Application of Tb$_4$O$_7$ Nanoparticles for Lasalocid and Salicylate Determination in Food Analysis

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ABSTRACT: The usefulness of Tb$_4$O$_7$ nanoparticles (NPs) as analytical reagents using sensitized luminescence as a detection system is described for the first time, and the results obtained are compared with those obtained using Tb(III) ions. Two drugs used in veterinary practice, namely, lasalocid (LAS) and salicylate (SAL), have been chosen as model analytes to carry out this study. The experimental conditions for these systems have been optimized, and their analytical features were obtained. The detection limits obtained for LAS and SAL using Tb$_4$O$_7$ NPs were 1.0 and 4.0 ng mL^{-1}, respectively, which were comparable to those obtained using Tb(III) ions: 1.8 and 1.0 ng mL^{-1}, respectively. However, precision data, with relative standard deviation values in the range 2.3−3.8% using the NPs and 3.5−6.5% using Tb(III) ions, were slightly better for LAS with Tb$_4$O$_7$ NPs. The practical analytical usefulness of Tb$_4$O$_7$ NPs as luminescent reagents has been shown by performing the determination of LAS in tap water, feed premix, and egg samples, obtaining recoveries in the range of 80.0−105.0%.

KEYWORDS: Tb$_4$O$_7$ nanoparticles, sensitized luminescence, lasalocid and salicylate, food samples

INTRODUCTION

Lanthanide-sensitized luminescence is widely used for analytical purposes,[1,2] which can be attributed to the capability of Tb(III) and Eu(III) ions to form highly luminescent chelates with organic compounds by means of intramolecular energy transfer processes from the ligand to the lanthanide ion. Some features of these systems are the high spectral selectivity of the luminescence emission, which happens through the resonance levels of the lanthanide ion, the long decay time, and the large Stokes shift. Also, luminescence emission happens at a relatively long wavelength, which avoids the background fluorescence from sample matrices that have shorter lifetimes and usually emit at shorter wavelengths.

The huge impact of nanotechnology in the analytical field has given rise to the development of luminescent lanthanide nanoparticles (NPs) based on the incorporation of a luminescent lanthanide chelate into organic polymer NPs, such as polystyrene NPs, or inorganic NPs, such as silica, zirconia, or titania NPs.[3] Composite Tb(III)−acetylacetonate NPs have been used as reagents for the determination of salicylate (SAL) in pharmaceutical formulations and human plasma,[4] obtaining a detection limit of 3.5 ng mL^{-1}. The method involves an energy transfer process between SAL and the composite NPs, because of the spectral overlap between the fluorescence spectrum of SAL and the excitation spectrum of the NPs. Also, an efficient energy transfer process from benzoic acid to Tb(III) or Eu(III) has been demonstrated in benzoic acid-functionalized lanthanide-doped calcium fluoride NPs,[5] but the potential analytical use of these NPs has not been described. Two methods have been reported for the determination of fluoroquinolones[6] and catecholamines[7] in pharmaceutical, urine, and serum samples based on the positive effect of silver NPs on the luminescence emission of the complexes of Tb(III) with these analytes, which is attributed to the interaction of the excited state of the luminescent complexes and the surface plasmon electrons of silver NPs.

Nanostructures of some terbium oxides, such as Tb$_2$O$_3$,[8−10] have found application in laser technology because of their relatively high luminescence. However, other terbium oxides, such as Tb$_4$O$_7$ NPs, do not show luminescence. These NPs, which contain at least some Tb(IV), along with the more stable Tb(III), have been used as precursors for the synthesis of lanthanide nanophosphors[11] and superconductor materials,[12] but to the best of our knowledge, they have not been used for analytical purposes up to date. Thus, the study reported here constitutes the first attempt to use Tb$_4$O$_7$ NPs as analytical reagents by checking their capability to obtain terbium-sensitized luminescence in the presence of lasalocid (LAS) and SAL. These compounds have been chosen as model analytes because they form luminescent complexes with Tb(III), which have been used for the development of very sensitive analytical methods.[13−15] A systematic study has been performed to compare the analytical features of the corresponding methods involving the use of Tb(III) ions and Tb$_4$O$_7$ NPs as analytical reagents.

The structures of SAL and LAS, the latter being an antibiotic used for the prevention of coccidiosis, are shown in Figure 1. It can be seen that LAS contains a SAL moiety and a long chain substituent, which provides it with ionophore activity. The use of both drugs for veterinary purposes is allowed in Europe, according to the Commission Regulation 37/2010,[16] which defines the maximum residue limit (MRL) for LAS. However, no limit has been defined for the veterinary use of SAL, which is restricted to topical treatments. SAL could also be present as a
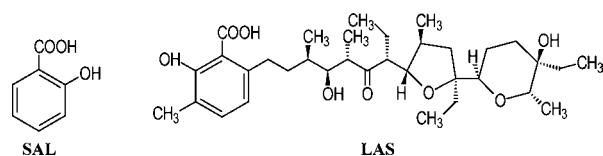
Figure 1. Chemical structures of SAL and LAS.

metabolite of acetylsalicylate, but its use is not allowed in milk- and/or egg-producing animals for human consumption. The determination of LAS in water and feed premix samples using Tb(III) as a reagent has been previously described,[13] but it has not been applied to the analysis of egg samples. However, the availability of a simple and fast method to detect the presence of LAS in these samples is of great interest because LAS can be present in egg samples even 9 days after treatment.[17] Shorter withdrawal periods would lead to LAS concentrations above its MRL with potential adverse effects on human health. With the aim of showing the analytical usefulness of Tb_4O_7 NPs, the method described here, involving the use of these NPs, has been applied for the first time to the determination of LAS in egg samples with satisfactory results.

Several liquid chromatographic methods have been described for LAS determination using fluorimetry[18,19] or mass spectrometry[20,21] as detection systems. These methods are the best option for multiresidue determination in biological matrices, in spite of the expensive instrumentation required. However, the results obtained in this study show the usefulness of a sensitized luminescence method, using a simple spectrofluorimeter, for the control of the poultry treatment with LAS for the prevention of coccidiosis. The high sensitivity and selectivity of the proposed method allow its application to the analysis of premix feed and egg samples after simple extraction and cleanup steps.

■ MATERIALS AND METHODS

Apparatus. An SLM Aminco (Urbana, IL) model 8100 photon counting spectrofluorometer, equipped with a 450 W xenon arc lamp and two R928 photomultiplier tubes, was used to perform luminescence measurements using a conventional 1 cm path-length quartz cell. The detector high voltage was set at 650 V with gain 100 for all of the optimization studies to compare the signals obtained in each instance. This voltage value was then changed to obtain suitable instrumental conditions to choose the best calibration conditions for each system.

Reagents. All reagents were of analytical grade and used as purchased. Stock solutions of LAS and SAL (1000 mg L^{-1} each) were prepared by dissolving the standards in the minimum volume of methanol and then raised up to mark with distilled water. Tb_4O_7 nanopowder (<100 nm, 99.5%) and Tb(III) nitrate pentahydrate (99.9%) were obtained from Aldrich (St. Louis, MO). A 0.019 M Tb(III) and a 4.75 mM Tb_4O_7 (equivalent to 19 mM in terbium) stock solutions were prepared in water and 2-propanol, respectively. A 1.5 mM tri-*n*-octylphosphine oxide (TOPO) solution was prepared by dissolving the appropriate amount of TOPO (99%, Aldrich, Gillingham, Dorset, United Kingdom) in ethanol and then adding water dropwise until mark to be finally 80% in ethanol. A 0.625 M sodium hydroxide solution was prepared to adjust the pH of the alkaline medium. A 0.2 M solution of disodium ethylenediaminete-tracetate (EDTA) was prepared by dissolving the appropriate amount of EDTA (Aldrich) in 0.625 M sodium hydroxide. Stock solutions of the surfactants cetyltrimethylammonium bromide (CTAB) (10^{-2} M) and Triton X-100 (0.1% w/v) (Fluka, Buchs, Switzerland) were prepared in distilled water. Imidazole (99%, Aldrich, Steinheim, Germany), hexamethylenetetramine (hexamine, Merck, Schuhard, Germany), and ammonium acetate (Aldrich) were also used.

Imidazole (0.1 M, pH 6−8), hexamine (0.1 M, pH 5.5−7.5), and ammonium acetate (0.1 M, pH 6.0) buffer solutions were prepared by dissolving the appropriate amount of the compound in water and adjusting the pH with either hydrochloric acid or sodium hydroxide. High-performance liquid chromatography (HPLC)-grade 2-propanol and methanol (Panreac, Castellar del Vallès, Spain) were also used. Supel-select HLB SPE tubes (60 mg/3 mL; Supelco, Bellefonte, PA) were used to perform the cleanup of egg samples. The AVATEC feed premix sample was kindly donated by MIPROMA Productos Zoosanitarios S.L. (Seville, Spain).

Experimental Procedures. *Determination of LAS.* Solutions containing LAS (3−4000 ng mL^{-1}), Tb_4O_7 NPs (62.5 μM, equivalent to 0.25 mM terbium ions), TOPO (50 μM), Triton X-100 (0.08%), hexamine buffer solution (1 mM, pH 6.2), and 2-propanol (2.5%) were prepared in 5 mL volumetric flasks. The luminescence intensity was measured at λ_{ex} 318 nm and λ_{em} 545 nm after 5 min, using an 8 nm bandwidth for both excitation and emission slits.

Determination of SAL. Solutions containing SAL (20−1250 ng mL^{-1}), Tb_4O_7 NPs (0.11 mM, equivalent to 0.44 mM terbium), TOPO (75 μM), Triton X-100 (0.04%), imidazole buffer solution (3.25 mM, pH 7.2), and 2-propanol (2.5%) were prepared in 5 mL volumetric flasks. The determination of SAL also can be carried out in alkaline medium preparing solutions containing SAL (40−2500 ng mL^{-1}), Tb_4O_7 NPs (0.19 mM, equivalent to 0.75 mM terbium), EDTA (2.5 mM), CTAB (75 μM), and NaOH (0.055 M) in 5 mL volumetric flasks. The luminescence intensity was measured in both instances under the instrumental conditions above-described.

Determination of LAS in Poultry-Related Samples. Tap Water. A volume (2.5 mL) of sample was mixed with the reagents using the experimental conditions above-described.

Feed Premix. An amount (0.5 g) of the AVATEC premix sample was extracted with 25 mL of ethanol acidified with 0.5 mL of concentrated HCl in an ultrasonic bath at 40 °C for 20 min. Then, the extraction was continued for 1 h using mechanical stirring at room temperature. The suspension was filtered, the filter was washed with ethanol, and the solution was raised up to 100 mL with this solvent. This solution was 100-fold diluted with water to match the dynamic range of the calibration curve, and 0.25 mL of this solution was treated following the above-mentioned procedure for LAS determination using Tb_4O_7 NPs as reagent.

Egg. An amount (0.4 g) of homogenized sample spiked with appropriate LAS amounts to be in the final range of 75−150 μg kg^{-1} (0.5, 0.66, and 1.0 MRL)[16] and allowed to equilibrate for 30 min was extracted with 1.5 mL of an ethanol solution acidified with HCl (at a final concentration of 11%). The suspension was placed in an ultrasonic bath at 40 °C for 20 min and then centrifuged at 537g for 15 min, and the supernatant was kept. This extraction was repeated, and both extracts were combined and diluted to 50 mL with distilled water. Afterward, a cleanup step was performed by a solid-phase extraction (SPE) procedure using polymeric HLB cartridges previously conditioned using 6 mL of methanol and 6 mL of acidified water with HCl (pH 1.2). Once the samples were applied, cartridges were washed using 5 mL of a 5% ethanol aqueous solution. Finally, LAS retained was eluted using 1 mL of a 95% ethanol/5% 10^{-2} M NH$_3$ solution. A volume (700 μL) of this solution was treated with 35 μL of 0.1 M HCl to adjust the pH and was subjected to the procedure above-described for LAS determination.

■ RESULTS AND DISCUSSION

Preliminary Studies on the Luminescent Systems. Several assays were carried out to study the potential usefulness of Tb_4O_7 NPs as an analytical reagent for LAS and SAL determination. The results obtained were compared with those obtained in the presence of Tb(III) ions, using the same equivalent terbium concentration in all instances. This consideration has been made from a theoretical point of view, bearing in mind the oxide stoichiometry. It is quite improbable that the analytes can get into the core of Tb_4O_7

NPs, so the available terbium concentration would be lower for Tb$_4$O$_7$ NPs than for Tb(III) ions. The insolubility of Tb$_4$O$_7$ NPs in water made necessary to study their dispersion in different solvents, such as 2-propanol, methanol, ethanol, toluene, and polyethylene glycol solutions, finding the best results with 2-propanol, which was also used in the assays using Tb(III).

Although it is known that the luminescence-sensitized intensity can be improved in the presence of a synergetic agent and a micellar medium, preliminary assays were carried out in their absence to compare the behavior of the NPs and Tb(III) with the selected analytes. Figure 2 shows the emission
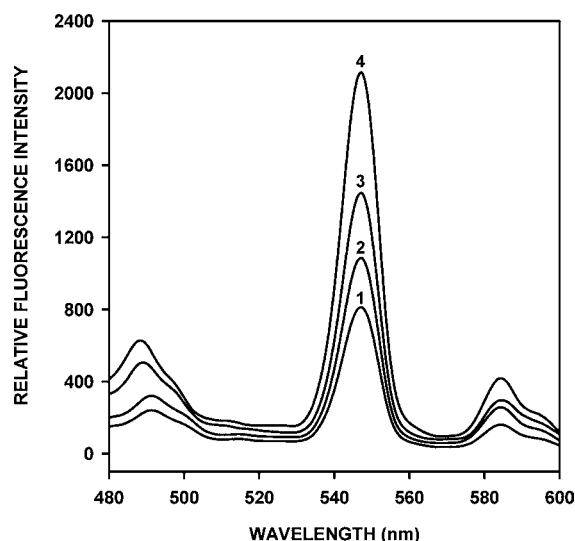

Figure 2. Emission spectra obtained for LAS (1 and 3) and SAL (2 and 4) using Tb(III) ions (1 and 4) and Tb$_4$O$_7$ NPs (2 and 3) as reagents (λ_{ex} = 318 nm). Conditions: [equivalent terbium] = 0.25 mM, pH 6.0, and [2-propanol] = 2.5%. [LAS] = [SAL] = 1000 ng mL^{-1}.

spectra obtained for LAS and SAL in the presence of Tb$_4$O$_7$ NPs and Tb(III) ions (λ_{ex} 318 nm), in which can be seen that all of the systems show the typical bands described for terbium-sensitized luminescence with maximum emission at 545 nm. The luminescence intensity obtained for NPs-LAS system was higher than that obtained for the Tb(III)−LAS system, but an opposite effect was observed for SAL. The presence of a synergetic agent and a micellar medium increased the luminescence of these systems, as it is described as follows.

Optimization of Variables. Variables affecting the systems were optimized using the univariate method. Those values yielding the best luminescence signals with minimum standard deviation for the variables involved were taken as optimal.

The study of the influence of pH in the range of 5.5−8.0 showed that this variable has a similar effect in the luminescence intensity obtained for both LAS and SAL using Tb$_4$O$_7$ NPs or Tb(III) ions. The optimum pH range was 6.0−6.5 for LAS systems and 7.0−7.5 for SAL systems. Hexamine, imidazole, and ammonium acetate buffer solutions were assayed to adjust the pH of LAS and SAL systems using Tb$_4$O$_7$ NPs or Tb(III) ions. A 1.0 mM hexamine buffer solution (pH 6.2) was chosen for LAS systems, and a 3.25 mM imidazole buffer solution (pH 7.2) was selected for SAL systems.

Taking into consideration that the presence of a synergistic reagent such as TOPO and a surfactant such as Triton X-100 notably improve the luminescence of the Tb(III)−LAS system,[13] the influence of the concentration of these variables on the LAS and SAL systems was comparatively studied using Tb$_4$O$_7$ NPs and Tb(III) ions. The results obtained showed that TOPO, at the concentration range of 4 × 10^{-5}−8 × 10^{-5} M, has a similar positive effect on both LAS and SAL systems using NPs or Tb(III) ions. The study of the effect of Triton X-100 concentration, above its critical micellar concentration, showed that the luminescence intensity of LAS systems was independent of this variable in the range 0.04−0.12%, whereas the optimum concentration range for SAL systems was 0.03−0.06%. The luminescence intensity of all of the systems in the presence of both TOPO and Triton X-100 was about 10 times
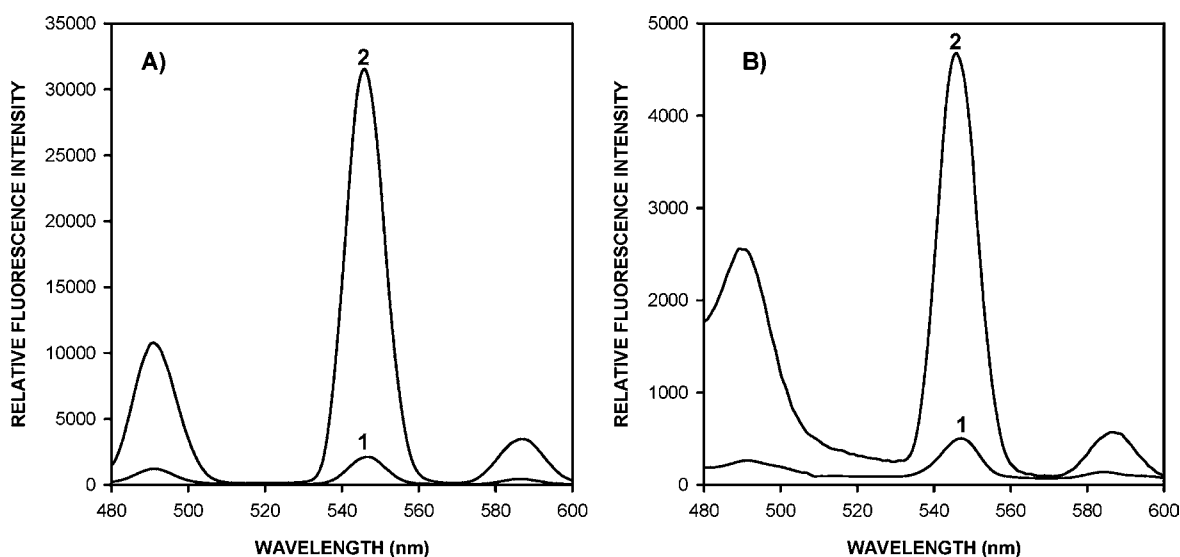

Figure 3. Emission spectra obtained for LAS (A) and SAL (B) systems in the absence (1) and the presence (2) of TOPO and Triton X-100. Conditions: In A and B, [2-propanol] = 2.5%. In A, [LAS] = 1000 ng mL^{-1}, [Tb$_4$O$_7$ NP] = 62.5 μM, and [hexamine] = 1 mM, pH 6.2; in A.2, [Triton X-100] = 0.08% and [TOPO] = 50 μM; in B, [SAL] = 1000 ng mL^{-1}, [Tb$_4$O$_7$ NP] = 0.11 mM, and [imidazole] = 3.25 mM, pH 7.2; and in B.2, [Triton X-100] = 0.04%, and [TOPO] = 75 μM.
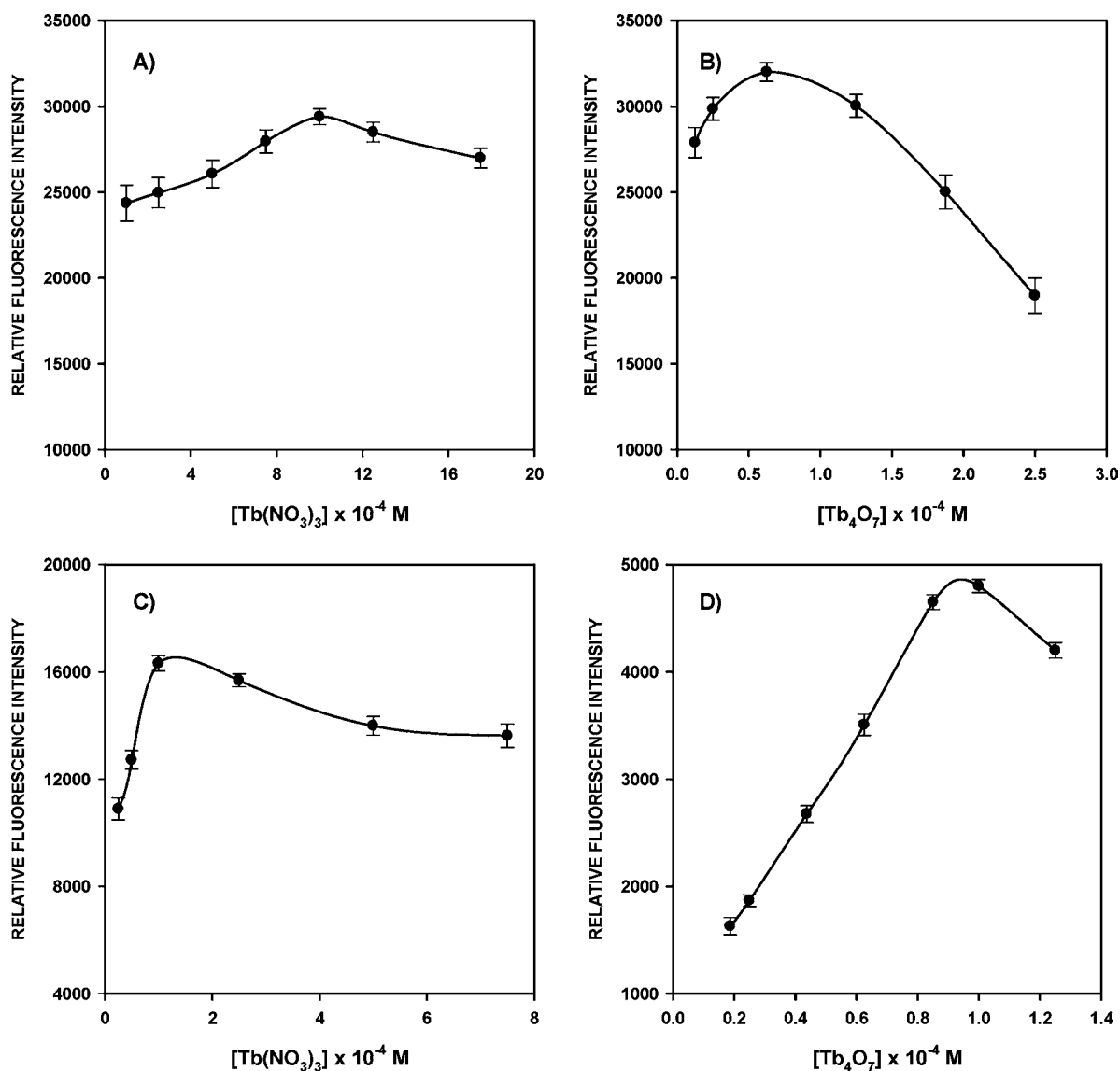
Figure 4. Influence of Tb(III) ion (A and C) and Tb_4O_7 NPs (B and D) concentrations on LAS (1000 ng mL^{-1}) (A and B) and SAL (1000 ng mL^{-1}) (C and D) systems. In A and B, [Triton X-100] = 0.08%, and [hexamine] = 1 mM, pH 6.2. In C and D, [Triton X-100] = 0.04%, and [imidazole] = 3.25 mM, pH 7.2. In all instances, [TOPO] = 5×10^{-5} M, and [2-propanol] = 2.5%.

higher than that obtained in their absence. This positive effect is shown in Figure 3 for both LAS and SAL systems in the presence of Tb_4O_7 NPs.

Figure 4 shows the comparative study of the influence of terbium ions and terbium NPs on both LAS and SAL systems. As can be seen, the luminescence obtained for the Tb_4O_7 NPs–LAS system was higher than that obtained for Tb(III)–LAS, but an opposite behavior was observed for the SAL systems. The optimum Tb_4O_7 NP concentration ranges for LAS and SAL systems were 3×10^{-5}–10×10^{-5} M and 8×10^{-5}–12×10^{-5} M, respectively, whereas the optimum Tb(III) ions concentration ranges were 8×10^{-4}–16×10^{-4} M and 8×10^{-5}–12×10^{-5} M, respectively. These results show that the optimum NPs concentration for LAS system is about 10 times lower than the Tb(III) concentration, whereas this difference is not observed for the SAL system. This behavior could be explained by the fact that the chemical structure of LAS is more planar than that of SAL, which favors a more efficient energy transfer process.

The study of the influence of the 2-propanol percentage on the LAS and SAL systems, in the range of 2.5–20%, showed

that the maximum signal was obtained for both Tb(III)–LAS and Tb_4O_7 NPs–LAS systems in the range of 2.5–10% 2-propanol. However, the luminescence intensity of both systems decreased at higher 2-propanol percentages, which could be attributed to the capability of the solvent to destruct the micelles, which shield the chelates and favor the intramolecular energy transfer process.[13] The presence of 2-propanol on the SAL systems, using Tb(III) ions or NPs, caused a more negative effect since the luminescence decreased up to 8 times when the percentage was increased from 2.5 to 10%. This difference could be mainly attributed to the fact that LAS is more hydrophobic than SAL, because of the presence of the relatively long chain substituent group in the LAS molecule.

As the determination of SAL using Tb(III) ions in alkaline medium has been previously described,[15] the potential usefulness of Tb_4O_7 NPs as a reagent in this medium also has been studied. The performance of terbium-sensitized luminescence in alkaline medium requires the addition of a strong chelating agent, such as EDTA, to prevent the precipitation of the lanthanide hydroxide, and the use of a

Table 1. Analytical Features of the Optimized Systems

system	Tb(III)−LAS	Tb₄O₇−LAS	Tb(III)−SAL	Tb₄O₇−SAL
linear range (ng mL⁻¹)	5−300	3−700	3−250	20−1250
	300−5000	700−4000	250−2500	
slope ± SD	144 ± 2	44.0 ± 0.8	97 ± 2	9.7 ± 0.8
	6.4 ± 0.2	4.72 ± 0.08	13.9 ± 0.9	
Y-intercept	$(1.9 ± 0.2) \times 10^1$	$(1.0 ± 0.3) \times 10^1$	$(2.0 ± 0.9) \times 10^1$	$(5.0 ± 0.4) \times 10^1$
	$(-1.0 ± 0.2) \times 10^1$	$(-1.3 ± 0.8) \times 10^2$	$(9 ± 1) \times 10^2$	
regression coefficient (r)	0.9991	0.998	0.996	0.998
	0.992	0.999	0.990	
LOD (ng mL⁻¹)	1.8	1.0	1.0	4.0
precision (% RSD)	6.5 (10.0)[a]	3.8 (5.0)	4.4 (5.0)	3.6 (50.0)
	4.3 (100.0)	2.3 (100.0)	3.5 (100.0)	2.8 (200.0)

[a]Analyte concentration assayed, ng mL⁻¹ (n = 10).

cationic surfactant, such as CTAB, to improve the luminescence signal. Several assays were carried out to study the capability of the LAS systems, using Tb(III) ions and Tb₄O₇ NPs, to obtain sensitized luminescence in alkaline medium, but the results obtained were not satisfactory. Thus, the comparative study using Tb(III) and NPs was only carried out for the SAL systems. Several experimental variables, including pH and EDTA, CTAB, Tb(III) ions, and NPs concentrations were optimized, obtaining that the luminescence intensity using the NPs was about 10 times lower than that obtained using Tb(III) ions. These results show that the analytical usefulness of Tb₄O₇ NPs in alkaline medium is limited.

Analytical Features. The features of the analytical methods developed for LAS and SAL determinations under the optimal experimental variables, using Tb(III) ions and Tb₄O₇ NPs, are summarized in Table 1. The wide dynamic ranges of the calibration graphs required the use of two different instrumental sensitivities, except for the NPs−SAL system, obtaining two linear ranges that show different slopes and y-intercepts. The regression coefficients obtained in all instances are indicative of the good calibration linearity. Both the detection (LOD) and the quantification (LOQ) limits were calculated according to IUPAC recommendations.[22] The LOQ values correspond to the lowest analyte concentrations of the dynamic range of the calibration curves. The low values obtained for these parameters show that both Tb(III) ions and Tb₄O₇ NPs can be used as reagents for the sensitive determination of LAS and SAL. As can be seen, the LOD for LAS with Tb₄O₇ NPs was about two times lower than that obtained using Tb(III) ions. Table 1 also shows the precision data, expressed as the percentage of relative standard deviation (% RSD), for the four systems. The precision values obtained using NPs were slightly lower than those obtained using Tb(III) ions.

The selectivity of the Tb₄O₇ NPs−LAS system was studied by assaying different antibiotics of veterinary use belonging to the ionophore group, such as monensin and salinomycin, or to other antibiotic groups such as aminoglycosides, sulfonamides, tetracyclines, fluoroquinolones, macrolides, and colistin (Table 2). The maximum concentration tested for all of these compounds was 10 μg mL⁻¹, when a 100 ng mL⁻¹ LAS concentration was assayed. A compound was considered not to interfere at a given concentration when the analytical signal obtained in the presence of this substance was within one standard deviation of the value obtained in its absence. All of the compounds assayed were tolerated at higher concentration levels than that of LAS, so that they do not interfere with LAS determination at the permitted MRL for these compounds in

Table 2. Influence of Other Antimicrobial Agents on the 0.1 μg mL⁻¹ LAS Determination by Using Tb₄O₇ NPs

compd	maximum tolerated interferent:analyte ratio[a]
monensin	75
gentamycin	50
streptomycin	40
neomycin	40
salinomycin	25
sulphamerazine	15
spectinomycin	10
marbofloxacin	5
sulphanilamide	5
tylosin	5
colistin	5
erythromycin	4
tetracycline	4
chlortetracycline	4
oxytetracycline	4

[a]The maximum tolerated ratio assayed was 100-fold of the analyte concentration.

veterinary samples. Although the interaction of fluoroquinolones with terbium ions has been previously reported,[6,23,24] their use is not allowed in animals producing eggs according to 37/2010 regulation. However, marbofloxacin, which was assayed as model compound belonging to this group, was tolerated in a 5-fold excess. The presence of tetracyclines, which can be used in egg-producing animals, caused a decrease of the analytical signal. The use of calcium ions, which have been reported to complex tetracyclines,[25] was checked out to avoid this interference, finding that the tolerated ratio increased to 4-fold that of LAS concentration in the presence of 7.0 mM calcium ions. A 0.4 μg mL⁻¹ tetracycline concentration, which corresponds to the tolerated ratio, is well above its MRL, thus being the selectivity of this method ensured for this purpose.

Applications. The proposed method for LAS determination using Tb₄O₇ NPs was applied to the analysis of tap water, feed premix (the commercial brand name AVATEC), and egg samples (Table 3). The feed premix sample is used for the therapeutic control of LAS in poultry and poultry-related food production. The analysis of tap water was directly performed without any sample treatment, whereas an extraction step with acidified ethanol, following previously described methods,[13,18] was required for the feed premix sample analysis and an additional SPE cleanup step for the egg analysis. The standard addition method was applied in all instances. LAS was

Table 3. Determination of LAS in Poultry-Related Food Samples

sample	content[a,b]	added[a]	found[a,b]	recovery (%)
		LAS concn[a]		
			recovery (%)	
tap water		40	42 ± 3	105.0
		60	57 ± 4	95.0
		80	78 ± 4	97.5
feed premix	13.9 ± 0.5	2	2.00 ± 0.09	100.0
		4	3.9 ± 0.1	97.5
		6	6.1 ± 0.2	101.7
hen egg		75	60 ± 4	80.0
		100	91 ± 7	91.1
		150	144 ± 5	96.0

[a]Units: water samples (ng mL^{-1}), feed premix (%), and egg sample (μg kg^{-1}). [b]Mean ± SD ($n = 3$).

not detected in the water and egg samples, while the content found in the feed premix sample (13.9 ± 0.5)% was slightly lower than the nominal value reported (15%) in the technical sheet provided by the manufacturer.[26] A recovery study was carried out by adding three different amounts of LAS to each sample. The results were in the range of 80.0−105.0% with a mean value of 96.0%. The mean recovery obtained for the feed premix sample was 99.7%, very similar to that previously reported using Tb(III)[13] (100.9%).

The present work has demonstrated for the first time the usefulness of Tb$_4$O$_7$ NPs to be sensitized by two model analytes, LAS and SAL, which have previously proven its ability to produce sensitized luminescence using Tb(III) ions.[13−15] The similar analytical features of the corresponding methods, using Tb(III) and Tb$_4$O$_7$ NPs, show that these NPs are suitable alternative reagents for LAS and SAL determination using sensitized luminescence. Also, the analytical applicability of these NPs has been demonstrated by the results obtained in the analyzed samples, which enables their use for the analytical control of poultry-derived foods during and after therapeutic treatment with LAS.

The commercial availability of these NPs facilitates their use as analytical reagents, as the synthesis step is avoided. Also, the cost of the assay using Tb$_4$O$_7$ NPs is lower than using Tb(III) ions as the amount required is 10 times lower, and the prices for both reagents are similar.

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Notes
The authors declare no competing financial interest.

■ ABBREVIATIONS USED

NPs, nanoparticles; LAS, lasalocid; SAL, salicylate; MRL, maximum residue limits; MS, mass spectrometry; TOPO, tri-*n*-octylphosphine oxide; EDTA, ethylenediaminetetracetate; CTAB, cetyltrimethylammonium bromide; HPLC, high-performance liquid chromatography; SPE, solid-phase extraction; LOD, limit of detection; RSD, relative standard deviation

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