CCLXI. THE USE OF PURE PHOSPHOTUNGSTIC ACIDS IN THE PRECIPITATION OF BASES.

I. METHOD OF CHECKING THE PURITY OF 1:24-PHOSPHOTUNGSTIC ACID.

II. THE INFLUENCE OF HYDRION CONCENTRATION AND SOME OTHER FACTORS ON THE PRECIPITATION OF BASES.

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Introduction.

With the exception of fractionation of phosphotungstates of bases by acetone-water mixture, no attempts seem to have been made to modify the use of phosphotungstic acid [Drechsel, 1887]. It has been shown by one of us [Peters, 1930] that some bases are characterised by the hydrion concentration at which precipitates appear with neutralised phosphotungstic acid (sodium phosphotungstate). Extended use was made of these observations in concentrating vitamin B_1 by Kinnersley and Peters [1930]. As the methods there employed seemed likely to have general application, a further study was undertaken.

The primary object of our work was practical, but it was hoped also to clear the ground for theory as to the conditions governing precipitation and composition of the precipitates. Much has been learnt, but it is clear that the work only constitutes a preliminary survey of a complicated field. Fundamental observations upon the separation of complexes at various c_H are being made by Britton and his colleagues [1932], to which this problem is clearly related. Previously, the purest commercial acid had been used. Here we have concentrated mainly upon the 1 : 24-phosphotungstic acid, though some observations have been made with the 1 : 18-acid. The results are not the same as those with the commercial acids.

PART I. METHOD OF CHECKING THE PURITY OF 1:24-PHOSPHOTUNGSTIC ACID (By H. BARNES).

Phosphotungstic acid, even of the best commercial grades, is a mixture, usually considered to approximate to 10 % of the 1 : 18-acid and 90 % of the 1 : 24-acid. Throughout, the ratios expressed refer to the ratio $P_2O_5 : WO_3$.

Among the numerous acids described in the literature [Scheibler, 1872; Sprenger, 1880; Kehrmann, 1887; Kehrmann and Fraenkel, 1891; Brandhorst and Kraut, 1888; Gibbs, 1877; Péchard, 1889], 1 : 12, 1 : 16, 1 : 21, 1 : 22, 1 : 18 and 1 : 24, the last two are the most clearly established. They have been prepared for the purpose of this work by the method of Wu [1920] from Kahlbaum's highest grade of sodium tungstate. A less reliable sample upon one occasion gave a 1 : 24-acid of green-yellow tint, possibly owing to the presence of sodium molybdate [see Folin and Marenzi, 1929]. The final product was best recrystallised from a small amount of water allowed to evaporate over sulphuric acid in a desiccator at room temperature, giving large octahedra. The usual yield of the purest acid from 200 g. of tungstate was 20 g. of the 1 : 24-acid. The yield was improved upon one occasion by cooling all the reagents thoroughly at the ether extraction stage. It was found important to store the crystals in a closely stoppered bottle to prevent the efflorescence which easily alters their composition. Even with considerable precautions the original analysis figures were not given after storing for a few weeks.

Only one form of the 1 : 18-acid was used in this work, obtained from an ammonium salt like Wu's B form by ether extraction, and consisting of pale greenish-yellow thick rhombohedral plates.

Analysis of the samples used.

In order to check the purity of the 1 : 24-acid used, a new method was devised, after unsuccessful work with other analytical methods. A short description of the experiments best indicates the difficulties and may be helpful to others. No success was obtained by the application of colorimetric phosphate methods to mixtures of sodium tungstate and potassium phosphate.

The following stages can be recognised in most previous work.

(*a*) The decomposition of the complex acid. Different investigators have used widely varying reagents from 20 % boiling sodium hydroxide for 30 minutes to dilute hydrochloric acid. Evidence is scanty for the efficacy of any particular method. Experiments performed during the progress of this research suggest that different acids require different treatment for disintegration.

(*b*) Precipitation (i) of the two acids together as a mixture of mercurous tungstate and mercurous phosphate; (ii) of the phosphate as magnesium pyrophosphate; (iii) of the tungstate as tungstic oxide, or as a compound with (iv) tannic acid or (v) benzidine.

Gibbs's method [1880][1]. After decomposition the tungstic and phosphoric acids were precipitated together in boiling acid solution with mercurous nitrate, as a mixture of mercurous tungstate and mercurous phosphate, the excess acid was neutralised with mercuric oxide, and the needles so obtained after washing with dilute mercurous nitrate solution and igniting gave the sum of P_2O_5 and WO_3. Gibbs states that no phosphorus was lost during the ignition, but von Knorre [1908] considers that some phosphorus was left in the filtrate from the mercury precipitation. On a second portion Gibbs determined the phosphate as $Mg_2P_2O_7$, after precipitation as magnesium ammonium phosphate. This latter method was worked out for Gibbs by Gooch [1879] and it is clear from his results that the separation of phosphoric and tungstic acids in this way is a matter of considerable difficulty. The phosphate always seems to be in error by + 6 to 8 % due to the adsorption of sodium tungstate from the solution on to the precipitate. This error is only slightly reduced, by taking up the magnesium ammonium phosphate precipitate in hydrochloric acid, and reprecipitating with magnesia mixture. Gibbs determined the WO_3 by difference.

[1] The earlier worker [Scheibler, 1872], has not given a method of analysis.

The rather unsatisfactory nature of these methods was shown by a re-investigation of the effect of sodium tungstate on the estimation of potassium hydrogen phosphate (Sørensen), as $Mg_2P_2O_7$, after precipitation as magnesium ammonium phosphate. The original work of Gooch was amply confirmed—the error in the first precipitation always being of the order of 10 to 20 %. Several reprecipitations were then tried, and only after four such reprecipitations of the magnesium ammonium phosphate could the error be reduced to approximately 1 %. In the experimental procedure adopted, the mixtures of sodium tungstate and potassium hydrogen phosphate were made up in amounts approximately equivalent to a 1 : 20-phosphotungstic acid, the solution was heated to boiling and magnesia mixture cautiously added with constant stirring. After cooling an excess of ammonia was added, and the mixture allowed to stand at least 4 hours before filtration. Table I gives the results.

Table I.

Amount of $Na_2WO_4, 2H_2O$ taken g.	Amount of KH_2PO_4 taken g.	No. of precipitations	$Mg_2P_2O_7$ found g.	KH_2PO_4 corresponding to $Mg_2P_2O_7$ found g.	Errors %
1·5641	0·0902	1	0·0876	0·1071	18·7
1·7697	0·1099	4	0·0912	0·1114	1·4
1·0718	0·1099	4	0·0914	0·1117	1·6

Naturally such a method is very tedious and subject to considerable manipulative error.

Other methods. Kehrmann [1887], after boiling the complex acid for half an hour with sodium hydroxide, precipitated the phosphate as magnesium ammonium phosphate, using magnesium and ammonium nitrates instead of the corresponding chlorides and, after evaporating down the ammoniacal filtrates, precipitated the tungstic oxide from them as such by addition of hydrochloric acid. Sprenger [1880] proceeded in a similar manner, but used tannic acid to precipitate the tungstic oxide.

A critical survey of the literature with some experimental work was published by von Knorre [1908]. He considered the method of Kehrmann for splitting up the complex acid, namely boiling with sodium hydroxide for half an hour, as far too vigorous, and he himself appears merely to have added hydrochloric acid. He also confirmed Gooch's experience on the difficulty of obtaining a pure magnesium ammonium phosphate. He states without giving experimental details that the determination of tungsten as oxide is not accurate in the presence of magnesium salts. With regard to the tannic acid method of Sprenger, von Knorre's experiments show that, using pure solutions of sodium tungstate, the results are always too low. In addition the exact conditions for a good precipitation by this method are difficult to obtain—the appearance of colloidal solutions being frequent. Von Knorre himself precipitates the tungstate first as benzidine tungstate in acid solution, adding a little sulphuric acid to help coagulation of the precipitate. On ignition the

benzidine tungstate gives tungstic oxide, and benzidine sulphate is volatilised. Phosphate is determined in the tungsten-free filtrate, as $Mg_2P_2O_7$. This method is open to several criticisms. First, the benzidine tungstate must be precipitated in acid solution, and the presence of acid will tend to re-form the complex acid from the sodium phosphate and tungstate, these being partly split again upon making alkaline for the phosphate precipitation; secondly, as von Knorre's experiments show, a small amount of phosphate is precipitated along with the benzidine tungstate, making reprecipitation necessary to free it completely from phosphate.

Wu's method [1920]. The essential stages for estimation of P_2O_5 are hot sodium hydroxide, precipitation as magnesium ammonium phosphate, ignition, digestion with HCl and reprecipitation of phosphate; for WO_3, ignition and determination of P_2O_5 in residue. Since P_2O_5 was found to be partly volatile, it is not clear why no P_2O_5 is lost in the first process.

This method was tried with the exception that the precipitations of magnesium ammonium phosphate were made in hot solutions. Table II shows some results with control mixtures of phosphate and tungstate[1]. The amount of phosphate (calculated as $Mg_2P_2O_7$) after subtraction of adherent sodium tungstate was not equal to the phosphate originally taken. After the digestion stage, the amount recovered however was in good agreement.

The incomplete removal of tungstic oxide by the digesting process in our hands is shown in the first experiment in Table II and a complete phosphate analysis according to Wu is shown in the second experiment of the same table.

Table II.

Na_2WO_4, $2H_2O$ g.	KH_2PO_4 g.	$Mg_2P_2O_7$ on 1st precipitation g.	Error in 1st precipitation %	WO_3 remaining after HCl g.	Corrected error %	$Mg_2P_2O_7$ on 2nd precipitation g.	Error in 2nd precipitation %
1·5641	0·0902	0·0876	18·7	0·0015	16·8	—	—
0·7833	0·1315	0·1214	12·9	0·0033	8·9	0·1067	0·8

The methods of separation of these mixtures proved so tedious and uncertain that a trial was made of ignition.

The ignition method. It has been stated [Rosenheim and Jaenicke, 1917] that upon heating to a dull red heat, these phosphotungstic acids give off most of their water, leaving behind only metaphosphoric acid and tungstic oxide. The latter should not volatilise until about 1400°, whereas the former should be volatilised by about 800°. By heating the phosphotungstic acid first at 540° to constant weight and then at 1000°, for the best samples practically theoretical figures could be obtained for water and tungstic oxide.

The acid was heated in a crucible supported on a small silica stand in an electric furnace; the temperature was regulated by a variable resistance and was read off directly on a calibrated thermocouple. Heating for some days at

[1] Wu did not publish any figures for controls.

540° and 1000° to drive off water and phosphorus compounds respectively was required. The process however needs little attention. Table III shows the results. There is some uncertainty as to the exact temperature of volatilisation of metaphosphoric acid, and it seems to depend to some extent upon conditions. We have therefore calculated the results expected in two ways, giving respectively the theoretical figures upon assumption that (*a*) the whole of the water disappears at 540°, leaving behind P_2O_5, and that (*b*) one molecule remains, forming metaphosphoric acid.

Table III.

Acid taken g.	H_2O lost at 540° g.	H_2O %	WO_3 left at 1000° g.	WO_3 %
2·3431	0·3864	16·48	2·0995	81·54
1·2880	0·2101	16·31	—	—
3·6913	0·6071	16·45	2·9908	81·02
2·1354	0·3466	16·24	1·7342	81·21
	Average	16·37		81·38

Formula for 1:24-acid: $3H_2O.P_2O_5.24WO_3.60H_2O$.
(*a*), (*b*) Calculated for $60H_2O + 3H_2O$ on assumption (*a*) that all H_2O volatilises at 540° and (*b*) that 1 molecule H_2O remains with P_2O_5.

	Observed	Calculated (*a*)	Calculated (*b*)
%	%	%	%
H_2O	16·37	16·56	16·31
WO_3	81·38	81·40	81·34

The figures are so close to the theoretical that there can be little doubt that the method is of value for the 1 : 24-acid. Curiously enough it proved impossible to control it by heating mixtures of sodium tungstate and phosphate; the conditions of volatilisation seemed to be altered. One experiment upon the 1 : 18-acid suggested that it might be also of value for this acid, but it seemed to take longer to attain constant weight.

Conclusion.

The purity of 1 : 24-phosphotungstic acid can be checked by heating successively to constant weight at 540° and 1000°.

PART II. THE INFLUENCE OF HYDRION CONCENTRATION AND SOME OTHER FACTORS ON THE PRECIPITATION OF BASES

(By H. BARNES and R. A. PETERS).

We have studied the influence of concentration of base and of phosphotungstate together with salts upon the initial precipitation. Most work has been done with creatinine, rather less with histidine, and least with a few other bases. More attention has been paid to the 1 : 24-acid, whose greater stability makes it a more useful reagent.

Methods.

Initial point of precipitation. An aqueous solution of the base (1·0 cc.) was treated with 0·1 cc. Na phosphotungstate solution (p_H 6·0). Acid (usually sulphuric) of convenient strength was added from a graduated pipette until a faint permanent cloud was observed. Observation of the initial point was aided by the use of a point source of light in a dark room, using a comparison tube. The sharpness of this point varied with the substance, being very definite with histidine. With creatinine it was much influenced by the speed of addition of the acid (see below).

p_H *range of precipitation.* The base was dissolved in 5·0 cc. water and divided into 1·0 cc. samples (Ostwald pipette). After adding 0·1 cc. of phosphotungstate (90 %) to each, and the requisite sulphuric acid of appropriate strength, the tubes were centrifuged after standing for half an hour. The liquid was used for the determination of p_H and the residue washed, dried and weighed.

Hydrion concentration. As the hydrogen and quinhydrone electrodes were inapplicable with the unstable phosphotungstates, observations were made colorimetrically, or where necessary with the glass electrode. In the latter case duplicate observations agreed to within 0·03 p_H.

Chemical substances.

Creatinine. Commercial samples were purified (1) by way of the zinc chloride compound and (2) by repeated purification from acetone [Edgar and Hinegardner, 1923]. A theoretical value was obtained for nitrogen.

Histidine. More than one sample was used, some from commercial sources, and others prepared in the laboratory by the methods of Raistrick [see Cole, 1926], and of Vickery and Leavenworth [1928]. The melting-points of the compounds varied, but no essential difference was found in the precipitation behaviour. A pure specimen of the dihydrochloride melting at 242° gave the same results as others.

Guanidine sulphate. Recrystallised from commercial sources.

Aminoguanidine sulphate, M.P. 207°. The specimens of *as*-dimethylguanidine and of arginine nitrate were from commercial sources and of doubtful purity.

Phosphotungstate solutions.

An aqueous solution of the phosphotungstic acid was neutralised with NaOH to p_H 6·0 approx., usually in presence of a trace of bromocresol purple. For weaker concentrations 10 % NaOH is sufficient. For the 90 % solutions, which are nearly saturated, it is best to dissolve in the minimum amount of water and add slowly 20 % NaOH cooling the solution between each addition. The use of stronger NaOH or taking to p_H 7·5, or allowing the solution to get hot is apt to cause decomposition. The weaker solutions appeared to keep indefinitely at room temperature, but the stronger ones often deposited crystals after standing. It is not known whether these have a different composition.

Creatinine.

This base was chosen because it was relatively easy to obtain, and had an initial precipitation point lying between p_H 5·0 and 6·0. This point was investigated with amounts of phosphotungstate (*a*) much less than and (*b*) more than were required to precipitate amounts of dissociated creatinine up to 8·0 mg. per cc. (0·0707 *M*). For (*a*) 0·1 cc. of 10 % phosphotungstate was added to 1·0 cc. solution containing the base, and for (*b*) 0·1 cc. of 90 %. As the amounts of acid needed to start precipitation varied, the concentra-

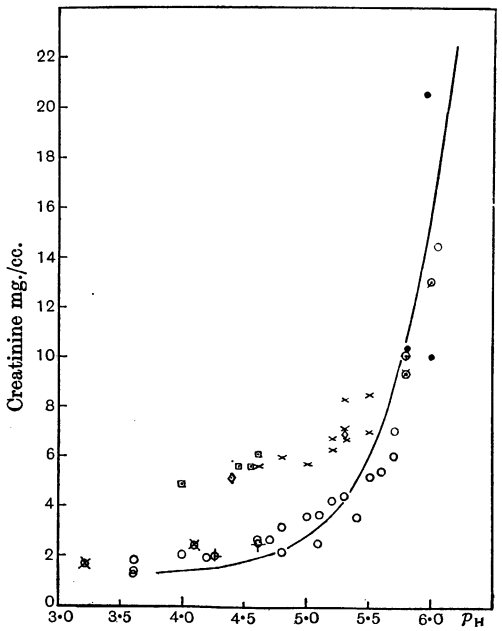

Fig. 1. Variation of initial point of precipitation of creatinine with concentration. Concentrated phosphotungstate solution (1:24). Ordinates creatinine concentration mg./cc., 11·3 mg./cc. = 0·1 *M*. Abscissae p_H.

⊙ × Points at 20°. ◈ 10°. ⊡ 31°. ⊠ Na_2SO_4 added. ⊕ NaCl added.

tions of phosphotungstate at the moment of precipitation might be as an extreme 4·5–8·0 %, and one-tenth of this respectively. With (*b*) points above 7–8 mg./per cc. creatinine, were well defined and could be obtained to within 0·1 p_H. Below this the conditions of precipitation became very important. The lowest points in Fig. 1 (circles) were obtained by one observer working to standard conditions, and represent an extreme. Other points (crosses) are observations of others. When the acid is added very slowly, there is no question that the initial point tends to be pushed to the acid side. The area containing the points is really an indeterminate zone. In (*a*) (Fig. 2) the same phenomena are to be seen. The weaker concentration of phosphotungstate is associated

with a shift of approximately 0·6 p_H for 10–20 mg. creatinine concentration. A few points are included with the 1 : 18-acid.

The results with the 10 % phosphotungstate can be compared directly with those previously published for the commercial acid [Peters, 1930] and are seen to be considerably further to the alkaline side. Commercial acid p_H 2·5, 2·0 mg./cc.; p_H 4·0, 10 mg./cc., and for 1 : 24-acid p_H 4·0 and 5·8; for 1 : 18-acid the values are p_H 5·2 and 6·2. With the 1 : 24-acid above 6·0 mg./cc. creatinine, the initial point becomes largely independent of concentration of base. We have included in the diagram points found in the presence of added sodium chloride and sulphate up to 2·5 % concentration

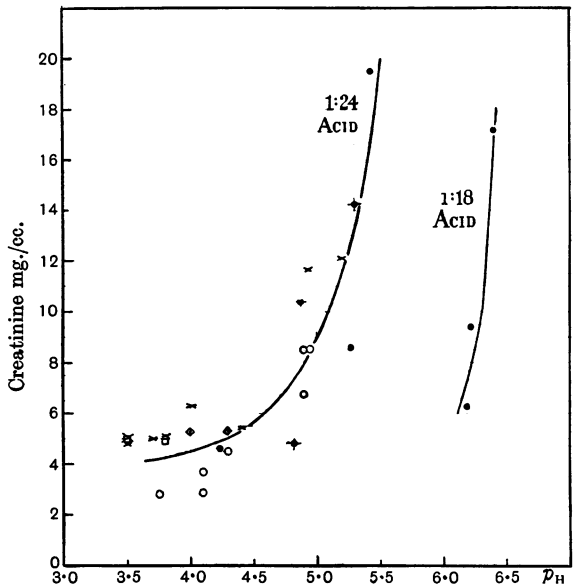

Fig. 2. Variation of initial point of precipitation of creatinine with concentration, for 1:24-phosphotungstate (dilute) and 1:18-phosphotungstate (dilute 10 %). Data as Fig. 1.

and also variations of temperature of ± 10°. The differences so introduced were slight if at all existent, and were negligible so far as these experiments were concerned. Nor did it make a significant difference if HCl were substituted for H_2SO_4. That traces of creatine could not influence results was also shown upon two occasions by adding creatine; this effectually controlled the possibility of traces of creatine in the creatinine or formed in solution.

Estimation of the p_H range of precipitation showed that with a concentration of 5 mg./cc. base most of the precipitate appeared upon titrating from p_H 6·0 to 2·8; hence the practical issue is that if we collect all the phosphotungstate precipitated between p_H 6·0 and 2·5, we shall have all the creatinine except that which is soluble, some 1·6 mg./cc. or rather more depending upon the concentration of phosphotungstate used.

From the theoretical standpoint, the important general points seem to be (1) the constancy of the initial precipitation point at about p_H 6·1 for both acids when sufficient phosphotungstate is present, (2) the fact that the zone of precipitation extends from p_H 6·0 to 2·0 approximately, over 4·0 units of p_H. The special points are (3) the tendency for the initial precipitation point to shift to the acid side in the case of the 1 : 24-acid with decreasing concentration of phosphotungstate, and (4) the tendency for precipitation to take place more definitely near the initial point with the 1 : 18-salt. Dealing first with the general points, the $p_{K'}$ for creatinine has been shown by Cannan and Shore [1928] to be 4·91 at 15°. The precipitation is therefore roughly co-extensive with the range of dissociation of creatinine. This suggests that it is related to this dissociation. Now the complex precipitated might be either a salt-like compound with phosphotungstate, or some complex with the phosphotungstate molecule as a whole. We could exclude the formation of a salt-like compound upon passing from an acid to an alkaline p_H, if it happened that the dissociation of the phosphotungstic acid was complete at p_H 2·0. It would then be clear that a precipitate formed at p_H 5·0 must consist of a complex of Na phosphotungstate together with dissociated creatinine. In the absence of data in the literature, the dissociation curve of the complex acids has been briefly investigated to settle this point.

Dissociation curves of the phosphotungstic acids. The dissociation curve for the 1 : 24-acid as determined by titration is given in Fig. 3. Up to p_H 7·0 some 19 g.-mol. Na are taken up per 1·0 g.-mol. of the acid and at approximately p_H 7·8 24 g.-mol. Na, or an amount of Na corresponding to each unit of WO_3. It is curious and suggestive that the sudden change in the character of the dissociation curve starts at p_H 7·0 and becomes intense at p_H 7·8, when 24 acidic groups have been neutralised. The points are here apt to become uncertain, and there is evidence of change of equilibrium, because some time must be allowed to elapse after addition of alkali before the final p_H is reached; immediately after titration the p_H is much more alkaline than the equilibrium point. Further investigation is needed, but the above is sufficient to show that the phosphotungstic acid molecule must be regarded as a polyacidic micelle; the dissociation curves of the different acidic centres overlap to form an almost continuous straight line over the range p_H 2·0–7·0. The behaviour is rather analogous to that of a protein, though uncomplicated by basic dissociations. Since gradual neutralisation of the acid occurs over the whole range p_H 2·0–7·0, we cannot exclude the possibility that salt-like compounds between dissociated phosphotungstic acid and base contribute to the precipitate formation. Two kinds of compounds can be pictured, if we assume that no actual change is taking place in the phosphotungstate molecule itself, (1) some molecules form creatinine phosphotungstates, while others remain uncombined; (2) each phosphotungstate molecule acts as a polyacidic micelle, and takes up a certain proportion of base. Though the data do not admit of decision, we think that (2) is more likely. This would give precipitates, con-

sisting of Na-creatinine-phosphotungstate with some undissociated groups in the molecule according to the p_H.

The dissociation curve for the 1 : 18-acid was briefly investigated and proved to be different. Our supplies were limited and did not admit of detailed study, but there seemed to be no buffering action between p_H 2·0 and 7·0. The difference provides one avenue to explore in explaining the variations in precipitation behaviour between the two acids.

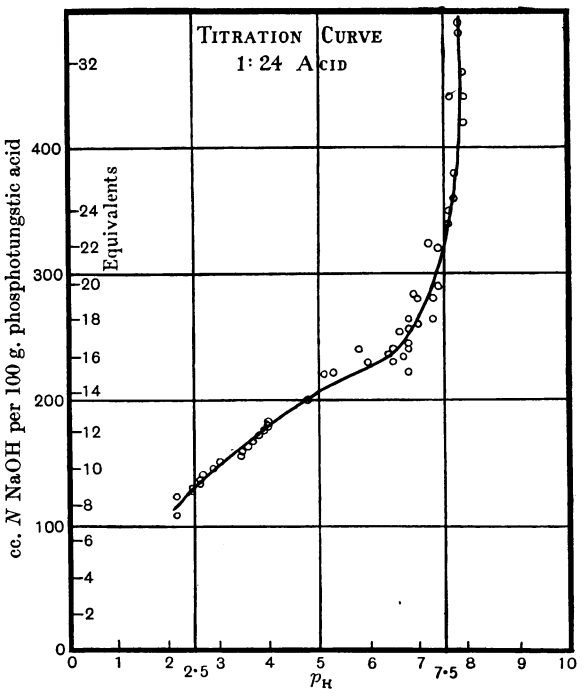

Fig. 3. Titration curve 1:24-acid. *M* equivalents NaOH.

Diagrammatically we should have

Phosphotungstic acid—undissociated groups (*n*)
Phosphotungstic acid—organic base (creatinine) (*m*)
Phosphotungstic acid—Na (*p*)

Here *n*, *m*, *p* represent whole numbers, the values of which vary according to the p_H and the concentration of phosphotungstate and Na.

Composition of the precipitates.

We have tested whether the precipitates contain sodium and found this present. They are clearly different from those formed by bases in acid solution. For instance according to Drummond [1918] 1 g. of creatinine gives in acid solution less than 10 g. phosphotungstate. In our case, 1 g. often gave 12–13 g. Experiments upon the relative proportion of Na and creatinine have been

made as follows. Approximately 100 mg. samples of creatinine have been treated with 1·5 cc. H_2O, 1·5–2·0 cc. 90 % Na phosphotungstate (p_H 6·0) added, and the p_H adjusted to 5·0 and 3·0 approximately. After standing for one hour or more, the precipitates were removed by centrifuge and the precipitate washed once with 1·0 cc. H_2O containing a trace of phosphotungstate at the p_H used. If taken within 1 hour, precipitation has not reached completion. They were not dried, owing to the possibility of introducing changes in composition and variations in the usual practical procedure. They were extracted as in the vitamin B_1 work by grinding with baryta and H_2O 4 times. The resulting solution was acidified with H_2SO_4 to Congo red, heated and made up to a known volume after filtration. One-fifth was taken for the estimation of creatinine by Folin's method, and the remainder evaporated to dryness and incinerated; the resulting Na_2SO_4 was determined by drying to constant weight in a crucible. Controls, in which known amounts of NaOH and creatinine were added to phosphotungstate at a given p_H and the whole was extracted with baryta, gave a recovery of 95–98 % for creatinine but not more than 75 % for sodium.

The experiments, some 10 in all, showed that Na was always found to be present in the baryta extracts, but the ratio creatinine/Na varied rather widely from the extremes of 10/1 to 4/1. The proportion of Na in the precipitates appeared to decrease when the precipitates were allowed to stand for periods over 1 hour. The fact that the weight of precipitate is some 12–13 times that of the creatinine taken is most simply explained upon the view that the precipitates contain on the average 5 molecules creatinine per molecule of phosphotungstic acid. Upon the assumption that creatinine is here monobasic, one molecule attached at one acidic grouping, using data from the dissociation curve, we may calculate that approximately at p_H 3·0 the ratio of creatinine/Na should be 4·7 and 3·4 at p_H 5·0. Incomplete recovery of Na will not explain the differences from the observed ratios, so that we are faced with the conclusion that upon balance each creatinine molecule displaces more than one Na. At p_H 5·0 replacement of 2Na would lead to a ratio of 12/1 and at p_H 3·0 a higher ratio still. The increase in the proportion of creatinine/Na upon standing must be due either to a continued replacement of Na in the precipitate by creatinine (a kind of base exchange like that of Ca in fuller's earth [compare Phelps, 1931]), or to the formation of phosphotungstates of more than one kind. In several cases crystals formed, so that the compounds must be well defined. Their evident complexity makes caution advisable in framing hypotheses.

If however the precipitation is due to dissociated creatinine, as seems most probable, the amount dissociated at a given p_H is known from the usual relation[1] $p_k + \log \frac{a}{1-a} = p_H$, where $p_k = 4{\cdot}91$. We can calculate for any p_H the total concentration needed to give an amount of dissociated creatinine equal

[1] p_k, apparent dissociation constant; a = ratio $\frac{\text{dissociated acid}}{\text{total acid}}$.

to the known solubility; upon hypothesis this should be proportional to the amount which just forms a precipitate. The curve in Fig. 1 is so calculated for a solubility of dissociated creatinine phosphotungstate of 1·25 mg./cc. The agreement with the points determined is sufficiently close to suggest that the fundamental assumption may be justified. In Fig. 2, a similar curve is drawn for a solubility of 4 mg./cc. In the latter case, there is a suggestion of discontinuity in the determined points, which was supported by the results of an attempt to determine the solubility product relations for low dilutions of phosphotungstate.

The concentration of phosphotungstic acid required to initiate precipitation at a constant p_H.

1·0 cc. solution (Ostwald pipette), containing 20 mg. dry creatinine, was treated with 0·264 cc. *N*/3 HCl unless otherwise stated to bring to p_H 4·90 approx. To this was added from a micro-burette, 4·95 % Na phosphotungstate solution. The p_H was then determined colorimetrically using a comparator. All solutions were stored in bottles protected from air with soda lime tubes to eliminate CO_2 as much as possible. Na phosphotungstate p_H 4·90 was made by neutralising 10 % 1 : 24-phosphotungstic acid in the ratio of 1·02 cc. 0·2 *N* NaOH to 1·0 cc. acid. Table IV A gives some results for immediate precipitation and IV B for precipitation upon standing for 24 hours.

Table IV. *Precipitation of creatinine by sodium phosphotungstate at constant* p_H.

In all cases 1·0 cc. creatinine solution (20 mg. creatinine) used.

0·264 cc. *N*/3 HCl added in Exps. 1, 2, 3, 8, 9, 10.
0·15 cc. „ Exps. 4, 6, 7.
0·08 cc. „ Exp. 11.
0·14 cc. „ Exp. 12.

Exps. 11, 12 with 90 % Na phosphotungstate, the remainder with 4·95 %.

Exp.	cc. phospho-tungstate	p_H	Conc./cc. Crea-tinine (C)	Phospho-tungstate (P)	C × P	
A. Temp. 18·5 ± 0·5°. Immediate precipitation.						
1	0·605	4·8	10·7	15·6	167	
2	0·385	5·0	12·15	11·6	140	
3	0·300	4·9	12·8	9·5	122	
4	0·144	5·3	15·5	5·51	85	
5	0·150	5·3	15·4	5·71	85	
B. Temp. 25·5 ± 0·5°. Slow precipitation or no precipitate (22 hours).						
6	0·02	5·3	17·1	0·085	1·45	Precipitation
7	0·01	5·3	17·2	0·043	0·7	Very small precipitation
8	0·05	4·9	15·3	0·189	2·9	Precipitation
9	0·035	4·9	15·3	0·133	2·04	Very small precipitation
10	0·02	4·9	15·3	0·077	1·2	No precipitation
11	0·10	6·0	17·0	70·6	1200	No precipitation
12	0·10	5·6	16·1	72·5	1160	Very small precipitation

For immediate precipitation, we have for p_H 4·8–5·0 a product of creatinine and phosphotungstate of 122–167 mg./cc., but at p_H 5·3 a product of 85,

i.e. less phosphotungstate seems to be required to produce an immediate precipitate at p_H 5·3. This suggests that the precipitates are complex, but no emphasis can be placed upon these figures; they are not true equilibrium points, because, upon standing for 24 hours, the precipitation tends to progress and beautiful crystals appear, of at least two different forms, needles and cubic prisms. These merit further study. Provided that the p_H is within the precipitation zone, surprisingly little phosphotungstic acid will suffice to give crystals in 24 hours, amounts far below those giving visible precipitates even within an hour (see Table IV B). It should be emphasised that outside the indeterminate zone, phosphotungstate and creatinine may be kept together in solution almost indefinitely without crystallisation or precipitation (see especially Exp. 11).

As a working hypothesis, it is suggested that the main precipitation is related to the presence of dissociated creatinine. The complete theory will have to take into account as well as the p_H the concentrations of creatinine and phosphotungstate, and probably the equilibrium between more than one form of compound.

Note. There is one possibility, which requires mention. It has been recently shown that at a liquid interface, there is an apparent shift of dissociation constant [Peters, 1931]; similar conclusions follow from work on charcoal adsorption for the solid interface [Phelps and Peters, 1929; Phelps, 1931]. This is likely to happen also with these precipitates, and probably provides a partial explanation of the change in character upon standing.

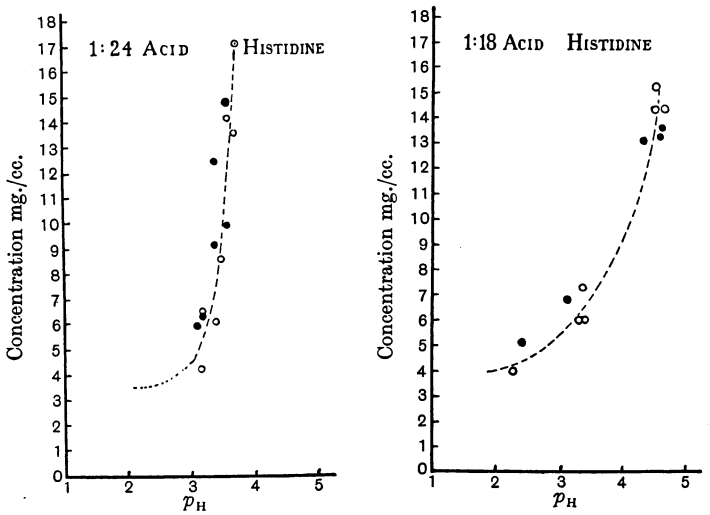

Fig. 4. Variation of initial point of precipitation of histidine (hydrochloride) with concentration. 1:24- and 1:18-acids. Concentration mg./cc., 9·1 mg./cc. = 0·1 *M*.
○ = 10 % acid. ● = 90 % acid.

Histidine.

The curves shown in Fig. 4 have been obtained by the same technique as in the case of creatinine, histidine hydrochloride being used. The curves for the 1 : 24- and 1 : 18-acids have the same position, differing in this from

creatinine. They bear the same relation to the dissociation curve p_H 1·0–4·0 (one of the dissociating groups of histidine) which we find in creatinine, but the same explanation does not apply. There is now much evidence for belief in the zwitterion constitution of the amino-acids [Bjerrum, 1923; Birch and Harris, 1930; Borsook and Wasteneys, 1930]. According to this view the group dissociating over this range is the —COOH grouping. Hence, we cannot take the simple explanation adopted for creatinine; we must believe that for histidine a precipitation of the compound formed over the range of dissociation of the —NH group is inhibited as long as the —COOH group is ionised. The matter could be tested by conductivity determinations. We should of course get precisely the same curve, but the initial point of precipitation would coincide with the dissociation of the —COOH group. This might explain the coincidence of the precipitation curves for the two different acids, because the compounds would already exist in solution at the p_H at which precipitation started.

Other bases.

We have not explored the field of the relation of chemical constitution to p_H of precipitation at all fully, and give the incomplete data in the belief that they may be of value to other workers. Fig. 5 represents the most probable value of the main precipitation point. Some considerable difference is made in some cases by the method of addition. For instance, for aminoguanidine sulphate, a very pure specimen, addition of Na phosphotungstate at p_H 5·0 to a solution containing 11 mg./cc. caused an opalescence. This became definite precipitation at p_H 7·4. The data should be studied in relation to those for the commercial acid. The points for this are seen to lie often intermediately between the very different points given by the 1 : 18- and 1 : 24-acids. With guanidine, as previously indicated, precipitation occurs on the alkaline side in low concentrations for the commercial acid and for the 1 : 24-acid, of which the commercial largely consists. It is more sharply defined for the 1 : 24-acid. Introduction of the amino-group modifies the precipitation, shifting the points to the alkaline side at a low concentration.

This alkaline precipitation by guanidine introduces the last complication which we have met, and seems to need another explanation, as it is related to the dissociation in the opposite sense. If $p_{K'_b}$ for guanidine is 8·5, then this compound only precipitates in low concentration when undissociated, and must be forming a complex. It is probably significant that this precipitation is coincident with the sudden bend in the dissociation curve of the 1 : 24-acid.

We may describe the scope of the theoretical problem by saying that it must be possible to decide from a knowledge of the compound whether it will form phosphotungstates either (*a*) with dissociated basic groups (example creatinine), or (*b*) with dissociated groups which precipitate only when the

influence of other groups ceases to operate (example histidine), or (*c*) complexes with undissociated basic groups, insoluble in alkaline solutions upon the lines of guanidine. A complete solution should enable us to predict much about the constitution of an unknown base precipitable by phosphotungstate from its behaviour to c_H, and conversely the most likely method of selecting a base from some biochemical mixture. Fortunately the complication introduced by guanidine is not important unless precipitation starts at an alkaline p_H. It is

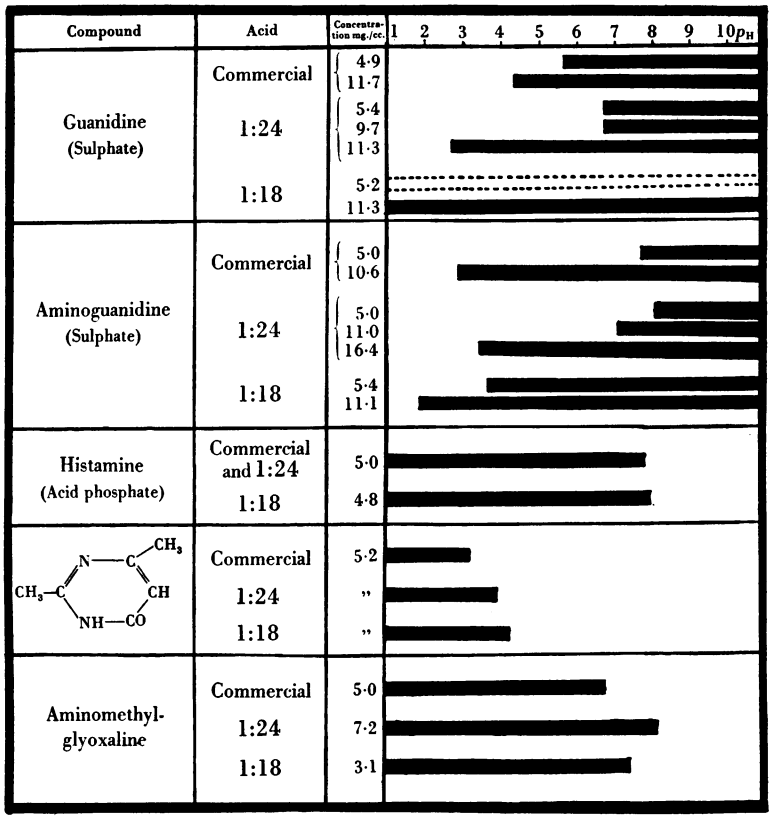

Fig. 5. Zones of precipitation (p_H), marked as black lines, for various compounds with three different phosphotungstic acids.

likely that substances attached to guanidine-like compounds are difficult to free from phosphotungstate with baryta. This may explain the losses sometimes found at a phosphotungstic stage. For instance Kinnersley and Peters [1925] found it to be quite impossible to recover vitamin B_1 from phosphotungstic acid precipitates at an early stage in their work.

The precipitates at acid p_H will usually dissolve at an alkaline p_H, upon addition of NaOH at a point some 2·0 p_H units more alkaline than the initial precipitation point, but there is a tendency to decomposition.

Some practical issues.

The applications of this work to the means of (1) characterising and (2) separating bases may be shortly considered in the light of these facts.

(1) It must now be realised that the original method of precipitating in the presence of minimum amounts of phosphotungstate must be rigorously watched and estimates of p_H of initial precipitation made at concentrations above the indeterminate zone. Solubility of the phosphotungstate matters much less in the case of substances like histidine or those like vitamin B_1 in which the vitamin compound is very insoluble. The latter precipitates at a dilution of less than 1 : 100,000 at p_H 5·0 [Kinnersley, O'Brien and Peters, 1932]. Presumably here the vitamin must precipitate very close to the beginning of its dissociation curve. (2) Several points are important in dealing with separations. The method used by Kinnersley and Peters [1930] for the separation of vitamin B_1, where the phosphotungstate is kept in minimum concentration, gives good results and has the great practical advantage that the amounts of phosphotungstate used are much smaller than the amounts needed to precipitate all the bases. This saves an expensive reagent. Their method, determined empirically, of working at different dilutions and gradually increasing the strength in successive fractionations is theoretically sound. Provided that a base precipitates between p_H 2·0 and 7·0, it should be possible to separate it by the use of suitable values of p_H and varying precipitations at various dilutions. These methods might be combined with fractionation in very acid solution. It is of course essential that the phosphotungstate should be very insoluble.

Precipitates at p_H 2–7 will give Na as well as organic bases upon decomposition. If therefore complete freedom from base is required, such precipitates should be treated with $N/10$ acid to free them from Na before decomposition with baryta. It is interesting to note that these principles of separation have been successfully applied at one stage in the separation of vitamin B_4 [Barnes, O'Brien and Reader, 1932], the vitamin appearing in the precipitate more acid than p_H 3·0.

Summary.

The precipitation of creatinine and of histidine HCl by pure phosphotungstic acids has been studied in detail and some data about the precipitation of certain other bases have been determined. The dissociation curves of the acids have also been investigated, and the data considered to some extent theoretically, but especially in their practical bearing upon newer methods of separating bases by the sodium phosphotungstate technique.

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