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Stabilisation of copper(I) polypyridyl complexes toward aerobic oxidation by zinc(II) in combination with acetate anions: a facile approach and its application in ascorbic acid sensing in aqueous solution†

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A new and facile approach to stabilise copper(I) complexes in aqueous solution by the addition of zinc(II) ions in combination with acetate ions (OAc^-) was demonstrated. This stability enhancement toward the aerobic oxidation of copper(I) species was investigated by various techniques including UV-vis spectroscopy, ^{1}H-NMR, FT-IR, and ESI-MS. Our experimental results together with DFT calculations led to a proposed structure of [(**adpa**)Cu-OAc-Zn(OAc)$(H_2O)_2]^{+/2+}$. It was also postulated that zinc(II) with its Lewis acidity may attract electrons from the Cu centre through the bridging ligands (OAc^-), resulting in the lower reactivity of Cu(I) with O_2. In addition, this strategy was shown to be applicable to ascorbic acid detection by monitoring a change in the redox states of copper complexes using fluorescence spectroscopy. Moreover, it was demonstrated that the method was sensitive and accurate for the quantitative analysis of ascorbic acid in vitamin C tablets.

Introduction

Copper is an abundant and redox-active metal. It is frequently employed as a redox catalyst in a wide range of both synthetic and biological reactions.[1–4] In nature, copper containing enzymes serve as mediators for substrate conversion. Typically, Cu(II) centres in the enzymes will be reduced to Cu(I) reactive species for further catalytic processes. Interestingly, there are several enzymes which can generate Cu(I) active intermediates using ascorbic acid (vitamin C, AsH_2) as a reducing agent such as dopamine β-hydroxylase (DβH), peptidylglycine α-hydroxylating monooxygenase (PHM) and ascorbate oxidase (AO).[5,6] Likewise, synthetic metal complexes show high potential in AsH_2 oxidation.[7–12] Hence, redox active metals including copper are potentially applicable as a sensor probe for AsH_2 by monitoring the changes in the redox state of the metal.

Ascorbic acid (vitamin C, AsH_2) is an essential antioxidant playing a vital role in biological processes. In addition, the AsH_2 level positively correlates with several diseases including cardiovascular disease, viral infection and Alzheimer's disease as well as anticancer therapy.[13–16] On the other hand, the lower level of AsH_2 can lead to the development of diabetes or cognitive impairment.[17–19] Also, AsH_2 has been used as an additive substance in food, beverages and pharmaceutical formulations for oxidative protection. Because of its advantages in food quality control and healthcare, the development of a simple method for AsH_2 detection has gained considerable attention. Recently, a number of research studies reported sensors for AsH_2 based on materials such as quantum dots, Au nanoparticles and C-dots/Fe(III).[20–26] However, these materials could be suffering from reproducibility and characterisation. On the other hand, molecular probes (*e.g.*, metal complexes) can be characterised in terms of molecular structures, chemical properties and mechanistic studies with routine spectroscopic techniques. In addition, these methods are quite repeatable.

Unfortunately, the utilisation of Cu complexes in the field of molecular sensors *via* a redox reaction (Cu(II) → Cu(I)) is usually hampered by the instability of the Cu(I) species in aqueous solution under aerobic conditions.[27,28] Therefore, new strategies for the stabilisation of the active species (Cu(I))

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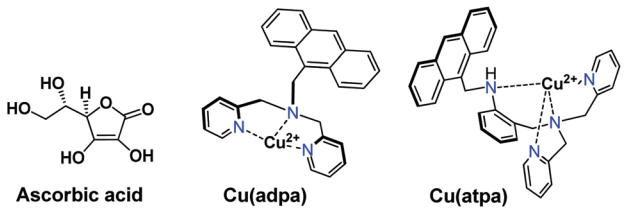


Chart 1 Ascorbic acid (AsH_2) and copper complexes used in this study.

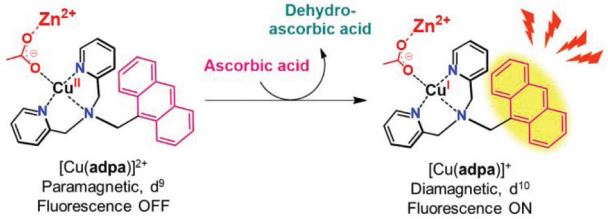


Fig. 1 Proposed acetate-bridged Zn(II)–Cu(I/II)**adpa** complex and the mechanism for AsH_2 detection.

are of particular importance for developing a simple, rapid and accurate sensor for the detection of antioxidants. It has been demonstrated that copper(I) can be stabilised by metal–π interactions relying on the ligand attributes with conjugated π systems or by coordinating solvents (*i.e.* acetonitrile).[29–32] We initially tried to stabilise Cu(I) using acetonitrile as the solvent due to its facile procedure. Unfortunately, our Cu(I) complex, Cu^{I}(**adpa**) (Chart 1) was not stable in aqueous solution despite the presence of acetonitrile.

Herein, we report a new approach to stabilise Cu(I) polypyridyl complexes toward aerobic oxidation by Zn(II) ions in combination with acetate anions (OAc^-) as shown in Fig. 1. We proposed that the positively-charged Zn^{2+} may stabilise Cu(I) by attracting electrons through acetate bridging ligands. This secondary coordination sphere modulation was inspired by an imidazolate-bridged Cu–Zn model for superoxide dismutase (CuZnSOD) enzymes.[33–37] Our simple and easy approach was then applied for the accurate detection of AsH_2 in vitamin C tablets with good sensitivity. To the best of our knowledge, this is the first example of copper complexes as fluorescent probes *via* a reversible redox reaction for AsH_2 detection.

Results and discussion

Reaction of the Cu(II) complex and ascorbic acid

To establish distinct spectroscopic patterns between Cu(I) and Cu(II) species and confirm the successful reaction of our copper complexes and AsH_2, the reaction of Cu^{II}(**adpa**) and AsH_2 was initially performed under a N_2 atmosphere in CH_3CN. Upon the addition of AsH_2 to the solution of Cu^{II}(**adpa**), a color change from blue to pale yellow was observed. Monitoring of this reaction by UV-vis spectroscopy and ^{1}H-NMR confirmed that Cu^{II}(**adpa**) was reduced to Cu(I) species in concomitance with the oxidation of AsH_2 as evidenced by the observed production of dehydroascorbic acid (Fig. S1 and S2†). It should be noted that the same result was obtained when the reaction was carried out in the presence of O_2, and the Cu(I) species seemed to be stable for at least 5 h. In contrast, when the solvent was changed to H_2O/CH_3CN (7 : 3 v/v) under ambient conditions, Cu^{I}(**adpa**) could not be completely formed and gradually reoxidised by air to Cu(II), suggesting its instability in aqueous solution. However, performing the reactions in aqueous solution is essential for the detection of biological substances including natural reducing agents owing to their solubility in water.

Cu(I) stability enhancement toward aerobic oxidation by the modulation of the secondary coordination sphere

From the heteronuclear metalloenzymes in nature, we learn that Zn^{2+} not only serves as a mediator for catalytic substrate conversion, but also plays a crucial role in the stabilisation of enzymes and proteins.[38] A synthetic model for CuZnSOD demonstrated the vital role of Zn^{2+} in controlling the redox potentials of Cu(II) ions through the imidazole (Im) bridge for the desired catalytic reaction.[36] From this inspiration, we came up with the idea that Zn^{2+} in combination with bridging ligands may help to facilitate Cu(II) reduction and stabilise our Cu(I) species in aqueous solution under aerobic conditions. Thus, we first proved our assumption by the addition of Zn^{2+} and Im in the reaction of Cu^{II}(**adpa**) + AsH_2 in H_2O/CH_3CN (7 : 3 v/v). As a result, it seemed that the reduction of Cu(II) to Cu(I) was facilitated as the Cu(II) d–d band was dramatically decreased when monitored by UV-vis spectroscopy. However, the reaction started to be cloudy after 15 min which hampered a prolonged monitoring of this reaction. Then, we also tried to add histidine (His) instead of Im, and found that Cu(II) was fully reduced to Cu(I). However, His was not well-dissolved in our solvent system. It should be noted that His contains not only the Im side chain, but also a carboxyl group which can serve as a bridging ligand. Therefore, we sought for other alternatives, and thought that acetate anions (OAc^-) might be a good candidate since they have been demonstrated as a bridge between two metal centres in dinuclear complexes.[39–43] To test our hypothesis, the reduction of Cu^{II}(**adpa**) by AsH_2 (1 mol equiv.) in H_2O/CH_3CN (7 : 3 v/v) was performed under aerobic conditions in the presence of 8 mol equiv. of $Zn(OAc)_2$, $Zn(NO_3)_2$ or $Zn(phen)_2$. Fig. 2 indicates that the d–d transition band of Cu(II) (λ_{max} = 632 nm) completely disappeared upon the addition of AsH_2 only in the presence of $Zn(OAc)_2$, and the spectrum was stable for at least 60 min before the d–d band started to come back, implying the regeneration of Cu(II). This could suggest that $Zn(OAc)_2$ can help facilitating the reduction of Cu^{II}(**adpa**) and stabilising the Cu(I) species as well. Moreover, the reversible d–d band of Cu(II) (Fig. S4†) confirmed that no transmetalation occurred between Zn^{2+} and Cu(**adpa**) during the redox reaction of Cu(**adpa**) in the presence of $Zn(OAc)_2$.

Furthermore, the importance of Zn^{2+} in stabilising Cu^{I}(**adpa**) was highlighted by comparative reactions using

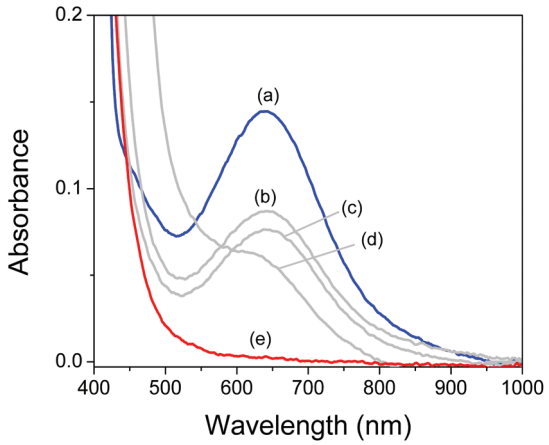


Fig. 2 UV-vis spectra of (a) 2 mM Cu^{II}(**adpa**); (b) Cu^{II}(**adpa**) + AsH_2 (1 mol equiv.); Cu^{II}(**adpa**) + AsH_2 (1 mol equiv.) in the presence of 8 mol equiv. (c) $Zn(NO_3)_2$, (d) $Zn(phen)_2$, and (e) $Zn(OAc)_2$ in H_2O/CH_3CN (7 : 3 v/v) at room temperature.

other redox inactive cations with OAc^- *i.e.*, Na^+, Mg^{2+} and Ca^{2+} instead of Zn^{2+} (Fig. 3 and S7†). It was found that in the presence of such metal ions, the Cu(II) d–d band was suddenly decreased upon the addition of AsH_2, suggesting that all metal ions with OAc^- are likely to facilitate the Cu(II) reduction by AsH_2. However, all Cu(II) spectra gradually returned after 5 min, except the one with $Zn(OAc)_2$ of which the Cu(I) species could be stabilised for at least 60 min. Therefore, it is conclusive that both Zn^{2+} and OAc^- are required to facilitate the reduction of the Cu(II) complex and stabilise the Cu(I) redox state. We also proposed that Zn^{2+} as a good Lewis acid may help to stabilise Cu(I) by the delocalisation of electrons through the OAc^- bridge, analogous to an imidazolate-bridged Cu–Zn model for CuZnSOD.[36]

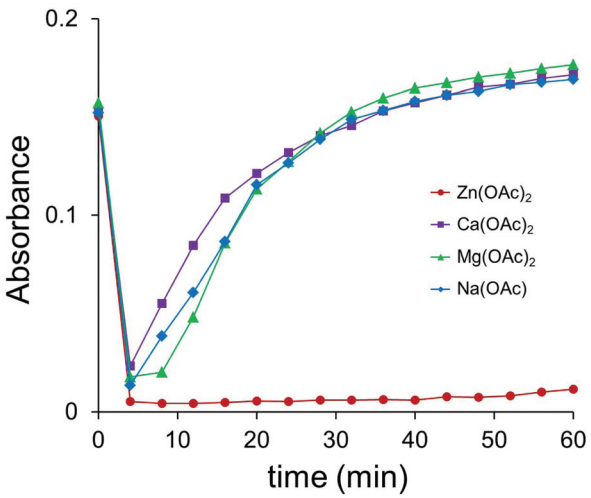


Fig. 3 Change in the absorbance of Cu(II) for the reactions of 2 mM Cu^{II}(**adpa**) with AsH_2 (1 mol equiv.) in the presence of various redox inactive cations as a function of time. Each reaction was performed in H_2O/CH_3CN (7 : 3 v/v) at room temperature.

It is worth mentioning that the influence of a Lewis acid on the redox potential of the metal centre has been previously demonstrated.[44–46] Therefore, the electrochemical behaviours of our Cu(I)/(II)(**adpa**) redox couple in the absence and presence of $Zn(OAc)_2$ were studied by cyclic voltammetry. It was found that the reduction potential of Cu^{II}(**adpa**) did not change significantly after adding $Zn(OAc)_2$, but Cu(I) seemed to be reoxidized less than that in the absence of $Zn(OAc)_2$. In addition, when the supporting electrolyte was changed from an acetic–acetate buffer to a non-coordinated electrolyte (potassium hexafluorophosphate, KPF_6), a similar result was obtained. These electrochemical results might suggest that the reoxidation of Cu(I) to Cu(II) is more difficult in the presence of $Zn(OAc)_2$.

To rule out the possibility that the pH change caused by Lewis acid Zn(II) ions may be responsible for the stability of Cu(I), a comparative study in the buffered solvent (acetic–acetate buffer solution, pH 5.6) was carried out (Fig. S18†). In the reaction of Cu^{II}(**adpa**) + AsH_2 in the absence of Zn(II), Cu(I) was not fully formed and gradually reoxidised to Cu(II) within 20 min. On the other hand, in the presence of Zn(II) under the same conditions, the Cu(I) complex was stabilised up to 40 min.

Proposed structure of $[(adpa)Cu\text{-}OAc\text{-}Zn(OAc)(H_2O)_2]^{+/2+}$

To shed some light on how $Zn(OAc)_2$ helps to stabilise the Cu^I species, we sought to investigate the interaction between Cu^{II}(**adpa**) and $Zn(OAc)_2$ by spectroscopic techniques, ESI-MS, and DFT calculations which led to a proposed structure of $[(adpa)Cu\text{-}OAc\text{-}Zn(OAc)(H_2O)_2]^{+/2+}$. To begin with, our first structural evidence on the proposed structure came from FT-IR analysis. Since the carboxylate group can coordinate to the metal ions in various ways, a number of research studies examined the vibrational frequencies of acetate salts to correlate with their structure and employed this relationship to predict the coordination mode of OAc^- in metal complexes.[41,42,47] Deacon and Phillips[41] reported that the frequency separation between the COO^- antisymmetric and symmetric stretching vibrations or the $\Delta\nu_{a-s}$ values for the species with different binding modes usually fall in the following order:

$$\Delta\nu_{a-s}(\text{unidentate}) > \Delta\nu_{a-s}(\text{ionic}) \sim \Delta\nu_{a-s}(\text{bridging}) > \Delta\nu_{a-s}(\text{bidentate}).$$

It should be noted that OAc^- has been reported as a bridging ligand in both homo- and heterometallic complexes.[39–43] The FT-IR spectrum of our precipitate obtained from the reaction between Cu^{II}(**adpa**) and $Zn(OAc)_2(H_2O)_2$ is shown in Fig. 4e in comparison with those of $Zn(OAc)_2(H_2O)_2$ (bidentate), Cu^{II}(**adpa**), Na(OAc) (ionic), and a solid mixture of $Zn(OAc)_2(H_2O)_2$ + Cu^{II}(**adpa**). It can be seen that our precipitate from the reaction showed two new peaks at 1561 and 1385 cm^{-1} which could be assigned to asymmetric and symmetric COO^- stretching, respectively. Since these new signals did not match with the starting material $Zn(OAc)_2(H_2O)_2$ and free OAc^- (ionic), it suggested that OAc^- might form a new

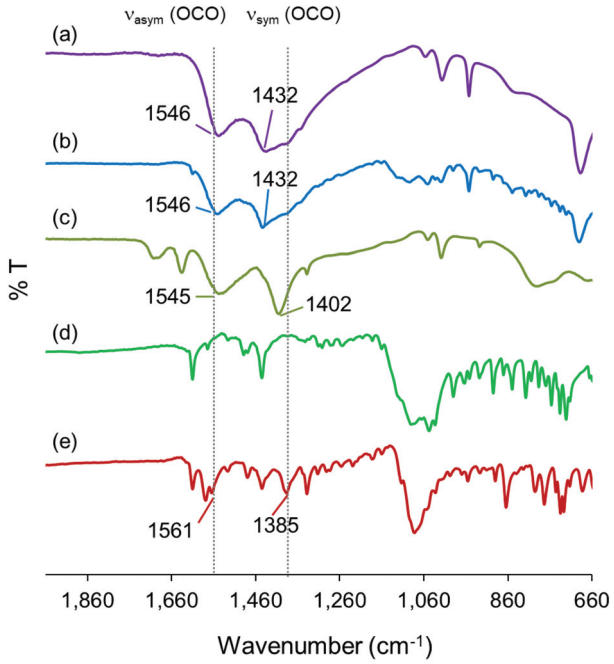


Fig. 4 Comparison of the FT-IR spectra of (a) $Zn(OAc)_2(H_2O)_2$; (b) solid mixture of $Zn(OAc)_2(H_2O)_2$ + Cu^{II}(**adpa**); (c) Na(OAc); (d) Cu^{II}(**adpa**) and (e) precipitate from the reaction of Cu^{II}(**adpa**) + $Zn(OAc)_2(H_2O)_2$.

coordination. Notably, the shifts in these vibrational frequencies are quite similar to those observed for acetate bridging in several carboxylate-bridged copper complexes (*e.g.* Cu–Cu, Cu–Ca).[42,48–50] The $\Delta\nu_{a-s}$ value of 176 cm^{-1} from our sample is quite close to that from the heterometallic carboxylate-bridged Cu–Ca complex (180),[42] but significantly different from that of bidentate OAc^- in $Zn(OAc)_2(H_2O)_2$ ($\Delta\nu$ = 114). These data pointed that OAc^- might serve as a bridging ligand between Zn^{2+} and Cu^{II}(**adpa**). To support this hypothesis, an optimised structure of [(**adpa**)Cu-OAc-Zn(OAc)$(H_2O)_2]^{2+}$ was obtained from the DFT calculations with the CPCM(UFF)/B3LYP/6-311+G(d,p) basis set. It was found that the O–C–O angle of the acetate bridge was 121.8° which is relatively close to that of the heterometallic bidentate acetate bridge of the Cu–Ca complex (121.5(3)°),[43] Zn–Ca (122.9, 123.9 and 124.3°) and Zn–Mg (123.9 and 124.9°).[39]

To gain further support for the proposed structure of [(**adpa**)Cu-OAc-Zn(OAc)$(H_2O)_2]^{2+}$ in the solution phase, we also monitored the Cu^{II}(**adpa**) + $Zn(OAc)_2(H_2O)_2$ reaction by UV-vis spectroscopy and ^{1}H-NMR. Interestingly, upon the addition of $Zn(OAc)_2(H_2O)_2$ to Cu^{II}(**adpa**), a slight shift in the d–d band of Cu(II) and a significant change in the ^{1}H-NMR signal of OAc^- were observed. The ^{1}H-NMR signal of OAc^- in Cu^{II}(**adpa**) + $Zn(OAc)_2(H_2O)_2$ was broadening and shifted to 2.6 ppm, indicating the coordination of OAc^- to Cu(II). For comparison, when Na(OAc) was added to Cu^{II}(**adpa**), the ^{1}H-NMR signal of CH_3COO^- (OAc^-) could not be observed (Fig. S8†). This suggested the difference in the coordination of OAc^- between these two systems. As a bridging ligand between the Zn(II) and Cu(II) complex, OAc^- may have a weaker paramagnetic influence of Cu(II) than that in the Cu^{II}(**adpa**) + Na(OAc) solution. In addition, the ESI-MS data clearly showed that OAc^- from $Zn(OAc)_2(H_2O)_2$ could bind to Cu(II) (see the ESI†). All of these results support the finding that the formation of [(**adpa**)Cu-OAc-Zn(OAc)$(H_2O)_2]^{2+}$ is feasible.

In the case of the Cu(I) species, the reaction of Cu^{II}(**adpa**) + $Zn(OAc)_2(H_2O)_2$ and AsH_2 gave rise to a prominent signal at *m*/*z* 512.1356 in the ESI-MS spectrum, corresponding to $[Cu^{I}$(**adpa**) + (OAc^-) + $H^+]^+$. It might be implied that OAc^- could coordinate to the Cu(I) centre in a similar manner to that found in the Cu(II) compound, Cu^{II}(**adpa**). Unfortunately, the interaction of the Cu(I) complex and $Zn(OAc)_2(H_2O)_2$ could not be investigated by other spectroscopic techniques due to the limitations of our system. Therefore, we turned our attention to studying this interaction and proposing the structure using DFT calculations. As a result, a DFT-optimised structure of [(**adpa**)Cu-OAc-Zn(OAc)$(H_2O)_2]^+$, together with its frontier orbitals, was obtained as shown in Fig. 5 and Fig. S13–15.† Markedly, a significant change in the highest occupied molecular orbital (HOMO) delocalisation on the copper(I) centre in the presence and absence of $Zn(OAc)_2(H_2O)_2$ was noted. In [(**adpa**)Cu-OAc-Zn(OAc)$(H_2O)_2]^+$, the HOMO orbital is delocalized to the Cu(I) centre substantially less than that of the Cu complex without $Zn(OAc)_2(H_2O)_2$. This result is consistent with our experimental finding that the Cu(I) complex is significantly less reactive toward O_2 in the presence of $Zn(OAc)_2$. However, when there is only OAc^- bound to the Cu(I) centre without Zn^{2+}, the HOMO is delocalised around the Cu(I) ion and its coordination site which is in agreement with our reactivity study that Cu^{I}(**adpa**) + NaOAc seemed to react with O_2 to

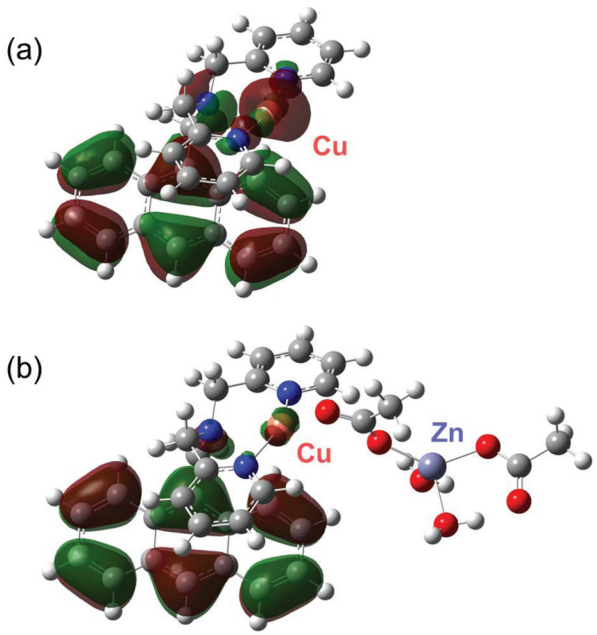


Fig. 5 Plots of the HOMOs of (a) Cu^{I}(**adpa**) and (b) Cu^{I}(**adpa**)/Zn $(OAc)_2{\cdot}(H_2O)_2$, computed at the CPCM(UFF)/B3LYP/6-311+G(d,p) level of theory.

generate the Cu(II) product. We account for the stability of Cu^{I}(**adpa**) in the presence of $Zn(OAc)_2$ by the role of Zn^{2+} as a good Lewis acid. Due to its electron-withdrawing properties, Zn^{2+} may help to attract electrons from the Cu(I) active site *via* the acetate bridge, leading to a less electron density on the Cu centre. This rationale was consistent with the fact that Cu^{I}(**adpa**) + $Zn(OAc)_2$ exhibited less reactivity toward O_2 when compared to Cu^{I}(**adpa**) itself. Given the proposed structure, we also speculate that the bridge through OAc^- and Zn^{2+} may help to provide a steric encumber which inhibits the oxidizing agent (*e.g.* O_2) to react with the Cu(I) centre.

All of these results are in agreement with our proposal that OAc^- coordinated to the copper centre and could serve as a bridging ligand between the Cu(I)/(II) and Zn^{2+} ions resulting in the stabilisation of the Cu(I) species under aerobic conditions. Next, this approach was tested for its applicability to AsH_2 sensing.

Application in ascorbic acid sensing by fluorescence

Our approach was applied to detect AsH_2 by fluorescence spectroscopy because of its high sensitivity. As a molecular sensor, Cu(**adpa**) contains a dipicolylamine Cu active site and anthracene as a sensory unit. A change in the redox states of the Cu centre would give a different fluorescence spectrum. As expected, Cu^{II}(**adpa**) did not show fluorescence signals owing to the disturbance of the paramagnetic species, Cu(II).[51] The addition of AsH_2 resulted in a significant fluorescence enhancement at 421 nm which is consistent with the conversion of Cu(II) to a diamagnetic Cu(I) complex as illustrated in Fig. 6.

Because ascorbic acid is a hexanoic sugar acid with two acidic protons (pK_a 4.04 and 11.34),[21] the effects of pH and interferences from common natural reducing agents (*i.e.*, reducing sugar, citric acid, and glutathione) were investigated next. The results showed that pH had a large influence on our AsH_2 detection in terms of both sensitivity and selectivity (Fig. S19†). At pH 4, a satisfactory fluorescence intensity and high selectivity for AsH_2 detection were achieved. However, the selectivity was significantly lower at a higher pH due to Cu chelation by glutathione.[52–56]

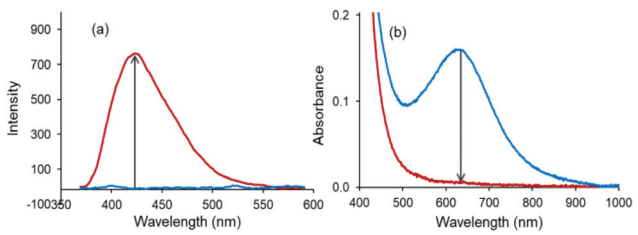
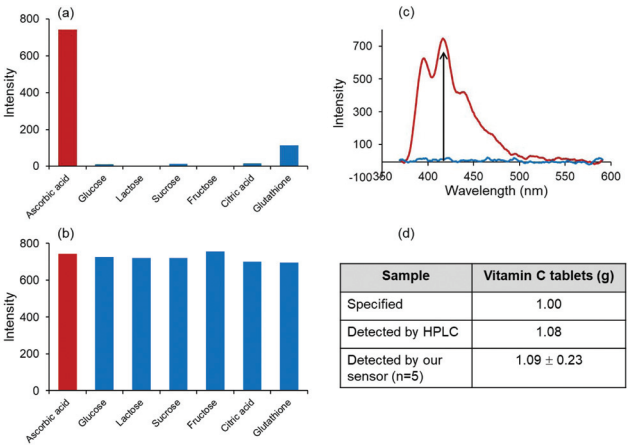


Fig. 6 Change in (a) the fluorescence signal (excitation wavelength = 340 nm, slit setting on the instrument = 10 and PMT = 500); and (b) UV-vis spectrum upon the addition of AsH_2 (1 mol equiv.) to the Cu^{II}(**adpa**) solution in the presence of Zn(II) in H_2O/CH_3CN (7 : 3 v/v) buffered with ABS at pH 5.6.



Sample	Vitamin C tablets (g)
Specified	1.00
Detected by HPLC	1.08
Detected by our sensor (n=5)	1.09 ± 0.23

Fig. 7 (a) Selectivity and (b) interference study for AsH_2 detection using our sensor; (c) fluorescence spectral change upon the addition of AsH_2 to Cu(**atpa**) + $Zn(OAc)_2$; and (d) detection of ascorbic acid in vitamin C tablets. The concentration of Cu(**atpa**) = 10 μM, $Zn(OAc)_2$ = 40 mol equiv. and λ_{ex} = 340 nm.

To decrease the influence of the chelation of Cu ions by analytes and to expand the pH range of our detection, we decided to prepare Cu^{II}(**atpa**) (Chart 1) for our further studies. As a tetradentate ligand, **atpa** was expected to bind Cu ions more tightly than a tridentate ligand, **adpa**. Hence, copper sequestration by glutathione will be less pronounced. Our results confirmed this assumption as shown in Fig. 7. In addition, our limit of detection (LOD) for AsH_2 was determined to be 163 nM which is relatively low ($3\sigma/S$; σ is the standard deviation for the blank solution, n = 10, and S is the slope of the calibration curve).

Moreover, our approach was then employed to measure the AsH_2 amount in vitamin C tablets to verify our accurate detection in real samples. The measurements by our sensor, Cu^{II}(**atpa**), in comparison with HPLC were done using the standard addition method. The result from our method was in good agreement with that from standard HPLC and was close to the amount specified on the vitamin C tablets. Our recovery was in between 101 and 104, and the relative standard deviation was 1.5–4.0. This demonstrated the high accuracy of our method.

Conclusions

In conclusion, we presented a new strategy to stabilise Cu(I) complexes. Our experiments demonstrated that a combination of Zn^{2+} ions and OAc^- can stabilise Cu(I) species in aqueous solution under aerobic conditions. This approach was subsequently applied to detect AsH_2 in vitamin C tablets with high accuracy. Our strategy would also attract interest in other areas such as catalysis for the control of stability and reactivity of metal complexes for mechanistic investigation. Undoubtedly, this approach would pave the way for designing copper complexes as molecular sensors for biological applications.

Experimental

Materials and methods

Solvents of HPLC grade were purchased from Merck and used without further purification. Milli-Q was prepared using ultrapure water systems. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories, Inc. NMR experiments were carried out using a 400 MHz Varian Mercury spectrometer. All UV-vis spectra were recorded by using a Varian Cary 50 probe UV-visible spectrophotometer with a quartz cuvette (path length = 10 mm). Fluorescence spectra were obtained using a Varian spectrofluorometer with Cary Eclipse, a pulsed xenon lamp and a photomultiplier tube detector. Mass spectra were obtained by Electrospray Ionization Mass Spectrometry (ESI-MS) on a Bruker Daltonics microOTOF. IR measurements were carried out on a Thermo, Nicolet 6700 FT-IR in ATR mode. Cyclic voltammetry was performed on an μAutolab Type III potentiostat under an N_2 atmosphere. This system contained a three-electrode cell: glassy carbon as the working electrode, Pt wire as the counter electrode and $Ag/AgNO_3$ (0.01 M) as the reference electrode. For HPLC analysis, the amounts of ascorbic acid in vitamin C tablets were determined using a Varian Prostar with a C-18 column (C18 4.6 × 250 mm, 5 μm, Phenomenex), pumped at a flow rate of 1.5 mL min^{-1}. Vitamin C tablets were purchased from PT Bayer Indonesia, Depok, Indonesia. All copper complexes and ligands were prepared according to modified published procedures.[57–61]

Caution! Perchlorate is a potentially explosive salt. Experiments should be carefully handled.

Computation methods

The density functional theory (DFT) method, the hybrid density functional theory of Becke's three parameter exchange functional[62] with the Lee–Yang–Parr correlation functional[63] (B3LYP), using the 6-311+G(d,p) basis set[64,65] was employed. All structure optimizations in aqueous solution using the B3LYP/6-311+G(d,p) method combination with the solvent effect of the polarizable continuum model (PCM)[66–72] using the CPCM (conductor-like PCM, water as a solvent) model[73–75] with the UFF molecular cavity model,[64] called the CPCM(UFF)/B3LYP/6-311+G(d,p) level of theory, were carried out. All calculations were performed with the Gaussian09 program.[76] The molecular graphics for relevant compounds and their frontier orbitals (HOMOs and LUMOs) were plotted and visualised using the GaussView 5.0.9 program.[77]

Reaction of Cu^{II}(adpa) and ascorbic acid (AsH_2) in CH_3CN

All solvents were deoxygenated prior to use by purging N_2 for at least 1 h. A stock solution of AsH_2 was prepared in a solvent mixture of 5% H_2O in CH_3CN (44 mM). To a solution of Cu^{II}(**adpa**) (2.0 mM) in CH_3CN was added the AsH_2 stock solution (0.55 mol equiv.). A color change of the solution from blue to pale yellow was observed. Monitoring of this reaction by UV-vis spectroscopy showed a complete reduction of Cu^{II}(**adpa**) (λ_{max} = 591 nm corresponding to a d–d transition band of d^9 Cu^{II} species) to Cu^{I}(**adpa**) (no d–d transition band). When exposed to air, this pale-yellow species was found to be stable for at least 5 h. An NMR sample was prepared as follows: to a solution of Cu^{II}(**adpa**) (10 mM) in CD_3CN (550 μL) was added AsH_2 (20 μL, 0.55 mol equiv. dissolved in 15% D_2O in CD_3CN). A color change from blue to pale yellow was noted, consistent with the formation of Cu^{I}(**adpa**). When compared to a broad ^{1}H-NMR spectrum of Cu^{II}(**adpa**), the pale-yellow species exhibited a sharp spectrum, supporting an assignment of diamagnetic d^{10} Cu^{I} species. An analysis of the ^{1}H-NMR spectrum also revealed the formation of dehydroascorbic acid (an oxidized form of ascorbic acid) after the reaction. When the reaction was carried out in the presence of O_2, the same result was obtained.

Generation of Cu^{I} species in the presence of $Zn(OAc)_2$

Unless otherwise noted, the following experiments were conducted under ambient conditions. A stock solution of AsH_2 (80 mM) was prepared in H_2O/CH_3CN (7 : 3 v/v). In a typical reaction, a solution of Cu^{II}(**adpa**) (2.0 mM) was combined with $Zn(OAc)_2$ (8.0 mol equiv.) in H_2O/CH_3CN (7 : 3 v/v). To this solution mixture was added the AsH_2 stock solution (1.0 mol equiv.). A color change of the solution from green to pale yellow was observed. The reaction was also monitored by UV-vis spectroscopy, which revealed a complete conversion of the spectrum for Cu^{II}(**adpa**) (λ_{max} = 632 nm) to the spectrum for a Cu^{I} complex (no d–d transition band). The Cu^{I} species seemed to be stable for at least 60 min. When $Zn(OAc)_2$ was changed to $Zn(NO_3)_2$ or $Zn(phen)_2$; however, the reduction was not completed as the d–d band of Cu^{II} was present to a significant extent. In fact, they gave the same result as observed in the reaction without $Zn(OAc)_2$. This indicated that the presence of OAc^- is necessary to help facilitate the reduction of Cu^{II}(**adpa**) and the stabilisation of Cu^{II}(**adpa**).

Attempts at characterising this new Cu complex in the presence of $Zn(OAc)_2$ by mass spectrometry have also been made. Samples for the ESI-MS analysis were prepared in 7 : 3 (v/v) H_2O/CH_3CN. A prominent peak at a mass-to-charge ratio (m/z) of 511.1332 (calc. m/z 511.13) was observed in the reaction of Cu^{II}(**adpa**) + $Zn(OAc)_2(H_2O)_2$ (1 : 8 mol equiv.), corresponding to $[Cu^{II}$(**adpa**) + $OAc]^+$. On the other hand, m/z at 512.1356 (calc. m/z 512.14) which corresponded to $[Cu^{I}$(**adpa**) + (OAc^-) + $(H^+)]^+$ was found when the solution mixture of Cu^{II}(**adpa**) + $Zn(OAc)_2(H_2O)_2$ was added to AsH_2 (5 mol equiv.). This supported the possibility of OAc^- being coordinated to Cu(II/I)**adpa**.

In addition to ESI-MS, the coordination of $Zn(OAc)_2(H_2O)_2$ on the Cu^{II} centre was also investigated by IR spectroscopy. The solid sample for IR analysis was prepared from the reaction of Cu^{II}(**adpa**) and $Zn(OAc)_2{\cdot}(H_2O)_2$ (8 mol equiv.) in 7 : 3 (v/v) H_2O/CH_3CN. After being stirred for around 10 min, the solution mixture was precipitated under reduced pressure to remove CH_3CN and obtain a dark green solid. The solid was filtered for subsequent analysis by IR spectroscopy in the ATR mode.

An NMR sample was prepared as follows: a solution of Cu^{II}(**adpa**) (10 mM) in CD_3CN (550 μL) was combined with $Zn(OAc)_2$ (8.0 mol equiv. dissolved in D_2O/CD_3CN 7 : 3 v/v). A

shift and broadening of a signal corresponding to OAc^- was noted, indicating the coordination of OAc^- to the paramagnetic Cu^{II} centre. To this solution was added AsH_2 (50 μL, 5.0 mol equiv. dissolved in D_2O/CD_3CN 7 : 3 v/v). The solution became pale yellow, consistent with the conversion to Cu^I(**adpa**). The yellow product gave a sharp spectrum, corresponding to the Cu^I species.

Effect of pH

Acetic–acetate buffer solutions (ABS) with pH 4.0–5.6 and phosphate buffer solution (PBS) with pH 6.0–8.0 were prepared in H_2O/CH_3CN (7 : 3 v/v). In a typical reaction, to a solution of Cu^{II}(**adpa**) (10 μM) in a buffer solution with the desired pH (2.00 mL) was added $Zn(OAc)_2$ (100 μL, 40 mol equiv. dissolved in H_2O/CH_3CN 7 : 3 v/v). To this solution mixture was then added a stock solution of AsH_2 (50 μL, 5 mol equiv. dissolved in H_2O/CH_3CN 7 : 3 v/v). It should be mentioned that a color change of the solution could not be observed with the naked eye at this low concentration. After being stirred for 2 min, the reaction was monitored by fluorescence spectroscopy, which revealed an emission band at λ_{max} = 423 nm. (Fluorescence parameters: excitation wavelength = 340 nm, slit setting on the instrument = 10 and PMT = 500).

To demonstrate the effect of pH on AsH_2 detection in the presence of a possible interference, a solution of glutathione (GSH, 5.0 mol equiv.) or a mixture of AsH_2 (5.0 mol equiv.) and GSH (5.0 mol equiv.) was used instead of AsH_2.

Reaction in the presence of other divalent metal ions

A stock solution of AsH_2 (80 mM) and $M(OAc)_n$ (M = Zn^{2+}, Na^+, Ca^{2+} or Mg^{2+}) was prepared in H_2O/CH_3CN (7 : 3 v/v). In a typical reaction, Cu^{II}(**adpa**) (2 mM) in 2.00 mL of H_2O/CH_3CN (7 : 3 v/v) was combined with $M(OAc)_n$ (8 mol equiv., 100 μL). After being stirred for 2 min, the solution was monitored by UV-vis spectroscopy. To this solution mixture was added the AsH_2 stock solution (1.0 mol equiv., 50 μL). An immediate color change of the solution from green to pale yellow was observed. The reaction was also monitored by UV-vis spectroscopy, showing a complete conversion of the spectrum for Cu^{II}(**adpa**) (λ_{mzx} = 635 nm) to the spectrum for a Cu^I complex.

Reaction in the presence of other bridging ligands

A solution mixture of Cu^{II}(**adpa**) (2 mM) and a bridging ligand (16 mol equiv. of imidazole, or histidine) was prepared in 2.00 mL of H_2O/CH_3CN (7 : 3 v/v). A solution of $Zn(NO_3)_2$ (8 mol equiv., 100 μL) was then added. To this reaction mixture was added the AsH_2 stock solution (1.0 mol equiv., 50 μL). The reaction was stirred for 2 min before being monitored by UV-vis spectroscopy.

Studies of selectivity and interferences by fluorescence spectroscopy

Acetic–acetate buffer solutions (ABS) with pH 5.6 were prepared in H_2O/CH_3CN (7 : 3 v/v). In a typical reaction, to a solution of Cu^{II}(**adpa**) or Cu^{II}(**atpa**) (10 μM) in the buffer solution (2.00 mL) was added $Zn(OAc)_2$ (100 μL, 40 mol equiv. dissolved in H_2O/CH_3CN 7 : 3 v/v). To this solution mixture was then added a stock solution of AsH_2 (50 μL, 5 mol equiv. dissolved in H_2O/CH_3CN 7 : 3 v/v). After being stirred for 2 min, the reaction was monitored by fluorescence spectroscopy (fluorescence parameters: excitation wavelength = 340 nm, slit setting on the instrument = 10 and PMT = 500 for Cu(**adpa**); PMT = 530 for Cu(**atpa**)).

For the selectivity study, the addition of AsH_2 was changed to glucose, lactose, sucrose, fructose, citric acid or glutathione. For the interference study, the addition of AsH_2 was changed to the mixture of AsH_2 (5.0 mol equiv.) and a possible interfering substance (5.0 mol equiv. of glucose, lactose, sucrose, fructose, citric acid or glutathione).

Sample preparation for the determination of AsH_2 in vitamin C tablets

Three tablets of vitamin C were dissolved in Milli-Q water (500.00 mL). The solution was then filtered with a 0.45 μM Millipore filter to remove insoluble components. An amount of the filtrate (31 μL) was then transferred to a volumetric flask and diluted to 10.00 mL with ABS (pH 5.6) in H_2O/CH_3CN (7 : 3 v/v). The amount of ascorbic acid in vitamin C tablets was determined by the standard addition method.

Conflicts of interest

There are no conflicts of interest to declare.

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