Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-022-04411-x

PAPER IN FOREFRONT

New terbium complex as a luminescent probe for determination of chlorogenic acid in green coffee and roasted coffee infusions

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Received: 12 August 2022 / Revised: 26 October 2022 / Accepted: 28 October 2022

Abstract

Green coffee is coming into vogue as a food that contains remarkable contents of antioxidants like chlorogenic acid (ChA) and induces mild stimulation to the consumer. While most methods for determination of ChA require chromatographic separation prior its quantitation, we present the first probe and a simple, sensitive and validated luminescence method for the determination of chlorogenic acid in green and roasted coffee infusion samples that does not require a chromatographic separation. ChA can remarkably quench the luminescence intensity of the Tb^{3+} complex with 1-(furan-2-ylmethyl)-4-hydroxy-*N*-(4-methylpyridin-2-yl)-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (R_3) in aqueous solution containing urotropine buffer at a near neutral pH 7.5 at $\lambda_{exc} = 315$ nm and $\lambda_{em} = 545$ nm. Under optimal conditions, the quenching of the luminescence intensity is directly proportional to the concentration of ChA in the range of 0.5–30 µg/mL, and the detection limit is 180 ng/mL. From measurements of luminescence decay time, it was determined that both static and dynamic quenching is induced upon coordination of ChA to Tb-R_3. The related quenching constants are $K_S = 5.97 \bullet 10^4\ M^{-1}$ and $K_D = 1.05 \cdot 10^4\ M^{-1}$. Finally, the method was applied successfully to the determination of ChA in real green and roasted coffee infusion samples and validated by HPLC to yield very closely matching concentrations of both methods. Therefore, this method can serve for a simple quality control of total ChA contents in coffee prior and after roasting.

Keywords Chlorogenic acid · Luminescence · Probe · Terbium complex · Quenching

Introduction

Chlorogenic acid (ChA)—3-(3,4-dihydroxycinnamoyl) quinic acid (see Scheme 1)—is one of the main substances contained in, e.g. coffee beans or blueberry leaves, and can be chemically regarded as an esterification product between quinic acid and caffeic acid. Being a polyphenol, it shows remarkable antioxidant, anti-carcinogenic, anti-inflammatory therapeutic and analgesic action [1, 2]. The content of chlorogenic acid in coffee depends on the growing area, soil, climatic, ripeness state of the coffee beans and storage conditions, etc. [1]. The properties of green coffee are still being studied, as are its effects on human health. It is a product that is especially recommended and known for its high content of chlorogenic acid. It contains about 5–12 g of chlorogenic acid per 100 g of grain [3]. Green coffee extract is mostly available as a nutraceutical in capsules but also as a supplement in beverages, chocolate and chewing gum. Most often, the total polyphenolic components, including chlorogenic acid, are determined by high-performance liquid chromatography (HPLC or UHPLC) coupled with photometric detection [2–6].

Few methods have been reported for the determination of chlorogenic acid including HPTLC [7, 8], spectrophotometric [9–11] and HPLC-spectrofluorometric [12] methods. While HPTLC provides high sensitivity, yet specialized equipment, photometry is simple, but displays too little sensitivity. HPLC provides superb separation and identification capability, especially when coupled with MS detection. However, HPLC instruments are quite expensive to purchase

Dedicated to Prof. Dr. Otto S. Wolfbeis on the occasion of his 75th birthday.

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Published online: 15 November 2022

and require regular maintenance, whereas luminescence provides a similarly sensitive detection with less instrumental demand. If luminescence is combined with an appropriate probe that shows high binding constants to its target and high brightness, trace analysis is achieved even with inexpensive instruments. Therefore, new probes and luminescent methods could represent a promising avenue for quantitation of ChA.

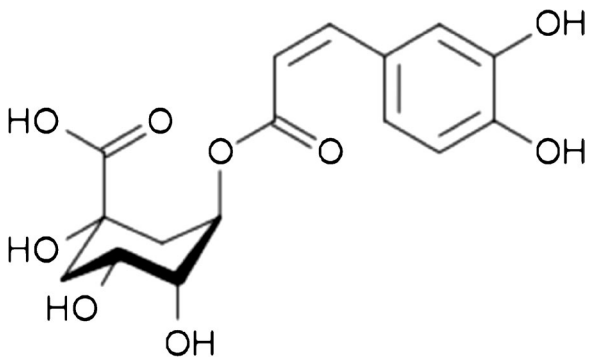
The analytical application of lanthanide-sensitized luminescence has created great interest for a long time. The main advantages of lanthanide chelates as probes in luminescence spectrometry include large Stokes' shifts, narrow emission bands and long luminescence lifetimes. Moreover, their luminescence can be considerably increased by coordination of so-called antenna ligands with high molar absorbance and high quantum yield [13–15]. Such complexes are ideal probes for luminescence assays based on changes of luminescence intensity or lifetime. In the last years, the analytical use of sensitized lanthanide luminescence as well as its response with decrease or enhancement of luminescence intensity or lifetime towards the presence of relevant biomolecules demonstrated their potential as useful probes [16–18].

As chlorogenic acid is known for its complexation ability towards, e.g. Fe^{3+}, we reasoned that ChA should also show coordination to lanthanide complexes with suitable stoichiometry and thereby have an impact on the luminescence properties of such a complex. The potential of those as luminescent probes for bioanalytical purposes has been recently described [17]. We then characterized three different new Tb-quinolone complexes as potential probes for chlorogenic acid due to their high molar absorbances, long lifetimes and high quantum yields. Furthermore, the complexation constants of ChA to the best Tb^{3+} probe and the stoichiometry upon coordination were determined. Importantly, we could derive a method for quantitation of ChA in real coffee samples based on the quenched emission of the Tb^{3+} ion, especially the hypersensitive $^5D_4 \rightarrow {}^7F_5$ transition at 545 nm. It works at a near neutral pH of 7.5 and has a wide working range and a low LOD. The suggested method is rapid, simple, sensitive and can be used for the determination and quality control of ChA in green and roasted coffee as validated by HPLC with photometric detection.

Materials and methods

Instrumentation

All luminescence measurements (luminescence spectra, excitation spectra and lifetimes) were carried out on a Cary Eclipse (Varian, Australia) luminescence spectrophotometer in the range (220–700 nm) equipped with a xenon flash lamp in a 1.00 cm quartz cell. The excitation and emission monochromator band widths were 5 nm. The excitation wavelength was set at 315 nm, and the luminescence was measured using the peak height at 545 nm. All measurements were performed at room temperature (21–23 °C) which should be kept at this level. Absorption spectra were recorded with a UV-2401 PC (Shimadzu, Japan) spectrophotometer.



Scheme 1. Structure of chlorogenic acid (ChA)

A pH meter (Lab 850, Schott Instruments GmbH, Germany) was used for pH adjustment. HPLC chromatograms were obtained using an Agilent Technologies 1200 Series chromatograph with isocratic elution under the conditions given in the "Preparation of the lanthanide complex" section. Peak areas were automatically integrated by the Agilent software.

Reagents

All of the used chemicals were of analytical grade or chemically pure and doubly distilled water was used, throughout. The standard solution of terbium (III) chloride ($1.00 \cdot 10^{-1}$ mol/L) was prepared from a high purity oxide. The concentration of the metal was determined by complexometric titration with Arsenazo I as the indicator.

The ligands R_1–R_3 were synthesized as described in [19]. The stock standard solutions of the ligands ($1.00 \cdot 10^{-3}$ mol/L) were prepared by dissolving accurate weights of the solid compounds in dimethylformamide. The stock standard solutions of the ligands were diluted to $1.00 \cdot 10^{-4}$ mol/L with water.

An accurately weighted standard sample of 50.0 mg of chlorogenic acid (from Acros, CAS 327–97-9) was dissolved in water, placed into a 50.0-mL volumetric flask, stirred and diluted to the mark with water. Herewith, a standard solution of 1.00 mg/mL was obtained. The stock standard solution of ChA was diluted to 100 µg/mL with water before being used. Similarly, standard samples of caffeine (from Sigma-Aldrich, CAS 58–05-2) and caffeic acid (from Sigma-Aldrich, CAS 331–39-5) were prepared. A green coffee sample "Gregincof" (Vietnam robusta) was supplied by

Buon Ma Thuot coffee company (Vietnam). A roasted coffee sample "Cerrado" (BRAZIL, arabica) was supplied by Kyiv Roasting Company (Ukraine).

An 2.85 mol/L (40%) urotropine buffer was prepared by dissolving 40.0 g of urotropine in a 100-mL volumetric flask with water and adjusting the pH to 7.5 with hydrochloric acid and then diluting with water to 100 mL. Ammonium acetate buffers of various pH were prepared to determine the pH-dependence of the luminescence emission of the Tb-R complexes.

Preparation of the lanthanide complex

The complex of a ligand with Tb^{3+} ions was prepared by mixing the respective ligand at a concentration of $1.00•10^{-5}$ mol/L and Tb^{3+} with $1.00•10^{-5}$ mol/L in a molar ratio of 1:1 in water at room temperature. The Tb-R_3 complex is water soluble up to a concentration of $1.00•10^{-3}$ mol/L as no precipitation was observed.

Luminescence spectra of Tb-R_3 complex in presence of different concentrations of ChA

0; 0.050; 0.100; 0.300; 0.500; 0.700; 1.00; 1.50; 2.00; 3.00 mL of ChA working solution (100 µg/mL), respectively, were added into volumetric flasks. 1.00 mL of a working terbium chloride solution ($1.00•10^{-4}$ mol/L), 1.00 mL of R_3 working solution ($1.00•10^{-4}$ mol/L) and 0.500 mL of urotropine buffer (40 %) were added to each of these volumetric flasks. The solutions were diluted with water to 10.0 mL and stirred. The luminescence intensity (F) was measured at $\lambda_{exc}/\lambda_{em}$ = 315/545 nm after 5 min. Concentrations of the samples were derived from the related calibration curve.

Luminescence determination of ChA in green coffee samples

The green coffee samples were prepared for HPLC and luminescent analysis based on the following procedure according to a published method [20]. 100 mg of ground green coffee sample were accurately weighed in 25 mL beakers, 10.0 mL of distilled water were added to each sample, and the samples were boiled for 30 min while stirring. Then, the coffee samples were cooled and the solution was filtered through a 0.45-µm filter. The clear filtrate was used for analysis and diluted as required. 0.300 mL of the filtrate were placed into a 10.0-mL volumetric flask. Further, 1.00 mL of a working terbium chloride solution ($1.00•10^{-4}$ mol/L), 1.00 mL of R_3 working solution ($1.00•10^{-4}$ mol/L) and 0.500 mL of urotropine buffer were added to each of these volumetric flasks. Then, water was added to make the volume up to 10.0 mL, and luminescence intensity was measured at $\lambda_{exc}/\lambda_{em}$ = 315/545 nm. The luminescence intensity of the control solution (F_0) which contains all components with the exception of ChA was recorded at the same time.

HPLC determination of ChA in green coffee

The HPLC conditions were as follows: column, reverse phase – ODS 150 × 4.6 mm, flow rate 0.6 mL/min, column temperature 40 °C, photometric detection wavelength 280 nm, mobile phase methanol:5.00 mM KH_2PO_4 (30/70), sample volume 10 µL. Note that the number of theoretical plates should not be less than 2000 as calculated based on the chlorogenic acid peak. The area under the peak was used for calibration.

Results and discussion

Spectral characteristics of the ligands and their Tb complexes

The absorption spectra of the ligands in aqueous solutions show two bands in the UV with peak wavelengths around 240 and 310 nm. The molar absorption coefficients (22,800–25,300 $L•mol^{-1}•cm^{-1}$) of these bands are high, and therefore, the ligands provide efficient absorption of excitation light. The triplet energy levels (E) of the ligands were calculated from phosphorescence spectra of the respective Gd complexes at 77 K (Table 1). This energy is higher than the energy of the level of the first excited Tb^{3+} ion state ($^5D_4$; 20,500 cm^{-1}) which enables an energy transfer from any ligand R to the lanthanide ion.

Figure 1a shows the excitation spectrum of the Tb-R_3 complex monitored at an emission wavelength of 545 nm. The excitation maximum is found at 315 nm for both, the Tb-R_1 complex and the Tb-R_3 complex, while the excitation maximum of the Tb-R_2 complex is more longwave at 346 nm. The emission bands of the Tb-R_3 complex (Fig. 1b) are located at 490, 545, 585 and 620 nm, obviously generated through the $^5D_4 \rightarrow {}^7F_6$, $^5D_4 \rightarrow {}^7F_5$, $^5D_4 \rightarrow {}^7F_4$ and $^5D_4 \rightarrow {}^7F_3$ transitions, respectively. The other two Tb complexes with R_1 and R_2, respectively, show a very similar pattern of emission bands.

The quantum yields of the complexes with R_1–R_3 were determined in urotropine buffer of pH 7.5 relative to quinine sulphate at λ_{exc} = 315 nm. They are given in Table 1 and vary from 0.18 to 0.32 with Tb-R_3 having the highest quantum yield. Moreover, Tb-R_3 has the longest luminescence lifetime of almost 1 ms. The lifetimes of the other two complexes are also in a typical range for luminescent Tb-complexes. Organic solvents were found to decrease the luminescence intensity of Tb-R_3 by 40–70% (see Fig. S1 in the supplementary information) as compared to pure water. The higher quantum yield and longer lifetimes suggested

the use of complex Tb-R_3 as the most suitable probe for the luminescent determination of ChA.

The luminescence spectra of the Tb-R_3-ChA complex are similar to those of free Tb-R_3, but the luminescence intensity is increasingly quenched in the presence of increasing amounts of ChA (Fig. 1b). In presence of 30 µg/mL of ChA, only 16% of the original intensity remains. On comparing the quenching ratio of all complexes (Table 1, last column), it is obvious that Tb-R_3 is quenched most efficiently among all three complexes in presence of ChA. This additionally suggests the use of the Tb-R_3 complex as a probe for ChA by means of luminescence quenching. Moreover, the interaction of the Tb-R_3 complex with ChA causes a remarkable bathochromic shift of 20 nm of the absorption maximum from 310 to 330 nm (Fig. 2). This points to a strong coordination interaction between Tb-R_3 with ChA.

Effect of pH and of stoichiometry

The complexation of Tb(III) with the ligands occurs in a wide range of pH values from 4 to 11 (Fig. 3). The highest luminescence intensity of the complex Tb–R_3 is observed at a neutral pH range of pH 7.0–8.5 at constant concentration of $1.00•10^{-5}$ mol/L. Therefore, the pH of the solutions was maintained at 7.5 with urotropine buffer for further measurements.

Applying the restricted-logarithm method to the luminescence data, it was found that in case of molar ratios of Tb:R_3 of until 1:1 or at equimolar ratio of Tb:R_3, a complex with 1:1 stoichiometry and a lifetime (τ) of 948 µs is formed. If the ligand is present in excess, however, terbium forms a complex with R_3 in a 1:3 molar ratio. This is further supported by the much longer lifetime of 1130 µs that is detected for the Tb-$(R_3)_3$ complex. Hence, to permit the coordination of ChA in a quenching assay, equal concentrations ($1.00•10^{-5}$ mol/L) of Tb^{3+} and R_3 were regarded as optimal to form the luminescent probe and those were chosen for the further analytical experiments. When we then experimentally changed the concentration of ligand R_3 at constant concentrations of Tb^{3+} ($1.00•10^{-5}$ mol/L) and ChA (20 µg/mL), we also found that a concentration of $1.00•10^{-5}$ mol/L of both $Tb^{3+}$and R_3 is the optimum.

Analytical performance of Tb-R_3 for quantitation of chlorogenic acid

The strong luminescence quenching due to the presence of ChA pointed to set up a calibration plot that is based on a Stern–Volmer plot. Hence, the luminescence quenching of Tb-R_3 in 1:1 molar ratio was determined at various concentrations of the quencher (ChA) and validated in terms of linearity, accuracy,—and intra-day precision and specificity. F_0 and F were measured at $\lambda_{exc} = 315$ nm and $\lambda_{em} = 545$ nm, respectively, and a nonlinear Stern–Volmer plot was obtained (Fig. 4). The following equation was obtained as best fit: $F_0/F = 1.126 - 0.042c_{ChA} + 0.005c_{ChA}^2$ with a very good correlation coefficient of 0.9962. Here, F_0 and F are the relative luminescence intensities determined without and with ChA, respectively, and c_{ChA} is the concentration of chlorogenic acid in µg/mL. As can be seen from Fig. 4, the Stern–Volmer plot has an upward curvature and obeys the polynominal equation. This points to the presence of both, static and dynamic

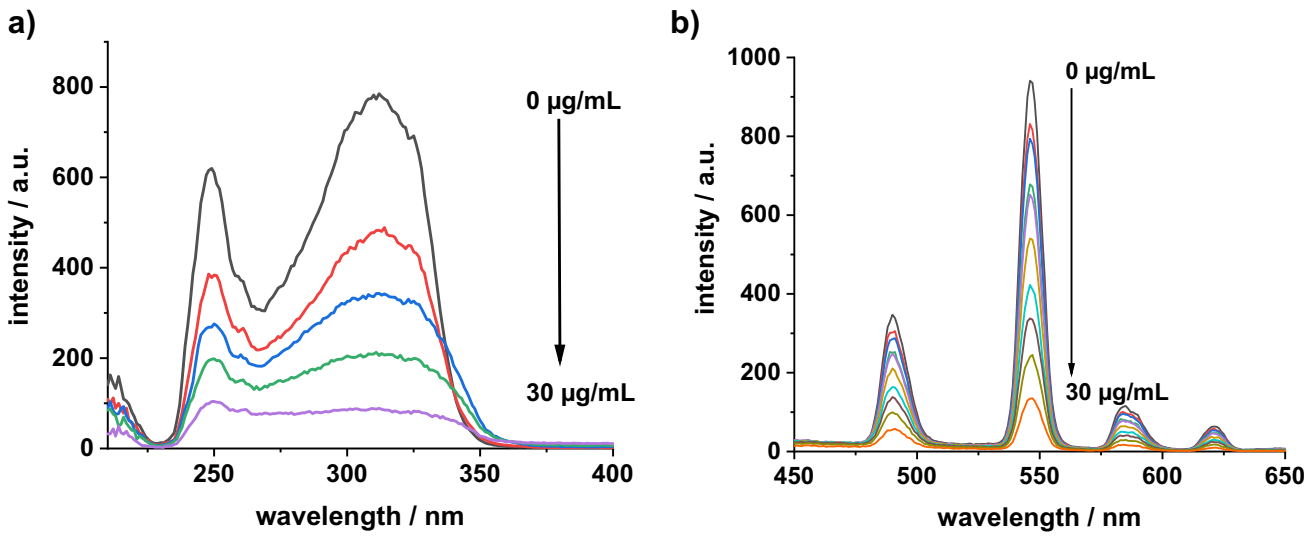
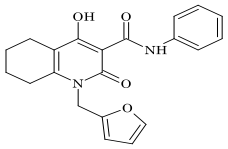
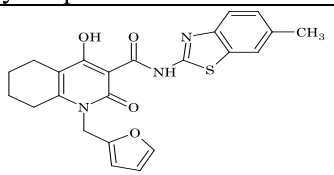
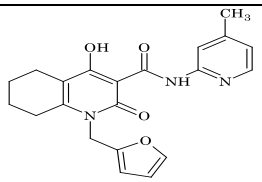


Fig. 1 Excitation spectra of the Tb-R_3 complex (**a**) and luminescence spectra of Tb-R_3 in the presence of various concentrations of ChA (**b**) ($c_{Tb}^{3+} = c_{R3} = 1.00•10.^{-5}$ mol/L; $\lambda_{exc}/\lambda_{em} = 315$ nm/545 nm)

Table 1 Triplet-state energy levels (E) of ligands R_1–R_3; excitation maxima (λ_{exc}), lifetimes (τ) and quantum yields (Φ) of the Tb complexes with these ligands ($c_{Tb}^{3+} = c_R = 1 \cdot 10^{-5}$ mol/L; pH 7.5; $\lambda_{em} = 545$ nm) and luminescence quenching factors of Tb-R complexes in the presence of ChA

R	Ligand	E [cm^{-1}]	λ_{exc} [nm]	τ [μs]	Φ	k*
1	 1-(furan-2-ylmethyl)-4-hydroxy-2-oxo-*N*-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide	20530	315	760	0.18	3.18
2	 1-(furan-2-ylmethyl)-4-hydroxy-*N*-(6-methyl-1,3-benzothiazol-2-yl)-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide	20550	346	875	0.29	2.07
3	 1-(furan-2-ylmethyl)-4-hydroxy-*N*-(4-methylpyridin-2-yl)-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide	20570	315	948	0.32	3.90

*Quenching ratio of the luminescence intensity of the terbium complex in absence of ChA to the luminescence intensity of the terbium complex in the presence of ChA ($c_{ChA} = 20$ μg/mL).

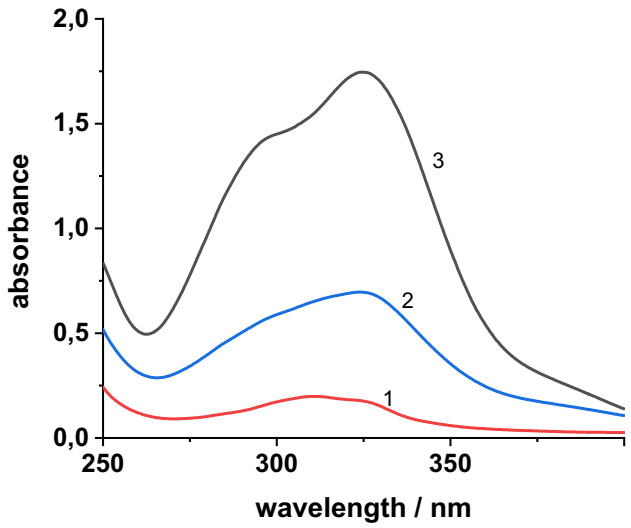


Fig. 2 Absorption spectra of Tb-R_3 (1), Tb-R_3-ChA (2), ChA (3) ($c_{Tb}^{3+} = c_{R3} = 1.00 \cdot 10.^{-5}$ mol/L, $c_{ChA} = 10.0$ μg/mL)

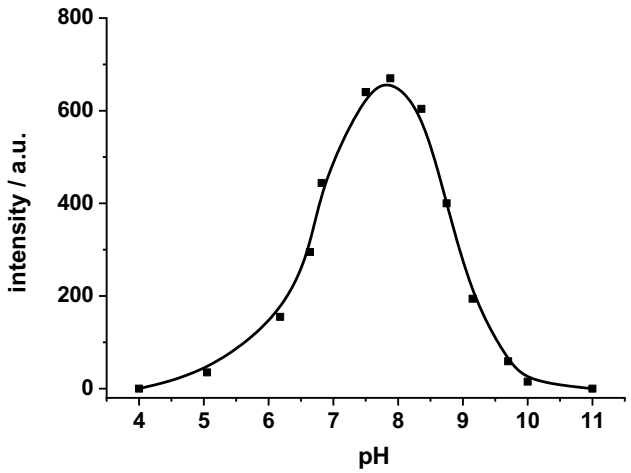


Fig. 3 Effect of pH on the luminescence intensity of the complex Tb-R_3 ($c_{Tb}^{3+} = c_{R3} = 1.00 \cdot 10.^{-5}$ mol/L)

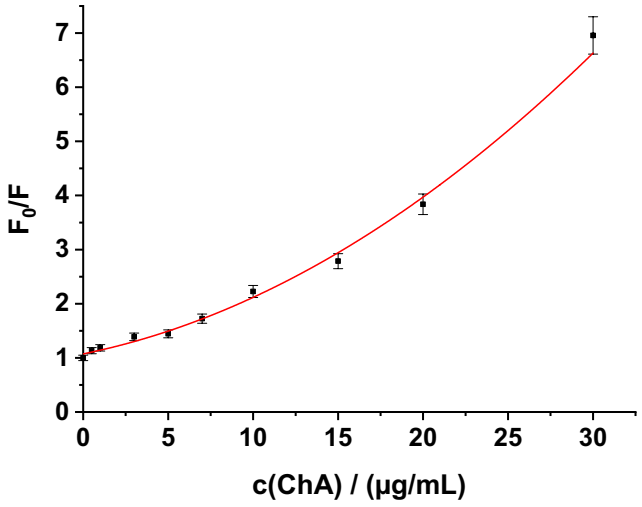


Fig. 4 Non-linear Stern–Volmer plot for the determination of ChA ($c_{Tb^{3+}} = c_{R3} = 1.00 \cdot 10^{-5}$ mol/L; $\lambda_{exc}/\lambda_{em} = 315/545$ nm) ($n = 3$)

quenching. More detailed data on the quenching mechanism, lifetime data and quenching constants are given in the "Determination of luminescence quenching mechanism" section. The quenching of luminescence intensity is proportional to the concentration of chlorogenic acid in the range of 0.5–30 µg/mL, and the detection limit is 180 ng/mL (Table 2).

The precision of the method was established by acquiring the luminescence quenching at a ChA concentration of 10 µg/mL. For a series of 12 measurements, the relative standard deviation was 2.4% for the intra-day and 2.8% for the inter-day analysis, respectively ($P = 95\%$ confidence level) for ChA indicating high precision.

Accuracy of the method was evaluated by carrying out a recovery study at three different concentration levels of ChA. The results of the recovery study indicate that the proposed method is very accurate for the estimation of ChA in green coffee (Table 3). The recovery shows no systematic trend towards lower or higher levels of ChA.

The interference of caffeine and caffeic acid which are contained in higher concentrations in green coffee [20, 21] was studied by addition of these compounds to a solution of 10 µg/mL of ChA. Then, this mixture was added to a solution of the Tb-R_3 complex, and the change of the luminescence intensity (ΔF) was determined as compared to a solution of ChA and Tb-R_3 with the same concentrations, but without the interferent being present. As shown in Table 4, caffeine and caffeic acid had a very little effect on the luminescent determination of ChA. Hence, a high specificity is achieved by the proposed method. This suggests that the Tb-R_3 complex is a reliable luminescent probe to determine ChA in green coffee**.**

Next, ChA was determined in real green and roasted coffee samples by luminescence quenching of Tb-R_3 and validated with HPLC with photometric detection as described in the "HPLC determination of ChA in green coffee" section. A representative HPLC chromatogram is displayed in Fig. 5 and shows ChA eluting at the highest absorption peak at a retention time of 5.50 min. Additionally, caffeine and caffeic acid are found in typical concentrations [3, 20, 21] of 1.4 mg/100 mg and 0,85 mg/100 mg, respectively, in the green coffee sample. 1.2 mg/100 mg of caffeine and 0.65 mg/100 mg of caffeic acid were found in the roasted coffee sample. A comparison of the related concentrations of ChA in mg/100 mg (i.e. in %) found in the coffee samples by luminescence quenching and HPLC is shown in Table 5. The concentrations of ChA obtained match very well within the range of errors of the individual methods. Importantly, the standard deviations are very low, even though the number of samples ($n = 5$) was not very high. This demonstrates that both methods work precisely and that the determination of ChA with Tb-R_3 also works reliably in real coffee samples. Additionally, the very similar contents of ChA found by both methods in real samples points to that Tb-R_3 seem to deliver the total content of all ChA derivatives found in

Table 2 Figures of merit of the luminescence determination of ChA with Tb-R_3 according to the non-linear Stern–Volmer plot

Parameter	Result
Quantitation range [µg/mL]	0.5–30.0
LOD [µg/mL]	0.18
Correlation coefficient (r)	0.9962
Accuracy ($n = 5$) [%]	100.6
Precision Inter-day ($n = 6$) [%] Intra-day ($n = 6$) [%]	2.4 2.8
Specificity	specific

Table 3 Recovery of ChA in solution ($n = 5$, $P = 95\%$)

Amount added [µg/mL]	Amount found [µg/mL]	Recovery [%]	RSD [%]
3	3.05 ± 0.06	101.7	1.6
10	10.09 ± 0.15	100.9	1.2
20	19.85 ± 0.27	99.3	1.1
		Average recovery: 100.6	

Table 4 Tolerance limits of various interferents on the determination of 10 µg/mL of ChA

Interferent	Interferent-to-analyte ratio	ΔI [%]
Caffeine	2:1	− 0.05
Caffeic acid	2:1	− 1.5

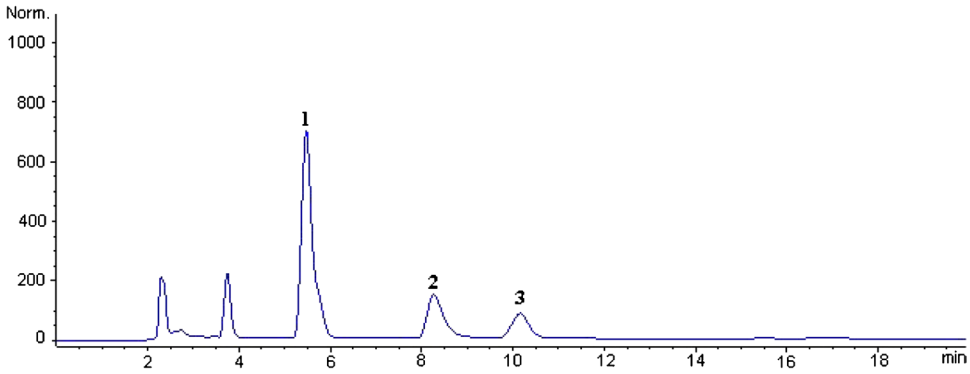


Fig. 5 Chromatogram of green coffee sample: ChA (1); caffeine (2); caffeic acid (3)

Table 5 Determination of ChA in coffee ($n=5$, $P=95\%$)

	Sample №	ChA [mg/100 mg]					
		HPLC			Luminescence		
		Found	$X_{av} \pm \Delta X$	RSD [%]	Found	$X_{av} \pm \Delta X$	RSD [%]
Green	1	6.62	6.59 ± 0.18	2.25	6.70	6.73 ± 0.27	3.25
	2	6.47			6.58		
	3	6.71			6.74		
	4	6.57			6.81		
	5	6.60			6.63		
Roasted	1	0.74	0.72 ± 0.02	2.78	0.82	0.79 ± 0.05	4.90
	2	0.73			0.75		
	3	0.71			0.84		
	4	0.73			0.76		
	5	0.69			0.78		

coffee [20, 21]. This is plausible because the luminescence response always occurs via coordination of the ChA derivatives with the free carboxylic acid of the quinic acid part of ChA, independently which coffee acid is esterified with the quinic acid part.

Another welcome aspect is that the luminescence method not only permits the determination of the higher concentrations of ChA but also the much lower concentrations of ChA that remain after roasting of the coffee. This permits the quality control of the content of ChA in both green and roasted real coffee samples (i.e. prior and past roasting) with the very same method and hence makes the luminescence quenching method more valuable.

Compared to known methods for determination of ChA (Table 6), the HPLC methods offer better detection limits than our luminescence method. However, HPLC is associated with purchasing more expensive analytical instrumentation that is more complicated to operate. Luminescence equipment requires less than a quarter of the costs of an HPLC system, and the sample preparation is rapid and easy. Compared to spectrophotometric methods, our luminescence method is more sensitive, provides a wider dynamic range and covers the concentration range of ChA without dilutions during sample preparation.

Determination of luminescence quenching mechanism

Luminescence quenching experiments were carried out to explore the quenching mechanism and to determine quenching constants. Chlorogenic acid has a carboxyl functional group, which makes it amenable to coordinate to Tb^{3+}. This group has a pK_a value of 3.55 [22] and is therefore deprotonated under the conditions (urotropine buffer of pH 7.5) used here. Hence, ChA can coordinate as a ligand that is countercharged with respect to Tb^{3+}. The coordinative binding is confirmed by the strong red-shift of Tb-R_3-ChA as compared to the absorption spectra of the free Tb^{3+}-R_3 complex and of ChA (Fig. 2). As a result, an energy loss of the Tb^{3+}-R_3 complex is observed which leads to the fluorescence quenching.

The decay time τ of the terbium emission of the Tb-R_3 complex is 948 µs. In the presence of increasing concentrations of ChA from 1 to 20 µg/mL, τ decreases from 880 to 700 µs (Fig. 6). This confirms a contribution of dynamic quenching to the overall quenching of Tb-R_3.

From a Stern–Volmer plot of τ_0/τ vs. c_{ChA}, where τ_0 is the average lifetime of the Tb-R_3 complex, the slope of the linear function gives the dynamic quenching constant.

Table 6 Overview on common methods for determination of chlorogenic acid in coffee samples

Method	λ[nm]	Linear range [µg/ml]	LOD [µg/ml]	Ref
HPLC–UV	325	0.07–12	-	3
HPLC–UV	326	0.05–1.25	0.013	4
MEKC-UV	325	25–900	0.98	5
HPLC–UV	330	9.69–1299	1.25	6
HPTLC-UV	366	20–300*	-	7
HPTLC-UV	330	12–240*	-	8
UV spectroscopy	324	48–176	16	9
NIR-vis spectroscopy	790	10–800	3.5	10
UV spectroscopy	330	0.5–5.0	0,2	11
Luminescence	545 (λ_{em})	0.5–30.0	0.18	This work

*- ng/band.

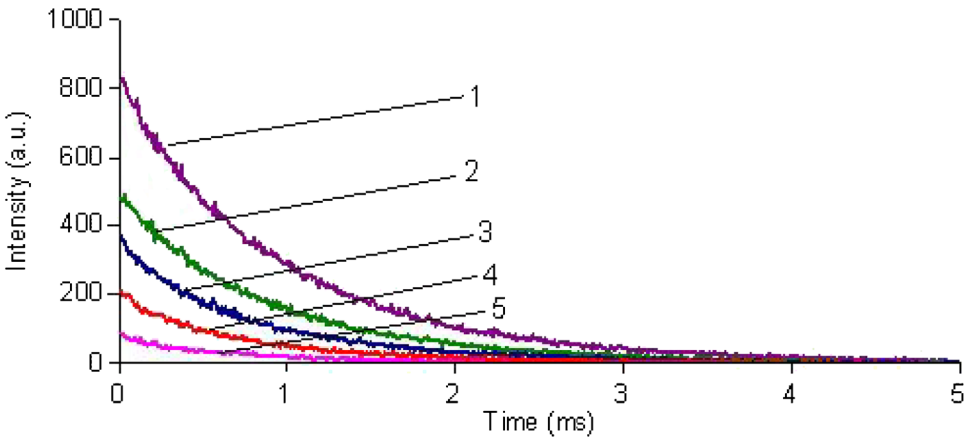


Fig. 6 Luminescence decay curves of the Tb-R_3 complex without (1) and in presence (2)–(5) of various concentrations of ChA ($c_{Tb^{3+}} = c_{R3} = 1 \cdot 10.^{-5}$ mol/L; c_{ChA}: (2) 1 µg/mL (τ = 880 µs); (3) 5 µg/mL (τ = 780 µs); (4) 10 µg/mL (τ = 725 µs); (5) 20 µg/mL (τ = 700 µs), $\lambda_{exc}/\lambda_{em}$ = 315 nm/545 nm)

The slope of the plot τ_0/τ vs. c_{ChA} is consistent with a K_D of $1.05 \cdot 10^4$ M^{-1}. This coincides very well with the concentration of the quencher of $1.0 \cdot 10^{-5}$ mol/L where 50% of the intensity is quenched and which should also equal K_D [23].

We then calculated K_s based on the luminescence intensity of the plot shown in Fig. 4. This plot was converted to a plot of F_0/F vs. c_{ChA}/(mol/L). Accordingly, one can calculate the dynamic and static quenching constants using $F_0/F = 1 + B_1 \cdot c_{ChA} + B_2 \cdot c_{ChA}^2$ [23].

B_1 of this plot equals $K_D + K_S$ and B_2 equals $K_D \cdot K_S$, where K_D is the dynamic quenching constant and K_S is the static quenching constant. This equals $F_0/F = 1 + (K_D + K_S) \cdot c_{ChA} + K_D \cdot K_S \cdot {}_{ChA}^2$. In the fit function, we calculated $F_0/F = 1.120 + 14{,}985 \cdot c_{ChA} + 6.265 \cdot {}_{ChA}^2$ which results in $K_D + K_S = 14{,}985$ M^{-1} and $K_D \cdot K_S = 6.265 \cdot 10^8$ M^{-2}. Consequently, we assign $K_S = 5.97 \cdot 10^4$ M^{-1} and $K_D = 1.05 \cdot 10^4$ M^{-1}. Hence, the quenching mechanism of ChA on the luminescence of Tb-R_3 complex is combined static and dynamic.

Conclusions

We describe the luminescence properties of three new terbium complexes with 1-(furan-2-ylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide ligands. The complex of one of these ligands (R_3) in 1:1 molar ratio with Tb^{3+} is a highly sensitive and selective probe for the determination of chlorogenic acid by luminescence quenching. This enables the reliable and accurate determination of ChA in urotropine buffer at near neutral pH as validated by a concomitant determination of ChA with HPLC. The ChA contents found by luminescence determination in real green and roasted coffee samples agreed very well with those of HPLC. Luminescence lifetime measurements show contributions of both static and dynamic quenching and the related quenching constants are $K_S = 5.97 \cdot 10^4$ M^{-1} and $K_D = 1.05 \cdot 10^4$ M^{-1}. The proposed method is simple, yet very accurate and reproducible and can be used for the quality control of ChA in coffee samples prior and after roasting.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00216-022-04411-x.

Funding Open Access funding enabled and organized by Projekt DEAL.

Declarations

Conflict of interest The authors declare no competing interests.

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