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Formation of $[Ru(bpy)_3]^{2+}$-Containing Microstructures Induced by Electrostatic Assembly and Their Application in Solid-State Detection of Electrochemiluminescence

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Abstract: Tris(2,2′-bipyridine)ruthenium(II) ($[Ru(bpy)_3]^{2+}$) is one of the most extensively studied and used electrochemiluminescent (ECL) compounds owing to its superior properties, which include high sensitivity and stability under moderate conditions in aqueous solution. In this paper we present a simple method for the preparation of $[Ru(bpy)_3]^{2+}$-containing microstructures based on electrostatic assembly. The formation of such microstructures occurs in a single process by direct mixing of aqueous solutions of $[Ru(bpy)_3]Cl_2$ and $K_3[Fe(CN)_6]$ at room temperature. The electrostatic interactions between $[Ru(bpy)_3]^{2+}$ cations and $[Fe(CN)_6]^{3-}$ anions cause them to assemble into the resulting microstructures. Both the molar ratio and concentration of reactants were found to have strong influences on the formation of these microstructures. Most importantly, the resulting $[Ru(bpy)_3]^{2+}$-containing microstructures exhibit excellent ECL behavior and, therefore, hold great promise for solid-state ECL detection in capillary electrophoresis (CE) or CE microchips.

Keywords: electrochemiluminescence · electrostatic interactions · ion conductors · ruthenium · self-assembly

Introduction

Self-assembly is the fundamental principle that produces structural organization on all scales and refers to the spontaneous organization of molecules, molecular clusters, and aggregate structures into two-dimensional (2D) arrays and three-dimensional (3D) networks by attractive forces or chemical-bond formation.[1] During the last few years, self-assembly in solution has attracted increasing research interest because it provides a means for the integration of molecular systems into functional mesoscopic devices and macroscopic materials.[2] Electrochemiluminescence (ECL), the production of light from electrochemically generated reagents,[3] has been widely studied during the last few decades owing to its versatility, simplified optical setup, very low background signal, and good temporal and spatial control, and it has become an important and valuable detection method in analytical chemistry.[4] It has found many applications such as in immunoassays and DNA analysis,[5] chemical sensing,[6] imaging,[7] lasers,[8] and optical studies,[9] and has also been used as detectors for chromatography[10] and capillary electrophoresis.[11] Ruthenium(II) tris(bipyridine) ($[Ru(bpy)_3]^{2+}$) is one of the most extensively studied and used ECL compounds owing to its superior properties, which include high sensitivity and stability under moderate conditions in aqueous solution.[12] Herein we develop a simple method for the preparation of $[Ru(bpy)_3]^{2+}$-containing microstructures by electrostatic self-assembly in solution that involves direct mixing of aqueous $[Ru(bpy)_3]Cl_2$ and $K_3[Fe(CN)_6]$ at room temperature. Such microstructures exhibit excellent ECL behavior, and the formation of these microstructures is heavily influenced by both the molar ratio and the concentration of the reactants.

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Results and Discussion

Figure 1 shows typical scanning electron microscopy (SEM) images of the resulting precipitate of sample 1 (see Experimental Section for preparation details). The low-magnification image indicates that the precipitate consists mainly of a large quantity of one-dimensional (1D) structures (Fig-

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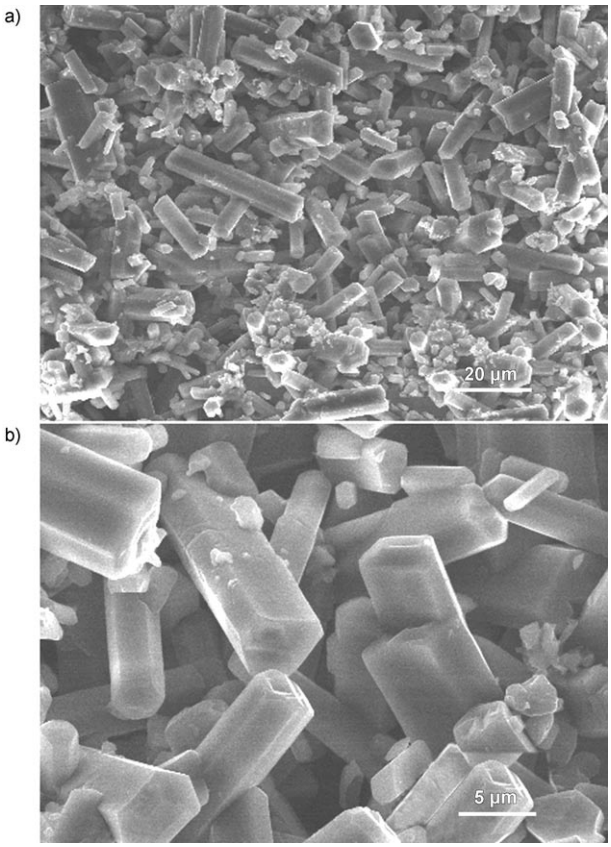

Figure 1. a) Low-magnification and b) high-magnification SEM image of the resulting precipitate of sample 1.

ure 1 a). The higher-magnification image further reveals that these 1D microstructures are pillar-shaped (Figure 1 b). Notably, such microstructures are robust enough to withstand the violent sonication process.

The chemical composition of the resulting precipitate was determined by energy-dispersive X-ray spectroscopy (EDS) and X-ray mapping of the precipitate on an indium tin oxide (ITO) coated glass slide. The EDS spectrum (Figure 2 a) shows four peaks corresponding to C, N, Fe, and Ru. The inset in Figure 2 a shows the corresponding SEM

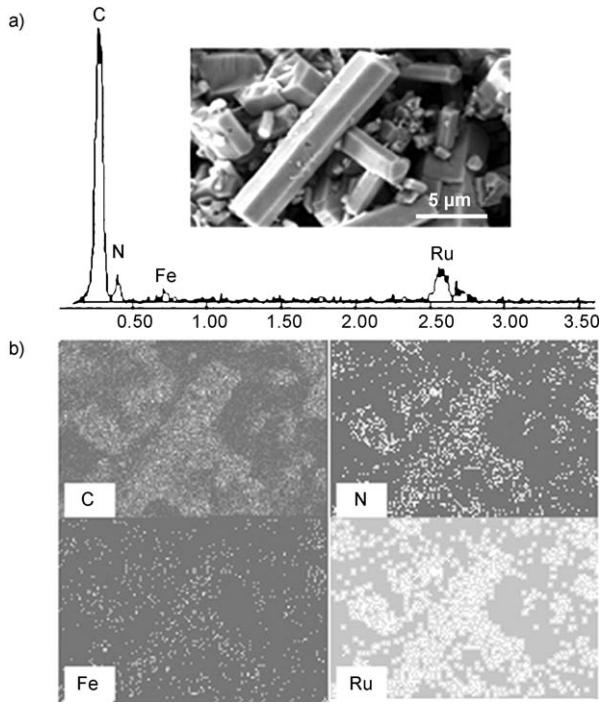

Figure 2. a) EDS spectrum and b) X-ray maps of the precipitate of sample 1. Inset: SEM image of the precipitate used for collecting the EDS spectrum and X-ray maps.

image of the precipitate used for to obtain the EDS spectra and the X-ray maps. The X-ray maps shown in Figure 2 b further reveals that these four elements are relatively uniformly distributed in the microstructures. These observations indicate that these microstructures are products formed from $[Fe(CN)_6]^{3-}$ and $[Ru(bpy)_3]^{2+}$. Quantitative elemental analysis of the microstructures indicates that the atom number ratio C/N/Fe/Ru in the microstructures is 101.6:29.8:3.1:2:98, thus revealing that the $[Fe(CN)_6]^{3-}$/$[Ru(bpy)_3]^{2+}$ stoichiometry is about 2:3, which is consistent with

Abstract in Chinese:

三联吡啶钌$[Ru(bpy)_3]^{2+}$由于具有灵敏度高及在水溶液中的稳定性好等优越性能而成为目前研究并使用得最多的具有电化学发光性能的化合物之一。本文中我们报道了一种基于静电组装技术制备含$[Ru(bpy)_3]^{2+}$的微结构的方法，即：在室温下直接混合$[Ru(bpy)_3]Cl_2$和$K_3[Fe(CN)_6]$水溶液，借助$[Ru(bpy)_3]^{2+}$阳离子和$[Fe(CN)_6]^{3-}$阴离子之间的静电作用，得到了微米尺寸的组装体。实验表明，反应物间的摩尔配比及其浓度对微结构的形成有强烈影响。最重要的是，本方法所合成的这种含$[Ru(bpy)_3]^{2+}$的微结构具有很好的电化学发光性能，因而在毛细管电泳或毛细管电泳微芯片的固态电化学检测方面有着很好的应用前景。

International Advisory Board Member

Erkang Wang *obtained his BS at the Univ. of Shanghai in 1952 and his PhD under Prof. J. Heyrovsky at the Czechoslovak Academy of Sciences, Prague, in 1959. He has received many national and international awards for his research, which involve electro- and bioelectrochemistry and environmental analysis. He was Visiting Prof. at many institutions, including the Univ. of Houston (1984–1985), Dijon Univ. (1988, 1992), and Kyoto Univ. (1989), and has presented over 150 seminars and 70 plenary and keynote lectures. Since 1994, he has been Director of the Changchun Scientific Research Center of Applied Chemistry.*

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the 2:3 molar ratio of $[Fe(CN)_6]^{3-}$ to $[Ru(bpy)_3]^{2+}$ used to prepare the microstructures. Considering the positive charge of $[Ru(bpy)_3]^{2+}$ and the negative charge of $[Fe(CN)_6]^{3-}$, we can suggest that electrostatic interactions between these two components result in the formation of the microstructures by self-assembly. Figure 3 shows the UV/Vis spectrum of

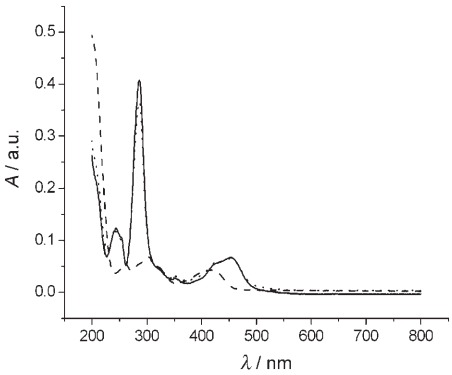

Figure 3. UV/Vis spectra of aqueous $[Ru(bpy)_3]Cl_2$ (——), aqueous $[K_3Fe(CN)_6]$ (– – –), and the microstructures of sample 1 suspended in water (-----).

aqueous $[Ru(bpy)_3]Cl_2$, aqueous $K_3[Fe(CN)_6]$, and the microstructures of sample 1 suspended in water. It is clear that the UV/Vis absorption spectrum of aqueous $[Ru(bpy)_3]Cl_2$ is quite similar to that of the resulting microstructure suspension in water, which further confirms that only electrostatic interactions are responsible for the formation of these $[Ru(bpy)_3]^{2+}$-containing microstructures.

Figure 4 shows the XRD patterns of the microstructures of sample 1, $[Ru(bpy)_3]Cl_2$, and $K_3[Fe(CN)_6]$. It is clear that the microstructures exhibit quite a different pattern from $[Ru(bpy)_3]Cl_2$ and $K_3[Fe(CN)_6]$, which indicates that the crystal nature of the microstructures is quite different from its two components. However, because of a lack of standard or referenced XRD data of this type of new structure, we cannot give detailed information on the crystal nature of the microstructures formed through electrostatic interactions.

We examined the effect of the molar ratio of the reactants on these microstructures by preparing another two samples at different $[Fe(CN)_6]^{3-}/[Ru(bpy)_3]^{2+}$ molar ratios; the conditions were otherwise identical to those used to prepare sample 1. When the molar ratio was decreased to 2:9, the resulting microstructures consisted mainly of irregular microparticles, and only a few pillar-shaped microstructures were observed in the same preparation (Figure 5a). When the molar ratio was increased to 2:1, the resulting microstructures consisted only of irregular microparticles (Figure 5b).

The effect of the concentration of the reactants on the microstructures was also examined. Figure 6 shows typical SEM images of another two samples obtained with different reactant concentrations; the conditions were otherwise identical to those used to prepare sample 1. When the concentration was decreased to one third, we obtained products that consisted mainly of irregular 1D microstructures (Figure 6a). When the concentration was increased by up to

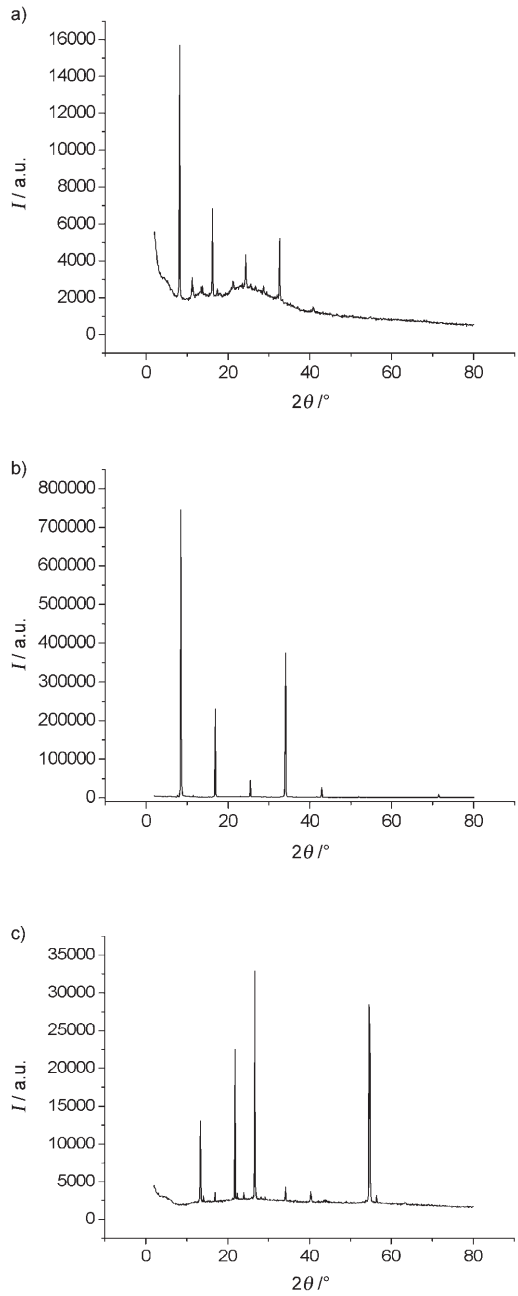

Figure 4. XRD patterns of a) the microstructures of sample 1, b) $[Ru(bpy)_3]Cl_2$, and c) $[K_3Fe(CN)_6]$.

threefold, the resulting microstructures consisted mainly of small irregular microparticles; at the same time, a few large irregular microparticles were also observed in the same preparation (Figure 6b).

To test the ECL behavior of the $[Ru(bpy)_3]^{2+}$-containing microstructures prepared, we acquired the cyclic voltammogram (CV), ECL intensity–potential curve, and ECL intensity–time curve of the microstructures of sample 1 coated on an ITO–glass electrode. Figure 7a shows the CV acquired at a scan rate of 50 mV s^{-1} in 50 mM phosphate buffer solution (pH 9.2). A pair of redox waves appeared at around 1.1 V (vs. Ag/AgCl), which is attributed to the one-electron redox

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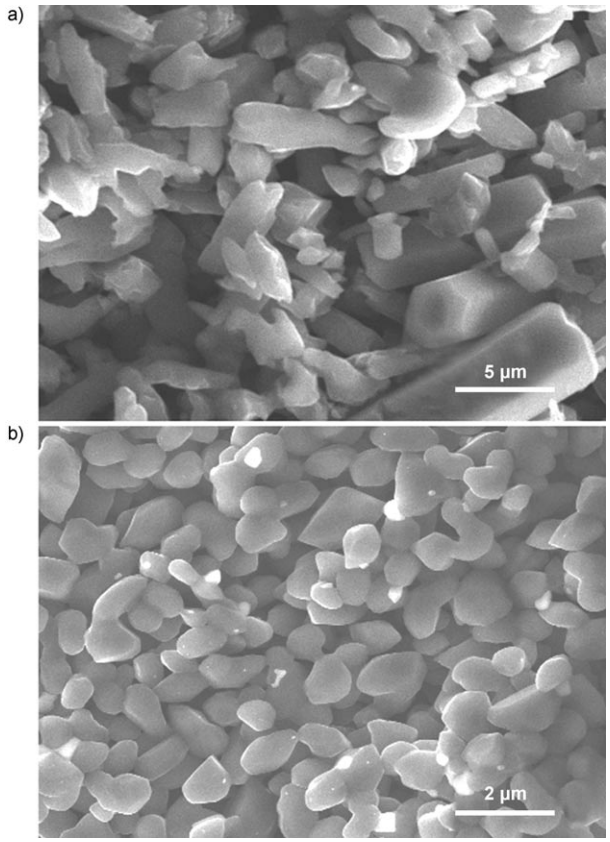

Figure 5. SEM images of the precipitate obtained with a) 2:9 and b) 2:1 $[Fe(CN)_6]^{3-}/[Ru(bpy)_3]^{2+}$ molar ratios under otherwise identical conditions used for the preparation of sample 1.

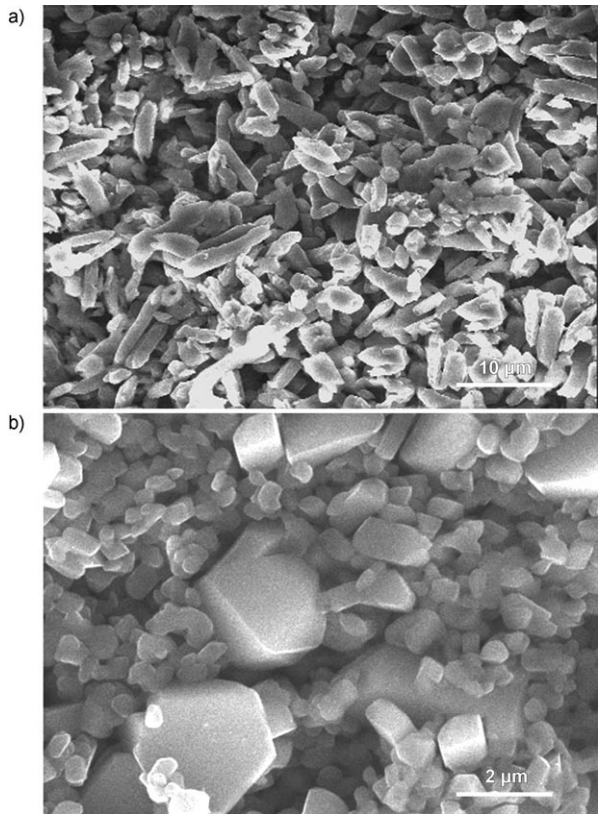

Figure 6. SEM images of the precipitate obtained at a) one third and b) threefold concentration of reactants under otherwise identical conditions used for the preparation of sample 1.

reaction of $[Ru(bpy)_3]^{2+}$.[13] Another pair of redox waves that appeared at 0.1–0.4 V is attributed to the redox reaction of $[Fe(CN)_6]^{3-}$.[14] Figure 7b shows the corresponding ECL intensity–potential curve. The onset of luminescence occurred at 0.9–1.0 V, which is consistent with the oxidation of $[Ru(bpy)_3]^{2+}$,[13] and then the ECL intensity rose steeply. Figure 7c shows the corresponding ECL intensity–time curve in phosphate buffer solution (pH 9.2) under continuous potential scanning for five cycles. The relative standard deviation was 0.8%. Interestingly, although no tripropylamine (TPA) was introduced into the solution, the ECL intensity was high (up to 1500 a.u.), and the ECL intensity of the first cycle was out of the measurement range of the ECL analyzer. Because $[Ru(bpy)_3]^{2+}$ is the only component that exhibits ECL in the microstructures, we can suggest that the ECL signals observed resulted from the $[Ru(bpy)_3]^{2+}$ components in the microstructures on the electrode surface. These observations suggest that the resulting $[Ru(bpy)_3]^{2+}$-containing microstructures exhibit excellent ECL behavior and, therefore, hold great promise for solid-state ECL detection in capillary electrophoresis (CE) or CE microchips. Notably, the precipitates of the other samples also showed quite good ECL behavior.

$[Fe(CN)_6]^{3-}$ is a redox-active anion and was used by Kelley and co-workers to construct a DNA detector with excellent sensitivity and reproducibility because it can significantly amplify the electrochemical signal generated by $[Ru(NH_3)_6]^{3+}$.[15] Shankaran and Narayanan prepared a copper hexacyanoferrate (CuHCF) precipitate, an ion conductor with redox-active sites, by mixing aqueous solutions of $Cu(NO_3)_2$ and $K_4[Fe(CN)_6]$.[16] We recently found that a mixture of $[PtCl_6]^{2-}$ and $[Ru(bpy)_3]^{2+}$ can also yield microstructures with good ECL.[17] However, unlike $[Fe(CN)_6]^{3-}$, $[PtCl_6]^{2-}$ is not redox-active and is very expensive. When the supramolecular $[Fe(CN)_6]^{3-}$–$[Ru(bpy)_3]^{2+}$ ion conductor was used for ECL detection, we could suggest that the $[Fe(CN)_6]^{3-}$ components contained in the microstructures provide effective redox-active sites that not only facilitate electron transfer between the $[Ru(bpy)_3]^{2+}$ components in the microstructures and the electrode,[16] but also significantly amplify the electrochemical and ECL signals generated by $[Ru(bpy)_3]^{2+}$.[15] On the other hand, the amount of the $[Ru(bpy)_3]^{2+}$ component is larger than that of the $[Fe(CN)_6]^{3-}$ component (the $[Ru(bpy)_3]^{2+}/[Fe(CN)_6]^{3-}$ stoichiometry is about 3:2). As a result, the couple of peaks at 1.1 V was two times larger than that at 0.2 V, the corresponding ECL intensity was high (up to 1500 a.u.), and the ECL intensity of the first cycle even exceeded the measurement range of the ECL analyzer in the absence of tripropylamine.

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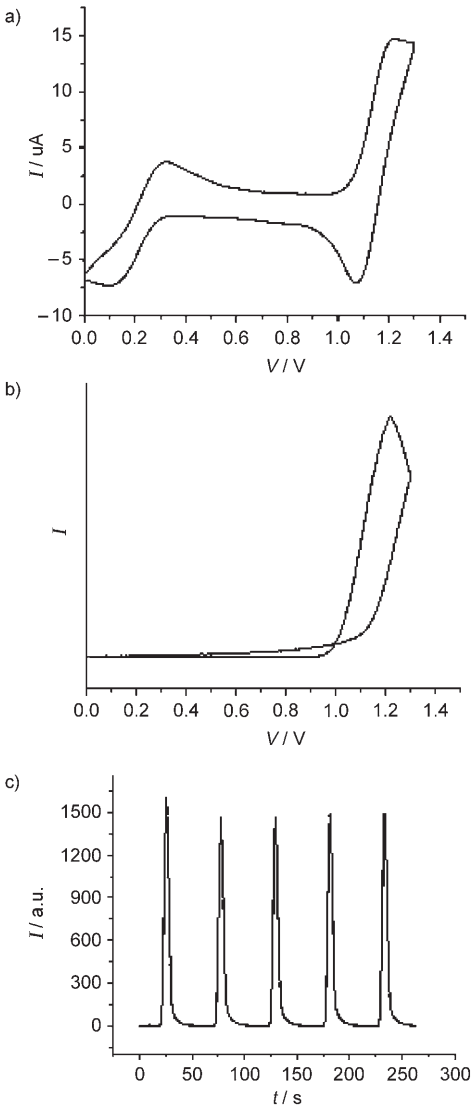

Figure 7. a) CV of the microstructures of sample 1 coated on an ITO electrode at a scan rate of 50 mV s.$^{-1}$ b) ECL intensity–potential curve of the microstructures of sample 1 coated on an ITO electrode in 50 mM phosphate buffer solution (pH 9.2). c) ECL intensity–time curve of the microstructures of sample 1 coated on an ITO electrode in 50 mM phosphate buffer solution (pH 9.2) with continuous cycling for 5 cycles.

Conclusions

$[Ru(bpy)_3]^{2+}$-containing microstructures have been prepared by electrostatic assembly of $[Ru(bpy)_3]^{2+}$ and $[Fe(CN)_6]^{3-}$ in aqueous solution at room temperature. It was found that that both the molar ratio and concentration of reactants have a strong influence on the formation of such microstructures. The electrochemical data indicate that these microstructures exhibit excellent ECL behavior and, thus, provide us with a new type of material for solid-state ECL detection in capillary electrophoresis or CE microchips.

Experimental Section

$[Ru(bpy)_3]Cl_2{\cdot}6H_2O$ and $[K_3Fe(CN)_6]$ were purchased from Aldrich and Beijing Chem. Co., respectively. All reagents were used as received without further purification. The water used was purified through a Millipore system. Sample 1 was prepared as follows: In a typical experiment, aqueous $[K_3Fe(CN)_6]$ (120 μL, 0.30 M) was introduced into aqueous $[Ru(bpy)_3]Cl_2$ (10 mL, 0.0054 M) ($[Fe(CN)_6]^{3-}/[Ru(bpy)_3]^{2+}$ molar ratio = 2:3) under vigorous stirring at room temperature. Several minutes later, a large amount of precipitate appeared. The resulting precipitate was collected by centrifugation, washed several times with water, and then suspended in water. The suspension was used for further characterization. For characterization by SEM, the suspension (20 μL) was placed on an ITO-coated glass slide and air-dried at room temperature. SEM measurements were made on a XL30 ESEM FEG scanning electron microscope at an accelerating voltage of 20 kV. UV/Vis spectra were collected on a CARY 500 Scan UV/Vis/near-IR spectrophotometer. The electrode for electrochemical and ECL analysis was constructed by placing the suspension (10 μL) on an ITO-coated glass electrode and air-dried at room temperature. A Model 800 electrochemical analyzer (CHI Instruments Co., Austin, TX) was used to perform cyclic voltammetry. ECL signals were monitored by an MCFL-A multifunctional chemiluminescent and bioluminescent analytical system (Remax Electronic Co., Ltd.) with the voltage of the photomultiplier tube set at 800 V.

Acknowledgements

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[1] J. H. Fendler, *Chem. Mater.* **2001**, *13*, 3196–3210.
[2] a) A. K. Boal, F. Ilhan, J. E. DeRouchey, T. Thurn-Albrecht, T. P. Russell, V. M. Rotello, *Nature* **2000**, *404*, 746–747; b) B. Frankamp, A. K. Boal, V. M. Rotello, *J. Am. Chem. Soc.* **2002**, *124*, 15146–15147.
[3] *Electroanalytical Chemistry* (Ed: A. J. Bard), Marcel Dekker, New York, **1977**.
[4] K. A. Fähnrich, M. Pravda, G. G. Guilbault, *Talanta* **2001**, *54*, 531–559.
[5] M. M. Richter, *Chem. Rev.* **2004**, *104*, 3003–3036.
[6] a) A. W. Knight, *TrAC Trends Anal. Chem.* **1999**, *18*, 47–62; b) W.-Y. Lee, *Mikrochim. Acta* **1997**, *127*, 19–39.
[7] a) R. M. Wightman, C. L. Curtis, P. A. Flowers, R. G. Maus, E. M. McDonald, *J. Phys. Chem. B* **1998**, *102*, 9991–9996; b) R. C. Engstrom, K. W. Johnson, S. DesJarlais, *Anal. Chem.* **1987**, *59*, 670–673.
[8] V. G. Kozlov, V. Bulovic, P. E. Burrows, S. R. Forrest, *Nature* **1997**, *389*, 362–363.
[9] F.-R. F. Fan, D. Cliffel, A. J. Bard, *Anal. Chem.* **1998**, *70*, 2941–2948.
[10] J. B. Noffsinger, N. D. Danielson, *J. Chromatogr. A* **1987**, *387*, 520–524.
[11] A. Arora, J. C. T. Eijkel, W. E. Morf, A. Manz, *Anal. Chem.* **2001**, *73*, 3282–3288.
[12] K. Honda, M. Yoshimura, T. N. Rao, A. Fujishima, *J. Phys. Chem. B* **2003**, *107*, 1653–1663.
[13] Z. Guo, Y. Shen, M. Wang, F. Zhao, S. Dong, *Anal. Chem.* **2004**, *76*, 184–191.
[14] Z. Wu, J. Tang, Z. Cheng, X. Yang, E. Wang, *Anal. Chem.* **2000**, *72*, 6030–6033.
[15] M. A. Lapierre, M. O'Keefe, B. J. Taft, S. O. Kelley, *Anal. Chem.* **2003**, *75*, 6327–6333.
[16] D. R. Shankaran, S. S. Narayanan, *Bull. Korean Chem. Soc.* **2001**, *22*, 816–820.
[17] X. Sun, Y. Du, L. Zhang, S. Dong, E. Wang, *Anal. Chem.* **2007**, *79*, 2588–2592.

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