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Flow cytometric studies of DNA-binding characteristics of an inorganic layered double hydroxide (LDH)

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Introduction

Layered double hydroxides (LDHs) consist of cationic metalhydroxide layers and exchangeable interlayer anions. Due to this negatively charged structure, biomolecules like DNA can be incorporated to form a DNA-LDH hybrid.

DNA removal from cell culture supernatants is often one of the major concerns in downstream processing. Due to the anion exchange capabilities of LDHs we tested their capability to bind DNA from aqueous solution.

1

Experimental

Several LDHs were tested towards their loading capacity of DNA by using UV-spectroscopy. LDH-suspensions (10mg ml^{-1}) were incubated with DNA solution (2,5 mg ml^{-1}) for a given period. The supernatant was analysed by UV-spectroscopy (adsorption isotherm). The loaded LDHs were also analysed by flow cytometry (long term kinetics), by labelling the DNA with propidiumiodide (PI).

As the first binding of DNA to the LDH was too fast to be measured we carried out short term kinetic measurements. Labelled DNA was injected to an aqueous LDH-suspension. The assembly for these measuerements is given below.

2

Used materials

LDH: Ceratofix™NA, Südchemie

Solution: H_2O

Model DNA: Herring sperm DNA (2,5 mg ml^{-1} in TE buffer)

DNA-binding dye: Propidium-iodide (50 µg ml^{-1})

Cytometer: Beckmann Coulter Epics XL

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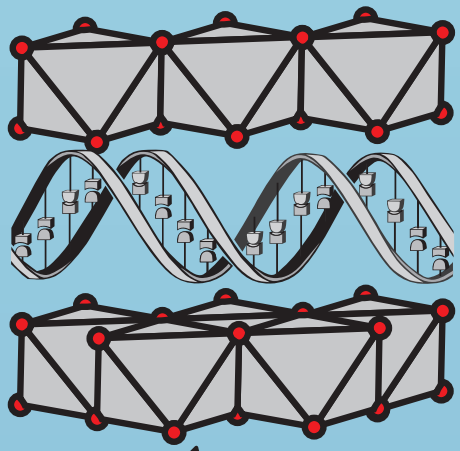

Assembly for short term kinetic measurements

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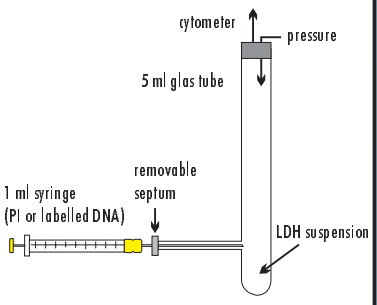

A standard 5 ml glass tube with a sideways small pipe ending with a removable septum was used for the injection of labelled DNA

Adsorption isotherm

DNA removal from aqueous solution

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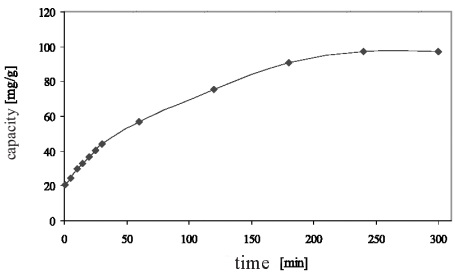
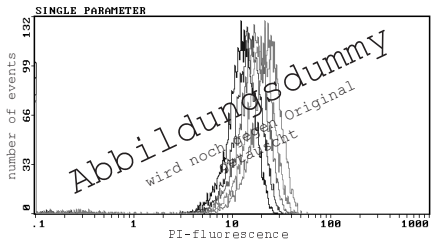


DNA quantification of the supernatant solution by UV-spectroscopy (260 nm)

6

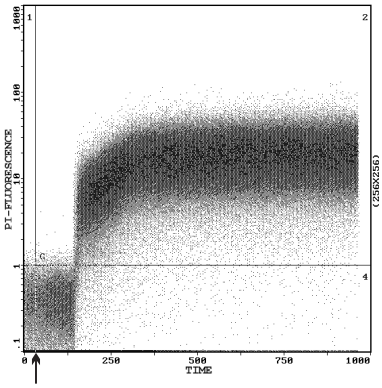
Long term kinetic measurement



Long term kinetic measurements were carried out by labelling the DNA-loaded LDH with PI after the given incubation time.

7

Short term kinetic measurement



Point of injection (10 sec)

Short term kinetic measurements were performed by injecting labelled DNA (500µl) to 1 ml aqueous LDH suspension.

Summary

We were able to show that LDHs are suitable for DNA removal from solutions.

Based on our results we can state that Ceratofix™NA has the highest capacity of 205 mg DNA per g LDH (data not shown).

To reveal the binding kinetics we performed spectroscopical in long term (0-5 h) and flow cytometric studies in long term (0-5 h) and short term (0-240 sec) experiments.

Flow cytometry has proven to be an excellent tool for measuring the bound DNA by fluorescence, directly, fast and reproducable.