Essentials in bioresearch

94-0510

Instrumentation, Chemistries, and Expertise for Bio-Synthesis and Separations

A New Strategy for Rapid Optimization of Protein Separations

Maximize Recovery and Specific Activity

Waters exclusive Auto·Blend™ method offers a systematic approach to optimizing protein separations for maximum recovery and resolution.[1,2] The Waters 650 Advanced Protein Purification System incorporates this technology into an instrument designed for fast protein liquid chromatography. This system is capable of precise, reproducible delivery and accurate blending of four buffers.

The Auto·Blend method utilizes this multi-buffer capability through the selection of blends that automatically produce buffer of the desired pH and ionic strength. By quickly screening a wide pH and ionic strength range, conditions that provide the best resolution and recovery are achieved.

Adjust pH and Ionic Strength Automatically

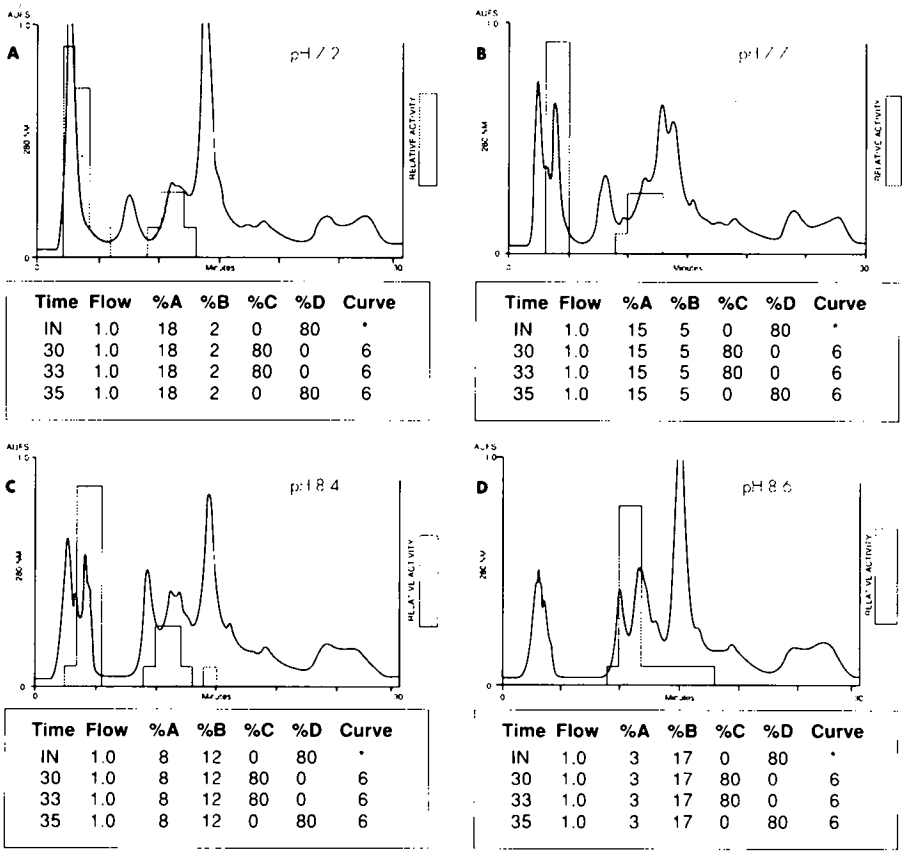
The conventional approach to chromatographic separations of proteins requires the careful preparation of buffers of predetermined pH and ionic strength. Separations in protein ion exchange chromatography, for example, are most conveniently optimized by eluting the protein of interest with an increasing salt gradient at several pH values. This conventional "optimization" requires the repetitive preparation of numerous pairs of pH buffers.

Auto·Blend capability makes optimizing separation protocols easy. A single set of stock solutions enables the protein chemist to select blends that produce the desired pH and ionic strength. By simply programming the 650 system to deliver different proportions of the four solutions (acid salt, basic salt, 1.0 M NaCl, and Milli-Q™ water), a systematic approach to screening for the best conditions is readily available. In addition, this approach makes chromatography more reproducible by reducing errors in buffer preparation and titration.

Protein Separations Respond to Subtle pH Changes

Complex biological samples typically contain many proteins with varying isoelectric points or surface charge properties. The pH of the buffer selected for the separation of these samples can dramatically affect both the recovery and resolution achieved. This is illustrated by examining the effect of pH on the protein mass and enzymatic activity profile for a crude mouse liver extract separated on a Waters Protein-Pak DEAE 8HR anion exchange column (Fig 1). By systematically varying the pH from 7.2 to 8.6, significant changes in the resolution of the total protein are seen, and various isozymes of lactate dehydrogenase are separated. There is a particularly large difference in the retention of the major activity peak (LDH-A_4) relative to the bulk of the protein between pH 8.4 and pH 8.6.

Figure 1: Purification of Mouse Liver Lactate Dehydrogenase



Time	Flow	%A	%B	%C	%D	Curve
IN	1.0	18	2	0	80	*
30	1.0	18	2	80	0	6
33	1.0	18	2	80	0	6
35	1.0	18	2	0	80	6

Time	Flow	%A	%B	%C	%D	Curve
IN	1.0	15	5	0	80	*
30	1.0	15	5	80	0	6
33	1.0	15	5	80	0	6
35	1.0	15	5	0	80	6

Time	Flow	%A	%B	%C	%D	Curve
IN	1.0	8	12	0	80	*
30	1.0	8	12	80	0	6
33	1.0	8	12	80	0	6
35	1.0	8	12	0	80	6

Time	Flow	%A	%B	%C	%D	Curve
IN	1.0	3	17	0	80	*
30	1.0	3	17	80	0	6
33	1.0	3	17	80	0	6
35	1.0	3	17	0	80	6

The Auto·Blend method was used to identify the best pH for isolation of lactate dehydrogenase (LDH) from mouse liver. The pH was systematically adjusted by programming changes within the gradient table.

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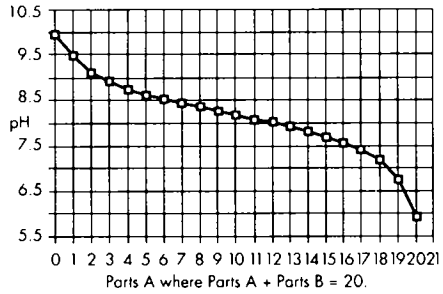
Easily Program the Desired pH

Waters has developed Auto•Blend tables to help get you started. These tables, generated by varying integral ratios of acidic and basic buffers (A and B), provide a reference point for adjusting pH to the desired level. By simply programming different proportions of A and B, the 650 will automatically blend buffer of a desired pH (see Table 1 for Tris buffers). Buffer concentration is maintained by keeping the sum of A and B constant. The ionic strength of the final buffer is controlled independently by varying the proportions of 1.0 M NaCl and water.

Table 1: Auto•Blend Table for Tris Buffer System

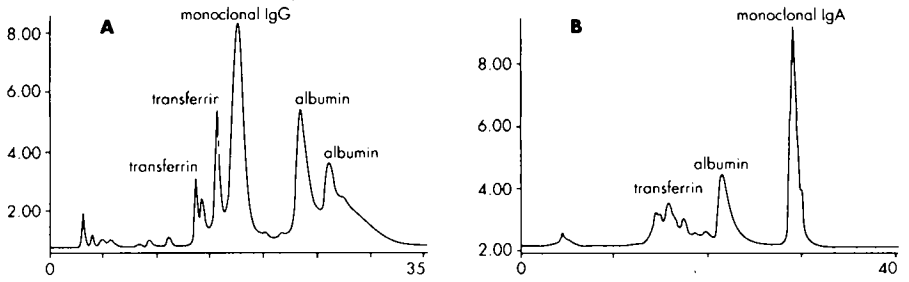


Data generated by precise instrument blending of 100mM stock Tris solutions with Milli-Q water to create 20mM Tris/Cl buffers of various pH values. Buffer lines A=100mM Tris HCl, B=100mM Tris base, C=1.0M NaCl, D=Milli Q water.

Simultaneous pH and Ionic Strength Gradients

Additional selectivity in ion exchange separations can be achieved by independent pH and ionic strength gradient profiles. For example, the purification of monoclonal antibodies present in ascitic fluids, tissue culture or serum-free media is best performed using a concurrent buffer pH and ionic strength gradient[3]. The Auto•Blend method simplifies this purification procedure as shown by the separation of monoclonal IgG from mouse ascites (Figure 2A). Due to the diversity of monoclonal subclasses and sample sources, modifications in the established separation protocol may be required. Independent programming of buffer pH and ionic strength on the 650 system significantly improves the separation and recovery of a monoclonal IgA from ascites (Figure 2B).

Figure 2: Purification of Monoclonal Antibodies



Eluent A = 100mM Tris HCl **Eluent B =** 100mM Tris Base **Eluent C =** 1.0M NaCl **Eluent D =** Milli-Q water

Time	Flow	%A	%B	%C	%D	Curve
Initial	1.0	6	14	0	80	*
30	1.0	19	1	30	50	6

Time	Flow	%A	%B	%C	%D	Curve
Initial	1.0	6	14	0	80	*
10	1.0	12	8	15	65	6
20	1.0	19	1	50	30	6

A: Separation of 2 ml of mouse ascites containing monoclonal IgG using an optimized concurrent pH and ionic strength gradient profile on a Protein-Pak™ DEAE 5PW anion exchange column
B: Auto•Blend optimized method for isolation of monoclonal IgA from mouse ascites.

Note: Peaks were collected and identified by SDS PAGE under reducing conditions.

The Best System for Fast Protein LC

The Waters 650 is the only system optimized for fast protein LC with built-in Auto•Blend capability. Use the 650 at any step of your protein purification protocol. Transfer your open column separations to an automated instrument and improve reproducibility as well as initial recovery and resolution. Perform final polishing using high resolution chemistry on the same instrument. Purify as much protein as you need by taking advantage of system and column capacity up to the multigram scale. Experience fast, error-free programming and editing of methods with instant, real time monitoring of system operating parameters and status.

References

1. Warren, W., et.al, (1989) American Biotechnology Laboratory, 7(6), 34-40.

2. Warren, W. et.al. (1990), Journal of Chromatography, 512, 13-22.

3. Gemski, M.J., et.al., (1985) BioTechniques 3(5), 378-384.

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