein concentration of the samples. Table 2 gives each protein solution with the concentration at which 98% of the PEG is reproducibly recovered. The reason for the differences observed between protein concentrations with albumin is unclear. The particular salts used in each preparation have been tested. Glycine with γ-globulin and 0.02 *M* sodium caprylate−0.02 *M* acetyl tryptophenate used in albumin preservation and which is also present in PPF showed no interferences. The buffers, sodium citrate and heparin used in AHF fractionation, also do not interfere. The erratic results at protein concentrations above those mentioned in Table 2 are probably due to the presence of TCA soluble protein degredation products caused by the many proteolytic enzymes in plasma. This requires further investigation.

SUMMARY

Polyethelene glycol is determined in a variety of biologic fluids. A stable, accurate, and sensitive method is described which is useful when PEG is used for protein purification, and in physiologic function and pharmacologic mechanism studies.

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Microdetermination of Total Sulfur and Inorganic Sulfate in Biological Materials[1]

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INTRODUCTION

Numerous methods for the microestimation of total sulfur in biological materials are described in the literature. In general, these methods involve decomposition of organic material by lengthy dry ashing (*3, 7, 17, 18*), oxygen flask combustion (*15, 19–21*), acid digestion in a specialized digestion/distillation apparatus (*10*), or other methods based on acid digestion (*2, 16*). In most of these methods sulfate reacts with barium, lead, or mercury and the salt thus formed is determined either by gravimetric or titrimetric measurements using various indicators (*5–8*). In the case of the Johnson and Nishita procedure (*10*), the reduction of sulfate to sulfide is followed by a subsequent reaction with amino dimethyl aniline to form methylene blue, which is measured spectrophotometrically at 670 nm. In many cases, these methods are subject to various interferences commonly associated with biological materials, e.g., several cations, nitrate and phosphate and are time consuming.

Klipp and Barney (*12*) and Bertolacini and Barney (*1*) reported the use of barium chloranilate for the indirect determination of inorganic sulfate based on the reaction of Ba^{2+} with SO_4^{2-}. The liberated ionized chromophoric chloranilate ions proportional to the sulfate present were measured in a 50% ethanol system at pH 3 and at a wavelength of 330 or 530 nm. However, these workers found that the use of a buffer to bring the final pH to 5.6 was necessary in order to avoid high blank readings due to the solubility of barium chloranilate at lower pH values. Wainer and Koch (*22*), modified the Klipp and Barney procedure to the direct determination

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of inorganic sulfate in urine and observed a threefold increase in sensitivity at 530 nm. They employed micro ion exchange columns of H^+-form resin to remove interfering cations. The method was not affected by the presence of K^+, Na^+, Mg^{2+}, NH_4^+, or PO_4^{2-} but Zn^{2+}, Cu^{2+}, Ca^{2+}, Fe^{3+}, and Pb^{2+} interfered with the determination (*13*). Some of these interfering elements are present in biological materials and can be effectively removed by ion exchange resins such as Dowex 50W-X8 and Rezxn H^+-forms resins.

In recent years, research in animal nutrition on the effects of minor elements in the diet of animals has been intensified (*4, 9, 11, 15, 17, 19, 20*). The importance of sulfate in animal nutrition has resulted in the need for a rapid, accurate, and sensitive method for sulfur determination in biological materials.

We report here a simple, rapid, and sensitive method for the microdetermination of total sulfur in whole animal blood, blood plasma, and urine, as well as inorganic sulfate in 10% trichloracetic acid (TCA), extracts, and urine. The method is based on a controlled nitric acid + perchloric acid mixture digestion of organic material in graduated digestion tubes using a Technicon BD-40 digestion apparatus. This is followed by removal of interfering cations by an ion exchange column, reaction of sulfate with barium chloranilate at pH 5.6, and spectrophotometric measurement of liberated chloranilate ions at 520 nm. Optimum digestion conditions and ideal pH control conditions are also reported.

MATERIALS AND METHODS

Apparatus and Reagents

Technicon BD-40 heating unit and control unit.
Beckman Model DU spectrophotometer.
Concentrated HNO_3, 70–71%, ACS reagent.
Concentrated $HClO_4$, 70–72%, ACS reagent.
Barium chloranilate, J. T. Baker No. B650.
5 *N* NaOH.
1 *N* and 0.5 *N* HCl.
50% CH_3COOH in H_2O.
1% Phenolphthalein in 95% ethanol.
pH 4.63 buffer, Fisher SO-B-100.
Ethanol 95%.
Dowex 50W-X8 H^+-form resin, 20–50 mesh.
Digestion tubes: Pyrex No. 7900, graduated at 25 and 50 ml.
Reaction tubes: Pyrex No. 8084, 15-ml graduated centrifuge tubes with glass stoppers.
Ion exchange columns: A. H. Thomas Co. micro Pregl filter tubes, No 5309-A.

Standard sodium sulfate solution (100 ppm S), weight 0.44325 g dried. Fisher Co., S-420 Na_2SO_4 and make up to 1 liter with H_2O.

Note. Double glass distilled water was used for making up all solutions and sample dilutions.

Preparation of Calibration Curve

Introduce 1 ml of pH 4.63 buffer solution into graduated centrifuge tubes. Accurately pipet aliquots of Na_2SO_4 solution (100 ppm S) to cover the range 20–100 μg S and add 5 ml 95% ethanol. Weigh 30 mg (±5 mg) barium chloranilate and add to the tubes. Make up volume to 10 ml with H_2O. Stopper tubes and shake 5–10 times over an interval of 10 min and centrifuge for 5 min at 500*g*. Pipet 5 ml supernatant into test tubes and add 5 ml 50% CH_3COOH. Mix tubes and after 15 min determine absorbance at 530 nm on a Beckman DU spectrophotometer against a reagent blank prepared in the same manner. Plot concentration vs absorbance as a linear graph. The reaction obeys Beer's Law over the range 20–100 μg S. A typical calibration curve is shown in Fig. 1.

Total Sulfur in Whole Animal Blood, Blood Plasma, and Urine

Pipet 1 ml whole blood, containing citrate as anticoagulant, blood plasma, or urine into digestion tubes. Add 3 ml conc. HNO_3 and 2 ml conc. $HClO_4$. Cover tubes with small funnels and digest for 3 hr at 250°C on the Technicon BD-40 digestion unit. Cool digests and dilute with 10 ml of

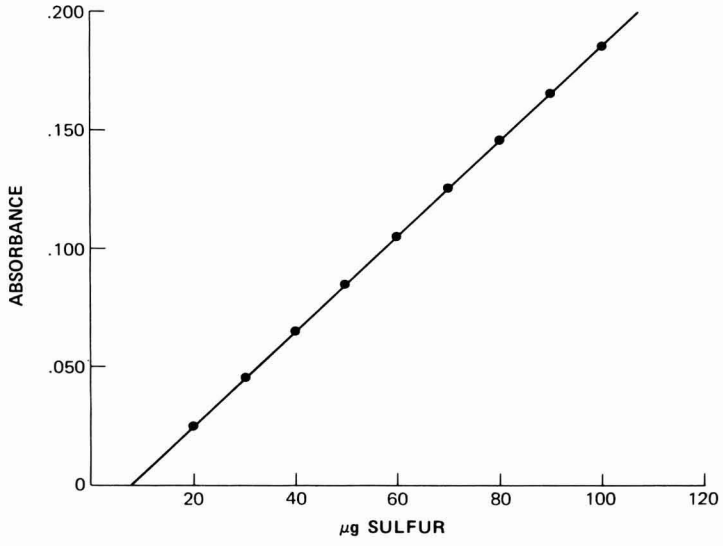

FIG. 1. Calibration curve for S.

H_2O. Add one drop phenolphthalein indicator and neutralize excess acid by addition of small amounts of 5 *N* NaOH until the pink color persists. Add 1 or 0.5 *N* HCl to just dispel the pink color. Make up to the 25 ml mark with H_2O. The pH of the solution should be 5–8 at this point.

Ion Exchange

Prepare micro ion exchange columns by using micro Pregl filter tubes. Place a glass wool plug at the bottom of filter tubes and add Dowex 50W-X8 resin to make columns measuring 10 × 20 mm. Condition columns before use by passing through 1 ml of 1 *N* HCl followed by 3 × 1 ml washings of H_2O. Place 15-ml centrifuge tubes containing 1 ml buffer (pH 4.63) under columns (prepared in a series of 10) to collect eluates. Pipet 1-ml aliquots of blood, blood plasma, or urine digests onto columns and elute with equal volume of H_2O. It should be noted that the pH of deionized solutions may be too low at this point (<pH 3). A few trials on aliquots of deionized digest will indicate the extent of buffering required (ideally pH 5–6). Normally, addition of one drop 5 *N* NaOH to centrifuge tubes will suffice and can be verified by measurement on a pH meter. Add 5 ml of 95% ethanol to buffered eluates followed by 30 mg barium chloranilate and then make up to 10 ml with H_2O. Prepare a blank in the same manner with an equal volume of digest blank. Carry out color development as described in the Preparation of Calibration Curve section.

Inorganic Sulfate in 10% TCA Plasma Extracts and Urine

TCA plasma extracts as well as urine may be analyzed for inorganic sulfate. Since the initial pH of TCA extracts is too low (<pH 2), it is necessary to buffer TCA aliquots with 5 *N* NaOH (two or three drops per 2 ml aliquot) in order to increase the pH to 5–6 prior to color development. A blank using 1 ml of the TCA extracting solution must be prepared in the same manner against which the absorbance of extracts is read. For urine samples, since inorganic sulfate is present in large amounts, take a 0.25-ml aliquot, and then deionize and buffer as above. Read absorbance against a reagent blank.

RESULTS AND DISCUSSION

In order to determine the effectiveness of the digestion procedure for complete recovery of organic sulfur, several organic analytical standard compounds (B.D.H. organic analytical standards) were digested under various conditions. Essentially complete recoveries of S in the organic analytical standard compounds were obtained. This was achieved after raising the digestion temperature to 250°C and increasing the volumes of HNO_3 and $HClO_4$ (Table 1). Mean recovery of S in the standards was 98.1% with an SD (standard deviation) of ±2.6. These digestion conditions were adopted as optimum and applied to the digestion of whole blood, blood plasma and urine samples. Mottershead (*16*) reported a

method for the determination of total sulfur in biological materials employing mixed nitric and perchloric acids along with potassium dichromate and ammonium metavanadate catalysts for digestion. The digestions, which were carried out in funnel-covered 50-ml flasks on a hot plate, could require 1–3 hr heating from 80 to 190°C for complete oxidation of organic material. The final measurement of sulfate was made on a Technicon AutoAnalyzer system by barium turbidimetry. This digestion technique seems subjective and less than ideal since possible interfering elements from the added catalysts remain in the digests. However, use of a Technicon heating unit and controller as described in this paper permits a more consistent control of digestion conditions.

Lamand (14) pointed out that accurate analysis of sulfur in biological materials is often difficult, requiring extreme care in methodology and technique. A sizable error can occur depending on the form of sulfur being estimated, e.g., heparinized blood will yield higher sulfur values since heparin contains sulfonic acid ester groups.

In view of the above, careful experimentation of recoveries of sulfur containing amino acids (cystine and methionine), added to urine, blood plasma, and citrated whole blood were undertaken. Results are summarized in Table 2. Mean recovery of S from the amino acids was 99.3% with and SD of ±3.2.

TABLE 1
RECOVERIES OF SULFUR IN ORGANIC ANALYTICAL STANDARDS

Digestion conditions	Compound	Percentage recovery[a]
1 hr 200°C 2 ml HNO_3 + 1 ml $HClO_4$	S-Benzyl isothiourea HCl	67.0
	Cystine	65.6
2 hr 200°C 2 ml HNO_3 + 1 ml $HClO_4$	S-Benzyl isothiourea HCl	89.6
	Cystine	84.7
3 hr 200°C 2 ml HNO_3 + 1 ml $HClO_4$	S-Benzyl isothiourea HCl	85.7
	Cystine	80.8
3 hr 250°C 3 ml HNO_3 + 2 ml $HClO_4$	S-Benzyl isothiourea HCl	98.2
		99.3[b]
	Cystine	95.5
		95.5[b]
		99.3[b]
	Benzyl disulfide	96.0
		96.0[b]
	Sulfamic acid	101.5
		101.5[b]

[a] Range of S per 25 ml diluted digest 1–2 mg.
[b] Replicated 1-ml aliquots of same digest.

TABLE 2
RECOVERIES OF SULFUR FROM CYSTINE AND METHIONINE ADDED TO BIOLOGICAL SAMPLES

Sample[a]	Percentage recovery[b]
1 ml Urine + cystine	99.5
1 ml Urine + methionine	99.8
1 ml Blood plasma + cystine	95.3
1 ml Blood + methionine	100.3
1 ml Whole blood + cystine	97.6
1 ml Whole blood + methionine	103.4

[a] Range of S per 25 ml diluted digest 1–2 mg.
[b] Total S found – biological S found (average of two determinations).

Precision data for replicate analyses of citrated sheep's whole blood, blood plasma, 10% TCA extracts, and urine are shown in Table 3 and agree with normally expected sulfur levels in these materials. Ten determinations were carried out for each lot of samples and standard deviations were calculated. Study of precision data in Table 3 shows that the method has the accuracy desired for routine sulfur analyses of biological materials. Based on our experience, criteria for obtaining accurate values for sulfur by barium chloranilate colorimetry are as follows:

(1) Digestion of biological material for 3 hr at 250°C in a mixture of 3 ml HNO_3 + 2 ml $HClO_4$.

(2) Careful buffering of deionized sample digests to pH 5–6 before carrying out color reaction. Verified on a pH meter for maximum accuracy.

(3) Taking spectrophotometric measurements of samples against equal volumes of digest blanks carried through the whole procedure.

(4) Use of highest purity water for preparing all reagent solutions in order to maintain the blank value to a minimum.

SUMMARY

A simple, rapid, and accurate method for determination of total sulfur and inorganic sulfate in biological materials by barium chloranilate colorimetry is presented. Criteria re-

TABLE 3
PRECISION OF S DETERMINATION, WHOLE BLOOD, BLOOD PLASMA, TCA EXTRACTS, AND URINE

Sample	Determinations	Mean (μg)	SD (μg)
Whole blood	10	1004	21
Blood plasma	10	543	4
TCA extracts	10	24	2
Urine (totals)	10	598	31
Urine (inorganic S)	10	374.4	19

quired for accurate determination of sulfur such as digestion conditions and pH control have been investigated. The method permits the carrying out of about 40 sulfur determinations daily. The method may also be applied to the micro determination of sulfur in organic compounds and other biological materials.

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MICROCHEMICAL JOURNAL 24, 298–299 (1979)

The Application of Triphenylmethane Dyes to Visualization of Selected Aliphatic Compounds in Thin-Layer Chromatography

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INTRODUCTION

The problem of visualizing organic substances on a thin layer is of serious importance from the analytical point of view. From the wider range of triphenylmethane dyes, there are only fuchsine dyes, which have found certain application as visualizing agents (2).

The aim of this work is to investigate the possibilities to apply new fuchsine, malachite green, and methyl violet as visualizing agents with selected aliphatic compounds tested by means of thin-layer chromatography.

EXPERIMENTAL

Kieselgel GF_{254} (E. Merek, West Germany), layer thickness of 0.3 mm, was used as an adsorbent, the activation period at 110°C lasting 30 min. The mobile phase was composed of chloroform and acetone at a volume ratio of 8:2.

On the adsorbent layer, changing microgram amounts of the examined substances were placed, preserving in each case the same volume of the applied solutions. After developing and drying at room temperature for 24 hr, the glass plates were visualized by spraying with the visualizing agents. On 100 cm^2 of a glass plate, 5 ml of the 0.01 N water solutions of the examined triphenylmethane dyes was used. With the investigated acids, the obtained R_f values were 0.33, with the alcohols, 0.62, and with the amines, 0.30.

RESULTS AND DISCUSSION

From the results obtained (Tables 1 and 2), the triphenylmethylene dyes are the most efficient visualizing agents with fatty acids, fatty alcohols, and aliphatic amines. New fuchsine, malachite green, and methyl violet all demonstrate the following order of diminishing efficiency with the applied groups of substances: acids–amines–alcohols. Also, the contrast indexes, which were determined by means of the already described

* Deceased.

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TABLE 1
DETECTABILITY (IN MICROGRAMS) OF HIGHER ALIPHATIC COMPOUNDS, VISUALIZED ON THIN LAYERS BY MEANS OF TRIPHENYLMETHANE DYES

Compound	Number of carbon atoms per molecule	Micrograms		
		New fuchsine	Malachite green	Methyl violet
Lauric acid	12	0.20	0.20	0.20
Palmitic acid	16	0.44	0.44	0.44
Arachidic acid	20	0.88	0.88	0.88
Lauryl alcohol	12	4.4	4.4	4.4
Cetyl alcohol	16	4.4	4.4	4.4
Arachidyl alcohol	20	2.2	2.2	2.2
Dodecylamine	12	0.44	0.44	0.44
Hexadecylamine	16	0.44	0.44	0.44
Eicosanamine	20	2.2	2.2	2.2

TABLE 2
THE CONTRAST INDEXES OF NEW FUCHSINE, MALACHITE GREEN, AND METHYL VIOLET

Compound	Contrast index					
	New fuchsine		Malachite green		Methyl violet	
	α (°)	h (cm)	α (°)	h (cm)	α (°)	h (cm)
Palmitic acid	30	1.1	30	1.0	29	1.2
Cetyl alcohol	89	0.4	87	0.4	80	0.4
Hexadecylamine	29	0.6	28	0.6	27	0.7

method (*1*), support the aforementioned statement, concerning the best visualizing effect with acids, then with amines, and finally, with alcohols.

The differences observed with visualizing and the contrast index values are at least partially due to the dissolving effect of the examined substances during developing and spraying of the chromatograms, as well as to the different adsorption power of the applied dyes on the chromatographically analyzed substances. All of the discussed dyes belong to the group of the preserving visualizing agents.

SUMMARY

Selected triphenylmethane dyes were used as new visualizing agents in thin-layer chromatography of higher fatty acids, higher fatty alcohols, and higher aliphatic amines.

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MICROCHEMICAL JOURNAL 24, 300–309 (1979)

Automatic Apparatus for the Determination of Carbon and Hydrogen in Organic Compounds[1]

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INTRODUCTION

The elemental analysis of carbon and hydrogen is still a technique widely used in organic chemistry, alongside more universal methods. Our laboratory, for instance, has to perform about 40 analyses of this type daily. This volume of work occupies the entire working day of an operator in order to respond to the demand. That is why it appeared necessary to design a simple and accurate method for the determination of carbon and hydrogen allowing automation so that intervention of the operator was limited to weighing the analytical samples.

Generally speaking, the first stage in the analysis of an organic compound is a combustion, leading to the quantitative formation of carbon dioxide and water, the species representing carbon and hydrogen, respectively. Their determination has already been the subject of numerous publications. The analysis can be completed by either the so-called reference methods (gas chromatographic) or absolute methods. Only the latter have been considered in this survey.

Determination of Carbon Dioxide

This is generally achieved by acidimetry. The use of a barium salt in aqueous solution as the absorption medium, then titration of the resulting acidity, have been described by numerous authors (*3, 5, 6, 9, 10*), but with this method it is imperative to have an intermittent flow of CO_2 and its simultaneous neutralization, in order to maintain the pH of the solution at a high enough level for complete absorption.

This system, besides being complicated, leads to variations in solution temperature prejudicial to stable pH measurement. A solution has been brought about (*1, 7, 8, 12*) through the absorption of CO_2 in an organic medium and in the presence of monoethanolamine. Under these conditions, the amine carbamate that is formed can be quantitatively deter-

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mined by acidimetry in an appropriate solvent. Thus Merz (*7*) achieves this determination in dimethylformamide by means of a standard solution of tetrabutylammonium hydroxide. The addition of this reagent requires previous determination of its strength, and also systematic renewal of the absorption solution to compensate for dilution.

A solution to the problem is the coulometric generation of the titrating reagent and this method has been applied notably by Boniface and Jenkins (*1*) to the determination of carbon in steel. The detection of the titration end-point then is generally photometric, but this method requires stopping the gas flow during the measurements, which modifies the dynamic equilibrium in the combustion unit and is liable to affect the accuracy of the results.

The study of a coulometric cell and a suitable titration medium allowed us to achieve the continuous determination of CO_2, thus eliminating all the drawbacks mentioned.

Determination of Water

This is conveniently carried out according to the principle already reported by Warf (*11*), Floret (*4*), and Merz (*7*). According to this method, water reacts with carbonyldiimidazole, the hydrolysis of which results in the quantitative liberation of CO_2, that is then determined in the same way as before. The combination of these two determinations has led us in a second step to develop an automatic apparatus for the determination of carbon and hydrogen.

PRINCIPLE

The sample is weighed into a silver capsule, then flash combusted in a stream of oxygen using a vertical combustion tube heated to 1050°C. The water is initially retained by freezing in a low-temperature trap, while the CO_2 is absorbed and determined acidimetrically in a titration cell using constant current coulometry.

The subsequently vaporized water when passed over solid carbonyldiimidazole liberates a fresh quantity of CO_2 which is determined as before in a second titration cell.

The use of a dual water trapping circuit and two titration cells allows, through the use of a multiport valve, the simultaneous determination of the carbon from one sample and the hydrogen from the preceding sample. This procedure can be easily automated.

EXPERIMENTAL

A drawing of the apparatus is shown in Fig. 1. It consists of four parts: (a) the combustion system, (b) the water-trapping system, (c) the carbon determination system, and (d) the hydrogen determination system.

a. Combustion system. The combustion system consists of a vertical

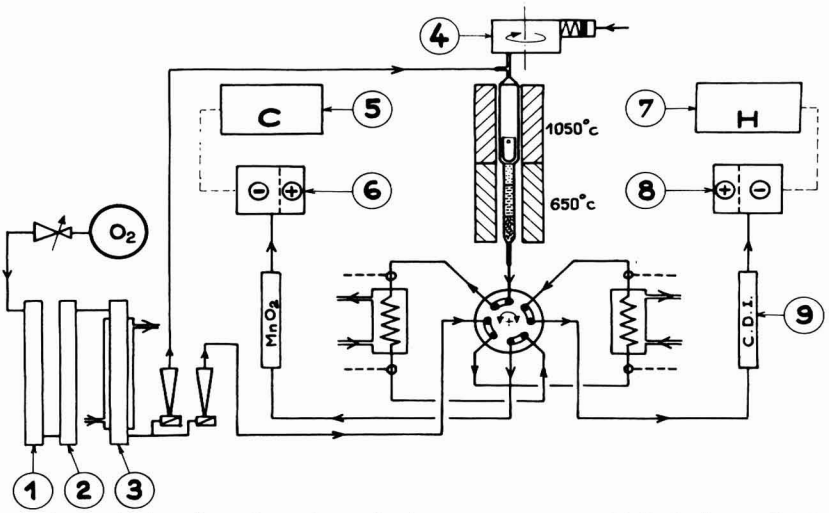

FIG. 1. Schematic drawing of a carbon–hydrogen apparatus. (1) Soda lime, (2) $MgClO_4$, (3) 5-Å molecular sieves, (4) sample dispenser, (5)–(7) coulometers, (6)–(8) titration cells, (9) carbonyldiimidazole tube.

tubular furnace with two temperature zones, containing the quartz combustion tube (Fig. 2), whose packing ensures complete combustion and also retains phosphorus, sulfur, fluorine, and halogens that might interfere in the subsequent determination. The oxygen required for the combustion circulates with a flow-rate of 30 ml/min after purification and drying over soda lime, magnesium perchlorate, and molecular sieve (5Å). A sample distributor (Carlo Erba Type 08.50.04550) is mounted on top of the combustion tube and allows storage of 23 samples.

b. Water-trapping system. The water-trapping system consists of a stainless-steel tube (Ø 1.6–3.2 mm) 500 mm long wound into a coil with 10 well-spaced turns and placed in a cooling chamber. Cooling is obtained by admitting expanded liquid nitrogen, while the subsequent heating results from the application to the ends of the coil of 5 V supplied by a 90 VA transformer. A thermocouple, connected to the control system, allows the temperature to be kept at either −50 or +200°C.

Using two identical traps, alternately cooled or heated, it is possible to determine simultaneously the carbon representing one sample and the hydrogen from the preceding sample. An eight port valve (Bimatic Carlo Erba) directs the combustion gases toward the cooled trap while the water resulting from the preceding combustion is vaporized in the second trap and directed to the hydrogen determination system.

c. Carbon determination system. After eliminating nitrogen oxides on a manganese dioxide filling, CO_2 is absorbed in the titration cell (Fig. 3) by a solution consisting of 6.4 g of tetrabutylammonium bromide in a mixture

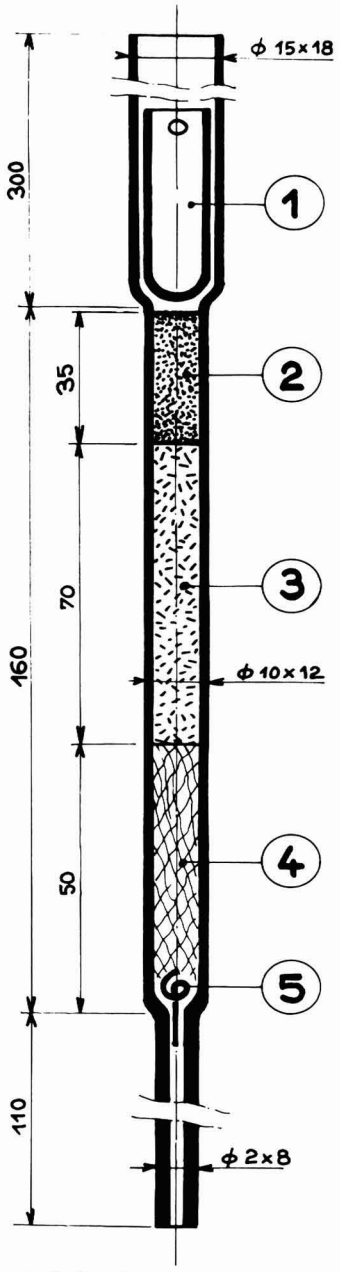

FIG. 2. Combustion tube. (1) Collecting cup, (2) magnesium oxide, (3) cobalto-cobaltic oxide, (4) silver wool, (5) silver wire Ø 1.5 mm.

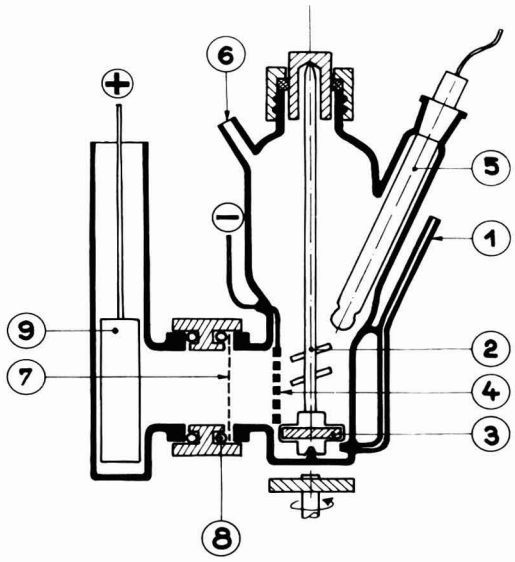

FIG. 3. Titration cell. (1) Gas inlet, (2) stirrer axis, (3) magnetic stirrer, (4) platinum cathode, (5) combined electrode, glass-reference, (6) gas outlet, (7) anionic membrane, (8) o-ring for membrane fixation, (9) platinum anode.

comprising 90 ml dimethylsulfoxide, 10 ml water, and 3 ml ethanolamine. The auxiliary compartment of the electrolysis cell is filled with a 0.2 *M* solution of tetrabutylammonium hydroxide in a mixture of dimethylsulfoxide–water 90–10 (by volume).

Electrolysis is carried out with a constant current of 20 mA and detection of the neutralization point is achieved using a pair of electrodes (Glass and $Ag/AgBr_2^-$).

A servosystem linked with the coulometer (Model Mipo Eraly[3]) makes it possible to stop the electrolysis at the preset neutralization potential.

The development of the reference electrode has stemmed from the work of Courtot-Coupez and Le Demezet (*2*) describing the reference system 10^{-3} *M* $Ag/AgCl_2^-$ in 10^{-1} *M* LiCl. The great similarity between the stability of the complexes of silver chloride and silver bromide in dimethylsulfoxide has allowed construction of the following system: 5.10^{-3} *M* $Ag/AgBr_2^-$ 0.2 *M* tetrabutylammonium bromide in the mixture dimethylsulfoxide–water, 90–10 (by volume).

d. Hydrogen determination system. The water previously kept at a low temperature is vaporized by heating, then directed toward the carbonyldiimidazole conversion tube by an oxygen flow of 100 ml/min. The CO_2

[3] Eraly, 67 rue A. Le Bourblanc, Noisy-le-Roi, 78590 France.

thus produced is then absorbed in a second titration cell where it is determined as previously.

The carbonyldiimidazole conversion tube is filled with 5 g of powdered reagent according to Floret (*4*) and heated to 40°C.

AUTOMATION OF ANALYSIS

The apparatus has been designed to operate without any intervention from the operator except for weighing the analytical samples. Thus the various control and command functions of the apparatus as well as the processing of results is performed by a Hewlett-Packard 9825A desk-top computer with a 15K byte built-in memory.

After entering the weights of the successive analytical samples and their identification into the memory, the analysis program may be shown schematically as follows:

Cool water trap No. 1 and simultaneously heat trap No. 2.

Introduce the first sample to be analyzed.

Adjust the coulometers to zero, then electrolyze until they have returned to their set potential.

Read the coulometer for the carbon determination and enter into store.

Transpose the carbon and hydrogen circuits by rotation of the multiport valve and transpose the heating and cooling of the two water traps.

Introduce the second sample, then determine as before.

Read both coulometers. Store the carbon reading.

Calculate and print the results for the first sample as percentage carbon and hydrogen.

After the last sample has been introduced into the apparatus there still remains one hydrogen analysis to be performed.

Therefore it is necessary to run one more complete cycle, thus requiring a sum total of 24 cycles for a series of 23 analyses (the capacity of the distributor).

At all times during an analysis cycle, except for the very short calculation time, the computer monitors the correct operation of the apparatus, for sufficient oxygen pressure, sufficient cooling nitrogen, and correct temperature of the combustion furnaces.

If the apparatus fails to function correctly, the computer will stop the analysis program.

As described, the program is only an illustration of the analytical procedure. A subprogram has been devised that allows a series of sample weights to be entered into the memory during the analysis cycle.

Weighing and Identification of Sample

The computer is coupled through an appropriate interface to a Mettler electronic balance Model ME 22. The weight of the sample to be analyzed is then entered into the computer, together with a specific code identifying

it with the sample. Thus it is possible to perform a series of 23 weighings, that will be analyzed later according to the method previously described.

As an indication, a series of 23 analyses is completed in 4 hr and the instrument is capable of analysing 69 samples per day, the last series being completed without the presence of the operator.

DISCUSSION

Titration Medium

Acidimetric titration using constant current coulometry requires 100% overall electrolysis efficiency (reduction of H^+ ions and formation of hydroxyl ions). Generally speaking, this quantitative efficiency may be obtained in an aqueous organic medium using polished platinum electrodes, but the choice of the supporting electrolyte is very important so as to avoid any interfering reductions on the one hand and to obtain on the other hand a medium possessing good conductivity.

Dimethylsulfoxide has been chosen as the main solvent for the following reasons: very good stability, high dielectric constant (48.9 at 20°C), low vapor pressure (0.6 mm Hg at 25°C), and can be used over a pH range sufficiently wide to allow the titration of weak acids. The addition of 10% water allows quantitative coulometric generation of hydroxyl ions and contributes to a decrease in the electrical resistance of the medium.

Testing various supporting electrolytes showed that the presence of alkaline ions (sodium or potassium) was prejudicial to a correct determination.

In fact, if the principle of the CO_2 determination is shown by the reaction:

$$CO_2 + R-NH_2 \longrightarrow R-NH-COOH \qquad (1)$$

the presence of alkaline ions leads to the reaction:

$$CO_2 + H_2O + 2\ Na^+\ (2\ K^+) \longrightarrow Na_2CO_3\ (K_2CO_3) + 2\ H^+ \qquad (2)$$

Reaction (2), promoted by the low solubility of alkaline carbonates in this medium, results in the appearance of an excess acidity compared with CO_2 involved.

Tetrabutylammonium bromide (free from alkaline ions) at a concentration of 0.2 *M* has been chosen as the supporting electrolyte.

Titration Cell

This is shown in Fig. 3. Each generating electrode consists of a plate of polished platinum, 50 × 30 mm for the cathode and 30 × 30 mm for the anode. The two compartments are electrically joined through an anionic membrane avoiding any diffusion of H^+ ions to the auxiliary compartment.

TABLE 1

ACCURACY AND REPEATABILITY FOR STANDARD COMPOUNDS

Standard compound	Number of determinations	Carbon (%)		Standard deviation	Hydrogen (%)		Standard deviation
		Calculated	Found		Calculated	Found	
Mannitol	10	39.56	39.63	0.058	7.75	7.63	0.092
Methylbenzimidazole	12	72.70	72.71	0.054	6.10	5.97	0.070
Benzoic acid	10	68.84	68.89	0.030	4.95	4.97	0.070

TABLE 2
CONSECUTIVE ANALYSES

Compound	Sample weight (mg)	Carbon (%)		Hydrogen (%)	
		Calculated	Found	Calculated	Found
Anthracene	1.877	94.34	94.38	5.66	5.78
	1.932		94.28		5.66
Methylbenzimidazole	2.238	72.70	72.71	6.10	6.20
	1.959		72.77		6.08
Research compound	2.156	47.13	47.18	4.32	4.37
	1.781		47.26		4.29
Phenacetin	2.440	67.02	66.98	7.31	7.12
	2.148		67.02		7.47
Mannitol	2.450	39.56	39.56	7.75	7.67
	2.388		39.62		7.81
Polyvinyl chloride	2.024	38.43	38.56	4.83	5.02
	2.280		38.57		4.92
Benzoic acid	1.853	68.84	68.94	4.95	5.00
	2.839		68.82		5.08
Calcium carbonate	2.553	12.00	12.04	—	—
	2.835		12.03		

Moreover the membrane plays a part in obtaining a low internal electric resistance.

Thus, under the above operating conditions, the voltage across the generating electrodes is about 8–9 V (with a current of 20 mA). This limits solution heating and allows the use of a glass electrode for potentiometric detection without any of the interferences associated with the electrical field, contrary to the opinion of many authors.

RESULTS

The results obtained with different reference products make quite clear on one hand the accuracy and repeatability of the method (Table 1) and on the other hand the precision of consecutive analyses of different compounds (Table 2).

SUMMARY

A microanalytical determination of carbon and hydrogen in organic compounds is achieved by an original technique of coulometric titration in a nonaqueous medium. The apparatus, designed for the purpose, wholly automatic and monitored by a computer, makes possible the analysis of 23 samples without any intervention from the operator. Each analysis takes about 10 min and the precision of the method results in relative standard deviations of about 0.1 and 1% for carbon and hydrogen, respectively.

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The Use of Iodate and Periodate with Iodide in the Potentiometric Titration of Arsenite, Sulfide, and Sulfite with Mercury (II)

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INTRODUCTION

This work was undertaken to explore the feasibility of the use of iodate and periodate in the indirect potentiometric titration of some reductants. The method developed for arsenite, sulfide, and sulfite involves addition of the sample of the reductant to a known, excess amount of iodate or periodate made acidic, thereby forming iodine. A known, excess amount of iodide is then added, which starts with the excess of oxidant. The excess of iodide is titrated with mercury (II) potentiometrically using a silver amalgam indicator electrode.

Some prior studies on the determination of reductants by potentiometric titrations are instructive:

Arsenite has been titrated with iodide even in dilute solutions (*1*). Peroxymolybdate has been used for the determination of arsenite and iodide (*5*). Dichromate and iodine have been used as oxidants, and arsenic (III), thiosulfate, tin (II), antimony (III), and iron (II) as reductants in titrations in formamide (*7*). Manganese (III) has been used in the determination of arsenic (III), tin (II), antimony (III), and hexacyanoferrate (II) (*11*). Arsenic (III) and thiocyanate have been determined using iodate and bromate titrations (*9*). Various reductants have been titrated with mercury (II) chloride (*6*). Iodide resulting from the action of sulfite on iodate has been used for the standardization of permanganate (*4*). Sulfide has been titrated with silver (I) using a silver sulfide-coated carbon electrode (*8*), but the electrode preparation is tedious. Arsenic (III) has been titrated with iodate (*3*) and also with bromate in 0.1 *M* osmic acid as catalyst (*2*). Under these conditions arsenic cannot be determined in the presence of antimony (III), as partial oxidation of the latter occurs. Sulfide, thiosulfate, and polysulfide have been titrated with mercury (II) chloride (*11*). The potentiometric titration approach now developed has several advantages, including application to reductants and oxidants over a wide range of concentrations, simplicity, rapidity, reproducibility, and high accuracy. The time for a single titration does not exceed 15 min. The end-point

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potential break averages 350 mV, which corresponds to 0.1 ml of 0.05 *M* mercury (II).

EXPERIMENTAL METHODS

The water used in this investigation was always twice distilled from all-glass equipment. The chemicals were all of the highest purity available. They were nitrate of mercury (II), potassium iodide, iodate, and periodate, nitric, sulfuric, and hydrochloric acids; hexamine, zinc oxide, disodium ethylenediaminetetraacetate (EDTA); sodium arsenite, sulfide, and sulfite, and methyl thymol blue and Eriochrome black T indicators.

Solutions

The 0.05 *M* EDTA solution was prepared normally and standardized against a standard 0.05 *M* zinc solution prepared from an oxide sample and nitric acid. The 0.0474–0.0498 *M* mercury (II) nitrate solutions were prepared from a nitrate sample dissolved by the aid of concentrated nitric acid (4 ml/liter) and standardized potentiometrically in hexamine buffer (pH 10) against EDTA using silver amalgam as the indicator electrode. The 0.0924–0.1004 *M* iodide solutions were prepared from potassium iodide, standardized against standard mercury (II) solution, and kept in a storing black bottle. The ammoniacal buffer (pH 10) was prepared by mixing 54 g of ammonium chloride with 350 ml of 25% ammonium hydroxide and diluting to 1 liter. The 0.025 *M* iodate was 5.35 g/liter containing not more than 6 ml of concentrated sulfuric acid. The 0.025 *M* periodate was 5.7504 g/liter containing not more than 5 ml of concentrated sulfuric acid. The 0.1178 *M* arsenite solution was prepared from sodium arsenite and standardized against standard potassium permanganate (*10*). The 0.0282, 0.0351, and 0.0595 *M* sulfide solutions were prepared from sodium sulfide and standardized against standard iodine (*10*). The 0.0946 *M* sulfite was prepared from sodium sulfite and standardized against potassium dichromate (*10*).

The silver amalgam electrode consisted of a spectroscopically pure silver rod 7 mm in diameter and 30 mm in length fitted to a glass tube by the aid of neutral polyethylene tubing and paraffin wax.

The titration system consisted of a borosilicate glass vessel fitted with a glass cover with ground joints for the electrode, the salt bridge with its end immersed together with the tip of the calomel electrode in saturated KCl, and the delivering tip of a 10-ml microburette (1 division = 0.02 ml); a magnetic stirrer (No. 9986, Type RH 12, Ruhromage); Scalamp galvanometer (W. G. Pye and Co. Ltd., Cambridge, Cat. No. 790115), connected to a Pye student potentiometer.

Procedures

(A) Effect of acidity. Transfer with continuous stirring, in the following

order, 1 ml of 0.118, 0.035, and 0.095 *M* arsenite, sulfide, and sulfite, respectively, 4 ml of 0.0253 *M* iodate or periodate, and 1–8 ml of (1:1) H_2SO_4 to make the total volume of 30 ml from 0.5 to 6 *N* with respect to acid. To the brown solution containing iodine equivalent to the reductant, add 5–10 ml of 0.1 *M* iodide and potentiometrically titrate the unreacted iodide equivalent to excess oxidant with mercury (II).

(B) Effect of concentration. Procedure A involves the use of an amount of oxidant in excess of that equivalent to a given reductant. Follow the same procedure, using the optimum acidity while changing the concentration of reductants.

RESULTS AND DISCUSSION

The completeness of the redox reactions involving, as oxidants, iodate and periodate and, as reductants, arsenite, sulfide, and sulfite, may be predicted by considering the standard reduction potentials of the half reactions involved. For instance,

$$IO_3^- + 5e + 6H^+ \rightarrow \tfrac{1}{2} I_2 + 3H_2O \quad E^\circ = +1.19 \text{ V},$$

and

$$AsO_4^{3-} + 2e + 2H^+ \rightarrow AsO_3^{3-} + H_2O \quad E^\circ = +0.57 \text{ V}.$$

The E° value for the redox reaction

$$IO_3^- + 5/2\ AsO_3^{3-} + H^+ \rightarrow \tfrac{1}{2} I_2 + 5/2\ AsO_4^{3-} + \tfrac{1}{2} H_2O$$

is then calculated to be $+1.19 - (+0.57) = +0.62$ V.

Similarly the E° values for the following redox reactions are computed:

$$IO_4^- + 7/2\ AsO_3^{3-} + H^+ \rightarrow \tfrac{1}{2} I_2 + 7/2\ AsO_4^{3-} + \tfrac{1}{2}H_2O \quad E^\circ = +0.24 \text{ V},$$
$$IO_3^- + 5/2S^{2-} + 6H^+ \rightarrow \tfrac{1}{2}I_2 + 5/2\ S + 3H_2O \quad E^\circ = +1.05 \text{ V},$$
$$IO_4^- + 7/2\ S^{2-} + 8H^+ \rightarrow \tfrac{1}{2}I_2 + 7/2\ S + 4H_2O \quad E^\circ = +0.67 \text{ V},$$
$$IO_3^- + 5/2\ SO_3^{2-} + H^+ \rightarrow \tfrac{1}{2}I_2 + 5/2SO_4^{2-} + \tfrac{1}{2}H_2O \quad E^\circ = +1.02 \text{ V},$$
$$IO_4^- + 7/2\ SO_3^{2-} + H^+ \rightarrow \tfrac{1}{2}I_2 + 7/2\ SO_4^{2-} + \tfrac{1}{2}H_2O \quad E^\circ = +0.64 \text{ V}.$$

By aid of the relation $E^\circ = 0.0591/n \log K$, where E° is the standard redox potential, n is the number of electrons required to reduce 1 mol of iodate = 5 or 1 mol of periodate = 7, and K is the equilibrium formation constant of the redox reaction, one calculates $\log K$ with iodate and periodate to be 52.45, 28.43; 88.74, 79.24; and 86.29, 57.81, respectively. By aid of the relation $\log a = \log K/(n_1 + n_2)$, where a is the degree of completion of the redox reaction and n_1 and n_2 are the numbers of electrons involved in the two half-reactions, one calculates a to be 7.49, 3.16; 12.68, 8.8; and 12.32, 8.42, respectively. Such values indicate that the above redox reactions should proceed quantitatively to completion and hence the accurate determination of arsenite, sulfide, and sulfite by the present method. Tables 1 and 2 list representative results obtained with experiments run in triplicate, by the present procedure. Negligible errors are

TABLE 1
DETERMINATION OF MILLIGRAM AMOUNTS OF REDUCTANTS USING IODATE[a]

No.	Reductants (mg)		Error (±%)	Titrant (mV/0.1 ml)
	Taken	Found		
1	9.95	9.94	0.10	353
2	19.90	19.80	0.50	356
3	29.85	29.83	0.07	356
4	39.80	39.97	0.43	354
5	49.74	49.96	0.42	308
6	59.70	60.06	0.60	294
7	3.68	3.72	0.52	271
8	6.99	6.97	0.40	281
9	10.49	10.42	0.66	294
10	14.00	14.00	0.00	305
11	17.50	17.49	0.06	325
12	20.99	20.92	0.36	340
13	2.98	2.98	0.00	370
14	5.96	5.94	0.33	378
15	8.94	8.92	0.22	380
16	11.92	11.90	0.16	382
17	17.89	17.87	0.11	370
18	23.81	23.72	0.50	330

[a] Nos. 1–6—0.50–3 ml 0.1178 *M* AsO_3^{3-} + 3–6 ml 0.0255 *M* IO_3^- + 5–8 ml 0.1004 *M* I^- against 0.0498 *M* Hg (II); Nos. 7–12—0.50–3.50 ml 0.0282 *M* S^{2-} + 2.0–3.50 ml 0.0255 *M* IO_3^- + 5–7 ml 0.0924 *M* I^- against 0.0498 *M* Hg (II); Nos. 13–18—0.25–2.00 ml 0.0946 *M* SO_3^{2-} + 3–5 ml 0.0152 *M* IO_3^- + 7–8 ml 0.1004 *M* I^- against 0.0498 *M* Hg (II).

involved whether iodate or periodate was used in determining milligram amounts of arsenite, sulfide, or sulfite in the ranges of 4–60, 2–21, or 3–30, respectively. In most cases the obtained potential breaks were of a considerably large order of magnitude, ranging from 280 to 400 mV/0.1 ml of titrant, thus allowing easy and accurate determination of end points. Such results were obtained under optimum conditions of acidity and concentration, that is, 2–3, 1–2, or 2 *N*, with respect to sulfuric acid with the above milligram amounts of reductants, respectively. We calculated the standard deviations in the determination of 19.9, 8.45, and 12.16 mg of arsenite, sulfide, and sulfite, respectively, after carrying out 10 experiments with each reductant, to be ±0.025, ±0.019, ±0.018 and ±0.022, ±0.013, ±0.015 with iodate and periodate, respectively. Corresponding relative percentage errors were 0.12, 0.13, 0.15 and 0.15, 0.19, 0.13. The silver amalgam, which acts as a mercury electrode, has several advantages over the J-type mercury pool electrode, namely, its very easy preparation and use, its being much less voluminous, and its surface being much more stable than that of the mercury pool, which may often lose some droplets under continuous stirring of the titrant.

TABLE 2
DETERMINATION OF MILLIGRAM AMOUNTS OF REDUCTANTS USING PERIODATE

No.	Reductant (mg)		Error (±%)	Titrant (mV/0.1 ml)
	Taken	Found		
1	3.98	3.95	0.75	366
2	4.97	4.95	0.40	387
3	14.93	14.97	0.26	330
4	19.90	19.90	0.00	340
5	39.80	39.84	0.10	320
6	59.69	59.59	0.02	350
7	2.11	2.14	1.42	354
8	3.38	3.40	0.54	323
9	5.07	5.04	0.50	343
10	8.45	8.44	0.12	309
11	13.53	13.43	0.70	343
12	16.91	16.89	0.12	288
13	5.96	5.94	0.33	370
14	8.94	8.92	0.22	378
15	11.92	11.92	0.00	384
16	17.88	17.84	0.22	380
17	23.84	23.77	0.29	401
18	29.81	29.81	0.00	378

[a] Nos. 1–6—0.20–3.0 ml 0.1178 *M* AsO_3^{3-} + 3–6 ml 0.0252 *M* IO_4^- + 7–10 ml 0.1004 *M* I^- against 0.0198 *M* Hg (II); Nos. 7–12—0.25–2.0 ml 0.0351 *M* S^{2-} + 1–3 ml 0.0254 *M* IO_4^- + 4–7 ml 0.0989 *M* I^- against 0.0474 *M* Hg (II); Nos. 13–18—0.5–2.50 ml 0.0946 *M* SO_3^{2-} + 3–6 ml 0.0252 *M* IO_4^- + 7–10 ml 0.1004 *M* I^- against 0.0498 *M* Hg (II).

SUMMARY

A new potentiometric method is adopted for the accurate microdetermination of arsenite, sulfide and sulfite. The reductant is added to a known excess of standard iodate or periodate properly acidified with sulfuric acid. To the brown solution containing iodine equivalent to the reductant, an excess of standard iodide solution is added followed by titrating unreacted iodide with mercury (II) potentiometrically using the silver amalgam as indicator electrode. The potential breaks which averaged 350 mV per 0.1 ml of 0.05 *M* titrant were sharp enough for the precise determination of end points, and hence the high accuracy of the present method. In addition, besides simplicity and rapidity the stoichiometry of the reaction between iodate or periodate with the above reductants is still maintained even with the very low concentration.

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Oxidation of Organic Substances with Compounds of Trivalent Manganese

XIII. Oxidation of Pyruvic Acid with Manganese(III) Sulfate in a Medium of Sulfuric Acid and with Hexaquomanganese(III) Ions in Noncomplexing Medium of Perchloric Acid[1]

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INTRODUCTION

Previous works in this series have dealt with the oxidation of α-hydroxyacids (*1*, *4*) considering conditions for analytical application of this reaction. In the framework of a systematic study of the oxidation of organic substances with compounds of trivalent manganese, this work is devoted to oxidation of α-ketoacids, where pyruvic acid was chosen as a model substance. It followed from a kinetic study of the oxidation of this substance by the diphosfate complex of trivalent manganese (*5*) that this reaction proceeds according to the following mechanism:

$$[Mn(H_2P_2O_7)_3]^{3-} + CH_3COCOOH \rightleftharpoons [Mn(H_2P_2O_7)_2CH_3COCOO]^{2-} + H_2P_2O_7^{2-} + H^+ \quad (1)$$

$$[Mn(H_2P_2O_7)_2CH_3COCOO]^{2-} \rightarrow [Mn(H_2P_2O_7)_2]^{2-} + CH_3\dot{C}O + CO_2 \quad (2)$$

$$CH_3\dot{C}O + Mn^{3+} \rightarrow CH_3{}^+CO + Mn^{2+} \quad (3)$$

$$CH_3{}^+CO + H_2O \rightarrow CH_3COOH + H^+ \quad (4)$$

where Eq. (2) is the rate-determining step of the reaction.

Only the oxidation of pyruvic acid with manganese(III) acetate has so far found analytical application. In this determination an excess of reagent at 50°C is used and the unreacted portion is determined iodometrically.

This work considers the possibility of analytically employing the oxidation of pyruvic acid by manganese(III) sulfate and hexaquomanganese(III) ions in noncomplexing perchloric acid medium. Both these

[1] Part XII. Oxidation of citric acid with hexaquomanganese(III) ions in noncomplexing medium of perchloric acid. *Coll. Czech. Chem. Commun.*, in press.

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strong oxidizing agents can be readily prepared from normally obtainable chemicals and their stability is sufficient even for indirect determinations (*3*). Considering the mechanism proposed for the oxidation of pyruvic acid by the diphosfate complex of trivalent manganese, it could also be assumed that the oxidation of this substance by the less stable sulfate complex of trivalent manganese or with hexaquomanganese(III) ions in noncomplexing perchloirc acid medium proceeds much more rapidly, so that elevated temperatures need not be used. The trivalent manganese reagent is sufficiently reactive, especially in noncomplexing perchloric acid medium, so that the relatively rapid and quantitative course of the reaction could be expected even in very dilute solutions and thus it could be used for the microdetermination of this α-ketoacid.

EXPERIMENTAL

Reagents

The 0.05 and 0.005 *M* solutions of manganese(III) sulfate in a medium of 6*M* H_2SO_4 and 0.1 *M* $MnSO_4$ and 0.005 and 0.0005 *M* solutions of hexaquomanganese(III) ions in media of 6 *M* $HClO_4$ and 0.4 *M* $Mn(ClO_4)_2$ were prepared as described in a previous work (*3*). The titer of 0.1, 0.01, and 0.001 *N* ferrous sulfate in 0.5 *N* H_2SO_4 was found daily using dichromate. Pyruvic acid solutions with various molarities were prepared daily by dissolving the exactly weighed amounts of the pa substance in the form of the sodium salt (Lachema, Brno) and diluting with distilled water to the mark.

Apparatus

Potentiometric titrations were carried out using a TTT1 titrator and an ABU1 automatic burette (Radiometer, Copenhagen), using a P101 platinum indicator electrode and a K4O1 calomel reference electrode.

Procedure

Oxidation of pyruvic acid with manganese(III) sulfate: study of the reaction stoichiometry in the presence of oxygen. Amounts of 20.00 ml of 0.05 and 0.005 *M* solutions of manganese(III) sulfate in 6 *M* H_2SO_4 and 0.1 *M* $MnSO_4$ were measured into ground glass flasks; 5.00 ml of 0.05 or 0.005 *M* pyruvic acid was added. After time *t* the unreacted residue of the reagent was determined by potentiometric titration with ferrous salt. A blank was carried out simultaneously.

Study of the reaction stoichiometry in the absence of oxygen. The procedure was completely analogous to that used above, but both reagent solutions were deaerated for 15 min prior to mixing by bubbling with nitrogen and the reaction was also carried out with constant bubbling of the reaction mixture with nitrogen.

The consumption of oxidizing agent in the flasks per mole of oxidized

acid was calculated from the difference in the consumptions for the blank and for the actual determination. The measurements were carried out at laboratory temperature.

Recommended Determination Procedure

An amount of 5.00 ml of a solution containing 13–40 mg of pyruvic acid is added to 20.00 ml of a 0.05 *M* solution of manganese(III) sulfate in 6 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate. After standing for 20 min at laboratory temperature, the amount of reagent consumed is determined by potentiometric titration with ferrous salt. A blank is carried out simultaneously. One milliliter of 0.01 *N* ferrous sulfate corresponds to 4.403 mg of pyruvic acid.

Oxidation of pyruvic acid by hexaquomanganese(III) ions in non-complexing perchloric acid medium: study of the stoichiometry of the reaction in the presence of oxygen. An amount of 5.00 ml of a 0.005 or 0.0005 *M* solution of pyruvic acid is added to 20.00 ml of 0.005 or 0.0005 *M* solution of hexaquomanganese(III) ions in 6 *M* perchloric acid and 0.4 *M* manganese(II) perchlorate. After time *t* the unreacted reagent residue was determined by potentiometric titration with ferrous sulfate. A blank was carried out simultaneously.

Study of the reaction stoichiometry in the absence of oxygen. The procedure was completely analogous except that the solutions of both reactants were bubbled with nitrogen and the bubbling was continued during addition of the pyruvic acid solution to excess reagent and during the reaction.

Recommended Determination Procedure

Semimicro level. An amount of 20.00 ml of a 0.005 *M* solution of hexaquomanganese(III) ions in 6 *M* perchloric acid and 0.4 *M* manganese(II) perchlorate is bubbled for 15 min and then 5.00 ml of a solution containing 1–5 mg pyruvic acid is added, which was prebubbled with nitrogen. During sample addition and during the reaction the reaction mixture must be constantly bubbled with nitrogen. After 20 min of reaction in excess reagent at laboratory temperature the unreacted part is determined by potentiometric titration with 0.01 *N* ferrous sulfate. A blank is carried out simultaneously. On milliliter of 0.01 *N* ferrous sulfate corresponds to 0.4403 mg of pyruvic acid.

Micro level. An amount of 20.00 ml of a 5.10^{-4} *M* solution of hexaquomanganese(III) ions in 6 *M* perchloric acid and 0.4 *M* manganous perchlorate is bubbled for 20 min with nitrogen and then, with constant bubbling with nitrogen, 5.00 ml of a solution containing 100–500 μg of pyruvic acid and which is also bubbled with nitrogen is added. After standing for 6 hr at laboratory temperature with constant bubbling with nitrogen, the unreacted excess of the oxidation agent is determined by

potentiometric titration with 0.001 *N* ferrous sulfate. A blank is carried out simultaneously. One milliliter of 0.001 *N* ferrous sulfate corresponds to 44.03 μg of pyruvic acid.

RESULTS AND DISCUSSION

The Oxidation of Pyruvic Acid by Manganese(III) Sulfate

Reaction stoichiometry. Table 1 gives the time dependence of the consumption of oxidizing agent per mole of pyruvic acid in the presence of oxygen and in the absence of oxygen. Each value is the average of three measurements, which do not differ by more than $\pm 0.4\%$.

It follows from this table that the studied reaction proceeds quantitatively and stoichiometrically with consumption of two equivalents of oxidizing agents per mole of pyruvic acid according to Eq. (5) and that the products do not undergo further oxidation.

$$CH_3COCOOH + 2Mn^{3+} + H_2O \rightarrow CH_3COOH + CO_2 + 2Mn^{2+} + 2H^+ \quad (5)$$

When using 0.05 *M* manganese(III) sulfate it is not necessary to carry out the reaction in an inert atmosphere, as oxygen does not interfere in this medium. When using 0.005 *M* reagent the reaction proceeds quantitatively after 2 hr and only in the absence of oxygen. The interfering effect of oxygen can be explained analogously to previous works (*1*, *4*) through its reaction with free radicals produced as reaction intermediates.

Analytical Use of the Reaction

In formulating to recommended procedure for the determination based on the oxidation of pyruvic acid with manganese(III) sulfate, the effect of the concentration of sulfuric acid and of manganese(II) sulfate on the stability (*3*) and on the reactivity (*1*, *4*) of the reagent used was considered. The correctness and reproducibility of the determination carried out ac-

TABLE 1
THE TIME DEPENDENCE OF THE OXIDATION OF PYRUVIC ACID WITH MANGANESE(III) SULFATE IN THE PRESENCE (a) AND ABSENCE (b) OF OXYGEN

Time (min)		5	10	15	20	30	60	120	240	360
Consumption of 0.05 *M* reagent (equiv/mol)	(a)	1.96	1.97	1.98	2.00	2.03	2.03	2.01	2.02	2.02
	(b)	1.99	2.00	2.02	2.02	2.03	2.03	2.02	2.01	2.02
Consumption of 0.005 *M* reagent (equiv/mol)	(a)	0.94	1.15	1.20	1.22	1.23	1.27	1.30	1.33	1.38
	(b)	1.57	1.86	1.87	1.87	1.88	1.90	2.00	2.01	2.01

TABLE 2
THE ACCURACY AND REPRODUCIBILITY OF THE DETERMINATION OF PYRUVIC ACID WITH MANGANESE(III) SULFATE

Pyruvic acid taken (mg)	Found (mg)[a]	Standard deviation (μg)
13.76	13.85	54
27.51	27.62	20
41.27	41.31	23

[a] These values represent the average of seven determinations, from which the standard deviation was found.

cording to the procedure recommended in the experimental section is given in Table 2.

Oxidation of Pyruvic Acid with Hexaquomanganese(III) Ions in Noncomplexing Perchloric Acid Medium

The oxidation of pyruvic acid with manganese(III) sulfate proceeds too slowly in dilute solutions for use on a micro level. Thus the reaction of this substance with hexaquomanganese(III) ions in noncomplexing perchloric acid medium, where a faster rate can be expected, was studied.

Reaction stoichiometry. The dependence of the consumption of oxidizing agent on time using a 0.005 *M* solution of hexaquomanganese(III) ions in the presence and absence of oxygen is given in Table 3. It follows from this table that, in the absence of oxygen and under the given conditions, the reaction proceeds quantitatively within 20 min and that the reaction products do not undergo further oxidation. In the presence of oxygen the reaction proceeds nonstoichiometrically, which can be explained by reaction of intermediate free radicals with oxygen dissolved in the reaction mixture.

TABLE 3
THE TIME DEPENDENCE OF THE OXIDATION OF PYRUVIC ACID BY HEXAQUOMANGANESE(III) IONS IN A MEDIUM OF PERCHLORIC ACID IN THE PRESENCE (a) AND ABSENCE (b) OF OXYGEN

Time (min)		5	10	15	20	30	60	120	240	360	1200
Consumption of 0.005 *M* reagent (equiv/mol)	a	1.27	1.28	1.30	1.32	1.33	1.33	1.35	1.39	1.40	1.39
	b	1.97	1.98	1.99	2.01	2.00	2.01	2.00	2.00	2.00	2.00
Consumption of 0.0005 *M* reagent (equiv/mol)	a	0.67	0.75	0.78	0.84	0.88	0.93	0.99	1.17	1.20	1.32
	b	1.13	1.25	1.32	1.40	1.47	1.55	1.65	1.95	2.00	2.01

TABLE 4
THE ACCURACY AND REPRODUCIBILITY OF THE SEMIMICRO DETERMINATION OF PYRUVIC ACID WITH HEXAQUOMANGANESE(III) IONS IN NONCOMPLEXING PERCHLORIC ACID MEDIUM

Pyruvic acid taken (mg)	Found (mg)[a]	Standard deviation (μg)
1.375	1.368	6
2.751	2.760	5
4.126	4.129	2

[a] These values represent the average of seven determinations, from which the standard deviation was calculated.

Analytical Use of the Reaction

Semimicro level. In the formulation of the recommended procedure for the determination of pyruvic acid with hexaquomanganese(III) ions on a semimicro level, factors affecting the stability (*3*) and the reactivity (*1*, *4*) of the reagent used were considered. The correctness and reproducibility of the determination carried out according to the procedure recommended in the experimental section is given in Table 4.

Micro level. In considering the applicability of this reaction on a micro level, the time dependence of the consumption of a 0.0005 *M* solution of hexaquomanganese(III) ions in oxidation of 5.00 ml of 0.0005 *M* pyruvic acid was studied. It was found (see Table 3) that the reaction proceeds quantitatively after 6 hr, while with back titration with 0.001 *N* ferrous sulfate yields well-developed curves even when the potential in the vicinity of the equivalence point equilibrates relatively slowly (after 10–15 min). The potential change at the equivalence point is around 950 mV (vs SCE) and is about 50 mV for 0.02 ml of 0.001 *N* ferrous sulfate.

The accuracy and reproducibility of the microdetermination of pyruvic acid according to the procedure recommended in the experimental section is given in Table 5.

TABLE 5
THE ACCURACY AND REPRODUCIBILITY OF THE MICRODETERMINATION OF PYRUVIC ACID WITH HEXAQUOMANGANESE(III) IONS IN NONCOMPLEXING PERCHLORIC ACID MEDIUM

Pyruvic acid taken (μg)	Found (μg)[a]	Standard deviation (μg)
137.5	138.9	4
275.1	269.5	5
412.6	408.7	4

[a] These values are the average of seven determinations, from which the standard deviation value was calculated.

In conclusion, it can be stated that the assumption of a more rapid reaction for the oxidation of pyruvic acid with more reactive forms of trivalent manganese compared with the diphosphate or acetate complex used so far was confirmed and the usefulness of the studied reaction for the microdetermination of pyruvic acid was verified. It can be assumed that the principle described above can also be used for the determination of other α-ketoacids.

SUMMARY

The oxidation of pyruvic acid with manganese(III) sulfate in a medium of sulfuric acid and with hexaquomanganese(III) ions in noncomplexing perchloric acid medium was studied. It was found that pyruvic acid is oxidized by both reagents to give acetic acid and carbon dioxide and the optimal conditions for the analytical application of this reaction on both a semimicro and micro level were found.

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The Oxidation of Organic Substances by Compounds of Trivalent Manganese

XIV. Oxidation of Malonic Acid by Manganese(III) Sulfate in Sulfuric Acid Medium and with Hexaquomanganese(III) Ions in Noncomplexing Perchloric Acid Medium

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INTRODUCTION

Previous works in this series dealt with the oxidation of substances belonging among α-hydroxyacids (*1*) and α-ketoacids (*3*) in an attempt to find optimum conditions for analytical use of these reactions. In the framework of a systematic study of the oxidation of organic substances by compounds of trivalent manganese, this work was devoted to the study of malonic acid as a representative of dicarbonic acids. In this group of substances, attention has so far been paid to the analytical use of the oxidation of oxalic acid by the diphosphate (*6*) and phosphate (*12*) complexes of trivalent manganese and also by manganese(III) sulfate (*11*) and acetate (*13*) and by hexaquomanganese(III) ions in noncomplexing perchloric acid medium (*10*). It is also well known (*14*) that higher dicarbonic acids are not oxidized to a marked degree by compounds of trivalent manganese.

The kinetics of the reaction of malonic acid with the diphosphate complex of trivalent manganese (*7*) and manganese(III) sulfate (*9*) have been studied and a mechanism for the reaction has been proposed. Only the reactions with the diphosphate complex of trivalent manganese (*8*) and with manganese(III) acetate (*13*) have been used analytically. A disadvantage of the first method is the use of an empirical oxidation equivalent and in the latter case it is necessary to use excess manganese(III) acetate at an elevated temperature.

Consequently, this work was devoted to the oxidation of malonic acid by manganese(III) sulfate and hexaquomanganese(III) ions in noncomplexing perchloric acid medium. Both these reagents can be readily prepared (*4*), they are sufficiently stable even for indirect determinations (*4*), and their reactivity is greater than that of the diphosphate or acetate complex of trivalent manganese (*2*) as trivalent manganese is bound in a

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weaker sulfate complex or exists directly as $[Mn(H_2O)_6]^{3+}$ ions. The increased reactivity of hexaquomanganese(III) ions in noncomplexing medium of perchloric acid results in a rapid reaction even in dilute solutions and enables use of this reagent for microdeterminations of organic substances.

In the study of the oxidation of malonic acid by both the above agents, the stoichiometry of this reaction was first found, followed by the effect of various factors on its rate, and then, on the basis of the obtained results, an optimal procedure for the analytical application was proposed.

EXPERIMENTAL

Reagents

Solutions of manganese(III) sulfate (0.05 and 0.005 *M*) in 6 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate and a 0.005 *M* solution of hexaquomanganese(III) ions in 6 *M* perchloric acid and 0.4 *M* manganese(II) perchlorate were prepared in the way already described (*4*). The titer of 0.1 and 0.01 *N* ferrous sulfate in 0.5 *N* sulfuric acid was found daily using dichromate. Malonic acid solutions of the required normality (equiv = mol/6) were prepared daily by dissolving a precisely weighed amount of the pa substance (Lachema, Brno) in redistilled water and diluting to the mark.

Apparatus

Potentiometric titrations were carried out using a TTT1 titrator and an ABU1 autoburette (Radiometer, Denmark) using a Pt indicator electrode and a saturated calomel reference electrode. The spectrophotometric measurement was carried out using a Spekord UV VIS instrument (Carl Zeiss, Jena) with 1- and 5-cm cuvettes. The temperature was maintained with a U 10 ultrathermostat (Mechanik Prüfgeräte, Medingen) with a precision of ±0.1°C.

Procedure

Oxidation of malonic acid with manganese(III) sulfate: Reaction stoichiometry. Amounts of 20.00 ml of 0.05 or 0.005 *M* manganese(III) sulfate solutions in 6 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate were measured into ground-glass stoppered flasks. Then 5.00 ml of 0.1 or 0.01 *N* solution of malonic acid (equiv = mol/6) was added to the reagent. After time *t* the excess reagent was determined by potentiometric titration with 0.1 or 0.01 *N* ferrous sulfate. Simultaneously a blank was carried out and the consumption of oxidant in equivalents per mole of malonic acid was found by subtracting the blank from the determined value.

Measurement in the absence of oxygen was carried out in an analogous manner, however, the solutions of reagent and malonic acid were prebubbled with nitrogen and the reaction mixture was bubbled with nitrogen

during the reaction. The measurement was carried out at laboratory temperature.

Measurement of the Reaction Half-time

Dependence of the reaction half-time on the concentration of H^+ ions. To 5.00 ml of a 0.02 *M* solution of manganese(III) sulfate in media with various concentrations of H^+ ions and 0.1 *M* manganese(II) sulfate, was added, with constant stirring, 5.00 ml of 0.02 *N* solution of malonic acid in a medium with the same concentration of H^+ and Mn^{2+} ions. The concentration of HSO_4^- ions was maintained in both solutions by additions of sodium sulfate at a constant value of 4.1 *M*. The mixture produced was rapidly transferred to a 1-cm cuvette and the absorbance was measured at 21,500 cm^{-1} in dependence on time. The recording was initiated simultaneously with reactant mixing. The reactant solutions were thermostatted at 20°C and freed of oxygen by bubbling with nitrogen. The reaction half-time was found as the time during which the absorbance of the reaction mixture fell to one-half.

Dependence of the reaction half-time on the concentration of HSO_4^- ions. The procedure was analogous to that of the previous procedure. The concentration of HSO_4^- ions was varied in the interval 0.6 to 4.1 *M* and the concentration of H^+ ions was maintained at a constant value of 4 *M* by addition of perchloric acid. The concentration of manganese(II) sulfate was 0.1 *M*.

Dependence of the reaction half-time on the concentration of Mn^{2+} ions. The measurement was carried out in a similar way as described above. The concentration of Mn^{2+} ions was varied in the range 0.02 to 0.10 *M*, while the concentrations of H^+ and HSO_4^- ions were maintained at constant values 4 and 4.1 *M*, respectively. The decrease in the concentration of sulfate ions caused by the decreasing concentration of manganese(II) sulfate was compensated by increasing additions of zinc sulfate. (It was found that Zn^{2+} ions do not influence the rate of the studied reaction.)

Dependence of the reaction half-time on reactant dilution. The measurement was carried out in the same way as described above. The concentrations of H^+ and HSO_4^- ions were 4 and 4.1 *M*, respectively; the concentration of manganese(II) sulfate was 0.1 *M* and the initial concentration of the reactants was varied over the range 5×10^{-3} to 12.5×10^{-3} *N*. A 5-cm cuvette was used in measurements on more dilute solutions.

Recommended Procedure for Analytical Use of the Reaction

Twenty milliliters of 0.05 *M* solution of manganese(III) sulfate in 2 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate is bubbled for 20 min with nitrogen. Then 5.00 ml of solution containing 4–13 mg of malonic acid is added, which was also prebubbled with nitrogen. The reaction mixture is

left to stand in an inert atmosphere for 20 min at laboratory temperature and then the unreacted excess of oxidizing agent is determined by potentiometric titration with 0.1 *N* ferrous sulfate. A blank is carried out simultaneously and the amount of substance determined is calculated from the difference in the consumption for the blank and for the actual determination. One milliliter of ferrous sulfate corresponds to 1.734 mg of malonic acid.

Oxidation of malonic acid with hexaquomanganese(III) ions in noncomplexing medium of perchloric acid: reaction stoichiometry. An amount of 5.00 ml of 0.01 *N* malonic acid (equiv = mol/6) was added to 20.00 ml of a 0.005 *M* solution of hexaquomanganese(III) ions in 6 *M* perchloric acid. At a given time *t* the unconsumed amount of reagent was found by potentiometric titration with 0.01 *N* ferrous sulfate. A blank was carried out simultaneously and the reagent consumption in equivalents per mole of malonic acid was computed from the difference of the ferrous sulfate consumptions for the blank and the actual determination. The reaction proceeded at laboratory temperature. The same reaction was then carried out in an inert nitrogen atmosphere, using reactants freed of oxygen.

Dependence of the reaction half-time on the concentration of H^+ ions. To 5.00 ml of a 5×10^{-3} *M* solution of hexaquomanganese(III) ions in a medium with a required concentration of H^+ ions and with 0.1 *M* manganese(II) perchlorate was added 5.00 ml of an equinormal solution of malonic acid in a medium containing the same concentrations of H^+ and Mn^{2+} ions, with constant stirring. Spectrophotometric recording was started simultaneously. The reaction mixture was rapidly transferred to a 5-cm cuvette and its absorbance was monitored in dependence on time at 21,500 cm^{-1}. The reactant solutions were thermostatted at 20°C and prebubbled with nitrogen to remove oxygen. The reaction half-time was determined as the time within which the reaction mixture absorbance decreased to one-half. The concentration of ClO_4^- ions was maintained constant at 6.8 *M* by additions of sodium perchlorate.

Dependence of the reaction half-time on the concentration of Mn^{2+} ions. The measurement was performed analogously as above. The concentration of Mn^{2+} ions was varied from 0.08 to 0.4 *M*, the concentration of H^+ ions was 6 *M*, and that of ClO_4^- ions was maintained at 6.8 *M* by additions of sodium perchlorate.

Dependence of the reaction half-time on the reactant dilution. The procedure was analogous as above. The concentration of H^+ ions was 6 *M*, that of ClO_4^- ions 6.8 *M*, and that of Mn^{2+} ions 0.4 *M*. The initial reactant concentration was varied from 5×10^{-3} to 1.0×10^{-3} *N*.

Recommended Procedure for Analytical Use of the Reaction

Twenty milliliters of 0.005 *M* solution of hexaquomanganese(III) ions in 6 *M* perchloric acid and 0.2 *M* manganese(II) perchlorate is bubbled with nitrogen for 20 min. Then 5.00 ml of a solution containing 400–1300 μg malonic acid, prebubbled with nitrogen, is added. The reaction mixture is bubbled with nitrogen during all operations. The solution is set aside for 2 hr at laboratory temperature and the unconsumed oxidant is potentiometrically titrated with 0.01 *N* ferrous sulfate. A blank is carried out in parallel and the amount of the substance to be determined is calculated from the difference of the consumptions for the blank and for the actual determination. One milliliter of 0.01 *N* ferrous sulfate corresponds to 173.4 μg malonic acid.

RESULTS AND DISCUSSION

Reaction Stoichiometry

It follows from the dependence of the oxidant consumption on time (see Table 1) that the oxidation of malonic acid with 0.05 *M* manganese(III) sulfate in the absence of air is quantitative and stoichiometric, six equivalents of the reagent being consumed per mole of malonic acid, and that the products formed are not further oxidized. The reaction can thus be expressed by the equation

$$CH_2(COOH)_2 + 6Mn^{3+} + 2H_2O \rightarrow 6Mn^{2+} + HCOOH + 2CO_2 + 6H^+ \quad (1)$$

The reaction must be performed in an inert nitrogen atmosphere, as its course is nonstoichiometric in the presence of oxygen. This fact can be explained, similar to the oxidation of tartaric (*1*), citric (*5*), and pyruvic (*3*) acids, by a reaction of temporarily formed free radicals with oxygen, leading to a decreased consumption of the oxidant.

TABLE 1

TIME DEPENDENCE OF THE OXIDATION OF MALONIC ACID BY MANGANESE(III) SULFATE IN THE PRESENCE (a) AND ABSENCE (b) OF OXYGEN

Time (min)		5	10	20	30	60	120	240	360
Consumption of	a	5.47	5.88	5.89	5.89	5.90	5.92	5.90	5.91
0.05 *M* reagent	b	5.80	5.99	5.99	6.01	6.00	6.01	6.02	6.00
(equiv/mol)									
Consumption of	a	1.20	1.63	2.58	3.44	4.31	4.88	5.09	5.20
0.005 *M* reagent	b	1.30	1.85	2.77	3.67	4.51	5.03	5.30	5.45
(equiv/mol)									

TABLE 2
DEPENDENCE OF THE HALF-TIME OF THE REACTION OF MALONIC ACID WITH MANGANESE(III) SULFATE ON THE H^+ ION CONCENTRATION[a]

$[H^+]$ (mol/liter)	0.5	1	2	3	4
Half-time (min)	5.3	5.5	6.3	7.0	7.5

[a] $[Mn(III)]_{t=0} = 0.01\ M$, $[HSO_4^-] = 4.1\ M$, $[Mn^{2+}] = 0.1\ M$, temperature 20°C.

It further follows from Table 1 that with 0.05 *M* manganese(III) sulfate the reaction is quantitative after 10 min, whereas with the 0.005 *M* reagent it is not quantitative even after 6 hr, which prevents its use on microscale.

Measurement of the Reaction Half-time

In the study of analytical conditions for use of the reaction, the effect of the concentrations of H^+, HSO_4^-, and Mn^{2+} ions on the reaction rate was followed; reaction half-time was selected as the measure of the reaction rate. It was found that the reaction rate increases with decreasing concentrations of these ions (see Tables 2–4), which is in agreement with the mechanism (*9*) assuming reversible formation of a complex of manganese(III) with the substance to be oxidized and decomposition of this complex in the rate-determining step with formation of a free radical which is immediately further oxidized. The pronounced decrease in the reaction rate with decreasing reactant concentrations (see Table 5) also indicates that this reaction could not be used on microscale.

Analytical Use of the Reaction

In proposing a procedure for an indirect titrimetric determination of malonic acid with manganese(III) sulfate, two opposite effects had to be

TABLE 3
DEPENDENCE OF THE HALF-TIME OF THE REACTION OF MALONIC ACID WITH MANGANESE(III) SULFATE ON THE HSO_4^- ION CONCENTRATION[a]

$[HSO_4^-]$ (mol/liter)	0.6	1.1	2.1	3.1	4.1
Half-time (min)	3.0	3.5	5.0	6.0	7.5

[a] $[Mn(III)]_{t=0} = 0.01\ M$, $[H^+] = 4\ M$, $[Mn^{2+}] = 0.1\ M$, temperature 20°C.

TABLE 4
DEPENDENCE OF THE HALF-TIME OF THE REACTION OF MALONIC ACID WITH MANGANESE(III) SULFATE ON THE Mn^{2+} ION CONCENTRATION[a]

$[Mn^{2+}]$ (mol/liter)	0.02	0.04	0.06	0.08	0.10
Half-time (min)	3.5	4.5	5.8	6.5	7.5

[a] $[Mn(III)]_{t=0} = 0.01\ M$, $[H^+] = 4\ M$, $[HSO_4^-] = 4.1\ M$, temperature 20°C.

TABLE 5
DEPENDENCE OF THE HALF-TIME OF THE REACTION OF MALONIC ACID WITH MANGANESE(III) SULFATE ON THE REACTANT INITIAL CONCENTRATION[a]

$[Mn(III)]_{t=0}$ (mol/liter)	12.5×10^{-3}	10×10^{-3}	7.5×10^{-3}	5×10^{-3}
Half-time (min)	5.0	7.5	12.5	22.0

[a] $[H^+] = 4\ M$, $[HSO_4^-] = 4.1\ M$, $[Mn^{2+}] = 0.1\ M$, temperature 20°C.

considered. With increasing concentrations of H^+, HSO_4^-, and Mn^{2+} ions the reagent stability increases (*4*), but, on the other hand, the reaction rate decreases. Therefore it has been recommended to use 0.05 *M* manganese(III) sulfate in 2 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate, which is both sufficiently stable and sufficiently reactive. The accuracy and reproducibility of the determination carried out according to the procedure described in the experimental section follows from Table 6.

Oxidation of Malonic Acid by Manganese(III) in Noncomplexing Perchloric Acid Medium

Reaction stoichiometry. As it has been found that the oxidation of malonic acid by 0.005 *M* manganese(III) sulfate is not completed even after 6 hr (see Table 1), attention was paid to the oxidation of malonic acid by 0.005 *M* solution of hexaquomanganese(III) ions in a perchloric acid

TABLE 6
ACCURACY AND REPRODUCIBILITY OF THE DETERMINATION OF MALONIC ACID WITH MANGANESE(III) SULFATE

Malonic acid		
Taken (mg)	Found (mg)[a]	Standard deviation (μg)
4.335	4.35	9
8.672	8.68	10
13.008	13.01	18

[a] The values given are the averages of seven determinations, from which the standard deviation was also calculated.

TABLE 7
TIME DEPENDENCE OF THE OXIDATION OF MALONIC ACID BY HEXAQUOMANGANESE(III) IONS IN NONCOMPLEXING PERCHLORIC ACID MEDIUM IN THE PRESENCE (a) AND ABSENCE (b) OF OXYGEN

Time (min)		5	10	20	30	60	120	360	1080
Reagent consumption	a	3.30	3.83	4.24	4.55	4.58	4.59	4.60	4.63
(equiv/mol)	b	4.06	4.73	5.30	5.78	5.88	6.00	6.01	6.07

TABLE 8
DEPENDENCE OF THE HALF-TIME OF THE REACTION OF MALONIC ACID WITH HEXAQUOMANGANESE(III) IONS ON THE H^+ ION CONCENTRATION[a]

$[H^+]$ (mol/liter)	3	4	5	6
Half-time (min)	14	13	12.5	11.5

[a] $[Mn(III)]_{t=0} = 2.5 \times 10^{-3}$ *M*, $[ClO_4^-] = 6.8$ *M*, $[Mn^{2+}] = 0.4$ *M*, temperature 20°C.

medium, where manganese(III) is not complexed and thus is more reactive.

The time dependence of the oxidation of malonic acid by this reagent is given in Table 7, showing that malonic acid is quantitatively oxidized after 2 hr by excess reagent at laboratory temperature. An inert atmosphere must be maintained for the same reasons as in the oxidation with manganese(III) sulfate (see above).

Six equivalents of the oxidant were consumed per mole of malonic acid; hence the reaction stoichiometry can again be expressed by Eq. (1).

Measurement of the Reaction Half-time

The effect of the concentrations of H^+ and Mn^{2+} ions and of the initial reactant concentrations on the reaction rate was studied by monitoring the reaction half-time. It has been found that the reaction rate increases with increasing concentration of H^+ ions, decreasing concentration of Mn^{2+} ions, and with increasing reactant concentrations (see Tables 8–10).

Analytical Use of the Reaction

In proposing a procedure for an indirect titrimetric determination of malonic acid with hexaquomanganese(III) ions the factors affecting the

TABLE 9
DEPENDENCE OF THE HALF-TIME OF THE REACTION OF MALONIC ACID WITH HEXAQUOMANGANESE(III) IONS ON THE Mn^{2+} ION CONCENTRATION[a]

$[Mn^{2+}]$ (mol/liter)	0.08	0.16	0.24	0.32	0.40
Half-time (min)	2.0	2.8	3.5	4.3	5.0

[a] $[Mn(III)]_{t=0} = 5 \times 10^{-3}$ *M*, $[H^+] = 6$ *M*, $[ClO_4^-] = 6.8$ *M*, temperature 20°C.

TABLE 10
DEPENDENCE OF THE HALF-TIME OF THE REACTION OF MALONIC ACID WITH HEXAQUOMANGANESE(III) IONS ON THE REACTANT INITIAL CONCENTRATION[a]

$[Mn(III)]_{t=0}$ (mol/liter)	5×10^{-3}	3.5×10^{-3}	2.5×10^{-3}	1.5×10^{-3}	1.0×10^{-3}
Half-time (min)	5	8	12	22	30

[a] $[H^+] = 6$ *M*, $[ClO_4^-] = 6.8$ *M*, $[Mn^{2+}] = 0.4$ *M*, temperature 20°C.

TABLE 11
ACCURACY AND REPRODUCIBILITY OF THE DETERMINATION OF MALONIC ACID WITH HEXAQUOMANGANESE(III) IONS IN NONCOMPLEX PERCHLORIC ACID MEDIUM

Malonic acid		
Taken (μg)	Found (μg)[a]	Standard deviation (μg)
433.6	436.8	4
862.7	869.8	5
1300.8	1300.3	2

[a] The values given are the averages from seven determinations, from which the standard deviation was also calculated.

rate of the reaction and the reagents stability (*4*) were taken into consideration. The accuracy and reproducibility of the determination carried out according to the procedure given in the experimental section follows from Table 11.

SUMMARY

The oxidation of malonic acid by manganese(III) sulfate in a medium of sulfuric acid and by hexaquomanganese(III) ions in a noncomplexing perchloric acid medium was studied.

The reaction stoichiometry was found and the effect of the concentrations of H^+, Mn^{2+}, and HSO_4^- ions and of the initial reactant concentrations on the course and rate of the reaction was studied.

The optimum conditions have been found for analytical use of the reaction, procedures have been proposed for the determination of malonic acid using the two reagents, and the accuracy and reproducibility of the determinations have been found.

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Thin-Layer Chromatographic Separation of Aldrin, Dieldrin, γ-Hexachlorocyclohexane, Malathion, Ethyl-Parathion, Pentachlorophenol, and of Chlordane, Dieldrin, γ-Hexachlorocyclohexane, Malathion, Ethyl-Parathion, and Pentachlorophenol from Each Other

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INTRODUCTION

Analysis of the organochlorine and organophosphorus pesticides has been performed largely and successfully by gas chromatography employing detector systems such as electron capture (*2, 5, 13*), thermoionic (*9*), flame ionization (*16*), and flame photometric (*3*). However, a number of investigators have also reported on the thin-layer chromatographic separation of the organochlorine pesticides as a group and the organophosphorus types as another separate group on silica gel (*1, 4, 6, 7, 10, 14, 15*), silica gel impregnated with silver nitrate (*11*), alumina (*8*), and basic zinc carbonate (*12*) while still fewer have reported on the thin-layer chromatographic separations of both groups of pesticides from mixtures containing members of each group (*17, 18*). The present paper reports such a procedure from mixtures containing four organochlorine and two organophosphorus pesticides.

MATERIAL AND METHODS

Apparatus

Thin-layer chromatographic equipment. A developing tank (27 × 27 × 7.5 cm) with glass top.

Syringe. Ten-microliter syringe (Hamilton & Company).

Electric dryer.

Spraying bottle.

Ultraviolet light source.

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TABLE 1

Mobile solvent systems	Ratio of solvent components	Maximum number separable	Pesticides separated[a]
n-Heptane		4	Ald,Chlo,BHC,(Para,Mala,PCP,Diel)
n-Hexane:methyl cyclohexane	50:50	4	BHC,Para,(Ald,Chlo,Diel),(Mala,PCP)
n-Hexane:cyclohexane	50:50	4	Para,(Ald,Chlo),(BHC,Diel),(Mala,PCP)
n-Hexane:benzene	50:50	4	BHC,PCP,(Ald,Chlo,Diel),(Para,Mala)
Benzene:cyclohexane	50:50	4	Para,(Mala,PCP),(Ald,Chlo),(BHC, Diel)
n-Hexane:*n*-heptane:cyclohexane	25:25:25	4	Para,(PCP,Mala),(Ald,Chlo),(BHC,Diel)
Benzene:methyl cyclohexane: triethanolamine	25:25:15	4	Para,(Mala,PCP),(Ald,Chlo),(Diel,BHC)
Benzene:xylene:2,2,4-trimethylpentane: methyl cyclohexane	25:25:25:25	4	Para,(Mala,PCP),(Ald,Chlo),(Diel,BHC)
Benzene:toluene:heptane:pentane	25:25:25:25	4	Para,(Mala,PCP),(Ald,Chlo),(Diel,BHC)
Cyclohexane:methyl cyclohexane: benzene:petroleum ether	25:25:25:25	4	Para,(Mala,PCP),(Ald,Chlo),(Diel,BHC)
n-Hexane:xylene:benzene:toluene: cyclohexane:mineral oil:acetic acid	25:25:25:25:25:13:5	4	Para,Mala,PCP,(Chlo,BHC,Ald,Diel)

Cyclohexane:*n*-hexane:toluene: benzene:methyl cyclohexane: heptyl alcohol	25:25:25:25:25:15	4	BHC,(Ald,Chlo),(Para,Diel),(Mala,PCP)
Xylene		5	Chlo,Diel,Para,(Mala,PCP),(Ald,BHC)
Cyclohexane:chloroform	80:20	5	BHC,Diel, Para,(Ald,Chlo),(Mala,PCP)
Cyclohexane:xylene:water	50:10:10	5	BHC,Diel,Para,(Ald,Chlo),(PCP,Mala)
n-Hexane:xylene:benzene:toluene: cyclohexane:methyl cyclohexane: heptane	50:50:50:50:50:50:12.5	5	Para,Mala,PCP,(Ald,Chlo),(Diel,BHC)
n-Hexane:xylene:benzene:toluene: cyclohexane:methyl cyclohexane: triethanolamine	25:25:25:25:25:25:10	5	Para,Mala,PCP,(Ald,Chlo),(Diel,BHC)
n-Hexane:benzene:cyclohexane:methyl cyclohexane:toluene:acetic acid.	25:25:25:25:25:10	5	Para,Mala,PCP,(Ald,Chlo),(Diel,BHC)
n-Hexane:xylene:benzene:toluene: cyclohexane:methyl cyclohexane	50:50:50:50:50:50	6	BHC,Diel,Para,Mala,(Ald,Chlo)

[a] Abbreviations: Ald, aldrin; BHC, γ-hexachlorocyclohexane; Chlo, chlordane; Diel, dieldrin; Mala, malathion; Para, ethyl-parathion; PCP, pentachlorophenol.

Reagents

Samples. The pesticides were supplied by the Environmental Protection Agency of the U.S. Governament and were 99.9% pure.

Thin-layer chromatography plates. 13252 alumina with fluorescent indicator, (No. 6063) 20 × 20-cm plates. (Eastman chromagram sheet for thin-layer chromatograph), 1381 silica gel with fluorescent indicator, (No. 6060) 20 × 20-cm plates. (Eastman chromagram sheet for thin-layer chromatography.)

Chromogenic Reagents

o-Tolidine 1% v/v in acetone.

Silver nitrate reagent. Silver nitrate (1.7 g) treated with 5 ml water and 10 ml 2-phenoxyethanol and diluted to 200 ml with acetone. Ammonium hydroxide (5 ml) may be substituted for the 10 ml 2-phenoxyethanol.

Recommended Procedure

The plates were spotted using a 10-μl syringe containing the relevant samples, which were dissolved in benzene individually and as mixture. The concentration of the samples plated was 20 ppm. The plates were dried, using an electric drier. Then they were placed in a developing tank containing the solvent system, benzene:cyclohexane:hexane:xylene: methyl cyclohexane:toluene (50:50:50:50:50:50). The tank was covered with a glass plate top and 1 hr was allowed for prior equilibration, after which the plates were developed by placing them in the tank for an hour at an optimum angel of 45° between the absorbent layer and the solvent surface. After the solvent had migrated to about 13.5 cm, the plates were removed and the solvent was then allowed to evaporate from the plate at room temperature or by using an electric drier, after which they were uniformly sprayed with 1% *o*-tolidine reagent perpendicular to the direction of the solvent flow. They were then dried with hot air and exposed to short-wave ultraviolet light for 20 min. Aldrin, dieldrin, and γ-hexachlorocyclohexane appeared as light purple and blue colors against a white background. After this, the plates were again sprayed using silver nitrate reagent and exposed to shortwave ultraviolet light for another 15 min. Ethyl-parathion appeared as a yellow spot and malathion as a white spot, while pentachlorophenol and chlordane appeared as purple spots. The background color changed gradually to black. Their R_f values were calculated.

RESULTS AND DISCUSSION

Table 1 records the mobile solvent systems and their respective ratios which separate more than three pesticides from mixtures containing seven on alumina plates. These results show that the maximum number of separations obtainable is six out of seven using the solvent system *n*-hexane:

TABLE 2
$R_f \times 100$ VALUES OF SINGLE COMPONENT PESTICIDE SAMPLES IN SOLVENT SYSTEM CONTAINING BENZENE:CYCLOHEXANE:HEXANE:METHYL CYCLOHEXANE:TOLUENE (50:50:50:50:50:50) USING 13252 ALUMINA WITH FLUORESCENT INDICATOR

Compounds	$R_f \times 10^2$
Aldrin	96
Chlordane	95
Dieldrin	61
γ-Hexachlorocyclohexane	70
Ethyl-parathion	27
Malathion	25
Pentachlorophenol	0

xylene:benzene:toluene:cyclohexane:methyl cyclohexane (50:50:50:50:50:50). Aldrin and chlordane failed to separate in this solvent system and, indeed, except in xylene and *n*-heptane, both compounds always tended to migrate together in all the other solvent systems recorded in this table.

Table 2 records the R_f values for the individual pesticides employing the *n*-hexane–xylene–benzene–cyclohexane–methyl cyclohexane solvent system and alumina plates. These results demonstrate that except for aldrin and chlordane which show similar R_f values, all the other pesticides are well separated from each other with sufficiently different R_f values. Pentachlorophenol did not migrate in this solvent system and remained at the origin.

Table 3 records the results of the simultaneous separation of six pes-

TABLE 3
$R_f \times 100$ VALUES FOR SIMULTANEOUS SEPARATION OF ALDRIN, γ-HEXACHLOROCYCLOHEXANE, DIELDRIN, ETHYL-PARATHION, MALATHION, AND PENTACHLOROPHENOL, USING 13252 ALUMINA WITH FLUORESCENT INDICATOR AND SOLVENT SYSTEM CONTAINING BENZENE: CYCLOHEXANE:HEXANE:XYLENE: METHYL CYCLOHEXANE:TOLUENE (50:50:50:50:50:50)

Compounds	$R_f \times 10^2$
Aldrin	96
γ-Hexachlorocyclohexane	70
Dieldrin	58
Ethyl-parathion	38
Malathion	24
Pentachlorophenol	0

TABLE 4
$R_f \times 100$ VALUES FOR SIMULTANEOUS SEPARATION OF CHLORDANE, γ-HEXACHLOROCYCLOHEXANE, DIELDRIN, ETHYL-PARATHION, MALATHION, AND PENTACHLOROPHENOL, USING 13252 ALUMINA WITH FLUORESCENT INDICATOR AND SOLVENT SYSTEM CONTAINING BENZENE: CYCLOHEXANE:HEXANE:XYLENE: METHYL CYCLOHEXANE:TOLUENE (50:50:50:50:50:50)

Compounds	$R_f \times 10^2$
Chlordane	97
γ-Hexachlorocyclohexane	70
Dieldrin	58
Ethyl-parathion	38
Malathion	24
Pentachlorophenol	0

ticides. In the absence of chlordane, aldrin is effectively separated along with dieldrin, γ-hexachlorocyclohexane, malathion, ethyl-parathion, and pentachlorophenol, while Table 4 records a similar separation for chlordane along with the previously mentioned components when aldrin is absent.

These investigation were extended to include further studies on the chromatographic behavior of the pesticides on neutral silica gel plates. The maximum number of individual pesticides separated on this solid phase from mixtures containing all seven was five using three solvent systems composed as follows: *n*-hexane:cyclohexane (50:50), benzene:xylene:2,2,4-trimethyl pentane:methyl cyclohexane (25:25:25:25), and benzene;toluene:*n*-heptane:*n*-pentane (25:25:25:25). Each of these solvent systems separated the same five components, γ-hexachlorocyclohexane, dieldrin, ethylparathion, aldrin (or chlordane), and malathion (or pentachlorophenol), from a mixture containing all seven on neutral silica gel. In all cases pentachlorophenol migrated from the origin, unlike its behavior on alumina plates, but with considerable tailing and resulting interference with the separation of malathion. Once again aldrin and chlordane migrated together on the silica gel plates as they did on alumina plates. Another interesting solvent system which separated five pesticides from mixtures containing seven on silica gel plates was *n*-hexane:2,2,4 trimethylpentane:diemthylformamide (25:25:15) which separated γ-hexachlorocyclohexane, dieldrin, pentachlorophenol, aldrin (or chlordane), and ethyl-parathion (or malathion). In this solvent system pentachlorophenol stayed at the origin and did not move, so that no tailing effect arose from this compound. However, once again aldrin and chlordane migrated together, as did the two organophos-

phorus pesticides malathion and ethyl-parathion which were not separated from each other.

SUMMARY

A thin-layer chromatographic method is reported for the separation of aldrin (or chlordane), dieldrin, γ-hexachlorocyclohexane, malathion, ethyl-parathion, and pentachlorophenol from each other. The procedure, however, does not separate aldrin and chlordane from each other although it does separate either pesticide from the other components. The method employs alumina plates with *n*-hexane:xylene:benzene:toluene:cyclohexane:methyl cyclohexane (50:50:50:50:50:50:) as developing solvent.

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MICROCHEMICAL JOURNAL 24, 341–349 (1979)

Use of the Vanadium(V)–Xylenol Orange Mixture as an Improved Reagent for the Spectrophotometric Determination of Traces of Hydrogen Peroxide

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INTRODUCTION

The mixed ligand complex formation in ternary systems such as $M^{m+}-Y-H_2O_2$ (M^{m+} and Y denote metal ion and organic reagent, respectively) has been employed to advantage in the spectrophotometric determination of traces of metal ions (*3, 7, 8, 11, 15, 17*). The utility of such the complexes in the determination of trace hydrogen peroxide was first suggested by Nordschow, who adopted the mixture of Ti(IV) and xylenol orange (denoted as XO) as a colorimetric reagent and determined the trace amount of hydrogen peroxide produced through the enzymic oxidation of glucose and urate in human fluids (*9, 10*). Detailed investigation of the Ti(IV)–XO–H_2O_2 system has further been done for this purpose (*4, 6, 12, 16*).

In our previous papers, we noticed the mixtures of Ti(IV)–XO and Ti(IV)–chromazurol S (denoted as CAS) as especially useful reagents for the colorimetric determination of hydrogen peroxide (*5, 6*). However, as previously pointed out, a difficulty arose in the use of these reagents because of interference of some foreign substances such as phosphate, tartrate, and cysteine. Such substances hinder the coloration due to the Ti(IV)–XO (or CAS)–H_2O_2 complex formation by their competitive coordination with Ti(IV).

In the course of our studies of the ternary systems involving hydrogen peroxide, it seemed that an attempt to avoid the interference of these substances can be successfully made in the case of the V(V)–XO–H_2O_2 system. Comparing this system to other systems, a difference in the mode of reaction was found for this system, that is, addition of hydrogen peroxide to the solution containing V(V) and XO brought about a significant decrease in the adsorption peak due to the V(V)–XO binary complex. The decrease in the absorbance (denoted as ΔA) was proportional to the concentration of added hydrogen peroxide, and the value of ΔA was little affected by the presence of several foreign substances.

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It is felt from these facts that the significant change in absorbance will serve as a very sensitive test for trace hydrogen peroxide without serious interference of foreign substances. This expectation prompted the present study concerning the absorption behavior of the V(V)–XO–H_2O_2 system and the applicability of the V–XO reagent to the spectrophotometric determination of trace hydrogen peroxide.

EXPERIMENTAL

Reagents

All the chemicals were reagent grade and were used without further purification.

Stock vanadium(V) solution (1.00 mM). Vanadium(V) pentoxide (0.0910 g) was dissolved in 4 ml of 0.5 *M* sodium hydroxide and then diluted with water to 1000 ml. The final solution was used as a stock solution of vanadium(V).

Stock XO solution (1.00 mM). Stock solution of XO was prepared by dissolving sodium salt of XO (0.7166 g) in 1000 ml of water.

Stock hydrogen peroxide solution (100 mM). A 30% hydrogen peroxide solution (2.6 ml) was diluted with water to 500 ml; the solution was standardized by titration with potassium permanganate.

V–XO reagent. The V–XO reagent was prepared by mixing 10 ml of each stock solution of vanadium(V) and XO and diluting with water to 100 ml.

Apparatus

Visible absorption spectra were recorded on a Hitachi double-beam spectrophotometer, Model 200-10, with a 10-mm quartz cell. The pH of the test solution was obtained by measurement with a Toshiba–Beckman pH meter, Model SS-2.

Procedure

To make a test solution, a known amount of hydrogen peroxide was diluted with water to 25 ml together with 10 ml of the V–XO reagent. The pH of the test solution was adjusted to 4.0 using acetate buffer. Then hydrogen peroxide was determined by subtracting the absorbance of the test solution from that of the V–XO reagent. Both the absorbances were obtained at 582 nm with reference to water.

RESULTS AND DISCUSSION

Absorption Spectra

Spectrophotometric studies of the complexes formation in the V(V)–XO–H_2O_2 system were done for the purpose of deciding the optimum conditions for the determination of hydrogen peroxide by using the V–XO reagent.

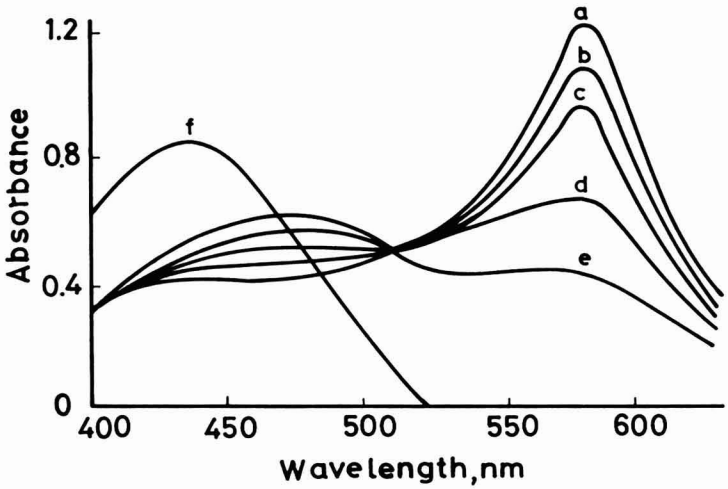

FIG. 1. Absorption spectra of V(V)−XO−H_2O_2 (a−e) systems and XO (f) at pH 4.0. [V(V)] and [XO], 4.0×10^{-5} *M*; [H_2O_2]: a, 0; b, 0.5×10^{-5} *M*; c, 1.0×10^{-5} *M*; d, 2.0×10^{-5} *M*; e, $3.0 \times 10^{+5}$ *M*.

In Fig. 1, the absorption spectrum of the V−XO reagent is shown as curve a. A sharp peak having a maximum at 582 nm is given over the range of pH 3 to 5, suggesting the formation of only one complex species between vanadium(V) and XO. At pH values above 5, the absorbance at 582 nm decreased with increasing pH and a broad peak appeared anew at around 500 nm at the same time. The feature of the former peak was similar to that found by Aleksandrov and Budevski, who determined the molar absorptivity of V(V)−XO complex to be 2×10^4 at 590 nm (*2*). Agarwala and Dey assigned the peak at 500 nm to the 1:1 complex of V(V) and XO, and the formation constant of which was determined to be $10^{4.5}$ (*1*). However, the broad shape of the latter peak observed in this experiment seems to imply the presence of some V(V)−XO complexes of different compositions in the V−XO reagent, so that it is preferable to use the V−XO reagent at lower pHs than 5.

On the addition of hydrogen peroxide to the V−XO reagent, the absorption peak at 582 nm was lowered significantly, and the magnitude of the decrease in absorbance (denoted as ΔA) was proportional to the concentration of hydrogen peroxide (Fig. 1, curves b−e). The value of ΔA per 1 *M* hydrogen peroxide was found to be 25,000. This value is relatively large compared with the molar absorptivity of the usual ternary complexes formed in the $M^{m+}-Y-H_2O_2$ systems, for example, about twice the molar absorptivity of the Ti(IV)−XO−H_2O_2 complex (*6*). This fact suggests the applicability of the V−XO reagent to the determination of traces of hydrogen peroxide with high sensitivity.

No clear account of the behavior observed in the V(V)−XO−H_2O_2 system as seen in Fig. 1 can be offered at the present time. Shtokalo and Kulik (*14*) found a similar behavior in the Zr(IV)−XO−H_2O_2 system and the results were explained on the basis of the ligand substitution in Zr(IV)−XO complexes by hydrogen peroxide. Such an explanation is not valid in the present case, i.e., neither the absorbance increase at 436 nm (Fig. 1) nor the spots on a thin-layer chromatogram corresponding to the liberation of XO from V(V)−XO complex was observed. On the other hand, the presence of the ternary complex (1:1:1 type) was previously detected in the Ti(IV)−XO−H_2O_2 system by the present authors (*5*, *6*). However, such a complex cannot be expected to exist under the present conditions from the fact that the absorbance at 582 nm in the V(V)−XO−H_2O_2 system reverts to its original value (i.e., absorbance at $[H_2O_2] = 0$) by a further addition of XO.

Then it becomes feasible to think that oxidation of XO bonded to vanadium(V) as a ligand takes place by the adjacent hydrogen peroxide, probably with the catalytic action of vanadium(V), as a result of which the absorption peak at 582 nm disappeared completely at when $[XO] \leqq [H_2O_2]$. The broad band around 460 nm may be attributed to some oxidation products of XO.

Since the results given in Fig. 1 indicate that the use of the V−XO reagent seems to be hopeful for the colorimetric determination of hydro-

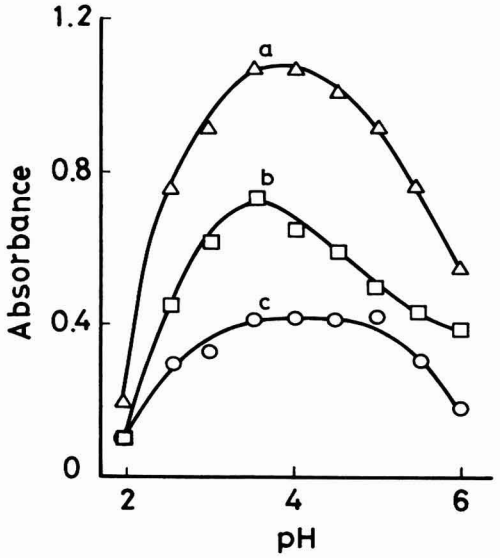

FIG. 2. Effect of pH on absorbance at 582 nm for V(V)−XO (a) and V(V)−XO−H_2O_2 (b) systems. Curve c was obtained by subtracting curve b from curve a. [V(V)] and [XO]; 3.8×10^{-5} *M*; $[H_2O_2]$; 1.6×10^{-5} *M*.

gen peroxide, subsequent experiments were performed to decide the appropriate conditions for the practical use of the reagent.

Effect of pH

Figure 2 shows the pH dependence of absorbance at 582 nm for the V(V)−XO and V(V)−XO−H_2O_2 systems (curves a and b, respectively), and that of ΔA (curve c). The last one was obtained by subtracting curve b from curve a. The constant value of ΔA was obtained in the pH range 3.5 to 4.5. Then pH value of 4.0 are employed as an optimum condition for the use of the V−XO reagent.

Effect of Composition of the Reagent

Since the value of ΔA was affected by the concentration ratio of XO to vanadium(V), the effect of XO concentration in the reagent on ΔA was examined keeping the concentrations of both vanadium(V) and hydrogen peroxide constant. As seen in Fig. 3, the value of ΔA remains constant irrespective of the XO concentration when $0.5 < [XO]/[V(V)] < 1.2$. Then the [XO]/[V(V)] ratio is fixed to be unity in the V(V)−XO reagent.

Time Course of the Absorbance

Time course of the absorbance was followed for the V(V)−XO and the V(V)−XO−H_2O_2 systems at different temperatures. In the latter system, the constant value of absorbance was obtained within 3 min at 35°C, but longer time was needed at lower temperatures (Fig. 4). This value remained virtually unchanged on standing the solution at room temperature

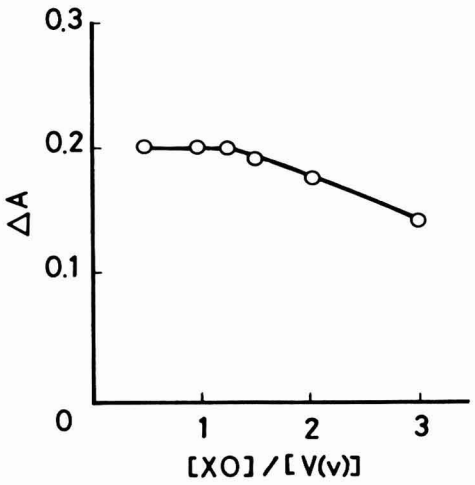

FIG. 3. Effect of concentration of XO and ΔA at pH 4. [V(V)]; 4.0×10^{-5} *M*; [H_2O_2]; 8.0×10^{-6} *M*.

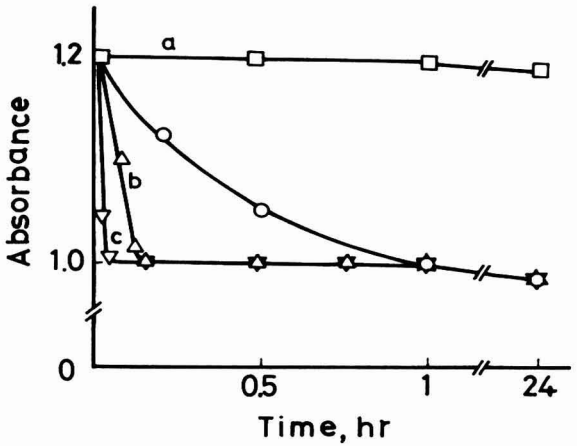

FIG. 4. Time dependence on absorbance of V(V)−XO (a) and V(V)−XO−H_2O_2 systems at pH 4. [V(V)] and [XO]; 4.0×10^{-5} *M*; [H_2O_2]; 8.0×10^{-6} *M*; temperature: b, 15°C; a and c, 25°C; d, 35°C.

for 24 hr. The reagent can be kept for 3 months in a refrigerator without any detectable change in absorbance.

Determination of Hydrogen Peroxide

Based on the above experimental results, the conditions for the colorimetric determination of hydrogen peroxide with the V−XO reagent were decided as listed in Table 1.

Plot of ΔA against the hydrogen peroxide concentration gives a straight line as shown in Fig. 5, from which the reagent is found to be applicable to the determination of hydrogen peroxide in the concentration range of $1 \times 10^{-6} \sim 8 \times 10^{-5}$ *M*. The data are reliable with a coefficient of variation below 2.1%.

Effects of Foreign Substances

Since all the results stated above suggest utility of the V−XO reagent in the determination of trace amounts of hydrogen peroxide, for example, in foods or biological samples, the effects of some foreign substances were further examined. The results are summarized in Tables 2 and 3.

TABLE 1
OPTIMUM CONDITIONS FOR THE DETERMINATION OF HYDROGEN PEROXIDE USING THE V−XO REAGENT

[V(V)] (m*M*)	[XO]/[V(V)]	pH	λ (nm)	Concn range (*M*)
0.04 ~ 0.08	0.5 ~ 1.2	3.5 ~ 4.5	582	$1 \times 10^{-6} \sim 8 \times 10^{-5}$

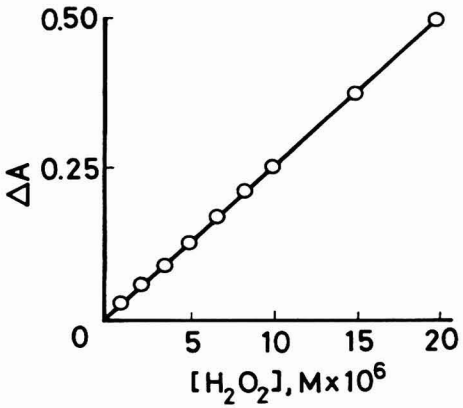

FIG. 5. Relation between ΔA and concentration of hydrogen peroxide at pH 4. [V(V)] and [XO]; 4.0×10^{-5} *M*.

As seen in Table 2, some inorganic ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, F^-, Cl^-, I^-, ClO_4^- and NO_3^- scarcely affect the absorption measurements even when present in large excess compared with hydrogen peroxide. On the other hand, a fairly large error appears in the presence of SO_4^{2-} and PO_4^{3-}. Cu^{2+}, Al^{3+}, and Fe^{3+} give rise to considerable errors, even at low concentration, due to the complex formation between these metal ions and XO. However, as mentioned above, the V–XO reagent provides appreciably large value of ΔA for hydrogen peroxide, so that the interference of these ions will be easily avoided by diluting the samples in practical analysis for human fluids.

As can be seen from Table 3, some organic compounds show only a

TABLE 2
EFFECT OF INORGANIC IONS ON THE DETERMINATION OF HYDROGEN PEROXIDE[a]

Inorganic ion	H_2O_2 found (%)	Inorganic ion	H_2O_2 found (%)
Na^+	100	F^-	100
K^+	100	Cl^-	100
Mg^{2+}	100	I^-	100
Ca^{2+}	100	ClO_4^-	100
Mn^{2+}	100	NO_3^-	100
Zn^{2+}	100	SO_4^{2-}	50
Cu^{2+}[b]	120	PO_4^{3-}	80
Al^{3+}[b]	113		
Fe^{3+}[b]	111		

[a] $[H_2O_2]$ added: $1.0 \times 10^{-5}M$; [inorganic ion] added: $1.0 \times 10^{-3}M$.
[b] $1.0 \times 10^{-6}M$.

TABLE 3
EFFECT OF ORGANIC COMPOUNDS ON THE DETERMINATION OF HYDROGEN PEROXIDE[a]

Organic compound	H_2O_2 found (%)
Acetic acid	100
Ascorbic acid	100
Citric acid	95
Glycolic acid	103
Glyoxylic acid	102
Lactic acid	101
Pyruvic acid	100
Tartaric acid	100
Amino acids[b]	100
Urea	100

[a] $[H_2O_2]$ added: $1.0 \times 10^{-5} M$; [organic compound] added, $1.0 \times 10^{-4} M$.
[b] Arg, His, Cys, Met, Cit, Asn, Asp, Gly, Ala, Thr, Ser, Orn, Trp, and Tyr.

minor effect on the determination of hydrogen peroxide. Among the compounds listed in Table 3, citrate and amino acids are known as important substances involved in several metabolic sequences. The fact that such compounds have practically no effect is worth noting, because these compounds induce a pronounced interference in the use of the Ti−XO reagent as stated in an earlier paper (*6*). The use of the V−XO reagent is recommended for the improved analysis of hydrogen peroxide in biological fluids.

Comparing the value of ΔA per 1 M hydrogen peroxide with the molar absorptivity in the determination of hydrogen peroxide in the usual way, the sensitivity in the present method is over three times higher than the peroxidase-4-aminoantipyrine phenol method, but a little lower than the peroxidase-3-methyl-2-benzothiazolinone hydrazone-dimethyl aniline method (*13*). In the usual cases using peroxidase, some reducible substances, such as ascorbic acid, are appreciably inhibitory to the color development of chromogens. On the other hand, no enzyme is needed in the present method, and actually ascorbic acid exerts no effect on the result in Table 3.

The usefulness of the V−XO reagent for the spectrophotometric determination of traces of hydrogen peroxide was thus proved. Further advantage of the present method is its availability for practical uses at a low cost because of no need of enzyme. Application of the method in biological and clinical fields seems to be hopeful.

SUMMARY

The reaction in V(V)−xylenol orange (XO)−H_2O_2 system was studied by spectrometry. On the addition of hydrogen peroxide to the mixture of vanadium(V) and XO (V−XO reagent), the absorption peak of V(V)−XO complex (λ_{max} = 582 nm) decreased significantly. The decrease in the absorbance (denoted as ΔA) was proportional to the concentration of

hydrogen peroxide. The constant values of ΔA were obtained under the condition of [XO]/[V(V)] = 0.5 ~ 1.2 and in the pH 3.5 ~ 4.5 region. Based on these results, the conditions for the use of the V−XO reagent in the colorimetric determination of hydrogen peroxide were examined in detail. The V−XO reagent was found to be useful for the trace analysis of hydrogen peroxide with high sensitivity, and the data were little affected by the presence of some inorganic and organic substances. The lower limit of the determination is about 1×10^{-6} *M*.

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Microdetermination of Copper with 4-*S*-Benzyl-1-*p*-chloro-phenyl-5-phenyl-2,4-isodithiobiuret (BPPTB)

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INTRODUCTION

Various chelating agents are reported for the determination of copper and have been reviewed in a recent monograph (*3*). In addition to these, recently (*6*) a few new oximes also have been reported for the solvent extraction and determination of copper.

Deshmukh and Kharat (*5*, *7*, *8*) have successfully established 4-*S*-benzyl-l-*p*-chloro-phenyl-5-phenyl-2,4-isodithiobiuret (BPPTB) as an analytical reagent for the determination of platinum metals. The extension of these studies reveals that it is possible to extract copper quantitatively with BPPTB in chloroform from an aqueous solution having pH 4.2. The extracted copper can directly be determined in organic phase by spectrophotometry at 368 nm. The method based on these observations is described in this paper. The developed method is simple, rapid, sensitive, and selective. It is possible to accomplish clear-cut separation of copper from several elements such as cobalt, nickel, zinc, manganese, and chromium which are generally associated with it in alloys and minerals.

EXPERIMENTAL METHODS

Copper perchlorate solution was prepared by dissolving sulfate-free copper hydroxide obtained from 2.5 g of copper sulfate (A.R., B.D.H.) in perchloric acid and was standardized gravimetrically with 2′-hydroxy-4-methoxy-5′-methylchalkone oxime (*4*). The reagent, BPPTB, was synthesized according to the reported method (*5*) and was purified by repeated crystallization from ethanol to get the melting point 127°C. Fresh solution in alcohol-free chloroform (*13*) was used. Perchloric acid stock solutions of interfering ions containing 0.01 to 0.1 mg of test ion per ml were used. The solutions of lower concentration were prepared by volumetric dilution of the stock solution. Spectrophotometric measurements were made with Beckman model DU-2 spectrophotometer with matched quartz cell with 10-mm light path. Absorption measurements were noted against a processed reagent solution in chloroform. The pH

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measurements were made with an ELICO pH meter Model LI-10 with a glass and calomel electrode assembly. The pH of the aqueous solution was adjusted with the help of perchloric acid and sodium hydroxide solutions.

RESULTS AND DISCUSSION

Absorption Spectra of BPPTB

The absorption spectrum of solution of BPPTB in chloroform against the pure chloroform blank shows strong absorbance at 305 nm. The absorptivity of the reagent at 305 nm is 1.96×10^4. BPPTB exhibits one peak of absorption at 258 nm when scanned in the aqueous solution at pH 12.0. The reference solution used is aqueous solution of pH 12.0. The value of molar extinction coefficient of the BPPTB at pH 12.0 is 2.80×10^4 at 258 nm. It follows Beer's law in the concentration range of 1.3×10^{-5} to 7.6×10^{-5} *M* in chloroform at 305 nm and 1.0×10^{-5} to 6.0×10^{-5} *M* in aqueous solution of pH 12.0 at 258 nm. It was also observed that 1.0 *M* sodium perchlorate in aqueous medium has no effect in the determination of BPPTB.

Distribution of BPPTB

A 10-ml solution of BPPTB of known concentration $[\text{BPPTB}]_{\text{tot}}$ in the chloroform was mixed with equal volume of aqueous layers of different pH and ionic strength of 0.4 *M*. After equilibration the concentration of the BPPTB in chloroform layer, $[\text{BPPTB}]_{\text{org}}$, was determined from the absorbance of the solution at 305 nm using similarly equilibrated chloroform solvent blank as a reference. The distribution ratio of the BPPTB is then:

$$D_{\text{R}} = [\text{BPPTB}]_{\text{org}}/[\text{BPPTB}]_{\text{tot}} - [\text{BPPTB}]_{\text{org}}. \tag{1}$$

The behavior of the reagent distribution as a function of the aqueous solution is similar to the distribution isotherm reported earlier (*5*).

Proton–Ligand Association Constants of BPPTB

If K_1^{H} and K_2^{H} are the respective proton–ligand association and protonation constants of the reagent BPPTB and P_{R} is the partition constant, then the distribution ratio D_{R} may be related as (*9*):

$$1/D_{\text{R}} = 1/P_{\text{R}}\left(1 + \frac{1}{K_1^{\text{H}} \cdot [\text{H}^+]} + \frac{1}{K_1^{\text{H}} \cdot K_2^{\text{H}} \cdot [\text{H}^+]^2}\right). \tag{2}$$

The BPPTB distribution data analysis yields the values of $\log K_1^{\text{H}}$, $\log K_2^{\text{H}}$, and $\log P_{\text{R}}$ as 10.18, 0.60, and 2.02, respectively, at ionic strength of 0.4 *M*. The value of $\log K_1^{\text{H}}$ obtained from potentiometric titration in 50% ethanol and from similar distribution data analysis in isoamyl–alcohol–

water system at 0.4 M ionic strength comes out to be 10.68 and 10.14, respectively, which are in good agreement with each other.

Absorption Spectra of Extractable Complex

The absorption spectrum of copper(II)–BPPTB complex in chloroform (Cu = 1.0×10^{-5} M) is shown in Fig. 1. The spectra was scanned against the similarly processed reagent as a blank. The colored complex shows a strong and sharp absorbance peak at 368 nm and hence all the absorbance measurements were carried out at this wavelength. The molecular extinction coefficient of the complex at 368 nm is 4.5×10^4, calculated on the basis of copper contents (1.0×10^{-5} M) with the absorbance of 0.450 ± 0.005 when 10-mm cells were used.

Extraction as a Function of pH

The solvent extraction behavior of Cu–BPPTB system was studied over the pH region of 1.0 to 12.0 (Fig. 2). It was observed that there is no extraction in the pH region of 1.0 to 2.5. The extraction commenced at pH 2.5 and it was quantitative at pH 4.0 to 6.6. Beyond this pH region the extraction has decreased. Therefore optimum pH for quantitative extraction is 4.0 to 6.6.

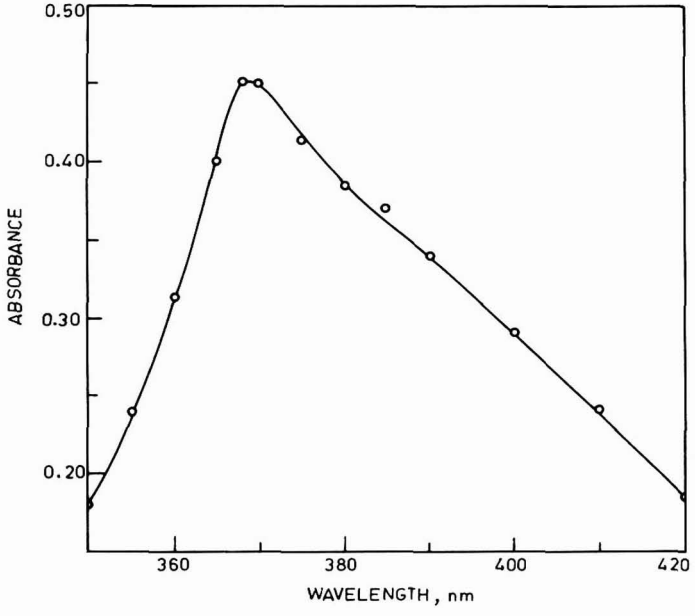

FIG. 1. Absorption spectrum of Cu(II)–BPPTB complex in chloroform.

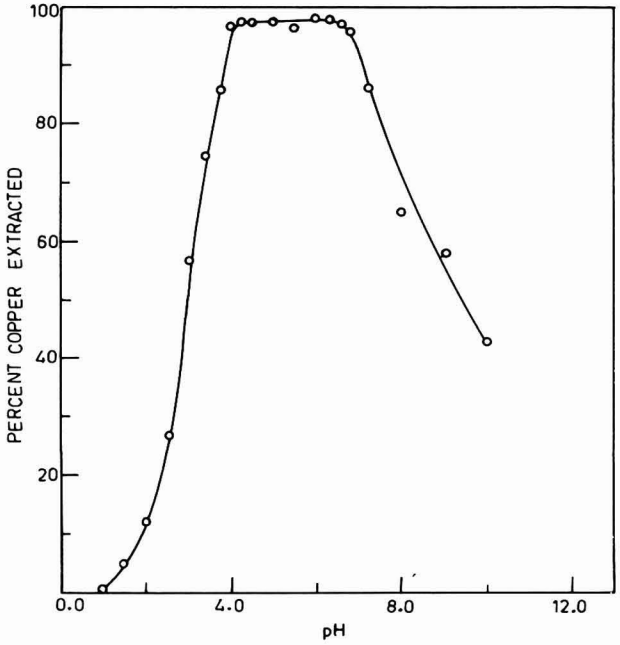

FIG. 2. Extraction of Cu(II) as a function of pH of aqueous phase.

Effect of Reagent Concentration

The concentration of the reagent was varied from 1.0×10^{-4} to 2×10^{-3} *M* in 10 ml of chloroform. It was observed that the extraction was quantitative with the reagent concentration of 1×10^{-3} *M*. It was incomplete with low concentration of the reagent. The extractions did not improve substantially at the higher reagent concentration.

Adherence to Beer's Law

The varying amounts of copper ranging from 0.1 to 3.0 μg/ml were taken and were extracted at pH 4.2 with 0.001 *M* BPPTB in chloroform. To observe the adherence of the system to Beer's law, their absorbance was measured at 368 nm. The light yellow copper–BPPTB system conformed to Beer's law at 368 nm over the concentration range of 0.2 to 2.8 μg of copper per ml. Optimum range (0.2–0.8 μg of copper per ml) suitable for analytical work was determined by Ringbom (*10*) plot (Fig. 3).

Effect of Metal Ion Concentration

By varying metal ion concentration in the range of 1.0×10^{-5} to 5.0×10^{-5} *M* in aqueous phase at pH 4.2 and extracting with 0.001 *M* BPPTB in chloroform, it was found that there is no appreciable effect on the distribution ratio of the complex. This reveals that the extracted species is monomeric in nature.

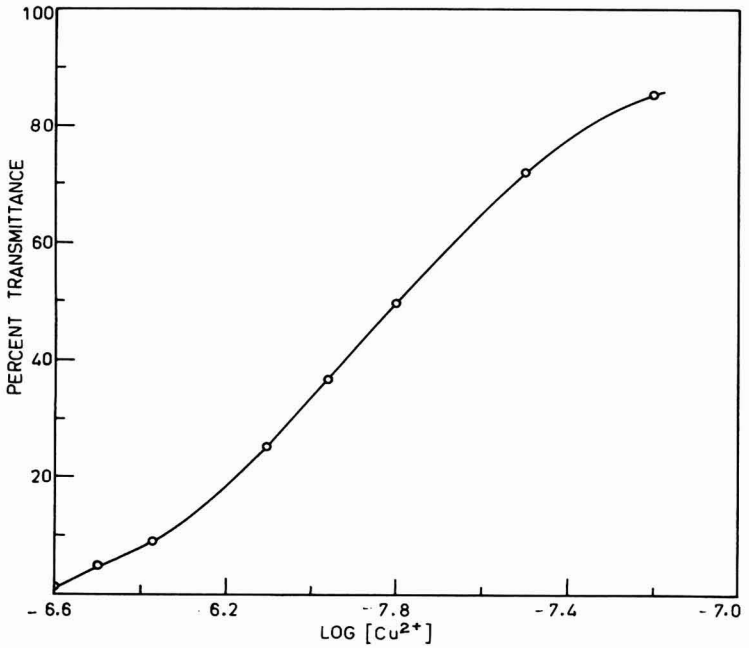

FIG. 3. Ringbom plot for Cu(II)–BPPTB system.

Effect of Ionic Strength

The distribution of copper was studied at various concentrations of sodium perchlorate (0.2 to 4.0 *M*) at pH 4.2, and it was observed that the extent of extraction of copper remains constant in the studied range.

Stability of the Color of the Complex

The absorbance of the colored complex was measured at elapsed intervals of 0.5, 5, 16, 24, 48, 96, and 120 hr. The value of the absorbance was found to be constant, viz, 0.450 until 48 hr. This shows that the complex was stable for at least 48 hr.

Period of Phase Equilibration

Varying the shaking period from 0.5 to 15.0 min when all other factors are constant, it was concluded that the extraction was quantitative in 1.5 min of equilibration. Hence in all measurements, extraction was carried out for at least 2 min.

Effect of Solvent

Among the several solvents tried, such as benzene, carbon tetrachloride, carbon disulfide, isobutanol, isoamyl alcohol, and chloroform, it was found that chloroform is the most efficient solvent for extraction of copper.

Effect of Foreign Ions

The effect of various diverse ions on the extraction behavior of copper was studied. The tolerance limits are reported in Table 1. The tolerance limit was set at the amount of ion that could cause ±2% error in the recovery of copper. The results show that chromium(III), manganese(II), cobalt(II), nickel(II), and zinc(II) did not interfere in the determination of copper at the experimental conditions. Fe(II) and Fe(III) showed some interference. Large numbers of anions did not interfere in the estimation of copper.

Precision

The absorbance of solution from 10 determinations of 1×10^{-5} *M* copper was 0.450 ± 0.005. The relative standard deviation was ±1.1%. Sandell's sensitivity was 0.0014 μg of Cu per cm^2. The method is simple, rapid, and sensitive. It is applicable at tracer concentration. The overall time of operative procedure is just 30 min.

Nature of the Extractable Species

The slope ratio method (*11*) as applied by earlier workers (*2*) has been applied for the determination of the stoichiometry of the extractable species. The method indicates 1:2 (Cu:BPPTB) composition of the extractable complex. This composition was supported by the plot of $\log D_{Cu}$ (distribution ratio of copper) versus $\log [BPPTB]_{org}$ (concentration of BPPTB in organic phase).

Equilibrium Constants

The formation of 1:2 (Cu:BPPTB) extractable chelate permits the representation of the following equilibria. The formation of neutral complex in aqueous phase and its extraction in organic phase:

$$Cu^{2+} + 2R^- \overset{\beta_{abs}}{\rightleftharpoons} CuR_2 \tag{3}$$

$$CuR_2 \overset{P_{Cu}}{\rightleftharpoons} CuR_{2_{org}} \tag{4}$$

Two phase equilibrium can be presented as

$$Cu^{2+} + 2R^- \overset{\beta_{ext}}{\rightleftharpoons} CuR_{2_{org}} \tag{5}$$

and the respective equilibrium constants can be written as:

$$\beta_{abs} = [CuR_2]/[Cu^{2+}] \cdot [R^-]^2 \tag{6}$$

TABLE 1
EFFECT OF DIVERSE IONS[a]

Foreign ion	Added as[b]	Tolerance limit (μg)
F^-	NaF	10,000
Cl^-	NaCl	19,000
SO_3^{2-}	Na_2SO_3	16,000
SO_4^{2-}	Na_2SO_4	48,000
$S_2O_3^{2-}$	$Na_2S_2O_3$	None
$S_2O_8^{2-}$	$Na_2S_2O_8$	16,000
$Mo_7O_{24}^{6-}$	$(NH_4)_6Mo_7O_{24}$	5,000
WO_4^{2-}	Na_2WO_4	2,500
PO_4^{3-}	KH_2PO_4	1,000
BO_3^{3-}	H_3BO_3	1,000
$COOH^-$	HCOOH	10,000
$C_2O_4^{2-}$	$H_2C_2O_4$	9,000
CH_3COO^-	CH_3COOH	10,000
$Citr^{3-}$	Citric acid	19,000
$Tart^{3-}$	Tartaric acid	15,000
$EDTA^{4-}$	EDTA (disodium salt)	3,000
NTA^{3-}	Nitrilotriacetic acid	2,000
Mal^-	Malonic acid	9,700
SSA^-	Sulfosalicylic acid	500
TGA^-	Thiogallic acid	5,000
TMA^-	Thiomalic acid	4,000
Tu	Thiourea	2,500
Py	Pyridine	5,500
Ur	Urea	2,000
O-phen	*O*-phenonthroline	100
Ag^+	$AgNO_3$	1,000
Au^{3+}	$HAuCl_4$	1,200
Cd^{2+}	$CdSO_4$	500
Co^{2+}	$CoSO_4$	30,000
Cr^{3+}	$CrCl_3$	5,200
Fe^{2+}	$FeSO_4$	50
Fe^{3+}	$Fe(NO_3)_3$	50
Hg^{2+}	$HgCl_2$	1,000
Mn^{2+}	$MnCL_2$	11,000
Ni^{2+}	$NiSO_4$	6,000
Os^{8+}	OsO_4	200
Pd^{2+}	$Pd(ClO_4)_2$	700
Pt^{4+}	H_2PtCl_6	400
Rh^{3+}	$RhCl_3$	100
Ru^{3+}	$RuCl_3$	100
Zn^{2+}	$ZnSO_4$	6,500

[a] [Cu] = 1×10^{-5} *M*, [BPPTB] = 1×10^{-3} *M*, λ_{max} = 368 nm, pH = 4.2.
[b] Water of hydration omitted due to conciseness.

$$\beta_{ext} = [CuR_2]_{org}/[Cu^{2+}]\,[R^-]^2 \quad (7)$$

$$P_{Cu} = [CuR_2]_{org}/[CuR_2] \quad (8)$$

and

$$\beta_{abs} = \beta_{ext} \cdot P_{Cu} \quad (9)$$

The conditional extractive stability constants may be written as:

$$\beta_2 = [CuR_2]_{org}/[Cu^{2+}] \cdot [HR]^2_{org} \quad (10)$$

Consideration of Eqs. (7 and 10) and values of K_1^H and P_R yields Eq. (11).

$$\beta_{ext} = \beta_2 \cdot P_R{}^2 \cdot K_1^{H2} \cdot [H^+]^2 \quad (11)$$

Next by considering Eq. (10) and presuming the presence of only limited species in aqueous phase one can get

$$\beta_2 = D_{Cu} \cdot [HR]^2_{org} \quad (12)$$

The values of β_2 and β_{ext} were determined by Yatsimirskii's and Leden's methods of graphical extrapolation (*11*) with suitable modification (*7*). The obtained values were checked by ligand number method (*12*) and with the help of Eq. (12). The calculations by Eq. (12) and by ligand number method are reported in Tables 2 and 3, respectively. The plot of $pR(-\log[R^-])$ (Table 3) versus $\bar{n}$ gives the values of $\log K_1$ and $\log K_2$. Values of the equilibrium constants are accumulated in Table 4.

The value of $\log \beta_{abs}$ was determined from the value of $\log \beta_{ext}$ obtained from different methods and by considering Eq. (9) and is reported in Table 4. The value of P_{Cu} was determined by consideration of theoretical (E_{th}) and practical (E_{obs}) extinction coefficients. The value of P_{Cu} turns out to be 36.0 which suggests maximum extraction of copper as 97.30%.

TABLE 2
DETERMINATION OF β_2 BY EQ. (12)[a]

$[HR]_{tot} \times 10^4$	Absorbance	D_{Cu}	$[HR]_{org} \times 10^4$	$\beta_2 \times 10^{-7}$
2.00	0.315	2.136	1.932	5.723
2.40	0.340	2.775	2.253	5.467
2.80	0.370	4.000	2.640	5.739
3.00	0.380	4.605	2.836	5.727
3.20	0.385	4.967	3.034	5.397
3.40	0.395	5.854	3.229	5.614
3.60	0.403	6.776	3.426	5.774
4.50	0.425	11.331	4.316	6.082
5.00	0.430	13.225	4.814	5.706
5.50	0.435	15.807	5.312	5.602
6.00	0.440	19.576	5.810	5.800
7.00	0.445	25.455	6.808	5.493

[a] $[Cu] = 1 \times 10^{-5}\ M$, pH = 4.2, $P_{Cu} = 36.0$, $\lambda_{max} = 368$ nm.

TABLE 3
DETERMINATION OF STABILITY CONSTANT BY LIGAND NUMBER METHOD[a]

$[HR]_{tot} \times 10^4$	Absorbance	$[MR_2']_{org} \times 10^5$	$\bar{n}$	$-\log [R^-]$ pR
0.20	0.040	0.087	0.17	12.74
0.30	0.065	0.141	0.28	12.57
0.50	0.120	0.260	0.52	12.35
0.80	0.175	0.378	0.76	12.14
1.00	0.220	0.476	0.95	12.04
1.20	0.235	0.508	1.02	11.96
1.40	0.255	0.551	1.10	11.89
1.60	0.275	0.595	1.19	11.83
1.80	0.300	0.649	1.30	11.78
2.00	0.315	0.681	1.36	11.73
2.40	0.340	0.675	1.47	11.65
2.80	0.370	0.800	1.60	11.58
3.00	0.380	0.822	1.64	11.55
3.20	0.385	0.832	1.66	11.52
3.40	0.395	0.854	1.71	11.49
3.60	0.403	0.871	1.74	11.47
4.00	0.420	0.908	1.82	11.42
4.50	0.425	0.919	1.84	11.37
5.00	0.430	0.930	1.86	11.32
5.50	0.435	0.940	1.88	11.28
6.00	0.440	0.951	1.90	11.24
7.00	0.445	0.962	1.92	11.17

[a] [Cu] = 1×10^{-5} *M*, pH = 4.2, P_{Cu} = 36.0, λ_{max} = 368 nm.

Procedure

An aliquot of copper perchlorate solution containing less than 100 μg of copper was taken and adjusted to pH 4.2 with perchloric acid and sodium hydroxide in a 10-ml volume. The solution was then introduced

TABLE 4
EQUILIBRIUM CONSTANTS OF COPPER–BPPTB CHELATE AT 30 ± 1°C

	Method			
Constant	Yatsimirskii's	Leden's	$D_{Cu} / [HR]^2_{org}$	Ligand number
$\log k_1$	3.96	3.85	—	—
$\log k_2$	3.66	3.55	—	—
$\log \beta_2$	7.62	7.40	7.75	8.00
$\log K_1$	—	—	—	12.35
$\log K_2$	—	—	—	11.65
$\log \beta_{ext}$	23.62	23.40	23.75	24.00
$\log \beta_{abs}$	22.06	21.84	22.19	22.44

into a separatory funnel and shaken with 10 ml of 1×10^{-3} *M* BPPTB in chloroform for 2 min. Layers were allowed to separate. The organic phase was withdrawn in a 10-ml volumetric flask and measured at 368 nm against the similarly processed reagent solution as a blank. The amount of copper was then obtained from the calibration curve.

SUMMARY

4-*S*-Benzyl-1-*p*-chloro-phenyl-5-phenyl-2,4-isodithiobiuret (BPPTB) forms bis complex with copper(II). The complex is easily extractable in chloroform. It exhibits a λ_{max} at 368 nm. The values of the equilibrium constants have been evaluated by different methods. The analytical application of this system has been studied. It was found that copper can easily be separated from large numbers of ions which are generally associated with it. A suitable procedure has been recommended for spectrophotometric microdetermination of copper.

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Ultramicrodetermination of Nitrogen in Organic Compounds

XI. Removal of Interference from Iodine for the Sealed-Tube Method

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INTRODUCTION

In ultramicrodetermination of nitrogen with the sealed-tube method (*1*, *4*, *5*), the results of analyses of organic compounds containing interference elements were satisfactory (*7*) because small quantities of sample and newly reduced copper were used for every analysis.

However, as the iodine content increases, the tendency to obtain low results for nitrogen increases. If the sample contains more than about 30% iodine, the results of nitrogen analyses are beyond the accepted limits of accuracy. To remove the interference from iodine, the samples were burned with various additives. We found that addition of 30 to 50 mg tungsten trioxide is most effective.

EXPERIMENTAL AND DISCUSSION

Table 1 shows the results of analyses of organic compounds containing iodine with no modification of the sealed-tube method (*4–6*). Almost all the iodine-containing compounds tended to give low nitrogen results.

We tried to develop a method based on absorption of iodine by heated silver in the form of wire, gauze, or granules (particle size 20–30 mesh). The results were unsatisfactory with silver wire and gauze, but better with silver granules which had been heated in air at 500°C for 1 hr then added at more than 1000 times the quantity of the iodine in the sample, as can be seen from Table 2. A small blank with silver granules should be measured prior to use, because porous silver granules give rise to large blank values. Iodine which is incorporated into silver granules may be more difficult to dislodge at high temperature, than that in silver wire and gauze.

We also tried to develop a method using alkali metal, such as barium, sodium, and potassium carbonate, and sodium and potassium hydroxide. Addition of about 30 mg barium carbonate was not effective. Addition of the other alakli was also not successful as the combustion tube eroded at high temperature, explosively breaking or cracking at its fine-tip end. If

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TABLE 1

RESULTS OF ANALYSES OF ORGANIC COMPOUNDS CONTAINING IODINE WITH NO MODIFICATION OF THE SEALED-TUBE METHOD

Sample	Calculated I (%)	Weight of sample (mg)	Calculated N (%)	N Found (%)	Difference for N (%)
N-(2-Ethyldithiophenyl)-4-iodobenzenesulfonamide		0.4920		2.98	−0.12
		0.3439		2.93	−0.17
	28.12	0.3717	3.10	3.10	0
		0.2745		3.19	0.09
		0.3836		2.98	−0.12
N,*N*′-(2,2′-Dithiodiphenyl)-bis(4-iodobenzenesulfonamide)		0.3431		3.43	−0.16
		0.2596		3.46	−0.13
	32.52	0.3273	3.59	3.35	−0.24
		0.3124		3.40	−0.19
		0.3058		3.51	−0.08
Tetra-*n*-buthylammonium iodide		0.2576		3.39	−0.40
		0.2865		3.19	−0.60
	33.97	0.4324	3.79	3.44	−0.35
		0.2804		3.40	−0.39
		0.3194		3.51	−0.28
		0.3417		3.44	−0.35
Tetra-*n*-propylammonium iodide		0.2935		4.10	−0.37
		0.3268		3.96	−0.51
	40.22	0.3534	4.47	4.25	−0.22
		0.3115		3.95	−0.52
		0.2958		3.86	−0.61
		0.5707		4.19	−0.28

TABLE 1—*(Continued)*

Sample	Calculated I (%)	Weight of sample (mg)	Calculated N (%)	N Found (%)	Difference for N (%)
Acetylcholine iodide		0.5506		4.63	−0.50
		0.3632		4.57	−0.56
	46.47	0.3205	5.13	4.44	−0.69
		0.4177		4.35	−0.78
		0.3402		4.51	−0.62
1-Methylquinolinium iodide		0.6761		4.49	−0.68
	46.81	0.5763	5.17	4.65	−0.52
Tetraethylammonium iodide		0.3552		5.01	−0.44
		0.4190		4.67	−0.78
	49.14	0.4333	5.45	4.99	−0.46
		0.2808		4.83	−0.62
		0.3688		4.96	−0.49
Quinolinium iodide	49.36	0.4237	5.45	4.70	−0.75
Pyridinium iodide	61.30	0.7937	6.76	6.07	−0.69

TABLE 2
RESULTS OF ANALYSES OF ORGANIC COMPOUNDS CONTAINING IODINE WITH ADDITION OF SILVER GRANULES[a]

Sample	Weight of sample (mg)	Iodine in sample weighed (mg)	Weight of Ag-granules added (mg)	Ratios of I to Ag (%)	N Found (%)	Difference for N (%)
Quinolinium iodide	0.7752	0.3826	152	0.25	5.06	−0.39
	0.2718	0.1342	220	0.06	5.42	−0.03
	0.2562	0.1265	211	0.06	5.50	0.05
1-Methylpridinium iodide	0.8861	0.5087	152	0.33	5.98	−0.40
	0.2244	0.1288	211	0.06	6.52	−0.19
Pyridinium iodide	0.7359	0.4511	152	0.30	6.35	−0.41
	0.4746	0.2909	233	0.13	6.67	−0.09
	0.2947	0.1807	258	0.07	6.61	−0.15

[a] Blank value with 200 mg:1.0 μl.

the combustion tube was not broken, it was taken out of the furnace after cooling to room temperature and satisfactory results were obtained as given in Table 3. However, use of alkali to remove the iodine was dangerous and destructive to laboratory equipment.

A final attempt was made using vanadium pentoxide and tungsten trioxide, because they are suitable additives in many case (*3*, *8*), and tungsten diiodide is formed when iodine vapor is passed over the red-hot tungsten or tungsten dioxide (*2*).

We used G.R. vanadium pentoxide and tungsten trioxide preheated for 1 hr in a current of air at 600°C for the former and 800°C for the latter. The samples were mixed with the above additives by rotating the combustion tube before heating in the furnace. Addition of vanadium pentoxide was often not successful as in the analogous case of the potassium hydroxide. As is given in Table 4 satisfactory results were obtained in the case where the cracking of combustion tube does not occur.

As can be seen from Table 5, addition of tungsten trioxide (30 to 50 mg) for analyses of organic compounds having iodine atoms gave necessarily good results that were reliable and compatible with the standards of ultramicrodetermination of nitrogen in organic compounds with the normal sealed-tube method.

SUMMARY

The interference from iodine in ultramicrodetermination of nitrogen in organic compounds with the sealed-tube method was investigated and attempts to minimize it are discussed. Use of 30 to 50 mg G.R. tungsten trioxide prepared by heating in a current of air at 800°C for 1 hr is recommended. Errors were almost always within ±0.3%.

TABLE 3

RESULTS OF ANALYSES OF ORGANIC COMPOUNDS CONTAINING IODINE WITH ADDITION OF ABOUT 30 mg POTASSIUM HYDROXIDE

Expt.	Sample	Weight of sample (mg)	Temperature (°C)	Volume of nitrogen (μl)	Pressure of N (mm Hg)	N Found (%)	Difference for N (%)
1	Tetra-*n*-buthylammonium iodide	0.3000	26.4	10.2	761.9	3.90	0.11
2	Tetra-*n*-propylammonium iodide	0.4518	26.3	18.0	761.9	4.57	0.10
3	Acetylcholine iodide	0.4995	26.5	22.8	761.9	5.23	0.10

TABLE 4

RESULTS OF ANALYSES OF ACETYLCHOLINE IODIDE WITH ADDITION OF ABOUT 50 mg VANADIUM PENTOXIDE (N%: 5.13)[a]

Expt.	Weight of sample (mg)	Temperature (°C)	Volume of nitrogen (μl)	Pressure of N (mm Hg)	N Found (%)	Difference for N (%)
1	0.5703	21.9	23.9	775.0	4.95	−0.18
2	0.5805	21.9	24.1	774.6	4.90	−0.23
3	0.5584	22.0	23.9	774.6	5.05	−0.08
4	0.5155	22.1	21.6	774.6	4.99	−0.14

[a] Blank value with 50 mg: 1.0 μl.

TABLE 5

RESULT OF ANALYSES OF ORGANIC COMPOUNDS CONTAINING IODINE WITH ADDITION OF TUNGSTEN TRIOXIDE[a]

Sample	Weight of WO_3 added (mg)	Weight of sample (mg)	Calculated N (%)	N Found (%)	Difference for N (%)
N-(2-Ethyldithiophenyl)-4-iodobenzenesulfonamide		0.3390		3.16	0.06
		0.5114	3.10	3.14	0.04
		0.4590		3.17	0.07
N,N'-(2,2'-Dithiophenyl)-bis(4-iodobenzenesulfonamide)		0.3140		3.69	0.10
		0.4601	3.59	3.85	0.26
	30[b]	0.4393		3.68	0.09
Acetylcholine iodide		0.3232		5.03	−0.10
		0.2528		5.43	0.30
		0.4289	5.13	5.16	0.03
		0.3948		5.35	0.22
		0.3478		5.23	0.10
Tetraethylammonium iodide		0.2968		5.36	−0.09
		0.2645	5.45	5.53	0.08
		0.3286		5.30	−0.15
		0.3609		5.40	−0.05
		0.5768		5.32	−0.13
Tetra-*n*-buthylammonium iodide		0.2537		3.81	0.02
		0.3246		3.79	0
		0.4382	3.79	3.82	0.03
		0.3911		3.86	0.07
		0.3600		3.80	0.01
Tetra-*n*-propylammonium iodide		0.3296		4.33	−0.14
		0.3329		4.49	0.02
		0.3592	4.47	4.57	0.10
		0.3826		4.58	0.11
		0.4515		4.46	−0.01
N-(2-Ethyldithiophenyl)-4-iodobenzenesulfonamide		0.3223		3.39	0.29
		0.2621	3.10	3.25	0.15
		0.2608		3.27	0.17
N,N'-(2,2'-Dithiophenyl)-bis(4-iodobenzenesulfonamide)		0.2505		3.71	0.12
		0.3059	3.59	3.70	0.11
		0.3642		3.74	0.15
Acetylcholine iodide		0.2457		5.29	0.16
		0.3191		4.97	−0.16
		0.3704	5.13	4.92	−0.21
		0.4493		5.18	0.05
	50[c]	0.3111		5.14	0.01
Tetraethylammonium iodide		0.4919		5.47	0.02
		0.5208		5.50	0.05
		0.2522	5.45	5.65	0.20
		0.3961		5.41	−0.04
		0.3051		5.49	0.04
Tetra-*n*-buthylammonium iodide		0.4445		4.09	0.30
		0.4267	3.79	3.80	0.01
		0.3230		3.86	0.07
		0.3999		3.87	0.08
		0.3725		3.83	0.04
Tetra-*n*-propylammonium iodide		0.4287		4.62	0.15
		0.3907		4.51	0.04
		0.5865	4.47	4.58	0.11
		0.4030		4.68	0.21
		0.4310		4.45	−0.02

[a] Mean error, 0.06_0%; standard deviation of error, 0.115_7%.

[b] Blank value, 0.3, 0.2, 0.2, 0.3, 0.2 μl; mean of blank value, 0.2 μl.

[c] Blank value, 0.3, 0.3, 0.3, 0.3, 0.2 μl; mean of blank value, 0.3 μl.

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MICROCHEMICAL JOURNAL 24, 367–377 (1979)

Application of the Reaction between 2,2′-Diquinoxalyl and Tin(II) or Titanium(III) to Kinetic Determination of Copper and Iron

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INTRODUCTION

Rates of various reactions are based upon catalytic processes and many of them are catalyzed with the copper and iron ions. In recent years the application of kinetic methods used for the determination of copper ions in substances having high purity grade increased. Kinetic methods for the determination of iron ions are well known for their high sensitivity.

As part of our work on the properties and applications of 2,2′-diquinoxalyl as a reagent in analytical chemistry, kinetic methods of ion determination were established, based upon oxidation of a product of the reaction between 2,2′-diquinoxalyl and tin(II) or titanium(III) ions. Other methods are known which take advantage of 2,2′-diquinoxalyl involved in redox reactions, which are applied in analytical fields such as spectrophotometry (*2–4*), potentiometry, volumetric analysis, and thin-layer chromatography (*4–6*).

Working on the reduction of 2,2′-diquinoxalyl with strong reductive agents in concentrated acid solutions a remarkable susceptibility to become oxidized was noticed with the reaction product in a reaction between 2,2′-diquinoxalyl and tin(II) or titanium(III), which enabled the reaction substrate to be obtained, i.e., 2,2′-diquinoxalyl once more. It was stated that oxidation can be accelerated with the trace amounts of copper and iron ions, and the course of oxidation can be checked with the given concentrations of the oxidizing agents, for instance of hydrogen peroxide.

REAGENTS AND EQUIPMENT

2,2′-Diquinoxalyl (1, 7). Its purity grade was tested by means of the UV spectrophotometric method (*9*) and application of thin-layer chromatography (*8*).

Hydrochloric acid and hydrogen peroxide. Spectroscopic grade.

The standard tin(II) solution (0.5 M). The solution was prepared by

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dissolving of 56.4 g diaqueous tin(II) chloride (analytical grade) in hydrochloric acid (1:1) in a measuring flask (vol 1000 ml). Concentration of the solution was determined through the bromometric method. Further solutions were prepared from the standard one by dissolving with hydrochloric acid of a demanded concentration.

The standard titanium(III) solution (0.01 M). The solution was obtained by dissolving 100 g titanium(IV) chloride in 500 ml of concentrated hydrochloric acid; the difference to 1000 ml was filled with water. From this solution 5 ml was transferred with a pipet to a measuring flask (vol 250 ml); the difference was filled with hydrochloric acid (1:1). The obtained titanium(IV) solution was the 0.01 *M* one. Titanium(IV) was then reduced to titanium(III) in a column filled with amalgamated cadmium. Concentration of the titanium(III) solution was determined potentiometrically with iron(III). From the standard solution the working ones were prepared by dissolving with hydrochloric acid of various concentrations.

The standard copper solution. The standard copper solution containing 0.1 mg of copper(II) in 1 ml was prepared from $CuSO_4 \cdot 5H_2O$, analytical grade.

The standard iron solution. The standard iron solution containing 0.1 mg of iron(III) in 1 ml was prepared from $FeCl_3 \cdot 6H_2O$, analytical grade. In each case triply distilled water was used. The gaseous carbon dioxide was bubbled through every solution and reduction was carried out in an oxygenless atmosphere.

Spectrophotometer Unicam SP1700.

Spectrophotometer Spekol.

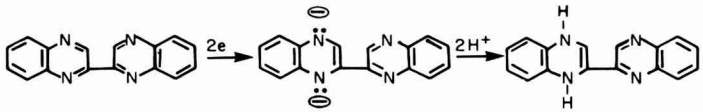
EXPERIMENTAL

The 2,2′-diquinoxalyl solutions in 6*N* hydrochloric acid were introduced into measuring flasks (vol 25 ml) in a quantity such as to obtain the 3.06×10^{-5} *M* concentration of a reagent in a working solution. These solutions were bubbled with the gaseous carbon dioxide for 3–4 min. Then the equimolar amounts of tin(II) or the doubled amounts of titanium(III) in 6 *N* hydrochloric acid were introduced to the a.m. solution. After obtaining the maximum absorbance values [after 45 min with tin(II), after 1 hr with titanium(III)], the hydrogen peroxide solution and the examined ions were introduced to the flasks which had earlier been bubbled with carbon dioxide.

Oxidation of the Product of a Reaction between 2,2′-Diquinoxalyl and Tin(II) or Titanium(III)

2,2′-Diquinoxalyl is a heterogenic compound, including nitrogen atoms in its rings. It can be reduced with the strong reductive agents in the hydrochloric acid atmosphere, which results in a blue compound, its coefficient of molar absorption at $\lambda = 685$ nm equal 3×10^4. Reduction of

2,2′-diquinoxalyl is a dielectron reaction and in this case the following course has been suggested (*4*):



The obtained compound, i.e., 2,2′-DQx_{red} demonstrates in a hydrochloric acid solution the constant absorbance value lasting slightly less than 1 hr and then a slow fainting of color is observed, due to the oxidation of the reaction product with oxygen from air. The final product of oxidation, derived from the blue-colored substance is the starting compound, 2,2′-diquinoxalyl (Fig. 1). Oxidation of the blue reaction product runs faster under the influence of the added hydrogen peroxide or in the presence of metal ions, such as Cu(II) or Fe(III). The catalytical influence of the above-mentioned ions on the examined course of reaction was established by means of hydrogen peroxide.

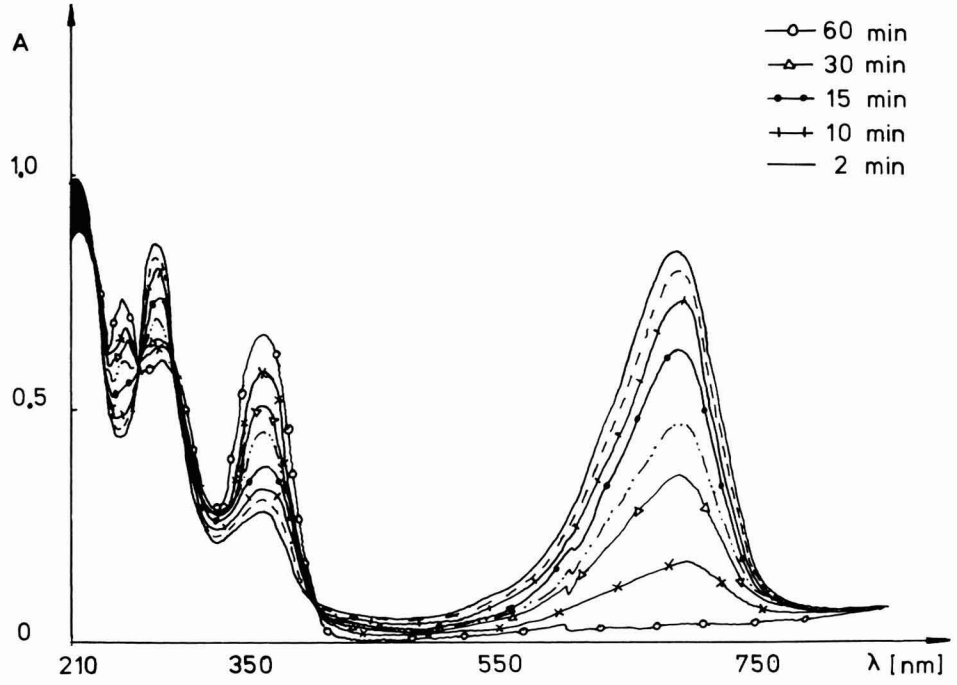


FIG. 1. The UV spectra showing the course of 2,2′-diquinoxalyl$_{red}$ oxidation by means of oxygen from air.

The Influence of Hydrogen Peroxide, Copper(II), and Iron(III) on Oxidation of 2,2'-DQx$_{red}$

The influence of hydrogen peroxide on oxidation of 2,2'-DQx$_{red}$ (concentration 3.06×10^{-5} *M*) was examined in the concentration range of hydrogen peroxide from 3.47×10^{-5} to 10.41×10^{-5} *M*. With the increasing value of the hydrogen peroxide concentration the oxidation process runs faster, with the most convenient concentration (from the analytical point of view) being 6.94×10^{-5} *M*. The influence of copper(II) and iron(III) on oxidation of 2,2'-DQx$_{red}$ was also examined without introducing hydrogen peroxide to the solutions. After obtaining the constant maximum absorbance value in a solution of a colored compound, copper(II) and iron(III) were introduced in amounts such as to have the ions concentration range from 0.4 to 2.0 μg/ml, with the constant 2,2'-diquinoxalyl concentration of 3.06×10^{-5} *M*.

It was found that after introducing copper(II) to a solution the slow diminishing of an absorbance value occurred, which depended upon the reaction time and the quantities of copper(II) used. The straight-line dependences were obtained of ΔA (the absorbance value difference between the "blind" and the normal sample) versus reaction times for the copper concentrations in the range from 0.4 to 2.0 μg (Fig. 2).

After introducing iron(III) to the 2,2'-DQx$_{red}$ solutions a fast decrease of absorbance is observed, which then stops at a certain value and preserves it for about 20 min (Fig. 2).

The determined formal potential of the oxidized 2,2'-diquinoxalyl (reduced 2,2'-diquinoxalyl/reduced in a 6 *N* hydrochloric acid) system equals +0.41 (*4*).

After introducing iron(III) to a solution reduction of iron(III) to iron(II) takes place and simultaneously an oxidation of 2,2'-DQx$_{red}$ to 2,2'-diquinoxalyl occurs, oxidation being the dielectron process.

The Catalytical Influence of Copper(II) on Oxidation of 2,2'-DQx$_{red}$ with Hydrogen Peroxide

Reduction of 2,2'-diquinoxalyl in a 6 *N* hydrochloric acid was run either with the help of tin(II) or titanium(III). After obtaining the maximum absorbance value copper(II) and hydrogen peroxide were introduced to the solutions. The following reagent concentrations were applied when measuring absorbance values: 3.06×10^{-5} *M* of 2,2'-diquinoxalyl, 6.94×10^{-5} *M* of hydrogen peroxide, and 0.4 to 3.5 μg of copper(II) in 10–25 ml. Simultaneously absorbance of the "blind" sample was measured, containing all the solution components excluding copper(II). The absorbance measurements were performed in measuring cells, their optical path length $d = 1$ cm, at the wavelength $\lambda = 685$ nm. From the curves of ΔA vs reaction time for various copper(II) concentrations [ΔA is the absorbance

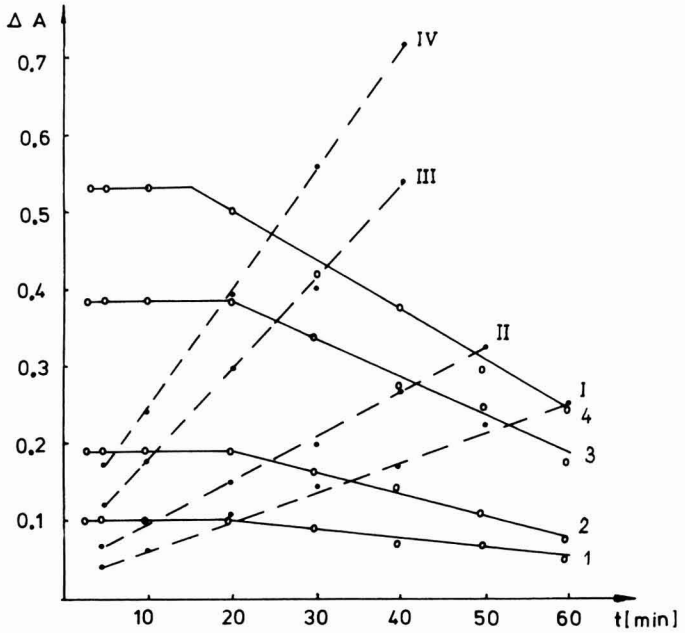


FIG. 2. The absorbance differences between the "blind" sample and the one containing the examined ions, which depends on the reaction time and on different concentrations of copper(II) and iron(III). Cu(II): I, II, III, IV curves—0.4, 0.8, 1.6, 2.0 μg/ml; Fe(III): 1, 2, 3, 4 curves.

difference between the "blind" and the copper(II) containing sample] the angle tangents were calculated between the straightline courses of dependences and the x axis (Fig. 3).

The obtained analytical curves enable quantitative determination of copper(II), based upon oxidation of 2,2′-DQx_{red} by means of hydrogen peroxide.

The catalytical reactions of oxidation performed with lower and higher concentrations of hydrogen peroxide proved to be less useful for analytical purposes.

The Influence of Iron(III) on Oxidation of 2,2′-DQx_{red} with Hydrogen Peroxide

The influence of iron(III) on oxidation of 2,2′-DQx_{red} was tested under conditions similar to when determining copper(II). The straightline dependence was found for the absorbance difference with two solutions, one examined without and one with the addition of iron(III) ions, the concentration of iron(III) ions ranging from 0.4 to 3.0 μg in 10–25 ml of a solution. The preliminary operation was to establish the shape of the analytical curve and in this case the method of constant time was applied

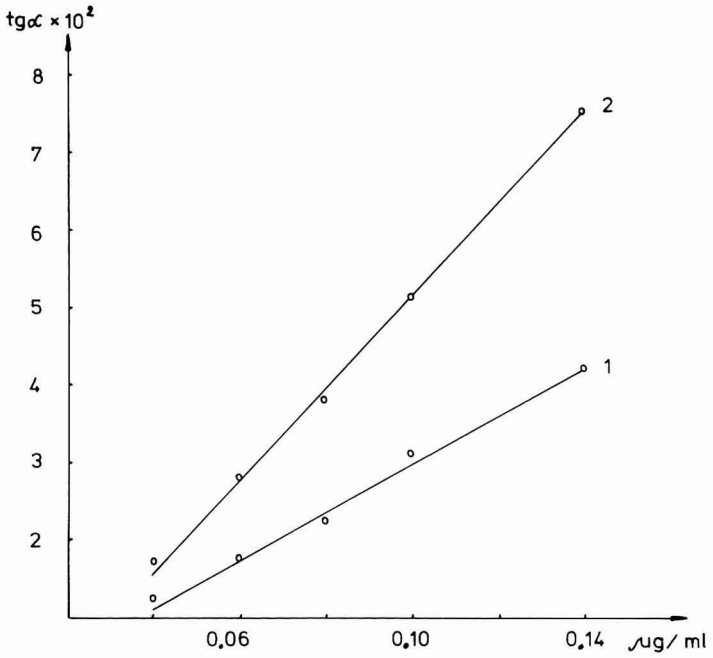


FIG. 3. Analytical curves of determination for copper(II) based upon the reaction of 2,2′-diquinoxalyl$_{red}$ oxidation: (1) 2,2′-diquinoxalyl–Ti(III); (2) 2,2′-diquinoxalyl–Sn(II).

(Fig. 4). The absorbance measurements were accomplished 4 min after adding hydrogen peroxide to the investigated solutions.

The lower and higher concentrations of hydrogen peroxide were also applied, but the repeatability of the results was unsatisfactory in those cases. For analytical purposes oxidation of 2,2′-DQx$_{red}$ with hydrogen peroxide was suggested with the hydrogen peroxide concentration 6.94×10^{-5} *M*.

The Influence of Hydrochloric Acid and the Strange Ions on Oxidation of 2,2′DQx$_{red}$ with Hydrogen Peroxide

Oxidation was carried out in hydrochloric acid, its concentration ranging from 4 to 8 *N*, and applying the 2,2′-diquinoxalyl concentration 3.06×10^{-5} *M*. Slight changes in the reaction course were established, when changing the hydrochloric acid concentration from 6 to 8 *N*, this effect being more evident with the lower concentrations of acid. For analytical purposes the 6 *N* hydrochloric acid was the most recommended.

It was firmly established that the following ions, NH_4(I), Na(I), Zn(II), Pb(II), Bi(III), As(V), As(III), Cr(III), Ni(IV), Co(II), Sb(III), Mn(II), introduce no analytical disturbance even in an amount 1000 times higher

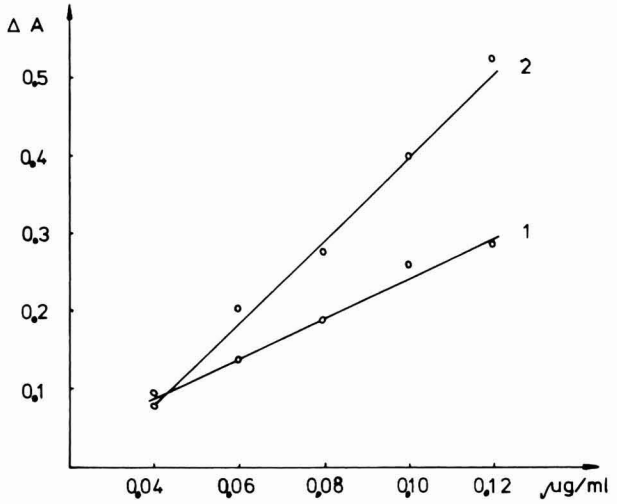


FIG. 4. Analytical curves of determination for iron(III) based upon the reaction of 2,2′-diquinoxalyl$_{red}$ oxidation: (1) 2,2′-diquinoxalyl–Ti(III); (2) 2,2′-diquinoxalyl–Sn(II).

than that of the determined ion. They also do not affect the oxidation of 2,2′-DQx$_{red}$, when copper and iron remain absent.

In the discussed reaction the following ions, Sb(V), Cr(VI), Au(III), cause a real and serious obstacle, which can be noticed in the form of the immediate absorbance decrease, and the obtained absorbance differences are proportional to the amount of introduced ions. Oxidation is also accelerated in the presence of Pt(IV) and the established dependences between the absorbance differences and the reaction time with different concentrations of this ion point to the possibility of kinetic determination of Pt(IV) based upon the oxidation of 2,2′-DQx$_{red}$.

Determination of the Trace Amounts of Copper and Iron in the Spectrally Pure Salts, Based upon the Kinetic Reaction of 2,2′DQx$_{red}$ Oxidation by Means of Hydrogen Peroxide

The established method was applied to the determination of trace amounts of copper and iron in the following, spectrally pure salts: $ZnSO_4 \cdot 7H_2O$, NH_4Cl, $(NH_4)_2SO_4$. Determination of iron in zinc sulfate was carried out without separating a matrix and after the extraction of iron with ethyl ether. Determination of copper in ammonium chlorate and sulfate was performed through extraction of copper with dithizone (10).

Determination of Iron and Copper

The 10-ml measuring flasks were filled with suitable amounts of 2,2′-DQx and tin(II) solutions in 6 *N* hydrochloric acid. After obtaining the

maximum absorbance values of a solution (i.e., after 45 min) solutions of the examined salts were introduced into the flasks. In one or two flasks no salt samples were introduced (the "blind" samples). To each flask the hydrogen peroxide solution was introduced in 0.5-min intervals. After mixing the flask volumes were filled with the 6 *N* hydrochloric acid.

With iron the constant time method was used and the absorbance value was measured after 4 min, preserving the same order and the same intervals as when adding hydrogen peroxide. The absorbance difference, ΔA, between the "blind" and the investigated sample was calculated and from the analytical curve the iron amount was made out.

With determination of copper absorbance of a solution was measured after 2, 4, 6, 8, and 10 min. The ΔA vs reaction time dependence was drawn and the angle tangents were measured, and from the analytical curve the copper amounts were deduced. The obtained results are given in Table 1.

DISCUSSION AND CONCLUSIONS

The colored product of the reaction between 2,2′-diquinoxalyl and tin(II) or titanium(III) ions (i.e., 2,2′-DQx_{red}) in a hydrochloric acid solution undergoes an easy oxidation toward 2,2′-diquinoxalyl. The related lightening of the solution color, i.e., the decrease of absorbance at the wavelength $\lambda = 685$ nm, is accelerated in the presence of copper(II) and iron(III) ions. Oxidation of 2,2′-DQx_{red} by means of hydrogen peroxide in a 6 *N* hydrochloric acid solution, catalyzed with the copper(II) and

TABLE 1
DETERMINATION OF COPPER AND IRON IN THE SPECTRALLY PURE SALTS[a]

Investigated salt (1)	Sample (g) (2)	Determined iron μg (3)	Determined iron % (×10⁻⁴) (4)	Investigated salt (5)	Sample (g) (6)	Determined copper μg (7)	Determined copper % (8)
$ZnSO_4 \cdot 7H_2O$ Determined with a matrix	0.4	0.47	1.2	NH_4Cl	4.0	0.45	1.12×10^{-5}
	0.4	0.52	1.3		5.0	0.60	1.2×10^{-5}
	0.5	0.63	1.26		4.0	0.52	1.3×10^{-5}
	0.5	0.56	1.12				
Extracted with ethyl ether	0.5	0.67	1.34	$(NH_4)_2SO_4$	8.0	0.60	6.0×10^{-6}
	0.5	0.63	1.26		10.0	0.68	6.8×10^{-6}

[a] According to the attested data the iron amount in $ZnSO_4 \cdot 7H_2O$ was lower than 1×10^{-4} and the copper amount in NH_4Cl equaled 1×10^{-5}, and in $(NH_4)_2SO_4$—5×10^{-6}.

iron(III) ions, their concentration ranging from 0.4 to 3.5 μg in a volume of 10–25 ml, enables the quantitative determination of those ions. In the investigated range of ion concentrations the convenient absorbance differences are observed with the solutions, in which the catalyzed and the noncatalyzed reactions take place.

To quantitatively determine copper(II) ions using the method based upon the catalyzed oxidation of 2,2′-DQx_{red} the tangents procedure was recommended, and for the quantitative determination of iron(III) ions the method of constant time (4 min) was applied. When simultaneously determining copper(II) and iron(III) no activating effect of one factor upon the other was found, and the measured absorbance difference, ΔA, is a mean value for both components.

The course of oxidation was investigated in the presence of hydrogen peroxide, copper(II), and iron(III) and when applying hydrogen peroxide along with the examined ions. The reaction parameters were also checked, such as the influence of hydrogen peroxide, hydrochloric acid, and the strange ions on the process. The correlation was established between the rate of oxidation of 2,2′-DQx_{red} and the hydrogen peroxide concentration, and the measurement of the absorption difference was suggested in the presence of a "blind" sample (containing H_2O_2 without the examined ions), which efficiently helped to eliminate the measurement error, due to the change of concentration and the decomposition rate of hydrogen peroxide in the given reaction conditions.

Usefulness of the established method, concerning determination of copper(II) and iron(III) ions, based upon the kinetic reaction of oxidation of 2,2′-DQx_{red} with hydrogen peroxide was investigated, to determine these ions in the spectrally pure salts. It is possible to determine iron in $ZnSO_4$ without separating a matrix, and with copper in the ammonium salts condensation of copper was gained through extractions, because the copper amount in the investigated salts created the necessity of using several gram samples of those salts.

The possibility of applying the established method to the determination of the trace amounts of copper and iron in the samples was also demonstrated, in which the quantities of the above-mentioned ions were in the range of $10^{-4}-10^{-5}$%. The established method of determination of the copper(II) and iron(III) ions shows sensitivity of the 0.04 μg/ml range of values. The relative error of this method is about 40%.

In addition some attempts were undertaken to establish the reaction order and the rate constants of the kinetic reactions.

Determination of the reaction order with noncatalytic and catalytic reactions was carried out with the different 2,2′-DQx_{red} and hydrogen peroxide concentrations and the time was measured, in which the equal absorbances of the solutions were observed.

On the basis of the obtained results it was established that the discussed reaction was the second-order one.

Measurements were performed for the following reagent concentrations: 2,2′-DQx, $c = 3.06 \times 10^{-5}\,M$; H_2O_2, $c = 6.94 \times 10^{-5}\,M$. The plots of log C_B/C_A vs time dependences were drawn and the straightline dependences were obtained in the time range from 0 to 4 min only, and from those dependences the angle tangents were calculated.

On the basis of the obtained data the initial stage of the reaction can be recognized as the second-order one, the first-order in respect of either 2,2′-DQx_{red} or hydrogen peroxide.

The presented results show the complexity of oxidation and can be regarded as the first step toward the investigation of the kinetics of the discussed systems.

SUMMARY

The possibility of application was examined of a reaction between 2,2′-diquinoxalyl and tin(II) or titanium(III) to catalytic determination of copper and iron. The reaction parameters, the influence of the hydrogen peroxide concentration, the hydrochloric acid concentration, and the presence of strange ions on oxidation of the reduced form of 2,2′-diquinoxalyl were tested.

The usefulness of the established method was proven for the determination of copper(II) and iron(III) ions in the spectrally pure salts, having the concentration range of those ions $10^{-4}-10^{-5}\%$. The sensitivity of the discussed method is of 0.04 μg/ml.

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N-*p*-Chlorophenylcinnamohydroxamic Acid: A New Reagent for the Microgram Determination of Vanadium(V)

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INTRODUCTION

N-Phenylbenzohydroxamic acid, PBHA, is widely used as an analytical reagent for solvent extraction and gravimetric determination of various metal ions (*2, 3, 7, 8, 10, 12, 13, 16–19*). *N*-Phenylcinnamo- and *N*-*p*-tolylcinnamohydroxamic acids are also used as reagents for the extraction and spectrophotometric determination of vanadium(V) with molar absorptivities of 6.3×10^3 and 6.5×10^3, respectively (*20*). Several other substituted hydroxamic acids were used for the determination of vanadium (*4, 5, 9, 11, 21*). Recently, *N*-(*p*-*N*,*N*-dimethylanilino-)-3-methoxy-2-naphthohydroxamic acid was reported to be a sensitive and selective reagent for vanadium. However, the results are not reproducible, and the reagent *N*-(*p*-*N*,*N*-dimethylanilino)-3-methoxy-2-naphthohydroxamic acid is not stable. The preparation is very tedious, and the reagent has poor solubility in organic solvents. Its sensitivity is also less than other substituted hydroxamic acids (*1*). Generally, titanium, molybdenum, tungsten, and zirconium interfere with vanadium determination. So far, most of these methods have not been extended for the determination of vanadium in natural resources, geological samples, and steel samples.

In the present investigation, a new reagent *N*-*p*-chlorophenylcinnamohydroxamic acid, (*N*-*p*-Cl-CHA), is reported as a selective and sensitive reagent for the microgram determination of vanadium(V). It has greater sensitivity than *N*-phenylcinnamo- and *N*-*p*-tolylcinnamohydroxamic acids. The vanadium content is also determined in natural resources, steel, and rock samples.

MATERIALS

Apparatus. Photometric measurements were made on VSU2-P spectrophotometer. The pH values of the solutions were measured on a Systronics digital pH meter Model 335.

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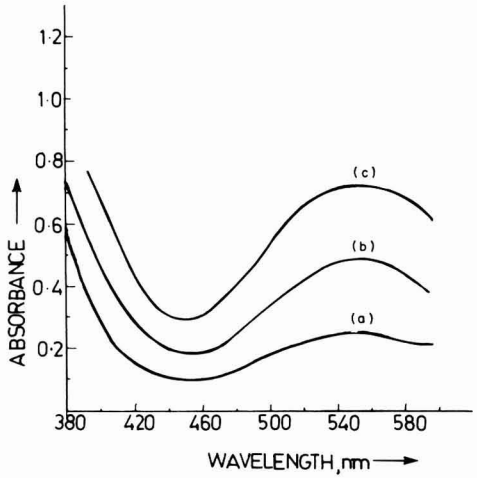

FIG. 1. Characteristic spectra of vanadium(V) with *N-p*-chlorophenylcinnamohydroxamic acid. (a) 1.86, (b) 3.72, and (c) 5.58 ppm of vanadium in 25 ml chloroform extract.

Chemicals and reagents. All the chemicals used were of G.R. or AnalaR grades from E. Merck and B.D.H., respectively, unless otherwise stated.

The reagent, *N-p*-chlorophenylhydroxamic acid (*N-p*-Cl-CHA) was synthesized as described elsewhere (*6*), mp 181°C. Its purity was checked by TLC, elemental analysis, and uv and ir spectra. Usually a 0.1% solution of the reagent in ethanol-free chloroform was used for all extractions. The chloroform was purified by the method of Vogel (*22*).

TABLE 1
SPECTRAL CHARACTERISTICS OF VANADIUM(V)–*N-p*-CHLOROPHENYLCINNAMOHYDROXAMATE COMPLEX IN CHLOROFORM[a]

Concentration of HCl (*M*)	Color of chloroform extract[b]	Molar absorptivity (ϵ) (M^{-1} cm^{-1} × 10^3)
2.0	V	3.3
4.0	BV	6.7
4.8	BV	6.7
5.6	BV	6.7
6.4	BV	6.7
7.2	BV	6.7
8.0	BV	6.7
8.8	BV	6.1
9.6	BV	5.9
10.4	V	5.5

[a] Vanadium = 3.72 ppm; wavelength of maximum absorbance = 540–550 nm.
[b] V, violet; BV, bluish-violet.

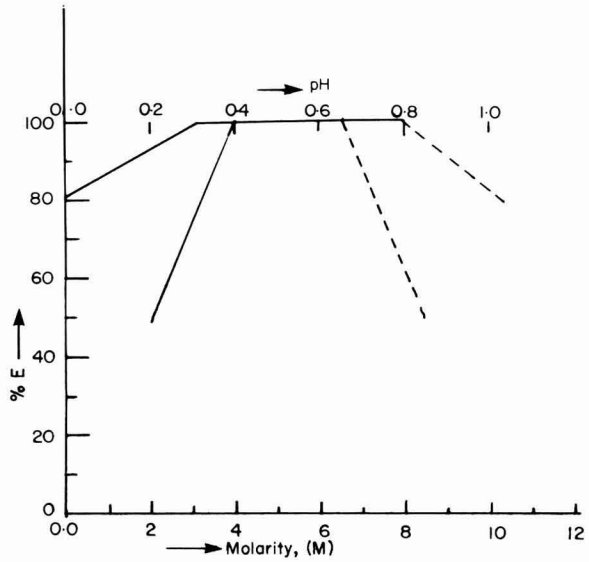

FIG. 2. Effect of pH and molarity of hydrochloric acid on the extraction of vanadium.

A standard solution of vanadium was prepared by dissolving 0.01159 g of ammonium metavanadate in 100 ml carbonate-free double-distilled water. Its vanadium content was determined volumetrically (*23*) and spectrophotometrically with PBHA (*13*).

PROCEDURE

Into a 150-ml separatory funnel, place 2 ml of vanadium solution (9.14×10^{-4} *M*), 3 ml of water, and 10 ml conc HCl. To this add 5 ml of a 0.1% chloroform solution of the reagent and shake vigorously for 4–5 min. Allow the two phases to separate. Separate the bluish violet chloroform extract and dry over anhydrous sodium sulfate. Repeat the extraction twice with 2 ml of reagent solution to ensure the complete extraction. Dilute the extract along with the washings to 25 ml. Measure the absorbance of the bluish violet extract at 540–550 nm against a reagent blank.

Determination of vanadium from natural resources. Depending upon the concentration of vanadium in samples, 20–50 g of sample was digested with an excess of $HClO_4$ and conc HNO_3. The hot solution was filtered and centrifuged to remove silicous matter. The filtrate was evaporated and diluted to 100 ml with conc HCl. Ten milliliters of each sample solution were mixed with 10 ml of blank solution in a 100-ml separatory funnel. A 0.1 *M* potassium permanganate solution was added dropwise until a pink color persists for 5 min. Ten milliliters of 0.1 *M* oxalate and citrate and 10 ml of 0.1 *M* sodium fluoride solutions were added. Then the extraction was carried out as usual.

TABLE 2
EFFECT OF pH ON THE EXTRACTION OF VANADIUM(V)–*N*-*p*-CHLOROPHENYLCINNAMOHYDROXAMATE COMPLEX IN CHLOROFORM[a]

Concentration of HCl (*M*)	pH	Percentage extraction	Distribution ratio (*D*)
2.0	0.85	49.9	1.0
4.0	0.65	100	*
4.8	0.60	100	*
5.6	0.55	100	*
6.4	0.45	100	*
7.2	0.35	100	*
8.0	0.30	100	*
8.8	0.2	91.5	11.3
9.6	0.1	87.5	7.2
10.4	0	81.4	5.1

[a] Vanadium = 3.72 ppm; maximum absorbance = 545 nm.
* Too high to measure:

$$\text{percentage extraction} = \frac{100\,D}{D + (V_{aq}/V_{org})},$$

assuming that the volumes of both phases (V_{org} and V_{aq}) were equal and the distribution coefficient D was determined from:

$$D = \frac{\text{Concentration of vanadium in organic phase}}{\text{Total vanadium taken} - \text{Vanadium extracted in organic phase}}.$$

RESULTS AND DISCUSSION

Absorption spectra. The absorption spectrum of the bluish violet vanadium complex has maxima at 540–550 (Fig. 1). The absorbance is reproducible at 545 nm. The chloroform solution of the reagent absorbs between 280 and 320 nm, but does not absorb between 400 and 600 nm. The spectral characteristic data are given in Table 1.

Distribution ratio of the reagent, *N*-*p*-chlorophenylcinnamohydroxamic acid in chloroform, was found to be 215.

Effect of acidity of HCl. The effect of acid concentration and pH was studied. The maximum extraction was observed between 4 and 8 *M* HCl (pH 0.65–0.3) (Fig. 2). As the concentration of HCl decreases or increases beyond 4 and 8 *M* the percentage extraction decreases. The maximum extraction was obtained with 4–8 *M* HCl. The data are given in Table 2.

Effect of the solvents. The extraction of the vanadium(V)–(*N*-*p*-Cl-CHA) complex in different solvents was studied. It showed that chloroform was the most suitable solvent causing the greatest molar absorptivity at 545 nm (wavelength of maximum absorbance) keeping other conditions similar. Extraction in benzene and carbon tetrachloride was poor. In amyl

TABLE 3
EFFECT OF SOLVENTS ON THE EXTRACTION OF VANADIUM(V)–*N*-*p*-CHLOROPHENYLCINNAMOHYDROXAMATE COMPLEX[a]

Solvent	Color of the extract[b]	Wavelength of maximum absorbance (nm)	ϵ (M^{-1} cm^{-1} × 10^3)
Chloroform	BV	540–550	6.7
Benzene	BV	540–550	6.4
Carbon tetrachloride	V	540	5.6
Amyl alcohol	LBV	*	*

[a] Vanadium = 3.72 ppm; concentration of HCl = 8*M*.
[b] BV, bluish-violet; LBV, light bluish violet; V, violet.
* The color disappears within 5 to 10 min, so the wavelength of maximum absorbance cannot be measured.

alcohol the extraction was very poor, the layers did not separate, the complex was not stable for longer periods, and the color disappeared within 5 to 10 min. The data are given in Table 3 and Fig. 3.

Validity of Beer's law. Beer's law is obeyed by the system in the range of 0.08–9 ppm of vanadium at 545 nm. The sensitivity of the reagent as defined by Sandell (*14*) is 0.008 μg V/ml using a 10-mm cell, and the molar absorptivity was 6.7 × 10^3 M^{-1} cm^{-1} at 545 nm.

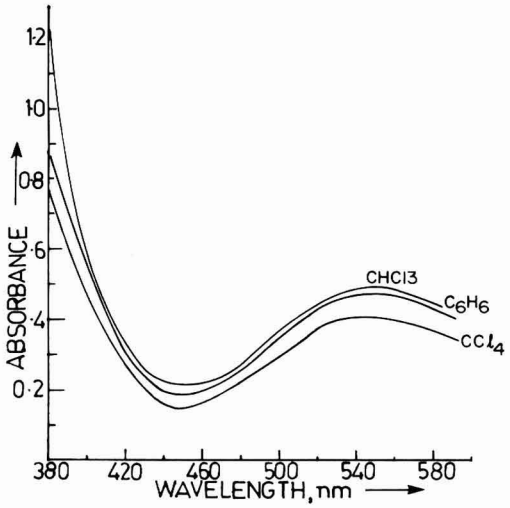

FIG. 3. Effect of solvent on the extraction of vanadium(V).

TABLE 4

EFFECT OF $HClO_4$ AND H_2SO_4 CONCENTRATION ON EXTRACTION OF VANADIUM(V)–*N-p*-CHLOROPHENYLCINNAMOHYDROXAMATE COMPLEX FROM CHLOROFORM[a]

Concentration of acid (*M*)	Acid	Color of complex extracted[b]	Wavelength of maximum absorbance (nm)	Percentage extraction	Distribution ratio (*D*)	ϵ (M^{-1} cm^{-1})
1.0	H_2SO_4	YB	460[c]	100	*	1.5×10^3
2.0	H_2SO_4	YB	460	100	*	1.5×10^3
4.0	H_2SO_4	Y	460	49.7	0.95	0.7×10^3
6.0	H_2SO_4	Y	460	4.97	0.09	0.7×10^2
4.0	$HClO_4$	B	520–530	73.1	2.7	4.1×10^3
6.0	$HClO_4$	B	520–530	82.9	4.9	4.6×10^3
8.0	$HClO_4$	VB	520–530	100	*	5.6×10^3
10.0	$HClO_4$	VB	520–530	90.2	9.3	5.1×10^3

[a] Vanadium = 3.72 ppm.

[b] YB, yellowish-brown; Y, yellow; B, brown; VB, violet-brown.

[c] There is no peak but a hump is observed.

* Too high to measure.

Effect of $HClO_4$ and H_2SO_4. Extraction with $HClO_4$ was initially violet but on shaking for 1–2 min the color changed to violet brown. The maximum absorbance was observed at 530 nm with 8 *M* $HClO_4$, the molar absorptivity being 5.6×10^3 M^{-1} cm^{-1}. With H_2SO_4 the extraction is brown, and the color intensity decreases as the concentration of H_2SO_4 increases. Complete extraction was obtained with 2 *M* H_2SO_4, and the molar absorptivity was 1.5×10^3 M^{-1} cm^{-1} at 460 nm. The data are given in Table 4.

Effect of reagent concentration. Extraction of vanadium was studied with varying concentrations of reagent under optimum conditions. The results showed that a single extraction with 5 ml of 0.1% reagent solution was enough for the quantitative extraction of vanadium. Lower concentrations gave incomplete extractions. Larger quantities of reagent can be used without difficulty as the reagent does not absorb between 400 and 600 nm and hence does not have any effect on the extracted species.

Stability, shaking time, and recovery. The vanadium complex is stable under a large excess of the reagent. The absorbance was unchanged for a period of 15 days. The extraction of vanadium is rapid, and a shaking time of 5 min is sufficient for complete extraction. A solution of 3.72 ppm of vanadium was extracted according to the procedure, and the aqueous phase was re-examined by repeating the extraction with another 5 ml of the reagent solution and measuring the absorbance of the organic phase. There was no difference between the absorbance of reagent blank and the organic phase on repetition. Further, the absence of vanadium in aqueous phase was confirmed by the atomic absorption technique. The analytical data on the extraction of vanadium are given in Table 5.

TABLE 5
ANALYTICAL DATA ON EXTRACTION OF VANADIUM(V)[a]

Vanadium taken (ppm)	Vanadium found (ppm)	(Theory − found)	Standard deviation[b] (σ)
0.93	0.92	−0.1	±0.02
1.86	1.85	−0.1	±0.01
3.72	3.72	±0.0	±0.01
5.58	5.60	+0.02	±0.02
7.44	7.45	+0.01	±0.01
11.16	11.15	−0.01	±0.01
24.18	24.20	+0.02	±0.02
48.36	48.35	−0.01	±0.02
100.00	100.02	+0.02	±0.02

[a] Concentration of HCl = 8*M*; maximum absorbance = 545 nm.
[b] Eight determinations.

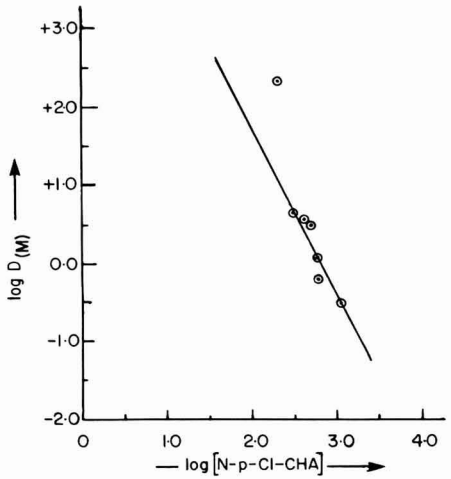

FIG. 4. Plots of distribution constant of metal [D_M] against [ligand], vanadium–(*N*-*p*-Cl-CHA), in chloroform.

Effect of diverse ions. The interference due to various diverse ions in the direct spectrophotometric determination of vanadium(V) was studied with *N*-*p*-Cl-CHA. Vanadium, 46.5 ppm in 25 ml of aqueous solution, was determined in presence of following ions: Ba^{2+} (30 mg), Ca^{2+} (30 mg), Cd^{2+} (30 mg), Co^{2+} (30 mg), Cu^{2+} (30 mg), Hg^{2+} (40 mg), Mn^{2+} (30 mg), Zn^{2+} (30 mg), Fe^{3+} (30 mg), Pb^{2+} (30 mg), Ni^{2+} (30 mg), Al^{3+} (30 mg), WO_4^{2-} (30 mg), UO_2^{2+} (40 mg), Ce^{4+} (40 mg), and Os^{6+} (50 mg). The interference of molybdenum (30 mg), titanium (30 mg), and zirconium (30 mg) was eliminated by adding NaF solution before extraction. The complexing ion fluoride has no effect on the extraction and determination of vanadium(V).

Stoichiometry of the complex. The extraction was carried out by taking

TABLE 6
ANALYSIS OF NBS AND BCS STANDARD SAMPLES

No.	Sample	Vanadium		
		Standard	New method found	Standard deviation (σ)
67	Manganese steel (NBS)	0.17–0.19	0.185	±0.01
117	Ferrotitanium (NBS)	0.05–0.08	0.062	±0.01
132	Steel (NBS)	1.60–1.68	1.69	±0.02
224	Cr–V steel (BCS)	0.240	0.24	±0.01
241/1	High Speed (BCS)	1.570	1.568	±0.01

TABLE 7
VANADIUM IN PHOSPHORITES OF MUSSOORIE PHOSPHATE DEPOSITES

No.	Sample No.	Vanadium (ppm)	Standard deviation
1	MS-I	320	±0.2
2	MD-I	320	±0.1
3	DV-I	310	±0.1
4	DV-II	300	±0.2
5	DV-III	310	±0.1

fixed amount of vanadium and then adding to it 0.5 ml of 8.472×10^{-4} *M* reagent solution. Then successively increasing the amount of the reagent, the distribution of vanadium in the aqueous and organic phases was determined. A graph was plotted between log D_M and −log (ligand) which gave a straight line of slope 2.0 (Fig. 4). It shows that the ratio of M:L, vanadium to ligand, is 1:2.

In addition, the ratio of vanadium to *N*-*p*-Cl-CHA (1:2) is also confirmed by Job's continuous variation and molar ratio methods.

Determination of vanadium in steel. To test the reliability of the method

TABLE 8
VANADIUM IN NATURAL RESOURCES

Sr. No.	Sample	Vanadium found (ppm)	No. of determinations	Standard deviation
1	Potato sample 1	5.72	10	±0.05
2	Potato sample 2	6.82	8	±0.09
3	Onion sample 1	7.45	10	±0.05
4	Onion sample 2	6.83	10	±0.02
5	Seawater	5.72	10	±0.01
6	Lake water	1.53	8	±0.02
7	Carrots	3.55	8	±0.02
8	Spinach	6.35	8	±0.02
9	Cabbage	5.75	10	±0.02
10	Nonripe mango skin	1.54	9	±0.03
11	Ripe mango skin	3.55	10	±0.03
12	Tobacco	2.05	9	±0.01
13	Corn leaves	1.05	9	±0.01
14	Tomato	1.55	8	±0.04
15	Rat liver	0.05	12	±0.01
16	Rat kidney	0.01	12	±0.01
17	Rat bone	0.01	12	±0.01
18	Rice	1.0	10	±0.02
19	Peas (leaves)	0.75	12	±0.01

five samples from the U.S. National Bureau of Standards and Bureau of Analysed Samples Limited were dissolved in conc HCl, oxidized with conc HNO_3, and evaporated to dryness. The residue was redissolved in conc HCl. Then a 0.1 *M* potassium permanganate solution was added dropwise until the pink color persisted for 5 min. The solution was finally diluted to 100 ml with 0.05 *M* HCl. The results presented in Table 6 show that vanadium can be determined precisely with the accuracy of ±0.02 ppm.

Determination of vanadium in phosphorites. Five samples from Mussoorie Phosphate Deposites were analyzed for their vanadium content. Solutions of phosphorite samples were prepared as "B" solutions by the Shapiro and Brannock method (*17*). The data are given in Table 7.

Determination of vanadium in natural resources. This method was found to be sensitive, convenient, and rapid for the extraction and spectrophotometric determination of vanadium. Generally plants have higher amounts of molybdenum and titanium which interfere in the direct determination of vanadium. In the present method these were eliminated by masking with NaF, and vanadium was determined precisely. The data on the vanadium content found in natural resources are given in Table 8.

SUMMARY

A new reagent, *N*-*p*-chlorophenylcinnamohydroxamic acid, for the rapid solvent extraction and spectrophotometric determination of microgram quantities of vanadium(V) is reported. Vanadium(V) is extracted from a chloroform solution of the reagent, *N*-*p*-chlorophenylcinnamohydroxamic acid, with 4–8 *M* HCl. The bluish violet extract has maximum absorbance at 545 nm. The extract obeys Beer's law at 545 nm, and the sensitivity of the reagent is 0.008 μg V/ml. The complex is stable for several days. The effects of acidity, reagent concentrations, and diverse ions are discussed.

ACKNOWLEDGMENTS

The authors are indebted to Prof. S. K. Banerjee, Head, Pharmacy Dept., M.S. University of Baroda, for providing the necessary facilities. They are grateful to Dr. A. K. Ganguly, Director, Chemical Group, B.A.R.C., Bombay, for permitting us to use AAS. One of the authors (R.D.R.) thanks U.G.C., New Delhi, for awarding the Junior Fellowship.

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BOOK REVIEWS

Protein Crosslinking, Biochemical and Molecular Aspects. Edited by MENDEL FRIEDMAN. Advances in Experimental Medicine and Biology, Volume 86A. Plenum Press, New York, 1977. xix + 760 pp. $59.50.

This is the first volume of the Proceedings of the Symposium on Protein Crosslinking held at the A.C.S. meeting in San Francisco, California in September 1976. It consists of forty-three contributions, many of which are concerned with the general topic currently discussed under the descriptor "affinity labeling."

Among the topics discussed are: disulfide crosslinks and thiol–disulfide interchange; mechanism of renaturation of proteins containing disulfide bonds; a very important and novel strategy for studying the antigenic structures of disulfide–containing proteins as exemplified by application to hen egg white lysozyme (by M. Z. Atassi and co-workers); crosslinking of antibodies; crosslinking of plant lectins, of ribosomes, of insulin, of keratin, of wool, of collagen, and of proteins to nucleic acids.

Among the techniques employed for crosslinking are: bifunctional antigens, hydrophobic and hydrophilic reagents, thermal crosslinking, ethylene glycol, cyanoborohydride-induced reductive amination, ionizing radiation-induced crosslinking, ultraviolet-light-induced crosslinking, photoaffinity labeling, and crosslinking by formaldehyde, bisulfite ion, peroxydisulfate, and mercaptoimidates.

In addition, there are discussions of the thermodynamics of crosslinks and the design and synthesis of novel crosslinking reagents. The volume represents a very wide breadth of interests and should be a valuable addition to a library collection. Further comments are presented at the end of the review of the second volume.

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Kirk–Othmer Encyclopedia of Chemical Technology, Vol. 1, 3rd ed. Editorial Board, HERMAN F. MARK, DONALD F. OTHMER, CHARLES G. OVERBERGER, AND GLENN T. SEABORG; Executive Editor, MARTIN GRAYSON; Associate Editor, DAVID ECKROTH. Wiley–Interscience, New York, 1978. xxix + 967 pp. $120.00; subscription price, $95.00 per volume.

There are eighty contributors to this monumental piece of work which deals with subjects and substances beginning with the letter A (abherents to alkanolamines). Two other volumes are planned to cover other subjects and substances beginning with the letter A, and other volumes down the alphabet.

There is a wealth of information, which should prove to be invaluable and should find a place in all libraries used by chemists. The price of the volume, and supposedly of those planned, is one that only libraries can afford.

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The Determination of Organic Compounds with *N*-Bromosuccinimide and Allied Reagents. By N. K. MATHUR AND C. K. NARANG. Academic Press, New York, 1975. ix + 166 pp. $13.75.

This book gathers the existing material on the use of the *N*-haloamides and *N*-haloimides for the determination of a number of different types of organic compounds and is most helpful in this respect. These include hydroxyl, phenols, amines, sulfonamides, unsaturation, hydrazines, amino acids, sulfur, etc. For the most part, the book is written as a review, but there are numerous procedures given.

Persons involved in organic reactions will find the book to be of considerable value.

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Laboratory Handbook of Paper and Thin-layer Chromatography. By J. GASPARIČ AND J. CHURÁČEK. John Wiley & Sons, New York, 1978. 362 pp., $42.50.

In recent years paper and thin-layer chromatography have changed largely from an art form to a scientific discipline, with the selection of a suitable solvent system now being derived from knowledge of the substances to be chromatographed. The authors' intent is to present up-to-date information on practical laboratory methods for applying paper and thin-layer chromatography to the analysis of a variety of chemical compounds. This volume is part of the Ellis Horwood Series in Analytical Chemistry, edited by R. A. Chalmers and M. R. Masson.

The volume includes 31 chapters, grouped into two parts, an appendix, a list of chemicals for which methods of analysis are presented, and a brief subject index. Part 1 presents the principles and techniques of paper and thin-layer chromatography, typical uses of these procedures, a list of commercial firms supplying chromatographic supplies, and a list of references to the chromatographic literature. Part 2 presents detailed discussions of procedures for the analysis of a variety of chemical compounds by paper or thin-layer chromatography. Each method is referenced, and in most cases complete instructions are provided to allow immediate laboratory use of the procedure. A valuable feature is the numerous tables of R_f values provided throughout the book. A highly useful appendix provides the recipes for 88 different detection reagents mentioned in the book.

The authors have successfully achieved their objective and have produced a good laboratory handbook. A special compliment must be paid to Z. Procházka who completed this excellent translation into English. There is much of interest in this book for those working with paper and thin-layer chromatographic procedures.

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Microscope Technique. By W. BURRELLS. Halsted Press, New York, 1978. xiv + 574 pp., $25.00.

This book is an expanded revision of Burrells' book originally published in 1961 under the title "Industrial Microscopy in Practice." It is a complete, well-written, and worthy addition to the literature of microscopy. It is not elementary and more space is given to matters and procedures not usually covered in detail. Methods for dealing with any microscopical prob-

lem are so clearly described that the laboratory worker can easily adapt them to any particular situation.

After a short introduction, 31 chapters cover every aspect of microscopy from the care of the instrument to phase, interference, reflecting, and electron microscopy plus the proton microscope. The chapter on methods for handling small amounts of material is a veritable course in microchemical techniques. This reviewer read the book straight through (it is better than many modern novels) making copious notes for the improvement of his own personal techniques.

It is amply illustrated and includes a glossary of terms as well as a comprehensive index. "Microscope Technique" is highly recommended to everyone who ever has any use under any circumstances for a microscope, either infrequently or all the time. It will serve as a refresher for the veteran and an instructor for the novice. It should be in every library—personal, industrial, academic, and even public—and the modest price makes this possible.

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Ion Chromatographic Analysis of Environmental Pollutants. By E. SAWICKI, J. MULIK, AND E. WITTGENSTEIN. Ann Arbor Science, Ann Arbor, 1978. 210 pp., $28.00

Ion chromatography is a brand new technique for measuring water-soluble anions and cations. The technique involves the use of ion exchange principles modified so as to allow the use of electrical conductance as a means for quantifying the eluted ionic species. The purpose of this book is to describe this new method and discuss its application in the analysis of many environment pollutants. The articles making up the contents of this book were originally presented at a Symposium on Ion Chromatographic Analysis of Environmental Pollutants.

The book consists of 15 articles and a detailed subject index. The first two articles discuss the technique of ion chromatography and its potential usefulness as an analytical procedure. The next 10 articles present the application of ion chromatography to the analysis of atmospheric pollutants such as sulfur dioxide, ammonium ion, and alkyl amines, and for the analysis of stationary source and mobile source emissions. Article thirteen examines the use of ion chromatography for organic elemental microanalysis and article fourteen looks at its application to the quantitative determination of inorganic salts in certifiable color additives. The last paper is an evaluation of the analytical perspective of ion chromatography. Following many of the papers is the discussion which followed at the symposium, which adds additional information about the subject presented in the paper.

This book performs its function of discussing this new technique and its applications to the analysis of environmental pollutants. The book and the technique described should be of interest to a variety of scientists faced with a need to analyze water-soluble cations and anions.

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Modern Methods for Trace Element Analysis. By M. PINTA. Ann Arbor Science, Ann Arbor, 1978. 492 pp., $29.50.

The level of concentration which is considered "trace" must constantly be redefined because of the continuous improvement in the sensitivity of analytical instruments and the development of new instruments and procedures. The availability of these instruments and

improved procedures have enabled investigators to complete studies previously impossible and to discover correlations unrecognized due to lack of means to detect small differences in concentration. The purpose of this text is to examine these new and improved instruments and techniques and describe how they are used.

The text is divided into 10 chapters, 11 appendices, an author index, and a subject index. The first chapter examines trace analysis by fluorometric means and presents methods for a large number of elements. Chapters two and three examine emission spectroscopy and its application to the analysis of a variety of natural and industrial materials. Flame and flameless atomic absorption spectrometry are discussed in chapters four and five, including the advantages and disadvantages of these two methods for trace elemental analysis. The use of atomic absorption spectrometry for trace analysis is covered in chapter six, with specific sections on analysis of minerals, water, biological materials, atmospheric samples, and industrial materials. Chapters seven and eight focus on atomic and X-ray fluorescence spectrometry methods and instrumentation. Activation analysis, using thermal or fast neutrons, high-energy charged particles, or high-energy γ-rays is carefully evaluated in chapter nine. Chapter ten is a short review of the application of trace analysis to environmental studies. The 11 appendices present tables of data and graphs keyed to the different analytical methods presented in the text.

The presentation of material is clear and straightforward, and the information is well documented. The author has prepared a highly useful review of the state-of-the-art in trace analysis.

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Ion-Selective Electrodes in Organic Elemental and Functional Group Analysis: A Review. By W. SELIG. Lawrence Livermore Laboratory, University of California, Livermore, California, 1978. iv + 127 pp.

This excellent, timely review is a "wind-fall" for those wishing to begin work with ion-selective electrodes. It reviews the literature on anions, cations, sufactants, and detergents. The functional groups reviewed include thiols, disulfides, and several others classified as divalent sulfur functions. The sections on compounds containing hydroxyl groups treat not only types but individual compounds, such as chymotrypsin, cyanocobalamine, saccharin, ephedrine, etc. Finally treated is the subject of ion-selective electrodes as detectors in gas and liquid chromatography. The survey is complete through *Chemical Abstracts*, Vol. 83 (1975).

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Applications of Ion-Selective Membrane Electrodes in Organic Analysis. By GEORGE E. BAIULESCU AND VASILE V. COŞOFREŢ. (Translation Editors: R. A. CHALMERS AND MARY R. MASSON). Halstead Press (Wiley), New York, 1977. 235 pp., $37.50.

This review of applications of ion-selective membrane potentiometry may be most appropriately described in a word as useful. Behind the modest format and the moderate length lies a very readable compendium of considerable scope. The readability is undoubtedly enhanced by both a lucid translation and the liberal inclusion of figures and tables, so that it is rare to find two contiguous pages of text without a helpful graph or table of some kind.

Part I of this volume is a brief discussion of the principles of potentiometry with selective electrodes. Although it covers such topics as selectivity coefficients and response times of the electrodes, it will not suffice as a framework for the naive reader and requires a basic knowledge of electrochemistry. Chapter 5 of Part I is of particular interest as it deals with the use of selective electrodes in nonaqueous or partially nonaqueous solutions.

Part II, comprising 12 chapters on applications, is the *raison d'ètre* of the book. Although the analysis of several classes of organics, including hydroxy compounds, sulfur compounds, carboxylic acids, and halides, is reviewed, this section is of particular merit from a biochemical and pharmaceutical point of view. The reader can find mention of ion-selective electrode methods for anionic and cationic detergents (Chap. 11), biguanides (Chap. 12), amygdalin (Chap. 14), ephedrine, acetylcholine, amino acids, and sulfa drugs (Chap. 15), and uric acid, penicillins, and vitamins (Chap. 16). The last chapter discusses some recent uses of the electrodes for determining enzymic activity; techniques for cholinesterases, glucosidases, chymotrypsin, asparaginase, and amino acid and glucose oxidases, among others, are outlined.

One caution (about the technique, not the book) is that the sensitivity of the method is usually comparable to a corresponding spectrophotometric assay (microgram per milliliter range); hence, the use of this technique would more probably be in the area of analytical quality control, rather than, for example, in the detection of drugs in biological fluids, where submicrogram per milliliter sensitivity is often required.

This work reviews over 650 references, some of them published as late as 1976. Several tests of the subject index revealed it to be thorough and valuable.

The book jacket represents this volume as the first English-language treatment of ion-selective electrode analysis. As such, it is certainly done well enough to merit a place in the library of analytical chemists.

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Particle Size Analysis. Edited by JOHN D. STOCKHAM AND EDWARD G. FOCHTMAN. Ann Arbor Science Publishers, Ann Arbor, 1977. xi + 140 pp., $24.00.

This book gives the papers originally presented at the 1975 meeting of the Midwest Chapter of the Filtration Society of England by members of the Fine Particles Research Section of the IIT Research Institute. The 12 chapters present an excellent introduction and guide to particle size analysis. It outlines the techniques of presenting particle size data and the basic principles of many of the instruments used to measure particle size. It does not give complete information as to the total details of particle size measurements. However, once the type of particle size information that is required for a given application has been established, it offers information on the selection of the instrument most suitable for this purpose. The book is well indexed and the cited references would give the reader the key to solving the particle size measurement problem. The basic coverage in the book includes the statistics of particle size measurements, particle sizing using the optical microscope, sizing with modern image analyzers, sedimentation methods, optical methods of particle size analysis, centrifugal particle size analysis, analyzers for particle size measurement of aerosols, and particle size measurements using "electrozone" counters. The book will be of great value to readers encountering a particle size problem.

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Announcement

8th International Microchemical Symposium

Graz, Austria

August 25–30, 1980

The goal of the Symposium is to emphasize the relations between the different disciplines of microchemistry and the evolution since the pioneer work performed by Fritz Pregl and Friedrich Emich in Graz and many others.

Inquiries should be directed to the President of the Symposium:

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Austria