Analytical techniques for determining monoclonal antibody charge

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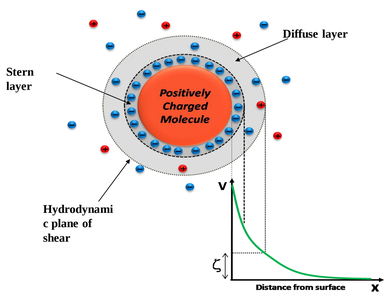
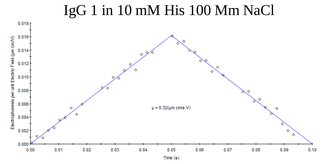
We have examined the electrophoretic mobility μ, zeta-potential and effective charge of a monoclonal antibody, mAb C, and made a comparative study of protein solution analytical methods and instrumentation that measure electrophoretic mobility such as: Wyatt *Möbiuζ*, Malvern Zetasizer Nano ZS, Spin Analytical MCE (membrane confined electrophoresis) and Beckman P/ACE MDQ CE (capillary electrophoresis). Due to volume restrictions associated with sub-cutaneous injections, it is desirable to administer highly concentrated monoclonal antibody solutions. In order to formulate drugs that contain high concentrated solutions of IgG proteins, it is important to understand and be able to predict their colloidal properties in the formulation buffer. The in-vivo analysis depicts the electrostatic driven interactions within and between the protein molecules that explain their affinity, specificity, solubility and stability. The most abundant antibody molecule in the plasma is the immunoglobulin γ-IgG molecule. In 1937, Arne Tiselius pioneered the world of electrophoresis and showed that in the blood serum that γ-IgGs, are the slowest moving proteins. A monoclonal antibody (mAb) is an engineered monospecific antibody that mimics a naturally produced human antibody. In blood plasma IgGs are weakly anionic at physiological pH, their apparent charge is low and it gives rise to a low electrophoretic mobility and by behaving like that IgGs solutions show poor solubility, gel formation and have high viscosity. Since charge-charge repulsion between mAb molecules is important for solubility, the charge on mAbs needs to be determined as part of the formulation development.

First principle electrophoretic techniques

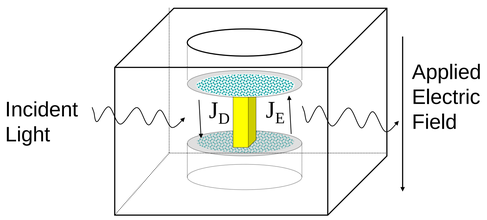


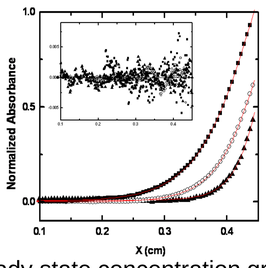
IgG 1 in 10 mM His 100 Mm NaCl



1) Dynamic Light Scattering

- **PALS gives the electrophoretic mobility μ_E as the migration velocity per unit electric field**
- **Derives protein's electrophoretic parameters:**
  - Effective charge Z*e
  - Zeta potential ζ
  - Isoelectric point
- **Möbiuζ capabilities**
  - Sensitivity: 1mg/mL Lysozyme (14 Kda), particle size: 1-1000 nm
  - Temperature range: 4 – 70 °C
  - No practical limit to the mobility measurement
  - Low sample volume: ~170μL





Steady state concentration gradient

2) Membrane Confined Electrophoresis

- **Steady State electrophoresis (SSE) measures the electrophoretic mobility μ_E at steady state from the concentration gradient c(x)**
- **Real-time Mobility electrophoresis (REM) uses the velocity of the concentration boundary to determine electrophoretic mobility μ_E**
- **Direct measurement of protein's e effective charge Z*e**
- **MCE capabilities**
  - Accuracy: "the gold standard for charge measurement"
  - Speed: fast REM and slow SSE
  - Wide salt concentration: 5-500 mM
  - Very low sample volume: ~20μL

Monoclonal Antibody charge

Analytical Technique	*Mobility (μm*cm/V *s)*	Z_{eff}	Z_{DHH}
MCE Mobility	**0.164**	**1.07**	**3.24**
MCE Steady state	**0.158**	**1.03**	**3.12**
Mobiuζ	**0.16**	**1.04**	**3.16**
Zetasizer	**0.305**	**2**	**6.02**
CE constant current	**0.226**	**1.46**	**4.39**
Calculated values from the amino acid sequence	**2.14**	**13.87**	**41**

- apparent valence Z_{eff}
- actual valence or Debye-Henry-Hückel Z_{DHH}

$$\xi \propto \mu_E \frac{\eta}{\varepsilon}$$

$$z_{eff} = \frac{\mu_E k_B T}{Dte}$$

$$z_{DHH} = z_{eff} \frac{(1+\kappa R_S)}{f(\kappa R_S)}$$

η solvent viscosity
D Diffusion coefficient
κ InverseDebyelength
R_S Sum of Stokes radii

Conclusions:

- It is not clear why the actual valences are so much less than the predicted values from the amino acid sequence. There are several publications and prior observations that show the measured IgG's charge to have large deviations from the expected charge
- Due to their strengths and limitations, the methods presented in this poster are complementary
- Dynamic light scattering, capillary and membrane confine electrophoresis techniques give same data for a monoclonal antibody in 100 mM NaCl 10 mM Tris buffer at pH 7.5

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