

Short Communication

On the polarography of cyanocobalamin

The amperometric titration of cyanocobalamin (vitamin B₁₂), with chromous ion in an ethylenediaminetetraacetate medium of pH 9.5 was used by BOOS, CARR AND CONN¹, to determine conclusively the equivalent weight of this compound. These authors observed an anodic polarographic wave at an $E_{1/2}$ of -0.311 V *vs.* S.C.E., which increased as the titration progressed, and they ascribed this wave to the oxidation of the reduced form of the vitamin. We have done experiments which confirm, in part, the results of BOOS, CARR AND CONN on the reaction of cyanocobalamin with chromous ion, but show that the afore-mentioned anodic wave is not ascribable to the oxidation of a reduction product of the vitamin, but to the oxidation of mercury of the dropping mercury electrode, in the presence of the cyanide ion which is liberated in the titration.

The experiments were done on a cyanocobalamin sample purchased from Nutritional Biochemicals Corp., and assayed according to the procedure of CORDS AND RATYCZ². The sample was 89% cyanocobalamin; the balance of the sample was presumed to be moisture. The amperometric titrations were performed with the dropping mercury electrode, and for each increment of reagent a complete polarogram was recorded on a Leeds and Northrup Electrochemograph, Type E. A conventional H-cell, made gas tight, was used for the titrations. When the pH of the EDTA supporting electrolyte was 9.6, the titrations yielded an average equivalent weight of 1300 for the vitamin, in good agreement with the results reported by BOOS, CARR AND CONN. However, duplicate results using a capillary for which $m^{2/3}t^{1/6}$ was 2.27 $\text{mg}^{2/3}\text{sec}^{-1/2}$ gave a diffusion current constant of 1.35 $\mu\text{A}/\text{mg cc}^{-1} \text{mg}^{2/3}\text{sec}^{-1/2}$ or 1.76 $\mu\text{A mmoles l}^{-1} \text{mg}^{2/3} \text{sec}^{-1/2}$.

When the pH of the titration medium was changed to 7.0, the anodic wave observed at pH 9.6 was shifted to a more positive potential and cyanide could be detected in an inert gas stream passing through the titration cell. The volatility of hydrocyanic acid from this titration medium permitted its collection by passing purified nitrogen first through the cell and then through two scrubbers containing 0.1 M sodium hydroxide during the titration and for one hour thereafter. As cyanide was removed from the titration cell, the anodic wave became indistinguishable from the mercury dissolution wave.

The cyanide trapped in the scrubbers was identified by the polarographic wave it produced. The E_f of this wave, (defined in ref. ³), was -0.286 V *vs.* S.C.E., in excellent agreement with the E_f of -0.284 V *vs.* S.C.E. found by NEWMAN, CABRAL AND HUME³, as an average value for the anodic mercury wave in the presence of cyanide at pH values above 10. Upon amperometric titration of the scrubber solutions with 0.0531 M mercuric nitrate, the height of this wave decreased and an estimate of cyanide could be made. Figure 1 shows the results of the two amperometric titrations

on a sample of cyanocobalamin which weighed 4.81 mg (corr.). The data indicate a recovery of 79% of the theoretical cyanide on the basis of one cyanide ion per cyanocobalamin molecule and 4.5 μ equiv. of reductant consumed by the sample. It should be pointed out, however, that these data give an equivalent weight of 936 for the vitamin, significantly lower than the value obtained at pH 9.6. This may be due to appreciable oxidation of the chromous reagent by hydrogen ion at the lower pH. If 1300 is taken as the equivalent weight of the vitamin, the recovery of cyanide is 96%.

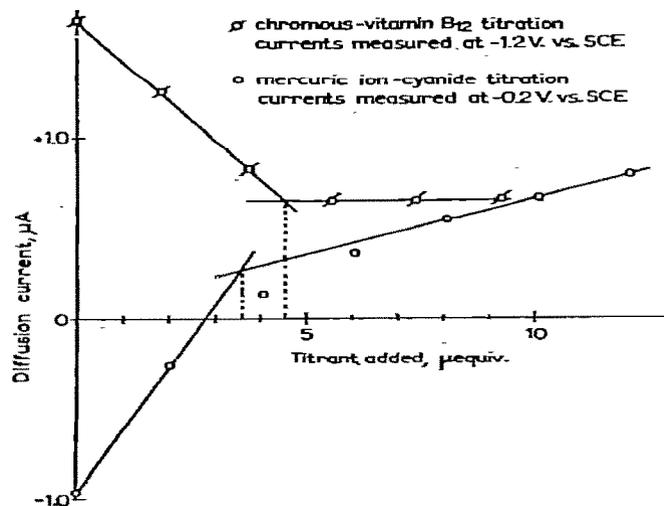


Fig. 1. Amperometric titration curves for cyanocobalamin and the cyanide liberated during its reduction.

While the lower equilibrium concentration of hydrocyanic acid at pH 9.6 precludes its volatilization and estimation from a titration at the higher pH, we believe that the results found at pH 7 are valid at pH 9.6. The observation by BOOS, CARR AND CONN, that the anodic polarographic wave disappears on air-oxidation of the titration product, can be explained by cyanide uptake of the reduced form of the vitamin during oxidation. To verify this explanation, we have compared the behavior of the reduced form of the vitamin on air-oxidation at pH 7 and pH 9.6. At the lower pH, in a solution from which cyanide had been removed, aeration oxidized the reduced form of the vitamin to a product whose brown color showed that it was different from that obtained on oxidation in the presence of cyanide. Furthermore, this product could be retitrated with chromous ion to an end-point, and the resulting solution gave no anodic wave. At the higher pH, where cyanide is retained, air oxidation of the reduced form of the vitamin gave a product which, we found, contrary to the experience of BOOS, CARR AND CONN, could be retitrated with chromous ion to an end-point, identical to the first. During this titration the anodic wave reappeared in its original form.

The fact that the E_p for the anodic wave in the titration mixture differs from that observed after the cyanide has been transferred to the scrubbers is not surprising, since there is a large amount of EDTA in the titration mixture, which may be pre-

sumed to affect the formula of the mercury complex formed when mercury is oxidized from the dropping mercury electrode, and through that, the potential of the wave. This view is supported by the polarograms observed on solutions of 0.1 M EDTA at pH 9.6 to which pure potassium cyanide had been added. The waves in this case had E_f values of -0.34 to -0.37 V vs. S.C.E., in agreement with an E_f of -0.33 V for the anodic wave obtained during vitamin reduction under the same conditions.

ACKNOWLEDGEMENT

We acknowledge with thanks the interest and assistance of Dr. ROBERT H. ABELES, who directed our attention to the area of this work.

Department of Chemistry,
The Ohio State University,
Columbus, Ohio (U.S.A.)

J. W. COLLAT
S. L. TACKETT

¹ R. N. BOOS, J. E. CARR AND J. B. CONN, *Science*, 117 (1953) 603.

² H. CORDS AND O. T. RATYCZ, *Drug Standards*, 27 (1959) 132.

³ L. NEWMAN, J. DEO. CABRAL AND D. N. HUME, *J. Am. Chem. Soc.*, 80 (1958) 1814.

Received February 8th, 1962

J. Electroanal. Chem., 4 (1962) 59-61

Book Reviews

Experiments for Instrumental Methods — Laboratory Manual, C. N. REILLEY AND D. T. SAWYER, McGraw-Hill Book Co., New York and London, 1961, X + 412 pages, \$ 5.95.

Not only is the use of instrumental methods, both for measuring and recording, growing continuously, but also, instruments become more complex and sophisticated in order to achieve an improved performance in stability, precision, reproducibility, etc. But, generally speaking, not every University or high school teaches the correct use of instrumental methods, *i.e.* the notion of systematic and accidental errors, noise, signal to noise ratio, limits of use for the different kinds of instruments, etc. This need has been felt by many authors, and several books and manuals have been published which deal with basic information and theory of instrumental methods. The book under review is of a similar kind, particularly written as a practical laboratory manual for performing several experiments using instrumental methods.

Each chapter gives, firstly, a short outline of the theory needed to carry out the experiments described correctly, then a good bibliography for widening the knowledge of the particular topic treated, and finally, a clear description of the experiment itself. The physico-chemical methods dealt with are as follows: potentiometry, conductometry, polarography, amperometry, electrolysis, emission spectroscopy, UV and IR absorption, column, paper and gas chromatography, ion exchange, radiochemical methods, elementary electronics and servicing of instruments.

As comment, and perhaps as a suggestion for a future new edition, a short introduction on transistorized electronics would be very welcome, because transistorized instruments are becoming more usual. Also, a short treatment of general principles, more or less common to all instruments, would be very useful.

This book has been printed by off-set methods, so that its price is relatively low, which is an advantage. Notwithstanding, the printing of the text and drawings are very clear and easy to read. The book can be warmly recommended as a laboratory manual for students.

J. Electroanal. Chem., 4 (1962) 61