INDIRECT SPECTROFLUORIMETRIC DETERMINATION OF MOSAPRIDE CITRATE IN PHARMACEUTICAL FORMULATIONS

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Abstract: New europium complexes of 3-oxo-1-hydroxy-quinoline-2-carboxylic acid amide derivatives (L₁⁻L₃), which are highly luminescent and do not require luminescence enhancers are reported. The luminescence intensity of the Eu-L₁⁻ complexes was enhanced by the addition of citrate ions in water solution. A sensitive luminescence enhancement system was developed for the determination of citrate ions on the base of Eu-9-fluoroor-1-hydroxy-5-methyl-3-oxo-6,7-dihydro-3H,5H-pyrido[3,2,1-ij]quinoline-2-carboxylic acid (2-piperazin-1-yl-ethyl)-amide (L₁) complex. This effect was applied to the determination of the drug, which is not a lanthanide luminescence sensitizer. The Eu-L₁-Cit complex with a components’ ratio 1:1:2 was proposed to be used as the analytical form for the luminescence determination of drug – mosapride citrate. The calibration curve is linear in the range of 1.0-25.0 µg/mL of mosapride citrate (LOD is 0.35 µg/mL). This method was applied for the determination of mosapride citrate in dosage form - tablets “Mosid MT” – 2.5 mg.

Keywords: mosapride citrate; luminescence enhancement; europium (III)

Mosapride citrate (MC), 4-amino-5-chloro-2-ethoxy-[N-[4-(p-fluorobenzyl)-2-morpholinyl]methyl]-benzamide, is a potent gastroprokinetic drug and it is used in gastrointestinal symptoms associated with chronic gastritis. MC is not yet official in any Pharmacopeia but there are several publications which describe methods for the determination of mosapride citrate in pure and in dosage forms. Most of the proposed methods for mosapride citrate analysis are HPLC (1) and spectrophotometric methods (2, 3).

The luminescence sensitization of lanthanides (Ln) in their complex compounds with organic ligands is widely used for the determination of drugs: fluoroquinolones (4), tetracycline (5), non-steroidal anti-inflammatory preparations (6), catecholamines (7) in the dosed forms and in various biological fluids.

In the last few years the possibility of analytical use of the lanthanide ions luminescence sensitization effect as well as their decrease/enhancement effects by some inorganic and organic anions, has been of special interest (8).

In this paper we have utilized the enhancement effect of sensitized luminescence by citrate ions for the determination of MC, which is not an Ln luminescence sensitizer.

EXPERIMENTAL

Apparatus

Luminescence and excitation spectra and lifetimes were measured with a Solar luminescence spectrometer (Belorussia) and an Aminco-Bowman Series 2 (SLM–Aminco, Rochester, New York) spectrometer with a 150-W xenon lamp. All of the

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measurements were made at room temperature (21–23°C). The pH values of solutions were measured on Lab 850 pH meter (Schott Instruments GmbH, Germany) with a glass electrode. Absorption spectra were recorded on a UV-2401 PC (Shimadzu) spectrophotometer.

MATERIALS AND METHODS

A standard 0.1 M solution of europium(III) chloride 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 3-oxo-1-hydroxyquinoline-2-carboxylic acid amide derivatives (L₁ – L₃) (Table 1) were synthesized as described elsewhere (9). The standard solutions of reagents (1×10⁻³ M) were prepared by dissolving accurate weights of the solid compounds in water.

The standard solution of sodium citrate (1×10⁻³ M) was obtained by dissolution of accurately weighed salt in water.

A standard solution of mosapride citrate, 1.0 mg/mL, was obtained by dissolving 50 mg in 30 mL of water, adjusting the pH to 7.5 with 0.1 M NaOH, and diluting to 50 mL. A working standard solution of mosapride citrate was obtained by dilution with the same solvent to final concentration of 100 µg/mL.

A urothropin buffer was prepared by dissolving 40.0 g of urothropin in water, adjusting the pH to 7.5 with HCl, and making up the volume to 100 mL.

A solution of ruthenium complex [Ru (2,2'-dipyridyl)_3]Cl₂◊6 H₂O (Fluka) was prepared in distilled water.

All of the used chemicals were of analytical grade or chemically pure, dissolved in doubly-distilled water.

Table 1. Spectroscopic and luminescence properties of reagents and their complexes with europium (Cₑₜ = 1×10⁻⁶ M; Cₑₐ = 5×10⁻⁴ M; pH 7.5)

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<tr>
<td>1</td>
<td>9-fluoro-1-hydroxy-5-methyl-3-oxo-6,7-dihydro-3H,5H-pyrido[3,2,1-ij]quinoline-2-carboxylic acid (2-piperazin-1-yl-ethyl)-amide</td>
<td>344</td>
<td>21010</td>
<td>0.42</td>
<td>292</td>
<td>3.33</td>
<td>1.38</td>
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<td>2</td>
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<td>21100</td>
<td>0.47</td>
<td>292</td>
<td>3.42</td>
<td>1.43</td>
</tr>
<tr>
<td>3</td>
<td>9-fluoro-1-hydroxy-5-methyl-3-oxo-6,7-dihydro-3H,5H-pyrido[3,2,1-ij]quinoline-2-carboxylic acid (2-ethylaminooethyl)-amide</td>
<td>347</td>
<td>20510</td>
<td>0.55</td>
<td>291</td>
<td>4.06</td>
<td>1.67</td>
</tr>
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Figure 1. Fluorescence decay curves for sensitized europium luminescence of Eu(III)-L complex in the presence of various concentrations of citrate ions, M: 1 ñ 0; 2 ñ 1◊10-5; 3 ñ 3◊10-5; 4 ñ 5◊10-5 (C_Eu = 1◊10-4 M; C_L = 5◊10-5 M; pH 7.5).

Figure 2. Excitation (a) and emission (b) spectra of Eu(III)-L complex in the presence of different concentrations of mosapride citrate (C_Eu = 1◊10-4 M; C_L = 5◊10-5 M; pH 7.5; λ_ex = 318 nm, λ_em = 612 nm).

Construction of calibration curve

Aliquots of 0.1, 0.2, 0.5, 0.7, 1.0, 1.5, 2.0 and 2.5 mL of a working solution of mosapride citrate (100 mg/mL) were placed in 10 mL volumetric flasks. One mL of a working 1◊10-3 M europium chloride solution, 0.5 mL of a standard, 1◊10-3 M reagent solution (L) and 0.5 mL of urothopin buffer were added to each flask. Simultaneously, a blank solution containing all the components except mosapride citrate was prepared. The solutions were diluted to volume with water and stirred. After 5 min the intensity of luminescence was measured at λ_em = 612 nm (λ_exc = 318 nm).

Figure 3. Dependence of the luminescence intensity of the Eu(III)-L complex on pH (C_Eu = 1◊10-4 M, C_L = 5◊10-5 M; pH 7.5).

Figure 4. Calibration curve for mosapride citrate determination (C_Eu = 1◊10-4 M, C_L = 5◊10-5 M; pH 7.5).
Twenty tablets of an analyzed drug were weighed to obtain the average tablet weight, and were then powdered. The powder equivalent to 5.0 mg of the active ingredient was placed into a 50 mL volumetric flask, mixed with 30 mL of water, adjusted to pH 7.5 with 0.1 M NaOH, stirred, diluted to volume with the same solvent and filtered to remove insoluble materials. To the filtrate (1 mL) in a 10 mL volumetric flask were added: 1.0 mL of a working 1◊10⁻³ M europium chloride solution, 0.5 mL of a standard, 1◊10⁻³ M reagent solution and 0.5 mL of urothropin buffer. The solution was made up to volume with water and luminescence intensity was measured at \(\lambda_{\text{em}} = 612\) nm (\(\lambda_{\text{ex}} = 318\) nm). After preparation of the standard solution by placing 1.0 mL of mosapride citrate stock solution (100 µg/mL) in a volumetric flask, and then addition of all components (with the exception of 1 mL of the filtrate solution) as described above, the \(I_0\) was recorded at the same time.

The content of mosapride citrate (\(X_1\)) in one tablet in milligrams was calculated by the formula:

\[
X_1 = \frac{I_1 \cdot m_0 \cdot b \cdot 5 \cdot 50 \cdot 10}{I_0 \cdot m_1} = \frac{I_1 \cdot m_0 \cdot b \cdot 0.1}{I_0 \cdot m_1}
\]

where \(I_1\) is the luminescence intensity of the assay, \(I_0\) is the luminescence intensity of the standard, \(m_0\) is the weight of standard in mg, \(m_1\) is the weight of powdered tablets in mg and \(b\) is the average tablet weight in mg.

**RESULTS AND DISCUSSION**

**Spectral characteristics**

The absorption spectra of ligands in aqueous solutions are characterized by the presence of three bands with high molar absorption coefficients (\(\varepsilon\)) in the UV region of the spectrum (Table 1). These coefficients give the possibility for effective absorption of light energy. The energies of triplet levels (T) of the ligands (20510 – 21100 cm⁻¹) were calculated from phosphorescence spectra of their Gd complex.
es at 77 K. These energies are higher than the energy of levels of the first excited states of Tb\(^{3+}\) (20500 cm\(^{-1}\)) and Eu\(^{3+}\) (17300 cm\(^{-1}\)) ions, resulting in possible energy transfer from ligands to lanthanide ions. The lifetimes (\(\tau\)) of the test complexes are rather long.

It has been established that ligands form luminescent complexes with europium ions. Table 1 summarizes the maxima of the excitation spectra (\(\lambda_{\text{exc}}\)) of all of the test complexes. The quantum yields of luminescence (F) for europium complexes with L\(_1\) ñ L\(_3\) were calculated according to Haas and Stein (10) with reference to [Ru(bipy)\(_3\)]\(^{2+}\) (F = 0.028) (11) at \(\lambda_{\text{exc}} = 318\) nm and pH 7.5 (Table 1).

Applying the restricted-logarithm method to the luminescence data, we found that with a deficiency of reagents or at equimolar ratio Eu forms complex compounds with L\(_1\) ñ L\(_3\) at the component ratio Eu:L\(_1\)–L\(_3\) = 1:1, but with an excess of reagents europium forms complex compounds with L\(_1\)–L\(_3\) at the component ratio Eu:L\(_1\)–L\(_3\) = 1:2.

It is known that one of the causes of quenching in lanthanide complexes is due to energy transfer processes from the metal center to the OH oscillators (water molecules bound to lanthanide center). Displacement of water molecules by a coordinating anion is signaled by an increase in the lifetime of the lanthanide excited state and the emission intensity (12).

It was found that introduction of second ligand - citrate ion in Eu(III)-L\(_1\) system with the component ratio 1:1 resulted in the increasing luminescence intensity of europium(III) ions. The complex Eu(III)-L\(_1\)-Cit exhibited the greatest luminescence intensity enhancement. Eu(III)-L\(_1\) complex is therefore promising for the determination of citrate ion, because the quantum yield of this complex is higher than that of Eu(III)-L\(_1\). This would enable lower limits of detection. The further researches were carried out with this complex.

It was established that luminescence intensity of the complex Eu(III)-L\(_1\)-Cit achieved the maximum in 5 min after preparation of solutions and remained constant for 30 min that proved its photo-stability. The interaction in Eu(III)-L\(_1\)-Cit system was proved by the increase of lifetime of the excited state D\(_0\), Eu(III) ions in ternary system (Fig. 1). Thus, the process involved in the europium emission enhancement when citrate ions were added to Eu(III)-L\(_1\) complex, is displacement of water molecules from inner sphere.

It was found that in aqueous solutions in the presence of mosapride citrate the luminescence intensity of europium (III) in Eu(III)-L\(_1\) complex was enhanced. Figure 2 shows the (a) excitation and (b) luminescence spectra of Eu(III)-L\(_1\) complex in the presence of different concentrations of mosapride citrate.

### Influence of pH and ligand amount of the components

The complexation of Eu(III) with the ligand occurs over a wide range of pH values from 3.0 to 11.0 (Fig. 3). The maximal luminescence intensity of the complexes Eu(III)-L\(_1\) and Eu(III)-L\(_1\)-Cit is observed at pH 7.5 ñ 9.0. The pH of solutions was maintained at 7.5 with urothropin buffer. The optimal concentration of Eu\(^{3+}\) was 1×10\(^{-4}\) M and the ligand – 5×10\(^{-4}\) M.

### Analytical performance

The proposed method was validated in terms of linearity, accuracy, inter- and intra-day precision and specificity (Table 2). We developed the method for the luminescent determination of mosapride citrate concentration by using complex Eu(III)-L\(_1\) and dissociation of mosapride citrate (into mosapride and citrate ions). The proposed method was evaluated by statistical analysis of the experimental data, by fitting the overall least squares line according to RI
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\[ a + bc \ (RI = 0.086 + 0.699c; \text{with correlation coefficient of } 0.99978) \]

where \( RI \) is the luminescence intensity of Eu(III)-L1-Cit calculated as \( RI = (I - I_0) / I_0 \), where \( I \), \( I_0 \) and \( I_0 \) are the relative luminescence intensities of the system without and with MC, respectively; and \( c \) is the concentration of MC, in \( \mu g/mL \). The calibration curve is linear in the range of 1.0 - 25.0 \( \mu g/mL \) of mosapride citrate (Fig. 4). The signal-to-noise ratio of 3 was considered as the limit of detection (LOD). The LOD for MC was found to be 0.35 \( \mu g/mL \).

The accuracy of the analysis was evaluated by a recovery study at three different levels, namely 80, 100 and 120%. The results of this recovery study indicate that the proposed method is accurate for estimation of the drug in tablet dosage form. (Table 3).

To establish the precision of the method we tested the analytical signal corresponding to an MC concentration of 10 \( \mu g/mL \). For a series of 10 measurements, the relative standard deviation was 2.3% for the intra-days and 3.7% for the inter-days analysis (\( p = 0.95 \)) for MC.

In order to confirm the specificity of the proposed method we studied the effect of some common excipients used in pharmaceutical preparations (glycerin, propylene glycol, sucrose, lactose, glucose, sorbitol, ethanol, sunset yellow, starch, magnesium stearate, talc, microcrystalline cellulose) by analyzing sample solutions containing a fixed amount of mosapride citrate (5 \( \times 10^{-5} M \)) with various amounts of each excipient. No interference could be observed with the proposed method.

This method was used to assay the active ingredient - mosapride citrate in a dosage form - tablets “Mosid MT” (Torrent, India) – 2.5 mg. The content of mosapride citrate in dosage form in milligrams was calculated by the standard sample method with the above formula. Three batches of mosapride citrate tablets were analyzed. The results are shown in Table 4.

In comparison with the spectrophotometric methods reported, as shown in Table 5, the method proposed in this paper offers higher sensitivity and a wider linear range. In addition, this method is quicker and simpler than the HPLC method. The Eu(III)-L1 probe shows a large Stokes’ shift of 300 nm which facilitates the separation of excitation and emission spectra. This can be very useful if simple luminescence instrumentation with filters or microplate readers are employed for detection.

**CONCLUSIONS**

In this work, we found that the complexes of 3-oxo-1-hydroxyquinoline-2-carboxylic acid amide derivatives with Eu\(^{3+}\) can sensibilize the intrinsic luminescence of the lanthanide ion. We determined the spectroscopic and luminescence characteristics of these complexes. When citrate ions were added to the above systems, the luminescence was significantly enhanced. The complex Eu(III)-L1 was used as a luminescent probe for the determination of citrate ions. This effect was applied for the determination of the drug, which is not an Ln luminescence sensitizer. The proposed luminescence method for the determination of mosapride citrate is simple, reliable and sensitive, with the advantage of a wide range of determination without the need of extraction or heating. This method can be successfully applied to the micro determination of mosapride citrate in pharmaceutical formulations.

**REFERENCES**


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