Journal of Chromatography, 266 (1983) 395–399
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 041

PURIFICATION OF NADP BY HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY

KARL-ARNE HANSSON
Pharmacia Fine Chemicals, Box 175, 75104 Uppsala (Sweden)

SUMMARY

The preparation of high-purity NADP was achieved by anion-exchange chromatography on Polyanion SI, a high-performance liquid chromatography matrix designed for biomolecular separations. The purification employed two gel materials which differed only in particle size (Polyanion SI-8 and -17 μm). All the chromatographic conditions were optimized on an analytical scale (8-μm exchanger) and then applied directly to preparative-scale chromatography (17-μm exchanger). Since the two gel matrices have been designed to be used in this way, "scaling up" meant only increasing the sample sizes and total volumes of the gradients. After a preparative run, the purity of the NADP was checked on an analytical column. The result indicated a higher purity reagent.

INTRODUCTION

With the recent progress made in biotechnology, there is an increasing need for high-purity reagents and test substances. The availability of new chromatographic media makes it possible to achieve better analytical separations, but often lacking are matrices designed for direct scale-up.

Although an analytical separation has been optimized, the scale-up procedure may introduce new problems. Such problems have been avoided in the present case by the use of Polyanion SI-8-μm material and its complement Polyanion SI-17 μm. During the synthesis of the 17-μm material, all parameters were chosen so as to provide the same separations as those achieved on Polyanion SI-8 μm but on a larger scale. In this way, the performance of the gels is so similar that scaling-up required only the programming of greater flow-rates. Chromatography on an analytical scale allowed minimal use of sample when determining the best chromatographic conditions. In this paper, optimal conditions are reported, along with chromatograms from analytical and preparative runs. A procedure for desalting of the purified material is also described.

EXPERIMENTAL

Apparatus

All equipment and gels were supplied by Pharmacia (Uppsala, Sweden). An

0021-9673/83/$03.00

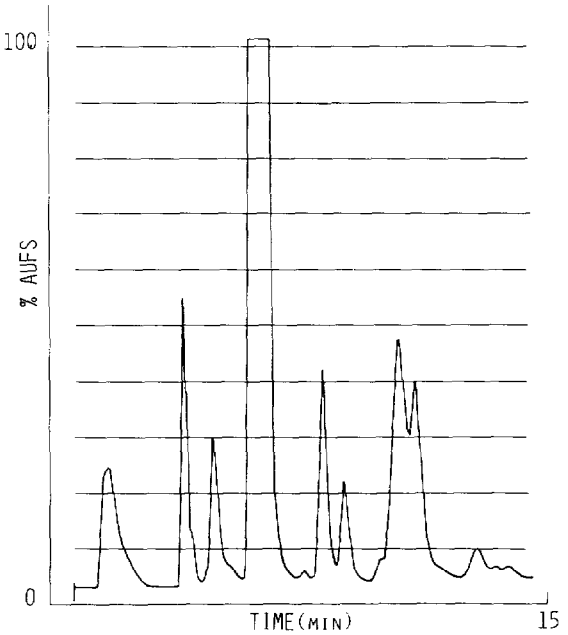

Fig. 1. Chromatogram of the optimal separation of a commercial grade NADP. Conditions: column, prepacked Polyanion SI HR 5/5 (0.5 × 5 cm); buffer A, 0.01 *M* phosphate pH 6.0; buffer B, 0.50 *M* phosphate pH 6.0; gradient, 0–100% B in 15 min; flow-rate, 1.0 ml/min (5.1 cm/min, linear); sample, 1.4 mg NADP in 500 μl of starting buffer; detection, 254 nm, 2.0 AUFS.

fast protein liquid chromatography (FPLC) system was used which consisted of two P-500 pumps, a Valve V-7 injector with a 10-ml Superloop, a GP-250 gradient programmer, a UV-1 monitor with a HR-10 cell, a FRAC-100 fraction collector and a REC-482 recorder. A prepacked column of Polyanion SI HR 5/5 (50 × 5 mm I.D.) was used for the optimization procedure, and bulk Polyanion SI-17 μm was dry-packed into a HR 10/10 column (100 × 10 mm I.D.), which was used for preparative chromatography. Desalting was performed in a HR 16/50 column (500 × 16 mm I.D.) containing Sephasorb® HP Ultrafine.

Reagents

All buffers and salts were obtained from Fluka (Buchs, Switzerland). The phosphate buffer was purified by the procedure described by Karkas *et al.*[1]. The NADP (nicotinamide–adenine dinucleotide phosphate, oxidized) was a commercial grade, Lot No. 150 1320 (Boehringer, Mannheim, G.F.R.).

Optimization

Optimal resolution (Fig. 1) was achieved in the pH range 4–6. The choice of eluting ion was not found to be critical; phosphate and acetate gave very similar results. In the example shown here, sodium phosphate (0.5 *M*) was used. If counter-ions with lower eluting power are used, it is necessary to use higher concentrations. For example, with ammonium acetate, the concentration of buffer B was increased to 3 *M* in order to achieve similar results to those obtained with sodium phosphate.

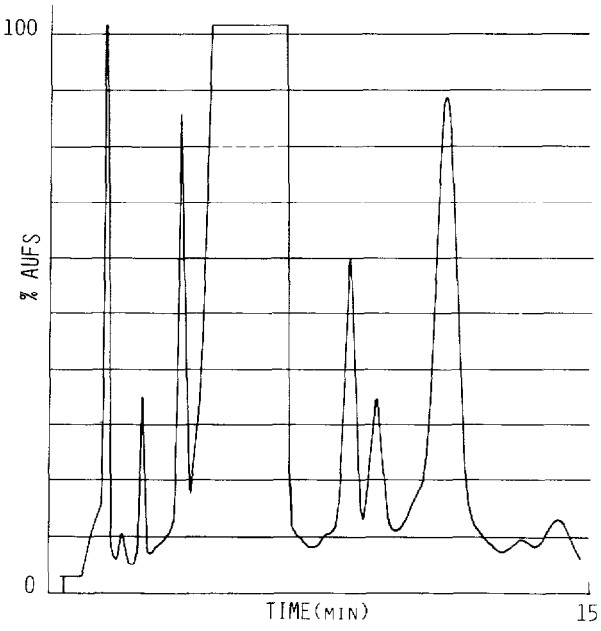

Fig. 2. Chromatogram of the preparative separation of a commercial-grade NADP. The fraction collected was the material which had a peak height greater than 80 % full scale. Conditions: column, Polyanion SI-17 μm in an HR 10/10 (1 × 10 cm); buffer A, 0.01 *M* phosphate pH 6.0; buffer B, 0.50 *M* phosphate pH 6.0; gradient, 0–100 % B in 15 min; flow-rate, 4.0 ml/min (5.1 cm/min, linear); sample, 10 mg NADP in 500 μl of starting buffer; detection, 254 nm, 2.0 AUFS.

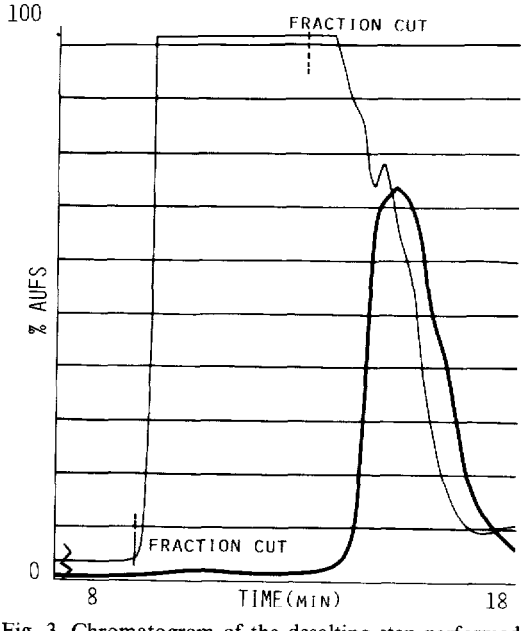

Fig. 3. Chromatogram of the desalting step performed on Sephasorb HP Ultrafine, UV absorbance is represented by the thin black line and conductivity by the thick black line. Separation conditions: column, HR 16/50 packed with Sephasorb HP Ultrafine; gel bed 46 × 1.6 cm; eluent, water–methanol (25:75); flow-rate, 4 ml/min; sample, 8-ml fraction from preparative chromatography; detection, 254 nm, 2.0 AUFS and conductivity.

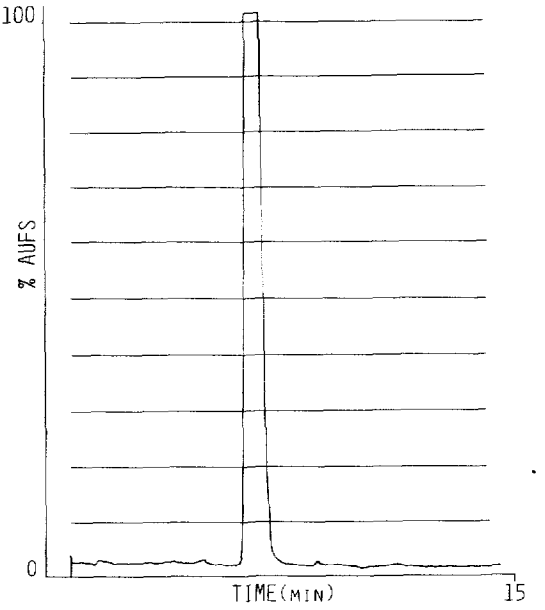

Fig. 4. Chromatogram of a purity check performed on material collected from the preparative separation.

Preparative separations

Polyanion SI-17-μm gel was dry-packed (tamp and fill) into a HR 10/10 column (10 × 1 cm I.D.). Approximately 5 g of material were used for the packing The column was equilibrated with start buffer and a 10-mg sample of NADP was applied to the column. In order to obtain the same gradient in the larger column, the flow-rate was increased by a factor of four (the linear flow velocity was identical). The main peak was collected as an $\approx$ 8-ml fraction. The chromatogram is shown in Fig. 2.

Desalting

Gel filtration was performed with Sephasorb HP Ultrafine packed into a 46 × 1.6 cm gel bed. (Sephasorb HP Ultrafine is similar to Sephadex LH-20 but it has a much higher matrix density and smaller particle size, 10–23 μm. This material provided high efficiency in the desalting step with minimal sample dilution.) The 8-ml fraction above was applied with a 10-ml Superloop. The NADP fraction was eluted, collected and dried. The gel-filtration chromatogram is shown in Fig. 3.

When ammonium acetate was used as the buffer, the fraction was desalted by freeze-drying. However, since some ammonium acetate remained, it was necessary to dissolve the sample in a small volume of water and freeze-dry a second time.

Purity check

The final step was to check the purity on the analytical column. An identical run to the optimized analysis was performed and the chromatogram is shown in Fig. 4.

RESULTS AND DISCUSSION

The procedure described provided an easy way to optimize the chromatographic conditions and then proceed to a scaled-up procedure, directly. The main advantages of this type of system are: (1) small amounts of sample are used during the optimization process, and (2) similarity of the two gel matrices simplified the scale-up procedure.

From the results reported here, it is reasonable to assume that the combination of Polyanion SI-8 μm and -17 μm could provide a suitable gel system for the purification of many biomolecules. With improved analytical methods, high quality and reproducible test substances for research and quality control are essential. To ensure the required purity of test substances, purification before use is often necessary. Polyanion SI-8 and -17 μm provide a convenient method for optimization and scale-up.

REFERENCE

1 J. D. Karkas, Y. Germershausen and R. Liou, *J. Chromatogr.*, 214 (1981) 267.