

Automation of Sample Preparation for CE-SDS-LIF of rMAbs with a Robotic Purification System

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Late Stage Analytical Development

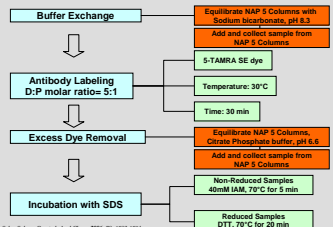
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INTRODUCTION

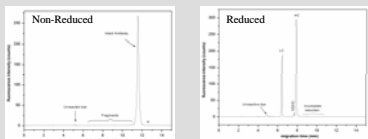
An increasingly used analytical method for protein biopharmaceuticals is capillary electrophoresis sodium dodecyl sulfate (CE-SDS) non-gel sieving analysis. However, CE-SDS with UV detection only provides the sensitivity of coomassie stained gels. To get the sensitivity of silver-stained gels the use of laser induced fluorescence is required. This requires the use of a manual and time-consuming dye-labeling protocol. The protein sample must be buffer-exchanged to provide the optimal conditions for fluorescence dye labeling followed by excess dye removal purification. A way to overcome this bottleneck is a method developed for a liquid handling robot. This allows the automation of the dye labeling protocol and SDS incubation as well as decreasing the amount of protein sample required by ten fold. This greatly increases thorough-put by reducing user input and decreases the amount of sample used. This approach is applied to rMAbs and comparability to previous methods will be demonstrated.

CURRENT METHODOLOGY

Sample Preparation Scheme for CE-SDS Analysis with LIF Detection



Results Using Standard Conditions



Salas-Solano, O., et al. Anal.Chem. 2006, 78, 6583-6594.

AUTOMATED SAMPLE PREPARATION

Phynexus System Advantages

- Automated Operation
- Sample size
 - 10-50ug on Phynexus vs. 500ug on NAP-5 Column
- Scalability
 - 1-12 samples per run on the Phynexus Robot
 - 96 per run on Standard Pipetting Robot
- Familiarity
 - PhyTip columns use the same G25 Sephadex Media as NAP-5 columns

Phynexus System



Overview of Automated Sample Preparation

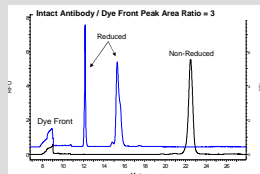
1) Removal of Glycerol from Phy-Tip gel bed



2) Sample Purification/ Buffer Exchange



Results Using Manufacturer Conditions



- Low Signal
- Low protein recovery during buffer exchange
- Issues during excess dye removal
- Poor protein recovery
- Limited tip capacity

OPTIMIZATION

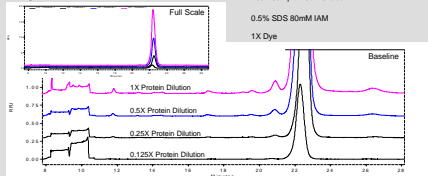
Changes to Phynexus Method

- Complete the removal glycerol from filtration gel
 - Deep Well Plates: 200uL to 2mL buffer volume
 - Increase tip wash to three washes at three 2mL wells from 2 washes in 200uL buffer
- Remove air-push from the method
 - Use the minimum air push to force sample into the gel bed. To avoid drying out the tip media

Buffer Exchange Optimization

Sample Volume	Protein Amount Loaded (ug)	Air-Chase	Elution Volume (uL)	Percent Recovery	RSD*
20	40	200	40	40	8
20	40	44	60	60	5
20	40	44	100	80	3
20	40	44	120	80	3

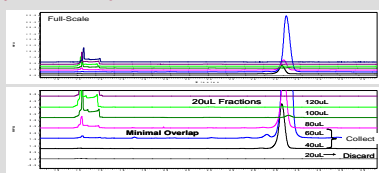
Dye Removal : 80uL Bed



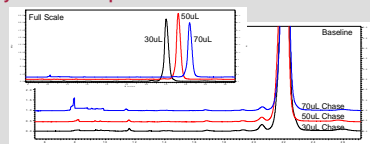
Conclusion: Poor Column Efficiency

Solution: Phynexus develops new 160uL bed PhyTip

Dye Removal Optimization: 160uL Bed

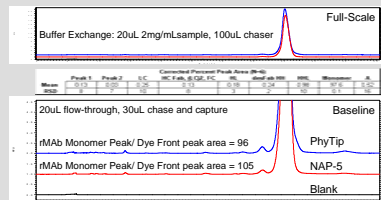


Dye Removal Optimization: Chaser

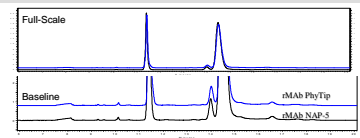


FINAL RESULTS

Manual Method vs. PhyTip: Non-Reduced Samples



Reduced Samples



CONCLUSIONS

Conclusion

The automated sample method for CE-SDS-LIF of rMAbs shown here, provides reproducible results that are comparable to the manual NAP-5 method with 10 times less sample

The automation and scalability of the Phynexus system will meet current and future analytical needs

ACKNOWLEDGEMENTS

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REFERENCES

Salas-Solano, O., et al. Anal.Chem. 2006, 78, 6583-6594.