Chinese Chemical Letters Vol. 14, No. 2, pp 185 – 187, 2003
http://www.imm.ac.cn/journal/ccl.html

A Capillary Electrophoresis Detection Scheme for Water-soluble Vitamins Based on Luminol – BrO^- Chemiluminescence System

Wei DENG, Wei Ping YANG, Zhu Jun ZHANG*, Hou Jiang ZHOU

College of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062

Abstract: A novel chemiluminescence detection scheme has been developed for detecting water-soluble vitamins following capillary electrophoresis (CE) separation. This detection was based on the inhibitory effect of vitamins on the CL reaction between luminol and BrO^- in basic aqueous solution. Detection of vitamins was accomplished with a borate–based background electrolyte at pH 9.2. The luminol was used as a component of the separation carrier electrolyte.

Keywords: Capillary electrophoresis, chemiluminescence, vitamins.

The detection of vitamins is of great interest due to the biological significance of these compounds. Several studies on the determination of vitamins have been performed using HPLC[1,2], CE[3,4].

Chemiluminescence (CL) analysis shows excellent sensitivity, wide linear range and requires simple instrumentation for assay of vitamins[5-7], but poor selectivity of CL greatly limits its application in analysis of complex systems. The research presented here describes some preliminary results on the determination of the water-soluble vitamins based on luminol - BrO^- CL reaction system following CE separation. It is based on the inhibitory effect of vitamins on the CL reaction between luminol and BrO^- in basic aqueous solution.

All the data were collected using a home-built CE – CL detection system. In brief, a 0 – 30 kV high power supply provided the separation voltage. A fused-silica capillary (60 cm × 75 μm I.D.) was used for separation. A 2 mm section of the end of the separation capillary was burned and then inserted into a reaction capillary (16 cm × 530 μm I.D.). The detection window was formed by burning 4 mm polyimide layer of the reaction capillary and was set in the front of the photomultiplier tube (PMT). A section of the end of the separation capillary was inserted within the middle of the detection window. The required CL reagent was delivered by a microsyring pump through a reagent capillary (20 cm × 250 μm I.D.). All the capillaries were fixed in place by a plexiglass or teflon connector, which was made in three - way (400 – 600 μm I.D.). The data acquisition and collection were processed using commercially available software (IFFM – A data analysis system, Xi'an). During the data collection and acquisition, the

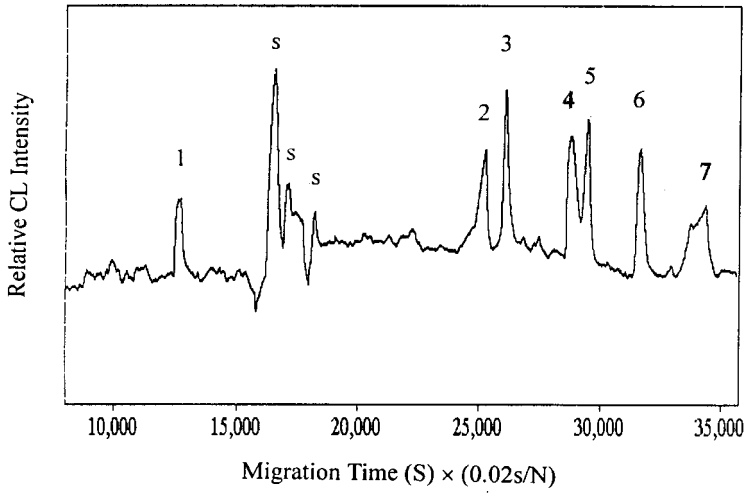
*E-mail: zzj18@hotmail.com

electropherograms were reversed to get the positive peaks. The capillary was rinsed with 0.1 mol/L sodium hydroxide for 2 min., and then with the separation buffer for 2 min.. The samples were introduced to the capillary electrokinetically by applying a 10 kV power for 9 s. After completion of this purging routine and sample injection, the microsyring pump was switched on to provide a constant flow of CL reagent to the reaction capillary.

Optimization of analytical conditions: 20 mmol/L sodium borate (pH 9.2) and 5 $\times 10^{-4}$ mol/L luminol were chosen as the electrolyte. 5×10^{-5} mol/L BrO^-, prepared by Br_2 dissolved in 0.1 mol/L NaOH solution, was used as the CL reaction reagent. The applied voltage was 15 kV.

Figure 1 showed the electropherogram for the separation of six water-soluble vitamins. The response to vitamins was linear in the concentration range of 5×10^{-6} - 1 $\times 10^{-4}$ mol/L. The relative standard deviation (RSD) for the analysis of vitamins was less than 1% for the migration time and 5% for the peak area. The limits of detection were from 1×10^{-6} to 5×10^{-6} mol/L for the 6 water-soluble vitamins.

Figure 1 Electropherogram of vitamins separation



1, V_{B1}; 2, V_{B6}; 3, V_{K1}; 4, V_C; 5, V_{B2}; 6, V_{B12}; 7, unknown; S, system peak.

The influences of foreign species were investigated by analyzing a standard solution of 1×10^{-5} mol/L V_{B1}, V_{B6}, V_{K1}, V_C, V_{B2}, V_{B12}, respectively. The tolerated ratio of a foreign species to 1×10^{-5} mol/L V_{B1}, V_{B6}, V_{K1}, V_C, V_{B2} and V_{B12} was 500 for the other vitamins and transition-metal ions. The method has been applied to the determination of vitamins in complex vitamin pills. The results are given in **Table 1.**

Table 1 Result of analysis of vitamin in drug sample

	Super Vita			Gold Theragran		
Vitamins*	V_{B1}	V_{B2}	Vc	V_{B1}	V_{B2}	Vc
Amount (mg/pill)	2.5	2.5	2.5	3	3.4	90
Amount found (mol/L)	2.3×10^{-6}	2.1×10^{-6}	2×10^{-5}	2.5×10^{-6}	3.0×10^{-6}	7.4×10^{-5}
Added (mol/L)	1.0×10^{-5}	1.0×10^{-5}	5.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	5.0×10^{-5}
Found (mol/L)	1.1×10^{-5}	9.6×10^{-4}	4.92×10^{-5}	1.05×10^{-5}	9.7×10^{-4}	4.8×10^{-5}
Recovery(%)	106	96	98	105	97	96

* V_{B6}, V_{K1}, V_{B12} were not determined.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (No. 20175039).

References

1. P. Moreno, V. Salvadó, *J. Chromatogr. A,* **2000**, *870* (1-2), 207.
2. I. Koshiishi, Y. Mamura, T. Imanari, *J. Chromatogr. A,* **1998**, *802* (2), 340.
3. L. Fotsing, M. Fillet, P. Chiap, P. Hubert, J. Crommen, *J. Chromatogr. A,* **1999**, *853* (1-2), 391.
4. M.W. Davey, G. Persiau, G. Bauw, M.V. Montagu, *J. Chromatogr. A*, **1999**, *853* (1-2), 381.
5. T. Pere-Ruiz, C. Martinez-Lozaro and A. Sanz, *Anal. Chim. Acta*, **1995**, *308*, 299.
6. Z. Zhang, W. Qin, *Talanta*, **1996**, *43*, 119.
7. W. Qin, Z. Zhang, H. Liu, *Anal. Chim. Acta.*, **1997**, *357*, 127.

Received 15 April, 2002