Generic CZE method for charge variant analysis of mAbs and complex biotherapeutics

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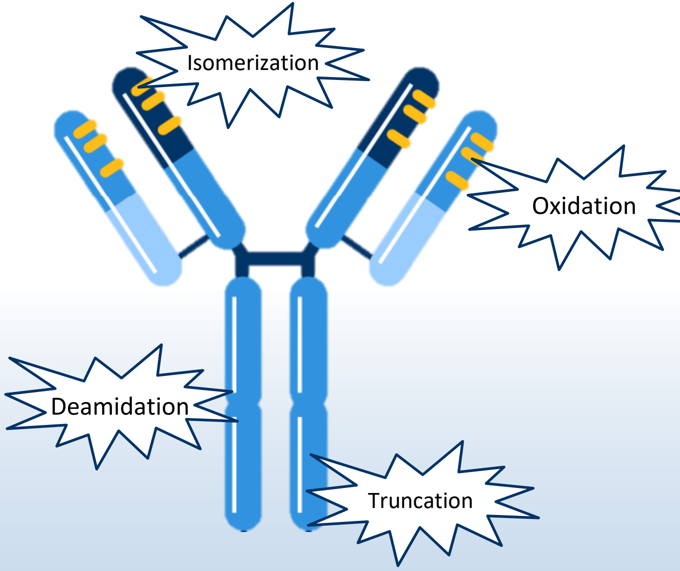
Charge heterogeneity & analysis techniques

- Charge heterogeneity is an important quality attribute with potential impact on safety and efficacy
- CE-based methods are powerful alternatives to LC
- Increasing number and complexity of molecules requires fast and efficient method development



Generic CE-based methods for charge variant analysis

CZE

- Charge to size ratio

iCIEF

- Isoelectric point

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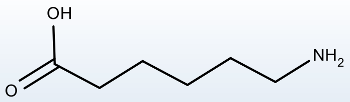
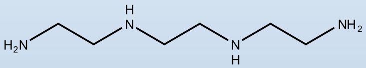
Approaches in CZE

- High pI and complex molecules often suffer from low resolution
- Robustness issues due to BGE components quality

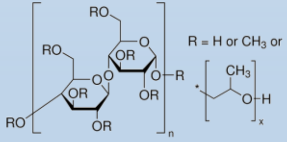
ε-aminocaproic acid (eACA)



Triethylenetetramin (TETA)



Hydroxypropyl-methylcellulose (HPMC)

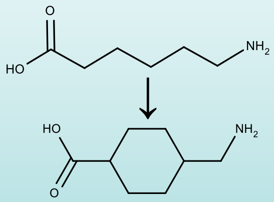


New BGE comprising
- Increased pH
- Replacement of eACA

Substitution by tranexamic acid

eACA

Tranexamic acid



Remaining robustness issues

Good’s buffer-based system
- Optimized additives
- Adapted ionic strength and capillary length

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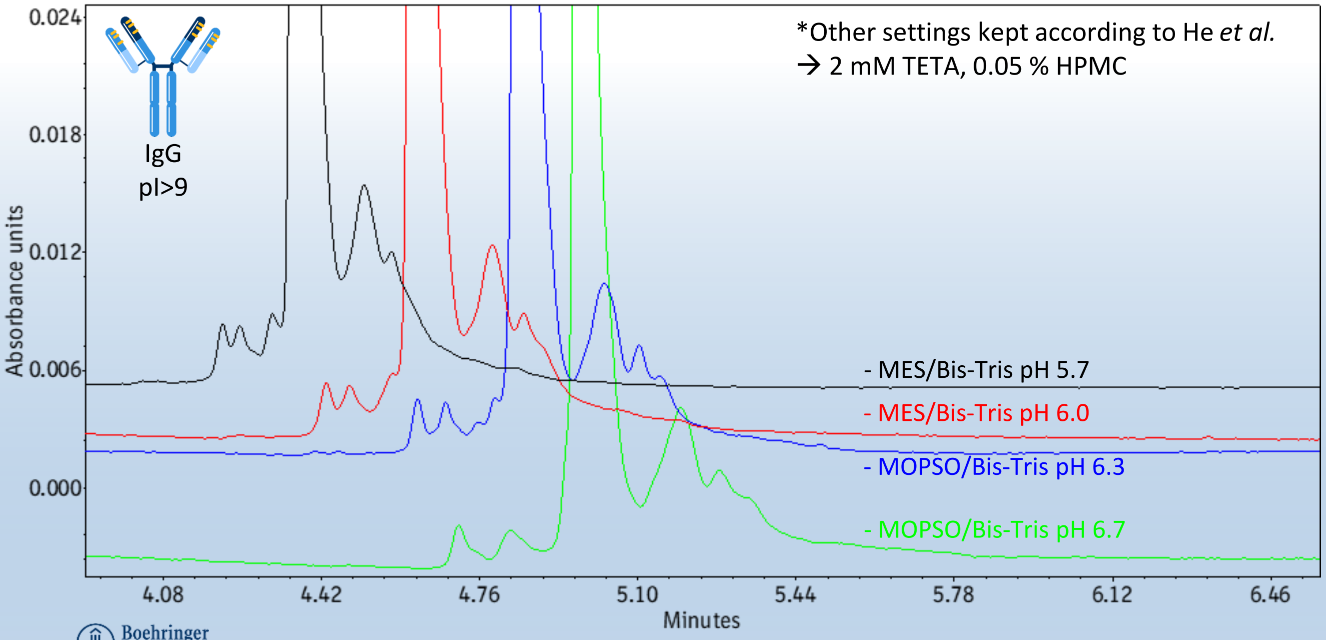
Method according to He *et al.* *J. Sep. Sci.* **34**, 548–555 (2011).

Good's buffer-based system

- Favorable properties for CE
  - High buffering capacity, low conductivity when used at respective pH
- Sulphonic acid derivate pH adjusted with basic polyol



→ Buffer system applicable

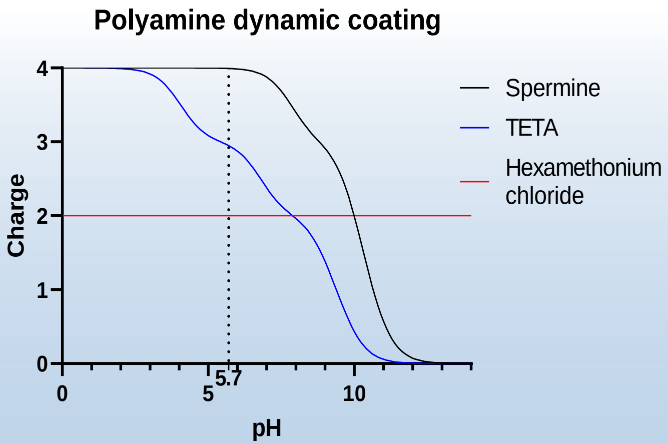
→ pH increased

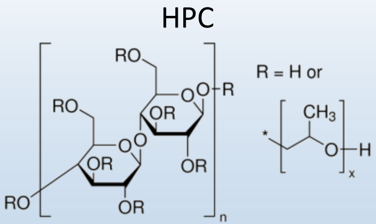
→ Resolution decreases at pH>6.3, vanishes at pH 7

Publication pending

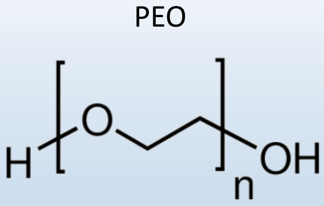
Dynamic coating & viscosity enhancing additives

- TETA: Loss of functionality at elevated pH
- Test of alternative polyamines spermine and hexamethonium chloride
- Alternatives hydroxypropyl-cellulose (HPC) or polyethyleneoxide (PEO)
- Different MW and concentrations tested





80, 370, 1000 kDa

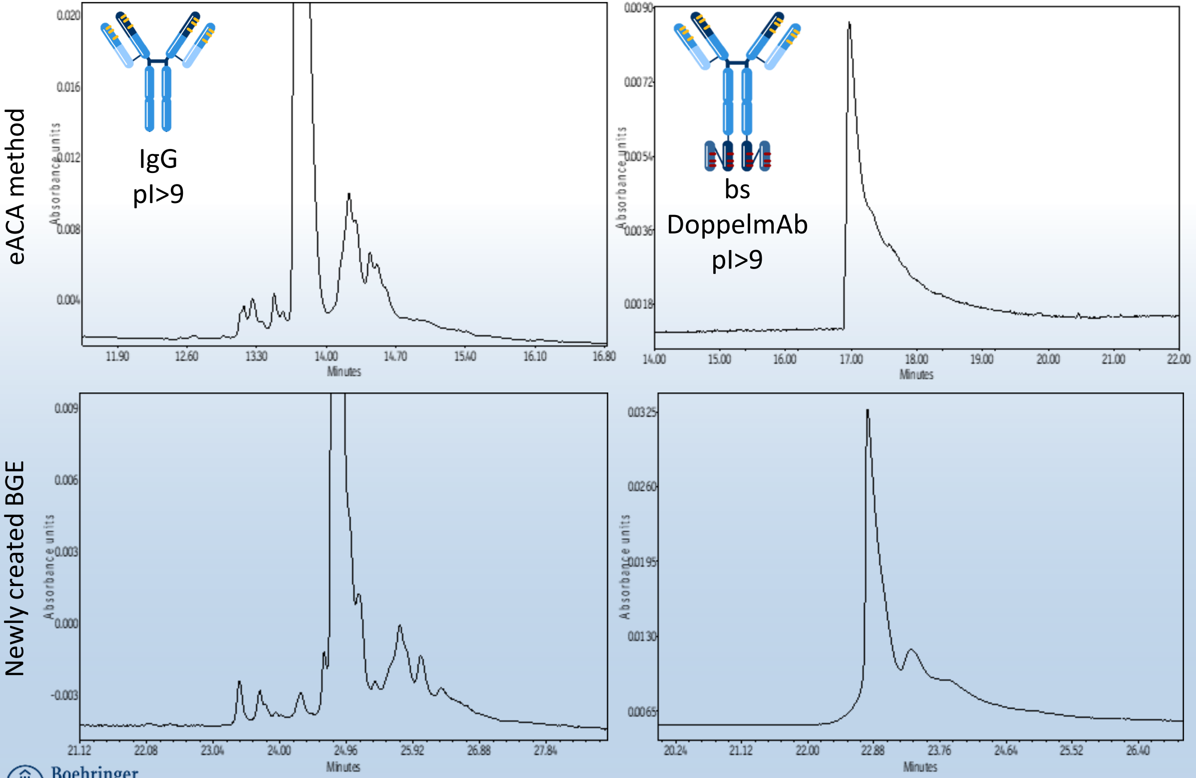


0.4, 100, 600 kDa

→ Spermine and mid-sized polymers HPC and PEO found to be superior under new conditions

Final method & comparison

- BGE comprising MOPS/Bis-Tris pH 6.3, spermine, PEO 100; 50 cm BFS capillary

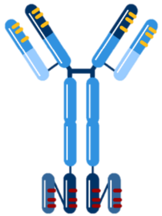


→ Increased resolution for most tested mAbs

→ Applicable to broad range of pI and formats

Method performance check – challenging molecule

- Charge methods IEC, iCIEF & eACA CZE failed
- Method validation possible?



		bs DoppelmAb	Acceptance criterion met
Linearity	10% - 200% target load	MP: r=1.00 APG: r=0.99 BPG: r=0.94	✓
Accuracy	Absolute difference to target % peak area	<1%	✓
Repeatability	%RSD	MP & APG: <4% BPG: <8%	✓
Specificity	BGE interference & stability samples	No interference, stability indicating	✓
Robustness	Hold-time	24 h holdtime	✓

Summary

- Increase in BGE pH improved separation, especially of high pI molecules
- Method performance indicates generic applicability to a broad pI range and complex formats
- eACA successfully replaced, further additives optimized
- Ongoing: Robustness testing of BGE components

Acknowledgements

Matthias Knape

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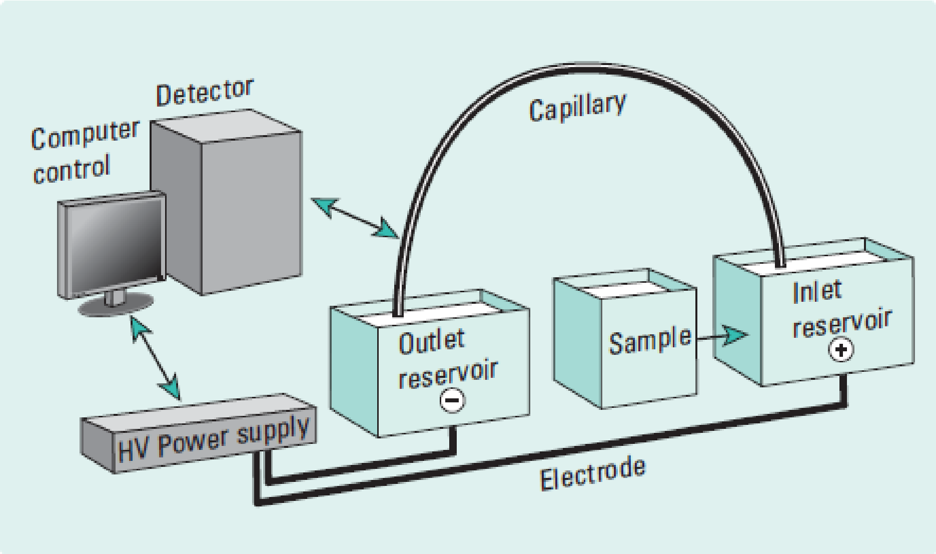
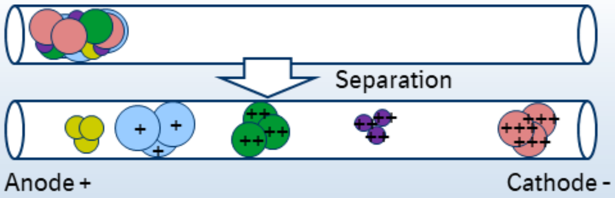
Anete Hornauer

Backup

Capillary zone electrophoresis - Method principle

- Separation by charge to size (resp. hydrodynamic radius) ratio
- In a electrolyte-filled capillary under voltage





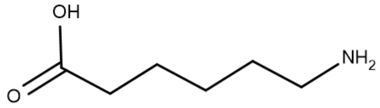
Separation due to resulting differences of migration velocity

→ CZE methods for charge variant analysis are described as fast, generic, high resolution *(Moritz 2017, Kahle 2018)*

Substitution of eACA by tranexamic acid

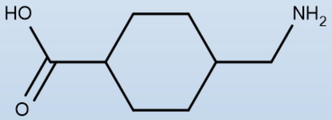
- Acid/Base Properties of Tranexamic acid virtually identical to εACA
- Sterically slightly different
- Might overcome robustness issues

A



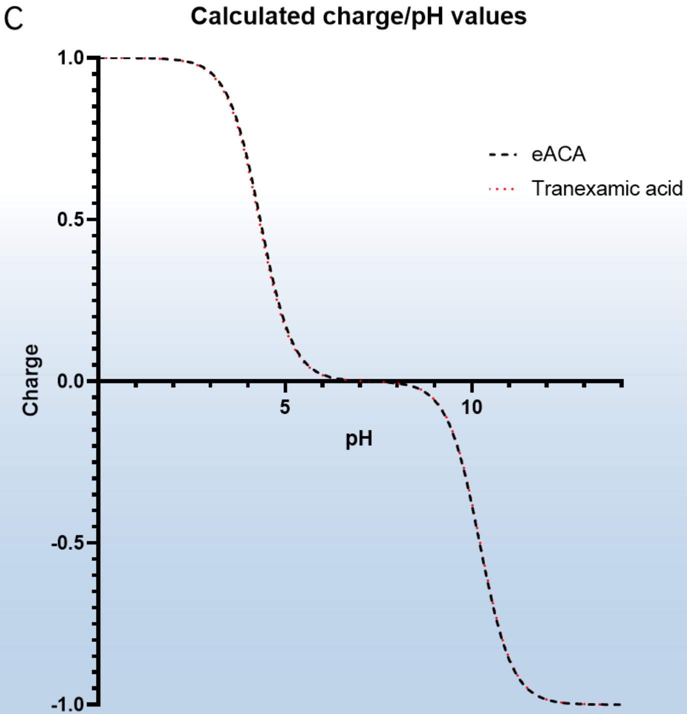
pKa_1=4.4 MW: 131.17 g/mol
epsilon-aminocaproic acid (εACA)

B



pKa_1=4.3 MW: 157.21 g/mol
Tranexamic acid

C



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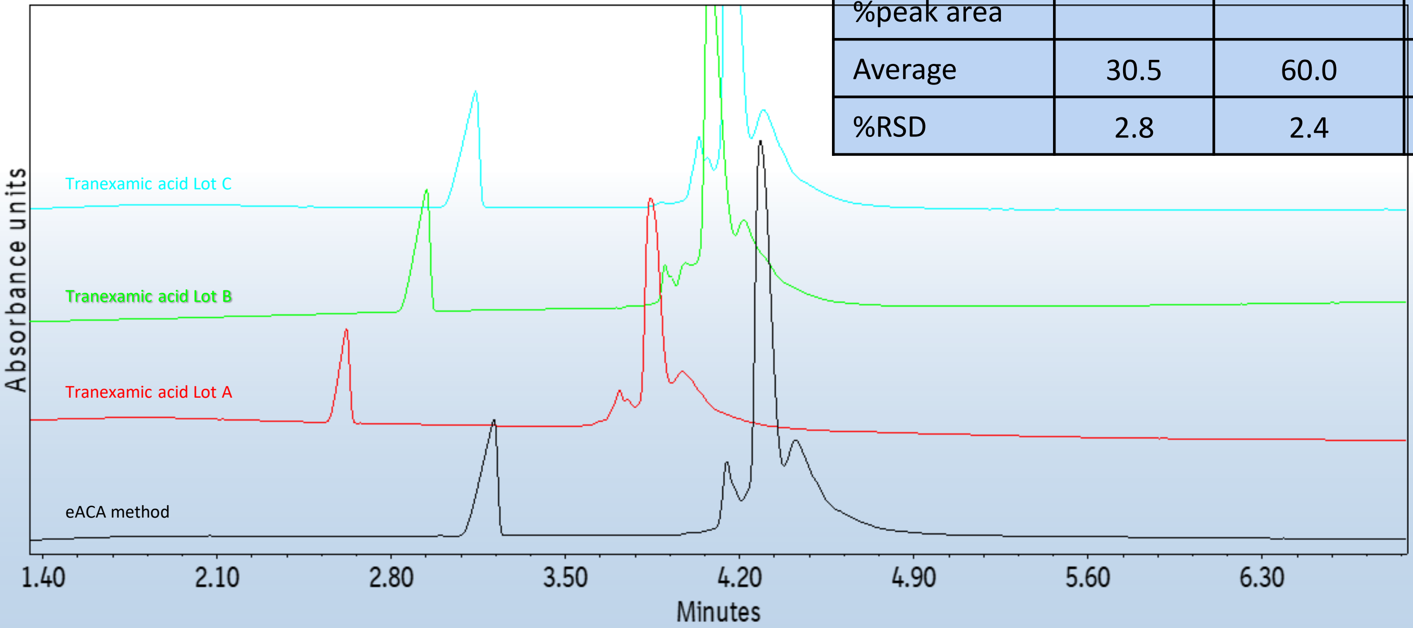
Tranexamic acid – Lot-to-Lot variability

- Poor reproducibility

Lots A,B,C %peak area	APG	Main	BPG
Average	30.5	60.0	9.6
%RSD	2.8	2.4	7.9



→ Not advantageous over eACA

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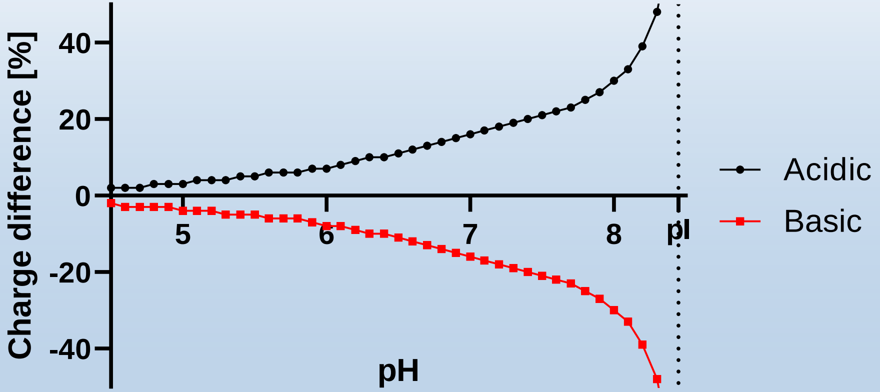
New BGE composition

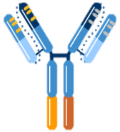
- Overcome robustness issues by replacing eACA by Good's buffer
  - Zwitterionic, larger than eACA → less conductivity
  - Buffer capacity in resp. adjustable pH range
  - No absorbance at 214 nm

- Increase pH to improve separation → not possible with eACA

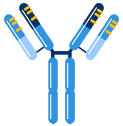


Relative charge difference of charge variants of Trastuzumab
- Acidic variant: N deamidation
- Basic variant: C-terminal K addition

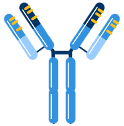
Comparison



Bs
ZweimAb
pI>9



IgG
pI>9



IgG
pI<7.5