

Sodium Accelerated Buffer System



The continual increase in sample numbers in busy labs means that it is often difficult for Quality Control or Contract Analysis Labs to maintain short turnaround times, particularly when instruments are already running at full capacity.

To address the need for faster analysis while retaining the quality of separation offered by dedicated amino acid analysers, an improved formulation of sodium citrate based buffers has been developed by Biochrom.

Introduction

The Accelerated Buffer System consists of a set of four buffers, with pH varying between 3.2 and 9.2, and a regeneration solution (sodium hydroxide).

The Accelerated Buffer System can be used for the analysis of both simple protein hydrolysate and oxidised protein hydrolysate samples.

Experimental conditions

The Accelerated Buffer System can be used on the Biochrom 31 Protein System or Biochrom 32 Oxidised Protein System, with no special conditions being required (the system is compatible with all Biochrom sodium columns).

The buffer flow rate and the ninhydrin flow rate remain the same as the other sodium high performance systems, i.e. 35 ml/hr and 25 ml/hr respectively.

Results

As shown on the standard chromatogram (figure 1), using this new system, Arginine elutes at around 45 mins. compared to 60 mins. for the standard oxidised high performance system.

The analytical programme has been specially developed to achieve optimum separation using the Accelerated Buffer System. Details of the experimental conditions are given in figure 2.

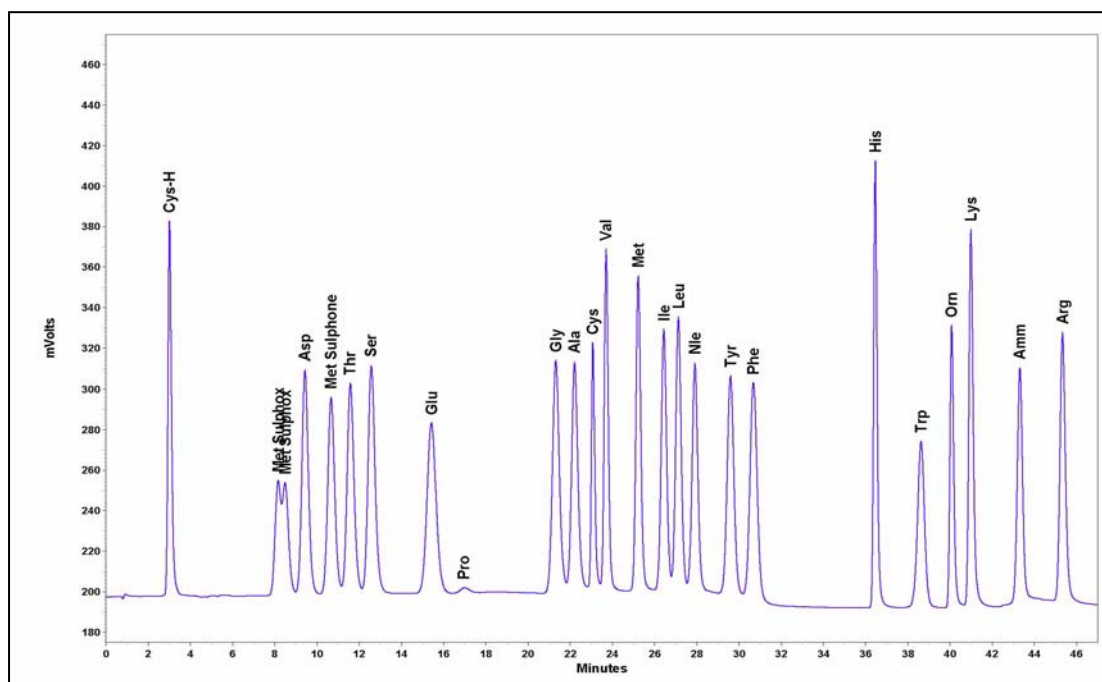


Figure 1: Oxidised Protein Hydrolysate Standard

The separation between the amino acids of interest, especially the sulphur containing amino acids methionine sulphoxide and methionine sulphone, is retained in this system.

The order of elution of methionine sulphone and aspartic acid, is reversed compared to the standard oxidised system.

Sample: <u>Oxidised Protein Hydrolysate Standard</u>	Amount Loaded: <u>5</u>	nmol
Column Type: <u>Peek</u>	Column Number: <u>u-1521</u>	Resin Batch: <u>11741</u>
Bed Length (mm): <u>200</u>	Diameter (mm): <u>4.6</u>	Instrument Serial Number: <u>Bio 30</u>
Test Number: <u>n/a</u>		
	Buffer	Nin
Flow Rate (ml/h):	<u>35</u>	<u>25</u>

	<u>Buffer</u>	<u>Molarity</u>	<u>pH</u>	<u>Batch No.</u>
Buffer 1 -	Accelerated Buffer A		3.25	13815
Buffer 2 -	Accelerated Buffer B		3.50	12241
Buffer 3 -	Accelerated Buffer C		4.40	13992
Buffer 4 -	Accelerated Buffer D		9.23	13810
Buffer 5 -	n/a	n/a	n/a	n/a
Buffer 6 -	Sodium Hydroxide Solution	0.40		13638
Reagent	Ninhydrin			13551
	Ultrasolve			13572

Optimisation guide

<u>Compound</u>	<u>Optimisation</u>
Met slx/Asp:	Decrease T1 Increase time of buffer 1 Increase equilibration time
Asp/Met sln:	Increase equilibration time
Met sln/Thr:	Increase T1 Increase T2 Decrease time of buffer 1
Gly/Ala:	Increase time of buffer 1 Increase time of buffer 2
Ala/Cys:	Increase time of buffer 1 Increase time of buffer 2
Cys/Val:	Decrease time of buffer 1
Ileu/Leu:	Increase time of buffer 3 Decrease T2
Leu/Nleu:	Increase time of buffer 3
Trp/Orn:	Increase T3

Title: Sodium Accelerated Buffer System
Filename: C:\Program Files\BioSys\Programs\Accelerated Buffers.prg
Nin Flow Rate: 25.0 ml/h

<u>No.</u>	<u>Time</u>	<u>Temp</u>	<u>Buffer</u>	<u>Pump</u>	<u>Nin</u>	<u>Rec</u>	<u>Commands</u>
1	01:00	40°C	1	35.0ml/h	ON	OFF	
2	00:00	40°C	1	35.0ml/h	ON	OFF	Reset
3	01:00	40°C	1	35.0ml/h	ON	OFF	Load
4	04:00	40°C	1	35.0ml/h	ON	ON	
5	05:00	51°C	2	35.0ml/h	ON	ON	
6	13:00	51°C	3	35.0ml/h	ON	ON	
7	18:00	94°C	4	35.0ml/h	ON	ON	
8	04:00	94°C	6	35.0ml/h	ON	ON	
9	04:00	94°C	1	35.0ml/h	ON	ON	
10	04:00	40°C	1	35.0ml/h	OFF	OFF	
11	04:00	40°C	1	35.0ml/h	ON	OFF	

Figure 2: Experimental conditions

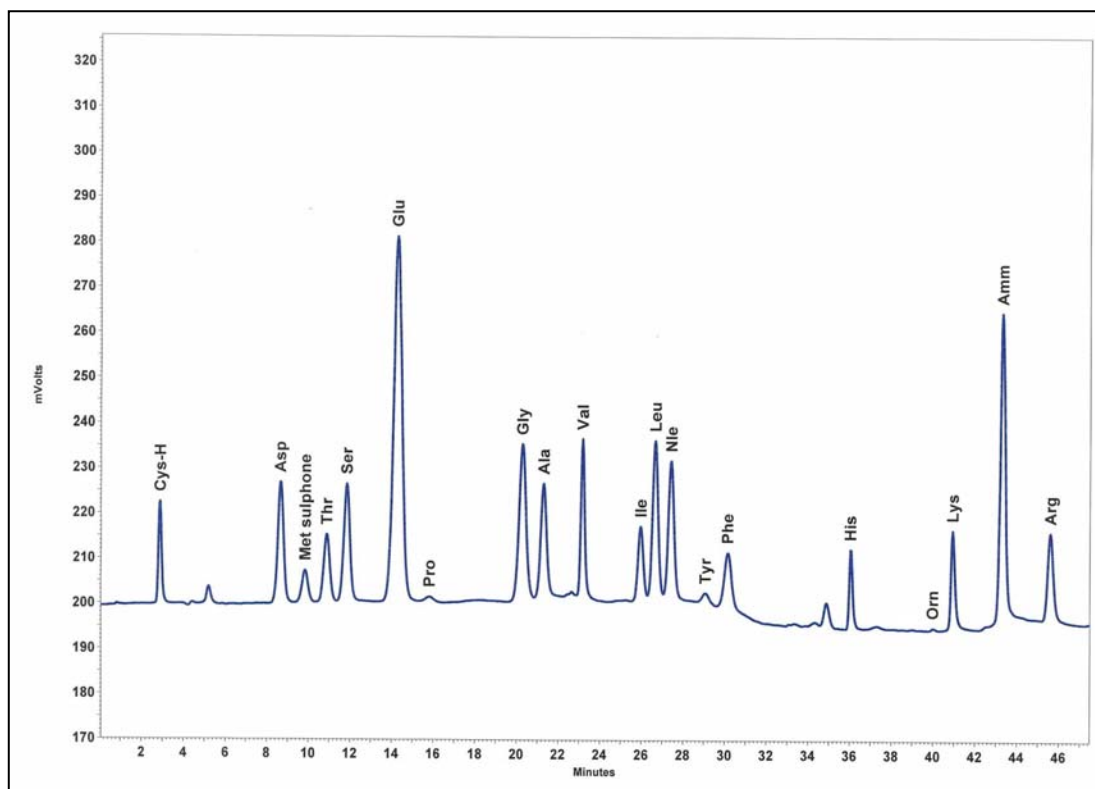


Figure 3: Oxidised Protein Hydrolysate Sample

In addition to a significant reduction in analysis time, other advantages of this system include a smoother baseline under Cystine which allows accurate quantification of this amino acid.

The system also offers more flexibility for the analysis of other less common amino acids as described in Application note B30-1.

Conclusion

The Accelerated Buffer System enables the total analysis time to be reduced by up to 30%, which is equivalent to 7 additional runs per day. By reducing the run times, the buffer and ninhydrin consumptions are also reduced. The Accelerated Buffer System is therefore an attractive alternative to the classic oxidised system, particularly for customers for whom speed of analysis is critical.

Further reading

Application Note: B30-1 Improved analysis of feedstuffs
 Application Note: B30-10 Rapid analysis of lysine for feedstuffs applications
 Brochure # 80-6000-27 Amino Acid Analysis of Food and Feedstuffs

Ordering information

80-2115-26 Accelerated Buffer Chemical Kit