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Highly sensitive and selective spectrofluorimetric determination of metoclopramide hydrochloride in pharmaceutical tablets and serum samples using Eu^{3+} ion doped in sol–gel matrix

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ABSTRACT

A simple, sensitive and selective spectrofluorimetric method for the determination of Metoclopramide hydrochloride (MCP) is developed. The MCP can remarkably enhances the luminescence intensity of the Eu^{3+} ion doped in sol–gel matrix at λ_{ex} = 380 nm in DMSO at pH 8.7. The intensity of the emission band of Eu^{3+} ion doped in sol–gel matrix increases due to energy transfer from MCP to Eu^{3+} in the excited state. The enhancement of the emission band of Eu^{3+} ion doped in sol–gel matrix at 617 nm was found to be directly proportional to the concentration of MCP with a dynamic range of $5 \times 10^{-9} - 1.0 \times 10^{-6}$ mol L^{-1} and detection limit of 2.2×10^{-11} mol L^{-1}.

1. Introduction

Metoclopramide (MCP), 4-amino-5-chloro-2-methoxy-N-(2-diethylamino-ethyl) benzamide, is a dopamine-receptor antagonist active for gastrointestinal motility. It is used as an anti-emetic in the treatment of some forms of nausea and vomiting and to increase gastrointestinal motility. It is also used in case of higher doses for the prevention of cancer chemotherapy-induced emesis [1].

In this perspective, the wide applications of MCP in both clinical and experimental medicine have prompted extensive interest in its determination. Current analytical methods employed for the determination of MCP can involve fluorimetry [2], spectrophotometry [3–10], chromatography [11–15], capillary electrophoresis [16,17], differential scanning calorimetry (DSC) and X-ray diffraction [18], gas chromatography–mass spectrometry (GC–MS) [19], potentiometry [20], voltammetry [21], fast stripping continuous cyclic voltammetry [22], square wave anodic stripping voltammetric [23] and ^{1}H NMR spectroscopy [24]. The chromatographic method is costly and also time-consuming, limiting its application. Other methods often are typically less sensitive or have their own intrinsic disadvantages such as technical complexity or require expensive instrumentation.

Recently, Al-Arfaj [25] developed a flow-injection (FI) methodology for the rapid and sensitive determination of MCP by using $Ru(bipy)_3^{2+}$ chemiluminescence (CL) and an electrochemiluminescent (ECL) sensor for the determination of MCP was developed based on $Ru(bipy)_3^{2+}$-doped silica (RuDS) nanoparticles dispersed in a perfluorosulfonated ionomer (Nafion) on a glassy carbon electrode (GCE) [26]. These methods are relatively complicated and expensive. In this work, the MCP concentration was determined by the optical sensor, europium doped in the sol–gel matrix. The absorption and emission spectra of MCP and europium were measured in the sol–gel matrix. In comparison with other techniques, Table 1, this method is simple, relatively free from interference with coexisting substances and can successfully be applied to the determination of the drug MCP in pharmaceutical tablets and in serum samples with satisfactory results.

2. Experimental

2.1. Chemicals and reagents

All chemicals used are analytical-reagents of higher grade. Pure MCP was either purchased from Sigma or supplied by the National Organization for Drug Control and Research (Cairo, Egypt) Fig. 1. Pharmaceutical preparations, Primpran, 10 mg (Aventis company) and Migracid, 5 mg (Cid company) were purchased from local market.

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Table 1
Comparison of spectrofluorimetric technique with some existing methods for the determination of MCP.

Method	Linear range	Detection limit	References
Chemiluminescent determination of metoclopramide hydrochloride	0.005–3.5 μg mL^{-1}	1 ng mL^{-1}	[25]
Spectrophotometric method	5–25 μg mL^{-1}	0.5 μg mL^{-1}	[8]
Flow injection-spectrophotometric determination of metoclopramide hydrochloride	0.5–85 mg L^{-1}	0.05 mg L^{-1}	[10]
Square wave anodic stripping voltammetric determination of metoclopramide hydrochloride	0.067–0.269 ng mL^{-1}	0.06 ng mL^{-1}	[23]
High-performance liquid chromatography	1–10 μg mL^{-1}	0.5 μg mL^{-1}	[35]
PVC matrix membrane sensor for potentiometric determination of metoclopramide hydrochloride	1×10^{-2} to 6×10^{-5} mol L^{-1}	4×10^{-5} mol L^{-1}	[20]
Optical sensor Eu^{3+} doped in sol–gel	5×10^{-9} to 1.0×10^{-6} mol L^{-1}	2.2×10^{-11} mol L^{-1}	Present work

Distilled water and pure solvents from Aldrich were used for the preparation of all solutions. A stock solution of MCP (1×10^{-2} mol L^{-1}) was freshly prepared and dissolved in ethanol and stored at 4 °C. The working standard solution of (5×10^{-4} mol L^{-1}) was freshly prepared by appropriate dilution with DMSO.

An Eu^{3+} ion stock solution, (1×10^{-2} mol L^{-1}) was prepared by dissolving $EuCl_3$, Aldrich, 99.99% in a small amount of ethanol in 100 mL measuring flask, then diluted to the mark with ethanol.

2.2. Apparatus

All luminescence measurements were carried out on a Shimadzu RF5301 spectrofluorophotometer in the range 290–750 nm. The absorption spectra were recorded with a Unicam UV–Visible double-beam spectrophotometer from Helios Company. It employs a Tungsten filament light source and a Deuterium lamp, which has a continuous spectrum in the ultraviolet region. The spectrophotometer is equipped with a temperature-controller cell holder. (All measurements were measured at Photoenergy Center, Faculty of Science, Ain Shams Univ.).

2.3. General procedure

2.3.1. Preparation of lanthanide Eu^{3+} doped in sol–gel matrix

A mixture consisting of Tetraethoxysilane (TEOS), C_2H_5OH and H_2O in a molar ratio of 1:5:1 was refluxed for 1 h to give precursor sol solution using a few drops of diluted HCl solution as a catalyst. Subsequently, appropriate amount of the $EuCl_3 \cdot 6H_2O$ (0.02 g) dissolved in 10 mL ethanol and the precursor solution were mixed and stirred together for 15 min until the mixture became homogeneous. The obtained europium-dispersed sol solution was casted into polystyrene cup with dimensions (3 cm, 0.2 cm, 0.8 cm) and kept at 25 °C in air for 2 weeks then was heated at 500 °C for 24 h to give solidified and transparent composite sample, Fig. 2 [27].

2.3.2. Preparation of MCP solutions

To 10 mL clean and sterilized measuring flasks, the standard solutions of MCP were prepared by different additions of 1×10^{-2} mol L^{-1} MCP stock solution to give the following concentrations of MCP, 5×10^{-4} to 1×10^{-9} mol/L. The solutions were diluted to the mark with DMSO at room temperature. The above solutions were used for subsequent measurements of absorption and emission spectra as well as the effect of solvents. The luminescence intensities were measured at $\lambda_{ex}/\lambda_{em}$ = 380/617 nm.

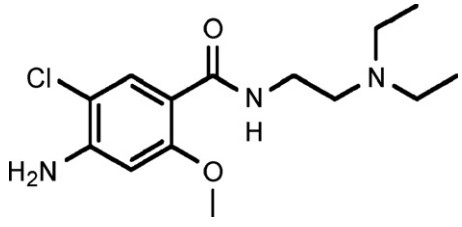

Fig. 1. Structure of MCP.

2.3.3. Calibration curve

After the preparation of the different standard solutions of MCP in DMSO as described above, the optical sensor Eu^{3+} doped in sol–gel matrix was immersed in each standard solution of MCP in the cell of the spectrofluorimetric device, then the luminescence spectrum was measured at the selected excitation wavelength. The optical sensor was rinsed after each measurement using DMSO.

2.3.4. Determination of MCP in tablets and in serum samples

Ten tablets each from Primpran and Migracid are carefully weighed and ground to finely divided powders. Accurate weights equivalent to 10 mg of each drug were accurately transferred to separate Primpran and Migracid to give each test solution in 50 mL beaker and dissolved in DMSO and solutions were left for about 10–15 min and filtered up using 12 mm filter papers then transferred to 100 mL volumetric flask and completed to the mark with DMSO to give the test solution.

A 1.0 mL of samples of serum collected from various real health state human was centrifuged for 15 min at 4000 rpm to remove proteins. The unknown amount of MCP in human serum samples was determined using the standard addition (spiking) techniques as follow; a known volume of the treated serum of the real health state human was transferred into a calibrated 10 mL measuring flask and diluted by DMSO. The luminescence intensity of the test solution was measured before and after addition of 1.0 mL of previously prepared serum solution. The change in the luminescence intensity was used for determination of MCP in serum sample.

3. Results and discussion

3.1. Spectral characteristics

3.1.1. Absorption spectra

The absorption spectra of MCP and MCP plus different concentrations of Eu^{3+} in sol–gel matrix are shown in Fig. 3. Comparing the spectrum of the drug with its spectra after the addition of different concentrations of Eu^{3+} ion into MCP in sol–gel matrix, a red shift was observed for the two bands at 265 and 298 nm by 11 and 13 nm, respectively. The absorbance is also enhanced, which indicates that MCP can form a complex with Eu^{3+} ion.

3.1.2. Emission and excitation spectra

The excitation spectrum of the complex Eu^{3+}–MCP (spectrum 1), as well as the emission spectra of different concentrations of MCP (spectra 2 and 3, inset), that of Eu^{3+} in sol–gel (spectrum 4) and the those of different concentration of MCP + Eu^{3+} in sol–gel

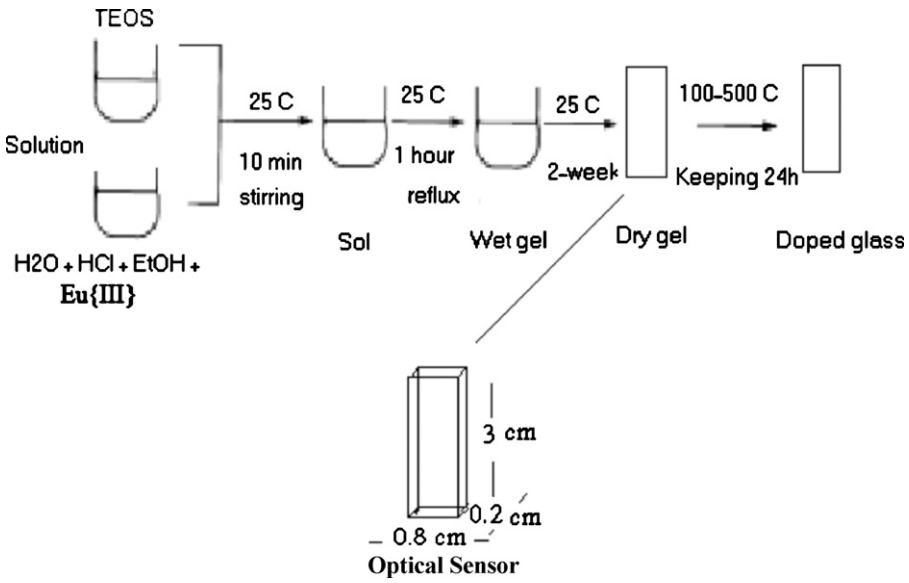

Fig. 2. Preparation of optical sensor Eu^{3+} ion doped in sol–gel matrix.

matrix (spectra 2* and 3*) are shown in Fig. 4. From spectrum 4 in Fig. 4, it can be seen that Eu^{3+} ion in sol–gel matrix has nearly two very weak peaks. Comparing spectra (2 and 3, inset) with (2* and 3*) in Fig. 4, after the addition of different concentrations of MCP into the Eu^{3+} ion in sol–gel matrix, show that MCP can form a complex with Eu^{3+} ion. The characteristic peaks of Eu^{3+} ion appear at ($^5D_0 \rightarrow {}^7F_0 = 580$ nm, $^5D_0 \rightarrow {}^7F_1 = 593$ nm, $^5D_0 \rightarrow {}^7F_2 = 617$ nm, $^5D_0 \rightarrow {}^7F_3 = 653$ nm and $^5D_0 \rightarrow {}^7F_{4,5} = 693{,}704$ nm).

Comparing spectrum 2* with 3* in Fig. 4. It can be seen that the characteristic peak of Eu^{3+} at 617 nm has remarkably been enhanced after the addition of MCP, which indicates that MCP effectively enhances the energy of MCP–Eu^{3+} complex.

3.2. Effect of experimental variables

3.2.1. Effect of the amount of MCP

The influence of the amount of MCP on the luminescence intensities of the europium ion doped in the sol–gel matrix was studied. The luminescence intensity of Eu–MCP complex was increased upon increasing the concentration of MCP till 5×10^{-4} mol L^{-1} then becomes constant. The experimental results showed that the luminescence intensity reached maximum and remained constant when MCP concentration is 5×10^{-4} mol L^{-1} in the DMSO preparations.

3.2.2. Effect of the amount of Eu^{3+}

The influence of the amount of Eu^{3+} ion on the luminescence intensities of Eu–MCP in sol–gel matrix was studied under the conditions established above. The luminescence intensity of Eu–MCP complex at 617 nm increased upon increasing the concentration of Eu^{3+} up to 2.0×10^{-4} mol L^{-1} then becomes constant. When the concentration of Eu^{3+} ion is 2.0×10^{-4} mol L^{-1}, the composition ratio for the Eu^{3+} to MCP is 1:2. Thus, 2.0×10^{-4} mol L^{-1} Eu^{3+} ion concentration was used for further analysis in the sol–gel matrix.

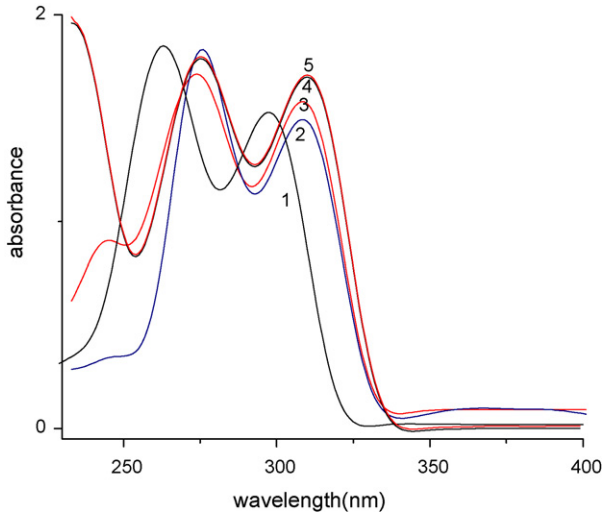

Fig. 3. The absorption spectra of (1) 5×10^{-4} mol L^{-1} of MCP in (2) 5×10^{-6}, (3) 5×10^{-5}, (4) 2×10^{-4} and (5) 1×10^{-3} mol L^{-1} of Eu^{3+} doped in sol–gel matrix.

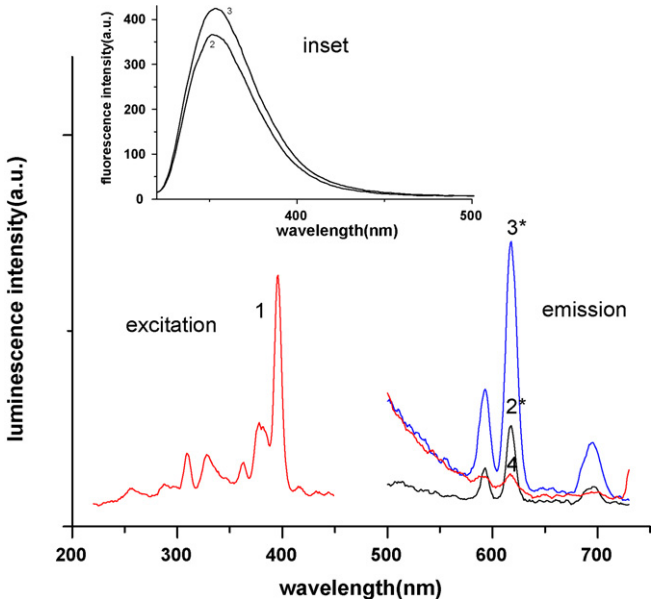

Fig. 4. The excitation spectrum (1) of Eu^{3+} – MCP, the emission spectra (2,3) of two different concentrations of MCP, emission spectrum (4) of Eu^{3+} in sol-gel matrix and emission spectra (2* and 3*) of Eu^{3+} in the presence of two different concentrations of MCP in sol-gel matrix, at $\lambda_{ex}/\lambda_{em} = 380/617$ nm.

Table 2
Freeze–thaw stability of MCP in pharmaceutical tablets and human serum ($n = 3$).

Drug	Normal concentration ($\times 10^{-7}$ mol L^{-1})	Found, average recovery ± SD ($\times 10^{-7}$ mol L^{-1})			Average, RSD (%)
		0 day	15 days	30 days	
Primpran, 10 mg (Aventis Company)	0.1	0.102	0.102	0.103	3.88
	1.0	1.0	1.03	1.04	3.75
	5.0	5.0	5.1	5.1	3.47
Migracid, 5 mg (Cid Company)	0.1	0.1	0.102	0.102	4.77
	1.0	1.01	1.03	1.05	4.67
	5.0	5.0	5.1	5.2	4.60
Serum sample	0.1	0.102	0.104	0.105	3.20
	1.0	1.0	1.01	1.04	3.10
	5.0	5.0	5.02	5.1	2.99

3.2.3. Effect of pH

The pH of the medium has a great effect on the luminescence intensity of the Eu–MCP. The pH has been adjusted using NH_4OH and HCl. The optimum pH value where the peak at 617 nm has the highest intensity was obtained at pH 8.7.

3.2.4. Effect of solvent

The influence of the solvent on the luminescence intensity of the Eu^{3+} in the complex of 5.0×10^{-4} mol L^{-1} of MCP with 1.0×10^{-3} mol L^{-1} of $EuCl_3$. 6 H_2O in the sol–gel matrix was studied under the conditions established above. The results show that there is no quenching in the emission intensity of Eu^{3+}–MCP in the sol–gel matrix in the presence of DMSO [28,29].

3.3. Stability studies

The processed pharmaceutical tablet and serum samples (200, 500 and 700 nmol L^{-1}) treated as sample preparation were kept at room temperature for 24 h and then the stability was determined. The freeze–thaw stability was determined after three repeated freezing and thawing cycles in day 0, 15 and 30, Table 2.

No significant loss of MCP (0.52%, RSD) was observed after storage of pharmaceutical tablet and serum samples at room temperature for at least 24 h, Table 3. Pharmaceutical tablet samples and serum samples were stable over at least three freeze–thaw cycles, Table 2, indicating that the pharmaceutical tablet and serum samples can be frozen and thawed at least three times prior to analysis (4.1%, RSD).

3.4. Elucidation of the complex structure

3.4.1. Structure

The Ln^{3+} ions may be classified as hard acids. Therefore, it is expected and observed that the most suitable order of coordination is O > N > S [30]. Furthermore, it is generally agreed that Ln^{3+}-ligand coordination occurs predominantly via ionic bonding interactions, leading to a strong preference for negatively charged donor groups that are also hard bases, and neutral donors that possess large ground-state dipole moments such as amide carbonyl oxygen. Water molecules and hydroxide ions are particularly strong ligands for Ln^{3+}.

The ion titration revealed that the complex formed is of M:L ratio (1:2) (Fig. 5), which indicates that the ligand may coordinate to the metal from different coordination sites, the ligand coordinates through either N adjacent to the carbonyl group, the O of the carbonyl group and or O of the methoxy group which are the more probable sites for coordinations but the more preferred coordination sites are the O of the methoxy group and the carbonyl oxygen because they have the highest negative charges as indicated by DFT calculations [31], therefore the predicated structure of the complex was shown in Fig. 6.

3.4.2. Evidences on the energy transfer from ligand to the Eu^{3+} ion

In general, energy is transferred via the triplet state of the organic ligand because the intersystem crossing is enhanced by the nearby, paramagnetic lanthanide ions, and because energy transfer via the singlet state is not fast enough to compete with the luminescence or the intersystem crossing, Fig. 7 and this was observed in Fig. 4 in which the decreasing in the fluorescence intensity of MCP

Table 3
Results of analysis of tablets by the proposed method and statistical comparison of the results with the reference method.

Tablet brand name	Nominal amount, added ($\times 10^{-8}$ mol L^{-1})	Found[a] (percent of label claim ± SD)				
		Reading	Average[b] found ($\times 10^{-8}$ mol L^{-1})	BP (LC)	Proposed method	
					Students t and F values	Average recovery ± RSD (%)
Primpran, 10 mg (Aventis Company)	20	20.02, 19.89, 20.1	20.03	99.98 ± 1.0	$t = 0.25$, $F = 5.65$	100.7 ± 0.61
	50	49.87, 50.05, 50.01	49.97		$t = 0.25$, $F = 6$	
	70	70.06, 70.08, 69.99	70.07		$t = 0.10$, $F = 1.2$	
Migracid, 5 mg (Cid Company)	20	19.94, 20.14, 20.11	20.06	99.90 ± 0.2	$t = 0.30$, $F = 1.3$	104.4 ± 0.40
	50	50.02, 50.02, 50.05	50.03		$t = 0.13$, $F = 3.7$	
	70	69.97, 70.10, 69.95	70.01		$t = 0.20$, $F = 1.7$	
Serum sample	20	20.09, 20.03, 19.97	20.03	99.99 ± 0.1	$t = 0.15$, $F = 3.6$	100.5 ± 0.52
	50	50.05, 49.89, 49.72	49.88		$t = 0.41$, $F = 2.1$	
	70	70.11, 70.15, 70.10	70.12		$t = 0.07$, $F = 10.5$	

Tabulated t value at the 95% confidence level is 4.303. Tabulated F value at the 95% confidence level is 19.

[a] Average of three determinations.

[b] Each reading was repeated three times (average was taking for three reading by three analysts).

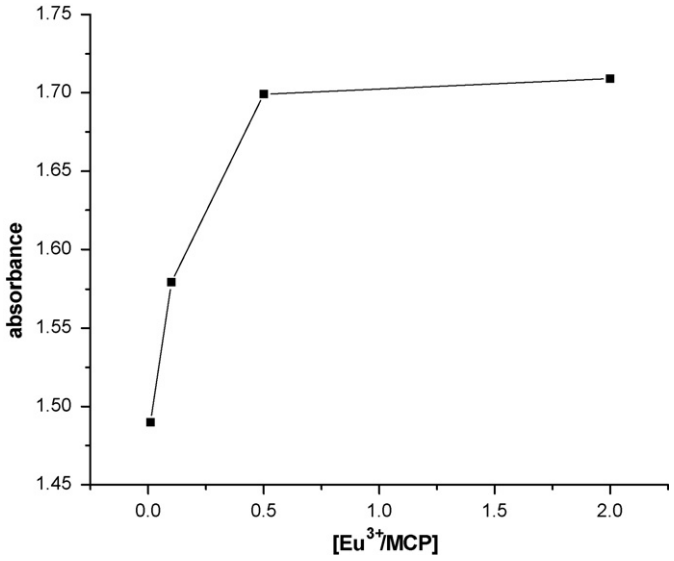

Fig. 5. Relationship between different molar concentration ratios of Eu^{3+}/MCP and the absorbance (molar ratio method).

(inset) and increasing in the luminescence intensity of Eu^{3+}–MCP complex.

3.5. Analytical performance

3.5.1. Method validation

3.5.1.1. Analytical parameters of optical sensor method. A linear correlation was found between luminescence intensity of MCP–Eu^{3+} complex at λ_{em} = 617 nm and concentration of MCP in the ranges given in Table 4. The six points (1000, 500, 170, 50, 10, 5 nmol L^{-1}) calibration curve was obtained by plotting the peak intensity of Eu^{3+} at λ_{em} = 617 nm versus the concentration of MCP and the graph was described by the regression equation:

$$Y = a + bX$$

where Y = luminescence intensity of the optical sensor at λ_{em} = 617 nm; a = intercept; b = slope and X = concentration in nmol mL^{-1}. Regression analysis of luminescence intensity data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values were presented in Table 4. The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [32] using the formulae: LOD = 3.3 S/b and LOQ = 10 S/b (where S is the standard deviation of blank luminescence intensity values, and b is the slope of the calibration plot) are also presented in Table 4.

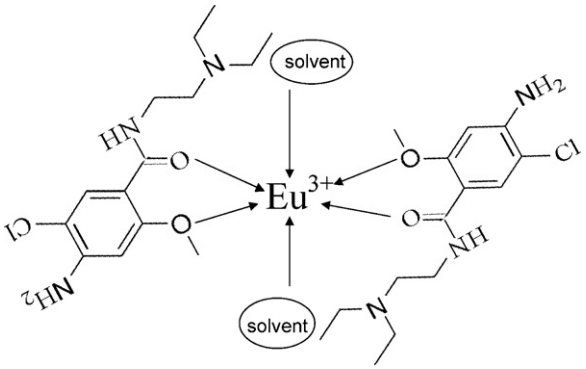

Fig. 6. The predicted structure of the complex.

Table 4
Sensitivity and regression parameters for optical sensor.

Parameter	Method
L_{em} (nm)	617
Linear range (mol L^{-1})	5×10^{-9} to 1×10^{-6}
Limit of detection (LOD) (mol L^{-1})	2.2×10^{-11}
Limit of quantification (LOQ) (mol L^{-1})	6.6×10^{-11}
Regression equation, Y[a]	
Intercept (a)	37.76
Slope (b)	2.2×10^{11}
Standard deviation	1.54
Variance (Sa^2)	2.37
Regression coefficient (r)	0.9551

[a] $Y = a + bX$, where Y is luminescence intensity, X is concentration in nmol L^{-1}, a is intercept, b is slope.

The low value of LOD indicate the high sensitivity of the proposed method.

3.5.1.2. Accuracy and precision of the method. To compute the accuracy and precision, the assays described under "general procedures" were repeated three times within the day to determine the repeatability (intra-day precision) and three times on different days to determine the intermediate precision (inter-day precision) of the method. These assays were performed for three levels of analyte. The results of this study are summarized in Table 5. The percentage relative standard deviation (%RSD) values were ≤0.08% (intra-day) and ≤0.15% (inter-day) indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and the taken concentrations of MCP. Bias {bias% = [(Concentration found − known concentration) × 100/known concentration]} was calculated at each concentration and these results are also presented in Table 5. Percent relative error (%RE) values of ≤2.5% demonstrates the high accuracy of the proposed method.

3.5.1.3. Selectivity. The proposed methods were tested for selectivity by placebo blank and synthetic mixture analysis. A placebo blank containing talc (250 mg), starch (300 mg), lactose (30 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (40 mg), sodium alginate (70 mg) and magnesium stearate (100 mg) was extracted with water and solution made as described under "analysis of dosage forms". A convenient aliquot of solution was subjected to analysis according to the recommended procedures. In the method of analysis, there was no interference by the inactive ingredients.

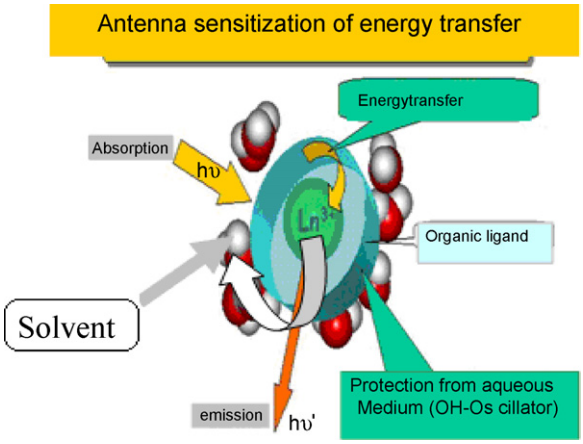

Fig. 7. Mechanism of the energy transfer from MCP to Eu^{3+}.

Table 5
Evaluation of intra-day and inter-day accuracy and precision.

Method	MCP taken[a]	Intra-day accuracy and precision ($n=3$)			Inter-day accuracy and precision ($n=3$)		
		MCP average found[a] ± CL	%RE	%RSD	MCP average found[a] ± CL	%RE	%RSD
Primpran, 10 mg (Aventis Company)	2.0	2.03 ± 0.23	1.50	0.09	2.06 ± 0.32	3.00	0.13
	3.0	2.95 ± 0.20	1.66	0.08	3.05 ± 0.31	1.66	0.12
	4.0	4.09 ± 0.21	2.25	0.08	4.12 ± 0.28	3.00	0.11
Migracid, 5 mg (Cid Company)	0.50	0.51 ± 0.16	2.00	0.07	0.49 ± 0.30	2.00	0.16
	1.00	1.02 ± 0.18	2.00	0.06	1.04 ± 0.41	4.00	0.13
	1.50	1.49 ± 0.13	0.60	0.05	1.53 ± 0.31	1.50	0.12
Serum sample	0.60	0.61 ± 0.40	1.60	0.16	0.62 ± 0.42	3.33	0.17
	0.80	0.82 ± 0.39	2.50	0.15	0.83 ± 0.39	3.75	0.15
	1.00	1.02 ± 0.36	2.00	0.14	1.03 ± 0.46	3.00	0.15

%RE: percent relative error, %RSD: relative standard deviation and CL: confidence limits were calculated from: $CL = \pm tS/\sqrt{n}$. (The tabulated value of t is 4.303, at the 95% confidence level; S = standard deviation and n = number of measurements.)
[a] The values are multiplied by 10^{-7} mol L^{-1} for method.

Table 6
Method robustness and ruggedness expressed as intermediate precision (% RSD).

Method	MCP taken[a]	Robustness		Ruggedness
		Parameter altered		Inter-analysts, (%RSD) ($n=2$)
		Concentration of Eu^{3+} [b] (%RSD)	Reaction time[c]	
Primpran, 10 mg (Aventis Company)	6.0	1.48	0.68	1.45
Migracid, 5 mg (Cid Company)	1.0	1.62	0.66	1.89
Serum sample	0.5	1.95	0.98	1.99

[a] The values are multiplied by 10^{-7} mol L^{-1}.
[b] Concentrations of Eu^{3+} were 2, 5 and 6×10^{-4} mol L^{-1}.
[c] The reaction times studied were 19, 20 and 21 min.

A separate test was performed by applying the proposed method to the determination of MCP in a synthetic mixture. To the placebo blank of similar composition, different amount of MCP of different products were added, homogenized and the solution of the synthetic mixture was prepared as done under "analysis of dosage forms". The filtrate was collected in a 100-mL flask. Five milliliters of the resulting solution was assayed ($n=3$) by the proposed method which yielded a % recovery of 99.30 ± 0.65 and 96.60 ± 0.85 for tablet and serum samples, respectively. The results demonstrated the accuracy as well as the precision of the proposed method. These results complement the findings of the placebo blank analysis with respect to selectivity.

3.5.1.4. Robustness and ruggedness. The robustness of the method was evaluated by making small incremental changes in the concentration of Eu^{3+} and MCP and contact time, and the effect of the changes was studied on luminescence intensity of the optical sensor. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD (≤1.75%). Method ruggedness was expressed as the RSD of the same procedure applied by two different analysts. The inter-analysts RSD were within 1.81% for the same MCP concentrations ranged from 1.4% to 2.1% suggesting that the developed method was rugged. The results are shown in Table 6.

3.5.1.5. Application to formulations. The proposed method was applied for the determination of MCP in two representative tablets Primpran, 10 mg and Migracid, 5 mg which containing other inactive ingredients and on serum sample of the health state human. The results in Table 3 show that the method is successful for the determination of MCP and that the excipients in the dosage forms did not interfere. The results obtained (Table 3) were statistically compared with the official British Pharmacopoeia (BP) method [33]. The average recovery and RSD for the tablet and serum samples in our method were found to be (102.5% and 0.51%) and (100.5% and 0.52%) respectively. Data obtained by BP method give average recovery 99.99% and RSD 0.1%, were also presented for comparison and show a good correlation with those obtained by the proposed

Table 7
Results of recovery study using standard addition method.

Tablet studied	MCP in sample/extract	Pure MCP[a] added	Total MCP[a] found	Pure MCP recovered (percent ± SD)
Proposed method				
Primpran, 10 mg (Aventis Company)	1.5	1.5	2.95	98.30 ± 0.55
	1.5	3.0	4.45	98.88 ± 0.45
	1.5	4.5	6.11	101.83 ± 0.70
Migracid, 5 mg (Cid Company)	1.0	1.5	2.46	98.4 ± 0.67
	1.0	3.0	4.15	103.8 ± 0.95
	1.0	4.5	5.43	98.7 ± 0.85
Serum sample	0.05	1.5	1.45	93.5 ± 1.35
	0.03	3.0	2.92	96.4 ± 0.35
	0.06	4.5	4.50	98.6 ± 0.65

[a] The values are multiplied by 10^{-7} mol L^{-1}.

method. The results obtained by the proposed method agreed well with those of reference method and with the label claim. When the results were statistically compared with those of the reference method by applying the Student's *t*-test for accuracy and *F*-test for precision on the tablets and serum samples, the calculated Student's *t*-value and *F*-value [34] at 95% confidence level did not exceed the tabulated values of 0.3, 0.4 and 6.0, 10.2, respectively, for two degrees of freedom. Hence, no significant difference exists between the proposed method and the reference method with respect to accuracy and precision, Table 3.

3.5.1.6. Recovery study. To further assess the accuracy of the methods, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure MCP at three different levels (15, 30 and 45 $\mu mol\,L^{-1}$) of the content present in the tablet powder (taken) and the total was found by the proposed method. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 98.87% and 103.3%, 93.50% and 98.60% with relative standard deviation in the range 0.45–0.95%, 0.35–1.35% for tablet and serum samples, respectively. Closeness of the results to 100% showed the fairly good accuracy of the method. The results are shown in Table 7.

4. Conclusion

The Eu^{3+} ion doped in sol–gel matrix has high sensitive and characteristic peaks in the presence of MCP. The intensities of these peaks were enhanced by increasing the concentration of MCP, due to energy transfer from MCP to the Europium ion and can be used for determination of MCP in pharmaceutical preparations and in serum samples.

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