Basic Biochemical Methods

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Preface

Laboratory experience in biochemistry is becoming increasingly important to students in the biological sciences. This text is designed to introduce the student to methods used in the isolation and quantitation of various cell fractions or compounds having biological significance.

This manual is intended for a laboratory course for advanced undergraduate and beginning graduate students in the biological sciences. Our students, among others, are majors in plant science, animal science, genetics, microbiology, neurobiology, and nutrition. Therefore, the experiments were designed for a broad spectrum of interest areas to teach good technique and the application of biochemical methods to these related fields. The students using this manual should have completed basic courses in biology and inorganic and organic chemistry, and they should have had an introductory lecture course in biochemistry.

The principles discussed in conjunction with the experiments presented here are addressed mostly to the methods themselves rather than to the theory or concepts involved. These discussions are held to a minimum to allow mostly for laboratory experiments in this text. To supplement this manual, *The Tools of Biochemistry* by Terrance G. Cooper is particularly valuable.

Many of the techniques taught here are introduced as part of an ongoing study rather than being presented as individual unrelated procedures. The experiments are therefore grouped in modules. Of these modules, "Proteins and Buffers" and "Enzymology" are designed for all students, as they include basic methods. The other modules are scheduled for three-week periods, and students should select those that fit into the time allotted and suit their interests.

Although some of these experiments require the use of various instruments which may not be available in all student laboratories, experiments may be selected from each module that require essentially only pipettes, a centrifuge, and a spectrophotometer.

The appendix is a compilation of equipment and supplies needed for each experiment and a guide to reagent and media preparation. Most commonly used biochemical procedures appear as self-contained units and can be followed directly from the protocols given; in this manner, the manual can serve as a reference text as well as a classroom guide.

The experiments in this manual are the result of over a decade of teaching an introductory course in biochemistry in the Department of Biochemistry, Molecular and Cell Biology at Cornell University. Many scientists who have taught with us over the years have contributed toward this effort. We are particularly indebted to Drs. June Fessenden-Raden, Lemuel D. Wright, Nancy I. Wurster, Virginia Utermohlen, David McCoy, and Alfred H. Merrill, Jr. and to the many students who helped us test and retest these experiments.

We also appreciate the assistance we received from Jeffrey A. Goliger with the section on recombinant DNA and from Joe Noto and Jennifer Lambert with preparation of the appendix. We thank Monica Howland for the art work and Marsha Cox for typing and preparation of the manuscript.

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The Laboratory Notebook

KEEPING RECORDS AND PRESENTATION OF DATA

Communication is an essential aspect of conducting laboratory experiments. Much of the value of collecting experimental results is lost if proper records are not kept and if the data are not written up clearly so that the information can be transmitted effectively to one's colleagues. It is important to record all experimental findings in a notebook at the time that the measurements and observations are made. The following is an outline describing a format suitable for a biochemistry laboratory.

1. *The Notebook.* A bound, quadrille ruled notebook with numbered pages is suitable. Several blank pages in the front should be reserved for a table of contents.
2. *Organization of the Subject Matter for Each Experiment.*
   (a) Brief descriptive title.
   (b) Date on which experiment is performed
   (c) Purpose of the experiment.

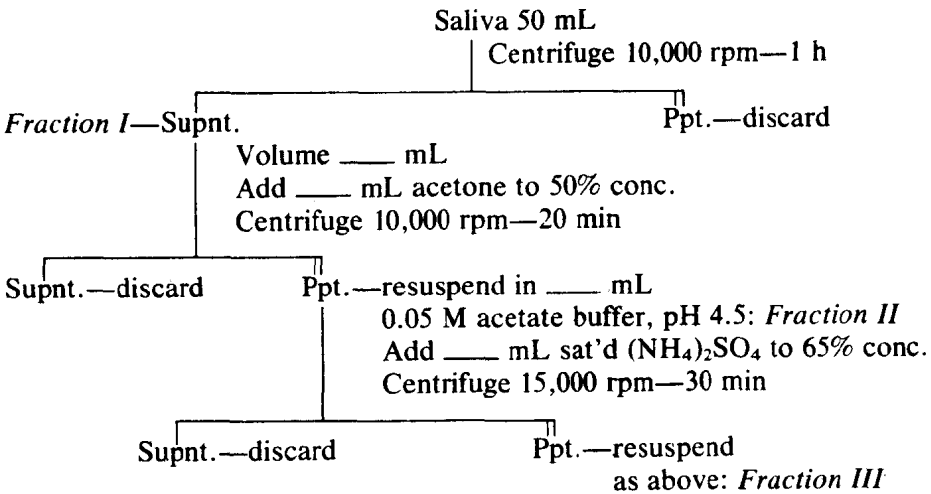
(d) Procedure or methods section. This may require an outline of the steps taken to perform the experiment or, if a published text is used, reference to the procedure can be made.

(e) Presentation of data.

(1) *Flow charts.* If an isolation scheme is to be followed, a flow chart provides a convenient diagram to follow. A procedure is given and space is made available to record data such as volume of the fractions, additions made, and information about centrifugation (See **Example** below).

(2) *Protocols.* A protocol consists of a chart which provides places to plan a series of tests and to record the results obtained. An example is shown below for establishing a standard curve. It is customary to show the tube numbers horizontally and to list the additions made to each tube vertically and in the order in which the additions are made. The variable is added first and water is added to bring the volume to a constant, 1.5 mL. (See Table 1.1)

EXAMPLE: PARTIAL PURIFICATION OF α-AMYLASE



(3) *Graphs and tables.* The data obtained can now be plotted to generate a standard curve (see Figure 1.1). The variable, bovine serum albumin (BSA) in this case, is placed on the abscissa, and the absorbance on the ordinate. The wavelength at which the measurements are made is indicated as

TABLE 1.1. PROTEIN DETERMINATION BY THE BIURET METHOD

Additions (mL)	Tube Number					
	1	2	3	4	5	6
Bovine serum albumin (2 mg/mL)	0	0.1	0.2	0.4	0.8	1.0
H_2O	1.5	1.4	1.3	1.1	0.7	0.5
Biuret reagent	1.5	→	→	→	→	→
$A_{540\ nm}$	0	0.025	0.050	0.105	0.210	0.265
mg/aliquot	0	0.2	0.4	0.8	1.6	2.0

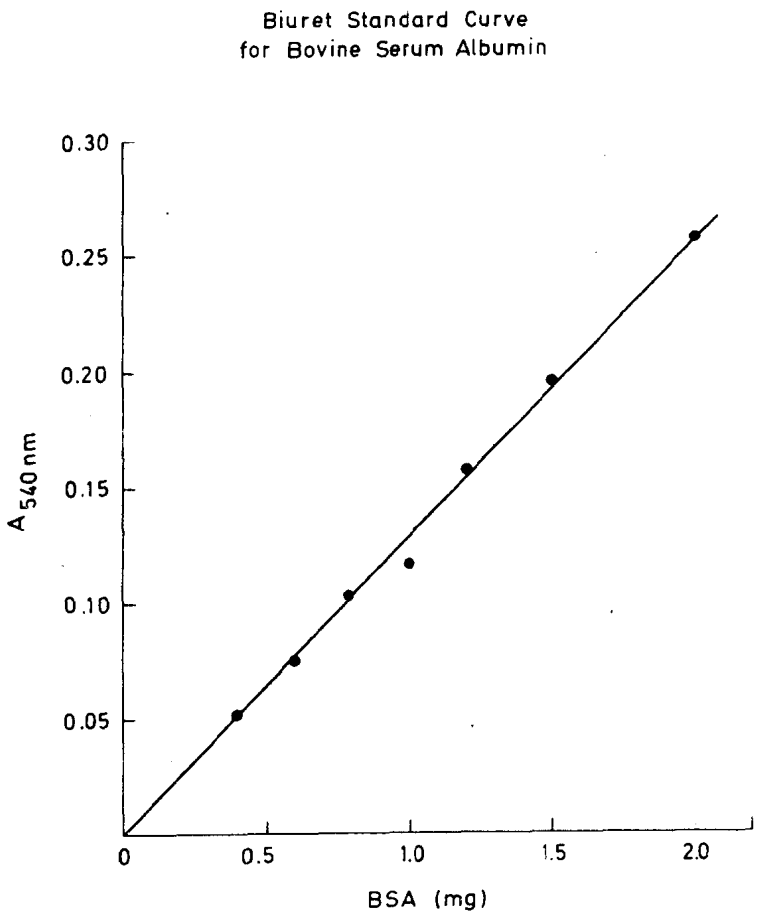


Figure 1.1. Standard curve for protein determination by the Biuret Method.

a subscript of A, and the units of protein (mg) are also included. A title should clearly indicate what is represented on the graph, and a legend, if required, should be included. Several curves can be accommodated if the same units are involved but graphs should not be cluttered or they become difficult to interpret.

Tables should likewise have titles. Headings for each column should be clearly marked and include units (e.g., mg/mL, percent, etc.), if applicable. It is advisable to list the variables down rather than across and the values or properties referring to the variable horizontally. Tables should be compact and should not contain procedural detail that can be written into the text.

3. *Calculations and Analysis of Data.* When measurements are made, it is important to take into account the *sensitivity* of the method and the instrument. Sensitivity refers to the differences or changes in measurement possible for the method or the instrument.

Accuracy is achieved when the observed value is close to the actual value. It is dependent on the quality of the reagents used and the care exercised in performing the experiment. *Precision* refers to how well experimental values agree with each other. Therefore, the two terms are not synonymous and should not be used interchangeably. For instance, in a game of darts, the dart hitting the center was thrown accurately. However, when several darts land in close proximity to one another, the shots were precise, but unless located in the center of the dart board, they were not accurate.

One should also be aware of the correct use of *significant figures*. A problem frequently arises because a series of mathematical manipulations is carried out on a calculator that provides an answer with a large group of numbers. The valid value should contain only the digits known with certainty, plus the first uncertain one. For example, if four protein determinations give values of 3.6, 3.7, 3.7, and 3.5 mg, the mathematical average is 3.63 mg and the standard deviation of the sum is ± 0.11. The standard deviation, S.D. $= \sqrt{s^2/n}$, where s is the difference between the individual observation and the mean, and n is the number of observations included in determining the mean. In this example then, we might be inclined to give the protein content of our sample as 3.63 ± 0.11 mg, indicating a possible range from 3.52 to 3.72 mg. However, only the first decimal place is certain, and accordingly the average value for the protein content should be reported as 3.6 ± 0.1 mg.

4. *Discussion and Interpretation of Results.* In this section, the results obtained should be interpreted. What do the facts suggest about the system studied? Can a general conclusion be reached? How do the results and interpretation thereof agree with previously published data?

The language of the discussion should be clear and the statements should be concise and to the point. The writing should be impersonal, the third person singular is used, and no personal pronouns should appear. The discussion should end with a brief summary or a conclusion stating the significance of the study. When the laboratory findings are to be submitted for publication, the journal for which the scientific paper is written should be consulted for the style and format to use.

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2

Proteins, Buffers, and Thin-Layer Chromatography

Week 1

Day 1.

(a) Biuret method.

(b) Warburg-Christian method.

Day 2.

(a) Lowry method.

(b) Warburg-Christian method.

(c) Protein-dye binding assay.

Week 2

Day 3. Amino acid titration.

Day 4.

(a) Buffer preparation and titration.

(b) TLC analysis of a mixture of amino acids.