

THE *IN SITU* IDENTIFICATION OF PHOSPHATE SPECIES IN MULTI-SPOT CHROMATOGRAMS BY NEUTRON ACTIVATION ANALYSIS

MARJORIE A. ROMMEL* AND ROY A. KELLER

Department of Chemistry, University of Arizona, Tucson, Ariz. (U.S.A.)

(Received September 7th, 1964)

As a preface to a theoretical treatment of multiple zones and spots, *i.e.*, more than one zone for what is commonly accepted to be a single species, KELLER AND GIDDINGS¹ presented an extensive review of the phenomenon. Among the examples cited was one reported by CURRY², who attributed multiple spots on paper chromatograms of sodium orthophosphates to proton exchanges of the type:



where B^{n-} is some Bronsted base. In paper chromatography the water present as the immobile phase and, normally, as a component of the mobile phase, can act in this capacity. It need not be the only base present. ERDEM³ employed CURRY's suggestion to explain the multiple spotting of orthoarsenates on paper. As KELLER AND GIDDINGS remarked, the proposal is not wholly acceptable for kinetic reasons. The most serious experimental omission is the verification that the two spots do indeed contain different phosphate species. CURRY based his identification of the spots on their relative size and intensity when the different sodium salts of phosphoric acid were chromatographed. What is needed is an independent, nonchromatographic identification of the species *in situ*. The availability of a nuclear reactor suggested neutron activation analysis.

THEORY

The naturally occurring stable isotopes of sodium and phosphorus, when bombarded with thermal neutrons, accept a fraction of these neutrons to produce radioactive species. The relationship valid during activation is⁴:

$$A_t = N\phi\sigma(1 - e^{-0.693t/T}) \quad (1)$$

where A_t is the activity in disintegrations per second at time t after start of the irradiation, N is the total number of nuclei present in the sample, ϕ is the neutron flux in neutrons/cm²·sec, σ is the activations cross section in cm², and T is the half life of the radioactive isotope. Here, t is the period of irradiation and A_t is the activity at the instant of removal of the sample from the reactor. The equation must be further

* Koppers Company Teaching Fellow, 1963; Continental Oil Company Research Fellow, 1964; present address, Rocketdyne, Analytical Research Laboratory, Canoga Park, Calif.

modified to include the fact that the radiation observed or the apparent activity, R_t , *i.e.*, the radiation admitted to a Geiger tube through an aperture, is some fraction of A_t . The proportionality factor is the detection coefficient or counting yield, Y (ref. 4), *i.e.*:

$$R_t = YA_t \quad (2)$$

which may be resolved into some eleven contributions. On introduction into eqn. (1):

$$R_t = YN\phi\sigma(1 - e^{-0.693t/T}) \quad (3)$$

In this particular problem the species in the solute spots are judged to be the same or different by a determination of the ratio of the number of atoms of each, *i.e.*:

$$\frac{R_t(\text{Na})}{R_t(\text{P})} = \frac{Y_{\text{Na}}N(\text{Na})\phi_{\text{Na}}\sigma_{\text{Na}}(1 - e^{-0.693t_{\text{Na}}/T_{\text{Na}}})}{Y_{\text{P}}N(\text{P})\phi_{\text{P}}\sigma_{\text{P}}(1 - e^{-0.693t_{\text{P}}/T_{\text{P}}})} \quad (4)$$

The paper strips holding the spots, each of which contain both sodium and phosphorus, were irradiated simultaneously at the same position in the reactor so that $\phi_{\text{Na}} = \phi_{\text{P}}$ and $t_{\text{Na}} = t_{\text{P}} = t$. The combined radioactivity of the two species present in a single spot was determined so that it seemed reasonable to assume that the detection coefficient was identical for both, *i.e.*, $Y_{\text{Na}} = Y_{\text{P}}$. The counter geometry was identical in both cases and both isotopes lose β -particles of nearly the same energy. Eqn. (4) becomes:

$$\frac{R_t(\text{Na})}{R_t(\text{P})} = \frac{N(\text{Na})\sigma_{\text{Na}}(1 - e^{-0.693t/T_{\text{Na}}})}{N(\text{P})\sigma_{\text{P}}(1 - e^{-0.693t/T_{\text{P}}})} \quad (5)$$

The nuclear properties of interest appear in Table I. Geiger-Müller counters are approximately 100 times more efficient for β -particles than for γ -rays⁵. The error in assuming sodium to be strictly a β -emitter is about 1%, which is much less than the error in the nuclear cross section. The outstanding advantages existent with this pair of elements are: (1) there is one naturally occurring isotope for each species so there is only one nuclear reaction to produce one daughter, (2) the nuclear cross sections are both sufficiently large and comparable that convenient activities can be produced in both species in a reasonable irradiation time, (3) the half lives are sufficiently different to give a sharp change in slope of the plot of the activity *vs.* time as the sodium decays away and (4) the half lives are sufficiently short to make the time required by the experiment reasonable. This last factor is also convenient in disposal and contamination problems, *i.e.*, one may decontaminate by sealing off the radiation area for a reasonable period of time.

Koch⁶ suggests some possible interferences. These are listed in Table II along with other pertinent data. The threshold energy, *i.e.*, the kinetic energy of the neutron just capable of making the reaction energetically possible⁵, is 1 MeV or above for all reactions except the last. Thermal neutrons, the kind encountered in the TRIGA reactor, are those with energies of $2.5 \cdot 10^{-8}$ MeV⁷ to $3.5 \cdot 10^{-8}$ MeV⁵. Thus the only feasible interference is from silicon. A measure of the probability of the reaction is best given by the product of the relative isotope abundance and the cross section, *i.e.*,

TABLE I

NUCLEAR PROPERTIES OF THE ISOTOPES OF THE REACTIONS $^{23}\text{Na} (n,\gamma) ^{24}\text{Na}$ AND $^{31}\text{P} (n,\gamma) ^{32}\text{P}$

	^{23}Na	^{31}P
Per cent abundance ⁴	100	100
Nuclear cross section for thermal neutrons (barns) ⁴ (Probable error = 20% ⁵)	0.536 ± 0.01	0.19 ± 0.01
Daughter	^{24}Na	^{32}P
Mode of decay ⁶	β^- , 1.39 MeV γ , 1.38 MeV 2.76 MeV	β^- , 1.712 MeV
Half life ⁴	14.97 h	341.3 h

0.003 (compared with 0.19 for phosphorus, a factor of 63 in difference). This coupled with the extremely small amount of silica in paper as contamination reduces the likelihood of interference from this reaction to an infinitesimal.

The mixture of radioactive ^{24}Na and ^{32}P is classified as a mixture of independent radionuclides with stable daughters (^{24}Mg and ^{32}S). Its total activity, which is the observed activity, R_t , as a function of time is:

$$\log R_t = \log[R_t(\text{Na})]_0 + \log[R_t(\text{P})]_0 - (\lambda_{\text{Na}} + \lambda_{\text{P}})t^*/2.303 \tag{6}$$

Quantities $[R_t(\text{Na})]_0$ and $[R_t(\text{P})]_0$ are the activities at the time of the first count which here is the instant of removal from the reactor ($t^* = 0$). In actuality this activity was not determined either because the sample was judged dangerous by the radiation officer or because of time consumed in transfer of the sample from the reactor site to the counter. The rates at $t^* = 0$ are the extrapolated values obtained from the activity-time plot and the known elapsed time from termination of irra-

TABLE II

POSSIBLE INTERFERING NUCLEAR ACTIVATIONS

Interfering reaction	Per cent abundance	Threshold energy (MeV)	Nuclear cross section of parent* (barns)
<i>^{23}Na analysis</i>			
$^{27}\text{Al}(n,\alpha)^{24}\text{Na}$	100	3.3	0.14 (13) 0.116 (14)
$^{24}\text{Mg}(n,p)^{24}\text{Na}$	78.6	4.9	0.22 (13) 0.19 (14.5)
<i>^{31}P analysis</i>			
$^{32}\text{S}(n,p)^{32}\text{P}$	95.02	~ 1	0.30 (6-10) 0.31 (15)
$^{35}\text{Cl}(n,\alpha)^{32}\text{P}$	75.4	1.0	0.19 (14.5)
$^{30}\text{Si}(n,\gamma)^{31}\text{Si}$ ↓ $-\beta^-$	3.05	Thermal	0.110
$^{31}\text{P}(n,\gamma)^{32}\text{P}$			

* The number in parentheses is the neutron energy for the cross section listed.

radiation to the first count. The constants λ_{Na} and λ_P are characteristic of the radioactive species, *i.e.*, $0.693/T$ where T is the half life. The graphical method of resolution of eqn. (6) into the separate activities is sufficiently well known so that it is not repeated here^{4,5}.

Fig. 1 is typical of the data and demonstrates the ease of resolution of the activities and the subsequent almost utopian character of the method. The dashed lines are the extrapolations of the linear portions of each curve to $t^* = 0$. The values of $[R_t(Na)]_0$ and $[R_t(P)]_0$ (found from the intercepts at $t^* = 0$) were used in eqn. (5) to calculate $N(Na)$ and $N(P)$ present in the solute zone.

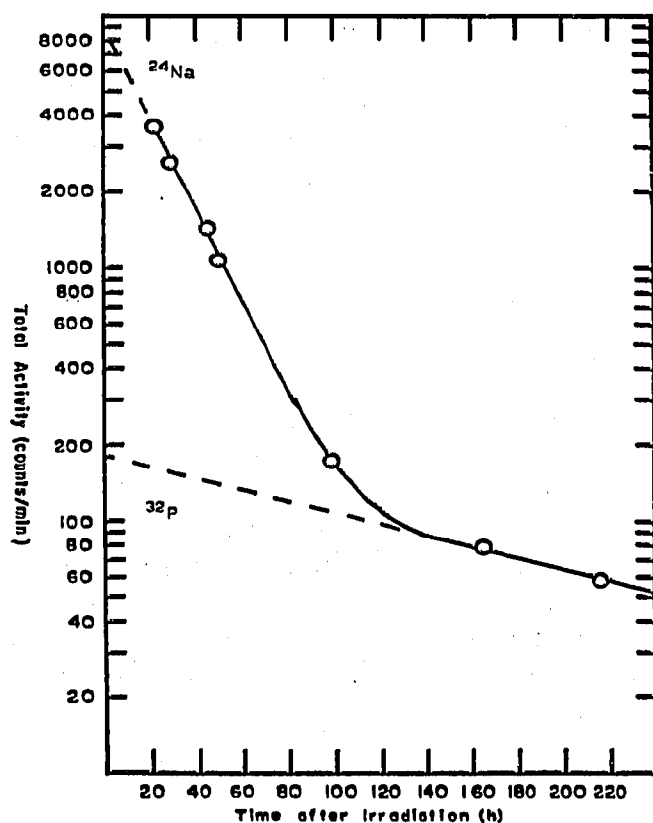


Fig. 1. Observed activity, R_t , of solute zones containing ^{24}Na and ^{32}P as a function of time.

EXPERIMENTAL.

The TRIGA reactor^{8,9} is a thermal, heterogeneous, enriched uranium reactor normally of 10 kW power level. The 12 in. thick graphite reflector holds a rotary specimen rack of forty 1-in. diam. \times 4 in. long, 50 cm³ capacity specimen tubes 3 in. from the core of enriched uranium-zirconium hydride rods. The unit is situated at the bottom of a 6 ft. diam. \times 20 ft. deep water filled tank. Specimens are transferred to and from the sample rack or a 1 in. diam. thimble in the center of the core by a pneumatic tube. The approximate neutron flux is 10^{11} neutrons/cm²·sec in the core (average value), $0.7 \cdot 10^{11}$ neutrons/cm²·sec at the specimen rack and $1.5 \cdot 10^{11}$ at the central thimble. Operation at 30 kW yields $8 \cdot 10^{11}$ neutrons/cm²·sec¹⁰. The University of Arizona reactor is currently licensed to operate at 100 kW for 2 h at a flux of about 10^{13} neutrons/cm²·sec.

The paper strip with the materials for activation was coiled with another unused strip of identical size to prevent transfer of material from one position on the formed strip to another (a potential source of double spots). The coiled paper was inserted in a polyethylene reactor sample cylinder which had been washed with detergent and rinsed with distilled water followed by acetone and dried. Careful cleaning removes contaminating substances which can activate to give a dangerously active container. Container and samples may be confiscated for storage for indefinite periods. This was delivered to the reactor operator for activation. The radiation officer monitored the container after activation and returned it if radiation was at a sufficiently low level. The reactor operator furnished the neutron flux, activation time, and the time of removal from the reactor.

Examination of the radioactivity of the strip chromatogram was performed with a Tracerlab (Waltham, Mass., U.S.A.) model SC-55S Auto/Step Chromatogram Scanner. The paper strip, affixed to a geared wand by cellophane tape, advanced under a slit collimator in discrete steps. The step length and slit width were both adjustable from 0 to 7 mm. Radiation passed through the slit to a TGC-2 end-window Geiger tube of 1.9 mg/cm² window thickness, 200 μ sec recovery time, and 1400 V operating potential. The signal passed to a model SC-71 Compu/Matic II Scaler. This could be programmed to determine the number of counts in a preselected time interval or the time interval required for a present number of counts or whichever came first. On completion of the count the data passed to a model SC-88 Auto/Computer consisting of a Translator and Lister. This unit printed out on a tape the sample number, count, time, and computed the counts per minute. On completion of the printing step, the counter reset itself and the scanner advanced the strip to the next position. The scanner was set to advance the strip 5.0 mm per counting site and the slit width set at 5.1 mm to allow some overlap of sites. The counting time was varied to compromise between random errors of short counting periods and the half life which made long counting periods undesirable. In general, periods of one minute were satisfactory. The performance of the equipment depended upon the line voltage. This was metered and controlled by a Metered Variac, Type W5MT3AW (General Radio Co., Concord, Mass.) and kept at 115 to 125 V.

The circumstances which lead to multiple spot formation are complicated and will be described in detail in another publication. One circumstance seems to be the sodium content of the paper which must be reduced by washing. Routinely the strips to be used were immersed in a four liter beaker of 2 M acetic acid for 5 min followed by three consecutive 10 min immersions in distilled water. If the last rinse showed no difference in pH from distilled water as measured with a short-range pH paper, the papers were termed acid free. The paper strips were hung from a glass rack and each side washed with 20 ml of 95 % ethanol delivered from a polyethylene wash bottle. This was repeated with ethyl acetate. The papers were allowed to dry in air. This procedure removed any traces of grease and oil introduced during cutting and handling. Polyethylene gloves were worn while manipulating papers to further prevent contamination. The sodium content of fingerprints is sufficient to give a high activity on irradiation.

Chromatography was performed on 55 cm \times 2.8 cm paper strips cut in the direction of machining from 47 \times 55 cm sheets of Whatman Filter Paper No. 1, Acid Washed. Two different packages of paper were used which are designated B-1 and B-2.

RESULTS

To test the reliability of the method, known sodium salts of orthophosphoric acid were placed on a paper strip and the Na/P ratio determined by neutron activation. Table III presents the results of two independent determinations.

TABLE III

SODIUM/PHOSPHORUS RATIO IN SODIUM ORTHOPHOSPHATES AS DETERMINED BY NEUTRON ACTIVATION ANALYSIS

Salt	Na/P	
	Trial 1	Trial 2
NaH ₂ PO ₄	1.0	1.2
Na ₂ HPO ₄	2.0	2.0
Na ₃ PO ₄	3.1	3.0

These same salts and phosphoric acid were chromatographed with 15 % water/85 % (pyridine-ethyl acetate, 45:100 v/v) as the forming solvent (mobile phase). Table IV presents the results of six chromatograms of the various solutes formed simultaneously in the same chamber for the same length of time. After radiocounting, the spots were sprayed with 0.4 % ammonium molybdate in 8 % nitric acid, dried, sprayed with 0.05 % benzidine hydrochloride in 10 % acetic acid and exposed to ammonia vapors as is outlined by BLOCK, DURRUM, AND ZWEIG¹¹. Regions containing phosphate species developed a blue color. The Na/P ratios were determined at the point of maximum radioactivity in each phosphate solute zone. All of the chromatograms were formed with the goal of producing widely separated solute spots. This required that the forming solvent should over-run the chromatogram, *i.e.*, drip off the paper strip. Thus the distance of the spot from the origin can be reported but not the R_F value. Fig. 2 is a photograph of the revealed chromatogram.

For phosphoric acid, there is sufficient evidence of sodium in the zone but nothing near the 1/1 ratio required by the primary salt. The species is predominately

TABLE IV

DATA OF THE CHROMATOGRAMS OF THE SODIUM ORTHOPHOSPHATES (FIG. 2)

Paper No.	Solution applied	Distance of phosphate zone from origin (cm)	Na/P
176	0.01 M H ₃ PO ₄	17.5	0.03
177	0.05 M H ₃ PO ₄	17.5	0.14
178	0.10 M H ₃ PO ₄	16	0.05
179	0.01 M NaH ₂ PO ₄	18.5	0.24
180	0.01 M Na ₂ HPO ₄	5	0.75
		18	0.19
181	0.01 M Na ₃ PO ₄	4.5	1.2
		18	0.19

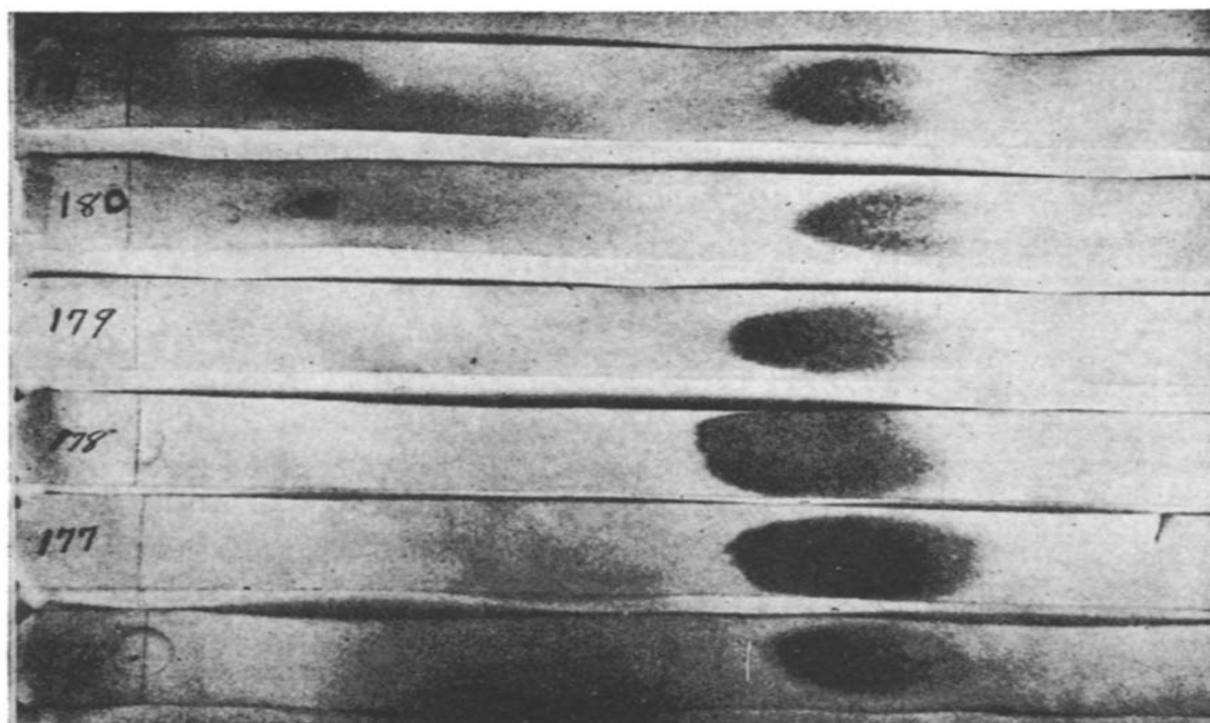


Fig. 2. Chromatograms of the sodium orthophosphates formed with pyridine-ethyl acetate/water (see Table IV).

H_3PO_4 . There is evidence in support of the contention that the sodium came from the paper. With the primary salt, the single spot has a higher Na/P ratio than the phosphoric acid but it is sufficiently less than unity that the conclusion is that sodium was extracted from the solute zone and protons introduced. The same conclusion applies to the secondary and tertiary salts. In addition, the second or slow spot in both papers 180 and 181 has a Na/P ratio much less than expected from the composition of the salt applied. In the sense that the Na/P ratios of the solute zones in multiple spot chromatograms differ, the species are different as proposed by CURRY. The two zones do not originate in a discontinuity in the mobile phase¹. However, the composition of these zones cannot be explained by a simple proton exchange. The production of the fast spot of low Na/P ratio, particularly significant in the case of the tertiary salt which contains no protons, strongly indicates a source of protons and sodium ions in the system and their participation in the chromatographic process. This source can only be the paper itself and the impurities therein.

A valid criticism of the foregoing discussion is that it is an error to assign a degree of protonation to the phosphate ion on the basis of the Na/P ratio since the pyridine can act as a base to accept a proton and behave as a cation, *i.e.*, the fast zone may be a pyridinium phosphate rather than phosphoric acid. This is very possible if one wishes to use this language of recognized stoichiometric formulas. However, chromatograms were also performed with butanol-water. The investigation with this solvent was not extensive because the time required for formation of the chromatograms was impractical (useful results were obtained in some cases only after 100 h of chromatography). Multiple spots were produced but the circumstances were different. Two spots were obtained from tertiary sodium phosphate and one

spot from the primary salt when formed with pyridine-ethyl acetate/water. Fig. 3 shows these salts chromatographed with 10% water in *n*-butanol; No. 323 is 0.01 *M* tertiary sodium phosphate (one spot) while No. 324 is sodium dihydrogen phosphate (two spots). The presence of pyridine in the forming solvent affects the multizoning but is not responsible for it. Table V gives the distance of the spots from the origin and the Na/P ratio as determined by neutron activation analysis. It is difficult to conceive that the sodium deficiency of the fast spot in No. 324 is compensated by any species other than protons.



Fig. 3. Chromatograms of the sodium orthophosphates formed with *n*-butanol-water (see Table V).

The occasion arose to employ eqn. (3) in an estimation of the sodium content of the paper. This required an estimate of Y and ϕ , the first of which is the greater problem. Unfortunately the facilities for direct calibration of Y were unavailable and a geometrical calculation had to suffice. This involved the distance from the paper to the Geiger tube window and the slit dimensions combined with the assumption that the sample is a point source spherical radiator at the center of the slit area. The oversimplified model showed that the fraction of the radiation sphere collected by the slit is at best 0.40 and it can be as low as 0.15. Thus the error in the detection coefficient is as much as 70% which overwhelms the 20% error in nuclear cross section and the estimated error in the neutron flux.

TABLE V

DATA OF THE CHROMATOGRAMS OF THE SODIUM ORTHOPHOSPHATES (FIG. 3)

Paper No.	Solution applied	Distance of phosphate zone from origin (cm)	Na/P
323	0.01 <i>M</i> Na ₃ PO ₄	5	0.80
324	0.01 <i>M</i> NaH ₂ PO ₄	5 12	1.2 0.38

Samples of both batches of paper, acetic acid washed and unwashed, were analyzed for sodium by neutron activation. Table VI reports the sodium content. The per cent by weight sodium is based on $Y = 0.40$; the upper limit for the content in μ equiv. is based on this same Y and the lower limit on a 70% error. The third column reports the sodium content of a section of a chromatogram, solute free, over which the forming solution had passed during chromatography. ULTEE AND HARTELL¹²

TABLE VI

SODIUM CONTENT OF CHROMATOGRAPHIC PAPER

Source	Unwashed		Washed		Chromatographed	
	Per cent by wt.	μ equiv.	Per cent by wt.	μ equiv.	Per cent by wt.	μ equiv.
B-1	0.33	42-140	0.06	8-26	0.005	1-5
B-2	0.74	96-320	0.08	11-35		

report a carboxyl content of 8 μ equiv./g for Whatman No. 1. The more extensive study of ACKERMAN AND KRÜGER¹³ reports ion exchange capacities of 2.2 to 9.0 μ equiv./g for a variety of German papers. This seeming agreement is deceiving for the two groups differ by a factor of ten for capacities of papers from the same manufacturers (they apparently did not duplicate grades since the numerical designations for the papers do not agree). If one presumes a capacity of 8 μ equiv./g, and this is a very tenuous assumption, then the unwashed paper has a sodium content far above the exchange capacity of the paper, is reduced to slightly above this capacity on washing, and is slightly below capacity after chromatography. In view of the suspicion which must be attached to the published values of the exchange capacity of paper, a better estimate of Y would not provide any more reliable conclusion. One can calculate the relative reduction in sodium content brought about by these procedures. In this case, eqn. (4) is employed and the doubtful terms, *i.e.*, detector coefficient and nuclear cross section, cancel. Had the irradiation of the three paper samples been carried out simultaneously, the right-hand side of eqn. (4) would involve the ratio of N -values only. This was not the case, however, so the flux and irradiation time had to be retained. Nonetheless it can be said with a great deal of confidence that washing the paper reduces the sodium content by a factor of 7 to 8 and chromatography reduces the sodium content by a factor of 12 below that of the washed paper. This labile sodium of the paper must play a part in the chromatographic process, most likely as an exchangeable ion, and the paper, the various phosphate anions, and the bases in the forming solvent, including water, must all complete in some complex fashion for protons.

ACKNOWLEDGEMENTS

The authors are indebted to the National Institutes of Health, RG-7046-Bio(C1) and the National Science Foundation G-14216 for financial support of this work, to Dr. E. N. WISE and Dr. J. T. YOKE, III of the Chemistry Department for their advice, and in particular to Dr. L. E. WEAVER and his group in the Department of Nuclear Engineering for making the reactor available and for their highly skilled assistance throughout the project.

SUMMARY

The solute zones obtained on paper chromatography of phosphates were analyzed for the sodium to phosphorus ratio by neutron activation analysis. Where

double spots were formed, the analysis indicated different phosphate species in the two zones. The work also indicated that the sodium in the paper plays an active role in the phenomenon, probably through some ion exchange mechanism.

REFERENCES

- 1 R. A. KELLER AND J. C. GIDDINGS, *J. Chromatog.*, 3 (1960) 205; in M. LEDERER (Editor), *Chromatographic Reviews*, Vol. 3, Elsevier, Amsterdam, 1961, p. 1.
- 2 A. S. CURRY, *Nature*, 171 (1953) 1026.
- 3 B. ERDEM, *Rev. Fac. Sci. Univ. Istanbul*, 20c (1955) 332, 346; *C.A.*, 50 (1956) 16530g.
- 4 R. T. OVERMAN AND H. M. CLARK, *Radioisotope Techniques*, McGraw-Hill, New York, 1960.
- 5 G. FRIEDLANDER AND J. W. KENNEDY, *Introduction to Radiochemistry*, John Wiley, New York, 1949.
- 6 R. C. KOCH, *Activation Analysis Handbook*, Academic Press, New York, 1960.
- 7 S. GLASSTONE, *Sourcebook on Atomic Energy*, D. Van Nostrand Co., Princeton, N.J., 1950.
- 8 H. ISBIN, *Proc. U.N. Intern. Conf. Peaceful Uses At. Energy, 2nd, Geneva, 1958*, Vol. 8, p. 561.
- 9 S. L. KOUTZ, T. TAYLOR, A. McREYNOLDS, F. DYSON, R. S. STONE, H. P. SLEEPER, JR. AND R. B. DUFFIELD, *Proc. U.N. Intern. Conf. Peaceful Uses At. Energy, 2nd, Geneva, 1958*, Vol. 10, p. 282.
- 10 E. N. WISE, *J. Chem. Educ.*, 39 (1962) A771.
- 11 R. J. BLOCK, E. M. DURRUM AND G. ZWEIG, *A Manual of Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1958.
- 12 A. J. ULTEE, JR. AND J. HARTELL, *Anal. Chem.*, 27 (1955) 557.
- 13 G. ACKERMAN AND G. KRÜGER, *Z. Anal. Chem.*, 191 (1962) 17.

J. Chromatog., 18 (1965) 349-358