

# LSC Standardization of $^{32}\text{P}$ in Inorganic and Organic Samples by the Efficiency Tracing Method

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## INTRODUCTION

The wide field of applications of  $^{32}\text{P}$  in Medicine, Agriculture, and Biochemistry requires the highest achievable accuracy in its standardization.

Since  $^{32}\text{P}$  is a pure beta emitter, liquid scintillation counting is the most appropriate method of measurement. Nevertheless, this method presents several difficulties, the wall adsorption in glass vials and the instability of phosphoric compounds in toluene-based scintillators, that very often make the detection efficiency unpredictable.

In order to overcome the adsorption problem, the commonly used procedures have recourse to a previous siliconing treatment of the vial walls<sup>1</sup> or to the addition of a variable amount of inactive carrier that in some cases does not produce the expected effect.<sup>2</sup>

On the other hand, the low solubility of phosphor-derived inorganic compounds in liquid scintillators can be overcome by synthesizing a  $^{32}\text{P}$  labeled organic compound. This allows sample preparation showing a high stability for toluene-based and other scintillation cocktails.<sup>3</sup>

In this chapter, a systematic study of the behavior of  $\text{H}_3^{32}\text{PO}_4$  aqueous samples was first made; either the vial walls were treated with silicon, or carrier was added in variable amounts. Secondly, in view of the results obtained with inorganic samples, a triphenyl-phosphine oxide compound labeled with  $^{32}\text{P}$  has been synthesized in an attempt to obtain better samples when incorporated into organic scintillators.

Finally, both the organic and inorganic stable samples have been used to standardize the  $^{32}\text{P}$  by the efficiency tracing method, with an experimental calibration curve of  $^3\text{H}$  efficiency obtained from a set of quenched standards.

## PREPARATION OF INORGANIC SAMPLES

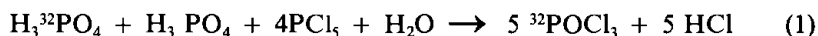
All the inorganic samples have been prepared from a carrier-free, aqueous solution of  $H_3\ ^{32}PO_4$ . These samples have been measured in INSTAGEL, since a very high water content can be incorporated without any problem. The volume of scintillator has been 10 mL to minimize the wall effect of high energy electrons.

Four different kinds of samples have been prepared and studied.

1. Type A samples were obtained by direct addition of  $H_3\ ^{32}PO_4$  into the liquid scintillator with no other process involved.
2. For the type B samples, the vial was previously immersed in silicone oil for 24 hr at room temperature before adding the  $H_3\ ^{32}PO_4$ .
3. Type C samples were prepared with six different amounts of carrier, by adding 6, 12, 24, 36, 48, and 60  $\mu g$  of inactive  $H_3PO_4$  to the scintillator volume before the active solution was incorporated.
4. Finally, for type D samples, the carrier adsorption was compelled by exposing the vial to an inactive solution of  $H_3PO_4$ , with 85% concentration, diluted with the same volume of water. After a 24 hr exposure at room temperature, the solution was removed and the vial was filled with the scintillator and the active solution.

## PREPARATION OF ORGANIC SAMPLES

After a structural analysis of several organic compounds that could be labeled with  $^{32}P$  and a previous work with tributyl-phosphate labeled samples,<sup>3</sup> the triphenyl-phosphine oxide was selected due to its low oxygen contents and ability to reduce wall adsorption.<sup>4</sup> The triphenyl phosphine oxide was obtained in two steps. In the first step, the  $^{32}POCl_3$  was synthesized from the  $H_3\ ^{32}PO_4$ , by the reaction<sup>5</sup>:



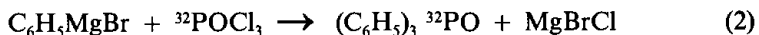
This process was carried out by mixing 10  $\mu Ci$  of  $H_3\ ^{32}PO_4$ , 0.08 mmol of  $H_3PO_4$  and 13 mmol of  $H_2O$  in a two-necked flask.

One neck was connected to a micro dropping funnel and the other one was equipped with a drying tube. A dry ice-acetone bath was used to cool the flask. In the funnel was placed 12.9 mmol of phosphorus pentachloride. The reaction started by adding small amounts of phosphorus pentachloride and allowing the mixture to thaw slowly, until the reaction became controlled without freezing. At that point, the remainder of the phosphorus pentachloride was rapidly added.

Once the reaction mixture was at room temperature, it was refluxed for 15 min and then immersed in a dry ice-acetone bath. Warm water circulated through the condenser to help distilling from the condenser into the flask. The

phosphoryl- $^{32}\text{P}$  chloride was obtained by cooling the flask at  $-5^\circ\text{C}$  and distilling under vacuum. The radiochemical yield was about 90%.

In the second step, the triphenyl phosphine oxide was produced through the reaction<sup>6</sup>



The 36 mmol of the Grignard agent were diluted to 100 mL of absolute ether, then 12 mmol of the phosphoryl  $^{32}\text{P}$  chloride were slowly added into the flask. The flask was cooled in a dry ice-acetone bath. Once the reaction mixture warmed to room temperature, it was heated at  $45^\circ\text{C}$ . The white precipitate was hydrolyzed, extracted with absolute ether, washed with water, and distilled under vacuum. The radiochemical yield attained 45%. The solid triphenyl phosphine oxide labeled with  $^{32}\text{P}$  was diluted in benzene and then dispensed to the measurement vials.

The  $^{32}\text{P}$  labeled samples obtained in this way have been measured with three different scintillators: a toluene-based solution composed of 1 L of toluene, 5 g of PPO, and 0.3 g of dimethyl POPOP and the commercial cocktails HISAFE II and INSTAGEL. Vials containing 10 mL of INSTAGEL and 15 mL of HISAFE II and toluenic scintillator were used.

## EQUIPMENT AND CHEMICALS

The chemicals used for the preparation of organic and inorganic samples were  $\text{PCl}_5$ ,  $\text{H}_3\text{PO}_4$  (85%),  $\text{Na}_2\text{SO}_4$ ,  $\text{C}_6\text{H}_5\text{MgBr}$  of analytical grade, an aqueous solution of  $\text{H}_3^{32}\text{PO}_4$  containing about 40 MBq/mL and an n-hexadecane- $^3\text{H}$  standard solution around 50 kBq/mL.  $\text{Cl}_4\text{C}$  was used as a quenching agent.

Glass vials with an internal diameter of 2.5 cm and very low Potassium contents were used in all the measurements and the scintillator volumes were dispersed by means of Brand instruments, having a calibration uncertainty lower than 1%.

Gilson micropipettes with an uncertainty calibration lower than 1% were used to incorporate radioactive samples into the scintillator volume.

The measurement system was liquid scintillation counting equipment from LKB, model Rackbeta 1219 Spectral. It has a 10  $\mu\text{Ci}$  source of  $^{226}\text{Ra}$  as external standard source for the quench determination.

## RESULTS OF SAMPLE PREPARATION

The liquid scintillation spectrometer checked the stability of the samples. A 24 hr period of thermal conditioning inside the counter has been always respected before starting the final measurements.

### Stability of inorganic samples

The time stability of type A samples was studied for 17 days. Figure 1 shows the time evolution of the counting rate in the  $^{32}\text{P}$  window from channel 531 to channel 830. This helped avoid the possible interference of the  $^{33}\text{P}$  contaminant commonly accompanying the  $^{32}\text{P}$ . Radioactive decay correction has been applied to take into account the  $^{32}\text{P}$  half-life of 14.29 days. A strong counting loss was observed in these samples. Initially, it amounted to more than 5% a day and seemed to reach the maximum degradation in 15 days; it showed a total count loss of more than 35%.

The instability is also evident in Figure 2, where the spectra were measured at 0, 6, and 17 days after the preparation. The spectra have been normalized in the figure to the highest count rate so that the radioactive decay of  $^{32}\text{P}$  is compensated and the degrading effect of wall adsorption becomes clear.

Type B samples were measured for 12 days. The count rate is shown in Figure 1, and the spectra taken at 0, 6, and 12 days appear on Figure 2. The effect of the wall treatment is not clear. The initial counting loss was even greater than for type A samples, but after five days, it seemed to moderate with strong, nonstatistical fluctuations. The spectral degradation also changed, corresponding to the wall treatment, but the sample still is clearly unstable.

The behavior of type C samples was studied for 12 days. Figure 3 shows the count rate evolution of samples containing 6, 24, and 60  $\mu\text{g}$  of carrier. The stability is good enough for values greater than 24  $\mu\text{g}$  with no systematic trend, and it is good enough for statistical fluctuations with 0.2% standard deviation in agreement with the experimental measurements. The 6  $\mu\text{g}$  sample shows a strong counting loss of 0.8% a day. The spectral evolution appears in Figure 4, where the degradation is only evident for the 6  $\mu\text{g}$  carrier sample.

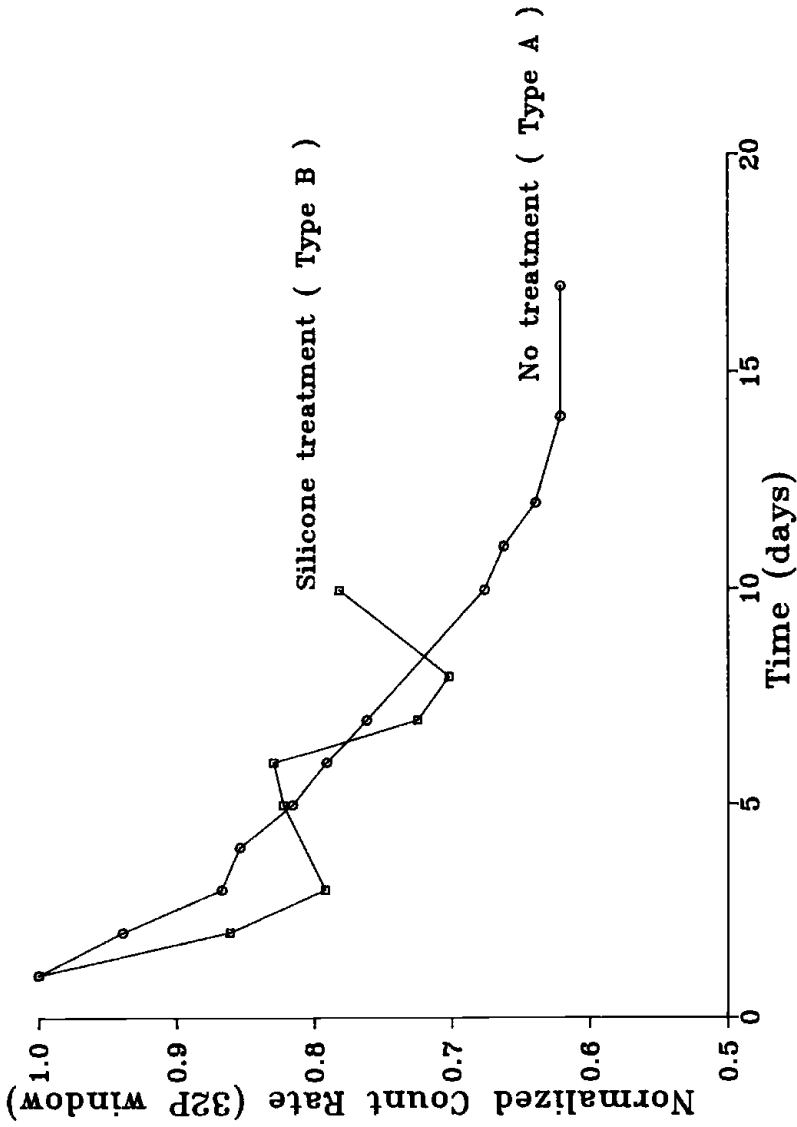
The type D sample also reveals a good stability for 20 days, better than 0.06% a day, even though it has a greater  $^{33}\text{P}$  contribution, due to its late preparation date, as can be appreciated in Figures 5 to 6.

### Stability of Organic Samples

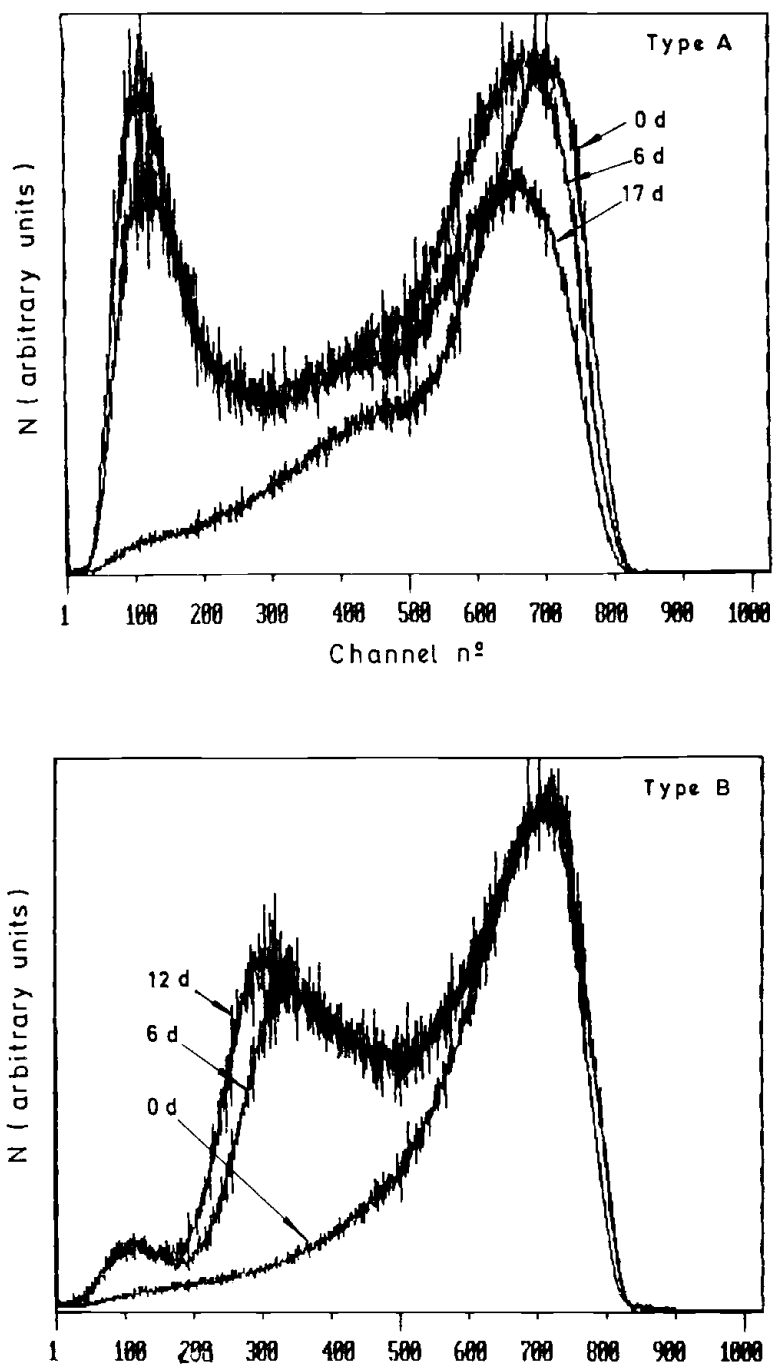
The behavior of the organic samples has been investigated in three different scintillators, in toluene and HISAFE II for 44 days and in INSTAGEL for 12 days. Counting rates refer to the same  $^{32}\text{P}$  window set for inorganic samples, and they have been corrected for  $^{32}\text{P}$  radioactive decay.

Figures 7 and 8 show the time evolution of the count rate and the spectra measured at 0, 20, and 44 days for the toluene and the HISAFE II scintillators. The stability in INSTAGEL along the 12 day term and the spectra at 0, 6, and 12 days are shown in the same figures.

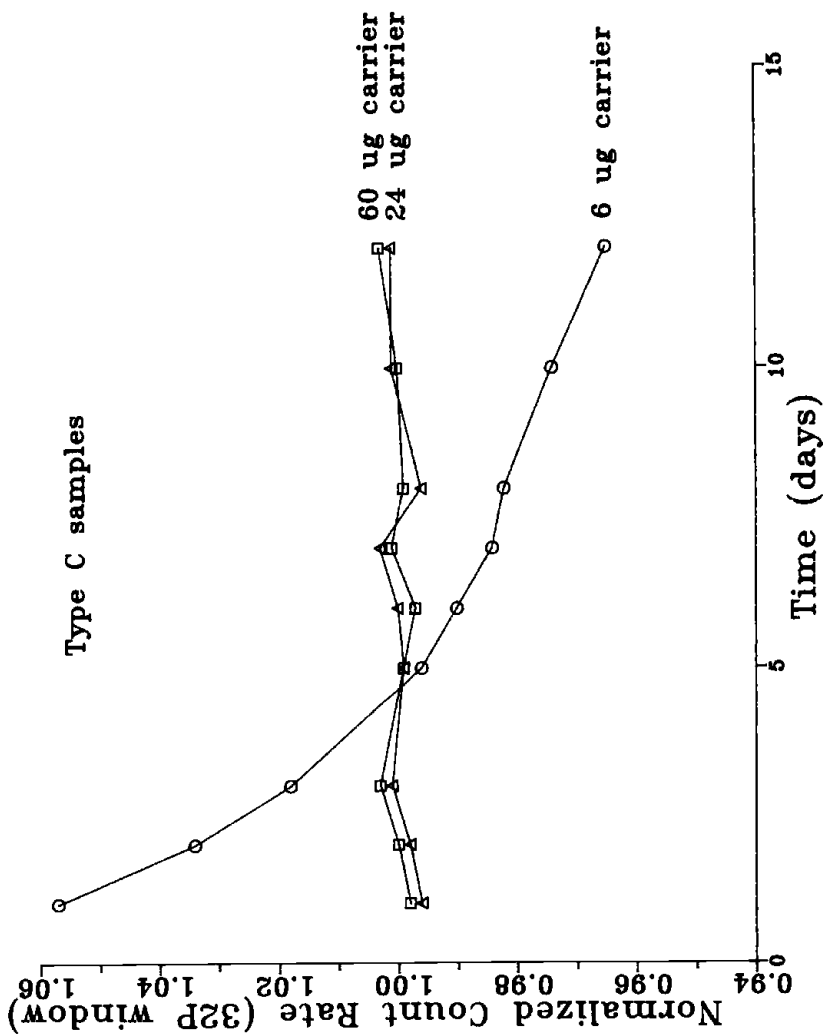
In consequence, the diphenyl phosphine oxide sample is stable enough in all three scintillators with no significant trend in the half-life corrected count rate values. The fluctuations observed are within the statistical limits, with stan-



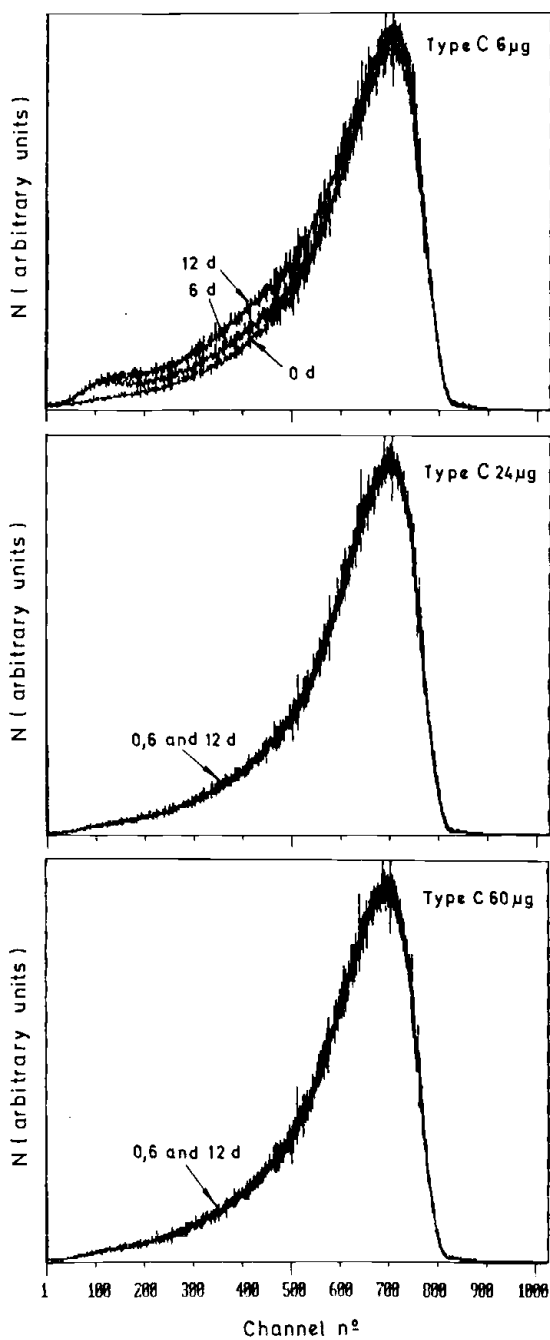
**Figure 1.** Time evolution of  $\text{H}_3^{32}\text{P}\text{O}_4$  samples in INSTAGEL. 0: Type A, no treated samples;  $\square$ : Type B, silicone treated walls. The count rates are half-life corrected and then normalized to the value in the first day.



**Figure 2.** Spectral evolution of  $H_3^{32}PO_4$  samples in INSTAGEL. A: Type A, no treated samples; B: Type B, silicone treated walls.



**Figure 3.** Time stability of  $\text{H}_3^{32}\text{P}\text{O}_4$ , type C, samples in INSTAGEL. ○: 6  $\mu\text{g}$  carrier; □: 24  $\mu\text{g}$  carrier; △: 60  $\mu\text{g}$  carrier. The count rates are half-life corrected and then normalized to the mean value in the measurement period.



**Figure 4.** Spectral evolution of  $H_3^{32}PO_4$ , Type C, samples in INSTAGEL, for 6, 24, and 60  $\mu g$  of carrier.

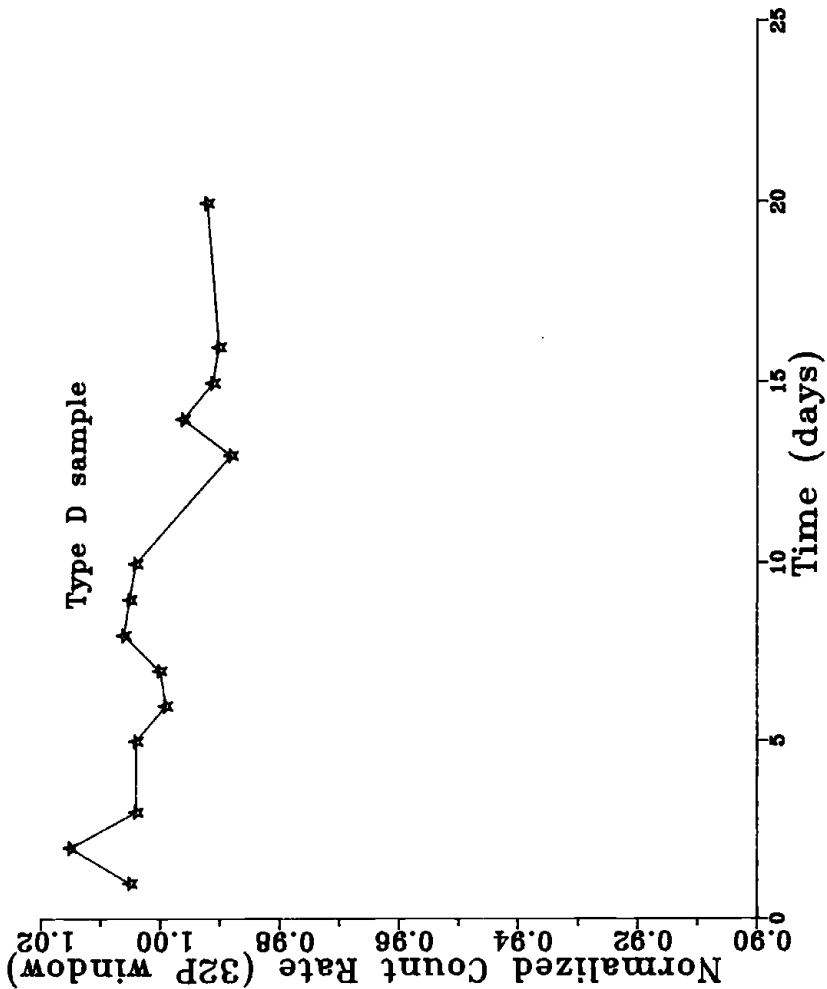


Figure 5. Time stability of  $\text{H}_3^{32}\text{PO}_4$ , type D, samples in INSTAGEL. The count rates are half-life corrected and then normalized to the mean value in the measurement period.

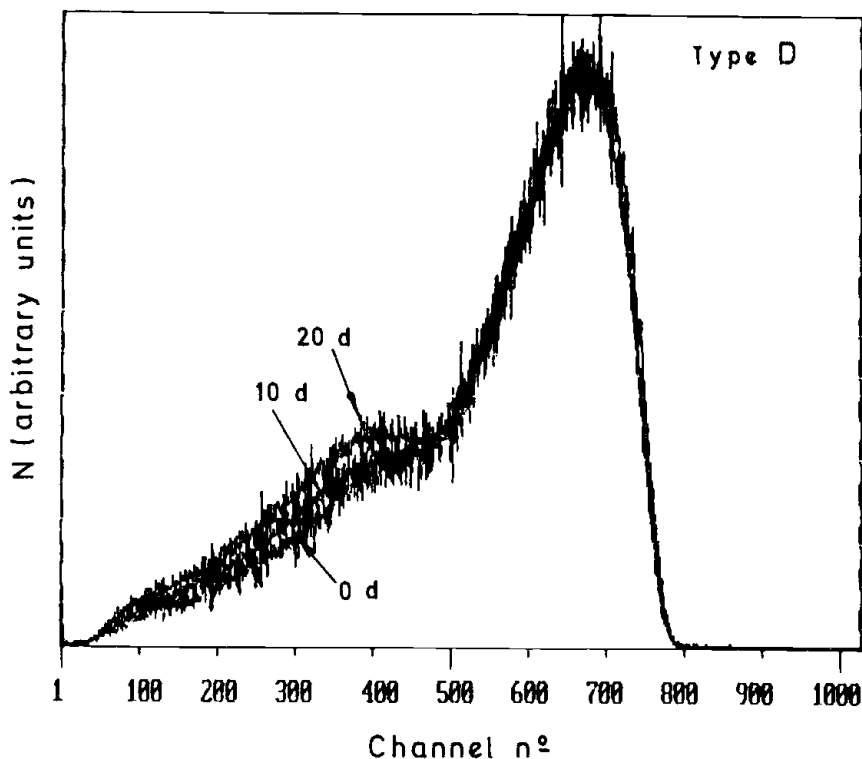


Figure 6. Spectral stability of  $H_3^{32}PO_4$ , type D, samples in INSTAGEL.

dard deviations of 0.4% in toluene, 0.3% in HISAFE II, and 0.15% in INSTAGEL, according to the total number of counts stored. The spectral changes in the three scintillators are due to  $^{33}P$  interference that is clearly observed in the long term samples.

### STANDARDIZATION OF $^{32}P$ BY THE EFFICIENCY TRACING METHOD

The organic sample in toluene as well as the type D inorganic sample in INSTAGEL have both been used to standardize the two  $^{32}P$  solutions by the efficiency tracing method.<sup>7</sup> Both samples were calibrated within 48 hr after preparation, so that the inorganic sample 0.06% unstability did not affect the final results.

The activity concentration of each radioactive solution can be obtained from the expression

$$A = \frac{N}{\epsilon V} \quad (3)$$

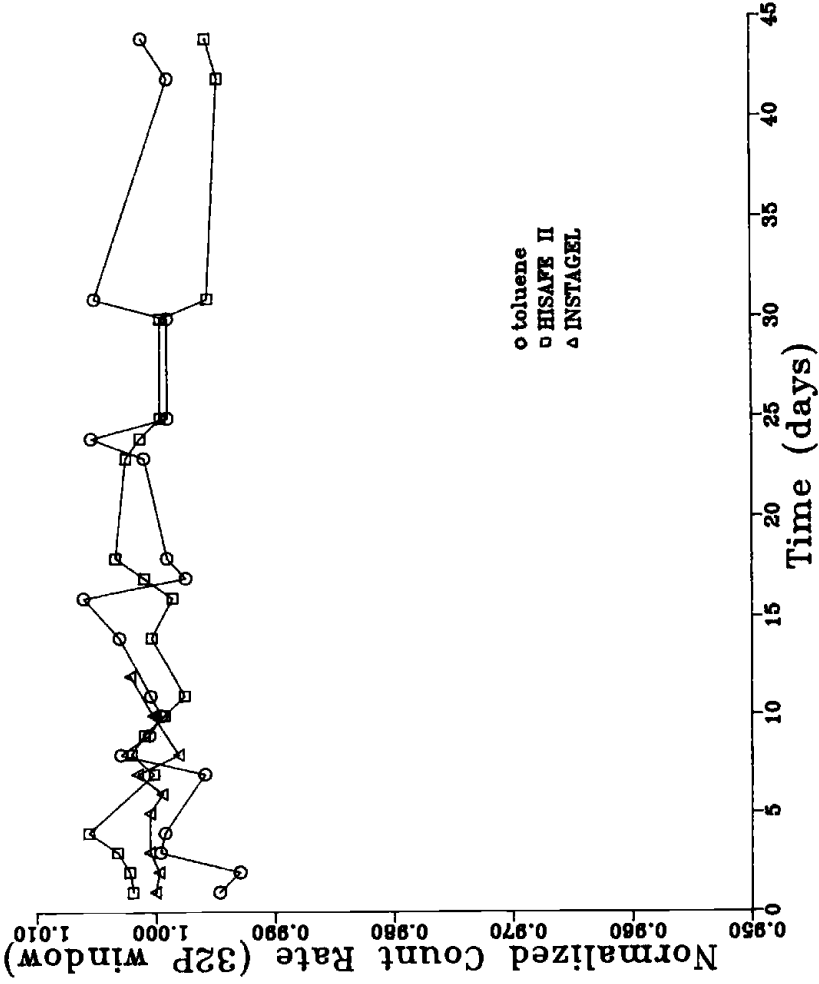
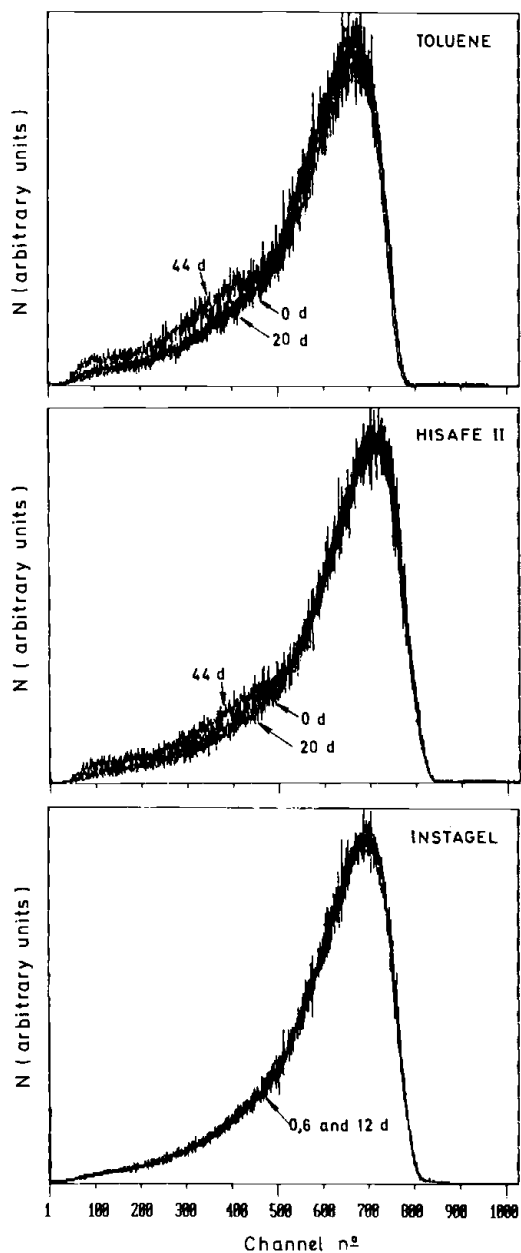


Figure 7. Time stability of  $(\text{C}_6\text{H}_5)_3\text{ }^{32}\text{P}_0$  samples in three scintillators. ○: toluene; □: HISAFE II; △: INSTAGEL. The count rates are half-life corrected and then normalized to the mean value in the measurements period.



**Figure 8.** Spectral stability of  $(C_6H_5)_3^{32}P_0$  samples in toluene, HISAFE II, and INSTAGEL.

where  $V$  is the volume of the active solution,  $N$  is the total count rate of the  $^{32}P$  spectrum, and  $\epsilon$  is the detection efficiency.

The radioactive solution has been dispensed with micropipettes GILSON, in

amounts of 100 or 200  $\mu\text{L}$ , depending on the freshness of the radioactive solution.

The number of total counts in each spectrum has been kept greater than 900,000, so that the statistical uncertainty is better than 0.1%.

The total counting efficiency  $\epsilon$  has been determined by the efficiency tracing method<sup>7</sup> which needs an experimental curve of a set of tritium standards and the experimental measurement of  $^{32}\text{P}$ .

The samples are affected by the  $^{33}\text{P}$  contamination to a different extent depending on the time elapsed before measurement. The partial count rates  $N_{32}$  and  $N_{33}$  of  $^{32}\text{P}$  and  $^{33}\text{P}$  have been determined by a least-squares fitting procedure to the decay function

$$N(T) = N_{32}e^{-0.693t/T_{32}} + N_{33}e^{-0.693t/T_{33}} \quad (4)$$

where  $T_{32} = 14.29$  days and  $T_{33} = 25.4$  days are the respective half-life values. Figure 9 shows the fit for the organic sample in toluene.

The counting efficiency was computed with the program EFFY<sup>8</sup> using the well known  $\beta$ -decay scheme of Figure 10 for  $^{32}\text{P}$ .<sup>9</sup>

Both  $^{32}\text{P}$  samples have been measured at different degrees of quench using increasing amounts of  $\text{Cl}_4\text{C}$  to check the consistency of the procedure. The measurements were made for figures of merit in the range 1.5 to 3.0 in INSTAGEL and 1.5 to 4.5 in toluene, representing a  $^3\text{H}$  equivalent efficiency between 45% and 15%.

Figure 11 shows the experimental and computed curves of efficiency vs figure of merit for the  $\text{H}_3^{32}\text{PO}_4$  sample in INSTAGEL and the  $(\text{C}_6\text{H}_5)_3^{32}\text{PO}$  sample in toluene. The maximum discrepancy is 0.8% for the inorganic sample and 0.5% for the organic sample.

An evaluation of the uncertainty factors of the  $^{32}\text{P}$  computed efficiency is shown in Table 1, according to the recommendations of the Comité International des Poids et Mesures.<sup>10</sup> The total composed uncertainty is 0.525%, which agrees very well with the experimental determinations. The activity concentration of each sample was computed by the Equation (3) and its total uncertainty for both samples is shown in Table 2.

## CONCLUSIONS

Several kinds of samples to calibrate  $^{32}\text{P}$  by liquid scintillation counting have been analyzed.

Inorganic samples of phosphoric acid,  $\text{H}_3^{32}\text{PO}_4$ , having more than 24  $\mu\text{g}$  carrier in 10 mL of scintillator are stable for more than 12 days with no counting loss observed. Samples whose vial walls have been previously saturated with carrier are also stable enough for 20 days, showing count losses lower than 0.06% a day.

Organic samples of triphenyl-phosphine-oxide,  $(\text{C}_6\text{H}_5)_3^{32}\text{PO}$ , synthesized in

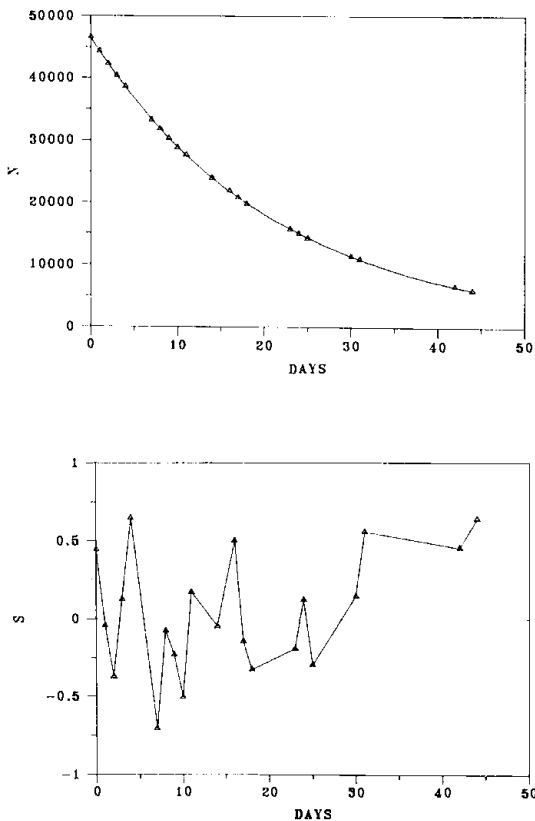


Figure 9. Total count rate fit and residuals for  $(C_6H_5)_3^{32}PO$  sample in toluene. N,S are stated in cpm and standard deviations, respectively.

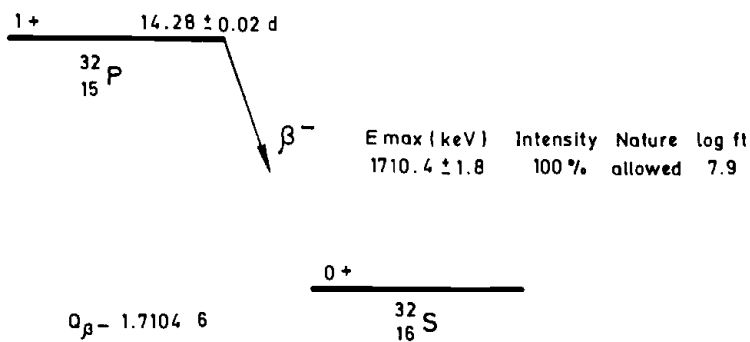


Figure 10. Decay scheme data of  $^{32}P$ .

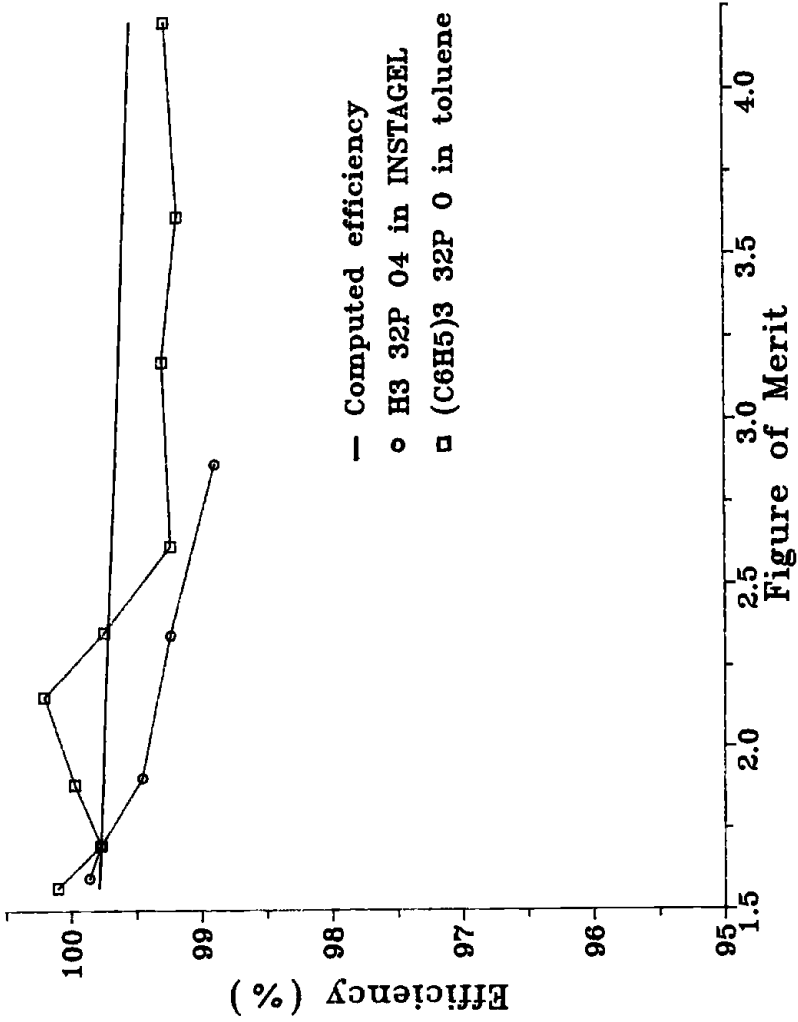


Figure 11. Counting efficiencies for  $\text{H}_3 \text{ }^{32}\text{PO}_4$  samples in INSTAGEL and  $(\text{C}_6\text{H}_5)_3 \text{ }^{32}\text{PO}$  samples in toluene. O: inorganic sample;  $\Delta$ : organic sample; - : computed by the efficiency tracing method.

**Table 1. Contribution of the Diverse Uncertainty Factors (1 Standard Deviation) of the  $^{32}\text{P}$  Efficiency Computed by the Efficiency Tracing Method**

Factor	Original Uncertainty	Resulting Uncertainty on $\epsilon$ ( $^{32}\text{P}$ )
$^3\text{H}$ standard calibration	3%	0.010%
Quench determination for $^3\text{H}$ standard	1%	0.003%
Counting ( $^3\text{H}$ )	0.3%	0.001%
Non detection probability ( $^3\text{H}$ , figure of merit 1-15)	2-7%	<0.100%
Volumetry	1%	0.325%
Quench determination for $^{32}\text{P}$ sample	1%	<0.0001%
Nuclear constants	—	0.300%
Phototube asymmetry	—	<0.250%
Ionization quench	—	0.100%
Composed uncertainty		0.525%

**Table 2. Estimated Uncertainty Factors (1 Standard Deviation) Associated to the Activity Concentration**

Factor	Uncertainty
$^{32}\text{P}$ computed efficiency	0.525%
Volumetry	1.0%
Counting	0.1%
Composed Uncertainty	1.38%

the laboratory show a very good long term stability, with no systematic trend, for 44 days in three different scintillators: toluene, HISAFE II, and INSTAGEL.

Both kind of samples have been calibrated by the efficiency tracing method. The discrepancies between experimental and computed values of the efficiencies are lower than 0.8% for inorganic samples and 0.5% for organic samples. Efficiency tracing allowed the  $^{32}\text{P}$  solutions to standardize at a total composed uncertainty of 1.4%.

## REFERENCES

1. Petroff C.P., P. Nair, and D. Turner. "The Use of Siliconized Glass Vials in Preventing Wall Adsorption of Some Inorganic Radioactive Compounds in Liquid Scintillation Counting," *Int. J. Appl. Radint. Isot.* 15:491 (1964).
2. Tykva, R. "Stability of Liquid Scintillation Counting for to Sample Sorption to the Counting Vial," *Int. J. Appl. Radint. Isot.* 26:495 (1975).
3. Rodríguez, L., A. Grau, J.M. Los Arcos, and C. Surález. "Preparación y Calibración por Centelleo Líquido de una Muestra de  $^{32}\text{P}$ ," CIEMAT Report 623 (1988).
4. Wigfield, D. and V. Srinivasan. "Liquid Scintillation Counting and Sample

- Adsorption. Structural Factors in Organic Molecules Controlling Likelihood of Adsorption," *Int. J. Appl. Radint. Isot.* 25:473 (1974).
5. Murray, A. and L. Willians. *Organic Syntheses with Isotopes*, Part II (New York: Interscience Publishers Inc., 1958) p. 1904.
  6. Grignard, V. and J. Savard. "Sur les dérivés Magnésiens de la Dichlorotriphenylphosphine et sur les Pentaphosphines," *Comptes-Rendus* 139:674 (1904).
  7. Grau, A. and E. García-Toraño. "Evaluation of Counting Efficiency in Liquid Scintillation Counting of Pure  $\beta$ -ray Emitters," *Int. J. Appl. Radint. Isot.* 33:249 (1982).
  8. E. García-Toraño and A. Grau. "EFFY, a Program to Calculate the Counting Efficiency of  $\beta$ -particles in Liquid Scintillation," *Comp. Phys. Comm.* 23:385 (1981).
  9. Lagoutine, F., N. Coursol, and J. Legrand. *Table de Radionucléides*, (Giff sur Ivette, France: Laboratoire de Métrologie des Rayonnements Ionisants, 1983).
  10. Kaarls, R. "Rapport du Groupe de Travail sur l-Expression des Incertitudes," Procès Verbaux du Comité International des Poids et Mesures, 49:A1-A12 (1981).

