Chinese Chemical Letters Vol. 14, No. 12, pp 1278 – 1280, 2003
http://www.imm.ac.cn/journal/ccl.html

DNA Separation by Capillary Electrophoresis with Ultraviolet Detection using Mixed Synthetic Polymers

Qian WANG, Xu XU*

State Key Laboratory of Organometallic Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032

Abstract: The mixtures of two polymers, poly (N,N-dimethylacrylamide) (PDMA) and polyvinylpyrrolidone (PVP) were synthesized and used as the separation medium for double-stranded and single-stranded DNA fragments by capillary electrophoresis with UV detector. On optimal conditions, 2%w/v PDMA + 2%w/v PVP can be used to separate the doublet 123/124bp in pBR322/*Hae* III Markers.

Keywords: Synthetic polymer, DNA separation, capillary electrophoresis, ultraviolet detection.

The separation medium is one of the most important factors for DNA separation by capillary electrophoresis (CE), whose characteristics and concentration have great influence on the separation. A number of water-soluble polymers have been used for this purpose and achieved good results [1,2]. Generally, these experiments were performed by CE with the laser-induced fluorescence (LIF) detector. Compared with LIF detector, UV detector commonly used in many laboratories, does not need any fluorescent reagent, yet suffers from lower sensitivity and higher sample load. All these are detrimental to DNA separation. Therefore, it is very difficult to achieve DNA single-base separation by CE with UV detector.

This paper presents a kind of DNA separation medium, the polymer solution of poly (N, N-dimethylacrylamide) and polyvinylpyrrolidone (PDMA/PVP), which can separate both dsDNA and ssDNA to single-base resolution using UV detector.

Experimental

Reagents: N,N,N′,N′-tetramethylethylenediamine (TEMED,99%); ammonium persulfate (98%); N,N-dimethylacrylamide;acrylamide(AA,99.9%); polyvinylpyrrolidone (PVP, K30); pBR322/*Hae*Ⅲ Markers (0.05 μg/μl).

Instruments: Bio-focus 3000™ Capillary Electrophoresis (Bio-Rad, USA). Electrophoresis buffer: NaH_2PO_4-Na_2HPO_4 (20 mmol/L，pH 7.0). Temperature: 25°C. UV detection wavelength: 260 nm. Running voltage: -4 kV. Injection: -4 kV×3 s.

* E-mail:xuxu@pub.sioc.ac.cn

Capillary: ∅ 50 μm × 36 cm (total length), one uncoated column and one column coated with linear polyacrylamide[3].

Results and Discussion

Separation of dsDNA in the polymer solution of PDMA/PVP with UV detection

Without fluorescent reagent in the separation medium, it was found that the polymer solution of 2%w/v PDMA + 2%w/v PVP could achieve the best result even with UV detection, as shown in **Figure 1**. The doublet 123/124bp in pBR322/*Hae*III Markers was separated and the R_s value was 0.85,while the required R_s value in DNA sequencing is above 0.59. Therefore, the polymer solution is believed to be a good medium for DNA separation. The similar results could be achieved with the uncoated capillary column, indicating the dynamic coating ability of the medium. Wang[2] *et al* reported that the solution of 4%w/v PDMA + 4%w/v PVP + fluorescent reagent could be used to achieve the single-base resolution with LIF detection, in which fluorescent reagent was necessary for dsDNA separation.

Separation of ssDNA in the polymer solution of PDMA/PVP with UV detection

After the denaturant formamide was added into the polymer solution of 2%w/v PDMA + 2%w/v PVP, the single-base separation could also be achieved for ssDNA if the formamide concentration was in the range of 10% ~ 30% (v/v). **Figure 2** shows the separation of ssDNA by 2%w/v PDMA + 2%w/v PVP containing 15% (v/v) formamide. The doublet 123/124 was also separated with R_s of 0.88.

Figure 1 Separation of pBR322/*Hae*III Markers by CE using a polymer solution of PDMA/PVP with UV detection.

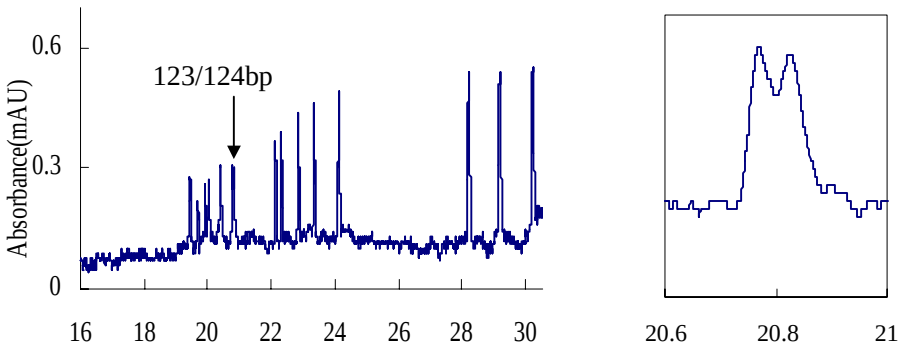
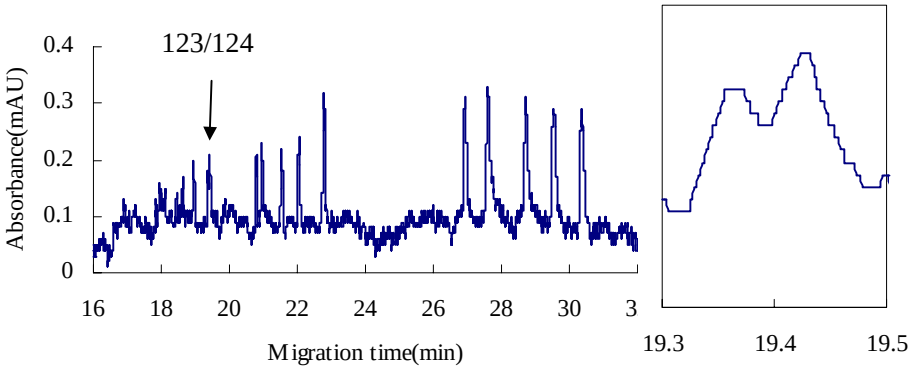


Figure 2 Separation of pBR322/*Hae*III Markers by CE using the polymer solution of PDMA/PVP (containing 15% v/v formamide) with UV detection.



Note: The other conditions are the same as those in **Figure 1**.

Acknowledgment

The Project is financially supported by the Innovation Fund (KSCX1-06) of Chinese Academy of Sciences.

References

1. L. Song , T. Liu , D. Liang , *et al*, *Electrophor.*, **2001**, *22*, 3688.
2. Y. Wang , J. Hao , D. Liang , *et al*, *Electrophor.*, **2002**, 23, 1460.
3. S. Hjertèn, *J. Chromatogr*. ,**1985**, 347, 19.

Received 2 December, 2002