International Journal of Applied Radiation and Isotopes, 1962, Vol. 13, pp. 7–10. Pergamon Press Ltd. Printed in Northern Ireland

A Chromatographic Method for the Study of the Stability of Labeled Sodium Polymetaphosphate

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(*Received* 7 *June* 1961)

Ascending chromatography method developed on the basis of the EBEL technique provided us with a good and easy technique for the differential analysis of the mixture of phosphates in the case of sodium polymetaphosphate labeled with radiophosphorus. This radiopharmaceutical, used extensively in clinical work for the treatment of bone cancer, is therapeutically efficient only with a high ratio of highly condensed molecules.

This method, carried out as a radiometric determination, gives us values close to the photocolorimetric method values.

UNE METHODE CHROMATOGRAPHIQUE POUR L'ETUDE DE LA STABILITE DU POLYMETAPHOSPHATE DE SODIUM MARQUE

Une méthode de chromatographie ascendante, développée sur la base de la technique d'Ebel, nous a fourni une bonne technique facile pour l'analyse différentielle du mélange de phosphates dans le cas du polymétaphosphate de sodium marqué au radiophosphore. Ce produit radiopharmaceutique, largement employé dans le travail clinique pour le traitement du cancère de l'os, a une efficacité thérapeutique seulement dans le cas d'une forte proportion de molécules largement condensées.

Cette méthode, achevée en la façon d'une mesure radiométrique, nous donne des valeurs proches à celles de la méthode photocolorimétrique.

ХРОМАТОГРФИЧЕСКИЙ МЕТОД ИССЛЕДОВАНИЯ СТАБИЛЬНОСТИ МЕЧЕНОГО ПОЛИМЕТАФОСФАТА НАТРИЯ

Разработанный по Эбелю метод восходящей хроматографии обеспечивает надежный и легкий способ дифференциального анализа смеси фосфатов в случае полиметафосфата натрия, меченого радиофосфором. Этот радиоактивный фармацевтический препарат, применяемый исключительно в клинических условиях для лечения костной саркомы, оказывает терапевтический эффект только при использовании его в высокой концентрации.

Данный метод, используемый в качестве радиометрического определения, позволяет получать значения концентраций фосфора, близкие к значениям, получаемым фотокалориметрическим методом.

EINE CHROMATOGRAPHISCHE METHODE ZUM STUDIUM DER STABILITÄT VON MARKIERTEM NATRIUMPOLYMETAPHOSPHAT

Eine ansteigende chromatographische Methode, entwickelt auf Basis der Ebel-Technik, erwies sich als gute und einfache Methode zur Differenzierungsanalyse eines Gemisches von Phosphaten bei Radiophosphor markiertem Natriumpolymetaphosphat. Dieses radioaktive Medikament, welches klinisch ausschliesslich zur Behandlung von Knochenkrebs verwendet wird, ist nur bei einem hohen Grad hochkondensierter Moleküle therapeutisch wirksam.

Diese radiometrische Methode lieferte uns sehr ähnliche Werte, wie die welche mittels photometrischer Methode gewonnen wurden.

A METHOD was developed for the analysis of of the composition of sodium polymetaphosphate labeled with radiophosphorus (P^{32}), used extensively in clinical work for the treatment of bone cancer where the highly condensed molecules ($n > 7$) are most effective[1].

This assay was carried out with sodium polymetaphosphate labeled with P^{32}, obtained according to the AUDRIETH[2] technique. The purpose of this work was to determine the stability of the radioactive material under various conditions of pH and times of storage.

The KARL-KROUPA[3] technique of ascending paper chromatography developed on the basis of the EBEL technique[4] was used for the chromatographic differential analysis of the mixture of phosphates. This assay was completed by radiometric analysis of the different zones of the chromatogram.

EXPERIMENTAL

Chromatography was accomplished by developing first with the basic solvent, then at a 90° angle from the original solvent direction with the acid solvent. Visualization of the spots was made possible by spraying the paper with the special chromatographic spray and developing according to the method described below. This technique provides a standard two-dimensional method for separating ring and chain phosphates into two distinct groups[5]. The elution step before the colorimetric determination of the phosphorus was avoided because of the possibility of a considerable loss of activity that can remain in the paper.

The sodium polymetaphosphate used was in a solution of 9.8 mg/ml with a specific activity of 0.4 mc/ml. The stability was tested 2 weeks and 4 weeks after its preparation by the AUDRIETH[2] procedure. The effect of pH was also studied, changing the pH of the original solution (6.5) to pH 8.5 and pH 5.0, respectively, by the addition of NaOH (0.01 N) or HCl (0.01 N). The average temperature during storage was 20°C.

(1) *Chromatography*

According to EBEL's[4] technique of ascending paper chromatography with a spot of about 30 gamma of phosphorus. Run first with basic solvent:

400 ml isopropyl alcohol
200 ml isobutyl alcohol
390 ml water
10 ml concentrated ammonium hydroxide

for 5 hr. Then dry and run again at a 90° angle from the basic solvent direction with acid solvent:

750 ml isopropyl alcohol
50 g trichloracetic acid
250 ml water
2.5 ml concentrated ammonium hydroxide.

Run the chromatogram in this solvent for 4 hr. Dry with hot air and put in an oven with a dish of water at 80°C for ½ hr. Dry again and spray with chromatographic solution:

5 ml 60 per cent perchloric acid
1 ml concentrated hydrochloric acid
1 g ammonium heptamolybdate tetrahydrate and water to 100 ml.

Dry and expose to steam in an oven or to an ultraviolet lamp until blue spots develop.

With the same technique, run a reference chromatogram with a standard solution containing ortho-, pyro-, tri-, trimeta- and tetrameta-phosphate (reagent grade), approximately 0.4 gamma of each phosphorus per μl and approximately 2 gamma of total phosphorus per μl.

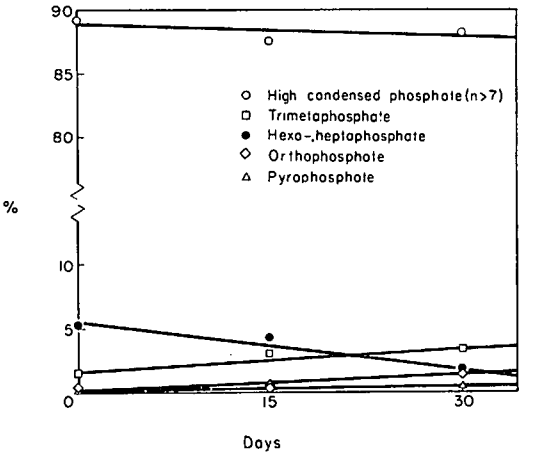

FIG. 1. Changes with time in distribution of P^{32}-activity in polymetaphosphate-P^{32} solution (pH 6.5, 20°C).

(2) *Colorimetric analysis*

After the development of the spots, due to the action of the perchloric acid, the paper becomes weak and must be handled carefully. Draw lines 1 cm apart with a pencil, so dividing the paper into squares of 1 cm^2. Cut the paper off in slices according to the drawing in the zone of the spots. Mark each one and transfer to 25 ml flask. After 1 ml of 8 N ammonia and 7 ml distilled water base have been added, allow to stand for 15 min. Add 3 ml of 8 N H_2SO_4. Put them in a boiling water bath for $\frac{1}{2}$ hr. After the flasks have cooled, add exactly 10 ml of benzene isobutyl alcohol (1:1) mixture to each, followed by 2 ml of 1% ammonium molybdate solution. The volume is made up to the 25 ml mark with distilled water. Phosphomolybdate complex is extracted by vigorous shaking for $\frac{1}{2}$ min. If it is necessary, transfer the entire organic layer to a centrifuge tube with a glass stopper and centrifuge. Then transfer exactly 5 ml of the organic solution into a 25 ml volumetric flask and dilute with about 10 ml of 2% H_2SO_4 (vol./vol.) in methanol (aldehyde-free). Then add 1 ml of reducing agent (0.5 ml of 25% $SnCl_2$ in concentrated HCl diluted to 100 ml with 1 N H_2SO_4). Mix and make up to the mark with H_2SO_4–methanol. The blue solution is mixed well by shaking. After 10 min read the absorbance against distilled water at about 650 mμ (red filter).

Perform the same operation with a slice of paper from far off the spots to constitute the blank.

(3) *Radiometric analysis*

Take exactly 1 ml of the organic solution and put into a planchet. Dry with an infrared lamp. Count with a β-counter. Make the calculations for the corresponding dilution, and refer to the activity of P^{32} in the original spot.

We found, for known standards, the values of r.f. (at 20°C) given in Table 1.

For the chromatographic analysis, Whatman Chromatographic paper No. 3MM was used.

The amount of phosphorus in the original spot was 40 gammas.

TABLE 1

	Acid solvent (pH 2.7)	Basic solvent (pH 8.8)
Orthophosphate	0.73	0.35
Pyrophosphate	0.34	0.24
Triphosphate	0.08	0.20
Tetraphosphate	0.08	0.17
Pentaphosphate	0.06	0.15
Hexaphosphate	0.04	0.13
Heptaphosphate	0.02	0.11
Trimetaphosphate	0.10	0.42
Tetrametaphosphate	0.05	0.31

The experimental results obtained by both the radiometric and colorimetric procedures on sodium polymetaphosphate-P^{32} solutions stored for 15 and 30 days and at pH values of 5.0, 6.5 and 8.0 are indicated in Fig. 1. Table 2 illustrates the changes with time in the distribution of P^{32} activity in a solution of sodium polymetaphosphate.

TABLE 2

Phosphate fraction	pH 5.0				pH 6.5				pH 8.5			
	% found at 15 days		% found at 30 days		% found at 15 days		% found at 30 days		% found at 15 days		% found at 30 days	
	Activity	Phosphorus	Activity	Phosphorus	Activity	Phosphorus	Activity	Phosphorus	Activity	Phosphorus	Activity	Phosphorus
High condensed $n > 7$	84.60	79.80	81.22	75.34	87.88	82.50	88.04	82.90	81.80	82.68	84.88	78.65
Hexa- and Hepta-	5.60	5.50	5.21	6.57	4.35	5.10	1.83	3.25	6.70	7.63	3.73	3.63
Penta-	1.60	1.70	1.56	1.50	1.23	6.85	1.19	2.55	2.90	2.54	1.20	1.81
Tetrameta-	1.60	1.60	2.95	4.33	2.35	4.35	2.00	3.15	2.65	1.27	4.48	3.02
Trimeta-	3.25	3.70	5.36	6.28	3.06	6.25	3.67	3.70	4.30	2.54	4.18	6.95
Ortho-	1.10	4.60	1.90	3.44	0.53	3.10	1.52	2.51	1.15	3.18	0.73	3.63
Pyro-	2.25	3.10	1.70	2.25	0.60	2.50	0.77	1.72	0.40	0.10	0.80	1.81

TABLE 3. Changes with time in distribution of P^{32} activity in polymetaphosphate solution stored at pH 6.5

Phosphate fraction	% found at 0 days		% found at 15 days		% found at 30 days	
	Activity	Phosphorus	Activity	Phosphorus	Activity	Phosphorus
High condensed *n* 7	89.01	83.60	87.88	82.50	88.04	82.90
Hexa- and Hepta-	5.06	6.01	4.35	5.10	1.83	3.25
Penta-	1.36	5.05	1.23	6.85	1.19	2.55
Tetrameta-	2.30	5.15	2.33	4.35	2.00	3.15
Trimeta-	1.85	4.86	3.06	6.25	3.67	3.70
Ortho-	0.32	2.15	0.53	3.10	1.52	2.51
Pyro-	0.10	1.26	0.60	2.50	0.77	1.72

DISCUSSION

The stability of the sodium polymetaphosphate incorporating P^{32} was very good even over a wide range of pH (5.0–8.5). The rate of hydrolysis was studied only at 15 and 30 days because those times are approximately once and twice the half-life ($t_{\frac{1}{2}} = 14.3$ days): these are the practical limits for the time of use for this kind of radiopharmaceutical.

Using the radiometric results (activity) for the basic information and colorimetric values for phosphorus found (phosphorus) for auxiliary purposes because of the simplicity and reproducibility of the former, we drew the following conclusions.

(a) The stability of the radioactive sodium polymetaphosphate is at a maximum at pH 6.5, and is slightly decreased at lower or higher pH values such as 5.0 or 8.5.

(b) At pH 5.0 the hydrolysis products in increasing concentrations are tetrameta-, trimeta- and ortho-phosphate. Hydrolysis occurs in the highly condensed fraction ($n > 7$) because of the effect of the acid pH.

(c) At pH 6.5 there is no change in the high condensed fraction and the increase in the ortho- and pyro-phosphate fractions may be due to the hydrolysis of the hexa-, hepta-, penta- and tetrameta-phosphate fractions in view of their decreasing activity.

(d) At pH 8.5 the lower fractions increase at the expense of the higher fractions.

(e) The increase in the activity of the highly condensed fraction at pH 8.5 may be due to decrease in the migration rate at the increased pH. The separation and differentiation of the spots is more difficult in a solution of high pH.

(1) It is possible to perform the radiometric analysis for P^{32} on an aliquot of the sulfuric acid solution prior to formation of the phosphomolybdate color complex.

(2) This method provides us with a rapid method for checking the composition of sodium polymetaphosphate-P^{32} without the tedious colorimetric determination, making only the radiometric assay of an aliquot of each dissolved spot. The precision of the method is within ± 5 per cent of the colorimetric determination.

REFERENCES

1. KAPLAN E. *et al. J. Nucl. Med.* **1,** 13 (1960).
2. AUDRIETH L. F. *Inorganic Syntheses* Vol. 3, p. 104 (1950).
3. KARL-KROUPA E. *Analyt. Chem.* **28,** 1091 (1956).
4. EBEL J. P. *Bull. Soc. chim. Fr.* **20,** 991, 998 (1953).
5. EBEL J. P. *Mikrochim. Acta* **1954,** 679.