Characterization of the protein solubilizer SimpleSol for imaged capillary isoelectric focusing (icIEF) analysis

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Abstract

Charge heterogeneity of therapeutic proteins is one of the critical quality attributes used to monitor a protein's quality and consistency during development and manufacturing. However, protein aggregation or precipitation can occur during isoelectric focusing (IEF), resulting in non-reproducible peak profiles. Urea is commonly used as a protein solubilizer to mitigate aggregation and precipitation during IEF analysis, however, urea solutions must be prepared immediately prior to running the sample, adding an additional step and extra time to sample analysis. Urea has other drawbacks, including potential induction of carbamylation, anodic gradient drift causing apparent pI values to change, and denaturing effects above 4M. To overcome these limitations, we have identified an alternative reagent to solubilize proteins for icIEF analysis on iCE and Maurice systems. In this poster we show that this reagent, SimpleSol, can effectively solubilize proteins for icIEF but is a significantly more stable agent than urea, eliminating the need for analysts to prepare urea fresh every time. SimpleSol is also stable when pre-mixed with methylcellulose as opposed to urea and is compatible with absorbance and native fluorescence detection on Maurice icIEF. In addition we show that SimpleSol had less of an impact on the acidic portion of the pH gradient formed during icIEF compared to urea, resulting in more stable pI values of protein peaks relative to using urea. Due to the benefits of solution stability, pH gradient formation, and ease-of-use, SimpleSol is well positioned as a valuable addition for analytical laboratories that routinely use additives to minimize aggregation and precipitation in icIEF analysis.

Materials and Methods

Maurice

cIEF cartridge

SimpleSol

Materials

- Maurice cIEF cartridge
- Maurice cIEF Method Development Kit (contains reagents used in this study, including SimpleSol)

Samples

Proteins were obtained from the following vendors: IgG1 kappa (Protos Immunoresearch, PN 523), Human recombinant EPO (R&D Systems, PN 286-EP), Alpha-amylase (Sigma-Aldrich, PN A4551), NISTmAb (Sigma-Aldrich, PN NIST8671)

Maurice cIEF Analysis Methods

IgG kappa – Sample prep: 0.35% methyl cellulose, 30% SimpleSol, 2.56% 8-10.5 Pharmalyte, 2.05% 3-10 Pharmalyte, Maurice pI markers 7.05 and 9.50, and 0.15 mg/mL IgG kappa. Separation conditions: 1500V for 1 minute, followed by 3000V for 10 minutes.

Human recombinant EPO – Sample prep: 0.35% methyl cellulose, 40% SimpleSol, 3% 2.5-5 Pharmalyte, 1% 5-8 Pharmalyte, 10 mM iminodiacetic acid (IDA), Maurice pI markers 3.38 and 5.85, and 0.3 mg/mL EPO. Separation conditions: 1500V for 1 minute, followed by 3000V for 12 minutes.

Maurice pI Marker Mix – Sample prep: 0.35% methyl cellulose, 4% 3-10 Pharmalyte, 10 mM arginine, 10 mM iminodiacetic acid (IDA), SimpleSol (14, 20, 24 or 30%) or Urea (1, 2, 3 or 4 M) and 2 uL of each Maurice pI marker (3.38, 4.05, 5.84, 6.14, 7.05, 8.40, 9.50, 9.99 and 10.17). Separation conditions: 1500V for 1 minute, followed by 3000V for 5 minutes.

Therapeutic Fusion Protein – Sample prep: 0.35% methyl cellulose, 1% Servalyte mix (3:3:4 ratio of 3-5, 5-8 and 2-9 Servalytes), 50% SimpleSol, 4 mM arginine, 5.6 mM iminodiacetic acid (IDA), Maurice pI markers 4.05 and 9.50, and 5 mg/mL of protein. Separation conditions: 1500V for 1 minutes, followed by 3000V for 12 minutes.

Samples were vortexed and centrifuged at 13,000g for 3 minutes. The top 160ul of the centrifuged solution was transferred to 96 well plates or sample vials, centrifuged and placed in Maurice. Data was analyzed with Compass for iCE software in Absorbance (0.005 second exposure) and Native Fluorescence (5, 10, 15, 20 second exposures).

SimpleSol is an Effective Solubilizing Agent

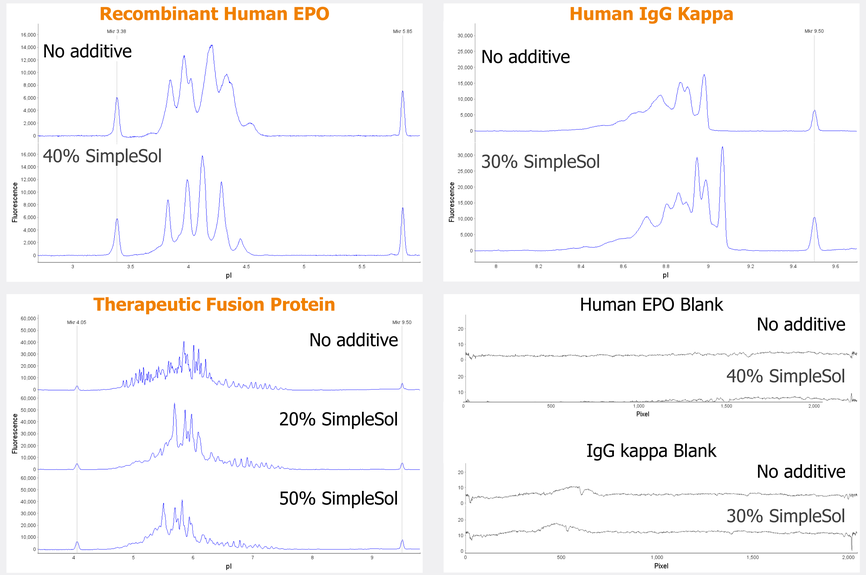


Figure 1. SimpleSol mitigates protein aggregation/precipitation during icIEF analysis. Maurice icIEF native fluorescence traces of Recombinant Human Erythropoietin (EPO), purified human IgG kappa monoclonal antibody, and a therapeutic fusion protein from an industry collaborator with and without SimpleSol. Injections comparing blank samples (no proteins) using the same conditions as used for EPO and IgG kappa shows that SimpleSol does not contribute to the background absorbance.

Injection Repeatability Using SimpleSol with EPO

Peak pI	Average	Std. Dev.	RSD
Peak 1	4.46	0.001	0.02%
Peak 2	4.29	0.002	0.04%
Peak 3	4.11	0.000	0.01%
Peak 4	3.93	0.001	0.02%
Peak 5	3.79	0.001	0.03%

Peak % Area	Average	Std. Dev.	RSD
Peak 1	5.57	0.08	1.50%
Peak 2	23.79	0.14	0.58%
Peak 3	30.29	0.32	1.07%
Peak 4	23.73	0.34	1.43%
Peak 5	13.16	0.37	2.83%

Tables 1 and 2. Replicate injections of samples with SimpleSol show great reproducibility. Quantitative analysis of percent peak area using Maurice icIEF native fluorescence signal for recombinant human EPO run with 40% SimpleSol. Quantitation shows very consistent percent peak areas with RSDs below 3% and pI values with RSDs below 0.05% for all five peaks. Peak 1 is the most basic peak and Peak 5 is the most acidic peak.

Comparison of SimpleSol and Urea

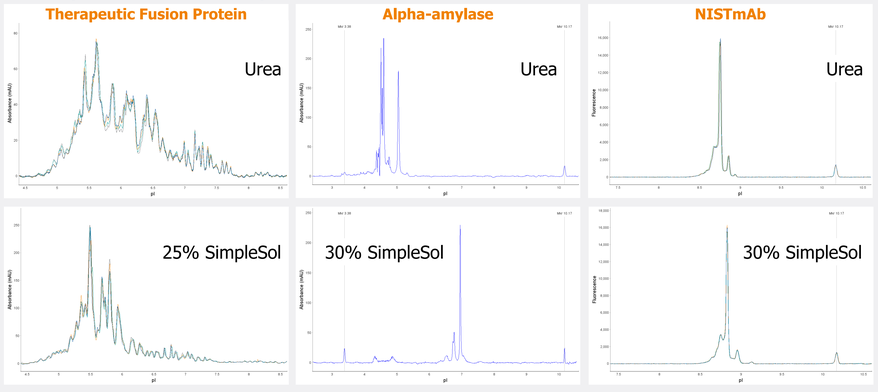
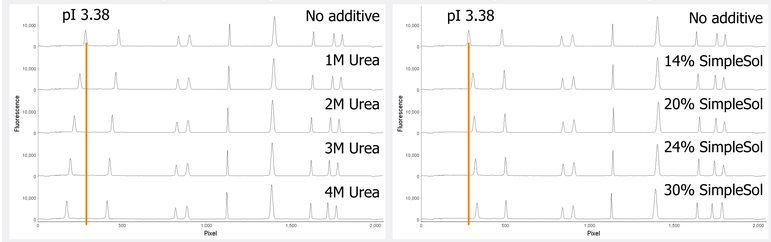


Figure 2. SimpleSol can be used for samples where urea shows insufficient data quality. Each sample was run on Maurice icIEF with either urea or SimpleSol as a solubilizing agent. Injection reproducibility for a therapeutic fusion protein was improved when using SimpleSol as compared to 4M urea (N = 4). Addition of SimpleSol dramatically reduced precipitation seen when using 4M urea for alpha-amylase. Increased resolution was observed for the NIST mAb reference material when replacing 3M urea with SimpleSol for the same icIEF conditions.

Minimal Impact on pH Gradient and pI With SimpleSol



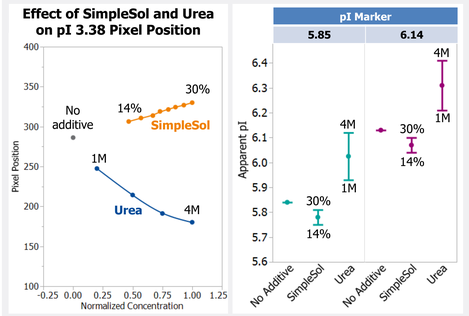
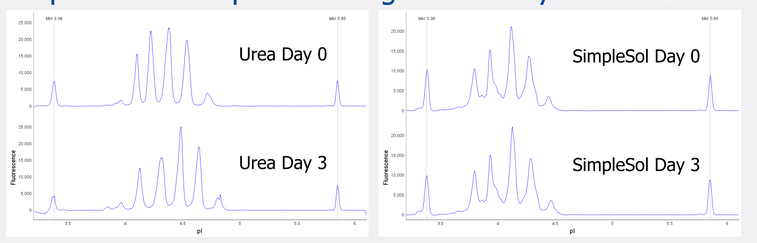


Figure 3. SimpleSol has a lesser effect on the acidic portion of a pH gradient in IEF compared to urea. Maurice native fluorescence traces of a mix of nine Maurice pI markers ranging from pI 3.38 to pI 10.17 was run in 4% 3-10 Pharmalyte, 10mM arginine, 10mM IDA, and 0.35% methyl cellulose with no additive or with either urea or SimpleSol at indicated final concentrations. Urea induces larger shifts in pixel position for the acidic pI marker 3.38, as evident in an average anodic shift of 106 pixels in samples with 4M urea relative to samples with no additive. 30% SimpleSol shows an average shift of 44 pixels. Additionally, a wide range of SimpleSol concentrations (14-30%) results in apparent pI values of smaller differences and closer in value to samples with no additive, as shown for pI markers 5.85 and 6.14.

SimpleSol Has Improved Reagent Stability Versus Urea



% Peak Area	Urea Day 0	Urea Day 3	SimpleSol Day 0	SimpleSol Day 3
Peak1	5.43	5.14	5.56	5.58
Peak2	24.52	22.67	25.13	25.17
Peak3	29.52	28.94	29.11	29.55
Peak4	24.30	24.68	23.61	23.28
Peak5	13.57	13.99	16.59	16.42
pI Value	**Urea Day 0**	**Urea Day 3**	**SimpleSol Day 0**	**SimpleSol Day 3**
Peak1	4.71	4.81	4.44	4.46
Peak2	4.53	4.64	4.27	4.29
Peak3	4.37	4.48	4.12	4.13
Peak4	4.22	4.30	3.93	3.93
Peak5	4.10	4.13	3.80	3.80

Figure 4. SimpleSol has better stability than urea for samples analyzed with icIEF. Human EPO was run with urea prepared fresh (Day 0) or aged 3 days at 4C or SimpleSol aged in parallel. EPO run with aged urea showed a change in peak profile, including shifts in apparent pI values and percent peak area compositions. Aged SimpleSol performed similarly for both timepoints. Peak 1 is the most basic peak and Peak 5 is the most acidic peak.

Conclusions

- The novel protein solubilizer SimpleSol can be used to mitigate aggregation/precipitation during icIEF for a variety of proteins.
- SimpleSol is compatible with icIEF analysis of proteins, including absorbance and fluorescence detection on Maurice, and produces highly reproducible data for pI and percent peak area.
- As a ready-to-use solution that is more stable than urea, SimpleSol eliminates the need to prepare reagents fresh for each protein analysis, and in some cases is a better solubilizer than urea.
- SimpleSol has less of an impact on gradient formation in IEF and thus has less of an effect on apparent pI calculations.