Articles

Potentiometric and Spectrophotometric Study of 3-Hydroxyflavone−La(III) Complexes

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Flavonoids are a large family of natural compounds that includes flavones, flavonols, and chalcones, among others. These compounds are investigated due to their varied biological properties and their capacity to complex metal ions. In this work, the complexation of four 3-hydroxyflavones with the lanthanum(III) ion was studied. A series of novel complexes of La(III) with the selected 3-hydroxyflavones, 3,7-dihydroxyflavone (L_1), 3,7,3′-trihydroxyflavone (L_2), 3,5,7,3′,4′-pentahydroxyflavone (quercetin) (L_3), and 3,5,7,2′,4′-pentahydroxyflavone (morin) (L_4), were studied by means of potentiometric and spectrophotometric methods at 26.3 °C and 0.1 M ionic strength in a water−dioxan (1:1, v/v) medium. Their formation constants were evaluated by the Calvin−Bjerrum and Irving−Rossotti methods. Complex 1:1 was found for all four ligands, whereas the 2:1 ligand−metal stoichiometry was found for only three of the four ligands. The obtained protonation constants of the ligands were: L_1, log $K^H{}_1 = 9.128 \pm 0.664$; L_2, log $K^H{}_1 = 11.091 \pm 0.134$, log $K^H{}_2 = 8.822 \pm 5 \cdot 10^{-4}$; L_3, log $K^H{}_1 = 9.310 \pm 0.119$, log $K^H{}_2 = 8.382 \pm 0.109$; L_4, log $K^H{}_1 = 10.021 \pm 0.355$, log $K^H{}_2 = 7.173 \pm 0.576$. The determined stability constants were: L_1−La(III), log $K_1 = 7.269 \pm 0.387$; L_2−La(III), log $K_1 = 9.164 \pm 0.020$, log $K_2 = 5.200 \pm 0.185$; L_3−La(III), log $K_1 = 8.143 \pm 0.284$, log $K_2 = 5.632 \pm 0.585$; L_4−La(III), log $K_1 = 7.407 \pm 0.596$, log $K_2 = 4.830 \pm 0.165$.

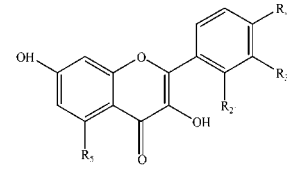
Introduction

Flavonoids are a large family of natural polyphenolic compounds widely distributed in plants. This family includes flavones, flavonols, and chalcones, among others. Beneficial effects of flavonoids on human health have gained increasing interest among researchers over the last few years. These compounds show varied biological properties including activity against HIV[1,2] and the dengue[3] and influenza virus[4] as well as antitumoral[5,6] and antioxidant[7] effects. Most of the pharmacologic effects of flavonoids seem to be associated with their antioxidative properties since flavonoids may act as free radical scavengers. Metallic ion complexes of these polyphenols can also prevent free radical formation.[8]

Furthermore, many flavonoids have metal ion complexation capacity.[9,10] Flavones, in particular, have been widely studied[11–14] because of this capacity.

Flavonoids exhibit two major absorption bands in the ultraviolet/visible region. The absorption in the (320 to 385) nm range is related with the cinnamoyl system (Band I), and the absorption in the (240 to 280) nm range corresponds to the benzoyl system (Band II). The spectra of the metal flavonoid complexes are shifted at higher wavelengths as compared to those of the uncomplexed flavonoids.

The biological applications of La(III) as an antitumoral,[15] cytotoxic,[16] and antibacterial[17] agent have been widely studied. Since lanthanum does not have these properties by itself, it has been found that some ligands, when complexed with La(III), increase their biological properties.[18] On the other hand, this metallic ion also has many industrial applications, among others, as a modifier in lead titanate films and stainless steels.[19,20]



System	Compound	R_5	$R_{2'}$	$R_{3'}$	$R_{4'}$
L_1	3,7-dihydroxyflavone	H	H	H	H
L_2	3,7,3′- trihydroxyflavone	H	H	OH	H
L_3	3,5,7,3′,4′-pentahydroxyflavone (quercetin)	OH	H	OH	OH
L_4	3,5,7,2′,4′-pentahydroxyflavone (morin)	OH	OH	H	OH

Figure 1. Chemical structure of the studied compounds.

Four 3-hydroxyflavones, also known as flavonols, were studied in this work: 3,7-dihydroxyflavone (L_1), 3,7,3′-trihydroxyflavone (L_2), 3,5,7,3′,4′-pentahydroxyflavone (quercetin) (L_3), and 3,5,7,2′,4′-pentahydroxyflavone (morin) (L_4) (see Figure 1). The formation of the complexes was determined spectrophotometrically. The molar ratio of each complex was evaluated by the Yoe−Jones method.[21]

Due to spectral overlap, the application of a linear graphical method for determining the apparent formation constants[22] developed previously is not posssible. Derivative methods have been applied to the quantitative assay of different compounds

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Table 1. Ligand Protonation Constants at 26.3 °C and 0.1 M NaCl Ionic Strength

ligand	log K_1^H	log K_2^H
3,7-dihydroxyflavone (L_1)	9.128 ± 0.664	−
3,7,3′-trihydroxyflavone (L_2)	11.091 ± 0.134	8.822 ± 5·10^{-4}
quercetin (L_3)	9.310 ± 0.119	8.382 ± 0.109
morin (L_4)	10.021 ± 0.355	7.173 ± 0.576

Table 2. Stability Constants of 3-Hydroxyflavone−La(III) Complexes at 26.3 °C and 0.1 M NaCl Ionic Strength

system	stoichiometry	log K_1	log K_2
3,7-dihydroxyflavone−La(III)	1:1	7.269 ± 0.387	-
3,7,3′-trihydroxyflavone−La(III)	2:1	9.164 ± 0.020	5.200 ± 0.185
quercetin−La(III)	2:1	8.143 ± 0.284	5.632 ± 0.585
morin−La(III)	2:1	7.407 ± 0.596	4.830 ± 0.165

and metals in the mixtures, as described in the literature.[23] However, neither first nor second derivative spectrometry can resolve the overlapping.

The apparent formation constants of the flavone−La(III) complexes were determined by means of a potentiometric method. The protonation constants of each flavonol, as well as the stability constants of their La(III) complexes, were determined potentiometrically using the Calvin−Bjerrum method, and calculations were performed according to the Irving−Rossotti method.[24]

Experimental Section

Reagents. The structures and numbering system of the four 3-hydroxyflavones studied are shown in Figure 1.

Quercetin and morin were purchased from Sigma. 3,7,3′-trihydroxyflavone and 3,7-dihydroxyflavone were synthesized, purified, and identified according to the method reported by Tanaka and Sujino.[25] $LaCl_3 \cdot 7H_2O$ was purchased from Tetrahedron. Methanol and 1,4-dioxan were provided by Merck.

Solutions. A set of solutions were prepared as described below:

Acid Solution. [(4·10^{-3} M HCl + 0.1 M NaCl) in 25 mL of dioxan−H_2O (1:1, v/v)] + 25 mL of dioxan−H_2O (1:1, v/v).

Ligand Acid Solutions. [(4·10^{-3} M HCl + 0.1 M NaCl + 7.3·10^{-4} M 3,7-dihydroxyflavone) in 25 mL of dioxan−H_2O (1:1, v/v)] + 25 mL of dioxan−H_2O (1:1, v/v).

[(4·10^{-3} M HCl + 0.1 M NaCl + 1.2·10^{-3} M 3,7,3′-trihydroxyflavone) in 25 mL of dioxan−H_2O (1:1, v/v)] + 25 mL of dioxan−H_2O (1:1, v/v).

[(4·10^{-3} M HCl + 0.1 M NaCl + 1.2·10^{-3} M quercetin) in 25 mL of dioxan−H_2O (1:1, v/v)] + 25 mL of dioxan−H_2O (1:1, v/v).

[(4·10^{-3} M HCl + 0.1 M NaCl + 1.2·10^{-3} M morin) in 25 mL of dioxan−H_2O (1:1, v/v)] + 25 mL of dioxan−H_2O (1:1, v/v).

Ligand and Metal Acid Solutions. [(4·10^{-3} M HCl + 0.1 M NaCl + 7.3·10^{-4} M 3,7-dihydroxyflavone) in 25 mL of dioxan−H_2O (1:1, v/v)] + La(III) 6·10^{-4} M in 25 mL of dioxan−H_2O (1:1, v/v).

[(4·10^{-3} M HCl + 0.1 M NaCl + 1.2·10^{-3} M 3,7,3′-trihydroxyflavone) in 25 mL of dioxan−H_2O (1:1, v/v)] + La(III) 6·10^{-4} M in 25 mL of dioxan−H_2O (1:1, v/v).

[(4·10^{-3} M HCl + 0.1 M NaCl + 1.2·10^{-3} M quercetin) in 25 mL of dioxan−H_2O (1:1, v/v)] + La(III) 6·10^{-4} M in 25 mL of dioxan−H_2O (1:1, v/v).

[(4·10^{-3} M HCl + 0.1 M NaCl + 1.2·10^{-3} M morin) in 25 mL of dioxan−H_2O (1:1, v/v)] + La(III) 6·10^{-4} M in 25 mL of dioxan−H_2O (1:1, v/v).

Apparatus. An Agilent 8454 diodo-array spectrophotometer was used to record the ligands and their complex spectra. A Hanna HI 8424 pH meter and a Cole Parmer 60061 glass electrode were used to measure pH.

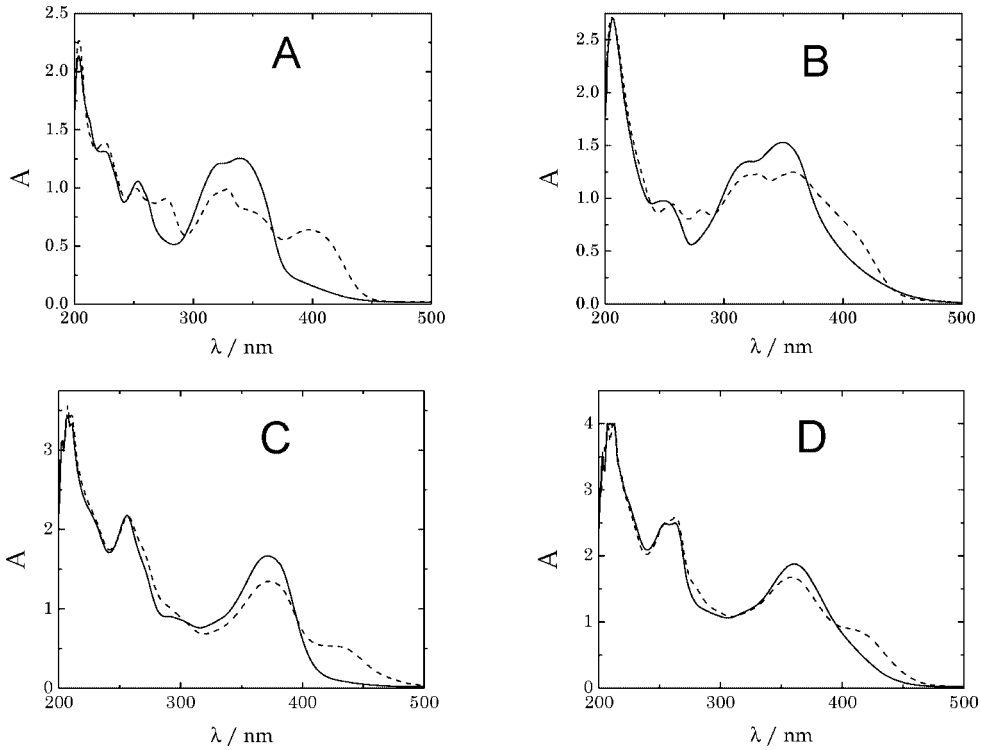

Figure 2. Spectral changes of the ligands upon the addition of the metallic ion solution. (A) [3,7-dihydroxyflavone (solid curve); 3,7-dihydroxyflavone + La(III) (dashed curve)], (B) [3,7,3′-trihydroxyflavone (solid curve); 3,7,3′-trihydroxyflavone + La(III) (dashed curve)], (C) [quercetin (solid curve); quercetin + La(III) (dashed curve)], and (D) [morin (solid curve); morin + La(III) (dashed curve)].

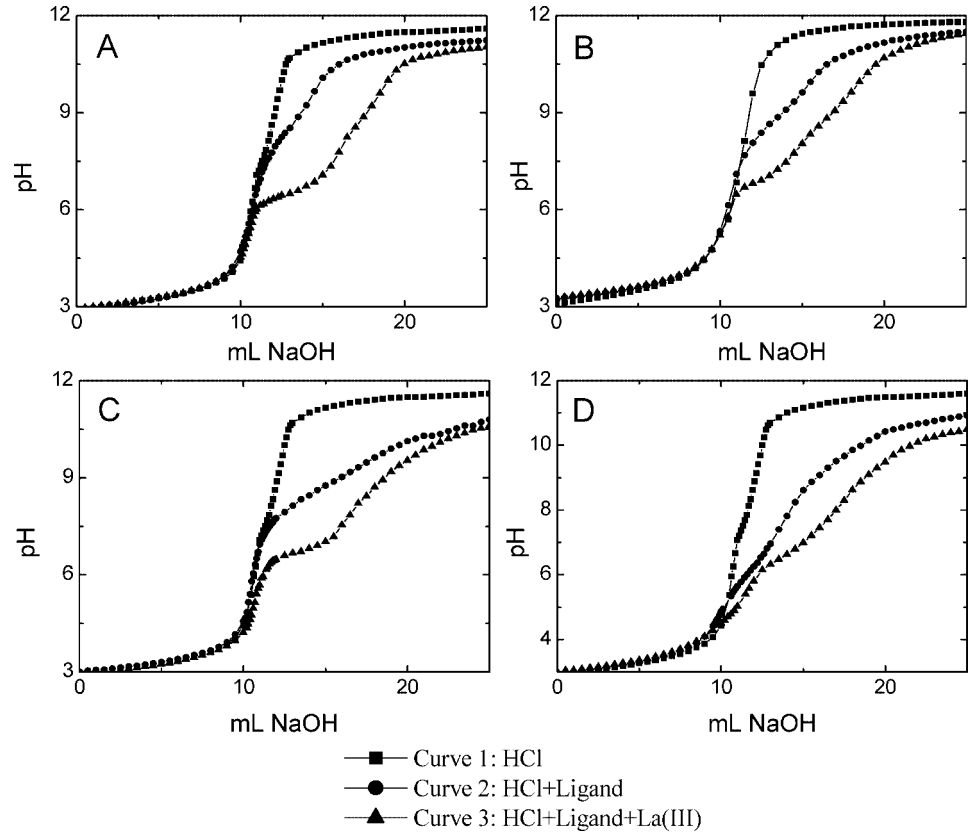

Figure 3. Potentiometric titration curves of (A) [3,7-dihydroxyflavone + La(III)], (B) [3,7,3′-trihydroxyflavone + La(III)], (C) [3,5,7,3′,4′-pentahydroxyflavone + La(III)], and (D) [3,5,7,2′,4′-pentahydroxyflavone + La(III)] in water/dioxan (1:1) at 26.3 °C and 0.1 M NaCl ionic strength.

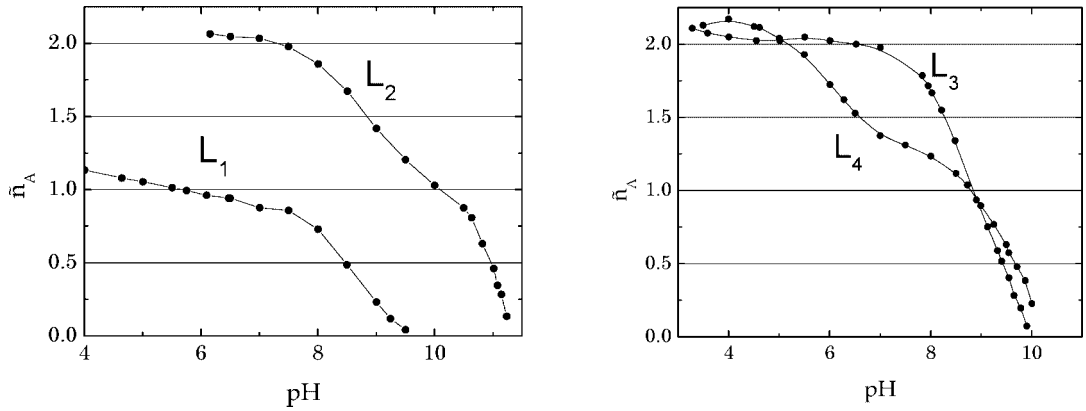

Figure 4. Formation curves of 3,7-dihydroxyflavone (L_1), 3,7,3′-trihydroxyflavone (L_2), 3,5,7,3′,4′-pentahydroxyflavone (L_3), and 3,5,7,2′,4′-pentahydroxyflavone (L_4) in water/dioxan (1:1) at 26.3 °C and 0.1 M NaCl ionic strength.

Procedure. The Yoe−Jones method is a spectrophotometric method used to determine the molar composition of metallic complexes.[21] Stoichiometries of the four complexes studied are listed in Table 2.

According to the Irving−Rossotti method,[24] three titrations were carried out: (1) acid titration; (2) ligand acid titration, and (3) metal/ligand acid titration. To determine the protonation constants, the solutions of the acid and of each ligand were titrated potentiometrically using $8.936 \cdot 10^{-3}$ M NaOH, at constant ionic strength (0.1 M) and temperature (26.3 °C). Potentiometric titration curves 1 and 2 (Figure 3) of each system were used to calculate the average values $\tilde{n}_A$. The equation used for the calculation is

$$\tilde{n}_A = y + \frac{(V_1 - V_2)(N + C_0)}{(V_0 + V_1)C_{L_0}} \quad (1)$$

where V_0 is the initial volume; N is the molarity of NaOH; C_{L_0} is the ligand concentration; C_0 is the initial concentration of the acid; and y denotes the number of dissociable protons initially present on the ligand. $(V_1 - V_2)$ is the measure of the displacement of the ligand curve relative to the acid curve where V_1 and V_2 are the volumes of alkali added to reach the same pH reading in both titrations.

The protonation constants were determined from the graph of $\tilde{n}_A$ vs pH, named the formation curve. The pH values at $\tilde{n}_A = 0.5$ and $\tilde{n}_A = 1.5$ designate the log $K^H{}_1$ and log $K^H{}_2$, respectively.

To determine the stability constants of the complexes, the HCl + NaCl + ligand + metal ion mixture was titrated potentiometrically using $8.936 \cdot 10^{-3}$ M NaOH at constant ionic strength (0.1 M) and temperature (26.3 °C) (curve 3 in Figure 3). $\tilde{n}_L$ values were calculated using the $\tilde{n}_A$ values and the equation given below

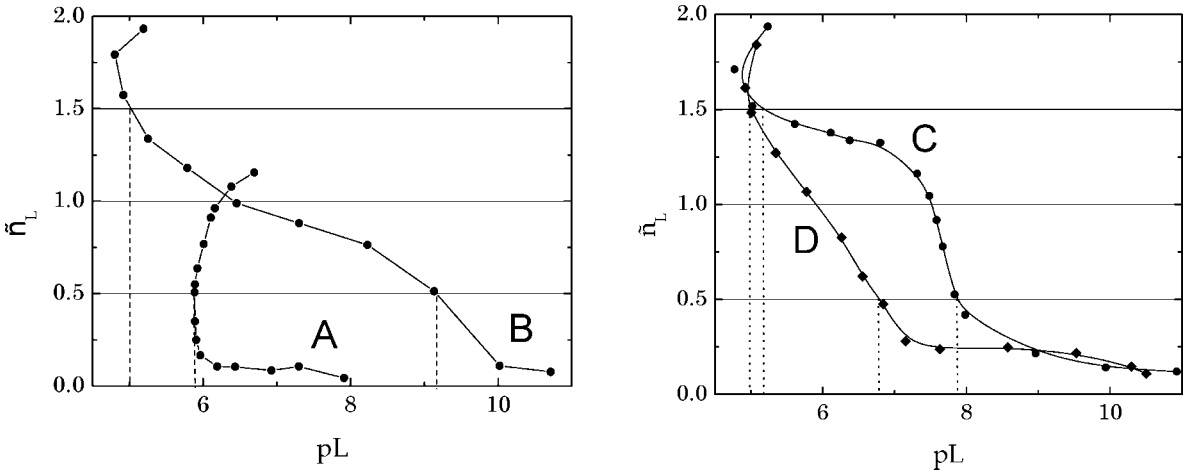

Figure 5. $\tilde{n}_L$ vs pL plots of: (A) [3,7-dihydroxyflavone−La(III)], (B) [3,7,3′-trihydroxyflavone−La(III)], (C) [3,5,7,3′,4′-pentahydroxyflavone−La(III)], and (D) [3,5,7,2′,4′-pentahydroxyflavone−La(III)] in water/dioxan (1:1) at 26.3 °C and 0.1 M NaCl ionic strength.

$$\tilde{n}_L = \frac{(V_3 - V_2)(N + C_0 + C_{Lo}(y - \tilde{n}_A))}{(V_0 + V_1)\tilde{n}_A C_M} \quad (2)$$

where V_0 is the initial volume; N is the molarity of NaOH; C_{L_0} is the ligand concentration; C_0 is the initial concentration of the acid; and C_M is the metal concentration. $(V_3 - V_2)$ is the measure of the displacement of the metal curve relative to the ligand curve where V_2 and V_3 are the volumes of alkali added to reach the same pH reading. pL values were calculated using the $\tilde{n}_L$ values and the equation

$$\mathrm{pL} = \log\left(\frac{1 + K_1^{H}[H] + K_1^{H}K_2^{H}[H]^2 + ... + K_1^{H}K_2^{H}...K_j^{H}[H]^j}{C_L - \tilde{n}_L C_M}\right) \quad (3)$$

The stability constants were determined from the $\tilde{n}_L$ vs pL curve, where the pL values at $\tilde{n}_L = 0.5$ and $\tilde{n}_L = 1.5$ designate the log K_1 and log K_2, respectively. All titrations were carried out at constant values of both temperature and ionic strength. The mole ratio of metal to ligand was kept at 1:1 for 3,7-dihydroxyflavone and 1:2 for the others, to reach the maximum coordination of the ligand in every case.

Results and Discussion

The formation of four 3-hydroxyflavone−La(III) complexes was indicated by the intensification of yellow color due to the bathochromic shift and was confirmed by recording the spectra of the ligand solution and of the same solution after La(III) addition (see Figure 2).

The stoichiometries of complexes formed were determined by the Yoe−Jones method. All four ligands formed a 1:1 complex, whereas only three formed a 2:1 ligand/metal complex (see Table 2).

The protonation constants for L_1, L_2, L_3, and L_4 and the stability constants for the complexes between the four 3-hydroxyflavones and La(III) were determined by carrying out three titrations of (1) acid, (2) ligand acid and (3) metal/ligand acid solutions, using the same temperature (26.3 °C) and ionic strengh (0.1 M) constant, according to the Irving−Rossotti method.[24] Every titration carried out is exhibited in Figure 3, where pH and NaOH volume data are plotted.

$\tilde{n}_A$ vs pH plots of L_2, L_3, and L_4 were obtained between 0 and 2, indicating that these three 3-hydroxyflavones have two protons dissociable from their OH groups (see Figure 4). On the other hand, the L_1 plot was between 0 and 1, so 3,7-dihydroxyflavone presents only one dissociable proton. Interpolation at 0.5 and 1.5 $\tilde{n}_A$ values gave log $K^{H}{}_1$ and log $K^{H}{}_2$ for each ligand. The corresponding values are listed in Table 1.

Similarly, the complex stability constants were determined from $\tilde{n}_L$ vs pL plots. The pL values at $\tilde{n}_L = 0.5$ and $\tilde{n}_L = 1.5$ designate log K_1 and log K_2, respectively, as described above. Figure 5 shows the formation curves for the four complexes studied, and their stability constant values are reported in Table 2.

Stability data of the complexes here considered show that 3,7,3′-trihydroxyflavone−La(III) is about 3 orders of magnitude more stable than that of quercetin. The coordination with metallic ions could involve oxygen atoms from the OH in C3 and carbonyl groups, forming a stable five-atom ring, as reported for chelates by De Souza and De Giovanni.[26] Quercetin and morin present greater stereochemical difficulties which could justify their lower stability constant values.

Conclusion

According to our present knowledge, there are few literature data concerning useful ligands of La(III) as analytical reagents. The results obtained from this study reveal that 3,7,3′-trihydroxyflavone is the most suitable ligand for lanthanum(III) complexation.

Moreover, the stability constant for the 3,7-dihydroxyflavone−La(III) complex (log $K_1 = 7.269 \pm 0.387$) is also a high value considering its 1:1 stoichiometry, slightly higher than that reported by Athawale[27] for the La(III)−(±)-norvaline system (log $K_1 = 5.36$).

It must be noted that flavones 3,7-dihydroxyflavone and 3,7,3′-trihydroxyflavone, which were synthesized in our laboratory, may find an application as analytical reagents for the lanthanum(III) metallic ion.

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