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Spectrophotometric determination of obidoxime chloride as its Pd(II) complex in water and injections*

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Abstract: It has been established that obidoxime chloride reacts with palladium(II) chloride in the pH range 3.9–8.0 and forms a yellow water-soluble (1:1) complex with maximum absorbance at 355 nm. By applying the methods of Sommer, Nash and Job involving non-equimolar solutions the conditional stability constant of the complex, at the optimum pH of 6.3 and an ionic strength $\mu = 0.55$ M, is found to be $10^{5.3}$. The molar absorptivity at 355 nm is 3.16×10^4 l mol^{-1} cm^{-1} at pH 6.3. Beer's law is obeyed up to 20 μmol l^{-1} obidoxime chloride concentration. The detection limit of the method is 0.36 μg ml^{-1}. The relative standard deviation ($n = 10$) varies over the range 1.10–2.66%. The proposed method was found to be suitable for the accurate and sensitive analysis of obidoxime chloride in water and injection solutions.

Keywords: *Obidoxime chloride: palladium(II) chloride; complexometry; colorimetry.*

Introduction

A number of mono and bis(pyridinium oximes) are known to be acetylcholinesterase reactivators and, as such, potential antidotes to poisoning with organophosphorus compounds such as pesticides or nerve gases [1, 2]. Only three such compounds (obidoxime chloride, PAM-2 and TMB-4) are used in clinical medicine [3, 4].

Recently the polarographic determination of obidoxime chloride (Toxogonin; 1,3-bis(4-hydroxyiminomethyl-1-pyridinio)-2-oxapropane dichloride) in water, in injections [5] and also in biological materials [6] was described.

The reactions between obidoxime chloride and the analytical reagents aquapentacyanoferrate(II) ions [7], and also nitrosylpentacyanoferrate(II) [8], have been studied spectrophotometrically as a basis for its determination. Christenson [9–12] has studied in detail the stability and decomposition products of obidoxime chloride using spectrophotometric methods.

The present paper reports the results obtained during a study of the reaction of obidoxime chloride with Pd(II), as the basis for its determination in water and in

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injections. This report is a continuation of the Authors' systematic studies on the behaviour of quinolinium oxime–palladium(II) complexes [13].

Experimental

Reagents

Obidoxime chloride (purity > 99.5%) was synthesized at the Laboratory of Organic Chemistry and Biochemistry, Faculty of Science, Zagreb. Toxogonin ampoules (Merck, FRG) containing 250 mg ml^{-1} were used. All other chemicals were of analytical-grade purity (Merck). Doubly-distilled water was used.

Solutions

For analytical purposes a freshly prepared 2×10^{-4} M aqueous solution of pure obidoxime chloride was used as the stock solution; this was stable for one week. A standard solution containing 71.80 μg ml^{-1} of Toxogonin was prepared by diluting the injection with water.

Palladium(II) chloride standard solution (2.081×10^{-2} M) was prepared as described previously [13], and then standardized gravimetrically by precipitation with dimethylglyoxime [14].

The ionic strength (μ) of the final solution for spectrophotometric determination was kept constant at 0.55 M by the addition of 2 M potassium chloride solution.

Britton–Robinson buffer solutions [15] covering the pH region 3.5–8.0 were made by mixing 0.08 M phosphoric acid, boric acid and acetic acid with the appropriate volume of 0.4 M sodium hydroxide and with enough 2 M potassium chloride to adjust the ionic strength to 0.2 M.

Apparatus

A Pye–Unicam SP-6-500 ultraviolet–visible spectrophotometer (Cambridge, UK) provided with 10-mm quartz cells was used. A Radiometer PHM 62 pH-meter, calibrated with appropriate standard buffer solutions, was employed. The pH values were determined with a saturated calomel–glass electrode system.

Procedure for calibration curve

Potassium chloride solution (2.25 ml) and palladium(II) chloride standard solution (0.40 ml) were placed in a 10 ml standard flask, and an aliquot of obidoxime chloride stock solution (0.05–1.00 ml) or standard Toxogonin injection solution (0.50 ml) was added. The pH was then adjusted by adding 5.00 ml of pH 6.3 Britton–Robinson buffer, and the solution diluted to volume with water. The solution was mixed and the absorbance measured after 15 min at 355 nm against a reagent blank. All measurements were made at room temperature (25 ± 0.5°C).

Results and Discussion

The characteristics of the complex

With palladium(II) chloride (R) it was found that obidoxime chloride produces a yellow complex soluble in Britton–Robinson buffer in the pH range 3.90–8.0. Absorption spectra were recorded over the wavelength range 250–550 nm. The complex shows a sharp absorbance maximum at 355 nm (Fig. 1, curve 3), which is therefore used

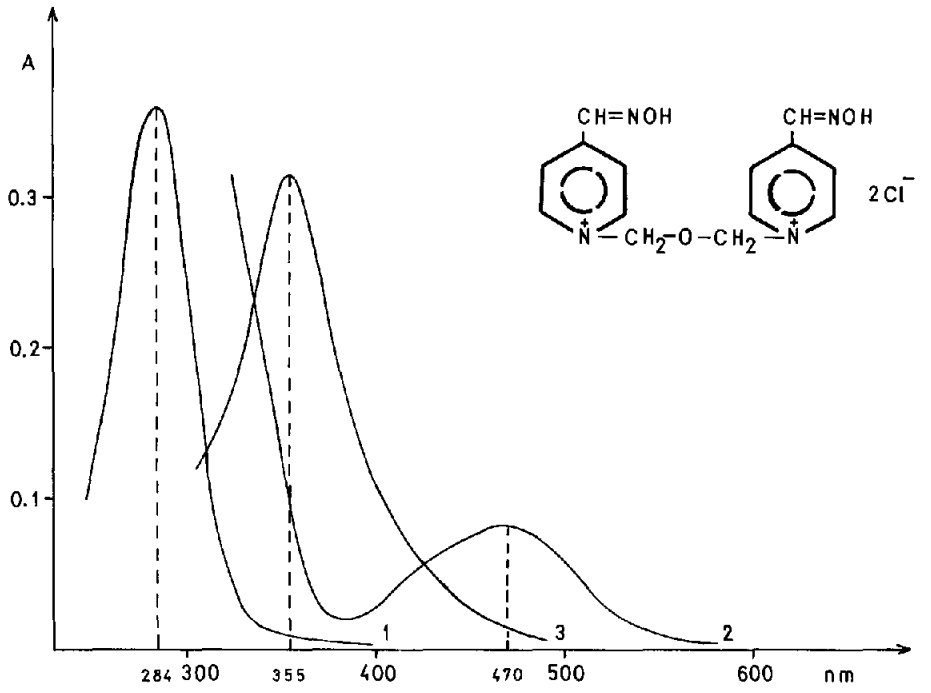

Figure 1
Absorption spectra of obidoxime (curve 1); palladium(II) chloride (curve 2); and complex (curve 3). [Obidoxime] = 1×10^{-5}M; [Pd(II)] = 4.5×10^{-4}M; pH = 6.30; $\mu = 0.55$.

for the analytical determinations. Obidoxime under the same conditions shows negligible absorbance at this wavelength (Fig. 1, curve 1). The reagent has a λ_{max} at 470 nm and, since it has an absorbance at 355 nm, all measurements were performed against a reagent blank, with a correction for the cell blank, as appropriate.

The reaction rate and the amount of the complex produced are considerably influenced by the pH of the reaction mixture (Fig. 2). The complex is only produced at pH values above 3.92. At higher pH values the absorbance gradually increases to reach a plateau at pH values between 5.90–6.50. Above pH 6.50 the absorbance decreases. Thus, pH 6.30 was used as the working pH. Because of its strong dependence on pH it can be concluded that the complex involves the ionic form of obidoxime. The shape of the absorption spectrum and the position of the absorption maximum of the complex formed do not vary with pH, which indicates that only one type of complex is formed.

Investigations on the effect of reagent concentration showed that the absorbance increases for molar ratios up to 40:1 of palladium(II)–oxime; the absorbance does not increase with further excess of reagent.

The effects of time and ionic strength on the course of the reaction are presented in Fig. 3. At low ionic strength the absorbance decreases very rapidly. At higher ionic strength maximum absorbance is observed after 15 min and is unchanged up to 120 min. Thus measurements were made at 15 min using an ionic strength $\mu = 0.55$.

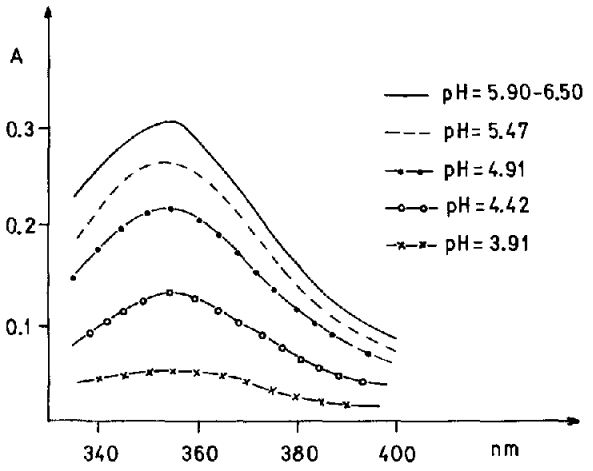

Figure 2
The effect of pH on complex formation. [Obidoxime] = 1×10^{-5}M; [Pd(II)] = 4.5×10^{-4}M; $\mu = 0.55$.

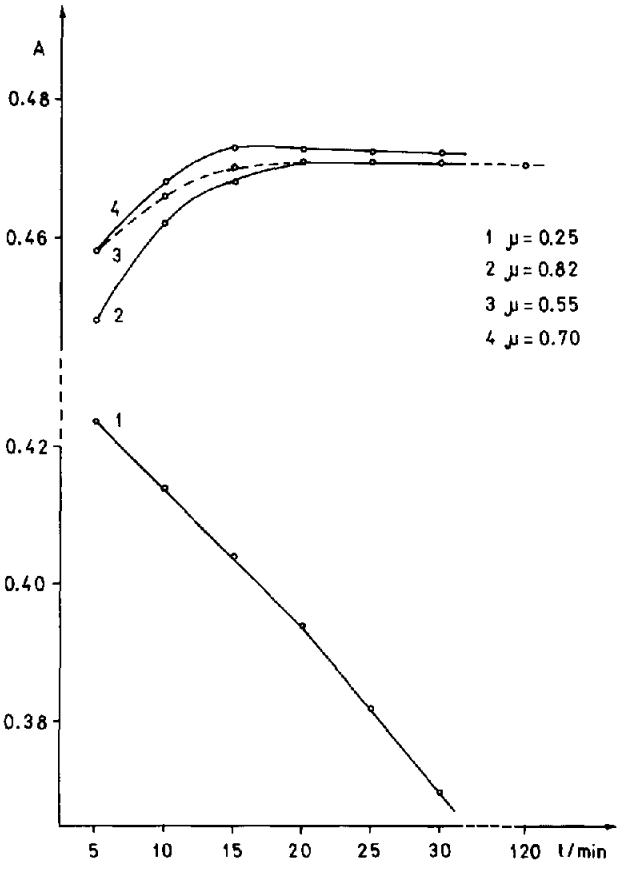

Figure 3
The influence of ionic strength and time on the course of the reaction. [Obidoxime] = 1.5×10^{-5}M; [Pd(II)] = 6.75×10^{-4}M; pH = 6.30.

The composition and conditional stability constant

By using Job's method of equimolar solutions [16, 17], the curves obtained display a maximum at a molar fraction of $x_{max} = 0.5$ which indicates the formation of 1:1 complex (Fig. 4). The curves obtained by the molar ratio method [18] show a break point at obidoxime–Pd(II) molar ratio 1:1 (Fig. 5). The Bent–French method [19] was used in order to determine the number of oxime ions in the complex. The absorbance of the complex was measured at constant excess of Pd(II) and with various concentrations of obidoxime (Fig. 6). The slope ($q = 1.00$–1.04) points to the participation of one oxime ion in the formation of the complex, which confirms the results of the previous methods.

The conditional stability constant of the complex was calculated according to the method of Sommer *et al.* [20] by using Job's curves of equimolar solutions. The results are presented in Table 1. By using Job's method of non-equimolar solutions [17] the curves obtained for five-fold and ten-fold excess of reagent (p) (Fig. 7) gave values for x_{max} obtained by projecting the peak maximum onto the abscissa and dividing it by the total volume of solution used in each case (12 ml). The conditional stability constant was then calculated in the following way:

$$K' = \frac{(p - 1)(1 - 2x_{max})}{c_{oxime}[(1 + p)x_{max} - 1]^2},$$

where $p = 5$ or 10 and $c_{oxime} = 3 \times 10^{-5}$ mol l^{-1}. The values of log K' are presented in Table 2.

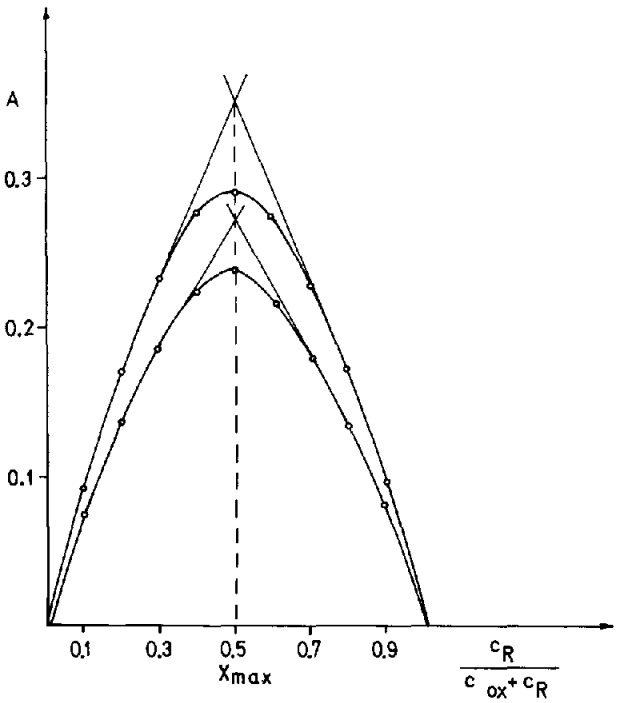

Figure 4
Job's curves of equimolar solutions at 355 nm (curve 1) and 370 nm (curve 2). [Obidoxime] + [Pd(II)] = 5×10^{-5}M; pH = 6.30; $\mu = 0.55$; $c_R = 5 \times 10^{-4}$M; $c_{ox} = 5 \times 10^{-4}$M.

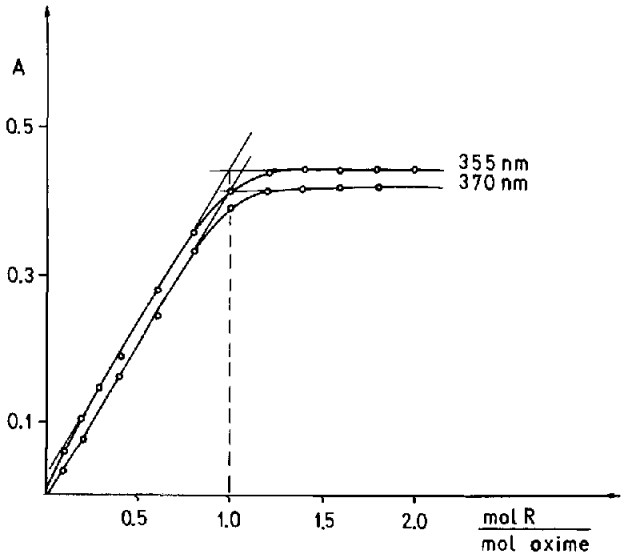

Figure 5
Molar ratio method. [Obidoxime] = 2×10^{-5}M; pH = 6.30; μ = 0.55.

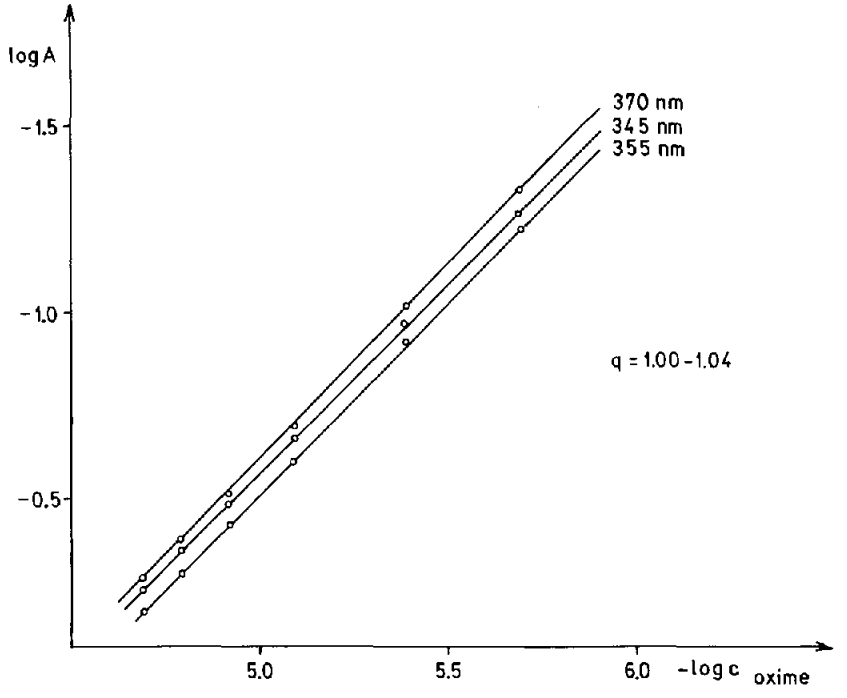

Figure 6
Bent–French's method. [Obidoxime] = 2×10^{-6}–2×10^{-5}M; [Pd(II)] = 8×10^{-4}M; pH = 6.30; μ = 0.55.

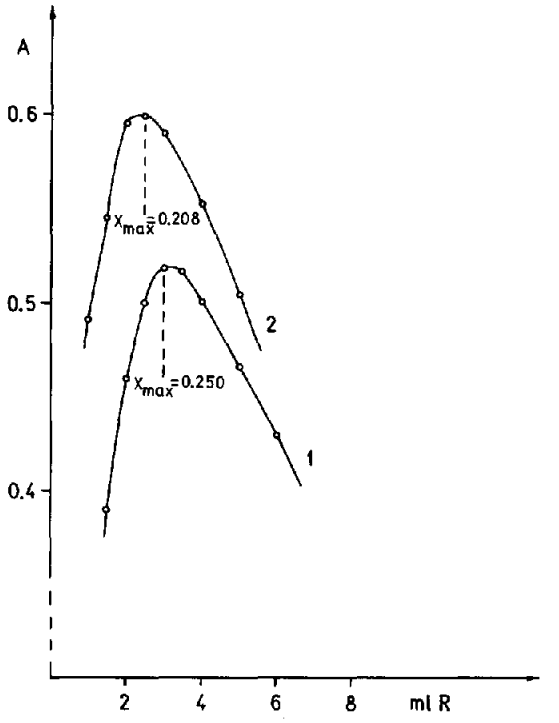

Figure 7
Job's curves of non-equimolar solutions. [Obidoxime] = 3×10^{-5}M; $p = 5$ (curve 1); $p = 10$ (curve 2); pH = 6.30; $\mu = 0.55$; R = $PdCl_2$.

Table 1
Conditional stability constant of the obidoxime complex calculated according to Sommer's method*

log $\bar{K}'$	log K'_{min}	log K'_{max}	SD†	RSD‡(%)
5.17	5.14	5.25	0.03	0.58

*Conditions: pH = 6.30; $\mu = 0.55$; $t = 25 \pm 0.5°$; $n = 12$.
†SD = Standard deviation.
‡RSD = Relative standard deviation.

Table 2
Conditional stability constant (K') of the obidoxime complex*

Job's method of non-equimolar solutions			Nash's method	
p†	x_{max}	log K'	λ (nm)	log K'
5	0.250	5.43	350	5.18
			355	5.24
10	0.208	5.45	360	5.20
Mean: 5.44 ± 0.01			Mean: 5.21 ± 0.03	

*Conditions: pH = 6.30; $\mu = 0.55$; $t = 25 \pm 0.5°$.
†$p = 5$: five-fold excess of reagent.
$p = 10$: ten-fold excess of reagent.

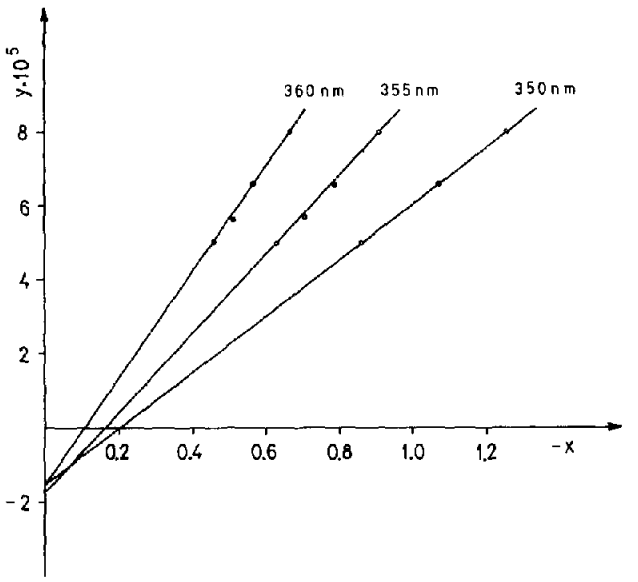

Figure 8
Nash's method. [Obidoxime] = 1.25–2 × 10^{-6}M; [Pd(II)] = 2 × 10^{-4}M; pH = 6.30; μ = 0.55.

By means of Nash's graphical method [21] (Fig. 8) a linear dependence was shown to exist for $y = f(-x)$, where $y = 1/c_{oxime}$ and $x = A_o/(A_o - A)$ (A_o = absorption of the reagent; A = absorption of the complex). This also indicates that the ratio of obidoxime to Pd(II) is 1:1. The intercepts on the ordinate represent the negative values of the conditional stability constant (Table 2). The results of these three methods are in good agreement.

Quantification and linearity of the calibration curve

Beer's law was verified in the Britton–Robinson buffer solution at pH = 6.30. A linear relationship between absorbance and concentration was established over the range 1–20 μmole l^{-1}. The molar absorptivity found for the complex was 3.16 × 10^4 l $mole^{-1}$ cm^{-1}. The regression equation was $y = 0.0313x - 0.0022$, the correlation coefficient being $r = 0.9999$ ($n = 7$), indicating excellent linearity.

The reliability of the method was checked at three different concentrations (Table 3). The relative standard deviation ($n = 10$) varied from 1.10 to 2.66% for concentrations of obidoxime chloride from 3 to 10 μmole l^{-1}. The lower limit of sensitivity of the method was found to be 0.36 μg ml^{-1}.

Application to dosage forms

The applicability of the method for the assay of simple dosage forms was examined by analyzing Toxogonin ampoules (Merck). The recovery was 100.7% ($n = 6$) relative to the labelled strength of this preparation; the relative standard deviation of the method applied to Toxogonin injection was 0.48% ($n = 6$). The results confirm the suitability of the proposed method for the routine analysis of pharmaceutical preparations, although further work would be required to examine the effect of degradation products [12].

Table 3
Spectrophotometric determination of obidoxime chloride with palladium(II) chloride*

Obidoxime chloride	3.00 μmole l^{-1}	5.00 μmole l^{-1}	10.00 μmole l^{-1}
Found	$\bar{x}$	3.01	5.03	9.97
	x_{min}	2.87	4.94	9.78
	x_{max}	3.11	5.13	10.21
	SD	0.08	0.08	0.11
	$S\bar{x}$	0.03	0.03	0.04
	RSD(%)	2.66	1.59	1.10

* Conditions: λ_{max} = 355 nm; pH = 6.30; (n = 10).

In conclusion, it may be considered that the proposed method, using palladium(II) chloride as an analytical reagent, can be suitable for the accurate and sensitive analysis of obidoxime both in pure and dosage forms.

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