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Flow-injection Chemiluminescence Determination of Trace Calf Thymus DNA

Min ZHOU, Yong Jun MA, Xiao Yong JIN, Xiu Lan TENG, Zi Yu ZHANG, Hui CHEN*

Institute of Chemistry, Northwest Normal University, Lanzhou 730070

Abstract: A flow injection procedure for the determination of calf thymus DNA (CT DNA) over the range 2.1×10^{-6}~2.1×10^{-1} μg mL^{-1} is described, based on measurement of the enhanced chemiluminescence emission of rhodamine B-Ce(IV) system, activated by imidazole-HCl buffer solution. This method is highly sensitive, with the linearity range broadened to five orders of magnitude. It has been applied to determine CT DNA in synthetic sample with satisfactory results.

Keywords: Chemiluminescence, flow-injection, calf thymus DNA, imidazole, rhodamine B.

DNA quantification is critical for many biological studies, since it is often used as a reference for measuring other parameters. Till now, many approaches for DNA determination have been proposed, including spectrophotometry [1,2], fluorometry [3-6] and resonance light scattering (RLS) method [7,8]. Chemiluminescence (CL) measurements in flow-injection (FI) have also been utilized to detect DNA [9,10], but most of FI-CL methods have a calibration range only of two orders of magnitude.

The present study found that CT DNA activated by imidazole-HCl buffer solution enhanced the CL signal of cerium (IV) – rhodamine B system in sulfuric acid. For the first time, the linearity range was broadened to five orders of magnitude, and the detection limit was decreased to 3.5×10^{-7} μg mL^{-1}.

Experimental

Reagents and apparatus

Stock solution of CT DNA was prepared by directly dissolving commercial CT DNA (Sino-American Biotechnology Company, SABC, China) in doubly deionized water and stored at 0-4℃. Twenty-four hours or longer time were needed for complete dissolution. The concentration was calculated according to the absorbance at 260 nm [11]. The final concentration of stock solution was 42 μg mL^{-1}.

Imidazole-HCl buffer solution (0.1 mol L^{-1}) was prepared by dissolving the reagent

* E-mail: myjlr@public.lz.gs.cn

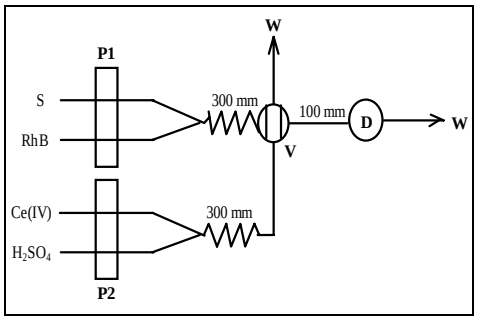
(from Jiangsu Guangyao Reagent Company, China) in water, in which 1 mol L^{-1} HCl was used to adjust the pH to 7.0.

The cerium (IV) solution (0.05 mol L^{-1}) was prepared by dissolving $Ce(SO_4)_2 \cdot 4H_2O$ (Xinhua Chemical Co., Beijing, China) in 0.2 mol L^{-1} H_2SO_4. A 100 mg L^{-1} rhodamine B solution was prepared by dissolving rhodamine B (Merck, Germany) in doubly deionized water.

All chemicals were of analytical grade without further purification. Doubly deionized water was used throughout.

The FI system, as shown in **Figure 1**, was an IFFL-D FI-CL analysis system (Xi'an Record Electric Ltd Corp, China).

Figure 1 FI manifold for CT DNA determination



Ce(IV) 0.04 mol L^{-1} in 0.2 mol L^{-1} H_2SO_4; RhB, rhodamine B, 100 mg L^{-1}; S, DNA sample; W, waster; P1,P2, peristaltic pump; D, CL detector; Volume of flow cell 150 μL

Procedure

Standard DNA solutions were prepared by mixing CT DNA with 0.1 mol L^{-1} imidazole-HCl buffer solution in the proportion of 1:1 (V/V) and incubated for 1 h at room temperature. Then the standard solutions were injected into the FI system one by one, as shown in **Figure 1**.

Results and Discussion

0.04 mol L^{-1} Ce(IV) in 0.2 mol L^{-1} H_2SO_4, 100 mg L^{-1} rhodamine B were used and 0.8 mol L^{-1} H_2SO_4 was chosen to use as a carrier. 0.1 mol L^{-1} imidazole-HCl buffer solution (pH7.0) induced the widest linearity range. A total flow rate was 11 mL min^{-1} (5.5 mL min^{-1} for each channel). All above conditions were recommended as optimum conditions.

Under above optimum conditions, the calibration graph between log CT DNA *versus* peak height was linear over the range 2.1×10^{-6}~2.1×10^{-1} μg mL^{-1} (r=0.9998, n=8). The relative standard deviation (R.S.D) for 2.1×10^{-3} μg mL^{-1} CT DNA was 1.1% (n=11). The detection limit, defined as three times the S.D. for the reagent blank signal, was 3.5×10^{-7} μg mL^{-1}.

The influence of foreign species on the determination of 2.1×10^{-3} μg mL^{-1} CT DNA (**Table 1**) was studied. The tolerance of each foreign species was taken as the largest

concentration yielding an error of <5% in the analytical signal of DNA.

Table 1 The interference of the maximum ratio of the foreign species on determination of CT DNA.

Foreign species	Maximum tolerable mole ratio*
EDTA	1000
Urea	1000
Na^+ Cl^-	1000
SDS	200
Mg^{2+} SO_4^{2-}	200

* 1000 is the greatest ratio tested

Determination of synthetic samples

According to the maximum tolerances of the foreign species, the sample containing 2.1×10^{-3} μg mL^{-1} CT DNA was prepared by mixing an appropriate amount of standard CT DNA solution with foreign species, then the intensity of CL was detected. The results are summarized in **Table 2.**

Table 2 Determination of CT DNA in synthetic sample

CT DNA concentration in synthetic sample (μg mL^{-1})	Found (μg mL^{-1})	Recovery %	RSD % (n=5)
2.1×10^{-3}	2.13×10^{-3}	101.4	1.4

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