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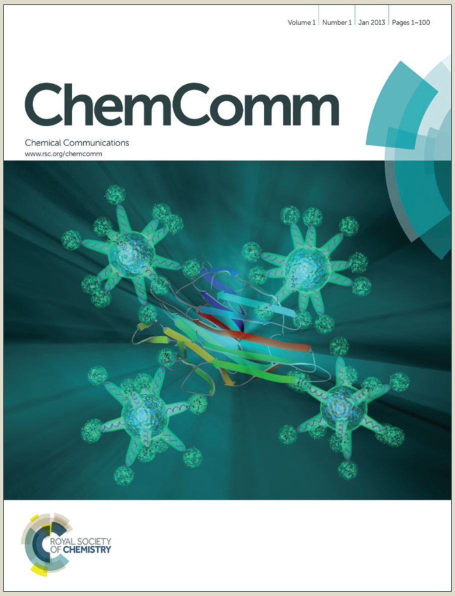

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Interception Copper-based Carbene Radical with α-Carbonyl Diazomethane Radical: C1/C1N2 Copolymerization

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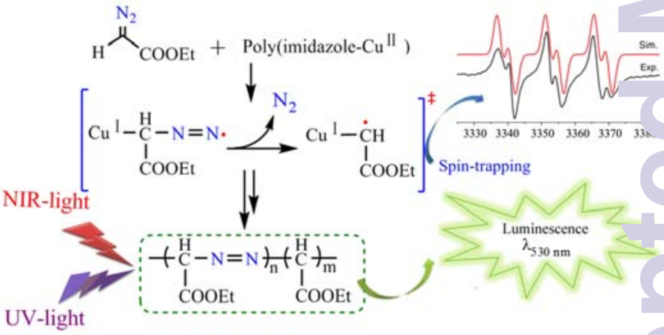
Poly(imidazole-Cu)-mediated C1/C1N2 copolymerization of ethyl diazoacetate involved copper-based carbene radical and α-carbonyl diazomethane radical intermediates, which could be captured by radical traps and detected by room-temperature EPR spectroscopy. The obtained C1/C1N2 copolymer could be used as a new kind of upconversion agent for non-invasive bioimaging study.

The elegance of natural metalloenzymes which use metal-stabilized ligand radicals as 8 enzymatic intermediates in biology has evoked a recent explosion of research on redox-active ligands.[1-5] Fischer-type carbene ligands are now recognized as the so-called "redox noninnocent ligands" and through an external or intra-molecular one-electron reduction on the carbene moiety could lead to the "carbene-radical" ligands.[6,7] A prominent example is the cobalt-based carbene radical,[8] which can undergo typical radical-type reactions such as C-C and C-H bond formation[6] and can also be intercepted by the radical inhibitor 2, 2, 6, 6-tetramethylpiperidine-1-oxy (TEMPO) *via* C-O bond formation.[9]

On the other hand, redox active transition metals are also suggested to play an important role in determining the formation of metal-ligand radical complexes.[10] Jeffrey M. Zaleski and coworkers reported photolysis of copper(II)-coordinated diazo complexes could generate a copper(I)-$L^{+\cdot}$ ligand radical species (L refers to ligand) through one-electron reduction at the copper(II) centre. Inspired by the imidazole-containing galactose oxidase, which performs high-efficiency oxidation of alcohols to aldehydes in biology,[11] we envisaged that the decomposition of ethyl diazoacetate (EDA) in the presence of the polymeric imidazole-copper(II) catalyst (poly(imidazole-Cu)), may also involve a copper(I)-carbene radical intermediate, which could be captured by radical traps through radical addition.



Scheme 1 Interception of copper-based carbene radical with diazomethane radical to produce C1/C1N2 copolymer: spin-trapping and fluorescent study.

Different from our previously reported copper(0)-catalyzed carbene polymerization,[12] which is one of the most important "C1 polymerization" and allows for synthesis of densely functionalized carbon-chain polymers,[13] the poly(imidazole-Cu) mediated decomposition of EDA produces an unusual copolymer with both the monomer unit [-CH(COOEt)-N=N-] and the carbene unit [-CH(COOEt)-] incorporated into the oligomer chain (Scheme 1). Thus, we supposed that once the copper-based carbene radical is generated, it could also be intercepted by its precursor diazomethane radical to afford the newly-developed "C1/C1N2 copolymerization" product.

Besides, the copolymer with a π-conjugated -CH-N=N- chromophore displays visibly luminescent upon excitation with both UV light and near-infrared (NIR) light, which is especially unique to an organic fluorescent substance. Recently, up-conversion materials,[14-17] which can convert NIR excitation to visible emission, have attracted considerable attention as they enable deeper tissue imaging with less cellular damage. The results indicate that the copolymer could be used as a new kind of up-conversion agents and offers possibility for non-invasive bioimaging study by utilizing NIR excitation light.

The poly(imidazole-Cu) catalyst was prepared by self-assembly of $CuSO_4$ and amphiphilic poly[(*N*-vinylimidazole)-*co*-(*N*-isopropylacrylamide)] in $CHCl_3/H_2O$ solution *via* coordinative convolution method[18] and its aggregation structure was characterized by SEM and EDS techniques (Fig. S1, ESI†).

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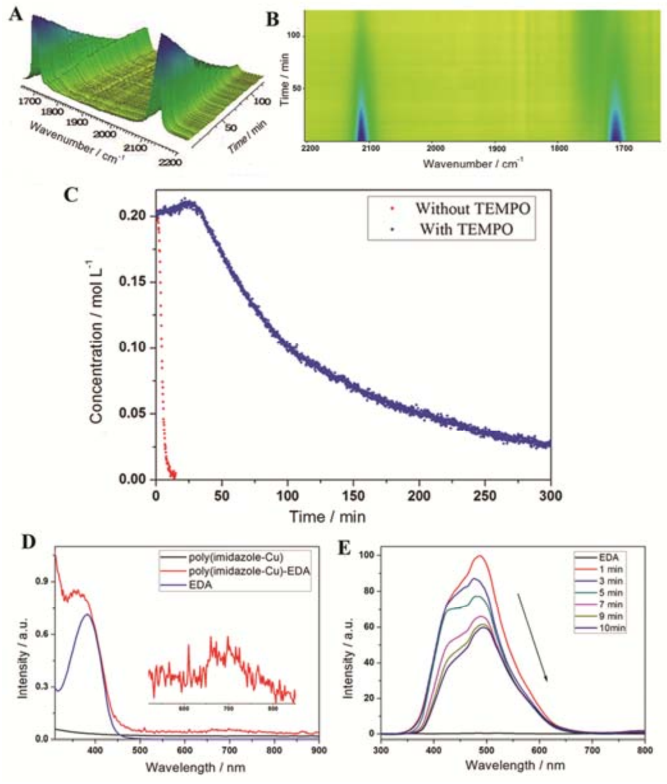

Fig. 1 (A) 3-D and (B) 2-D IR spectra of poly(imidazole-Cu)-catalyzed decomposition of EDA process. (C) Poly(imidazole-Cu)-catalyzed decomposition of EDA with and without addition of TEMPO. (D) UV-Vis spectra of poly(imidazole-Cu), EDA and the reaction mixture of poly(imidazole-Cu) and EDA in $CHCl_3$. (E) Fluorescent spectra of EDA and the reaction mixture of poly(imidazole-Cu) and EDA in $CHCl_3$ detected at different reaction time.

We start the investigation by tracing the process of poly(imidazole-Cu)-catalyzed decomposition of EDA *in situ* with online IR spectroscopy. Fig. 1A and 1B show the corresponding 3-D and 2-D IR spectra of the absorption bands between 2200 cm^{-1} and 1600 cm^{-1} upon the reaction time from 0 to 150 min. Besides the steady disappearance of EDA ($υ_{-CH=N2}$ at 2110 cm^{-1} and $υ_{-C=O}$ at 1705 cm^{-1}), the formation of another absorption band at 1740 cm^{-1} could also be observed. Since it is not possible to isolate the intermediates due to their high reactivity, we use the radical inhibitor TEMPO to affect the reaction process in order to verify the existence of the radical intermediates.

As shown in Fig. 1C, the reaction system of poly(imidazole-Cu)-EDA in toluene was treated without and with TEMPO (0.013 mol), and the decomposition of EDA ($υ_{-CH=N2}$ at 2110 cm^{-1}) was tracked with online IR spectroscopy. Although the decomposition of EDA could not be completely blocked, the reaction time of the run with TEMPO was prolonged to more than 300 min while without TEMPO, the reaction reached completion within 15 min. But no complete conversion of EDA could be reached in the presence of TEMPO. Since no reaction between poly(imidazole-Cu) and TEMPO was found, the results indicate that some intermediates with a structure that the radical primarily resided on the organic moiety must be formed.[9,19] Besides, another striking feature that can be seen on Fig. 1C is the initiation phase was also prolonged, which suggests that at least two separated radical processes exist within this system.

Ultraviolet-visible (UV-Vis) spectroscopy and fluorescent spectroscopy were also used to characterize the reaction system. The UV-Vis spectrum of EDA exhibits a strong absorption band centred at 380 nm, which is assigned to the n→π* transition of the diazo group (Fig. 1D). Upon addition of poly(imidazole-Cu), the spectrum of the reaction mixture shows a slight blue shift ($λ_{max}$=360 nm) of the diazo group due to complexation to the copper centre. And a small absorption band centred at 680 nm (ascribed to the copper d-d transition)[10] could also be observed. On the other hand, while no emission could be detected with EDA under irradiation with 360 nm light (Fig. 1E), the immediate appearance of the strong emission band ($λ_{max}$=500 nm) upon addition of poly(imidazole-Cu) and the gradually decreased emission intensity along with N_2 loss clearly indicate the formation of the excited state of copper-diazo species, which has a propensity for N_2 elimination.

Spin-trapping in conjunction with room-temperature (RT) electron paramagnetic resonance (EPR) spectroscopy was then used to detect the ligand-centred radical intermediates. Monitoring the reaction system of poly(imidazole-Cu) and EDA at 60 °C in the presence of *N-tert*-butyl-α-phenylnitrone (PBN) led to a clear EPR spectrum (Fig. 2A), which mainly consists of a doublet of triplets that is assigned to the spin adducts of PBN with radical intermediates.[20,21] However, the appearance of this EPR spectrum shows a discrepant splitting, which suggests that more than one radical species were trapped by PBN.

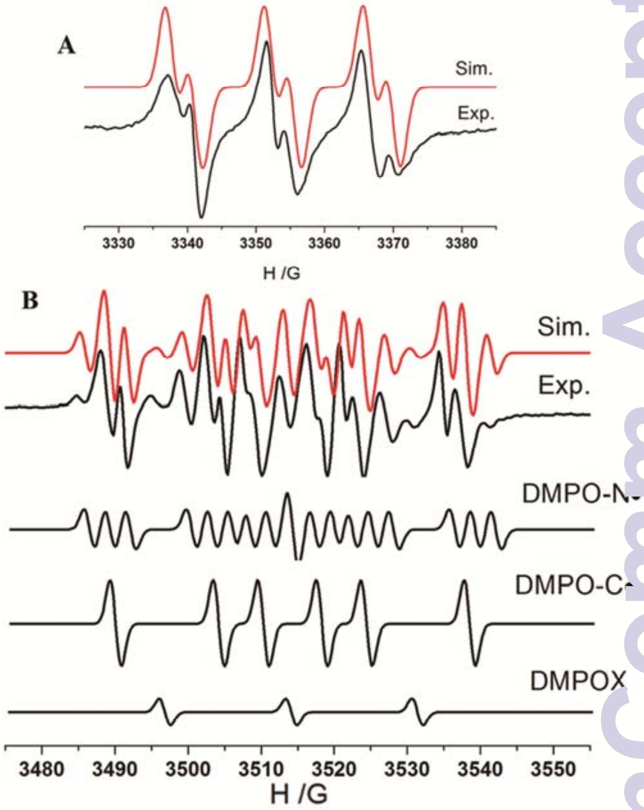

Fig. 2 EPR spectra of poly(imidazole-Cu)-catalyzed decomposition of EDA in the presence of PBN (A) and DMPO (B) (experimental (black line) and simulated spectrum (red line). The spectrum in (B) was simulated as a mixture of DMPO-C·, DMPO-N· and DMPOX radicals in a rough of 1:0.5:0.03 ratios. Detection conditions: 60 °C, PBN 10 mg or DMPO 10 μL, poly(imidazole-Cu) 30 mg, EDA 500 μL, toluene 5 mL. 2 μL of the solution was collected and detected by room-temperature EPR.

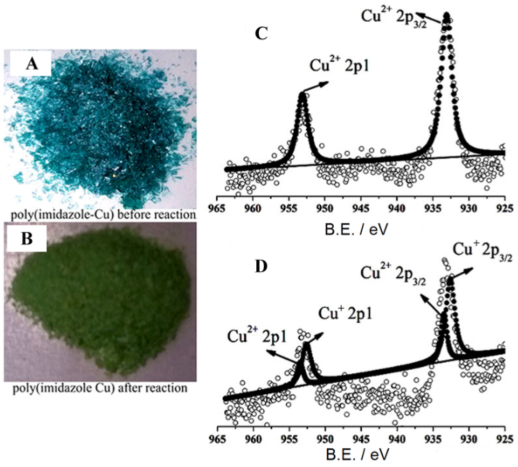

Fig. 3 Photographs of poly(imidazole-Cu) before (A) and after use (B); XPS spectra with fit for poly(imidazole-Cu) before (C) and after use (D).

To differentiate these radical intermediates, 5, 5-dimethylpyrroline-1-*N*-oxide (DMPO) was further used as the spin trap. It was amazing to observe an instant EPR spectrum with the reaction mixture in the presence of DMPO (Fig. 2B), which is well resolved and after simulation yields the hyperfine coupling constants (HFCs) of a^N=14.00 G, a^H=22.00 G, $a^{\gamma\text{-}N}$=2.80 G for a trapped N-centered radical (DMPO-N·), the HFCs of a^N=14.12 G, a^H=20.22 G for a trapped C-centered radical (DMPO-C·) and the HFC of a^N=13.70 G for an oxygenated DMPO radical (DMPOX).[22,23] Control experiments confirm that without EDA or poly(imidazole-Cu) catalyst, no EPR signals could be detected (Fig. S2, ESI†). We thus take the DMPO-N· and DMPO-C· signals as an indication of the trapped N-centred diazo radical and C-centred carbene radical adducts.[24,25] The appearance of the three-line DMPOX signal, which was also detected with the reaction system of oxygenation of iridium(II)-ethene (or “iridium(III)-ethyl radical”)[26] in the presence of DMPO could support the generation of the open-shell copper complexes with the poly(imidazole-Cu)-EDA system.

After reaction, we found the colour of poly(imidazole-Cu) changed from blue to green (Fig. 3A and 3B) and X-ray photoelectron spectroscopy (XPS) characterization on the Cu 2p peaks reveals the binding energy of Cu $2p_{3/2}$ shifted from 933.12 eV to 932.58 eV (Fig. 3C and 3D), which indicate copper(II) was reduced to Cu(I) by EDA.[18] Thus, the chemical change in copper is coupled to the generation of the diazo radical and carbene radical intermediates, which strongly suggest that the generation of these radical species on the copper catalyst occurs through an intra-molecular electron transfer process.[10]

DFT calculations were carried out to evaluate the electronic structures and spin-density distributions of the proposed radical intermediates (both using neutral catalyst model; Fig. 4A, copper-diazo radical; Fig. 4B, copper-carbene radical).[27-30] The calculated Mulliken spin density of copper-diazo radical indicates that 93.8% of spin density is localized on the diazo moiety (66.1% on the terminal nitrogen atom and 17.2% on the central nitrogen atom). And in copper-carbene radical, a significant amount of spin density (78.1%) is localized on the carbene-carbon atom while only 6.7% of spin density is on the copper atom, which is indicative of carbon-centred copper-carbene radical. Besides, the spin density is also delocalized over the carbonyl group with 15.6% of spin density is localized on the carbonyl-oxygen atom. These values are consistent with the results of EPR analyses and indicate a considerable ligand-centred radical character.

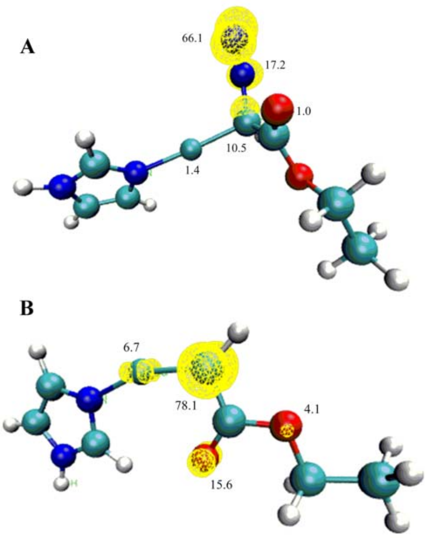

Fig. 4 Spin density plots of the DFT optimized copper-diazo radical (A) and copper-carbene radical (B).

Besides, we obtained an unusual copolymer with the monomer unit [-CH(COOEt)-N=N-] and the carbene unit [-CH(COOEt)-] incorporated into the oligomer chain from the reaction mixture (Table S1, ESI†). Elemental analyses reveal high nitrogen content of 4.05%~9.16% with the copolymer, which supports the incorporation of [-CH(COOEt)-N=N-] unit. The MALDI-TOF MS of the copolymer (Fig. 5) clearly shows a main set of peaks with repeating units of 114 Da and 86 Da, which corresponds to the structure of Na^+\{H-[CH(COOEt)-N=N-]$_n$[CH(COOEt)-]$_m$-OH\} (e.g., *m/z*=555.3 corresponds to Na^+\{H-[CH(COOEt)-N=N-]$_3$[CH(COOEt)-]$_2$-OH\}). The ^{1}H NMR spectrum of the copolymer shows the signals centred at chemical shift of 4.23 ppm and 3.98 ppm, which are assigned to the -OCH_2- group and the -CH-N=N- group (Fig. S3, ESI†). Both the FT-IR spectrum and the Raman spectrum show absorption bands at about 1580 cm^{-1}, which is attributed to the -N=N- group (Fig. S4, ESI†). The DSC curve of the obtained oligomer shows glass transition temperature at about -15 ^{o}C (Fig. S5, ESI†) and the TGA spectrum exhibits the good thermal-stability of the copolymer (Fig. S6, ESI†).

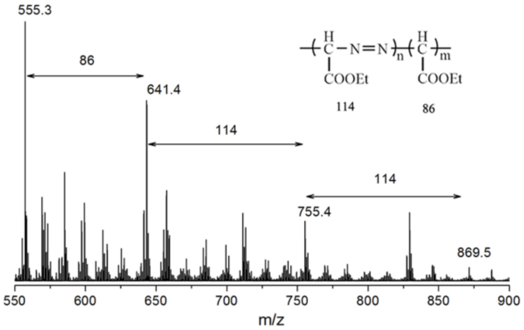

Fig. 5 MALDI-TOF mass spectrum of the copolymer obtained with poly(imidazole-Cu) catalyzed decomposition of EDA.

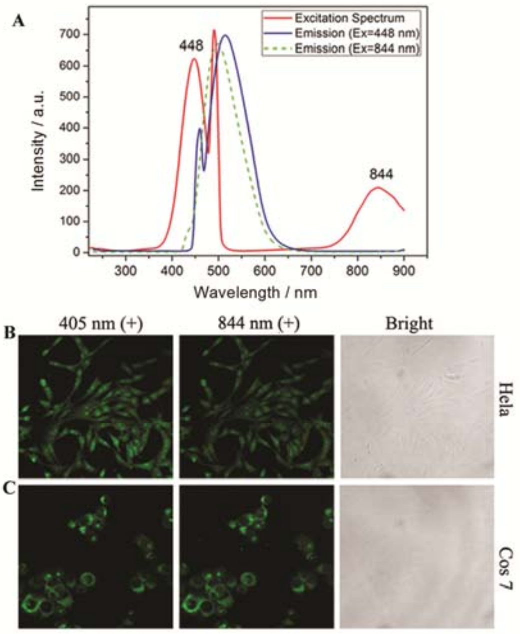

Fig. 6 (A) Fluorescence excitation and emission spectra of the copolymer. Confocal laser scanning microscopy images of (B) Hela cells and (C) Cos7 cells after incubation with the copolymer (500 µg ml^{-1}) for 4 h at 37 ^{o}C. Excitation filter: 405 nm/40 nm; emission filter: 535 nm/50 nm. The bright-field images outline the position of the cells.

Based on the spin-trapping results and the synthetic advances in obtaining the diazoacetate-containing copolymer, we supposed that the carbon-centred carbene radical could also be intercepted by its precursor nitrogen-centred diazo radical to generate the C1/C1N2 copolymer.

Carrying a π-conjugated -CH-N=N- chromophore, the copolymer displays visibly luminescent under both 448 nm and 844 nm excitation light (Fig. S7, ESI†). The emission spectra centred at about 530 nm could be obtained with both of the excitation bands (Fig. 6A), which indicates the copolymer could be used as fluorescent substance and novel NIR-induced optical material. We investigated the intracellular uptake and imaging of the copolymer in living cells by means of confocal laser scanning microscopy. As shown in Fig. 6B and 6C, two types of cell lines (Hela cervical carcinoma cells and Cos7 monkey kidney fibroblast cells) were incubated with the copolymer (500 µg ml^{-1}) for 4 h and then irradiated with 405 nm and 844 nm laser under an optical microscope, strong bioluminescence could be detected in all cases. The results support the feasibility of using the copolymer as a new kind of up-conversion agents for bioluminescence study and also offer new possibility for non-invasive bioimaging study by utilizing NIR excitation light.

Conclusions

In conclusion, we report the poly(imidazole-Cu)-catalyzed decomposition of EDA involved the copper-based carbene radical and diazo radical intermediates, which could be trapped by spin traps and detected by room-temperature EPR spectroscopy. The carbene radical could also be intercepted by the α-carbonyl diazomethane radical to produce the unusual C1/C1N2 copolymer, which displays unique fluorescence and could be used as a new ki of up-conversion agents for bioimaging study.

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