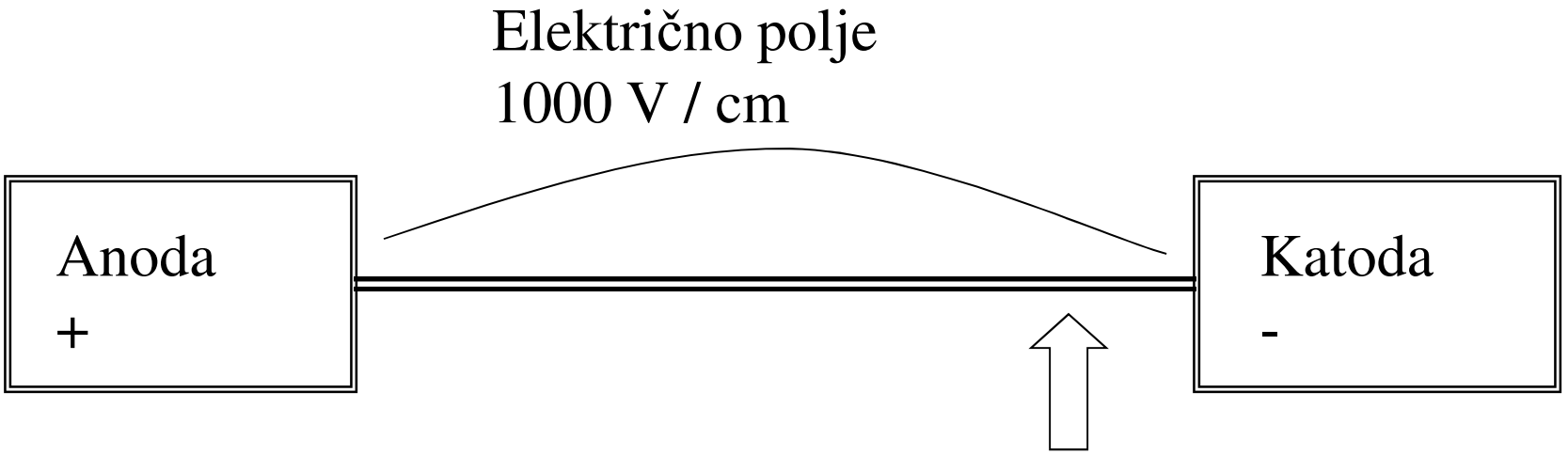
Kapilarna elektroforeza

Shema sistema



$$v = E \mu$$

hitrost = (jakost polja) (elektroforezna mobilnost)

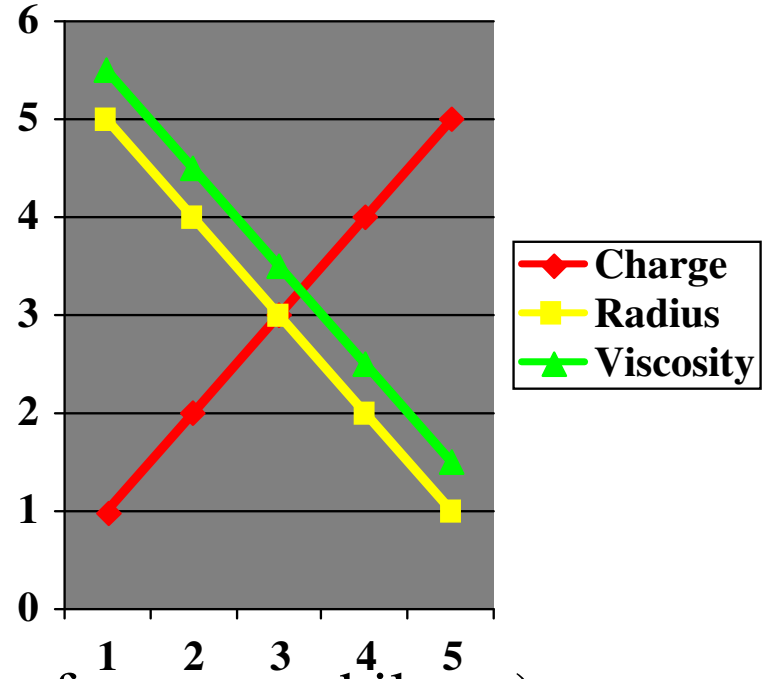
Elektroforezna mobilnost

$$U_{ep} = \frac{Z}{6 \pi n r}$$

Z = naboj analita
n = viskoznost medija
r = Stokesov radij

Vplivi na elektroosmotsko aktivnost

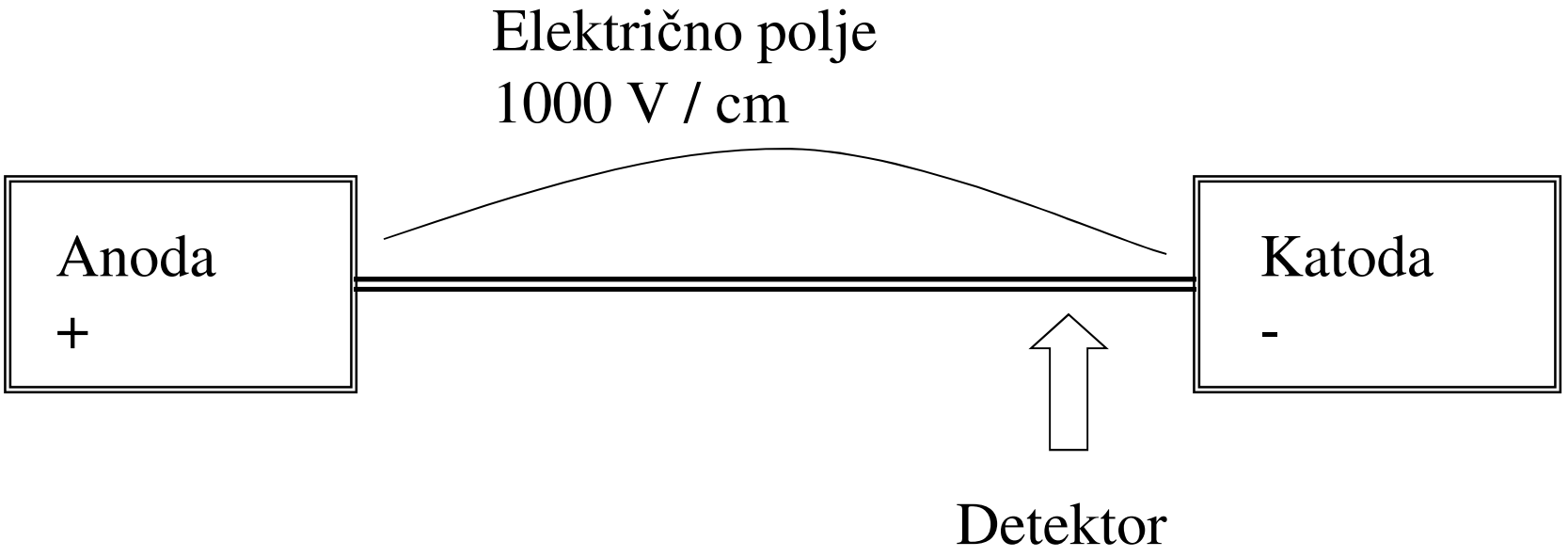
- naboj
- radij*
- viskoznost



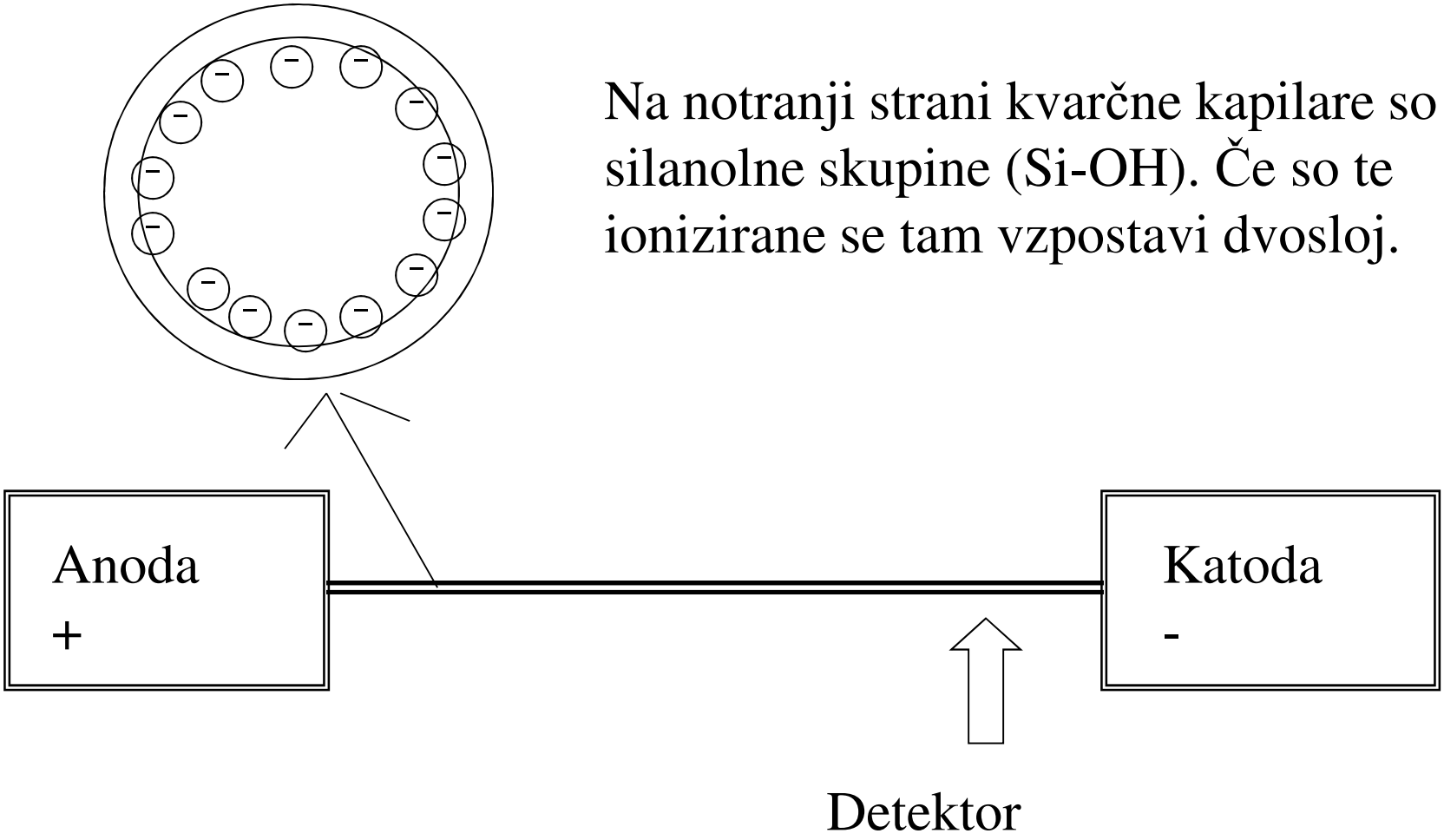
* Stokesov radij je odvisen od molekulske mase. Težje molekule imajo večji radij.

hitrost = (jakost polja) (elektroforezna mobilnost)

Pojav elektroosmotskega toka

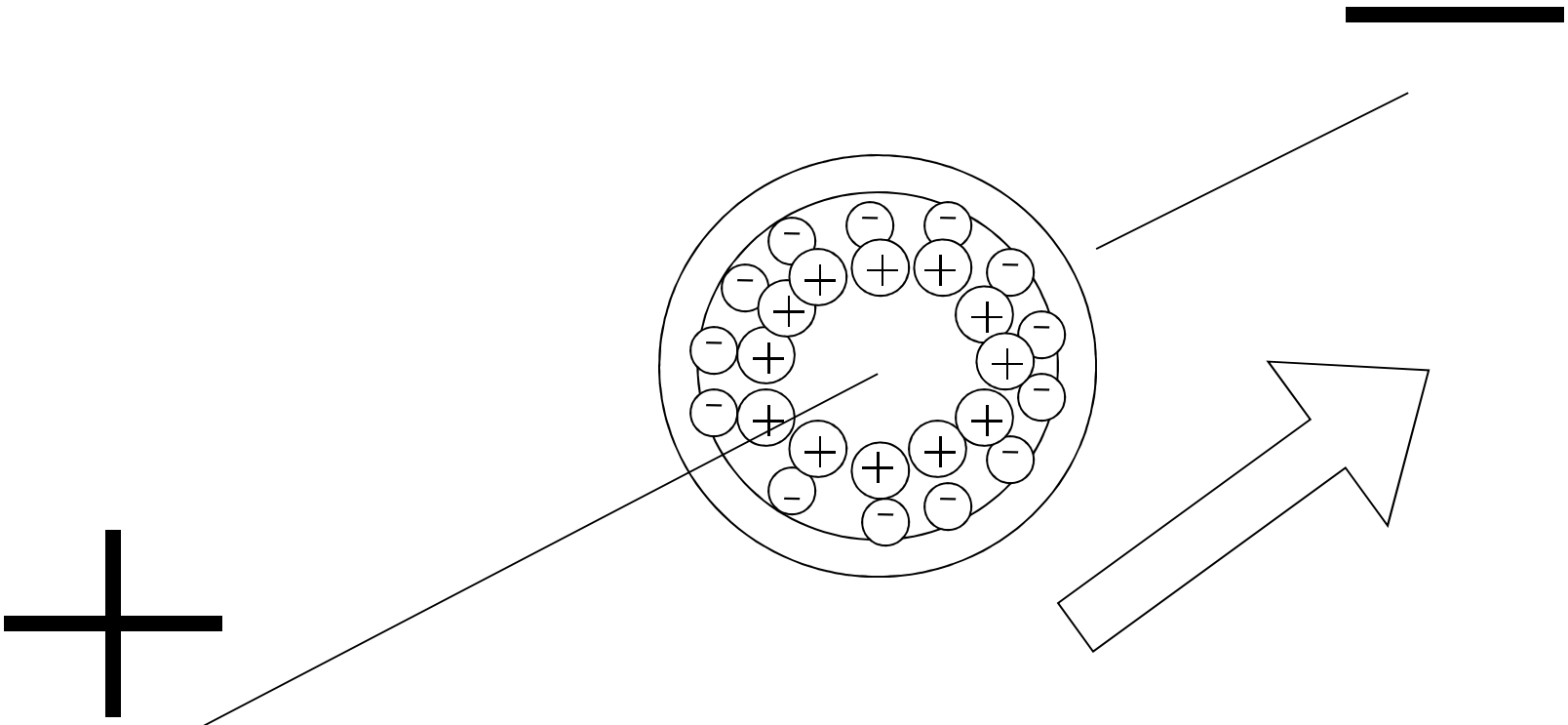


Pojav elektroosmotskega toka



Na notranji strani kvarčne kapilare so silanolne skupine (Si-OH). Če so te ionizirane se tam vzpostavi dvosloj.

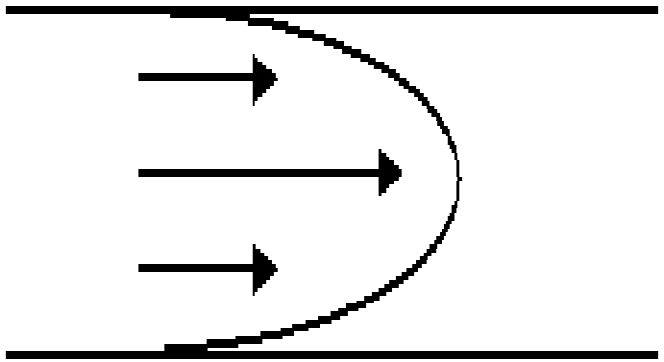
Pojav elektroosmotskega toka



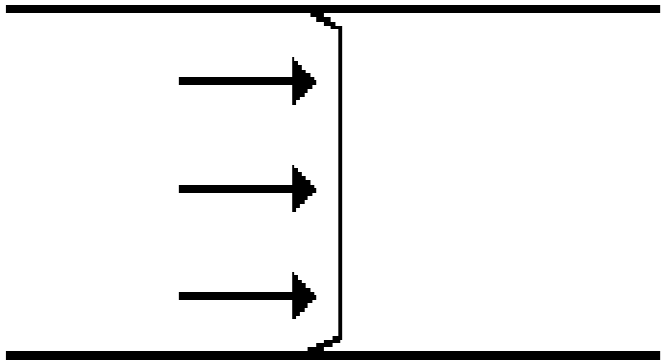
Ko priklopimo iztosmerno napetost, se pozitivno nabiti hidratizirani ioni pričnejo gibati proti katodi in s tem uztvarijo elektroosmotski pretok (cm/min).

Razlika v profilu toka med CE in HPLC

- **Profil EOF se razlikuje od tistega pri HPLC**
- **Pri pretoku, ki ga ustvarja nadtlak (HPLC) se zaradi trenja pretok on stenah upočasni.**
- **Pri EOF se tok ustvarja ob stenah zato sedaj dobimo laminaren tok.**

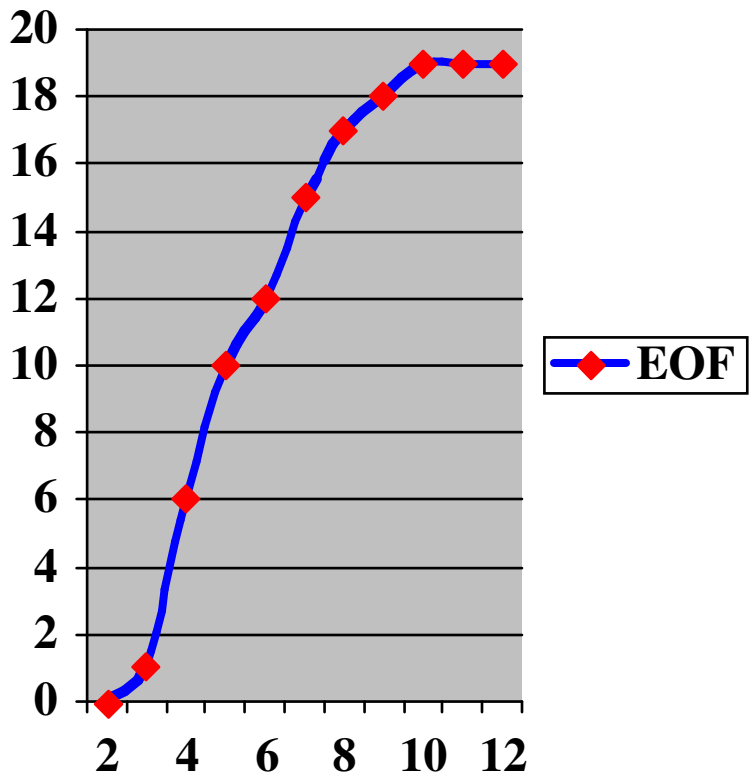


Parabolic or laminar flow



Plug flow

Vpliv pH na hitrost EOF.

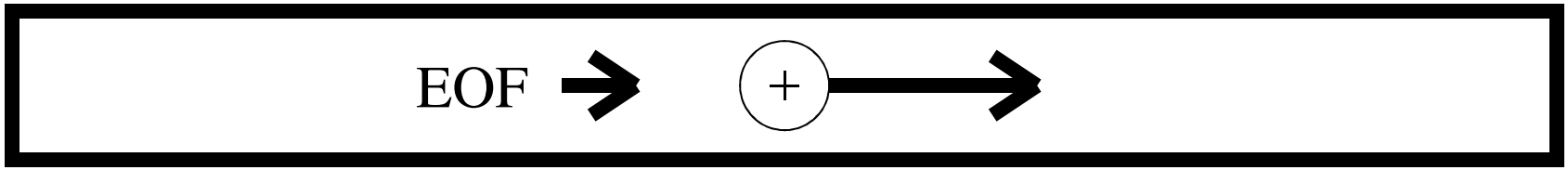


- Nizek pH, malo ioniziranih silanolnih skupin, počasen EOF.
- Z višanjem pH se poveča število ioniziranih silanolnih mest. EOF se povečuje.
- Pri visokem pH je ionizacija popolna in ni več vpliva na EOF.

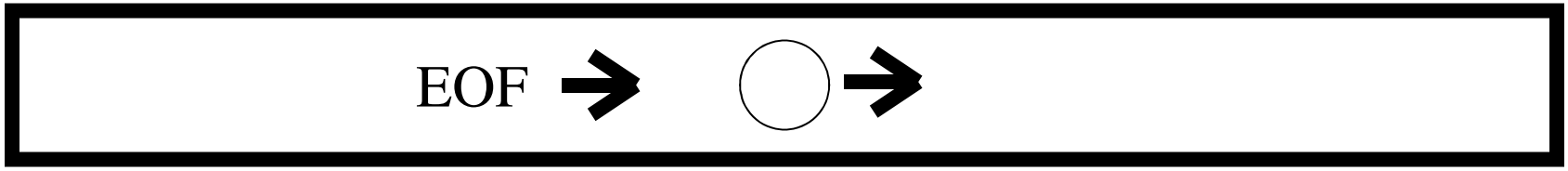
Skupna hitrost analita (u) je vsota elektroforetske in elektroosmotske hitrosti

$$u = (U_{ep} + U_{eo}) \boldsymbol{E}$$

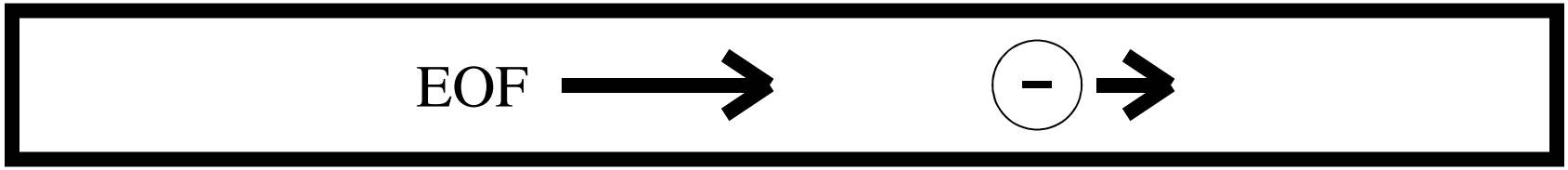
Kako se giba analit pri CE?



Analit s pozitivnim nabojem se giblje hitreje kot EOF



Nevtralen analit se giblje z isto hitrostjo kot EOF.
(To je način merjenja EOF.)

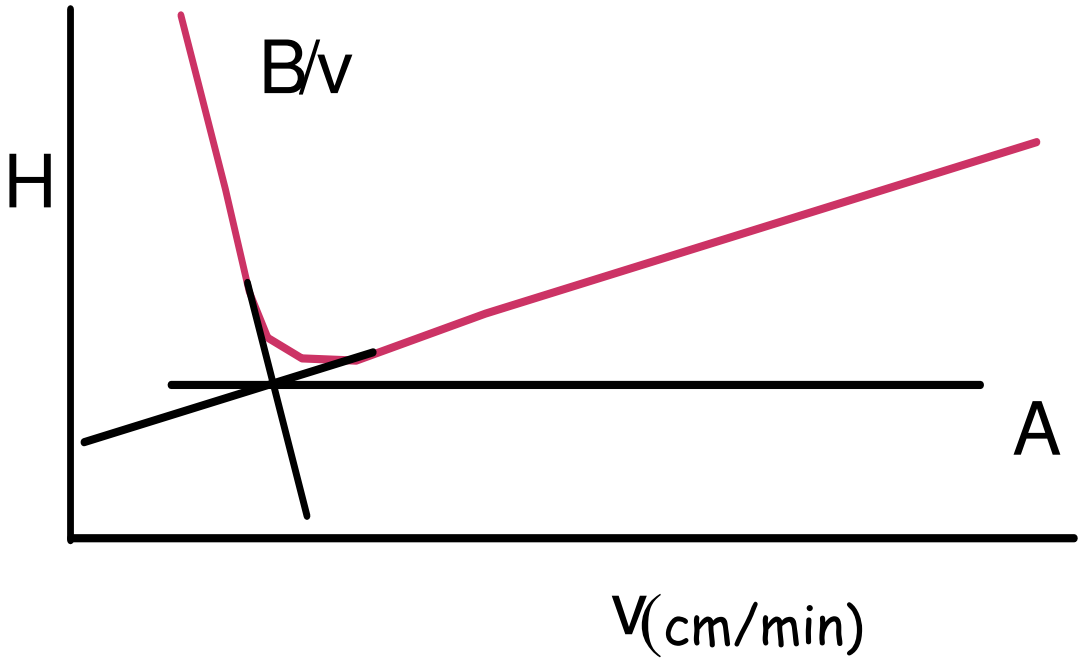


Analit z negativnim nabojem se giblje počasneje kot EOF

Širjenje kromatografskih vrhov

Uporaba mobilne faze pri optimalni hitrosti

$$H = A + B/v + C.v$$



Število teoretičnih prekatov

$$N = \frac{\mu U}{2 D}$$

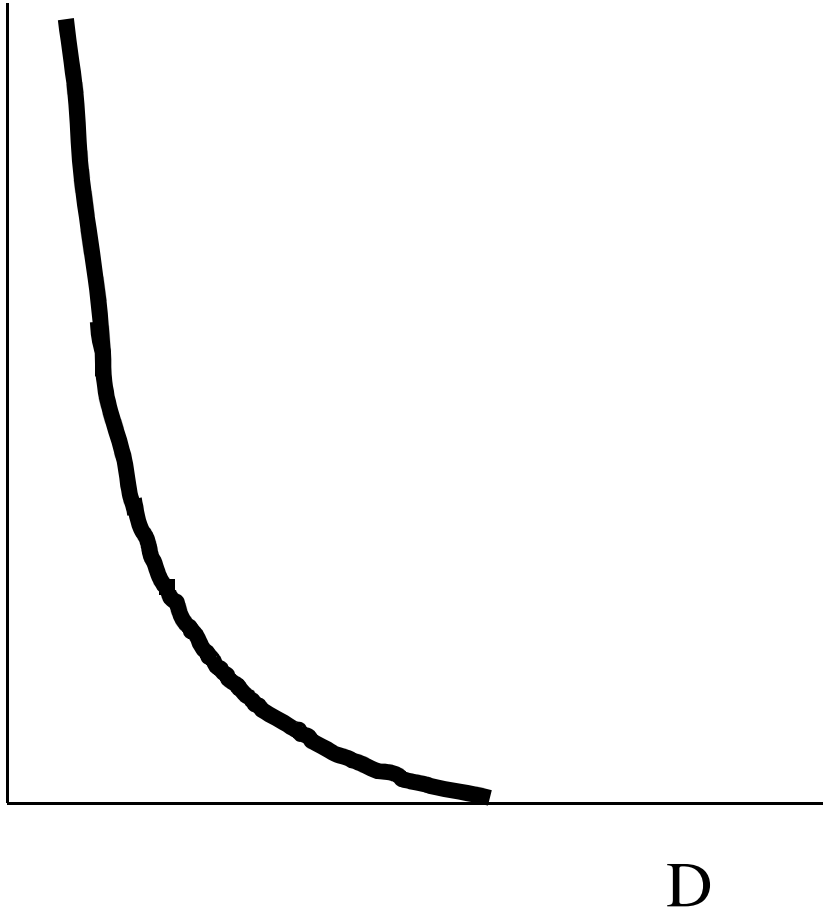
D- difuzijski koeficient

μ - mobilnost

U - napetost

Učinkovitost separacije (Y) and difuzijski koeficient (X)

- Hiter padec separacijske učinkovitosti pri povečanju difuzijskega koeficienta
- Včasih ni prednosti v primerjavi s klasično HPLC



Injeciranje

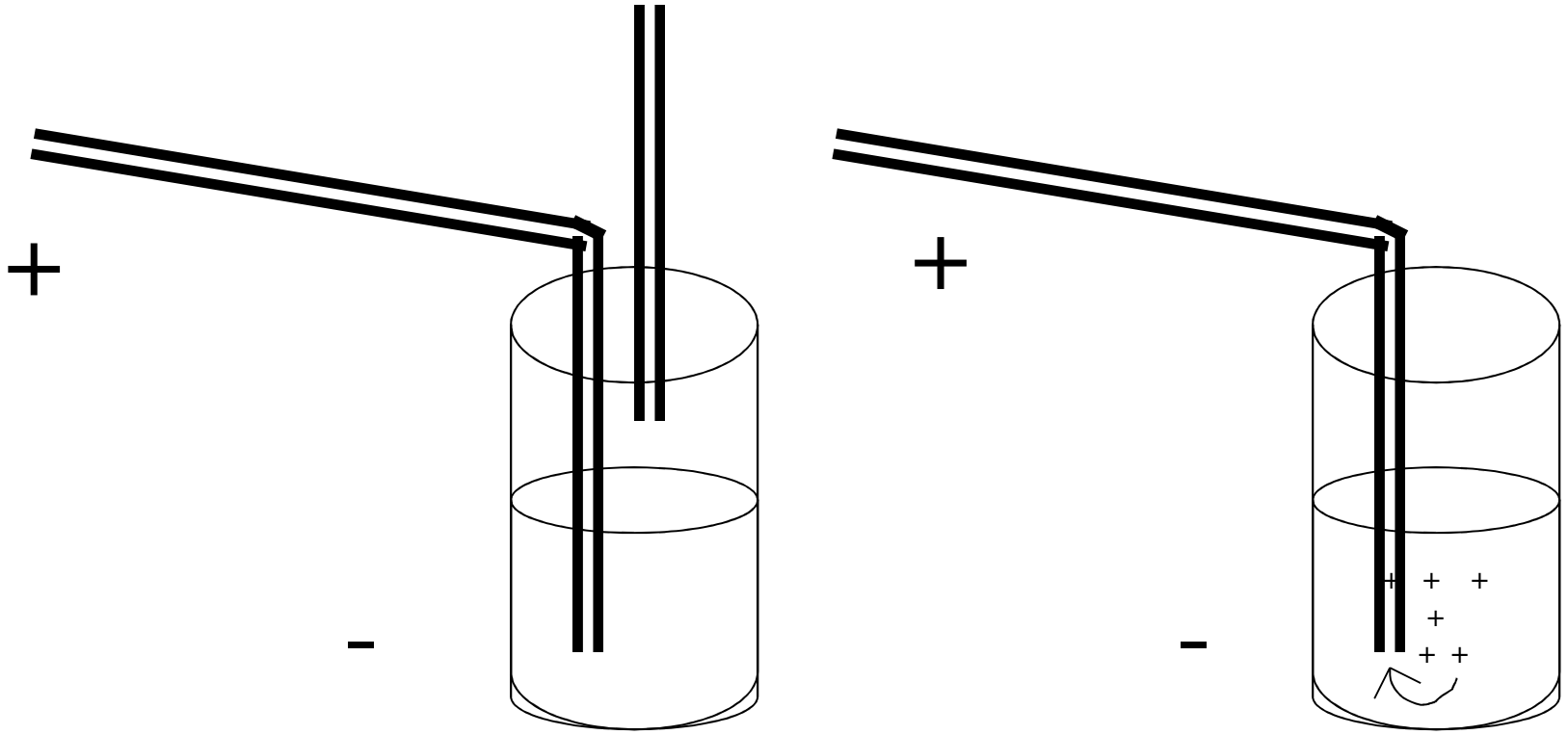
There are two principle methods:

- Pressure differential works by applying a pressure across the capillary while it it is dipping into the sample solution.
- Electrokinetic injection works by applying a voltage and allowing ions to migrate into the capillary because of their charge.

Injection volumes are typically very small:

- Typically if injection volumes exceed 1% of the column volume, separation efficiency severely suffers.
- Sample volume can be increased by focusing the ions inside the capillary. This technique uses a combination of additives to the medium and selectively applied charges.

Tlačno in elektrokinetično injeciranje



- Pri elektrokinetičnem injeciranju lahko selektivno predkoncentriramo vzorec.

Prednosti CE so:

- Večje število teoretičnih prekatov.
- Ni masnega prenosa analita na stacionarno fazo, zato je širjenje vrhov manjše
- S spreminjanjem pogojev na koloni lahko predkoncentriramo ali fokusiramo analit.

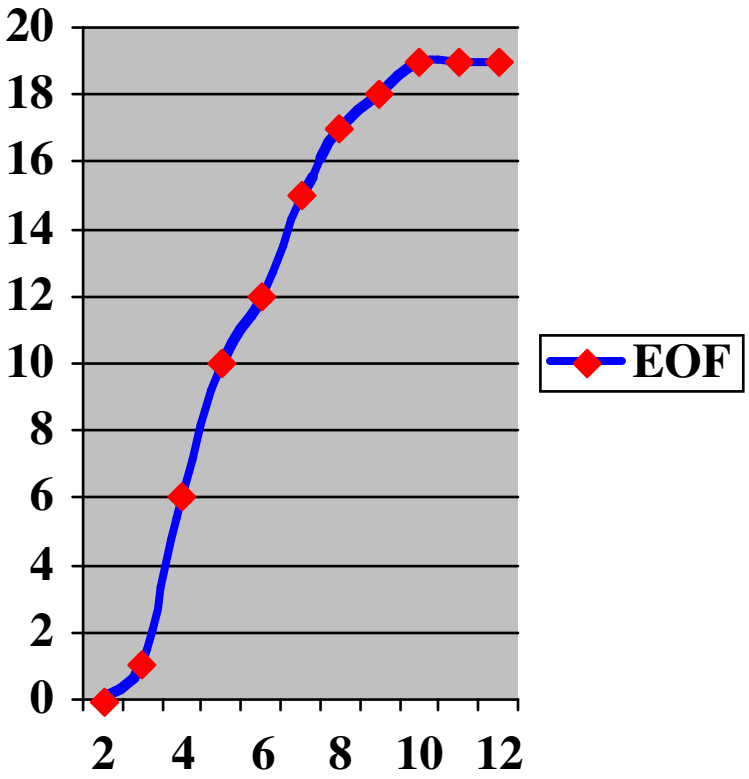
Obtaining Reproducible (Good) Results

- Column condition.
- Composition and pH of the medium.
- Viscosity of the medium.
- Operating temperature.

- Adequate sample volume.

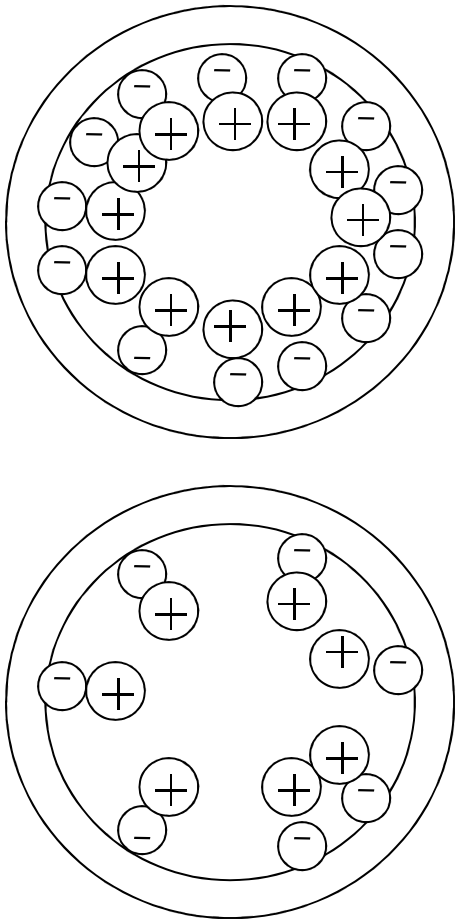
- Use of internal standards.

The pH must be tightly controlled to obtain reproducible EOF flow.



- Remember that the percentage of silanols that are ionized is dependent on the pH.

Column Condition



- As time goes on, certain molecules will block or otherwise neutralize the ionized silanol sites. This will change the EOF and alter retention times.
- It is also very important to condition the column properly before use. Follow the directions in the published method.

Internal Standards

- The main advantage of an internal standard is that it is subject to the same conditions as the analyte.

Detektorji

- UV/VIS
- Detektor na diodni niz
- Fluorescenčni
- MS

Aplikacije

- Določevanje molekul, ki niso primerne za HPLC.
- Kiralna separacija enantiomer.
- Določevanje zdravil v človeških tekočinah.
- Separacija bakterij

Kapilarna elektrokromatografija

- Kombinacija obeh tehnik
  - Kapilarna elektrokromatografija s polnjenimi kolonami
  - Micelarna elektrokromatografija