# 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.

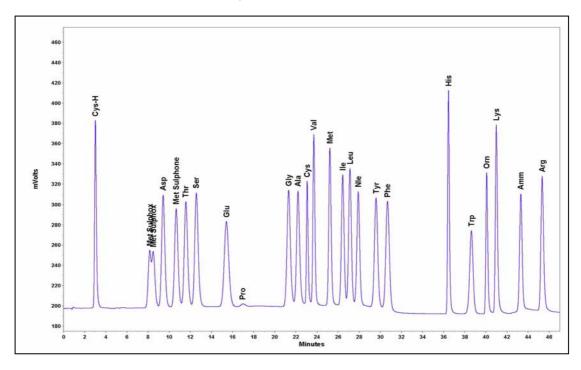


Figure 1: Oxidised Protein Hydrolysate Standard

Application Note: Bio30.14

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.

Column Type: Peek         Column Number: u-1521         Resin Batch: 11741           Bed Length (mm): 200         Diameter (mm): 4.6         Instrument Serial Number: Bio 30           Test Number: n/a           Buffer Nin           Flow Rate (ml/h): 35         25           Molarity pH Batch No.           Buffer 1 - Accelerated Buffer A Buffer 2 - Accelerated Buffer B 3.50 12241           Buffer 3 - Accelerated Buffer C 4.4.0 13992         3.50 12241           Buffer 4 - Accelerated Buffer D 9.23 13810         9.23 13810           Buffer 5 - n/a 10/a 13638         13638           Reagent Ninhydrin Ultrosolve         13551         13551           Ultrosolve         13572	Sample: Oxidised Protein Hydrolysate Standard					t Loaded: <u>5</u>	nmol
Buffer   Nin	Column Type: Peek Column Number: u-152				Resin E	eatch: 11741	
Buffer   Nin	Bed Length (mm): 200	Diamete	er (mm): 4.6		Instrum	ent Serial Number:	<u>Bio 30</u>
Buffer   Molarity   pH   Batch No.	Test Number: n/a						
Buffer   Molarity   pH   Batch No.							
Buffer         Molarity         pH         Batch No.           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           Buffer 6 - Sodium Hydroxide Solution         0.40         13638           Reagent         Ninhydrin         13551		Buffer	Nin				
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         Buffer 6 -       Sodium Hydroxide Solution       0.40       13638         Reagent       Ninhydrin       13551	Flow Rate (ml/h):	<u>35</u>	<u>25</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         Buffer 6 -       Sodium Hydroxide Solution       0.40       13638         Reagent       Ninhydrin       13551							
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         Buffer 6 -       Sodium Hydroxide Solution       0.40       13638         Reagent       Ninhydrin       13551	21.101			200.0	.100		
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         Buffer 6 -       Sodium Hydroxide Solution       0.40       13638         Reagent       Ninhydrin       13551	<u>Buffer</u>			<u>Molarity</u>	<u>pH</u>	Batch No.	
Buffer 3 -       Accelerated Buffer C       4.40       13992         Buffer 4 -       Accelerated Buffer D       9.23       13810         Buffer 5 -       n/a       n/a       n/a         Buffer 6 -       Sodium Hydroxide Solution       0.40       13638         Reagent       Ninhydrin       13551	Buffer 1 - Accelerated	Buffer A			3.25	13815	
Buffer 4 -       Accelerated Buffer D       9.23       13810         Buffer 5 -       n/a       n/a       n/a         Buffer 6 -       Sodium Hydroxide Solution       0.40       13638         Reagent       Ninhydrin       13551	Buffer 2 - Accelerated	2 - Accelerated Buffer B			3.50	12241	
Buffer 5 - n/a n/a n/a n/a Buffer 6 - Sodium Hydroxide Solution 0.40 13638 Reagent Ninhydrin 13551	Buffer 3 - Accelerated Buffer C				4.40	13992	
Buffer 6 - Sodium Hydroxide Solution 0.40 13638 Reagent Ninhydrin 13551					9.23	13810	
Reagent Ninhydrin 13551					n/a	n/a	
Ultrosolve 13572							
	Ultrosolve					13572	

## Optimisation guide

<u>Title:</u> Filen:	=)	Sodium Accelerated Buffer System  C:\Program Files\BioSys\Programs\Accelerated Buffers.prg					
	low Rate:		25.0 ml/h	you rogamisa to		v.sp.g	
<u>No.</u>	Time	Temp 40°C	Buffer	Pump	<u>Nin</u> ON	Rec	Commands
2	01:00 00:00	40°C	1	35.0ml/h 35.0ml/h	ON	OFF 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	Loud
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	

1		
	Compound	Optimisation
	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
	lleu/Leu:	Increase time of buffer 3
		Decrease T2
	Leu/Nleu:	Increase time of buffer 3
	Trp/Orn:	Increase T3

Figure 2: Experimental conditions

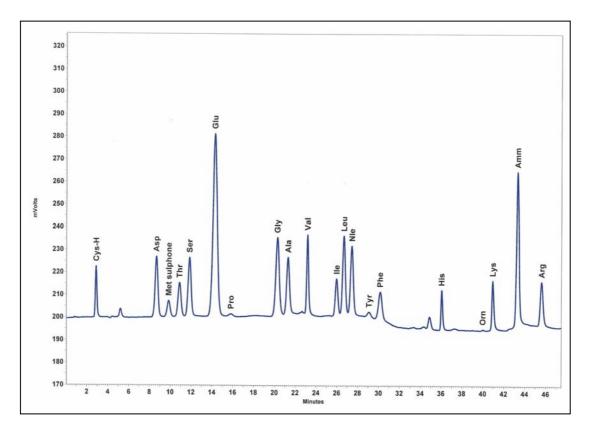


Figure 3: Oxidised Protein Hydrolysate Sample

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