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RESEARCH PAPERS

SODIUM AND CALCIUM GLYCEROPHOSPHATES

A SURVEY

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THE object of this paper is to draw attention to the unsatisfactory position now existing in which commercial supplies of glycerophosphates, although issued as complying with the standards of the British Pharmaceutical Codex, do not actually satisfy the full requirements of the monographs. In order to clarify this position the authors of the present paper produced some pure salts, following factory practice, and investigated them. Published work by other investigators in this subject may be referred to from the bibliography given at the end of the paper[1,2,3,4,5,6,7,8].

DIFFERENTIATION BETWEEN THE ISOMERS

In order to differentiate between the α and β-salts of glycerophosphoric acid an efficient method of assay was required. This was found in the Malaprade reaction of periodic acid on vic. glycols for the estimation of the α-isomer. This method was first applied by Fleury and Paris[9] to the assay of α-glycerophosphates, using arsenious acid for determining the excess of periodic acid, and was later adopted by Pyman and Stevenson[8]. But the present authors found the arsenious acid method somewhat difficult to manipulate, and they prefer the direct procedure of acidifying in the presence of excess of potassium iodide and determining the amount of iodine lost in the reduction of the periodic acid to iodic acid, in comparison with a standard blank. Both methods gave results in complete agreement.

0·3 g. of the crystalline sodium glycerophosphate (or 0·15g. if the α content exceeds 50 per cent.) is weighed, placed in a stoppered 250-ml. conical flask and dissolved in a minimum amount of water; 25 ml. of a periodic acid solution (prepared by dissolving 3·674 g. of $Na_3H_2IO_6$ in 37·5 ml. of N sulphuric acid and diluting to 500 ml.) is added, the flask is swirled and set aside for 10 minutes. The reaction that takes place is represented by the equation—

$$\begin{array}{l} CH_2{\cdot}O{\cdot}PO_3Na_2 \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + KIO_4 = KIO_3 + \begin{array}{l} CH_2{\cdot}O{\cdot}PO_3Na_2 \\ | \\ CHO \end{array} + HCHO + H_2O$$

After standing, 1 g. of sodium bicarbonate is added to the flask followed by 5 g. of potassium iodide and 10 ml. of dilute hydrochloric acid. The carbon dioxide evolved is allowed to displace the air, the flask is stoppered, and set aside for 10 minutes, and the solution finally titrated with 0·1N sodium thiosulphate. A blank must be carried out in a

similar manner and the difference between the two titrations noted. The reactions that take place on acidification are—

$$KIO_4 + 8HI = KI + 4H_2O + 4I_2$$
$$KIO_3 + 6HI = KI + 3H_2O + 3I_2$$

Thus when 1 molecule of potassium periodate is reduced to potassium iodate a loss of iodine is shown in the final titration.

Hence, from the first equation above, 1 gram-molecule of sodium glycerophosphate is equivalent to 1 gram-molecule of potassium periodate, or 1 gram-molecule of iodine, or 2 litres N/1 thiosulphate. Thus each ml. of 0·1N thiosulphate in the difference noted is equivalent to 0·0162 g. of the α-salt $C_3H_7O_6PNa_2,6H_2O$.

In a mixture of the isomers, the amount of α-sodium glycerophosphate can be found by the above method, and the amount of the β-salt determined by titrating the total glycerophosphate and deducting from that result the titre due to the α-salt. The indicator in the titration should be methyl yellow, which gives a much sharper end point than methyl orange. At this stage of the work it had been assumed that the samples crystallising from the liquors were pure mixtures of α and β-isomers and contained no other titratable matter.

Samples of calcium glycerophosphate were assayed in a similar manner, taking 0·125 g. of the salt, dissolving this directly in 25 ml. of the periodic solution and completing the titration in the manner already described; the difference between the titration of the blank and the test being recorded, 1 ml. of 0·1N thiosulphate being equivalent to 0·0105 g. of anhydrous calcium glycerophosphate.

When analysing sodium glycerophosphate liquors from the original combination, and also the mother liquors from which crops of crystals have been taken, it is essential that any free glycerin shall be completely removed, by repeated extraction with alcohol, because even traces of glycerin give rise to error in the assay for the α-salt.

Preparation of Pure α and β-Sodium Glycerophosphate

Crude sodium glycerophosphate was prepared by combining, under vacuum, two equivalents of glycerin with one equivalent of sodium acid phosphate and then hydrolysing the resultant diglyceryl ester with sodium hydroxide. The glycerin was removed by extraction with alcohol and, after separation, the alcohol that remained was expelled by evaporation. Samples from three bulk batches were adjusted to contain 50 per cent. w/w of $C_3H_7O_6PNa_2 5\frac{1}{2}H_2O$ and the solution analysed. The results are shown in Table I.

TABLE I

Batch	Sp. gr. at 15·5°C.	Assay by titration	Assay by ignition	Residue at 150°C.	α-isomer ($6H_2O$)
		per cent.	per cent.	per cent.	per cent.
A	1·274	50·0	50·7	35·4 (theory 34·3)	23·15
B	1·277	49·5	50·6	35·4	21·0
C	1·277	50·0	50·7	36·3	22·0

These figures show the general constancy of the combination.

The bulked solution of these three batches was evaporated and adjusted to a strength of 70 per cent. w/w of $C_3H_7O_6PNa_2,5\frac{1}{2}H_2O$ and allowed to crystallise during several days. The crystals were removed and washed with alcohol. The mother liquor was again set to obtain further crystals and this procedure repeated until six different crops had been collected. The liquor from the sixth crop was uncrystallisable.

These six crops of crystals, after washing with alcohol to ensure the removal of any adherent glycerin, were assayed for their α β-content. The results are shown in Table II.

TABLE II

Crop	α-salt ($6H_2O$)	β-salt ($5H_2O$)	Total
	per cent.	per cent.	per cent.
1	13·9	85·2	99·1
2	71·0	30·9	101·9
3	91·5	7·85	99·35
4	28·5	72·9	101·4
5	71·0	30·1	101·1
6	42·0	57·8	99·8

Each of the six samples satisfied all the chemical requirements of the B.P.C., but all failed to comply with the opening definition that " sodium glycerophosphate is the sodium salt of β-glycerophosphoric acid."

Table II shows that the first crop of crystals contain the highest proportion of the β-isomer, whereas the third crop consisted almost entirely of the α-variety. These two crops of crystals were taken separately and twice recrystallised; finally separated from their mother liquor by centrifuging then washed with alcohol and dried at 60°C.

The analysis of these recrystallised preparations (given in Table III) shows that two pure isomers were obtained, the α-isomer containing 6 molecules of water of hydration and the β-isomer 5 molecules.

TABLE III

Tests	1st crop (twice recrystallised) β-isomer	3rd crop (twice recrystallised) α-isomer
	per cent.	per cent.
Loss at 150°C.	29·75 (*theory 29·42*)	33·25 (*theory 33·34*)
Assay (titration)	99·30 $C_3H_7O_6PNa_2,5H_2O$	99·50 $C_3H_7O_6PNa_2,6H_2O$
Assay (gravimetric)	99·20 " "	99·20 " "
α-isomer	absent	99·00 " "
Sp. gr. of 50 per cent. w/w solution	—	1·271 at 15·5°C.

A saturated solution of the α-isomer contained the equivalent of 63·5 per cent. w/w of $C_3H_7O_6PNa_2,6H_2O$ and a saturated solution of the β-isomer contained 45·5 per cent. w/w of $C_3H_7O_6PNa_2,5H_2O$.

The crystals of the α-isomer were hard and of a semitransparent nature, quite distinct from those of the β-isomer, which were fragile and of needle-like appearance.

The uncrystallisable liquor, which represented 10 per cent. of the original combination, was diluted with sufficient water to keep any α and β-calcium glycerophosphate in solution and then treated with a solution of calcium acetate. The precipitate that formed was collected

and analysis indicated its being a di-ester $CHOH(CH_2O.PO_3Ca)_2$; the figures based on material dried at 150°C. are given in Table IV.

This calcium salt did not reduce periodic acid solution, and therefore was not a vic. glycol. An approximate computation showed that 100 g. of presumptive sodium glycerophosphate in the uncrystallisable mother

TABLE IV

	Calcium	Residue on ignition	Phosphorus
	per cent.	per cent.	per cent.
For suggested formula	24·4	77·5	18·90
For the precipitate	25·5	78·1	18·60

liquor consisted of 55 parts of di-ester and 45 parts of true sodium glycerophosphate; yet, when adjusted to a liquor containing 50 per cent. of apparent sodium glycerophosphate ($5\frac{1}{2}H_2O$) as estimated by titration, it agreed with the standards set in the B.P.C.

Calcium Glycerophosphate

Calcium salts were prepared from solutions of the pure α and β-salts of sodium glycerophosphate.

With the β-salt, the method employed was that of treating the solution with an excess of 30 per cent. w/v aqueous solution of calcium acetate, and following with an excess of alcohol sufficient to throw down the calcium glycerophosphate. This was collected, washed free from calcium and sodium acetates by means of alcohol, dried and then examined. It held one molecule of water of hydration ($1H_2O$) and was soluble to the extent of 0·96 g. of anhydrous material in 100 ml. of solution at room temperature.

With the α-salt, two methods of preparation were employed: one precisely as just described for the β-salt; the other, that of using more concentrated solutions (without the use of alcohol) and allowing the calcium glycerophosphate to crystallise from the supersaturated condition. In both cases the material was collected, washed with alcohol, dried and analysed. The precipitate effected by means of alcohol contained 2 molecules of water of hydration ($2H_2O$), and that depositing from the supersaturated solution only 1 molecule ($1H_2O$). The analytical figures for the 3 salts are given in Table V.

Solubility of the Two α-Salts in Water

Each salt was treated with distilled water, leaving a little of the salt undissolved. After about an hour's digestion 50 ml. of solution was withdrawn and titrated and the amount of calcium glycerophosphate in solution calculated. The solution of the $2H_2O$ salt contained 4·3 per cent. of the anhydrous salt and that of the $1H_2O$ salt 1·17 per cent. The suspensions were set aside for 3 days, with occasional shaking, and then again tested. The amount of $2H_2O$ salt in solution had dropped to 4·0 per cent., whilst that of the $1H_2O$ salt remained at 1·17 per cent. The

mixture containing the $2H_2O$ salt was now tested periodically until constant solubility was attained. This occurred at 1·26 per cent. of the anhydrous material after about 4 weeks' digestion at from 20° to 25°C.; considerable precipitation having taken place. The residuum of this digest was collected, washed with alcohol, dried and examined: it consisted of α-calcium glycerophosphate now associated with only 1 molecule of water of hydration. Thus a change in constitution has occurred, and the more soluble but unstable salt with 2 molecules of water has reverted to the less soluble but stable salt with 1 molecule of water.

These 2 α-hydrates were rendered anhydrous: the $2H_2O$ salt lost all the water at 140°C., but the $1H_2O$ salt had to be dried at 150°C. On exposing the anhydrous salts to the atmosphere, both absorbed moisture and returned to their original states of hydration, that is to say, to the $2H_2O$ and $1H_2O$ hydrates, and dissolved 1 in 25 and 1 in 100 of water respectively, just as they did before subjection to dehydration. Thus there are 2 hydrates chemically the same, but having different solubilities. Possibly, the structures of the two hydrates differ, and a different arrangement of the calcium and glycerophosphate ions and of the molecules of water in the lattices could explain the effects noted.

X-ray diffraction data of the two hydrates and of the corresponding dehydrated salts are given below.

TABLE V

X-RAY DIFFRACTION DATA OF THE TWO HYDRATES OF α-CALCIUM GLYCEROPHOSPHATE. INTERPLANAR SPACINGS IN Å WITH APPROXIMATE RELATIVE INTENSITIES

Monohydrate	Dehydrated Monohydrate	Dihydrate	Dehydrated Dihydrate
13·0 vs.	14·8 vvs.	14·2 vs.	10·9 vvs. broad line
6·7 w.	about 4·0 s. diffuse band	7·1 mw.	4·19 mw.
about 4·0 w. diffuse band			
5·5 mw.	2·73 w.	5·17 m.	3·86 ms.
4·64 s.	1·99 w.	3·49 m.	3·48 ms.
3·34 mw.		2·22 vw.	3·27 w.
2·89 m.		2·05 w.	3·04 mw.
2·75 w.		1·81 w.	2·57 w. broad
2·33 w.		1·72 w.	2·11 w. broad
2·10 vw.			1·85 vw.
1·83 w.			1·73 vw.

s Strong. m Medium. w Weak.

These experimental data demonstrate the distinct structural difference of the two hydrates and of their respective dehydrated salts. It is interesting to compare the effect of dehydration on the diffraction patterns, and in particular on the strong high spacing lines, in the 2 cases. For the dehydrated monohydrate the pattern is almost non-existent except for a strong diffuse band and an extremely strong line of higher spacing than the maximum spacing line of the original monohydrate. This suggests that the water molecules are important in holding the calcium glycerophosphate units of the structure together, and that on their removal these units drift apart and the structure becomes much less ordered. On the other hand, the dehydrated dihydrate has a good strong pattern with a decrease in spacing of the maximum spacing line from that of the original dihydrate. This suggests that on removal of the water mole-

cules the remaining calcium glycerophosphate units can pack together in a highly ordered crystal structure which can, however, readily revert to that of the original hydrate.

Commercial Samples of Calcium Glycerophosphate

Samples of this salt were obtained from the principal manufacturers and subjected to the B.P.C. tests: the results are given in Table IV. The last three samples in the table were prepared by the present authors.

TABLE VI

Analytical Data of Samples of Calcium Glycerophosphate

Sample	Loss at 150°C.	Assay by titration	Assay by ignition	α-isomer	Solution 1 in 50
	per cent.	per cent.	per cent.	per cent.	
A	7·6	93·0	99·46	79·76	Soluble ; but flocculent. Precipitate overnight.
B	10·8	94·60	98·80	78·92	Soluble but within 1 hour commenced to flocculate.
C	7·5	94·18	101·16	79·64	Not complete ; also commenced to flocculate on standing.
D	10·5	93·90	97·85	51·91	Soluble ; flocculated on standing.
α-salt $2H_2O$ ...	13·9	98·90	99·63	99·15	Soluble ; only very slight flocculation overnight.
α-salt $1H_2O$ ...	7·0	99·33	99·5	100·90	Not complete but dissolves quickly 1 in 120 to a clear solution.
β-salt $1H_2O$ ...	9·8	100·22	100·0	absent	Not complete but dissolves slowly 1 in 120.
B.P.C. Standards	Not more than 15·0	94·50	98·0	....	Dissolves with slight turbidity.

All figures are based on material dried at 150°C. ; B.P.C. directs drying at 130°C.

Reference to Table VI will show that 3 out of the 4 samples analysed failed to meet the standard set for titratable matter, and yet reached the requirement (or practically so) as regards ash. This at once brings up the question as to whether the preparations or the standards are wrong; and when the different solubilities are also taken into account, emphasis is added to the question. It is clear that there are some difficulties in manufacture, for otherwise there would be greater uniformity in the products, and it may be asked whether the requirements of medicine are such that more precise standards, to secure a closer approach to the pure salt, should be set up or whether such differentiation is of little therapeutic importance and wider limits should be allowed: such wider limits being, of course, rather arbitrary and merely arranged to suit good commercial practice. But on the question of solubility there is room for debate: insisting upon a degree of solubility which is not a stable property of the salt serves no useful purpose, but rather the reverse. If a pharmacist looks to a salt being soluble 1 in 50 of water and then, when using it in compounding, finds precipitation takes place, he is perturbed and at a loss; but if he knows that an ultimately stable solution can only exist at 1 in 100 some of his difficulties will not arise. Again, if there is no sound therapeutic reason for preferring one or other of the isomers, then degree of solubility in water is of still less importance since both salts will be readily soluble in the acid gastric juice.

Proposed Changes to the B.P.C. Monographs for Sodium and Calcium Glycerophosphate

Resulting from their present investigation the authors suggest that the following changes might be made in the B.P.C. monographs.

Sodium Glycerophosphate

1. To omit the chemical formula and also the description referring to the material as " the sodium salt of β-glycerophosphoric acid." Instead, the description to be " Sodium glycerophosphate may be the sodium salt of α-glycerophosphoric acid $C_3H_7O_6PNa_2,6H_2O$ or the sodium salt of β-glycerophosphoric acid $C_3H_7O_6PNa_2,5H_2O$ or any mixture of these isomers."

2. The assay to remain as at present, but referred to the material dried at 150°C. Standards to be altered to " Not less than 98 per cent. and not more than 102 per cent. of $C_3H_7O_6PNa_2$ calculated on the material dried at 150°C."

3. Loss at 150°C. not to exceed 34·5 per cent. This admits the hexahydrate with 33·34 per cent. of water and allows a little additional moisture.

Sodium Glycerophosphate 50 per cent. Liquor

1. To be described as " An aqueous solution containing about 50 per cent. w/w of α-sodium glycerophosphate hexahydrate, or a mixture of the α and β-isomers."

2. The assay to refer to the equivalent of anhydrous sodium glycerophosphate in the liquor. The limits to be 32·6 per cent. and 36·0 per cent. of $C_3H_7O_6PNa_2$.

These limiting figures are derived from the following considerations —50 per cent. of the hexahydrate is represented by 33·6 per cent. of anhydrous salt, which becomes 32·6 per cent. when allowing for a purity limit of 98 per cent. Similarly 50 per cent. of the pentahydrate is equivalent to 35·3 per cent. of anhydrous salt, and this, calculated to the upper limit of 102 per cent. allowed for the crystalline salt, becomes 36·0 per cent.

3. The specific gravity range to be from 1·255 to 1·300. This covers the two limits of the assay and also allows for the presence of 2 per cent. of glycerin.

All the other present limits, including that for glycerin, could remain since no difficulty has been experienced in meeting these standards.

Calcium Glycerophosphate (Dihydrate)... Now included in the B.P.C.

1. The loss on drying to be determined at 150°C.

2. The assay should be the method of Bennett and Campbell[10] in which igniting with ammonium nitrate and reigniting with nitric acid is adopted. The present authors found this method very satisfactory.

3. 1 g. of the salt should dissolve in 50 ml. of water at a temperature below 20°C. within a few minutes. On further dilution to 100 ml. and standing overnight no more than a very slight precipitate should develop.

The test for titratable matter and other limiting tests allow ample margin for the manufacturers' difficulties in production.

Calcium Glycerophosphate (Monohydrate)... Not recognised in the B.P.C.

1. It is recommended that official recognition be given to both α and β-salts both approximating to the monohydrates.

These are the salts which are stable in water, and which satisfy all the official requirements at present set out for the dihydrate with the single exception of solubility; the present requirement being that of an unstable condition.

1 g. of the salt should dissolve in 130 ml. of water within a few minutes. Both salts will dissolve in a smaller quantity of water, but the rate of dissolution is slow with the β-salt.

SUMMARY

1. A method has been described for the determination of the α-isomer of glycerophosphoric acid.

2. The preparation and properties of pure crystalline α- and β-sodium glycerophosphate have been described.

3. The isolation of "an impurity," occurring from a side reaction in the primary combination and which seems to approximate to a di-ester, is reported.

4. Two types of α-calcium glycerophosphate have been prepared: a dihydrate which is unstable in water and a monohydrate which is stable.

5. The properties of the two hydrates of the calcium salt have been investigated and differences in their physical behaviour are reported.

6. The preparation and properties of β-calcium glycerophosphate have been described.

7. Commercial samples of calcium glycerophosphate have been examined and their departure from official standards noted.

8. Suggestions for the official monographs have been made.

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