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Cd(II) and Cu(II) complexes of polydentate Schiff base ligands: synthesis, characterization, properties and biological activity

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Abstract

We report the synthesis of the Schiff base ligands, 4-[(4-bromo-phenylimino)-methyl]-benzene-1,2,3-triol (A_1), 4-[(3,5-di-*tert*-butyl-4-hydroxy-phenylimino)-methyl]-benzene-1,2,3-triol (A_2), 3-(*p*-tolylimino-methyl)-benzene-1,2-diol (A_3), 3-[(4-bromo-phenylimino)-methyl]-benzene-1,2-diol (A_4), and 4-[(3,5-di-*tert*-butyl-4-hydroxy-phenylimino)-methyl]-benzene-1,3-diol (A_5), and their Cd(II) and Cu(II) metal complexes, stability constants and potentiometric studies. The structure of the ligands and their complexes was investigated using elemental analysis, FT-IR, UV–Vis, ^{1}H and ^{13}C NMR, mass spectra, magnetic susceptibility and conductance measurements. In the complexes, all the ligands behave as bidentate ligands, the oxygen in the *ortho* position and azomethine nitrogen atoms of the ligands coordinate to the metal ions. The keto-enol tautomeric forms of the Schiff base ligands A_1–A_5 have been investigated in polar and non-polar organic solvents. Antimicrobial activity of the ligands and metal complexes were tested using the disc diffusion method and the strains *Bacillus megaterium* and *Candida tropicalis*.

Protonation constants of the triol and diol Schiff bases and stability constants of their Cu^{2+} and Cd^{2+} complexes were determined by potentiometric titration method in 50% DMSO–water media at 25.00 ± 0.02 °C under nitrogen atmosphere and ionic strength of 0.1 M sodium perchlorate. It has been observed that all the Schiff base ligands titrated here have two protonation constants. The variation of protonation constant of these compounds was interpreted on the basis of structural effects associated with the substituents. The divalent metal ions of Cu^{2+} and Cd^{2+} form stable 1:2 complexes with Schiff bases.

The Schiff base complexes of cadmium inhibit the intense chemiluminescence reaction in dimethylsulfoxide (DMSO) solution between luminol and dioxygen in the presence of a strong base. This effect is significantly correlated with the stability constants K_{CdL} of the complexes and the protonation constants K_{OH} of the ligands; it also has a nonsignificant association with antibacterial activity.

Keywords: Schiff base ligands; Metal complexes; Antimicrobial activity; Stability constants; Potentiometric studies; Luminol chemiluminescence

1. Introduction

The condensation of primary amines with carbonyl compounds yields Schiff bases [1,2]. In the recent years, there has been considerable interest in the chemistry of transition metal complexes of Schiff bases [3–5]. This is due to the fact that Schiff bases offer opportunities for inducing substrate chirality, tuning the metal centred electronic factor, enhancing the solubility and stability of either homogeneous or heterogeneous catalysts [6–11]. Schiff base complexes have been amongst the most widely studied coordination compounds in the past few years, since they are becoming increasingly important as biochemical, analytical and antimicrobial reagents [12]. Schiff bases derived from a large number

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of carbonyl compounds and amines have been used [13,14]. It has been shown that Schiff base complexes derived from 4-hydroxysalicylaldehyde and amines have strong anticancer activity, e.g., against *Ehrlich ascites carcinoma* (EAC) [15]. It is well known that some drugs have greater activity when administered as metal complexes than as free organic compounds [16].

A large number of reports are available on the chemistry and the biocidal activities of transition metal complexes containing O,N and S,N donor atoms. The transition metal complexes having oxygen and nitrogen donor Schiff bases possess unusual configuration, structural lability and are sensitive to molecular environment [16]. The environment around the metal centre "as coordination geometry, number of coordinated ligands and their donor group" is the key factor for metalloproteins to carry out specific physiological functions [17].

Among different physicochemical properties of organic compounds, protonation constants and stability constants determined in mixed solvents provide an important basis for speculation about whether substituent effects influence their acidity and basicity. It is also accepted that the knowledge of the stability constants of such Schiff base-metal complexes may eventually help to throw some light on the inactivation of essential trace metals in biological systems. It was therefore, of interest to determine the protonation and stability constants of some Schiff base-metal complexes in DMSO–water media of different compositions potentiometrically.

In the present paper, the preparation, characterization and antimicrobial properties of the various Schiff bases and their Cu(II) and Cd(II) complexes shown in Fig. 1 are reported. The study further deals with the potentiometric determination of the protonation constants of the Schiff bases and stability constants of the complexes by the potentiometric titration method in 50% DMSO–water media at 25.00 ± 0.02 °C and ionic strength 0.10 M sodium perchlorate. Data were evaluated using a computer program BEST [18]. The effects of substituents on the protonation constants of Schiff bases and on the stability constants of Schiff base-metal complexes have also been investigated.

We also report and discuss the inhibition by the Schiff base complexes of cadmium of the intense chemiluminescence reaction in dimethylsulfoxide (DMSO) solution between luminol and dioxygen in the presence of a strong base [19]. This is believed to be the first study of the inhibition of luminol chemiluminescence to be carried out in a non-aqueous solvent system.

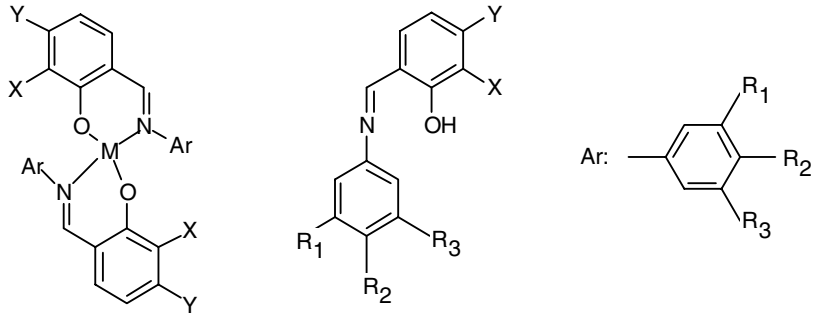
2. Experimental

2.1. Materials

All chemicals used in this study were obtained commercially and used as supplied unless otherwise stated. 3,5-Di-*t*-butyl-4-hydroxyaniline was prepared according to a known procedure [20] and used without further purification.

2.1.1. Potentiometric titrations

Stock solutions of these Schiff bases were prepared in purified DMSO [21]. Doubly distilled conductivity (Millipore system) water was used as aqueous medium as well as for preparation of DMSO–water mixtures. All



M: Cu(II) and Cd(II)

Groups	A_1	A_2	A_3	A_4	A_5
X	OH	OH	OH	OH	H
Y	OH	OH	H	H	OH
R_1	H	$C(CH_3)_3$	H	H	$C(CH_3)_3$
R_2	Br	OH	CH_3	Br	OH
R_3	H	$C(CH_3)_3$	H	H	$C(CH_3)_3$

Fig. 1. Proposed structures of the ligands and their metal complexes.

the other chemicals used were of A.R. grade and were used without further purification. Stock solutions of 0.03 M $Cu(ClO_4)_2 \times 6H_2O$ and $Cd(ClO_4)_2 \times 6H_2O$ were standardized using an appropriate indicator by EDTA titrations [22]. Sodium hydroxide solutions were prepared as 50% aqueous dimethyl sulfoxide solutions and its concentration and absence of carbonate were frequently checked by means of Gran plots [23] using potassium hydrogen phthalate (Merck) as the acid. 0.1 M acid solutions prepared from Merck p.a. perchloric acid were titrated against standardized 0.1 M sodium hydroxide solution [24]. The ionic strength of each solution was adjusted to 0.10 M by the addition of $NaClO_4$ as supporting electrolyte.

2.1.2. Effects of cadmium complexes on luminol chemiluminescence

12 mM Luminol (Sigma–Aldrich) stock solution was prepared by dissolving luminol in DMSO and protected from light by a foil wrapper. 1.0 mmol dm^{-3} solutions of cadmium acetate (Sigma–Aldrich) and the cadmium complexes were made up in DMSO (Avocado), were stored at 4 °C and thawed by gentle warming before use.

Luminol chemiluminescence in DMSO requires the presence of only air and a strong base [19]; a reagent was prepared comprising DMSO saturated with air and sodium hydroxide and containing 400 mg dm^{-3} Tween 65 (Fluka). The solvent was stirred for 30 min with the surfactant and an excess of NaOH pellets while simultaneously bubbling with air. It appeared that suspended small particles of solid sodium hydroxide were an essential component of the reagent, for if these were allowed to settle and the clear solution decanted for use as reagent, light emission was very feeble. As the results show, the presence of the surfactant gave tolerable reproducibility from the same batch of reagent, but blank values of different batches varied.

2.2. Procedures

2.2.1. Preparation of Schiff base ligands (A_1–A_5)

The Schiff base ligands were prepared by the condensing the amine derivatives (1 mmol) with the carbonyl compounds (1 mmol) in EtOH (40 ml) by boiling the mixture under reflux for 5–6 h. The precipitated ligand was filtered off, recrystallized from acetone/hexane and dried in a *vacuum* desiccator.

2.2.2. Preparation of Schiff base complexes

The appropriate quantity of Schiff base ligand (1 mmol) was dissolved in absolute EtOH (15 ml). To this solution was added a solution of $Cd(ClO_4)_2 \cdot 6H_2O$ or $Cu(ClO_4)_2 \cdot 6H_2O$ (0.5 mmol) in absolute EtOH (15 ml) [1:2 molar ratio, M:L]. The mixture was stirred for 3 h at 80 °C. The precipitated complex was then filtered off, washed with cold ethanol and dried in a vacuum dessicator.

Caution: As the perchlorate salts are explosive, care is essential.

2.2.3. Preparation of microbial culture

Fifteen compounds were evaluated for their in vitro antibacterial activity against *Bacillus megaterium* DSM 32 and antifungal activity against *Candida tropicalis* FMC 23 by the agar-well diffusion method. Bacteria were inoculated into Nutrient Broth (Difco) and incubated for 24 h and the fungi studied incubated in Malt Extract Broth (Difco) for 48 h. In the agar-well diffusion method, Mueller Hinton Agar (Oxoid) for bacteria and Malt Extract Broth (Difco) sterilized in a flask and cooled to 45–50 °C was distributed (20 ml) to sterilized petri dishes after injecting 0.01 ml cultures of bacterium prepared as mentioned above and allowed to solidify. The dilution plate method was used to enumerate microorganisms (10^5 bacteria per ml) and fungi (10^3–10^4 per ml) for 24 h [25]. By using a sterilized cork borer (7 mm diameter), wells were dug in the culture plates. Compounds dissolved in $CHCl_3$ were added (0.2 μl) to these wells. The petri dishes were left 4 °C for 2 h and then the plates were incubated at 30 °C for bacteria (18–24 h) and at 25 °C for fungi (72 h). At the end of the period, inhibition zones formed on the medium were evaluated as millimeters (mm) diameter. The control samples were $CHCl_3$ only.

2.2.4. Physical measurements

Elemental analyses (C, H, N) were performed by the TÜBITAK Instrumental Analysis Laboratory (Besevier, Ankara, Turkey) using a Carlo Erber 1106 elemental analyser. Infrared spectra were obtained using KBr discs (4000–400 cm^{-1}) on a Shimadzu 8300 FT-IR spectrophotometer. The electronic spectra in the 200–900 nm range were obtained on a Shimadzu UV-160 A spectrophotometer. Magnetic measurements were carried out by the Gouy method using $Hg[Co(SCN)_4]$ as calibrant. Molar conductances of the Schiff base ligands and their transition metal complexes were determined in DMSO ($\sim 10^{-3}$ M) at room temperature using a Jenway Model 4070 conductivity meter. The mass spectral analyses were carried out on a VG ZabSpec spectrophotometer. ^1H and ^{13}C NMR spectra were taken on a Varian XL-200 NMR instrument. TMS was used as internal standard and deuterated dimethyl sulfoxide as solvent. The metal contents of the complexes were determined by an Ati Unicam 929 Model AA Spectrometer in solutions prepared by decomposing the compounds in aqua regia and then subsequently digesting in concentrated HCl.

2.2.5. Potentiometric titrations

The pH measurement of proton-ligand and metal–ligand systems of Schiff bases were made with a Orion

EA 940 pH meter, equipped with a Mettler Toledo Inlab 412 combined glass electrode and Orion 960 automatic titrator, containing carbonate-free sodium hydroxide at a known (~0.1 mol dm^{-3}) concentration at 25.00 ± 0.02 °C with ionic strength 0.10 M ($NaClO_4$). Temperature was maintained constant inside the cell at 25.00 ± 0.02 °C, by the circulating water by a Haake thermostatted bath (precision ±0.02). All potentiometric measurements in this study were carried out in water–DMSO mixtures containing 50% DMSO because of low solubility of Schiff bases and possible hydrolysis in aqueous solutions.

The potentiometric cell was calibrated before each experiment to obtain $-\log[H^+]$ values (pH) for the titration medium [26]. The ion products ($K_w = [H^+][OH^-]$) were calculated at a constant ionic strength of 0.10 M with $NaClO_4$ in 50% aqueous DMSO solutions based on measurements of $[OH^-]$ and pH in several series of experiments. The standardization of the combined pH electrode was also checked in the alkaline range by addition of excess NaOH. By assuming the E^0_{cell} value determined in the acidic range to be reliable and the $[OH^-]$ concentration of a base added in excess, we calculated the reproducible values of pK_w for the examined 50% aqueous dimethyl sulfoxide solution [27,28]. The pK_w value obtained is 15.48 in this medium.

Potentiometric titrations were carried out at constant temperature and in an inert atmosphere of nitrogen with CO_2-free standardized 0.1 M NaOH in a 50.0 ml solution containing 0.1 M $NaClO_4$: (i) 2.5×10^{-3} M $HClO_4$ (for cell calibration); (ii) 3.0×10^{-3} M $HClO_4 + 1.5 \times 10^{-3}$ Schiff base (for the protonation constant of the Schiff base); (iii) 3.0×10^{-3} M $HClO_4 + 1.5 \times 10^{-3}$ M Schiff base + 7.5×10^{-4} M Cu$(ClO_4)_2$/Cd$(ClO_4)_2$ (for the stability constant of the complex).

2.2.5.1. Data processing. The protonation and stability constants of A_1–A_5 Schiff bases were evaluated by iterative non-linear least squares fit of potentiometric equilibrium curves through mass balance equations for all the components expressed in term of known and unknown equilibrium constants using a computer program BEST [18]. All the models converged at $\sigma < 0.03$ pH units of the observed pH values, which is considered to be an acceptable fit. The equilibrium constants reported in this paper were obtained as averaged values of three titrations. Selection of the equilibrium models was based on critical evaluation of the least squares fitting results, namely analysis of the statistical parameters.

2.2.6. Effects of cadmium complexes on luminol chemiluminescence

All chemiluminescence measurements are determinations of emission at 480 nm (10 nm bandwidth) using a Shimadzu RF-150X spectrofluorimeter, with the shutter closed and auto-shutter off (i.e., no photoexcitation). The instrument photomultiplier was set for high sensitivity. Solutions under examination were held in disposable polystyrene cuvettes (Fisher, Loughborough, UK), 4.0 ml capacity. In the interests of reproducibility, the following protocol for measuring the light emission was observed strictly for each determination.

The chemiluminescence reagent as described above was inverted and then shaken five times in its flask to give reasonable homogeneity; 2.0 ml was pipetted into the cuvette and the determination begun (time = 0 s) by adding 200 μl of the DMSO solution under investigation (or, for blanks, 200 μl of DMSO alone). The cuvette was inverted and shaken five times. At the 60 s point, 100 μl of 12 mM luminol in DMSO solution was added, followed by two inversions and 10 shakes. The cuvette was then installed in the cuvette holder of the spectrofluorimeter and at the 90 s point, the first luminescence reading was recorded. Readings were then recorded every 15 s, until the 10th reading has been recorded at the 225 s point. Light emission was measured in the arbitrary units of the spectrofluorimeter data output.

2.2.6.1. Data processing. An index of light emission was obtained by summing the 10 readings taken. Means and standard deviations of this measure were calculated for each solution examined. The means were ranked and the statistical significance of the differences between means adjacent in the series was evaluated using the Student *t*-distribution [29].

Linear correlation between light emission and other properties was measured by Pearson's correlation coefficient, computed by MSExcel. The significance of the correlation coefficients was tested at $p = 0.10$ using statistical tables [30].

3. Results and discussion

3.1. Spectroscopy

Condensation of the aldehydes with primary amines readily gives rise to the corresponding imines, which were easily identified by their IR, ^1H and ^{13}C NMR spectra, where replacement of the carbonyl by the imine group results in: (i) lowering of the energy of the ν(C=O) stretch in the IR spectrum and (ii) a shift to higher field of the CH=N proton signal in the ^1H NMR spectrum. The imines prepared in this way are formed in nearly quantitative yields and are of high purity. All compounds, except 3,5-di-*t*-butyl-4-hydroxyaniline compound, are very stable at room temperature in the solid state. On the other hand, the electron withdrawing or – attracting groups at the *ortho* and *para* positions of the ligands are able to produce different electronic and steric effects. Therefore, it may be possi-

ble that the *t*-butyl groups on the aniline ring and the hydroxy or methoxy groups *meta* to OH in the salicylidene moiety lead to a decrease in stability of the complexes. The yields of the complexes are lower than those of the ligands. Further stirring and heating did not increase the yield of the complexes containing the *t*-butyl groups. The low yields may be due to the steric hindrance around the coordination centre. All the ligands and their complexes are soluble in common organic solvents such as $CHCl_3$, EtOH, MeOH, THF, etc. Solution conductivity measurements were performed to establish the charge of the complexes. The results show that all compounds are non-electrolyte [31]. The results of the elemental analyses, given in Table 1, are in accord with the composition suggested for the ligands and their metal complexes.

The infrared spectral data of the ligands (A_1–A_5) and their metal complexes are given in Table 2. In the ligands, the bands in the 3500–3420 cm^{-1} range may be assigned to ν(O–H) stretching. For the free ligands, the broad bands in the 2800–2700 cm^{-1} range are assigned to the OH group vibration (*ortho* position) associated intramolecularly with the nitrogen atom of the CH=N group [32]. These bands disappear in the complexes, as a result of proton substitution by cation coordination to oxygen. For the ligands, the strong bands observed in the 1640–1615 cm^{-1} range are assigned to the azomethine group vibration. These bands are slightly shifted towards lower frequencies in the complexes, and this change in the frequencies shows that the imine nitrogen atom coordinated to the Cd(II) and Cu(II) ions. The medium intensity bands observed for all ligands in the 1350–1275 cm^{-1} range can be attributed to the phenolic stretch. These bands are observed for the complexes at lower wave number by ca. 10–20 cm^{-1} relative to the free ligands suggesting involvement of the oxygen atom of the C–O moiety in coordination [33]. In all of the present complexes a medium and/or weak band observed in the 510–415 cm^{-1} range can be attributed to the ν(M–N) and ν(M–O) [34] modes.

In the case of the ligands and their metal complexes, it is particularly important to establish whether the molecules retain the imine character of their phenol precursor. The most useful techniques to investigate the tautomeric forms (Fig. 2) of these ligands are UV and NMR spectroscopy, while IR seems of limited value here because location of the ν(C=O) and ν(C–O) stretches in the spectra is obscured by the abundance of aromatic skeletal modes. In order to investigate the keto-enol tautomeric forms of the free ligands, the electronic spectra were measured in heptane, chloroform and ethanol. In heptane, the ligands exhibit maxima in 320–278 nm range. However, in chloroform and ethanol, new bands in the 464–337 nm range were observed. The former set (~278 nm) in heptane have been assigned to the enolimine tautomer and the latter to the ketoamine tautomer of the Schiff bases [35].

Electronic spectra have been measured in EtOH and the numerical data are given in Table 2. Hydrogen-bond forming solvents (EtOH) thus favour the formation of the ketoamine. The interaction of enolimine with a hydrogen bond forming solvent would presumably reduce the O–H bond strength and facilitate proton transfer to the nitrogen centre. In like manner, the bands in the 464–337 nm range are also assigned to the n–π* transition of the azomethine group. In the spectra of the complexes, the bands of the azomethine chromophore n–π* transition are shifted to lower frequencies indicating that the imine nitrogen atom is involved in coordination to the metal ion. The bands at higher energies

Table 1
Some analytical and physical data for the imine ligands and their complexes

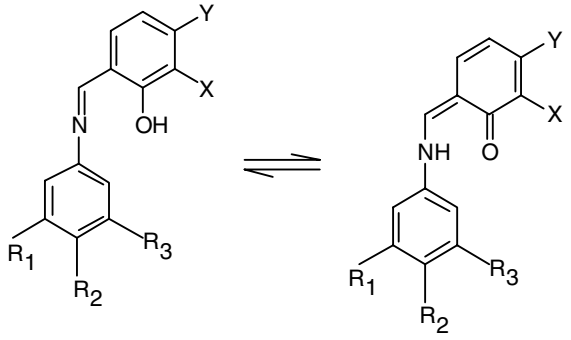
Compound	Colour	μ_{eff} (as B.M.)	Yield (%)	m.p. (°C)	Found (Calc.) (%)				Λ_M[a]
					C	Λ_M[a]	H	M	
A_1	orange		84	201	50.67(50.65)	4.49(4.54)	3.22(3.24)		1.8
$Cu(A_1)_2$	brown	1.79	74	>250	46.35(46.32)	4.17(4.18)	2.63(2.64)	9.48(9.43)	7.4
$Cd(A_1)_2$	yellow	Diamag.	70	>250	42.95(42.91)	3.87(3.85)	2.51(2.48)	15.55(15.49)	7.0
A_2	yellow		85	204	70.53(70.58)	3.88(3.92)	7.58(7.56)		1.2
$Cu(A_2)_2$	dark brown	1.8	60	>250	65.03(64.99)	3.64(3.61)	6.74(6.71)	8.27(8.19)	6.8
$Cd(A_2)_2$	yellow	Diamag.	70	>250	61.16(61.13)	3.36(3.40)	6.34(6.31)	13.72(13.64)	9.0
A_3	orange		87	156	74.04(74.00)	6.15(6.17)	5.68(5.73)		1.5
$Cu(A_3)_2$	light brown	1.81	68	>250	65.20(65.17)	5.46(5.43)	4.70(4.66)	12.40(12.32)	6.9
$Cd(A_3)_2$	orange	Diamag.	79	238	59.50(59.52)	5.01(4.96)	4.28(4.25)	19.99(19.93)	8.0
A_4	orange		90	184	53.47(53.42)	4.86(4.79)	3.47(3.42)		1.0
$Cu(A_4)_2$	dark brown	1.8	65	>250	48.37(48.33)	4.32(4.34)	2.83(2.79)	9.90(9.84)	8.5
$Cd(A_4)_2$	yellow	Diamag.	65	201[b]	44.89(44.92)	4.04(4.03)	2.64(2.59)	16.27(16.20)	10.0
A_5	yellow		86	163	73.87(73.90)	4.16(4.11)	7.88(7.92)		1.9
$Cu(A_5)_2$	dark brown	1.81	70	>250	67.81(67.78)	3.74(3.77)	7.02(6.99)	8.65(8.55)	7.3
$Cd(A_5)_2$	orange	Diamag.	68	124[b]	63.58(63.60)	3.57(3.53)	6.60(6.56)	14.27(14.19)	7.6

[a] $\Omega^{-1}\,cm^2\,mol^{-1}$.
[b] Decompose.

Table 2
Infrared[a] and electronic spectral data for the Schiff base ligands and their metal complexes (cm^{-1})

Compound	ν(OH)^x	ν(OH)	ν(CH$_3$)^y	ν(O–H···N)	ν(CH=N)	ν(C–OH)	ν(M–N)	ν(M–O)	λ_{max} (nm)
A_1		3450 br		2800 m	1620 s	1290			420, 375, 339, 280, 240
A_2	3610 s	3380 br	2980 s	2790 m	1620 s	1280			425, 348, 294, 255
A_3		3400 br	2910 s	2700 m	1641 s	1283			359, 320, 281, 226
A_4		3375 br		2745 m	1633 s	1300			464, 368, 317, 279, 271
A_5	3600 s	3410 br	2975 s	2740 m	1625 s	1265	503 w	434 w	430, 275, 225
$Cd(A_1)_2$					1605 m	1270	505 w	428 w	418, 354, 287, 238
$Cd(A_2)_2$	3608 s		2978 s		1610 m	1276	497 w	415 w	357, 289, 234
$Cd(A_3)_2$			2910 s		1614 s	1275	500 w	434 w	389, 334, 271, 245, 220
$Cd(A_4)_2$					1620 s	1294	503 w	444 w	438, 354, 305, 262, 260
$Cd(A_5)_2$	3600 s		2975 s		1613 s	1260	510 w	424 w	422, 354, 349, 283, 238
$Cu(A_1)_2$					1612 s	1275	490 w	448 w	624, 380, 324, 275, 216
$Cu(A_2)_2$	3609 s		2977 s		1618 s	1273	480 w	442 w	633, 412, 285, 254, 215
$Cu(A_3)_2$			2909 s		1613 s	1270	495 w	420 w	610, 370, 341, 295, 271, 227
$Cu(A_4)_2$					1624 s	1250	497 w	430 w	612, 363, 325, 294, 270,221
$Cu(A_5)_2$	3598 s		2975 s		1610 s	1262	485 w	418 w	627, 429, 327, 220

[a] br (broad), s (strong), m (medium), w (weak). x: Sterically hindered phenol, y: CH_3 and CH_3 group of the *t*-butyl group.



Enolimine Ketoamine

Fig. 2. Tautomeric forms of the ligands.

(~290–206 nm range) are associated with benzene π–π* transitions. The spectra of the complexes show intense bands in the high-energy region at 302–372 nm which can be assigned to charge transfer L → M bands [36]. Although the precise nature of this transition involving the phenolate group is not fully clear [it has been considered to be an O(phenolate) → metal(II) LMCT [37] or as a metal(II) → π* (phenolate) MLCT [38] transition], it is agreed that a higher energy component should exist near 330 nm [39]. *t*-Butyl groups on the aniline moiety occupy a more significant region of space in the vicinity of the metal centre due to the presence of the branched carbon. Therefore, it is quite plausible that a sterically induced increase in the average metal–ligand separation results in a hypsochromic shift in the c.t. absorption maximum. The spectra of the Cu(II) complexes contain one absorption band in the 633–610 nm range which may be assigned to the d–d transition of the Cu(II) ion suggesting that they are four-coordinated complexes.

Magnetic measurements were recorded at room temperature and the effective magnetic moment (μ_{eff}) values of the complexes are presented in Table 1. The magnetic moment of the copper(II) complexes was observed in the range of 1.79–1.81 B.M. which corresponds to a single unpaired electron with a very slight orbital contribution. The geometries of the Cu(II) complexes are square-planar [40]. The structures of the monomeric complexes are supported by magnetic moment data. The Cd(II) complexes of the ligands A_1–A_5 were found to have diamagnetic character and tetrahedral geometry around the metal ions.

Additional structural information can be deduced from the ^{1}H and ^{13}C NMR spectra. The ^{1}H- and ^{13}C-chemical shifts for the Schiff base ligands are given in Table 3. It is noteworthy that the influence of substitution on the chemical shifts of the aniline moiety is weak. When a bromine atom is present on the aniline residue, a significant deshielding of the proton signals can be observed, due to the strong electron-withdrawing effect of this substituent. However, the OH groups on the salicylidene moiety increases the electron density of the aromatic rings, due to the resonance or mesomeric effect. In Schiff base ligands A_1–A_5, there is the proton donor OH group and one proton acceptor group in the *ortho* position. Due to presence of the OH groups in ortho position to imine group, formation of a few intramolecular hydrogen bonds is possible. It is important to emphasize the ^{1}H resonance of the O–H group in the 10.10–12.30 ppm range. The signal due to the OH proton disappears in D_2O solution.

The formulation of the ligands are deduced from analytical data, ^{1}H and ^{13}C NMR and further supported by mass spectroscopy. The relatively low intensities of the molecular ion peaks, $[M]^+$, are indicative of the ease of fragmentation of the compounds, and this may reflect the number of heteroatoms present in each structure. The spectra of the ligands A_1–A_5 show peaks at m/e 308, 358, 227, 292 and 342

Table 3
The ^{1}H (^{13}C) NMR data (as ppm) for the Schiff base ligands using $CDCl_3$ as solvent

Compound	CH_3	*t*-Bu	Ar	CH=N	OH
A_1			7.20–7.74 (112.81–152.45)	8.94 (163.15)	10.45–11.70
A_2		1.51 (32.00)	7.14–7.19 (110.10–156.66)	8.38 (166.75)	10.10–12.15
A_3	2.28 (27.30)		7.18–7.60 (116.13–157.17)	8.90 (162.52)	10.60–11.05
A_4			7.32–7.72 (113.40–154.61)	8.92 (165.05)	10.55, 10.30
A_5		1.62 (32.95)	7.04–7.29 (115.20–155.00)	8.39 (169.90)	10.10–12.30

CH_3 coupling constant $J \sim 3$ Hz.

$[M]^+$, respectively. All the ligands decompose in a similar way.

3.2. Antimicrobial activity

Synthesized compounds were tested for in vitro antimicrobial activity by the agar-well diffusion method. The antibacterial and antifungal activities of the ligands and their complexes against one bacterium (*B. megaterium*) and one fungus (*C. tropicalis*) are presented in Table 4. Also included is the activity of the solvent $CHCl_3$. It was observed that all the compounds tested showed antibacterial and antifungal activity. The complex $Cu(A_1)_2$ of the ligand A_1 does not show activity against *C. Tropicalis*. On the other hand, the complex $Cu(A_2)_2$ has most activity against the same fungi. However, the ligands and their Cu(II) and Cd(II) complexes are effective against the bacterium *B. megaterium*.

3.3. Crystallization

We have already made numerous efforts to crystallize any complex of the compounds for X-ray diffraction studies. However, up till now, no single crystals have been obtained. In this way, it would be possible to determine the coordination of the ligands.

Table 4
Antimicrobial effects of the ligands and their metal complexes[a]

Compound	Inhibition zone (mm[b]) *C. tropicalis*	Inhibition zone (mm[b]) *B. megaterium*
Control ($CHCl_3$)		
A_1	15	18
A_2	10	14
A_3	7	12
A_4	13	13
A_5	12	15
$Cd(A_1)_2$	18	23
$Cd(A_2)_2$	17	21
$Cd(A_3)_2$	11	20
$Cd(A_4)_2$	13	18
$Cd(A_5)_2$	24	21
$Cu(A_1)_2$	15	19
$Cu(A_2)_2$	26	22
$Cu(A_3)_2$	21	17
$Cu(A_4)_2$	23	20
$Cu(A_5)_2$	26	18

[a] Conc. of compounds is 50 μg/cm^3.
[b] Including diameter of disc (6 mm).

3.4. Protonation constants of Schiff bases

The study of complex formation by the studied Schiff bases cannot be carried out in aqueous solution because of the nature of the compounds involved. These metal complexes as well as the ligands themselves are insoluble in water. This solvent has been most widely used for potentiometric determination of stability constants. The mixture DMSO–water 50:50% was the chosen solvent for our study. In such a medium, the studied Schiff bases and their metal complexes are soluble giving stable solutions. The use of this mixed solvent has some advantages over pure DMSO. Thus, pure DMSO is very hygroscopic and controlling its water content is difficult [41]. This fact would affect reproducibility of our experiments. However, DMSO–water 50:50% mixture has only a small hygroscopic character. A further advantage is its compatibility with the standard glass electrode, so that the pH measurements may be carried out in a similar way to that employed in a purely aqueous solution. In contrast, the glass electrode has a slow response and irreproducible behaviour in pure DMSO (10). For these reasons, the use of pure DMSO is not recommended for potentiometry. Another advantage of the DMSO–water 50:50 mixture is its large acidity range ($pK_w = 15.48$) which allows the investigation of deprotonation equilibria of weak acids which could be hardly studied in water [42,43].

The stoichiometric protonation constants of the investigated Schiff bases (A_1–A_5) were determined in 50% DMSO–water mixture at 25 °C and these constants are tabulated in Table 5. As the titration curve of the ligand in Fig. 5, which is drawn based on A_1 Schiff base, it can be seen that there are two end-points at $a = 1$ and $a = 2$. According to the results obtained from this titration curve it can be concluded that the Schiff bases studied here have two protonation constants. The $\log K_{OH}$ and $\log K_{NH}$ values are related to the protonation of no. 2 phenolic oxygen and imine nitrogen, respectively, as Fig. 4.

This is also illustrated in the species distribution of the A_1 ligand in Fig. 3. At pH < 4, the ligand exists in the fully protonated form H_2L^+ and in the form HL.

Table 5
Successive protonation constants of A_1–A_5 Schiff bases in 50% DMSO–water mixture (μ = 0.100 M $NaClO_4$, 25.00 ± 0.02 °C)

Schiff bases	Log K_{OH}	Log K_{NH}
A_1	7.65 ± 0.02	1.75 ± 0.02
A_2	8.24 ± 0.02	2.54 ± 0.02
A_3	11.55 ± 0.02	1.80 ± 0.02
A_4	11.50 ± 0.02	1.53 ± 0.02
A_5	8.54 ± 0.02	2.84 ± 0.02

As the pH is increased, the ligand loses its protons from imine nitrogen to become HL, which is the predominant species in pH range of 3–6. As conditions become more alkaline the second phenolic hydroxyl group begins deprotonation to the free ligand L^- anion which is predominant at pH > 9.

Previous studies about Schiff bases report that the log protonation constants of imine nitrogen of Schiff bases are about 3–5 [26,4]. In this study, however, the protonation constant logarithms of the Schiff bases studied are to be found between 1.5 and 2.84. This can be explained by the fact that there is an aromatic triol or diol attached to the imine nitrogen and such substituted phenyl groups inductively reduce the electron density of imine nitrogen.

From Table 5, log K_{OH} of A_2 Schiff base is 8.24 and that of A_1 Schiff base is 7.65 and log K_{NH} of A_2 Schiff base is 2.54 and that of A_1 Schiff base is 1.75. Substitution by hydroxyl instead of bromine causes a 0.59 unit increase for log K_{OH} and a 0.79 unit increase for log K_{NH}, which means a lowering of the acidity. This can be explained by electron release of the hydroxyl group in the benzene ring with resonance. Since tertiary methyl groups are attached at the meta position relative to the imine nitrogen, it is assumed that they have no effect on the value of the protonation constant of the imine nitrogen.

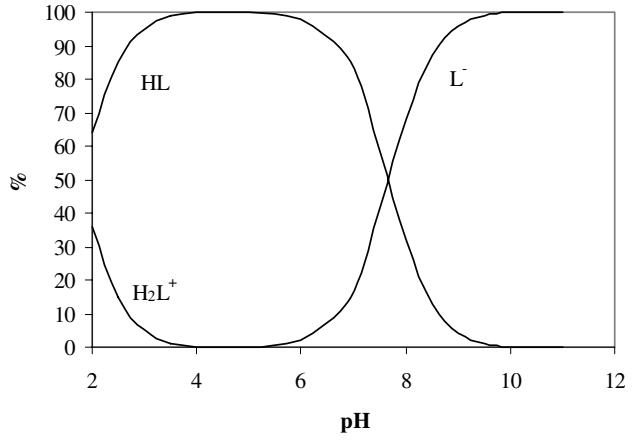


Fig. 3. Species distribution diagram for the systems A_1 Schiff base (L) as a function of pH. μ = 0.100 mol l^{-1} $NaClO_4$, t = 25.0, $T_L = 1.5 \times 10^{-3}$ mol l^{-1}, % = percentage concentration of species.

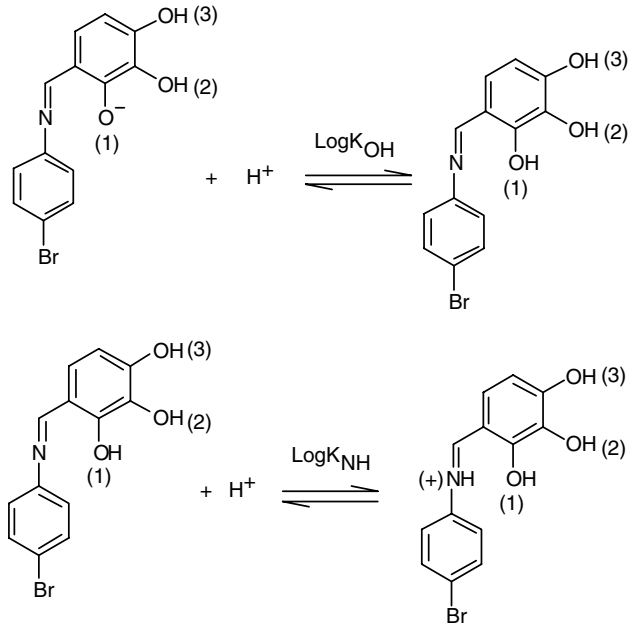


Fig. 4. The log K_{OH} and log K_{NH} equilibrium reactions of the ligand A_1.

Another remarkable situation about protonation constant values of the Schiff bases is that values of log K_{OH} protonation constants of the A_3 and A_4 Schiff bases are 3 units greater than others. This may be because of the attachment at the meta position of the OH group, which is inductively an electron acceptor and an electron donor with resonance. At the meta position, OH cannot donate electrons with resonance, so it just acts as an electron acceptor.

In the case of A_3 Schiff base and A_4 Schiff base, the log K_{NH} values are found to be 1.80 and 1.53, respectively, and the log K_{OH} values are found to be 11.55 and 11.50, respectively. It can be seen that there is a relationship between alkyl substitution on the amino nitrogen and phenolic oxygen and increasing log K_{NH} and log K_{OH} values. The electron-releasing effect of the alkyl groups is the main factor in these examples. However, the log K_{OH} value of A_4 Schiff base is only slightly smaller than the log K_{OH} value of A_3 Schiff base because the substituents are located far from the reaction site.

3.5. Stability constants of the Schiff base complexes

The potentiometric titration curves of A_1 Schiff base with equivalents of ligand to metal ion for Cd^{2+} and Cu^{2+}, are shown in Fig. 5. All metal ions depress the titration curve of the free ligand by the release of protons according to the abilities of the metal ions to bind to the ligand Schiff bases. As the titration curves of the complexes formed by cadmium and copper together with A_1 Schiff base are examined, two inflection points can be observed at $a = 2$ and $a = 4$ for Cd^{2+}, and at

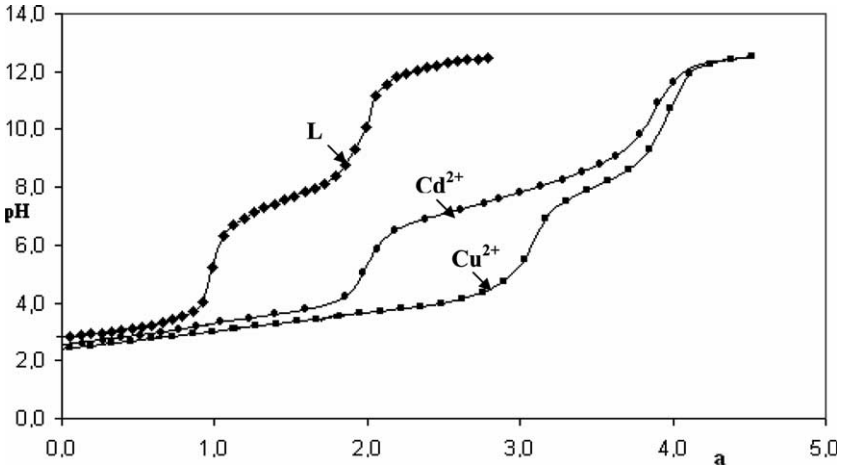


Fig. 5. Potentiometric titration curves for A_1 Schiff base (L) and 1:2 stoichiometries of Cu^{2+} and Cd^{2+} to A_1 Schiff base (ML_2) as a function of added NaOH. (a = Moles of base added per mole of metal ion (or ligand) present.)

$a = 3$ and $a = 4$ for Cu^{2+}. The presence of an inflection point on the titration curves at $a = 3$ and $a = 2$ for Cu^{2+} and Cd^{2+}, respectively, can be explained by the formation of protonated complexes. This is also illustrated in the species distribution of the ligand in Figs. 6 and 7. In order to investigate change with pH in the concentration of the complexes, which Schiff bases formed with Cu^{2+} and Cd^{2+}, the stability constant values in Tables 6 and 7 are evaluated, using SPE computer program (1) and the species distribution curves are drawn. For example, in Fig. 6, if the distribution diagram for Cu–A_1 system is examined it will be seen that the complex form with CuL_2H_2 proton is dominant in the region up to pH 3.5. This species forms 93% at pH 2. Between pH 4 and 8 CuL_2H complex is dominant and above pH 9 CuL_2 complex forms. This CuL_2 complex forms 95.6% ratio at pH 11.6. Moreover, from the distribution diagram it can be seen that very few CuL and CuLH complexes form. Similarly, as the distribution diagram in Fig. 7 is examined for Cd–A_1 system, complex form CdL_2H_2 seems to be dominant at pH < 6. This complex forms 42.5% at pH 3.9. Above pH 9, CdL complex is dominant.

The stoichiometric stability constants of metal(II) (Cu^{2+} and Cd^{2+}) complexes of the investigated Schiff Bases were determined in 50% DMSO–water mixture at 25 °C and these constants are tabulated in Tables 6 and 7. All the metal ions were found to combine easily with A_1, A_2, A_3, A_4 and A_5 Schiff bases to form deprotonated (ML_2) and multiprotonated (MH_nL_n) in

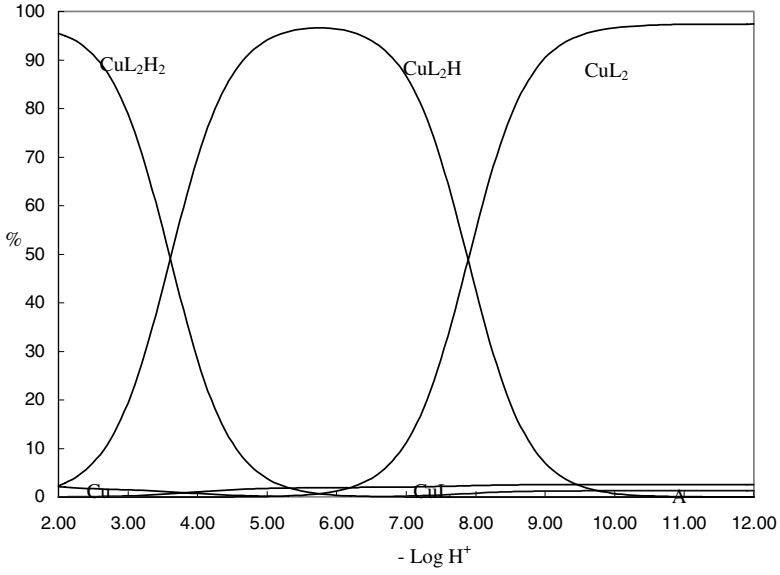


Fig. 6. Species distribution diagram for the systems Cu–A_1(L) Schiff base system in 1:2 molar ratio as a function of pH. $\mu = 0.100$ mol l^{-1} $NaClO_4$, $t = 25.0$, $T_L = 3.0 \times 10^{-3}$ mol l^{-1}, $T_{Cu} = 1.5 \times 10^{-3}$ mol l^{-1}, % = percentage concentration of species.

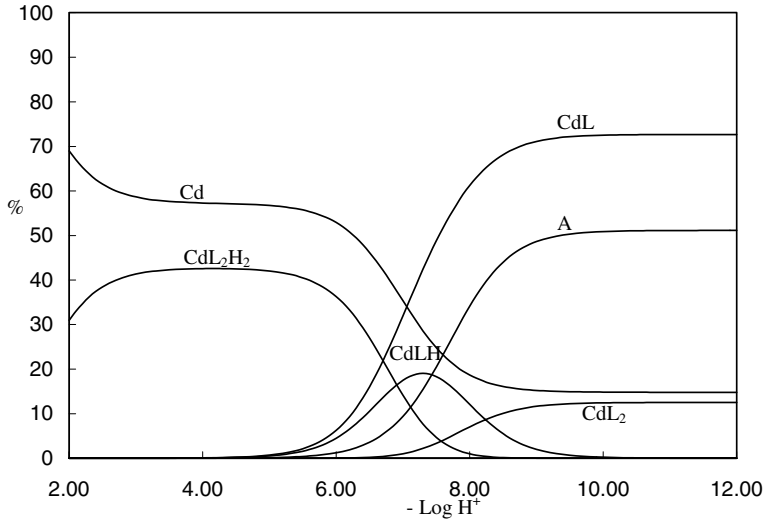


Fig. 7. Species distribution diagram for the systems Cd–A_1(L) Schiff base system in 1:2 molar ratio as a function of pH. $\mu = 0.100$ mol l^{-1} $NaClO_4$, $t = 25.0$, $T_L = 3.0 \times 10^{-3}$ mol l^{-1}, $T_{Cd} = 1.5 \times 10^{-3}$ mol l^{-1}, % = percentage concentration of species.

concentrations depending on the pH of the solution. When the values in the table are examined, the following order is obtained for stability constants of mononuclear complexes (ML) with the Schiff bases: $A_3 > A_4 > A_5 > A_2 > A_1$. When the rank orders of stability constants and of protonation constants are compared, it is seen that there is correlation between them. The linear correlation coefficients (r) between the logarithms of the stability constants of the complexes and the protonation constants of the ligands are set out in Table 8. For both cadmium and copper complexes, correlation between the complex stability constants and the ligand protonation constant K_{OH} attains or approaches statistical significance [30] in almost every case and is greatest for the complexes of the type MH_2L_2, i.e., stability of these complexes is greatest when the ligand is protonated under most conditions. Unsurprisingly, this does not apply to complexes of the ML_2-type, which display a negative correlation between the two constants. None of the stability constants correlates significantly with K_{NH}. The magnitude of stability constants for all ligands was found to be $Cu^{2+} > Cd^{2+}$, in agreement with increasing acidity of the metal ion.

3.6. Effects of the cadmium complexes on luminol chemiluminescence

In DMSO solution, luminol reacts with dissolved oxygen in the presence of a strong base with intense chemiluminescence [19]. Because of the relatively simple reaction conditions, this phenomenon has found great favour as a demonstration of chemiluminescence [44], but analytical applications have almost universally made use of the related reaction in aqueous solution.

Each of the five cadmium complexes studied here had the effect of significantly ($p < 0.01$) diminishing the chemiluminescence of luminol in DMSO solution, as shown in Fig. 8. Chemiluminescence diminished along the series $A_1 > A_2 > A_4 > A_3$, in each case the signal being significantly less than that obtained in the presence of the preceding member of the series ($p < 0.01$ for $A_2 > A_4$, but $p < 0.05$ for $A_1 > A_2$ and $A_4 > A_3$). The observed difference in emission between the two complexes with the most powerful inhibitory effect ($A_3 > A_5$) were not significant at the concentration used, in part because of the imprecision of relatively low signals.

Table 6
Logarithms of the stability constants of Cu(II) complexes of A_1–A_5 Schiff bases, in 50% DMSO–water mixture ($\mu = 0.100$ M $NaClO_4$, 25.00 ± 0.02 °C)

Schiff Bases	Log K_{ML}	Log β_{MHL}	Log β_{ML_2}	Log β_{MHL_2}	Log $\beta_{MH_2L_2}$
A_1	8.09 ± 0.02	6.25 ± 0.02	13.94 ± 0.02	21.83 ± 0.02	25.44 ± 0.02
A_2	8.25 ± 0.02	6.44 ± 0.02	13.73 ± 0.02	21.76 ± 0.02	26.03 ± 0.02
A_3	8.49 ± 0.02	7.05 ± 0.02	13.67 ± 0.02	22.01 ± 0.02	28.25 ± 0.02
A_4	8.38 ± 0.02	6.98 ± 0.02	13.65 ± 0.02	21.96 ± 0.02	28.12 ± 0.02
A_5	8.33 ± 0.02	6.32 ± 0.02	13.83 ± 0.02	22.06 ± 0.02	26.35 ± 0.02

Definitions of stability constants: $K_{ML} = [ML]/[M^{2+}][L]$; $\beta_{MHL} = [MHL]/[M^{2+}][L][H^+]$; $\beta_{ML_2} = [ML_2]/[M^{2+}][L]^2$; $\beta_{MHL_2} = [MHL_2]/[M^{2+}][L]^2\ [H^+]$; $\beta_{MH_2L_2} = [MH_2L_2]/[M^{2+}][L]^2\ [H^+]^2$ (L = ligand in all definitions).

Table 7
Logarithms of the stability constants of Cd(II) complexes of A_1–A_5 Schiff bases, in 50% DMSO–water mixture (μ = 0.100 M $NaClO_4$ t = 25.00 ± 0.02 °C)

Schiff Bases	Log K_{ML}	Log β_{MHL}	Log β_{ML_2}	Log β_{MHL_2}	Log $\beta_{MH_2L_2}$
A_1	3.38 ± 0.02	6.77 ± 0.02	5.31 ± 0.02	13.54 ± 0.02	20.45 ± 0.02
A_2	3.69 ± 0.02	7.03 ± 0.02	5.18 ± 0.02	13.41 ± 0.02	20.72 ± 0.02
A_3	3.86 ± 0.02	7.12 ± 0.02	5.09 ± 0.02	13.87 ± 0.02	22.15 ± 0.02
A_4	3.78 ± 0.02	7.09 ± 0.02	5.13 ± 0.02	13.78 ± 0.02	22.03 ± 0.02
A_5	3.73 ± 0.02	6.86 ± 0.02	5.26 ± 0.02	13.65 ± 0.02	21.01 ± 0.02

Definitions of stability constants as in Table 6.

Table 8
The linear correlation coefficients (r) between the logarithms of the stability constants of the complexes and the protonation constants of the ligands (n = 5 in each case)

	Cadmium		Copper	
	Log K_{OH}	Log K_{NH}	Log K_{OH}	Log K_{NH}
Log $\beta_{MH_2L_2}$	0.9942***	−0.4827	0.9957***	−0.4800
Log β_{MHL_2}	0.8810**	−0.4988	0.5284	0.0529
Log β_{MHL}	0.8286*	−0.2857	0.9840***	−0.5916
Log β_{ML_2}	−0.8818**	0.3400	−0.8446*	0.2398
Log K_{ML}	0.7708	0.0860	0.8637*	−0.0843

* r Significant at $p < 0.10$.
** r Significant at $p < 0.05$.
*** r Significant at $p < 0.01$.

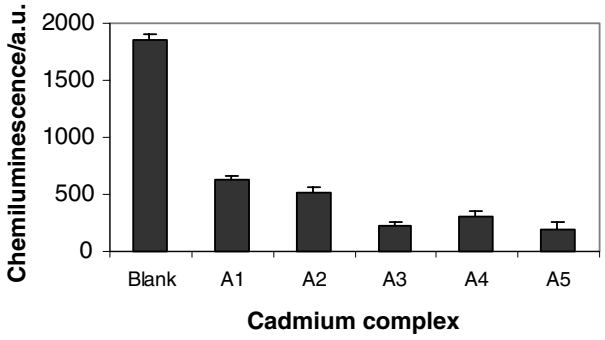


Fig. 8. Effect of cadmium complexes each at a final concentration of 100 μM on the chemiluminescence of luminol in DMSO solution (mean + SD; n = 3 or 4).

The reagent used in this experiment was saturated with sodium hydroxide. The novel complexes studied were all water-labile, i.e., subject to nucleophilic attack, and in DMSO solution they would have been subject to attack by the sodium hydroxide present; base would be consumed and metal ions would dissociate from the ligands to which they were initially co-ordinated. The concentration of sodium hydroxide in the reagent was not accurately known but the limit of solubility is more than 100 mM (4 mg ml^{-1}). Only a small proportion of this would be consumed by the postulated reaction between the complexes and sodium hydroxide. Therefore the likely fall in base concentration would not seem to be sufficient to explain the inhibition of chemiluminescence that occurred.

Metal ions released by the breakdown of the complex might have interfered in some way with the chemiluminescence process. So luminol chemiluminescence was also measured in the presence of 100 μM cadmium acetate (Fig. 9). Although all the cadmium complexes significantly diminished the luminol emission, cadmium acetate at the same metal ion concentration as the complexes did not. There have been numerous previous observations of the effects on luminol chemiluminescence of cadmium ions, but only in aqueous solution. Thus, cadmium(II) ions enhance luminol-hydrogen peroxide chemiluminescence [45] in the presence of 8-hydroxy-5-quinolinesulfonic acid. In contrast, a detection reaction for cadmium(II) ions in an electrophoresis buffer has been based on their reduction of luminol-hydrogen peroxide chemiluminescence signals [46] by displacement of cobalt(II) ions on which the light emission depends. However, the emission being inhibited in that case was dependent on the presence of a metal ion, whereas luminol chemiluminescence in DMSO is regarded as being independent of the d-block catalysts that are effective in aqueous solutions [19]. So the same mechanism would not be expected to be operative in the present study.

The absence of effect of cadmium ion suggests that the light-emitting reactions are blocked either by the whole complexes or by released ligands. Luminol chemiluminescence proceeds by a radical mechanism [47] and is known to be inhibited if radicals are removed from the reaction medium [19]. The Schiff base ligands A_{1-5} are

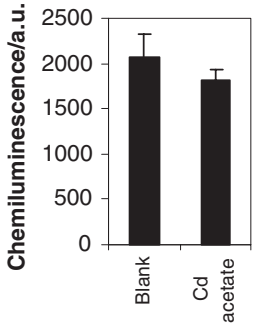


Fig. 9. Effect of cadmium acetate at a final concentration of 100 μM on the chemiluminescence of luminol in DMSO solution (mean + SD; n = 3).

all polyphenolic in character and the radical scavenging potential of such compounds is well-known [48,49]. The complexes containing benzene-1,2,3-triol ligands (A_1, A_2) suppressed luminol chemiluminescence less than those containing benzene-1,2-diol ligands (A_3, A_4); the single complex (A_5) containing a benzene-1,3-diol ligand gave the greatest signal loss.

In addition to their direct radical scavenging effect, the different ligands might have effects which depend on the stability of their complexes. So we examined the relationship between the stability constants of the complexes and their effect on the chemiluminescence reaction. There is a significant ($p = 0.10$) correlation ($r = 0.8310$, $n = 5$, critical value 0.805 [30]) between the effect of the complexes $Cd(A_{1-5})_2$ on luminol chemiluminescence and the logarithm of the formation constant K_{CdL} (i.e., they correlate negatively with intensity of the signal). The more stable the complex the more it inhibits the chemiluminescence, suggesting that the radical scavenging capacity is greatest in the intact complex. So, it seems, although the cadmium ion does not affect the light emission directly, it does so indirectly through its coordination of the ligands.

There are nonsignificant correlations ($r = 0.4410$–0.7473, $n = 5$) with the logarithms of the other formation constants, but the fact that it is $\log(K_{CdL})$ that most strongly correlates with the chemiluminescence is consistent with the finding reported above that the CdL complex is dominant at pH > 9, the prevailing conditions in the medium of the chemiluminescence reaction. The strongly basic conditions of the chemiluminescence reaction also explain the relatively high (though not significant) correlation ($r = 0.6354$, $n = 5$) between the effects on chemiluminescence and the logarithms of the protonation constants K_{OH} (which range from ~8–12) but not K_{NH} (~2, $r = 0.1772$, $n = 5$). Inhibition of chemiluminescence is thus associated with greater values of K_{OH} which lead to a greater degree of protonation at high pH.

The effect on chemiluminescence of the cadmium complexes is negatively correlated to a reasonable degree (but nonsignificantly) with their antimicrobial activity for *B. megaterium* ($r = -0.6096$, $n = 5$), but not at all for *C. tropicalis* ($r = -0.0316$, $n = 5$). This seems to arise because the effect on chemiluminescence of the complexes is correlated to the logarithms of their formation constants K_{CdL} and of the protonation constants K_{OH} of the ligands, which are negatively correlated with antibacterial activity to a greater degree than are the effects of the complexes on chemiluminescence. Thus, antibacterial activity of the complexes correlated with the formation constants K_{CdL} for *B. megaterium* and the coefficient ($r = -0.7980$, $n = 5$) closely approached significance at $p = 0.10$; this was not the case for the fungus *C. tropicalis* ($r = -0.3830$, $n = 5$). The correlations with the protonation constants K_{OH} are even more striking. Thus the activities against *B. megaterium* correlate with K_{OH} with coefficients of −0.8608 for complexes and −0.8349 for uncoordinated ligands, both significant at $p < 0.10$. The corresponding values for *C. tropicalis* are −0.7635 and −0.4924, which are not significant.

Because of the relationship between the inhibition of chemiluminescence and stability, we suggest that this procedure might make a useful screening test for newly synthesized compounds. It is rapid (4 min per test) and inexpensive to perform. In addition, it requires no special apparatus and both reagents and instrumentation are widely available. The value of the procedure as a predictor of antibacterial activity is also worth further investigation. But the most intriguing question raised by this study is how the radical scavenging behaviour of the polyphenolic Schiff bases is affected by coordination to a metal ion; this mechanism has possible relevance to the design of antioxidants for use in therapeutics and in food preservation.

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