PHYSICAL METHODS of Organic Chemistry

SECOND EDITION

PART III

TECHNIQUE OF ORGANIC CHEMISTRY

Volume I—Part III

PHYSICAL METHODS of Organic Chemistry

Second Completely Revised and Augmented Edition

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PART THREE

INTRODUCTION

Organic chemistry, from its very beginning, has used specific tools and techniques for the synthesis, isolation, and purification of compounds, and physical methods for the determination of their properties. Much of the success of the organic chemist depends upon a wise selection and a skillful application of these methods, tools, and techniques, which, with the progress of the science, have become numerous and often intricate.

The present series is devoted to a comprehensive presentation of the techniques which are used in the organic laboratory and which are available for the investigation of organic compounds. The authors give the theoretical background for an understanding of the various methods and operations and describe the techniques and tools, their modifications, their merits and limitations, and their handling. It is hoped that the series will contribute to a better understanding and a more rational and effective application of the respective techniques. Reference is made to some investigations in the field of chemical engineering, so that the results may be of assistance in the laboratory and help the laboratory chemist to understand the problems which arise when his work is stepped up to a larger scale.

The field is broad and some of it is difficult to survey. Authors and editor hope that the volumes will be found useful and that many of the readers will let them have the benefit of their criticism and of suggestions for improvements.

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TECHNIQUE OF ORGANIC CHEMISTRY

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PHYSICAL METHODS OF ORGANIC CHEMISTRY

From the Prefaces to the First and Second Editions of Parts I and II

In recent years, the science of physics has become increasingly important to the organic chemist. Physics has given much greater precision to the concept of atoms, bonds, and structural formulas, and it has made possible the development of new, and the improvement of older, methods for the examination of chemical systems. With the increasing number and complexity of physical methods for the treatment of organic chemical problems there has resulted a specialization of research workers in the methods which they employ, and the selection of a research problem is frequently governed more by the physical method to be used than by the chemical nature of the problem. Some workers have made themselves familiar with several methods in order to deal with their individual problems. In other cases, however, physical methods have been used without adequate preparation.

The chemist, in order to acquaint himself with a certain physical method, has in the past been compelled to search through periodicals and specialized books. The present work has been compiled with the hope of relieving him of much of this burden. It has been the object of the authors to provide a description of tested methods, the theoretical background for understanding and handling them, and the information necessary for a critical evaluation of the experimental results.

Because of the diversity of the methods discussed, no attempt has been made to secure a uniformity of presentation which might have been desirable for formal reasons. In some chapters a discussion of theory was unnecessary, in some a relatively brief theoretical treatment sufficed, and in other chapters a rather complete exposition of the theory appeared necessary. Some methods have been treated in monographs, while for others no comprehensive modern presentation is available. Therefore, a rather severe selection and delimitation of material was exercised in some chapters and a more complete treatment given in others.

The book is also calculated to appeal to the student who seeks to increase his understanding of the methods described, although he may not practice them himself. For him, chapters like those on x-ray and electron diffrac-

tion should be adequate, but the practical application of these techniques will require the use of the supplemental literature to which reference is made.

The title, *Physical Methods of Organic Chemistry*, has been called too narrow. "Organic Chemistry" distinguishes the methods described from those physicochemical methods which, though essential in other fields, are less important for, or not applicable to, organic chemistry—for example, methods employing very high temperatures. We do not, of course, suggest that the methods described are applicable to organic problems only. Though it is gratifying that workers in other fields have found the book useful, it is our chief object to provide information on the physical methods used by chemists, physicochemists, physicists, biologists, and other research workers in dealing with organic chemical problems.

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Preface to Part III

The first edition of this treatise appeared in 1945 (Part I) and 1946 (Part II). It was followed by a second edition in 1949 (Parts I and II); in this, new chapters were added and others were rewritten, substantially revised, or enlarged.

As can be expected in a rapidly expanding field, it has again become desirable to present newly developed methods, and a new edition was considered. Closer analysis, however, showed that large sections of the second edition of *Physical Methods of Organic Chemistry* do not require revision, and it did not seem right to link the new with the unchanged so that owners of the second edition would have to duplicate material on their shelves in order to gain access to the new and the revised chapters. The second edition is, therefore, expanded and rejuvenated by the present Part III rather than made obsolete by a new edition.

Physical Methods of Organic Chemistry, Part III, contains the following new chapters in fields not treated or only touched upon in the first two parts: Electron Microscopy, by F. A. Hamm; Microspectroscopy, by E. R. Blout; Determination of Streaming Birefringence, by R. Signer; Measurement of Dielectric Constant and Loss, by J. G. Powles and C. P. Smyth; Radio-frequency Spectroscopy, by B. P. Dailey; and Neutron Diffraction, by J. M. Hastings and L. Corliss. Additions to chapters contained in Part II have been supplied by T. E. McGoury and H. Mark, Viscometry of Dilute Polymer Solutions; D. Harker, Determination of Crystal Structure of Organic Compounds by X-Ray Diffraction; L. O. Brockway, Electron Diffraction by the Sector-Microphotometer Method; J. F. Bonner, Determination of Radioactivity by Scintillation Counting; and a new presentation of Determination of Magnetic Susceptibility has been contributed by P. W. Selwood.

A. W.

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CHAPTER XXXII

ELECTRON MICROSCOPY

F. A. HAMM, *Eastman Kodak Company*

I. INTRODUCTION

Busch[1] was the first to demonstrate the feasibility of an electromagnetic lens which would overcome the limitations imposed upon light microscopy by the equation:

$$d = k\lambda/(n \sin \alpha) \qquad (1)$$

[1] H. Busch, *Ann. Physik*, **81**, 974 (1926).

where λ is the wave length of the illumination, n is the refractive index of the medium in which the objective lens is immersed, α is one-half the angular aperture of illumination to the objective, and k is a constant determined by the particular conditions of illumination; d is the limit of resolution for the light microscope. Jelley[2] has outlined the usefulness of the light microscope. For resolution of structures finer than about 0.5 micron, illumination of shorter wave length is needed. Although protons, x-rays, and electrons have been used, only electrons have gained practical importance in

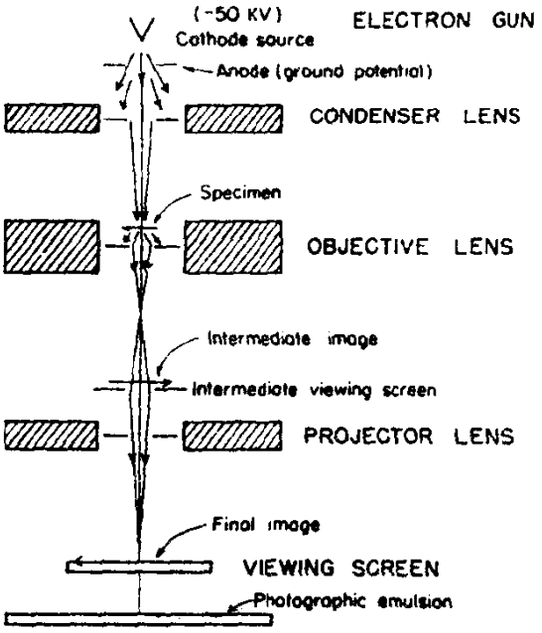

Fig. 1. Schematic representation of an electromagnetic electron microscope.

microscopy. In the applications of the electron microscope described in Section III, for the most part 50 k.v. electrons have been used with a wave length of about 0.054 Å. Hall has described the relative merits of using electrons of different accelerating voltages as well as the commercially available instruments. His recent book and the others listed under General References should be consulted for a detailed description of the physical principles involved in the design of electron microscopes as well as for discussions of general instrumentation. For current developments, the reader is referred to the *Proceedings of the Electron Microscope Society of America* published each year in the *Journal of Applied Physics*.

[2] E. E. Jelley, in A. Weissberger, ed., *Physical Methods of Organic Chemistry (Technique of Organic Chemistry*, Vol. I). Interscience, New York-London, 1949, Part I, Chapter XV.

Figure 1 illustrates the general concept of image formation in the electron microscope, specifically in a completely electromagnetic instrument of the RCA-EMU type. The horizontal lines within each electromagnetic lens intercept electrons of relatively high angular aperture; these lines represent the lens apertures. The final viewing screen serves as a shutter and is tilted out of the beam during the recording of the image. The projector lens uses electron rays of very small angular aperture and forms an image at high magnification so that the final image is in focus for any position of the photographic emulsion; the *depth* of focus is essentially infinite. In practice it is usually conveniently located several inches below the phosphorescent screen.

An intermediate projector lens[3] may be inserted just below the objective lens so that the image is formed in *three* stages. Greater flexibility in varying the final magnification and simplicity in using the electron microscope for diffraction studies are the major advantages. This lens and the self-biased electron gun not shown in Figure 1 are now used routinely in many laboratories.

The theoretical limit of electron microscope resolution is largely determined by spherical aberration and diffraction. In fact, this limit is a compromise between the two effects. To a fair approximation, this limit is determined by the equation:

$$d \cong C_s^{1/4} \lambda^{3/4} \tag{2}$$

The electron wave length is represented by λ and the spherical aberration constant by C_s. Based on the diffraction effects inherent in the small angular apertures of illumination normally used (*ca.* 1×10^{-4} radian) and the spherical aberration constants (*ca.* 0.05 cm.) typical of present day objective lenses, a theoretical instrumental resolving power of 5 to 10 Å. is usually the optimum resolution. In routine electron micrography, resolutions of the order of 50 to 100 Å. may be considered a good average.

Many factors may adversely affect the formation of the image. Electron scattering by the specimen, specimen instabilities, lens defects, and faulty electrical power supply regulation play an important role. Inadequate contrast is often a serious limitation in the study of fine detail even in a high quality image. Also, contrast—especially edge contrast caused by Fresnel diffraction—is *less* for an in-focus image than for an out-of-focus image. The fundamental aspects of contrast are discussed in Section II.3.

The electron microscope is useful from the limit of light microscope

[3] J. Hillier, *J. Applied Phys.*, 21, 785 (1950).

resolution to the dimensions of very large organic molecules. While, however, light optics can be used to record a specimen under dynamic conditions, the electron microscope specimen is static, since it is desiccated and immobilized. Combined use of both techniques may therefore be desirable. For example, the growing of crystals in their mother liquor, the extrusion of synthetic polymers, the passage of fibers over spindles during weaving, the action of lubricants during motion of bearings or pistons, etc., may be photographically recorded by means of photomicrographic or flash camera techniques, while the fine detail may be observed with the electron microscope. Section III of this chapter illustrates the electron microscope approach to some of these examples.

During recent years a growing majority of scientists have shifted their attention from achieving the optimum in electron microscope resolution toward a better understanding of micrographic detail in the region from several hundred to several thousand angstroms. While the 50 Å. region is certainly of great interest, a better understanding of structures just below the limit of optical resolution is likewise important. Later sections of this chapter will illustrate that, in examining organic materials, one is seldom concerned with fine structure approaching in size the limit of resolution of the instrument. Thus, instrument magnifications from 5000 to 20,000 diameters with subsequent optical enlargements of from 5 to 10 diameters represent the bulk of electron micrography. The average human eye can readily resolve 0.2 to 0.5 mm. so that details of the order of 50 Å. may conveniently be studied in a good quality micrograph enlarged to a final magnification of 100,000×.

At present more than 500 electron microscopes are in use. In this chapter only sparse information is given on the instrument and its manipulation. On the other hand, the preparation of specimens, the specimen-image relations, and the interpretation of the electron micrographs of organic materials are emphasized. Considerable emphasis is placed on electron-specimen interactions because this important subject is often not well understood. This discussion leads to an evaluation of the factors that account for the formation and the quality of the image. Alterations in the specimen leading to erroneous interpretation are discussed together with techniques to avoid them.

A few general aspects of specimen preparation are discussed in Section II. However, because electron microscopy has become highly diversified and specialized, a number of specific applications are given in Section III. These, together with the interpretations of the electron micrographs, serve to illustrate the value of the electron microscope.

No electron micrograph can give valid information unless the specimen is truly representative of the gross sample. In this regard, one must bear in mind that an electron microscope specimen grid may contain only several micrograms of material and represent an area only 4×10^4 square microns (6×10^{-5} square inch). Relatively few organic chemists carry out their own electron microscope program, whether in routine service work or in fundamental research. It is hoped that this chapter will assist in the understanding and evaluation of the electron micrographs by the chemist experienced in other aspects of the problem, so that he will be better able to judge how extensive an electron microscope program should be planned.

II. GENERAL

Because the 50 k.v. electron penetrates only about 0.2 mm. of air at normal pressure, the electron microscope must be operated at such a pressure that the mean free path of the electron is greater than its path from the source (electron gun) to the final image recorded by the photographic emulsion. For this reason, pressures of the order of 0.1 μ of mercury or less are normally used. The vapor pressure of the specimen must therefore be considered; liquids (in the normal sense) and certain solids are therefore precluded as specimens.

1. Specimen Mounts

With the exception of replicas (see Section II.2) most objects are particulate in nature and therefore often need support in the instrument. The specimen particles are usually mounted on 100–200 Å. thick films of nitrocellulose, cellulose acetate, or Formvar (polyvinyl formal). These supporting films are usually referred to as substrates. A convenient method of preparation is to cast a dilute solution of the polymer, 10% or less, in amyl acetate, dioxane, or ethylene dichloride on clean, motionless water by allowing a drop of the solution to spread on the water surface. A 0.2% ethylene dichloride solution of Formvar has been extensively used. Also, thin films on grease-free glass, such as light microscope slides formed by dipping the glass in the appropriate solution followed by draining and drying, may be floated off on water by scoring the edges and gently sliding the combination at a small angle into water. If properly prepared, these plastic organic substrates are quite stable to the electron beam and introduce no inherent structure (micelles) of their own above the 50 Å. level.

Occasionally substrates having less structure, lower solubility, or greater

chemical inertness may be required. For example, the micrography of single synthetic macromolecules with molecular weights varying from several hundred thousand to several million, dispersed on organic substrates, is seriously impaired by the background structure of the supporting film.[4] Similarly, strong acids holding pigment particles in suspension may hydrolyze the usual organic films. In these cases substrates may be prepared[4] by evaporating *in vacuo* materials such as SiO, SiO_2, Be, Al-Be alloys, etc. Films calculated to be 40 to 150 Å. thick condensed from the vapor state on glass or cleaved mica may again be conveniently floated free on water. Freshly cleaved mica presents an extremely smooth condensing surface.

The substrates must receive some support when used in the instrument. For this purpose, they are mounted on round, $^1/_8$-inch diameter meshes (grids) made of copper, nickel, or stainless steel. The substrates must be scored at some time during their preparation to give square or circular areas commensurate in size with the specimen grids. Usually the small areas of supporting film floating on water are picked up directly from beneath by raising the specimen grids up through the water. The particulate matter such as polymer latices, dyestuffs, fibers, catalysts, etc., are pipetted onto the coated grids and after drying are ready for examination in the electron microscope. When the dispersion of aggregates into the component particles presents a difficulty, the solid matter may be milled in a solution of the plastic and the mixture then cast on glass or water. The shearing forces of the polymer solution are put to advantage, especially if a viscous solution is first used; such a system requires dilution with solvent so as to yield a final mixture that spreads to a final film thickness of about 100 Å. In specimens of this kind the dispersed solid is an integral part of the substrate. This procedure has often been used in the study of organic pigments.

In general, organic specimens should be less than 0.5 micron thick for stability, and preferably of the order of 5×10^{-2} to 1×10^{-1} micron thick to permit detection of fine detail. Biologists at present are utilizing microtome sections only about 250 Å. thick.

2. Replicas

Many organic materials can be examined in their natural state. However, even relatively thin materials such as natural and synthetic fibers, paint films, and photographic film base, varying in thickness from several

[4] J. J. Comer and F. A. Hamm, *Anal. Chem.*, 24, 1006 (1952).

microns to 20 or 30 microns, absorb so much energy from the electron beam that opacity and thermal degradation become prohibitive. In such cases the original samples cannot be used but replicas of their surface must be used as specimens. The books by Wyckoff and Hall listed under General References describe the various kinds of replica techniques now considered acceptable and only a brief discussion of this subject is given here. Special applications are discussed in Section III.

Replica techniques are *indirect* methods for examining *surface* topography. A replica is a thin film (50 to 200 Å.) of material of high electron

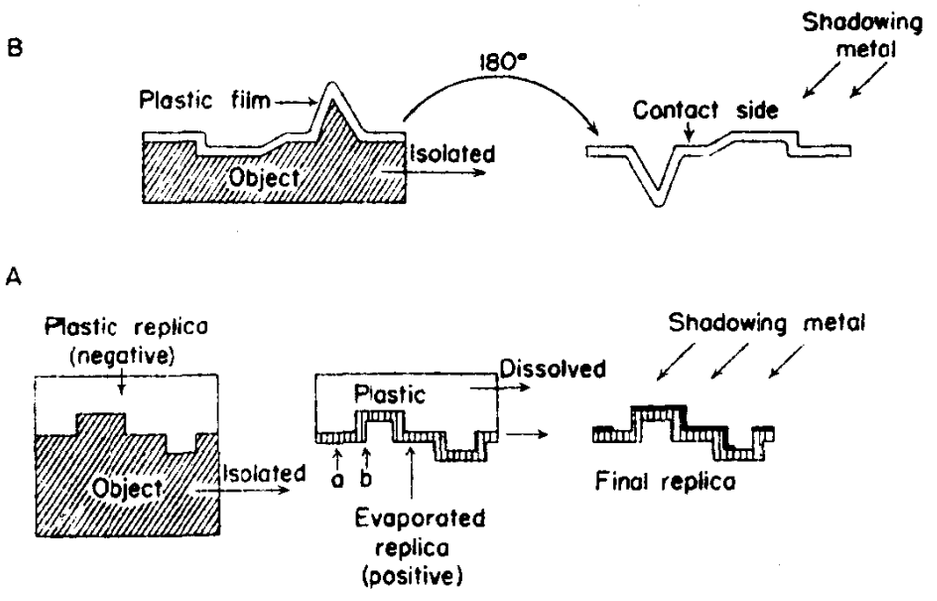

Fig. 2. Schematic illustration of the preparation of common types of replicas: (A) Two-step or negative-positive combination producing finally a positive replica. (B) One-step type producing a negative replica.

transmittance that may be positioned in precise contact with the original surface and later isolated and examined directly in the electron microscope. The usual materials are Formvar or collodion-type substances cast from solution, or $SiO_2(SiO)$ condensed in a high vacuum from the vapor state. In preparing the cast replicas from solution, the original sample is flooded or immersed in a solution of the plastic (analogous to those used for preparing substrates), suspended vertically for quick draining, and allowed to dry in a relatively moisture-free atmosphere. Separation may be effected by gently teasing the replica from the original surface, usually under water, or by dissolving the original sample. If the replica is damaged because of a particular procedure required for its isolation from the original material, an intermediate hydrophilic stripping layer may be used.

Comparatively little use has been made of this latter technique. The added step in specimen preparation and the introduction of undesirable structure above the 50 Å. level by evaporated films of sodium chloride, for example, have caused many electron microscopists to avoid this step. However, the swelling of organic materials such as cotton, cellulose acetate fibers, photographic film base, etc., before dissolution, or the mechanical difficulties encountered in applications to metallographic specimens make the use of a hydrophilic stripping layer highly desirable. The need for a material that could be used as a stripping layer only a few angstroms thick and that would impart no discernible structure to the subsequent replica appears to have been filled. Victawet 35B fulfills these requirements; its application is given in more detail in Section III.1.B.

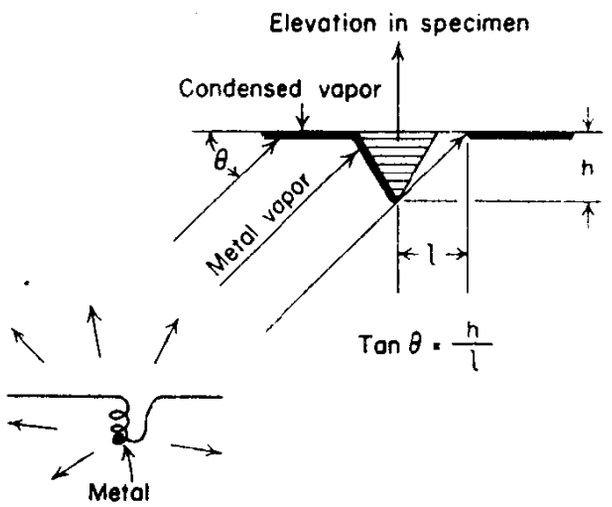

Fig. 3. Schematic illustration of the metal shadow-casting technique. The angle θ is exaggerated. As in the evaporation steps in Figure 2, the system is under high vacuum.

Figure 2 illustrates the major steps in preparing the common types of replicas. Section II.3A treats image contrast quantitatively. Qualitatively, points *a* and *b* in Figure 2A show that the replica exhibits some contrast because the electron path through it varies in length depending on whether the electrons strike normal to a flat surface or traverse a sloping surface where the path length is greater. The relatively simple procedure illustrated in Figure 2B produces a *negative* replica; the elevations and cavities are reversed with respect to the original surface.

Shadowing of the replica is almost always imperative in order to provide adequate contrast in the image. Metals such as U, Pt, Pd, Au, and Cr are evaporated in a high vacuum at small angles to the plane of the specimen as illustrated in Figure 3. The metal vapor travels essentially in