Silver Nanoparticles Enhanced Luminescence of Terbium Complex in Solution for L-Dopa Determination

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Luminescent properties of a terbium (Tb³⁺)-L-3, 4-dihydroxyphenylalanine (L-dopa) complex by binding to colloidal silver nanoparticles (Ag NPs) have been presented. Luminescence intensity of the L-dopa complex was dramatically enhanced about 6–7 times by introducing Ag NPs. The Ag NPs concentration on the luminescent intensity was regarded as a main factor that balancing between an enhancing and a quenching effect of the Ag NPs. It was observed that changing the concentration of L-dopa causes the change in luminescence intensity. Under the optimized condition, the luminescence intensity of the system was linearly related to the concentration of L-dopa. Based on this observation, L-dopa-Tb³⁺ complex containing Ag NPs has been applied for the determination of L-dopa in pharmaceutical formulation. Linear responses of luminescence intensity were observed in the concentration range of 0.25 to 1.5 nM (r = 0.9934) of L-dopa with limit of detection 0.042 nM. The performance of the system was tested using 1.0 × 10⁻⁹ M of L-dopa, yielding a precision of 1.21% RSD for nine replicate measurements. The present method has been successfully applied to determine L-dopa in pharmaceutical samples.

Keywords: Terbium, L-3, 4-Dihydroxyphenylalanine, Luminescence, Silver Nanoparticles.

1. INTRODUCTION

Lanthanide ions such as terbium and europium ions and their complexes are interested in various nanotechnology and biotechnology applications such as fluorescence materials, electro luminescence devices, fluorescence labels and probes.¹–⁴ Lanthanides in an aqueous solution are known to be either non-fluorescent or weakly fluorescent due to their low molar absorptivities and poor quantum yields.⁵ However, luminescence property of lanthanide ions, in particular terbium (Tb³⁺) and europium (Eu³⁺) ions, can be dramatically enhanced by forming a chelate with ligands that have a broad intense absorption band. In this process, an efficient intramolecular energy transfer occurs from the excited triplet state of the ligands to the emitting level of the lanthanide ion.⁶–⁸ This sensitization process is not diffusion controlled because the lanthanide ion combines with ligands as donors by coordinate linkage. These ions form stable luminescent complexes with bidentate or multidentate ligands, especially when negatively charged oxygen donating groups are involved in ligand structures.⁹ It is well reported that lanthanide sensitized luminescence provide large Stokes shift, narrow emission bands and long luminescence lifetimes.¹⁰

In recent years, it has been well established that metallic nanostructures can modify the spectral properties of fluorophores.¹¹–¹³ Metal–fluorophores interactions that greatly enhance the luminescence intensity are termed as metal-enhanced fluorescence (MEF).¹² It is concluded in the study of MEF that fluorescent species are excited with an external light source and the energy from the electronically excited states is partially transferred or coupled to surface plasmons in metallic nanostructure.¹⁴ The coupled emission may be consequently emitted by the metal–fluorophore “unified system” with the identical spectral characteristics of the selected fluorophore.¹⁵ Recently many applications of MEF have been demonstrated including various bioassays.¹⁶–¹⁷ Solution-based MEF has also been presented by the Institute of Fluorescence, University of Maryland Biotechnology Institute.¹⁸–¹⁹

In this work, the luminescence of Tb³⁺ and Tb³⁺-L-3, 4-dihydroxyphenylalanine (L-dopa) complex by binding to silver nanoparticles (Ag NPs) in solution has been presented. L-dopa is an important neurotransmitter and is used...
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2. EXPERIMENTAL DETAILS

2.1. Reagents and Solution

L-dopa, terbium chloride (TbCl₃ • 6H₂O), tris (hydroxymethyl) amino methane, silver nitrate and sodium borohydride were obtained from Sigma-Aldrich. Other reagents used for this study were of analytical grade purity from a standard supplier. The primary stock solutions of the L-dopa (1.0 × 10⁻² M) were prepared using 0.1 M hydrochloric acid and deionized (DI) water. Stock solution of terbium chloride (1.0 × 10⁻² M) was prepared using DI water. The prepared stock solutions were stored in refrigerator at 4 °C. Tris-HCl buffer was prepared by dissolving appropriate amount of tris (hydroxymethyl) amino methane in 500 mL DI water and pH was adjusted using 0.1 M HCl. 1.0 mM of silver nitrate and 2.0 mM of sodium borohydride solution were prepared by dissolving appropriate amount of tris (hydroxymethyl) amino methane in 500 mL DI water and pH was adjusted using 0.1 M HCl. 1.0 mM of silver nitrate and 2.0 mM of sodium borohydride solution were prepared. Working solutions were prepared daily before use by required dilution of the stock solution.

2.2. Preparation and Characterization of Ag NPs

Ag NPs were prepared in accordance with the procedure described in a literature with some modification. Briefly, 30 mL of 2 mM NaBH₄ aqueous solution was taken in an Erlenmeyer flask and chilled in an ice bath under vigorous stirring then 10 mL of 1 mM AgNO₃ aqueous solution was added drop wise until the color of the colloidal solution was turned into light yellow. The color changing of the solution indicates the complete reduction of silver ions. This colloidal solution was stored at 4 °C before using. The prepared Ag NPs were characterized by UV-visible spectrum using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) and transmission electron microscopic (TEM) images using a Transmission electron microscope (TEM, Hitachi-7100, Japan) with an accelerating voltage of 100 kV. The TEM image of Ag NPs showed that the average diameters of the particles are approximately 15 ± 2 nm according to the scaling as depicted in Figure 1. The prepared Ag NPs exhibited intense absorption maximum at about 400 nm as shown in Figure 2(e) which is the characteristic plasmonic band of Ag NPs.

2.3. Apparatus and Procedure

Luminescence measurements were conducted using a spectrofluorometer (F-4500, Hitachi, Japan) equipped with a 150 W xenon lamp and photomultiplier tube (Model R 928, Hamamatsu, Japan). A pH meter (Orion, 520A, USA) was used for pH adjustment. UV-visible spectra were recorded by the UV-visible spectrophotometer. All experiments were performed at room temperature.

The following procedure was adopted for luminescence measurements. A certain volume of terbium chloride, 1.0 mL of buffer (pH, 8.5), appropriate volume of L-dopa solution were added to a 10 mL-volumetric flask. The mixture was diluted with 5 mL double DI water and allowed to stand for several minute to form complex and then a certain volume of colloidal solution of Ag NPs was added to the complex. The luminescence spectra of Tb³⁺, L-dopa-Tb³⁺ complex and L-dopa-Tb³⁺-Ag NPs were measured in

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for the treatment of neural disorders such as Parkinson’s disease. L-dopa is capable to form a strong chelate with Tb³⁺ ions because it contains an active group in the form of –COOH and –OH in the structure. It is already reported that terbium and europium ion can bind to form complex with similar organic ligand such as norfloxacin, nalidixic acid, ciprofloxacin, gatifloxacin. It was observed that terbium and europium ion can bind to form complex of –COOH and –OH in the structure. It is already reported as pharmaceutical formulation was to be tested.

The following procedure was adopted for luminescence measurements. A certain volume of terbium chloride, 1.0 mL of buffer (pH, 8.5), appropriate volume of L-dopa solution were added to a 10 mL-volumetric flask. The mixture was diluted with 5 mL double DI water and allowed to stand for several minute to form complex and then a certain volume of colloidal solution of Ag NPs was added to the complex. The luminescence spectra of Tb³⁺, L-dopa-Tb³⁺ complex and L-dopa-Tb³⁺-Ag NPs were measured in
3. RESULTS AND DISCUSSION

3.1. Absorption and Luminescence Spectra

UV-visible spectra of Tb\(^{3+}\), L-dopa, L-dopa-Tb\(^{3+}\), and L-dopa-Tb\(^{3+}\)-Ag NPs were recorded separately in order to comprehend the formation of complex and interaction of the complexes with Ag NPs. Figure 2(a) shows the characteristic UV spectra of Tb\(^{3+}\) with absorbance maximum at around 218 nm and L-dopa (Fig. 2(b)) with absorbance maxima at around 221 nm and 279 nm. As shown in Figure 2(c), the absorption maxima of the L-dopa found to be red shifted from 221 to 242 nm and 279 to 294 nm after reaction with Tb\(^{3+}\) solution in presence of tris-HCl buffer. This wavelength shifting indicates the formations of L-dopa-Tb\(^{3+}\) complex. The UV-visible absorption spectra of the L-dopa-Tb\(^{3+}\) complex containing colloidal solution of Ag NPs display further shifting of absorbance maxima of the L-dopa with a new absorbance maximum at around 400 nm (Fig. 2(d)). This observation clearly indicates the interaction of Ag NPs with the L-dopa-Tb\(^{3+}\) complex.

Tb\(^{3+}\) in aqueous medium emits very weak luminescence (Fig. 3(a)) due to weak absorption of metal ion itself. It can act as an energy acceptor from the lowest triplet state of organic ligand having strong absorption in the UV region.\(^{26}\) Therefore, enhanced luminescence of Tb\(^{3+}\) is occurred from the ligand-Tb\(^{3+}\) interaction. When the L-dopa-Tb\(^{3+}\) complex in solution was excited at 279 nm near the absorption wavelength of L-dopa, the enhanced emission band was observed at 490 nm and 545 nm (Fig. 3(b)) which are characteristic luminescence peaks of Tb\(^{3+}\) corresponding to the transitions of \(5D_{4} \rightarrow 7F_{5}\) and \(5D_{4} \rightarrow 7F_{6}\) respectively. The most intense peak was located at 545 nm. Thus the peak at 545 nm was chosen for the luminescence measurement throughout this work. Intramolecular energy transfer from L-dopa to Tb\(^{3+}\) may cause the enhancement of native weak luminescence of Tb\(^{3+}\) originating from the intra-chelate energy transfer from the triplet state of the organic ligand (L-dopa) to the excited energy levels of Tb\(^{3+}\) ion.\(^{27}\) The addition of the colloidal solution of Ag NPs to the L-dopa-Tb\(^{3+}\) complex enhanced the luminescence intensity spectacularly by 6–7 times (Fig. 3(c)) compared to that of the L-dopa-Tb\(^{3+}\) complex (Fig. 3(b)).

The interaction of Ag NPs with fluorophores has been reported to alter the free space absorption condition, which dramatically change the spectra from those observable in the absence of metallic nano surfaces.\(^{28}\) The spherical particles can enhance the extent of resonance energy transfer to the fluorophores through the interaction of the excited-state fluorophores and surface plasmon electron in the metal NPs.\(^{29}\) In this concept, the L-dopa-Tb\(^{3+}\) complex may interact with the surface plasmon electrons of Ag NPs. Fluorophores (Tb\(^{3+}\)) may be in close proximity with the Ag NPs in that state. In this condition, when Ag NPs containing the L-dopa-Tb\(^{3+}\) complex solution are excited, two processes may arise:

1. non-radiative coupling from the excited fluorophores (Tb\(^{3+}\)) to the surface plasmonic electrons of Ag NPs, which are subsequently radiated by the NPs themselves, and
2. enhancement of the absorption of light by the fluorophores due to increased electric field between and around the NPs.\(^{29}\)

Figure 3(c) is consistent with the reported speculation. The excitation spectra of the L-dopa-Tb\(^{3+}\)-Ag NPs system.
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3.2. Optimization of Reaction Parameters

The influence of pH of the L-dopa-Tb\(^{3+}\)-Ag NPs system on the luminescence intensity was investigated. The results showed that the luminescence intensity is increasing with the pH value from 7.0 to 8.5 and decreasing slowly above 8.5. Therefore pH 8.5 was selected as the optimum pH value for this experiment.

Concentrations of a colloidal solution of Ag NPs influenced the luminescence intensity of the system. The concentration effect was examined by varying the concentration of the colloidal solution from 2.2 \(\times\) 10\(^{-5}\) to 2.8 \(\times\) 10\(^{-4}\) M. The maximum intensity of the system was observed at 1.15 \(\times\) 10\(^{-4}\) M colloidal solution (Fig. 4) with good reproducibility, and this solution of Ag NPs was therefore used as the optimum concentration. It is reported that structural parameters of NPs such as size, shape and concentration have been reported to affect the fluorescence enhancement of lanthanide ions such as Tb\(^{3+}\) and europium. The dependence of the system’s luminescence intensity on the concentration of the colloidal solution indicates a dependence on the Ag NP concentration. It was therefore suggested that metal NPs can enhance or quench the luminescence.

When the colloidal solution of Ag NPs was added to the Tb\(^{3+}\)-L-dopa complex, the local refractive index around the terbium complex might have been changed and the electric dipole transition rate thereby modified. A colloidal solution concentration of more than 1.15 \(\times\) 10\(^{-4}\) M may distort the local field arising from the self-interaction of plasmon electrons of Ag NPs. This effect might have quenched the luminescence intensity.

3.3. Analytical Characteristics

Under the aforementioned optimal conditions, luminescence intensity of the L-dopa-Tb\(^{3+}\) complex containing Ag NPs system produced linear relationship with the concentration of L-dopa in the range of 0.25 to 1.5 nM with regression equation \(I = 82 + 811 \times C_{\text{L-dopa}}\) \((r = 0.9934)\). The limit of detection was calculated as defined by IUPAC, \(C_{\text{LOD}} = 3 \times Sb/m\) (where \(S_b\) is the standard deviation of the blank signals and \(m\) is slope) from the calibration curve below nano molar scale as 0.042 nM. The relative standard deviation (RSD) for 9 repeated measurements of 1.0 \(\times\) 10\(^{-9}\) M of L-dopa was 1.21%.

3.4. Interferences

The effects of some potentially interfering ions that can coexist in pharmaceutical samples were investigated. They can suppress or enhance the luminescence signal. Effect of the potential interferents was therefore investigated by
4. CONCLUSIONS

The luminescence of L-dopa-Tb$^{3+}$ complex in solution was highly enhanced by adding Ag NPs. Under the optimized condition, the luminescence intensity was enhanced linearly with the concentration of L-dopa. Therefore the Ag NPs enhanced luminescence of terbium-L-dopa complex has been applied for the determination of L-dopa in pharmaceutical formulation with satisfactory results.

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References and Notes


Table 1. Results for determination of L-dopa in pharmaceutical samples and recovery test.

<table>
<thead>
<tr>
<th>Sample Labeled</th>
<th>Proposed method</th>
<th>Added (×10⁻³ M)</th>
<th>Found (×10⁻³ M)</th>
<th>Recovery ± RSD (%)</th>
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<tbody>
<tr>
<td>L-dopa 1000</td>
<td></td>
<td>1.0</td>
<td>0.98 ± 0.12</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>1.98 ± 1.53</td>
<td>99.3</td>
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<tr>
<td></td>
<td></td>
<td>3.0</td>
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<tr>
<td></td>
<td></td>
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<td>3.97 ± 1.36</td>
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<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>4.92 ± 1.23</td>
<td>98.4</td>
</tr>
</tbody>
</table>

*Relative standard deviation for six replicate measurements (n = 6).

Active ingredients in one tablet.

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