**Roundtable Session 2 – Table 8 - SDS-CGE and CGE Method Development and Application**

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**Abstract:**

SDS-CGE and CGE-LIF are powerful techniques for separating and analyzing therapeutic proteins (mAb, ADC, AAV etc), DNA and RNA. The two techniques are commonly employed for assessing size heterogeneity of protein and DNA during manufacturing and storage. Both techniques are valuable in biopharmaceutical development and quality control. The roundtable will focus on the discussion of technology development and application advances in SDS-CGE and CGE-LIF for biopharmaceutical analysis in recent years.

**Discussion Questions:**

1. What SDS-CGE gels do you use in your lab? What are the advantages and disadvantages of different SDS-CGE gel?

2. What therapeutic proteins do you test with SDS-CGE? What are the specific challenges and solutions for each modality?

3.What CGE gels do you use for plasmid DNA and RNA separation? What are the advantages and disadvantages of these gels?

4. What therapeutic DNA and RNA do you test with CGE-LIF? What are the specific challenges and solutions for each application?

5. Do you develop in-house SDS-CGE or CGE-LIF gel, and why?

6. Method development in sample preparation for SDS-CGE/UV and CGE-LIF?

**Notes:**

Welcome everyone this is table 8

We can talk about CGE for protein and RNA analysis

Introductions….

**~Starting**

-Most of you here are familiar with SDS-CGE and for awhile we called it CGE-SDS and why do we call it this similar to how we say SDS-PAGE which raises the point of, does anyone have a opinion on what the correct term should be.

-It should be Just CGE but because we call it SDS-PAGE that stuck with the name. but it should be just CGE because you can use other surfactants.

-For CGE we also use it for protein labeling for high sensitivity such as using intercalating dye.

-Either ADC, Protein, mRNA many different things are used other than SDS. So really the term should just be CGE and then it differentiates based on the surfactant or dye used.

**~New topic**

-Biorad and other companies compete for gels, such as Beckman Sciex and each one had different resolutions and peak shapes. At the moment Sciex has the agreed better gel.

-Now Maurice has a gel of their own as well.

-If you have run these different gels how do they compare to each other? Such as reproducibility and baseline noise.

-Is anyone using the Maurice CE-SDS for separation?

-Yes, we have been using it abit, it had a dip in the low weight region. But results were comparable, but we still use the Sciex PA800 gel, we never used Biorad gel.

-Back in the day for academia I used my own gel and could target specific protein weight ranges, but it is not QC friendly.

-Back in 2005 in the early days there were some problems.

-Sciex gel the high weight region can be pretty broad and have some waviness to the base line, I tried mixing 90% Sciex gel with 10% Biorad gel. This controls the degree of conjugation using positive charged dextran. Then the peaks become more sharp.

-If there are only a few mabs this might be fine but if there are many then this can be a problem to only use one gel and this can cause peak shape and resolution issues if it's not optimized.

-We use microCE from Revvity and saw batch to batch reproducibility issues with the gel.

**~New Topic**

-In CE there is more freedom to change method parameters but with the Chip there is less flexibility to change things. Is there any recommendations to change this or make the process more controlled.

-You need to talk to the vendor.

-The lot-to-lot charge and variability is high for revvity we want to try our own gel to reduce these lot-to-lot variability, do you know any other vendors we can try to fix this issue?

-Gel is a critical reagent, like for ampholytes this can be a issue when something goes wrong with it. Its better to be able to have at least two vendors but its not always possible.

-You can use something to demonstrate comparability between a new gel and old gel to have a back up possible.

-I did a comparison of Maurice gel and PA800 sciex gel, the sciex gave better separation but maybe the Maurice could have a better baseline. And even if there are some differences as long as it is understood you can try different gels with different technologies.

-Any Vendor should provide a COA for the reagents, and you can do a audit to check the vendors products and ask them for help.

-The Revvity seems to have issue with conductivity, and the vendor says they are checking it out, but they are blaming other parts of their process for why this is an issue.

-It's best to talk with the Vendor since they might not fully understand the issue.

-What is wrong with the Gel?

-It seems to be migration shifts or new peaks and sometimes resolution, almost anything.

-First thing we do is polymer characterization to understand the polymer and conductivity. If the conductivity is correct and the polymer is well controlled this shouldn't be a issue, its about the polymer. Resolution should be very solid.

-They don't have good control of the process, and they tried in-house controls, but it doesn't seem to work.

-Others have also seen this with the Labchip gel from revvity.

-If you compare labchip to other technologies it has very good sensitivity, but it doesn't have a great cleaning process such as with sciex PA800 using acid and base wash between samples but the chip doesn't have this, for dirtier samples this can be a larger issue on labchip.

-On the labchip scale the viscosity of the gel is likely different than a larger capillary such as for Sciex PA800 making hard to try to use another product.

-That's correct but its hard to use other gels or settings for labchip as its not as flexible to control the method parameters.

-When there is a failure, you can usually trace it back to a bad lot of gel and then work with the vendor so both the vendor and customer can change parameters and learn from it to tighten specs to make sure it fixes some of the issues.

-okay lets move on.

**~New Topic**

-So what platforms do people normally use? Such as for GMP?

-For QC its usually always Sciex.

-What about Maurice? Do people use the CE-SDS cartridge?

- We saw lower purities on the Maurice than the PA800+ something wrong with the reagents and method that causes method induced fragmentation, shorter capillary.

-If you compare Maurice with Sciex instruments, the PA800 has pressure on both ends to prevent current from going down. The Maurice uses a lower voltage to separate 15 kV for PA800 vs about 5 kV for Maurice.

-If you compare Maurice gel and Sciex gel?

-Maurice gel- peak tailing on main peaks, couldn't detect HMMS due to this.

-So far the PA800 is really still the work horse for these reasons as well as being so QC friendly.

**~New Topic**

-So for many different kinds of modalities Mab, Fusion, ADC, AVV what are unique challenges for these different modalities. How do you overcome them?

-Complex proteins with high Glycosylation

-Well you can use labeled dye to quantify free protein such as seen in the previous presentation, why is the protein peak so much smaller than the polysaccharide conjugate.

-The larger the molecule the farther the migration time, why did the free protein migrate later than these massive polysaccharides?

-For the ADC separation, the H0 50KDa which has the ability to conjugate 3 of the 10K PEGS. By adding the PEGS it widens the size differences of each of these DARs giving better separation.

-The light Chain 1 is 21KDA, L0 is 30KDa. By adding a large enough alkylate you can force the different DARs to separate better.

-If your trying to develop a method for heavily glycosylated proteins, should you try to degly first?

-And now with all the vendors that sell enzymes for that there can be lot to lot variability as well.

-Does anyone do degly?

-Yes, I was trying to do DAR CGE by degly but by using the basic gel from Sciex and capillary. We didn't want to deviate too much and got good L0 and L1 species but not the heavy chain species and went to the huge alkylant.

-Does degly work for ADC?

-Yes, you can but it might not really be useful, and we use it for other methods such as for mass methods to clean up the profile and get accurate mass.

-If so what about AAV protein and the conjugation can be low also there can be a lot of salt which causes issues such as low sensitivity but now with NFD this can be helpful for this problem. But this is only for the BioPhase only, we want it for the PA800 as well. But they want to sell the biophases but they are hearing that a lot of people want NFD for PA800.

-If we cant transfer this to QC then what's the point which makes it hard for BioPhase method creation.

-Maybe next year a new PA800 NFD can be made.

-Biophase is likely the way they will go.

-The bridging can be difficult, is that the main issue?

-For me it's the prep, I don't like full trays where the PA800 you can do just part of a row of vials. So if it's a large run the BioPhase is fine but for only a few samples this can be a pain. And this

isn’t even needed with how many samples most people get. And it’s very hard to bridge these technologies do to the HC/LC differences too.

-QC doesn’t really need a BioPhase because the number of samples they get doesn’t require it.

-For development its different such as screening studies which can have a lot of samples, which can be painful on PA800, which shows that Sciex should make both NFD for PA800 and Biophase.

-QC would be afraid of system suit failures for some capillaries and what does that mean exactly.

-Is the Sciex gel good tho?

-The gel is good with no issues other than the baseline roll.

-Only an issue with pressure injections with a peak at 20 minutes. However, we don’t use pressure injections much.

-Only time issues were seen were with those pressure injections and Sciex sent specific lots for this.

**~New Topic**

-Yesterday we talked about plasmids, mRNA, Proteins. In terms of the gel we have RNA gel.

-What gel do you use? What is your opinion on it for these different types of gel.

-We tried different gels and some were working better than others for plasmids. Some of them are very large molecules so for bigger plasmids some gels worked better than others. Talked to Sciex and some kits were made for some isoforms but it didn’t work for others that would be in the same run. With OC its so large it can be a pain with the way it migrates and can come out sooner than the linear even though it shouldn’t.

-The kit was not as impressive for the Open Circle.

-Ok what about RNA separation. Do you use fragment analyzer?

-Does Sciex have a kit?

-Yes, it goes to 9Kb from 19Kb

-There are companies doing clinical trials with the Sciex kit but I haven’t heard as much about them.

**~ New Topic**

-So let’s talk about sample preparation.

-For nrCGE such as HMMS some people try to optimize the method.

-We say the nrCGE is only for fragments and we don’t care as much about the HMMS because of the gel wave at the end, and we rely on SEC to track aggregate species.

-Do we think Aggregate is covalent dimer or is it antibody + a heavy chain or light chain. And we try to characterize this to see if its Heavy + light chain. Such as monomer plus a 92k weight.

-You can form a thioether link between HC +LC which can cause this for rCGE.

-They call them non covalent aggregates.

-They used SHS additive during denaturation to get rid of these non-covalent aggregates.

-Its thioether link so it wont fall apart during denaturation.

-You run these samples by SEC and CGE when you see these peaks, you can collect fractions from SEC to try to see what these aggregate species are, and usually we see its dimer but sometimes it could be something else.

-You can do the fraction collection on SEC and then reinject into the CGE to confirm that it is the peak you were looking for as well.

-Toward the end of the run the species tend to come out closer together even if the weights are very different as can be seen with a molecular weight ladder meaning the peak after intact on nrCGE could be some kind of dimer.

-I think most of the dimer is non-covalent unless we are missing something so it should be separating when denatured.

**~Ran out of time, round table talk finished**