



*Anal. Bioanal. Chem. Res., Vol. 6, No. 2, 311-317, December 2019.*

## Fluorescence Chemosensing of $Mg^{2+}$ by Phenylhydrazone of a Difluorenylpiperidin-4-one

P. Sumathi<sup>a,b</sup> and Israel V. Muthu Vijayan Enoch<sup>c,\*</sup>

<sup>a</sup>R & D Centre, Bharathiar University, Coimbatore, Tamil Nadu, India

<sup>b</sup>Department of Chemistry, Muthayammal College of Arts and Science, Namakkal District, Tamil Nadu, India

<sup>c</sup>Nanotoxicology Research Lab, Department of Nanoscience, Department of Chemistry, Karunya Institute of Technology and Sciences, Coimbatore, India

(Received 30 August 2018 Accepted 28 December 2018)

Magnesium is an abundant element in the environment. Magnesium ion sensing by fluorescence spectral method is of importance due to the need for the detection of the metal in the human body and the environment. In this paper, we report the  $Mg^{2+}$  ion sensing behavior of the phenylhydrazone derivative of a difluorenylpiperidin-4-one. The preparation method of this compound is simple. The compound shows an enhanced absorption and fluorescence in the presence of  $Mg^{2+}$  ions, strikingly different from the other metal ions, which do not show significant spectral changes. The stoichiometry and the binding strength of the  $Mg^{2+}$  complex of the phenylhydrazone are determined. The compound shows an association constant of  $3375.36 M^{-1}$  on binding to  $Mg^{2+}$ . The range of detection and the competitive binding behavior of  $Mg^{2+}$  are reported. The phenylhydrazone of the difluorenylpiperidin-4-one shows appreciable selectivity and sensitivity of detection for  $Mg^{2+}$  ions.

**Keywords:**  $Mg^{2+}$  sensing, Piperidin-4-one, Phenylhydrazone, Fluorescent chemosensor, Association

### INTRODUCTION

Being sensitive and selective, fluorescent chemosensors are effective in detecting metal ions and they are easy to operate [1-3]. Magnesium is the fourth most abundant metal in the human body and plays several vital roles *viz.*, maintenance of the integrity of membranes, providing nervous tissue conduction, hormone secretion, improvement of the neuromuscular excitability, and aiding muscle contraction [4-9]. The range of concentration of  $Mg^{2+}$  in mammalian cells is 14-20 mM [10]. Depletion of the amount of  $Mg^{2+}$  might lead to congestive heart failure, muscle dysfunction, lung cancer [11-13]. On the contrary, elevated  $Mg^{2+}$  levels leads to diseases like Alzheimer's disease and hypertension [14]. There have been reports on  $Mg^{2+}$  ion sensors in the literature [15-20]. However, development of newer and easy-to-synthesize  $Mg^{2+}$  ion

sensors is of importance due to the above mentioned necessity for  $Mg^{2+}$  ion detectors.

Piperidones form the core of the structures of several antipsychotics [21], antihistaminis [22], antinociceptive drugs [23], anti-microbials [24] and antifungal agents [25], piperidine derivatives take place as crucial part of molecular structures of popular drugs [26]. The phenylhydrazone of difluorenylpiperidin-4-one exhibits interesting fluorescence properties and has been reported to exhibit molecular logic operation based on photonic and proton inputs [27]. In this paper, we report the  $Mg^{2+}$  sensing behavior of phenylhydrazone of 3-methyl-2,6-di-(9H-fluorenyl) piperidin-4-one based on absorption and fluorescence spectral characteristics.

### EXPERIMENTAL

#### Materials, Methods, and Instrumentation

All the chemicals were purchased from commercial

\*Corresponding author. E-mail: drisraelenoch@gmail.com

suppliers. Fluorene-2-carboxaldehyde, 2-butanone, ammonium acetate, and the salts of  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Al}^{3+}$  were procured from Merck, India. The solvents were purchased from Hi-Media, India.

The solvent (ethanol) was distilled before use. The spectral measurements were done on the following instruments:  $^1\text{H}$  and  $^{13}\text{C}$  NMR: Bruker Avance 400 MHz spectrometer, Mass spectrum: Finnigan mat 8280 mass spectrometer, UV-Visible spectra: Jasco V 630 spectrophotometer, fluorescence spectra: Jasco FP8300 spectrofluorometer with a xenon source lamp, IR: Perkin Elmer system one FTIR/AT. The reference solution in NMR was tetramethylsilane and the solvent used was deuterated chloroform ( $\text{CDCl}_3$ ). The UV-Vis spectra were measured using 1 cm path length cuvettes and the spectra were recorded placing appropriate blank solutions (without the fluorophore) as the reference. IR spectrum was measured by pelletizing the compound with KBr. pH measurements were made using a Elico LI 120 pH meter.

The detections of the metal ions were operated in aqueous solutions of pH 7.4, acetonitrile-water (1% v/v). The stock solution of 1 was prepared in acetonitrile. Stock solutions of  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Al}^{3+}$  were made at a concentration of  $1 \times 10^{-3} \text{ mol dm}^{-3}$  and test solutions were prepared by appropriately diluting them in twice-distilled water. The spectra were recorded at room temperature and for recording the fluorescence spectra, the excitation wavelength was set up at 305 nm. A linear plot was made using the modified Benesi-Hildebrand equation [28]:

$$\frac{1}{I - I_0} = \frac{1}{I - I_0} + \frac{1}{I' - I_0} \frac{1}{K[\text{Mg}^{2+}]}$$

where  $I_0$  and  $I$  refer to the intensity of fluorescence of the chemosensor before and after the addition of metal ions in different concentrations, respectively.  $I'$  refers to the intensity at maximal concentration and  $K$  is the association constant.  $[\text{Mg}^{2+}]$  refers to the concentration of  $\text{Mg}^{2+}$  ions.

### Synthesis of Phenylhydrazone of 3-Methyl-2,6-di-(9H-fluorenyl)piperidin-4-one

The compound 3-phenyl hydrazone of 3-methyl-2,6-di-

(9H fluorenyl)piperidin-4-one (compound 1) was synthesized in two steps following a procedure reported by us previously [27].

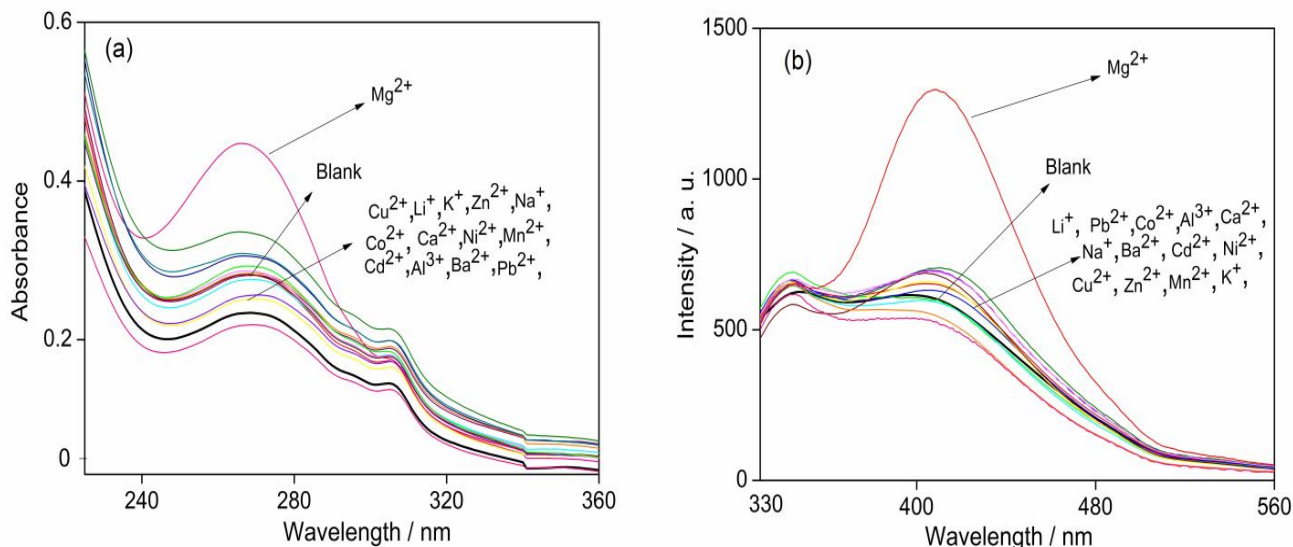
Fluorene-2-carboxaldehyde (2 moles, 2.5 g), butan-2-one (1 mol, 0.4615 g), and ammonium acetate (1 mol, 0.4933 g) were heated in ethanol for about 3 h. The product was extracted with dry diethyl ether (Yield 62%, m. p.: 80 °C), the phenylhydrazone of 3-methyl-2,6-di(9H-fluorenyl) piperidin-4-one was synthesized by refluxing equimolar mixture of the piperidone and phenyl hydrazine in ethanol for 90 min (Scheme 1). The product was recrystallized by ethanol (m. p.: 158 °C, yield: 70%). IR ( $\text{KBr}$ ,  $\text{cm}^{-1}$ ): 3437 (NH, str), 2849, 2918 (Ar), 1565 (C-N str), 1415 (C-C str).  $^1\text{H}$  NMR (DMSO, ppm): 1.232 (d, 3H, methyl), 2.845 (d, 2H,  $\text{CH}_2$ ), 3.462-3.695 (CH, positions 2, 6), 2.183 (m, 1H, CH), 6.667-7.892 (m, Ar), 5.029 (broad, NH), 9.147 (s, hydrazo NH) for hydrazone & 3.981 (s,  $\text{CH}_2$ ) for fluorenyl ring.  $^{13}\text{C}$  NMR Chemical shift against DMSO in ppm:  $\delta$  60.50 (CH), 39.23 ( $\text{CH}_2$ ),  $\delta$  69.59 (CH),  $\delta$  13.23 (C- $\text{CH}_3$ ),  $\delta$  118.32-148.56 (aromatic).

## RESULTS AND DISCUSSION

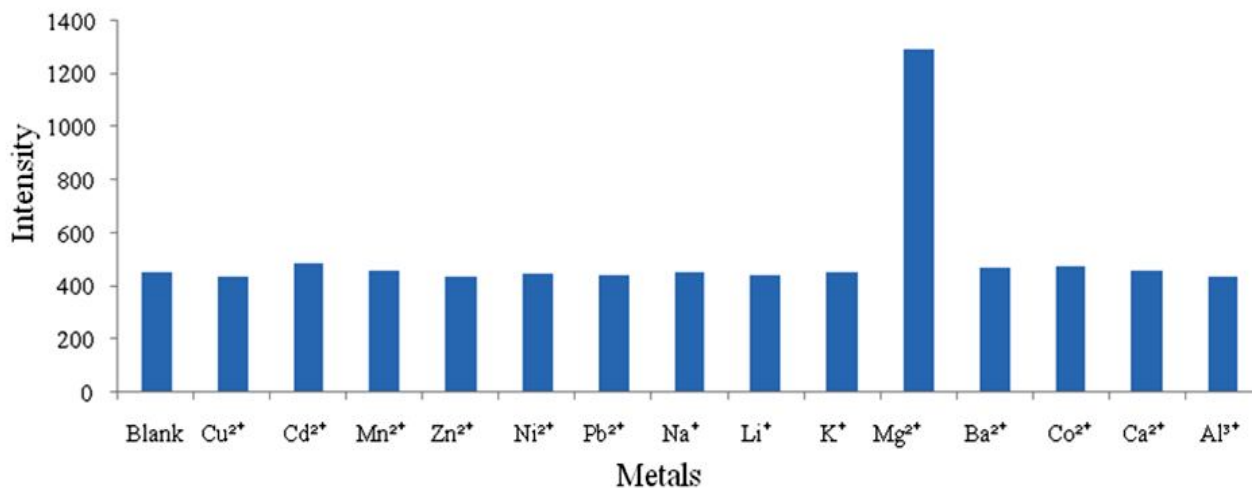
Compound 1 was synthesized as per the procedure given in the experimental section. The structure of the compound was characterized using  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopic techniques [27].

### Selectivity

In order to function as a probe for detecting metal ions, the chemosensor should be selective [29]. Such a selectivity in the recognition of the required metal ion from a variety of cations is an attractive tool as it finds applications in several areas [30]. The metal ion recognizing behavior of compound 1 was evaluated in acetonitrile/water (1%) by observing the changes in the absorption and fluorescence spectra. Experiments were carried out at pH 7.4 to keep the sensor compound suitable for application at physiological pH and to real water samples of the environment. The absorption spectra (Fig. 1a) showed a prominent band at 272 nm and a shoulder at 305 nm. The spectral band of 1 on the addition of  $\text{Mg}^{2+}$  ions showed a vivid hyperchromic shift of the shorter wavelength band. The spectra of 1, when the rest of the metal ions ( $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,



**Fig. 1.** (a) UV-Vis absorption spectra of 1 (in CH<sub>3</sub>CN/H<sub>2</sub>O) in the presence of various metal ions of Cu<sup>2+</sup>, Cd<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Na<sup>+</sup>, Li<sup>+</sup>, K<sup>+</sup>, Ba<sup>2+</sup>, Co<sup>+</sup>, Ca<sup>2+</sup>, Al<sup>3+</sup> and Mg<sup>2+</sup> ions. (b) Fluorescence spectra of 1 (in CH<sub>3</sub>CN-H<sub>2</sub>O, 1% CH<sub>3</sub>CN, pH 7.4) in the presence of various metal ions of Cu<sup>2+</sup>, Cd<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Na<sup>+</sup>, Li<sup>+</sup>, K<sup>+</sup>, Ba<sup>2+</sup>, Co<sup>+</sup>, Ca<sup>2+</sup>, Al<sup>3+</sup> and Mg<sup>2+</sup> ions. The concentration of the metal ions and compound 1 is 1 × 10<sup>-5</sup> mol dm<sup>-3</sup>.

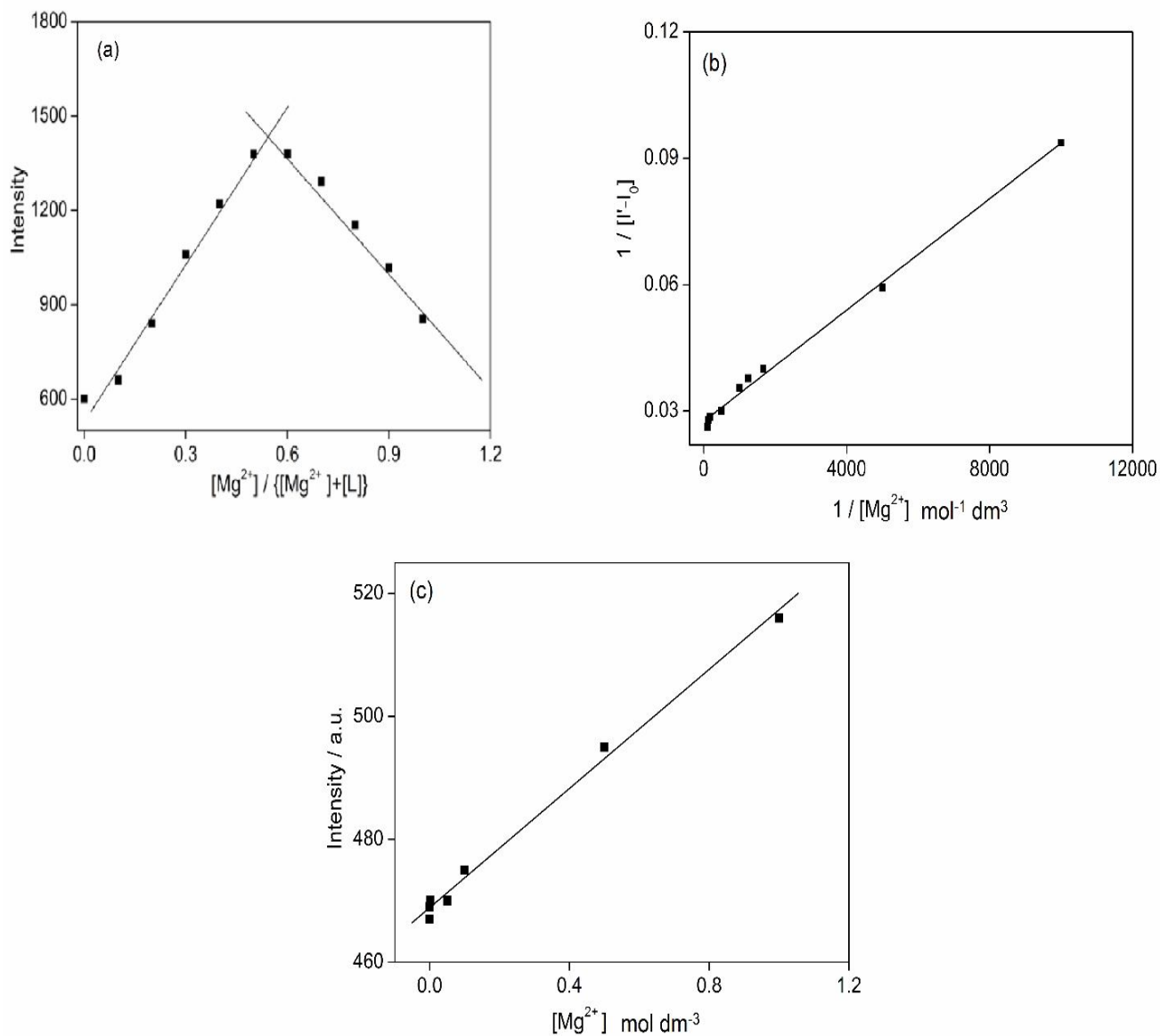


**Fig. 2.** Fluorescence response of 1 upon addition of various metal ions.

Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and Al<sup>3+</sup>) showed insignificant shifts of absorption bands. These results indicated the chelation of the Mg<sup>2+</sup> and 1.

Figure 1b shows the changes of fluorescence intensity of 1 (1.0 × 10<sup>-5</sup> M) in the presence of various metal ions

(Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and Al<sup>3+</sup>). Upon the interaction of the different metal ions with 1, the fluorescence intensity and the wavelength changes were insignificant. However, among the tested metal ions, Mg<sup>2+</sup> induced a pronounced

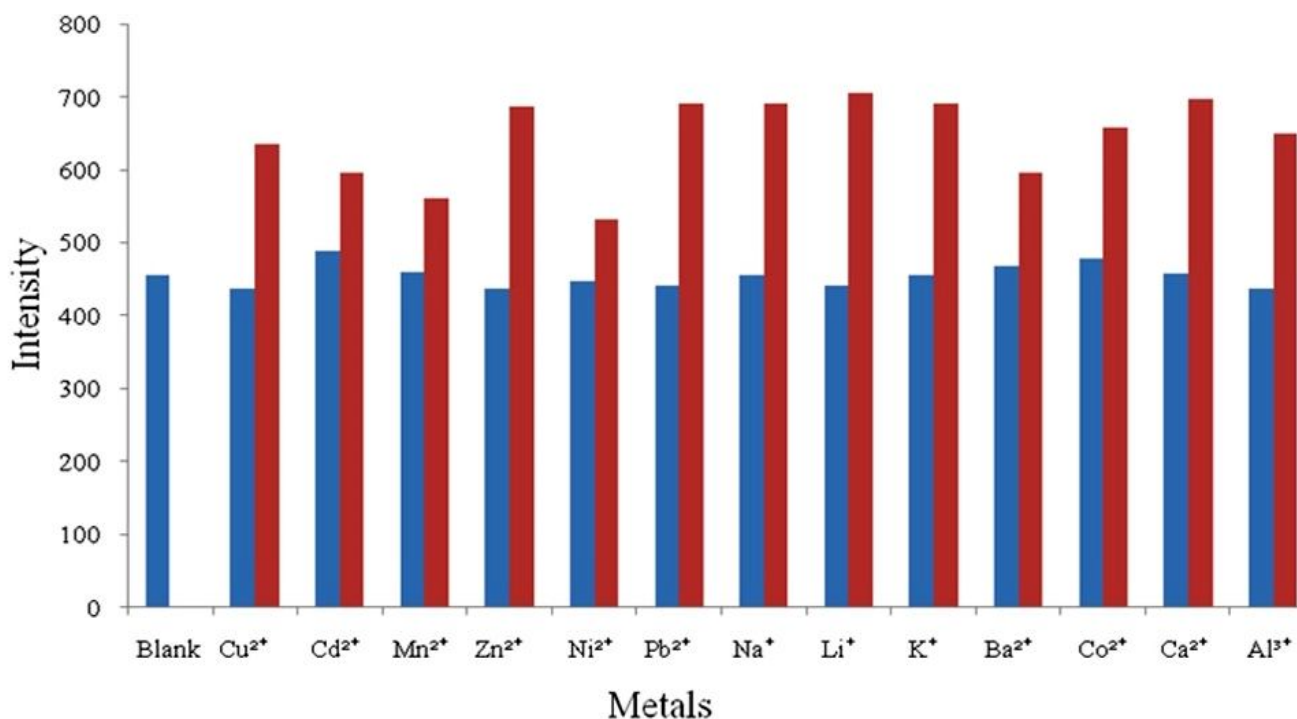


**Fig. 3.** (a) Job's plot for 1:1 complex of 1 and  $Mg^{2+}$  ion (in  $CH_3CN/H_2O$ ), (b) Benesi-Hildebrand plot for complex of 1 with  $Mg^{2+}$ , (c) The  $Mg^{2+}$  detection limit of 1.

enhancement of fluorescence. Hence, the fluorescence spectral properties of the metal ion-added 1 showed that the compound 1 was selective towards  $Mg^{2+}$  ion binding. The striking difference was observed in the 1- $Mg^{2+}$  ion solution. The intensity changes of the fluorescence of 1, upon the addition of metal ions are shown in Fig. 2.

### Spectral Characteristics of Compound 1 Added with $Mg^{2+}$

The fluorescence filtration experiments of 1 with  $Mg^{2+}$  were done in acetonitrile/water (1%) solution. As shown in the Job's plot as in Fig. 3a, the binding of  $Mg^{2+}$  with the sensor 1 induced the fluorescence intensity of the spectral



**Fig. 4.** Fluorescence response of 1 upon addition of  $Mg^{2+}$  in the presence of other metal ions (in 1%  $CH_3CN/H_2O$ , pH 7.4). Concentration of the test solutions is  $1 \times 10^{-5} \text{ mol dm}^{-3}$ .

band at 420 nm to get quenched up to the value of 0.6 of the mole fraction of  $Mg^{2+}$  in the ligand-added solutions. The inflection point fell around 0.5, suggesting that the complex formed was of the stoichiometry 1:1 (compound 1:  $Mg^{2+}$ ). The association constant was determined by doing a plot of the inverse of the concentration of  $Mg^{2+}$  against the inverse of the incremental differences of the intensity of fluorescence at various concentrations Fig. 4, employing the following Eq. (1) [31]:

$$\frac{1}{F - F_{\min}} = \frac{1}{K(F_{\max} - F_{\min})[Mg^{2+}]} + \frac{1}{(F_{\max} - F_{\min})} \quad (1)$$

where  $F_{\min}$ ,  $F$ , and  $F_{\max}$  are the fluorescence intensities of 1 in the absence of  $Mg^{2+}$  ion, at varying  $Mg^{2+}$  concentration, and at the saturation concentration of the complex respectively, and  $K$  is the binding constant.

The linearity suggested again the formation of 1:1 complex and the calculated association constant was  $3375.36 \text{ M}^{-1}$ . Further, the range of detection of  $Mg^{2+}$  ions by

1 was studied in the following way: a plot made as per the following equation [32]:

$$LOD = \frac{K S_b}{m} \quad (2)$$

where  $S_b$  is the standard deviation of the blank measures,  $m$  is the calibration sensitivity and is the method detection limit. The calculated lower detection limit of  $Mg^{2+}$  ions by compound 1 is  $1 \times 10^{-8} \text{ mol dm}^{-3}$  (Fig. 3c).

### Competitive Binding of $Mg^{2+}$ to Compound 1 in the Presence of other Metal Ions

Preferential binding to chemosensors over the other interfering metal ions is desired for chemosensors [33]. Therefore, the selective association of  $Mg^{2+}$  by 1 was studied by performing competitive binding experiments. By treating 1 with one equivalent of  $Mg^{2+}$  in the presence of 1 equivalent of various metal ions, the interference of the other metal ions with  $Mg^{2+}$  was measured. The competitive

binding shown by the fluorescence intensity differences are shown in Fig. 4. It was quite observable that the intensity of fluorescence was not affected by the miscellaneous competitive metal ions. This observation further suggested that the compound 1 could selectively detect  $Mg^{2+}$  with insignificant competitive binding by other metal ions.

## CONCLUSIONS

The compound phenylhydrazone of 3-methyl-2,6-di-(9H-fluorenyl)piperidin-4-one was synthesized using a reported procedure and screened for its metal ion sensing property. The compound selectively binds to  $Mg^{2+}$  ions and the selectivity and sensitivity are good to be supporting it as an  $Mg^{2+}$  ion sensor. The stoichiometry and association constant of the  $Mg^{2+}$  complex of the compound are 1:1 and 3.5283 (logK), respectively. The compound shows  $Mg^{2+}$  binding in the presence of competitive metal ions. The lower detection limit of  $Mg^{2+}$  is  $1 \times 10^{-8}$  mol dm<sup>-3</sup>.

## REFERENCES

- [1] G. Tamilselvan, M. Kumaresan, R. Sivaraj, P.M. Selvakumar, I.V.M.V. Enoch, *Sens. Actuators B* 229 (2016) 181.
- [2] P.R. Queen, R. Sivaraj, P.M. Selvakumar, F.G.D. Banos, G. Villora, J.P. Ceron-Carrasco, H. Perez-sanchez, I.V.M.V. Enoch, *RSC Adv.* 6 (2016) 15670.
- [3] E.J. Antony, R. Masilamani, P.R. Queen, P.M. Selvakumar, I.V.M.V. Enoch, *J. Fluoresc.* 25 (2015) 1031.
- [4] A. Hartwig, *Mutat. Res.* 475 (2001) 113.
- [5] B.O. Rourke, P.H. Backx, E. Marban, *Science* 257 (1992) 245.
- [6] L.J. Dai, G. Ritchie, D. Kerstan, H.S. Kang, D.E.C. Cole, G.A. Quamme, *Physiol. Rev.* 81 (2001) 51.
- [7] D. Zhai, J. Yang, Z. Guo, Q. Wang, J. Ouyang, *RSC Adv.* 4 (2014) 46800.
- [8] F.I. Wolf, A. Torsello, A. Fasanella, A. Cittadini, *Mol. Asp. Med.* 24 (2003) 11.
- [9] C. Schmitz, A. Perraud, C.O. Johnson, K. Inabe, M.K. Smith, R. Penner, T. Kurosaki, A. Fleig, A.M. Scharenberg, *Cell* 114 (2003) 191.
- [10] A.M.P. Romani, *Arch. Biochem. Biophys.* 512 (2011) 1.
- [11] O.B. Stepura, A.I. Martynow, *Int. J. Cardiol.* 131 (2009) 293.
- [12] J.H.F. de Baaij, J.G.J. Hoenderop, R.J.M. Bindels, *Physiol. Rev.* 95 (2015) 1.
- [13] F.H. Nielson, H.C. Lukaski, *Magnes. Res.* 19 (2006) 180.
- [14] R.M. Toyuz, *Front. Biosci.* 1 (2004) 1278.
- [15] J. Kim, T. Morozumi, H. Nakamura, *Org. Lett.* 9 (2007) 4419.
- [16] Y. Liu, M. Han, H. Y. Zhang, L.X. Yang, W. Jiang, *Org. Lett.* 10 (2008) 2873.
- [17] G. Farruggia, S. Iotti, L. Prodi, M. Montalti, N. Zaccheroni, P.B. Savage, V. Trapani, P. Sale, F.I. Wolf, *J. Am. Chem. Soc.* 28 (2006) 344.
- [18] Y. Dong, J. Li, X. Jiang, F. Song, Y. Cheng, C. Zhu, *Org. Lett.* 13 (2011) 2252.
- [19] P.S. Hariharan, S.P. Anthony, *RSC Adv.* 4 (2014) 41565.
- [20] M.H. Kao, T.Y. Chen, Y.R. Cai, C.H. Hu, Y.W. Liu, Y. Jhong, A.T. Wu, *J. Lumin.* 169 (2016) 156.
- [21] Y. Chen, S. Wang, X. Xu, X. Lu, M. Yu, S. Zhau, S. Lu, Y. Qiu, T. Zhang, B. Lui, G. Zhang, *J. Med. Chem.* 56 (2013) 4671.
- [22] J. Janssens, L. Stokbroek, *J. Med. Chem.* 28 (1985) 1925.
- [23] F. Mathia, S. Marchalin, D. Vegh, M. Bobosikova, M. Halinkovicova, *Acta Chem. Slov.* 5 (2012) 145.
- [24] J. Emile, G. Motte, M. Madeleine, M. Albert, WO 2013/160435, A1.
- [25] S. Ranjan, F. Shirazi, P. Madhukar, WO 2010/109299, A1.
- [26] M.T. Gulluioglu, Y. Erdogdu, S. Yurdakul, *J. Mol. Struct.* 540 (2007) 834.
- [27] J.M.C. Xavier, S. Chandrasekaran, I.V. Enoch, V. Gunasekaran, *Appl. Spectrosc.* 67 (2013) 1042.
- [28] H. Benesi, J. Hildebrand, *J. Am. Chem. Soc.* 71 (1949) 2703.
- [29] W.F. Li, H.C. Ma, C. Lu, Y. Ma, C.X. Qi, Z.W. Zhang, Z.M. Yang, H.Y. Cao, Z.A. Ley, *RSC Adv.* 5 (2015) 6869.
- [30] G. Li, F.R. Tao, H. Wang, Y.C. Li, L.P. Wang, *Sens.*

- Actuators B 211 (2015) 325.
- [31] S. Poomalai, T.S. Govindaraj, S. Soundrapandian, M.S. Paulraj, I.V.M.V. Enoch, Luminescence 33 (2018) 538.
- [32] M. Sumithra, R. Sivaraj, G.T. Selvan, P.M. Selvakumar, I.V.M.V. Enoch, J. Lumin. 185 (2017) 205.
- [33] J. Zhang, B.J. Zhao, C. Li, X.F. Zhu, R.Z. Qiao, Sens. Actuators B 196 (2014) 117.