1969

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Recommended Citation

Rice, Gary, "Kinetics of the Decomposition of the Cupric Ion Sulfhydryl Bond in Beef Plasma Albumin" (1969). *Honors Theses*. 355.
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Chemistry
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KINETICS OF THE DECOMPOSITION OF THE CUPRIC ION - SULFHYDRYL BOND IN BEEF PLASMA ALBUMIN

Presented to
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In Partial Fulfillment
of the Requirements of
Honors Special Studies H491

by
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May 29, 1969

#218

PURPOSE: The purpose of this work was to study the kinetics of the binding of cupric ion to beef plasma albumin, in particular the decomposition of the Cu^{++}--BPA complex which absorbs light at 375 millimicrons.

THEORY: The work of Klotz, Urquhart, Klotz, and Ayers[1] has shown that the characteristic absorption band at 375 mu produced by the binding of cupric ion to beef plasma albumin is due to a copper-sulfhydryl bond. They have further demonstrated that the disappearance of this absorption band with time is accompanied by irreversible changes in the structure of the protein. The intensity of the initial absorption was shown to increase with increasing pH up to about pH 8, where it appeared to decrease again, possibly because of the increased rate of decomposition of the complex at high pH.

PROCEDURE: The solutions to be used were all prepared in .20M acetate buffer. The BPA solutions were made up as 6×10^{-4} M, and the copper solutions as 6×10^{-3} M. Some experiments were carried out with more dilute solutions, but were generally unsatisfactory. The prepared reagents were mixed 1:1 by pumping them individually through a polystaltic pump. Mixing was carried out in a tee joint, and the mixture run into a flow cell in position to be

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[1] I. M. Klotz, J. M. Urquhart, T. A. Klotz, and J. Ayers, *Journal of the American Chemical Society* 77, 1919, (1955).

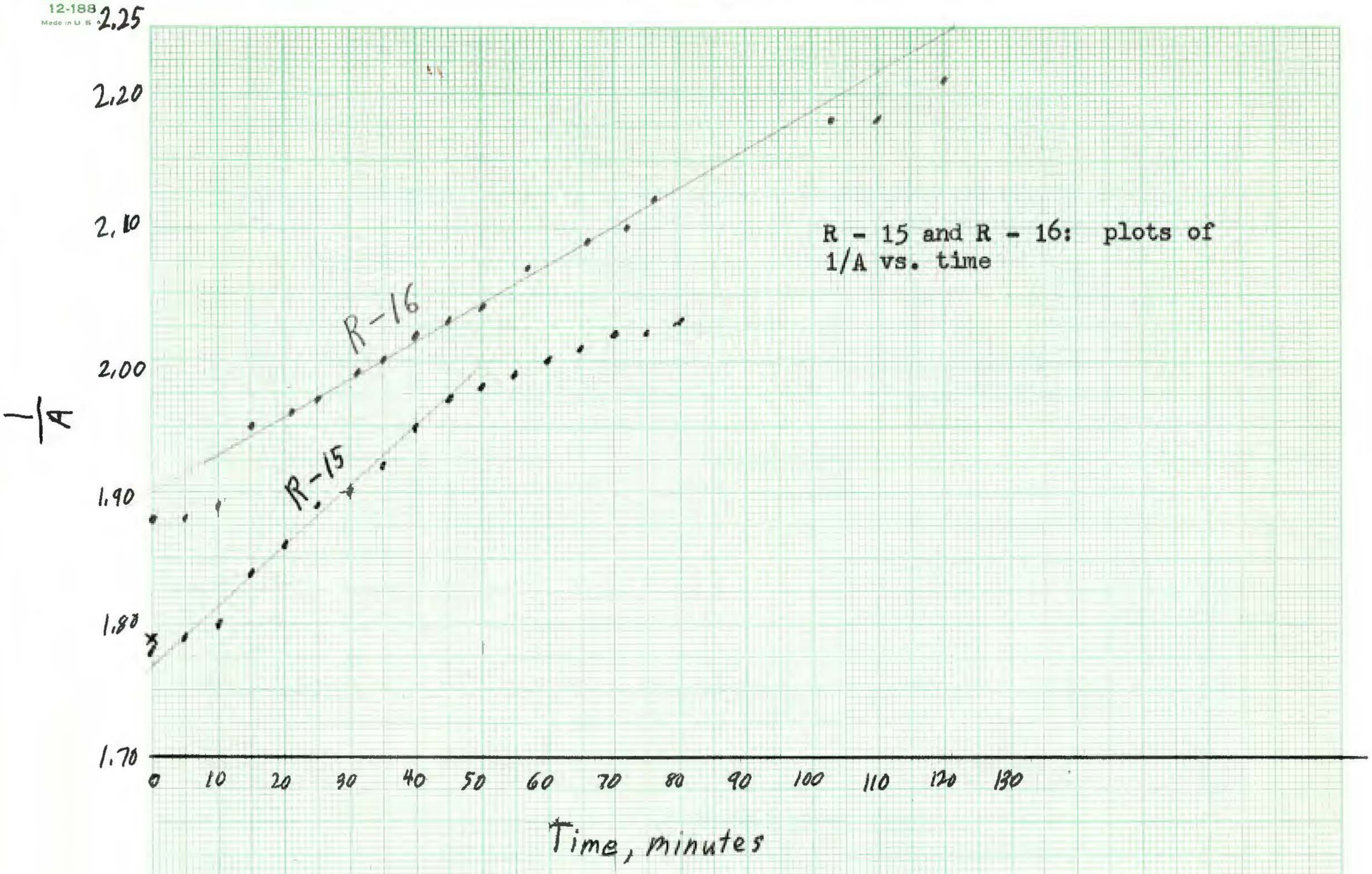
read. All absorbance readings were made with a Beckman model DU spectrophotometer. Readings were taken for up to 4½ hours after mixing. The data will be found in the notebook accompanying this report.

CALCULATIONS: A reaction is shown to be first order if a plot of log(B) against time is linear, where B is any quantity proportional to the concentration of the reactant in question. The reaction is shown to be second order if a plot of 1/B against time is linear. If it is assumed that the absorbance of a protein-copper solution at 375 m_u is proportional to the concentration of the copper-sulfhydryl bonded complex, it should be possible to follow the disappearance of that complex with time by taking the absorbance of the solution periodically. The kinetics of the initial formation of the complex should also be susceptible to this technique, but the competing reaction, the very small changes in absorption, and the rapidity of the reaction cause great difficulty with the procedure followed here. Several plots of 1/A and logA are shown on plates I - III. All plots show some initial nonlinearity, probably because some of the absorbing specie was still being formed. The break on the plots of R - 15 was caused by precipitation of the complex; the solution was exposed to the atmosphere on this particular run, and a cloudy precipitate was formed in the top of the cell and subsequently settled to the bottom, as opposed to the precipitations encountered in some of the earlier runs, which were more or less homogenous precipitations.

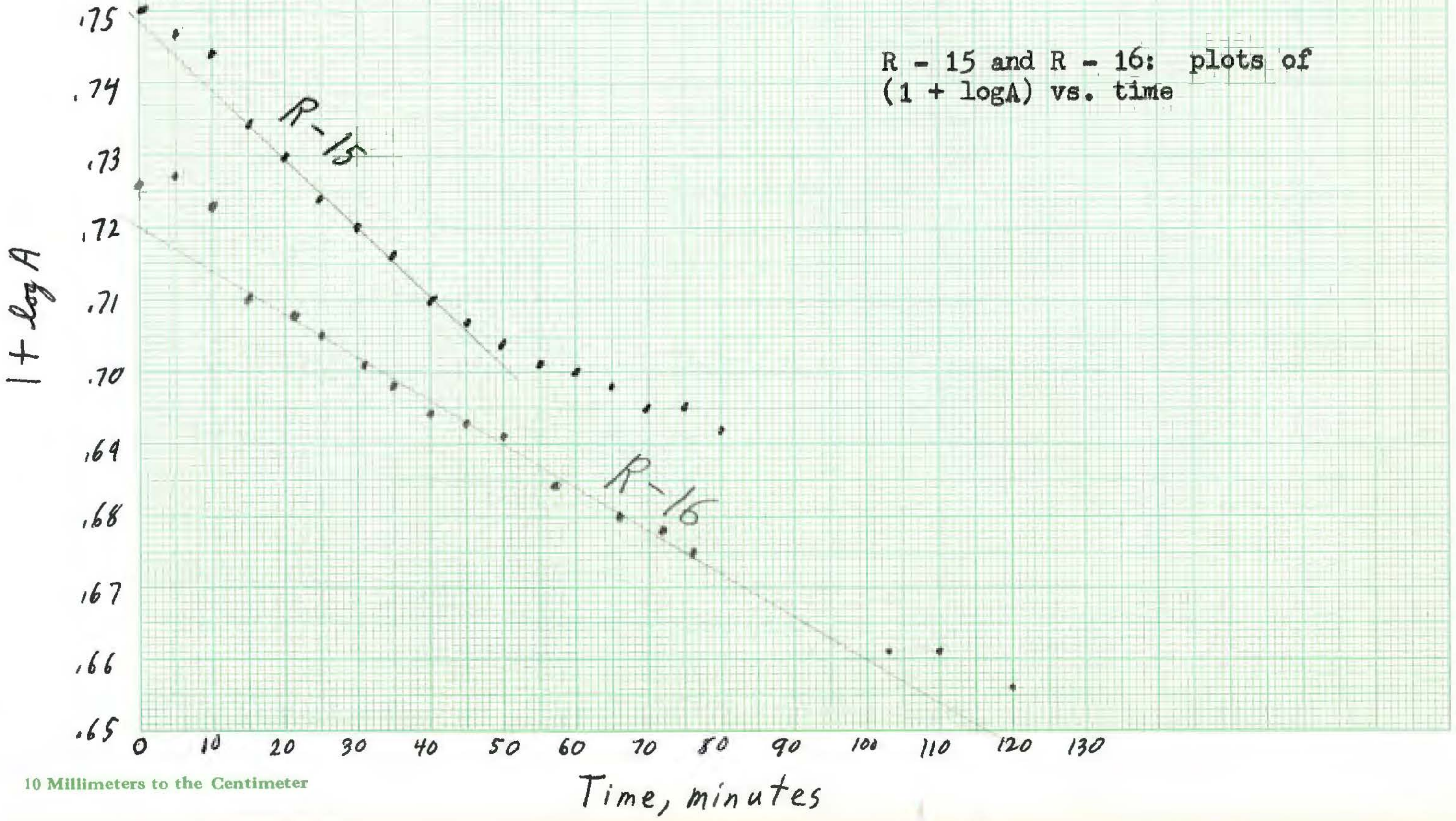
In plateIV, it was assumed that the product formed by the decomposition of the complex also absorbed at 375 m_u, and that its absortivity was approximately 1/3 that of the reactant, or $A_r = A - \frac{1}{3}A_o$, where A_r is the absorbance of the reactant. The plots obtained are generally similar to the plots obtained by using the total absorbance A of the solution. It can be seen that all plots could conceivably be called linear, with the possible of exception of those on plate III, which are too scattered to be really informative.

<u>CONCLUSION</u>: No attempt will be made here to assign the order of the reaction by the graphs obtained. It would seem that the reaction did not proceed nearly to completion in any of the runs carried out. Thus, the seemingly contradictory linearity of all types of plots would result from the linear appearance of a small portion of a curved line. Further investigation of the reaction over longer periods of time or at higher reaction rates appear to be necessary before the order of the reaction can be determined.

PLATE I

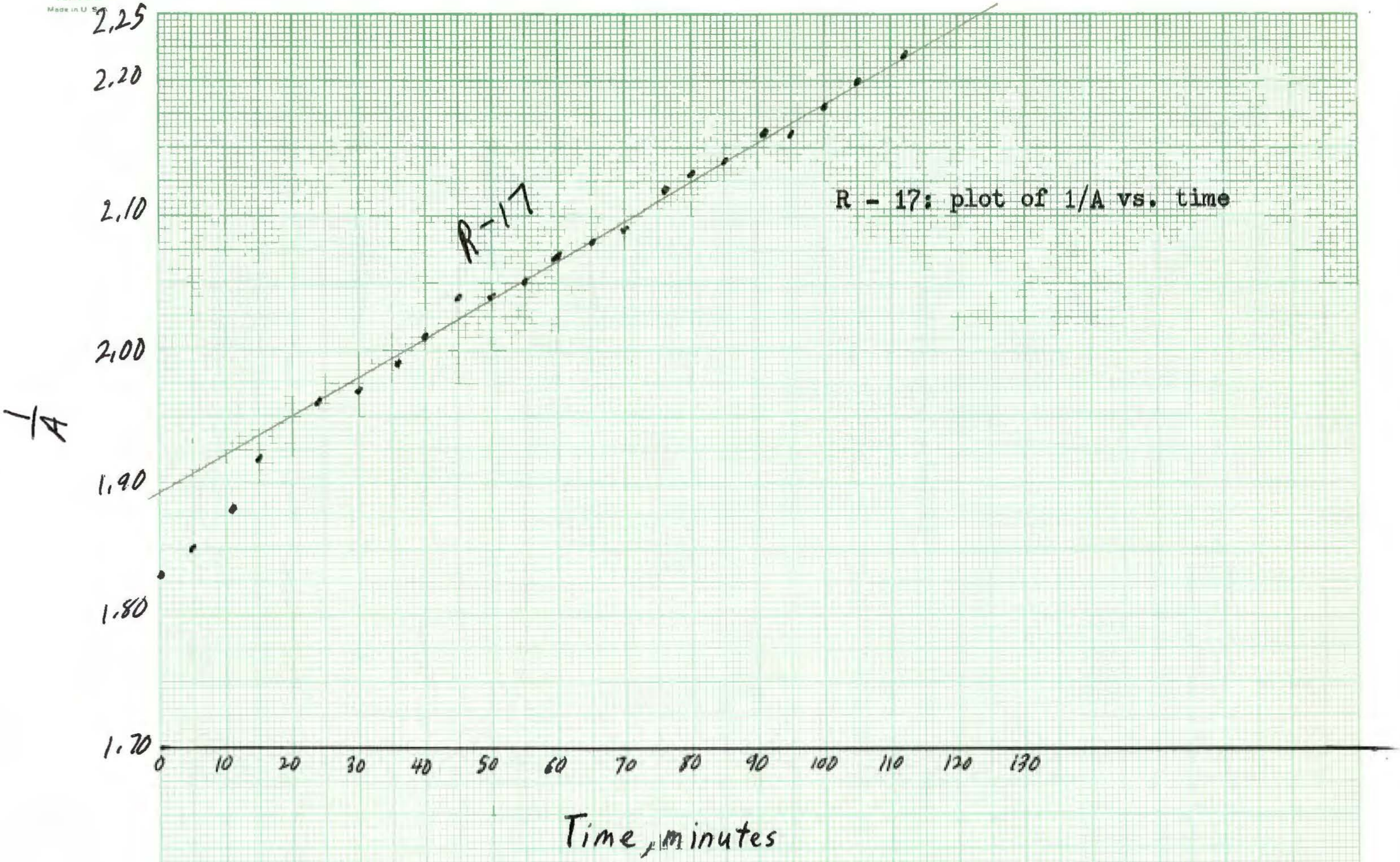


R - 15 and R - 16: plots of 1/A vs. time



R - 15 and R - 16: plots of (1 + logA) vs. time

PLATE II


R - 17: plot of 1/A vs. time

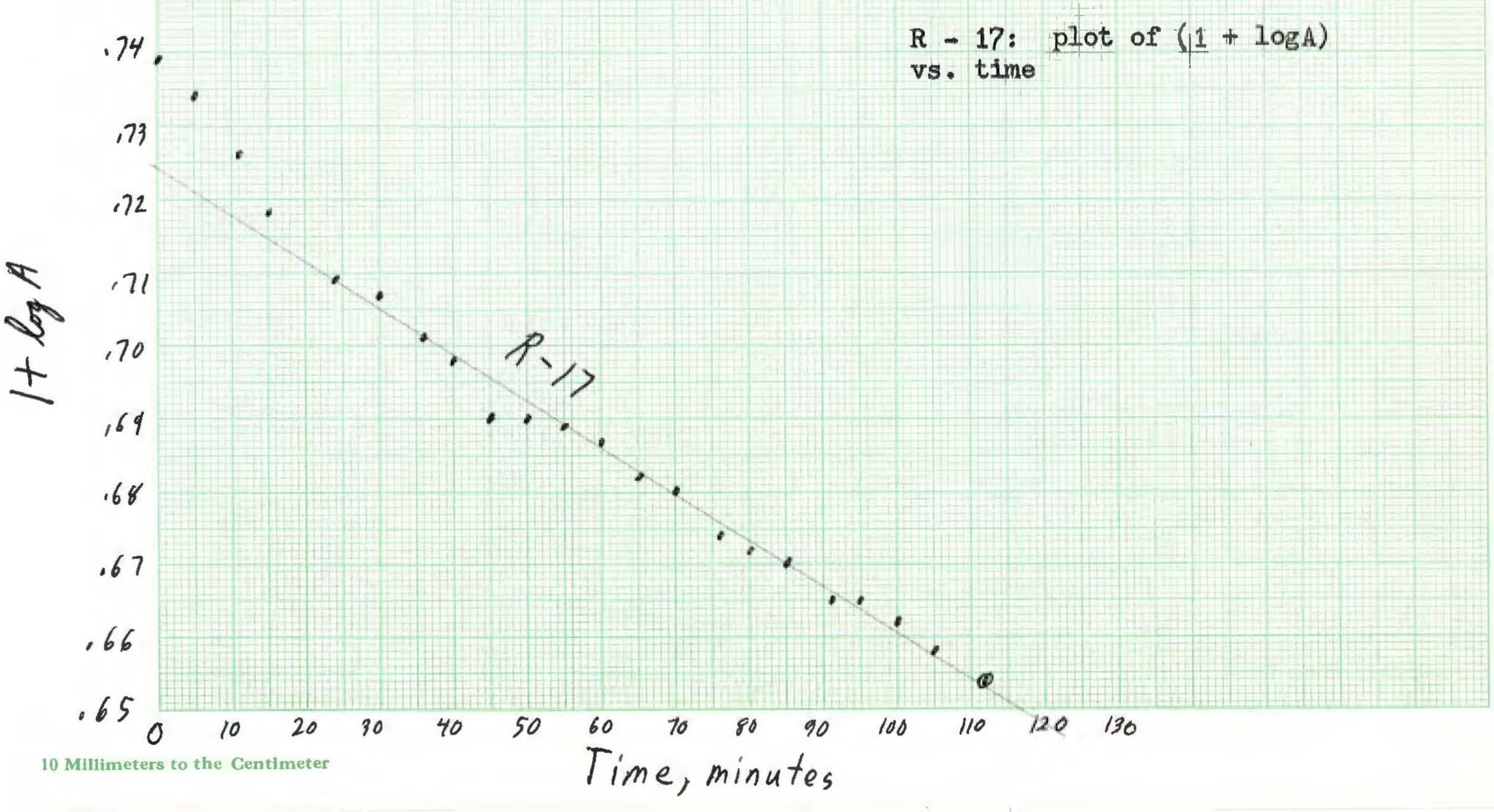
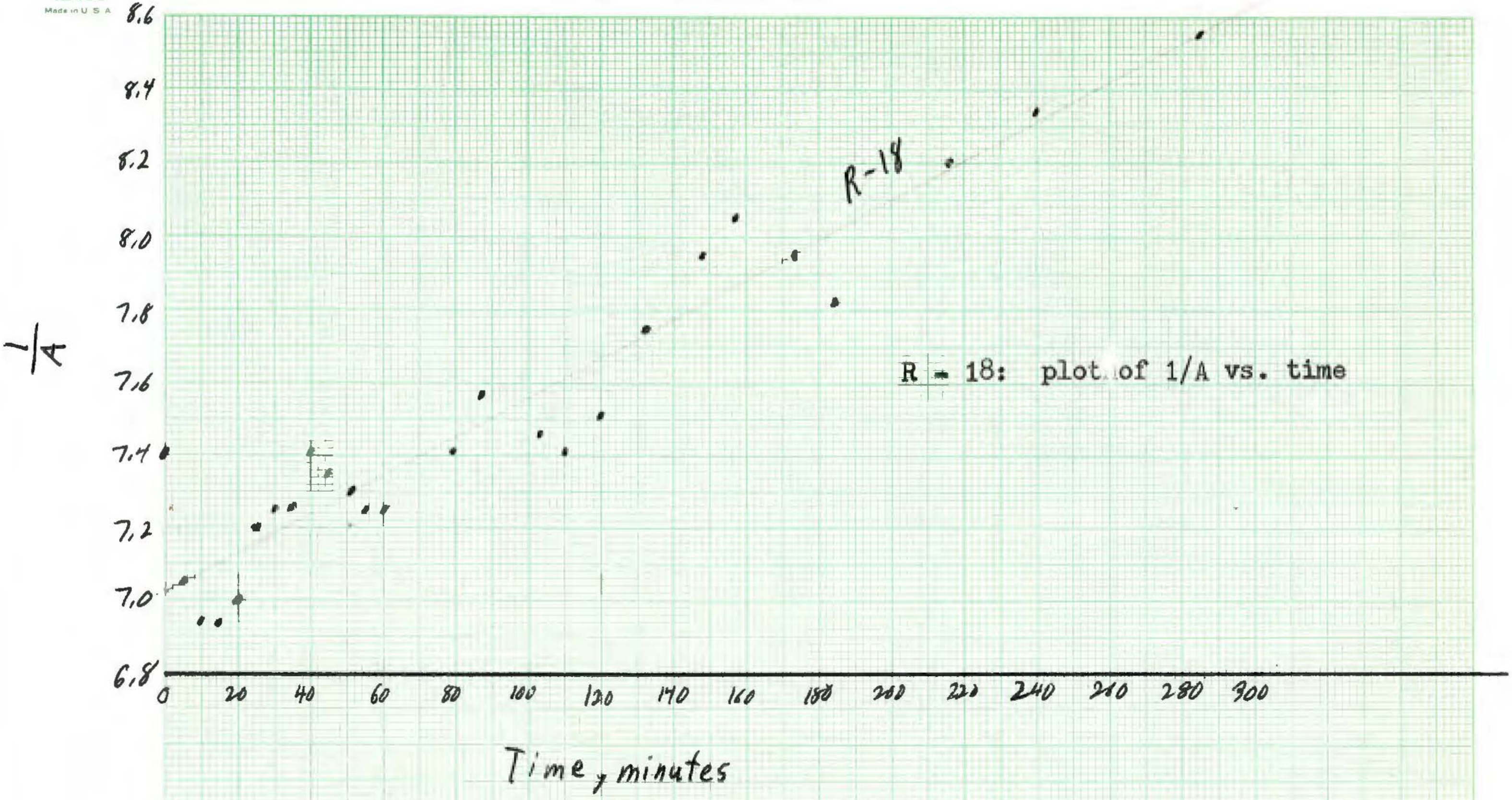
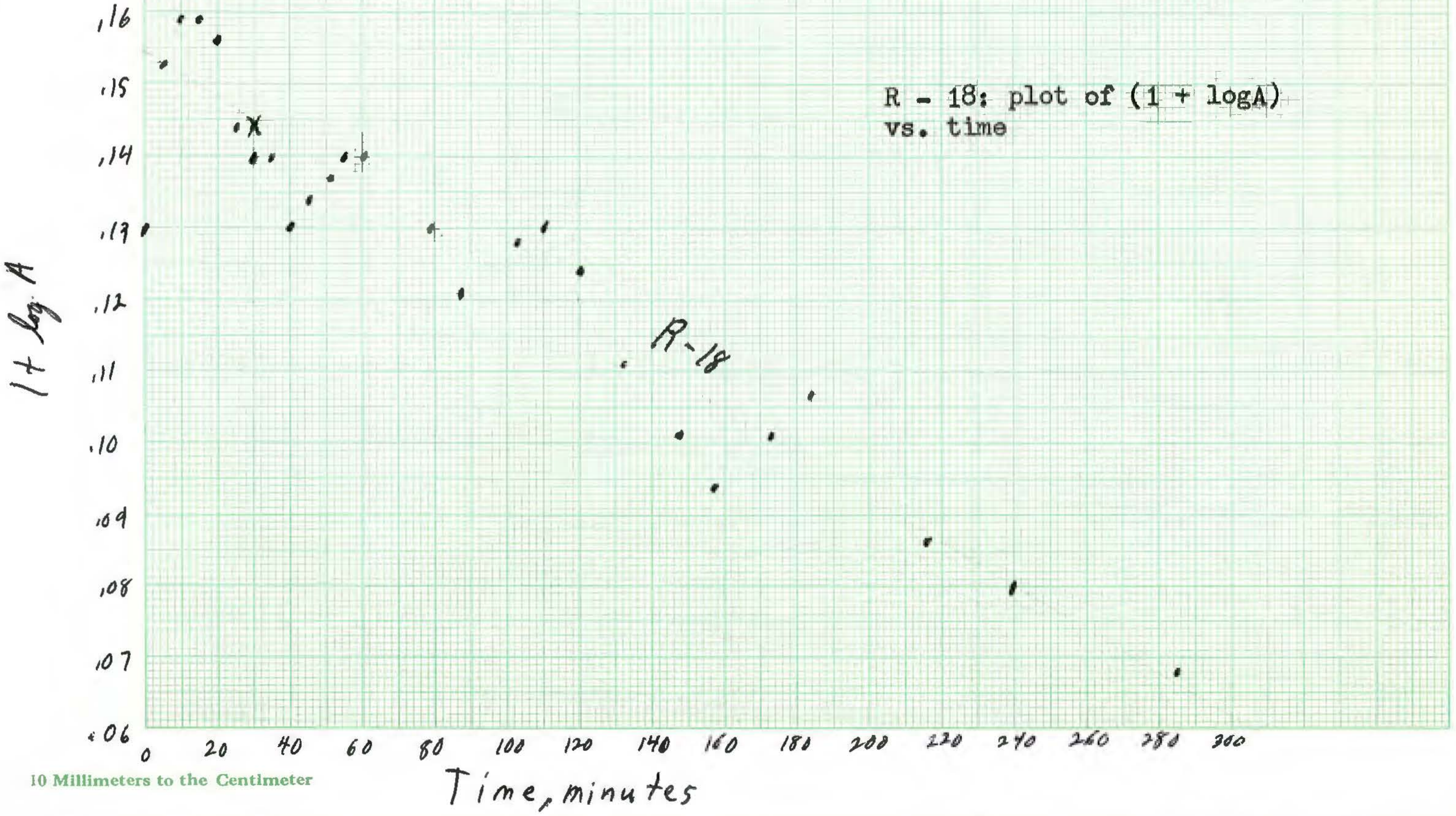

R - 17: plot of (1 + logA) vs. time

PLATE III

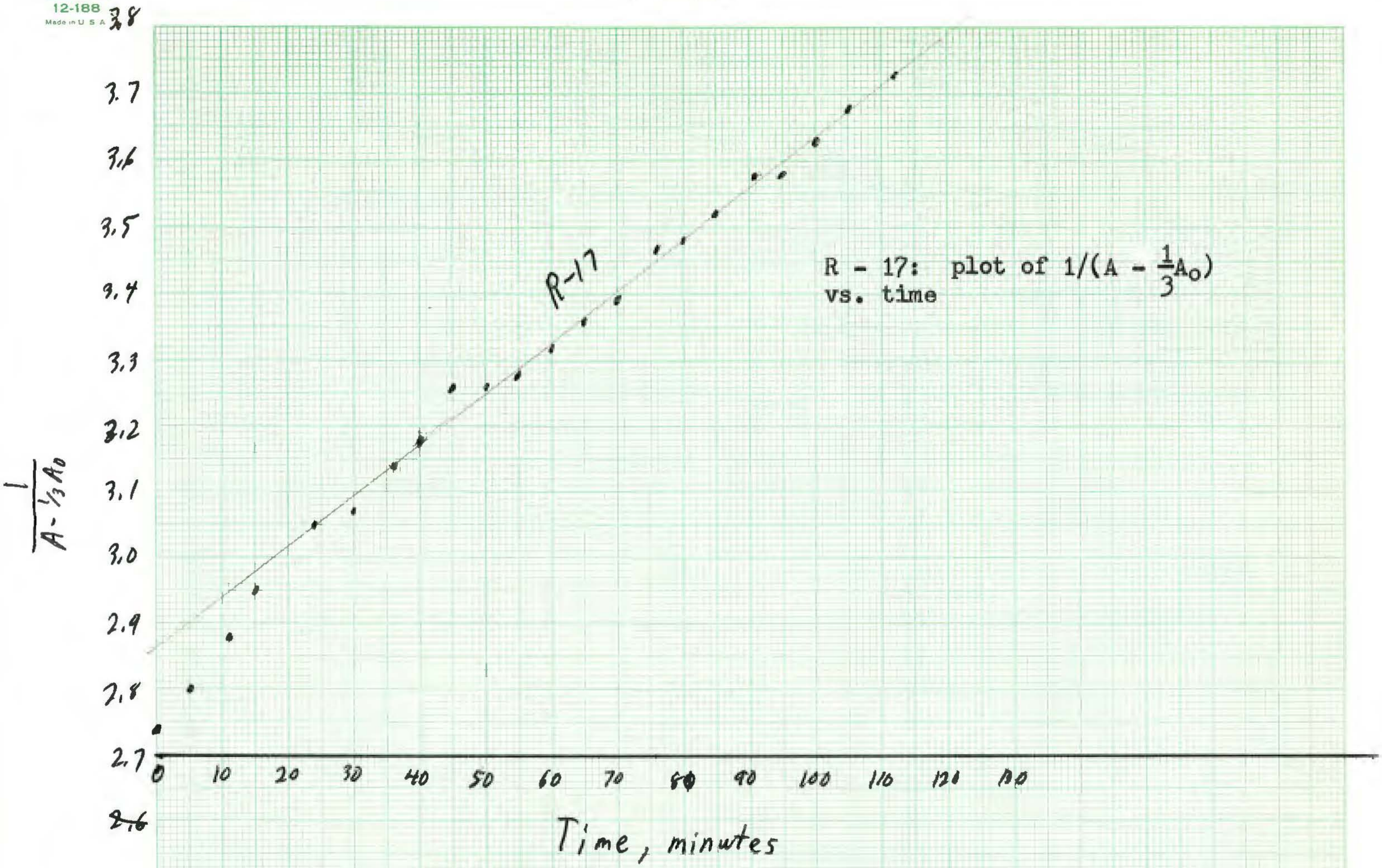


R - 18: plot of 1/A vs. time

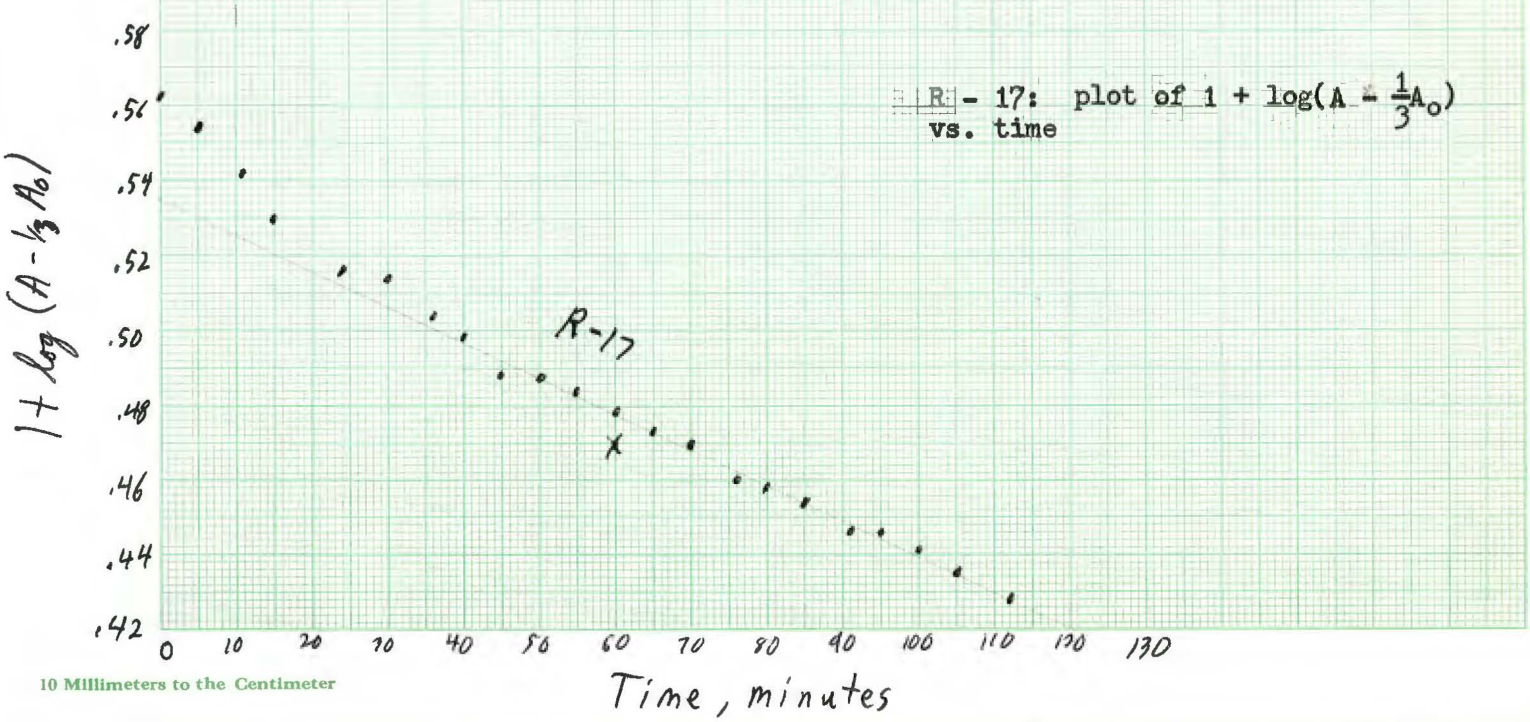


R - 18: plot of (1 + logA)
vs. time

PLATE IV



R - 17: plot of $1/(A - \frac{1}{3}A_o)$
vs. time



R - 17: plot of $1 + \log(A - \frac{1}{3}A_o)$
vs. time