STABILISATION OF PENICILLIN-SALT SOLUTIONS WITH SODIUM CITRATE

by

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In a recent paper I described the stabilising effect of sodium citrate (disodium salt dissolved in physiological NaCl solution) on the rate of destruction of sodium penicillin solutions by heat. Only very dilute concentrations of sodium penicillin were used and it was shown that sodium citrate in concentrations of M/1 to M/100 stabilises sodium penicillin solutions at 100° C, and at room temperature for twenty three days. Further observations using sodium citrate in 0.9% NaCl solution, the concentration ranging from M/200 to M/1000, proved that an optimal stabilising effect on sodium penicillin solutions containing 5 units per ml was produced by concentrations of M/300 to M/400.

In this article I propose to report on the results obtained with higher concentrations of sodium penicillin solutions and the correlation between molar concentration of sodium citrate and the stabilising effect. It will be seen that even in higher concentration of sodium and calcium penicillin the stabilisation by citrate is being maintained.

The stability of penicillin salts in aqueous solutions is conditioned by several factors of which p_H, temperature, purity of the manufacture, concentration of the salts, and the addition of buffer-containing impurities are the most important ones.

An excellent survey about this problem is given by the book "Penicillin, its properties, uses and preparations" (1946) from which I take the following quotations. BENEDICT and co-workers (1945 and 1946) working with crystalline sodium penicillin II found that the maximum stability is at p_H 6, at which value sodium penicillin solution, in a concentration of 100 units per ml had a half life (that means the time in which it had lost half its original activity) of fourteen days.

Rise of *temperature* increases the hydrolytic action responsible for the inactivation of aqueous penicillin solutions whereby dilute solutions are more resistent against heat than solutions of a higher concentration. KIRBY (1944) who tested samples from eight American manufactures in solutions containing only 1 unit per millilitre found that most of them retained full potency at about 22° C for 10 to 12 days. GREEY and MACDONALD (1945) reported for a stronger solution containing 1000 units per ml a half life of about twenty four hours at 56° C.

WINTERBOTTOM (1946) found that a solution containing 270 units calcium penicillin per ml in physiological NaCl solution at p_H 6.5 displayed no significant loss of potency during a week at 25° C or during two months in a refrigerator whilst a ten times stronger solution of calcium penicillin at p_H 6.2 lost about 40% of its activity in one month. SMITH (1946) using samples of crude sodium penicillin (containing 470 units per mg) in solutions of 12 500, 62 000, and 250 000 units per ml found that at 24° C the most dilute solution lost about 4% of its activity per 24 hours; the next stronger solution showed a loss of 8% per day, and the strongest solution 25% per day.

DENSTON (1946) who investigated the effect of the *purity* of penicillin on its stability in solutions reported that a solution of pure sodium penicillin II, containing 20 000 units per ml retained 90% of its potency after four days at room temperature and 70% after five weeks in a refrigerator. This solution could be boiled for five minutes without appreciable loss in activity. Solutions of a manufacture with only 300 units per mg retained a potency of 90% for three or four days, and at least 60% for five weeks in a refrigerator. The suggestion that the greater stability of impure samples

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may be related to a buffering effect of the impurities is supported by DENSTON's observations that a buffered solution of crystalline sodium penicillin at p_H 7 kept its potency for fourteen days at room temperature.

PREVIOUS WORK ON STABILISATION OF PENICILLIN SOLUTIONS

Attempts of using substances for stabilising penicillin solutions were made by many authors. SMITH (1946) testing several buffering substances found that CALGON brand of sodium hexametaphosphate in a 0.5 % solution was most effective. Pure sodium penicillin II dissolved in this solution containing 1000 units per ml had a half life at 24° C of forty five days compared with fourteen days as shown by BENEDICT for a crystalline sodium penicillin II solution at p_H 6 in a concentration of 100 units per ml. PULVERTAFT and YUDKIN (1946), in a recent paper, described the stabilising effect of phosphate on the rate of destruction of penicillin by heat. Working with three samples of penicillin varying in degree of potency and amounts of unknown impurities, they showed that the addition of phosphate, especially to dilute solutions was able to preserve the potency of penicillin for long periods at room temperature and that phosphate stabilises penicillin solutions at 100° C to a considerable extent. They came to the conclusion that the stabilising effect is due to a specific action of the PO_4 ion and not to the control of the p_H.

Sodium or potassium citrate has been used extensively for simultaneous oral administration as a buffer in the attempt of preventing inactivation of penicillin by the gastric juice. (FREE, PARKER, and BIRO; SZENT-GYÖRGYI and co-workers; BUSHBY and HARKNESS, 1946).

METHOD

The agar-cup method (FLEMING), as described in my previous article was used. Standard curves were constructed with each batch of the penicillin manufacture under test daily, dilutions of the standard having concentrations of two, one, 0.5, and 0.25 units per ml. The samples to be tested after being kept at 100° C or room temperature were diluted to contain approximately 1 unit per ml. The average of the diameters of the zone of inhibition from at least three assays was determined and all tests repeated thrice, thus providing at least nine results for comparison with the standard curve.

The stabilising effect of the sodium citrate –0.9 % NaCl mixture was assessed by the estimation of the rate of destruction of penicillin by heat and under room temperature condition. The solutions to be tested for stabilisation at room temperature were kept in ampoules and boiled for 15 minutes. Control assays were carried out with phosphate and distilled water as solvents.

T.R.C. sodium penicillin tablets, sodium penicillin "Roche", and sodium penicillin, The Dist. Co. Ltd., Liverpool were used for the tests.

RESULTS

OPTIMAL MOLAR CONCENTRATIONS OF SODIUM CITRATE IN 0.9 % NaCl SOLUTION FOR STABILISATION OF PENICILLIN CONCENTRATIONS OF 5,100, AND 1000 UNITS PER MILLILITRE

The results are shown in the following table, giving the average values of nine estimations. The p_H of most of the solutions was determined before and after the heating.

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TABLE I

5, 100, AND 1000 UNITS PER ml KEPT AT 100° C FOR 30 MINUTES

Citrate-concentration	pH before heating	pH after heating	5 u/ml percentage of penicillin remaining	units of penicillin remaining	pH before heating	pH after heating	100 u/ml percentage of penicillin remaining	units of penicillin remaining	pH before heating	pH after heating	1000 u/ml percentage of penicillin remaining	units of penicillin remaining
M/1	7.4	7.4	40	2.0	. .	. .	. .	. .	7.4	7.4	26.6	266
M/50	6.6	6.6	54	2.7	6.6	6.6	60	60	6.8	6.9	67.6	676
M/100	6.4	6.6	58	2.9	6.4	6.4	65	65	6.7	6.7	72.1	721
M/200	6.3	6.3	60	3.0	6.3	6.3	72	72	6.5	6.7	75.8	758
M/300	5.8	5.9	72	3.6	5.7	5.8	73	73	6.1	6.3	81.5	815
M/400	5.8	5.8	78 | M/450 86	3.9 | M/450 4.3	5.6	5.6	75	75	6.1	6.2	80.7	807
M/500	5.7	5.8	58	2.9	5.6	5.7	65	65	6.0	6.2	85	850
M/600	5.6	5.6	56	2.8	5.4	5.5	60	60	6.0	6.1	64	640
M/700	. .	. .	54	2.7	. .	. .	. .	. .	5.8	6.0	62	620
M/800	5.2	5.4	54	2.7	. .	. .	. .	. .	5.7	5.8	75.3	753
M/900	. .	. .	44	2.2	. .	. .	. .	. .	5.6	6.0	82	820
M/1000	5.0	5.1	44	2.2	. .	. .	. .	. .	5.5	5.9	82.3	823
Control with dist. water, buffered to pH 6.3	6.3	6.5	5	0.25	6.3	6.4	75	75	pure dist. water 5.3	6.3	85.6	856

Solutions 5 u/ml sodium penicillin T.R.C. tablets 392 were used for testing. The samples were boiled in ampoules 30 minutes at 100° C. Molar concentrations of citrate ranging from M/1 to M/1000 were used. It will be seen from the table that there was an optimal concentration of M/400 to M/450 at a p_H 5.8, above and below which the rate of destruction was greater.

In using higher concentrations of penicillin salts for our experiments it was to be born in mind that together with the penicillin the amount of impurities containing an unknown addition of buffering substances must necessarily increase too and this to an incalculable extent. The possibility of the impurities interfering with sodium citrate chemically must be taken in consideration. (We found a trace of phosphate in T.R.C. tablets). Irregular results were, therefore, to be expected.

Still, with sodium penicillin solutions containing 100 u/ml there was an optimal molar concentration of citrate reached: M/400 at p_H 5.6. But, in distilled water buffered with phosphate to p_H 6.3–6.4 the result was the same. In order to ascertain how far the p_H factor in itself was reponsible for the stabilisation, solutions of 100 u/ml sodium penicillin T.R.C. 392 (A) in Citrate M/400 solution at p_H 6.6 to 6.9, (B) in distilled water adjusted with phosphate to p_H 6.6, and (C) in distilled water p_H 4.0, were kept 15, 30, 45 and 60 minutes at 100° C. The results were as follows:

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PERCENTAGE OF PENICILLIN REMAINING

After heating at 100° C for	(A)	(B)	(C)
15 minutes . .	75	75	—
30 ,, . .	60	70	37
45 ,, . .	50	35	—
60 ,, . .	25	12.5	—

It will be noted that there was no difference between (A) and (B) after 15 minutes and the stabilising effect was better in (B) after 30 minutes, but weaker in (C). After 45 and 60 minutes the stabilising effect of the citrate-solution exceeds that of buffered dist. water.

That a fair amount of buffering agents is present in T.R.C. tablets was already observed by PULVERTAFT and YUDKIN (1946).

This was bound to become more evident with higher concentrations of sodium penicillin and thus the results obtained with solutions containing 1000 u/ml represent irregular and rather confusing figures. Molar concentrations of M/500 and M/1000 produce about the same optimal values. The buffering agents included in the impurities apparently influence the results to a considerable extent. The samples of redistilled water at my disposal coming from different sources, and even freshly distilled water were acid, usually at p_H 4. In one assay 1000 u/ml sodium penicillin T.R.C. 392 was dissolved in redist. water and showed a p_H 4.8 before boiling. After 50 minutes heating at 100° C the solution had a p_H 6.4 and preserved a penicillin potency of 87 %. Sodium citrate M/1000 at p_H 6.6 before and 6.7 after heating at 100° C for 50 minutes kept 1000 u/ml of the same sample stable (99 % potency).

With solutions of sodium penicillin T.R.C. 392,1000 u/ml being dissolved in M/400, M/600, M/800, and M/1000 citrate solutions the following values of p_H were obtained:

	Sodium citrate			
	M/400	M/600	M/800	M/1000
p_H before	5.9	5.6	5.3	5.1
p_H after boiling for 30 minutes .	6.6	6.6	6.6	6.6

The percentage of remaining penicillin was as follows:

M/400	M/600	M/800	M/1000
100 %	90 %	100 %	100 %

In order to ascertain whether the stabilising effect of citrate in these experiments is purely or mainly related to the p_H factor or due to a chemical or catalytic action of the citrate ion, it became necessary to compare solutions with the same p_H.

1000 units per ml were dissolved (A) in M/400 citrate solution p_H 6.4 to 6.8, and (B) in distilled water adjusted with phosphate buffer to 6.6. The results after heating at 100 ° C were as follows:

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Minutes	Percentage of penicillin remaining	
	(A)	(B)
15	100	100
30	80	80
45	80	56
60	80	48

The stabilising effect of M/400 citrate solution thus definitely exceeds that of dist. water adjusted to the same p_H.

The rate of destruction by heat of a solution containing 1000 u/ml of Calcium penicillin Imperial Chemical (pharmaceutic) Ltd., Manchester, No AF 45/14 (A) in citrate solution of molar concentrations ranging from M/10 to M/100 000 and (B) in distilled water at p_H 4 is shown in the following table. Both solutions were heated at 100° C for 50 minutes.

(A)	p_H before	p_H after heating	Percentage of penicillin remaining
M/10	7.2	7.2	37
M/100	6	6.2	85
M/1000	5.6	5.8	20
M/10 000 . . .	5.6	5.9	10
M/100 000. . .	5.6	5.9	0
(B)	5.6	6.0	10

Judging from these experiments the optimal p_H for this batch of calcium penicillin solution appears to be 6 to 6.2, but though the solution in distilled water adjusted itself to p_H after boiling it shows a loss of potency of 90 % compared with a loss of activity of only 15 % in the M/100 citrate solution.

With 20 000 u/ml sodium penicillin tablets T.R.C. 392 — solutions dissolved (A) in citrate M/1000, p_H 6.4 and (B) in distilled water p_H 4 — which had a p_H 6.4 after dissolving the tablet — the results after boiling for 10 minutes were as follows:

Percentage of penicillin remaining in (A) 96 %

Percentage of penicillin remaining in (B) 92 %

The difference after this short

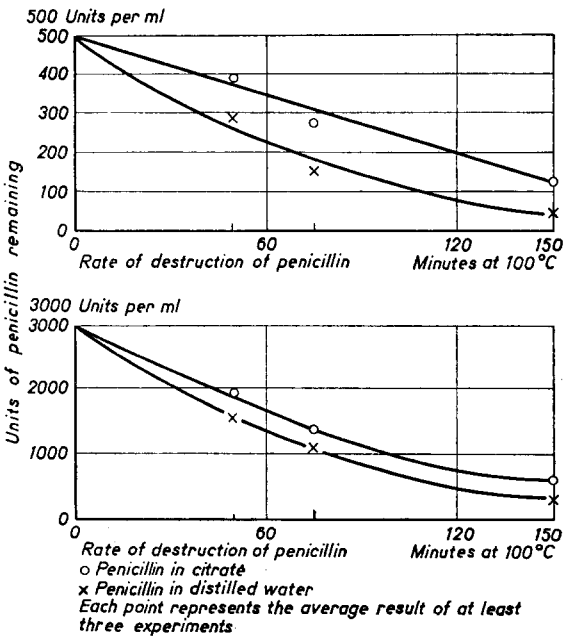

Fig. 1. Stabilising effect of citrate on sodium penicillin solutions

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exposure to heat is, therefore, negligeable. The buffering effect of the impurities in the T.R.C. tables seems to be sufficient to ensure a stability against boiling the solution for 10 minutes.

The stabilising effect of citrate in the optimal molar concentrations of M/400 on 500 u/ml, and M/1000 on 3000 u/ml of sodium penicillin T.R.C. solutions compared with solutions in distilled water adjusted to p_H 6.5 is illustrated in Fig. 1.

It will be noted that in citrate solution 500 units of sodium penicillin preserve an activity of 25.4 % even after boiling for 150 minutes, the potency in buffered water being 9.4. With 3000 units the difference between the two solutions is minimal, most likely because the amount of buffering agents present already in the impurities is adding its preserving effect.

It may be remarked that PULVERTAFT and YUDKIN (1946) using the same manufacture, but tablets containing only 45 units per mg found a complete destruction of the activity of sodium penicillin dissolved in M/10, M/100, and M/1000 phosphate in their experiments with 5000 u/ml after these solutions have been kept at 100° C for 150 minutes.

STABILISATION OF PENICILLIN SALT SOLUTIONS AT ROOM TEMPERATURE

Ampoules were filled with penicillin salt solutions dissolved (A) in distilled water and (B) in M/40 Citrate-NaCl Mixture, then kept at 100° C for 15 minutes and afterwards at room temperature. The samples remained sterile as proved by several tests for sterility.

The manufactures used for these assays were: T.R.C. tablets 330, sodium penicillin 1000 u/ml for (A), sodium penicillin "Roche" 5000 u/ml and sodium penicillin Dist. Comp. Liverpool, containing 800 units per mg in the concentration of 50 000 units per ml for (B).

Days	Percentage of Penicillin remaining		
	(A) 1000 u/ml	(B) 5000 u/ml	50 000 u/ml
15	75	100	100
25	18	80	67.5
38	16	73	
58	8	20	
72			12.5
87	0	18	
103			10

Whereas 1000 units sodium penicillin dissolved in buffered dist. water lost 82 % of their activity after 25 days, 5000 u/ml in the citrate mixture lost only 20 % after 25 days and 27 % after 38 days. 50 000 units per $^0/_{00}$ dissolved in the same mixture preserved 67.5 % of potency for 25 days.

DISCUSSION

The destruction of aqueous penicillin salt solutions by hydrolytic action is accelerated by rise of temperature in accordance to the well known law of physics. Citrate

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inhibits this action whereby the optimal molar concentration varies with the concentration of the penicillin solution. The stability of the various samples of penicillin salts as now on the market is much higher than previous work on their stability suggested. Still, the addition of sodium citrate appears to be of a considerable advantage in preserving the potency of penicillin solutions even in higher concentrations.

The results reported in this paper intimate that p_H is a very important but not the only factor responsible for the stabilising effect of sodium penicillin and that sodium citrate most likely acts as a chemical or enzymatic substance interfering with the hydrolytic process. Preliminary assays with substituting citric acid by other aliphatic hydroxyacids such as tartaric acid yielded promising results, thus supporting the suggestion that the inhibition of the hydrolytic destruction of penicillin can be compared with the inhibition of the breakdown of lactic acid to pyvuric acid by α-hydoxybutyric acid or tartaric acid. Further investigations will be necessary to throw more light on this question.

The practical applications of the findings as reported in this article are obvious. The stabilising effect exerted by sodium citrate on penicillin-salt solutions in such concentrations as used for clinical treatment implies the possibility of sterilising those solutions in ampoules. Such ampoules can be kept at room temperature with a relatively small loss of potency. It will no doubt be possible to find the appropriate molar concentration of sodium citrate balancing the buffering substances contained in the impurities of the various manufactures.

Attempts of using the sodium penicillin-citrate mixture for oral treatment on which a separate article will soon be published showed that higher levels of penicillin in the plasma could be obtained than previous workers reported. With volunteers the level reached after oral administration of 20 000 and 40 000 units sodium penicillin in citrate solution was between 0.1 and 0.2 units per ml.

I wish to thank Mr W. KAUFMANN, technical assistant to the bacteriological laboratory of Glyn Hughes Hospital for his excellent help and cooperation.

SUMMARY

Sodium citrate has a considerable stabilising effect on penicillin salts solutions even in higher concentrations.

The optimal molar concentration of sodium citrate varies with the concentration of the penicillin salt solutions and appears to be conditioned by the amount of buffers included in the impurities of the various samples.

Penicillin salt solutions dissolved in sodium citrate solution can be sterilised by boiling with a small loss of potency.

The mode of action is discussed.

RÉSUMÉ

Le citrate de sodium manifeste un effet stabilisateur considérable vis-à-vis des sels de pénicilline en solution, même lorsque ces sels sont à concentrations élevées.

La concentration moléculaire optimum du citrate de sodium varie avec la concentration de la solution du sel de pénicilline et paraît dépendre de la quantité de tampon apportée par les impuretés des divers échantillons.

Des solutions d'un sel de pénicilline en présence de citrate de sodium peuvent être stérilisées par ébullition sans perte appréciable de leur activité.

Le mode d'action du citrate de sodium est discuté.

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ZUSAMMENFASSUNG

Natriumcitrat hat eine beträchtliche stabilisierende Wirkung auf Penizillinsalzlösungen, sogar bei höheren Konzentrationen.

Die optimale molare Konzentration von Natriumcitrat ändert sich mit der Konzentration der Penizillinsalzlösungen und wird anscheinend durch die Menge der Puffer in den Unreinheiten der verschiedenen Proben bedingt.

Penizillinsalzlösungen, die in Natriumcitratlösung gelöst sind, können durch Kochen mit geringem Wirkungsverlust sterilisiert werden. Die Wirkungsweise wird diskutiert.

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