THE INFLUENCE OF IODIDE CONCENTRATION IN THE IODIMETRIC TITRATION OF PENICILLIN

By T. Canbäck, I. Ehrlen, K. Ilver, F. Reimers and S. Wester

Communication from the Scandinavian Pharmacopœia Council

Received March 10, 1950

In the analysis of penicillins according to the method described by J. F. Alicino[1], the penicillins are decomposed in alkaline solution to penicilloinates which are titrated iodimetrically. It appears from the works available on the iodimetric titration of penicillin that the iodine consumption is dependent on *p*H. Titrating at *p*H 6·24, A. M. Wild[2] found an iodine consumption of *c.* 8·2 equivalents, while when titrating in acid solution, as described by Alicino and others, the consumption was almost 9 equivalents. V. Pedersen[3] investigated the dependence of the iodine consumption on the acidity at *p*H values between 1 and 8 and found a dependency as depicted in Figure 1. B. Örtenblad[4] repeated these experiments and arrived at a completely different result. He did not find the marked increase in iodine consumption at *p*H values between *c.* 2 and 4·5. A series of investigations were carried out at the instigation of the Scandinavian Pharmacopœia Council, Penicillin Sub-Committee, both in the laboratories of the Danish and the Norwegian Pharmacopœia Commissions, and also in Sweden in the Control Laboratories of the Pharmaceutical Society. In the first mentioned, Pedersen's results were confirmed, while Örtenblad's results were confirmed in the two last mentioned laboratories.

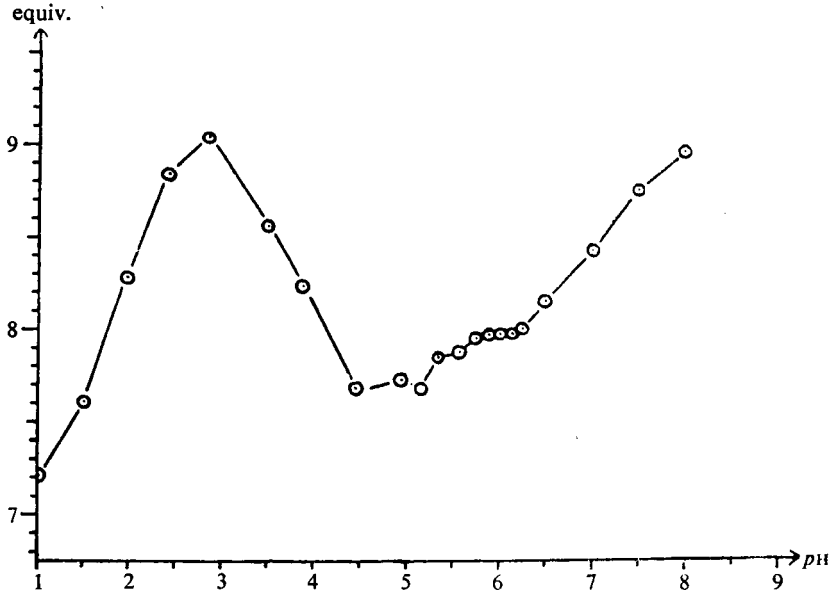

Fig. 1. Interdependence between iodine consumption and *p*H according to V. Pedersen[3].

In these experiments, the same analytical procedure and the same penicillin samples were used, and it is therefore obvious that the deviations must be caused by circumstances not prescribed in the outline of the analytical procedure. A detailed discussion of all experimental steps showed that the difference was probably due to the fact that the Norwegian and Swedish laboratories used 0·01N iodine containing 0·2 per cent. of potassium iodide, while in the Danish laboratory 0·01N iodine was used which was prepared according to Ph.Dan. 1948 and contained 2·2 per cent. of potassium iodide.

It has been found previously by Wild (*loc. cit.*) that during titration at *p*H 6·24 the iodine consumption decreases with increasing concentration of iodide. If the difference in iodide concentration should be the cause of the discrepancy between Pedersen's and Örtenblad's results, the effect of changes in the iodide concentration should be different at different *p*H values. The investigations into the influence of the iodide concentration carried out partly in the Control Laboratory of the Pharmaceutical Society in Stockholm, partly in the laboratory of the Pharmacopœia Commission in Copenhagen, have confirmed this assumption and also the fact that the disagreement found earlier must be a consequence of differences in the iodide concentration.

Our experiments were carried out in the following manner. The solutions used contained 0·0500 g. of benzylpenicillin salt in 100 ml.; 0·0800 g. in the case of procaine benzylpenicillin. The procaine salt was brought into solution either by shaking with water or by means of 1 ml. of methyl alcohol and subsequent dilution with water. To 5·00 ml. of penicillin solution 1·00 ml. of N sodium hydroxide was added and the penicillin decomposed by standing for 20 minutes. Then 5·0 ml. of buffer mixture, 1·00 ml. of N hydrochloric acid and 10·00 ml. of 0·01N iodine were added. After storage in the dark for 20 minutes at *c.* 20°C. titration was performed, using 0·01N thiosulphate with 2 drops of mucilage of starch as indicator. The blanks were carried through in the same way, however, omitting the treatment with N sodium hydroxide and the subsequent addition of N hydrochloric acid.

At *p*H values between 2·5 and 6, phthalate buffer (Ph.Svec. 1946) was used, and at *p*H values between 6·5 and 7 a phosphate buffer (Ph.Svec. 1946). In order to attain so high a buffer capacity that the *p*H would not change too much towards lower values during oxidation with iodine, we have used buffer mixtures of a concentration six times higher than stated in Ph.Svec. 1946. *p*H values 1·5 and 2 were obtained by addition of hydrochloric acid.

*p*H determinations on the titrated solutions were carried out by means of a glass electrode. The following penicillin samples were used:

(A) 2 samples of crystalline benzylpenicillin sodium purified from other penicillins, one by recrystallisation from butyl alcohol and acetone, the other by preparation through the di-*iso*propyl etherate.

(B) 2 samples of crystalline benzylpenicillin sodium—commercial.

(C) 1 sample of impure crystalline benzylpenicillin calcium.

(D) 2 samples of crystalline procaine benzylpenicillin—commercial.

The results obtained in the Danish laboratory and in experiments with

the Danish penicillins were in good agreement with the results obtained in experiments with Swedish penicillins, carried out in the Swedish laboratory. As examples of these results Figures 2 and 3 present curves

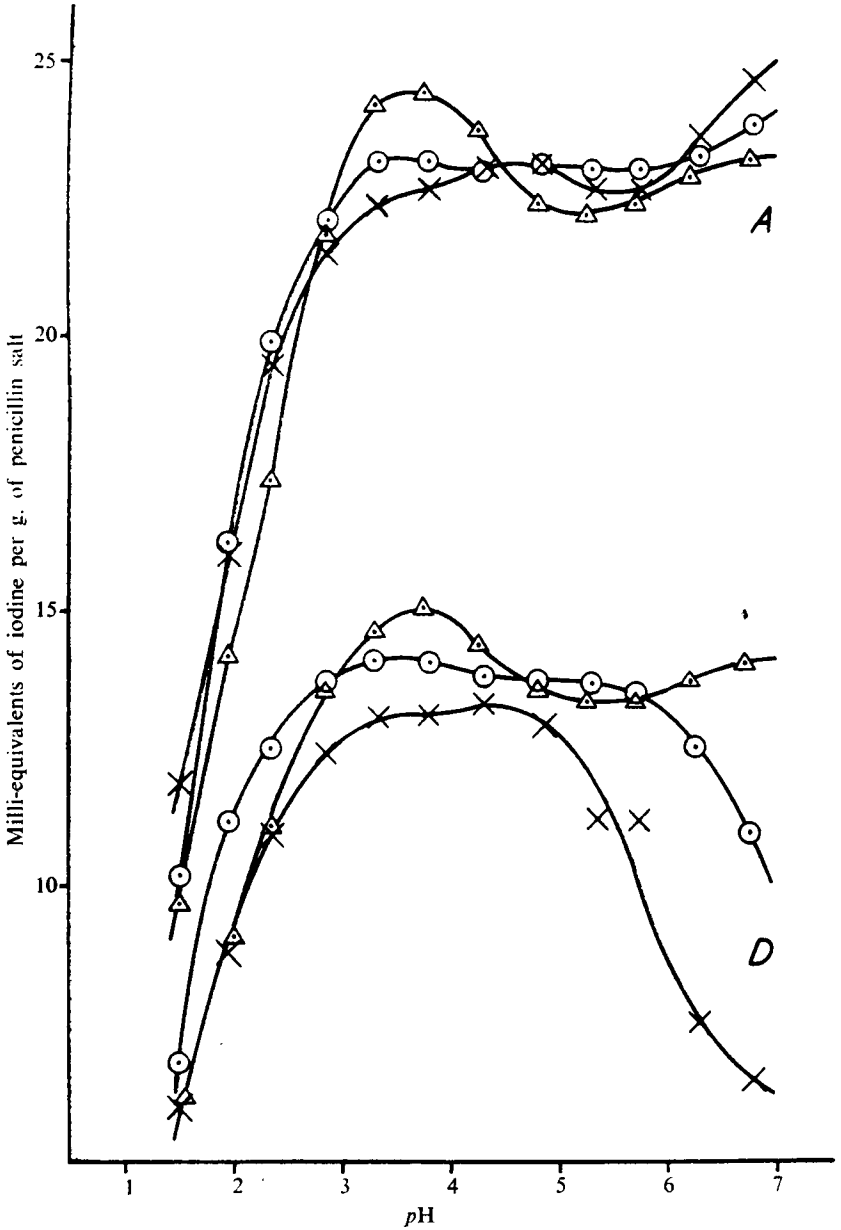

FIG. 2. Interdependence between iodine consumption and *p*H for pure crystalline benzylpenicillin sodium (A) and for crystalline procaine benzylpenicillin (D).
x——x 0·0125N KI in 0·01N I.
o——o 0·050N KI in 0·01N I.
Δ——Δ 0·200N KI in 0·01N I

showing the interdependence between iodine consumption (calculated as milli-equivalents of iodine per g. of penicillin salt) and *p*H for one preparation of each of the four groups A, B, C, and D determined with

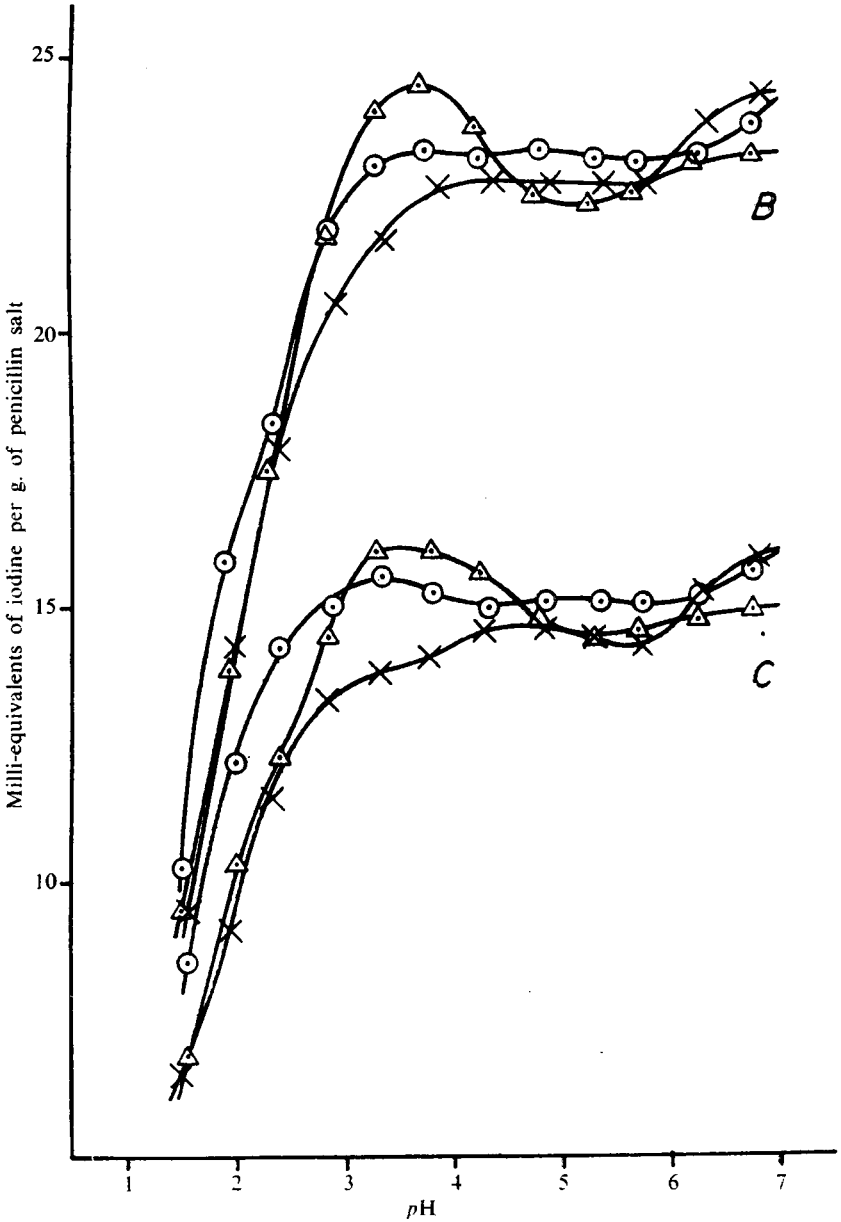

FIG. 3. Interdependence between iodine consumption and *p*H for crystalline benzylpenicillin sodium (commercial) (B) and impure crystalline benzylpenicillin calcium (C).

x——x 0·0125N KI in 0·01N I.
o——o 0·050N KI in 0·01N I.
Δ——Δ 0·200N KI in 0·01N I.

0·01N iodine, which was 0·0125N, 0·05N, and 0·20N, respectively, with respect to potassium iodide.

The curves of Figures 2 and 3 show almost the same effect of changes in the iodide concentration in experiments with the penicillin salts A, B, and C, while the course of the curve of the procaine salt D deviates at higher *p*H values.

In all cases, a significant increase in the iodine consumption as determined at a *p*H around 3·5 is found when 0·01N iodine with a high iodide

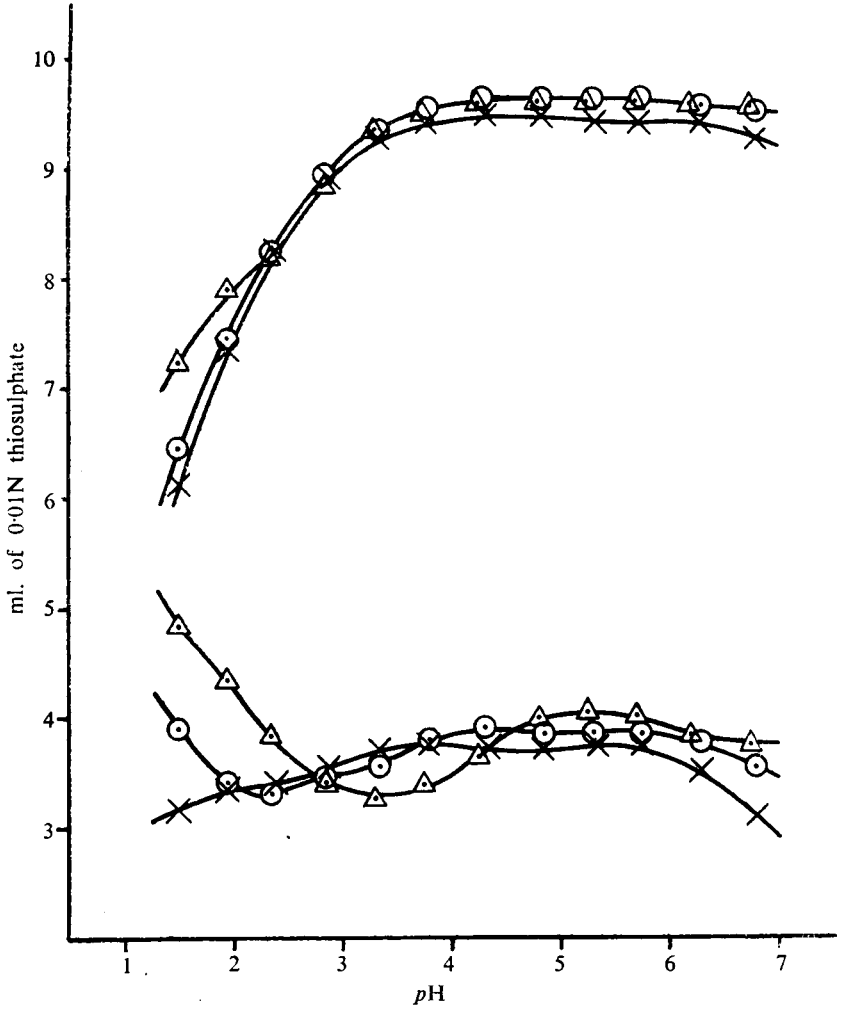

FIG. 4. Consumption of 0·01N thiosulphate in the analysis of 2·5 mg. of benzylpenicillin sodium (Upper graph).

Below: consumption by titration after treatment with sodium hydroxide.
Above: consumption when determining the blank.

x——x 0·0125N KI in 0·01N I.
o——o 0·050N KI in 0·01N I.
Δ——Δ 0·200N KI in 0·01N I.

concentration is used. At *p*H values below *c.* 3 the iodine consumption decreases, mainly because of the fact that the iodine consumption in the determination of the blank increases. This appears from Figure 4, which, as an example, shows the consumption of 0·01N thiosulphate in the analysis of purified benzyl penicillin sodium (A), both during titration of the penicilloic acids formed by decomposition and during the determination of the blank. Penicillin salts B and C lead to similar curves.

Analogously, the reduction in iodine consumption in the analysis of benzylpenicillin procaine at *p*H values above *c.*5, using 0·01N iodine with a low iodide concentration, must be due to an increased iodine consumption in the determination of the blank. Using 0·01N iodine which is 0·0125N with respect to potassium iodide, the titrated mixture turns opaque during determination of the blank. The colour change becomes somewhat less sharp when the determination is carried out at *p*H values above *c.*5. The same does not happen when the potassium iodide concentration is 0·2N and appears only at *p*H values above *c.*6 when the potassium iodide concentration is 0·05N. Opalescence does not appear when titrating the procaine salt after treatment with sodium hydroxide.

These experiments show clearly that it is necessary to prescribe the potassium iodide concentration in the titration solution to be used for the iodimetric titration of penicillin.

If it is desirable to apply a 0·01N iodine with a concentration of iodide in which a change of *p*H causes only a small change of the results, it is obviously not practical to use an iodide concentration greater than 0·05N while, on the other hand, for the titration of the procaine salt it is unfavourable to choose a concentration considerably below 0·05N.

It has been the purpose of the present investigation exclusively to show the interdependence between iodine consumption and *p*H when applying 0·01N iodine with varying iodide concentrations. It has not been the intention to determine the exact iodine consumption under different experimental conditions. For this reason we have not carried out control analyses of the penicillin samples applied in order to determine exactly their total content of penicillin.

SUMMARY

The potassium iodide concentration in 0·01N iodine used in the iodimetric titration of penicillin is of decisive importance for the result obtained. If it is desirable to use an iodide concentration for which small changes of *p*H involve only small changes of the result, it would not be practical to choose a concentration higher than 0·05N. At the same time, the iodide concentration used in the titration of procaine salts should not be considerably below 0·05N. These conclusions are based on the curves A—D of Figures 2 and 3.

We wish to express our thanks to Dr. B. Ortenblad for informing us of his results with the titrations of penicillin at different *p*H values, and

for his permission in the present paper to discuss his experiments which have not yet been published. We also wish to express our gratitude to Mr. P. Mørch and to Dr. H. F. Meldahl, who very kindly placed the samples of penicillin salts at our disposal.

REFERENCES

1. Alicino, *Industr. Engng. Chem., Anal. Ed.*, 1946, **18**, 619.
2. Wild, *J. Soc. chem. Ind.*, 1948, **67**, 90.
3. Pedersen, *Arch. Pharm. Chemi*, 1948, **55**, 625.
4. Örtenblad, *private communication*.