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A study on photophysical properties of some Vitamin K_3 derivatives

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Abstract

Photophysical properties of menadione sodium bisulfite (MSB), menadione nicotinamide bisulfite (MNB) and menadione dimethylpyrimidinol bisulfite (MPB) were studied in their aqueous solutions. They are oil-soluble vitamins and not naturally fluorescent, however, it is possible to gain fluorescent signal by the use of phosphate buffer solutions containing sodium dodecyl sulfate (SDS) at optimum concentration. The quantum yields of fluorescence emission, Φ_f, were measured as $\sim 10^{-4}$, 0.0085 and 0.053 for MSB, MNB, MPB, respectively. Fluorescence quenching with Co^{2+} ions yielded the quenching rates of 1.7×10^9, 4.6×10^{10}, and $1.5 \times 10^{10}\ M^{-1}\,s^{-1}$ for MSB, MNB and MPB, respectively. Calculated Gibbs free energy (ΔG) values (≤ -5 kcal/mol) of the compounds suggest that electron transfer could be the reason of quenching with Co^{2+} ions. Studied derivatives were found to give two electrons. Equilibrium constants (K_{eq}) derived from ΔG values pointed out strong association between the pairs of MSB, MNB and MPB complexes. They were irradiated by using not only artificial Xe lamp of fluorescence spectrophotometer, but also by using direct and concentrated sunlight and degradation (%), rate constant (k_p) and half-lives ($t_{1/2}$) were compared. Photodegradation (%) of Vitamin K_3 derivatives varied depending on the irradiation source. Photostability tests pointed out MPB as the most stable derivative.

Keywords: Vitamin K_3; Photophysical property; Surfactant; Photodegradation; Concentrated sun light

1. Introduction

Vitamin K is a generic term for a group of fat-soluble vitamins consisting of 2-methyl-1,4-naphthaquinone. They are relatively heat resistant (≤100 °C) and stable under ambient air conditions. However, they are very sensitive to alkali medium and light. Wilson et al. [1] have shown that menaquinone-1 and phylloquinone gives stable trioxanes when irradiated with visible light under aerobic conditions. Naturally occurring compounds with Vitamin K activity have a common 2-methyl-1,4-naphthaquinone ring but differ in the structure of side chain. Vitamin K is present in vegetables and seem to play an active role in photosynthetic mechanism. They also play an important role in cellular respiration as electron transporters and in oxidative phosphorylation [2–5], act as effective electron carriers and electron transfer agents [4–7].

Menadione is a naphthaquinone derivative and it is known as Vitamin K_3 (VK_3) and has antihemorrhagic activity. There are five commercial VK_3 products in market used for feed fortification: menadione sodium bisulfite (MSB), menadione sodium bisulfite stabilized (MSB stab), menadione dimethylpyrimidinol bisulfite (MPB), menadione sodium bisulfite complex (MSBC) and menadione nicotinamide bisulfite (MNB). MSB, MPB and MNB contain minimum 51.5, 44 and 44% menadione, respectively [8]. MSB is a water-soluble derivative of menadione and is used extensively poultry and swine feeds [9]. It has limited stability to light, moisture, heat and pH values higher than neutrality [10–12]. At pH lower than neutral, the isomerization of MSB occurs, resulting in a product that has no antihemorrhagic activity. Considering the menadione activity and its stability BASF, Inc. have announced MSB stab and MPB as the most economical VK_3 sources [13]. Unlike BASF, Marchetti et al. [10] have stated that MNB, which is an organic salt combining menadione and nicotinamide, shows better stability towards physical and chemical factors once it is added to premixes or foods.

The advantages of surfactant and phosphate buffer usage has been stated in literature. It increases both the stability and the fluorescence intensity of the VK_3 [4–6]. Berzas Nevado et al. [6] have used β-cyclodextrin and micellar dodecyl sulfate (SDS) to enhance the solubility of MSB in water

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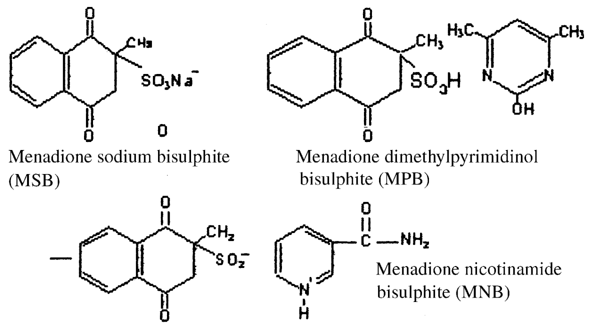

Scheme 1. Open structures of MSB, MPB and MNB.

and monitored the fluorescence intensity change. Although they found an increase in solubility for both of the media, in contrast to β-cyclodextrin, they could observe no fluorescent signal in MSB solution containing SDS.

We now report the photophysical characteristics, equilibrium constants and photostability of menadione complexes, MSB, MNB and MPB (Scheme 1), in phosphate buffer solution containing SDS at the optimum concentration. By the use of SDS and phosphate buffer medium, it was possible to increase the solubility and to obtain the fluorescence signal for all of the three VK_3 derivatives (MSB, MNB and MPB). Photostability tests carried out by the use of two different irradiation sources and three different methods pointed MPB as being the most stable derivative among them.

2. Experimental details

2.1. Reagent

MSB, MNB and MPB were supplied from Oxyvit and used without any further purification. Menadione contents of these complexes were as given above. Phosphate buffer (pH 7 ± 0.2) and SDS, used in the preparations of solutions, $CoCl_2{\cdot}6H_2O$, used in quenching experiments and KCl, used in polarographic measurements were supplied from Merck and used without any further purification. High quality pure water, obtained from Elga apparatus working in reverse osmosis principle was used. Solutions were used either as soon as they were prepared or stored in dark at 4 °C. The reference standard for fluorescence quantum yield was anthracene ($Q_f = 0.27$ in EtOH [14]).

2.2. Instrumentation

The absorbance spectra were measured by using JASCO V-530, UV-Vis spectrophotometer and the fluorescence emission spectra were recorded at PTI-QM1 fluorescence spectrophotometer. Concentrated sun light (CSL) experiments were performed with Fix Focus FF 3,5-HTC GmbH (Germany) instrument with a reflective surface area of $2.66\,m^2$. The radiation intensities were determined by using Vilbert Loumart radiometers at 254 and 312 nm radiation wavelengths, and compared with measured intensity of direct sun light (DSL) using the same radiometers. DSL were performed at ambient air. The Polarographic measurements and cyclic voltammetric studies were done by the use of Metrohm 746 VA Trace Analyzer.

3. Results and discussion

3.1. Determination of optimum SDS concentration

The 0.1 M SDS solution was prepared in phosphate buffer and used as stock solution. The solubility of MSB in water was found to be better than that of MNB and MPB. The solubility of them was increased by the addition of certain amounts of SDS into the aqueous medium. The concentrations of MSB, MNB and MPB in phosphate buffer were in the range of $(1–3) \times 10^{-5}$ M. The 2 ml of VK_3 solutions were titrated with the addition of 20 μl of stock SDS at each time. After each SDS addition, absorption and emission spectra of VK_3 derivatives were recorded and the intensity increase was monitored up to a certain SDS concentration above which it remained constant. Dilution effect was taken into account for the calculation of the SDS concentration in the medium. Optimal SDS concentrations for MSB, MPB and MNB were found to be 7.3×10^{-3}, 6.8×10^{-3}, and 6.6×10^{-3} M, respectively (Fig. 1). The fluorescence emission intensities of MSB, MNB and MPB were enhanced about 80, 70 and 65%, respectively, in the SDS containing aqueous solutions with respect to the aqueous solutions without SDS.

3.2. Spectroscopic studies

UV-Vis absorption spectra of MNB and MPB have shown three bands in aqueous solution of phosphate buffer containing SDS at optimum concentration, and the UV-Vis absorption spectrum of MSB has shown two bands. Absorption maxima and corresponding molar absorptivity constants (ε) are given in Table 1.

Berzas Nevado et al. [6] have examined the effect of micellar SDS on MSB solubility. They reported that it has been possible to get solubilization of MSB in an aqueous medium, but no fluorescence signal has obtained. They used a buffer solution (pH 6.2) that has been prepared by mixing appropriate amounts of citric acid and sodium hydroxide. In our

Table 1
UV-Vis spectroscopic data (λ, nm and ε, $mol^{-1}\,cm^{-1}$) of MSB, MNB and MPB in phosphate buffer containing optimum SDS concentration

Compound	λ_1	ε_1	λ_2	ε_2	λ_3	ε_3
MSB	231	5920	266	850	–	–
MNB	229	7010	263	3035	307	630
MPB	229	6086	267	2930	298	945

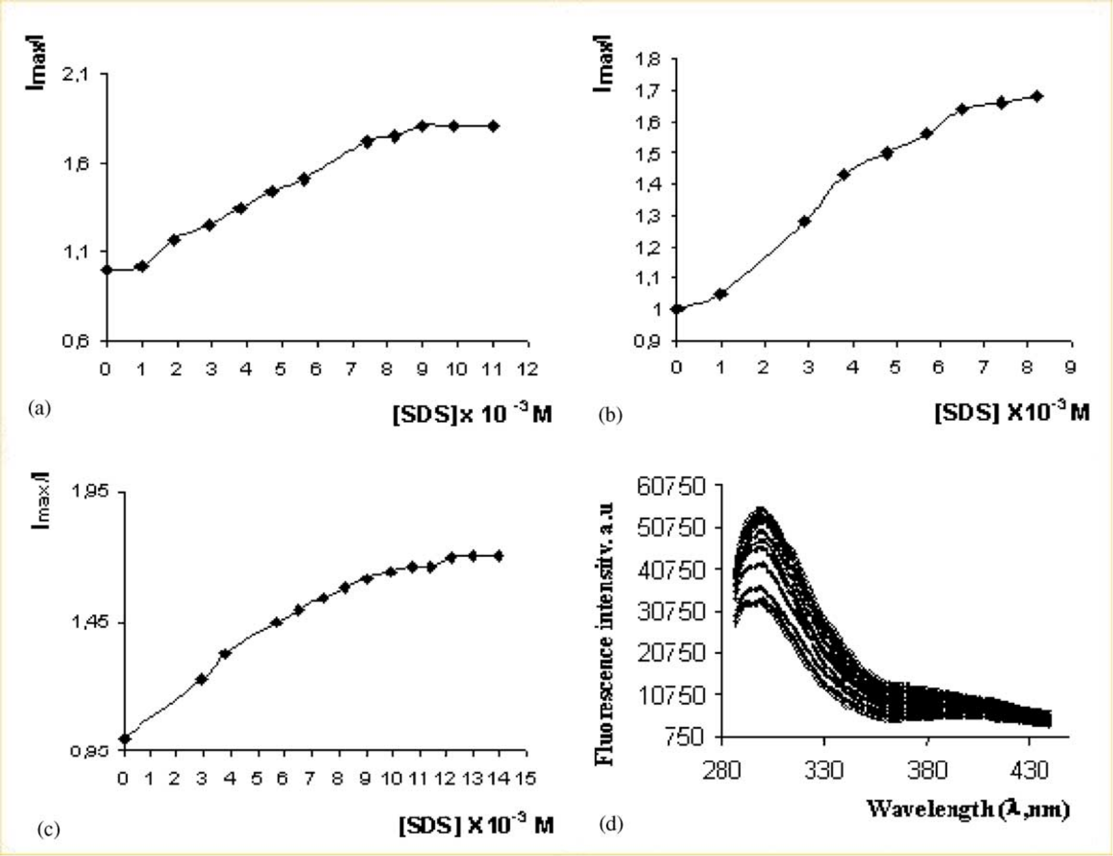

Fig. 1. (a)–(c) Fluorescence intensity ratios vs. SDS concentrations for MSB, MNB and MPB, respectively. (d) Increase of MNB fluorescence intensity with the addition of SDS ($\lambda_{ext} = \lambda_2$).

study we have used a phosphate buffer (pH 7 ± 0.2) medium and SDS together and were able to obtain fluorescence signal. The fluorescence quantum yields (Φ_f), of MSB, MNB and MPB were calculated with reference to the absorption and fluorescence emission spectra of anthracene. The calculated Φ_f values were corrected for the index of refraction differences. Indexes of refraction were taken as 1.329 and 1.354 for water and ethanol, respectively. The equation used in calculation of fluorescence quantum yield is given below, where Φ_f is the quantum yield, A the absorption intensity, S the integrated emission band area and n the solvent reflective index, u and s refer to the unknown and standard, respectively.

$$\Phi_{fu} = \Phi_{fs} \frac{S_u}{S_s} \frac{A_s}{A_u} \frac{n_u^2}{n_s^2} \qquad (1)$$

The radiative life times, τ_0, were calculated by the formula, $\tau_0 = 3.5 \times 10^8/(\upsilon_{max}^2 \varepsilon_{max}\, \Delta\upsilon_{1/2})$, where υ_{max} is the wave number per centimeter (cm^{-1}), ε_{max} the molar extinction coefficient at the selected absorption wavelength and $\Delta\upsilon_{1/2}$ the half width of the selected absorption in wave number unit (cm^{-1}). Fluorescence lifetimes are estimated as $\tau_f = \tau_0 \Phi_f$, the rates of fluorescence as $k_f = 1/\tau_0$ and deactivation rate constant as $k_d = (k_f/\Phi_f) - k_f$ [15]. Calculated values are given in Table 2.

Φ_f of MSB was calculated as around 10^{-4} (λ_{ext} = 267 nm). This value was 0.0085 and 0.053 for MNB (λ_{ext} = 263 nm) and MPB (λ_{ext} = 267 nm), respectively. The reason for the low fluorescence intensity of the studied vitamins may be the intersystem crossing from the low-lying $S_{n\pi^*}$ level to the triplet $T_{\pi\pi^*}$ one, which is typical for benzaldehyde and for azoheterocycles [16].

Table 2
Photophysical properties of MSB, MNB and MPB (fluorescence quantum yields, Φ_f, radiative life times, τ_0, fluorescence life times, τ_f, fluorescence rate constants, k_f, deactivation rate constants, k_d, quenching rate constants, k_q) in phosphate buffer containing SDS at optimum concentration

Compound	Φ_f	τ_0 (s)	τ_f (s^{-1})	k_f (s^{-1})	k_d (s^{-1})	k_q (M^{-1} s^{-1})
MSB	~10–4	1.2×10^{-10}	1.2×10^{-14}	8.3×10^9	8.3×10^{13}	1.7×10^9
MNB	0.0085	1.4×10^{-10}	1.2×10^{-12}	7.1×10^9	8.5×10^{11}	4.6×10^{10}
MPB	0.053	1.5×10^{-10}	8.0×10^{-12}	6×10^9	1.0×10^{11}	1.5×10^{10}

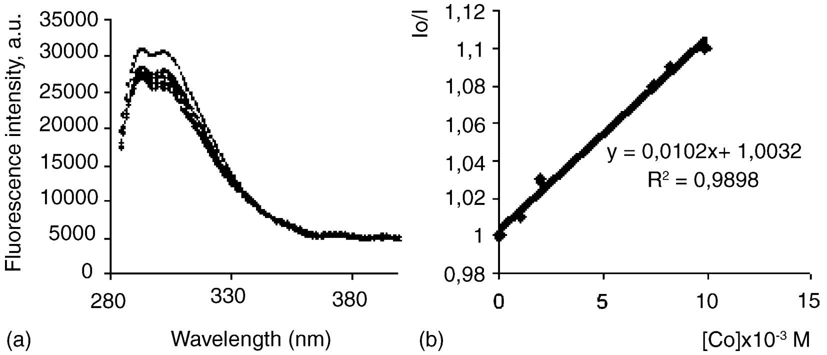

Fig. 2. Fluorescence emission quenching of: (a) MNB with increasing Co^{2+} concentration with the range of (1–9) × 10^{-3} M, in phosphate buffer solution containing optimum SDS concentration. (b) Stern–Volmer plot for MPB.

Fluorescence emission quenching of MSB, MNB and MPB was studied at increasing concentrations of Co^{2+} ions. Approximately 10^{-5} M solutions of MSB, MNB and MPB were prepared in phosphate buffer containing corresponding optimum SDS concentration. The 2 ml of VK_3 solutions were titrated with the addition of 20 µl of stock 0.1 M Co^{2+} at each time. The resulting Co^{2+} ion concentrations were in the range of (1–9) × 10^{-3} M (Fig. 2). Quenching rate constants (k_q) were calculated from Stern–Volmer plots. The quenching rate constants of MNB and MPB were approximately 25- and 9-fold of MSB, respectively. The efficient fluorescence emission quenching of the VK_3 derivatives could be explained by their electron donation to cobalt ions.

3.3. Polarographic measurement and cyclic voltammetric studies

The differential pulse polarograms were recorded using a polarographic analyzer. The cell was equipped with a three-electrode system; all the potentials were referred to Ag/AgCl reference electrode while the auxiliary electrode was a platinum wire. The working electrode was a mercury drop electrode. Electrolyte used was 3 M KCl solution. Standard addition method was used. The 20 ml phosphate buffer solution, 100 µl VK_3 derivatives, 500 µl 3 M KCl were used for polarographic measurements. The number of transferred electrons is calculated by the following formula:

$$n = \left[\frac{\log \frac{1}{I_\mathrm{d}} - 1}{E_{1/2} - E_u} \right] 0.059 \tag{2}$$

where n is the electron number, $E_{1/2}$ the half wave potential and I_d the diffusion current. According to this formula, Faraday numbers (the number of moles of transferred electrons) were found as 2 for all of the three VK_3 derivatives.

The redox potentials of MSB, MNB and MPB were measured by the use of cyclic voltammetry with a Ag/AgCl reference electrode. It is well known that quenching data can be analyzed by the use of Eq. (3) [17]

$$\Delta G\ (\mathrm{kcal/mol}) = 23.06 \left[E_{(D^+/D)} - E_{(A/A^-)} \right] - E_\mathrm{D}^* \tag{3}$$

where the free energy change for an encounter pair undergoing electron transfer is computed by using redox potentials, and where E_D^* is the excitation energy of the excited state participating in quenching. Computed free energies are provided in Table 3. All of which were found to be lower than −5 kcal/mol (the "rule of thumb" [18]). This fact points to the possibility of electron transfer between the vitamin molecule and Co^{2+} ion.

The calculated ΔG values can be used to determine stability constants for MSB, MNB and MPB complexes. The stability of a complex in solution refers to the degree of association between the two species involved in the state of equilibrium. Qualitatively the greater the association, the greater the stability of the complex. The magnitude of the (stability or formation) equilibrium constant (K_eq) for the association, quantitatively expresses the stability. Since $\log K_\mathrm{eq}$ is directly proportional to the free energy ΔG of the complex equation (4) can be used and at room temperature (25 °C) Eq. (5) is gained:

$$\Delta G = -2.303 RT \log K_\mathrm{eq} \tag{4}$$

$$K_\mathrm{eq} = 10^{-\Delta G/1.36} \tag{5}$$

The calculated K_eq values are given in Table 3. They point out a strong association between the pairs of VK_3 derivatives in phosphate buffer containing SDS at optimum concentration.

3.4. Photostability of VK_3 derivatives

Photostabilities of MSB, MNB and MPB in solutions of phosphate buffer containing SDS were determined on

Table 3
Half wave potentials, $E_{1/2}$, Gibbs free energies, ΔG and equilibrium constants of MSB, MNB and MPB in phosphate buffer containing SDS at optimum concentration

Compound	$E_{1/2}$ (V)	ΔG (kcal/mol)	K_eq
MSB	−1.343	−71.08	1.82×10^{52}
MNB	−1.339	−72.69	2.8×10^{53}
MPB	−1.346	−72.24	1.38×10^{53}

exposure to Xe lamp of PTI, and with the use of DSL and CSL irradiations.

The data were acquired at 301, 345 and 302 nm for MSB, MPB and MNB, respectively, for Xe lamp irradiation in fluorescence spectrophotometer. These wavelengths correspond to the emission maxima of studied VK_3 derivatives in the phosphate buffer containing SDS. The 40 min of monitoring was performed. According to the emission spectra analysis, photostability obtained for SDS–phosphate buffer medium was better than phosphate buffer medium without SDS (Fig. 3). It is seen that 23 and 54% of MPB was degraded, during 40 min of irradiation, when the photodegradation in the presence and absence of SDS media are compared, respectively. The use of micellar SDS solution not only enhances the solubility but also increases the photostability. MPB is determined as the most stable VK_3 derivative under Xe lamp irradiation.

Photostability of the so-called vitamins were tested under DSL (1 sun) and CSL (100–120 suns) also. The 50 ml solutions of MSB, MNB and MPB in SDS–phosphate buffer medium were prepared; half of the solutions were used for DSL and left over quantities were used for CSL photostability tests. The photodegradation of VK_3 derivatives under DSL irradiation pointed MPB as the most stable derivative also. Unlike Xe lamp irradiation MPB degradation was much lower than the other two. Only 6.6% of it was photodegraded at the end of 40 min. Another interesting result obtained with DSL irradiation was the acceleration of MNB photodegra-

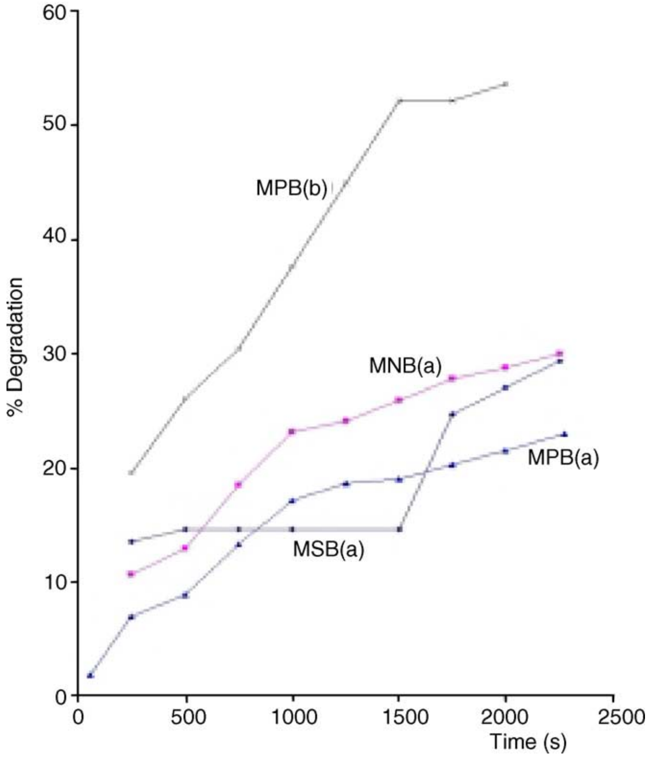

Fig. 3. Photodegradation (%) of MSB, MNB and MPB under Xe lamp irradiation in the: (a) presence of SDS at optimum concentration; (b) absence of SDS. MNB: $\lambda_{\text{ext}} = 263$ nm, $\lambda_{\text{emis}} = 302$ nm; MSB: $\lambda_{\text{ext}} = 267$ nm, $\lambda_{\text{emis}} = 301$ nm; MPB: $\lambda_{\text{ext}} = 267$ nm, $\lambda_{\text{emix}} = 345$ nm.

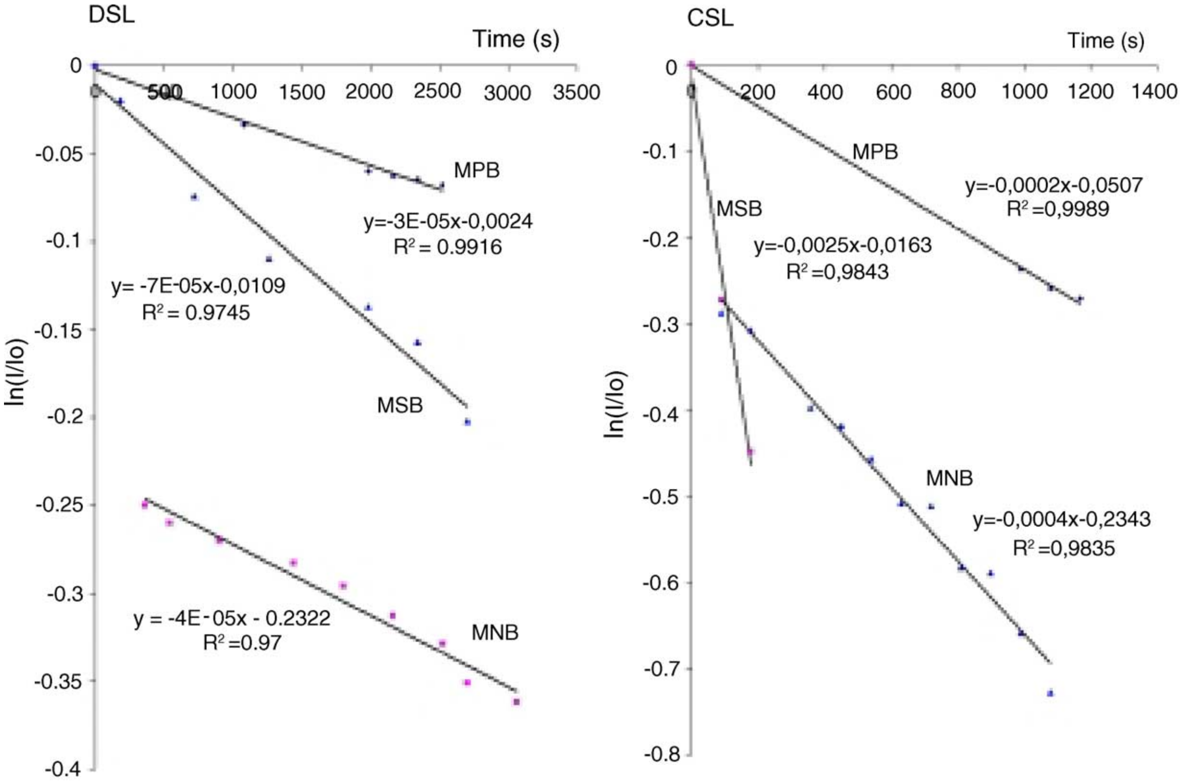

Fig. 4. First-order relations for photodegradation of MSB, MNB and MPB under DSL and CSL irradiation.

Table 4
Rate constants and half-lives for photodegradation of MSB, MNB and MPB ($t_{1/2}$ values obtained in seconds were converted into hours)

Irradiation source	Compound	k (s^{-1})	$t_{1/2}$ (h)
DSL	MSB	7×10^{-5}	2.75
	MNB	4×10^{-5}	4.81
	MPB	3×10^{-5}	6.41
	MSB	2.5×10^{-3}	0.077
CSL	MNB	4×10^{-4}	0.48
	MPB	2×10^{-4}	0.96
	MSB	2×10^{-4}	0.96
Xe lamp	MNB	2×10^{-4}	0.96
	MPB	1×10^{-4}	1.92

dation and MNB becoming the least stable VK_3 derivative. This situation was observed for CSL irradiation also.

The plateau observed in photodegradation of MSB may be attributed to the formation of MSB isomerization product [3]. MPB was found to be the most stable derivative for all the three different irradiation sources.

The rate constants for apparent consumption of VK_3 derivatives were obtained from the relation

$$-\ln\left(\frac{I}{I_0}\right) = k_p t \qquad (6)$$

where I_0 and I are the emission intensity of the compound at times zero and t, k_p the first-order rate constant (s^{-1}). Half-lives $t_{1/2}$ (s) were also calculated using Eq. (7) which was derived from Eq. (6) by placing I with $I/2$

$$t_{1/2} = -\frac{\ln(1/2)}{k_p} = \frac{0.693}{k_p} \qquad (7)$$

First-order rate constants estimated from the regression $\ln(I/I_0)$ vs. time (s), shown in Fig. 4, and the half-lives for photodegradation of MSB, MNB and MPB together with rate constants are given in Table 4.

4. Conclusions

It was found that by the use of surfactants such as SDS, it is possible to increase photostability and observe fluorescence signal of VK_3. Therefore, all experiments were carried out in phosphate buffer containing corresponding SDS concentration. The quantum yields of fluorescence emission, Φ_f, were measured as $\sim 10^{-4}$, 0.0085 and 0.053 for MSB, MNB and MPB, respectively. The fluorescence quenching with Co^{2+} ions has yielded quenching rates of $1.7 \times 10^9\ M^{-1}\ s^{-1}$ for MSB, $4.6 \times 10^{10}\ M^{-1}\ s^{-1}$ for MNB and $1.5 \times 10^{10}\ M^{-1}\ s^{-1}$ for MPB. MPB was found to be more stable than MNB, at photostability tests carried out by the use of two different irradiation sources and three different methods.

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