Drug Fate & Metabolism

Methods and Techniques Volume 2

Edited by Edward R. Garrett and Jean L. Hirtz

DRUG FATE AND METABOLISM

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VOLUME 2

Edited by

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PREFACE

Historically, the major emphasis in drug development was on the isolation and synthesis of active principles and the evaluation of their safety and efficacy in animals and man. The fate of drugs in the body, which includes their absorption, distribution, metabolism, and elimination, was not emphasized. Systematic studies on the fate of drugs in the body have been conducted only within the last several decades.

Such studies were inhibited by the inadequacies of analytical techniques and methods to isolate, identify, and assay the drugs and their metabolites in the biological tissues and fluids of the organism. Drug metabolism studies were performed as long as a century ago, on quinine (1869), salicylic acid (1877), and morphine (1883) with the simple techniques then available. However, such studies were infrequently done and in limited depth until B. B. Brodie elaborated a general method for the discriminatory extraction of drugs and metabolites from biological fluids during World War II in connection with the United States' antimalarial screening program. At about the same time, L. C. Craig developed countercurrent distribution procedures for separation and identification purposes. By modern standards, methods of quantification then available, which included colorimetry, fluorometry, and ultraviolet absorption spectroscopy, were insensitive and in the microgram per milliliter range.

Pharmacokinetics, the study of the time course of a drug's absorption, distribution, metabolism, and elimination, is another aspect in the fate of drugs and was of even later vintage. Its maturation also depended on the development of sensitive and reliable assays in biological fluids. Probably the first publication in this field of adequate sophistication was on ethanol in 1922. The basic principles of pharmacokinetics were elaborated by Torsten Teorell of Uppsala in 1937, and the first book on the subject was published by F. H. Dost of Berlin, later of Giessen, in 1953. However,

this field did not truly flower until the 1960s, and its initial blossoming was observed at the first international conference on the subject in 1962 held under the initiative of Ekkehard Krueger-Thiemer at Borstel Forschungsinstitut in Germany.

The burgeoning of these studies on drug fate in the organism was fertilized by the development of radiometric techniques when radiolabeled drugs became available. Gas-liquid chromatography, now the most widely used method, provided a simple and inexpensive technique to separate and quantify drugs and their metabolites. Sensitive detectors were developed to provide picogram monitoring of nonlabeled materials. Other analytical and separative methods of high sensitivity and precision became commonplace in the laboratory. Instrumentation became available and less expensive for detection (NMR, spectrofluorimetry, infrared, gas and mass spectrometry, immunoassay) and for separation (thin-layer and high pressure liquid chromatography, etc.).

Today, it can be stated that the sensitivity of analytical detection no longer limits investigation into the fate of drugs. Separation and purification still is a rate-determining factor in assay development and demands a multidisciplined expert in biological, physical, organic, and analytical chemistry.

Similarly, the theoretical bases of pharmacokinetics and the technology of its applications have been expanded and refined within the last two decades. The generalized use of computers has permitted quantification of the models used to describe the totality of processes contributing to the time course of the drug in the body and to relate this time course to that of observed pharmacodynamics and pharmacological and toxicological action. The foundations of a modern pharmacology have been laid down, upon which structure personalized dosage regimens can be predicted for individualized optimum treatment with minimum toxicities; it is upon these premises that action and toxicities in one species can be predicted from studies performed on another.

The insights gained into the mechanisms of drug action provide clues to molecular modification that can best embody the active principal of action. Metabolic engineering can be construed as that practice which modifies the design of the molecule to take advantage of extant metabolic pathways to prolong or shorten the time of drug presence in the body. The clinical awareness that the rate and extent of drug release from a dosage form can perturb the availability and delivery of therapeutic agents has led to the necessity of establishing standards for bioequivalences of formulations. Pharmacokinetics now serves as a basis for these biopharmaceutical necessities.

It is therefore not suprising that the study of drug absorption, distribution, metabolism, and excretion constitutes a large part of the modern research for new and more efficient therapeutic agents. Governmental regulatory agencies in various countries now require precise data on the fate of new drugs and their formulations in animals and man and are increasingly insistent on stricter compliance.

Although there are several books dealing separately with drug metabolism, drug disposition, pharmacokinetics, and the like, a proper compendium has been lacking which encompasses the various fields and provides a delineation and appropriate critique of the useful methods and techniques that can be applied in them.

One of the editors (JLH) published (1968) a book on the analytical techniques (Les Méthodes Analytiques dans les Recherches sur le Métabolisme des Médicaments, Masson, Paris) which was later translated by editor ERG into English (Analytical Metabolic Chemistry of Drugs, Marcel Dekker, New York, 1971). The reception of this book was gratifying and prompted us to bring out the present more comprehensive and modernized series of volumes which includes other methods and techniques in the study of drug fate, not only analytical procedures. Since this ambitious goal exceeded the expertise of only one or a few authors, a multi-author series was projected. Experts were chosen who were highly respected in their fields. We reserved the right, and exercised it, to edit and revise to maintain a reasonable level of homogeneity in conformance with the objectives of the series. We hope we have succeeded.

The intent of these volumes is to review all the techniques, physical, chemical, biological, medical, and mathematical, which can be applied to the study of drug fate in the organism. It is addressed primarily to the research scientist and is devoted to methods, with only the minimal theory given for perspective, appreciation, and proper evaluation of results. The intent was not to compete with the many fine theoretical texts available, but to provide a broad spectrum of information that can be readily utilized by the research worker.

The practical use of these methods is explored fully. The limitations are explained. Necessary precautions and sources of error are delineated. Examples are given of applications in the study of the fate of drugs. When possible, each chapter includes tables that condense the appropriate literature on the particular topic. Each chapter has a selected, adequate, but not exhaustive, bibliography. For a more complete bibliographic survey, the reader is referred to the series edited by editor JLH (The Fate of Drugs in the Organism: A Bibliographic Survey, Marcel Dekker, New York: Vol. 1, 1974; Vol. 2, 1975; Vol. 3, 1976; Vol. 4, 1977).

It was deemed proper to include chapters on methods that would not be modern methods of choice but are of historical importance in evaluating the significance and limitations of the earlier studies in these fields. Whenever possible, a critique is provided, the future development is predicted, and the utility of a considered technique is evaluated.

It is our sincere hope that these endeavors of our dedicated authors will serve the desired purpose.

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(Other volumes in preparation)

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Chapter 1

VOLTAMMETRIC METHODS

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I. INTRODUCTION

The well-documented [1-7] electroanalytical methods for the assay of bulk chemicals, intermediates, and dosage forms are often methods of choice since their relative simplicity and rapidity facilitate the large number of analyses involved in quality assurance studies. The electroanalytical procedures of potentiometry, coulometry (constant current and potential), and voltammetry (polarography and amperometry) typically employed are applicable to the analysis of raw materials and finished products with usual required sensitivities of 2 to 5 μg/ml (10^{-5} M).

The assays of drugs in biological fluids, however, require specific assays with sensitivities in the submicrogram range and are frequently performed with spectrophotometric or chromatographic methods and, more recently, by radioimmunoassay (RIA) rather than by electrochemical methods. Examples of early electrochemical methods used to measure drugs directly in biological fluids are the direct current polarographic assays of 2-ethyl-4-thioureidopyridine [8] and 1,4-benzodiazepines [9,10]. These assays were relatively insensitive (detection limits of >10 μg per ml biological fluid) and were inherently nonspecific in that compounds of similar structure such as metabolites and endogenous biological materials could interfere.

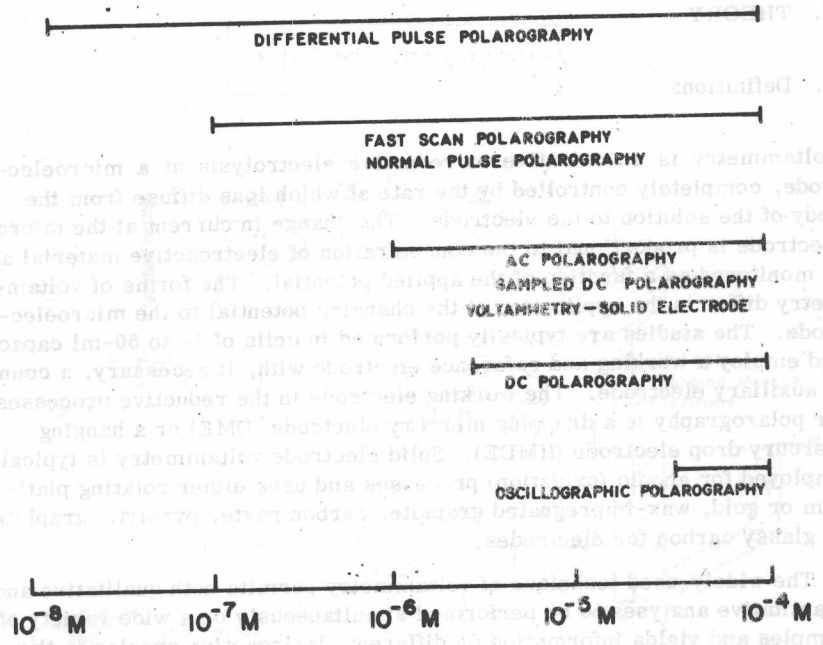

FIG. 1. Range of practical usefulness of voltammetric techniques.

However, recent advances in instrumentation have resulted in newer forms of polarography, capable of greater sensitivity (see Fig. 1) and specificity. Voltammetric methods can now routinely assay drugs with specificity in biological fluids at concentrations as low as 10 ng/ml [11]. The low electroactive background signal from the biological specimen relative to that of the species to be analyzed facilitates analyses on simple extracts, protein-free filtrates, diluted specimens (e.g., urine), or eluted thin-layer chromatography (TLC) separated extracts.

The purpose of this chapter is to review the basic theory involved in voltammetric methods, especially the newer polarographic methods used for drug analysis in biological samples. Specific attention will be given to the compounds and derivatives which can be assayed and the preparation of the sample for analysis. Section VI will review the literature of the voltammetric measurement of drugs in biological fluids. These examples will serve to demonstrate the utility of voltammetric methods in toxicological analysis, in bioavailability and pharmacokinetic studies on drugs, and in metabolite identification.

II. THEORY

A. Definitions

Voltammetry is an oxidative or reductive electrolysis at a microelectrode, completely controlled by the rate at which ions diffuse from the body of the solution to the electrode. The change in current at the microelectrode is proportional to the concentration of electroactive material and is monitored as a function of the applied potential. The forms of voltammetry differ in the application of the changing potential to the microelectrode. The studies are typically performed in cells of 1- to 50-ml capacity and employ a working and reference electrode with, if necessary, a counter of auxiliary electrode. The working electrode in the reductive processes for polarography is a dropping mercury electrode (DME) or a hanging mercury drop electrode (HMDE). Solid electrode voltammetry is typically employed for anodic (oxidation) processes and uses either rotating platinum or gold, wax-impregnated graphite, carbon paste, pyrolytic graphite, or glassy carbon for electrodes.

The widely-used technique of voltammetry permits both qualitative and quantitative analyses to be performed simultaneously on a wide variety of samples and yields information on different electroactive species in the same system. The technique is versatile, has a wide linear dynamic range, and yields accurate reproducible and easily interpretable results.

B. Direct Current Polarography

Jaroslav Heyrovsky developed direct current polarography as one of the first instrumental analytical techniques in 1922 and applied it to inorganic analysis [12] for which he was awarded the Nobel Prize in 1959. Shikata first demonstrated the usefulness of the technique in organic chemical analysis [13] in 1925 by the polarographic analysis of nitrobenzene. The advances of organic polarography are evidenced by texts [1, 14] and reviews [15, 16].

In its simplest form, DC polarography is a voltammetric process in which a linearly varying DC potential ramp is applied between two electrodes: one small and easily polarizable (the working electrode) and the other large and relatively resistant to polarization (the reference electrode). The components of a typical polarographic cell are shown in Figure 2. In addition to the DME (the working electrode) and the saturated calomel electrode (the reference electrode), a platinum wire serves as an electrode to monitor potential. The purpose of this "auxiliary electrode" is to com-