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Synthesis, characterization and antimicrobial studies of Cd(II), Hg(II), Pb(II), Sn(II) and Ca(II) complexes of curcumin

Abstract: Cd(II), Hg(II), Pb(II), Sn(II) and Ca(II) complexes of curcumin have been prepared and characterized on the basis of their analytical, spectral and conductance data. In all the complexes, curcumin behaved as a monobasic bidentate ligand in which the intramolecularly hydrogen-bonded enolic proton is replaced by the metal ion. The antifungal (with *Aspergillus niger, Aspergillus flavus, Aspergillus heteromorphus* and *Penicillium verruculosum*) and antibacterial (with *Bacillus cereus*) studies reveal that metal complexation considerably increased the activity of curcumin, and among the complexes, Sn(II) complex exhibited maximum activity except for *A. flavus* where Hg(II) complex is more active.

Keywords: antifungal and antibacterial activities; curcumin; metal complexes; spectral studies.

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Introduction

Turmeric, derived from the plant *Curcuma longa*, is a gold-colored spice commonly used in the Indian subcontinent not only for health care but also for the preservation of food and as a yellow dye for textiles (Lampe, 1918; Kapoor et al., 2008). Extensive research within the past half century has proven that most of these activities are due to curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (Danie et al., 2004; Miriyala et al., 2007). Curcumin has been shown to exhibit antioxidant (Ak and Gulcin, 2008), anti-inflammatory (Jurenka, 2009), antiviral (Singh et al., 2010), antibacterial (Martin et al., 2009), antifungal (Negi et al., 1999; Wang et al., 2009), and anticancer (Wilken et al., 2011) activities and thus has a potential against various malignant diseases, diabetes, allergies, arthritis, Alzheimer's disease (Mishra and Palanivelu, 2008) and other chronic illnesses. The biological activity of curcumin is associated with phenolic and methoxy groups in conjunction with the 1,3-diketone-conjugated diene system (Anand et al., 2008). Recent studies have shown that metal complexation enhances the biochemical activities of curcumin (Thompson et al., 2004; Tajbakhsh et al., 2008; Lou et al., 2010; Zhao et al., 2010; Modi, 2011). Even though the literature is extensive on the antimicrobial studies of curcumin (Hariharan, 2012; Singh and Jain, 2012; Hamed et al., 2013) and its transition/inner transition metal complexes (Sumathi et al., 2012; Refat, 2013), reports are scanty on such studies of its complexes with main group metals. In continuation of our previous studies on metal complexes of curcumin and its derivatives (John and Krishnankutty, 2005, 2011), we report here the synthesis, structural characterization and some biological properties of Cd(II), Hg(II), Pb(II), Sn(II) and Ca(II) complexes of curcumin.

Results and discussion

The observed elemental analytical data (Table 1) of the metal complexes of curcumin (HL) suggest [ML_2] stoichiometry. All the complexes behave as nonelectrolytes [specific conductance <10 Ω^{-1} cm^{-1}; 10^{-3} m solution in dimethylformamide (DMF)]. Magnetic measurements indicate that all the complexes are diamagnetic in nature, as expected. The spectral data of the complexes are in conformity with Figure 1.

Infrared spectra

The infrared (IR) spectra of curcumin show a strong band at 1619 cm^{-1} and a broad band in the range 2800–3500 cm^{-1}

Table 1 Physical and analytical data of the metal complexes of curcumin.

Complex/ molecular formula	Color	Yield (%)	M.p. (°C)	Elemental analysis: found (calculated) (%)		
				C	H	M
$[CdL_2]$ $C_{42}H_{38}CdO_{12}$	Greenish yellow	58	>300	59.40 (59.55)	4.50 (4.49)	13.15 (13.28)
$[HgL_2]$ $C_{42}H_{38}HgO_{12}$	Dark red	82	>300	53.76 (53.93)	4.03 (4.07)	21.55 (21.46)
$[PbL_2]$ $C_{42}H_{38}O_{12}Pb$	Reddish brown	62	184	53.40 (53.55)	3.99 (4.04)	21.84 (22.01)
$[CaL_2]$ $C_{42}H_{38}CaO_{12}$	Red	56	170	65.26 (65.12)	4.88 (4.91)	5.15 (5.17)
$[SnL_2]$ $C_{42}H_{38}O_{12}Sn$	Black	43	>300	59.06 (59.18)	4.44 (4.46)	13.85 (13.94)

due to the stretching of the chelated carbonyl and the intramolecularly hydrogen-bonded enol functions, respectively (John and Krishnankutty, 2011). The absence of any band assignable to a normal α,β-unsaturated carbonyl group in the region 1640–1740 cm^{-1} indicates the existence of the compound entirely in the enolic form (Bellamy, 1980). In the IR spectra of the metal complexes, the band at 1619 cm^{-1} of the ligand is absent and, instead, a strong band assignable to the stretching of the coordinated carbonyl moiety appeared at ~1580 cm^{-1}. The broad band in the region 2800 to 3500 cm^{-1} disappeared in the spectra, indicating the replacement of enolic proton by the metal cation during complexation (Nakamoto, 1997; John and Krishnankutty, 2005). However, spectra of all the complexes exhibited bands at ~3400 cm^{-1} due to the stretching of the OH group in the phenyl ring (Nakamoto, 1997). This suggests that only the enol proton is replaced by metal ion and the phenolic OH is excluded from coordination. The prominent band at ~975 cm^{-1} is typical of a *trans* -CH=CH- group, which remained unaltered in the spectra of metal complexes (Nakamoto, 1997; John and Krishnankutty, 2005). That the carbonyl groups are involved in bonding with the metal ion as in Figure 1 is further supported by the appearance of two medium-intensity bands at ~420 and ~470 cm^{-1} assignable to $\nu_{M\text{-}O}$ (Nakamoto, 1997). Important bands that appeared in the spectra are given in Table 2.

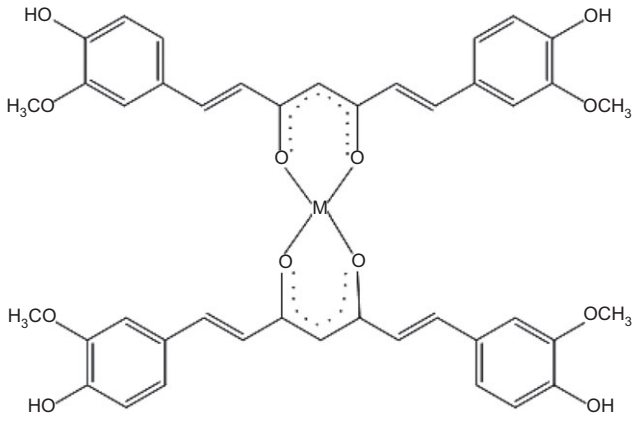

Figure 1 Structure of the metal complexes of curcumin. M=Cd(II), Hg(II), Pb(II), Sn(II) and Ca(II).

^{1}H NMR spectra

The ^{1}H NMR spectrum of curcumin displayed a one-proton signal at δ 16.30 ppm due to the intramolecularly hydrogen-bonded enolic proton (Roughley and Whiting, 1973; Jiang et al., 2011). The trans orientation of the -CH=CH- group is evident from the observed *J* values (16 Hz). In the ^{1}H NMR spectra of the diamagnetic complexes, the low field signal due to the enol proton of the ligand disappeared, indicating its replacement by the metal ion during complexation (John and Krishnankutty, 2005). The methine proton signal shifted appreciably to low field due to the deprotonation of the OH group. The integrated intensities of various signals agree well with Figure 1 of the complexes. That the phenolic (~δ 10 ppm) and methoxy (~δ 3.7 ppm) groups on the aryl ring are not involved in bonding with the metal ion is clearly indicated (John and Krishnankutty, 2005) in the spectra where the signals remain unaltered. The assignments of various proton signals observed are assembled in Table 2.

Mass spectra

The mass spectrum of curcumin (Baar et al., 1998; John and Krishnankutty, 2005) showed an intense molecular ion peak at *m*/*z* 368. The fast atom bombardment (FAB) mass spectra of the metal complexes showed intense molecular ion peaks corresponding to their formulae. Peaks correspond to successive removal of aryl groups, $[ML]^+$, L^+ and fragments of L^+ are also present in the spectra. Important fragments appeared in the spectra and their probable assignments are given in Table 3.

Table 2 IR and ^{1}H NMR spectral data of the metal complexes of curcumin.

Complex	IR stretching bands (cm^{-1})			^{1}H NMR spectral data (δ, ppm)			
	Chelated C=O	CH=CH *trans*	M-O	Methine	CH=CH	Phenyl	Aryl substituents
$[CdL_2]$	1574	970	422, 479	6.78	8.16, 8.26	7.02–7.98	10.02, 3.73
$[HgL_2]$	1582	978	418, 466	6.74	8.12, 8.20	6.90–7.80	10.04, 3.71
$[PbL_2]$	1589	978	424, 476	6.78	8.14, 8.22	6.98–7.78	10.06, 3.75
$[CaL_2]$	1585	966	424, 467	6.68	8.10, 8.20	7.12–8.02	10.04, 3.70
$[SnL_2]$	1586	980	426, 474	6.82	8.14, 8.20	7.22–7.90	10.08, 3.68

Ultraviolet spectra

The ultraviolet (UV) spectrum of curcumin shows two broad bands with maxima at 380 and 270 nm due to the various $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions (Bong, 2000). The spectra of the complexes bear close resemblance to that of the free ligand, indicating that no structural alteration of the ligand took place during complexation. However, the two free ligand spectral absorption maxima are shifted to longer wavelength in the spectra of the complexes. This indicates the involvement of the metal ion (John and Krishnankutty, 2005).

Antimicrobial studies

Various biological activities exhibited by curcumin have been the subject of numerous physiological and clinical studies (Negi et al., 1999; Ak and Gulcin, 2008; Anand et al., 2008; Mishra and Palanivelu, 2008; Jurenka, 2009; Martin et al., 2009; Wang et al., 2009; Singh et al., 2010; Wilken et al., 2011). In the present study, the antifungal and antibacterial activities of the metal complexes of curcumin were investigated. The data obtained in the presence of nistatin and the medium DMF are included in Table 4. The results clearly suggest that curcumin showed appreciable antifungal activity compared to nistatin. Metal complexation considerably increased the activity of the free ligand. Among the complexes, Sn(II) complex exhibited maximum activity toward *Aspergillus niger, Aspergillus heteromorphus, Penicillium verruculosum* and *Bacillus cereus*, whereas Hg(II) complex showed maximum activity toward *Aspergillus flavus*.

Conclusions

The Cd(II), Hg(II), Pb(II), Sn(II) and Ca(II) complexes of curcumin with $[ML_2]$ stoichiometry were synthesized and characterized by UV, IR, ^{1}H NMR and mass spectral data. In complexes, curcumin behaved as monobasic bidentate in which the intramolecularly hydrogen-bonded enolic proton is replaced by the metal ion. The compounds were investigated for their possible antimicrobial activities. The results clearly reveal that Sn(II) complex shows maximum activity toward *A. niger, A. heteromorphus, P. verruculosum* and *B. cereus*, whereas Hg(II) complex shows maximum activity toward *A. flavus*.

Table 3 Mass spectral data of the metal complexes of curcumin.

Assignments	*m/z*				
	$[CdL_2]$	$[HgL_2]$	$[PbL_2]$	$[CaL_2]$	$[SnL_2]$
P^+	846	935	941	774	853
$[ML]^+$	479	568	574	407	486
$[P\text{-}Ar]^+$	723	812	818	651	730
$[P\text{-}2Ar]^+$	600	689	695	528	607
$[P\text{-}3Ar]^+$	477	566	572	405	484
$[P\text{-}4Ar]^+$	354	443	449	282	361
L^+	367	367	367	367	367
Ligand fragments	244, 123	244, 123	244, 123	244, 123	244, 123

Table 4 Antimicrobial activity (diameter inhibition zone in millimeters) of curcumin (HL) and its metal complexes.

Compound	*P. verruculosum*			*A. niger*			*A. heteromorphus*			*A. flavus*			*B. cereus*		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
DMF	11	11	11	10	10	10	10	10	10	12	12	12	11	11	11
Nystatin	12	12	12	12	12	12	13	13	13	13	13	13	13	13	13
HL	14	16	20	15	16	21	15	16	22	13	14	19	15	17	24
$[CdL_2]$	18	19	23	13	15	20	18	21	24	20	22	27	19	20	26
$[PbL_2]$	15	17	22	13	14	19	15	17	22	19	21	26	14	16	20
$[HgL_2]$	17	18	23	19	21	25	17	19	24	24	26	31	17	18	25
$[SnL_2]$	22	23	27	20	22	26	19	21	26	15	18	24	20	21	27
$[CaL_2]$	17	19	22	15	17	23	15	17	21	14	14	21	16	17	23

a=500 ppm; *b*=1000 ppm; *c*=5000 ppm.

Experimental

Methods, instruments and materials

Carbon and hydrogen percentages were determined by microanalyses (Heraeus Elemental analyzer, RSIC, Central Drug Research Institute, Lucknow, India) and metal contents of complexes by atomic absorption spectroscopy (Perkin Elmer 2380, Euclid, OH, USA). The UV spectra of the compounds in methanol (10^{-4} mol/L) were recorded on a UV-Visible spectrophotometer (UV-1601 PC, Shimadzu, Kyoto, Japan), IR spectra (KBr discs) on an FTIR spectrophotometer (FT-IR-8101 A, Shimadzu, Kyoto, Japan), ^{1}H NMR spectra ($CDCl_3$ or DMSO-d_6) on a NMR spectrometer (Mercury Plus 300 MHz NMR spectrometer, Varian, USA) and mass spectra on a JEOL USA SX-102 mass spectrometer (FAB using argon and *meta*-nitrobenzyl alcohol as the matrix). Molar conductance of the complexes was determined in DMF (~10^{-3} mol/L) at 28±1°C. Magnetic susceptibilities were determined at room temperature on a Guoy type magnetic balance, Sherwood Scientific Ltd., UK.

Synthesis of curcumin

Curcumin was prepared by the condensation of vanillin with acetylacetone-boric oxide complex in ethylacetate medium in the presence of tri(*sec*-butyl)borate and *n*-butylamine as reported (Pabon, 1964).

Synthesis of metal complexes

A methanolic solution of metal acetate (0.001 mol, 25 mL) was added slowly with stirring to a methanolic solution of curcumin (0.002 mol, 25 mL). The mixture was refluxed gently for ~1 h and the volume was reduced to half. The precipitated complex on cooling to room temperature was filtered, washed with 1:1 aqueous methanol, recrystallized from hot methanol and dried in vacuum.

Determination of antimicrobial activity

The four different fungal strains used were *A. niger, A. flavus, A. heteromorphus* and *P. verruculosum*. The organism selected for antibacterial study was *B. cereus*. The well diffusion method (Prescott et al., 1990; Belkys et al., 2006) was employed for both antifungal and antibacterial studies. Nutrient agar medium was used for maintaining pure bacterial culture and to lawn the bacteria for detecting the antimicrobial activity. It was prepared by dissolving peptone (0.5 g), beef extract (0.3 g), NaCl (0.5 g) and agar (2 g) in 100 mL of distilled water. Potato dextrose agar (PDA) was used to maintain pure fungal culture and to lawn fungus for detecting antimicrobial activity. It was prepared by dissolving PDA (0.36 g) and agar (2 g) in 100 mL of distilled water. Normal saline was used as a suspension of fungal/bacterial spores for lawning. It was prepared by dissolving NaCl (0.95 g) in 100 mL of distilled water. Solutions of the test compounds were prepared in DMF, and for sterilizing, all the media used were autoclaved at 121 °C for 20 min. The Petri plates were made with PDA media for antifungal activity. The PDA solution was poured into the Petri plates after autoclaving at 121 °C for 20 min. Plates were allowed to dry for 20 min. Using an agar punch, wells (10 mm) were made on these plates. The fungal suspensions of spores were prepared in normal saline.

To prepare the mat growth of fungi on Petri plates, this spore suspension was swabbed on the surface of the plates and the compounds in DMF were added into each well at different concentrations (500 ppm, 1000 ppm, 5000 ppm) for each sample. Each plate has a well for the control (solvent DMF) and one for the reference antibiotic nistatin. These wells were properly labeled, and the plates were incubated at room temperature for 24–28 h. The antifungal activity was detected by measuring the diameter of the inhibiting zone around each well (mm). The greater diameter shown by the compound other than the control indicates their antifungal activity. In the case of antibacterial activity, a uniform lawn of bacteria *B. cereus* was spread evenly in the Petri plates as in the case of fungi. Compounds in DMF were placed in the well and the antibacterial activity was measured from the diameter of the zone of inhibition (mm).

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