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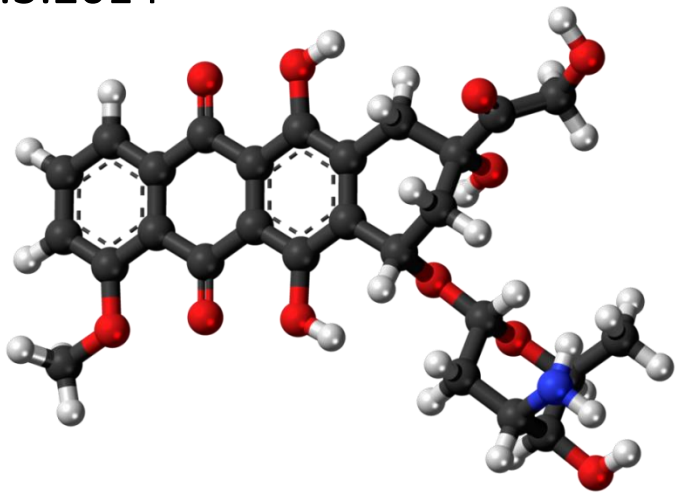
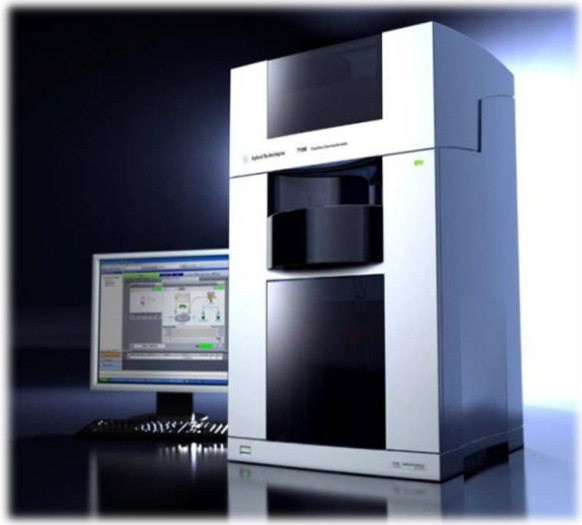
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: **THE ANALYSIS OF DOXORUBICIN AND APOFERRITIN BY CAPILLARY ELECTROPORESIS**

Školitel: Romana Konečná

Datum: 7.3.2014





Reg.č.projektu: CZ.1.07/2.3.00/20.0148

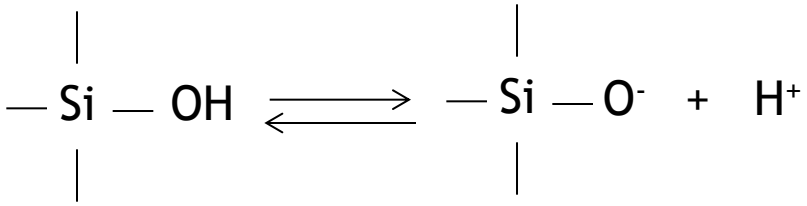
Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

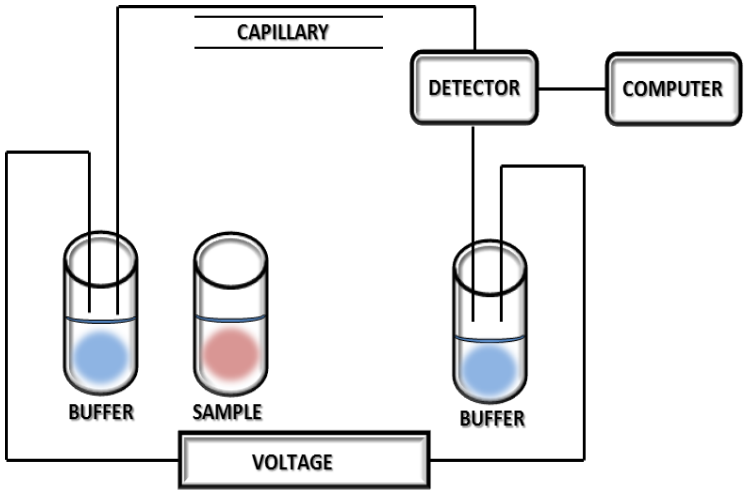
CAPILLARY ELECTROPHORESIS - CE

Electromigration separation method

Electroforetic mobility

$$m = v / E$$

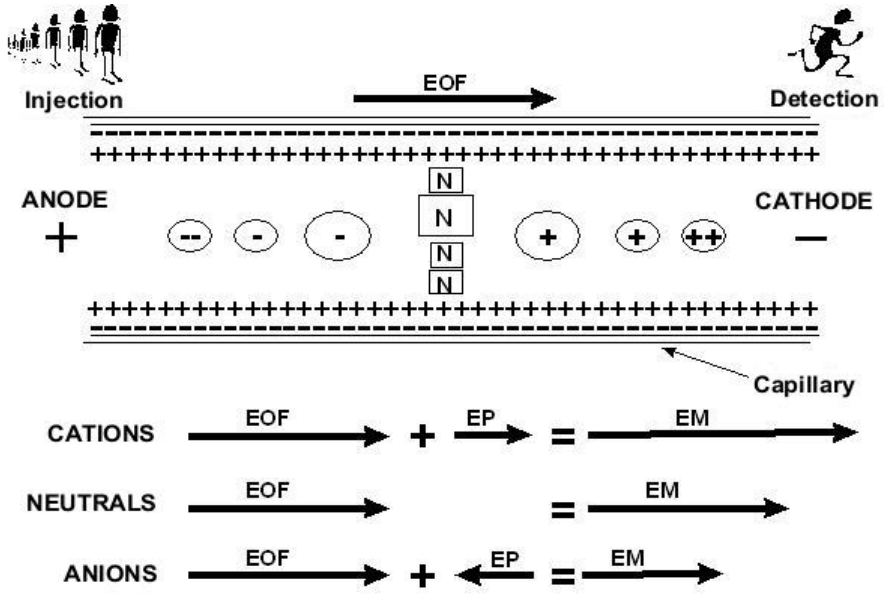




Electroosmotic flow

↑ pH - hight electroosmotic flow

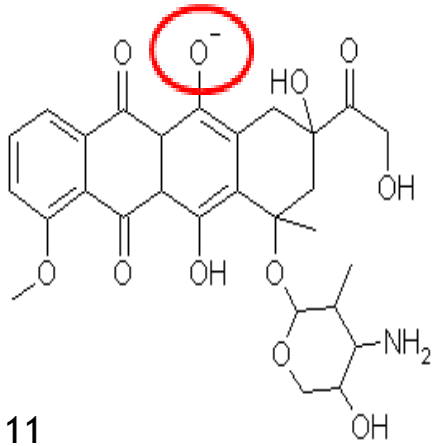
↓ pH - low electroosmotic flow



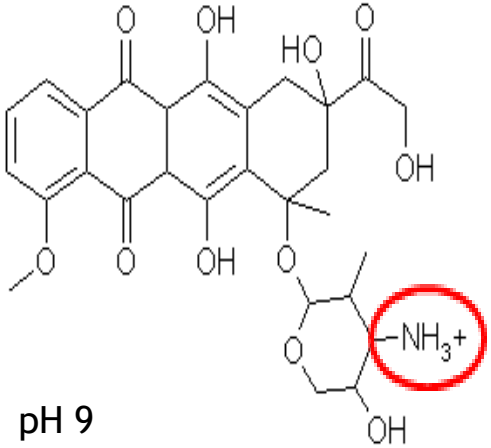
DOXORUBICIN

- $C_{27}H_{29}O_{11}$
- fluorescence properties (ex. 480nm, em. 600nm)
- mol. mass 543.22 g/mol

Structure of DOX in anionic and cationic form

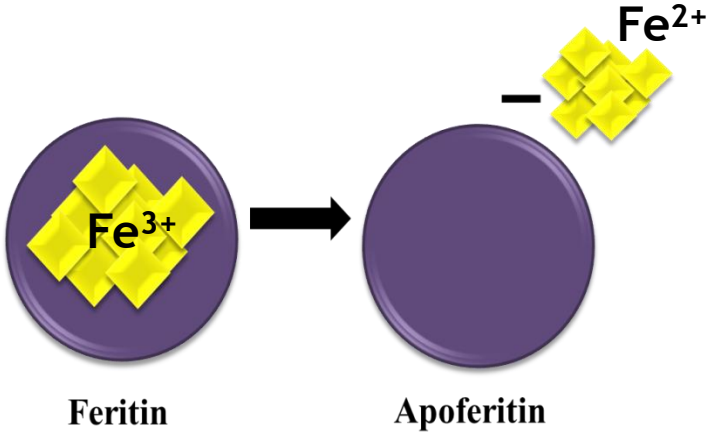


pH 11

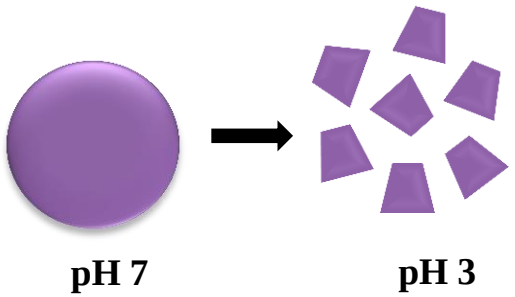


pH 9

APOFERRITIN



Structure of apoferritin depends on the pH



Cumulative dose 550 mg/m^2 of body surface area

- encapsulation DOX to apoferritin = ↓ cumulative dose

AIMS OF THE EXPERIMENT

1. **DOXORUBICIN**
    - **1.1. Characterization of DOX fluorescence in various environments**
    - **1.2. CE-LIF of DOX with pH matching to BGE pH**
    - **1.3. Electrophoretic mobilities of DOX under conditions matching the pH of BGE and DOX zone**
    - **1.4. CE-UV of apoferritin**

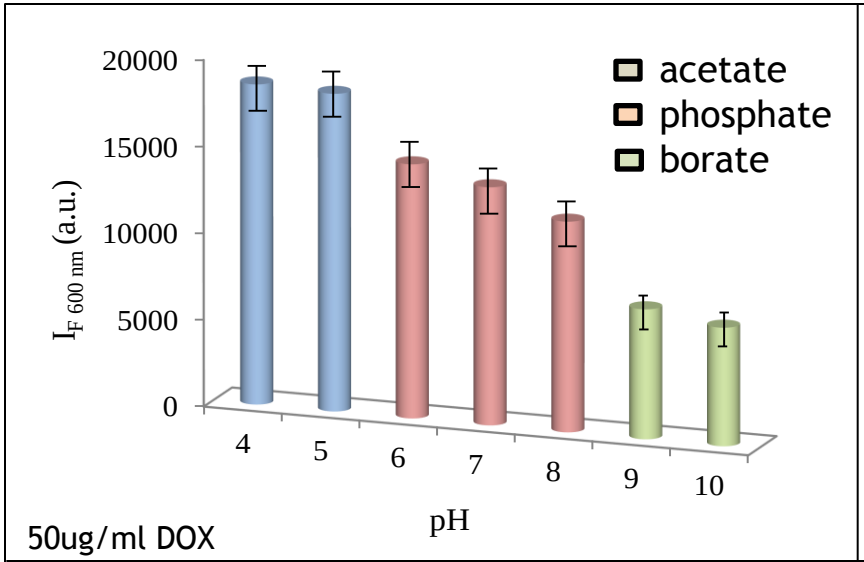
Study of DOX and apoferritin CE method provided us with enough information for subsequent separation of the complex APODOX

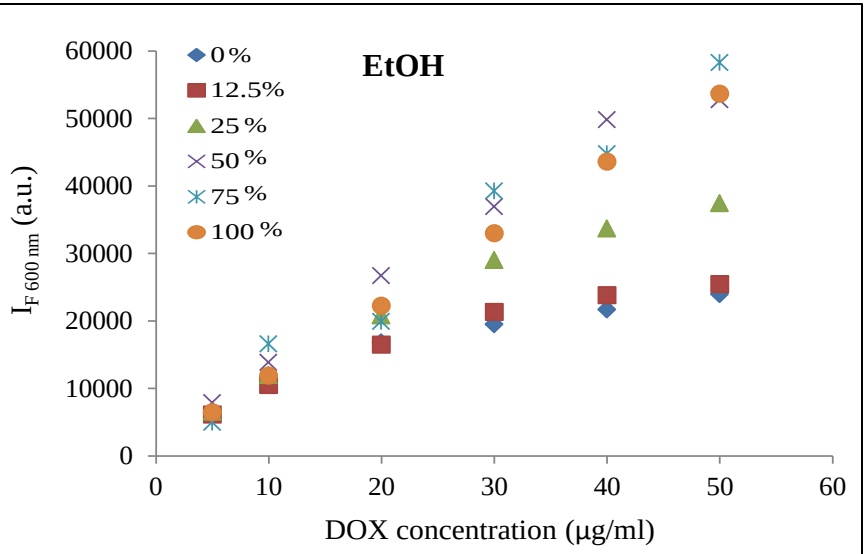
2. **APODOX**
    - **2.1. The apoferritin complex with dox**
    - **2.2. pH triggered DOX release**

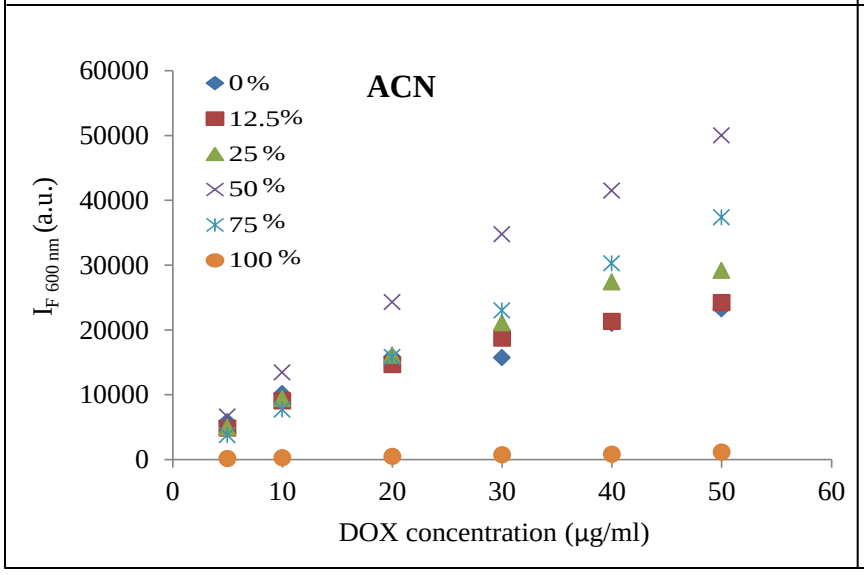
RESULTS

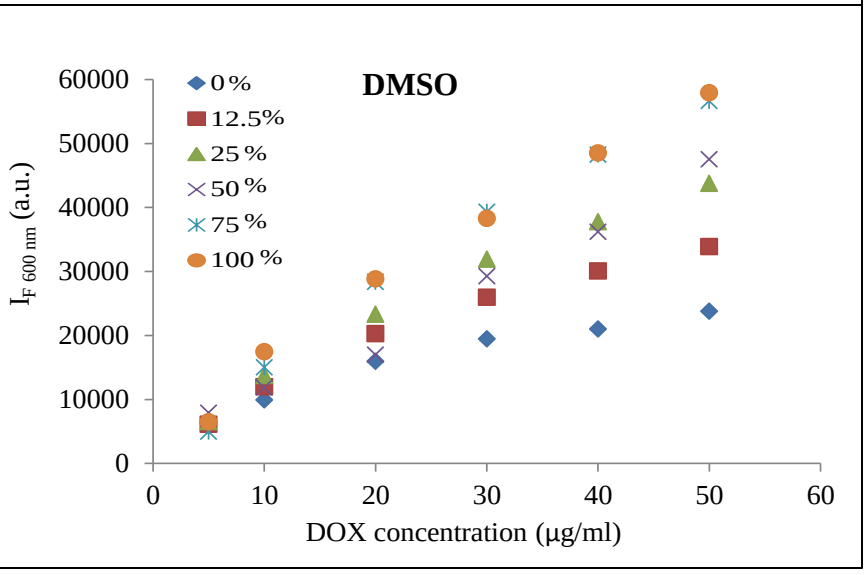
1. DOXORUBICIN

1.1 Characterization of DOX fluorescence in various environments







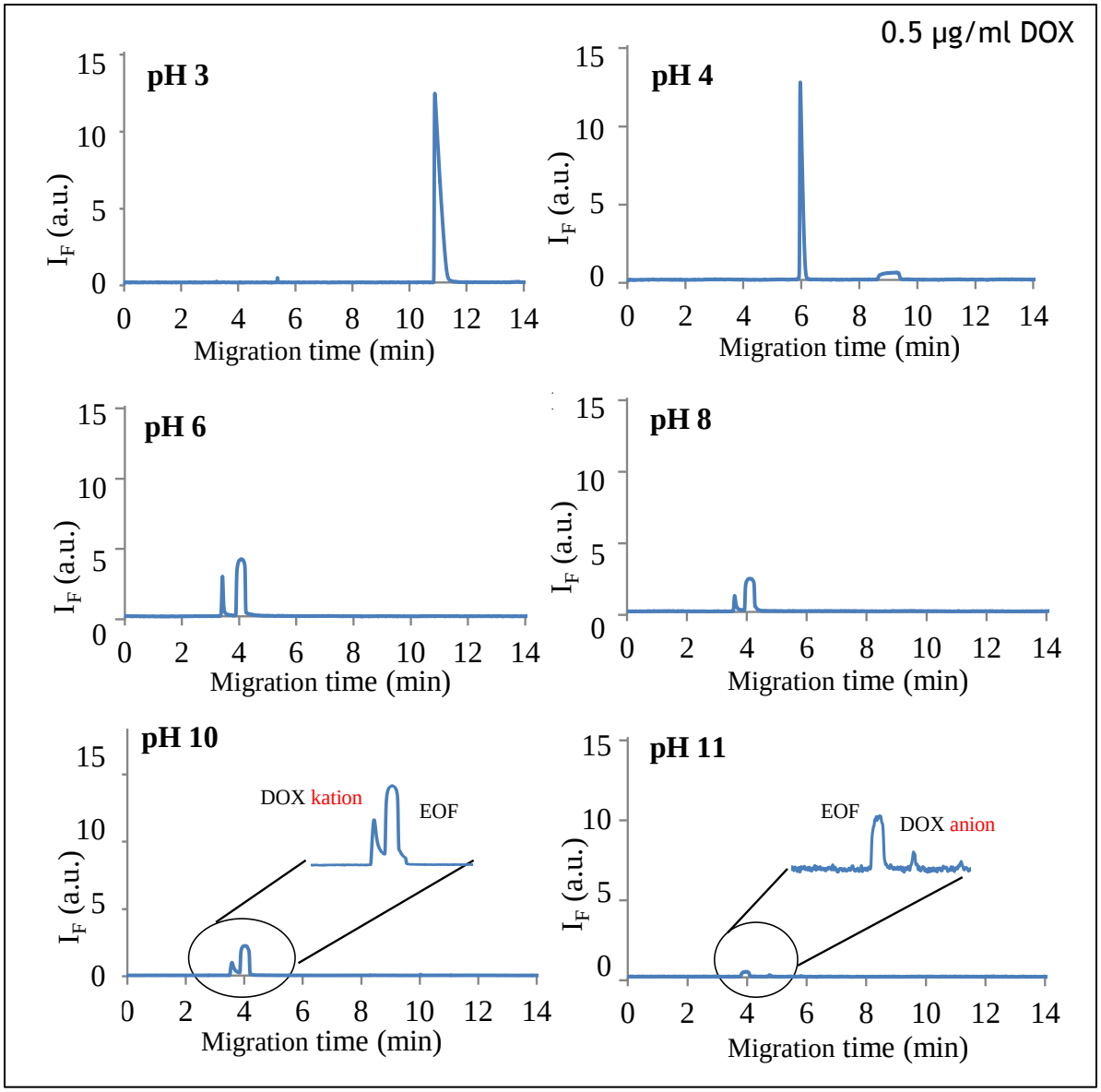


1.2 Separace CE-LIF of DOX with pH matching to buffer pH

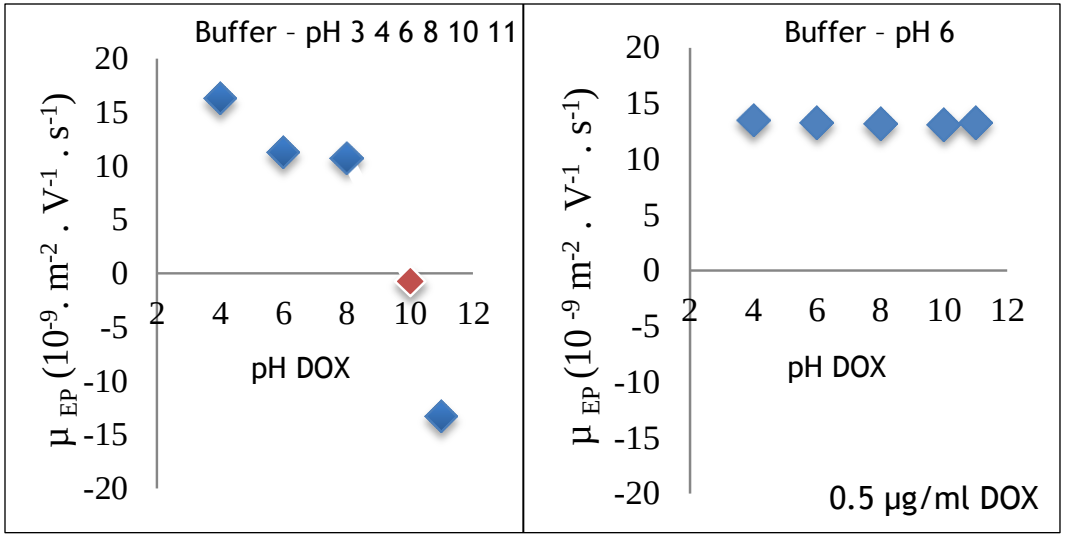
- To determine the ionic form of DOX and its pI, phosphate buffer with pH 3, 4, 6, 8, 10, or 11 was used as a separation electrolyte and coumarine 334 was employed as an EOF marker.

- pH as low as 3 caused very slow EOF and therefore only DOX peak was obtained, however, the increasing pH above this value, peaks of both DOX and coumarine 334 were detected.

- DOX migrated as a cation in the pH up to 10. When the pH was increased to 11, the peak of DOX occurred after the EOF marker, which confirmed the anionic form of DOX.



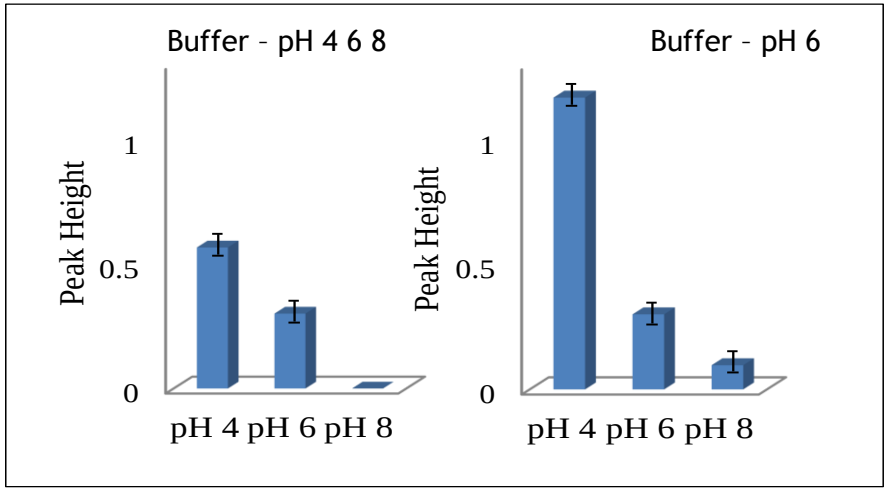
1.3 Electrophoretic mobilities of DOX under conditions matching the pH of BGE and DOX zone



$$\mu_{AP} = \frac{L \times \ell}{t_{mig} \times 60 \times V} \quad (10^{-9} m^2 \cdot V^{-1} \cdot s^{-1})$$

$$\mu_{EOF} = \frac{L \times \ell}{t_{EOF} \times 60 \times V} \quad (10^{-9} m^2 \cdot V^{-1} \cdot s^{-1})$$

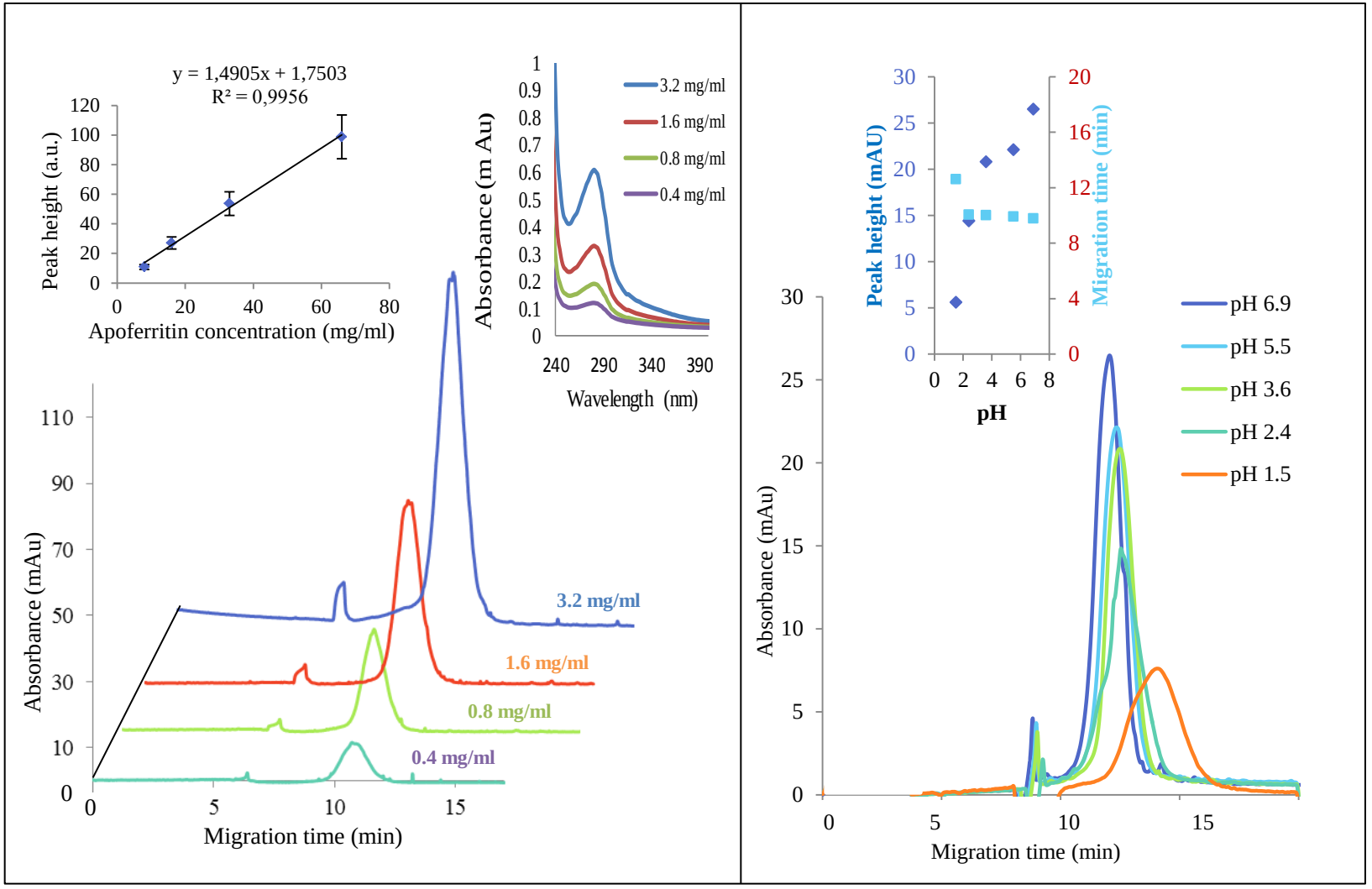
$$\mu_{EP} = \mu_{AP} - \mu_{EOF} \quad (10^{-9} m^2 \cdot V^{-1} \cdot s^{-1})$$



Sample preconcentration

- the pH of BGE was kept constant (6) and the pH of sample zone was 4, 6, and/or 8. In the case of sample zone pH 4, 2-fold increase in DOX peak height was observed.
- The signal of DOX measured in the presence of solution of pH 8 was negligible (when matching the BGE and sample zone pH), however using the described non-matching conditions, the detection of DOX was possible

1.4 CE-UV of apoferritin

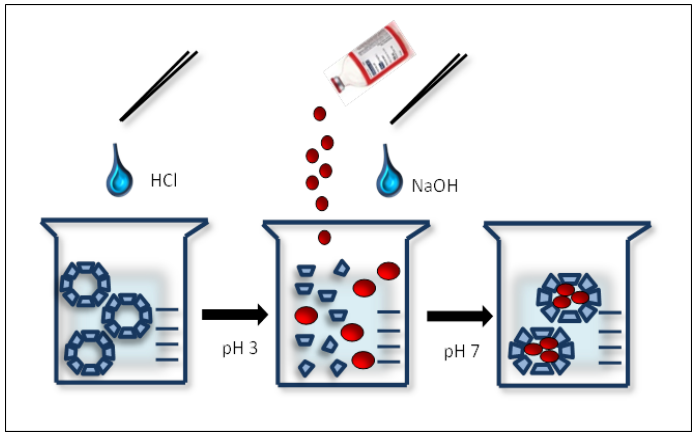


- The pI of apoferritin is 4.4 and therefore under used CE conditions, the protein was in an anionic form and migrated after the EOF
- The peak height of the protein decreased with its pH. On the other hand, the influence of the pH on the migration time was not so significant

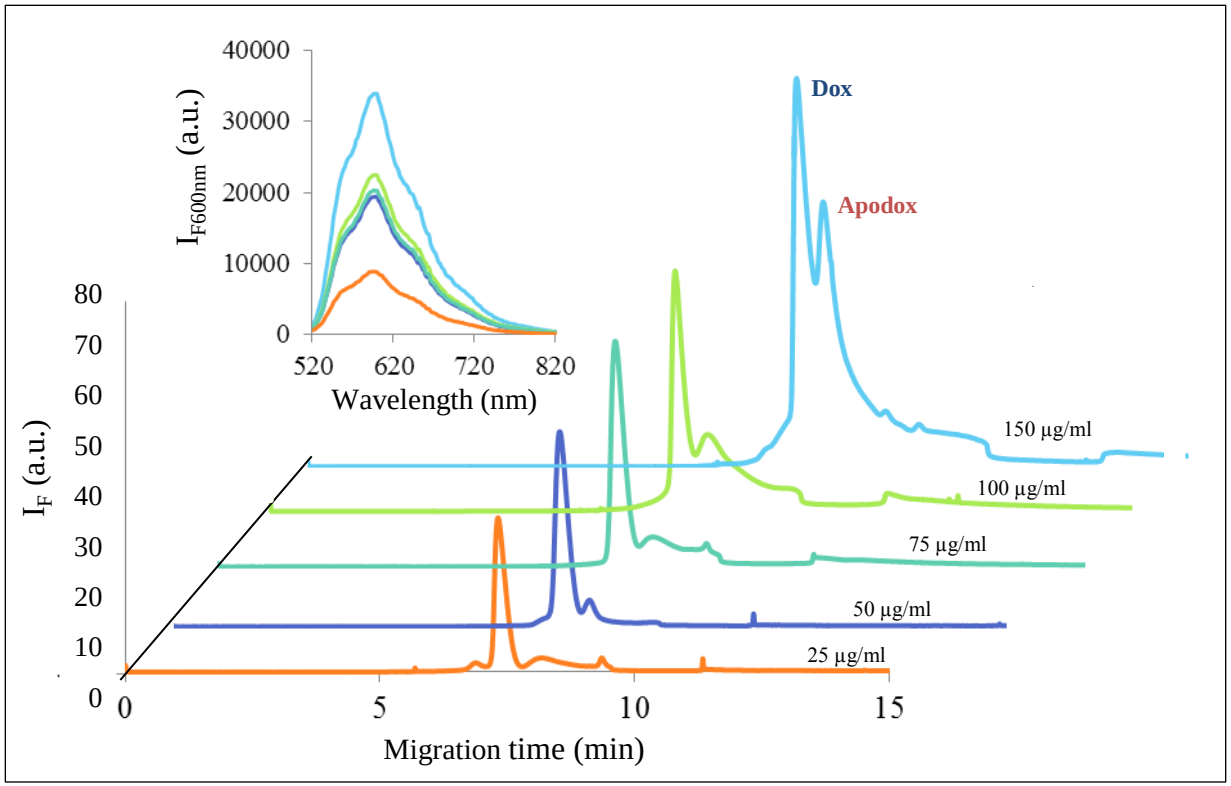
Buffer phosphate, 20mM, pH 7

2. APODOX

2.1 The apoferritin complex with dox



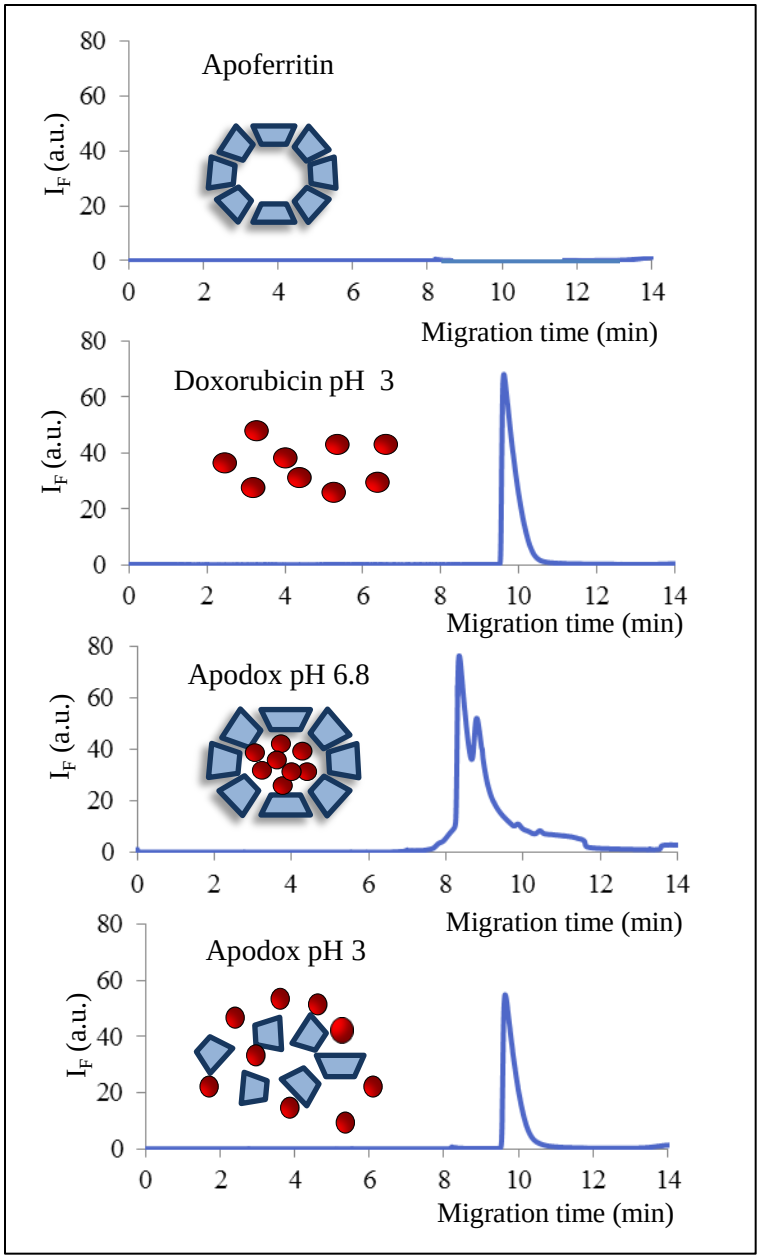
- To determine the encapsulation of DOX into the apoferritin cavity a set of APODOX samples was prepared using a different amount of applied DOX (25 – 150 µg/mL)



- The two major signals were identified. The first peak represents DOX molecule adsorbed on the apoferritin surface and released by electric field. The second peak labeled "APODOX" is attributed to DOX encapsulated into the cavity of apoferritin.

Buffer phosphate, 20mM, pH 7

2.2 pH triggered DOX release



- CE-LIF of apoferritin (1.6 mg/ml)
  and schematics of apoferritin structure
- CE-LIF of DOX (3 µg/ml) at pH 3
  schematics of DOX molecules
- CE-LIF of APODOX at pH 6.8
  scheme of DOX encapsulated in apoferritin
- CE-LIF of APODOX at pH 2
  schematic APODOX dissasembling by low pH

Buffer phosphate, 20mM, pH 7

CONCLUSION

- The fluorescence properties of DOX are strongly dependent on the environment and the increasing pH as well as the presence of water causes the fluorescence quenching

- Encapsulation of the DOX into the protective molecule such as apoferritin decreases its toxicity and therefore broadens its applicability

- CE-LIF has proven to be an effective tool for monitoring of the encapsulation into the apoferritin cavity as well as controlled pH triggered release

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Thank you for your attention!

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