

BIOCHEMICAL LABORATORY METHODS FOR STUDENTS OF THE BIOLOGICAL SCIENCES

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PREFACE TO THE SECOND REVISED EDITION

Sans laboratoires les savants sont les soldats sans armes.—PASTEUR.

THIS manual was first issued in 1927, and was followed three years later by the companion text, "Outlines of Biochemistry," from the pen of Dr. Ross Aiken Gortner. In this edition a few minor changes were made in the order of experiments to conform to that of the text. With the appearance of the latter it has been unnecessary to preface certain experiments with a discussion of the underlying principles and to cite those references which do not bear directly upon the laboratory technique but deal rather with the wider applications of the principles of the experiment. More space has thus been made available for additional experiments on some of the newer phases of biochemistry. The photomicrographs of the osazones and other sugar derivatives were selected from typical laboratory results rather than from the more perfect crystals which might be obtained under the most favorable and unusual conditions. As was true of the first edition, attempt has been made not to encroach upon the field adequately covered by several standard manuals of physiological chemistry.

The author wishes to acknowledge the aid and encouragement given him by Dr. Ross Aiken Gortner, Chief of the Division of Agricultural Biochemistry in the University of Minnesota. To his colleague, Dr. Henry B. Bull, he is indebted for the description of the micro-cataphoretic method; and to Mr. Webster W. Benton, Dr. Robert Jeffrey, and Mr. Tellef Senum for their aid in checking certain experiments and references. Acknowledgment is also due The Chemical Catalog Company and The Williams and Wilkins Company for permission to use certain data.

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BIOCHEMICAL LABORATORY METHODS

CHAPTER I

THE COLLOIDAL STATE

I. LYOPHOBIC SOLS

Success in the preparation of lyophobic sols depends upon the relative absence of electrolytes and of organic matter and dust particles. Satisfactory preparations have been made with ordinary distilled water but the following precautions may be required. The vessels and stirring rods should be of hard glass (Pyrex or Jena), cleaned with chromic acid solution and thoroughly rinsed or steamed for some time. The *specially distilled water* should be freshly prepared and condensed in quartz, block tin, or hard glass tubes. The supply of water in the condenser should be cut down so that the volatile substances present will not be condensed and the water will issue steaming. Pipets should not be put into stock solutions; each student should transfer to his own test tubes the estimated quantities of reagents.

A. CONDENSATION METHODS

Reduction

Expt. 1. Gold Sol by Formaldehyde.—Place 120 cc. specially distilled water in a 300-cc. Pyrex beaker and bring to boiling. While heating add 2.5 cc. of gold chloride solution and the amount of 0.18 *N* potassium carbonate solution needed to neutralize. This is generally 3.5–4.0 cc. but should be determined by an independent titration of the gold solution using sensitive litmus paper as an outside indicator; a faint alkaline reaction ($\text{pH}=7$ to 7.5) is the desired end-point. As soon as the boiling point is reached, remove the flame. Add the formaldehyde solution rapidly, but a drop at a time, with stirring. As soon as a definite pink color appears, stop adding the reagent; stir vigorously. A rapid change in color results; the sol

should be bright red by transmitted light and a muddy color by reflected light. No blue color should be evident when the sol is examined by transmitted light. However, it is often observed that the first color produced is a violet rather than a pink, but this should disappear with the rapid stirring immediately after the addition of formaldehyde is stopped.

A modification of the above procedure is to substitute for the potassium carbonate a solution containing $1\frac{1}{2}$ per cent each of sodium carbonate and sodium bicarbonate.

Gold chloride solution.—Dissolve 3.43 gm. of pure gold in *aqua regia* in a casserole and evaporate to dryness on a steam bath. Add a small quantity of concentrated hydrochloric acid, and again evaporate to dryness; then add distilled water and evaporate to dryness a third time. Finally dissolve the resulting chlorauric acid, $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, in sufficient specially distilled water to make 1 liter. This gives a concentration of 6 gm. per liter. Solid gold chloride sold in ampules for this purpose is also satisfactory.

Formaldehyde solution.—Dilute 0.6 cc. of 37–40 per cent formaldehyde with 200 cc. of specially distilled water. The solution should be freshly prepared.

*¹ZSIGMONDY, R. Die hochrothe Goldlösung als Reagens auf Colloide. *Z. anal. Chem.*, 40, 697–719 (1901).

*SCHULZ, F. N., und ZSIGMONDY, R. Die Goldzahl und ihre Verwertbarkeit zur Charakterisierung von Eiweissstoffen. *Beitr. Chem. Physiol. Pathol.*, 3, 137–160. Cf. 141 (1903).

*SVEDEBERG, T. Die Methoden zur Herstellung kolloider Lösungen anorganischer Stoffe. S.73–77. Theodor Steinkopff, Dresden, 1909.

*GREY, F. T. Preparation of colloidal gold for the Lange test. *Biochem. J.*, 18, 448–50 (1924).

WEISER, H. B., and MILLIGAN, W. O. Von Veimarn's precipitation theory and the formation of colloidal gold. *J. Phys. Chem.*, 36, 1950–1959 (1932).

Expt. 2. Nuclear Gold Sol.—The “nucleus sol” is first prepared: To 100 cc. specially distilled water is added 2 cc. of the gold solution (Expt. 1), and the potassium carbonate solution. Five cubic centimeters of a saturated solution of white phosphorus in ether is diluted to 100 cc. with anhydrous ether. This solution is added to the diluted gold solution slowly with stirring until a clear brown color results. Heat and note the change of color to red.

The sol proper is prepared by adding 2.5 cc. gold chloride reagent to 100 cc. distilled water and enough potassium carbonate

¹The asterisk (*) is used to indicate the original reference or references from which each experiment is obtained or expanded.

solution to just neutralize. The solution is then heated to boiling, 4 cc. of the "nucleus sol" is added, followed by 4-5 cc. of a 0.03 per cent solution of formaldehyde. The sol is then boiled for a minute; it should have the properties described in the previous experiment.

*MUKHERJEE, J. N., and PAPACONSTANTINON, B. C. The coagulation of gold hydrosols by electrolytes. The change in colour, influence of temperature, and reproducibility of the hydrosol. *J. Chem. Soc.*, 117, 1563-1573 (1920).

Expt. 3. Gold Sol by Phosphorus.---To prepare a gold sol by phosphorus Zsigmondy combines two methods, his own formaldehyde method (Expt. 1) and that of Faraday, who used a solution of white phosphorus in ether. By this combination, the sol obtained has a high degree of dispersion and is very sensitive to electrolytes. Place 120 cc. of specially distilled water in a 300-cc. Pyrex beaker; add 2.5 cc. of gold chloride solution and 3-3.5 cc. of freshly prepared 0.18 *N* potassium carbonate solution (Expt. 1). Next add, drop by drop, 0.5 cc. of a saturated solution of white phosphorus in ether. Reduction takes place at room temperature but the reaction is slow, the liquid first becomes brownish red and then gradually changes to a bright red, without the slightest turbidity either in transmitted or reflected light. A saturated solution of phosphorus in carbon tetrachloride may be used in place of the phosphorus in ether. The production of the sol can be hastened by cautious warming.

*FARADAY, M. Experimental relations of gold (and other metals) to light. *Phil. Trans. Roy. Soc., London*, 147, 145-181. Cf. 159-160 (1858). Received Nov. 15, 1856.

SVEDBERG, T. Die Methoden zur Herstellung kolloider Lösungen anorganischen Stoffe. S. 65-66. Theodor Steinkopff, Dresden, 1909.

*ZSIGMONDY, R. Colloids and the ultramicroscope. Translated by J. ALEXANDER. 1st ed., pp. 126-128. John Wiley & Sons, New York, 1909.

Expt. 4. Gold Sol by Phenylhydrazine.---Place 300 cc. of specially distilled water in a 500-cc. Pyrex beaker and add 1.25 cc. of gold chloride solution (Expt. 1). Then add 0.2-0.5 cc. of freshly prepared phenylhydrazine hydrochloride solution, a drop at a time, from a pipet, allowing a few seconds to intervene and stirring between each addition. Note the changes in color. Continue to add the reducing solution, drop by drop, and observe the color changes when 5 cc. have been added and again after the addition of 10-12 cc. The solution should now be a deep blue color. Explain this series of color changes. Allow the solution to stand for 48 hours or longer, and observe the precipitate which separates. What is this?

Phenylhydrazine hydrochloride.—The phenylhydrazine hydrochloride should be perfectly white. This salt rapidly decomposes and darkens unless it is very pure and dry. It is prepared from phenylhydrazine² which has been freshly purified by distillation under diminished pressure (p. 71). Dissolve the phenylhydrazine in 12 volumes of 95 per cent ethyl alcohol and precipitate as the hydrochloride by the addition of a slight excess of concentrated hydrochloric acid. Filter the hydrochloride on a Büchner funnel, sucking as dry as possible; wash thoroughly, first with a mixture of ethyl alcohol and ether, and then with ether, until the salt is snow white. Dry on a filter paper in a warm place for half an hour, and then at 100° C. for an hour. Preserve the dry salt in a tightly stoppered amber-colored bottle. If only an impure sample of phenylhydrazine hydrochloride is available, it may be purified in the following manner: Dissolve 25 gm. of the impure salt in boiling distilled water; slightly acidify with hydrochloric acid; decolorize the solution with the vegetable decolorizing carbon, Norit, at boiling temperature, and filter at once. Allow the clear solution to stand over night in a refrigerator at 1 or 2° C., so that crystallization may take place. Filter on a Büchner funnel, and dry as described above. To prepare the solution for this experiment dissolve 1 gm. of phenylhydrazine hydrochloride in 250 cc. of distilled water.

*GUTBIER, A., und RESENSCHECK, F. Über das flüssige Hydrosol des Goldes. II. *Z. anorg. Chem.*, 39, 112–114 (1904); or SVEDBERG, T. Die Methoden zur Herstellung kolloider Lösungen anorganischer Stoffe. S. 112–114. Theodor Steinkopff, Dresden, 1909.

*MULLIKEN, S. P. A method for the identification of pure organic compounds. Vol. I, p. 32. John Wiley & Sons, New York, 1905. The preparation of pure phenylhydrazine hydrochloride is described in the footnote.

Expt. 5. Gold Sol by Tannin.—Place 200 cc. of specially distilled water in a Pyrex beaker; add 1 cc. of gold chloride solution (Expt. 1) which has been neutralized to litmus paper by the addition of freshly prepared 0.18 *N* potassium carbonate solution, and 1 cc. of a 1 per cent tannin solution. Heat the mixture gradually to boiling, stirring constantly. A cherry red color develops as it becomes hot. If the red color does not appear immediately after heating, add more gold chloride and tannin alternately; heat and stir. Observe the sol in both transmitted and reflected light. Ordinary distilled or tap water may also be used for this experiment.

²Phenylhydrazine is poisonous. Its vapors should not be breathed; if it comes in contact with the skin, it produces an intolerable itching. Dilute acetic acid will remove it.