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# Detection of Ultra Trace Amount Gossypol with Chemiluminescence Using Capillary Electrophoresis as Injection Techniques

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**Abstract:** Detection of ultra trace amount gossypol is reported. It is based on luminol-hexacyanoferrate(III) chemiluminescence reaction with gossypol as a sensitizer. The detection limit ($3\sigma$)of gossypol reaches $5.21\times10^{-16}$ mol/L.

**Keywords:** Capillary electrophoresis, chemiluminescence, gossypol.

Gossypol is a polyphenolic yellow compound naturally occurring in various parts of cotton plants[1]. The compound has been associated with a wide range of important applications including oral male contraceptive[2], anticancer[3-5], and other biological activities[6,7]. Therefore, the determination of gossypol in biological fluids and pharmaceutical preparations is of great importance. But up to now, only HPLC[8,9] and UV-visible[10] methods have been reported for its quantitatively analytical description in the literature.

Now the detection of ultra trace amount of chemical compounds in diverse matrices is becoming more and more popular in the scientific community[11-15] and CL is famous for its high sensitivity[16]. Therefore, we engaged in studying the chemiluminescence (CL)of gossypol, and successfully detected the gossypol in the concentration of $5.21\times10^{-16}$ mol/L using capillary electrophoresis as injection techniques.

## Experimental

The capillary electrophoresis-chemiluminecence detector was laboratory-built. A 0-30 kV power supply provided the separation high voltage. The HF treated end of the separation capillary(28 cm×50 μm. I.D) which was inserted into a reaction capillary (17 ×530 μm. I.D). They were held in place by a Plexiglas four-way joint. The required CL reagents were delivered by gravity through a reagent capillary (40 cm×320 μm. I.D). A 2 cm detection window was formed on the reaction capillary by burning off the polyimide coating. The CL emission was collected with BPCL, which was operated at 750 V. The photocurrent was magnified then recorded using a computer. All the capillaries used were purchased from Hebei Yongnian Reifeng Chromatogram Factory

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Unless otherwise stated, all chemicals used were of analytical-reagent grade. The concentration of the stock gossypol solution was $2.468\times10^{-4}$ mol/L. $2.7\times10^{-3}$ mol/L luminol (Shanxi Normal University) was dissolved in $2\times10^{-2}$ mol/L sodium dihydrogen phosphate ($NaH_2PO_4$) buffer. The phosphate buffer was adjusted to pH 8.5 with 0.1 mol/L sodium hydroxide (NaOH). The post column reagent solution was adjusted to pH 13.0 with 1.0 mol/L NaOH. Standard solutions of the gossypols were prepared daily serial dilution of stock solutions. All solutions were filtered through 0.22μm membrane filters before use.

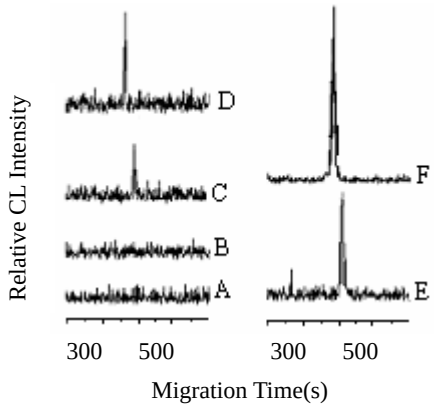
Before daily use, the electrophoresis capillary was washed with 0.1 mol/L NaOH, 0.1 mol/L HCl, water, and then equilibrated for 30 min with electrolyte. Sample volumes of 10 nL were introduced by gravity injection in 10 s.

## Results and Discussion

For capillary electrophoresis, choosing proper buffer solution is very important to CL. Hence we experimented the effect of acetate, sodium tetraborate and phosphate, and found that the CL signal could be greatly enhanced in phosphate buffer solution, while there was weak CL in acetate and sodium tetraborate buffer solution. So the phosphate buffer was used. Besides that, there are also some other factors which could effect the CL such as the amount of $K_3Fe(CN)_6$, luminol and so on. By experiment, $1.0\times10^{-4}$ mol//L $K_3Fe(CN)_6$ and $4.5\times10^{-4}$ mol/L luminol were used.

Under the optimum conditions described above, various amounts of gossypol (**Figure 1**) were injected. The detection limit of gossypol was $5.21\times10^{-16}$ mol/L(3 σ ).

**Figure 1** Injection of various amounts of gossypol




Curve A: blank. curve B: water. curve C: injecting $8.3\times10^{-16}$ mol/L of gossypol solution. curve D: injecting $8.3\times10^{-15}$ mol/L of gossypol solution. curve E: injecting $8.3\times10^{-14}$ mol/L of gossypol solution. curve F: injecting $8.3\times10^{-13}$ mol/L of gossypol solution. Electrophoresis electrolyte, $4.5\times10^{-4}$ mol/L luminol+$1.25\times10^{-3}$ mol/L phosphate buffer(pH 8.5). Post reagent: $4.4\times10^{-4}$ mol/L potassium ferricyanide +$2.5\times10^{-3}$ mol/L phosphate buffer (pH13).

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