501. *Some Factors which might affect the Accuracy of the Iodometric Estimation of Penicillin.*

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Iodide concentration, and other factors, are shown to affect the results of iodometric estimation of penicillin.

The iodometric estimation of penicillin [1,2] is not stoicheiometric; the uptake of iodine after hydrolysis has been reported [1] to be 8·97 equivs. per mole (in 15 min. at room temperature). During another investigation it was noted that variation from the standard method of estimation [2] sometimes led to considerable inaccuracy.

Rates of uptake of iodine at various iodide-ion concentrations and pH values have been determined at 0°. Graphs of the results (see Figure) show breaks which indicate that a number of consecutive reactions are involved, and that variation of the conditions, particularly of the iodide concentration, may affect different stages of the reaction in different ways.

At a low iodide concentration of 0·075M the uptake of iodine per mole of penicillin is not affected appreciably by a two-fold dilution of the reaction mixture. In solutions of much higher iodide concentration (1·5M), however, the effect of a two-fold dilution is to increase the rate of uptake at intermediate times. An increased rate of reaction on dilution of the mixture is also observed (at pH 5·33) when potassium chloride (final concentration 1·5M) is added to the diluted mixture, in which case the ionic strength of the solution is somewhat higher than in the undiluted mixture.

Katzin and Gebert [3] have investigated the iodine–iodide–tri-iodide equilibrium in the range of iodide concentrations 0·02—0·08M and at various ionic strengths. They find that changing the ionic strength in the range 0·1—1 has little effect on the equilibrium constants K_c at 20° and 25°, although the activity coefficients vary somewhat.

$$[I_3^-]K_c = [I_2][I^-] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (i)$$

Results of the present investigation are explicable if each intermediate product P_n is oxidised at such a rate that

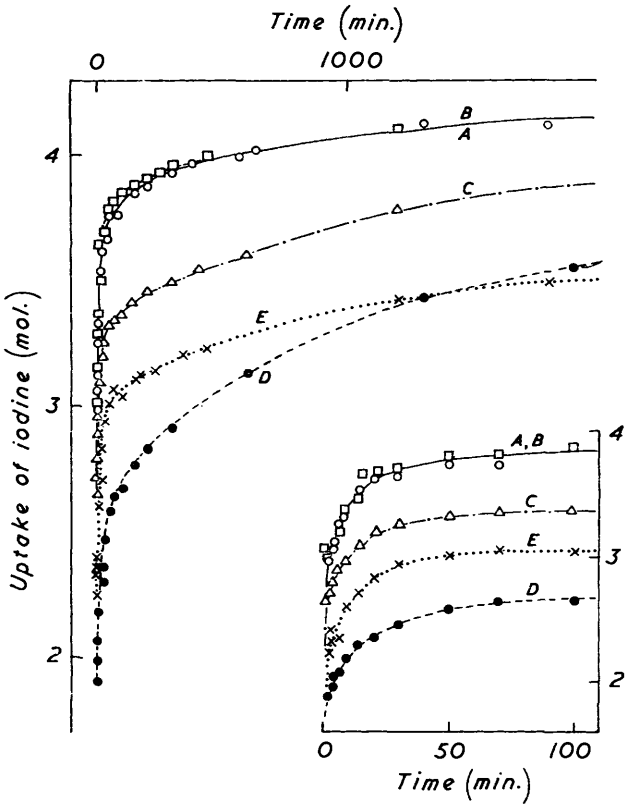
$$-\partial[P_n]/\partial t = [P_n] \, . \, \Phi_n([I_2], -) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (ii)$$

where Φ_n is a function which may depend on pH, etc. Provided that conditions (i) and (ii) are fulfilled simultaneously for all steps in the reaction sequence, the uptake of iodine per mole of penicillin will be independent of dilution, for all values of t.

[1] Alinico, *Ind. Eng. Chem. Anal.*, 1946, **18**, 619.
[2] Ministry of Health Conference, *Analyst*, 1949, **74**, 550.
[3] Katzin and Gebert, *J. Amer. Chem. Soc.*, 1955, **77**, 5814.

In solutions of higher iodide concentration, however, the effects of both the formation of higher polyiodide ions and of larger changes in the activity coefficients must become more important. Although conclusions concerning a mechanism derived from rates measured in such concentrated solutions are not necessarily valid, the faster reaction in the dilute solution might indicate a reversible reaction involving iodide ions.

Rate of uptake of iodine in runs B—E *of Table* 1, *and* (A) *under conditions of section* (*i*).



Estimation of penicilloic acid during the hydrolysis of penicillin by enzymes requires that either the enzyme be inactivated or that the time of contact with iodine be reduced to a minimum. When the standard method of estimation is varied, it seems important to determine both the amount and the rate of uptake of iodine at a number of penicilloic acid concentrations, for the final iodine–iodide ratio will differ from case to case.

Experimental

Materials.—Penicillin G sodium salt (crystalline; Commonwealth Serum Laboratories, Melbourne, Australia) (6·066 g.) was treated in water with M-sodium hydroxide (50 ml.) at 50° for 30 min.; the mixture was cooled, adjusted to pH 7·0 with 1·1M-hydrochloric acid and made up to 250 ml. with water. One drop of chloroform was added, and the mixture was kept at 4°. The supernatant liquid remained clear for several months. 0·5M-Phosphate buffer solutions were prepared from phosphoric acid, potassium dihydrogen phosphate, and disodium hydrogen phosphate, and the pH values were determined with both glass and quinhydrone electrodes. 0·05M-Iodine containing potassium iodide (50·0 g./l.) was used. Solutions containing appreciably less iodide deposited iodine on long storage at 0°.

Kinetic Measurements.—Runs were carried out in volumetric flasks which were cooled by immersion in ice–water contained in a heavily lagged bath. The temperature of the mixtures

varied between 0° and 1° during the reaction, but essentially no better temperature control was obtained when the ice-water was stirred. Runs, but not all readings, were duplicated.

(i) 0·5M-Phosphate buffer solution (pH 6·92; 120·0 ml.), water (20·0 ml.), and the penicillin hydrolysis mixture (10·00 ml.) were mixed and kept overnight in the ice-water bath. 0·05M-Iodine (50·0 ml.), cooled similarly, was added, and the whole mixed rapidly. Aliquot parts (10·00 ml.) were removed at intervals and added immediately to known amounts of sodium thiosulphate solution. The mixture was cooled by the addition of crushed ice, and the excess of thiosulphate was titrated with iodine.

Parallel runs were carried out with the omission of the penicillin. The loss of iodine was negligible over a period of several weeks. On the other hand, oxidation of iodide in the more acidic conditions was rapid immediately after the initial mixing and in some cases this oxidation was more rapid than the uptake of iodine. This effect could be eliminated by flushing the flasks with carbon dioxide before the iodine solution was added. The uptake of iodine (as moles of iodine per mole of penicillin) is plotted against time in the Figure (curve A). Uptake after 16 hr. at room temperature was 4·42 mol. The initial iodine concentration was 0·01354M-I_2.

(ii) Runs *B*—*E* (Table 1) were carried out as described in section (i). In each case penicillin hydrolysis mixture (10·00 ml.) and 0·5M-phosphate buffer solution were present. The uptake of iodine per mole of penicillin is shown in curves *B*, *C*, *D*, and *E*.

TABLE 1.

Run	*B*	*C*	*D*	*E*
Water added (ml.)	220	20	20	220
KI added (g.)	—	10·0	50·0	50·0
Initial [I] (M)	0·00684	$0·0135_1$	$0·0127_1$	0·00660

(iii) Results of additional runs at different pH values, with 0·5M-phosphate buffer solution (120 ml.) and penicillin hydrolysis mixture (10·00 ml.), are given in Table 2.

TABLE 2. *Uptake of iodine (moles per mole of penicillin).*

pH of buffer	5·33	5·33	3·42	3·42	3·42		5·33	5·33	3·42	3·42	3·42
H_2O added (ml.)	20	220	20	20	220		20	220	20	20	220
KI added (g.)	50·0	50·0 *	—	50·0	50·0		50·0	50·0 *	—	50·0	50·0
[I] (10^3M)	12·76	6·36	12·25	11·49	5·96		12·76	6·36	12·25	11·49	5·96
Time (min.): 2	1·93	2·11	3·18	1·25	1·14	250	3·19	—	3·90	—	2·56
3	2·02	2·19	3·28	1·26	1·41	360	—	—	—	2·64	—
4	2·08	2·28	3·35	1·46	1·57	400	3·29	3·79	—	—	2·79
6	2·20	2·42	3·43	1·66	1·73	450	—	—	3·96	—	2·77
9	2·31	2·56	3·49	1·77	1·87	600	—	—	—	2·78	2·93
14	2·44	2·71	3·60	1·92	1·98	700	3·45	3·94	3·98	—	—
20	2·60	2·86	3·64	1·99	2·00	1300	—	4·06	4·00	3·11	3·36
30	2·71	3·02	3·72	2·06	2·12	1800	—	—	4·01	—	3·58
50	2·85	3·20	3·75	2·13	2·21	3000	3·86	4·17			
70	—	3·29	3·78	2·20	2·32	7600	—	4·34			
100	2·99	3·37	3·81	2·28	2·54	14,600	4·38	—			
150	3·07	3·50	—	—	2·41	17,300	—	4·57			
160	—	—	3·86	—	—	23,000	4·48	—			
200	—	3·62	—	2·44	2·56						

* KCl (47·5 g.) was also added.

(iv) 0·5M-Phosphate buffer solution (pH 3·42; 120·0 ml.), water (220 ml.), and penicillin hydrolysis mixture (10·00 ml.) were treated as described in (i). After each titration the pH of the aliquot portion was determined with a glass electrode system. The uptake of iodine is given in Table 3.

TABLE 3.

Time (min.)	2	3	4	6	9	14	21	30	50
I uptake (mol.) ...	2·97	3·12	3·25	3·37	3·49	3·61	3·68	3·73	3·80
pH	3·45	3·48	3·45	3·49	3·44	3·38	3·42	3·46	3·45
Time (min.)	70	100	150	200	375	580	1300	1800	
I uptake (mol.) ...	3·83	3·86	3·89	3·90	3·94	3·97	4·01	4·01	
pH	3·46	—	3·40	3·40	3·43				

Initial iodine concentration 0·00609M-I_2.

(v) To a mixture of 0·1M-phosphate buffer solution (20 ml.) and crushed ice was added 0·00856M-penicillin hydrolysis mixture (10·00 ml.), and the mixture was titrated with 0·005M-iodine. The uptake of iodine (mol.) at various pH values was: pH 7·0, 1·26; pH 6·0, 1·06; pH 5·0, 1·01; pH 4·0, 1·15.

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