

Oxime-containing acetylcholinesterase reactivators and their complexes with Pd(II) and Pt(II) ions: recent developments

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Abstract: The ability of oxime-containing acetylcholinesterase reactivators BT-07 (K005), BT-08 (K033), BT-07-4M (K074), and obidoxime (H_2LX_2 , $X = Cl^-$, Br^-) to bind palladium(II) or platinum(II) cations was evaluated in aqueous solutions (pH 7.4) at metal-to-ligand molar ratio varying from 1:10 to 10:1. Spectral changes were monitored within a week after mixing the reagents in the range from 220 nm to 500 nm. Results showed that depending on chemical structure and reaction time, oximes form two complex species of composition, $[MHL]^{3+}$ or $[M_2L]^{4+}$ ($M = Pd(II)$, $Pt(II)$). It was also found that complexation of platinum(II) ions proceeds slower as compared to palladium(II). Conditional stability constants of new complex species were determined spectrophotometrically using an advanced chemometric procedure, based on the resolution of overlapping bands technique.

Key words: Bis-quaternary pyridinium aldoximes, acetylcholinesterase reactivators, palladium(II) complexes, platinum(II) complexes, UV-Vis spectroscopy

1. Introduction

Organophosphorus compounds (OPCs) are used in agriculture as insecticides (chlorpyrifos, methyl parathion, dimethoate, azinphos-methyl, etc.) and can present serious health risks in the case of improper handling or production and transportation accidents.^{1–4} Chemical warfare nerve agents (sarin, soman, tabun, VX, etc.), also belonging to OPCs, are classified as weapons of mass destruction according to UN Resolution 687. Both groups are irreversible inhibitors of acetylcholinesterase, responsible for the breakdown of acetylcholine in the synapse.^{4–9} Its inhibitory effect is based on phosphorylation or phosphonylation of the serine hydroxy group at the esteric site of the active center of the enzyme and its inhibition is the life-limiting factor.¹⁰

Currently adopted medical countermeasures against poisoning caused by OPCs consist of two approaches: i) prophylaxis (pretreatment) with a carbamate-containing reversible inhibitor of acetylcholinesterase to protect partially the enzyme activity, or ii) postexposure antidote treatment with combinations of an oxime-containing cholinesterase reactivator, cholinolytic, and anticonvulsant.^{11–13}

The most effective cholinesterase reactivators (ChRs) represent mono- or bis-quaternary pyridinium aldoximes that contain a variety of substituents in the pyridinium rings and/or different bridges (as type/length)

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between the rings.^{14–16} The reactivation ability of oximes has been extensively studied in experimental *in vivo* and *in vitro* assays.^{17–24} The overall process of interaction of the phosphorylated enzyme and cholinesterase reactivators is very complex and highly depends on the chemical structure of the inhibitor, type of phosphorus residue at the enzyme active center, structure of oxime compound, and conformational and spatial interactions between active groups of cholinesterase reactivators and phosphorus residue in the enzyme molecule.²³ Nowadays, from the wide variety of quaternary pyridinium aldoximes synthesized, only four compounds, 2-PAM, obidoxime, HI-6, and TMB-4, are approved as drugs of the first choice in the case of nerve agent (soman, sarin, or tabun) poisoning and are commercially available.^{25–28} To the so-called “big four” of ChRs, HLö-7 should also be added as a subject for intensive studies.^{22,29} It should be strongly stressed that there are difficulties in ChR application due to their fast elimination from the organism and the noncomplete recovery of enzymatic activity; the antidotal efficacy of known reactivators is also different against various OPCs and universal antidotes are still not developed.

On the other hand, it is well known that complexation of biologically active compounds can be applied as an efficient method to improve properties of initial compounds *via* various mechanisms.^{30–32} From a chemical point of view, quaternary pyridinium aldoximes are potential ligands able to bind metal ions, but only a few studies in this field are available. It was found in 1969 that the presence of some metal ions such as Mn(II), Mg(II), Co(II), Zn(II), and Ni(II) amplifies the reactivation ability of 2-PAM and the authors suggested possible complex formation, although no detailed study was performed.³³ We monitored the UV-Vis spectra of 2-PAM in the presence of a variety of divalent metal ions and were able to detect changes only in the case of Pd(II) and Pt(II) ions, which allowed us to make some conclusions regarding these particular ions. On the other hand, the absence of changes in 2-PAM spectra with Mn(II), Mg(II), Co(II), Zn(II), or Ni(II) ions does not mean that we have to exclude the formation of complex species, just spectrophotometrically we were not able to obtain such evidence and we refrain from drawing a parallel between Pd(II) coordination compounds and the other species containing divalent metal ions mentioned above.

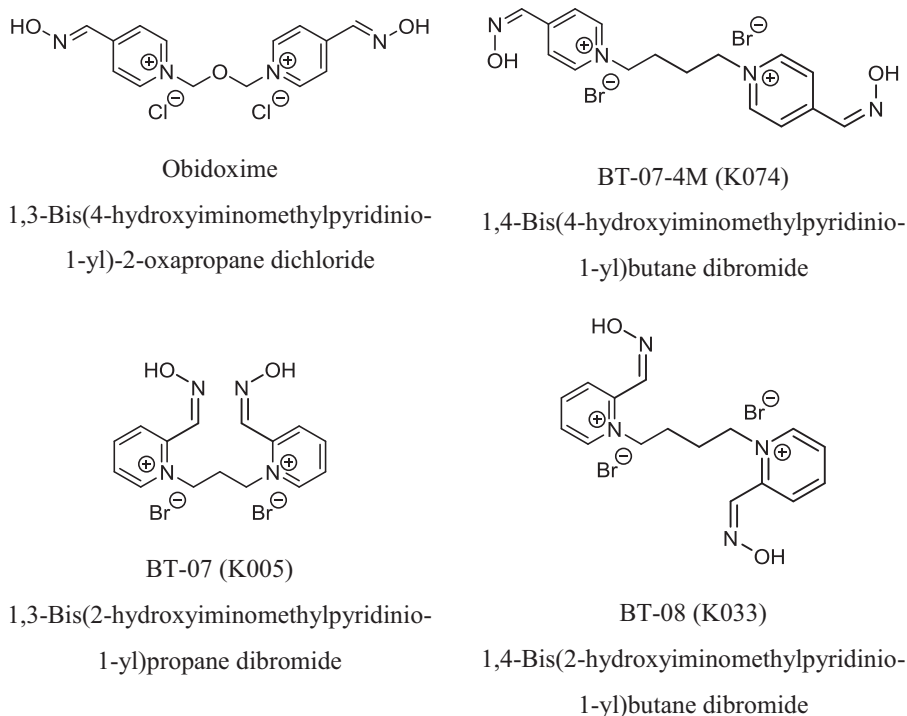
Later, coordination of some pyridinium aldoximes with pentacyanoferrate(II) or Pd(II) ions was monitored with respect to their determination in dosage forms and pharmaceutical formulations.^{34–39}

Recently we have shown that despite the limited reactivation ability of complexes in *in vitro* reactivation assay of paraoxon-inhibited rat brain acetylcholinesterase⁴⁰ Pd(II) ions form two species with obidoxime depending on reaction time. We admit that obidoxime complexes showed lower reactivation ability in this case, but it is still unclear what will be the reality in *in vivo* conditions or if changing the inhibitor structure.

In the present paper we report our further studies on the capacity of bis-quaternary pyridinium aldoximes belonging to the so-called K-oximes, after Czech researcher Kamil Kuća, namely BT-07 (K005⁴¹) (**1a**), BT-08 (K033⁴²) (**1b**), BT-07-4M (K074⁴¹) (**1c**), and obidoxime (**1d**) (Scheme), to coordinate palladium(II) (**1a–1c**) or platinum(II) cations (**1a–1d**).

2. Results and discussion

In the present research we studied the complexation properties of particular acetylcholinesterase reactivators (BT-07, BT-08, BT-07-4M, obidoxime), which differ in the position of oxime groups (*2-* / *4-*), as well as in the origin and in the length of bridges between both pyridinium rings (propyl, butyl, oxy-bis-methylene). Some of the studied compounds (obidoxime, for example) could be obtained commercially; on the other hand, there is a protocol to prepare a variety of bis-quaternary pyridinium aldoximes according to well-known synthetic



Scheme. Chemical structures of oxime-containing ligands.

procedures. The properties and purity of the final products are already well documented^{41,42} and are not subject of the present research.

Therapy for OPC intoxication generally faces two main problems: i) the low ability of ChRs to cross the blood–brain barrier and ii) fast ChR metabolism, leading to formation of inactive derivatives eliminated by the organism. Both processes result in low recovery of enzymatic activity and require regular patient treatment with ChR.⁴³ One of the strategies that can be applied to improve the biological activity of drugs is their modification by complexation in order to ensure a gradual decomposition of active species and in such a way to increase their availability in the organism. With this main purpose we evaluated the coordination ability of the ChRs mentioned above to bind palladium(II) or platinum(II) cations in order to find possible specific conditions for the isolation of metal-containing complex species of definite composition.

Currently UV-Vis spectroscopy is the most suitable method for the monitoring of complexation reactions and binding constant determination when ligands absorb in the UV-Vis area for several reasons: it works at relatively low concentrations, which avoids complications connected to association phenomena; measurements are possible in a variety of solvents (including water); the mathematical apparatus for complex formation is well defined by Beer's law; and the new chemometric approach allows to determine the binding constant without any approximations.⁴⁴ This is the reason for selecting UV-Vis spectroscopy in the case of ChRs to study their coordination ability towards Pt(II) or Pd(II) cations.

The UV-Vis spectra of oximes at pH 7.4 (Figure 1) indicate that ligands absorb in the range of 290–305 nm (BT-07 (**1a**), BT-08 (**1b**)) or 280–290 nm (BT-07-4M (**1c**), obidoxime (**1d**)), respectively, depending on the positions of oxime groups (2- or 4-). Partial conversion of oxime to oximate takes place at pH 7.4, seen by the less intensive absorbance band recorded in the wavelength interval from 340 to 360 nm, and this is in agreement with data published previously.^{38,45,46} In the presence of an excess of sodium carbonate, deprotonation of

ligands is completed and gives rise to absorbance of oximate anions (**13a–13d**) in the range from 330 nm to 360 nm due to the $\pi - \pi^*$ transitions within the aromatic system.^{47,48}

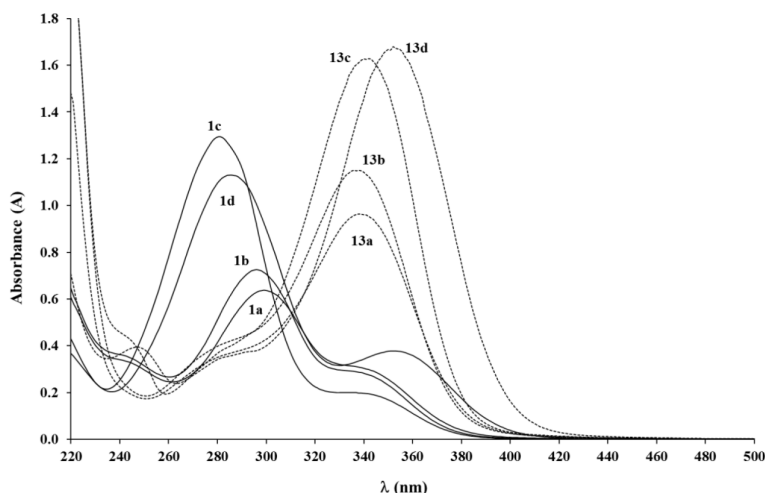


Figure 1. UV-Vis spectra of oximes at pH 7.4 (thick lines) and in the presence of base excess (dotted lines); $(\text{H}_2\text{L})^{2+} = 4 \times 10^{-5}$ M.

Addition of increasing amount of Pd(II) ions to oxime solutions leads to gradual deprotonation of ligands, resulting in decreased absorbance in the range of 280–300 nm and an increased one at ca. 350 nm, both accompanied by a shoulder at ca. 420 nm. The spectra of reaction mixtures containing Pd(II) ions and BT-07 (**1a**), BT-08 (**1b**), and BT-07-4M (**1c**) immediately after mixing the reagents (0 h) are shown in Figure 2.

Spectral changes occurring in Pd(II)-containing reaction mixtures within a week after mixing the reagents were further monitored. Data revealed that side reactions began to occur after 48 h, especially in the presence of high metal excess, disturbing the equilibrium and complicating the reaction systems. Time-dependent spectra of the Pd(II)-BT-08 system are given as an example (Figure 3).

Spectrophotometric study showed that deprotonation of oximes occurs in aqueous solutions (pH 7.4) in the presence of palladium(II) cations and formation of complex species takes place, resulting in the appearance of a new red-shifted absorption shoulder.

We performed analogous 1-week monitoring studies on the coordination ability of ligands **1a–1d** towards platinum(II) ions, as well. Two main points should be mentioned. First, the complexation reaction takes place more slowly than in the case of Pd(II) cations and equilibrium is reached within 24 h after mixing the reagents. Second, side reactions proceed faster, disrupting the equilibrium within the next 24 h (or 48 h after mixing the reagents). Similarly to Pd(II)-containing systems, side reactions were more pronounced at higher metal(II) excess. Time-dependent spectra of Pt(II)-BT-07-4M are shown as an illustration (Figure 4).

Summarizing, we were not able to observe the complete shift of the equilibrium towards Pd(II)/Pt(II)-containing species of selected ligands **1a–1d** (H_2L^{2+}) even in the presence of very high metal(II) excess, which makes the spectra of pure complexes unknown. Moreover, time-dependent side reactions limit the number of spectral data that can be used in calculation processing. The pH-dependent conversion of oximes to corresponding oximate species under the given reaction conditions should also be taken into account. All processes mentioned above exclude the application of standard procedures for determination of the solution

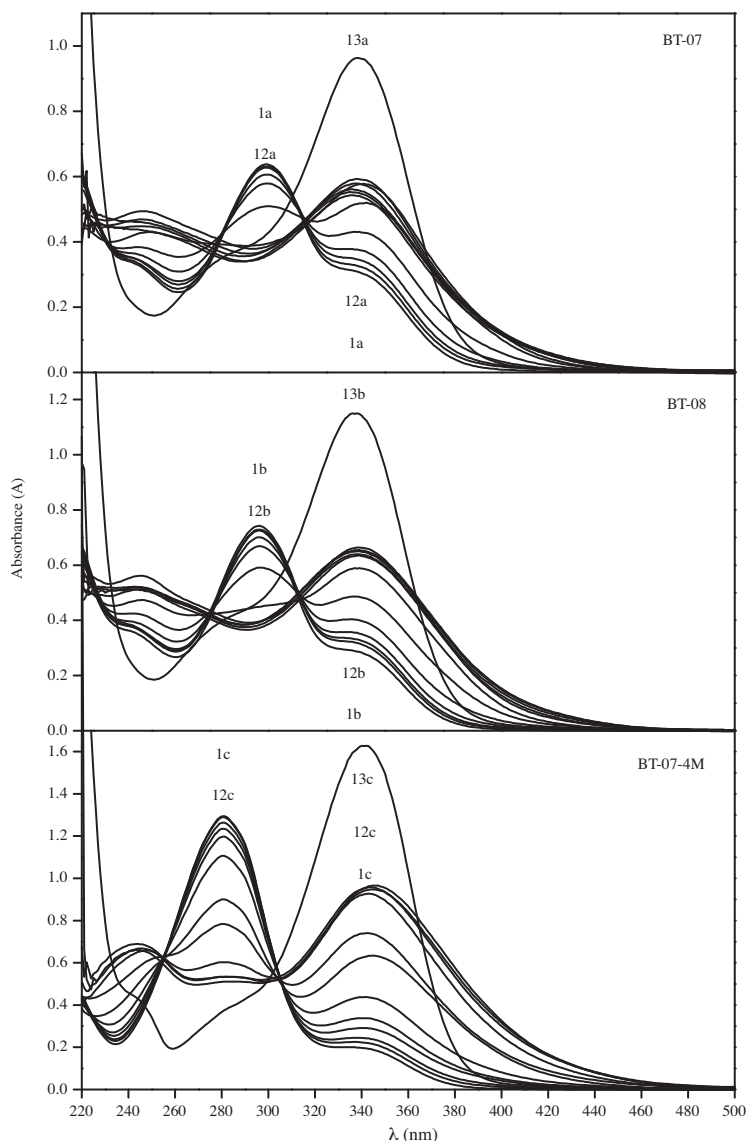


Figure 2. UV-Vis spectra of reaction mixtures containing Pd(II) ions and **1a–1c** (0 h) at various metal-to-ligand molar ratio, pH 7.4 (**1–10**, **2a–2c**; **1–8**, **3a–3c**; **1–6**, **4a–4c**; **1–4**, **5a–5c**; **1–2**, **6a–6c**; **1–1**, **7a–7c**; **2–1**, **8a–8c**; **4–1**, **9a–9c**; **6–1**, **10a–10c**; **8–1**, **11a–11c**; **10–1**, **12a–12c**); $(\text{H}_2\text{L})^{2+} = 4 \times 10^{-5}$ M.

equilibrium parameters.⁴⁰ For that reason, we took the advantage of the FiNAL procedure, which is based on resolution of overlapping bands and is a technique specially developed for quantitative analysis of such undefined, from the viewpoint of classical spectrophotometry, systems.^{49–51}

Using the FiNAL protocol we calculated molar parts of the free ligands (considering their conversion to corresponding oximates), as well as molar parts of Pd(II)/Pt(II)-containing complex species. Accounting also for time-dependent side reactions, spectral data used for stability constant computations were limited to the following metal-to-ligand molar ratios: Pt(II)-BT-07 – from 1:10 to 2:1, Pt(II)-BT-08 and Pt(II)-BT-07-4M – from 1:10 to 4:1, and Pt(II)-obidoxime – from 1:10 to 10:1. The whole spectral dataset was used to evaluate the conditional stability constants (β') of Pd(II)- H_2L^{2+} species.

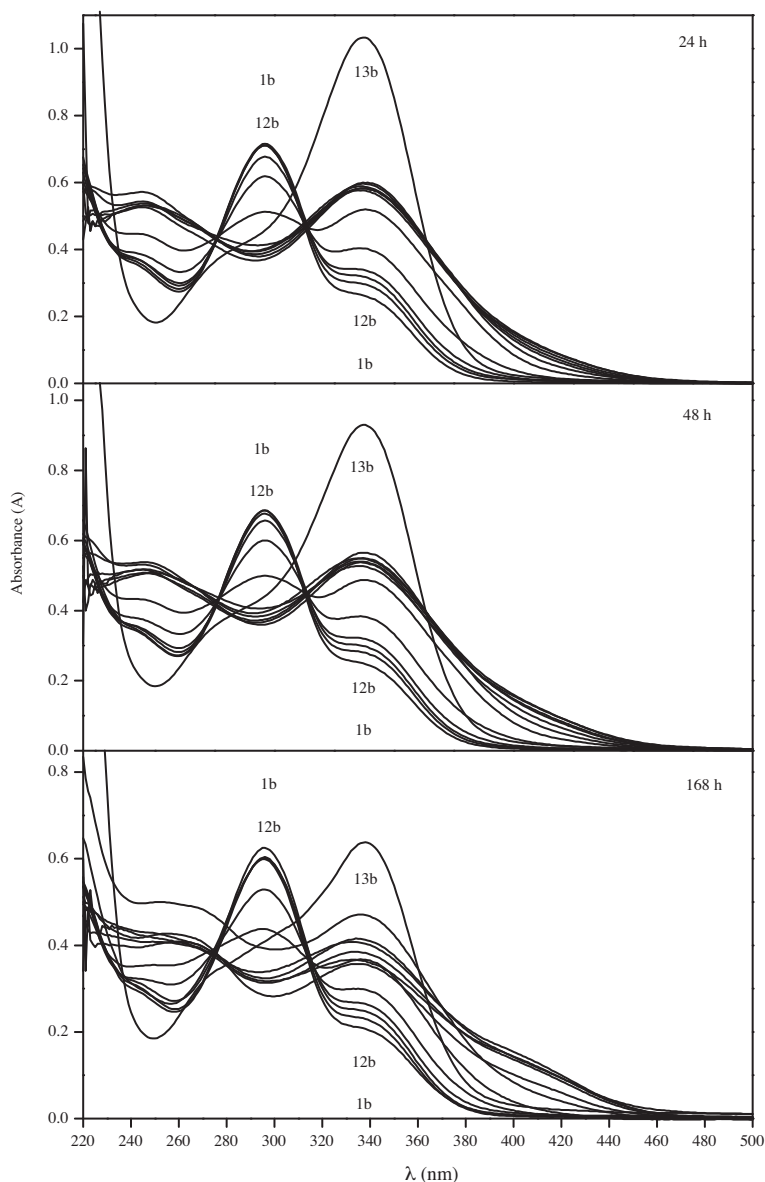
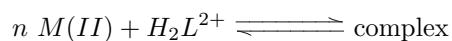


Figure 3. Time-dependent spectra of BT-08 (**1b**) in the presence of Pd(II) ions, pH 7.4, $(H_2L)^{2+} = 4 \times 10^{-5}$ M: 24 h (top); 48 h (middle); 168 h (bottom).

Having the stoichiometry of a complex process, corresponding conditional stability constants were calculated using the following reaction:



Details on the mathematical apparatus of the procedure and on evaluation of stability constants can be found in the literature.^{40,49–51} Data on calculated conditional stability constants of oxime complex species at pH 7.4 are summarized (Table) and spectra of the complexes of BT-07-4M are shown (Figure 5).

Experimental results showed that oximes BT-07 and BT-08 immediately coordinate two palladium(II) cations, forming complex species of composition $[Pd_2L]^{4+}$ stable up to 24 h. Ligand BT-07-4M forms two

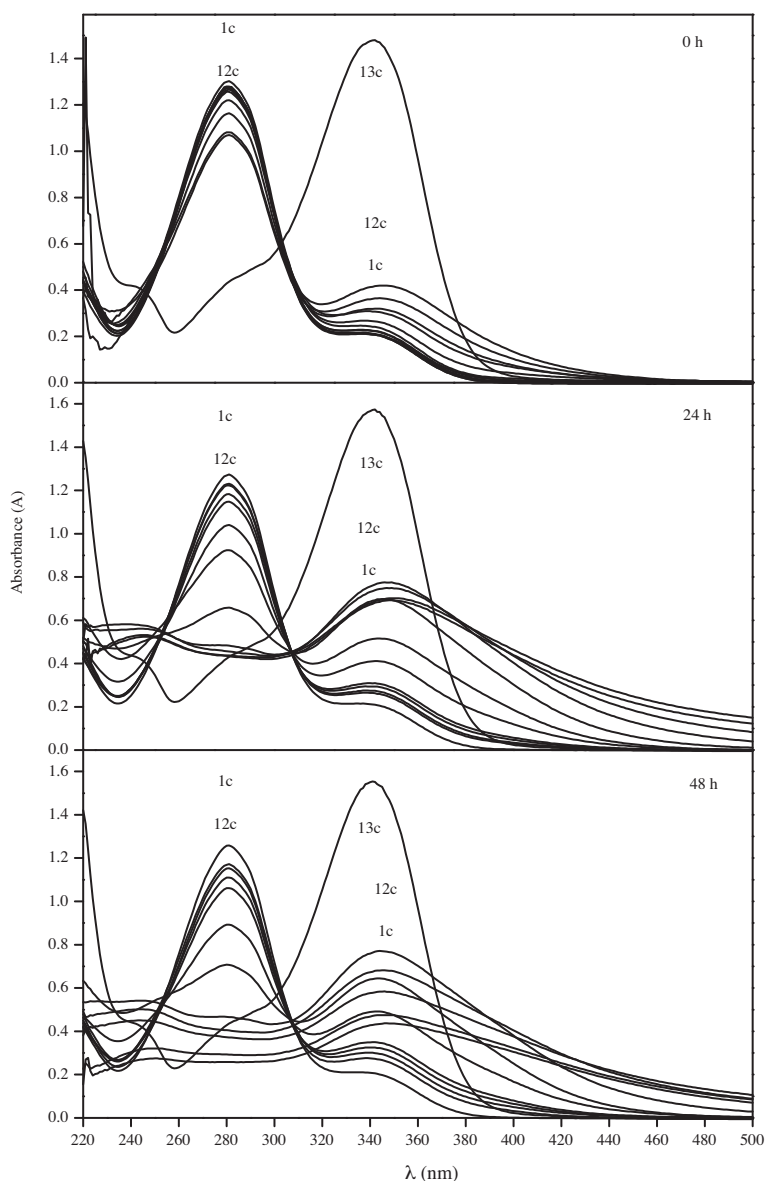


Figure 4. Time-dependent spectra of BT-07-4M (**1c**) in the presence of Pt(II) ions, $(\text{H}_2\text{L})^{2+} = 4 \times 10^{-5} \text{ M}$: 0 h (top), 24 h (middle), 48 h (bottom).

Table. Conditional stability constants (β') of complex species observed (R^2 : correlation coefficient).

Ligand	Pd(II)			Pt(II)		
	Complex	$\lg \beta'$	R^2	Complex	$\lg \beta'$	R^2
BT-07	$[\text{M}_2\text{L}]^{4+}$	9.93	0.9958	$[\text{MHL}]^{3+}$	5.91	0.9643
BT-08	$[\text{M}_2\text{L}]^{4+}$	8.66	0.9947	$[\text{MHL}]^{3+}$	6.61	0.9941
BT-07-4M	$[\text{MHL}]^{3+}$ $[\text{M}_2\text{L}]^{4+}$	6.32 8.28	0.9869 0.9941	$[\text{MHL}]^{3+}$	6.28	0.9699
Obidoxime ²⁷	$[\text{MHL}]^{3+}$ $[\text{M}_2\text{L}]^{4+}$	6.52 9.34	0.9874 0.9927	$[\text{MHL}]^{3+}$	6.26	0.9888

complexes of composition $[\text{PdHL}]^{3+}$ (0 h) and $[\text{Pd}_2\text{L}]^{4+}$ (24 h), respectively, similarly to obidoxime having both oxime groups on 4-positions in pyridinium rings.⁴⁰ At the same time, results revealed that coordination

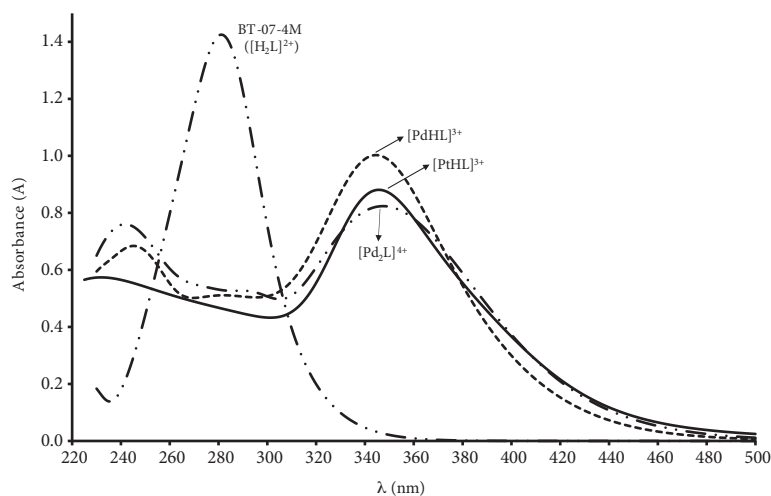


Figure 5. Calculated spectra of BT-07-4M and its complexes with palladium(II) and platinum(II) cations.

of Pt(II) cations proceeds more slowly as compared to Pd(II), forming only complexes of composition $[\text{PtHL}]^{3+}$ within 24 h after mixing the reagents. We could not observe formation of species $[\text{Pt}_2\text{L}]^{4+}$, most likely due to the side reactions occurring faster than in the case of Pd(II) ions.

To the best of our knowledge, very limited data exist in the literature regarding the complexation ability of ChRs. For the first time we report equilibrium data on complex formation between quaternary oximes and metal(II) ions. In summary, study of the coordination ability of bis-quaternary pyridinium aldoximes BT-07, BT-07-4M, BT-08, and obidoxime revealed that interaction with Pt(II) or Pd(II) ions is a complicated process accompanied by formation of positively charged complex species existing in equilibrium. Calculation of stability constants under the selected conditions suggests formation of considerably stable species, but at the present stage of the research no specific conditions for isolation of observed complexes in solid state have been found. From the experimental data available to date it is highly speculative to propose a coordination mode of the ligands studied. Nevertheless, we were able to observe two complex species formed by ChRs and to determine stability constants of Pt(II) and Pd(II) complexes of ChRs. Calculations were performed using the FiNAL protocol, because the spectra of free ligands and their complexes overlap upon complexation, and it is possible to be done only applying advanced data processing. The experimental data obtained are a good starting point to search for various possibilities of shifting equilibrium towards particular complex species in order to isolate compounds of definite composition, which will enable their structural characterization and the performance of further reactivation assays.

In conclusion, the complexation of bis-quaternary pyridinium aldoximes with Pd(II) cations at pH 7.4 leads to formation of $[\text{PdHL}]^{3+}$ or $[\text{Pd}_2\text{L}]^{4+}$ complex species depending on the chemical structure of the ligands and on the reaction time. It is shown that complexation of oxime-containing ChRs obidoxime, BT-07, BT-07-4M, and BT-08 with Pt(II) cations (pH 7.4) leads to the formation of mononuclear complex species of composition $[\text{PtHL}]^{3+}$. The coordination reaction of platinum(II) cations is a slower process compared to that of palladium(II) cations, but all complex species found possess considerable stability under the given reaction conditions.

3. Experimental

Bis-quaternary pyridinium aldoximes **1a–1d** were kindly provided by the Research Laboratory of Military Toxicology (Military Medical Academy, Sofia, Bulgaria). Diammonium tetrachloropalladate ((NH₄)₂PdCl₄) and diammonium tetrachloroplatinate ((NH₄)₂PtCl₄) were supplied by Sigma-Aldrich (Germany). Britton–Robinson buffer (pH 7.4) was freshly prepared before experiments by mixing 8 × 10⁻² M phosphoric acid, boric acid, and acetic acid with an appropriate volume of 4 × 10⁻² M NaOH.^{52,53} All reagents were of analytical grade; deionized water (18.2 MΩ.cm) was used in experiments.

Complexation reactions were followed spectrophotometrically (220–500 nm) on a Shimadzu UV-1800. Stock solutions of reagents (4 × 10⁻² M) were prepared in Britton–Robinson buffer (pH 7.4). Appropriate volumes of these solutions were mixed to obtain series of mixtures containing metal-to-ligand molar ratio varying from 1:10 to 10:1 (**2a–2d** and **12a–12d**) in a final volume of 1 mL. The spectra of oximates (**13a–13d**) were recorded after addition of an excess of carbonate to solutions of **1a–1d**. The final concentration of ligands studied was kept constant at 4 × 10⁻⁵ M. Spectra of metal-containing species were recorded against solutions containing corresponding concentrations of palladium(II)/platinum(II) ions. Spectral changes were monitored immediately after mixing the reagents and for up to 1 week. Calculation of molar parts of observed species and of complexes' spectra was performed using the FiNAL chemometric procedure.^{49–51}

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