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ORIGINAL PAPER

# Determination of Meloxicam Using Europium Sensitized Luminescence in the Presence of Co-Luminescence Reagents

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**Abstract** A sensitive time- resolved luminescence method for the determination of meloxicam (MX) in methanol and in aqueous solution is described. The method is based on the luminescence sensitization of europium ($Eu^{3+}$) by formation of ternary complex with MX in the presence of 1,10- phenanthroline as coligand, Tween-80 as surfactant and gadolinium ion as a co-luminescence reagent. The signal for Eu- MX-1, 10- phenanthroline is monitored at $\lambda_{ex}$=360 nm and $\lambda_{em}$=620 nm. Optimum conditions for the formation of the complex in aqueous system were 0.01 M TRIS buffer, pH 8.0, 1,10- phenanthroline ($6.0\times10^{-6}$ M) , $Gd^{3+}$ ($7.0\times10^{-6}$ M), Tween-80 (0.28%) and 1.75 mM of $Eu^{3+}$ which allows the determination of 20–800 ppb of MX with limit of detection (LOD) of 7 ppb. The relative standard deviations of the method range between 0.1 and 1.1% indicating excellent reproducibility of the method. The proposed method was successfully applied for the assay of MX in pharmaceutical formulations, plasma and in urine samples. Average recoveries of 99.8±1.1%, 100.2±0.9% and 100.9±1.1% were obtained for MX in tablet, plasma and urine sample respectively.

**Keywords** Sensitized luminescence · Meloxicam · Urine · Plasma · Co-luminescence

## Introduction

Meloxicam (MX) (Fig. 1), 4-hydroxy-2-methyl-N-(5-methyl-1, 3-thiazol-2-yl)-2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide belongs to the oxicam derivatives, a class of enolic acids that have anti-inflammatory, analgesic and antipyretic activity. It is a used for the treatment of rheumatoid arthritis, osteoarthritis and other joint diseases. The principle advantage of meloxicam is its long half-life, which permits the administration of a single daily dose [1]. Meloxicam is a selective non-steroidal anti-inflammatory drug (NSAID) that inhibits the enzymatic activity of COX-2 more strongly than that of COX-1. This preferential operation causes meloxicam to inhibit synthesis of prostaglandins without the unfavorable gastric mucosa side effects of non-selective NSAIDS. However, a COX-2 inhibitor might increase the risks of life-threatening heart or circulation problems, including potential heart attack or stroke. Besides its primary functions as anti-inflammatory agents meloxicam is emerging as a useful agent, in cancer treatment and Alzheimer's disease [2, 3].

Different methods have been reported in the literature for the determination of MX in pharmaceutical formulations and biological fluids. These include high performance liquid chromatography [4–6], spectrophotometry [7], voltammetry [8], capillary zone electrophoresis [9] and chemiluminescence [10]. Due to their sensitivity and selectivity, spectrofluorimetric methods are well suited for the determination of trace amounts of drugs in biological materials and in some dosage forms. The methods based on the native fluorescence of these compounds are not always sensitive and indirect methods such as ion pair formation of the drug with safranin [7] and alkaline hydrolysis of the drug with fluorescence derivatisation of the alkaline hydrolytic products o with 7-chloro-4-nitrobenz-2-oxa-1, 3-diazole (NBD-Cl) has been proposed [11]. Luminescence spectrometry, on the other hand, offers possibilities of sensitive and selective detection, such as the use of lanthanide-sensitized luminescence. Lanthanide ions, such as europium and terbium, are weakly fluorescent in

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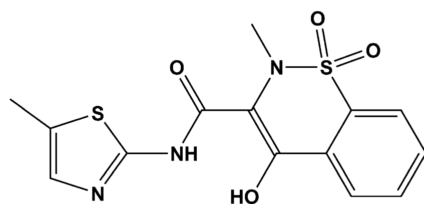


**Fig. 1** Chemical structure of meloxicam

aqueous solution due to their low molar absorptivities and low fluorescence quantum yields. When chelated with organic ligands, the luminescence quantum yield is greatly enhanced. The most common accepted explanation for the luminescence sensitization is that excitation light is absorbed and collected by the organic ligand, which serves as an antenna chromophore. This phenomenon is called the "antenna effect" which view such complexes as light conversion molecular devices because they are able to transform light absorbed by the ligand into light emitted by the ions *via* an intramolecular energy transfer process [12].

The efficiency of the energy transfer is governed by the nature of the ion, the ligand, the ligand–ion bond and the solvent. The energy gap between the excited and ground-state levels of the lanthanide ion and the rigidity of the molecular structure also contribute to the enhancement of the luminescence intensity of the system [12]. Lanthanide sensitized luminescence is very attractive, because the intramolecular energy transfer between the absorbing ligand and the lanthanide ion result in the emission of a strong narrow band luminescence with a large stokes shift and long decay time . Recently, our group reported the determination of various drugs in pharmaceutical formulations and biological fluids using lanthanide sensitized luminescence [13–15]. As part of our ongoing interest on developing simple and robust methods for drug analysis, we report a novel method for the essay of MX in pharmaceutical formulations and in biological fluids. The method is based on the luminescence enhancement and sensitization of europium brought about by complexation with MX. The luminescence properties were investigated in methanol and in aqueous system. Factors that affect complexation as well as the effect of co-ligands and co-luminescence agents on the luminescence properties of MX-$Eu^{3+}$ has been carefully studied. The method was then used to determine the concentration of MX in tablets, plasma and urine samples.

## Experimental

### Apparatus

Luminescence measurements were recorded with a Perkin Elmer LS-55 Luminescence spectrometer using a 1 cm quartz cell. The spectrometer was equipped with a xenon discharge flashlamp. Both the operation and data processing were controlled by the FL WinLab Software. The instrument was operated in the phosphorescence mode (time resolved mode ) with a delay time of 0.04 ms and a gate time of 1.0 ms. The excitation and emission slits were both 10 nm with a scan speed of 800 nm $min^{-1}$. The excitation and emission wavelengths were set at 360 nm and 620 nm respectively. Absorption spectra were recorded on a Varian CARY 50 Conc UV visible spectrophotometer with 1 cm matched quarts cell. All pH measurements were performed using pH 320 TUV.

### Reagents

All reagents used in this work were prepared from analytical grade reagents and were used without further purification. All solvents used were of HPLC grade. Ultra pure water (Milli-Q Millipore Corporation) was used.

### Preparation of Standard Solutions

A stock solution of MX ($1.1\times10^{-3}$ M) was prepared by dissolving pharmaceutical purity grade (99.4%) meloxicam donated by Jordanian Pharmaceutical Company, (Amman, Jordan) into enough methanol (MeOH) to make a final volume of 25.00 mL in a volumetric flask. Working standards were prepared by appropriate dilution with buffer. Europium (III) chloride solution ($1.0\times10^{-2}$ M) was prepared in methanol or deionized water. 1, 10-phenanthroline ($1.0\times10^{-3}$ M) was prepared in deionized water. Stock solutions of various surfactants were prepared by dissolving 1.00 g of each of the surfactant in 100.0 mL deionized water in a volumetric flask. The surfactants used in this study include Tween-20 (TW-20), Tween-80 (TW-80), Triton X-100, cetyl bromide and sodium dodecyl sulphate (SDS) (All from Sigma, Germany). Standard solutions of zinc chloride, terbium chloride, lanthanum chloride, gadolinium chloride, samarium chloride were prepared in highly pure water.

### Preparation of Urine and of Plasma Samples

Urine and plasma samples were individually spiked with convenient amounts of MX standard solution. The plasma sample was first de-proteinised by centrifuging with acetonitrile. The final MX concentration was in the range of 200–800 ppb. The samples were then treated in the same manner as for the standard samples.

### Preparation of Drug Samples

Two commercial (Coxicam and Mobic) coated tablets containing 15 mg MX were analyzed. The products also

contain excipients such as: microcrystalline cellulose, lactose, colloidal silicon dioxide, sodium citrate, crospovidone and magnesium stearate, Five MX tablets were accurately weighed in order to find the average mass of each tablet. Then the tablets were finely powdered in a mortar. An equivalent amount of the powder containing a known amount of active material was weighed and sonicated for 15 min filtered into a volumetric flask and completed to the mark with methanol to make a stock solution of 1000 ppm. Working solutions of 100 ppm in methanol were prepared. Appropriate aliquots from the working solution were taken for the determination of MX.

### Method

A systematic variation of the parameters that affect complex formation of Eu-MX-phen was investigated. Into a 10 ml volumetric flask was added 0.75 mL of 1, 10-phenanthroline ($1.0\times10^{-4}$ M), 0.5 mL of MX ($1.0\times10^{-4}$ M) in buffer, 0.28 mL of TW-80 and 1.8 mL of stock Eu (10 mM) the contents were diluted to the mark with buffer. The luminescence was measured at 620 nm using an excitation wavelength of 360 nm.

## Results and Discussion

### Spectral Characteristics

The absorption spectra of MX and europium meloxicam (Eu- MX) complex were recorded in 0.01 M TRIS buffer in methanol. From the absorption spectra, it was observed that both MX and Eu- MX complex exhibited absorption in the ultraviolet region with peaks at 270 nm and 360 nm with a maximum absorptivity at 360 nm. A slight increase in the absorptivity was noticed for the complex when compared to MX alone. The high molar extinction coefficient ($\varepsilon$=6.6×10$^4$ M$^{-1}$cm$^{-1}$) for the band at 360 nm of the $Eu^{3+}$-TRIS -MX complex when compared with $Eu^{3+}$ -TRIS complex indicates the high efficacy of absorption of light energy by MX and hence will function as an efficient antenna chromophore.

In order to assess the applicability of lanthanide sensitized luminescence for the determination of MX, the system of (MX-$Eu^{3+}$-TRIS) in methanol was investigated where MX was used to sensitize the luminescence of europium ion. The luminescence intensity of europium in the presence of TRIS is very weak, but when MX is added to $Eu^{3+}$ solution; an intense well known structured emission of $Eu^{3+}$ was observed as shown in Fig. 2. The luminescence enhancement is attributed to the efficient energy transfer of excitation energy from the MX moiety to the encapsulated $Eu^{3+}$ ion which can overcome the ions low intrinsic extinction coefficient. Successful sensitization processes requires the ligand to be excited in the ultra violet region in order to provide efficient energy transfer to the rare earth ion. The previous results agree with the successful sensitization process since MX contains efficient chromophore that absorb in the UV region. This absorption band may be assigned to the efficient n→π* transition [16], where the spectral properties of some oxicams (including MX) were investigated in various solvents. It has been reported that MX at low concentrations in methanolic solutions exists in the anionic form which exhibit absorption band at 365 nm ascribed to n→π* electronic transition [16].

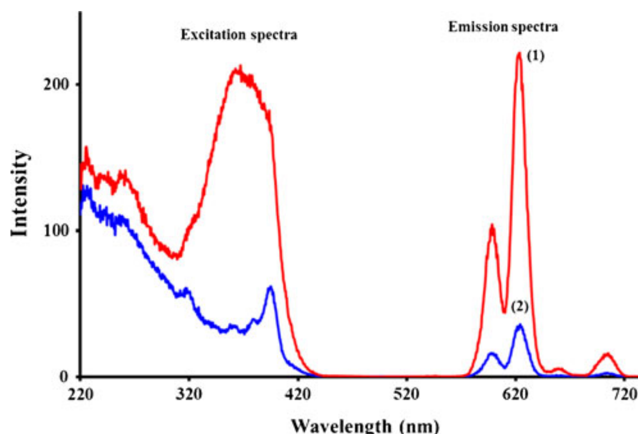


**Fig. 2** Luminescence excitation and emission spectra of: (1) $Eu^{3+}$ -Tris in presence of MX in methanol, (2) $Eu^{3+}$ -Tris in methanol system. [$Eu^{3+}$]=1.75 mM, [MX]=2 ppm, [TRIS]=0.01 M, $\lambda_{ex}$ of $Eu^{3+}$ -Tris-MX complex. $\lambda_{ex}$=225 nm, 255 nm, 358 nm, $\lambda_{em}$=597 nm, 622 nm, 650 nm, 697 nm

These spectral properties as well as the β-diketone structure of MX supported its use as a sensitizer of $Eu^{3+}$ luminescence, since β-diketones were used efficiently in previous studies for this purpose [17, 18]. The absorption spectrum of MX and the excitation spectrum of Eu–MX complex exhibited an excellent agreement between the absorption and excitation spectra ($\lambda_{max}$=360 for absorption, $\lambda_{excitation}$=358), is an indication of the efficient sensitization process and that the antenna chromophore is the only photo physical pathway leading to the observable luminescence in the system [19]. It is also noteworthy here to mention that there is a complete overlap between the absorption spectrum of MX and the excitation spectrum of $Eu^{3+}$–MX complex. In addition, the triply charged lanthanide cation is known to form stable chelates with ligands when negatively charged donating groups are involved [20], which is true for MX anion.

The luminescence emission spectrum of MX-$Eu^{3+}$-TRIS reveals the well-known bands of $Eu^{3+}$ luminescence at about 597, 622, 648 and 699 nm, corresponding to ${}^5D_0 \rightarrow {}^7F_0$, ${}^5D_0 \rightarrow {}^7F_1$, ${}^5D_0 \rightarrow {}^7F_2$ and ${}^5D_0 \rightarrow {}^7F_3$transitions. Among

these bands, the hypersensitive transition $^5D_0 \rightarrow {}^7F_1$ around 615 nm (orange red emission) is the strongest and hence the peak height of the emission band observed at 622 nm was used to measure the luminescence intensity of the MX-$Eu^{3+}$-TRIS complex. Moreover, a large Stoke's shift of 38000 $cm^{-1}$ between the absorption and emission spectra of the complex was observed indicating that there was no overlap of the $Eu^{3+}$ emission bands with the antenna chromophore absorption band. In previous studies conducted by Serra *et al*. [21] on the luminescence of a new $Tm^{3+}$ β-diketone ligand 3-phenyl-2, 4-pentadionate (PPA), it was suggested that the energy absorbed by the phenyl group attached to C3 in the PPA ligand was transferred to the metal ion through the enolate conjugated system. Since MX contains a conjugated system (phenyl group) in the oxicam nucleus, it is therefore reasonable to predict that a similar transfer of energy from the phenyl group to the europium ion will be realized as for (PPA- $Tm^{3+}$) system.

The luminescence intensity was observed to increase with an increase in concentration of TRIS reaching a maximum at 10 mM after which the intensity started to decline. This behavior indicated that an optimum concentration of TRIS buffer is required for maximum complex formation between $Eu^{3+}$ and MX. By exceeding the optimum concentration, the buffer molecules start to compete with MX ligand for $Eu^{3+}$ binding sites. This may result in less Eu–MX complex being formed and hence a decrease in luminescence intensity. Therefore the optimum concentration of TRIS buffer in this study was maintained at 10 mM.

Since luminescence sensitization is believed to occur via complex formation between europium and MX, it is necessary to optimize the concentration of $Eu^{3+}$ that will afford maximum complexation and hence maximum intensity of the emission line, of europium ions. The luminescence intensity of the system increased gradually with an increase in concentration of $Eu^{3+}$ reaching a maximum at 1.75 mM. This is a typical result, as an excess of $Eu^{3+}$ is required for complex formation. Therefore the concentration of $Eu^{3+}$ for this study was maintained at 1.75 mM.

### Study of the System in Aqueous Medium

Since our interest is to extend our study to include the determination of MX in biological fluids, we investigated the complexation behavior of MX- $Eu^{3+}$ in aqueous system. First the luminescence spectrum of MX and its Eu complex was determined in aqueous system in 0.01 M TRIS buffer and in the presence of 0.1% TW-80 surfactant. The concentration of MX and Eu were maintained at 2 ppm and 1.75 mM respectively. A weak luminescence signal was observed in Eu-TRIS-MX complex. Addition of TW-80 resulted in a fivefold enhancement of the luminescence signal of the complex. A possible explanation for this behavior may be due to the quenching effect of water molecules in the first coordination shell of the lanthanide ion which can seriously affect the emission process [22].

A number of surfactants (Tween-20, Tween-80, Triton X-100, and sodium dodecyl sulfate (SDS)) were investigated. The concentration of each surfactant was maintained at 0.1%, which is above the critical micelle concentration of all of the used surfactants. Among these surfactants Tween-80 afforded the highest intensity of the luminescence signal. The intense characteristic europium luminescence indicated that the surrounding MX ligand absorb and transfer energy more efficiently to the chelated europium ion in the presence of Tween-80. This may be attributed to the increase in the hydrophobicity of the environment in the presence of the surfactant and a decrease in quenching of the $^5D_0$ excited state of the $Eu^{3+}$ ion through non- radiative decay paths provided by OH oscillators of coordinated water molecules [22, 23]. The effect of Tween-80 concentration was optimized to enhance the signal further. The luminescence intensity was observed to increase with an increase in concentration of Tween-80 from 0.0 to 0.30% and then it decreased at higher concentration of Tween-80. As the concentration of Tween-80 increases the number of micelles formed increases until it reaches the required level to solubilize the whole compound (solubilizate) after that the excess amount of the surfactant will causes a reduction in the rate of solubilization. Therefore concentration of Tween-80 in this study was maintained at 0.28%.

### Effect of Addition of Co-ligands

One of the possible effective strategies to improve luminescence intensity of MX-$Eu^{3+}$ chelates is the substitution of water molecules in the coordination sphere with synergic agents. Several co-ligands were investigated in this study such as trioctylphosphine oxide (TOPO), ethylenediamine tetraacetic acid (EDTA), 1,10-phenanthroline (phen) and 4,7-diphenyl phenanthroline with a concentration of $1\times 10^{-5}$ M of each co-ligand. As shown in Fig. 3 the maximum luminescence sensitization was observed in the presence of phen followed by 4, 7-diphenyl phenanthroline which had the second highest intensity and then TOPO. Next the optimum concentration of phen was studied by monitoring the luminescence intensity of the complex in various concentrations of phen ranging from (0–12) μM. It was observed that the luminescence intensity of the complex increased with an increase in the concentration of phen for the range of 0–6 μM and reached a maximum at 6 μM, after which a slight decrease in the luminescence intensity was observed.

1, 10-phenanthroline acts as a co-ligand to promote the formation of MX-Eu complex by coordination to the

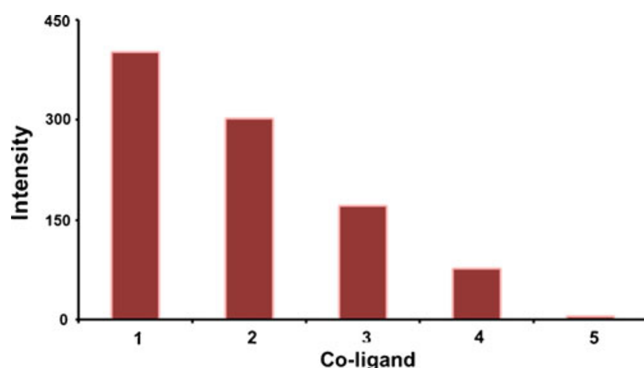


**Fig. 3** The effect of type of co-ligands on the luminescence spectra of MX-$Eu^{3+}$ system. [$Eu^{3+}$]=1.75 mM; [TRIS]=0.01 M; [Tween-80]= 0.275% (1) 1, 10 -phenanthroline, (2) 4, 7-diphenyl phenanthroline, (3) EDTA, (4) TOPO, (5) without co-ligand

europium ion through the lone pair of its nitrogen atoms. In addition it has the ability to remove water molecules from the coordination sphere. A high concentration of phen exceeding the optimum value results in competition between MX and phen for $Eu^{3+}$ ion and hence will result in a lesser enhancement of MX-Eu complex relative to europium blank. In this study we selected 6.0 μM to be the optimum concentration of phen.

## Effect of pH

As was previously reported, MX ($pK_{a1}$=1.09 and $pK_{a2}$= 4.18) structural forms are highly pH dependent in the aqueous medium, where the deprotonated form $MX^-$ is predominant at pH above 4.18 [24]. Since $MX^-$ is the form preferred for complexation with $Eu^{3+}$, it is necessary to investigate the influence of varying pH of the system on sensitization process. The effect of pH on the luminescence properties of the MX-Eu complex was studied by measuring the luminescence of the complex in 0.01 TRIS buffer of various pH from 2 to 9 while keeping the other experimental factors constant. As shown in Fig. 4, an increase in the luminescence intensity was observed when the pH increases in the range 3 to 8.0 reaching a maximum at pH 8.0. On increasing the pH further, a decrease in the luminescence intensity was observed.

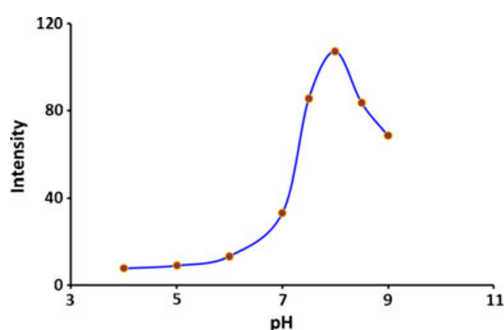


**Fig. 4** The influence of changing pH on luminescence spectra of MX-$Eu^{3+}$ complex in aqueous medium; [$Eu^{3+}$]=1.75 mM; [TRIS]= 0.01 M; [Tween-80]=0.275%, [1, 10-phenanthroline]=6 μM

This is a typical result, as the deprotonated form of MX exists at pH above 4.18 and is necessary for complexation to occur. At higher pH the luminescence intensity decreases due to the competitive hydrolysis of europium forming Eu $(OH)_3$ which is expected to predominate at a pH > 8.0. This was also clearly noticed when a solution of pH 10 was prepared and a precipitate of $Eu(OH)_3$ was observed.

## Effect of Addition of Co-luminescent Agents

The effect of addition of co-luminescence agents such as $Tb^{3+}$ , $Ln^{3+}$, $Gd^{3+}$, $Sm^{3+}$ and $Zn^{2+}$ with a concentration of 4 μM was also examined. Addition of gadolinium ions gave the highest luminescence intensity in this system as shown in Fig. 5. The reasonable explanation for this behavior is that $Gd^{3+}$ possess a relatively stable half-filled 4f shell and the luminescence level of $Gd^{3+6}P_{\frac{7}{2}}$ is probably higher than the triplet state of MX in the complex of ($Gd^{3+}$-MX), so that the energy of the later cannot be transferred to $Gd^{3+}$, but can be transferred to the luminescence level of $Eu^{3+}$ in the ($Eu^{3+}$-MX) complex. This process can be preceded by intermolecular energy transfer due to the short distance between the two complexes in the micelle. Each ($Eu^{3+}$-MX)-Tween-80 molecule is surrounded by ($Gd^{3+}$-MX)-Tween-80 molecules in the system. Therefore the fluorescence intensity of $Eu^{3+}$-is considerably enhanced. Similar results were reported by Yang [25] in his study on the co-luminescence system of Sm-Gd-BPMPHD-CTMAB. Furthermore, these results indicated that most of the lanthanides do not interfere, whereas lanthanum and samarium produce a quenching effect on the luminescence intensity.

In order to get further enhancement for the system under study, the influence of the concentration of $Gd^{3+}$- in the range (2–8 μM) was assessed. When the concentration of $Gd^{3+}$- added was 7 μM, the intensity reached maximum. Therefore 7 μM was employed as the optimum $Gd^{3+}$ concentration for this study.

## Analytical Figures of Merit

In order to probe whether sensitization is brought about by the presence of MX ligand, emission intensities in the presence of various concentration of MX were measured while keeping the concentration of $Eu^{3+}$ constant. An increase in the luminescence intensity was observed with an increase in the concentration of MX in the range studied.

The analytical performance of the proposed system was studied using the optimum conditions discussed above. The luminescence intensity *I*, versus MX concentration was

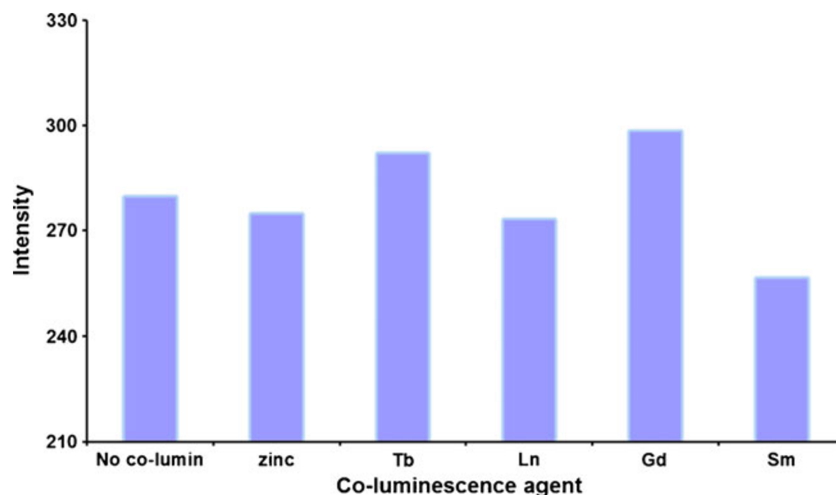


**Fig. 5** The effect of co-luminescence agents on the luminescence spectra of MX-$Eu^{3+}$ system. [$Eu^{3+}$]= 1.75 mM; [TRIS]=0.01 M; [Tween-80]=0.275%, [1, 10-phenanthroline]=6 μM

found to be linear over the range studied. The calibration equation was: $I = 194C + 0.75$, with a correlation coefficient, $R^2$, of 0.999. The detection limit (signal to noise ratio of 3) was 7 ppb and the quantification limit was 23 ppb. The figures of merit of the proposed method compared favorably with most of the published methods for the determination of MX (Table 1). In addition, the method offered an advantage over other luminescence methods, in that its emission is at a longer wavelength, free from interference from short-emitting species present in the matrices.

The reproducibility of the method was obtained for standard solutions of MX and was found to be less than 1.4% ($n$=5) showing excellent precision.

## Analytical Application

To examine the applicability of the method, the proposed method was first used to determine the concentration of MX in Mobic (Boehringer Ingelheim Pharma, Ingelheim am Rhein Germany) and Coxicam (Riyadh Pharma, Riyadh Saudi Arabia) tablets. Six samples prepared as described above were analyzed. The results of this analysis are shown in Table 2. It is clear from these results that excellent recoveries and precision are obtained for these samples.

The method was further applied to the determination of MX in plasma and in urine samples. 500 μl of the deproteinised serum samples was diluted 10 fold in deionized water. 1 ml of the diluted sample was spiked with appropriate amount of MX to give final concentration of the drug ranging from 350–750 ppb. Appropriate aliquots of the plasma were then treated in the same manner as for the standard solutions. Excellent recoveries ranging from 98.6–100.9% were obtained (Table 2). These recoveries indicate no interference with the plasma matrices.

Fresh urine samples were collected, filtered diluted with deionized water and spiked with appropriate amounts of

**Table 1** Comparison with other methods for the determination of meloxicam

| Method | Linear range [mol $L^{-1}$] | LOD [mol $L^{-1}$] | Reference |
|---|---|---|---|
| HPLC with UV detection | $1.47\times10^{-7}$–$4.3\times10^{-6}$ | $2.8\times10^{-9}$ | [4] |
| HPLC with UV detection | $2.8\times10^{-4}$–$1.42\times10^{-3}$ | $1.0\times10^{-5}$ | [6] |
| MB Complexation | $2.8\times10^{-6}$–$1.42\times10^{-5}$ | $7.7\times10^{-9}$ | [6] |
| DDQ Complexation | $1.1\times10^{-4}$–$4.6\times10^{-4}$ | $9.6\times10^{-6}$ | [6] |
| Method A | $5.7\times10^{-6}$–$2.8\times10^{-5}$ | $3.1\times10^{-7}$ | [7] |
| Method B | $2.8\times10^{-6}$–$2.8\times10^{-5}$ | $1.9\times10^{-7}$ | [7] |
| Method C | $1.1\times10^{-5}$–$3.4\times10^{-5}$ | $2.8\times10^{-7}$ | [7] |
| Spectrofluorimetry [FL] | $1.1\times10^{-6}$–$3.4\times10^{-6}$ | $2.4\times10^{-8}$ | [7] |
| Iron Complexation | $5.7\times10^{-6}$–$5.6\times10^{-4}$ | $1.3\times10^{-6}$ | [26] |
| Spectrophotometric FIA | $1.4\times10^{-6}$–$5.7\times10^{-6}$ | $1.1\times10^{-7}$ | [26] |
| Spectrofluorimetry [FL] | $2.8\times10^{-6}$–$2.8\times10^{-8}$ | $3.4\times10^{-7}$ | [11] |
| Electrochemical | $1.0\times10^{-8}$–$5.0\times10^{-6}$ | $2.0\times10^{-9}$ | [8] |
| Flow-injection CL | $2.2\times10^{-7}$–$2.8\times10^{-5}$ | $2.2\times10^{-8}$ | [10] |
| This work | $2.8\times10^{-10}$–$2.3\times10^{-9}$ | $2.0\times10^{-11}$ | |

**Table 2** Application of the proposed method to the assay of tablets, and urine samples

| Concentration of MX claimed | MX concentration found | % Recovery ± RSD |
|---|---|---|
| 0.35 ppm | 0.349 ppm | 99.7±0.3 |
| 0.75 ppm | 0.749 ppm | 99.8±1.1 |
| 0.55 ppm | 0.549 ppm | 99.8±1.1 |
| 15.0 mg (Mobic) | 15.06 mg | 100.4±1.5 |
| 15.0 mg (Coxicam) | 14.98 mg | 99.8±0.9 |
| 0.35 ppm (spiked plasma) | 0.346 ppm | 98.9±0.9 |
| 0.55 ppm (spiked plasma) | 0.542 ppm | 98.6±0.97 |
| 0.75 ppm (spiked plasma) | 0.75 ppm | 100.0±0.79 |
| 0.55 ppm (spiked urine) | 0.551 ppm | 100.2±0.5 |
| 0.35 ppm (spiked urine) | 0.353 ppm | 100.9±0.7 |
| 0.75 ppm (spiked urine) | 0.753 ppm | 100.4±1.7 |

MX standards. A series of urine samples containing MX ranging from 350–750 ppb were prepared and treated in the same manner as for the standard MX solutions. The sensitivity and detection limit achieved by the developed method allows the determination of MX in spiked urine samples. Excellent recoveries ranging from 100.2–100.4% were obtained (Table 2). These recoveries indicate no interference with the urine matrices. It was necessary to dilute the plasma and urine samples 10 fold in order to avoid interference from the matrix.

It has been previously reported in studies [27] conducted in rat urine and bile that meloxicam underwent extensive metabolism with only small amounts of unchanged drug excreted in the urine (<0.5%). Principal routes of biotransformation of this involved the oxidation of the 5-methyl group of the N-heteroaryl-carbamoyl side chain to yield the 5-hydroxymethyl derivative (33%) and the 5-carboxyl derivative (16%). Oxidative cleavage of the benzothiazinering yielded an oxamic acid metabolite in urine (23.5%). The first two metabolites have been detected both in vitro and in vivo in different species such as humans, thoroughbred horses, rats, mice and mini-pigs [28]. This method was able to detect the trace concentrations of meloxicam in human urine.

## Conclusion

The development of a sensitive and robust method for the determination of MX in pharmaceutical preparations and biological fluids is reported. The proposed method is based on sensitization of europium luminescence in the presence of 1, 10-phenanthroline and TW-80 through the formation of the ternary complex Eu-MX-phen-Gd. The enhancement of the europium luminescence upon complexation with MX has enabled the assay of this drug with high sensitivity and selectivity. The procedure was successfully applied for the determination of MX in tablets, plasma and in urine samples with excellent reproducibility and no interference was observed from excepients commonly found in pharmaceutical preparations and from the matrix in plasma and urine samples.

The proposed method has the advantage of being simpler, more rapid, and suitable for automation. It is sensitive to the low amount of meloxicam (detection limit is 7 ppb). As a result, the proposed method represents a good analytical alternative for the determination of meloxicam in pharmaceutical formulations and in biological fluid.

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