AMINO ACID ELIMINATION IN CHROMATOGRAPHIC MOLASSES SEPARATION SYSTEMS

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I. Introduction

Chromatographic separation processes for the recovery of sucrose from sugar beet molasses operate by means of several different chemical mechanisms. For example, large molecules such as dextran and other polysaccharides are separated from sucrose by *size exclusion*. As a molasses solution travels through a bed of low-crosslinked cation exchange resin, the relatively small sucrose molecules diffuse through resin beads and are somewhat retarded in flow through the bed. Large molecules such as dextran and other polysaccharides are excluded from resin and thus travel through the bed more rapidly than sucrose while smaller molecules such as the invert sugars, glucose and fructose, will travel through the bed more slowly than sucrose. Sucrose can therefore be separated from other carbohydrates based on this size exclusion mechanism.

A different separation mechanism operates in the case of strongly ionized salts such as inorganic sulfates, chlorides, and nitrates as well as salts of simple organic acids. Here, although the ions in solution are small in size, the resin matrix tends to reject strong electrolytes, and they travel ahead of the sucrose which diffuses through resin beads. Therefore, ionic salts are readily separated from sucrose by this *ion exclusion* mechanism. Removal of strongly ionized materials such as the inorganic salts is typically very efficient with up to 95% of such electrolytes removed in the raffinate fraction.

The free amino acids, which occur at significant levels in sugar beet molasses, are small molecules and might be expected to travel through a separation resin column at rates near or lower than that of sucrose. However, because of their zwitterionic nature, amino acids present a interesting variety of behaviors during chromatographic sucrose recovery. This behavior has been the subject of extensive study in our laboratory and partial results were given in a previous communication.[1]

II. Results and Discussion

Because amino acids possess both acidic (carboxylic acid) and basic (amine) functional groups they can, depending on solution pH, exist as the anion, cation, or the zwitterion (both positive and negative charge). This is represented for the simplest amino acid, glycine, by the following equilibrium equations:

$$\underset{\text{(Anion)}}{H_2N\text{-}CH_2CO_2^-} + H^+ \rightleftharpoons \underset{\text{(Zwitterion)}}{H_3N^+\text{-}CH_2CO_2\text{-}}$$

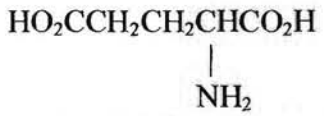
$$\underset{\text{(Zwitterion)}}{H_3N^+\text{-}CH_2CO_2^-} + H^+ \rightleftharpoons \underset{\text{(Cation)}}{H_3N^+\text{-}CH_2CO_2H}$$

In basic solution the amino acid will be present primarily as the carboxylate anion but as acidity increases the zwitterionic form will become predominant. Finally, in stronger acid, the ammonium ion (cation) will predominate. A key parameter of amino acid chemistry is the *isoelectric point,* which is defined as the pH at which the concentration of the zwitterionic form is highest. Isoelectric points of common amino acids are given in Table 1.

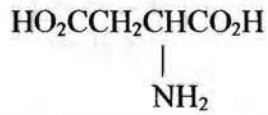
Table 1. Isoelectric Point of Amino Acids.

Amino Acid	Isoelectric Point
Glutamic Acid	2.76
Aspartic Acid	3.24
Tyrosine	5.65
Serine	5.68
Isoleucine	6.02
Leucine	5.98
Alanine	6.02
Asparagine	5.41
Proline	6.10
Valine	5.97
Threonine	5.16
Lysine	9.82
Arginine	10.76

Many of the amino acids at significant levels in sugar beet molasses (alanine, tyrosine, serine, threonine, leucine, and isoleucine) have isoelectric points in the range of 5.5 to 6.0. This means that at the neutral to slightly basic pH levels common in molasses the zwitterion, will be the important species in solution. Since the zwitterion has a net ionic charge of zero, these amino acids tend to behave somewhat like uncharged small molecules and travel with sucrose in the separation process (as will be shown in the following amino acid elimination data). The amino acids with isoelectric points higher than 8.0 (lysine and arginine) are not present at significant levels in sugar beet molasses but two of the major amino acids in beets, glutamic acid (1) and aspartic acid (2), have isoelectric points of 2.7 and 3.2 respectively due to the fact that they each have two carboxylic acid functional groups. These two amino acids would exist predominantly as the zwitterion only in acidic solutions and instead at normal molasses pH are farther toward the basic or anion end of their acid-base equilibria. Glutamic and aspartic acids therefore behave in the separator like strongly ionized compounds and are nearly completely eliminated in the raffinate stream with other salts.



Glutamic Acid
1



Aspartic Acid
2

A. Amino Acids in Conventional Simulated Moving Bed (SMB) Operation.

Table 2 gives levels of the major α-amino acids in a typical feed molasses and product (extract) from a conventional SMB separation system (this data is also shown as a bar chart in Fig. 1). Levels are included for γ-aminobutyric acid, which is not one of the common α-amino acids found in proteins but is a significant constituent of molasses and behaves similarly to the α-amino acids. In addition, values are given for percent of entering amino acid that is eliminated in the raffinate. Note that glutamic and aspartic acids, due to their more strongly ionic nature, are eliminated at 90% or higher levels to the raffinate stream and are present at substantially lower levels in extract than in molasses. In contrast, for most of the other amino acids elimination values range from 13 to 74% and levels in extract are actually higher than those in molasses, on a solids basis. In this example, amino acids in the extract total nearly 3%/RDS and are a significant fraction of the non-sugars remaining in extract.

Table 2. Amino Acid Levels in Conventional SMB Separation.

Amino Acid	Feed Molasses (g/100 RDS)	Extract (g/100 RDS)	Elimination (g/100 g entering)
Glutamic Acid	0.60	0.071	94
Aspartic Acid	0.46	0.093	90
Tyrosine	0.30	0.51	13
Serine	0.27	0.48	20
Isoleucine	0.25	0.38	22
γ-aminobutyric acid (GABA)	0.24	0.25	63
Leucine	0.23	0.27	39
Alanine	0.21	0.30	28
Asparagine	0.20	0.10	74
Proline	0.18	0.11	69
Valine	0.18	0.29	16
Total	3.12	2.85	

Another interesting feature of amino acid behavior during SMB recovery of sugar from molasses is a tendency for some amino acids to accumulate in the separator internal inventory. When a separation system reaches an equilibrium condition the exiting quantity of every component equals the entering quantity of the same component. However, the quantity and concentration of a component recirculating in the internal recycle stream depends on the quantities entering and exiting as the equilibrium condition is approached. As an example of this, the overall sucrose purity of the internal recycle stream may be increased by limiting sucrose in extract removal during system equilibration. The increased internal purity may then be used to allow removal of a higher purity

extract stream during equilibrium operation. In the same way, amino acids that are not easily removed in the raffinate stream may accumulate to higher levels within the separator system. The concentration factors for some of the amino acids, however, are much higher than those for major components like sucrose. Table 3 (or bar chart Fig. 2) gives levels for major amino acids in molasses and in a composite of the internal recycle stream during typical SMB operation.

Table 3. Amino Acid Levels in the Separator Internal Recycle Stream.

Amino Acid	Feed Molasses (g/100 RDS)	Internal Recycle (g/100 RDS)	Ratio (Internal/Feed)
Glutamic Acid	0.55	0.22	0.40
Aspartic Acid	0.43	0.24	0.56
Tyrosine	0.37	0.39	1.05
Serine	0.29	0.82	2.83
Isoleucine	0.31	0.29	0.94
γ-aminobutyric acid (GABA)	0.63	0.59	0.94
Leucine	0.28	0.27	0.96
Alanine	0.18	0.20	1.11
Asparagine	0.14	0.72	5.14
Proline	0.17	0.13	0.76
Valine	0.19	0.20	1.05
Threonine	0.07	0.65	9.29

Note that the two easily-removed amino acids, glutamic and aspartic acids, are in lower levels in the recycle stream than in molasses. Most of the other amino acids are at levels comparable to the feed stream but asparagine and the two low molecular weight hydroxyl-containing amino acids, serine and threonine, accumulate in the separator to levels of three to nine times the levels present in feed molasses. In this particular set of data, serine, threonine, and asparagine, which are far from being the predominant amino acids in molasses, become the major amino acids present in a composite of the internal recycle stream. The tendency for serine and threonine to accumulate in the internal separator inventory has been observed in other tests of the recycle stream composite composition.

B. Amino Acids in Coupled Loop SMB Operation.

The ARi Coupled Loop SMB system is designed to improve the elimination of betaine from sugar-containing product as well as improve the recovery of betaine. This is achieved by operating the first loop under conditions which allow sucrose to be collected in the leading or "upgrade" fraction (along with salts and high molecular weight materials) while the later, or "betaine", fraction contains much of the betaine and other compounds that are retarded in passing through the resin bed. The

second loop then operates more like a conventional ion exclusion system with separation of sucrose from the salts and high molecular weight materials to produce the final extract, or sugar-containing fraction, and raffinate, or non-sugar fraction. One might expect that the amino acids with isoelectric points in the 5.5 to 6.0 range, being low in molecular weight, would lag enough behind sucrose to be removed in the betaine fraction. However, it turns out that, with the exception of possibly leucine and isoleucine, most of these amino acids still behave enough like sucrose in chromatographic mobility that they travel through the entire Coupled Loop system with sucrose and end up in the extract fraction. Results of amino acid analysis of one typical set of weekly composite samples from a Coupled Loop system are given in Table 4 (or bar chart in Fig. 3). In this particular test, betaine elimination from the extract was approximately 88% but, except for the usual easily removed glutamic and aspartic acids, amino acid elimination was not very high. The only exceptions are leucine and isoleucine which are typically only 20-40% eliminated in a conventional separator but are 40-50% eliminated in Coupled Loop systems. Since leucine and isoleucine are two of the more important amino acids in molasses, increased elimination of them is an advantage but certainly nowhere near the benefit of the higher betaine elimination.

Table 4. Amino Acids in Coupled Loop SMB Operation

Amino Acid	Feed Molasses (g/100 RDS)	Extract (g/100 RDS)	Elimination (g/100 g entering)
Glutamic Acid	0.48	0.00	100
Aspartic Acid	0.44	0.06	85
Tyrosine	0.27	0.45	11
Serine	0.27	0.33	26
Isoleucine	0.27	0.30	43
γ-aminobutyric acid (GABA)	0.43	0.48	42
Leucine	0.24	0.27	44
Alanine	0.10	0.22	14*
Total	2.23	2.11	

*Levels too low for accurate material balance calculation.

C. Amino Acids in Block Displacement SMB Operation.

An older technology for betaine removal and improved betaine elimination is referred to as the Block Displacement method. This method simply involves the direct displacement of a selected non-sugar portion of the separator internal recycle stream with water. This displacement can occur for all or part of a system step and is repeated at the same portion of the recycle profile during each step. The removed material is then evaporated as a betaine byproduct stream. The Block Displacement technique is relatively easy to implement and operate but is not as efficient as the Coupled Loop process in betaine removal and recovery. Amino acid removal by the Block Displacement technique may vary with exact operating parameters but typical results from weekly composite samples

collected from a factory system are given in Table 5 (or bar chart in Fig. 4).

Table 5. Amino Acid Elimination in the Block Displacement SMB Operation

Amino Acid	Feed Molasses (g/100 RDS)	Extract (g/100 RDS)	Elimination (g/100 g entering)
Glutamic Acid	0.52	0.13	81
Aspartic Acid	0.49	0.11	87
Tyrosine	0.25	0.13	64
Serine	0.21	0.25	23
Isoleucine	0.25	0.12	65
γ-aminobutyric acid (GABA)	0.39	0.15	72
Leucine	0.21	0.09	69
Alanine	0.23	0.10	70
Valine	0.21	0.11	63
Total	2.76	1.19	

This data shows that, with the exception of serine, the elimination of amino acids in Block Displacement operation is generally better than either the conventional SMB or Coupled Loop systems. In this particular set of data, glutamic and aspartic acids are unusually high in extract, but even including those higher levels the total of measured amino acids in extract is only about 1.19 g/100 RDS or about one third to one half of the level in conventional or Coupled Loop system extract. Even though the Block Displacement method is not as efficient in betaine elimination, the direct removal of a portion of the recycling non-sugar profile with its relatively high levels of amino acids evidently results in a somewhat better elimination of most of those amino acids that are generally more difficult to remove.

III. Experimental

Amino acids were analyzed by conversion to phenylthiocarbamyl derivatives by reaction with phenylisothiocyanate.[2,3] Derivatives were analyzed by high performance liquid chromatography (HPLC) using the Waters Pico Tag™ method (trimethylamine/sodium acetate/60% acetonitrile gradient).

IV. References

1. Rearick, D. Eugene and Cheri McKay; Proceedings of the 1996 Conference on Sugar Processing Research, New Orleans, LA, April 14-17, 1996, p. 481-491.

2. Bidlingmeyer, B.A., S.A. Cohen, and T.L. Tarvin, *J. Chromatogr.*, **336**, 93 (1984).

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