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Spectrophotometric investigation of complex formation of an oxime PAM-4Cl with palladium(II) and its analytical application*

K. KARLJIKOVIĆ-RAJIĆ,†‡ B. STANKOVIĆ‡ and A. GRANOV§

‡*Faculty of Pharmacy, Institute of Analytical Chemistry, Belgrade, Yugoslavia*
§*Institute of Bosnalijek Company, Sarajevo, Yugoslavia*

Abstract: The colour reaction of 4-hydroxyiminomethyl-1-methylpyridinium chloride (PAM-4Cl) and palladium(II) chloride has been investigated. The optimum reaction conditions, spectral characteristics, conditional stability constant and composition of the yellow water-soluble complex have been established. A new spectrophotometric method is proposed for the microdetermination of PAM-4Cl.

Keywords: *Oxime; PAM-4Cl; antidote; palladium(II) chloride; complex; spectrophotometry.*

Introduction

It is generally accepted that the best therapeutic or prophylactic approach to the problem of exposure to highly toxic organophosphorus compounds [1, 2] is the use of atropine in combination with oximes that act as cholinesterase reactivators. Among the mono- and bis-pyridinium oximes, only PAM-2Cl, obidoxime chloride and TMB-4 are used in standard therapy [3–5].

The present work is the continuation of systematic spectrophotometric studies on the behaviour of Pd(II) complexes of obidoxime chloride [6] and PAM-2Cl [7] in which spectrophotometric methods were developed for the determination of these oximes both in pure and dosage-forms (injections and tablets). The present report concerns the reaction of PAM-4Cl with Pd(II) as the basis for determination of the oxime in aqueous solution as well as for the comparison of the effect of the position of the hydroxyiminomethyl group on formation of the complex.

Experimental

Reagents

4-Hydroxyiminomethyl-1-methylpyridinium chloride (PAM-4Cl × H_2O) of purity >99.5% was synthesized at the Laboratory of Organic Chemistry, Bosnalijek Company (Sarajevo). All other chemicals were of analytical grade (Merck). Double-distilled water was used.

Solutions

For analytical purposes a freshly prepared 2×10^{-3} M aqueous solution of pure PAM-4Cl was used as the stock solution; it was stable for several days.

Britton-Robinson buffer solutions [8] and standard Pd(II) solutions were the same as those described previously [6].

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

Apparatus

A Uvicon 810/820 (Kontron, Switzerland) UV–vis spectrophotometer, with 10-mm fused-silica cells, was used. A Radiometer PHM 82 (Copenhagen) pH-meter, calibrated with appropriate standard buffer solutions, was employed.

Procedure for calibration curve

Potassium chloride solution (2 M, 1.00 ml) and palladium(II) chloride solution (2×10^{-2} M, 0.20 ml) were placed in a 10-ml standard flask; a portion (0.05–0.75 ml) of 4×10^{-4} M PAM-4Cl was added followed by 5.00

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†Author to whom correspondence should be addressed.

ml of buffer (pH 6.45), and the solution was diluted to volume with water. The solution was mixed and the absorbance at 342 nm was measured after 10 min against a reagent blank. All measurements were made at room temperature (25 ± 0.5°C).

Results and Discussion

Absorption spectra

The reaction between PAM-4Cl and Pd(II) chloride in Britton-Robinson buffer solutions in the pH range 3.35–7.60 was studied. The spectra of the solutions containing the water-soluble yellow complex of PAM-4Cl with Pd(II) were recorded over the wavelength range 250–380 nm. The complex gave a sharp absorption peak at 342 nm (Fig. 1, curve 3), where the absorbance of Pd(II) was low (curve 2) and that of PAM-4Cl was negligible (curve 1). Since the reagent absorbed at the wavelength of the complex, all measurements were performed against a reagent blank.

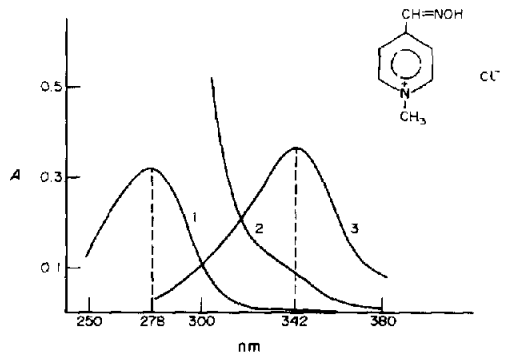

Figure 1
Absorption spectra of PAM-4Cl (curve 1); reagent (curve 2) and complex (curve 3); $c_{PAM\text{-}4Cl} = 2 \times 10^{-5}$ M; $c_{Pd(II)} = 2 \times 10^{-4}$ M; pH = 6.45; μ = 0.30 M.

Because of the position of the hydroxyiminomethyl group on the pyridinium ring, the λ_{max} of the Pd(II) complex of PAM-4Cl, in comparison to that of the PAM-2Cl–Pd(II) complex [7], is shifted bathochromically by 15 nm. PAM-4Cl alone exhibits a λ_{max} at 278 nm which is 16 nm lower than was observed for PAM-2Cl alone.

Reaction conditions

The complex is only produced in solutions with pH values above about 4. The optimum pH for a constant maximum absorbance at 342 nm was found to be pH 6.35–7.01 (Fig. 2). The shape of the spectra was independent of pH, indicating the formation of only one type of complex under these conditions. A Britton-Robinson buffer of pH 6.45 was used to provide the working pH. The most significant effects of pH on the shape of the absorption spectra of the complexes can be observed for the PAM-4Cl complex in Fig. 2. In the pH range 3.92–4.96, where the spectra of the PAM-2Cl–Pd(II) complex showed only a shoulder [7], the λ_{max} of the PAM-4Cl–Pd(II) complex was clearly observed, because of the difference between the λ_{max} of pure oximes

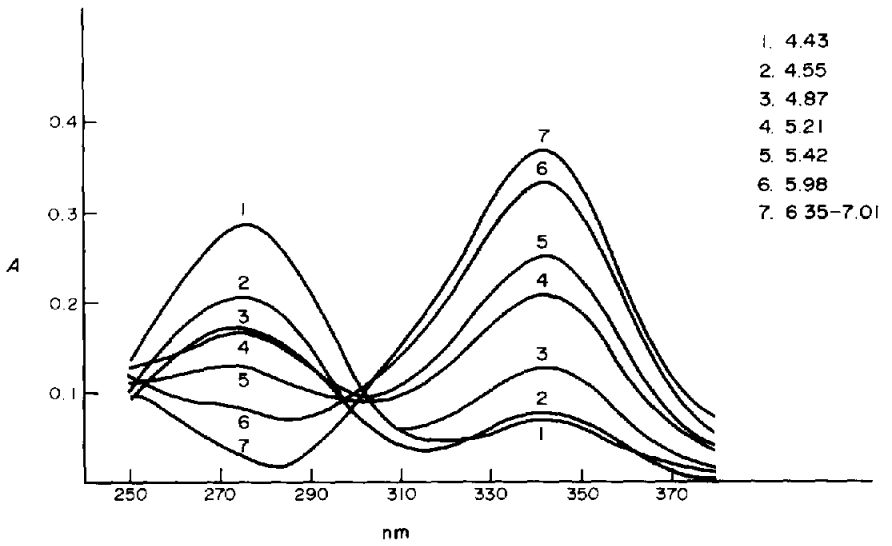

Figure 2
The effect of pH on the formation of the complex; $c_{PAM\text{-}4Cl} = 2 \times 10^{-5}$ M; $c_{Pd(II)} = 2 \times 10^{-4}$ M; μ = 0.30 M; pH = 4.43 (curve 1); pH = 4.55 (curve 2); pH = 4.87 (curve 3); pH = 5.21 (curve 4); pH = 5.42 (curve 5); pH = 5.98 (curve 6) and pH = 6.35–7.01 (curve 7).

and the λ_{max} of complexes; for PAM-4Cl the difference (64 nm) was twice that for PAM-2Cl.

At the working pH of 6.45, at least a 10-fold molar ratio of reagent to analyte (PAM-4Cl) was necessary for maximum complex formation.

In an experiment on the influence of ionic strength (0.15–0.8 M) on the course of the reaction of PAM-2Cl with Pd(II), it was found that at an ionic strength lower than 0.2 or higher than 0.4 the absorbance of PAM-4Cl complex decreased slightly. The best shape and the highest absorbance was obtained at an ionic strength of 0.3 M, which was the same as that previously selected for the PAM-2Cl–Pd(II) complex.

Under these conditions the absorbance of the complex reached about 95% of the maximum absorbance after 5 min. The complex was completely formed after 10 min and was unchanged up to 40 min. After this time the absorbance decreased slightly.

Physicochemical properties of the PAM-4Cl–Pd(II) complex

The composition of the complex was established by the continuous variation method [9, 10] and the molar ratio method [11]. Both methods showed that a 1:1 complex was formed. The results were confirmed by means of Nash's graphical method [12] and by Bent–French's method [13]; since the values of the slopes (q = 0.98–1.01 and p = 0.86–0.91) were obtained at three wavelengths, it was concluded that the complex was a monomer [13].

To determine the conditional stability constant (K'), the methods of Sommer [14], Asmus [15], Nash [12] and Job (with non-equimolar solutions) [9, 10] were used. The mean values of log K' obtained by these methods 5.14, 5.16, 5.06 and 5.12, respectively, were in good agreement. For the results obtained by Sommer's method the relative standard deviation (RSD) was 0.98% (20 replicates). The value of K' was slightly lower than that of the K' value of PAM-2Cl–Pd(II) complex obtained under the same experimental conditions.

Quantification of Beer's law and sensitivity

Under the optimum conditions described above, the calibration graph was rectilinear over the range 2–30 μM of PAM-4Cl and the corresponding equation of the regression line was $A = 1.83 \times 10^{-2}c + 5.86 \times 10^{-4}$ with a correlation coefficient of 0.9999 (n = 8). The complex had an apparent molar absorptivity ϵ of 1.83×10^4 l mol^{-1} cm^{-1} with a Sandell's sensitivity of 10.4 ng cm^{-2} per 0.001 absorption unit [16]. For comparison, the values for PAM-2Cl–Pd(II) were 1.04×10^4 l mol^{-1} cm^{-1} and 16.6 ng cm^{-2}, respectively.

The limit of determination of the proposed method was 0.38 μg ml^{-1}; the limit of detection (defined as the analyte concentration giving the signal equal to the blank signal, y_B, plus three standard deviations of the blank, $S_B(S_{y,x})$; $y = y_B + 3S_B$ [17]) was 0.02 μg ml^{-1}.

The lower limits of determination and detection obtained for PAM-4Cl, in comparison with those for PAM-2Cl, are due to the higher value of ϵ as well as to the lower value of $S_B(S_{y,x})$.

The precision of the method was determined at three different concentrations. The results (Table 1) show that the RSD varied from 0.65 to 1.20% for concentrations of PAM-4Cl of 5–20 μM.

The method, which is now used in clinical medicine for therapeutic drug monitoring [18] is a modification of that which was first introduced by Creasey and Green [19], and which involves the spectrophotometric determination of the corresponding oximate ions in ammonium hydroxide solution. The conditions in the proposed method are more suitable than those of the method [18] in current use because degradation of oxime does not occur; oxime degradation is the main disadvantage in the current method.

The proposed method, which uses Pd(II) as the analytical reagent, is simple and rapid as

Table 1
Test of the precision and accuracy of the proposed method for the spectrophotometric determination of PAM-4Cl with Pd(II) chloride*

PAM-4Cl	5 μM	10 μM	20 μM
Found $\bar{x}$	4.98	10.03	20.02
x_{min}	4.88	9.91	19.92
x_{max}	5.16	10.09	20.13
SD†	0.0597	0.0812	0.1301
$S_{\bar{x}}$	0.0188	0.0256	0.0411
RSD (%)	1.20	0.81	0.65
Calculated t-values	1.059	1.168	0.486
Tabulated t-value (ϕ = 9; P' = 0.05)	2.262	2.262	2.262

*At 342 nm; pH = 6.45 ± 0.05; μ = 0.3 M.
†Inter-assay standard deviation (n = 10).

well as accurate and sensitive, and can be successfully applied to the microdetermination of PAM-4Cl in aqueous solutions.

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