

Ultra Microanalysis of Ferric Ion Using Triazene and Capillary Electrophoresis

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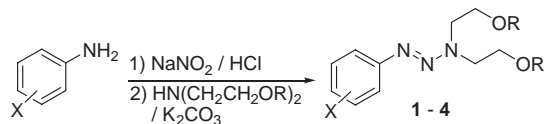
The triazene compound {2,2'-[3-(4-methoxyphenyl)triaz-2-ene-1,1-diyl]diethanol} was utilized for the high sensitive and quantitative detection of ferric ion.

Microanalyses of transition-metal ions are desired in diverse areas including biochemistry, environmental pollution, etc.¹ Recently, the selective detection of ferric ion is being intensively studied because it plays an important role in many biochemical processes in the cell² and is also well known as a fluorescence quencher due to its paramagnetic nature. Therefore, we tried to develop a simple and precise analysis for it using UV-vis spectroscopy. The triazenes possesses unique structure and three successive nitrogen atoms, and they are utilized in widespread areas including organic and polymer synthesis, and medicinal chemistry.³ Consequently, we focused on 1-aryl-3,3-dialkyltriazenes as new indicators. We designed and synthesized triazenes that specifically reacted with ferric ion. On the other hand, the field-amplified sample stacking technique in capillary zone electrophoresis, investigated by Mikkers et al., was a powerful microanalysis method.⁴ We assessed the properties of triazenes by measuring UV-vis spectra and capillary electrophoretic analysis to achieve ultra microanalysis of ferric ion.

The triazene compounds were synthesized according to the literature (Scheme 1).⁵ Thus, 3,3-bis(2-methoxyethyl)-1-phenyltriazene (**1**) was obtained in 94% yield employing aniline as the arylamine and bis(2-methoxyethyl)amine as the sondary amine. With the same procedure, 2,2'-[3-(phenyltriaz-2-ene-1,1-diyl)diethanol] (**2**) was synthesized from aniline and diethanolamine in 96% yield. Similarly 2,2'-[3-(4-methoxyphenyl)triaz-2-ene-1,1-diyl]diethanol (**3**) and 2,2'-[3-(4-nitrophenyl)-2-triazediethanol (**4**) were obtained in 63 and 64% yields, respectively.

To evaluate the functionality of ferric ion and other metal ions, the UV-vis spectra of the mixed methanol solution of the synthesized triazenes (**1-4**) and some multivalent metal ions were measured. Among them, only **3** reacted with ferric ion, and the mixtue of other compounds and other metal ions did not give any new absorption maxima (Figure 1).

After adding 1.0 equiv. of ferric ion to **3** in methanol, new absorption was observed (blue line). Surprisingly, an additional 1.0 equiv. of ferric ion resulted in the appearance of absorption maxima at 280 and 330 nm that increased nonlinearly (green line). The new absorption that might arise from a charge-transfer



Scheme 1. Triazene preparation.

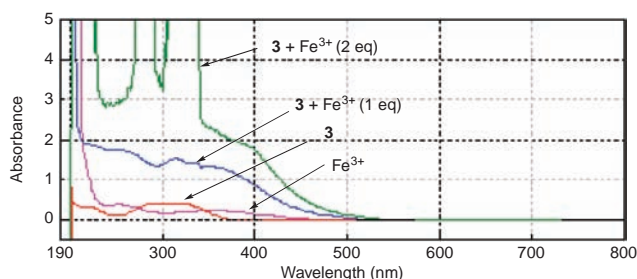
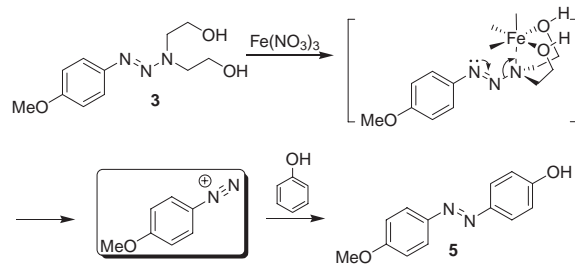


Figure 1. UV-vis spectra of triazene **3**, ferric ion, and their mixture.

complex could not be observed in the long-wavelength region. From the above results, this triazene could be useful to detect trace ferric ion. In this stage, it was thought that the mixture was transformed to different compounds possessing strong absorbancy, for example, a diazonium ion. Therefore, we performed the same reaction in large scale to confirm our speculation. The ferric nitrate solution was added to a methanol solution of **3**, and then phenol was added to the mixture to form 4-(4-methoxyphenylazo)phenol (**5**) in 47% yield. This result indicated that the reaction progressed via the diazonium cation as a reaction intermediate. We proposed a possible mechanism of diazonium ion generation from **3** as follows (Scheme 2). The ferric ion coordinated with the 3-nitrogen and the two oxygens of the hydroxyethylamino units of **3** and behaved as a Lewis acid. The following cleavage of 2- and 3-nitrogen bond occurred to produce a stable diazonium ion.

Furthermore, we synthesized stable diazonium salt: 4-methoxybenzene diazonium tetrafluoroborate (**6**) from *p*-anisidine, sodium nitrite, and tetrafluoroboric acid⁶ and its absorption spectrum was measured. Peak of spectrum was observed at 311 nm. The wavelength and molar absorptivity were almost the same as in the mixture of **3** and ferric ion (Table 1).

We tried to apply this system to the microscale determination of ferric ion using capillary electrophoresis, which was performed by Applied Biosystems model 270A system equipped



Scheme 2. Possible reaction mechanism for formation of diazonium cation from triazene (**3**) and ferric nitrate.

Table 1. Comparison of molar extinction coefficient

Compounds	$c/\text{mol L}^{-1}$	Abs	$\lambda_{\text{max}}/\text{nm}$	$\varepsilon_{\text{max}}/\text{M}^{-1}\text{cm}^{-1}$
6	1.70×10^{-5}	0.38	311	2.23×10^4
3 + Fe^{3+} (1 equiv.)	1.70×10^{-5}	0.38	313	2.24×10^4

with a capillary oven thermostated by circulating air. Separations were performed in an untreated fused-silica capillary (0.05-mm i.d.), which had a total length of 72 cm and a separation length of 50 cm. The electrophoretic solution was a 50 mM phosphate buffer (pH 6.8), and the applied voltage was set at +20 kV. Detection was carried out at 280 nm, and capillary temperature was maintained at 30 °C. Samples were prepared by addition of ferric ion solution of several concentrations to 5 mM triazene solution and then introduced by vacuum (5-inch Hg) injection for 1.5 s. The molar ratio of ferric ion and triazene was 1:1. Ferric nitrate was used as a ferric ion provider in the UV-vis spectra measurements. The migration time of **3** was ca. 6.2 min, and ferric ion was not detected at the used wavelength. The mixture of the aqueous solutions of ferric nitrate and **3** gave single peak at 3.6 min, indicating a quick and quantitative reaction (data not shown). The increased positive-charged material was produced from the triazene compound. We proposed a novel quantization method for ferric ion using the same manner.

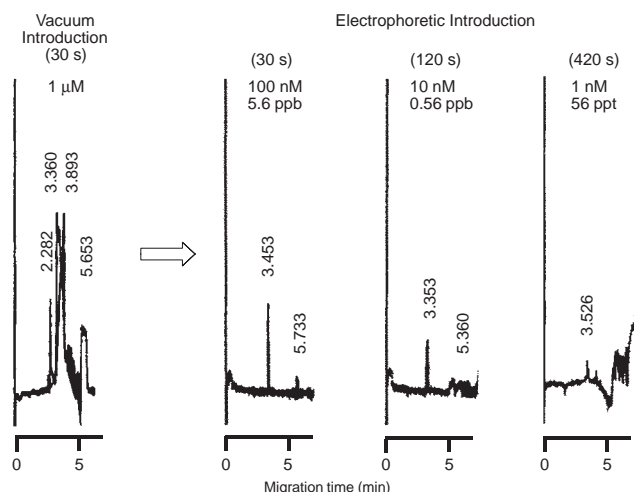
Next, we tried to make a calibration curve of the ferric ion as diazonium ion in the triazene solution. The peak responses of diazonium salt were plotted against ferric ion concentration. The curve was given by following equation:

$$y = 6.46 \times 10^4 x + 5.77 \times 10^3$$

And good linearity was obtained with correlation coefficients over 0.99 in a range of 10 μM –2 mM, calculated to 56 ppb–112 ppm. These results indicated the potential of this system for quantitative analyses of ferric ion.

However, the detection limit was relatively high concentration (10 μM) in the conditions. Accordingly, we investigated the enhancement of detection sensitivity using in-capillary sample condensation. When the 1.0 μM solution of sample was introduced by vacuum introduction for 30 s, diazonium ion was effectively concentrated by stacking phenomenon to provide sufficient signal at ca. 2.3 min. But interfering peaks also increased (Figure 2). Therefore, the method of introduction was changed to electrophoretic introduction method known as a sample stacking technique of on-line sample concentration. First, introductions of 100 nM samples by applying 5 kV for 30 s were investigated. They gave high sensitivity and excellent peak shape. Successively, we tried to analyze more diluted solutions. In the case of a 10 nM solution, 120 s of introduction time was required at the same applied voltage. For the further 10 fold dilute solution (1.0 nM), the concentrating time provided for 420 s and peaks of diazonium ion were still clearly detected. We achieved high-sensitive analysis of the solution containing ferric ion at 56 ppt. This identification limit was lower than that of recent reports by Hasegawa (3.3 nM),⁷ Ensafi (0.72 μM)⁸ and Cha (50 nM),⁹ respectively.

In conclusion, we synthesized coordinatable and easily reactable triazene with ferric ion and assessed it for analysis. The triazene reacted quantitatively with the solution containing ferric ion to afford diazonium salt. By using capillary electrophoresis, a highly sensitive analytical method of ferric ion with

**Figure 2.** Introduction-method detection limit of diazonium

an identification limit of 56 ppt employing an on-line condensation technique was developed. This method will be easy to prepare the reagent and the sample solution and to set up the device. It is considered that these results can be applied to biochemical analysis, clinical laboratory tests, and water examination, etc.

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