Biomimetic Sensor for Certain Phenols Employing a Copper(II) Complex

ARTICLE *in* ANALYTICAL CHEMISTRY · JULY 2010

Impact Factor: 5.64 · DOI: 10.1021/ac1004037 · Source: PubMed

5 AUTHORS, INCLUDING:

Mohammad Mobin Shaikh
Indian Institute of Technology Indore
325 PUBLICATIONS **5,321** CITATIONS
SEE PROFILE

Bankim Sanghavi
University of Virginia
17 PUBLICATIONS **976** CITATIONS
SEE PROFILE

Ashwini Srivastava
University of Mumbai
102 PUBLICATIONS **1,724** CITATIONS
SEE PROFILE

Pradeep Mathur
Indian Institute of Technology Indore
209 PUBLICATIONS **2,454** CITATIONS
SEE PROFILE

Anal. Chem. **2010,** *82,* 5983–5992

Biomimetic Sensor for Certain Phenols Employing a Copper(II) Complex

Shaikh M. Mobin,[†,‡,§] Bankim J. Sanghavi,[†] Ashwini K. Srivastava,[*,†] Pradeep Mathur,[*,‡,§] and Goutam K. Lahiri[*,‡,§]

Department of Chemistry, University of Mumbai, Vidyanagari, Mumbai-400098, India, and National Single Crystal X-ray Diffraction Facility and Department of Chemistry, IIT Bombay, Powai, Mumbai-400076, India

A new dimeric Cu(II) complex $[Cu(\mu_2\text{-hep})(hep\text{-}H)]_2 \cdot 2PF_6$ (1) containing a bidentate (hep-H = 2-(2-hydroxyethyl)pyridine) ligand was synthesized and characterized by single crystal X-ray diffraction studies. Each Cu ion in 1 is in a distorted square pyramidal geometry. Further 1 is used as a modifier in the construction of a biomimetic sensor for determining phenols [phenol (Phe), resorcinol (Res), hydroquinone (HQ), and catechol (Cat)] in phosphate buffer by using cyclic voltammetry (CV), chronocoulometry, electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), and square wave voltammetry (SWV). DPV has been proposed for trace determination of Phe and Res while SWV for HQ and Cat. The method has been applied for the selective and precise analysis of Phe in commercial injections, Res in hair coloring agents, HQ in photographic developers and cosmetics, and Cat in tea samples and guarana tablets. The calibration curves showed a linear response ranging between 10^{-6} and 10^{-8} M for all four of the analytes with detection limits (3σ) of 1.04×10^{-8}, 2.31×10^{-8}, 1.54×10^{-8}, and 0.86×10^{-8} M for Phe, Res, HQ, and Cat, respectively. The lifetime of the biomimetic sensor was 200 days at room temperature (at least 750 determinations). The catalytic properties of 1-CPE were characterized by chronoamperometry and were found to be in good agreement with Michaelis−Menten kinetics.

Dinuclear Cu(II) complexes containing μ-oxo bridging have drawn considerable attention in recent years for their potential application in the field of biomimetic chemistry to provide an enhanced understanding of the function of the biological sites and as potential catalysts for substrate oxidations.[1−6] Cu(II) complexes have been known for their structural diversity and functional models for the active centers of copper containing redox enzymes.[7−10] The construction of a biomimetic sensor is a formidable challenge in the development of analytical procedures for the determination of various analytes.[10−12] Biomimetic sensors are well documented as efficient catalysts for the oxidation of phenolic substrates to quinones.[7−13] Catechol oxidase is a copper-containing enzyme which catalyzes the oxidation of diphenols by molecular oxygen with the production of quinones.[11,12] Recently, the formation of copper complexes with the ligand containing pyridine, imidazole, and imine donors are of great interest, which not only stem from their fascinating structures but also show interesting material properties.[14−17]

Phenol (Phe) is a good antiseptic and a neurologic agent which has application in pharmaceutical industries as injections. Dihydroxy phenols have proved their own worth. Hydroquinone (HQ) is a major component in most photographic films and cosmetics as a skin whitener. Catechol (Cat) is one of the most important phenols, and its analysis in tea and guarana tablets is important as it is toxic in nature. Resorcinol (Res) is also an important component of innumerable hair colors on the market. Thus, phenols are of tremendous importance, but their overdose is toxic causing giddiness, deafness, salivation, sweating, and convulsions. Thus, their trace analysis is of significant importance.

Various methods have been employed for the trace level determinations of Phe, Res, HQ, and Cat.[18−22] However, these

* To whom correspondence should be addressed. Fax: +91-022-26528547. E-mail: aksrivastava@chem.mu.ac.in, akschbu@yahoo.com (A.K.S.).

† University of Mumbai.

‡ National Single Crystal X-ray Diffraction Facility, IIT Bombay.

§ Department of Chemistry, IIT Bombay.

(1) Karlin, K. D.; Tyeklar, Z. *Bioinorganic Chemistry of Copper*; Chapman & Hill: New York, 1993.

(2) Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E. *Chem. Rev.* **1996**, *96*, 2563–2605.

(3) Latour, J. M. *Bull. Soc. Chim. Fr.* **1988**, 508–523.

(4) Sorrell, T. N. *Tetrahedron* **1989**, *45*, 3–68.

(5) Vigato, P. A.; Tamburini, S.; Fenton, D. *Coord. Chem. Rev.* **1990**, *106*, 25–170.

(6) Kitajima, N. *Adv. Inorg. Chem.* **1992**, *39*, 1–77.

(7) Neves, A.; Rossi, L. M.; Bortoluzzi, A. J.; Szpoganicz, B.; Wiezbicki, C.; Schwingel, E. *Inorg. Chem.* **2002**, *41*, 1788–1794.

(8) Neves, A.; Rossi, L. M.; Bortoluzzi, A. J.; Mangrich, A. S.; Haase, W.; Werner, R. *J. Braz. Chem. Soc.* **2001**, *12*, 747–754.

(9) Neves, A.; Erthal, S. M. D.; Drago, V.; Griesar, K.; Haase, W. *Inorg. Chim. Acta* **1992**, *197*, 121–124.

(10) dos Anjos, A.; Bortoluzzi, A. J.; Szpoganicz, B.; Caro, M. S. B.; Friedermann, G. R.; Mangrich, A. S.; Neves, A. *Inorg. Chim. Acta* **2005**, *358*, 3106–3114.

(11) Sotomayor, M. D. P.; Tanaka, A. A.; Kubota, L. T. *Electrochim. Acta* **2003**, *48*, 855–865.

(12) Sotomayor, M. D. P.; Tanaka, A. A.; Kubota, L. T. *J. Electroanal. Chem.* **2002**, *536*, 71–81.

(13) Ebarvia, B. S.; Cabanilla, S.; Sevilla, S. F., III. *Talanta* **2005**, *66*, 145–152.

(14) Balamurugan, R.; Palaniandavar, M.; Halcrow, M. A. *Polyhedron* **2006**, *25*, 1077–1088.

(15) Kim, E. H.; Kim, D. I.; Lee, H. S.; Byun, J. C.; Choi, J. H.; Park, Y. C. *Polyhedron* **2007**, *26*, 85–92.

(16) Gao, G. G.; Xu, L.; Wang, W. J.; An, W. J.; Qiu, Y. F.; Wang, Z. Q.; Wang, E. B. *J. Phys. Chem. B* **2005**, *109*, 8948–8953.

(17) Salimi, A.; Alizadeh, V.; Hadadzadeh, H. *Electroanalysis* **2004**, *16*, 1984–1991.

(18) Pistonesi, M. F.; Di Nezio, M. S.; Centurión, M. E.; Palomeque, M. E.; Lista, A. G.; Fernández Band, B. S. *Talanta* **2006**, *69*, 1265–1268.

10.1021/ac1004037
Published on Web 06/22/2010

methods face drawbacks like being expensive, laborious, and require pretreatment. On the other hand, modern electroanalytical techniques are very sensitive, selective, and seldom require pretreatment, preseparation, etc. The availability of various modified electrodes at relatively low cost has provided considerable impetus to the use of electroanalytical techniques for analysis. The present study is concerned with the use of electroanalytical techniques for trace determination of phenols.

The electrochemistry of Phe,[23−25] Res,[26,27] HQ,[28,29] and Cat[30−32] has been well studied. Carbon paste electrodes (CPEs) are very popular due to their wide anodic potential range, low residual current, ease of fabrication, easy surface renewal, low cost, etc. Chemically modified electrodes (CMEs) are used to lower the order of magnitude of detection limits as compared to the plain carbon paste electrode (PCPE). Various modifiers like nanomaterials,[32−36] copper complexes,[37,38] macrocycles,[39,40] etc. have been employed successfully as modifiers for the carbon paste electrode.

The construction of a Cu(II) complex based biomimetic sensor is the most developing area in current research based on sensors. An up-to-date literature survey reveals that many copper containing oxidase complex based biomimetic sensors have been employed for the trace determination of phenols due to their redox properties.[41,42]

In view of the desirable characteristics of Cu-complexes, it is likely that electrochemical processes may occur in a facile manner. The present work aims at studying the electrochemical behavior of Phe, Res, HQ and Cat for trace determination employing various voltammetric techniques using synthesized Cu complex as biomimetic sensor. The developed methods have been employed for the selective and precise analysis of Phe in commercial injections, Res in hair coloring agents, HQ in photographic developers and cosmetics, and Cat in tea samples and guarana tablets, and ultimately the recovery studies have been carried out for all the aforementioned phenols. The catalytic activity and response time for phenols were obtained using chronoamperometry. To the best of our knowledge this is the first example of an alcoholic −OH group (present in **1**) based biomimetic sensor being developed for trace determination of phenols.

EXPERIMENTAL SECTION

Materials and Instrumentation. All chemicals were of A. R. grade and were used as received without any further purification. Phe, HQ, Res, and Cat were purchased from Fluka. HCl was procured from Merck, India. The commercially available starting materials, $Cu(OC(=O)CH_3)_2 \cdot 2H_2O$, 2-(2-hydroxyethyl)pyridine (hep-H), sodium hexaflouro phosphate ($NaPF_6$), ascorbic acid, acetaminophen, uric acid, pyridoxine HCl, folic acid, epinephrine, aspirin, caffeine, 4-bromophenol, and reagent grade solvent methanol, were used as received. Aspirin and uric acid were first dissolved in 0.001 N NaOH. Graphite powder, ascorbic acid, acetaminophen, uric acid, aspirin, 4-bromophenol, and caffeine were purchased from S. D. fine, India. Pyridoxine HCl, folic acid, epinephrine, and mineral oil were purchased from Fluka. Metol, methyl paraben, poly(ethylene glycol), and *o*-aminophenol were procured from Aldrich. Elemental analyses were carried out with a Perkin-Elmer 240C elemental analyzer. FT-IR spectra of complexes as KBr pellets were recorded using a Nicolet spectrophotometer. A UV−visible spectrophotometer used for validating the method was procured from Shimadzu, Japan. The pH measurements were performed using an ELICO LI 120 pH meter.

All voltammetric, chrono, and electrochemical impedance studies (EIS) measurements have been performed using GPES software, version 4.9.005, and Frequency Response Analyzer, software version 2.0, respectively, and were performed on an Eco Chemie, Electrochemical Workstation and model Autolab PGSTAT 30. An Ag/AgCl electrode and a platinum electrode were used as the reference and counter electrodes, respectively, to measure all the potentials. A plain carbon paste electrode (PCPE) and modified $[Cu(\mu_2\text{-hep})(\text{hep-H})]_2 \cdot 2PF_6$(**1**)-CPE (**1**-CPE) were used as working electrodes.

Synthesis of $[Cu(\mu_2\text{-hep})(\text{hep-H})]_2 \cdot 2PF_6$ (1). A solution of hep-H (123 mg, 1 mmol) in methanol (25 mL) was added to a solution of $Cu(OC(=O)CH_3)_2 \cdot 2H_2O$ (199 mg, 1 mmol) in methanol (25 mL). An aqueos solution of $NaPF_6$ (168 mg, 1 mmol) was added to the above reaction mixture, and the resultant solution was stirred for 6 h at room temperature. The solution was then passed through the filter paper in order to remove any unreacted materials. The filtrate was allowed to stand at room temperature for crystallization. Dark blue single crystals of **1** were obtained within 3 days by slow evaporation of the solvent. mp, 228−230 °C. Yield, 290 mg (91%). Anal. Calcd for $C_{28}H_{34}N_4O_4F_{12}P_2Cu_2$, (MW = 907.61): C, 37.05; H, 3.78; N, 6.17. Found: C, 37.09; H, 3.84; N, 6.12. IR (KBr, cm^{-1}):

(19) Figueiredo, E. C.; Tarley, C. R. T.; Kubota, L. T.; Rath, S.; Arruda, M. A. Z. *Microchem. J.* **2007**, *85*, 290–296.
(20) Lin, Y.-H.; Yang, Y.-H.; Wu, S.-M. *J. Pharm. Biomed. Anal.* **2007**, *44*, 279–282.
(21) Rodrigues, G. D.; de Lemos, L. R.; da Silva, L. H. M.; da Silva, M. d. C. H.; Minim, L. A.; Coimbra, J. S. d. R. *Talanta* **2010**, *80*, 1139–1144.
(22) Xiao, W.; Xiao, D. *Talanta* **2007**, *72*, 1288–1292.
(23) Lin, H.; Ji, X.; Chen, Q.; Zhou, Y.; Banks, C. E.; Wu, K. *Electrochem. Commun.* **2009**, *11*, 1990–1995.
(24) Santos, W. J. R.; Sousa, A. L.; Sotomayor, M. P. T.; Damos, F. S.; Tanaka, S. M. C. N.; Kubota, L. T.; Tanaka, A. A. *J. Braz. Chem. Soc.* **2009**, *20*, 1180–1187.
(25) Kim, G.-Y.; Cuong, N. M.; Cho, S.-H.; Shim, J.; Woo, J.-J.; Moon, S.-H. *Talanta* **2007**, *71*, 129–135.
(26) Han, L.; Zhang, X. *Electroanalysis* **2009**, *21*, 124–129.
(27) Wang, Z. H.; Li, S. J.; Lv, Q. Z. *Sens. Actuators, B* **2007**, *127*, 420–425.
(28) Zhao, D.-M.; Zhang, X.-H.; Feng, L.-J.; Jia, L.; Wang, S.-F. *Colloids Surf., B* **2009**, *74*, 317–321.
(29) Zhang, Y.; Zeng, G.-M.; Tang, L.; Huang, D.-L.; Jiang, X.-Y.; Chen, T.-N. *Biosens. Bioelectron.* **2007**, *22*, 2121–2126.
(30) Sun, W.; Li, Y.; Yang, M.; Li, J.; Jiao, K. *Sens. Actuators, B* **2008**, *133*, 387–392.
(31) Lin, H.; Gan, T.; Wu, K. *Food Chem.* **2009**, *113*, 701–704.
(32) Shan, D.; Zhang, J.; Xue, H.-G.; Zhang, Y.-C.; Cosnier, S.; Ding, S.-N. *Biosens. Bioelectron.* **2009**, *24*, 3671–3676.
(33) Yang, T.; Zhou, N.; Zhang, Y.; Zhang, W.; Jiao, K.; Li, G. *Biosens. Bioelectron.* **2009**, *24*, 2165–2170.
(34) Dai, H.; Wang, Y.; Wu, X.; Zhang, L.; Chen, G. *Biosens. Bioelectron.* **2009**, *24*, 1230–1234.
(35) Beitollahi, H.; Karimi-Maleh, H.; Khabazzadeh, H. *Anal. Chem.* **2008**, *80*, 9848–9851.
(36) Zhu, S.; Fan, L.; Liu, X.; Shi, L.; Li, H.; Han, S.; Xu, G. *Electrochem. Commun.* **2008**, *10*, 695–698.
(37) Inês Rosane de Oliveira, W. Z.; Neves, A.; Vieira, I. C. *Sens. Actuators, B* **2008**, *129*, 424–430.
(38) Hasebe, Y.; Gu, T. *J. Electroanal. Chem.* **2005**, *576*, 177–181.
(39) Vaze, V. D.; Srivastava, A. K. *Electrochim. Acta* **2007**, *53*, 1713–1721.
(40) Gaichore R. R.; Srivastava A. K. *Anal. Lett.* In press.
(41) Inês Rosane de Oliveira, W. Z.; Renata El-Hage de Barros Osório, M.; Neves, A.; Vieira, I. C. *Sens. Actuators, B* **2007**, *122*, 89–94.
(42) Sergeyeva, T. A.; Slinchenko, O. A.; Gorbach, L. A.; Matyushov, V. F.; Brovko, O. O.; Piletsky, S. A.; Sergeeva, L. M.; Elska, G. V. *Anal. Chim. Acta* **2010**, *659*, 274–279.

Table 1. Crystallographic Parameters for 1

identification code	**1** (150 K)
empirical formula	$C_{28}H_{34}N_4O_4F_{12}P_2Cu_2$
formula weight	907.61
wavelength	0.710 73 A
crystal system, space group	orthorhombic, *Pbcn*
a	14.580 6(5), Å
b	9.496 8(3), Å
c	24.917 6(6) Å
α	90
β	90
γ	90
V Å^3	3450.32(18)
Z, calculated density	4, 1.747 mg/m^3
absorption coefficient	1.432 mm^{-1}
F(000)	1832
crystal size	0.36 mm × 0.32 mm × 0.27 mm
θ range for data collection	3.04−25.00°
index ranges	$-15 \le h \le 17$, $-11 \le k \le 9$, $-28 \le l \le 29$
reflections collected/unique	16 840/3 034 [*R*(int)= 0.0371]
data collection instrument	Oxford XCalibur-S
absorption correction	multiscan
maximum and minimum transmission	0.6985 and 0.6267
refinement method	full-matrix least-squares on F^2
data/restraints/parameters	3034/0/241
goodness-of-fit on F^2	1.087
final *R* indices [$I > 2\sigma(I)$]	R1 = 0.0418, wR2 = 0.0941
R indices (all data)	R1 = 0.0567, wR2 = 0.1021
largest difference peak and hole	0.771 and −0.520 e A^{-3}

3560(br), 3120(w), 2385(w), 2360(w), 1609(m), 1571(w), 1486(m), 1448(s), 1312(w), 1187(w), 1167(w), 1069(s), 1049(m), 1032(w), 841(s), 788(m), 773(w), 558(s).

X-ray Crystallography. Single crystal X-ray structural studies of **1** was performed on a CCD Oxford Diffraction XCALIBUR-S diffractometer equipped with an Oxford Instruments low-temprature attachment. Data was collected at 150(2) K using graphite-monochromoated Mo Kα radiation ($\lambda_\alpha = 0.710\ 73$ Å). The strategy for the data collection was evaluated by using the CrysAlisPro CCD software. The data were collected by the standard "phi-omega" scan techniques and were scaled and reduced using CrysAlisPro RED software. The structures were solved by direct methods using SHELXS-97 and refined by full matrix least-squares with SHELXL-97 refining on F^2.[43]

The positions of all the atoms were obtained by direct methods. All non-hydrogen atoms were refined anisotropically. The remaining hydrogen atoms were placed in geometrically constrained positions and refined with isotropic temperature factors, generally $1.2U_{eq}$ of their parent atoms. All the H-bonding interactions, mean plane analyses, and molecular drawings were obtained using the program Diamond (version 3.1d). The crystal and refinement data are summarized in Table 1, and selected bond distances and bond angles are shown in Table S-1 in the Supporting Information.

Chronoamperometry. Kinetic data for oxidation of Cat in pH 7.0 phosphate buffer (0.1 M) was obtained by using chronoamperometry for **1**-CPE at a potential of 0.7 V. The results were obtained by gradually increasing the concentration of Cat in the range of 0−80 μM under stirring conditions. From the plot of *i* vs *t*, the slopes for the initial 0.8 s were measured after each increment of Cat. The data were then fitted with the Michaelis−Menten model, and the apparent kinetic parameters viz. Michaelis constant (K_M), maximum enzyme velocity (V_{max}), turnover number (k_{cat}), and specificity constant (k_{cat}/K_M) were estimated. The same technique was employed for all the other substrates.

Construction of the PCPE and Biomimetic Sensor (1-CPE). PCPE was prepared by mixing graphite with mineral oil at the composition of 70:30 (w/w) using a mortar and pestle and was allowed to homogenize for 48 h.[44,45] **1**-CPE was prepared by mixing 25 mg (10% w/w) of **1** with 150 mg of graphite powder (60% w/w) and 75 mg of mineral oil (30% w/w). The resulting **1**-CPE was used for all the analysis.

Procedures for Sample Preparation for Determination of Phenols in Real Samples. Phenol was analyzed in commercial injection samples. The sample (water based phenol injections) was dissolved in water and used as such. The oil based samples were treated as follows: The sample was dissolved in 10 mL of ether and extracted with successive 1 mL quantities of 2 M sodium hydroxide until the extraction is complete. The combined extracts were then boiled for 2 min, cooled, and diluted to 250 mL with water. Appropriate aliquots were taken from both the injection samples and diluted to 25 mL with supporting electrolyte. The method was validated by the procedure given in Pharmacopoeia.[46]

Res was extracted from all the hair coloring samples by liquid liquid partition.[47] The cream was accurately weighed and dissolved in 1:1 (v/v) ethanol−water by stirring for 5−6 min. This alcoholic mixture was then extracted with ether (20 mL), and this extract was evaporated. The dry residue was dissolved in 1:1 (v/v) ethanol−water, and the solution was transferred to a 50 mL volumetric flask and diluted to volume with the same solvent mixture. Appropriate quantities were then taken for the analysis.

Hydroquinone was determined in photographic developers and cosmetics. An accurate amount ranging from 5 to 7 g of each powder photographic developer sample was transferred into a 50 mL calibrated flask, dissolved, and diluted to volume with water.[48] An aliquot of 10 mL of this solution was treated initially by passing through a 0.8 cm (i.d.) × 15 cm (length) glass column packed with cation-exchange resin (Merck strongly acid, type I), where the metol was totally retained. The column was eluted with water, and the effluent was collected in a 25 mL calibrated flask. A 2 mL aliquot of this solution was reacted with 1 mL of 8.0 M formaldehyde solution at pH 4.5 promoting the formation of the sodium α-hydroxymethanesulfonate adduct. After the elimination of both interfering agents (metol and sodium sulfite), a 1 mL aliquot was transferred into a glass cell containing 10 mL of 0.1 M phosphate buffer (pH 7.0) solution and voltammograms were recorded. HQ was also analyzed in cosmetics as follows:[49] 50 mg of sample was accurately weighed into a glass centrifuge tube, 10 mL of methanol was added, and the tube was heated at 40 °C in a water-bath with stirring until sample dissolution was complete. After sample

(43) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2008**, *A64*, 112–122. *Program for Crystal Structure Solution and Refinement*; University of Goettingen: Goettingen, Germany, 1997.

(44) Adams, R. N. *Anal. Chem.* **1958**, *30*, 1576–1576.

(45) Švancara, I.; Vytřas, K.; Kalcher, K.; Walcarius, A.; Wang, J. *Electroanalysis* **2008**, *21*, 7–28.

(46) *British Pharmacopoeia*; 2003.

(47) Wang, L.-H.; Kuo, Y.-P. *Chromatographia* **1999**, *49*, 208–211.

(48) Vieira, I. C.; Fatibello-Filho, O. *Talanta* **2000**, *52*, 681–689.

(49) Sirajuddin; Bhanger, M. I.; Niaz, A.; Shah, A.; Rauf, A. *Talanta* **2007**, *72*, 546–553.

cooling and centrifugation, the supernatant was transferred into a 10 mL calibrated flask and made up to volume with water. Because of the high concentration of hydroquinone present in the cream, the solution had to be diluted. This was done by taking 100 μL of the previous solution and making it up to a volume of 25 mL with the pH 7.0 phosphate buffer for the analysis.

Cat was analyzed in tea and guarana tablet samples.[50] Various tea samples were purchased from local market and treated as follows: The tea sample (about 0.50 g) was exactly weighed, and the catechol was extracted with 60 mL of 20% (v/v) methanol solution for 20 min at 80 °C. The mixture was filtered and the volume made up to 100.0 mL for further measurement. The guarana powder samples were analyzed by taking one aliquot of 1.0 g of each sample of guarana powder that was extracted with 100 mL of ethanol at room temperature overnight, and then it was filtered through quantitative paper filter. After this step, an aliquot of 1 mL of the filtered solution (diluted with supporting electrolyte) was added to the cell containing the electrolyte.

Sea and sewage water samples were collected from various locations in India. Sea water samples were collected in polypropylene bottles from various locations in India. In order to preserve the sample, three drops of concentrated nitric acid were added for each 100 mL of water. Sewage samples were collected from different drains in and around Mumbai, India. Samples (100 mL) were collected in polypropylene bottles at the outlet of the drains. These collected samples were acidified, stored in a freezer, and analyzed in a way similar to that for the seawater. A total of 10 aliquots of both sea and sewage water samples were taken in order to guarantee the representation of the samples. The only sample cleanup used was filtering through a 0.22 μm PVDF syringe filter (Millex, Millipore Corporation), and no extraction steps were undertaken prior to the voltammetric measurements. An appropriate volume of sample was taken and made up to 25 mL with phosphate buffer (pH 6.0 for Phe and Res; pH 7.0 for HQ and Cat). The recovery tests were performed on sea and sewage water samples by spiking them with standard phenol solutions.

RESULTS AND DISCUSSION

The dimeric complex $[Cu(\mu_2\text{-hep})(\text{hep-H})]_2 \cdot 2PF_6$ (**1**) has been synthesized by the reaction of 2-(2-hydroxyethyl)pyridine (hep-H) with the methanolic solution of copper acetate dihydrate and aqueous solution of PF_6 under magnetic stirring for 6 h at room temperature. Subsequent slow evaporation of the solvent at room temperature yields single crystals suitable for X-ray diffraction, which establishes the dimeric structure of **1** as has been evidenced by its single crystal X-ray structure (Figure 1). **1** has been characterized with the aid of elemental analysis, IR, and UV−visible spectroscopic studies and yielded the correct elemental analysis. Strong IR bands confirm the presence of O−H streching with characteristic absorption at 3560 (broad) cm^{-1}.

The compound **1** crystallizes in the orthorhombic *Pbcn* space group with a crystallographically imposed inversion center (Table 1). The asymmetric unit consists of one Cu atom, one hep^- ligand, one hep-H group, and two half symmetric free PF_6 molecule. The crystal structure of **1** reveals the dinuclear feature of the complex with the overall composition of one $[Cu(\mu_2\text{-hep})(\text{hep-H})]_2^{2+}$ cation and two PF_6 anions (Figure 1). Each Cu^{II} ion is in a pentacoordinated N_2O_3 environment, bonded to two bridging μ-alkoxy oxygen atoms and one nitrogen atom of hep^- ligand and another nitrogen atom from the hep-H ligand and the fifth coordination site is occupied by the elongated one oxygen atom of unprotonated hep-H ligand. The basal positions of each Cu ion in **1** are composed of O(1), O(1A), N(1) of hep^-, and N(2) of hep-H. The Cu(1)−O(1), Cu−O(1A), Cu(1)−N(1), and Cu(1)−N(2) bond lengths are 1.906(1), 1.927(2), 1.954(1), and 1.992(2) Å, respectively (Table S-1 in the Supporting Information). The axial position is filled with an O(2) donor of the hydroxyl group of hep-H with an elongated Cu(1)−O(2) distance of 2.572(1) Å due to the Jahn−Teller effect. This results in a distorted square pyramidal geometry around each of the copper ions. The central Cu_2O_2 ring with two different types of Cu−O bond distances is nonplanar unlike the similar dimeric unit reported by us recently[51,52] in which central Cu_2O_2 is planar. The slight bend in the Cu_2O_2 is due to the bulkier hep-H ligand compared to the $^-$OAc group. The Cu···Cu distance in **1** is 3.017(1) Å. The cation **1** signifies the structural model for the active site of the phenolic group since it possess a dicopper−dioxygen (Cu_2O_2) unit containing without a deprotonated alcoholic (OH) group axially coordinated to each Cu(II) center.

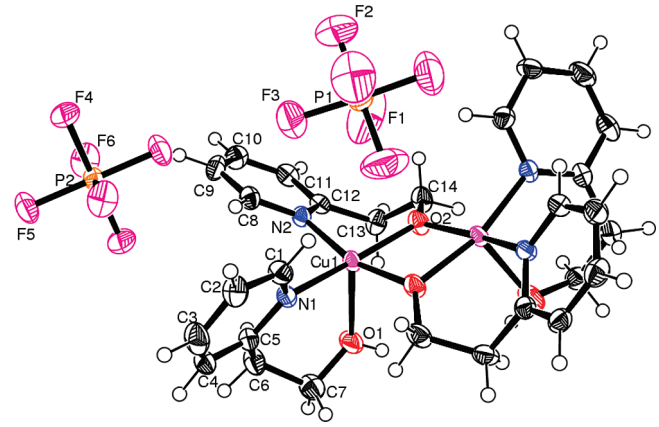

Figure 1. ORTEP view of **1** showing the molecule in the asymmetric unit with anisotropic displacement drawn at 50% probability.

The packing diagram of **1** along the *c*-axis reveals various intermolecular C−H···F and O−H···F interactions in conjunction with one intramolecular C−H···O interactions (Figure S-1 and Table S-2 in the Supporting Information). The presence of various intermolecular interactions in **1** prompted us to explore the possibility of the sensing properties via electrochemical pathways.

Recently we have reported several neutral dicopper−dioxygen complexes $[(OR)Cu(\mu\text{-hep})_2Cu(OR')]\cdot 2H_2O$ in presence of ancillary ligands [OR=OR′ = bidentate acetate, *n*-propionic acid, and trifluoro acetic acid and the bidentate mixed OR= acetate/OR′ = *n*-propionic acid],[51,52] which were applied in a single-crystal to single-crystal (SCSC) transformation from the dimeric copper complex to the tetrameric copper complex on exposure to alcohol vapors. This sensing property of the above-

(50) Magna, A.; Salomao, A. A.; Vila, M. M. D. C.; Tubino, M. *J. Braz. Chem. Soc.* **2003**, *14*, 129–132.

(51) Shaikh, M. M.; Srivastava, A. K.; Mathur, P.; Lahiri, G. K. *Inorg. Chem.* **2009**, *48*, 4652–4654.

(52) Shaikh, M. M.; Srivastava, A. K.; Mathur, P.; Lahiri, G. K. *Dalton Trans.* **2010**, *39*, 1447–1449.

mentioned complexes prompted us to employ them as a modifier in the carbon paste electrode for determination of phenols but could not yield good selectivity, sensitivity, and response time. Thus we decided to synthesize a new ionic complex **1** in absence of any ancillary ligand. **1** consists of $[Cu(\mu_2\text{-hep})(\text{hep-H})]_2^{2+}$ cation possessing a dicopper−dioxygen unit and axially coordinated alcoholic OH group [Cu(1)−O(2) distance of 2.572(1) Å]. This alcoholic OH group can be easily available to form an active site for phenols to bind two Cu(II) centers.

Electrochemical Studies: Optimization of 1-CPE Variables. Differential pulse (DPV) and square wave voltammetry (SWV) were performed on all the phenols. It was observed that the highest sensitivity in terms of peak height was obtained for Phe and Res employing DPV while that for HQ and Cat by using SWV. The optimum conditions for analysis were established by recording the peak current response of phenols on various factors such as (i) the composition of the modifier, (ii) effect of pH, (iii) various supporting electrolytes, and (iv) pulse height and the frequency range (SWV).

The amount of the modifier (**1**) on the surface of the electrode plays a predominant role in the voltammetric oxidation of phenols. The response of the various composition of **1** in the range of 5−20% (w/w) into carbon paste was studied. It was observed that the oxidation peak current for phenols increased with the increase in percentage of **1** up to 10% beyond which saturation in the anodic peak current occurs. As a result, 10% of **1** was selected as the optimum amount for preparation of the modified electrode (**1**-CPE). The **1**-CPE was prepared (in the same fashion in which PCPE was prepared) in the composition of graphite/**1**/mineral oil (60:10:30% w/w/w).

The influence of the pH on the oxidation peaks of all phenols (Phe, HQ, Cat, and Res) was investigated individually in the pH range of 2−12 using Britton−Robinson (BR) buffer. It was observed that the peak potential of phenols shifted to less positive values with the increase in pH. The maximum peak current was obtained for Phe and Res at pH 6.0 (DPV) while that for HQ and Cat at pH 7.0 (SWV). These pH values were thus employed for further studies. Various buffers were then employed, viz., acetate, BR, citrate-phosphate, HEPES, and phosphate at the optimized pH values. The best peak sensitivity was observed in the case of phosphate buffer which was employed for further studies. The concentration of phosphate buffer was then optimized employing it in a range of 0.01−0.5 M. It was observed that the best response in terms of peak current was obtained at 0.1 M of the buffer.

The differential pulse voltammogram was recorded by scanning the potential toward the positive direction in the range of about +0.4 to +1.1 V for Phe and Res and at a pulse height in the range of 10−100 mV in phosphate buffer (pH 6.0). However, the best shape of voltammogram was obtained at 50 mV pulse height; hence, it was employed for further studies. SWV was carried out by studying the effects of frequency, pulse amplitude, and scan increment parameters on the peak current response to HQ and Cat oxidation in 0.1 M phosphate buffer solution pH 7.0. The influence of the frequency from 10 to 300 Hz on the **1**-CPE response was studied. The highest current response was obtained at 100 Hz for HQ and 80 Hz for Cat with 100 mV pulse amplitude. The effect of the scan increment was investigated in the range of 0.5−4.0 mV. The current increased up to 3.0 mV and remained constant at higher values, which was chosen for further studies.

Differential pulse voltammograms of Phe and Res and square wave voltammograms of HQ and Cat at PCPE and **1**-CPE employing the above optimized conditions are given in Figure 3. A 5-fold enhancement is obtained at **1**-CPE for Phe and 4 times enhancement for Res as compared to PCPE. The 16-fold enhancement in peak current was obtained for HQ and 14-fold enhancements for Cat at **1**-CPE as compared to PCPE, which reveals that the **1**-CPE has a high selectivity for phenols.

Cyclic Voltammetry, Chronocoulometry, and Electrochemical Impedance Spectroscopy. The surface areas of all the four electrodes of the same nominal bore size were found out using the 6 mM of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{2-}$ system in 0.1 M KNO_3. The surface areas of both the electrodes were calculated using the Randles−Sevcik equation and were found to be 0.0039 cm^2 for PCPE and 0.0164 cm^2 for Cu(II)−PE.

Comparative cyclic voltammograms for Cu(II) salt and **1** are as shown in Figure S-2 in the Supporting Information. The effect of the scan rate on the electrochemical behavior of Cu(II) salt and **1** reveal that when the potential was varied from −0.5 to +0.5 V, the peak potentials changed gradually (Figures S-3 and S-4 in the Supporting Information). The plot of i_p vs $v^{1/2}$ gave a straight line plot which implies that the redox reaction is diffusion controlled on the PCPE.

The electrochemical behavior of phenols was investigated using the plain carbon paste electrode and **1**-CPE by cyclic voltammetry. In this study, cyclic voltammetric measurements were obtained by sweeping the potential between an approximate range of −0.6 to +1.3 V for Phe, Res, HQ, and Cat, respectively, vs Ag/AgCl(aq) (3 M KCl) at a scan rate of 100 mV/s. It was seen that Phe and Res gave a completely irreversible peak at +0.777 and +0.814 V, respectively, while HQ and Cat both give a redox peak. The $E_{p,a}$ and $E_{p,c}$ for HQ were +0.471 and +0.053 V while that for Cat were +0.430 and −0.052 V, respectively. Figure 2 shows the cyclic voltammograms obtained using a plain carbon paste electrode and **1**-CPE based biomimetic sensor in 0.1 M phosphate buffer solution (pH 6.0 for Phe and Res; pH 7.0 for HQ and Cat). As can be observed, the peak response to the oxidation of phenols on employing **1**-CPE was much better than that of the PCPE. The probable reaction process could be described using the following equations:

$$\text{Phenols(reduced)} \longrightarrow \text{Phenols(oxidised)} + 2e^- + 2H^+ \quad \text{----------(1)}$$

$$2\text{Cu(II)} + 2e^- \rightleftharpoons 2\text{Cu(I)} \quad \text{----------(2)}$$

$$\text{Phenols(reduced)} + 2\text{Cu(II)} \longrightarrow \text{Phenols(oxidised)} + 2\text{Cu(I)} + 2H^+ \quad \text{----------(3)}$$

Reaction 1 is irreversible for Phe and Res and reversible for HQ and Cat. Cu(II) acts as an electrocatalyst for oxidation of phenols. These reactions are highly facile in the presence of molecular oxygen.

The effect of scan rate on all phenols has been studied (Figures S-5 and S-6 in the Supporting Information). The straight line plot of i_p vs $v^{1/2}$ reveals that the oxidation of all phenols of interest is diffusion controlled. At different scan rates ranging from 10 to 2000 mV/s, it can be seen that the anodic peak shifts to a more positive value for Res, Phe, Cat, and HQ and the cathodic

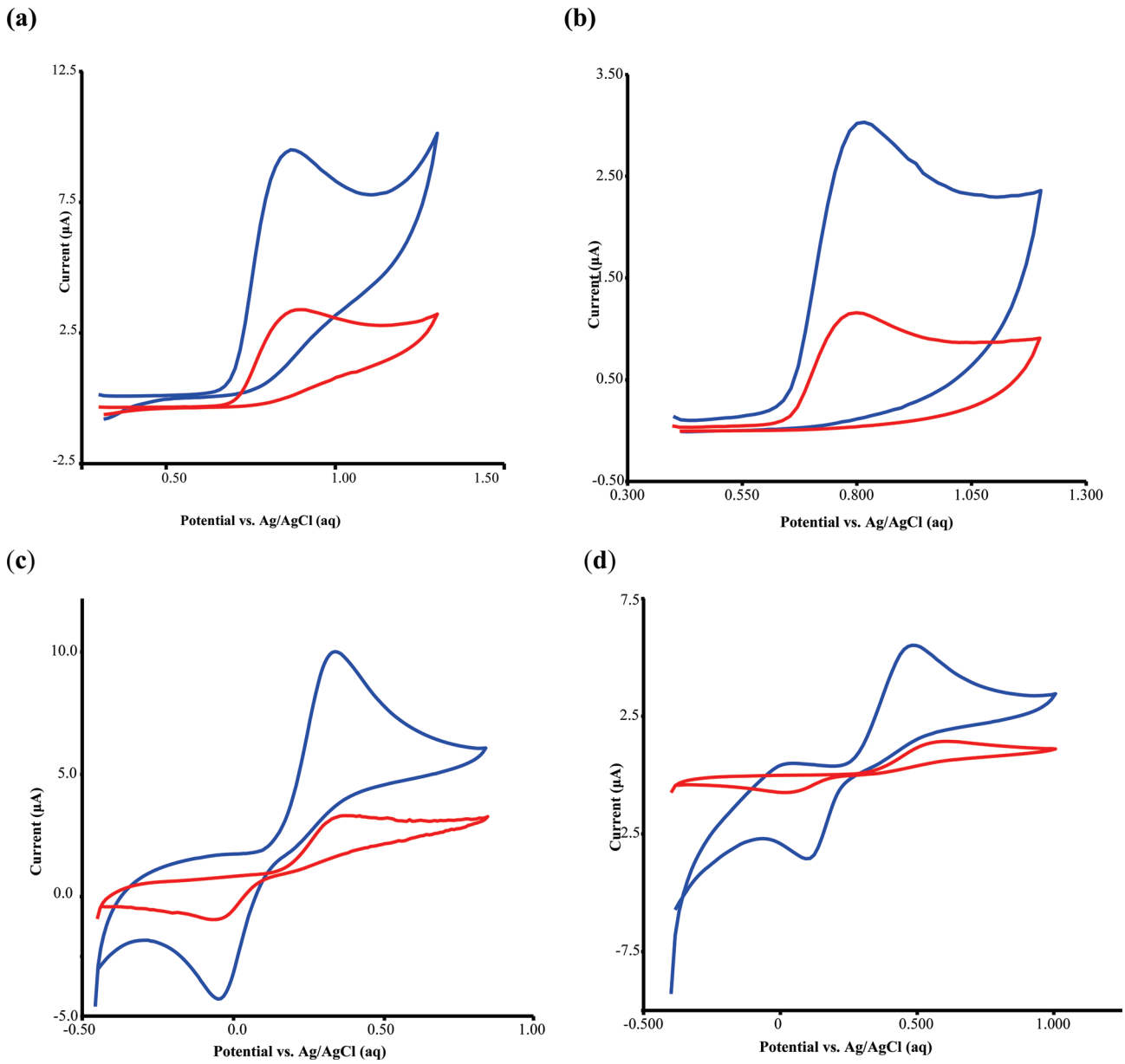

Figure 2. Cyclic voltammograms at scanning electrode potential with 100 mV/s (a) for Phe, 3.54×10^{-6} M at PCPE (red —) and Cu(II)-PE (blue —) between +0.30 and +1.3 V in phosphate buffer solution (pH 6.0). (b) For Res, 3.54×10^{-6} M at PCPE (red —) and Cu(II)-PE (blue —) between +0.4 and +1.2 V in phosphate buffer solution (pH 6.0). (c) For HQ, 1×10^{-6} M at PCPE (red —) and Cu(II)-PE (blue —) between −0.45 and +0.8 V in phosphate buffer solution (pH 7.0). (d) For Cat, 3.54×10^{-6} M at PCPE (red —) and Cu(II)-PE (blue —) between −0.6 and +1.0 V in phosphate buffer solution (pH 7.0).

peak for HQ and Cat shifts to the more negative values with increasing scan rates with a concurrent increase in peak current.

Electro-oxidation of Phe, Res, HQ, and Cat at the PCPE and **1**-CPE was characterized by employing chronocoulometry for the determination of the kinetics and mechanism of electrode reactions. The calculated parameters (diffusion coefficient and surface coverage) on employing double-potential step chronocoulometry[53] have been presented in Table 2. As can be seen from the Table, the value of Q_{ads} for **1**-CPE are more than that of PCPE, confirming that **1**-CPE makes the accumulation of all phenols on the electrode surface more effective.

In an attempt to clarify the difference between electrochemical performance of PCPE and **1**-CPE, electrochemical impedance spectroscopy was used. The Nyquist plots[54] for PCPE and **1**-CPE show a significant difference in the response for PCPE and **1**-CPE as shown in Figure 4. It reveals that **1**-CPE considerably affects the surface impedance properties. A semicircle with a larger diameter is observed for PCPE in the frequency range of 10^{-2} to 10^{6} Hz; however, the diameter of the semicircle diminished with the employment of modifier **1**-CPE (Figure 4). This implies that charge transfer resistance of the electrode surface decreases, and the charge transfer rate increased on employing **1**-CPE. A Warburg at 45° is also observed for both the electrodes.

(53) Anson, F. C. *Anal. Chem.* **1966**, *38*, 54–57.

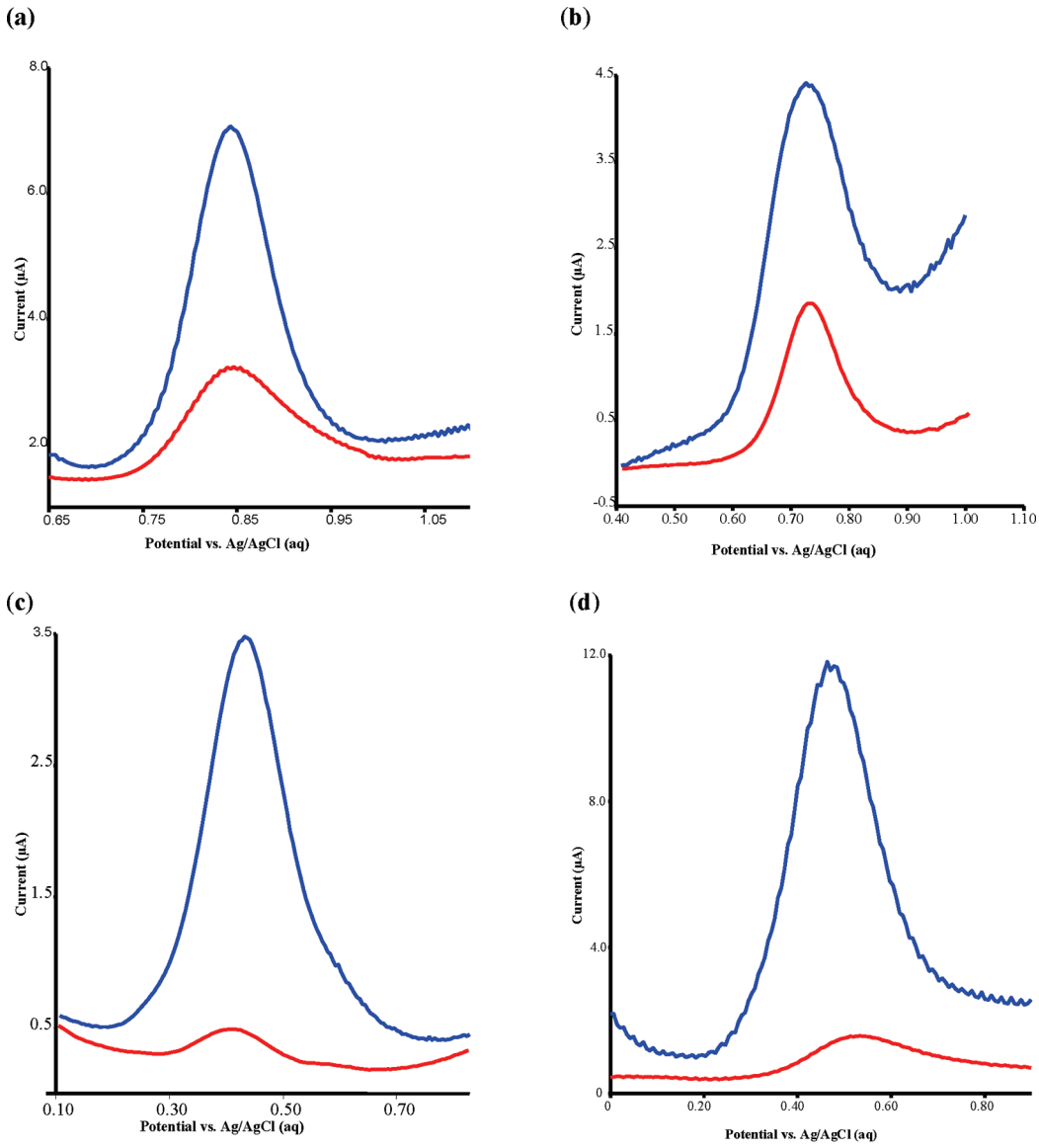

Figure 3. Differential pulse voltammogram at pulse height 50 mV of (a) Phe at PCPE (red —) and Cu(II)-PE (blue —). The concentration of Phe is 7.23×10^{-7} M in phosphate buffer solution (pH 6.0). (b) RES at PCPE (red —) and Cu(II)-PE (blue —). The concentration of Res is 1.33×10^{-6} M at pulse height 50 mV in phosphate buffer solution (pH 6.0). Square wave voltammogram (c) of HQ at PCPE (red —) and Cu(II)-PE (blue —). The concentration of HQ is 1.33×10^{-7} M at pulse height 50 mV and frequency 100 Hz in phosphate buffer solution (pH 7.0). (d) Cat at PCPE (red —) and Cu(II)-PE (blue —). The concentration of Cat is 7.81×10^{-7} M at pulse height 50 mV and frequency 80 Hz in phosphate buffer solution (pH 7.0).

Table 2. Chronocoulometry Data for 4×10^{-5} M Phe, Res, HQ, and Cat

molecule	electrode	slope (μC/s$^{-1/2}$)	Q_{ads} (μC)	surface coverage (10^{-10} mol/cm^2)	diffusion coefficient (10^{-6} cm^2/s)
Phe	PCPE	0.083	0.096	1.276	5.91 ± 0.33
	1-CPE	0.352	1.652	5.221	6.09 ± 0.49
Res	PCPE	0.089	0.117	1.561	6.93 ± 0.17
	1-CPE	0.379	1.865	5.892	7.02 ± 0.84
HQ	PCPE	0.092	0.146	1.937	7.33 ± 0.37
	1-CPE	0.391	1.958	6.186	7.48 ± 0.50
Cat	PCPE	0.088	0.152	2.016	6.67 ± 0.38
	1-CPE	0.367	1.898	5.999	6.58 ± 0.41

Determination of Michaelis−Menten Parameters by Chronoamperometry. The apparent Michaelis−Menten parameters, viz, Michaelis constant (K_M), maximum enzyme velocity (V_{max}), turnover number (k_{cat}), and specificity constant (k_{cat}/K_M) were calculated to study the catalytic effect of **1**-CPE on the substrate by employing chronoamperometry in a rapidly stirred solution.[55] This approach allowed the **1**-CPE catalyzed reaction to be monitored directly in the solution without a lag-phase. The catalysis process is based on diffusion layer approach. Figure 5a is a plot of initial rate (derived from the slope of current vs time over the first 0.8 s of chronoamperometric measurement) vs substrate concentration which reveals that the reaction rate increases linearly at low substrate concentrations. However, at high substrate concentrations, the Michaelis−Menten process is obeyed which implies an enzyme like catalytic process. Figure 5b is a Lineweaver−Burk plot obtained from the data in Figure

(54) Orazem, M. E.; Tribollet, B. *Electrochemical Impedance Spectroscopy*; Wiley: New York, 2008.
(55) Kamin, A.; Willson, G. S. *Anal. Chem.* **1980**, *52*, 1198–1205.

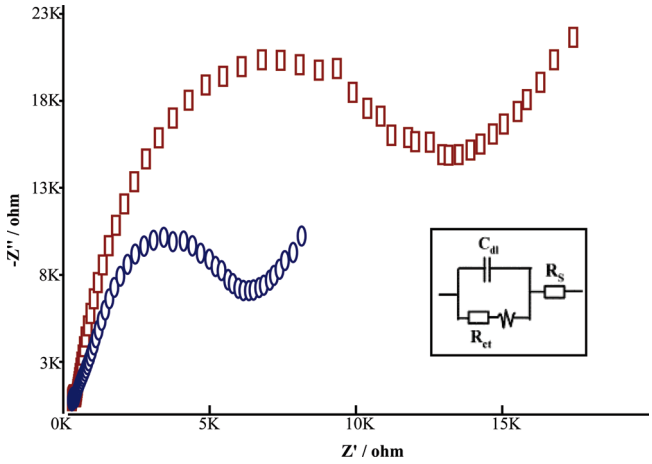

Figure 4. Nyquist plots for EIS measurements of PCPE (□□□) and **1**-CPE (○○○) in the frequency range 10^{-2} to 10^{6} Hz. In the box on the right lower side, an equivalent circuit was used for data fitting.

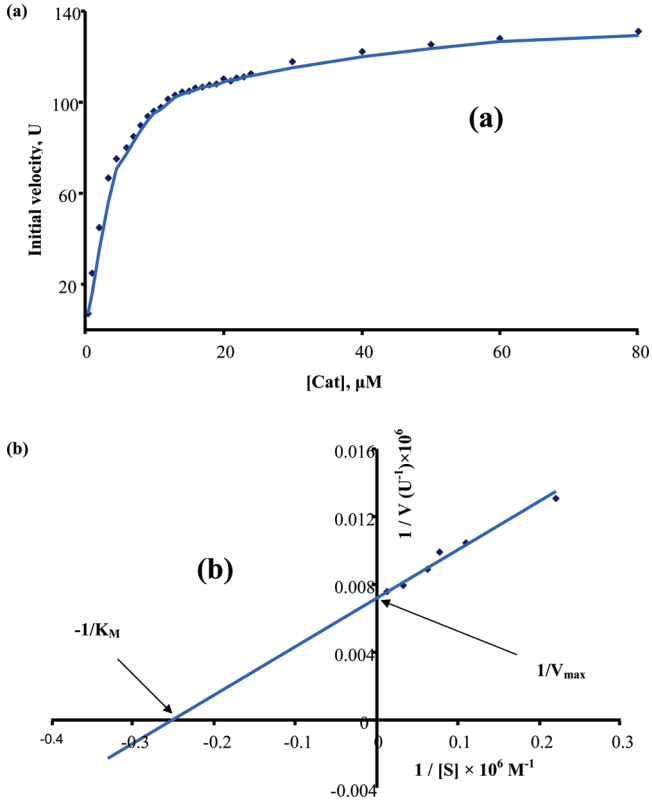

Figure 5. (a) Michaelis plot showing the initial reaction velocity in enzyme units (U), as determined by chronoamperometry at +0.65 V after addition of Cat for **1**-CME. (b) Lineweaver−Burk plot for the oxidation of Cat on **1**-CME.

5a. K_M and V_{max} values were obtained from this plot. The turnover number (k_{cat}) of the catalyst was determined by recording the initial rate of the oxidation of substrate (20 μM) in the presence of varying quantities of **1** (ranges 2−300 nmol) until saturation was observed. k_{cat} was calculated employing the formula $V_{max} = k_{cat}[E]_t$. $[E]_t$ is the concentration of the active sites that is obtained from the number of active sites (E_t) within **1**. It was observed that the initial rate increased until about 115 nmol of **1** was added to the substrate (Figure S-11 in the Supporting Information). Since **1** is a dinuclear complex, we get $E_t = 57.5$ nmol in 25 mL of solution which in turn gives $[E]_t$.

In Table 5, the catalytic activity of various substrates has been described with respect to their kinetic parameters. From the kinetic parameters, it can be seen that catalytic effect of **1** is maximum on Cat. The order for catalytic effect of **1** on phenols under study is Cat > HQ > Phe > Res. Few other molecules, viz., epinephrine, uric acid, 4-bromophenol, etc., have been employed to study the catalytic effect of **1** on them. Figure S-12 in the Supporting Information shows DPV plots of a few molecules on PCPE and **1**-CPE in pH 6.0 phosphate buffer. The values obtained for K_M, V_{max}, k_{cat}, and k_{cat}/K_M suggest **1** is an efficient catalyst for substrates having an −OH group.

Interference Studies, Validation, and Sample Analysis. Under optimal experimental conditions, the interferences of some metal ions and organic compounds especially phenols have been evaluated. The tolerance limit for interfering species was considered as the maximum concentration that gave a relative error in terms of ΔI_p less than ±5.0% at a concentration of 1.0×10^{-6} M of Phe, Res, HQ, and Cat. The results are tabulated in Table S-3 in the Supporting Information. Five replicates of each experimental set were performed for the calculation of the % RSD.

The statistical parameters are given in Table 3. The detection limits were calculated for all the phenols employing the IUPAC nomenclature of 3σ, where σ is the standard deviation of the peak currents of the blank at oxidation potentials for the respective phenol. The statistical data is presented in Table 3. The calibration curves based on voltammograms (Figures S-7−S-10 in the Supporting Information) showed a linear response ranging from $\sim 10^{-6}$ to 10^{-8} M for all the four analytes with detection limits of 1.04×10^{-8}, 2.31×10^{-8}, 1.54×10^{-8}, and 0.86×10^{-8} M for Phe, Res, HQ, and Cat, respectively.

Repeatability, reproducibility, precision, and accuracy of analysis using the proposed procedure were identified by performing five replicate measurements for standard solutions containing 2.17×10^{-7} M Phe, 1.89×10^{-7} M Res, 3.09×10^{-7} M HQ, and 2.55×10^{-7} M Cat over a single day (intraday assay) ($n = 5$) and for 5 days over a period of 1 week (interday assay). Satisfactory mean percentage recoveries (% R) and relative standard deviations (% RSD) were achieved (Table S-4 in the Supporting Information). The recoveries obtained confirmed both the high precision of the proposed procedure and stability of Phe, Res, HQ, and Cat solutions. The robustness of the proposed procedure (Table S-5 in the Supporting Information) was examined by studying the effect of a small variation of pH from 5.8 to 6.2 (Phe and Res) and 6.8 to 7.2 (HQ and Cat). The effect of varying the frequency over a narrow range on the recovery was obtained for HQ and Cat. As can be seen from Table S-5 in the Supporting Information, the % R of Phe, Res, HQ, and Cat were good and did not show a significant change when the critical parameters were modified. For further evaluation of the validity of the proposed method, the recovery tests for all phenols in sea and sewage water were carried out and the results are given in Tables S-6 (seawater) and S-7 (sewage water) in the Supporting Information. The various successive standard additions made are as given in the tables. It was observed that average % recovery varied between 98.0 and 102% for all phenols in the case of all samples. Recovery results

Table 3. Statistical Data for Phe, Res, HQ, and Cat[a]

no.	technique	molecule	LWR (M)	LRE	r	LOD (M)	% RSD
1	DPV	Phe	$(3.34 \times 10^{-8}) - (5.13 \times 10^{-6})$	$Ip(\mu A) = 1.2293 \times 10^{-6}C\ (M) + 0.017$	0.9991	1.04×10^{-8}	3.15
2	DPV	Res	$(7.66 \times 10^{-8}) - (5.81 \times 10^{-6})$	$Ip(\mu A) = 0.5194 \times 10^{-6}C\ (M) + 0.034$	0.9977	2.31×10^{-8}	2.43
3	SWV	HQ	$(5.41 \times 10^{-8}) - (1.81 \times 10^{-6})$	$Ip(\mu A) = 0.5135 \times 10^{-6}C\ (M) + 0.037$	0.9996	1.54×10^{-8}	3.28
4	SWV	Cat	$(2.83 \times 10^{-8}) - (26.23 \times 10^{-6})$	$Ip(\mu A) = 0.9921 \times 10^{-6}C\ (M) + 0.0076$	0.9979	0.86×10^{-8}	2.01

[a] LWR = Linear working range; RSD = relative standard deviation; r = correlation coefficient; LOD = limit of detection; LRE = linear regression equation.

Table 4. Determination of HQ in Photographic Developers and Commercial Cosmetics Using the Official Method and Biomimetic Sensor[a]

sample	a	b	c
photographic developer 1[b]	6.5	6.47 ± 2.11	6.38 ± 2.74
photographic developer 2[b]	7.0	6.95 ± 1.19	6.73 ± 2.18
medicated cosmetic sample[c]	40	39.73 ± 1.92	38.102 ± 2.37
medicated cosmetic sample[c]	60	59.36 ± 0.16	57.381 ± 1.23
medicated cosmetic sample[c]	55	54.871 ± 1.22	53.312 ± 1.87

[a] a, Amount of HQ in the sample; b, amount of HQ obtained by the proposed method ± % RSD (n = 5); c, amount of HQ obtained by the standard method ± % RSD (n = 5). [b] In % w/w. [c] In milligrams/liter (mg/L).

Table 5. Apparent Michaelis–Menten Parameters Obtained for the Oxidation of Following Substrate Molecules on 1-CPE

no.	substrate molecule	K_M (μM)	V_{max} (U)[a] (μM/min)	k_{cat} (min^{-1})	k_{cat}/K_M ($\mu M^{-1}\ min^{-1}$)
1	Phe	8.25	2.94	1.278	0.155
2	Res	8.66	2.41	1.048	0.121
3	HQ	4.82	6.77	2.943	0.611
4	Cat	3.89	7.89	3.430	0.882
5	epinephrine	4.33	7.33	3.186	0.736
6	uric acid	7.67	3.39	1.470	0.192
7	4-bromophenol	6.27	4.55	1.978	0.315
8	pyridoxine hydrochloride	9.88	2.73	1.189	0.120
9	ascorbic acid	11.64	1.44	0.626	0.054
10	acetaminophen	12.32	1.26	0.548	0.044
11	folic acid	14.93	0.95	0.413	0.028
12	acetyl salicylic acid	18.31	0.74	0.322	0.017
13	caffeine	21.81	0.22	0.096	0.0044

[a] 1 unit (U) represents 1 μmol of substrate formed per minute at 298 K.

were not affected significantly, and consequently the described method is reliable for the assay of all phenols in sea and sewage water samples from various locations.

The applicability of the voltammetric sensor in the determination of all phenols mentioned in this article was evaluated on a variety of matrixes. Phe was analyzed in injection samples while Res in hair colors and hair tonics employing DPV. HQ was analyzed in photographic developers as well as cosmetics while Cat was analyzed in tea samples and guarana tablets. The details of each are as given in the Experimental Section and Tables S-8 (Phe) and S-9 (Res) in the Supporting Information, in Table 4 (HQ), and Table S-10 (Cat) in the Supporting Information. All the samples were validated by comparing them with the official methods, i.e., Phe,[46] Res,[47] HQ,[48,49] and Cat.[50] By application of a paired t-test in the results obtained by this procedure and those claimed in the labels, it was found that all results are in agreement at the 95% confidence level and within an acceptable range of error.

Under optimal conditions, the response time (defined as the time when 95% of the steady-state current is reached) of the biomimetic sensor was obtained by chronoamperometry. The response time is 11, 10, 7, and 7 s for Phe, Res, HQ, and Cat, respectively. The rapid response indicates a fast mass transfer of the substrate across the **1**-CME.

Simultaneous Determination of Phenols in Their Respective Media at 1-CPE. The proposed DPV method has been employed for the simultaneous determination of Phe and Res in pH 6.0 phosphate buffer (0.1 M). Also simultaneous determination of HQ and Cat have been carried out in pH 7.0 phosphate buffer (0.1 M) employing SWV. The individual studies of each molecule showed that the oxidation peak potentials obtained for Phe and Res were 0.85 and 0.73 V, respectively (ΔE_p = 120 mV), indicating the potential for their simultaneous determination. The simultaneous determination was carried out by following variations: (i) increasing concentration of Phe in presence of fixed concentration of the Res (1×10^{-6} M) and vice versa (Figure S-13 in the Supporting Information) and (ii) increasing concentrations of Phe and Res simultaneously in the range of 1×10^{-7} to 1×10^{-6} M (Figure S-15a in the Supporting Information). Thus, both the molecules can be analyzed in the presence of each other. Similarly, simultaneous determination of HQ (E_p = 0.41 V) and Cat (E_p = 0.50 V) (Figures S-14 and S-15b in the Supporting Information) was also carried out.

In order to evaluate the validity of the simultaneous determination of phenols employing **1**-CPE, recovery tests for Phe and Res (pH 6.0 phosphate buffer) in sea and sewage water samples were carried out. Similarly, recovery tests were performed for HQ and Cat (pH 7.0) simultaneously. The % R obtained were in good agreement with those obtained for individual phenols as given in Tables S-6 and S-7 in the Supporting Information. This suggests the feasibility of the proposed electrode in simultaneous determination of Phe and Res (by DPV) and HQ and Cat (by SWV) in sea and sewage water samples.

Storage Capacity of the Biomimetic Sensor (1-CPE). The catalytic stability of the **1**-CPE sensor over time was checked during a period of 200 days (about 750 measurements). In between measurements, the sensor was kept at room temperature and in the dry state. A graph of i_p vs days reveal that a small loss in catalytic activity (approximately 5%) was measured after 200 days of storage. These results indicate a significant stability of the sensor and capacity for repeated measurement to be performed on the same electrode.

Table S-11 in the Supporting Information shows comparison of the biomimetic sensor's performance characteristic for measurement of Cat and HQ obtained by **1**-CPE and the reported

biomimetic sensors.[41,42,56,57] The lower limit of detection, low K_m values, short response time, and high V_{max} and k_{cat} values obtained for **1**-CPE suggest better catalytic activity of **1**-CPE on Cat and HQ. Moreover 1-CPE has been employed for various samples including sea and sewage water, cosmetics, and photographic developers. The presence of newly synthesized **1** as a modifier is distinctive to another modified electrode reported earlier due to its potential application as a biomimetic sensor with high sensitivity.

CONCLUSIONS

We have synthesized and fully characterized a new dinuclear copper(II) complex which contains an alcoholic OH group coordinated axially to each copper center forming a Cu_2O_2 nonplanar ring, which mimics the intermediate proposed in the mechanism of catechol oxidation through the enzyme catechol oxidase. The ability of this complex to mimic the active site of the enzyme was successfully used to construct a biomimetic sensor. To the best of our knowledge this is the first example of an alcoholic −OH group based biomimetic sensor being developed for trace determination of phenols. This is a first of its kind sensor where no oxidase or tyrosinase type ligand has been employed as a sensor material.

The method has been applied for the selective and precise analysis of Phe in commercial injections, Res in hair coloring agents, HQ in photographic developers and cosmetics, and Cat in tea samples and guarana tablets. This proposed method is free of common interferences associated with the molecules of interest and consequently is recommended for trace determination of Phe, Res, HQ, and Cat in clinical as well as quality control laboratories with great confidence. Moreover, the proposed methods have been employed for the trace simultaneous determination of Phe and Res in pH 6.0 phosphate buffer and HQ and Cat in pH 7.0 phosphate buffer. This sensor has practical application in quantitative analysis of certain acids, vitamins, and drugs having a −OH group. Further studies are planned for developing screen printed electrodes employing **1**-CPE for determination of phenols and other substrates considered in the present investigation. Also, the copper complex (**1**) has a potential to be employed for trace level detection of neurotransmitters, viz., glutamate, dopamine, etc., and even for peroxide and nitrite.

(56) Lakshmi, D.; Bossi, A.; Whitcombe, M. J.; Chianella, I.; Fowler, S. A.; Subrahmanyam, S.; Piletska, E. V.; Piletsky, S. A. *Anal. Chem.* **2009**, *81*, 3576–3584.

(57) Kafi, A. K. M.; Lee, D.-Y.; Park, S.-H.; Kwon, Y.-S. *Thin Solid Films* **2008**, *516*, 2816–2821.

ACKNOWLEDGMENT

A.K.S. and B.J.S. are thankful to the University Grant Commission-Special Assistance Programme, New Delhi, India, for providing financial assistance for the ongoing project. We are also grateful to Prof. M. Sudersanan, Adjunct Professor, University of Mumbai, for his valuable suggestions.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review February 13, 2010. Accepted May 31, 2010.

AC1004037