

Synthetic, Spectroscopic And Antibacterial Studies Of Fe(II),Co(II),Ni(II),Cu(II),and Zn(II)Mixed Ligand Complexes Of Nicotinamide And Cephalexin antibiotics

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Abstract

Mixed ligand complexes of bivalent metal ions, viz; M= Co(II), Ni(II), Cu(II) and Zn(II) of the composition [M(Ceph)(NA)₃]Cl in 1:1:3 molar ratio, (where Ceph = Cephalexin and NA = Nicotinamide have been synthesized and characterized by repeated melting point determination, Solubility, Molar conductivity, determination the percentage of the metal in the complexes by flame(AAS), FT-IR, magnetic susceptibility measurements and electronic spectral data. The ligands and their metal complexes were screened for their antimicrobial activity against six bacteria(gram +ve) and (gram -ve).

Key words:(Cephalexin antibiotics, Nicotinamide ,mixed ligand) Complexes, Antibacterial activities, and spectral studies

1. Introduction

Semi-synthetic β -lactamic antibiotics are the most used anti-bacteria agents, produced in hundreds tons/year scale. It may be assumed that this situation will even increase during the next years, with new β -lactamic antibiotics under development. The wider use of antibiotics in humans and animals and in areas other than the treatment and prophylaxis of disease have resulted in a serious problem of drug resistance. Various strategies have been worked out and tried to cope with the resistance problem and enhance the activity, or broaden the spectrum of drugs [1].In 2003 Juan Anacona and Ibranyellis[2],Synthesized cephalexin with transition and d10 metal ions have been investigated. The complexes [M(cepha)Cl].nH2O [M= Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II)] were characterized by physicochemical and spectroscopic methods. The IR and 1 HNMR spectra of the complexes suggest that cephalexin behaves as a monoanionic tridentate ligand. In vitro antibacterial activities of cephalexin and the complexes were tested .

Icbudak and co-workers [3], are reported the mixed-ligand p-hydroxybenzoate complexes of Ni(II),Cu(II) and Zn(II)with nicotinamide andN,Ndiethylnicotinamide were synthesized and characterized by elemental analysis, magnetic susceptibility measurements and mass spectrometry. When a metal ion combines with a ligand (Drug), the resulting substance is said to be a complex. If the ligand (Drug) which combine with the metal forms one or more rings with the metal, the resulting structure is said to be a metal chelate and the process is known as chelation [4]. El-Said and co-workers [5], Synthesized Mixed ligand complexes of Zn(II) and Cd(II) containing ceftriaxone (Naceftria) or cephradine (Hcefphr) antibiotics and other ligands have been prepared and characterized by elemental analysis, spectral, biological and thermal studies.

The complexes have the general formulae: [Zn(ceftria)(diamine)(OAc)].xH₂O, [M(ceftria)(L)(H₂O)₂].xH₂O,[Cd₂(cephr)(diamine)Cl₃(H₂O)].xH₂O,[Cd₃(cephr)(μ HL)Cl₅(H₂O)].2H₂O where diamine= 2,2'-bipyridyl or o-phenanthroline; M= Cd(II) or Zn(II), L= glycine,



proline or methionine and x=0-6. Ceftriaxone chelates to the metal ions as a bidentate monoanion ligand through the β - lactam carbonyl and carboxylate group. The complexes containing O,N donor atoms are very important owing to their significant antibacterial and anticancer activity [5-9]. The study of mixed ligand complex formation is relevant in the field of analytical chemistry, where the use of mixed ligand complexes allows the development of methods with increased selectivity and sensitivity. They have also great importance in the field of biological and environmental chemistry [10]

The work presented in this paper concerns the preparation, characterization and biological evaluation of Fe(II),Co (II), Ni (II),Cu(II), Zn(II)Mixed Ligand Complexes Of Nicotinamide and Cephalexin in 1:3:1 ratio.

2. Experimental

2.1. Chemicals

All chemicals used were of reagent grade (supplied by either Merck or Fluka , and used as received. , ethanol, methanol and dimethylforamaide , and KBr, from (B.D.H). Cephalexin mono hydrate powder DSM (Spain) and Nicotinamide from Riedial-Dehaen.

2.2. Instrumentals

UV-Vis spectra were recorded on a (Shimadzu UV- 160A) Ultra Violet-Visible Spectrophotometer. IR- spectra were taken on a (Shimadzu, FTI R- 8400S) Fourier Transform Infrared Spectrophotometer (4000- 400) cm⁻¹ with samples prepared as KBr discs. Metal contents of the complexes were determined by atomic absorption(A.A)technique using a Shimadzu AA 620G atomic absorption spectrophotometer. The Chloride contents of complexes were determined by potentiometric titration method using (686-Titro processor-665. Dosimat Metrohn Swiss). Conductivities were measured for 10⁻³M of complexes in DMSO at 25°C using (conductivity meter, Jewnwary, model 4070). Magnetic measurements were recorded on a Bruker BM6 instrument at 298°K following the Farady's method .. In addition melting points were obtained using (Stuart Melting Point Apparatus). The proposed molecular structure of the complexes were drawing by using chem. office prog, 3DX (2006).

(2.3) Synthesis of (Mixing ligands) complexes with some metal ions

(2.3.1) Synthesis of [Cu(Ceph)(NA)₃]Cl complex: [2, 11]

A solution of (Ceph) (0.366 gm, mmole) in methanol (5 ml) with KOH (0.05 gm) and a solution of (NA) (0.366 gm, mmole) in methanol (5 ml) were added to stirred of Cu(II) Chloride dihydrate (0.17 gm, mmole) in methanol (5 ml). The resulting mixture was stirred for (4 hours). Then the mixture was filtered and washed with an excess of ethanol and dried at room temperature during (24 hours). A green solid was obtained, m.p (196-200 °C) ,yield 87% . (2.3.2) Synthesis of $[Fe(Ceph)(NA)_3]Cl$, $[Co(Ceph)(NA)_3]Cl$, $[Ni(Ceph)(NA)_3]Cl$, and $[Zn(Ceph)(NA)_3]Cl$, complexes .

The method used to prepare these complexes was similar method to that mentioned in preparation of $[Cu(Ceph)(NA)_3]Cl$ complex in paragraph (2.3.1), but obtained a solution complexes with Fe(II), Co(II), Ni(II), dried them, and washed with an excess of ethanol and dried at room temperature during (24 hours). yield 83% . (2.3.1) Synthesis of $[Cu(Ceph)(NA)_3]Cl$ complex:

A solution of (Ceph) (0.366 gm, mmole) in methanol (5 ml) with KOH (0.05 gm) and a solution of (NA) (0.366 gm, mmole) in methanol (5 ml) were added to stirred of Cu(II) Chloride dihydrate (0.17 gm, mmole) in methanol (5 ml). The resulting mixture was stirred for (4 hours). Then the mixture was filtered and washed with an excess of ethanol and dried at room temperature during (24 hours). A green solid was obtained, m.p (196-200 °C), good yield. The complexes of the mixed ligands (cephalexin monohydrate, as primary ligand with nicotinamide as a secondary ligands with M(II) = Fe(II), Co(II), Ni(II), Cu(II), and Zn(II) are prepared by shown in (**Figure. 1**).

2.4. Antimicrobial activities

Antimicrobial activities of the ligands and **synthes**ed complexs have been carried out against the pathogenic bacteria like Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa, using nutrient agar medium by disc diffusion method [8]. The test solution were prepared in DMSO and soaked in filter paper of 5



mm diameter and 1mm thickness. These discs were placed on the already seeded plates and incubated at 37 o C for 24 h. The diameters (mm) of the inhibition zone around each disc were measured after 24 hours. [2-12].

(Figure. 1): Schematic representation of synthesis of Mixed ligand complexes

3. Results and Discussion

3.1. Characterization of Metal Complexes.

Generally, the complexes were prepared by reacting the respective metal salts with the ligands using 1:1:2 mole ratio, i.e. one mole of metal salt: one mole of cephalexin and two moles of nicotinamide.

The formula weights and melting points, are given in (Table I). Based on the physicochemical characteristics (Table I), it was found that all the complexes were non-hygroscopic, stable at room temperature and appears as powders with high melting points. The solubility of the complexes of ligands was studied in various solvents (Table 2). They are not soluble in water . All complexes are soluble in dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) solvent (Table 2). The complexes were analyzed for their metal by atomic absorption measurements and chloride contents were determined by standard methods [16]. (Table-1) for



all complexes gave approximated values for theoretical values. The observed molar conductance (Table 1) values measured in DMSO in 10^{-3} M solution lie in the .(33-37 Ω^{-1} cm² mol⁻¹) range, indicating their electrolytic nature with(1: 1). [13].

The atomic absorption measurements and Chloride contents (Table-1) for all complexes gave approximated values for theoretical values.

FT-IR spectra data:

In general, the complexes exhibited very comparable I.R features suggesting that they were of similar structure. Relevant bands were given in (Table-3).

FT-IR of [Fe(Ceph)(NA)₃]Cl(1), [Co(Ceph) (NA)₃] Cl (2) ,[Ni(Ceph)(NA)₃]Cl(3), [Cu(Ceph)(NA)₃]Cl(4), [Zn(Ceph)(NA)₃]Cl(5) complexes:[15-18]

The (FT-IR) spectrum for the free ligand (Ceph.), which exhibits band at (3275), and (3367, 3159) cm⁻¹ due to v(N-H) primary amine stretching vibration, on complexation this band has been shifted to higher frequency (3367), (3410), (3475), (3402), and (3541) cm⁻¹ for complexes (1), (2), (3), (4), and (5), :[15] showing that the coordination is through the Nitrogen atom of amine group. The band at (1699) cm⁻¹ is due to v(C=O) cm⁻¹ stretching vibration for (amide), and the band at (1759) cm⁻¹ is due to v(C=O) cm⁻¹ stretching vibration for (COOH), but in the complexes was at (1667), (1666), (1697), (1757, 1707), and (1759) cm⁻¹ for complexes (1), (2), (3), (4), and (5). The band at (1689) cm⁻¹ stretching vibration is due to v (C=O) for β -Lactum group, but these band has been shifted to lower frequency (1678), (1666), (1681), (1670, 1624), and (1666, 1631) cm⁻¹ for complexes (1), (2), (3), (4), and (5), showing that the coordination is through the Oxygen atom of β-Lactum group. :[17-18] The bands at (1595), and (1398) cm⁻¹ were assigned to stretching vibration (COOH) asymmetric and symmetric stretching vibration, respectively, on complexation these bands have been shifted to lower and higher frequencies [(1620), (1620), (1604), (1604), and (1616) cm⁻¹ for $v_{as}(COO^-)$], and $[(1396), (1392), (1392), (1379), and (12392) cm⁻¹, for <math>v_s(COO^-)]$ for the compounds (1), (2), (3), (4), and (5), showing that that the coordination with metal was occurred through the oxygen atom of carboxylate ion. The pyridyl C-C and C-N stretching vibration absorptions are observed in the form of medium bands between 1400 and 1575 cm-1. The bands in the region of 1600–1230 cm-1 can be assigned to the CH in-plane or out-of - plane bend, ring ,breathing and ring deformation absorptions of the pyridine ring of nicotinamide. The bands at (493), (497), (455), (487), and (459)cm⁻¹ were assigned to v(M-O) for compounds (1), (2), (3), (4), and (5), indicating that to the carbocylic oxygen, and oxygen of β-Lactam group of the ligand are involved in coordination with metal ions. The bands at (540), (563), (563), (549), and (536) cm⁻¹ ¹ were assigned to v(M-N) for compounds (1), (2),(3),(4),and (5) respectively, indicating that the amine nitrogen is involved in coordination with metal ions.

The UV-Visible Spectroscopy and Magnetic measurements for the mixed ligand complexes : $[Fe(Ceph)(NA)_3]Cl$, $[Co(Ceph)(NA)_3]Cl$, $[Ni(Ceph)(NA)_3]Cl$, $[Cu(Ceph)(NA)_3]Cl$, and $[Zn(Ceph)(NA)_3]Cl$ complexes:

The magnetic moments of the complexes shown in Table (4).were calculated from the measured magnetic susceptibilities after employing diamagnetic corrections. The electronic spectral data of



the complexes are summarized in (4) together with the proposed assignments and suggested geometries. The results obtained are in good agreement with other spectra and the literature.[19-26] [Fe(Ceph)(NA)₃] Cl:

The(U.V- Vis) spectrum, exhibits two peaks , the first high intense peak at (278 nm)(37037cm⁻¹)(ϵ_{max} =1645 molar⁻¹.cm⁻¹) is due to the (C.T) , while the second weak peaks at(990 nm)(10101 cm⁻¹)(ϵ_{max} =18 molar⁻¹.cm⁻¹), which assigned to (${}^5T_{2g} \rightarrow {}^5E_g$), transition and μ eff = 4.97 μ B, which suggests an octahedral geometry around the central metal ion. :[19-21] [Co(Ceph)(NA)₃] Cl:

The (U.V- Vis) spectrum, exhibits four peaks . The assignment of the electronic spectral bands, their positions, and the spectral parameters for Co(I1) $4T1(F) \rightarrow 4T2g(F)$ $\upsilon 1= (992 \text{ n nm})$ 10080 cm⁻¹ , ($\varepsilon_{max} = 14 \text{ molar}^{-1} \cdot \text{cm}^{-1}$), $4T1(F) \rightarrow 4A2$ g, $\upsilon 2= (833 \text{nm})$ 12004cm⁻¹, ($\varepsilon_{max} = 5 \text{ molar}^{-1} \cdot \text{cm}^{-1}$), ,(F) $\rightarrow 4T1$ g , $\upsilon 3= (728 \text{nm})$ 13736 cm⁻¹, ($\varepsilon_{max} = 7 \text{ molar}^{-1} \cdot \text{cm}^{-1}$), $\upsilon_2/\upsilon_1 = 1.19$, $\upsilon_1/\upsilon_2 = 0.38$, $\Delta/B = 10.38$ from Tanabe-Sugano diagram for d⁷ octahedral field the value of 10Dq equal to 10.080 cm⁻¹, $B' = 950 \text{ cm}^{-}$, $\beta = 0.978 \text{ cm}^{-1}$, The Co(II) complex under present investigation process interelectronic repulsion parameter (B') 950 cm⁻¹. The Racha parameter (B) is less than free ion value (971) suggesting a considerable orbital overlap and delocalization of electrons on the metal ion. The nephelauxetic ratio (β) for the present Co(II) complex (0.974). This is less than one, suggesting partial covalency in the metal ligand bond. suggest the octahedral geometry for Co(II) complex and fourth high peak at (268 nm)(37313 cm⁻¹)($\varepsilon_{max} = 1489 \text{ molar}^{-1} \cdot \text{cm}^{-1}$) is due to the (C.T), The room temperature magnetic moment ($\mu_{eff} = 4.54 \, \mu \text{B}$) corresponded to octahedral symmetry.

[Ni(Ceph)(NA)₃] Cl:

The (U.V- Vis) spectrum, exhibits three peaks. The assignment of the electronic spectral bands for, Ni(II) are ${}^3A2g \rightarrow {}^3T2$,(F) $\upsilon 1$ =(993nm) 10070cm-1)(ε max =19 molar-1.cm-1), 3A2g (F) \rightarrow 3T1g(F) $\upsilon 2$ = (800nm) 12500 cm⁻¹)(ε max =11 molar⁻¹.cm-1) , and (267nm) 37453 cm-1 for ligand filed transitions ,10Dq=10070 cm⁻¹, B'=966 cm⁻¹, β =0.93. Ni (II) complex exhibited a value of μ eff = 3.18 μ B, which suggests an octahedral geometry around the central metal ion. [2,19,22,23]

[Cu(Ceph)(NA)₃] Cl:

The (U.V- Vis) spectrum, exhibits two peaks , the first peak at (271 nm)(36900 cm⁻¹) ($\epsilon_{max} = 1883 \text{ molar}^{-1}.\text{cm}^{-1}$) is due to the (LMCT), while the second weak peaks at (994 nm)(10060 cm⁻¹)($\epsilon_{max} = 30 \text{ molar}^{-1}.\text{cm}^{-1}$), which assigned to ($2Eg \rightarrow 2T2g$) transition in an octahedral geometry . [2,13,24,] Cu(II) complex exhibited a value of μ eff =1.86 μ B, which suggests an octahedral geometry around the central metal ion. [2,21,25]

[Zn(Ceph)(NA)₃] Cl:

The (U.V- Vis) spectrum, exhibits one high peak at (270 nm)(37037 cm⁻¹)(smax =1556 molar⁻¹.cm⁻¹) is due (C.T) The Zn(II), complex did not display any peak in the visible region, no ligand field absorptions band was observed, therefore the bands appeared in the spectrum of complex could be attributed to charge transfer transition. in fact this result is a good agreement with previous work of octahedral geometry. Magnetic susceptibility measurements for Zn(II) (d¹⁰)(white complex) showed diamagnetic as expected from their electronic configuration . [2,15,21]

Discussion of the biological effect results for prepared compounds:



The newly synthesized metal complexes were screened in vitro for their antibacterial activity against bacteria: Staphyloccus aureus, E-coli , Klebsiella, Salmonella Typhi, Acinetobacter baumannii and Pseudomonas. The zone of inhibition of the ligands and their complexes against the growth of bacteria were given in Table (5), (Figure. 2) were tested utilizing the agar diffusion technique [26]. The antibacterial activity results revealed that the ligands and there complexes (DMF) . mixed ligand metal—shown weak to good activity when compared to the Control complexes have higher biological activities compared to the free ligands—. The increased inhibition activity of the metal complexes can be explained on the basis of Tweedy's chelation theory [27] . In metal complexes, on chelation the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π - electrons over the whole chelate ring. The ring of nicotinamide moiety makes the complexes more lipophillic[28] .

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Table (1) The physical properties of the compounds

				М.р°с (de) °c	$\frac{\Lambda_{\mathbf{m}}}{\Omega^{-1} \text{ cm}^2 \text{ mol}^2}$	Metal%	Cl%
Compounds (Chemical Formula)	M. wt Calc	Color	Yield %			Theory (exp)	Theory (exp)
[Fe(Ceph)(NA) ₃]Cl [FeC ₃₄ H ₃₆ N ₉ O ₇ S] Cl	806.07	Brown	73	101	37	6.9 3 (7.0)	4.40 (4.92)
[Co(Ceph)(NA) ₃]Cl [Co C ₃₄ H ₃₆ N ₉ O ₇ S] Cl	809.16	Brown	83	100	35	7.28 (7.1)	4.39 (4.65)
[Ni(Ceph)(NA) ₃]Cl [Ni C ₃₄ H ₃₆ N ₉ O ₇ S] Cl	808.92	Deep Bro	80	300 Dec	33	7.26 (7.16)	4.39 (4.72)
[Cu(Ceph)(NA) ₃]Cl [Cu C ₃₄ H ₃₆ N ₉ O ₇ S] Cl	810.13	Green	90	200	34	7.83 (8.87)	4.37 (4.62)
[Zn(Ceph)(NA) ₃]Cl [Zn C ₃₄ H ₃₆ N ₉ O ₇ S] Cl	811.13	Yellow	82	200	34	8.04 (8.82)	4.36 (4.72)

 $\Lambda m = Molar Conductivity$, Dec. =decomposition,

Table (2) The solubility of the mixed ligands complexes in different solvents

Compounds	H_2O	DMF	DMS	MeOH	EtOH	Aceton	C_6H_6	CCl ₄	CHCl	2-propar	Petroleum ether
[Fe(Ceph)(NA) ₃]Cl	+	+	+	÷	÷	÷	1	-	-	-	-
[Co(Ceph)(NA) ₃]Cl	+	+	+	÷	÷	÷	1	-	-	-	-
[Ni(Ceph)(NA) ₃]Cl	-	+	+	÷	÷	÷	-	-	-	-	-
[Cu(Ceph)(NA) ₃]Cl	+	+	+	÷	÷	÷	-	-	-	-	-
[Zn(Ceph)(NA)3]Cl	-	+	+	÷	÷	÷	-	_	-	_	-

(+) Soluble , (−) Insoluble , (÷) Sparingly



Table(3):Data from the Infrared Spectra for the Free Lingands and there Metal Complexes (cm-1).

Compound		υ(N-H)	υC=O)	υ(C=C	v(C	บล) sy	υΔ	v(C	υ((υ(0	υ(0	υ(0	υ(N	υ(N
	(N-H)	Secondary a		β-lacta	an	C	C		ar			ar	ali		
	orimary a							-COO)asy							
Cephalexir	3275	3219	1759	1689		15	13	197	15	12	12	30	28		
		3049													
Nicotine ami	3367, 3				16			-	15	12		30	27		-
Fe(Ceph)(NA)	3367	3186	1667 ove	1678		16	13	224	15	12	12	30	27	5.	4
Co(Ceph)(NA)	3410	3323 3190	1666 ove	1666		16	13	243	14	12	12	30	38	5	4
Ni(Ceph)(NA)	3475	3414 3236	1697	1681		16	13	224	15	12	12	30	28	5	4
Cu(Ceph)(NA)	3402	3266 3167	1757 1707	1670 1624		16	13	227	15	12	12	30	28	5.	4
Zn(Ceph)(NA)	3541	3468 3236	1759	1666, 1		16	13	224	15	12	11	30	28	5	4



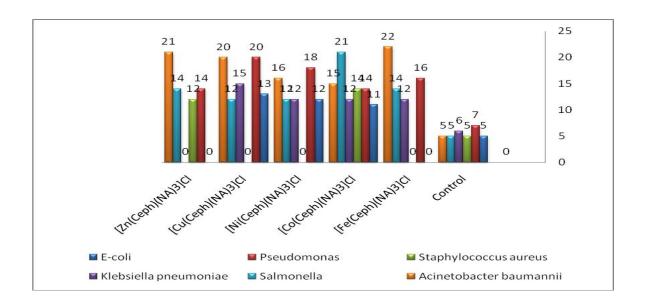
Compound	λnm	ν́ cm	ε _{max} (molar ⁻¹ .cm ⁻¹)	Assignments	μ _{eff} (μ Β)	Probable figur
Ceph	279	35842	770	π→π*	-	-
NA	220	45454		$\pi \rightarrow \pi^*$	-	-
	261	38314	1104	$\pi \rightarrow \pi^*$		
[Fe(Ceph)(NA) ₃] Cl	278	3703′	1645	C-T	4.97	Octahedra
	990	1010	18	${}^{5}\mathrm{T}_{2\mathrm{g}} \rightarrow {}^{5}\mathrm{E}_{\mathrm{g}}$		
[Co(Ceph)(NA) ₃] Cl	268	37313	1489	C-T	4.54	Octahedra
	728	13730	7	$3A2g \rightarrow 4T1g(P)$		
	833	12004	5	$3A2g \rightarrow 4T1g(P)$		
	992	10080	14	$3A2g(F) \rightarrow 3T1g(F)$		
[Ni(Ceph)(NA) ₃]Cl	267	3745	1319	L-F	83.1	Octahedra
	800	12500	11	$3A2g \rightarrow 4T1g(P)$		
	993	10070	19	$^{3}\text{A2g} \rightarrow ^{3}\text{T2,(F)}$		
[Cu(Ceph)(NA) ₃] Cl	271	36900	1883	CT	1.86	Octahedral
	994	10060	30	$2Eg \rightarrow 2T_{2g}$	1.00	
[Zn(Ceph)(NA) ₃]Cl	270	3703′	1556	C-T	0.00	Octahedral

Table (4) Electronic data for the mixed ligands complexes

Table(5):Biological activity of the mixed ligands complexes(Zone of inhibition) (mm)

Compound	E-col	Pseudomonas	Staphylococcus aure	Klebsiella pneumonia	Salmonella Typhi	Acinetobacter baumannii
Control	5	7	5	6	5	5
[Fe(Ceph)(NA) ₃]Cl	0	16	0	12	14	22
[Co(Ceph)(NA) ₃] Cl	11	14	14	12	21	15
[Ni(Ceph)(NA) ₃] Cl	12	18	0	12	12	16
[Cu(Ceph)(NA) ₃] Cl	13	20	0	15	12	20
[Zn(Ceph)(NA) ₃] Cl	0	14	12	0	14	21





(Figure. 2) Chart of biological effects of some of the studied complexes

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