Journal of Pharmaceutical & Biomedical Analysis
Vol. 4, No. 1, pp. 23–29, 1986
Printed in Great Britain

0731–7085/86 $3.00 + 0.00

Direct determination of zinc in insulin preparations by differential pulse polarography

L. L. LEWIN, P. SANZ PEDRERO* and M. R. VILARIÑO

Departamento de Físico-Química y Técnicas Instrumentales, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain.

Abstract: A rapid and direct differential pulse polarographic method for the determination of zinc in insulin preparations is described. Optimum conditions were studied using 0.2 M acetate buffer (pH 4.1) as the supporting electrolyte. Agreement between the results by direct analysis and those by the procedure involving pressurized digestion was satisfactory.

Keywords: *Differential pulse polarography; zinc; insulin preparations; digestion of protein.*

Introduction

A quantitative method is needed for the determination of zinc in pharmacopoeial insulin preparations because the element is an essential component of zinc–insulin crystals. Zinc is usually determined by the dithizone method or by atomic absorption spectrophotometry [1, 2]. Polarography and related techniques have rarely been used in the analysis of insulin preparations.

Differential pulse polarography (DPP) has been shown to be very useful for the determination of traces of zinc [3, 4]. High sensitivity and selectivity combined with inexpensive instrumentation make DPP suited for this task. Normally organic material must be destroyed before the electrochemical determination of trace metals [5–7]. Among various digestion procedures, digestion under pressure has been preferred for trace metal analysis because of its low blank values [5, 7]. However, for very simple matrices direct analysis can be carried out [8].

The aim of the present work was to develop a simple and direct procedure for the determination of zinc in insulin preparations by DPP.

Experimental

Insulin preparations

The preparations (Table 1) were purchased through normal commercial sources.

*To whom correspondence should be addressed.

Table 1
Sources of commercial preparations of insulin

Commercial description	Characteristics	Source
Monotard	Pork insulin, crystalline/ amorphous (7:3) (40 units ml^{-1})	Novo
Semilente MC	Pork insulin, amorphous (40 units ml^{-1})	Novo
Lente MC	Beef and pork insulin crystalline/amorphous (7:3), (40 units ml^{-1})	Novo
Ultralente MC	Beef insulin, crystalline (40 units ml^{-1})	Novo
Insulatard	NPH pork insulin (40 units ml^{-1})	Nordisk
Rapitard MC	Beef and pork insulin crystalline/soluble (3:1), (40 units ml^{-1})	Novo
Actrapid MC	Pork insulin injection (40 units ml^{-1})	Novo
Velosulin	Pork insulin injection (40 units ml^{-1})	Nordisk
Mixtard	Velosulin/Insulatard (3:7), (40 units ml^{-1})	Nordisk
Meztardia	Pork insulin/NPH pork insulin (5:5), (40 units ml^{-1})	Nordisk
Humulina regular	Human insulin injection (40 units ml^{-1})	Lilly

Reagents

Standard Zn(II) solutions were prepared from zinc chloride (Titrisol Merck). Sodium acetate, acetic acid and other reagents were of Suprapur or Analytical grade (Merck, Darmstadt, FRG).

Ultrapure water was obtained from a Millipore–Milli-Q water purification system (Millipore, Molsheim, France), after previous distillation. Mercury was triple-distilled and nitrogen was of high quality >99.998% N_2 (Sociedad Española de Oxígeno, Madrid, Spain).

All glass equipment was cleaned with hydrochloric and nitric acid solutions before use.

Apparatus

Differential pulse polarography was carried out with a Princeton Applied Research 384 polarographic analyzer connected to a REOO82 digital plotter and a model 303 static mercury drop electrode (S.M.D.E.), which includes the Pt counter electrode and the Ag/AgCl reference electrode, all from EG.&G. PARC (Princeton, NJ, USA).

pH was measured with a Radiometer PHM82 Standard pH meter (Radiometer Copenhagen, Denmark).

Micropipettes of 100, 200 and 250 μl (Scientific Manufacturing Industries, Emeryville, CA, USA), were used for adding the standard metal solution to the polarographic cell.

A clear supernatant liquid was obtained from the insulin preparations by centrifuging at 2000 r.p.m. for 5 min with an ECCO centrifuge (Pacisa, Madrid, Spain).

For the pressurized digestion of insulins a Berghof Labortechnik digestor system (Berghof GmBH, Tubingen, FRG) was used.

Procedures

Differential pulse analysis. The polarographic solution was deoxygenated by passing nitrogen through the solution for 5 min.The polarogram was then recorded from −0.70 to −1.30 V using a Ag/AgCl reference electrode. The following instrument settings were used: drop time 2.0 s, drop area 1.77×10^{-2} cm^2, scan rate 2 mVs^{-1}, and pulse amplitude −50 mV. The working temperature was 20–23°C.

The standard addition method was adopted; Zn(II) concentrations were calculated after the third standard addition by linear regression analysis. Blank values were determined by recording the polarogram of 0.2 M acetate buffer.

Direct determination of zinc. Samples (100–200 μl) of the insulin preparation were well shaken and added to 10 ml of the supporting electrolyte (0.2 M acetate bufer, pH 4.1); the zinc content was determined directly by DPP.

Determination of zinc in the supernatant liquid. Samples (2 ml) of the insulin preparation were centrifuged and the zinc content of 200 μl of the clear supernatant was determined as described above.

Digestion procedure. Zinc insulin samples were digested by a modification of the method of Oehme and Lund [5].

Aliquots of 1 ml of the insulin sample and 1 ml of a mixture of nitric, perchloric and sulphuric acids (2:1:1, v/v/v) were added to a quartz vessel inserted in the PTFE liner. The pressure vessel was closed and the temperature was raised slowly over 30 min to 230°C. After 2 h the heating was stopped and the vessels were cooled. After pressure digestion, the remaining acids were evaporated by placing the PTFE vessels in the heating block. After the sample had been evaporated to dryness at 230°C in a stream of nitrogen, the residue was dissolved in 25 ml of a solution of 0.2 M acetate buffer adjusted to pH 4.1 with 1 M sodium hydroxide. Aliquots (10 ml) of the solution were added to the polarographic cell and the zinc content was determined by the DPP method. The blank values were measured by evaporating and neutralizing the acid mixture and subjecting the solution to DPP described above.

Results and Discussion

The conditions for electrochemical determination were studied in order to improve the sensitivity and reproducibility.

The choice of pH for the supporting electrolyte was made by taking into account the characteristics of insulin [9, 10] and also the necessity to ensure that the hydrogen-ion concentration should not interfere with the polarographic peak of Zn(II). For the range of pH 3.7–5.6 the optimum value was found to be pH 4.1.

The dependence of the peak-current $(\Delta i)_{max}$ on the temperature, electrode surface, pulse amplitude and concentration was studied. The results obtained were in good agreement with the general equation [11] for the maximum pulse current in a diffusion controlled current. For the range 20–45°C, the temperature coefficient was 0.86%/degree and the peak potentials were not influenced by temperature. The range of pulse amplitude was −5 to −150 mV and the optimum value, in respect of sensitivity and peak performance, was found to be −50 mV (Fig. 1).

The reversibility analysis was based upon the diagnostic criteria of Parry and

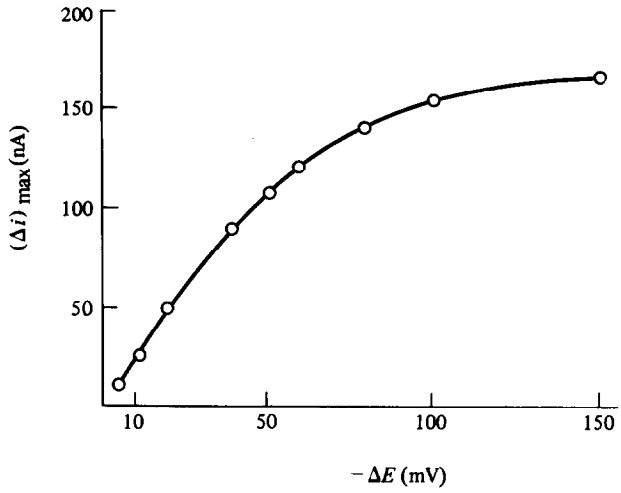

Figure 1
Peak current $(\Delta i)_{max}$ as a function of pulse amplitude (ΔE).

Osteryoung [11] and Birke *et al.* [12]. Both treatments indicated that the electrode process was reversible and involved two electrons. Table 2 shows the experimental results of the application of the criteria of Birke *et al.* on the Zn(II)/Zn(Hg) system of Zn(II) standard solution and Semilente insulin solution. Results were in good agreement and the Semilente insulin matrix did not seem to affect the Zn(II)/Zn(Hg) system.

The Zn(II) reduction peak was observed at -0.974 ± 0.006 V against Ag/AgCl. The lowest detectable concentration was 1.10 ng/ml based on a signal-to-noise ratio of 3. The relationship between peak current and zinc concentration was linear in the range of 0.01–10 μg/ml.

The regression data for working concentration ranges were:

From 0.1 to 1 μg/ml: $y = 0.2240x + 0.002$ ($n = 6$); SD of gradient 2.56×10^{-3}; SD of intercept 1.82×10^{-3}; $r = 0.9997$.

From 1 to 10 μg/ml: $y = 0.2179x - 0.002$ ($n = 6$); SD of gradient 7.44×10^{-4}; SD of intercept 4.6×10^{-3}; $r = 0.99998$.

Zinc determination in standard solutions

The applicability of this method was examined by the analysis of standard solutions for which the zinc concentrations were known. Results of zinc determinations, relative standard deviation (RSD) and recoveries are given in Table 3. RSD values were lower than the limit criterion of 10% [13] and recoveries were satisfactory.

Zinc determination in insulin preparations

In the direct analysis of insulins well-defined peaks of zinc were obtained and no interfering peaks were observed (Fig. 2). There were no differences when the analysis were carried out with a sample that had been subjected to the digestion procedure.

Results of the direct zinc determination in insulin preparations are given in Table 4. For some insulin preparations analysis of the zinc content in solution is required [1, 2]. The recovery was calculated from results obtained on samples spiked with known amounts of zinc. Although the precision and recovery of the procedure were satisfactory,

Table 2
Evaluation of electrode process reversibility using the diagnostic criteria of Birke *et al.**†

	System	$E_p^c - E_p^a$ mV	$\lvert I_p^a/I_p^c \rvert$	Diagnosis
Standard solution of Zn(II)	Zn(II)/Zn(Hg)	52	1.10	Reversible
Semilente insulin solution	Zn(II)/Zn(Hg)	52	1.09	Reversible
Diagnostic criteria values for conditions used		50	1.0	Reversible

*Conditions: pulse amplitude 50 mV; drop time 2 s; scan rate 2 mV s^{-1}; and drop area 1.77 $\times 10^{-2}$ cm^2.
†E_p^c, cathodic peak potential; E_p^a, anodic peak potential; I_p^a, anodic peak current; I_p^c, cathodic peak current.

Table 3
Direct analysis of zinc in standard solutions

Sample	Zinc content ($\mu g\ ml^{-1}$)	Zinc found* ($\mu g\ ml^{-1}$)	RSD† (%)	Recovery (%)
1	100.0	99.86	2.9	99.9
2	50.0	49.92	1.8	99.9
3	10.0	10.38	3.0	103.8
4	3.2	3.18	2.0	99.3

*Each value is the mean of five determinations.
†RSD, relative standard deviation.

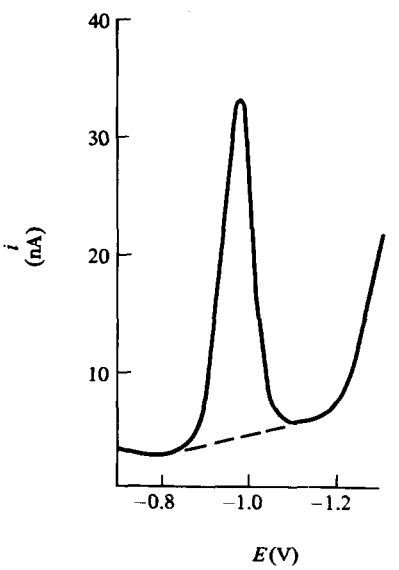

Figure 2
Differential pulse polarogram of zinc in Humulina regular insulin.

Table 4
Direct analysis of zinc in insulin preparations

Sample	Zinc found* (μg/ml)	RSD (%)	Recovery (%)	Zinc content expressed as a proportion of total zinc content (%)
Monotard‡	75.61	2.7	102.1	
Monotard supernatant‡	47.78	2.8	99.5	63.2
Semilente‡	82.19	3.2	101.0	
Semilente supernatant‡	51.06	3.0	99.5	62.1
Lente‡	79.24	2.1	99.6	
Lente supernatant‡	47.95	1.6	100.7	60.5
Ultralente‡	79.58	3.1	99.4	
Ultralente supernatant‡	45.65	2.5	100.6	57.4
Insulatard§	9.30	2.1	99.3	
Rapitard§	8.56	8.5	100.5	
Actrapid§	7.80	5.9	100.9	
Mixtard§	8.34	3.3	99.0	
Velosulin§	7.08	3.9	100.4	
Meztardia§	13.34	5.7	100.3	
Humulina regular§	7.23	7.2	100.7	

*Each value is the mean of five determinations.
†RSD, relative standard deviation.
‡The quantity added for recovery was 50 μg to 1 ml of sample.
§The quantity added for recovery was 10 μg to 1 ml of sample.

Table 5
Determination of zinc in insulin samples previously digested

	Semilente	Lente	Insulatard	Actrapid	Velosulin	Humulina regular
Zinc found (μg ml^{-1})	82.2	90.4	10.3	7.3	7.1	7.2
Quantity added (μg ml^{-1})	50	50	10	10	10	10
Spiked sample zinc found (μg ml^{-1})	127.9	145.2	20.7	16.9	17.4	17.7
Recovery (%)	91.4	109.5	103.5	96.0	103.3	105.0

the insulin preparations were digested to confirm that zinc determined by the direct procedure was the total zinc content. The digestion procedure was tested by analyzing some insulin samples which had been spiked with known amounts of zinc (Table 5). There was agreement between the results by direct analysis and those by the procedure involving digestion.

Conclusions

From the results of this work it appears that the direct zinc determination by DPP in various types of insulin preparation is efficient, inexpensive and rapid.

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[*First received for review 5 June 1984; revised manuscript received 9 August 1984*]