
ASSESSMENT OF DIFFERENT METHODS FOR CLEANING UP A FLUORESCENT LABELLED POLIOVIRUS SAMPLE AND ANALYSIS WITH LASER INDUCED FLUORESCENT CAPILLARY ELECTROPHORESIS

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The application of analytical separation techniques is not common in virology. By introducing capillary electrophoresis (CE) in virology we want to replace classical material-consuming virological procedures by material and reagent-saving, automated analytical methods.

Initially a CE method was developed for the analysis of poliovirus with UV-CE using a 12-experiment Plackett-Burman design (Oita I. et al., 2009). The identification of a poliovirus peak was performed by an indirect confirmation method.

To increase the limit of detection (LOD) in the future, we want to label the virus with a fluorescent tag. The fact of increasing the LOD will be important in further research such as morphogenesis studies or viral-cellular interactions in cell free extracts.

Pre-experimental data showed the possibility of direct labelling of poliovirus with the fluorophore fluoresceine isothiocyanat (FITC) and detection with an Ar⁺ - laser (ex/em = 488 nm/520 nm).

A 1000 to 2000 molecular excess of FITC is needed to reach a sufficient amount of labelled and detectable virus.

Analysis of poliovirus without any sample clean up resulted in electropherograms overloaded by FITC and masking all analytes. An efficient and reproducible method was required for FITC removal with a high recuperation of labelled viral particles being the main prerequisite.

We had to take into account the applicability of the method for possible presence of labelled subviral particles and single viral proteins.

Four methods were assessed for cleaning up a FITC labelled poliovirus sample, i.e.

- Dialysis
- Size exclusion chromatography on self-filled sephadex columns.
- Sucrogradient ultracentrifugation
- Ultrafiltration

Based on virus recovery, reproducibility, processing time and cost, the best method will be chosen.

Oita, I., Halewyck, H., Pieters, S., Dejaegher, B., Thys, B., Rombaut, B. and Vander Heyden Y., J. Pharm. Biomed. Anal. Doi: 10.1016/j.jpba.2008.09.049. Improving the capillary electrophoresis analysis of poliovirus using a Plackett-Burman design