TOSOH

Application Note

Rapid Analysis of Plasmid Topoisomers by Anion Exchange Chromatography

Recombinant plasmid DNA (pDNA) is increasingly used as a raw material in gene therapy (e.g. in lentiviral and AAV vector production) and as an active ingredient for DNA vaccination. Pharmaceutical grade plasmid DNA must meet specifications concerning both host-related impurities as well as homogeneity [i. e. the content of pDNA topoisomers: covalently closed circular (CCC), open circular (OC) and linear (L), as well as di- or multimeric variants]. During large-scale plasmid fermentation, plasmids are maintained predominantly in a supercoiled, covalently closed circular form. During a downstream process, some of the plasmids might become nicked and they will be transformed into open circular and linear forms.

For a fast and successful characterization of pDNA samples, Schuchnigg et al. developed an HPLC method with a high resolving power based on the TSKgel® DNA-NPR™ anion exchange HPLC column[1]. TSKgel DNA-NPR is packed with 2.5 µm hydrophilic non porous polymer beads modified with a weak anion exchange group. The non porous particle offers fast mass transfer, a key to achieve high resolution. The small particle size and the fast mass transfer of non porous beads can be exploited to speed up the analysis. By using pBR322, one of the first widely used *E. coli* cloning vectors, we exemplarily demonstrate that the method can be easily transferred from HPLC to UHPLC systems.

Material and Methods

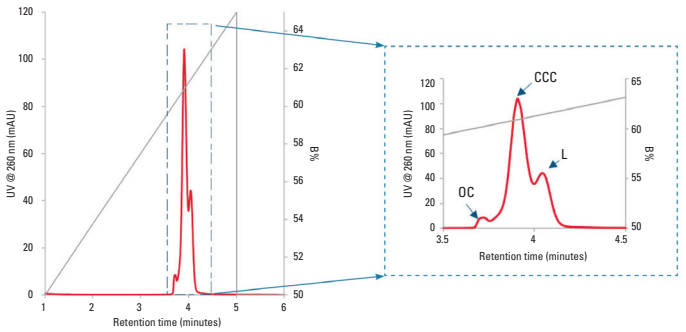
Column:	TSKgel DNA-NPR, 2.5 µm, 4.6 mm ID × 7.5 cm
Mobile Phase:	A: 20 mmol/L Tris/EDTA, pH 9.0
	B: A + 1.0 mol/L sodium chloride
Gradient:	50% B to 65% B in 5 min
Flow rate:	1.0 mL/min
UHPLC system:	Ultimate® 3000RS
Detection:	UV @ 260 nm
Temperature:	25 °C
Injection. vol:	5 µL
Sample:	pBR322 (NEBiolabs)

The plasmid pBR322 has a length of 4,361 base pairs, which results in a molecular weight of 2.83×10^6 Da. In order to classify the topoisomers properly, the linear form was prepared by incubation with the single-cutting restriction enzyme EcoRI (NEB). pBR322 has a single EcoR1 restriction site at position 4359. For the digest, 1 unit of restriction enzyme per µg DNA was used. The sample was incubated at 37 °C for 60 min. The reaction was stopped by heating to 65 °C for 20 min.

Fast Separation of Plasmid Topoisomers

The chromatographic analysis of pBR322 on TSKgel DNA-NPR is shown in *Figure 1*. The plasmid can be analyzed within a 5 min linear gradient from 50% to 65% mobile phase B at a flow rate of 1.0 mL/min. The plasmid elutes in three peaks representing the different species: supercoiled, open circular and linear. The highest peak corresponds to the supercoiled plasmid.

Figure 1. AEX analysis of pBR322 topoisomers on TSKgel DNA-NPR



The linear form of the plasmid was analyzed at the same conditions as the plasmid. *Figure 2* shows the corresponding chromatogram with a single peak, representing the EcoR1 digested linear form. *Figure 3* shows an overlay of the two chromatograms that confirms that peak three corresponds to the linear form.

Figure 2. AEX analysis of linear EcoR1 digested pBR322 on TSKgel DNA-NPR

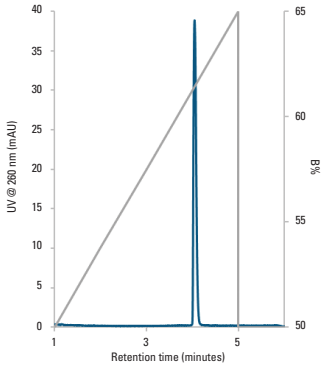
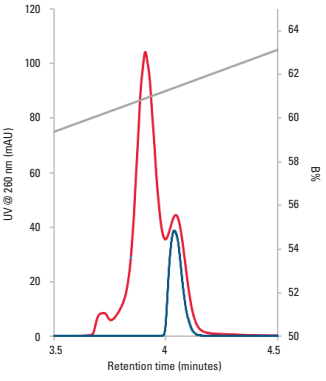


Figure 3. Overlay of pBR322 (OC, CCC, L) and linear pBR322 analyzed by anion exchange chromatography.



Conclusion

Analytical ion exchange chromatography on a TSKgel DNA-NPR column offers a simple and rapid method for discriminating between covalently closed circular, open circular and linear forms of plasmid DNA. Schuchnigg et al. showed that elution pattern changes with plasmid size. Therefore, it is recommended to confirm the position of the peak representing the linear form. The method is ideally suited to be used at various stages of pDNA R&D and manufacturing for pharmaceutical purposes and can be used in both HPLC and UHPLC systems.

References

(1) CHARACTERIZATION OF PLASMID DNA SAMPLES BY CHROMATOGRAPHIC METHODS; Hermann Schuchnigg, Patricia Cantarell, Christoph Pollak, Jochen Urthaler and Wolfgang Buchinger; Boehringer Ingelheim Austria GmbH, Poster HPLC 2008, Baltimore, MD, USA