ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
Εθνικόν και Καποδιστριακόν
Πανεπιστήμιον Αθηνών

Έλεγχος και Διασφάλιση Ποιότητας

Ενότητα 6: Qualification of Capillary Electrophoresis Instrument

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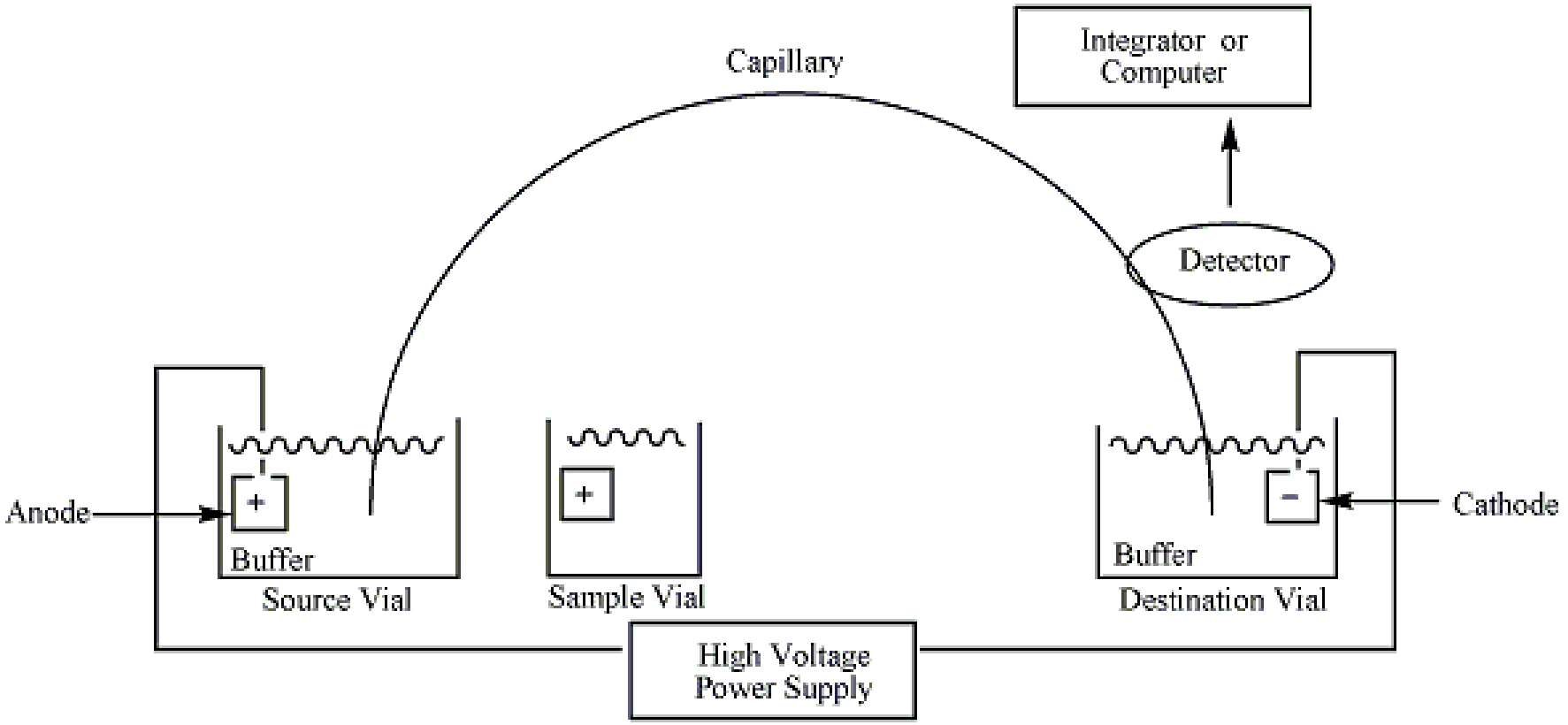
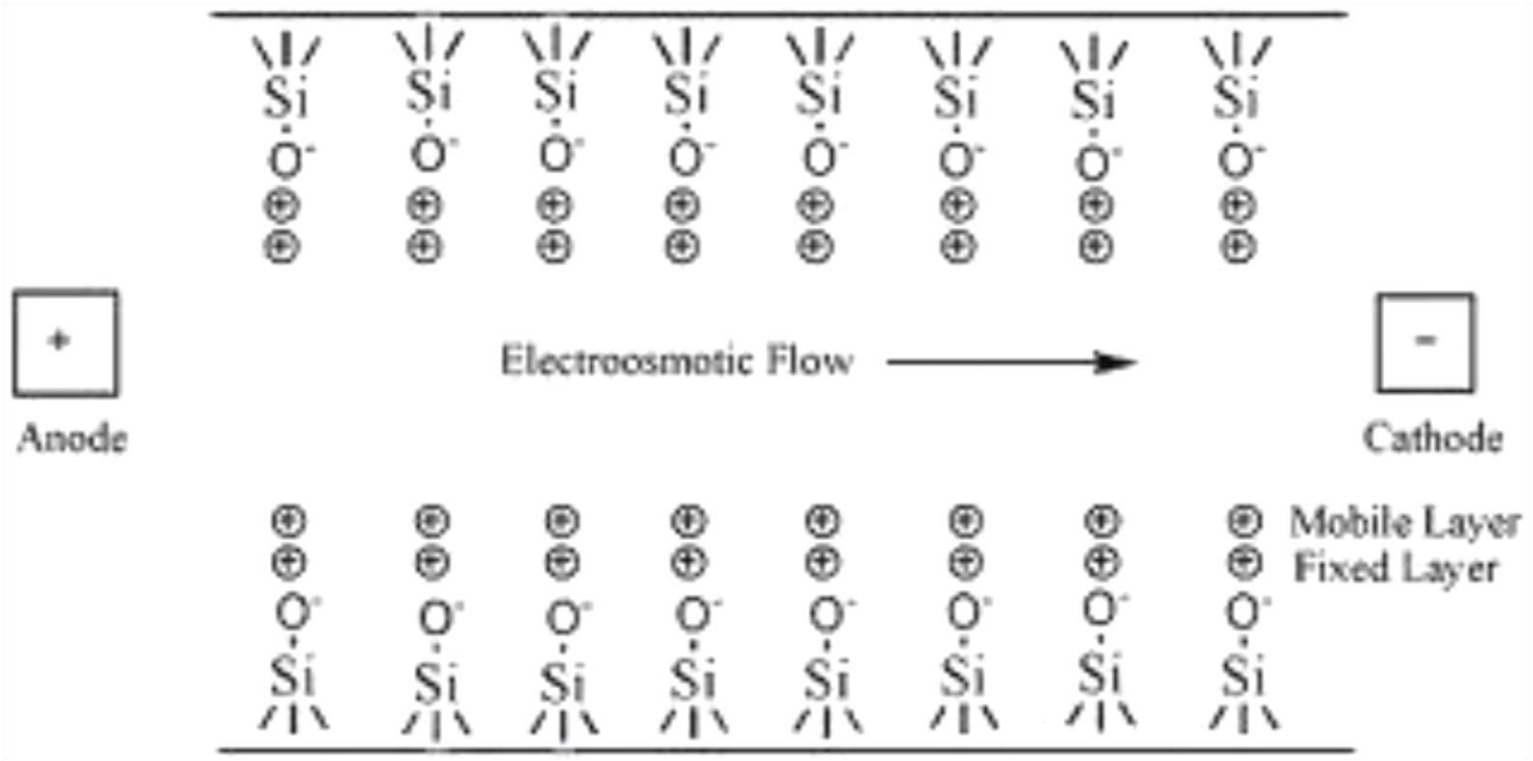
Τμήμα Χημείας

Εργαστήριο Αναλυτικής Χημείας

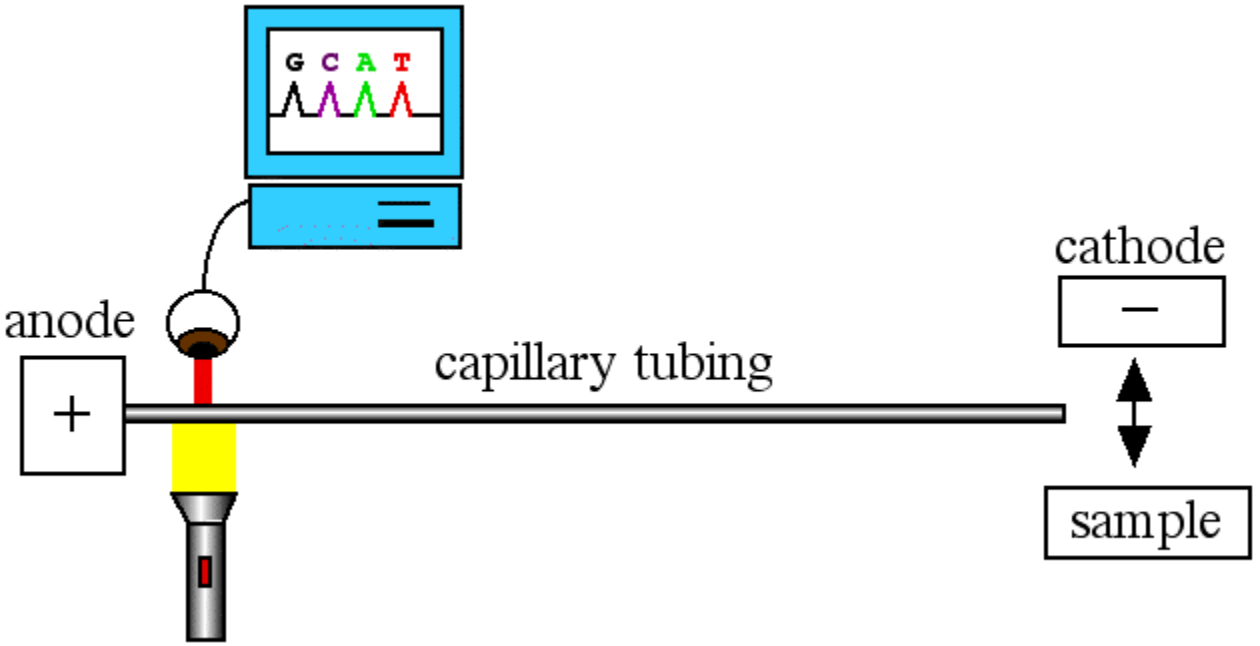
Principle of Capillary Electrophoresis
Αρχή Τριχοειδούς Ηλεκτροφόρησης



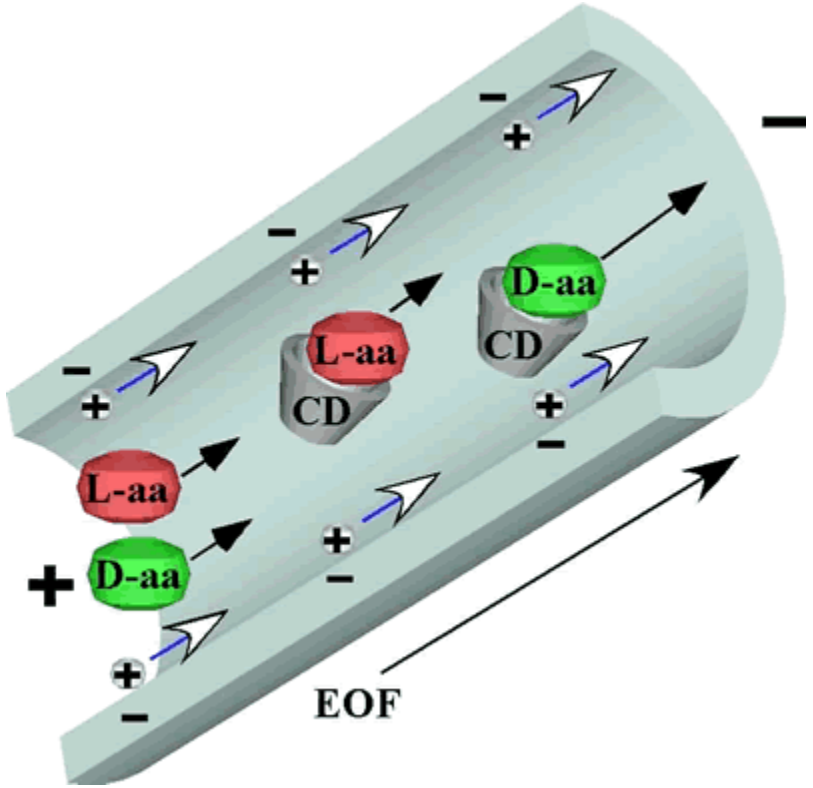
Internal Structure of Capillary
Εσωτερική Δομή Τριχοειδούς



Capillary Electrophoresis (CE)
Τριχοειδής Ηλεκτροφόρηση



Species Movement in CE
Τριχοειδής Ηλεκτροφόρηση-Κίνηση Σωματιδίων



Parameters to be tested in Qualification of CE Instrument

- Temperature module
  - Stability
  - Accuracy
  - Cooling system
- Voltage module
  - Stability
  - Accuracy
- Detector module
  - Noise (Drift)
  - Wavelength accuracy
  - Linearity
- Injection module
  - Accuracy (hydrodynamically)
  - Linearity
  - Precision

Temperature Module (1)

- The correct thermostating of CE is essential for reproducible results of migration time and peak area.
- The generation of heat inside the capillary is unavoidable in electrodriven separations depending on the applied voltage and the resulting electric current.
- This heat (Joule heating) results in an increase of the buffer temperature causing a radial temperature profile and can also include perturbations to the electro-osmotic velocity profile.

Temperature Module (2)

- Both effects lead to peak broadening.
- The viscosity of the electrolyte and the stability of solutions and samples are also dependent on the temperature.
- In turn, the stability of migration times and peak areas is affected.
- Therefore stability and accuracy of the temperature have to be tested in an OQ procedure.

Temperature Module (3)

- Additionally, the thermostating system should be tested to ensure heat transport away from the surface area of the capillary.
- Temperature stability can be easily measured if the used CE software comes with the possibility to record the temperature profile during a separation run.

Temperature Module (4)

- Otherwise, especially for older CE instruments, an external thermometer with a record function is required to ensure a relatively high measurement frequency (>1 Hz).
- An external thermometer is always required to assess accuracy.
- The measurement with an external thermometer can be difficult to implement in CE systems which use a liquid cooling system.

Temperature Module (5)

- Stability parameter
  - During a run without sample injection the temperature is recorded over 10 min testing at a low, a high and an often used value
  - Acceptance criterion: ± 0.1 ^{o}C
- Accuracy parameter
  - During the temperature stability testing an external thermometer measures the temperature in the thermostated area.
  - Acceptance criterion: ± 2 ^{o}C

Temperature Module (6)

- Cooling parameter
  - Depending on the capillary length a buffer and an adequate voltage is chosen.
  - On the basis of the arising current and the resultant power per unit length (W/m) one can decide whether the cooling system works sufficiently on not.
  - Acceptance criterion:
    - > 0.6 m (buffer 0.1 M phosphate pH 7.0, 30 kV/m) →3 W/m
    - <0.6 m (buffer 0.05 M phosphate buffer pH 7.0, 50 kV) →5 W/m

Temperature Module (7)

- In order to get reproducible electropherograms it is essential that the temperature is kept constant during separation.
- It is less important to accurately adjust the temperature to a certain value. Therefore the acceptance criterion for stability is set tight at ± 0.1 ^{o}C.
- On the contrary for temperature accuracy it is set at a wider interval of ± 2 ^{o}C.

Voltage Module (1)

- The voltage is a central parameter in CE.
- Hence, the stability and the accuracy have to be tested
  - They are directly responsible for the reproducibility of peak migration times.
- The stability of the voltage is tested during a run without sample injection (provided that voltage data during separation can be recorded) over 10 min testing at a high and an often used value.
  - Acceptance criterion: ± 0.5%

Voltage Module (2)

- The direct accuracy measurement of the voltage proves to be difficult as the electrodes are not easily accessible for an independent voltage measurement during a separation.
- The voltage accuracy can be more easily obtained indirectly using effective mobilities of the sample analytes.

Voltage Module (3)

- The following equation presents the relationship between the effective mobility (μ_{eff}) and the applied voltage (V).
- Total length (L), effective length (l), migration times of an EOF marker (t_{EOF}) and one of the sample peaks (t_s) are required to calculate the effective mobility.

$$\mu_{eff} = \mu_{app} - \mu_{eof} = \frac{L \times I \times \left(t_{eof} - t_s\right)}{V \times t_{eof} \times t_s}$$

Voltage Module (4)

- The EOF is measured with the widely used EOF marker acetanilide.
- A sample containing acetanilide, 3,5-dihydroxide benzoic acid and nicotinic acid is separated.
- The effective mobilities are determined and compared to a reference.
- Acceptance criterion ± 4%.

Detector Module (1)

- The well-established concept for HPLC detectors can be largely employed for CE systems, with wider acceptance criteria due to the shorter optical path length.
- Noise parameter
  - During a run without sample injection the absorbance is recorded over 10 min testing at an often used value and the standard deviation of the absorbance is assessed.
  - Acceptance criterion: $< 5x10^{-5}$ AU

Detector Module (2)

- Wavelength Accuracy parameter
  - The distinctive spectra (in the area less than 300 nm) of three sample substances (acetaminophen, 3,5-dihydroxybenzoic acid and nicotinic acid) are measured and evaluated by comparison with reference spectra.
  - Acceptance criterion: ± 3 nm
  - The CE systems use also as internal wavelength calibration a holmium oxide filter comparing the maxima in the region of 440-465 nm.

Detector Module (3)

- Linearity parameter
  - Using different concentrations of the three sample substances (0.1 – $5x10^{-3}$ mol/L) a linear regression is modelled between peak areas and concentration determining the coefficient of determination as criterion.
  - Acceptance criterion: $R^2 > 0.99$
  - A plot of residuals should also computed beside the linear regression, in order to properly recognize non-linearity and trends.

Injection Module (1)

- The common mode of injection in CE is the hydrodynamic, because of the higher repeatability (in comparison with the electrokinetic mode).
- Accuracy (hydrodynamically) parameter
  - Four weighted vials are filled with water and weighted again. One hundred injections of 50 mbar over 1 min from one vial into another vial at 20 ^{o}C are performed.
  - In the end all vials were weighted and two vials are used to compute the evaporation.
  - The injected amount is computed according to the Hagen – Poiseuille equation.

Injection module (2)

- The volumetric flow rate (V_f) is calculated by the volume (V) and the time flowing through a cylindrical tube and depends on the internal radius (r) of the tube, the pressure difference (Δp) between the two tube ends, the viscosity (η) of the streaming solution and the total length (l) of the tube.
- For the test bi-distilled water is pressed through the capillary at a frequently used pressure of 50 mbar thermostated at 20 ^{o}C and collected in the outer vial. In this case the viscosity of water is 1.008 mPa s.

$$V_f = \frac{dV}{dt} = \frac{\pi \times r^2 \times \Delta p}{8 \times \eta \times l}$$

Injection module (3)

- Acceptance criterion: ± 25%
- For example, the injected amount should be 7.7 mg ± 25% for a capillary length of 0.6 m injected 100 times for 1 min with 50 mbar.
- The weight of an inlet vial corrected for the evaporation should be reduced by this amount.

Injection module (4)

- Linearity parameter
  - Using different injection amounts (100 -500 mbar.s) a linear regression is modelled between the peak area and the product of time and pressure determining the coefficient of determination as criterion.
  - Acceptance criterion: $R^2 > 0.99$

Injection module (5)

- Precision parameter
  - The RSDs of peak areas and migration times are obtained from six consecutive runs of one sample.
  - Acceptance criterion
    - Migration time RSD < 1.5%
    - Peak area RSD < 2%

Holistic approach for PQ (1)

- Performing a run without injection, temperature stability and accuracy, as well as voltage stability and noise can be evaluated at the same time.
- Injection linearity and precision can be tested together using one injection setting of the linearity test for the precision test.
- Then three measurements of the linearity test and three additional ones with the same setting evaluated for the test of injection precision.

Holistic approach for PQ (2)

- Furthermore the wavelength accuracy can be obtained from the test of every parameter recording a spectrum of a suitable sample substance.

Τέλος

Χρηματοδότηση

- Το παρόν εκπαιδευτικό υλικό έχει αναπτυχθεί στο πλαίσιο του εκπαιδευτικού έργου του διδάσκοντα.
- Το έργο «**Ανοικτά Ακαδημαϊκά Μαθήματα στο Πανεπιστήμιο Αθηνών**» έχει χρηματοδοτήσει μόνο την αναδιαμόρφωση του εκπαιδευτικού υλικού.
- Το έργο υλοποιείται στο πλαίσιο του Επιχειρησιακού Προγράμματος «Εκπαίδευση και Δια Βίου Μάθηση» και συγχρηματοδοτείται από την Ευρωπαϊκή Ένωση (Ευρωπαϊκό Κοινωνικό Ταμείο) και από εθνικούς πόρους.

Σημειώματα

Σημείωμα Ιστορικού Εκδόσεων Έργου

Το παρόν έργο αποτελεί την έκδοση 1.0.

Έχουν προηγηθεί οι κάτωθι εκδόσεις:

- Έκδοση διαθέσιμη εδώ.

Σημείωμα Αναφοράς

Σημείωμα Αδειοδότησης

Διατήρηση Σημειωμάτων

Οποιαδήποτε αναπαραγωγή ή διασκευή του υλικού θα πρέπει να συμπεριλαμβάνει:

- το Σημείωμα Αναφοράς
- το Σημείωμα Αδειοδότησης
- τη δήλωση Διατήρησης Σημειωμάτων
- το Σημείωμα Χρήσης Έργων Τρίτων (εφόσον υπάρχει)

μαζί με τους συνοδευόμενους υπερσυνδέσμους.