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# Characterization of Lead (II)—Tris Complex

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**Abstract:** Tris, or known during medical use as tromethamine or THAM (tris (hydroxymethyl) aminomethane), is an organic compound with the formula $NH_2C(CH_2OH)_3$. It is extensively used in biochemistry and molecular biology as a component of buffer solutions such as in TAE (Tris, Acetic acid and EDTA (Ethylenediaminetetraacetic acid)) and TBE (Tris, Boric acid and EDTA (Ethylenediaminetetraacetic acid)) buffers, especially for solutions of nucleic acids. It contains a primary amine and thus undergoes the reactions associated with typical amines, e.g. condensations with aldehydes. Tris also complexes with metal ions in solution. Lead (II) chloride ($PbCl_2$) is an inorganic compound which is a white solid under ambient conditions. Lead (II) chloride is one of the most important lead-based reagents. In this research the tris -$Pb^{2+}$ complex was studied using a spectrophotometer. The study showed that complex is optical active and $Pb^{2+}$ coordinate a thermally stable complex with Tris buffer.

**Key words:** Absorption spectra, Lead (II) chloride, spectroscopy.

## 1. Introduction

Tris, or known during medical use as tromethamine or THAM (tris (hydroxymethyl) aminomethane), is an organic compound with the formula $NH_2C(CH_2OH)_3$. It is extensively used in biochemistry and molecular biology as a component of buffer solutions [1-7] such as in TAE (Tris, Acetic acid and EDTA (Ethylenediaminetetraacetic acid)) and TBE (Tris, Boric acid and EDTA (Ethylenediaminetetraacetic acid)) buffers, especially for solutions of nucleic acids. It contains a primary amine and thus undergoes the reactions associated with typical amines, e.g. condensations with aldehydes. Tris also complexes with metal ions in solution [8-12]. In medicine, tromethamine is occasionally used as a drug, given in intensive care for its properties as a buffer for the treatment of severe metabolic acidosis in specific circumstances [13-18]. Some medications are formulated as the "tromethamine salt" including hemabate (carboprost as trometamol salt), and "ketorolac trometamol" [19].

The useful buffer range for tris coincides with the physiological pH typical of most living organisms. This, and its low cost, make tris one of the most common buffers in the biology/biochemistry laboratory. Tris is also used as a primary standard to standardize acid solutions for chemical analysis. Tris is used to increase permeability of cell membranes [20-23]. It is a component of the Moderna COVID-19 vaccine [24] and Pfizer-BioNTech COVID-19 Vaccine for use in children 5 through 11 years of age [25].

Lead is a chemical element with the symbol Pb (from the Latin plumbum) and atomic number 82. It is a heavy metal that is denser than most common materials. Lead is soft and malleable, and also has a relatively low melting point. When freshly cut, lead is silvery with a hint of blue; it tarnishes to a dull gray color when exposed to air. Lead has the highest atomic number of any stable element and three of its isotopes are endpoints of major nuclear decay chains of heavier elements. Lead is a relatively unreactive post-transition metal. Its weak metallic character is illustrated by its amphoteric nature; lead and lead oxides react with acids and bases, and it tends to form covalent bonds. Compounds of lead are usually found in the +2 oxidation state rather than the +4 state common with lighter members of the carbon group. Exceptions are

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mostly limited to organolead compounds. Like the lighter members of the group, lead tends to bond with itself; it can form chains and polyhedral structures. Lead is a devastating and persistent neurotoxin that accumulates in soft tissues and bones. It damages the nervous system and interferes with the function of biological enzymes, causing neurological disorders ranging from behavioral problems to brain damage, and also affects general health, cardiovascular, and renal systems [26-31].

Lead (II) chloride ($PbCl_2$) is an inorganic compound which is a white solid under ambient conditions. It is poorly soluble in water. Lead (II) chloride is one of the most important lead-based reagents [32]. It also occurs naturally in the form of the mineral cotunnite. In solid $PbCl_2$, each lead ion is coordinated by nine chloride ions in a tricapped triangular prism formation—six lie at the vertices of a triangular prism and three lie beyond the centers of each rectangular prism face. The 9 chloride ions are not equidistant from the central lead atom, 7 lie at 280-309 pm and 2 at 370 pm [33]. $PbCl_2$ forms white orthorhombic needles.

## 2. Materials and Methods

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum. UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied. UV/Vis spectroscopy experiments were performed using a Varian Cary 100 Bio. Tris and $PbCl_2$ were mixed in 1 cm quartz cuvettes.

## 3. Results and Discussion

As known that Tris is form complexes with metal ions in solution [8], in this research the tris -$Pb^{2+}$ complex was studied using a Uv/Vis spectrometer. Lead (II) chloride is characterized by a strong absorption peak within 205 nm, while tris buffer is characterized by an absorption within 197 nm (Fig. 1, left). After adding Lead (II) chloride in solution containing tris, it indicates an additional peak at 255 nm (Fig. 1).

### *3.1 Titration Experiments*

For titration experiments, 100 μM concentrations of Lead (II) chloride were detected, and different concentrations of tris were added and the absorption spectra were taken. 100 μM Lead (II) chloride is characterized by significant absorption. During titration, the lead peak was reduced and the peak of the lead-tris complex was increased (Fig. 2). The spectrum passed on saturation at 3: 1 ratio of Tris: metal (300: 100 μM). At higher concentrations of lead the lead peak

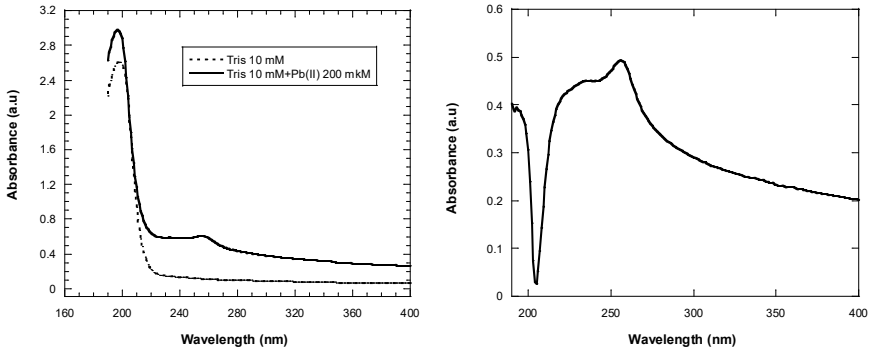



**Fig. 1 Left: dashed line: tris 10 mM, solid line: tris 10 mM + 200 μM $PbCl_2$, right: same experiment, solid line minus dashed line. pH 9.3.**

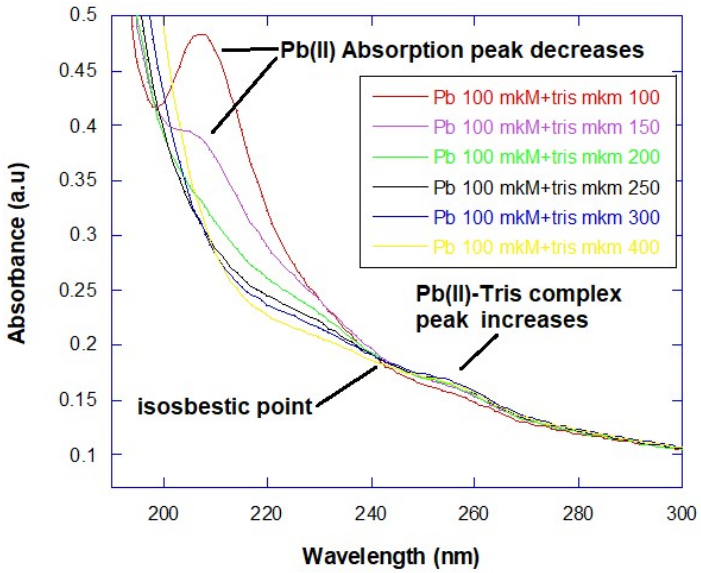



**Fig. 2 Fragment of titration experiments: $PbCl_2$ 100 µM, tris 100-400 µM, pH 9.3.**

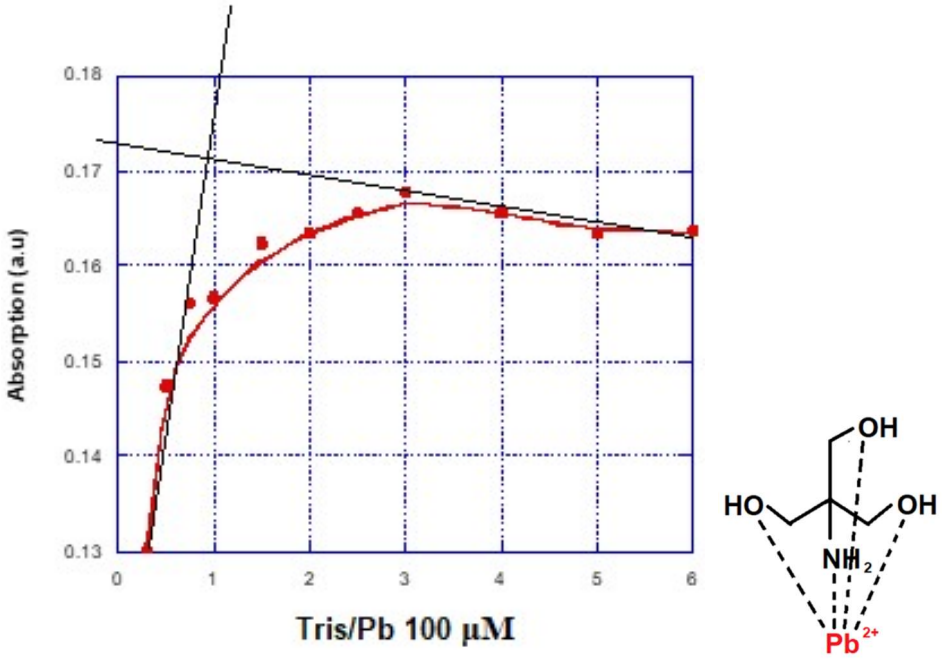



**Fig. 3 Left: Absorption of Tris/$Pb^{2+}$ ratio, $PbCl_2$ 100 µM, pH 9.3. Right: Supposed schematic form of $Pb^{2+}$ - Tris complex at pH 9.3.**

was again observed and increased. An absorption (y) and tris/lead ratio (x) graphs were constructed using a titration data (Fig. 3, left). From graph was determined the approximate optimal molecular ratio between tris and metal: the point of intersection of tangents, as shown 1: 1 ratio at pH 9.3. As known the amino group is involved as a main binding site [8] (Fig. 3, right).

*3.2 Melting Experiments*

To study the thermal stability of the complex, melting experiments were performed with a UV/Vis spectrometer at 255 nm wavelength. Experiments have shown that the complex has a thermal sufficiently stable structure: Tm ≈ 80 °C (10 mM tris and 5 µM $PbCl_2$) (Fig. 4, left). As a result shown, the peak of the complex at 99 °C has not completely disappeared (Fig. 4, right).

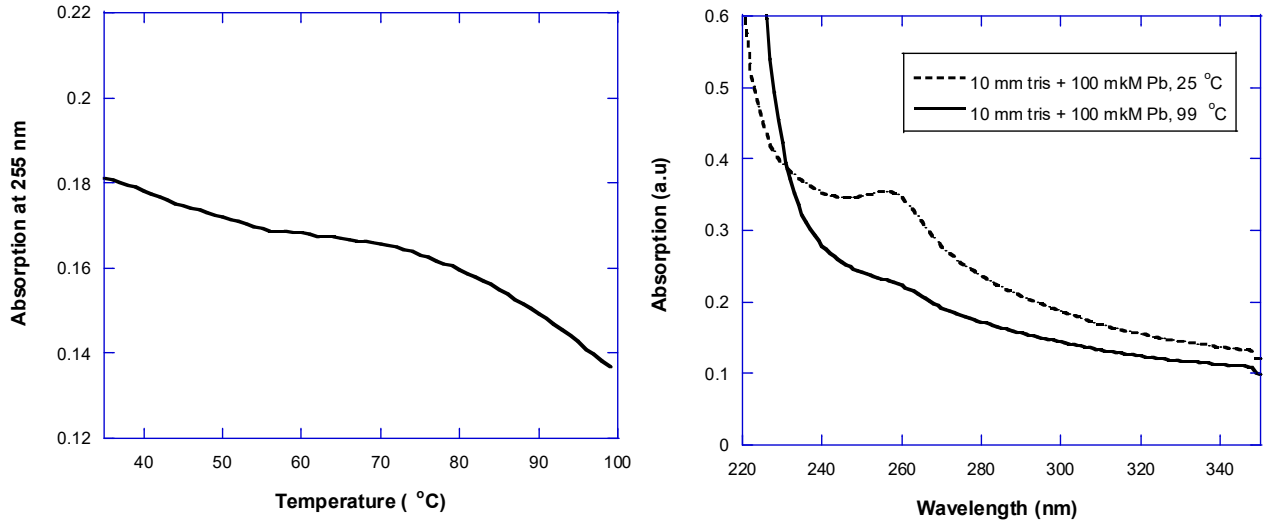



**Fig. 5 Left: Uv melting of Tris-$Pb^{2+}$ complex at 255 nm, 10 mM Tris and 5 µM $PbCl_2$. pH 9.3. Right: Absorption spectra of 10 mM Tris + 100 µM $PbCl_2$. Dashed line: 25 °C and solid line: 99 °C. pH 9.3.**

## 4. Conclusions

The study showed that $Pb^{2+}$ coordinate a thermally quite stable complex with Tris buffer (Tm ≈ 80 °C at 10 mM tris and 5 µM $PbCl_2$), the complex is characterized by absorption at ≈ 255 nm, titration experiments have shown that the supposed molecular ratio of the complex is 1: 1 at pH 9.3. This study will help Nucleic acids researchers with a spectrophotometer (absorbance at ≈ 260 nm) to avoid overlapping of the absorption signal in the presence of lead ions with Tris buffer.

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## Conflicts of Interest

The authors declare no conflict of interest.

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