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Use of palladium(II) chloride as colour-forming reagent in determination of pralidoxime chloride in water and tablets*

K. KARLJIKOVIĆ-RAJIC,†‖ B. STANKOVIĆ,† A. GRANOV‡ and Z. BINENFELD§

†*Faculty of Pharmacy, Institute of Analytical Chemistry, Belgrade, Yugoslavia*
‡*Institute of Bosnalijek Company, Sarajevo, Yugoslavia*
§*Laboratory of Organic Chemistry and Biochemistry, Faculty of Science, Zagreb, Yugoslavia*

Abstract: Pralidoxime chloride (PAM-2Cl) has been determined spectrophotometrically in Britton–Robinson buffer solution at pH = 6.45; the method is based on measurement of the absorbance of the Pd(II)-pralidoxime complex at 327 nm. Studies of the composition of the complex by Job's continuous variation method, the molar ratio method and Bent–French's method yielded a Pd(II):pralidoxime ratio of 1:1. The conditional stability constant (K') of the complex at the optimum pH of 6.45 and an ionic strength (μ) of 0.3 M was found to be $10^{5.2}$. The molar absorptivity was 1.05×10^4 1 mol^{-1} cm^{-1}. Beer's law was obeyed at concentrations up to 60 μM. The detection limit was 0.55 μg ml^{-1}. The relative standard deviation (N = 10) was 0.28–1.03%. The method was accurate and sensitive for the analysis of PAM-2Cl in water and tablets.

Keywords: *Pralidoxime chloride; palladium(II) chloride; complexiметry; spectrophotometry; tablets.*

Introduction

The nerve gases, as well as many of the organophosphorus insecticides and pesticides and their metabolites, function biologically by inhibition of acetylcholinesterase as a result of phosphorylation of the active site of the enzyme [1, 2]. Since certain mono- and bis-pyridinium oximes are reactivators of the inhibited enzyme, they are potential antidotes or protecting agents to poisoning with such compounds [3, 4].

The bis quaternary pyridinium aldoximes are effective reactivators. Of these substances the most successful in the treatment of poisoning in man is obidoxime chloride [5, 6], also known as Toxogonin. Pralidoxime chloride (PAM-2Cl) is 2-hydroxyiminomethyl-1-methylpyridinium chloride; it is available for therapeutic purposes, has undergone extensive clinical evaluation and is reported to be an effective antidote in man [7, 8].

Until now spectrophotometric methods based on the ultraviolet absorption of the drug

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‖To whom correspondence should be addressed.

in acidic and basic solutions have been often used for the determination of PAM-2Cl either in pharmaceutical preparations [9] or in biological materials [10–12].

The formation of complexes between PAM-2Cl and reagents such as amino-pentacyanoferrate(II) ions [13, 14] and nitrosyl-pentacyanoferrate(II) ions [15] has been studied spectrophotometrically as a basis for the determination of the drug and its dosage forms, and also for its estimation in biological materials [16, 17]. Recently the polarographic behaviour and determination of PAM-2Cl in water and dosage forms [18], as well as in biological materials [19, 20] have been reported from the authors' laboratory. The present work, which is a continuation of systematic studies on the behaviour of Pd(II) complexes of quinolinium oximes [21] and obidoxime [22], concerns the reaction of pralidoxime with Pd(II).

Experimental

Reagents

Pralidoxime chloride (purity >99.5%) was synthesized at the Laboratory of Organic Chemistry, Bosnalijek, Sarajevo. Standard and sustained-release (retard) tablets containing 500 and 250 mg of PAM-2Cl, respectively, were obtained from a commercial source (Bosnalijek Company, Sarajevo). All other chemicals were of analytical-grade purity (Merck). Double-distilled water was used.

Solutions

For analytical purposes a freshly prepared 2×10^{-3}M aqueous solution of pure PAM-2Cl was used as the stock solution; it was stable for several days.

Standard tablet solutions containing 1 mg ml^{-1} of PAM-2Cl were prepared by dissolving each tablet in double-distilled water and then filtering the solution into a 250-ml or 500-ml standard flask.

Britton–Robinson buffer solutions and the standard Pd(II) solution were the same as described previously [22].

The ionic strength (μ) of the final solution for spectrophotometric determinations was kept constant at 0.3 M by the addition of 2 M potassium chloride.

Diluted hydrochloric acid solution (1:100, v/v) was used in the industrial referee method (Bosnalijek Co., Sarajevo) for analysis of tablets.

Apparatus

The apparatus was the same as described previously [22]; in addition, use was made of a Uvicon 810 (Kontron, Switzerland) ultraviolet-visible spectrophotometer.

Procedure for calibration curve

Potassium chloride solution (1.00 ml) and palladium(II) chloride standard solution (0.30 ml) were placed in a 10-ml standard flask and an aliquot (0.08–1.50 ml) of 4×10^{-4} PAM-2Cl was added. The pH was then adjusted by adding 5.00 ml of Britton–Robinson buffer (pH 6.45) and the solution was diluted to volume with water. The solution was mixed and the absorbance at 327 nm was measured after 10 min against a reagent blank. All measurements were made at room temperature (25 ± 0.5°C).

Procedures for tablets

Method A. An aliquot (0.50 ml) of sample tablet solution containing about 69 $\mu g\ ml^{-1}$ of PAM-2Cl was used in the same procedure as described for the calibration curve.

Method B. One millilitre of sample tablet solution containing about 69 μg ml^{-1} was placed in a 25-ml standard flask and diluted to volume with diluted hydrochloric acid solution. The solution was mixed and the absorbance was measured at 293 nm against a reference solution at room temperature.

Results and Discussion

Absorption spectra

The reaction of pralidoxime chloride with palladium(II) chloride was investigated over the pH range 3.30–8.0 in Britton–Robinson buffer solutions; spectra were recorded at 250–500 nm. It was found that PAM-2Cl produced a yellow soluble complex with an absorbance maximum at 327 nm (Fig. 1, curve 3), which was therefore used for the analytical determinations. Under the same conditions PAM-2Cl had a λ_{max} at 294 nm and showed negligible absorbance at 327 nm (Fig. 1, curve 1). Since the reagent had an absorbance (Fig. 1, curve 2) at the wavelength of maximum absorbance of the complex, all measurements were performed against a reagent blank with a correction for the cell blank, as appropriate.

Effect of pH

The pH of the reaction mixture had a considerable influence on the absorbance (Fig. 2) of the complex. At pH values lower than 3.92 no complex formation was observed. In the pH range 3.92–4.96 the spectra showed only a shoulder but the absorbance gradually increased to reach a plateau at pH 6.20–6.90. Thus pH 6.45 was used as the working pH. Above pH 6.90 the absorbance decreased. The λ_{max} of the complex formed did not change at pH values above 5; that indicated that only one type of complex was formed.

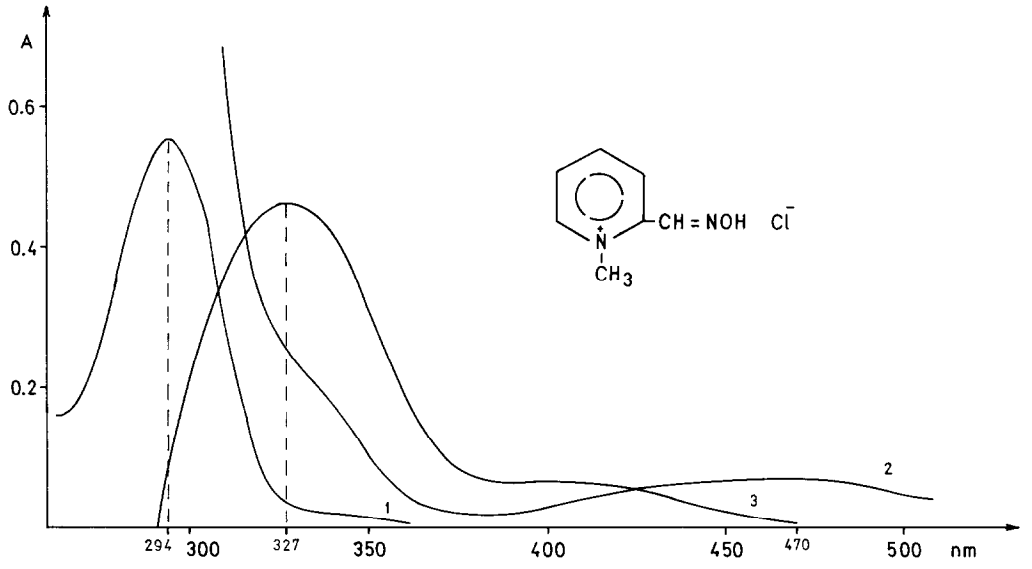

Figure 1
Absorption spectra of pralidoxime (curve 1); palladium(II) chloride (curve 2); and the complex (curve 3). [Pralidoxime] = 4.5 ×10 10^{-5}M; [Pd(II)] = 4.5 × 10^{-4}M; pH = 6.45; μ = 0.30 M.

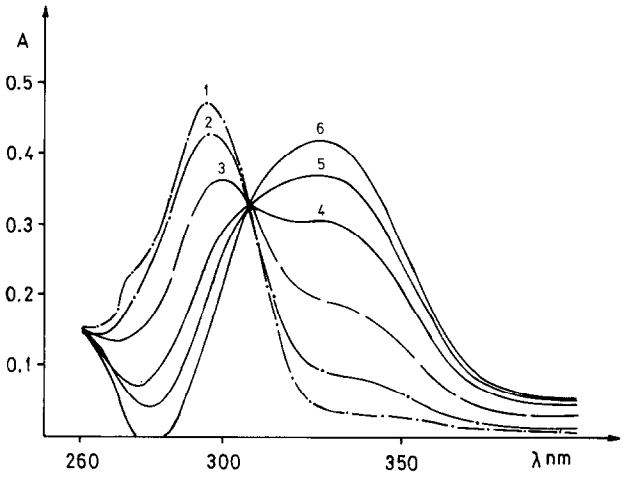

Figure 2
The effect of pH on complex formation. [Pralidoxime] = 4×10^{-5}M; [Pd(II)] = 4×10^{-4}M; μ = 0.30 M; pH = 3.36 (curve 1); pH = 3.92 (curve 2); pH = 4.45 (curve 3); pH = 4.96 (curve 4); pH = 5.39 (curve 5); pH = 6.20–6.90 (curve 6).

At the optimum pH range for complex formation the spectra indicated that PAM-2Cl had been completely bonded as the complex.

Optimum conditions of complex formation

Investigations on the effect of palladium(II) chloride concentration showed that at least a 10-fold molar excess was required to obtain maximum absorbance; the absorbance did not increase with a greater excess of reagent.

Full development of the colour was observed after 10 min and was unchanged up to 40 min. After this time the absorbance slowly decreased.

Little influence of ionic strength (range 0.15–0.8 M) on the course of the reaction was observed. The best shape of the spectra was obtained at an ionic strength of 0.30 M. The most significant differences between the optimum conditions for complex formation of obidoxime [22] and pralidoxime with Pd(II) can be observed from the effects of reagent concentration and ionic strength.

The composition of the complex

The stoichiometric ratio of PAM-2Cl and Pd(II) in the complex was determined by Job's method of continuous variation [23, 24]. The curve displayed a maximum at a molar fraction of $X_{max} = 0.5$ which indicated the formation of a 1:1 complex (Fig. 3). The curves obtained by the molar ratio method [25] showed a discontinuity at a pralidoxime–Pd(II) molar ratio of 1:1. The results were confirmed by means of the Nash graphical method [26] where a linear dependence was shown to exist for $y = f(-x)$ (Fig. 4) where $y = 1/C_{PAM-2Cl}$ and $x = A_o/(A_o - A)$ (A_o = absorption of the reagent; A = absorption of the complex).

The composition of the complex was also determined by the Bent–French method [27]. The results obtained by this method confirmed that the molar ratio PAM-2Cl:Pd(II) was 1:1; since the values of the slopes (q = 0.99–1.00 and p = 0.92–0.93)

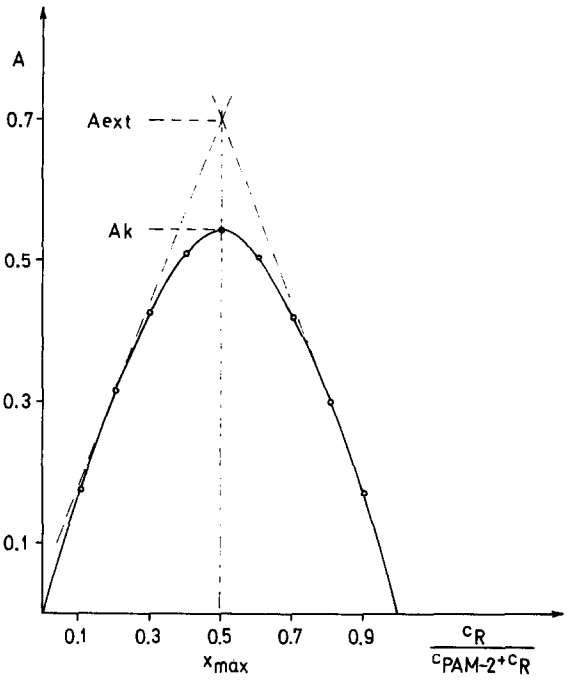

Figure 3
Job's curve of equimolar solutions at λ_{max} = 327 nm. [Pralidoximel] + [Pd(II)] = 1.6 × 10^{-4}M; pH = 6.45; μ = 0.30 M; C_R = 1.6 × 10^{-3}M; $C_{PAM-2Cl}$ = 1.6 × 10^{-3}M.

were obtained at three wavelengths, it was concluded that the complex is a monomer [27].

The conditional stability constant of the complex

By applying Sommer's [28] and Asmus's [29] methods, on the basis of data obtained with Job's curve of equimolar solutions (Fig. 3), the conditional stability constant has been determined (Table 1). In accordance with Job's method of non-equimolar solutions for 5-, 10- and 15-fold excess of reagent (p), the values of x_{max} were obtained by projecting the peak maximum of the curves on to the abscissa and dividing it by the total volume of the solution used in each experiment (12 ml). The constant was calculated using the same equation as presented in previous work [22]. The values of log K' are given in Table 1. By means of Nash's method (Fig. 4) the values of the constant were obtained as the negative intercept on the ordinate. The results are shown in Table 1.

The mean values of log K' obtained by four different methods (5.10–5.35) are in good agreement.

Quantification of Beer's law

A linear relationship between absorbance and concentration was established over the range 3.2–60 μM. The molar absorptivity for the complex was 1.05 × 10^4 1 mol^{-1} cm^{-1}. The regression equation was y = 1.042 × $10^{-2}x$ − 9.869 × 10^{-4} and the correlation coefficient (r) was 0.999 (N = 7), indicating excellent linearity. The detection limit was 0.55 μg ml^{-1}.

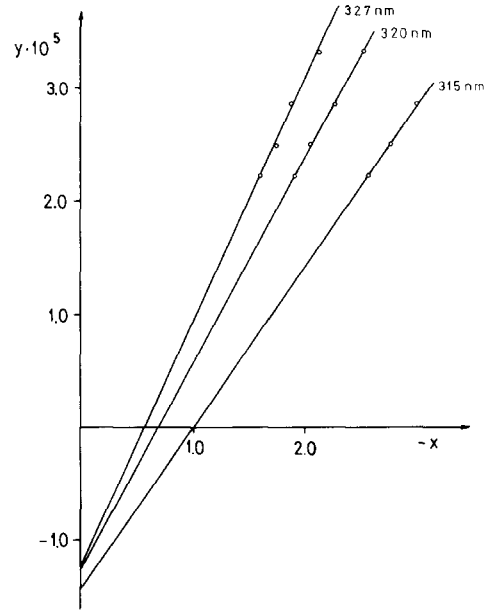

Figure 4
Nash's method. [Pralidoxime] = 3.0–4.5 × 10^{-6}M; [Pd(II)] = 6 × 10^{-4}M; pH = 6.45; μ = 0.30 M.

Table 1
Conditional stability constant of the pralidoxime complex*

Sommer's method			
log $\bar{K}$	SD	$S_{\bar{x}}$	RSD†(%)
5.35	0.0865	0.0261	1.62
(N = 11)			
Job's method of non-equimolar solutions			
P‡	x_{max}	log K'	
5	0.250	4.95	
10	0.158	5.09	
15	0.113	5.26	
		log $\bar{K}'$ = 5.10 ± 0.15	
Nash's method			
λ nm	log $\bar{K}'$	log $\bar{K}'$	
315	5.21		
320	5.05	5.10 ± 0.09	
327	5.05		
Asmus's method			
A_{ext}	A_k	log K'	
0.700	0.540	5.27	

*Conditions: pH = 6.45 ± 0.05; μ = 0.30 M; temperature = 25.0 ± 0.5°C.

†SD = standard deviation; RSD = relative standard deviation.

‡P = 5, P = 10 and P = 15 represent a 5-fold, 10-fold and 15-fold excess of reagent, respectively.

The precision of the method was determined at three different concentrations. The results (Table 2) show that the relative standard deviation (RSD) varied from 0.28 to 1.03% for concentrations of PAM-2Cl of 10–40 μM.

Analysis of pharmaceuticals

The method proposed for PAM-2Cl was applied to determine the content uniformity of this oxime in standard and sustained-release tablets obtained from a commercial source. Results were compared with those by the industrial referee method, which involved ultraviolet spectrophotometric determination in diluted hydrochloric acid solution [9]. Table 3 shows the comparison of results by these methods; each method gave results with a similar relative standard deviation. The student's *t*-test gave no significant difference between the means obtained by these methods. Excipients in PAM-2Cl tablets (e.g. talc, maize starch, zein and magnesium stearate) did not influence the absorbance values even if present in large excess.

The method, which uses Pd(II) as analytical reagent, is simple and rapid as well as accurate and sensitive, and can be successfully applied to the determination of pralidoxime chloride both as the substance and its dosage forms.

Table 2
Spectrophotometric determination* of pralidoxime chloride with palladium(II) chloride

PAM-2Cl		10 μM	20 μM	40 μM
Found	$\bar{x}$	10.06	20.03	39.96
	$\bar{x}_{min}$	9.88	19.95	39.72
	$\bar{x}_{max}$	10.16	20.15	40.10
	SD†	0.1037	0.0788	0.1127
	$S\bar{x}$	0.0328	0.0249	0.0356
	RSD‡ (%)	1.03	0.39	0.28

*At 327 nm and pH 6.45 ± 0.05.
†Inter-assay standard deviation (N = 10).
‡Relative standard deviation.

Table 3
Content uniformity of PAM-2Cl in tablets

	Sustained-release tablets (250 mg)		Standard tablets (500 mg)	
	Method A*	Method B†	Method A*	Method B†
Found	242.80 mg	244.75 mg	503.20 mg	505.25 mg
Recovery	97.1%	97.9%	100.6%	101.1%
SD‡	10.36 mg	10.64 mg	8.96 mg	9.05 mg
RSD	4.26%	4.35%	1.78%	1.79%
Sx	3.27	3.36	2.84	2.86
Calculated t-values	0.394		0.483	
Tabulated t-values (O = 18; P = 0.05)	2.101		2.101	

*Method A with Pd(II) at λ = 327 nm.
†Method B in diluted HCl solution at λ = 293 nm.
‡Inter-assay standard deviation (N = 10).

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