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Favipiravir determination by the enhancement of the emission of Sm^{3+} optical sensor

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ABSTRACT

The efficiency of excited-state interaction between Sm^{3+} and the industrial product Favipiravir (Fav) has been studied in different solvents and various pH values. The results indicated high luminescence intensity peak at 645 nm of Samarium complex in DMF without any change in solvent pH value. The photophysical properties of the Emissive Sm^{3+} Complex have been elucidated, the Samarium was used as optical sensor for the assessment of Fav in the pharmaceutical tablets λ_{ex}=370 nm with a concentration range (16-600) µg/mL of Fav, correlation coefficient of 0.97 and detection limit of 8 µg/mL. This method is simple, accurate and can successfully be applied to the determination of Fav in pharmaceutical preparation samples with remarkably satisfactory results.

Keywords: Favipiravir. Samarium (III), enhancing luminescence, Optical sensor, COVID-19.

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INTRODUCTION

Favipiravir was discovered by Toyama Chemical Co., Ltd. in Japan. It is a modified pyrazine analog that was initially approved for therapeutic use in resistant cases of influenza (Abdelnabi *et al*., 2021; Gordon *et al*., 2021).

The antiviral targets RNA-dependent RNA polymerase (RdRp) enzymes, which are necessary for the transcription and replication of viral genomes (Abdelnabi *et al*., 2021; Graham, *2021*; Holman *et al.*, 2921). Not only does favipiravir inhibit replication of influenza A and B, but it has shown promise in the treatment of avian influenza, and it may be an alternative option for influenza strains that are resistant to neuramidase inhibitors (Gordon *et al*., 2021; Abdelnabi *et al*., 2021). Moreover, Favipiravir has been investigated for the treatment of life-threatening pathogens such as Ebola virus, Lassa virus, and now COVID-19 (Imran *et al*., 2021; Kabinger *et al*., 2021; Khoo *et al*., 2021).

Only few methods were performed for the determination of Fav. Like HPLC method (Mahase, 2021; Malone and Campbell, 2021), the bioanalytical assay using a liquid chromatography tandem mass spectrometry LC-MS/MS (Ahlqvist *et al*., 2021) and Spectrofluorimetric method (Menendez-Arias, 2021).

In this work, Fav concentration was determined by the complexation between Fav as a ligand and the Sm^{3+} Ion and the possibility of the enhancement of the Sm^{3+} luminescence sensitized by Fav was established and investigated. The absorption and emission spectra of Fav and (Fav-Sm^{3+}) complex were measured in DMF without any change in pH value.

METHODOLOGY

1. Materials

Pure standard Favipiravir (Fig. 1) was supplied from Hetero, India. Sm $(NO_3)_3.6H_2O$ delivered from Sigma Aldrich, USA.

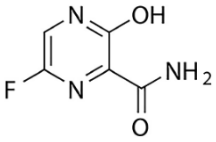

Fig. 1. Chemical structure of Favipiravir.

2. Reagents

All chemicals used are of analytical grade and pure solvents were purchased from (Sigma Aldrich). A stock solution of Fav (10^{-2} mol.L^{-1}) was freshly prepared by dissolving 15.71 mg in 10 mL pure DMSO. More diluted solution (1.0×10^{-4} mol.L^{-1}) was prepared by appropriate dilution with DMF. Stock and working solutions were stored at 5±3°C when are not in use. Sm^{3+} Ion stock solution (10^{-2} mol.L^{-1}) was prepared by dissolving 220 mg Sm $(NO_3)_3.6H_2O$ (delivered from Sigma Aldrich) in small amount of ethanol in 50 mL measuring flask, then dilute to the mark with ethanol. The working solution of Sm^{3+} Ion of 1×10^{-4} mol.L^{-1} was obtained by appropriate dilution with DMF. The pH value was adjusted by using 0.1 mol.L^{-1} of NH_4OH/HCl solutions.

3. Apparatus

All luminescence measurements were carried out on Shimadzu RF5301 (PC) spectro-fluorophotometer in the Range 290–750 nm. The absorption spectra were recorded with a Unicam UV-Visible double beam spectrophotometer from Helios. The spectrophotometer is equipped with a temperature-controller cell holder. All pH measurements were made with a pHs-JAN-WAY3040 ion analyzer.

4. General Procedure

To 10 mL measuring flasks, solutions were added in the following order: 0.15 mL of 1×10^{-2} mol.L^{-1} Fav solution and 0.1 mL of 1×10^{-2} mol.L^{-1} Sm^{3+} solution to give 1.5×10^{-4} mol.L^{-1} of Fav and 1×10^{-4} mol.L^{-1} of Sm^{3+}. The Mixture was diluted to the mark with DMF and pH value was adjusted by using 0.1 mol.L^{-1} of NH_4OH/HCl solutions. The above procedure was used for the subsequent measurements of absorption, emission spectra and effect of pH and solvents. The luminescence intensity was measured at λex/λem=370/645 nm.

5. Determination of Favipiravir in Pharmaceutical Preparations

Five tablets of Favipiravir 200 mg were carefully weighed and grounded to finely divided powders. Accurate weights equivalent to 157.1 mg Favipiravir were dissolved in 100 mL DMSO and mixed well and filtered up using 12 mm filter papers. Then transfer 0.15 mL from Fav solution and 0.1 mL of 1×10^{-2} mol.L^{-1} Sm^{3+} Solution into 10 mL DMF. The concentration of the drug was determined by using different concentrations from the corresponding calibration graph.

RESULT AND DISCUSSION

1. Absorption and Emission Spectra

The absorption spectra of Fav and (Sm^{3+} - Fav) complex are shown in Figure (2). Comparing the spectrum of the drug with its spectra after the addition of Sm^{3+} ion into Fav in DMF solvent, a red shift was observed and the absorbance is also enhanced which indicates that Fav can form a complex with Sm^{3+} ion.

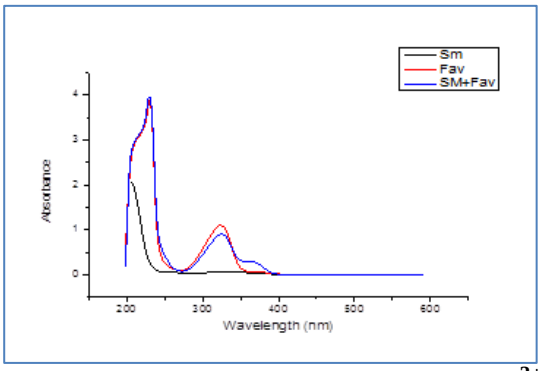

Fig. 2. The absorption spectra of Fav and Sm^{3+}-Fav complex.

The emission spectra of (Sm^{3+}– Fav) complex in different concentrations of Fav are shown in Figure (3).

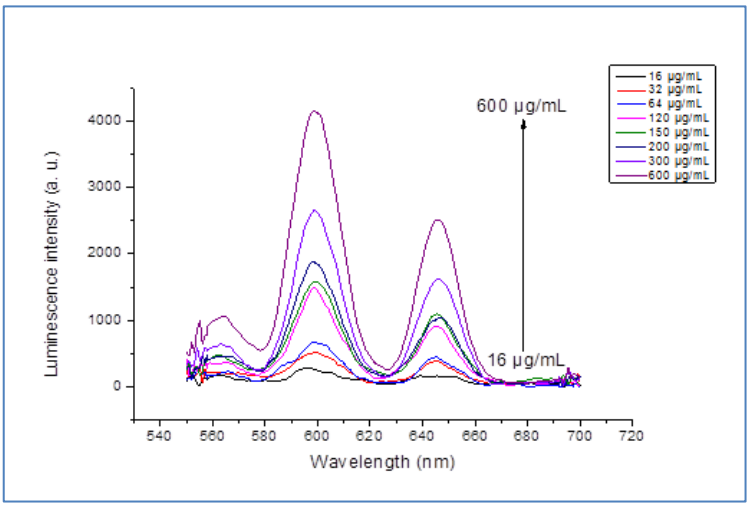

Fig. 3. The emission spectra of (Sm^{3+}– Fav) complex in different concentrations of Fav.

After the addition of different concentrations of Fav into the Sm^{3+} ion in DMF, the intensity of the characteristic peak at 645 nm of Sm^{3+} was enhanced indicating that Fav can form a complex with Sm^{3+} ion. The characteristic peaks of Sm^{3+} ion appear at ($^4G_{5/2} \rightarrow {}^6H_{5/2}$=564 nm, $^6H_{7/2}$=599 nm, $^6H_{9/2}$=645 nm, and $^6H_{11/2}$=707 nm), respectively.

2. Effect of Experimental Variables

2.1. Effect of pH

The pH of the medium has a vital effect on the luminescence intensity of the Sm-Fav. The pH has been adjusted using NH_4OH and HCl solutions. The optimum pH value where the peak at 645 nm has the highest intensity was obtained at pH value of DMF solvent as is without any change (Fig. 4. a & b).

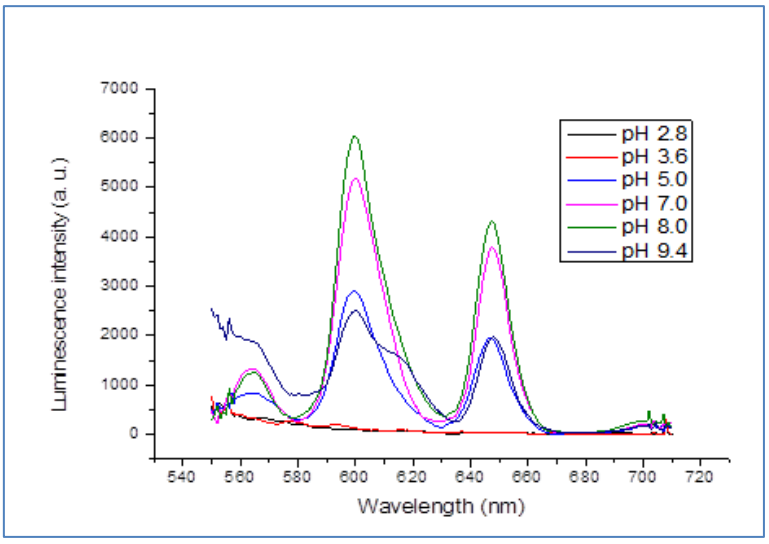

Fig. 4. a. The emission spectra of (Sm^{3+}– Fav) complex in different pH value.

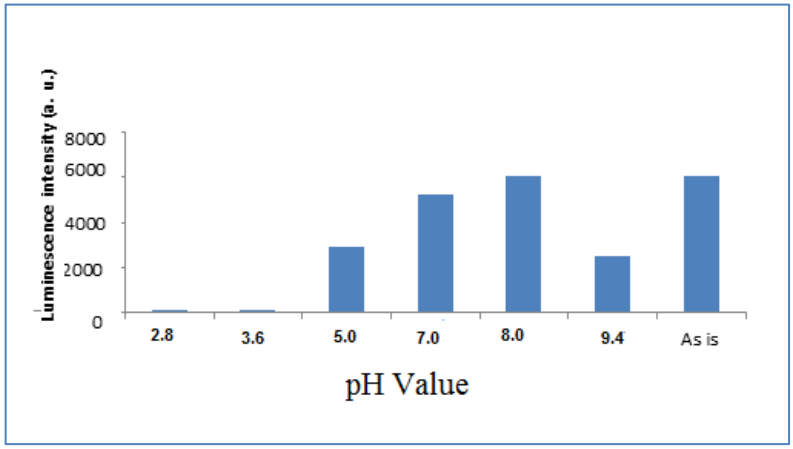

Fig. 4. b. The pH effect on the emission spectra of (Sm^{3+}– Fav) complex.

2.2. Effect of Solvent

The influence of the solvent on the luminescence intensities of the solutions containing 1.5×10^{-4} mol.L^{-1} of Fav and 1.0×10^{-4} mol.L^{-1} Sm^{3+} was studied under the conditions established above. The results show the enhanced emission of Sm^{3+}-Fav in DMF. This can be attributed to the formation of an hydrous solvates of Sm^{3+}-Fav complex Introducing solvent molecules in the first coordination sphere of Sm^{3+}-Fav leads to the enhancement of the intensity of all transitions ($^4G_{5/2} \rightarrow {}^6H_{5/2}$=564 nm, $^6H_{7/2}$=599 nm, $^6H_{9/2}$=645 nm, and $^6H_{11/2}$=707 nm), respectivly (Fig. 5. a & b).

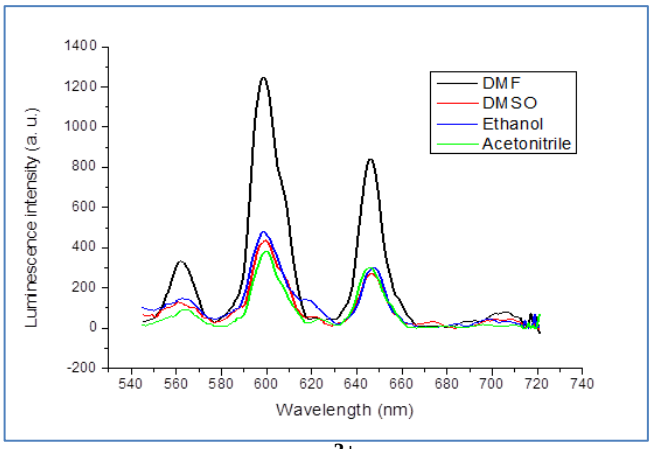

Fig. 5. a. The emission spectra of (Sm^{3+}– Fav) complex in different solvent.

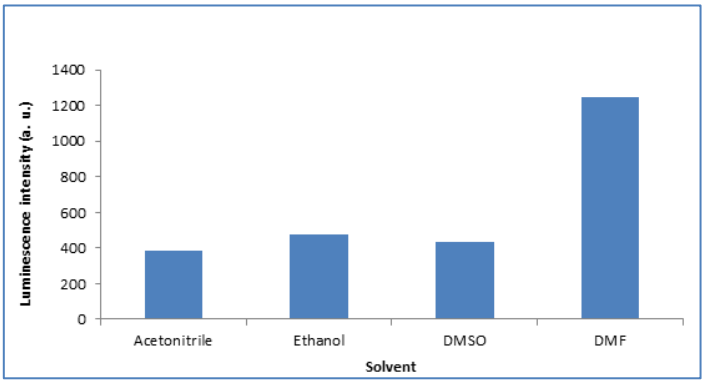

Fig. 5. b. The solvent effect on the emission spectra of (Sm^{3+}– Fav) complex.

By increasing the radiative rate, Sm^{3+} excited states became less sensitive to deactivation processes, ultimately resulting in a more efficiently emissive Sm^{3+} complex. Also, the luminescence intensities for the complexes in DMF solutions were stronger than in ethanol as hydroxyl solvent. This may be due to vibrational energy transfer to the solvent molecules.

It is well know that the excited state of the lanthanide ions is efficiently quenched by interactions with high-energy vibrations like O-H groups there by the luminescence of this complex in –OH containing solvents can be quenched easily because of the O-H oscillators (Attia *et al*., 2011, 2012, 2019).

3. Analytical Performance, Method Validation

Analytical parameters of optical sensor method indicated a linear correlation between luminescence intensity of (Fav– Sm^{3+}) complex at λ_{em}=645 nm and concentration of Fav in the ranges given in Table (1) and Figure (6). The calibration curve was obtained by plotting the peak intensity of Sm^{3+} at λ_{em}=645 nm versus the concentration of Fav and the graph was described by the regression equation: Y= a + bX

Where, Y=luminescence intensity of the optical sensor at λ_{em}=645 nm; a=intercept; b=slope and X=concentration in $\mu g.mL^{-1}$.

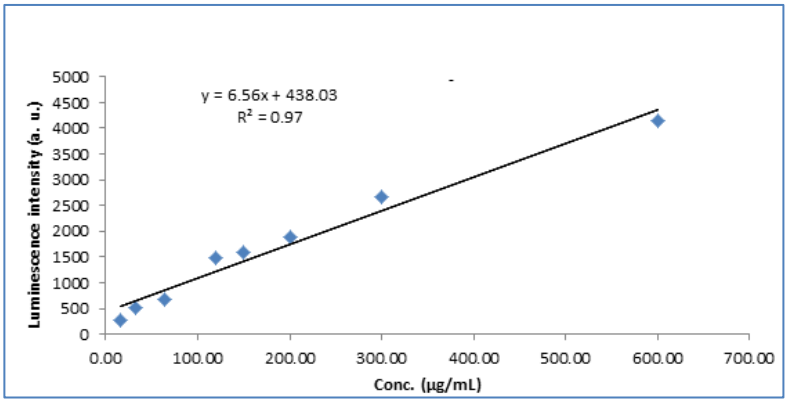

Fig. 6. Linear relationship between luminescence intensity of Sm^{3+}-Fav and different concentrations of Fav.

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 (1). The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines (ICH , 2005), using the formulae: LOD 3:3S=b and LOQ 10S=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 (1). The low value of LOD indicates the high sensitivity of the proposed method when compared by other methods (Table 2).

Table 1: Sensitivity and regression parameters for Sm-Fav chemosensor.

Parameter	**Results**
λ_{ex} / λ_{em}	370 / 645 nm
Linear range	(16-600) µg/mL
Intercept (a)	438.03
Slope (b)	6.56
Regression coefficient (R^2)	0.97
Limit of detection (LOD)	8 µg/mL
Limit of quantitation (LOQ)	16 µg/mL

Table 2: Comparison of spectrofluorimetric technique with some existing methods for the determination of Favipiravir.

Method	Linear range (µg/mL)	Detection limit (µg/mL)	Reference
HPLC-UV	10–100	1.20	Mahase (2021)
HPLC-UV	10-100	----------	Malone and Campbell (2021)
LC-MS/MS	0.00078-0.20	0.00078	Ahlqvist *et al*. (2021)
LC-MS/MS	0.10-20	----------	Malone and Campbell (2021)
Spectrofluorimetric	0.04-0.28	0.0094	Menendez-Arias (2021)
Sm-Fav optical sensor (Chemosensor method)	16-600	8	Present work

3.1. 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. The results of this study are summarized in Table (3). The percentage relative standard deviation (% RSD) values were 0.77% (intra-day) and 0.85% (inter-day) for Favipiravir tables, (%RSD) Indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and the taken concentrations of Fva. Bias (bias%= ((Concentration found - known concentration) x 100/ known concentration)) was calculated and these results are also presented in Table (3). Percent relative error (%RE) values of 1.05% (intra-day) and 0.78% (inter-day) for Favipiravir tablets demonstrates the high accuracy of the proposed method.

Table 3: Evaluation of intra-day and inter-day accuracy and precision.

	Sample Conc. µg/mL	Conc. Found µg/mL	RSD %	RE %	Average RE%
Day I	100	101.26	0.77	1.26	1.05
		100.19		0.19	
		101.72		1.72	
Day II		100.65	0.85	0.65	0.78
		99.43		-0.57	
		101.41		1.41	

3.2. Selectivity

The proposed method was tested for selectivity by placebo blank and synthetic mixture analysis. A placebo blank containing (talc, starch, lactose, calcium carbonate, calcium dihydrogen orthophosphate, methylcellulose, sodium alginate and magnesium Stearate) 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.

3.3. Application to Formulations

The proposed method was applied to the determination of Fav in one representative tablet of Favipiravir that was purchased from the local market and containing other inactive ingredients. The assay results was 101.05 % ± 0.77 which indicated that the method is successful for the determination of Fav and that the

excipients in the dosage forms did not interfere.

3.4. Stability

No significant loss of Fav (Assay % ± % R.S.D 101.05 ± 1.47. for tablet samples) was observed after storage of pharmaceutical tablet at 5±3ºC for at least 7 Days.

Conclusion

The Sm^{3+} Ion in DMF has high sensitive and characteristic peaks in the presence of Fav. The proposed method for the determination of Fav offers simple, rapid and sensitive technique for the analysis of Fav in DMF solvent without any adjustment in pH value and with a linear range of (16-600) $\mu g.mL^{-1}$ and detection limit of 8 $\mu g.mL^{-1}$. The developed optical sensor is selective, accurate and attractive for routine control analysis of the drug.

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تحديد فافيبيرافير عن طريق تحسين انبعاث المستشعر البصري Sm^{+3}

نجلاء ياسين ، عبد العزيز النادي ، محمد سعيد عطيه
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المستخلص

تمت دراسة كفاءة تفاعل الحالة المثارة بين Sm والمنتج الصناعي Favipiravir (Fav) في مذيبات مختلفة وقيم حموضة مختلفة. تم الحصول على ذروة كثافة اللمعان العالية عند ٦٤٥ نانومتر من مجمع Samarium في DMF دون أي تغيير في قيمة الأس الهيدروجيني للمذيب. تم توضيح الخصائص الفيزيائية الضوئية لمركب Emissive Sm ، وتم استخدام Samarium كمستشعر ضوئي لتقييم Fav في الأقراص الصيدلانية $\lambda ex = 370$ نانومتر مع نطاق تركيز (١٦-٦٠٠) ميكروغرام / مل من Fav ، معامل الارتباط لـ ٠.٩٧ وحد كشف يبلغ ٨ ميكروجرام / مل. هذه الطريقة بسيطة ودقيقة ويمكن تطبيقها بنجاح لتقدير Fav في عينات التحضير الصيدلاني بنتائج مرضية بشكل ملحوظ.