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4-(3,5-DICHLORO-2-PYRIDYLAZO)-1-3-DIAMINOBENZENE-GRAPHITE PASTE ELECTRODE FOR END-POINT DETECTION IN THE AUTOMATIC POTENTIOMETRIC TITRATION OF ZINC(II) WITH EDTA

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Summary—A simple and sensitive potentiometric sensor for end-point detection in the automatic titration of Zn(II) with EDTA was prepared and studied. The sensor was based on a conventional carbon paste which was mixed during preparation with 4-(3,5-dichloro-2-pyridil-azo)-1,3-diaminobenzene (3,5-C12PADAB). pH effects, buffer concentration, reagent content into carbon paste and the presence of foreign salts on the electrode response were studied. Titration curves with sharp end-point breaks were obtained in Zn(II) concentration range from 0.5 to 6550 ppm. The electrode was easily made and with very inexpensive materials. A titrimetric method for the determination of zinc in insulin by automatic potentiometric end-point detection is described. It was applied to the determination of the metal in two commercial pharmaceutical preparations. The results were in good agreement with those obtained by the U.S. Pharmacopeia standard method.

Interest in the development of not only determinative method but also of automatic analytical processes, is increasing more and more due to both considerable cost reduction in quality control of manufactured products and the possibility of continually monitoring a manufacturing process.[1,2]

As regards electrochemical determinations, potentiometric sensors are among the most attractive devices since it is possible to make them in different sizes with low cost materials and with robust designs.

In this paper a very simple potentiometric sensor for end-point detection in the titrations of zinc ions with EDTA is described. Such a sensor was prepared by mixing graphite-Nujol paste with 3,5-C12PADAB.

The carbon-paste electrodes were introduced for voltammetric measures by Adams[3] and their use for chemically modified electrodes is being investigated.[4] A carbon paste was adapted to manufacture ion-selectives electrodes by Ruzicka and Lam.[5] From then onwards, information about the use of this material in the development of potentiometric sensors is somewhat scarce.

The 3,5-C12PADAB compound has been used in our laboratory as a very sensitive reagent for the spectrophotometric determination of Pd(II),[6,7] and as metallochromic indicator for the complexometric titration of Cu(II) (and other compounds) with EDTA.[8] This reagent possesses four nitrogen donor atoms which coordinate with a few metallic ions, yielding positively-charged chelates, which are very stable, soluble in acid solutions but insoluble in neutral or alkaline media. Therefore, it was expected that a highly sensitive potentiometric sensor, made by doping a conventional carbon paste with 3,5-C12PADAB could be achieved. The new potentiometric sensor designed was of a very simple construction. It allowed easy incorporation or replacement of the active material and it could be made in different sizes and configurations. Owing to both the small quantity of reagent incorporated into the carbon paste and the materials used, its manufacture was accomplished at a very low cost.

EXPERIMENTAL

Apparatus

All potentiometric titrations were made with an Orion Automatic Titrator (960 Model Autochemistry System, Orion Research Inc., Cambridge, MA), by using 3,5-C12PADAB–graphite paste electrodes in conjunction with a double junction Ag/AgCl electrode (Orion 90-02).

Reagents

Standard zinc solution. (0.1*M*) 6.537 g of metal (99.99% pure) were dissolved in 50 ml of hydrochloric acid solution (1 + 1). Then, the solution was evaporated to about 5 ml by gentle heating. Finally it was cooled, transferred to a 1000-ml volumetric flask and diluted to the mark with distilled water.

*Standard EDTA solution (ca. 0.1*M*).* The disodium salt (37.22 g) was dissolved in copper-free distilled water and the solution was diluted to 1 l. with distilled water. It was standardized by potentiometric titration with standard Zn(II) solution.

3,5-C12PADAB reagent. The reagent was synthesized and purified as previously described.[6]

*Ammonia–ammonium chloride buffers (ca. 1.2*M*).* Several buffer solutions (pH 8–10) were prepared. Portions (6.75 g) of ammonium chloride, placed into separate 250-ml beakers, were dissolved in about 80 ml of distilled water. Concentrated ammonia was added to each portion until the desired pH was reached. The final pH adjustment was made after dilution to 100 ml.

All reagents were of analytical grade and all solutions were diluted further, as required.

Preparation of modified carbon paste electrode. First, a stock of pure carbon paste was made by hand-mixing 5 g of spectral-grade graphite powder and 5.5 g of Nujol oil in a mortar. Several portions of modified paste of 1 g each were prepared by mixing measured quantities of 3,5-C12PADAB and pure carbon paste. Pastes with 0.1–10% reagent were prepared and studied. The electrode was constructed in the following way. A tinned copper disk (about 7.5 mm of diameter) was soldered to one end of a copper wire (160 mm long, 1 mm of diameter). The wire, that provided the electric contact, was placed inside of a glass tube (150 mm long, 8 mm i.d.). The copper disk was fixed with a blob of epoxy resin at approximately 2 mm from one end of the glass tube, forming a cavity. Portions of the modified-graphite paste were packed into that cavity. Details about the electrode are shown in Fig. 1. The electrode was connected to the automatic titrator by means of a banana plug screwed to the free end of the copper wire. Smooth graphite surfaces were obtained by polishing it on a smooth glass surface. The excess of paste was scraped off with a glass spatula.

Electrode conditioning. The electrode prepared as described above did not need any special conditioning before use. Generally, the first titrations carried out with a recently prepared electrode gave erroneous results. So, whenever a new electrode was used, before titrating the samples, it was "activated" by titrating two or three portions of standard Zn(II) solution.

Storage of electrode. When the electrode was not in use, it was stored in a fresh and dry place, with the electroactive end cover with a Mylar film. In order to test the electrode durability, a pair of them were thus stored for more than six months and were checked weekly. These electrodes worked correctly, they were always ready to be used and no ageing effects were observed.

General procedure for Zn titrations. Transfer a measured volume of sample solution to a 100-ml beaker; neutralize, if necessary with litmus paper and diluted sodium hydroxide or hydrochloric acid solution and finally adjust to pH 10 by adding ammonia–ammonium chloride buffer as follows.

When the zinc ion concentration in the sample to be titrated lies between 0.5 and 100 ppm, add 1 ml of buffer every 50 ml of solution to ensure only a slight excess of ammonia. If larger amounts of Zn are present, add buffer dropwise until dissolving the zinc hydroxide formed with the first buffer drops. Then, add 1 ml of buffer each 50 ml of sample solution. Connect both the working and reference electrode, stir the solution with a magnetic stirrer and start the titration when a stable potential (±1 mV) is attained.

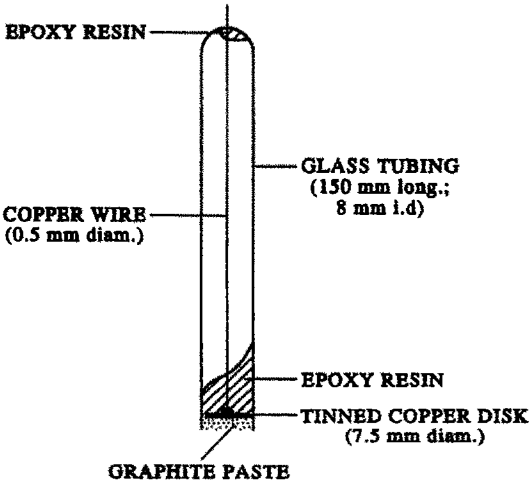

Fig. 1. Schematic representation of a longitudinal section of the graphite–paste electrode.

Recommended procedure for Zn determination in commercial insulin. Transfer a measured volume of sample into a 100-ml beaker and heat gently under a fume hood until the volume is reduced to approximately 1 ml. Then, add carefully 20 ml of a fresh mixture of concentrated HCl and HNO_3 (1 + 1) and heat in a hot plate until almost dry. Let it cool, add 5 ml of 30% H_2O_2 and 2 ml of concentrated HNO_3 and heat using a hot plate until dried. Cool, add 50 ml of distilled water and adjust the pH between 3 and 6 with diluted NaOH solution. Then, add 1 ml of the ammonia–ammonium chloride buffer (pH 10), immerse the electrodes in the solution and start the titration with 0.05*M* EDTA solution when a steady potential (±1 mV) is attained.

RESULTS AND DISCUSSION

3,5-C12PADAB electrode

Preliminary experiments showed that it was possible to use the electrode as potentiometric end-point indicator in Zn(II) titrations with EDTA. Considering that the electrode in some way responded to changes of Zn(II) concentration, the possibility of using it as an ion-selective electrode to carry out direct potentiometric measurements of the Zn(II) concentration was taken into account. Thus, experiments tending to evaluate the electrode response as a function of *p*Zn were carried out. The reagent and the Zn(II)–3,5-C12PADAB complex were soluble in acid medium (pH < 4.5). In this medium, however, 3,5-C12PADAB rapidly diffused from the paste towards the solution; so, no definite or stable potentials could be attained. That is why the experiments were carried out in alkaline solutions. Besides, since we were particularly interested in obtaining operational and not thermodynamic conclusions, they were performed at constant ionic strength. Under such conditions, $-\log a_{Zn(II)}$ is directly proportional to $-\log [Zn(II)]$. Ionic strength was adjusted to two levels (0.1 and 0.5) by adding an inert electrolyte (KNO_3).

In order to carry out the measures, several series of solutions were prepared at different pH (8, 9 and 10) by mixing zinc nitrate and ammonia–ammonium chloride buffer. Buffer concentration was varied in each series so as to obtain different Zn(II):ammonia ratios. After calculating the ionic strength due to the species added in each case, μ was finally adjusted to the desired value with KNO_3 solution. The free Zn(II) concentration was calculated with the stability constants for the zinc(II)–ammonia complexes. The total Zn range covered was 3–14.

The electrode showed neither Nernstian response nor reproducible potentials for Zn ions over the concentration range assayed. Although in the presence of Zn(II) a steady potential (to ±1 mV) was quickly reached, the potentials repeatedly measured for the same solution, or for different solutions with the same pH, zinc concentration, and buffer concentration varied from measurement to measurement by more than ±30 mV. Similar results were obtained when the electrode was immersed, between measurements, in 0.1*M* EDTA solution and subsequently washed with water to remove any zinc(II) adsorbed from the electroactive surface. Because of the negative results obtained the electrode was only used as a sensor for end-point detection.

Zn(II) titrations

In ammonia–ammonium chloride buffered solutions (pH 8–10) automatic direct EDTA titrations of Zn(II) could be carried out in the range from 0.5 to 6550 ppm. Titration curves showed satisfactory potential jumps, which were coincident with the theoretical equivalence points in the entire range of concentrations. At pH values below 8 the changes of potential at end-point were too small (of about 5–8 mV) with poor accuracy and precision. Figure 2

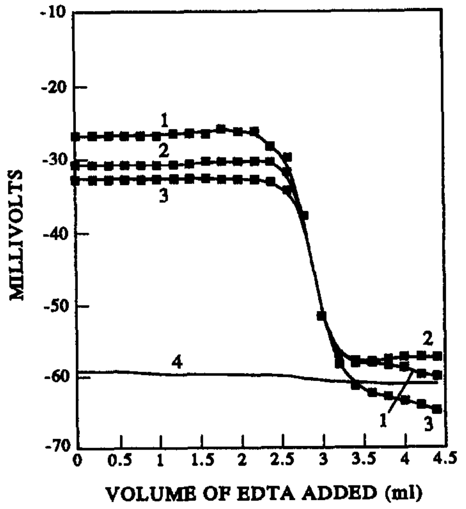

Fig. 2. Titrations of 20 ml of Zn(II) solutions at pH = 10. (1) Zn(II) $3.92 \times 10^{-2}M$ EDTA $2.7 \times 10^{-1}M$. (2) Zn(II) $1 \times 10^{-3}M$ EDTA $6.9 \times 10^{-3}M$. (3) Zn(II) $1 \times 10^{-5}M$, EDTA $6.9 \times 10^{-5}M$. (4) Response of pure carbon paste electrode in the titrations 1, 2 and 3.

illustrates some typical Zn(II) titrations with EDTA. As shown in curve 4, (Fig. 2), the response of the electrode is doubtless due to the presence of 3,5-C12PADAB in the paste.

Effect of 3,5-C12PADAB content

In the 0.7 to 7% (w/w) range, the 3,5-C12PADAB content into graphite paste had no significant influence on the electrochemical behavior of the electrode. A paste containing 2.8% 3,5-C12PADAB was the most commonly used.

Influence of foreign salts

The influence of the saline content on electrode behavior was studied by titrating Zn(II) solutions (0.5–6550 ppm) with variable content of different salts. No effect on the reproducibility of end-points was observed when titrations were performed in the presence of 0–2*M* KCl, NaCl, NaAcO, $NaClO_4$, Na_3PO_4 or sodium trichloroacetate.

Titrations in solutions containing some inert electrolyte were more satisfactory than those performed in their absence. In pure distilled water, the potential before end-point tended to be highly scattered and the potential changes at the end-point were smaller.

Effect of different buffer concentrations

In the pH range 8–10, high buffer concentrations adversely affected the response of the electrode owing to the formation of zinc(II)–ammonia complexes, which yielded a persistent drift of the electrode potential. At low buffer concentration (that is, low ammonia concentration) symmetrical titrations curves, with inflexion points coincident with the end-point, were obtained. Accordingly, a slight excess of buffer is specified in the recommended procedure for the determination of zinc.

Interferences

The effects of diverse anions and cations on the zinc titrations were studied. Solutions at two levels of Zn(II) concentration (2 and 100 ppm) containing different amounts of several ions were employed. The cations tested were added as their chlorides or nitrates. Nitrate, chloride, perchlorate, sulphate, acetate, phosphate and trichloroacetate anions produced no interference even at a 2*M* level. Citrate, tartrate ions and proteins seriously interfered due to the high stability of their complexes formed with Zn(II).

As it was expected, the method was subjected to the interference of many cations, the same documented for Zn determination via EDTA tritations.[9]

So, Cu^{2+}, Ni^{2+}, Co^{2+}, Ca^{2+} and Mg^{2+} caused interference even in 1:1 molar ratio with respect to Zn. Ca^{2+} and Mg^{2+} interfered because they consumed titrating reagent, but the electrode was not sensitive to these ions. Carrying out back-titrations in which the excess of EDTA was back-titrated with a standard Zn(II) solution, calcium and magnesium did not interfere.

Cobalt(II), copper(II) and nickel(II) interfered differently since the electrode also responded to changes in the concentration of these ions. Thus, for example, the electrode could be used to detect end-point in Cu(II) titrations with EDTA, but this was a special case since the presence of 3,5-C12PADAB into the paste had no influence on the response of the electrode. It was found that Cu(II) could be titrated with EDTA by using a pure carbon paste electrode for end-point detection.

In this case, the graphite paste electrode responded as an inert electrode, by yielding titrations curves like those obtained by using a Pt electrode or a solid graphite electrode. It was due, certainly, to the potential established by the Cu(II)/Cu(I) couple.

These interference problems limit the possible applications of the electrode for direct Zn(II) titrations in samples containing the ions or substances above-mentioned. Previous separations or masking steps are required before zinc can be determined in such samples. In spite of these disadvantages, the results obtained indicate that the electrode may be applied to some areas of chemical analysis.

Accuracy and precision

To evaluate the accuracy and repeatability of the method, 20 solutions having different concentrations of Zn were used. For each concentration level, six titrations were carried out at pH 10. The concentration range covered was from 0.5 to 6550 ppm. The best results were achieved when the titrating reagent was 10-fold more concentrated than Zn solutions. Table 1 presents some typical results obtained.

Applications

The 3,5-C12PADAB–graphite paste electrode was applied to the determination of Zn in two Zn–insulin pharmaceutical preparations. The samples chosen were commercially available formulations labelled "Betasint Insulin Zinc Protamine" (slow insulin) and "Biobras Regu-

Table 1. Some typical results obtained in EDTA titrations of pure Zn(II) solutions by using the 3,5-Cl2PADAB–graphite paste electrode as potentimetric indicator of end-point

Zn(II) taken (*ppm*)	0.540	26.75	261.1	522.0	3800	6532
	0.539	26.74	261.2	521.6	3801	6533
	0.538	26.74	261.3	521.8	3802	6532
Zn(II) found (*ppm*)	0.540	26.72	261.2	522.1	3802	6532
	0.540	26.73	261.1	522.1	3802	6533
	0.541	26.75	260.8	522.0	3802	6533
	0.541	26.76	260.9	522.0	3802	6532
Mean value (*ppm*)	0.540	26.74	261.1	521.9	3802	6532
Standard deviation	0.001	0.01	0.18	0.19	0.60	0.52

lar Insulin" (fast insulin). The samples were analysed by both the present method and the U.S. Pharmacopeia reference method (AAS) for 10 replicas in each case. The results obtained are shown comparatively in Table 2.

CONCLUSIONS

The 3,5-C12PADAB–graphite paste electrode was extremely simple to prepare. Entire assembly required no more than 20 min. In addition, very inexpensive materials were used for its construction and fresh electroactive mixtures could be easily replaced in a reproducible manner. It was useful for end-point detection in automatic titrations of zinc ions with EDTA in the range of *ca.* $8 \times 10^{-6}M$–$0.1M$. The potentiometric titration curves showed well defined end-points, even for the lowest concentration level. The electrode described in this work was found to be reliable, robust, sensitive and stable over long periods under normal laboratory conditions.

The present method offers the following advantages over both the AAS reference method and direct potentiometric measurement of Zn concentration.

(a) Zn can be determined in a wide interval of concentrations with an accuracy better than 0.1% and a reproducibility (coefficient of variation) lower than 0.18%. This allows applying the method in samples with high Zn content without carrying out extensive dilutions which diminish accuracy and precision in most instrumental methods.

(b) It does not require external standards for calibrating the instrument. In order to apply this method an EDTA solution of known concentration is needed as a unique reference. This reagent is inexpensive. It can be acquired with a high degree of purity or can be easily purified at the level of primary standard.[10]

(c) The total cost per determination is low. Comparing the prices of the equipment used in both cases, maintenance expenses, reagents used and so on, this method is more advantageous than the AAS one.

(d) The electrode showed a long useful life and a highly reliable response, without significant variations of sensitivity in the total range of Zn concentrations assayed. These characteristics are rarely reached by most of the ion-selective electrodes proposed for direct determinations of ions.

(e) Even when the preparation of the insulin sample can be considered somewhat tedious and time-consuming, the titration in itself does not require too much time (about 7 min). Besides, as it is automatic, it only requires the operator's attention to program the titrator and place the sample. Taking into account the possibility of joining an autosampler to the titrator, the method becomes specially attractive to be used in those laboratories where many samples are to be processed, without considerably increasing the cost.

Table 2. Results obtained in the complexometric determination of zinc in commercial insulin by using the 3,5-Cl2PADAB–graphite paste electrode as potentiometric sensor of end-point

Sample	Zn found (µg/*ml*)*	
	Method	
	This work	Reference method
Slow insulin	17.40 ± 0.20	17.26 ± 0.20
Fast insulin	$0.023 \pm 5 \times 10^{-3}$	0.023 ± 0.01

*Mean of 10 determinations ($n = 10$) on five samples.

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