VALIDATION OF THE CAPILLARY ELECTROPHORESIS METHOD FOR THE FORENSIC INK ANALYSIS

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Method principles

Apparatus

CE system:

- HP 3D CE System (Hewlett Packard, USA)
- Beckman P/ACE 5500 (Beckman Instruments Inc., Fullerton, CA, USA)
- Capillary:
- fused silica capillary of 50 µm i.d. and 375 µm o.d. (Polymicro Technologies, Phoenix, USA)
- 37 / 45.5 cm length for HP 3D CE
- 37 / 45 cm length for Beckman P/ACE 5500

Separation Conditions

A new capillary conditioning procedure: flushing with NaOH solution (1 M) for 20 minutes, then with water for 10 minutes and finally with the separation buffer for 20 minutes.

Rinsing between runs procedure: HCl (1 M), methanol, NaOH (1M) each for 1 minute and with the separation buffer for 2 minutes.

Injection: pressure of 30 mbar for 4 s

Separation: +24kV (42µA) for 15 minutes, at a constant temperature of 35°C

Separation buffer: (pH = 10), 42 mM SDS, 0.35 mM Brij-35, 10.5 mM 3-AP, 5.25 mM HCl, 30% of acetonitrile

Detection: a photo-diode array detector in the range of 200 to 600 nm at a bandwidth of 20 nm

Ink samples preparation

- 5 microdots of inked paper punched out from paper using a steel needle (i.d. 0.45 mm and o.d. 0.6 mm)
- placed in an insert of 50 µL volume + add 2.5 µL of pyridine solution (different pyridine-water ratio solution may be used for different ink types)
- placed in the ultrasonic bath, with water at room temperature, for 5 minutes
- the extract transferred to another injection vial
- the pyridine-water solution evaporated under nitrogen flow (0.6 L/min, 5 minutes)
- the remaining solute dissolved with 0.5 µL of MEE and diluted with 1.0 µL of Brij-Formic Acid dilution solution and again placed in the ultrasonic bath for 5 minutes

Separation Buffer preparation

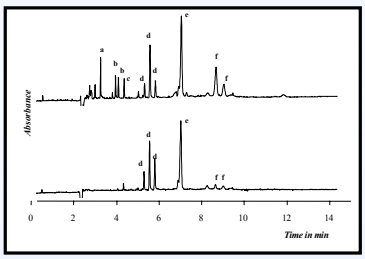
- 0.2100g of Brij-35 and 6.0556g of SDS weighted in the 100mL beaker on the analytical balance (±0.0001g) and dissolved using a small amount of water
- transferred to a volumetric flask for 500mL with 150mL of acetonitrile in it
- added 10.5mL of 0.5M solution of 3-AP and 2.625mL of 1N standard HCl solution
- the flask filled with deinozed water up to the mark

Because of the buffer stability a larger volume of the buffer can be prepared at the same time.

SELECTIVITY

possibility to distinguish and identify various different dyes was proved by performing the analysis of 22 ink dyes in 4 mixtures. Good separation was achieved for all dyes. All dyes could be identified by their characteristic spectra recorded by diode-array detector.

Figure 1. The electropherograms of chosen ink dyes mixture.



Dyes mixture composition:

a)Solvent Orange 3

b)Rhodamin B

c)Acid Red 52

d)Methylviolet

e)Victoria Blue B

f) Solvent Black 3

ROBUSTNESS

the sensitivity of the method to small deliberate deviations from the method

Sample age

Table 1. Reproducibility of migration time for samples age from: 0 to 4 hours.

	EOF	Peak1	Peak2	Peak3	Peak4	Peak5
Ink #7 extracted from white notebook paper						
mean [min]	2.574	5.009	5.209	5.391	7.097	7.616
SD [min]	0.002	0.011	0.012	0.014	0.013	0.020
RSD%	0.08	0.22	0.23	0.26	0.19	0.26

Table 2. Reproducibility of migration time for fresh samples and samples 24h old.

	EOF	Peak1	Peak2	Peak3
Ink #25 extracted from white notebook of paper				
mean [min]	2.652	7.350	7.524	7.675
SD [min]	0.028	0.442	0.473	0.501
RSD%	1.04	6.02	6.28	6.53

Buffer pH

Table 3. Influence of the separation buffer pH on migration times.

buffer	pH	EOF	peak1	peak2	peak3
Typical buffer	9.9841	2.876	7.496	7.635	7.754
pH ↑ buffer	10.061	2.957	8.395	8.562	8.719
pH ↓ buffer	9.8996	2.850	7.652	7.796	7.919

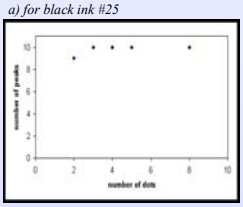
The change of 10% of concentration of buffer components cause the change of the migration time but it does not influence the separation. We predicted that 1% of change in the buffer component concentration will cause maximum 1% change in migration time. In practice the error of buffer components concentration due to the buffer preparation is expected much smaller than 1%. Therefore the deviation of migration time caused by differences in pH of the separation buffer due to the buffer preparation error is less than 1%.

SENSITIVITY

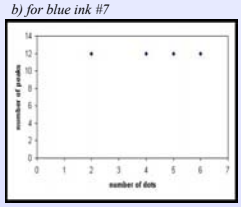
LOD (limits of detection) can be understand as the minimum number of microdots taken from paper to perform successful CE analysis. In order to compare inks the obtained electropherograms must show the ink profile, which represents the character of the ink. For the investigated inks 2 dots is sufficient to characterize the ink.

Figure 2. *The correlation between dots number and significant peaks number*

a) for black ink #25



b) for blue ink #7



REPEATABILITY AND REPRODUCIBILITY

The repeatability within one day work, on one capillary

Sample: 5 dots of ink line, black ink#25

Number of repeated measurements: 10

Table 4. Repeatability of migration time [min]

	EOF	Peak1	Peak2	Peak3
Mean value	2.619	6.642	6.760	6.863
SD[min]	0.03	0.10	0.10	0.10
RSD%	1.2	1.5	1.5	1.4

Table 5. Repeatability of peak area ratio

	Peak1/Peak2	Peak2/Peak2	Peak3/Peak2
Mean value	0.64	1.00	0.68
SD	0.01	---	0.03
RSD%	1.9	---	4.2

The reproducibility for two different capillaries

Sample: 5 dots of ink line, black ink#25

Number of repeated measurements: 31

Number of days: 7 days

Table 6. Repeatability of migration time [min]

	EOF	Peak1	Peak2	Peak3
Mean value	2.677	6.929	7.059	7.172
SD[min]	0.09	0.35	0.37	0.38
RSD%	3.5	5.1	5.2	5.3

Table 7. Repeatability of peak area ratio

	Peak1/Peak2	Peak2/Peak2	Peak3/Peak2
Mean value	0.66	1.00	0.67
SD	0.03	---	0.03
RSD%	3.9	---	4.2

Inks discrimination

The comparison of inks in CE analysis is based on visual comparison of ink extract electropherograms recorded at different wavelengths. The ink differentiation is based on ink profiles.

Definition of the ink profile

- ✓ *The number of significant peaks (A significant peak is a peak that is higher at least three times then the noise level) in electropherograms can be detected at different wavelengths.*
- ✓ *The spectrum of the peak or the peak area ratio for a peak at different wavelengths*
- ✓ *The peak area ratio for different peaks*

Requirements to obtain a characteristic ink profile

- ✓ *90% of peaks that characterized the ink must show up on the electropherogram.*
- ✓ *The peak spectrum has to be recognizable at all wavelengths.*
- ✓ *The peaks used to calculate the peak area ratio have to be at least three times larger than the noise level.*

The criteria to distinguishing between inks

- ✓ *The ink profile is significantly different from the other, otherwise the peak ratio comparison is necessary.*
- ✓ *The peak area ratio difference for corresponded peaks of two inks is larger than three time relative standard deviation of this ratio, generally 15%.*