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Environmentally Benign Synthesis, Characterisation and Antimicrobial Activities of Co (II)-Amino Acid Complexes

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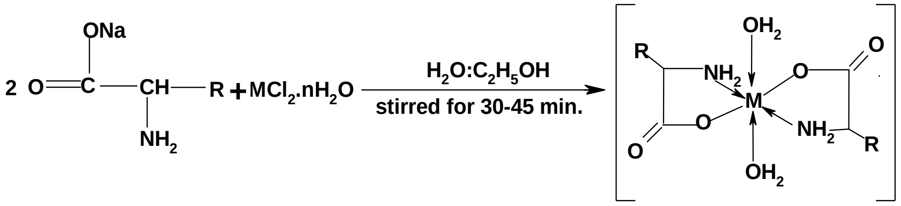
ABSTRACT

A rapid, clean and environmentally benign exclusive synthesis of some novel Co(II) complexes with non-essential biologically active α-amino acids vis. L-Asparagine (Asn or N), L-Glutamine(Gln or Q) and L-Aspartic acid or Aspartate (Asp or D) efficiently in aqua-ethanol medium with excellent yields has been described here. The elemental analysis and conductometric measurement of the investigated complexes reveal their 1:2 metals to ligand stoichiometry and non-electrolytic nature. The spectrochemical studies reveal that the complexation of mono-negative bidentate ligands with Co (II) ion undergoes through N of α-amine and β-carboxylate oxygen yielding six coordinated complexes with octahedral geometry around the central metal ion. The synthesized complexes were found to be more active compared with their respective free ligands against the same microorganisms and under the identical experimental conditions and in some cases better activity compared to the standard.

Graphical Abstract



Keywords: Cobalt (II) complexes, α-amino acids, antimicrobial activity, octahedral geometry.

INTRODUCTION

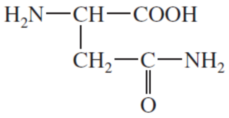
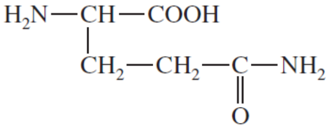
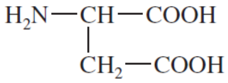
The twenty common α-amino acids are the standard amino acids (L-amino acids) that are the structural and chemical units of nearly all proteins and thus all living organism and are essential for various biochemical processes [1]. These are very good chelating agents and can coordinate to transition metal ions through their amino or carboxylic groups. During the last few decades the complexation of transition metal ions with amino acids have been studied extensively [2-3].

The amino acid-metallic ion interactions are found to be responsible for enzymatic activity and stability of protein structures. The metal-amino acid complexation is important field of study as they can be used as representative model systems to understand the metal-protein interaction in biological systems and metabolic enzymatic activities [4].

Cobalt (II) is a biologically active, essential ion; its chelating ability and positive redox potential allow participation in biological transport reactions along with the most potent antiviral, antitumor and anti-inflammatory actions [5]. These biological activities have provided an impetus to the study Co(II) complexes of some novel amino acids in general and have prompted us to update the structural-activity correlations [6]. Therefore we considered it necessary to study the effects of the possible varying structures of Co (II) - complexes of some amino acids and their anti-microbial activity, as this would yield information useful for designing anti-microbial agents.

The present work is in continuation of our research on coordination complexes of drug derivatives with transition metals and evaluating their biological activity [7-8]. The aim of the present study was to evaluate the antimicrobial properties of eco-friendly synthesized a range of cobalt (II) complexes with different amino acids comparing with free ligands ligands (L-*Asn*, L-*Gln* and L-*Asp*)[Table 1].

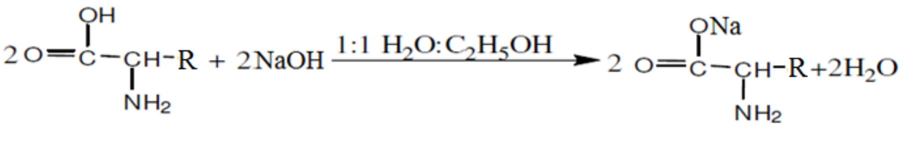
Table 1: Amino Acids used as Ligands

ame	**L-Asparagine**	**L-Glutamine**	**L-Aspartic Acid**
Structure	 $H_2N-CH(CH_2-C(=O)-NH_2)-COOH$	 $H_2N-CH(CH_2-CH_2-C(=O)-NH_2)-COOH$	 $H_2N-CH(CH_2-COOH)-COOH$
Symbol	N	Q	D
Abbreviation	*Asn*	*Gln*	*Asp*
Isoelectric Point	5.4	5.7	2.8

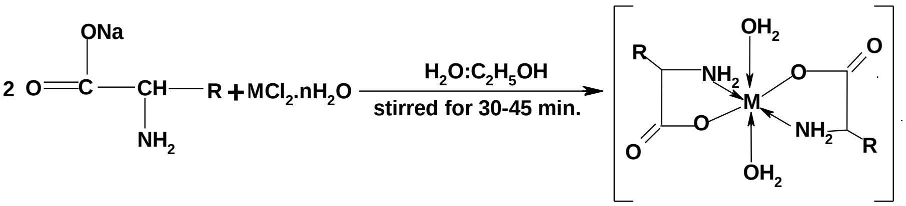
MATERIALS AND METHODS

The salt $CoCl_2.2H_2O$ used in this study was of AR grade and purchased from E. Merck. The amino acids *L-Asparagine (Asn or* N), *L-Glutamine (Gln or* Q*) and L-aspartic acid or Aspartate (Asp or* D*)* were purchased from Loba Chemie which were used as received without further purification. The metal salt and amino acid solutions were prepared by direct dissolution in doubly distilled water.

Synthesis of Co-Complexes: A solution of $CoCl_2.2H_2O$ (1 mmol) in 1:1 mixture of ethanol and water (10 mL) was added to a solution of the ligand (4 mmol) in 20 mL H_2O/ethanol(50%) mixture containing 0.33 mL 30% NaOH (for deprotonation of the amino acids, scheme-1) [4] using stoichiometric amount (1:2) [(metal: 2(Na^+ L)] molar ratio. The reaction mixture was stirred for several minutes (30-45 minutes) at room temperature. After 1-2 h a coloured crystalline solid was obtained which was filtered and washed with water-ethanol then triethyl ether .The solids were recrystalized from (H_2O: DMSO) (30:70) volumes' mixture and dried in vacuum over anhydrous $CaCl_2$ (Scheme-2) at 60^OC. The yields range from 80 to 90 %. The compounds were found to be soluble in DMSO and hot water.



Scheme 1: Deprotonation of used amino acid ligands



where R = $CH_2.CONH_2$ for N (*Asn*), $CH_2.CH_2.CONH_2$ for Q (*Gln*), R = $CH_2.COOH$ for D (*Asp*) and M = Co(II)

Scheme 2: Schematic representation of synthesis of the Co(II)-Complexes

Instrumentation: Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. All compounds were analysed satisfactorily for C, H and N using Carl-Ebra 1106 elemental analyser in micro-analytical laboratory. Metal content was estimated complexometrically by standard procedure [9-10]. Molar conductance measurements were conducted using 10^{-3}M solution of the complexes in water on Elico-CM 82 Conductivity Bridge at room temperature. Guoy balance at room temperature using mercuric tetrathiocyanato cobaltate (II) as the calibrant. Diamagnetic corrections were applied in compliance with Pascal's constant [11]. The pH of the solutions was measured by using Elico Li-120 pH meter.

The electronic absorption spectra of all the complexes in DMSO solution (10^{-3} M) in the ultraviolet and visible region were recorded on Shimadzu UV/VIS-160 spectrophotometer. The IR spectra were recorded in KBr discs on a Perkin Elmer Spectrometer-577 in wave number region 4000-200 cm^{-1}. The proton nuclear magnetic resonance spectra of complexes were taken in $CDCl_3$ or D_2O (wherever solubility permitted this) using Hitachi R-600 FT NMR spectrophotometer. Tetramethylsilane (TMS) was employed as the internal reference.

Antibacterial screening by Agar cup method: The strains used were *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus subtilis*, and one Methicillin resistant *S. aureus* clinical isolate for bacteria and *C. albicans* for fungi. In the agar cup method, a single compound can be tested against number of organisms or a given organism against different concentrations of the same compound. The method was found suitable for semisolid or liquid samples and was used in the present work. In the agar cup method, a plate of sterile nutrient agar with the desired test strain was poured to a height of about 5 mm, allowed to solidify and a single cup of about 8 mm diameter was cut from the centre of the plate with a sterile cork borer. Thereafter, the cup was filled with the sample solution of known concentration and the plate was incubated at 37^0C for 24 h. The extent of inhibition of growth from the edge of the cup was considered as a measure of the activity of the given compound. By using several plates simultaneously, the activities of several samples were quantitatively studied. All the tests were performed in triplicate.

RESULTS AND DISCUSSION

Characterization of metal complexes: The synthesis of mixed ligand Co(II) complexes may be represented in scheme 2. All the complexes are coloured, non-hygroscopic and thermally stable solids (Table 2). These properties indicate the strong metal-ligand bond. The complexes are insoluble in common organic solvents such as ethyl alcohol, acetone, etc., but are fairly soluble in DMSO and in hot water. The elemental analysis data (Table 2) of metal complexes are consistent with their general formulation as 1:2, monomeric complexes of the type $[Co (L)_2(H_2O)_2]$. The molar conductance values of the complexes in DMSO at 10^{-3} M concentration are very low indicating their non-electrolytic nature [12]. The melting points or decomposition temperatures for the complexes are shown in table-2. Most of the complexes decomposed before melting.

Magnetic studies: **T**he magnetic moments of the investigated metal complexes were calculated from the measured magnetic susceptibilities after employing diamagnetic corrections and revealed their paramagnetic nature. The observed values for effective magnetic moment (μ_{eff}) in BM, reported in Table 3, suggest the octahedral geometry for cobalt (II) complexes [13]. The magnetic moments of the complexes investigated were slightly greater than spin only values due to spin-orbit coupling and supports the conclusions.

Table 2: Empirical formula, molecular weight, colour, decomposition temperature and pH of the investigated cobalt complexes

Complex	Empirical Molecular	formula weight	Colour	Decomposition temperature (^{O}C)	pH	% Yield
[Co(Asn)$_2$2H$_2$O]	[Co(C$_4$H$_7$N$_2$O$_3$)$_2$]	320.93	Blue	223	**7**	80
[Co(Gln)$_2$2H$_2$O]	[Co(C$_5$H$_9$N$_2$O$_3$)$_2$]	348.93	Blue	208	6	86
[Co(Asp)$_2$2H$_2$O]	[Co(C$_4$H$_6$NO$_4$)$_2$]	322.93	Lilac	217	**6**	90

Table 3: Elemental analysis data, molar conductance and magnetic moments of investigated cobalt complexes

Complex	Elemental analysis Found (Calcd.)				Molar conductance (ohm^{-1} cm^2 mol^{-1})	μ_{eff} (in BM)
	%M	%C	%H	%N		
[Co(Asn)$_2$2H$_2$O]	18.40 (18.36)	30.05 (29.91)	4.40 (4.36)	17.50 (17.45)	0.18	3.87
[Co(Gln)$_2$2H$_2$O]	17.02 (16.88)	34.44 (34. 39)	5.20 (5.16)	16.15 (16.04)	0.24	3.86
[Co(Asp)$_2$2H$_2$O]	18.30 (18.25)	29.80 (29.73)	3.75 (3.72)	8.60 (8.67)	0.34	3.89

Electronic Spectral Studies: The electronic spectra of the ligands showed three absorption bands at 187-196, 210-216, and 223-232 nm assigned as the $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the major chromophores, NH$_2$ and COO−, present in the ligand molecules. On coordination, however, shifts were observed in these bands in addition to *d-d* transitions bands (Table 4). These in conjunction with the magnetic moment of the complexes were used to propose probable geometry of the complexes obtained.

The electronic spectra of the investigated complexes in DMSO were recorded in the UV-visible region. The spectra of the investigated cobalt (II) complexes displayed two bands at 526 and 476-480 nm, assigned to $^4A_{2g} \rightarrow {}^4T_{2g}$ (F) and $^4A_{2g}$ (F) $\rightarrow {}^4T_{1g}$ (P) *d-d* transitions. This is the indicative that the investigated complexes were the mononuclear complexes with 6-coordinate octahedral geometry [14]. This proposed geometry was corroborated by their magnetic moment value of 3.86 -3.89 BM. The important electronic spectral bands along with their assignments of the isolated ligands and the complexes under investigation are listed in Table 4.

Table 4: Electronic spectral bands and proposed geometry of the Co (II) complexes

Compound	nm (ε, cm^2, mol^{-1})		μ_{eff} (in BM)	Proposed geometry of complexes
	Ligand bands	d-d bands		
Asn	187, 200, 210	--	--	--
Gln	192, 200, 215	--	--	--
Asp	196, 212, 232	--	--	--

[Co(Asn)$_2$2H$_2$O]	--	526, 476	3.87	O_h
[Co(Gln)$_2$2H$_2$O]	--	526, 480	3.86	O_h
[Co(Asp)$_2$2H$_2$O]	--	526, 476	3.89	O_h

Infra-red spectral Studies: The IR spectra of the metal complexes were recorded in KBr discs over the range 4000-400 cm^{-1}. These spectra were complex due to the presence of numerous bands with varying intensities, making the interpretation task quite difficult. However, an attempt has been made to assign some of the important bands on the basis of reported infrared spectra of several N- and O-donor ligands, and their metal complexes (Table 5).

Table 5: Relevant IR bands for the compounds

Compound	**ν_s(NH$_2$)**	**ν_s(COO$^-$)**	**ν_{asy}(COO$^-$)**	**ν(M-N)**	**ν(M-O)**
Asn	3410m	1648s	1585s	--	--
Gln	3390s	1645s	1594s	--	--
Asp	3380w	1650s	1583s	--	--
[Co(Asn)$_2$2H$_2$O]	3410s	1593s	1580s	515	635
[Co(Gln)$_2$2H$_2$O]	3405m	1455s	1582s	545	652
[Co(Asp)$_2$2H$_2$O]	3143br	1561s	1684s	566	665

w: weak; m: medium; s: strong; br: broad

The infrared spectrum of the free ligands exhibited a broad band at 3380-3410 cm^{-1} which was assigned to the NH$_2$ stretching frequency. The intense bands at 1645-1650 and 1583-1594 cm^{-1} was observed and is attributed to COO$^{-}_{asy}$ and COO$^{-}_{sy}$ stretching frequencies, respectively [15-16].

For the investigated cobalt complexes the COO$^-$ asymmetric stretching frequencies were shifted to lower frequencies compared with that of the ligand. Bands in the region of 635-656 cm^{-1} indicate the formation of M-O bond and further support the coordination of the ligands to the central metal ion via the oxygen atom of the carboxylate group [17]. Hypsochromic shifts were observed for the -NH$_2$ frequencies on coordination for the investigated complexes. This indicates the bond elongation on coordination. It therefore, suggests probable square planar geometry for the complexes. New bands in the spectra of the complexes at 515-566 cm^{-1} were assigned to (M-N) stretching frequency. The participation of the lone pair of electrons on the N of the amino group in the ligand in coordination is supported by these band frequencies [18]. Similarly, at 3580-3615 cm^{-1} broad sharp band, of the investigated complexes, obviously shows the presence of coordinated water molecules in the complexes.

^{1}H-NMR Spectral Studies: The NMR spectroscopy is very important tool of investigation of structure of an unknown compound. This was studied using TMS as standard reference. The ^{1}H-NMR spectra in the ligands and of the investigated complexes were of the type (I) and (II).

In all the ligands, signal of -COOH appeared at -0.95τ (off the scale) but in metal complexes these signals do not appear, probably due to replacement of H$^+$ by metal ion. The signal due to protons of NH$_2$ appeared perturbed (decreased) probably due to coordination by N of NH$_2$ to metal ion. The NMR spectral study clearly shows the formation of complexes by the ligands. The NMR spectral results are presented in Table 6.

Table 6: Relevant ^{1}H-NMR signals for the compounds

Type of protons (in Ligands)	Nature of signal	Chemical shifts	
		δ	τ
-COOH	singlet	10.95	-0.95
$-CONH_2$	Broad singlet	8	2
$-NH_2$	Broad singlet	6	4
=CH	quartet	4.2	5.8
$-CH_2$	pentate	2.1	7.9
(In Complexes)			
-COOH	--	--	--
$-CONH_2$	Broad singlet	8	2
$-NH_2$	Broad	6.2	3.8
=CH	quartet	4.2	5.8
$-CH_2$	pentate	2.1	7.9

Proposed Structures: On the basis of the above observations, it is tentatively suggested that transition metal (II) investigated complexes show an octahedral geometry [Figure 1] in which the ligands act as mono-negative bidentate [N & O donor] ligands.

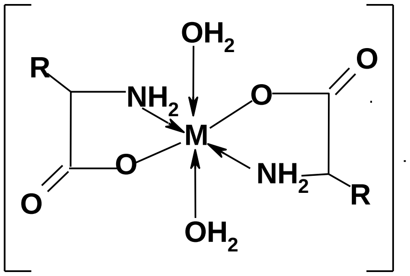


Figure 1: Proposed octahedral structure for investigated complexes

APPLICATIONS

Anti-bacterial Activity: The difference in antimicrobial activities of the investigated complexes and ligands with the standard were studied and the results are presented in Table 7.

The investigated complexes were found to be more active compared with their respective free ligands against the same micro-organisms and under the identical experimental conditions and in some cases better activity compared to the standard. The increase in the biological activity of the complexes may be due to the effect of the metal ion on the normal cell process.

A possible mode of toxicity increase may be considered in the light of chelation theory [19-20]. The chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor group and possible π-electron delocalisation within the whole chelate ring system that is formed during coordination. Such chelation could enhance the lipophilic character of the central metal atom and hence the hydrophobic character and lipo-solubility of the complex favouring its permeation through the lipid layers of the cell membrane [21-22]. This enhances the rate of uptake or entrance and thus the antimicrobial activity of the testing compounds.

Accordingly, the antimicrobial activity of the present investigated complexes can be referred to the increase of their lipophilic character which in turn deactivates enzymes responsible for respiration processes and probably other cellular enzymes, which play a vital role in various metabolic pathways of the tested micro-organisms. Apart from this, other factors such as solubility, conductivity and dipole moment (influenced by the presence of metal ion) may also be the possible reasons for increasing their antimicrobial activity [23-27].

Table 7: Antimicrobial activities of Ligands and its Co (II)-complexes

Compound	***E. coli***	***P. aeruginosa***	***P. vulgaris***	***S. aureus***	***B. subtilis***	***C. albicans***
Asn	8.09	6.04	6.07	7.10	7.05	8.05
Gln	8.03	7.02	6.07	6.09	7.01	8.03
Asp	6.02	6.07	6.0 0	6.01	6.01	8.01
$[Co(Asn)_2 2H_2O]$	7.56	5.58	5.89	6.68	6.95	7.96
$[Co(Gln)_2 2H_2O]$	7.28	6.80	6.05	6.03	6.35	7.95
$[Co(Asp)_2 2H_2O]$	6.01	6.04	6.02	5.97	6.00	7.35
Umequine	10.05	6.02	8. 25	9.52	10.15	9.05

CONCLUSIONS

The cobalt complexes of titled amino acids have been prepared in an environmentally benign protocol and characterized by elemental analysis, conductivity measurements, magnetic moment, and spectral analysis. Elemental analysis of the complexes revealed 1:2 metals to ligand stoichiometry and non-electrolytic nature. Physico-chemical studies revealed that the ligands undergoes complexation with cobalt (II) ion and gets coordinated through nitrogen of the α-amine and β- carboxylate oxygen. The ligand behaves as a mono-negative bidentate donor (N & O) yielding six coordinated cobalt (II) complexes with octahedral geometry around the central metal ion. The synthesized complexes were found to be more active compared with their respective free ligands against the same bacteria and under the identical experimental conditions. Therefore, the present results revealed the importance of amino acids complexes and can be associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health and suggest their potential application as potential antibacterial agents, in the field of disinfection, food packaging and piping of drinking water.

Present methodology offers very attractive features such as simple experimental procedure, higher yields and economic viability, when compared with other method as well as with other catalysts and will have wide scope in organic/inorganic syntheses.

REFERENCES

[1] N Farrell, Metal complexes as drugs and chemotherapeutic agents, *Comprehensive Coordination Chemistry*, **2003**, 9, 809-840.

[2] Z H Chohan, M Arif, M A Akhtar, C T Supuran, *Bioinorganic Chemistry and Applications*, **2006**, 1-11.

[3] K Nomiya, S Takahashi, R Noguchi, S Nemoto, T Takayama, M Oda, *Inorganic Chemistry*, **2000**, 39, 3301-3311.

[4] A Legler, A Kazachenko, V Kazbanov, O Per' yanova, *Pharmaceutical Chemistry*, **2001**, 35, 35-36.

[5] T Aiyelabola, I Ojo, O Akinkunmi, *International Journal of Chemistry*, **2012**, 4, 49-59.

[6] A Stanila, A Marcu, D Rusu, M Rusu, L David, *Journal of Molecular Structure*, **2007**, 834-836, 364-368.

[7] K P Srivastava, Anuradha Singh, Suresh Kumar Singh, *IOSR Journal of Applied Chemistry*, **2014**, 7, 4(1), 16-23.

[8] K P Srivastava, Anuradha Singh, *IOSR-Journal of Applied Chemistry;* **2016**, 9, 11 (III), 01-06**.**

[9] A I Vogel, *Textbook of Quantitative Inorganic Analysis*, 5th edn., Longmans Green and Co, UK Ltd., London, **1989**.

[10] A I Vogel, *Quantitative Inorganic Analysis*, 4th edn. ELBS, London, **1965**.

[11] R L Dutta, A. Syamal, *Elements of Magneto Chemistry*, 2nd edn, Affiliated East West Press, New Delhi, **1993**.

[12] W J Geary, *Coord. Chem. Rev*., **1971**, 7, 81.

[13] A D. Lieher, *J. Phys. Chem.*, **1967**, 67, 1314.
[14] C. J. Ballhausen, *An Introduction to Ligand Field Theory*, McGraw-Hill, NY, USA, **1962**.
[15] D. Pavia, G. Lampman, G. Kriz, *Introduction to Spectroscopy, A Guide for Students of Organic Chemistry*, pp. 22–368, Brooks and Cole, New York, NY, USA, 3rd edition, **2001**.
[16] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, K. Nakamoto, Ed., pp. 66–74, Wiley Interscience, New York, NY, USA, **2009**.
[17] W Kemp, *Organic Spectroscopy*, pp.22–38, Macmillan, Hong Kong, **1991**.
[18] L J Bellamy, *The Infrared Spectra of Complex Molecules*, Chapman & Hall, London, UK, **1975**.
[19] B G Tweedy, *Phytopathology*, **1964**, 55, 910.
[20] T. O Aiyelabola, I .A Ojo, A. C Adebajo et al., *Advances in Biological Chemistry*, **2012,** 2, 268–273.
[21] I Bertini, H B Gray, E I Stiefel, J. S. Valentine, *Biological Inorganic Chemistry: Structure and Reactivity*, University Science Books, Sausalito, Calif, USA, 1st edition, **2007.**
[22] M Carcelli, P Mazza, C Pelizzi, G Pelizzi, F Zani, *J. Inorg. Biochem.*, **1995**, 57, 43.
[23] A Stanila, A Marcu, D Rusu, M Rusu, L David, **2007**, *J Mol Struct*, 834, 364-368.
[24] M.R.A Kumar, S.Shanmukhappa, B. E. Rangaswamy, M.Revanasiddappa, *J. Applicable. Chem*, **2014**, 3(4), 1440-1446.
[25] V. Biradar, M. R. Karekal, M. B. H. Mathada, *J. Applicable. Chem*, **2015**, 3(1), 99-109.
[26] F. Rehman, M. Bhardwaj, U.K. Jetley, *J applicable Chem*, **2014**, 3 (2), 560-571
[27] M. B. Halli, Sadu Suryakant, S. Mallikarjun Kinni, *J. Applicable Chem*, **2015**, 4 (2), 467-475.

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