

ABSTRACT

This study was carried out to demonstrate the ability of a WorkBeads™ 17/100 SEC prepacked column to rapidly and efficiently desalt proteins. In this application a method for fast desalting is described.

-
- new agarose prepacked column
 - fast, efficient desalting and buffer exchange
 - compatible with biologic samples
-

INTRODUCTION

Speed is often an important parameter in chromatographic procedures, especially for less complex separations, such as removal of salt from a protein preparation or buffer exchange. A desalting step is often required prior to enzymatic digestion. This study will demonstrate how the prepacked WorkBeads™ 17/100 SEC column was used for rapid desalting of a protein prepared for use in an enzymatic digestion. Different linear flow rates, from 1.0 to 4.0 cm/min, were used.

WorkBeads™ 17/100 SEC is a matrix produced from agarose according to a new method. The rigidity and chemical stability of the product make it ideal for high flow rates or use with extreme pH values.

EXPERIMENTAL

The protein chosen for study was alcohol dehydrogenase, ADH, EC 1.1.1.1, No. 39002. A 6 mg/ml solution of ADH was prepared and reduced using 3 mmol dithiothreitol in a Tris buffer. The concentration of the buffer was 0.25 M containing 6 M guanidinium hydrochloride and 0.25 M EDTA. The pH was adjusted to 8.5.

For each injection, 20 μ l of sample was used, which corresponds to 60 μ g of protein and 12 mg of salt.

with a final concentration of 20 mM. The pH was adjusted to 8.35, using concentrated acetic acid. All chemicals used had a high grade of purity.

The flow rate was varied between 1.0 cm/min and 4.0 cm/min, which is equivalent to 0.5 - 2.0 ml/min since the cross-sectional area of the column is 0.5 cm². The column (8 x 300 mm) was prepacked with WorkBeads™ 17/100 SEC. The particle size distribution was 15-20 μ m (d_{50} of - 17 μ m by volume).

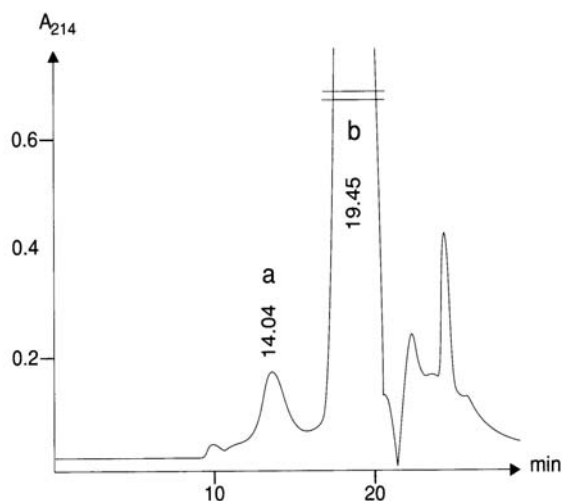


Figure 1. Desalting of ADH using WorkBeads™ 17/100 SEC at a flow rate of 1.0 cm/min. a) ADH, b) salt.

RESULTS AND DISCUSSION

ADH was prepared as if it would be treated with trypsin (1). A digestion of this type requires high concentrations of guanidinium hydrochloride and dithiothreitol for unfolding and reduction of the protein.

Salts must be removed prior to digestion. The most practical way to remove salt is by size-exclusion chromatography. In this procedure salt is effectively separated from the protein, and after that the buffer can be easily evaporated.

Fig. 1 shows that when 60 μ g protein and 12 mg of salt in 20 μ l of buffer was injected onto the column, a separation at a flow rate of 1.0 cm/min produced excellent results. After elution, the fraction containing ADH was shown to be entirely free of salt.

The retention time for ADH was 14.04 min; this corresponds to a retention volume of 7.02 ml. When the flow rate was raised to 2.0 cm/min, the separation was equally satisfactory and the retention time was reduced by half. When the flow rate was further increased to 4.0 cm/min, the separation was still very good (Fig.3). This means that complete separation was achieved at the retention time of 3.24 minutes for ADH

and the elution of one column volume required 7.5 minutes.

In order to investigate the influence of temperature on the resolution, these separations were duplicated at 4°C using the thermostating kit. The results obtained in this case were similar to those at 2°C, indicating that WorkBeads™ is perfectly stable over a wide range of working temperature conditions.

CONCLUSIONS

The purpose of the study was to demonstrate a common desalting step using a WorkBeads™ 17/100 SEC, 8 x 300 mm, column. The results show that the column performs excellently, with retained separation capacity even at very high flow rates, different temperatures and ionic strengths.

REFERENCES

1. Renlund, S, Klintrot, PM, Nunn, j., Shrimser, L, Wernstedt, C. and Hellman, U. *J. Chromatogr.*, 512 (1990) 325.
2. The data was produced in the research laboratory at Inovata AB in Stockholm, Sweden.

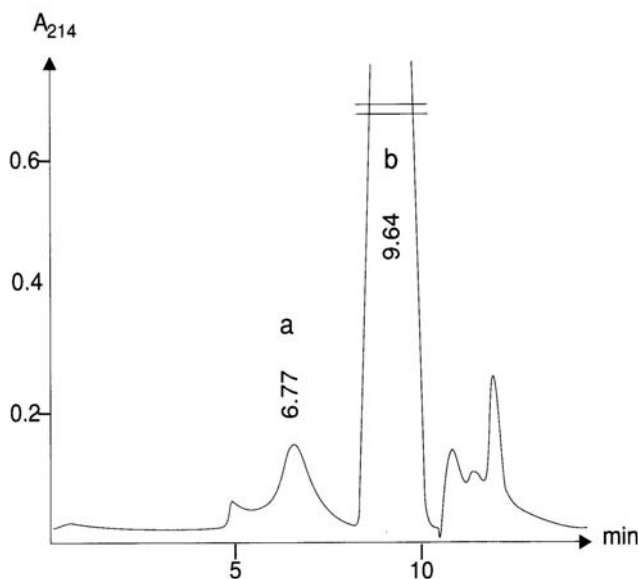


Figure 2. Desalting of ADH using WorkBeads™ 17/100 SEC at a flow rate of 2.0 cm/min. a) ADH, b) salt.

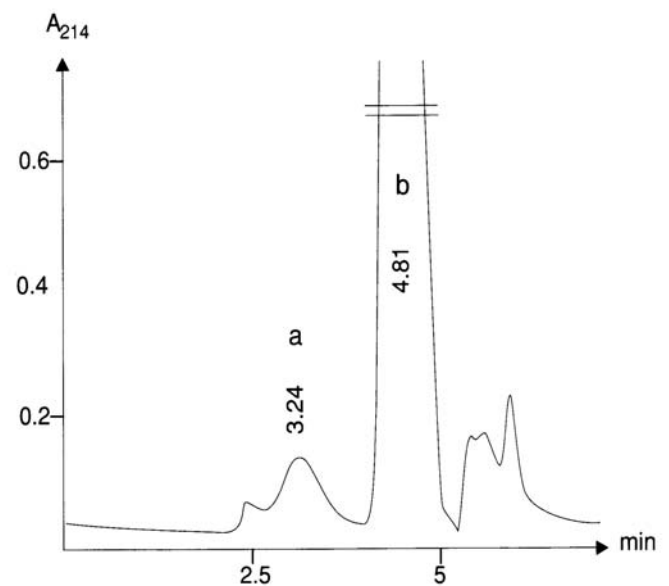


Figure 3. Desalting of ADH using WorkBeads™ 17/100 SEC at a flow rate of 4.0 cm/min. a) ADH, b) salt.

WorkBeads is a trademark of Bio-Works Company Limited.



Bio-Works Company Limited.
Visit us at www.bio-works.net
Email: info@bio-works.net