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Exploring the Role of HEPES Buffer in the Synthesis and Characterization of Lanthanide-Nucleotide Complexes

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Abstract. Lanthanide-nucleotide complexes are of interest due to their unique luminescent properties and have potential applications in a wide range of fields from medical imaging to environmental sensing. Given the sensitive nature of biochemical reactions, the choice of reaction conditions including pH plays a crucial role in determining the synthesis yield. One of the buffering agents that has gained prominence in this synthetic methodology is HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). The development of lanthanide and nucleotide materials in the field of diagnostics has been widely carried out This study investigates the role of HEPES buffer in maintaining stable pH conditions, which are critical for the successful formation of lanthanide-nucleotide complexes. Terbium complexes with adenosine triphosphate ligands have been successfully synthesized with a maximum reaction time of 60 minutes. The complex was characterized using a UV-Vis spectrophotometer where the absorption peak at a wavelength of 257.5 nm indicates the occurrence of π-π* electron transitions. There is an absorption band at a wavelength of 349 cm^{-1} in the TbATP complex using an FT-IR spectrophotometer, this can be associated with the vibration of the Tb-N bond, which confirms the formation of a terbium complex with adenosine triphosphate. A sharp infrared absorption band at a wavelength of 630 cm^{-1} indicates the presence of vibrations of the O-H bond bound to the terbium ion, namely the formation of a Tb-OH complex bond, indicating that the phosphate group in ATP is involved in the formation of the complex.

Introduction

Lanthanide metals from La to Lu have similar chemical properties, unique 4f electronic structures, different catalytic, magnetic, and optical properties. Lanthanide ions (Ln^{3+}) have been coordinated with various ligands to improve their properties so that they can be used as imaging contrast agents, catalysts, and biosensors (Xu et al., 2018). One of the ligands that can bind lanthanides is nucleotides by binding to the hydroxyl group in ribose sugar, oxygen atoms in phosphate groups, and oxygen atoms and nitrogen atoms in purine and pyrimidine bases (Hoffmann et al., 2017;Santi et al., 2020). Monodentate or multidentate coordination can occur depending on the nature and size of the metal and the reaction conditions such as solvent and pH (Zhou et al., 2021). The aromatic N atoms of nucleobase residues have quite good affinity for metal ions, for example, Hg^{2+}, Cd^{2+}, Pt^{2+}, and Pd^{2+}. The oxygen of the phosphate group preferentially interacts with "hard" metal ions such as Fe(III) (Z. Liu et al., 2019), Al(III), Mn(II), Mg(II), Na(I), or Ln(III) (Gan et al., 2021).

The combination of different lanthanide metals and nucleotides will produce various interesting materials. Research that has been done using terbium (Tb^{3+}) with AMP has detected Ag^{+} ions up to a concentration of 60 nM (Tan & Chen, 2011). Tb^{3+} ions have been synthesized with GDP to form a stable Coumarin@GDP-Tb polymer using carboxycoumarin in Tris-HCl buffer, this complex shows blue emission of coumarin and green emission of Tb^{3+} with an excitation wavelength of 290 nm which can be used to detect sulfide ions (Zeng et al., 2021).

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(Sun et al., 2023) have developed a nucleotide and Tb^{3+} based probe for rapid, enzyme-free, and isothermal detection of Ag^{+}. This complex has been successfully applied to water samples and can detect Ag^{+} ions in situ in food and environmental monitoring. The development of lanthanide and nucleotide materials in the field of diagnostics has been widely carried out, but what is different in this study is the synthesis of a terbium complex with adenosine triphosphate ligands by adding HEPES buffer, a zwitterionic molecule that maintains a stable pH level. This buffer is very effective in biological systems because it interferes minimally with enzymatic reactions. In addition, this buffer has low UV absorption, making it suitable for spectroscopic studies. The characteristics of the complex compounds obtained were determined using an FT-IR spectrophotometer and a UV-Vis spectrophotometer where it is expected that the complex material has optical properties that have the potential to be developed as a sensor material in the future.

Experimental

Material and Methods

The materials used in this study were ATP (Xi'an Finest Nutra), $TbCl_3$ (Merck), aquabidest, HEPES (Intralab ekatama), Universal pH paper, 5 mL serological pipette, 20 mL serological pipette, Eppendorf tube (1.5 mL), blue tip, Sensi Nitrile Disposable Gloves, 10 ml vial bottle, 100 ml glass bottle, Aluminum foil, Tissue, NaOH, HCl, Silica gel.

The instrumentation used in this study was a Shimadzu IRPrestige-21 Fourier Transform Infrared (FT-IR) Spectrophotometer, a Shimadzu UV-2600 UV-Vis spectrophotometer, and equipment such as a magnetic stirrer, centrifuge, micropipette, analytical balance, and glassware commonly used in chemical laboratories.

Procedures

Synthesis of Tb-ATP complex compound

In the initial stage, the synthesis of trivalent terbium metal ($TbCl_3$) with adenosine triphosphate (ATP) ligand will be carried out using 5 mL of $TbCl_3$ (5 mM) added to 5 mL of ATP (10 mM) (B. Liu et al., 2014) and added with HEPES buffer pH 7.4 and stirred for 2 hours. The Tb-ATP complex product was centrifuged at 14,000 rpm for 10 minutes, followed by washing three times with aquabidest and centrifuged, so that a white complex compound product was obtained

Characterization of complexes with several instruments

The Tb-ATP complex material obtained was characterized by several instruments, namely FT-IR Spectrophotometer and UV-Vis Spectrophotometer. FT-IR Spectrophotometer is used to determine functional groups of metals, ligands, complex compounds and to determine the interactions formed from the complex, UV-Vis Spectrophotometer is used in determining the maximum reaction time and complex formation.

Result and Discussion

Characterization of complex compounds using UV-Vis spectrophotometer

Figure 1 shows the interaction of the terbium complex with adenosine triphosphate ligand using a UV-Vis spectrophotometer. The presence of an absorption peak in the wavelength region of 257.5 nm indicates the occurrence of π-π* electron transition in the metal complex to adenosine triphosphate (Yin et al., 2022).

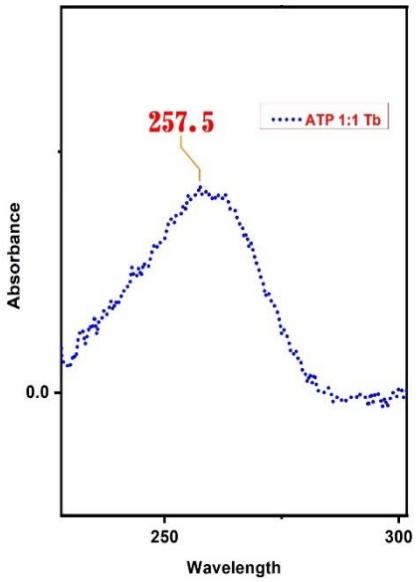

Figure 1. Absorption spectrum of Terbium(III) complex compound with Adenosine triphosphate ligand.

Determination of the reaction time of the TbATP complex

The UV-Vis absorbance spectrum of the formation of the Tb-ATP metal complex was observed with a time variation of 0-150 minutes (Figure 2). At the beginning of the reaction, the absorbance was observed to increase rapidly until the 10^{th} minute, then increased absorbance until reaching a maximum reaction time of 60 minutes, which indicates maximum absorption at a reaction time of 60 minutes between Tb^{3+} ions and ATP.

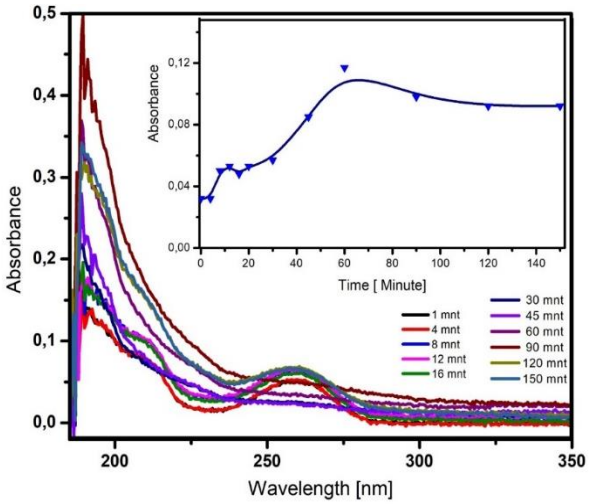

Figure 2. Absorbance graph of Tb-ATP at various time variations.

Characterization of complex compounds using FT-IR spectrophotometer

Figure 3 shows the red infrared absorption spectrum of $TbCl_3$ metal, ATP, and TbATP complex. The significant difference between the TbATP spectrum and the spectrum of $TbCl_3$ metal and ATP ligand indicates the interaction and formation of new chemical bonds. There is an absorption band at a wavelength of 349 cm^{-1} in the TbATP complex, this can be associated with the vibration of the Tb-N bond, which confirms the formation of a terbium complex with adenosine triphosphate involving a nucleobase group that has a nitrogen atom (Santi et al., 2024).

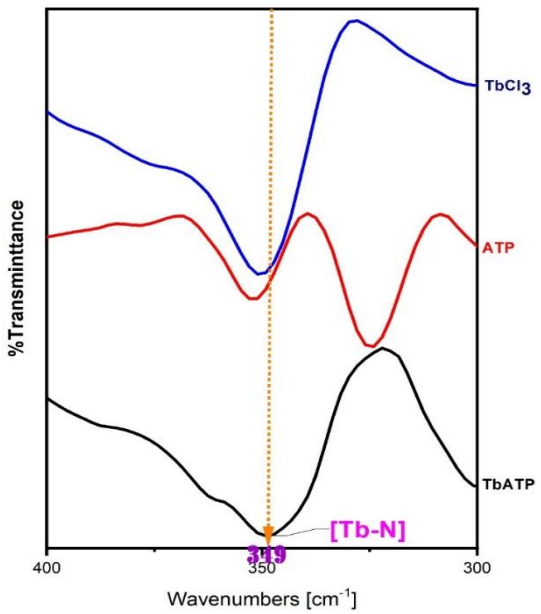

Figure 3. FT-IR spectra of $TbCl_3$, ATP, and TbATP.

While Figure 4 shows a sharp red infrared absorption band spectrum at a wavelength of 630 cm^{-1} indicating the presence of vibrations of the O-H bond bound to the terbium ion, namely the formation of a Tb-OH complex bond indicating that the phosphate group in ATP is involved in the formation of the complex, when compared to the metal $TbCl_3$ there is no absorption peak in this area while in the ATP ligand there is a weak absorption peak at a wavelength of 640 cm^{-1}. This shift indicates a change in the chemical environment of certain functional groups due to interaction with terbium ions (Wang et al., 2023).

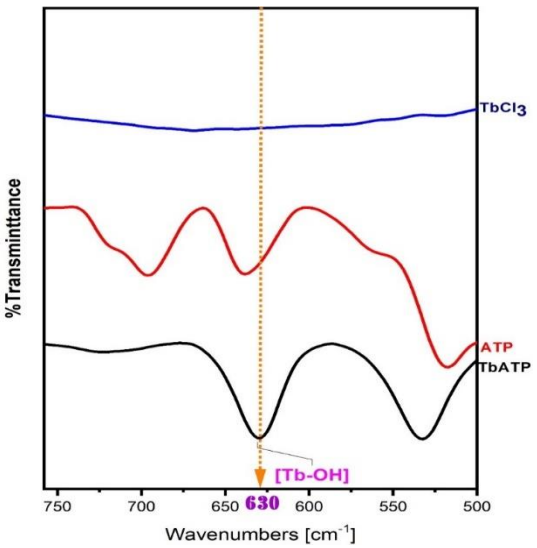

Figure 4. FT-IR spectra of $TbCl_3$, ATP, and TbATP.

Adenosine triphosphate (ATP) molecule is a complex organic molecule, consisting of a nitrogenous base (adenine), ribose sugar, and three phosphate groups. In the synthesized complex (Figure 5), ATP will bind through a coordination bond with the terbium ion through its donor atom, such as oxygen in the phosphate group or nitrogen in the nitrogenous base (Santi et al., 2021) (Mitta et al., 2019).

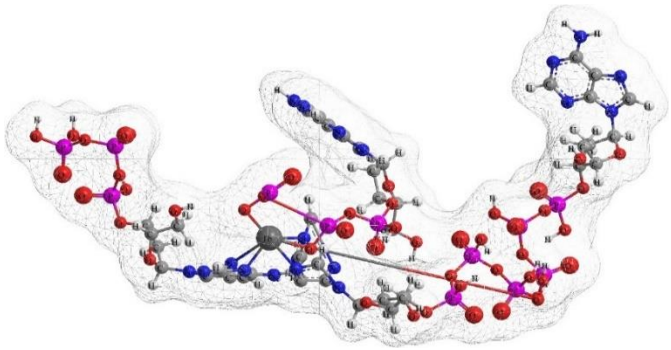

Figure 5. Complex structure of terbium with adenosine triphosphate.

Conclusion

Terbium complex with adenosine triphosphate using HEPES buffer has been successfully synthesized with a maximum reaction time of 60 minutes. Characterization of the complex using a UV-Vis spectrophotometer where the peak absorption wavelength of 257.5 nm indicates the occurrence of π-π* electron transitions. There is an absorption band using an FT-IR spectrophotometer, which indicates the occurrence of Tb–N bond vibrations and Tb–OH complex bonds, which confirms the formation of a terbium complex with adenosine triphosphate. Based on the characteristics of the terbium complex, it has the potential to be a sensor material in the future.

Conflict of Interest

The authors declare that there is no conflict of interest.

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