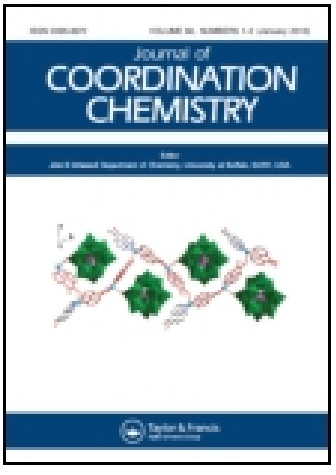

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Highly sensitive and selective fluorescent probe for determination of Cu(II) in aqueous solution

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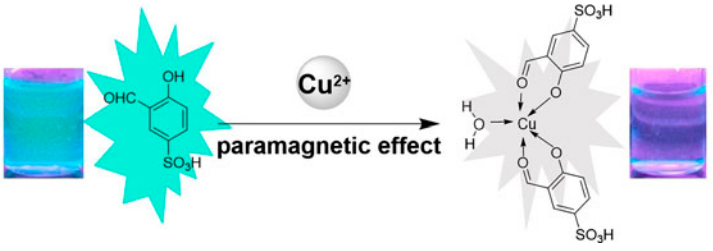

The sensor displayed selectivity for copper ions, as evidenced by a green fluorescence to colorless change, was characterized using fluorescence spectroscopy and its potential application to bioimaging was also illustrated.

A 5-sulfosalicylaldehyde compound, which displays high selective response for copper ions in aqueous solution, was synthesized by salicylaldehyde and concentrated sulfuric acid with high yields. The probe displayed selectivity, as evidenced by a green fluorescence to colorless change, characterized using fluorescence spectroscopy. Its potential application to bioimaging was also illustrated.

Keywords: Water solubility; Fluorescent; 5-Sulfosalicylaldehyde

1. Introduction

Fluorescent probes for metal ions, especially transition metal ions, have found widespread use not only in environmental monitoring but also in biological studies [1–4]. Highly sensitive fluorescent probes for metal ion analysis have attracted attention. Fluorescence offers significant advantages over other methods for metal ion detection and measurement, because of its high sensitivity, selectivity, and instantaneous response [5, 6]. Because of its significance in biological systems and metal pollutants, copper is one of the most interesting transition metal ions. It is well known that copper is a trace element in the organism, existing in the forms of Cu(I) and Cu(II). Although Cu(II) is a catalytic cofactor of many metalloenzymes such as SOD (superoxide dismutase), cytochrome C oxidation enzyme,

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and tyrosinase, increasing evidence suggests that it is also involved in several human diseases, including senile dementia, Wilson's disease and Menkes disease were caused by the presence of abnormal levels [7, 8]. At the same time, copper is widely used in industry and agriculture, including electroplating and electronics industries, cooking utensils and water distribution systems, fertilizers, bactericides, fungicides, algicides, and antifouling paints, and animal feed additives and growth promoters [9, 10]. Consequently, effective detection of Cu^{2+} in water or physiological samples is of toxicological and environmental concern [11–13]. Cu(II) fluorescent probes, which have been synthesized and applied to Cu(II) detection, are rhodamine derivatives [14–18], BODIPY derivatives [19], and coumarin derivatives [20–22] with the problem of complicated multi-step synthesis, low yield, and poor solubility. Herein, we report a soluble chemosensor with one-step synthesis for detecting Cu^{2+} by fluorescence spectroscopy. As a widely used organic intermediate, salicylaldehyde is accessible and inexpensive [23]. When joined with sulfo group, the water solubility of salicylaldehyde was greatly improved [24]. With these considerations in mind, the chemosensor employed is based on a salicylaldehyde derivative, which was prepared by the reaction of salicylaldehyde and concentrated sulfuric acid [25].

2. Experimental

2.1. *Materials*

All chemicals and solvents were of analytical grade and bought from Sigma-Aldrich or Beijing City without further purification. Deionized water was used to prepare all aqueous solutions. The solutions of Hg^{2+}, Er^{3+}, Al^{3+}, Ba^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Eu^{3+}, La^{3+}, Sm^{3+}, Fe^{3+}, Gd^{3+}, Nd^{3+}, Ho^{3+}, Sn^{4+}, Yb^{3+}, Ce^{4+}, and Zr^{3+} were prepared from their chloride salts. The solution of Ag^{+} was prepared from the nitrate salt. Solution of VO^{2+} was prepared from the sulfate salt. All spectroscopic measurements were performed in HEPES (10 mM, pH 7.0) buffer. HEPES buffer solutions were obtained by adding 1 M NaOH solution into 10 mM aqueous HEPES using a Mettler Toledo pH meter. The probe was dissolved in deionized water to prepare stock solutions with concentrations of 2.0 mM.

2.2. *Synthesis of the compound*

Salicylaldehyde (probe) (3.053 g, 25 mM) was added dropwise into 25 mL concentrated sulfuric acid in an ice-water bath. The reaction mixture was then stirred for 18 h at 40 °C. After cooling to r.t., the reaction mixture was poured into 100 mL ice water slowly while stirring. After that, 25 g sodium carbonate was added subsequently under stirring to neutralize the mixture and then the carnation solid was precipitated. The precipitate was filtered off and washed by absolute EtOH, followed by acetone several times. After drying in high vacuum, 3.89 g white powder was obtained in a yield of 77%.

2.3. *Characterization of compound*

^{1}H NMR (300 MHz, 25 °C, DMSO): δ 6.92 (t, 1H), 7.68 (q, 1H), 7.87 (s, 1H), 10.22 (d, 1H), 10.85 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 119.5, 121.9, 132.5, 135.4, 136.2, 163.42, 198.32; Elemental analysis (Calcd %) for $C_7H_6O_5S$: C, 41.59; H, 2.99. Found: C, 41.62; H, 2.97. Electrospray ionization mass spectra (ESI-MS) *m/z* 201.08, $[probe\text{-}H]^{-}$ (figure S1 see online supplemental material at http://dx.doi.org/10.1080/00958972.2014.935355).

2.4. *Physical measurements*

UV–visible spectra were recorded on a Cary 50 Bio UV–visible spectrophotometer in a 4.5 mL (1 cm in diameter) cuvette with 2 mL solution. Fluorescence spectra were measured on a Cary Eclipse fluorescence spectrophotometer. All data were treated with the Origin 8.0 program. Absorption maxima, λ_{max}, is given in nm. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE-300 and 75 MHz NMR spectrometer, respectively. Chemical shifts are given in parts per million downfield from tetramethylsilane (0.0 ppm) for spectra. ESI-MS was measured with a LC-MS 2010A (Shimadzu) instrument.

2.5. *Measurement procedure*

The UV–vis procedures were as follows: into a 10 mM, pH 7.0 HEPES buffer solution containing 60.0 μM probe, ion sample was gradually titrated. All UV–vis spectral data were recorded at 20 s after ion addition.

The fluorescence procedures were as follows: into a 10 mM, pH 7.0 HEPES buffer solution containing 60 μM probe, ion sample was gradually titrated. All fluorescence spectral data were recorded at 20 s after ion addition.

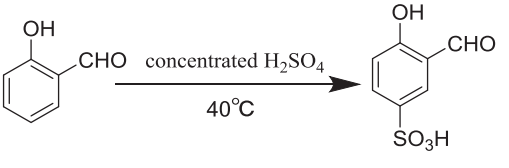
2.6. *Detection range*

Fluorescence spectra were measured from 372 to 750 nm with excitation at 370 nm, and the sensitivity for Cu^{2+} was 10^{-7}–10^{-6} M L^{-1}. The main bands in the UV–vis spectrum were centered at 325 and 378 nm. The detection threshold for Cu^{2+} was 10^{-6}–10^{-5} M L^{-1}.

3. Results and discussion

3.1. *Selectivity over metal ions*

The effect of a wide range of environmentally and physiologically active metal ions on 5-sulfosalicylaldehyde was investigated using fluorescence spectra of solutions containing 5-sulfosalicylaldehyde and the ion in the HEPES (10 mM) pH 7.0 aqueous buffer. The results showed that metal ions, such as K^{+}, Ca^{2+}, Mg^{2+}, Pb^{2+}, Hg^{2+}, Mn^{2+}, Er^{3+}, Eu^{3+}, Zn^{2+}, Ba^{2+}, Cd^{2+}, Ho^{3+}, Ni^{2+}, Co^{3+}, La^{3+}, Yb^{3+}, Fe^{3+}, Gd^{3+}, Nd^{3+}, Ce^{4+}, VO^{2+}, Sn^{4+}, and Zr^{3+} did not respond as well as Cu^{2+} [figure 1(a)]. At the same time, the competition of other metal ions on the determination of Cu^{2+} was examined. Figure 1(b) displays the fluorescence responses of the probe-Cu^{2+} to the presence of various metal ions. The emission quench of probe in response to Cu^{2+} was primarily unaffected in the presence of various competitive species (scheme 1).



Scheme 1. The synthesis of 5-sulfosalicylaldehyde.

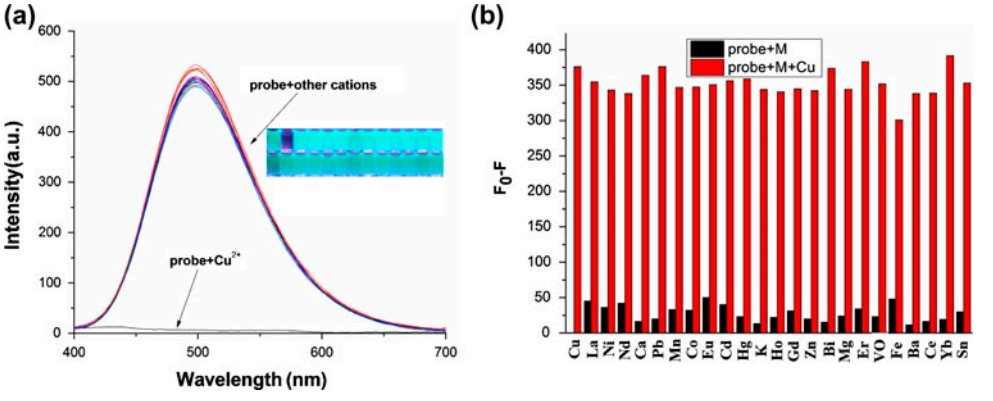


Figure 1. (a) Fluorescence spectra of probe (60 μM) with various analytes (1.5 mM) in solution (HEPES, pH 7.0) (λ_{ex} = 370 nm, slit: 5 nm/5 nm), inner: photograph showing the fluorescence change for Cu^{2+} (colorless) and the other cations (green) under illumination with a 365-nm UV lamp; (b) Fluorescent intensity of the probe (60 μM) upon the addition of 60 μM Cu^{2+} in the presence of 1.5 mM metal ions (La^{3+}, Ni^{2+}, Nd^{3+}, Ca^{2+}, Pb^{2+}, Mn^{2+}, Co^{3+}, Eu^{3+}, Cd^{2+}, Hg^{2+}, K^{+}, Ho^{3+}, Gd^{3+}, Zn^{2+}, Bi^{3+}, Mg^{2+}, Er^{3+}, VO^{2+}, Fe^{3+}, Ba^{2+}, Ce^{4+}, Yb^{3+} and Sn^{2+}) in pH 7.0 HEPES. Black bar: probe + various cations. Red bar: probe + various cations + Cu^{2+} (λ_{ex} = 350 nm, λ_{em} = 498 nm, slit: 5 nm/5 nm) (see http://dx.doi.org/10.1080/00958972.2014.935355 for color version).

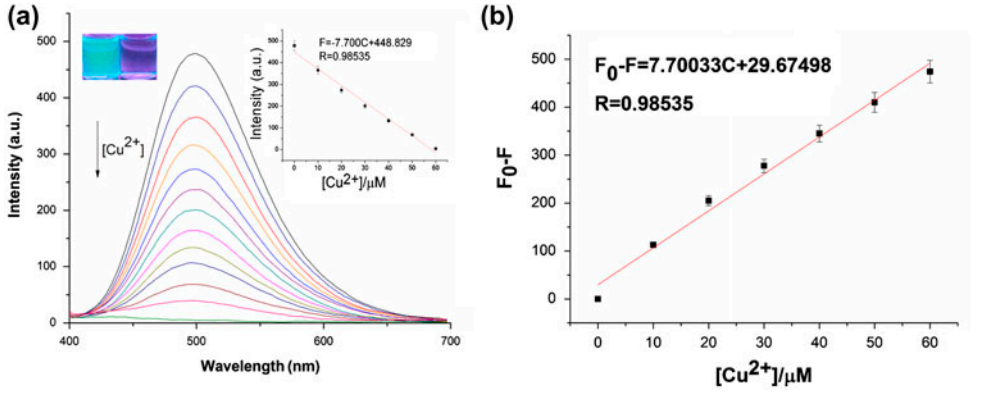


Figure 2. (a) Fluorescence spectral changes for probe (60 μM) in HEPES buffer solution (pH 7.0) (λ_{ex} = 370 nm, slit: 5 nm/5 nm) upon addition of Cu^{2+}; Cu^{2+} was added gradually with [Cu^{2+}] = 0–60 μM; (b) Plot of the fluorescence intensity (at 498 nm) as a function of the concentrations of Cu^{2+}.

Table 1. A comparison table about the detection limits for Cu^{2+}.

Method	Analyte	Signal output	Solvent	Detection limit (μM)
Ref. [27]	Cu^{2+}	Fluorescence	HEPES buffer	1.89
Ref. [28]	Cu^{2+}	Fluorescence	MeOH–H_2O	0.20
Ref. [29]	Cu^{2+}	Fluorescence	HEPES buffer	4.00
This work	Cu^{2+}	Fluorescence	HEPES buffer	0.15

3.2. *The fluorescence spectra of detecting Cu^{2+}*

The changes in the fluorescence spectra of probe (60 μM) in the absence or presence of Cu^{2+} (0–60 μM) in HEPES (10 mM, pH 7.0) buffer are displayed in figure 2(a) ($\lambda_{ex} = 370$ nm). The fluorescence intensity decreased dramatically upon increasing Cu^{2+} concentration. A linear correlation existed between the emission intensity and the concentration of Cu^{2+} within the range from 0 to 60 μM [figure 2(a), inset]. To investigate the detection limit of the probe for Cu^{2+}, probe (60 μM) was treated with various concentrations of Cu^{2+} (0–60 μM) and the relative fluorescent intensity at 498 nm was plotted as a function of Cu^{2+} concentration [figure 2(b)]. The detection limit, based on the definition by IUPAC ($C_D L = 3$ Sb/m) [26], was found to be 0.15 μM from 10 blank solutions. The probe shows a certain sensitivity towards copper which is comparable to other reported Cu^{2+} chemosensors [27–29] (table 1).

3.3. *pH dependent of the determination*

The pH responses for the probe and its Cu^{2+} complexes were investigated using solutions with pH values between 2.0 and 13.0. The probe has stronger fluorescence emission at 498 nm in the range of pH 7.0–13.0 compared with pH 2.0–6.0. Upon addition of Cu^{2+}, partial or no fluorescence quenching takes place at pH 7.5–13.0. Furthermore, the titration gradient of probe for Cu^{2+} was tested in quasi-neutral pH values (6–8) (see figure S2). The result showed that pH 7.0 is appropriate for its determination. The neutral pH 7.0 was thus used for further study (figure 3).

3.4. *Time-dependence in the detection process of Cu^{2+}*

Time-dependent fluorescence spectra of probe for Cu^{2+} were studied and the results are shown in figure 4. Upon addition of Cu^{2+}, the fluorescence intensity at 498 nm decreases

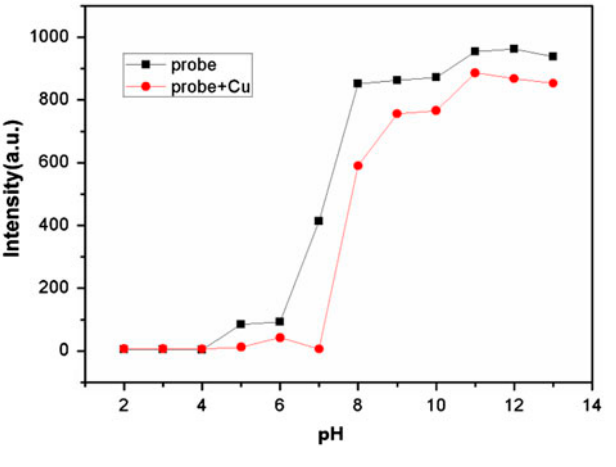


Figure 3. The fluorescence intensity at 498 nm of free probe (60 μM) and probe (60 μM)-Cu^{2+} under different pH conditions, respectively.

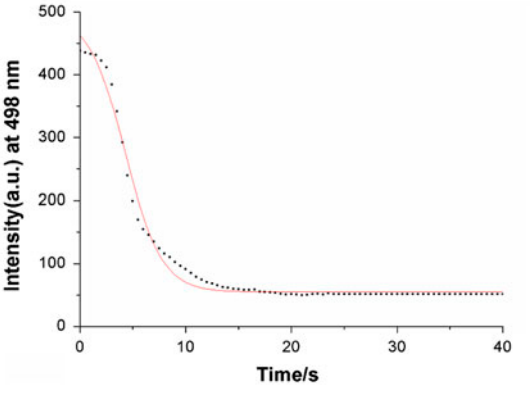


Figure 4. Reaction time profile of probe and Cu^{2+}.

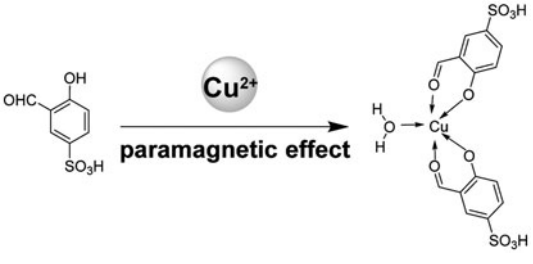


Figure 5. Proposed mechanism of the detection.

rapidly with increasing reaction time under the selected reaction conditions. Therefore, a 20 s reaction time was selected for the following experiments.

3.5. *Proposed mechanism*

A proposed mechanism for the detection and the structures of the probe with and without the addition of Cu^{2+} are shown in figure 5. The probe combines an electron donating (D, hydroxyl) and an electron accepting (A, benzene ring) fragment, keeping donor and acceptor in π conjugation. The $n\rightarrow\pi^*$ charge transition in the molecule causes strong fluorescence [30, 31]. For Cu^{2+}, the paramagnetic ions with unfilled *d* shells can quench via an electron or energy transfer [32]. After coordination with Cu^{2+}, the strong communication between the probe and Cu^{2+} which caused ligand-to-metal charge transfer band at 378 nm, displayed in the absorption spectra of the copper complex, obstructed the $n\rightarrow\pi^*$ charge transition and induced fluorescence quenching [33, 34]. In order to clarify the coordination mechanism, we first carried out a UV–vis titration experiment. It is seen in figure 6 that the absorption intensities of 325 and 378 nm changed with increased Cu^{2+} concentration, and this ended when the concentration of Cu^{2+} reached 30 μM, indicating the formation of 1 : 2 stoichiometry between Cu^{2+} and probe. Mass spectrometry analysis of a product obtained from the reaction of probe with Cu^{2+} in CH_3OH shows binding between probe and Cu^{2+}; a

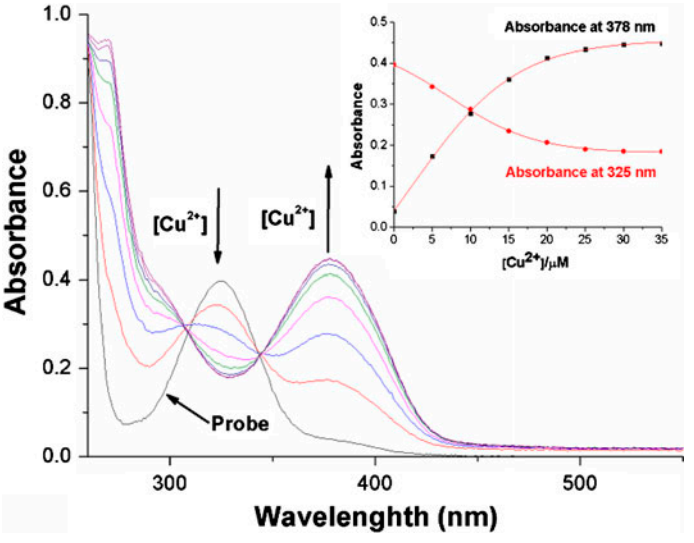


Figure 6. UV–vis spectra of probe (60 μM) in HEPES (pH 7.0) upon addition of $CuCl_2$ (ranging from 0 to 30 μM). Inset: Job's plot of the reaction between the probe and Cu^{2+} in HEPES (pH 7.0) solutions.

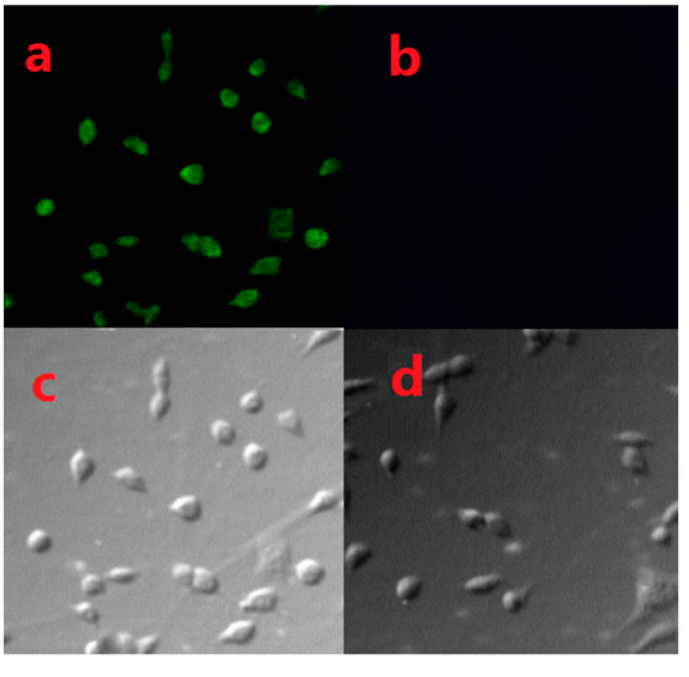


Figure 7. Confocal fluorescence images of HepG2 cells: (a) fluorescence image of HepG2 cells with adding probe (30 μM) and its brightfield image (c); (b) fluorescence image of HepG2 cells incubated with 30 μM probe for 30 min at 37 °C; and then incubated with 15 μM $CuCl_2$ for 30 min at 37 °C and its brightfield image (d).

peak at $m/2z = 279.08$, corresponding to $[2\text{Probe-Cu-}H_2O+2\text{ K}]^{2+}$, is clearly observed (figure S3), which is consistent with 1 : 2 stoichiometry between Cu^{2+} and probe. The complex discussed above involving the ligand and copper ion is similar to forms reported previously [35–38].

3.6. *Cellular imaging*

The ability of the probe to mark Cu^{2+} within living cells was also evaluated by laser confocal fluorescence imaging using an Olympus FV1000 laser scanning microscope. The optical window at the green channel (450–550 nm) was chosen as a signal output. As shown in figure 7(a), under selective excitation at 405 nm, HepG2 cells incubated with 30 μM probe for 30 min at 37 °C showed green fluorescence. In a further experiment, HepG2 cells displayed no fluorescence when the cells were first incubated with 30 μM of probe for 30 min at 37 °C and then incubated with 15 μM $CuCl_2$ [figure 7(c)]. These cell experiments show the good cell-membrane permeability of probe and it can thus be used to mark Cu^{2+} within living cells.

4. Conclusion

We have developed a good water soluble fluorescent chemosensor based on salicylaldehyde for the detection of Cu^{2+}. The probe displayed excellent selectivity, sensitivity, and an unprecedented speed of response to Cu^{2+} in aqueous media with a 2 : 1 binding mode. Upon addition of Cu^{2+} to the probe at neutral pH, a striking fluorescence quenching was determined. Because of its accessibility and good cell-membrane permeability, the probe has potential applications in both environmental and physiological systems for Cu^{2+} detection.

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Supplemental data

Supplemental data for this article can be accessed here http://dx.doi.org/10.1080/00958972.2014.935355.

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