Measurements of Sodium Removal from Ringer's Solution by Use of a Desalting Apparatus

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Mass spectrometry is increasingly used for the analysis of large biomolecules. These samples are often obtained from biological matrices with high salt content. These salts are responsible for unacceptably high noise in the mass spectra of these samples. Rapid and efficient desalting techniques are needed. In this article, the BAS Rapid Desalting Dialyzer was shown to remove at least 99.7% of the sodium from a Ringer's solution. The flow conditions chosen were shown to affect salt removal in a predictable manner.

The mass spectral analysis of large molecules in biological matrices requires the removal of salts in order to get adequate signal-to-noise ratios. The BAS Rapid Desalting Dialyzer®, ***F1***, uses a microdialysis membrane (MW cut-off 29,000) to remove low molecular weight molecules from samples. The large surface area-to-volume ratio, coupled with the use of counter current flow, accelerates the dialysis process effectively removing salts and small molecules from the matrix. These salt-free samples can be directly injected into the mass spectrometer, showing a significant signal-to-noise improvement over samples which contain salts (1). In this work, the amount of sodium removed from a Ringer's solution under four different flow conditions was quantified.

Experimental

The Rapid Desalting Dialyzer (Bioanalytical Systems, Inc., West Lafayette, IN) was used to remove salts from a Ringer's solution (Injection Ringer's, McGaw, Inc., Irvine, CA, pH 5.8, 3379.5 ppm NaCl, 156.41 ppm KCl and 90.18 ppm $CaCl_2$) under the four different flow conditions shown in ***T1***. The desalting device was flushed with NANO water for 30 minutes before use, and flow conditions were given 60 minutes to become established before sample collection began.

The Ringer's solution was pumped into the desalting device using a 2.5 mL syringe and a BAS syringe pump (Bioanalytical Systems, Inc., West Lafayette, IN). Filtered NANO water (Barnstad, Boston, MA) was pumped counter current to the sample with an ALTEX pump, Model 110A. (Altex Scientific Inc., Berkley, CA). Samples were collected into polypropylene containers at the four flow conditions given in ***T1*** and frozen until analysis.

Samples were analyzed for Na using flame atomic emission spectroscopy (AES) with a Smith Hieftje 4000 AA Spectrophotometer (2). The spectrophotometer was equipped with a sodium hollow cathode lamp and an air/acetylene flame for atomization of the sample. The instrument was interfaced with a computer and was operated using ThermoSPEC/AA Ver.4.1 software. The emission wavelength was set to 588.9 nm with a narrow band pass of 0.4 nm to avoid interference from nearby Na emission lines.

Standards for the AES analysis were prepared by dilution of the Ringer's solution to concentrations spanning the AES detection range for sodium (0.3 to 0.02 ppm). Standards were linear in this range, and quantitation was performed using standard linear regression analysis. Dilutions of the outer membrane flow samples and dialysates were made to assure that the samples were in the same concentration range as the standards. All dilutions were made with NANO water into polypropylene containers or glassware rinsed in 6N nitric acid and precautions were taken to avoid outside sources of Na contamination. Five repeat measurements were made on each sample and the average and standard deviations were calculated.

Results and Discussion

The efficiency of the desalting device for the removal of sodium from Ringer's is shown in ***F2***. Overall, 99.7+% of the sodium is removed under all flow conditions. Lower perfusate flow rates (2 µL/min) resulted in better salt removal than high flow rates (5 µL/min). Salt removal at higher outer membrane flow rates (500 µL/min) was better than at lower flow rates (200 µL/min). These findings are consistent with the idea that lower perfusion flow rates and faster outer membrane flow represent conditions with the highest concentration gradient across the membrane to optimize the removal of salt. Flow conditions of 2 µL/min perfusate flow and 500 µL/min outer membrane flow were the most efficient, resulting in 99.9+% removal of sodium. All flow conditions, however, were satisfactory, eliminating at least 99.7% of the sodium.

Conclusion

The Rapid Desalting Dialyzer effectively removed over 99.7% sodium under all flow rates tested for the device.

Reference

1. *Emmett, M., Caprioli, R. "Micro-Electrospray Mass Spectrometry: Ultra-High-Sensitivity Analysis of Peptides and Proteins." Am. Soc. Mass Spectrom. 5 (1994): 605-613.*
2. *"Flame and Plasma Atomic Emission Spectrometry" (Chapter 8) in Spectrochemical Analysis by J.D.Ingle, Jr. and S. R. Crouch, 1988, Prentice Hall, Englewood Cliffs, NJ, pages 225-256.*

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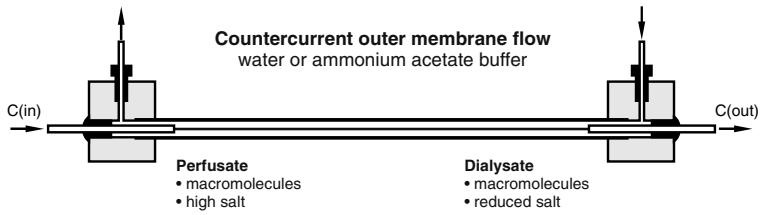
T1

Flow conditions used for characterization of a desalting device.

Flow Conditions	Perfusate Flow Settings µL/min	Outer Membrane Flow Settings µL/min
A	2	200
B	2	500
C	5	200
D	5	500

F1

Diagram of the BAS Rapid Desalting Dialyzer®.



F2

*Percent sodium removed after desalting a Ringer's solution under the four flow conditions (A-D) in **T1**.*