QUANTITATIVE INORGANIC CHROMATOGRAPHY

PART XII. AUTOMATIC ANALYSIS OF PHOSPHORUS-ANION MIXTURES BY ANION EXCHANGE CHROMATOGRAPHY

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Ion exchange chromatography can be made an automatic procedure by passing the effluent from the column through a system which continuously monitors the concentration of the solutes being separated. The detector for the monitoring system may be based on absorptiometric[1], conductivity[2], refractive index[3], flame photometric[4] or polarographic[5] methods and procedures. If radioactive tracers are used, continuous measurement of the effluent activity is made employing Geiger-Müller or scintillation counters[6] depending upon the counting efficiency required.

SPACKMAN, STEIN AND MOORE[1] have described a system which makes the analysis of amino-acids by ion exchange chromatography an automatic procedure. Essentially this involves mixing the column effluent with ninhydrin and continuously measuring the colour produced with a flowing colorimeter. Since the colour intensity for a given molar concentration depends on the amino-acid, this system has to be calibrated for each amino-acid. LUNDGREN AND LOEB[7] have described the automation of the anion exchange separation of condensed phosphates, originally described by GRANDE AND BEUKENKAMP[8], using the Technicon Autoanalyser. In this system, the column effluent containing condensed phosphates is pumped through a glass coil immersed in oil at 95° after being mixed with 6.66 *N* sulphuric acid, to hydrolyse the polyphosphates to orthophosphate. The liquid stream, now containing phosphorus only as orthophosphate, is passed through a dialyser and mixed with solutions of ammonium molybdate and hydrazine sulphate. The intensity of the molybdenum blue complex colour produced is measured continuously with a flowing colorimeter and since phosphorus is present only as orthophosphate the system does not have to be calibrated for each phosphorus anion.

We have modified the analytical system described by LUNDGREN AND LOEB[7] so that analysis of mixtures containing lower phosphorus anions, thiophosphates, amido- and imido-phosphates as well as polyphosphates is made a fully automatic procedure.

THE ANALYTICAL SYSTEM

In the analytical system, the phosphorus concentration in the sample stream is continuously monitored using a colorimetric procedure based on the molybdenum blue method for the determination of phosphorus. This method depends on the condensation of orthophosphoric and molybdic acids to give phosphomolybdic acids which, on

reduction, give an intensely blue coloured complex known as molybdenum blue, the intensity of the colour being proportional to the amount of phosphate ion incorporated in the complex. Since only orthophosphoric acid condenses with molybdic acid to form the complex acids, all the phosphorus present in the colorimetric system must be as orthophosphate. In the first part of the analytical system therefore, all the phosphorus must be quantitatively converted to orthophosphate and this may involve oxidation, hydrolysis, or both.

EXPERIMENTAL

The analytical system is based on the Technicon Autoanalyser and consists of a peristaltic proportionating pump to mix liquid streams in specified proportions, two double coil heating baths (with adjustable thermoregulators), a time delay coil, a flowing colorimeter fitted with silicon photocells and a potentiometric recorder (Elliott Dynamaster). The proportionating pump was fitted with 15 channel end blocks so that 15 liquid streams could be pumped simultaneously and by stretching tubes of various internal diameter between these end blocks, various liquid flow rates could be obtained.

The oxidation cycle

The effluent from the column is pumped at 0.42 ml/min and mixed with sodium hypochlorite solution (50 ml B.D.H. reagent grade sodium hypochlorite solution per litre) which is pumped at 1.2 ml/min (Fig. 1). Air is introduced at the point where the liquid streams meet so that the liquid stream is segmented to prevent diffusion. To en-

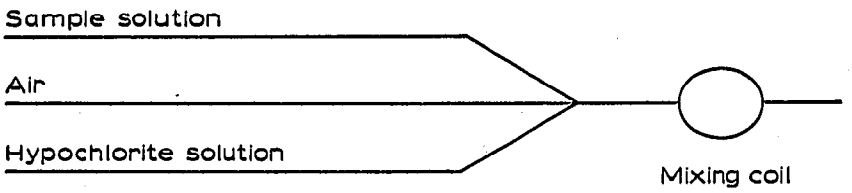

Fig. 1. The oxidation cycle.

sure that the liquid in each segment is homogeneous, the liquid stream is passed through a glass coil, positioned so that the axis of the coil is horizontal, where the pulsing motion of the segments ensures complete mixing of the liquid in each segment. The liquid stream then passes through a glass coil (40 ft. long), immersed in oil at 95°, and through a second glass coil (40 ft. long), at room temperature to effect the oxidation.

Owing to pressures which are built up inside these coils, especially in the one maintained at 95°, the flow rate of the liquid stream leaving the second coil is erratic and, if further reagents are introduced into this stream, the dilution will be erratic and valueless traces obtained. This difficulty can be overcome by passing only a part of the stream leaving the second coil through the pump at a constant rate so that, when it is mixed with further reagent streams, the dilution is uniform and reliable traces are obtained (Fig. 2).

Averaged over a fairly short interval of time, the sample stream recycled through the pump will be a constant fraction of that leaving the heating bath. Since the rate of change of phosphorus concentration in the column effluent is comparatively small, any errors introduced by the erratic flow rate of the sample stream leaving the heating

bath will be negligible. So that all the air will be lost before the sample stream is recycled through the pump, the pumping rate must be less than the lower limit of the

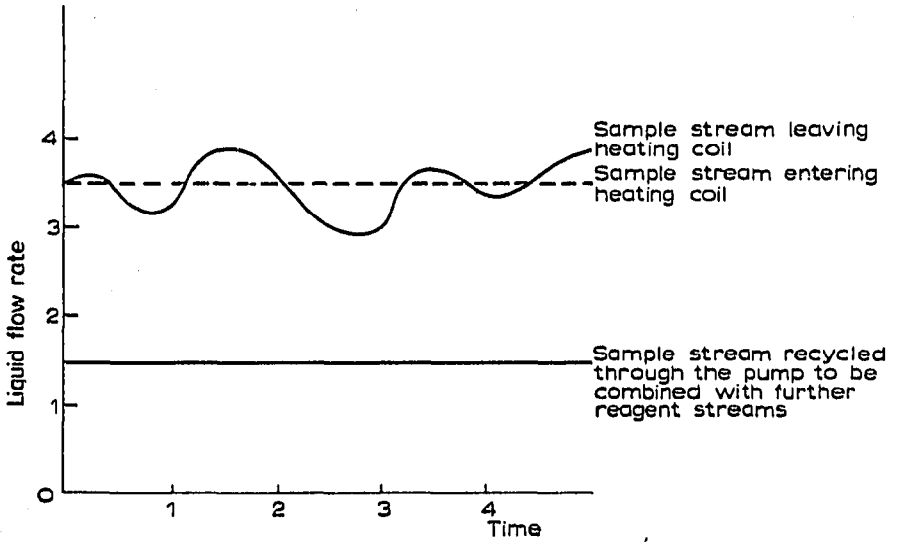

Fig. 2. Pumping rate traces.

flow rate of the stream leaving the heating bath. This is determined by trial and error. The average value of $(a + x)$ (see Fig. 3) will be equal to the rate at which liquid is being pumped into the heating bath.

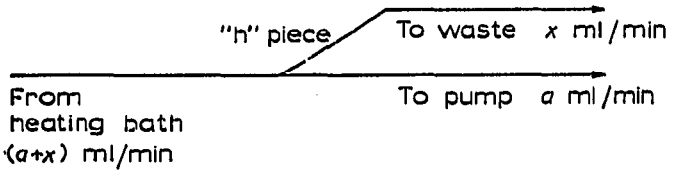

Fig. 3. Stream splitter for constant pumping speeds.

The hydrolysis cycle

The fraction of the sample stream retained in the analytical system is pumped at 0.32 ml/min and mixed with 10 *N* sulphuric acid containing 10 ml/l of saturated bromine water which is pumped at 1.2 ml/min. The liquid is resegmented with air and passed through a mixing coil before it enters a heating coil (2 glass coils, each 40 ft. long) maintained at 95°. After leaving the heating coil the liquid stream is cooled in a water-jacketed coil.

The colorimetric cycle

Once again in order to counteract surging in the sample stream, only a part (0.32 ml/min) is retained in the analytical system. This is mixed with a 1.25 % ammonium molybdate solution in 1.00 *N* sulphuric acid (0.80 ml/min) and segmented with air. After passing through a mixing coil, 0.05 % hydrazine sulphate solution is injected at 1.6 ml/min into the sample stream through an 'h' piece. This stream is pumped through a mixing coil and into a heating coil (40 ft. long) maintained at 95° to effect the development of the phosphomolybdenum blue complex.

The sample stream next passes through a cooling coil and then through the flowing colorimeter (silicon photocells, 801 mμ filters and a 10 mm flow cuvette). The percentage transmittance of the liquid stream flowing through the cell is read from a recorder chart (see Fig. 4). The flow time of the complete system is approximately one hour.

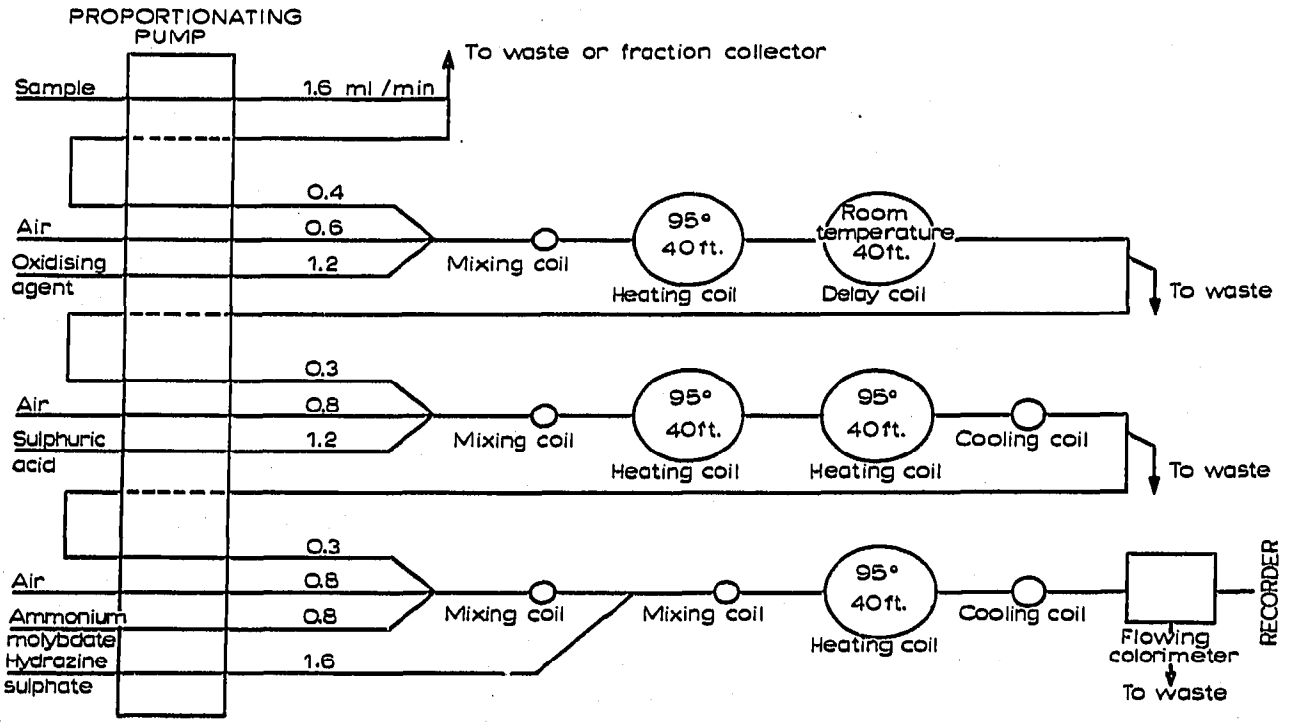

Fig. 4. Technicon Autoanalyser Module for phosphate anions.

Reagents

(*a*) *For the quantitative conversion of all phosphorus anions to orthophosphate.* Polyphosphates are readily hydrolysed to orthophosphate by strong acids and so 10 *N* sulphuric acid was chosen as the reagent for the hydrolysis cycle.

The choice of reagent in the oxidation cycle is more difficult because some lower oxyanions of phosphorus are only oxidised in neutral solution and others only in acid solution. In order to oxidise any anions which may only be oxidised in strong acid solution, saturated bromine water (10 ml/l) was added to the sulphuric acid used as reagent in the hydrolysis cycle. Several oxidising agents were used as reagents in the oxidation cycle, but only a solution of sodium hypochlorite (50 ml B.D.H. reagent grade sodium hypochlorite solution per litre) was found to quantitatively convert anions containing P–P bonds to orthophosphate.

To show that this reagent system quantitatively converts phosphorus anions to orthophosphate, samples of each anion to be studied were pumped through the analytical system and the optical density of the final sample stream was compared with that of an equivalent sample which had been converted to orthophosphate externally. External conversion to orthophosphate was effected by boiling with bromine water (10 ml of a saturated solution) in neutral solution for 1 hour, and then with bromine water (10 ml saturated solution) and 10 *N* sulphuric acid (10 ml) for a further hour.

The sample solutions were made 0.1 *M* with respect to potassium chloride and buffered to (1) pH 6.8, and (2) pH 11.4, in order to simulate anions in the column effluent.

The agreement between the optical density of the sample internally converted to orthophosphate, and that of the external standard (Table I) demonstrates that the

TABLE I

DIFFERENCE BETWEEN THE OPTICAL DENSITY OF THE EXTERNAL STANDARD AND THAT OF THE INTERNALLY CONVERTED SAMPLE

Anion	*Difference at*	
	pH 6.8	*pH 11.4*
$H_2PO_2^-$	+ 0.5 %	+ 0.5 %
HPO_3^{2-}	− 1.5 %	+ 0.9 %
PO_3S^{3-}	0.0 %	− 0.5 %
$PO_2S_2^{3-}$	+ 1.0 %	0.0 %
Pyrophosphate	− 0.5 %	0.0 %
Hypophosphate	0.0 %	—
Diphosphite	− 0.5 %	—
P^4–P^3–P^4	− 1.5 %	—
Monoamidophosphate	0.0 %	0.0 %
Trimetaphosphate	− 0.5 %	− 1.0 %

reagent system described is satisfactory for the quantitative conversion of these inorganic phosphates and lower phosphorus anions to orthophosphate, and we see no reason why it should not be satisfactory for other more complicated inorganic phosphorus anions.

(*b*) *For the colorimetric determination of phosphorus by the molybdenum blue method.* For the estimation of orthophosphate in the colorimetric system, the modification of the molybdenum blue method described by LUNDGREN[9] was used. In this system, the phosphomolybdic acid (formed by condensation of phosphoric acid and molybdic acid) is reduced by hydrazine sulphate in acid solution at 95°, to the phosphomolybdenum-blue complex.

(i) Ammonium molybdate solution: 1.25 % w/v ammonium molybdate in 1.0 *N* sulphuric acid.

(ii) Hydrazine sulphate solution: 0.05 % w/v aqueous hydrazine sulphate solution.

Calibration of the module

The module was calibrated by sampling solutions of sodium dihydrogen phosphate (Analar R) varying in concentration from 1 to 50 p.p.m. of phosphorus through the module.

A plot of absorbance of the final solution against concentration of phosphorus (Fig. 5) shows that Beer's law holds up to a concentration of 25 p.p.m. phosphorus in the sample solution (the transmittance corresponding to the limit is 75 %). Owing to the design of the module, only a fraction of the phosphorus entering the module passes through the colorimetric cycle, the remainder going to waste. In fact, therefore, Beer's law only holds within a much narrower range of concentration of phosphorus.

If absolute figures for the amount of phosphorus inany sample are required, it is necessary to run a standard with each batch of samples, because dilution factors vary

from day to day (owing to stretching of the manifold tubes). In the work described relative rather than absolute figures for phosphorus concentration were required, and so day to day calibration of the module was not necessary.

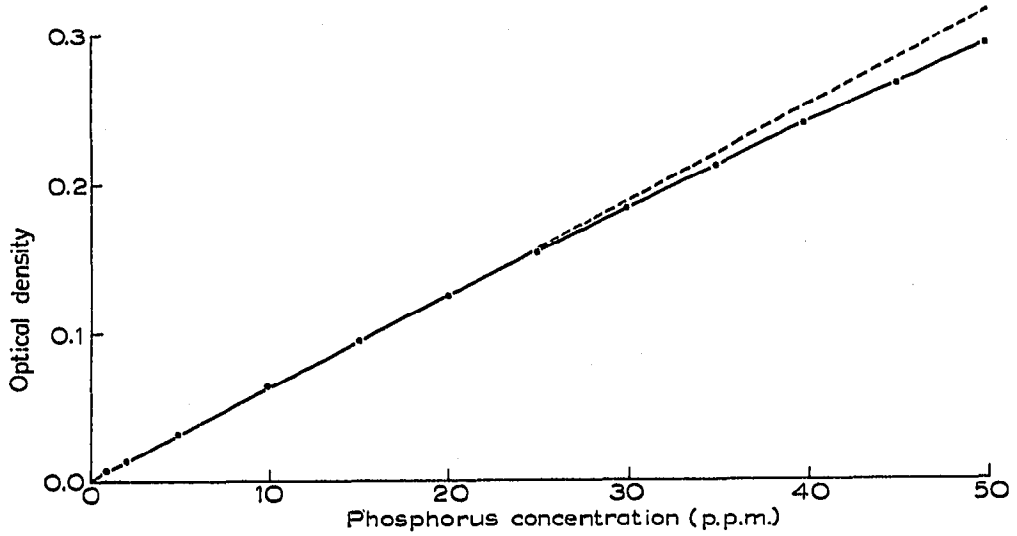

Fig. 5. Calibration graph for phosphorus as Molybdenum Blue.

The absorption spectrum of the final solution

The absorption spectrum of the molybdenum-blue complex obtained when a solution containing 100 p.p.m. phosphorus is sampled through the module was measured using a Unicam SP. 500 spectrophotometer. The spectrum indicates that the maximum absorption is at 817 mμ and hence 801 mμ filters (supplied by Technicon Instruments Ltd.), and silicon photocells were used in the colorimeter.

Anion-exchange chromatography

The ion-exchange columns were prepared in an identical manner to that described in previous publications[10,11], while the eluant gradients were obtained by the method described previously[7,8]. Without altering the analytical system, the column flow rate can be varied above a minimum of 0.42 ml/min, by pumping the column effluent at the required rate and rejecting all but that amount at a glass T-piece inserted in the liquid stream (see Fig. 6). The maximum permissible flow rate is governed by the porosity of the column, since pumping at too great a speed causes cracks and air-bubbles in the column.

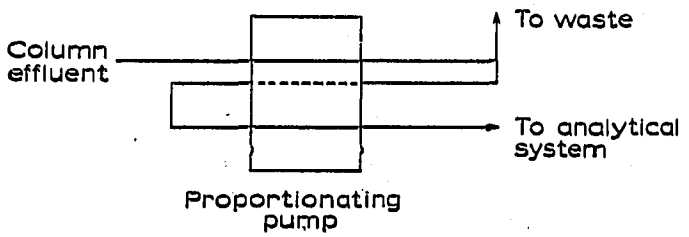

Fig. 6. Sample stream splitter.

The amount of phosphorus which can be applied to the column is limited by:

(i) the maximum load for which efficient chromatography can take place;

(ii) the fact that the minimum transmittance of the effluent must not be less than 75 %, otherwise Beer's law is not obeyed, and the calculated value of the phosphorus concentration would be subject to a systematic error.

Normally, the second factor is the limiting one, and thus the maximum load for any phosphorus anion depends on the time over which it is eluted from the column. (This will quite naturally depend on the dimensions of the column, the eluting gradient, and the affinity of the resin for the anion.)

Chromatographic separations

The following separations described in Table II and shown in Figs. 7 and 8 are typical of the results obtained.

TABLE II

SEPARATION DETAILS OF VARIOUS PHOSPHATE ANIONS

Figure No.	*Column dimensions*		*Resin Dowex-1*	*Temp.* (°C)	*pH*	*Flowrate* (*ml/min*)
	Length (*cm*)	*Diameter* (*cm*)				
7	50.0	1.5	× 8% D.V.B.	18	6.8	1.6
8	14.0	1.0	× 10% D.V.B.	~2*	11.4	1.6

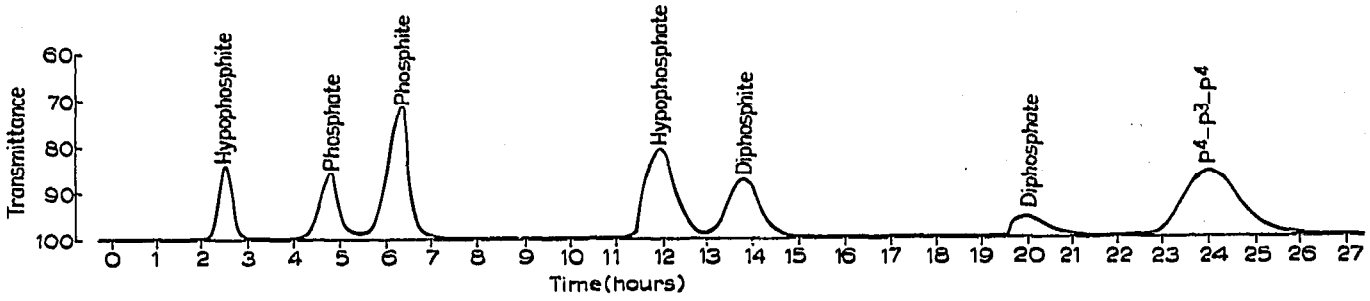

Fig. 7. Separation of lower phosphorus-containing anions.

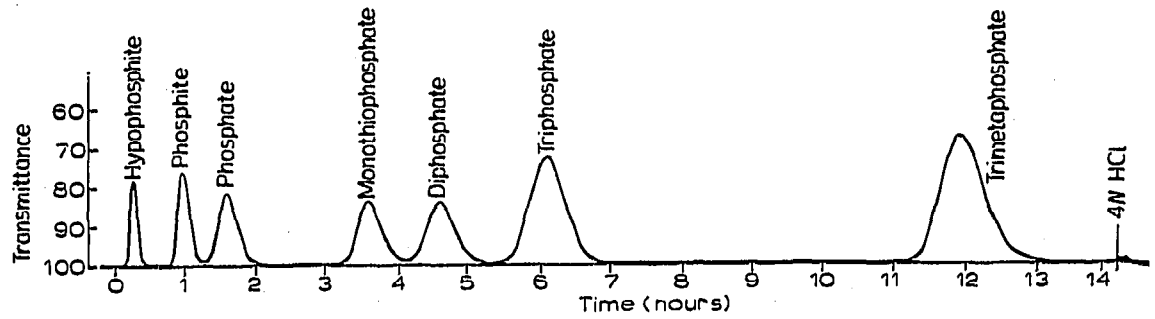

Fig. 8. Separation of simple and complex phosphorus-containing anions.

Quantitative evaluation of elution curves

The recorder pen of the Autoanalyser traces the variation with time of the transmittance rather than the absorbance of the solution in the colorimetric system, which means that elution curves cannot be quantitatively interpreted, simply by measuring peak areas with a planimeter. There are, however, several good approximation methods by which the peak area on an absorbance scale can be evaluated from the Autoanalyser trace:

(i) Assuming that the variation of phosphorus concentration in the column effluent is a Gaussian function of time.

(ii) Division of each of the peaks into narrow strips of equal width followed by calculation and summation of strip absorbances.

Since the phosphorus anions are monitored as orthophosphate in the colorimetric cycle, the distribution of phosphorus amongst the species represented by the peaks in the elution trace is simply given by the distribution of peak areas on an absorbance scale, provided that the absorbance of the molybdenum blue complex is within the limit of linearity with concentration of phosphorus.

According to Beer's law:

$\log_{10} T_0/T$ = Absorbance = $k.c$

where:

T_0 is the transmittance of the reagent blank;

T is the transmittance of the solution in the colorimetric system;

c is the concentration, in this case of the molybdenum-blue complex;

k is a constant depending on the extinction coefficient of the absorbing species and the length of the absorbance cell.

It should be noted that T is the true percentage transmittance of the solution. (When a range expansion factor other than 1 is used, the value for transmittance obtained from the recorder chart scale, T^c, must be corrected to the equivalent value for an expansion factor of unity.) Let the expansion factor be x (for the Range Expander unit supplied x may be 1, 2, 4 or 10).

The corrected transmittance is given by

$$T_b{}^c \left(1 - \frac{1}{x}\right) + \frac{T^c}{x}$$

where T^c is the value of the transmittance on the chart scale, and $T_b{}^c$ is the value of the transmittance on the chart scale for which balance was obtained.

(i) Calculation of absorbance peak areas assuming the elution peaks to be Gaussian. The phosphorus load (L) corresponding to an elution peak is given by:

$$L = \int_{-\infty}^{+\infty} c.\, \mathrm{d}t$$

where c = concentration.

Since flowrates in this system must be assumed to be constant, the variable volume may be replaced by time. When the elution peak is assumed Gaussian, the variation of concentration with respect to time is given by the formula

$$c = A \cdot \mathrm{e}^{-h^2 (t-m)^2}$$

where:

A is the maximum value of c for the peak,

m is the value of t corresponding to the peak maximum (retention time),

t is time,

h is a measure of the time interval during which the concentration is greater than $A/2$.

If such a time interval is defined by b, then,

$$h = \frac{2}{b}\sqrt{\log 2}$$

For a given phosphorus load, the values of A, m, and h will depend on the column parameters, the eluant gradient, and the ionic species being eluted.

$$\therefore L = \int_{-\infty}^{+\infty} e^{-h^2(t-m)^2}\, c.dt$$

$$= \frac{A\sqrt{\pi}}{h}$$

$$= Ab\sqrt{\frac{\pi}{4\log 2}} = \text{const. } A.b$$

Since the column load can be adjusted so that the phosphorus concentration in the column effluent is such that the concentration of the molybdenum blue complex remains within the limit of linearity with absorbance, c may be redefined as the absorbance. Thus, the phosphorus load corresponding to an elution peak will be proportional to the product of the peak absorbance, and the time interval during which the absorbance is greater than one half the maximum absorbance of the peak. These two parameters can readily be obtained from the Autoanalyser trace by using the general conversion formula:

$$\text{Absorbance} = T_b{}^c\left(1 - \frac{1}{x}\right) + \frac{T^c}{x}$$

To use this method for the calculation of the phosphorus load, L:

(a) the transmittance of the base-line $T_b{}^c$ and peak maximum $T_p{}^c$ are measured and corrected to their equivalents for an expansion factor of unity, say T_0 and T_p respectively. The value $\log_{10} T_0/T_p$ is the absorbance value of the peak maximum A;

(b) the transmittance (when $x = 1$) corresponding to an absorbance half that of the peak maximum is determined using the identity

$$\log_{10} T_p/2 = \log_{10} T_0 - \tfrac{1}{2}\log_{10} T_0/T_p$$

The width of the peak on the recorder trace when the transmittance ($x = 1$) is equal to $T_p/2$ is measured. This is b. The distribution of phosphorus among the peaks of an elution trace is then simply obtained by measuring the distribution of the product, $A.b$, for the peaks.

(*ii*) *Division of the peaks into narrow strips of equal width, followed by calculation and summation of absorbances.* Using the same nomenclature as under (*i*), an approximation to the absorbance peak area is obtained by dividing the peak obtained or the recorder chart into narrow strips, each of equal width, a, and measuring the transmittance, T, at the sides of each strip.

By adding the values of $\log_{10}(T_0)-\log_{10}(T)$ for all the strips comprising the peak, a value is obtained which, to a first approximation, is proportional to the corresponding load of phosphorus; the constant of proportionality being the same for all peaks in the elution trace, the degree of approximation being determined by the number of strips into which the peak is divided.

$$L = K \sum_{t=t_1}^{t=t_2} \log T_0/T$$

where t_2 and t_1 are times for the end and beginning of the peak respectively.

T is the transmittance at time t, and K includes the factor a.

For an expansion factor, x,

$$L = K \sum_{t=t_1}^{t=t_2} \log_{10} [T_b{}^c (x - 1) + T_0{}^c]/[T_b{}^c (x - 1) + T^c]$$

(When the base-line transmittance $T_0{}^c$ is not the same on either side of the peak, the average of $T_0{}^c$ at t_1 and $T_0{}^c$ at t_2 is substituted for $T_0{}^c$.)

This method of calculating the absorbance peak area is obviously very tedious, but it can be made a useful method by using a computer to evaluate the general expression for the phosphorus load given the values of $T_0{}^c$ and T^c for each peak. For the results which follow, an I.B.M. 1620 machine was programmed to process these data; the programme details together with more observations concerning this method will be published in a subsequent paper. It was considered necessary in this paper to outline the type of results which can be obtained comparing methods (i) and (ii).

RESULTS

To test these methods, a solution containing sodium orthophosphate and orthophosphite was prepared by weighing, and several aliquots of this solution subjected to separation on an ion-exchange column, under the conditions given in Table II and Fig. 7. Each run was analysed, and the results are given in Tables III and IV, which are a

TABLE III

CALCULATION OF THE PHOSPHATE TO PHOSPHITE RATIO ASSUMING THAT THE ELUTION PEAKS ARE GAUSSIAN*

Run No.	*Peak absorbances*		*Peak widths at half height*		*Ratio* P^5/P^3
	Phosphite	*Phosphate*	*Phosphite*	*Phosphate*	
1	0.0800	0.0669	19.5	47.1	2.02
2	0.0705	0.0596	20.9	51.0	2.06
3	0.0713	0.0602	19.8	50.2	2.14
4	0.0738	0.0617	20.0	50.8	2.12
5	0.0770	0.0656	20.3	50.0	2.10
6	0.0763	0.0607	20.9	52.3	1.99
7	0.0770	0.0632	19.9	51.0	2.10
8	0.0753	0.0613	20.1	51.2	2.07
9	0.0731	0.0605	21.0	52.3	2.06
			20.3 ± 0.5	50.7 ± 1.5	2.07 ± 0.04

* These results were obtained with K. W. C. BURTON, whose help is gratefully acknowledged.

TABLE IV

"STRIP" SUMMATION METHOD USING AN I.B.M. 1620

Run No.	*Absorbance phosphite*	*Absorbance phosphate*	*Ratio P^5/P^3*
1	1.4935	3.1092	2.082
2	1.4359	2.9619	2.063
3	1.3885	2.9622	2.133
4	1.4450	3.3039	2.096
5	1.5433	3.2115	2.081
6	1.5400	3.1100	2.019
7	1.4890	3.1183	2.094
8	1.4981	3.0649	2.046
9	1.5184	3.1271	2.059
			2.07 ± 0.03

direct comparison of methods (i) and (ii) applied to each separation. The weighed ratio of phosphate to phosphite was 2.07 : 1, and the results in the final column of each table compare very favourably with this value. These results, together with the programme, will be discussed at greater length, with many other observations on the system in a subsequent paper.

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SUMMARY

The development of a system, based on the Technicon Autoanalyser, is described for the quantitative determination of the phosphorus concentration in the column effluent from ion-exchange column separation of phosphorus anions. Quantitative interpretation of the elution peaks by the Gaussian approximation and by integration of peak areas are compared.

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