

PHYSICO-CHEMICAL STUDIES ON STABILITY OF PENICILLIN SALTS

Part V. Application of Polarographic and Counter-Current Distribution Methods

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IN earlier papers in this series¹⁻³ it was shown by spectrophotometric studies that trace contamination by penicillenic acid (strong absorption at 320 m μ) was responsible for the low life of sodium penicillin G. The application of polarographic and counter-current distribution methods for the study of stability of penicillin salts is described here.

POLAROGRAPHY

Polarograms were taken of a number of commercial samples of sodium penicillin G according to standard procedures. The sample (0.083 g.) is dissolved in water (100 ml.) and the solution is diluted ten times for the actual determination. Ammonium chloride (1 N., 10 ml.) and ammonium hydroxide (1 N. 10 ml.) are mixed and a stream of hydrogen gas is passed to remove dissolved oxygen. Cobaltous chloride solution (0.015 M., 2 ml.) and sample (3 ml.) are then added. Hydrogen gas is again passed for a few minutes. The polarogram is manually recorded at a sensitivity of 1/100 which is kept constant for all runs. The drop time of dropping mercury electrode is 4 secs. The non-polarisable electrode was a large pool of mercury with a potential of + 0.0716 V. vs. saturated calomel electrode. The polarograph employed was of Lange's type B. The applied potential vs. current in arbitrary units is shown in polarograms (Fig. 1).

RESULTS AND DISCUSSION

The catalytic waves for sulphydryl groups are taken in ammoniacal ammonium chloride solution containing divalent or trivalent cobalt. In such medium cobaltous cobalt gives a well-defined single wave for which E_1 is about - 1.2 V. This wave (blank, curve 1) is recorded for all the samples. In the case of pure samples of penicillin the curve (curve 2) is very similar to the blank.⁴ In impure samples (showing a higher absorption at 320 m μ) the catalytic wave appears at about - 1.4 V and reaches a maximum at - 1.6 V and then decreases as the potential is further increased. This has a half-wave potential of - 1.46. This polarogram is very similar to

that of cysteine under the same conditions where the catalytic wave appears at - 1.4 V and reaches a maximum at - 1.8 V.⁵ The quantitative proportionality of the wave height with respect to SH group present is brought out in curves 3 and 4 where a badly deteriorated batch was used at different concentrations. The absence of a catalytic wave in the region - 1.4 to - 1.6 V is an indication of the initial purity (i.e., freedom from penicillenic acid) and consequent storage stability. In a number of determinations with samples of different E values at 320 m μ polarograms of varying catalytic heights were obtained.

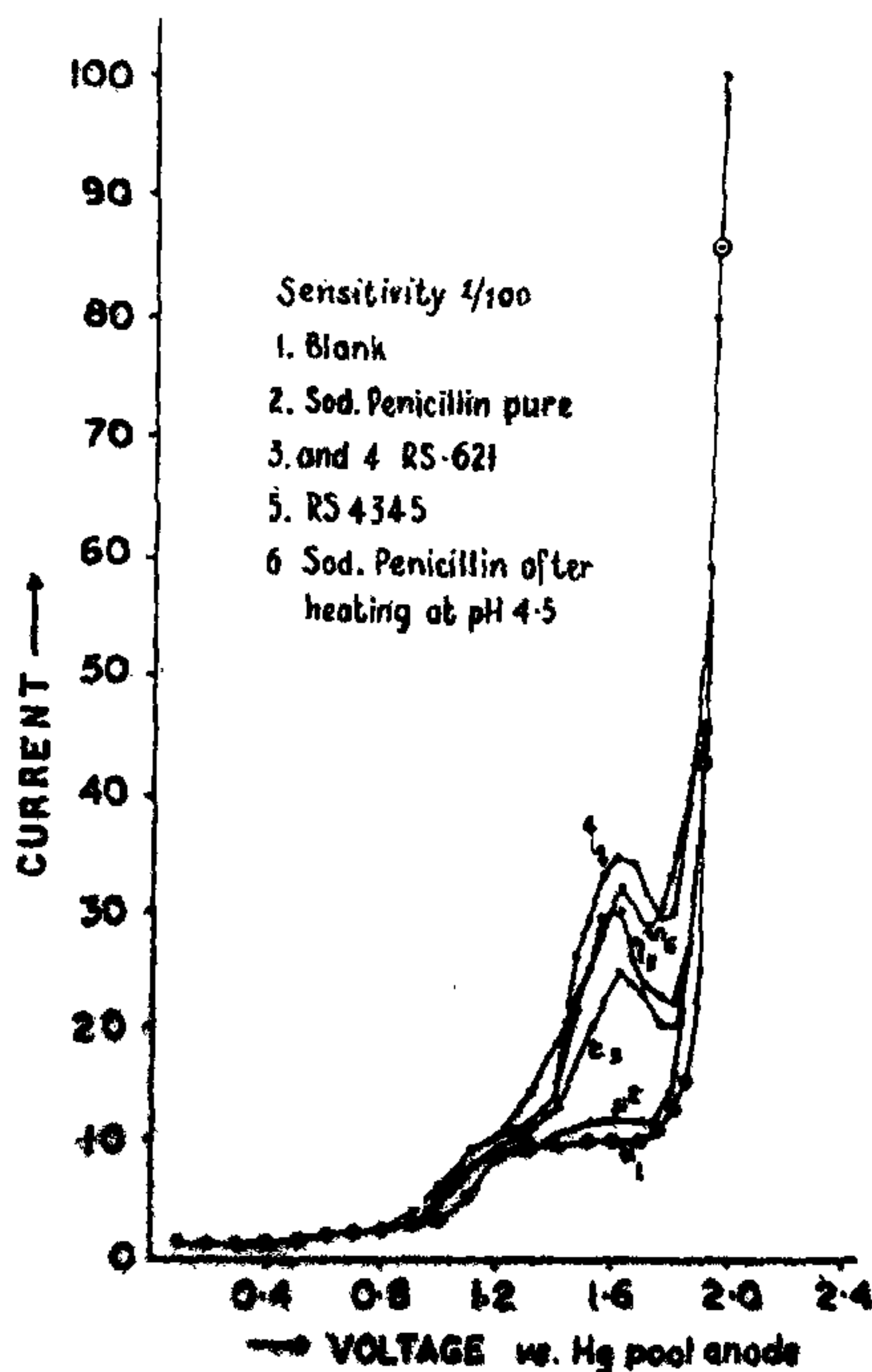


FIG. 1

The identity of the impurity causing the catalytic wave at -1.4 to -1.6 V as penicillenic acid was independently established by decomposing a pure sample of penicillin by heating a solution at pH 4.5 and running the polarogram (curve 6). The half-wave potential of the solution after heating at pH 4.5 was -1.44 V, closely agreeing with the value obtained (-1.46 V) for impure penicillin.

COUNTER-CURRENT DISTRIBUTION STUDIES WITH PENICILLIN

Counter-current studies on penicillin have been mainly aimed at distinguishing the distribution patterns of different penicillins with a view to find the homogeneity of the product and its freedom from admixture with other penicillins.^{7,8} There has not been any report so far in literature about the application of this method for the evaluation of the purity of penicillin and its admixture with decomposition products as contaminants. To get the distribution pattern of benzyl penicillin, penicillenic acid and penicilloic acid, a badly deteriorated batch containing both these impurities was chosen for this preliminary study. The solvent system was chloroform, 2 M phosphate buffer pH 5.0 (prepared from potassium dihydrogen phosphate 272 g. and sodium hydroxide 18 g. per litre). The substance (75 mg.) was taken and 50 transfers were carried out in Craig's semiautomatic counter-current apparatus (Quickfit and Quartz). Penicillenic acid was determined by absorption at $320\text{ m}\mu$ and penicilloic acid by colorimetric method using arsenomolybdate.⁹ Penicillin was determined both by iodimetry and absorbance at $260\text{ m}\mu$.

The distribution pattern is given in Fig. 2. The curve for penicillin given here is the calculated curve which was found to be very similar to the experimental curve, the maxima being in 28th tube in the system. Curves for penicillenic acid and penicilloic acid are the experimental curves, from which it can be seen that penicillenic acid has a maxima in the 3rd tube and penicilloic acid in the end tubes with a maxima at 47th tube. The observed K value

for penicillenic acid is 0.49 and for penicilloic acid it is 23.5.

Comparing the spectrophotometric method reported earlier¹⁻³ with the polarographic and counter-current methods described here it can be seen that the ultra-violet absorption method is by far the simplest and dependable method

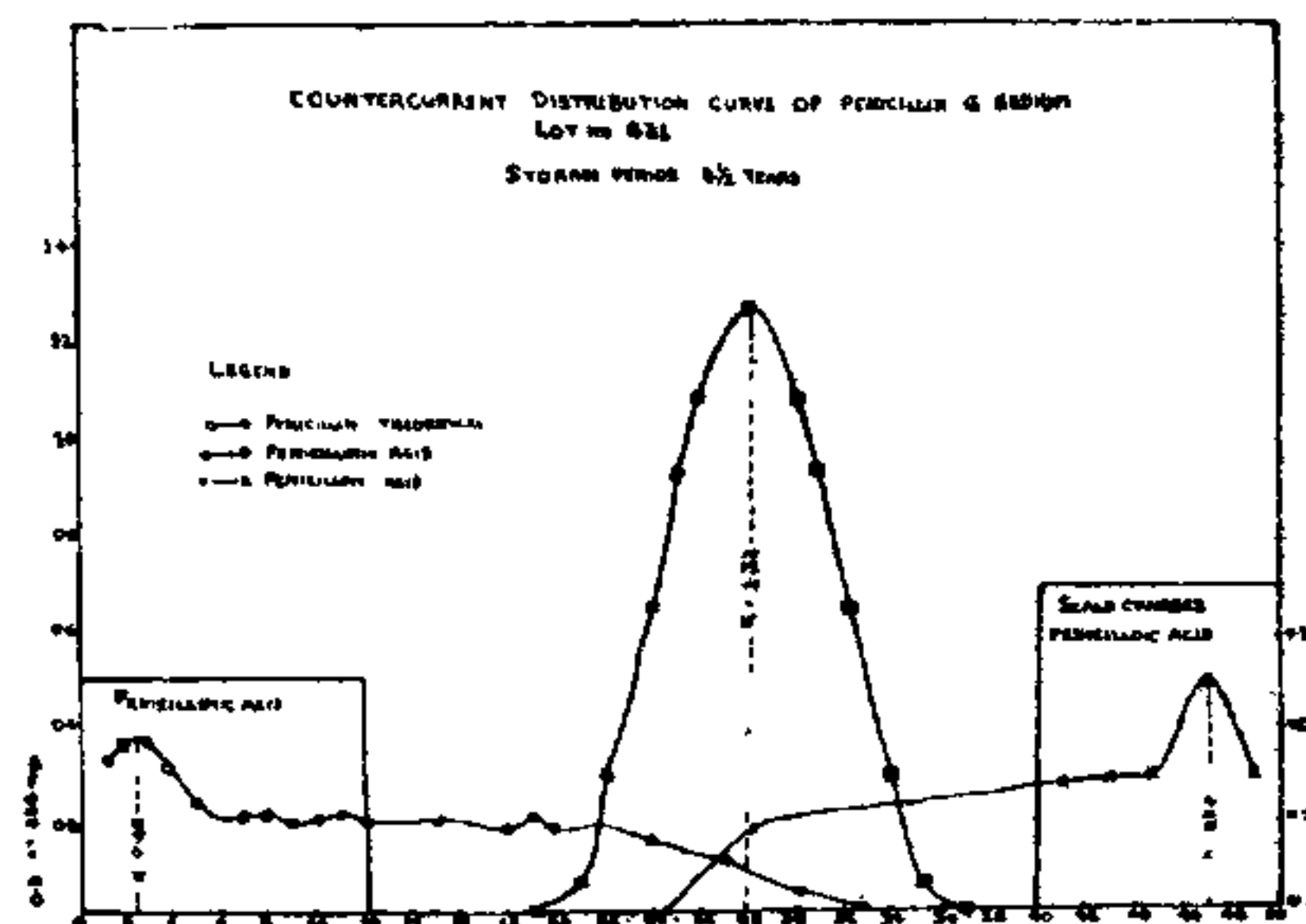


FIG. 2

to evaluate the initial and final purity of sodium penicillin G. Polarography is the next best method for detecting penicillenic acid even in trace amounts. Counter-current distribution is also shown to separate the impurities such as penicillenic acid and penicilloic acid from penicillin G.

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