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Photocharacterization of a novel fluorescent Schiff Base and investigation of its utility as an optical Fe^{3+} sensor in PVC matrix

Ozlem Oter [a], Kadriye Ertekin [a,*], Rafet Kılıncarslan [b], Mahmut Ulusoy [b], Bekir Cetinkaya [b]

[a] University of Dokuz Eylul, Faculty of Arts and Sciences, Department of Chemistry, 35160 Tinaztepe, Izmir, Turkey
[b] University of Ege, Faculty of Science, Department of Chemistry, 35100 Bornova, Izmir, Turkey

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Abstract

In this work photoluminescent properties of the newly synthesized Schiff base; *N*-(4-dimethylaminophenyl)-3,5-di-*t*-butyl salicylaldimine (DBS) were investigated in different solvents (dichloromethane (DCM), tetrahydrofuran (THF) and ethanol (EtOH)) and in a polymer matrix (polyvinyl chloride (PVC)) by absorption and emission spectroscopies. Quantum yield (ϕ_F), Stoke's shift ($\Delta\lambda_{ST}$; nm) and molar extinction coefficient $\left(\varepsilon;\ cm^{-1}\ M^{-1}\right)$ of the DBS dye were declared. PVC doped dye was found to exhibit a significant response for Fe^{3+} in direction of decrease in fluorescence intensity.

The response to Fe^{3+} ions was evaluated in terms of the effect of pH, salinity and cross sensitivity to other cations. The response time, reversibility, limit of detection, linear concentration range for the metal ion and repeatability characteristics of the sensing reagent also have been studied.

Keywords: Schiff base; Heavy metal sensor; Iron; Fe^{3+}; Fluorescence spectroscopy

1. Introduction

The discovery, design and synthesis of new organic fluorescent molecules and their interaction with cations, anions or biomaterials lead exciting advances in sensing, imaging and diagnostic. Owing to the active role in aquatic redox processes and medical analysis, iron is found to be one of the most important elements in metabolic processes, environmental investigations and biological materials. Different dyes have been used for spectral iron detection either in immobilized or free form.

Pulido-Tofino et al. used immobilized pyoverdin pigment (on controlled pore glass) which selectively reacts with Fe(III) [1]. Zhang et al. performed spectrophotometric determination of iron by using Eriochrome Cyanine-R as extractant and color-developing agent in organic solvents [2]. Sarradin and co-workers applied ferrozine method to flow injection analysis (FIA) to perform iron analysis [3]. The iron-sensitive organic chromophores Phen Green FL, Phen Green SK and Calcein have been intensely studied and commercialized by Molecular Probes™ Invitrogen Detection Technologies. Phen Green FL and Phen Green SK are cell membrane-permeable fluorescent diacetate derivatives of phenanthroline and are capable of detecting Fe^{2+}, as well as Cu^{2+}, Cu^{+}, Hg^{2+}, Pb^{2+}, Cd^{2+} and Ni^{2+} [4,5]. Cabantchik and co-workers have exploited the fluorescence quenching of Calcein at neutral pH to follow intracellular release of Fe^{2+} from transferin [6].

In the present work, a newly synthesized fluorescent DBS dye has been characterized in different solvents and PVC, and, used for sensor preparation in PVC matrix. Its possible use for optical sensing of Fe^{3+} and typical sensor characteristics such as dynamic working range, sensitivity, limit of detection, and

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* Corresponding author. Dokuz Eylul Universitesi Fen-Edebiyat Fakultesi Kimya Bolumu, 35160 Tinaztepe, Buca, Izmir, Turkey. Tel.: +90 232 4128691; fax: +90 232 453 4188/2153.

E-mail addresses: kadriye.ertekin@deu.edu.tr, kadriye.yusuf@superonline.com (K. Ertekin).

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selectivity has been investigated. The interferences of various cations on the response of Fe^{3+} have also been studied.

2. Experimental

2.1. Materials

The polymer membrane components, polyvinyl chloride (PVC) (high molecular weight) and the plasticizer, bis-(2-ethylhexyl) phthalate (DOP), were obtained from Fluka. Absolute ethanol, THF, DCM, hydrochloric acid (HCl) and chloroform ($CHCl_3$) were of analytical grade. Solvents for the spectroscopic studies were used without further purification. *N*,*N*-Dimethyl-1,4-phenylenediamine was purchased from Aldrich.

Buffer components and metal salts were of analytical grade (Merck and Fluka).

The pH values of the solutions were checked using a digital pH meter (WTW) calibrated with standard buffer solutions of Merck. All the experiments were carried out at room temperature; 25 ± 1 °C. 8-Hydroxypyrene-1,3,6-trisulfonate (HPTS) was used as reference for fluorescence quantum yield calculations of the DBS dye. The synthesis of DBS dye has been performed in our laboratories. Schematic structure of the employed dye molecule is shown in Fig. 1.

2.2. Apparatus

Absorption spectra were recorded using a Shimadzu UV-1601 UV–vis spectrophotometer. Steady-state fluorescence emission and excitation spectra were measured using Varian Cary Eclipse Spectrofluorometer with a Xenon flash lamp as the light source. The elemental analysis was carried out in the Laboratory of the Scientific and Technical Research Council of Turkey (TUBITAK). IR spectra were recorded on a Perkin Elmer Spectrum RXI FTIR spectrometer as KBr pellets, while ^1H and ^{13}C NMR spectra were recorded at 297 K on Varian at 400 MHz (^1H) and 100.56 MHz (^{13}C).

2.3. Synthesis of the DBS dye

3,5-Di-*tert*-butyl-2-hydroxybenzaldehyde (3,5-DTB) was synthesized according to literature procedure [7a,b]. The DBS dye was synthesized by the reaction of 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde (5 mmol) in 20 mL absolute ethanol and *N*,*N*-dimethyl-1,4-phenylenediamine (5 mmol) in 10 mL ethanol. The mixture was refluxed for 3 h, followed by cooling to room temperature. The solid was filtered off and recrystallized from CH_2Cl_2/EtOH (1:3) and dried under vacuum. Yield: 1.52 g (86%).

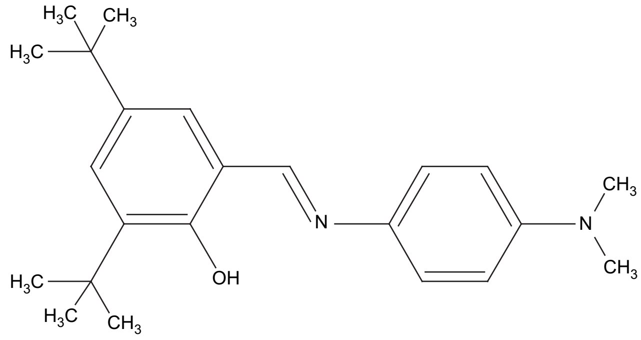

Fig. 1. Schematic structure of the *N*-(4-dimethylaminophenyl)-3,5-di-*t*-butyl salicylaldimine (DBS) dye.

^1H NMR of DBS ($CDCl_3$, 400 MHz, δ): 1.37 (s, 9H, $C_6H_2{-}5{-}C(CH_3)_3$); 1.52 (s, 9H, $C_6H_2{-}3{-}C(CH_3)_3$); 3.00 (s, 6H, $N{-}CH_3$); 6.77 (d, $J = 9.2$ Hz, 2H, $N{-}C_6H_4$); 7.22 (d, $J = 2.4$ Hz, 1H, $HC{-}C_6H_2$); 7.30 (d, $J = 4.8$ Hz, 2H $N{-}C_6H_4$); 7.43 (d, $J = 2.0$ Hz, 1H, $HC{-}C_6H_2$); 8.66 (s, 1H, $CH{=}N$); 14.13 (s, 1H, $C_6H_2{-}2{-}OH$). ^{13}C NMR of DBS ($CDCl_3$,100.56 MHz, δ): 158.23 ($CH{=}N$); 159.42; 149.89; 140.50; 137.87; 136.96; 127.34; 126.46; 122.34; 119.04; 113.15 (C_{aren}); 40.91 ($N{-}CH_3$); 35.33 and 34.43 ($C(CH_3)_3$); 31.80 and 29.76 ($C(CH_3)_3$). Anal. Calc. for $C_{23}H_{32}N_2O$: C, 78.36; H, 9.15; N, 7.95. Found: C, 79.74; H, 8.65; N, 8.10%.

Other selected analytical data for the DBS dye are shown below

Compound	F.W. (g/mol)	Color	m.p. (°C)	Isolated yield (%)	ν (cm^{-1})	
					(O–H)	(C=N)
DBS	352.51	Orange	102	86	3414	1618

2.4. Cocktail preparation protocols and emission–excitation spectra

The optode membranes were prepared to contain 120 mg of PVC, 240 mg of plasticizer, 7.2 mg of DBS dye (2 mmol-dye/kg polymer), equivalent amount of potassium tetrakis (4-chlorophenyl)borate and 1.5 mL of THF. The prepared cocktails contained 33% PVC and 66% plasticizer by weight which is quite common [8–10].

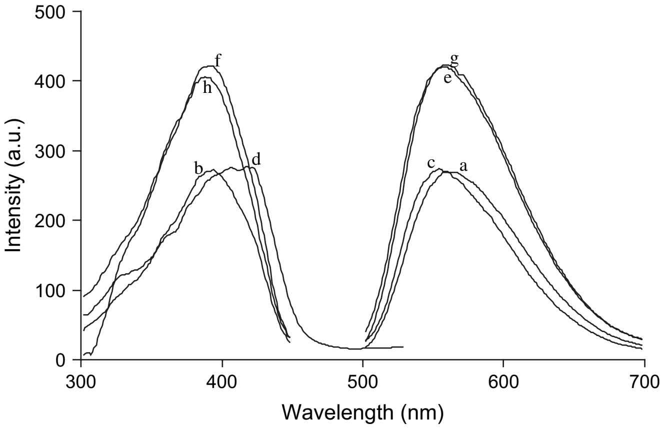

Fig. 2. Emission and excitation spectra of DBS in different solvents and PVC. (a) Emission in DCM: $\lambda_{ex} = 395$ nm, $\lambda^{em}_{max} = 562$ nm; (b) excitation in DCM: $\lambda_{ex} = 562$ nm, $\lambda^{ex}_{max} = 394$ nm; (c) emission in PVC: $\lambda_{ex} = 410$ nm, $\lambda^{em}_{max} = 554$ nm; (d) excitation in PVC: $\lambda_{ex} = 554$ nm, $\lambda^{ex}_{max} = 418$ nm; (e) emission in EtOH: $\lambda_{ex} = 390$ nm, $\lambda^{em}_{max} = 559$ nm; (f) excitation in EtOH: $\lambda_{ex} = 559$ nm, $\lambda^{ex}_{max} = 388$ nm; (g) emission in THF: $\lambda_{ex} = 390$ nm, $\lambda^{em}_{max} = 557$ nm; and (h) excitation in THF: $\lambda_{ex} = 557$ nm, $\lambda^{ex}_{max} = 390$ nm.

Table 1
Absorption spectrum related data of DBS

Dye	Solvent	ε (molar extinction coefficient, $cm^{-1} M^{-1}$)	n (refractive index)	λ_{max}^{abs}
DBS	EtOH	32 541	1.3590	385
DBS	DCM	32 392	1.4241	389
DBS	THF	31 850	1.4070	388
DBS	PVC	6 507 557	1.5200	399

The resulting cocktails were spread onto a 125 mm polyester support (Mylar™ type) in order to obtain the sensing films. The most important function of the polyester is to act as a mechanical support because the thin PVC films are impossible to handle. The polyester support is optically fully transparent, ion impermeable and exhibits good adhesion to PVC. The films were kept in a desiccator in the dark. This way the photostability of the membrane was ensured and the damage from the ambient air of the laboratory was avoided. Each sensing film was cut to 1.2 cm width and fixed diagonally into the sample cuvette and the excitation and fluorescence emission spectra were recorded.

3. Results and discussion

3.1. Quantum yield calculation

The fluorescence quantum yield of DBS in EtOH and PVC matrices was calculated by William's method. For this purpose, the emission spectra of 5 different concentrations of reference standard (HPTS) were recorded by exciting at 410 nm and 390 nm. By the similar way, the emission spectra of the 5 different concentrations of the DBS dye were recorded. The integrated fluorescence intensities were plotted *vs* absorbance for the reference standard and the dye, respectively. The ratio of gradients of the plots is important and is proportional to the quantum yield. The linearized plots of the dye in EtOH and PVC matrices can be described by equations and the relevant correlation coefficients of $\left[y = 2006.5x, \quad R^2 = 0.9922\right]$ and $\left[y = 76\,335x, \quad R^2 = 0.9935\right]$. For quantum yield of standard HPTS, the equations are $\left[y = 9\,71\,979x, \quad R^2 = 0.9988\right]$ and $\left[y = 3\,910\,150x, \quad R^2 = 0.9999\right]$ in EtOH and PVC, respectively. The (ϕ_F) values were calculated according to the following equation where ST and X denote standard and sample, respectively, Grad is the gradient from the plot and n is the refractive index of the solvent [11].

$$\phi_X = \phi_{ST}\left(\frac{\mathrm{Grad}_X}{\mathrm{Grad}_{ST}}\right)\left(\frac{n_X^2}{n_{ST}^2}\right)$$

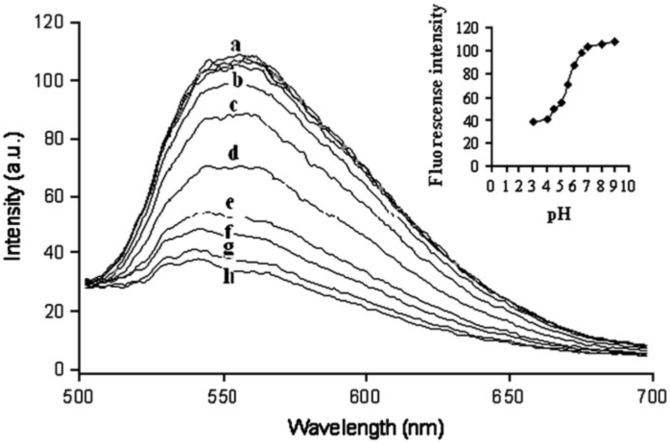

Fig. 3. pH induced emission spectra of the DBS membrane after exposure to buffer solutions of: (a) pH = 9.0 – 7.0, (b) pH = 6.5, (c) pH = 6.0, (d) pH = 5.5, (e) pH = 5, (f) pH = 4.5, (g) pH = 4.0, and (h) pH = 3.0 ($pK_a = 5.57$).

3.2. Spectral data and photophysical constants

The florescent DBS dye exhibited excellent photostability in all the solvents and plasticized PVC. The absorption, emission and excitation spectra of the DBS dye were recorded in the solvents of different polarities and PVC matrix. The gathered excitation–emission spectra of the DBS are shown in Fig. 2. Absorption spectra related to molar extinction coefficients and maximum absorption wavelengths of the dye in employed solvents and PVC are given in Table 1.

Absorption wavelength of DBS in PVC $\left(\lambda_{max}^{abs}\right)$ was found to be shifted to about 11 nm with respect to absorption maximum in THF $\left(\lambda_{max}^{abs}\right)$ solution. Red shift of absorption of DBS may be related to the enhanced conjugation in immobilized polymer phase by hindrance of rotational and vibrational motions. Fluorescence quantum yield of DBS in PVC, $\phi_F = 0.0254$, was increased about 12-fold with respect to ϕ_F of DBS in DCM, and, molar extinction coefficient of DBS in PVC matrix, $\varepsilon = 6\,507\,557$ was increased about 200-fold, with respect to ε of DBS in DCM. These data can be taken as proofs that the dye molecule DBS fluoresces better in immobilized PVC matrix (see Table 2).

In all the employed solvents and PVC the Stoke's shift values, $\Delta\lambda_{ST}$ (the difference between excitation and emission maximum), calculated from the spectral data were quite high and was found to spread in the wavelength range of 136–176 nm. Stoke's shift is important for fluorescence and optical sensor studies because the high Stoke's shift value allows the emitted fluorescence photons to be easily distinguished from the excitation photons, leading to the possibility of very low

Table 2
Emission and excitation spectra related data of DBS

Dye	Matrix	λ_{ex}^{em} (excitation wavelength for emission)	λ_{ex}^{ex} (excitation wavelength for excitation)	λ_{max}^{em} (maximum emission wavelength)	λ_{max}^{ex} (maximum excitation wavelength)	$\Delta\lambda_{ST}$ (Stoke's shift)	ϕ_F (quantum yield)
DBS	EtOH	388	559	559	388	171	0.00214
DBS	DCM	394	562	562	394	168	
DBS	THF	390	557	557	390	167	
DBS	PVC	410	554	554	418	136	0.0254

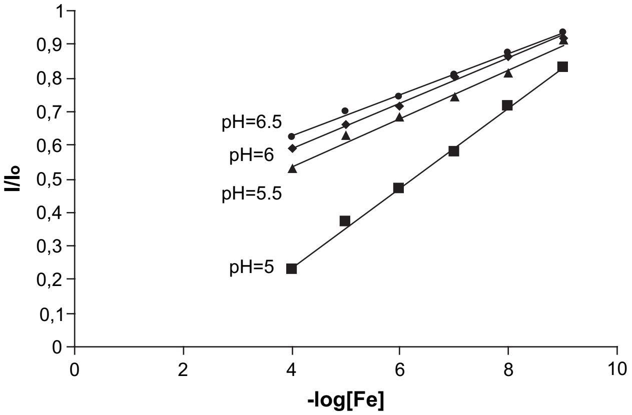

Fig. 4. pH dependent response of the DBS dye doped membrane to Fe^{3+} at $pH = 6.5, 6.0, 5.5,$ and 5.0.

background signals and permits the usage of DBS dye in the construction of fiber optic sensor.

Promising spectral properties such as visible region excitation (410 nm) and emission wavelength (554 nm), high molar extinction coefficient ($6\,507\,557\ cm^{-1}\ M^{-1}$), satisfactory quantum yield in immobilized form (0.0254) and intensity-based response to Fe^{3+} cations encouraged us to use the DBS dye in optical sensor design for iron sensing.

3.3. pH dependence of DBS dye

The pH dependence of the dye doped membrane was investigated in buffered solutions in the pH range of 3.0–9.0 iron free and Fe^{3+} containing solutions. Buffer solutions were prepared with 0.02 M CH_3COOH, 0.02 M NaH_2PO_4, 0.01 M BES or 0.02 M boric acid at desired pH. The emission maximum of the DBS dye at 559 nm decreases as the pH of the buffer varies from 9.0 to 3.0. The pH dependence of the dye is probably due to the protonation–deprotonation equilibrium of the terminal hydroxyl and the central nitrogen groups. Fig. 3 shows the pH dependency of the membrane in Fe^{3+} free buffer solutions in the pH range of 3.0–9.0. The pK_a value of the immobilized indicator is calculated to be $pK_a = 5.57$ by using non-linear fitting algorithm of Gauss–Newton–Marquardt method.

$$pK_a = pH + \log[(I_x - I_b)/(I_a - I_x)]$$

where I_a and I_b are the intensities of acidic and basic forms and I_x is the intensity at a pH near to the pK_a.

Fig. 4 shows the pH dependent response of the sensor slide to Fe^{3+} at different pH values. In borate buffer around pH 9.0, no response to 1 mM Fe^{3+} was observed, while around $pH = 5$, membrane was highly sensitive to Fe^{3+}. Therefore $pH = 5$ was chosen as optimum working pH.

3.4. Response to Fe^{3+} ions

The spectral response of the dye doped membrane to Fe^{3+} was investigated in buffered solutions at pH 5.0. Doubly distilled water was used throughout the studies. Stock solution of Fe^{3+} (0.1 M) was prepared from metal nitrate and diluted with 0.01 M BES (*N*,*N*-bis(2-hydroxyethyl)-2-amino-ethanesulfonic acid) buffer.

The dye doped membrane exhibited remarkable fluorescence intensity quenching on exposure to Fe^{3+} ions at $pH = 5.0$ while the effects of the other responding ions (Cu^{2+}, Sn^{2+} and Hg^{2+}) were less pronounced. The dynamic response to Fe^{3+} ions was monitored as a change in the relative fluorescence intensity at 559 nm as the membrane was exposed to buffer solutions containing different concentrations of Fe^{3+}. Spectral response and characteristic calibration curves obtained for 6 different concentrations of Fe^{3+} are shown in Figs. 5 and 6, respectively.

We also tested whether it has been possible to determine the 2 oxidation states of iron: Fe^{2+} and Fe^{3+}. Calibration sets for speciation of iron ions Fe^{2+} and Fe^{3+} were prepared separately. Fig. 7 shows the non-linearized comparative response of the DBS membrane to Fe^{2+} and Fe^{3+} in the concentration range of 01^{-9}–10^{-4} M at $pH = 5.0$.

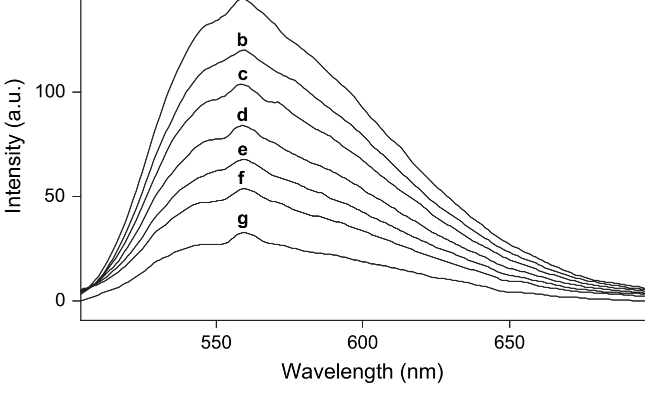

Fig. 5. Emission spectra of sensor membrane in BES-buffered solutions at $pH = 5.0$ after exposure to different concentrations of Fe^{3+}ions. (a) $pH = 5.0$ iron free BES buffer, (b) 10^{-9} M, (c) 10^{-8} M, (d) 10^{-7} M, (e) 10^{-6} M, (f) 10^{-5} M, and (g) 10^{-4} M Fe^{3+}.

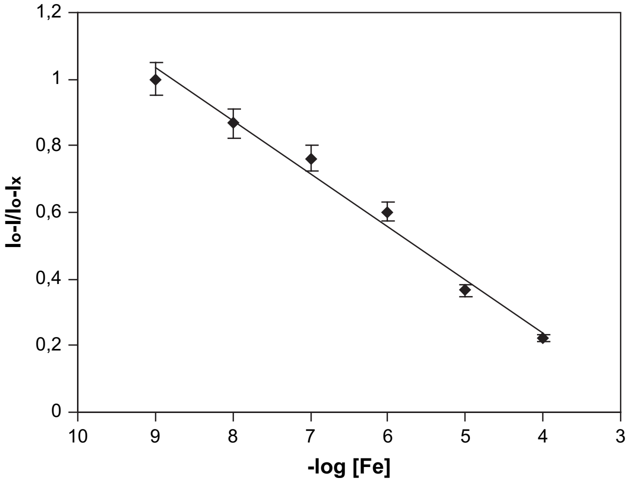

Fig. 6. Linearized calibration curve of DBS membrane after exposure to Fe^{3+} solutions at $pH = 5.0$.

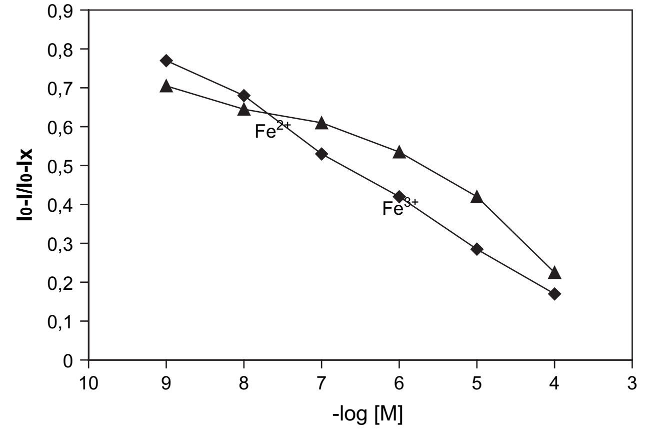

Fig. 7. Response of the DBS membrane to Fe^{2+} and Fe^{3+} in the concentration range of $01^{-9}–10^{-4}$ M at pH = 5.0.

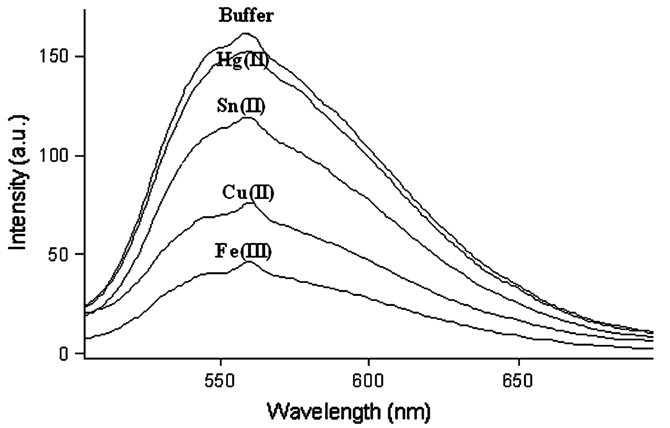

Fig. 9. Emission spectra of DBS membrane in the presence of 1 mM Fe(III), Cu(II), Sn(II) and Hg(II) ions at pH 5.5 in separate solutions.

The non-linearized calibration plots reveal that the slope of the calibration curve of Fe^{3+} is nearly same as the slope of the Fe^{2+}. This result confirms that Fe^{2+} and Fe^{3+} cannot be distinguished quantitatively in the region assessed.

3.5. Response and reproducibility

Fig. 5 shows the fluorescence spectra of DBS doped film after exposure to Fe^{3+} solutions in the concentration range of $10^{-9}–10^{-4}$ mol/L. The membrane exhibited a 77% relative signal change in the direction of decrease in fluorescence intensity. Regeneration was accomplished at pH = 5.0 in 0.1 M BES buffer solution with 100% efficiency.

The detection limit, which is the concentration of analyte producing an analytical signal equal to 3 times of the standard deviation of the blank signal, was found to be 10^{-9} M for Fe^{3+}.

Response time (τ_{90}) changed between 3 and 8 min for 10^{-9} M and 10^{-4} M, Fe^{3+}, respectively. The sensor was fully reversible and no drifts were observed after the fifth cycle (Fig. 8). The reproducibility of the optical responses was assessed by repeatedly introducing a sample of 10^{-5} M Fe^{3+} in buffer at pH 5.0 and a 0.1 M BES buffer at pH 7.0. Between the 1st and 5th cycles, the level of reproducibility of the upper signal level achieved was quite good with a standard deviation of 154.16 ± 1.329.

PVC matrix is one of the most durable matrices for aqueous media. Due to the lipophilic characteristics of the DBS dye and its compatibility with PVC matrix, there has been no leaching from membrane to aqueous media.

3.6. Selectivity studies

In order to determine the selectivity of the proposed method, the influence of a number of cations were investigated. Tests were performed in 1 mM iron free metal ion solutions of Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, Sn^{2+}, Cr^{3+} and Hg^{2+} at pH 5.0 in BES-buffered separate solutions. The membrane exhibited remarkable spectral change on exposure to 10^{-3} M Cu^{2+} and Sn^{2+} ions at pH 5.0, while the effects of other metal ions (Co^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, Cr^{3+} and Hg^{2+}) were negligible

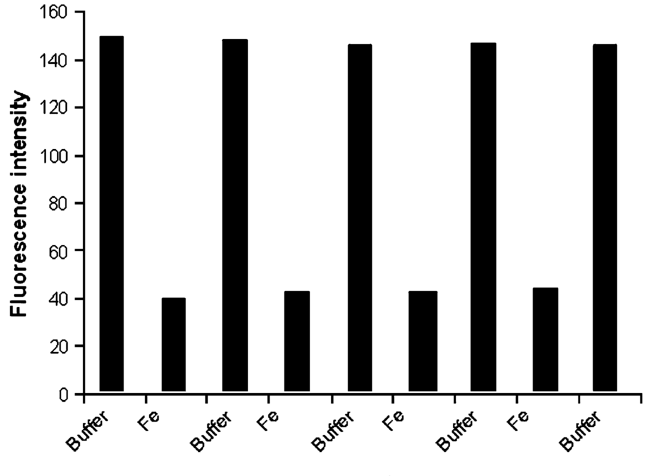

Fig. 8. Response of the DBS membrane to Fe^{3+} in the concentration range of $0.0–10^{-5}$ M at pH = 5.0. Reproducibility of the upper signal level is 154.16 ± 1.329 for 5 measurements.

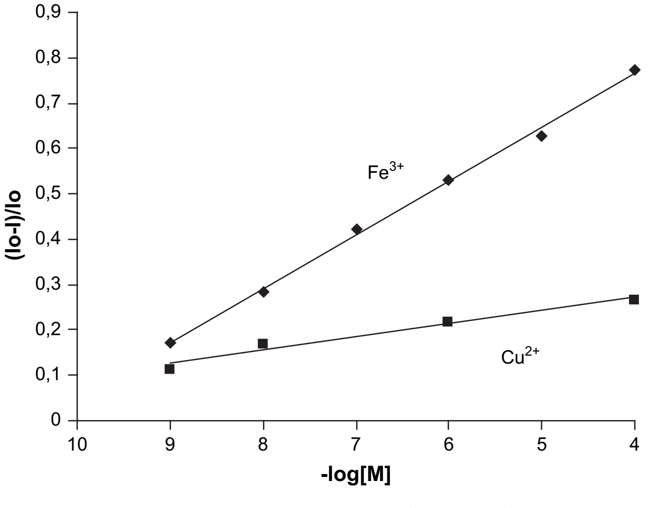

Fig. 10. Linearized response of DBS dye to Fe^{3+} and Cu^{2+} at pH = 5.0.

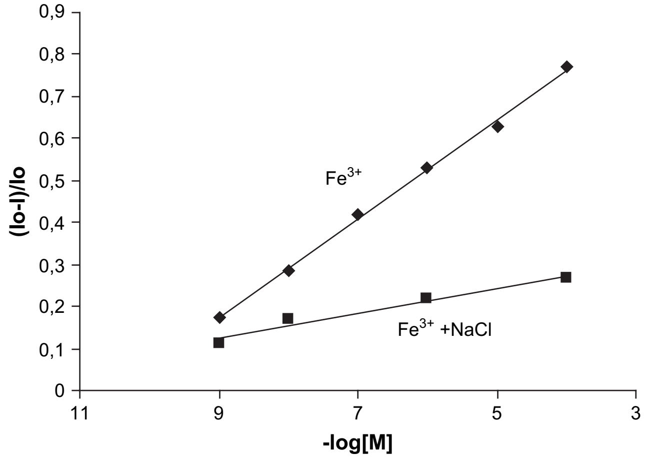

Fig. 11. Calibration curve of the DBS membrane in BES-buffered and 135 mM NaCl-containing solutions at pH 5.0.

(Fig. 9). The relative signal intensity changes were 52.0% and 25.2% for Cu^{2+} and Sn^{2+}, respectively.

Fig. 10 shows comparative response of DBS dye to Fe^{3+} and Cu^{2+}. Linearized plots can be defined with the equations: $y = -0.1184x + 1.237$, $R^2 = 0.9976$, and $y = -0.0291x + 0.3881$, $R^2 = 0.9679$ for Fe^{3+} and Cu^{2+}, respectively. The linearized plots reveal that, slope of the "Fe^{3+}calibration curve" is 5 times higher than the "Cu^{2+}calibration curve". The solution of 10^{-4} M of Cu^{2+} causes an approximately 20% error in determination of 10^{-4} M Fe^{3+}.

3.7. Effect of salinity

The iron concentration-dependent spectral response of sensor membrane is affected by the ionic strength of the buffer solution. By making the ionic strength of the buffer solution to 135 mM with NaCl, the relative signal change of the emission spectrum of the sensor in contact with this solution decreased by around 50%. Calibration curve of the membrane in BES-buffered and 135 mM NaCl-containing solutions at pH 5.0 is shown in Fig. 11.

4. Conclusion

The selective determination of Fe^{3+} was accomplished by use of the newly synthesized DBS dye in plasticized PVC matrix. No response at the working wavelength was observed from other potential heavy metal interferents. The membrane responds to Fe^{3+} by changing fluorescence intensity reversibly at 554 nm when excited at 410 nm, and is sensitive to Fe^{3+} in the 10^{-9}–10^{-4} M range for an average exposure time of 5 min. The membranes are sensitive to pH as well, and the determination of Fe^{3+} should be performed in buffered solutions, ideally at pH 5 as the fluorescent DBS dye can be offered as an optical Fe^{3+} sensor due to the visible excitation (410 nm) and emission wavelength (554 nm), high molar extinction coefficient (6 507 557 cm^{-1} M^{-1}), large Stoke's shift (136 nm) and satisfactory quantum yield (0.0254) in immobilized form.

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References

[1] Pulido-Tofino P, Barrero-Moreno JM, Perez-Conde MC. Talanta 2000;51(3):537–45.
[2] Zhang ZX, Xie YY, Shi Y. Spectrosc Spectral Anal 1999;19(2):241–3.
[3] Sarradin PM, Le Bris N, Le Gall C, Rodier P. Talanta 2005;66(5): 1131–8.
[4] Petrat F, Rauen U, de Groot H. Hepatology 1999;29(4):1171–9.
[5] Kuhn MA, Hoyland B, Carter S, Zhang C, Haugland Richard P. Proc SPIE Int Soc Opt Eng 1995;2388:238.
[6] Picard V, Epsztejn S, Santambrogio P, Cabantchik ZI, Beaumont C. J Biol Chem 1998;273(25):15382–6.
[7] (a) Hodgson DJ. Prog Inorg Chem 1975;19:173;
(b) Larrow JF, Jacobsen EN, Gao Y, Hong Y, Nie X, Zepp CM. J Org Chem 1994;59:1939.
[8] Seiler K, Simon W. Anal Chim Acta 1992;266:73.
[9] Bakker E, Simon W. Anal Chem 1992;64:1805.
[10] Lerchi M, Bakker E, Rusterholz B, Simon W. Anal Chem 1992;64:1534.
[11] Williams ATR, Winfield SA, Miller JN. Analyst 1983;108:1067.