

off, washed with acetic anhydride until the filtrate was almost colorless and then recrystallized from glacial acetic acid. After another recrystallization from the same solvent with the aid of a small amount of nuchar small white prisms were obtained; yield 0.15 g., m. p. 270–272°.

*Anal.*⁶ Calcd. for $C_{10}H_{10}O_4N_4$: C, 47.97; H, 4.02. Found: C, 47.56; H, 4.23.

2,5-Dicyanopyrazine.—Attempts to totally dehydrate the diamide of pyrazine dicarboxylic acid-2,5 by a twenty-hour reflux with thionyl chloride, heating with phosphorus pentoxide at 320° for seven hours, and a straightforward distillation with the latter reagent, brought about little but decomposition. The following procedure was successful.

Eight-tenths gram of the diamide of pyrazine dicarboxylic acid-2,5² together with 10 g. of phosphorus pentoxide and 35 ml. of freshly distilled nitrobenzene were placed in a 50-ml. flask fitted with an air condenser, precautions against moisture being taken. The mixture was refluxed for four hours. The residue was filtered off, and washed three times with hot nitrobenzene; the combined filtrates were evaporated down to 2 ml. and then chilled. The crystals thus obtained were filtered off and recrystallized from ethyl ether with the aid of a pinch of nuchar. The yield was 0.03 g., m. p. 188–189°; white glistening plates soluble in benzene, nitrobenzene and ethyl ether; insoluble in petroleum ether and water.

*Anal.*⁷ Calcd. for $C_6H_2N_4$: C, 55.38; H, 1.55. Found: C, 55.57; H, 2.03.

(6) Microanalysis by Wm. Saschek, N. Y.

(7) Microanalysis by Arlington Laboratories, Fairfax, Va.

DEPARTMENT OF CHEMISTRY
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Polarographic Analysis of Streptomycin

BY GABOR B. LEVY, PHILIP SCHWED AND J. WARREN SACKETT

In the recovery and purification of streptomycin,¹ as in all similar work (for instance with penicillin), the availability of a rapid and precise method of analysis (assay) is of fundamental importance. Such a method has not been available. Therefore, the development of a chemical method of analysis has been investigated.

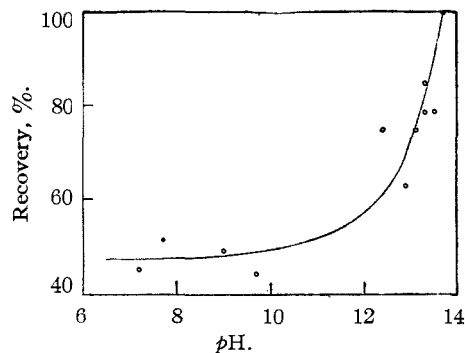


Fig. 1.

It was found that in alkaline medium, well-defined polarographic waves can be obtained with streptomycin solutions under certain conditions.

(1) Carter, *et al.*, *J. Biol. Chem.*, **160**, 337 (1945); also Peck, *et al.*, *This Journal*, **67**, 1866 (1945).

By rigid standardization a method was obtained by which solutions of partially purified² streptomycin, which contain 200 μ /ml. or more, can be analyzed.

As a supporting electrolyte we used tetramethylammonium hydroxide (3% in the final solution) the use of which eliminates maxima and which maintains the pH at a sufficiently high level. This is important since the lowering of the pH causes a rapid diminishing of the diffusion current and concurrently appearance of irregularities. The level of pH 13.6 to 13.8 when resulting from tetramethylammonium hydroxide in 3% concentration does not act destructively on streptomycin at 13.6° within an hour. The influence of the pH on polarographic analysis is represented in Fig. 1 and it is evident that pH conditions should not be varied.

The effect of temperature on the diffusion current is 3%/°C. around room temperature which is comparable to the effect found in other polaro-

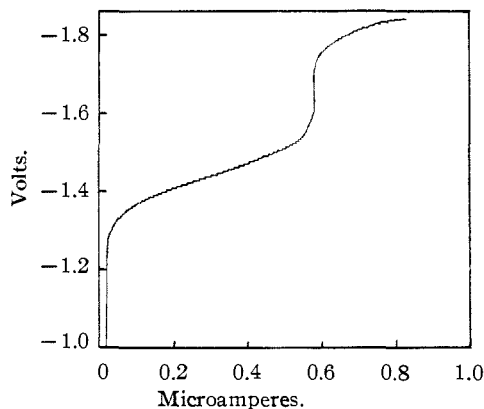


Fig. 2.

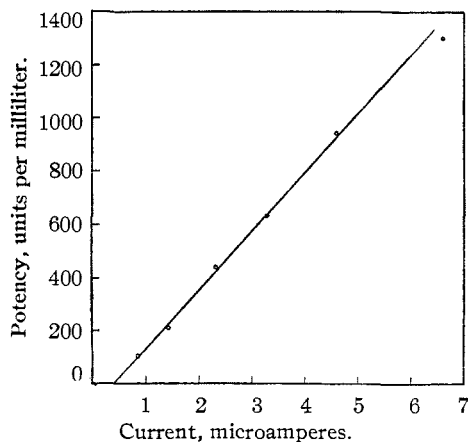


Fig. 3.

(2) Broth containing streptomycin and in some instances salt solutions contain materials which interfere with the analysis by producing additional polarographic waves. Preliminary work indicated that after precipitation of the impurities by lead ions and the removal of the excess lead by sulfuric acid, solutions were obtained which could be analyzed.

graphic analyses.³ At elevated temperatures, *e. g.*, 40°, erratic results were obtained and the analysis could not be performed. The effect of solvents such as methanol and ethanol is marked and special calibration curves were made for solutions containing such solvents. The complete removal of dissolved oxygen is particularly important at lower levels of streptomycin concentration. Since sulfite interferes with the analysis, for this purpose nitrogen is bubbled through the sample.

In aqueous solutions at 13.6° in 3% tetramethylammonium hydroxide experimental points obtained in calibration (Fig. 2) over the range of 100–1000 μ /ml. fell on a straight line with an average deviation of only ± 13 units. A typical polarogram is given in Fig. 3. It appeared from comparisons that in the electrochemical reaction one electron is involved, assuming reversibility. The half-wave potential is -1.45 v. measured against a mercury anode. This potential is subject to some shifting. The diffusion coefficient was found to be 3×10^{-6} cm.² sec.⁻¹, which is of the order to be expected. Nevertheless, the possibility of a catalytic wave is not to be excluded.

When the results of the polarographic analyses are compared with the corresponding microbiological assay results, very close agreement is found. This is true of solutions of low and high purity and in streptomycin produced in these laboratories as well as some originating from other sources. Twenty-five consecutive samples of streptomycin whose approximate purity varied from 70–800 μ /mg. were assayed both by the polarographic and the microbiological methods. The average deviation of the polarographic values from those obtained by the microbiological method was $\pm 6\%$, which is well within the accuracy of the latter method. In only two instances were the deviations greater than 14.5%, namely, -31 and 25% , and these were in streptomycin received from outside sources.

It appears, therefore, that a more precise and considerably more rapid method for the analysis of streptomycin than available heretofore has been developed. Thus, although in two instances we found unexplained deviations, and although we are not certain of the nature of the electrochemical reaction on which the analysis is based, we feel justified in presenting this method at this time. In doing so we have two aims. First, we wish to make available to others working in the field a rapid method of analysis for streptomycin by which the time required for the analysis is reduced to fifteen minutes. Second, we hope that by the extensive use of the method by others, an answer may be found sooner to the few points mentioned above and more generally to some aspects of the streptomycin problem.

We wish to acknowledge gratefully the interest

(3) *E. g.* Kolthoff and Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941.

and advice of Dr. A. J. Liebmann and the cooperation of the microbiological assay department under G. A. Snyder.

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The Use of Calcium Carbide in the Synthesis of Isopropylidene Glycerol

BY M. MARTIN MAGLIO AND CHARLES A. BURGER

Fischer's¹ method for the synthesis of isopropylidene glycerol has recently been modified by Newman and Renoll,² so that the compound may be prepared quite easily in almost quantitative yield. There are several objections to the latter procedure, however, in that an extremely lengthy reaction time and special apparatus are required.

The present authors have succeeded in developing a method which eliminates these objections, although resulting in slightly smaller yields. The procedure consists chiefly in the inter-reaction of acetone and glycerol at reflux temperatures in the presence of an inert diluent, a surface active agent and a desiccant, calcium carbide. Of particular interest is the fact that in our method no acidic catalyst is employed. The surface active agent used, Advawet 15,³ is the sodium salt of a sulfonated petroleum hydrocarbon mixture and is essentially neutral.

The physical properties of our twice-distilled product compare very favorably with those for Fischer's thrice-distilled.

Procedure.—A charge consisting of 110 g. of 95% glycerol, 200 ml. of solvent naphtha no. 4, 240 g. of acetone, 0.4 g. of Advawet 15 and 42 g. of calcium carbide was placed in a 1-liter, three-necked, round-bottomed electrically heated flask furnished with a thermometer in one side neck, a reflux condenser in the other side neck and a mercury-sealed mechanical agitator in the center neck. The charge comprised one solid and two liquid phases. The charge was maintained at 58° and was agitated and refluxed for one hour when an additional 42 g. of desiccant was added (calcium carbide). The mixture was refluxed for an additional three hours, the liquid layer being homogeneous after this time. The cooled reaction mixture was neutralized with 1.5 g. of powdered, freshly fused sodium acetate and then 3 g. of sodium carbonate. After gravity filtration, the filtrate was fractionally distilled at atmospheric pressure, employing a ten-inch Vigreux column, to remove the solvent and the excess, unreacted acetone. Subsequently, distillation at reduced pressure resulted in an 84% yield (126 g.) of colorless isopropylidene glycerol, b. p. 79–81° (11 mm.), n_D^{25} 1.4325, d_4^{25} 1.0624. The odor of the finished product is foul, but it may be eliminated completely by means of activated carbon or some similar absorbent.

A longer reaction time increases the yield by as much as 5%. If the Advawet 15 is not used, the reaction time is somewhat longer and the yield is reduced to 80%.

RESEARCH LABORATORIES

ADVANCE SOLVENTS AND CHEMICAL CORPORATION

NEW YORK, N. Y.

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(1) E. Fischer and Pfähler, *Ber.*, **53**, 1606 (1920).

(2) M. S. Newman and M. Renoll, *THIS JOURNAL*, **67**, 1621 (1945).

(3) Advawet 15 is a surface active agent sold by the Advance Solvents and Chemical Corporation.