UV and fluorescence spectral changes induced by neodymium binding of 
\( \text{N, N'} - \text{ethylenebis[2-(o-hydroxyphenolic)glycine]} \) and 
\( \text{N, N'} - \text{di(2-hydroxybenzyl)ethylenediamine-N, N'} \) diacetic acid

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Abstract

In 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 7.4 and room temperature, the binding of neodymium to \( \text{N, N'} - \text{ethylenebis[2-(o-hydroxyphenolic)glycine]} \) (EHPG), or \( \text{N, N'} - \text{di(2-hydroxybenzyl)ethylenediamine-N, N'} \) diacetic acid (HBED) had been studied from 210 to 330 nm by means of difference UV spectra. Two peaks at 240 and 292 nm appear in difference UV spectra after neodymium binding to EHPG or HBED. The 1:1 stable complex can be confirmed from spectral titration curves. The molar extinction coefficient of \( \text{Nd–EHPG} \) and \( \text{Nd–HBED} \) complexes are 
\[ \Delta_{\text{Nd–EHPG}} = (12.93 \pm 0.21) \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}, \]
\[ \Delta_{\text{Nd–HBED}} = (14.45 \pm 0.51) \times 10^5 \text{ cm}^{-1} \text{ M}^{-1} \] at 240 nm, respectively. Using EDTA as a competitor, the conditional equilibrium constants of the complexes are 
\[ \log K_{\text{Nd–EHPG}} = 11.89 \pm 0.09 \]
\[ \log K_{\text{Nd–HBED}} = 12.19 \pm 0.15, \] respectively. At the same conditions, fluorescence measurements show that neodymium binding to EHPG leads to a quenching of the fluorescence of EHPG at near 310 nm. However, there is no obvious fluorescence change of HBED at 318 nm with the binding of neodymium to HBED.

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1. Introduction

Chemistry of transferrin is important for understanding the role of metals in health, disease, therapy, and diagnosis. Transferrin has two remarkably similar domains, in which iron is octahedrally coordinated to two oxygens from two tyrosine residues, one nitrogen from an imidazole ring of histidine residues, one oxygen from aspartic acid residue and a carbonate anion (the so-called “synergistic anion”) adjacent to arginine residue in an unknown state of protonation [1,2]. Both EHPG and HBED (Fig. 1) can form stable sexadentate complexes with lanthanide ions, similar to transferrin [3,4], which played important roles to determine transferrin how to coordinate with metal ions [5]. Lanthanides have been known for their diversity in biological effects, and the application of lanthanides in medicine has high potential [6,7]. In agriculture, lanthanides have been used to increase the production of crops and to promote the growth of livestock in China for many years [8]. So it is of interest to study the interaction of EHPG or HBED with lanthanide ions.

In this paper, we have observed that the difference UV spectra and fluorescence spectra of the complexes of EHPG or HBED with neodymium ion, a lanthanide, and obtained the conditional equilibrium constants of the complexes of EHPG or HBED with it. There are different effects on the fluorescence of HBED and EHPG during neodymium binding to HBED and EHPG.

2. Materials and methods

2.1. Materials

\( \text{N, N'} - \text{ethylenebis[2-(o-hydroxyphenolic)glycine]} \) (EHPG), \( \text{N, N'} - \text{di(2-hydroxybenzyl)ethylenediamine-N, N'} \) diacetic acid (HBED), \( 4-(2\text{-hydroxyethyl})\text{-1-piperazineethanesulfonic acid (Hepes)} \), disodium ethylenediaminetetraacetic acid (EDTA), were all analytical grade reagents and used without further purification. The purity of \( \text{Nd}_2\text{O}_3 \) is more than 99.9%.

2.1.1. Stock solutions

A stock solution of neodymium was prepared by dissolving weighed \( \text{Nd}_2\text{O}_3 \) in hydrochloric acid, which was standardized by compleximetric titration with EDTA using xylenol orange as
indicator in HAc/NaAc buffer at pH 5.5. The solutions of EHPG or HBED were prepared by dissolving weighed samples. Each solution was diluted to a definite volume with double distilled water. The stock solutions were standardized by compleximetric titration with standardized zinc at the same conditions as above.

2.2. Methods

Difference UV spectra were recorded on a Hewlett-Packard 8453 spectrophotometer. All experiments were carried out in 0.01 M Hepes, pH 7.4, at 25 °C, by using a jacketed cell holder connected to an external circulating water bath. A solution containing EHPG or HBED was titrated with a solution of neodymium to determine a value of the molar extinction coefficient $\Delta_{\text{Nd}}$ of the Nd–EHPG or Nd–HBED complex to use in the calculation. The sample was also titrated with a solution of neodymium ion that contained various concentrations of EDTA as a competing ligand to measure the equilibrium constants. To correct for dilution during each titration and normalize the results from different titrations, the absorbance data were converted to molar extinction coefficient ($\Delta_1\varepsilon$) by dividing the absorbance by the analytical concentration of EHPG or HBED.

Fluorescence spectra were measured on a Perkin-Elmer LS-50B fluorescence spectrophotometer. Excitation and emission bands were set at 8 and 10 nm on these experimental conditions, respectively. All experiments were carried out at the same conditions as for difference UV spectra. Spectra were recorded at 2 min intervals after the addition of neodymium.

3. Results and discussion

3.1. Difference UV spectra

Difference UV spectra can be used to measure the binding of metal ions with EHPG or HBED [3,5]. A set of difference UV spectra caused by the addition of aliquots of neodymium ($5.48 \times 10^{-4}$ M) solution to 2 ml EHPG ($4.17 \times 10^{-5}$ M) in 0.01 M Hepes at pH 7.4, 25 °C is shown in Fig. 2. It can be seen from Fig. 2 that two maxima peaks at 240 and 292 nm, and there was obvious increase at 240 and 292 nm with the addition of the solution of neodymium. After the addition of the solution of neodymium get to a certain value, the increase would reach maximum. The dramatic increase in absorbance in the UV region at 240 and 292 nm are attributed to perturbations of the $\pi-\pi^*$ transitions of the aromatic ring, due to the coordination of phenolic hydroxyl which are completely protonated at pH 7.4 [9]. So the absorption spectrum reflects the differences in absorptivities between the neodymium complex and the protonated form of the ligand. These phenomena were easily observed
in the spectra. Dividing the absorbance at 240 nm by the analytical concentration of EHPG or HBED, one obtained molar extinction coefficient ($\Delta \epsilon$), which normalize the results from different titrations and correct the dilution effect. $\Delta \epsilon$ at 240 nm were plotted as a function of the molar ratio of neodymium to the analytical EHPG concentration, shown in Fig. 3. It can be seen that there is a sharp break near $C_{Nd}/C_{EHPG} = 1$, showing the 1:1 ligand stoichiometry of the Nd–EHPG complex. From the slope of the initial line at $C_{Nd}/C_{EHPG} < 1$, molar extinction coefficient $\Delta \epsilon_{Nd–EHPG} = (12.93 \pm 0.21) \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}$ at 240 nm according to Fig. 3 and many times parallel titration experiments.

At the same conditions, the binding of neodymium to HBED can also be monitored by difference UV spectra, which is similar to Fig. 2. $\Delta \epsilon$ at 240 nm were plotted as a function of the molar ratio of neodymium to the analytical HBED concentration, shown in Fig. 4. From the plot, 1:1 stable Nd–HBED complex was confirmed and the molar extinction coefficient $\Delta \epsilon_{Nd–HBED} = (14.45 \pm 0.51) \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}$ was obtained.

### 3.2. Binding constants

Neodymium and EHPG or HBED can both form stable complex, which has obvious positive peak at 240 and 292 nm. Neodymium and EDTA can also form stable complex, but the absorbance of the complex at 240 nm can be neglected. So EDTA can be used as competing ligand during the titration. Assuming that the decrease of absorptivity at 240 nm, and at a certain $C_{Nd}/C_{EHPG}$ value is attributed to the transformation of Nd–EHPG to Nd–EDTA, the system being described by mass balance equations for neodymium ion, EHPG, and EDTA, the concentrations of species for EDTA, EHPG, and neodymium, can be calculated as following equations [9–11]:

\[
[\text{Nd–EDTA}] = \frac{\Delta \epsilon_{\text{obs}}(a) - \Delta \epsilon_{\text{obs}}(b)}{\Delta \epsilon_{Nd}} [\text{EHPG}]_t
\]

where $\Delta \epsilon_{\text{obs}}(a)$, $[\text{EHPG}]_t$ is the concentration of complex of Nd–EHPG, free EHPG, and total EHPG, respectively. $[\text{Nd–EDTA}]$, $[\text{EDTA}]_t$ is the concentration of Nd–EDTA complex, free EDTA and total EDTA, respectively. $\Delta \epsilon_{Nd}$ is molar extinction coefficient of Nd–EHPG complex.

The conditional binding constant, $K_{Nd–EDTA}$ of Nd–EDTA complex is $10^{13.68}$ [12]. Using Eq. (6) we can calculate the binding constant, $\log K_{Nd–EHPG} = 11.89 \pm 0.09$ from Fig. 3. In the calculation, the data used were at the range of $C_{Nd}/C_{EHPG}$ ratio less than 1.0.

In a similar way, we can obtained $\log K_{Nd–EDTA} = 12.19 \pm 0.15$ from Fig. 4.

### 3.3. Fluorescence spectra

Fig. 5 is the fluorescence spectra of EHPG ($4.17 \times 10^{-5}$ M) in 0.01 M Hepes, at pH 7.4 and room temperature. There is a fluorescence peak at near 310 nm. With the addition of neodymium the fluorescence at 310 nm was quenched gradually. Dividing the fluorescence intensity at 310 nm by the analytical concentration of EHPG, one obtained molar fluorescence intensity ($F_M$)

\[
[F_M] = \frac{\text{Fluorescence at 310 nm}}{[\text{EHPG}]_t}
\]
which normalize the results from different titrations and correct the dilution effect $F_M$ at 310 nm was plotted as a function of the molar ratio of neodymium to the analytical EHPG concentration is shown in Fig. 6. It can be seen that there is a sharp break near $C_{Nd}/C_{EHPG} = 1$, showing the 1/1 ligand stoichiometry of the Nd–EHPG complex. The binding of neodymium leads to a reduction in molar fluorescence intensity. At same experimental conditions a fluorescence peak at 318 nm can be observed for HBED. However, there is no obvious change of fluorescence intensity at 318 nm during the titration of neodymium in 0.01 M Hepes, at pH 7.4 and room temperature. $F_M$ at 318 nm was plotted as a function of the molar ratio of neodymium to the analytical HBED concentration is also shown in Fig. 6. The difference of fluorescence property is maybe attributed to the differences between two free ligands in 0.01 M Hepes at pH 7.4. The hydrogen-bonded ring structures in HBED belong to N···H–O type, while that in EHPG are O···H–O type [9,10]. With the addition of neodymium (compare with Fig. 5), intramolecular hydrogen bonds, N···H–O type hydrogen bond in HBED and O···H–O type in EHPG, are broken.

4. Conclusions

We have monitored the different spectra of EHPG or HBED in 0.01 M 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (Hepes), pH 7.4 and 25 °C. It can be drawn that neodymium can form stable complex with EHPG or HBED by 1/1 ratio. From the Figs. 3 and 4, the molar extinction coefficient of Nd–EHPG and Nd–HBED complexes are

$$\Delta \varepsilon_{Nd-EHPG} = (12.93 \pm 0.21) \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}, \quad \Delta \varepsilon_{Nd-HBED} = (14.45 \pm 0.51) \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}$$

at 240 nm, respectively. The conditional equilibrium constants of the complexes are obtained through calculation, $\log K_{Nd-EHPG} = 11.89 \pm 0.09, \log K_{Nd-HBED} = 12.19 \pm 0.15$. Neodymium binding to EHPG leads to a quenching of the fluorescence of EHPG at near 310 nm, but no obvious fluorescence change of HBED at near 318 nm is observed with the binding of neodymium to HBED.

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