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Determination of Zinc in Pharmaceutical Products by Neutron Activation Analysis

MICHEL MARGOSIS and JOSEPH P. F. LAMBERT*

Abstract □ Nondestructive neutron activation analysis was applied as a rapid, efficient, and specific method for the determination of zinc at either low or major concentration levels in various pharmaceutical products in bulk or dosage forms. This technique yielded results that compare well with more conventional methods and offers decided advantages over them.

Keyphrases □ Zinc in pharmaceuticals—determination □ Impurity interference—zinc determination □ Neutron activation analysis—zinc

Official methods for the analysis of zinc involve ashing followed by gravimetry (1) or complexometric (2, 3) or alkalimetric titration (1). Zinc in insulin is determined by a dithizone colorimetric procedure subsequent to extraction (2); in zinc bacitracin, it is determined by complexometric titration with edetic acid (3). However, there is no official procedure for determining zinc in various zinc bacitracin dosage forms such as ointments. The advent of atomic absorption spectroscopy has increased the efficiency and specificity of the determination of zinc, but in many laboratories this technique has been limited to simple dilute aqueous systems.

Table I—Nuclear Properties of Zinc for Activation with Thermal Neutrons

Stable Nuclide	Abundance, %	Cross Section, Barns	Radionuclide Formed	Half-life ($t_{1/2}$)	Energy of Principal γ-Ray, Mev.
^{64}Zn	48.89	0.46	^{65}Zn	245 days	1.115
^{66}Zn	27.81				
^{67}Zn	4.11				
^{68}Zn	18.56	1.0	^{69}Zn	52 min.	None
^{68}Zn	18.56	0.1	^{69m}Zn	13.8 hr.	0.439
^{70}Zn	0.62	0.10	^{71}Zn	2.3 min.	0.12
^{70}Zn	0.62	0.01	^{71m}Zn	4.1 hr.	

The application of neutron activation analysis (NAA) to zinc in pharmaceutical products offers increased utility, specificity, and accuracy (4). Advantages of NAA accrue from the facts that the technique: (*a*) is normally unaffected by the complex organic matrixes composing drug systems; (*b*) requires no physicochemical separation procedure; and (*c*) is both qualitative and quantitative in a presumptive assay method at either microconcentration or macroconcentration levels. This study shows the application of NAA for the deter-

Table II—Determination of Zinc in Various Pharmaceutical Compounds

Sample	Percent Zn Found: ^{69m}Zn[a]	^{65}Zn	AA[b]	EDTA[c]	Theoretical (or Limits), %	Reference Number
Zinc bacitracin (Batch 1)	3.10	5.87	5.46	5.81	<10	(3)
Zinc bacitracin (Batch 2)	4.44	5.00	5.12	5.54	<10	(3)
Zinc bacitracin (Batch 3)	3.38	6.39	5.89	6.13	<10	(3)
Zn oxide	80.3	83.7	76.3	—	80.34	(2)
Zn stearate	12.1	12.6	11.1	—	12.5–14.0	(2)
Zn undecylenate	14.5	15.2	15.0	—	14.83–15.44	(1)
Zn phenolsulfonate	14.8	12.0	11.7	—	11.70–12.29	(1)
Zn metal (wire)	100.2	—	—	—	100.0	

[a] Unreliable results attributed to high analyzer dead-time. [b] Atomic absorption method. [c] Edetic acid titration method.

Table III—Determination of Zinc in Various Pharmaceutical Dosage Forms

Sample	^{69m}Zn Found, mg./g.	^{65}Zn Found, mg./g.	Zn Expected[a], mg./g.
Zn bacitracin ointment 1[b]	—	0.453	0.4–0.5
Zn bacitracin ointment 2	—	0.427	0.4–0.5
Zn bacitracin ointment 3	—	0.408	0.4–0.5
Zn bacitracin ointment 4	—	0.514	0.4–0.5
Zn bacitracin ointment 5	—	0.461	0.4–0.5
Zn bacitracin ointment 6	—	0.439	0.4–0.5
Neomycin tablet (Zn stearate)	0.732/tablet	0.767/tablet	0.78/tablet
Zn undecylenate ointment 1[c]	30.7	31.0	30.1
Zn undecylenate ointment 2[c]	30.7	29.9	30.1

[a] The expected zinc in the zinc bacitracin ointment is estimated on the basis of 500 units/g. ointment, about 50 units of activity/mg., and about 5–6% zinc in the zinc bacitracin used in making the ointment. [b] The six bacitracin ointments are from different lots, types, and/or manufacturers. [c] Duplicate analysis from a different weighed sample from the same tube.

mination of zinc in such diverse products as antibiotics and insulin and in such salts as the stearate, undecylenate, and phenolsulfonate, which are used as a lubricant, antifungal agent, and astringent, respectively.

EXPERIMENTAL

Irradiation Containers—Snap-cap polyethylene vials of approximately 1- or 5-ml. capacity[1] were used for all samples.

Standards—A stock solution of zinc standard was prepared by dissolving 100.0 mg. of reagent grade metal in about 10 ml. of dilute nitric acid and diluting to 100 ml. with water. Aliquots of 1.00 or 2.00 ml. were transferred to the polyvials for comparison with samples containing milligram quantities of zinc. A secondary dilution of the stock standard solution containing 50.00 mcg./ml. was prepared for comparison with samples of lower zinc content.

Samples—Several milligrams of zinc salts and about 1 g. of ointment were accurately weighed into the polyvials. Tablets were individually placed in polyvials; 1.00-ml. aliquots of insulin samples were transferred to the 5-ml. polyvials and evaporated overnight at 60° on a block hot-plate with an airflow through the hood.

Apparatus—Irradiations were performed in the high flux exposure tube of a 1-Mw. nuclear research reactor (swimming pool type)[2], which provides a thermal neutron flux of approximately 10^{13} n./cm.^{2}-sec. Gamma-ray spectra were obtained with a 1024-channel pulse height analyzer[3] (PHA), using a 7.62 × 7.62-cm. (3 × 3-in.) NaI(Tl) scintillation detector[4] housed in a 6-ton steel shield, and a 7.62 × 7.62-cm. (3 × 3-in.) NaI well-type detector. The detector, which was covered with a thick plastic β-absorber, had a resolution of 8% at 662 kev. The PHA had a gain setting of 10 kev. per channel, and four independent counts could be stored prior to readout. The readout equipment consisted of a teletype printer and an X-Y recorder[5].

Procedure—Three 5-ml. or four 1-ml. polyvials were packaged together with a standard into an irradiation bucket and lowered into the exposure tube. Samples containing milligram amounts of zinc were irradiated for 3 min., whereas the other samples (antibiotic ointments and insulin) were irradiated for 20 min. After a group irradiation, the samples were allowed to cool several hours or overnight and were placed in larger polyethylene containers. ^{69m}Zn-activity was then measured by counting from 4 to 20 min. at a maximal distance of 5 cm. from the detector, depending upon the total activity of the samples, so as to obtain a PHA dead-time lower than 30%. After several more days of cooling, ^{65}Zn-activity of samples and standards was measured again by counting in the well-type detector. In all measurements, the Covell method (5) for photopeak analysis was used to quantitate the zinc activity.

[1] Olympic Plastics, Los Angeles, Calif.
[2] At the Naval Research Laboratory, Washington, D. C.
[3] Northern Scientific model 610.
[4] Harshaw 12SW12-W 3/H.
[5] Moseley model 7590.

RESULTS AND DISCUSSION

Elemental zinc is a mixture of five stable nuclides which react with thermal neutrons to produce radionuclides (Table I), two of which are useful in this application (6). The ^{69m}Zn-nuclide, with a 14-hr. half-life and a major photopeak at 0.44 Mev., is produced in substantial quantity, as shown in Fig. 1, allowing a shorter analysis

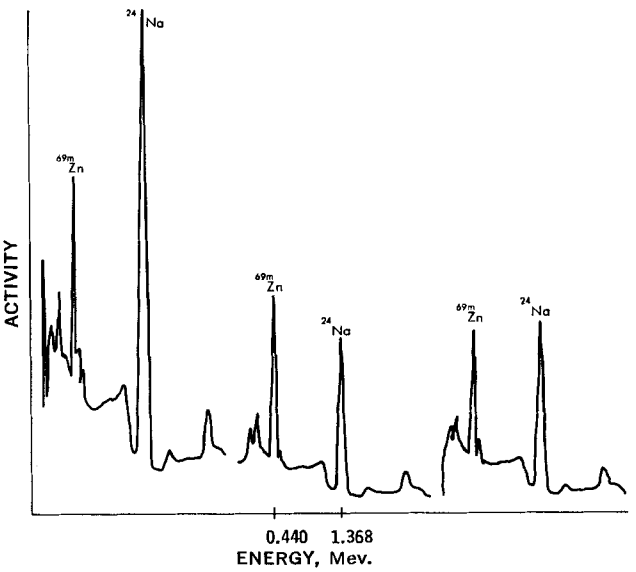

Figure 1—*Spectra of ^{69m}Zn from three different zinc bacitracin ointments.*

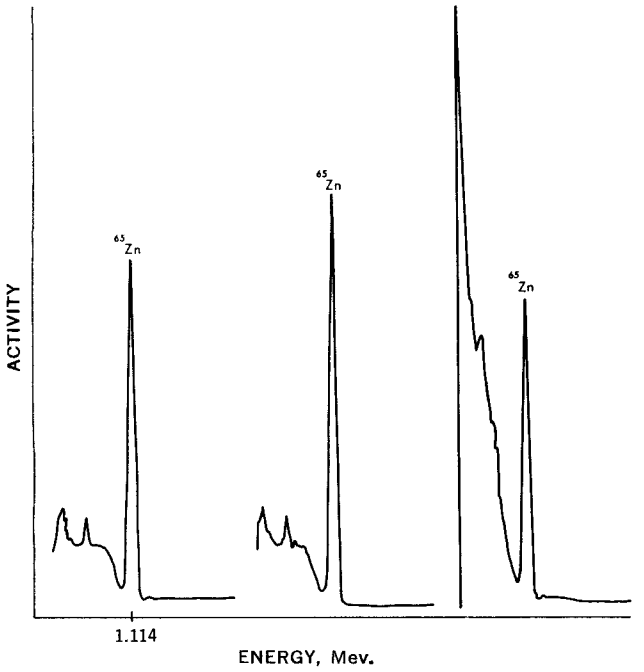

Figure 2—*Spectra of 245-day half-life ^{65}Zn from zinc bacitracin bulk, standard zinc sulfate solution, and a zinc bacitracin ointment.*

time while retaining the nondestructive feature of the technique. However, within the time of irradiation, impurities are also activated, with the combined effect of increasing both analyzer dead-time and background interference. Some of this interference is reduced by allowing a cooling/wait period of several hours before counting. This permits shorter-lived foreign nuclides to dissipate and correspondingly reduces the analyzer dead-time to a more practical level. Yet, it appears that in samples of lower concentration of zinc (*i.e.*, less than 0.5 mg./g. in ointments), the background is still large enough to lower the results generally by 10–25%, rendering them less reliable as evidenced by a lack of reproducibility and also by comparison to the ^{65}Zn-analysis. Although the Covell (5) method for spectral analysis is used, accuracy and precision still depend upon the purity of the photopeaks and the number of channels selected for integration, particularly with a sodium iodide detector.

To achieve maximal accuracy and precision, the ^{69m}Zn-nuclide is allowed to decay for several days to an insignificant activity level so that the 245-day half-life ^{65}Zn-nuclide can be measured at 1.12 Mev. (Fig. 2). By that time, the PHA dead-time is low enough for use of the well-type detector, which possesses superior geometry, and the background interference has been so reduced as to optimize conditions for measurement by the Covell method.

Results of analysis of several zinc-containing drugs by NAA are listed in Table II and are compared to results obtained by atomic absorption spectroscopy and by complexometric titration with edetic acid. The poor results obtained with ^{69m}Zn in the case of these compounds, particularly the zinc bacitracins, are mostly attributed to high-analyzer dead-time as well as some Compton contribution from ^{24}Na.

Various antibiotic ointments from different commercial sources, together with a neomycin tablet and one zinc undecylenate ointment, were analyzed for zinc (Table III). As previously mentioned, more reliable results are obtained by exploiting the longer-lived ^{65}Zn-radionuclide. Table IV lists results obtained from the analysis of various insulin samples and compares them to those obtained by

Table IV—Determination of Zinc in Insulin by NAA (Found, mcg./ml.)

Sample	Type[a]	NAA (^{65}Zn)	Atomic Absorption	Dithizone Colorimetric Method Lab I	Lab II
1	IZS-40	86.3	91	87	86
2	GZI-40	130	123	123	134
3	GZI-40	132	130	137	133
4	PZI-80	184	—	179	174
5	IZS-80	175	191	186	190
6	ISO-40	10.9	—	10	9
7	ISO-80	17.9	20	18	17
8	IZS-40	88.7	93	84	88

[a] IZS = insulin zinc suspension, GZI = globin zinc insulin, PZI = protamine zinc insulin suspension, and ISO = isophane insulin suspension.

atomic absorption spectroscopy and the dithizone colorimetric method. In this analysis, it was found necessary to evaporate the initial aliquot to dryness prior to irradiation, because the heat generated by the reactor caused loss of sample. There is good correlation among the results of all three methods.

Since the counting time is relatively short compared to the half-life of the nuclides, a decay factor correction is not necessary and has been omitted. Elemental zinc is then quantitated by direct comparison of activity to concentration; other variables are compensated for by irradiating samples and standards at the same time.

Additive interferences from accompanying nuclear reactions (7) with epithermal and fast neutrons producing ^{65}Zn or ^{69m}Zn from copper, nickel, gallium, or germanium are negligible, chiefly because the quantity of these elements, if present at all in these products, is very limited; the probability of such reactions is extremely small because of the relative abundance and cross section of these reactions.

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